Effect of harvest time and frequency on biomass quality and biomethane potential of common reed (*Phragmites australis*) under paludiculture conditions

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Keywords

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Abstract

This study examined the effect of harvest time (from May to September) and dry matter partitioning on biomethane potential and methane yield per unit area of a *Phragmites australis* cultivation under paludiculture conditions. The experimental site is part of a larger experimental platform (San Niccolò, Pisa) located within the Massaciuccoli Lake Basin in Central Italy (Tuscany, IT). The study also took into account the double cut strategy by evaluating the regrowth from June to September.

Biomethane potentials ranged from 384 to 315 and from 412 to 283 NL CH_4 $kgVS^{-1}$ (normal liters of methane per kg of volatile solids) for leaves and stems, respectively. About digestion kinetics, maximum daily production rate (R_{max}) was significantly affected by harvest time and not by plant partitioning. Along the harvest season, biomethane yield per unit area was mostly driven by the biomass yield showing an increasing trend from May (1659 Nm^3 ha^{-1}) to September (3817 Nm^3 ha^{-1}). The highest value was obtained with the double harvest option (4383 Nm^3 ha^{-1}), although it was not statistically different from the single harvest carried out in September. Owing to its remarkably

lower yields, *P. australis* cannot be considered along the same lines as crops conventionally used for biogas production, but it may represent an interesting option for paludiculture cropping systems by coupling peatland restoration with bioenergy production. September harvest management seemed the most feasible option, although further investigation on crop lifespan is needed for the different harvest options.

1. Introduction

Peatlands are efficient systems for carbon and nutrient storage on a global scale, as they cover only 3% of global land area but store more than 30% of total organic carbon [1]. Although natural peatlands are net nutrient sinks, their drainage for agricultural use does turn these ecosystems into net sources of CO_2, CH_4 and N_2O [2, 3]. Indeed, Couwenberg et al. [4] estimated that agricultural drained peatlands can release up to 50 t ha^{-1} $year^{-1}$ of CO_2 and up to 60 kg ha^{-1} $year^{-1}$ of N_2O, which is 265 times more potent than CO_2 over the 100-year horizon [5]. Moreover, peatland drainage is responsible for both internal and external eutrophication [6] and land subsidence [7].

Conversely, peatlands rewetted for paludiculture may contribute to reduce nutrient losses to the nearby environment and to climate change mitigation in two ways: (i) by reducing greenhouse gas emissions from soils and (ii) by replacing fossil resources with the production of renewable biomass alternatives [8]. Paludiculture, defined as the cultivation on wet or re-wetted peatlands to produce biomass for bioenergy, raw materials and other supply chains [9], is a relatively new peatland restoration approach. After rewetting, peat formation is stimulated and positive effects on greenhouse gases, carbon balance, and ground-water and surface-water quality have been observed [10]. Moreover, the harvest of biomass crops contributes to the removal of nutrients from surface water and soil, thereby reducing the risk of contamination of superficial water bodies [11].

Phragmites australis (Cav.) Trin ex Steud. (common reed) is a helophyte with a wide distribution, from cold temperate regions to the tropics and its biomass has been tested for several bioenergy supply chains as well as for industrial uses (i.e. thatching, green building) [12]. It is one of the most promising species for cultivation in permanent saturated soils, since it is highly productive under these conditions [13]. Winter harvested biomass has traditionally been used in district heating plants in Northern Europe, although its biofuel quality is rather low due to high ash content [14]. However, under Mediterranean conditions, there is not much room to improve biomass quality for combustion by harvest time management, since the nutrient content of winter harvested reed is not consistently reduced as a result of milder and mostly frost-free winters [15]. Conversely, we can maximize the amount of nutrient taken up from the peat/water system by selecting accurately the harvest time [16, 17].

Depending on the purpose for which common reed is cropped, different management strategies can be hypothesized, involving different harvest frequencies and harvest times that can significantly affect biomass characteristics. For instance, early harvesting increases the "greenness" of perennial grasses, thus increasing the potential suitability for anaerobic digestion, owing to lower C/N ratio, lignification and higher protein content [18, 19, 20, 21] . In fact, opening up the biogas sector to perennial grasses could encourage their introduction into European agriculture, thus helping to enhance the environmental performances of biogas production [22, 23]. From the adoption of the 2020 EU energy strategy, a wide support to biogas producers has been provided, thus increasing the profitability of biogas plants and, despite criticism, maize has become the most important energy crop for anaerobic digestion, although its cultivation is supported by a large use of inputs (e.g. herbicides, fertilizers) [22, 24]. For these reasons, the use of perennial grasses as biogas substrates can increase the sustainability of this energy sector, leading to a more extensive land use and to a profitable exploitation of marginal soils, as it has been ascertained by several authors [20, 21, 23, 25, 26]. Remarkable methane potentials have been often reported for perennial grasses, although kinetics of anaerobic digestion should also be considered, since rapid methane production is needed to achieve satisfying methane yields in real-scale plants [27].

