

Effect of Harvest Time and Frequency on Biomass Quality and Biomethane Potential of Common Reed (Phragmites australis) Under Paludiculture Conditions

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

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16

17 **Keywords**

18 Biogas; anaerobic digestion; perennial grasses; fiber components; digestion kinetics; peatland
19 cultivation

20

21 **Abstract**

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

27 Biomethane potentials ranged from 384 to 315 and from 412 to 283 NL CH₄ kgVS⁻¹ (normal liters of
28 methane per kg of volatile solids) for leaves and stems, respectively. About digestion kinetics,
29 maximum daily production rate (R_{max}) was significantly affected by harvest time and not by plant
30 partitioning. Along the harvest season, biomethane yield per unit area was mostly driven by the
31 biomass yield showing an increasing trend from May (1659 Nm³ ha⁻¹) to September (3817 Nm³ ha⁻¹).
32 The highest value was obtained with the double harvest option (4383 Nm³ ha⁻¹), although it was
33 not statistically different from the single harvest carried out in September. Owing to its remarkably

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

39

40

41 **1. Introduction**

42 Peatlands are efficient systems for carbon and nutrient storage on a global scale, as they cover only
43 3% of global land area but store more than 30% of total organic carbon [1]. Although natural peatlands
44 are net nutrient sinks, their drainage for agricultural use does turn these ecosystems into net sources
45 of CO₂, CH₄ and N₂O [2, 3]. Indeed, Couwenberg et al. [4] estimated that agricultural drained
46 peatlands can release up to 50 t ha⁻¹ year⁻¹ of CO₂ and up to 60 kg ha⁻¹ year⁻¹ of N₂O, which is 265
47 times more potent than CO₂ over the 100-year horizon [5]. Moreover, peatland drainage is responsible
48 for both internal and external eutrophication [6] and land subsidence [7].

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

58 *Phragmites australis* (Cav.) Trin ex Steud. (common reed) is a helophyte with a wide distribution,
59 from cold temperate regions to the tropics and its biomass has been tested for several bioenergy supply
60 chains as well as for industrial uses (i.e. thatching, green building) [12]. It is one of the most promising

61 species for cultivation in permanent saturated soils, since it is highly productive under these
62 conditions [13]. Winter harvested biomass has traditionally been used in district heating plants in
63 Northern Europe, although its biofuel quality is rather low due to high ash content [14]. However,
64 under Mediterranean conditions, there is not much room to improve biomass quality for combustion
65 by harvest time management, since the nutrient content of winter harvested reed is not consistently
66 reduced as a result of milder and mostly frost-free winters [15]. Conversely, we can maximize the
67 amount of nutrient taken up from the peat/water system by selecting accurately the harvest time [16,
68 17].

69 Depending on the purpose for which common reed is cropped, different management strategies can
70 be hypothesized, involving different harvest frequencies and harvest times that can significantly affect
71 biomass characteristics. For instance, early harvesting increases the “greenness” of perennial grasses,
72 thus increasing the potential suitability for anaerobic digestion, owing to lower C/N ratio, lignification
73 and higher protein content [18, 19, 20, 21] . In fact, opening up the biogas sector to perennial grasses
74 could encourage their introduction into European agriculture, thus helping to enhance the
75 environmental performances of biogas production [22, 23]. From the adoption of the 2020 EU energy
76 strategy, a wide support to biogas producers has been provided, thus increasing the profitability of
77 biogas plants and, despite criticism, maize has become the most important energy crop for anaerobic
78 digestion, although its cultivation is supported by a large use of inputs (e.g. herbicides, fertilizers)
79 [22, 24]. For these reasons, the use of perennial grasses as biogas substrates can increase the
80 sustainability of this energy sector, leading to a more extensive land use and to a profitable
81 exploitation of marginal soils, as it has been ascertained by several authors [20, 21, 23, 25, 26].
82 Remarkable methane potentials have been often reported for perennial grasses, although kinetics of
83 anaerobic digestion should also be considered, since rapid methane production is needed to achieve
84 satisfying methane yields in real-scale plants [27].