The use of common reed for anaerobic digestion has been considered by several studies, mainly focused on feedstock obtained from natural habitats, in the perspective of natural resource management and/or with main focus on other activities (i.e. thatching) [28, 29, 30, 31]. Nonetheless, the common reed biomass quality has not yet been extensively explored especially in relation to different cutting times. Therefore, the aim of this study was to assess the suitability for anaerobic digestion of common reed in the perspective of its use as a paludiculture crop, by analyzing the influence of harvest time and frequency, on biomass partitioning and composition, biochemical methane potential and digestion kinetics.

2. Materials and methods

2.1 Field experiment and samples collection

A local ecotype of common reed [*Phragmites austrialis* (Cav.) Trin. ex Steud.] was cultivated since April 2012 in Vecchiano (43° 49' 59.5'' N; 10° 19' 50.7'' E), about 10 km from Pisa (Italy) within a paludiculture system in the Natural Park of Migliarino-San Rossore-Massaciuccoli. This system lies within a larger phyto-treatment area (15 ha), described by Giannini et al. [32], using eutrophic waters gathered from the surrounding reclamation district, in which the water table level is artificially lowered by pumping to allow for conventional farming [33]. Contrastingly, the water table level in the paludiculture system is kept markedly higher than in the surrounding watershed because of the continuous supply of water to be treated, and it ranges from 0-5 cm to 10-20 cm below the soil surface, during winter and summer respectively. The paludiculturae system is crossed by channels providing for both drainage and irrigation, depending on seasonal rainfall abundance. Regarding the eutrophic status of inlet waters, average nitrogen concentrations range from 7.14 mg L^{-1} to 8.13 mg L^{-1}, while average phosphorus concentrations vary between 0.24 and 1.07 mg L^{-1}. About the soluble forms of the nutrients, Soluble Reactive Phosphorus (SRP) averages 0.15-0.22 mg L^{-1}, while nitrates range from 1.41 to 3.23 mg L^{-1}.

The climate of the site is classified as Hot-summer Mediterranean (Csa) according to Köppen-Geiger climate classification [34]. According to the soil classification of the USDA [35], the soil is a Histosol, consisting primarily of organic materials (peat with average depth of 3-4 m) as reported in [32].

Common reed was planted in April 2012 in the paludiculture system at a density of two rhizomes per square meter (1.0 × 0.5 m spacing, 20,000 rhizomes ha^{-1}) and, from 2012 to 2013, it was harvested once a year in late summer (September). In 2014, the crop was harvested at 5 different times (n=3) from May to September (PHR1-PHR5) (Table 1). Resprouting from the cut in June was also considered, by carrying out a second harvest in September (PHR-2R). Comparing 2014 with climatic long-term means (1990-2014), the average of maximum daily temperatures was slightly lower (24.5 *vs* 25.7 °C) and the rainfall was markedly more abundant (489 *vs* 379 mm), while the average of minimum temperatures was in line (13.5 °C).

At each harvest time, biomass fresh weight was determined in a 2 m^2 sampling area within each plot (10 x 3 m). Plant subsamples (10 stems) were partitioned into leaves and stems. Inflorescences, when present, were pooled with leaves due to their low proportion in the overall biomass. Subsequently, leaves and stems were weighed and their dry matter content (DM) was determined by oven drying at 65 °C until constant weight, in order to assess the overall dry biomass yield (Mg ha^{-1}) and its partitioning. Where double harvests were performed, biomass from first and second harvests was pooled in order to get the overall biomass yield of the double harvest system (PHR2+ PHR-2R).

2.2 Samples preparation and biochemical analyses

Samples for chemical analyses were prepared for each field replication by milling dry biomass in a Retsch SM1 rotor mill equipped with a 1 mm grid (Retsch, Haan, Germany). Fresh subsamples for Biochemical Methane Potential (BMP) determination were obtained from raw, partitioned biomass, milled and then stored at -20°C. Total solids (TS) and volatile solids (VS) were determined according to standard methods [36]; nitrogen concentration (% w/w) and C/N ratio were assessed by elemental analysis (Vario EL II, Elementar Analysensysteme GmbH, Hanau, Germany). Concentrations (% w/w) of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were determined with Van Soest method using the FiberCap™ 2021/2023 system (FOSS

Analytical AB, Höganäs, Sweden). Hemicellulose (HEM) was calculated as the difference between NDF and ADF, and cellulose (CEL) as the difference between ADF and ADL.

2.3 Biochemical Methane Potential (BMP) assay and kinetics analysis

Biogas assays were carried out in an experimental device composed by static batch reactors (2 L) operating under mesophilic conditions (37 ± 0.5°C) , in which temperature (Pt100) and pressure (piezo-resistive transducers) were automatically and continuously measured and recorded every 3 minutes by a Programmable Logic Controller (PLC) connected to a computer (Ragaglini et al., 2014). The assays were conducted in triplicates on fresh samples from leaves and stems of six different cuts of common reed (PHR1-PHR5 and PHR-2R). The inoculum ([TS] = 78.1 g kg^{-1}; [VS] = 55.7 g kg^{-1}; pH 7.9) was gathered from the methanogenic stage of a mesophilic anaerobic digester fed with energy crops, agricultural residues and manures, then sieved through a 1 mm mesh and left for 5 days at 37°C in order to reduce the amount of readily available organic matter and to be degassed [37].

In each reactor, 300 g of inoculum was suspended in a basal test medium, prepared according to the ISO 11734 standard, up to a final filled volume of 1 L. The substrates were added to the batches according to a ratio between the inoculum and the substrate (I:S) of 2:1 on the basis of VS content. Once the reactors were loaded with the different substrates, the reactors were sealed and flushed with N_2, in order to obtain anaerobic conditions. Subsequently, they were incubated under mesophilic conditions as long as the further production of biogas became negligible. Three blank experiments were also carried out with inoculum and medium only.

The Biochemical Biogas Potential (BBP) was calculated according to the ideal gas law and to the molar volume of ideal gases at standard temperature and pressure conditions (1 bar, 273.15 K). The composition of biogas was measured at discrete intervals (3, 6, 10, 20, and 45 days) by gas chromatography (micro-GC Agilent 3000, Agilent Technologies Inc., Shanghai, China). For estimating the cumulative methane production in each batch, and thus calculating the Biochemical Methane Potential (BMP), both the pressure reduction due to biogas removal at each sampling time and the biogas composition of the sampled gas were considered, as described by [21]. Methane yields per hectare were calculated as products of dry matter yields, VS concentrations and BMP for each biomass component at each harvest time.

The kinetics of anaerobic digestion of common reed substrates were examined by regression on time of the daily-cumulated methane measured in each reactor using a five-parameters Modified Gompertz function. The function and its first and second derivative were used to calculate kinetic parameters: the time (days) when 50% and 95% of methane production was reached (respectively, T_{50} and T_{95}), the maximum daily production rate (R_{max}, NL CH_4 day^{-1}) and the mean daily production rate from the beginning of the assay to T_{50} (R_{50}, NL CH_4 day^{-1}) [21, 27].

2.4 Statistical analyses

All statistical analyses were performed using the R software (version 3.3.1). Accumulated biomass and methane yields per hectare were compared for the different common reed cuts by one-way ANOVAs, while biomass quality and anaerobic digestion parameters were compared by two-way ANOVAs considering harvest times and plant organs as fixed factors. When significant differences were evidenced, pairwise comparisons were made via Tukey's test at the 0.05 p-level using the agricolae and the TukeyC packages [38, 39]. Pearson's correlation coefficients (r) were calculated for common reed leaves and stems, in order to point out the main factors that influenced biogas and methane production and kinetics, testing as predictors biomass quality parameters and digestion parameters. Curve fitting and model parameterization were performed using the "nlsList" function of the "nmle" package [40].

3. Results

3.1 Dry biomass yield

Common reed stands sprouted by the end of March. Aboveground biomass accumulation was 6.4 Mg ha^{-1} d.m. in May, then it increased up to 19.4 Mg ha^{-1} d.m.in September ($p<0.001$). The second cut carried out in September from plots previously harvested in June yielded 7.4 Mg ha^{-1} (Fig. 1a). Over the growing season, the proportion of stems on the overall biomass decreased from May to September. Conversely, a complementary decrease in leaves proportion was observed from June to September (Fig. 1b). For resprouted plants (PHR-2R), we found an opposite pattern between leaves and stems, with the latter being less than 50%.

3.2 Biomass quality

All the considered biomass quality parameters varied according to both the plant part and the harvest time; the interaction between the two factors was significant ($p<0.001$). Both nitrogen and ash concentrations were higher in leaves than in stems at each harvest time, showing downward trends with the harvest date delay, with the only exceptions of nitrogen concentration in leaves at PHR4, that was lower than at PHR5, and ash concentration of leaves at PHR1, that was not statistically different from that of stems (Fig. 2). In particular, N ranged from 1.41% (PHR1) to 0.63% (PHR5) in stems and from 3.78% (PHR1) to 1.77% (PHR4) in leaves. In PHR-2R, the N concentration in both organs was similar to that of PHR5 (3.35% and 0.96% in leaves and stems, respectively). Accordingly, the C/N ratio increased along the season from 34.3 to 77.2 in stems, while in leaves it slightly increased from PHR1 to PHR3 (13.9-16.0), it peaked in PHR4 (26.7) and then decreased in PHR5 (15.4). In PHR-2R, the C/N ratios were 50.4 and 14.6 in stems and leaves respectively. From PHR1 to PHR5, the ash concentration in leaves varied over time from 7.20% to 6.12%, while in stems it ranged from 6.95% to 3.32%; PHR-2R showed intermediate concentrations (5.92 and 4.78%, in leaves and stems respectively).