85 The use of common reed for anaerobic digestion has been considered by several studies, mainly
86 focused on feedstock obtained from natural habitats, in the perspective of natural resource

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

93

94 **2. Materials and methods**

95 **2.1 Field experiment and samples collection**

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

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

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

121 At each harvest time, biomass fresh weight was determined in a 2 m² sampling area within each plot
122 (10 x 3 m). Plant subsamples (10 stems) were partitioned into leaves and stems. Inflorescences, when
123 present, were pooled with leaves due to their low proportion in the overall biomass. Subsequently,
124 leaves and stems were weighed and their dry matter content (DM) was determined by oven drying at
125 65 °C until constant weight, in order to assess the overall dry biomass yield (Mg ha^{-1}) and its
126 partitioning. Where double harvests were performed, biomass from first and second harvests was
127 pooled in order to get the overall biomass yield of the double harvest system (PHR2+ PHR-2R).

128

129 **2.2 Samples preparation and biochemical analyses**

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

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

140

141 **2.3 Biochemical Methane Potential (BMP) assay and kinetics analysis**

142 Biogas assays were carried out in an experimental device composed by static batch reactors (2 L)
143 operating under mesophilic conditions ($37 \pm 0.5^\circ\text{C}$), in which temperature (Pt100) and pressure
144 (piezo-resistive transducers) were automatically and continuously measured and recorded every 3
145 minutes by a Programmable Logic Controller (PLC) connected to a computer (Ragagini et al., 2014).
146 The assays were conducted in triplicates on fresh samples from leaves and stems of six different cuts
147 of common reed (PHR1-PHR5 and PHR-2R). The inoculum ($[\text{TS}] = 78.1 \text{ g kg}^{-1}$; $[\text{VS}] = 55.7 \text{ g kg}^{-1}$;
148 $\text{pH } 7.9$) was gathered from the methanogenic stage of a mesophilic anaerobic digester fed with energy
149 crops, agricultural residues and manures, then sieved through a 1 mm mesh and left for 5 days at 37°C
150 in order to reduce the amount of readily available organic matter and to be degassed [37].

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

158 The Biochemical Biogas Potential (BBP) was calculated according to the ideal gas law and to the
159 molar volume of ideal gases at standard temperature and pressure conditions (1 bar, 273.15 K). The
160 composition of biogas was measured at discrete intervals (3, 6, 10, 20, and 45 days) by gas
161 chromatography (micro-GC Agilent 3000, Agilent Technologies Inc., Shanghai, China). For
162 estimating the cumulative methane production in each batch, and thus calculating the Biochemical
163 Methane Potential (BMP), both the pressure reduction due to biogas removal at each sampling time

164 and the biogas composition of the sampled gas were considered, as described by [21]. Methane yields
165 per hectare were calculated as products of dry matter yields, VS concentrations and BMP for each
166 biomass component at each harvest time.

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

173

174 **2.4 Statistical analyses**

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

185

186 **3. Results**

187 **3.1 Dry biomass yield**

188 Common reed stands sprouted by the end of March. Aboveground biomass accumulation was 6.4 Mg
189 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

190 carried out in September from plots previously harvested in June yielded 7.4 Mg ha⁻¹ (Fig. 1a). Over
191 the growing season, the proportion of stems on the overall biomass decreased from May to September.
192 Conversely, a complementary decrease in leaves proportion was observed from June to September
193 (Fig. 1b). For resprouted plants (PHR-2R), we found an opposite pattern between leaves and stems,
194 with the latter being less than 50%.