Regarding fiber components (NDF, ADF, ADL), all parameters showed higher concentrations in stems than in leaves at each harvest time. In stems, NDF varied from 77.8% in PHR1 to 82.4% in PHR5, while in PHR-2R the concentration was similar to that of PHR1 (77.3%). In leaves, the NDF concentration was rather stable, ranging from 63.6% in PHR1 to 64.5% in PHR5 without significant differences. ADF in stems raised from 49.0% in PHR1 to 60.8% in PHR4 and then slightly decreased in PHR5 (59.0%). On the contrary, ADF in leaves constantly increased from PHR1 to PHR5 (32.5-35.8%). In PHR-2R, a markedly lower ADF concentration than in PHR5 was observed in stems (53.5%), while in leaves the value was in line with those recorded along the season under single harvest management (35.0%). ADL increased from PHR1 to PHR 5 in both organs, ranging from 3.1% to 6.5% in leaves and from 6.5% to 9.0% in stems. As observed for the other fiber components, in PHR-2R the lignin concentration of stems was much lower than in PHR5 (7.1%). A similar result was observed in leaves, as their lignin concentration in resprouted plants was close to that of PHR3 (5.0%).

Hemicellulose concentration (HEM) was higher in leaves than in stems at all harvest times, with the exception of PHR1. In stems, hemicellulose decreased from PHR1 (28.8%) to PHR4 (20.4%), then increased in PHR5 (23.3%); in leaves, it slightly decreased from PHR1 to PHR2 (31.1%-29.3%), then it remained constant. Analogously, PHR-2R hemicellulose concentration was higher in leaves (28.7%) than in stems (23.8%). Cellulose (CEL) was much higher in stems than in leaves along the whole study. In detail, cellulose in stems increased from PHR1 (42.5%) to PHR4 (52.3%), then it decreased in PHR5 (50.0%). In contrast, cellulose concentrations in leaves were rather stable at all the considered harvest times, ranging from 29.3% to 30.3%. The PHR-2R concentration of cellulose in leaves was not different from those of the other harvest times (30.0%), while in stems it was lower than in PHR5 and close to that of PHR2 (46.4%).

3.3 Digestion kinetics and Biochemical Methane Potential

The digestion kinetics of leaves and stems at different harvest dates is illustrated as methane potentials over time and methane production rates over time in Figure 3. The time when half of the methane potential was reached (T_{50}) was not significantly affected by the harvest time, while significant differences between plant parts were observed (Tab.2). Indeed, during the first days of the experiment, the T_{50} averaged 7.2 and 6.3 days in leaves and stems, respectively. Also T_{95} was significantly dependent on plant part, as leaves took 29.6 days to reach the 95% of methane production, while stems required only 25.2 days. T_{95} was also affected by harvest time, although the two treatments (plant part and harvest time) did not interact each other. Both in leaves and in stems, T_{95} was remarkably high in PHR1, then it decreased in PHR2 and subsequently raised at the following harvest times. Regarding PHR-2R, T_{95} was close to PHR2 in leaves (26.74 days), while it was not distant from the mean of the considered harvest times in stems (25.53 days). The maximum daily production rate (R_{max}) depended only on the harvest time, since the differences between the organs were not significant (Table 3). Considering the weighted average between leaves and stems, the highest R_{max} was registered in PHR1 (25.60 NL kgVS^{-1} day^{-1}), then it decreased along the season to 19.09 NL kgVS^{-1} day^{-1} (PHR5). In PHR-2R, the highest methane production rate was similar to that of PHR1 (25.12 NL kgVS^{-1}day^{-1}). The methane production rate during the first days of the digestion (R_{50}) differed according to both harvest time and plant part, showing a significant interaction between the two factors. Indeed, in leaves R_{50} decreased from 22.71 NL kgVS^{-1} day^{-1} in PHR1 to 16.52 NL kgVS^{-1} day^{-1} in PHR4, then it remained almost stable in PHR5 (16.62 NL kgVS^{-1} day^{-1}); the digestion rate from the beginning of the assay to T_{50} was close to the mean of the harvest times in PHR-2R (18.94 NL kgVS^{-1} day^{-1}) (Fig. 4). In stems, a similar trend was observed from PHR1 to PHR5, ranging from 18.86 to 13.00 NL kgVS^{-1} day^{-1}. R_{50} in PHR-2R was higher than the mean of the other harvest times (20.28 *vs* 17.06 NL kgVS^{-1} day^{-1}) (Fig. 4).

The overall biogas production (BBP) was significantly affected by both harvest time and plant part, although the two treatments did not show a significant interaction. In general, BBP was higher in leaves than in stems, although this difference was not significant in PHR4 and PHR-2R. Averaged over harvest times, BBP of leaves and stems was 378.20 NL kgVS^{-1} and 324.34 NL kgVS^{-1}, respectively. In leaves, biogas potential in PHR1 and PHR2 was significantly higher than in other harvest times, while in stems PHR1 and PHR-2R showed the highest values, although PHR5 only was significantly lower. Analogously, BMP differed significantly according to harvest time and plant part (Table 2). Leaves showed higher values than stems at all the considered harvest times (269.90 *vs* 213.95 NL CH4 kgVS^{-1}). Contrastingly, the biogas potential of the two organs was similar after crop regrowth. In both leaves and stems, the highest values were observed in PHR1, while the lowest were observed in PHR5 and PHR-2R was intermediate. The methane concentration of biogas (MC) did not vary according to the harvest time, while leaves exhibited consistently higher MC values than stems (71.4% *vs* 66.0%).

3.4 Correlations between biomass quality and biogas

Regarding correlations among biogas parameters, both leaves and stems showed positive correlations between BBP and digestion rates (R_{max} and R_{50}). BMP was positively correlated with R_{50} in both plant parts, while a significant correlation with R_{max} was observed in leaves only (Fig. 5). In stems, a positive correlation between MC and T_{95} was also highlighted. In both organs, the ash concentration did not show any significant correlation with the considered parameters, thus it was not shown in the correlation matrix (Fig.6). BBP and R_{max} were negatively correlated with ADL, while both the digestion rates R_{max} and R_{50} were negatively correlated with NDF. Conversely, ADF in leaves and stems was positively correlated with ADL and HEM.

In stems, NDF negatively correlated with BBP and BMP, while it positively correlated with C/N. Nitrogen concentration was negatively correlated with ADF, ADL and CEL, while C/N and NDF showed positive correlations with these parameters. Positive correlations were found also between ADF and CEL and between ADL and CEL, while the correlation between HEM and CEL was negative.

In leaves, both BBP and BMP showed negative correlations with ADF and positive correlations with HEM. Moreover, a significant negative correlation between ADL and BMP was observed. T_{95} was positively correlated to NDF, while R_{max} and R_{50} were negatively correlated with ADF. ADL negatively correlated with R_{50} as well as with HEM.

3.5 Methane yields per hectare

Methane yields per hectare increased significantly ($p<0.001$) with crop maturity from PHR1 (1659 Nm^3 ha^{-1}) to PHR5 (3817 Nm^3 ha^{-1}) (Fig.7). However, the highest value was observed combining the methane yield of common reed harvested in June (PHR2) with that of its regrowth harvested in September (PHR-2R) (4383 Nm^3 ha^{-1}), although it did not differ significantly from PHR5. Along the period of observation, the contribution of leaves to the overall methane productions per unit area was about 50% in PHR1 and PHR3, 56% in PHR2 and 43% in PHR4 and PHR5. In PHR-2R, leaves contributed about 56% of the total methane production. Considering the overall double harvest management (PHR2+ PHR-2R), the contribution of the regrown biomass after the first cut was about 39%.

4. Discussion

The observed pattern in aboveground biomass accumulation along the season (May-September) was similar to that often described in literature, although some differences can be highlighted. For instance, [41] reported an almost continuous increase in aboveground biomass of common reed in Sweden from May to August, when the yield peaked. The same pattern was also observed by [42] in their study conducted in Germany, in which they found the highest yield in August, while [43] in North-Eastern Germany found a biomass yield peak in July. Since the phenology and crop productivity of common reed are highly dependent on temperature [44], the unlimited supply of water provided by the paludiculture conditions, and the high amounts of nutrients due to the eutrophication of the drainage water make possible a longer vegetative season under Mediterranean conditions, thus explaining the biomass peak recorded in September. Positive effects of climate conditions on crop growth can also be inferred looking at the biomass yield values recorded per unit area. In our conditions, the productivity peak of the crop was 19.4 Mg ha^{-1}d.m., whereas at higher latitudes [43] registered 18.7 Mg ha^{-1} d.m., and [41] and [42] reported about 10 Mg ha^{-1} d.m. In autumn and winter, after the yield peak, a lower proportion of green leaves and a markedly higher dry matter concentration were observed, suggesting that inferior characteristics for biogas purposes were reached, while lower dry biomass yields were also recorded (data not shown). Moreover, the moisture concentration of biomass in autumn approached the threshold level for thermochemical conversion (< 25%), while it was further from levels commonly accepted for ensiling (> 50%), what is the most common storage method for biomass addressed to anaerobic digestion [45].

Harvest time typically influences both biomass yield and quality of perennial grasses, thus being a major determinant of methane yields per unit area of energy crops [20, 21, 25]. Common reed showed a higher percentage of leaves at the beginning of the growing season than later, as observed for other grasses [20, 21, 23, 25, 46]. However, stem biomass was higher than leaf biomassat for all the considered harvest times, with the remarkable exception of the biomass regrown after the cut in June, due to the reduced stem elongation and the high juvenility of the crop [18].

As observed in a similar study conducted by [20] on the effect of harvest time on reed canary grass composition, the concentrations of nitrogen and ash in leaves and stems of young plants were the highest and then they quickly decreased due to carbon accumulation. These results are in line with another study carried out on the same experimental area [15]. Nonetheless, a sharp decrease in nitrogen concentration of leaves was observed from July to August, followed by an increase in September. This is likely due to the panicle formation phase occurring in July and thus to the translocation of nitrogen compounds to the plant apix [47]. Indeed, panicles are very rich in nitrogen, up to 12 times more than internodes [48]. Afterwards, favorable and non-limiting conditions may have fostered nutrient uptake before the end of the vegetative season. The eutrophic conditions of waters to be treated and the high availability of nutrients in soil can also justify the higher overall nitrogen concentrations in comparison to values generally reported by literature. [28] found N percentages ranging between 0.6-1.2% in Estonia at summer harvest.

Usually plant nitrogen content is positively correlated with methane yields and production rates [27], as well as with methane concentrations in biogas [24]. In this study, a clear role of N concentration was not highlighted. Separated plant parts did not show marked variations in N concentration from May to September, although these differences were statistically significant both in leaves and in stems, possibly because N was not a limiting factor in this environment. Thus, evaluating leaves and stems separately, biomethanation was determined mostly by other factors. The C/N ratio was mainly dependent on these nitrogen variations and was higher in stems than in leaves along the whole season. [31] also found that the nitrogen role in biomethane production from common reed biomass was unclear. In fact, nitrogen can also form plant components that can negatively influence biomethane yields, such as nitrates and lignin-bound proteins, and high concentrations of nitrates have occasionally been found in common reed according to the growing conditions, although they are usually below 100 ppm [17, 31]. However, specific hypotheses at this regard cannot be drawn from this study, while the influence of plant organs was clearer, since higher methane concentrations were observed in leaves.

Along the growing season, the stem contribution on the total dry matter increased, while the ADL concentration of leaves at crop maturity (PHR5) was almost equal to that of stems at juvenile stages (PHR1). The NDF and ADF content found at crop maturity (PHR5) were in line with those observed by other authors [49]. Lignin is known to negatively affect biomethanation due to its recalcitrance during anaerobic digestion and to its hampering action on the digestion of degradable compounds, as already observed in common reed [31] and other perennial grasses [19, 20, 23]. This study makes no exception, since lignin was found to be negatively related to biogas and biomethane potential and to digestion rates. However, in stems the most important negative correlation of fiber components with anaerobic digestion parameters was that of the whole NDF and not just lignin, while a role of hemicelluloses and celluloses was not evidenced. This may be due to the lignin role in providing resistance for enzymatic digestion to the other components by forming a complex matrix involving the whole fibers [19, 50, 51]. According to the literature, mature biomass typically has higher fiber contents, thus implying lower digestibility than in younger plants, in which the hampering due to physical lignin structures is less pronounced [52]. At the opposite, significant correlations were not shown for NDF in leaves, while negative correlations for lignin and ADF and a positive role of hemicelluloses were found. This can be explained in terms of higher importance of each single fiber component, likely due to a less tight lignification and a higher availability of degradable compounds, and particularly hemicelluloses, as already observed in other studies [18, 19].

Considering their experimental BMPs, cellulose and hemicelluloses are recognized as high-potential substrates and their reduced availability is typically acknowledged as the most important limiting factor in anaerobic conversion of biomasses (Triolo et al., 2012; Monlau et al., 2013). In particular, modifications of cellulose crystallinity and physicochemical properties of hemicelluloses have been proposed as factors influencing the digestion of both structural and non-structural carbohydrates [19, 51].

The lignification level in the resprouted biomass was lower than that of the crop harvested in September for the first time. However, this difference was higher in stems than in leaves, leading to similar BMPs and kinetics at the second harvest in the two plant parts. Similar results were also found in reed canary grass by [20], in whose study the leaves at the second cut (end of September) had a lignin concentration similar to that at the first cuts carried out in full summer, while the ADL content in stems was similar to that at the first harvests carried out in spring. In this sense, the double cut strategy could guarantee a lower recalcitrant fiber content [46, 51].

Rapid stem growth occurring at early stages of the growing season of grasses generally leads to low concentration of non-structural carbohydrates and then it typically increases over time after the formation of new leaves, while it tends to decrease when the photosynthetic rate is restricted by drought and other stress conditions [53]. Thus, non-structural carbohydrates may have played a role in determining a lower methane content in stems compared with leaves [24, 54] and in increasing the degradation rates of stems. Indeed, stems showed generally lower values of T_{50} and higher values of R_{max} than leaves [27].

The separate anaerobic digestion of different grass organs at different harvest times has already been considered in a previous study regarding reed canary grass [20]. In this case, the specific methane yield decreased with crop maturity in both plant parts, ranging from 384 to 315 NL CH4 $kgVS^{-1}$ for leaves and from 412 to 283 NL CH4 $kgVS^{-1}$ for stems. Compared with these results, common reed showed overall lower productivity both in leaves and stems. Comparing whole plant data reported in literature from Northern Europe, our results are in line with data from on common reed harvested from mid to late summer. [16] reported specific methane yields of about 180 NL CH4 kgVS^{-1}, while [55] showed biogas potential values ranging from 400 to 500 NL CH4 kgDM^{-1} with a maximum methane content of 55-60%. [56] presented higher BMP values, that approached 250 NL CH4 kgVS^{-1}, while [19] found lower methane potentials (190-200 NL CH4 kgVS^{-1}) from biomass harvested in the autumn season. [29] reported higher potentials for green reeds compared with dry reeds, and values higher than 250 NL CH4 kgVS^{-1} when green reeds were finely chopped (<5 mm). In substantial agreement with these results, the methane potential of common reed, averaged across all the tested harvest dates, was about 240 NL CH4 kgVS^{-1}. In detail, the weighted averages for the whole plant ranged from 283 NL CH4 kgVS^{-1} in May to 209 NL CH4 kgVS^{-1} in September, while the crop regrowth (PHR-2R) achieved 244 NL CH4 kgVS^{-1} .

Methane yield per hectare was predominantly influenced by biomass production, since the BMP varied only slightly according to the harvest time (coefficient of variation = 12%), while the biomass yields varied more largely (coefficient of variation = 30%). Comparing our results with those of other studied candidate crops for biogas production (e.g. x*Festulolium*, *Phalaris arundinacea*), *Phragmites australis* showed lower methane yields, due to generally lower BMPs. In particular, [26] found values exceeding 5000 Nm^3 ha^{-1} in two-cut strategies and 6000 Nm^3 ha^{-1} in three-cut strategies in festulolium, a very digestible species, whose specific methane yields averaged 393 NL CH_4 kgVS^{-1} . Reed canary grass, which is also tolerant to high water table level, showed higher maximum values under double harvest management (~5500 Nm^3 ha^{-1}), while the highest yield observed under single harvest management by [20] was similar to that of PHR5 (~3700 Nm^3 ha^{-1}). In literature, values ranging from 5000 to 9000 Nm^3 ha^{-1} are typically reported for maize, which is commonly acknowledged as the reference crop for biogas production. Yields up to 6000 Nm ha^{-1} have been reported for *Miscanthus* under European continental conditions [22, 23, 24], while at lower latitudes giant reed showed higher potentials (up to 9452 Nm^3 ha^{-1}) and a better response to double cutting

[21]. However, the attitude of these last species to thrive under paludiculture conditions still has to be fully evaluated [32, 57].

All these results considered, we can infer that the double harvest strategy for common reed did not show remarkable advantages compared to a single harvest, since the methane production per unit area was almost equal to that of the single harvest with the highest yield (September). According to the observed nitrogen concentrations in the double cut strategy could achieve about 430 kg N ha^{-1} could be removed by common reed, while the single cut strategy could only remove 320 kg N ha^{-1}. Differently, about phosphorus, there was not a remarkable difference between the two strategies (double cut: 30 kg P ha^{-1} vs single cut: about 28 kg P ha^{-1}). Nevertheless, these options should be evaluated also in the long term by considering the effect of a double harvest on the plantation life span and overall productivity including economics, energy and nutrient balances, with particular regard to phosphorus.

Moreover, also the summer harvest can shorten the crop lifespan. Many authors reported a depressive effect of the summer harvest, since the beds have not yet translocated all resources to rhizomes to guarantee a vigorous resprout in the next vegetative season [41, 58].

In real-scale plants, anaerobic digestion of common reed biomass can be hampered by C/N ratios, since the observed values were consistently higher than those considered optimal for the process. Such disadvantage can be overcome by co-digestion with N-rich feedstocks (e.g. manures, slurries) as many researches carried out at lab-scale seem to prove [30, 59]. However, there is often no significant market for such applications, since the production costs are usually too high [60]. According to our knowledge, there are no commercial plants using reed as a co-substrate at present and the possible co-benefits of co-digesting such substrate are not yet exploited. For instance, at district scale, added value could be given to the nutrient uptake from paludiculture crops, in order to remove nutrients from eutrophic waters. At the same time, fertilizers coming from the digestate made in biogas production could be reused out of the paludiculture system in order to close the nutrient cycles [16]. In this perspective, the anaerobic digestion of biomass from *P. australis* could allow farmers to continue their activity on peatland while providing services beneficial to the ecosystem.

5. Conclusions

In addition to the provided environmental services such as restoration of water regimes (no drainage), improvement of water quality, reduction of GHG emissions, slowing down mineralization of the organic matter and soil subsidence, paludiculture can contribute to a sustainable production of biomass on former degraded, unproductive and marginal lands. The crucial point for the success of paludiculture cropping systems is the choice of the crop to use, because it has to meet different needs such as longevity, harvestability, productivity and attitude to produce bioenergy [61].

Our results showed that *Phragmites australis* can be used as a productive crop for biogas production under paludiculture conditions, thus allowing to couple bioenergy production with valuable environmental services. Since the nitrogen concentrations were rather stable along the season, harvesting in September could maximize bioenergy production while achieving environmental goals at the same time thanks to a high nutrient uptake.

The double harvest strategy, although potentially able to guarantee higher methane yields per unit area, should be better investigated at farm scale since it can short the life span of the plantation and it implies higher management costs (fuel, machinery) and higher environmental impacts (emissions).

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Figure captions

Figure 1. Dry biomass yields (a) and partitioning (b) of common reed harvested at different times; PHR1–PHR5 refer to first cuts, while PHR-2R refer to regrowth from PHR2. For biomass yields, significance level of ANOVA is reported (***, $p< 0.001$); values with the same letter are not significantly different ($p\geq0.05$). Standard errors are shown as vertical bars.

Figure 2. Seasonal changes in chemical composition of common reed biomass; the secondary axis separates second cut (PHR-2R) from first cuts (PHR1-5). Upper case letters are for comparison between organs within the same date; lower case letters are for comparison among dates within the same organ. Values with the same letter are not significantly different ($p\geq0.05$). Standard errors are shown as vertical bars.

Figure 3. Kinetics of fermentation of common reed harvested at different times; PHR1–PHR5 refer to first cuts, while PHR-2R refer to regrowth from PHR2. Cumulative methane production of leaves (a) and stems (c), daily methane production rates of leaves (b) and stems (d) estimated as the first derivative of cumulate production curves. T_{50} (●),T_{95} (□), R_{max} (▲) and their standard error bars are also reported.

Figure 4. Biochemical Biogas Potential (BBP), Biochemical Methane Potential (BMP), average MC (Methane Content) of biogas, and methane production rate from the beginning of the assay to its half (R_{50}) for the considered substrates. Upper case letters are for comparisons between leaves (grey bars) harvested at different times, while lower case letters are for comparisons between stems (white bars); values with the same letter are not significantly different. For each harvest time, significance of difference between leaves and stems is indicated by asterisks ($p<0.05$). Standard errors are shown as vertical bars.

Figure 5 Pearson's r correlation between anaerobic digestion parameters of (a) leaves and (b) stems of common reed. Bold values show significant correlations ($p<0.05$).

Figure 6 Pearson's r correlation between anaerobic digestion parameters and characteristics of (a) leaves and (b) stems of common reed. Bold values show significant correlations ($p<0.05$).

Figure 7 Methane yields per hectare obtained at different harvest times from May to September (PHR1–PHR5) and combining a first harvest in June with a second harvest in September (PHR2+R). Standard errors and significance level of ANOVA are reported (***, $p< 0.001$). Values with the same letter are not significantly different ($p<0.05$).

Compliance with Ethical Standards

The authors declare that they have no potential conflict of interest, since they work for independent, public research institutions (Scuola Superiore Sant'Anna, University of Pisa and CRIBE), which are not financially involved in energy crops and bioenergy production. The study was funded by the public funds specified in the Acknowledgements section. The research did not involve any animals or human beings.

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Parameter	Unit	Value
pH		5.0
EC	(dS m^{-1})	1.46
sand (USDA)	(%)	56
silt (USDA)	(%)	25
clay (USDA)	(%)	19
bulk density	(g cm^{-3})	1.44
SOM (Walkey-Black)	(%)	30.1
N_{tot} (Kjeldahl)	(g kg^{-1})	13.2
P_{avail} (Olsen)	(mg kg^{-1})	79
K_{exch}*	(g kg^{-1})	516
CEC	(meq $100g^{-1}$)	75
Fe**	(g kg^{-1})	12.2
Al**	(g kg^{-1})	5.5

*determined by atomic absorption; **extractable with ammonium oxalate.

Table 1. Physical and chemical characteristics of soil in the paludiculture system (0-30 cm depth).

Harvest time	Date	TS (% of FM)	
		Leaves	Stems
PHR1	16 May 2014	44.9%	37.6%
PHR2	11 June 2014	46.7%	38.3%
PHR3	16 July 2014	61.2%	50.3%
PHR4	29 August 2014	57.0%	55.9%
PHR5	24 September 2014	60.9%	59.2%
PHR-R	24 September 2014	55.9%	47.9%

Table 2. Harvest date and total solids content (TS) on the fresh matter (FM) of leaves and stems at first harvests (PHR1–PHR5) and second harvest (PHR-R) of common reed.

Source of variation	df	BBP	BMP	MC	T_{50}	T_{95}	R_{max}	R_{50}
Harvest time (T)	5	***	***	*ns*	*ns*	**	**	***
Plant part (P)	1	***	***	***	***	**	*ns*	**
T x P	5	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*

Table 3. Significance of the effects of harvest time (T), plant part (P) and their interaction on anaerobic digestion parameters. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$