195

196 **3.2 Biomass quality**

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

211 Regarding fiber components (NDF, ADF, ADL), all parameters showed higher concentrations in
212 stems than in leaves at each harvest time. In stems, NDF varied from 77.8% in PHR1 to 82.4% in
213 PHR5, while in PHR-2R the concentration was similar to that of PHR1 (77.3%). In leaves, the NDF
214 concentration was rather stable, ranging from 63.6% in PHR1 to 64.5% in PHR5 without significant
215 differences. ADF in stems raised from 49.0% in PHR1 to 60.8% in PHR4 and then slightly decreased

216 in PHR5 (59.0%). On the contrary, ADF in leaves constantly increased from PHR1 to PHR5 (32.5-
217 35.8%). In PHR-2R, a markedly lower ADF concentration than in PHR5 was observed in stems
218 (53.5%), while in leaves the value was in line with those recorded along the season under single
219 harvest management (35.0%). ADL increased from PHR1 to PHR 5 in both organs, ranging from
220 3.1% to 6.5% in leaves and from 6.5% to 9.0% in stems. As observed for the other fiber components,
221 in PHR-2R the lignin concentration of stems was much lower than in PHR5 (7.1%). A similar result
222 was observed in leaves, as their lignin concentration in resprouted plants was close to that of PHR3
223 (5.0%).

224 Hemicellulose concentration (HEM) was higher in leaves than in stems at all harvest times, with the
225 exception of PHR1. In stems, hemicellulose decreased from PHR1 (28.8%) to PHR4 (20.4%), then
226 increased in PHR5 (23.3%); in leaves, it slightly decreased from PHR1 to PHR2 (31.1%-29.3%), then
227 it remained constant. Analogously, PHR-2R hemicellulose concentration was higher in leaves
228 (28.7%) than in stems (23.8%). Cellulose (CEL) was much higher in stems than in leaves along the
229 whole study. In detail, cellulose in stems increased from PHR1 (42.5%) to PHR4 (52.3%), then it
230 decreased in PHR5 (50.0%). In contrast, cellulose concentrations in leaves were rather stable at all
231 the considered harvest times, ranging from 29.3% to 30.3%. The PHR-2R concentration of cellulose
232 in leaves was not different from those of the other harvest times (30.0%), while in stems it was lower
233 than in PHR5 and close to that of PHR2 (46.4%).

234

235 **3.3 Digestion kinetics and Biochemical Methane Potential**

236 The digestion kinetics of leaves and stems at different harvest dates is illustrated as methane potentials
237 over time and methane production rates over time in Figure 3. The time when half of the methane
238 potential was reached (T_{50}) was not significantly affected by the harvest time, while significant
239 differences between plant parts were observed (Tab.2). Indeed, during the first days of the
240 experiment, the T_{50} averaged 7.2 and 6.3 days in leaves and stems, respectively. Also T_{95} was
241 significantly dependent on plant part, as leaves took 29.6 days to reach the 95% of methane

242 production, while stems required only 25.2 days. T_{95} was also affected by harvest time, although the
243 two treatments (plant part and harvest time) did not interact each other. Both in leaves and in stems,
244 T_{95} was remarkably high in PHR1, then it decreased in PHR2 and subsequently raised at the following
245 harvest times. Regarding PHR-2R, T_{95} was close to PHR2 in leaves (26.74 days), while it was not
246 distant from the mean of the considered harvest times in stems (25.53 days). The maximum daily
247 production rate (R_{max}) depended only on the harvest time, since the differences between the organs
248 were not significant (Table 3). Considering the weighted average between leaves and stems, the
249 highest R_{max} was registered in PHR1 (25.60 NL kgVS⁻¹ day⁻¹), then it decreased along the season to
250 19.09 NL kgVS⁻¹ day⁻¹ (PHR5). In PHR-2R, the highest methane production rate was similar to that
251 of PHR1 (25.12 NL kgVS⁻¹ day⁻¹). The methane production rate during the first days of the digestion
252 (R_{50}) differed according to both harvest time and plant part, showing a significant interaction between
253 the two factors. Indeed, in leaves R_{50} decreased from 22.71 NL kgVS⁻¹ day⁻¹ in PHR1 to 16.52 NL
254 kgVS⁻¹ day⁻¹ in PHR4, then it remained almost stable in PHR5 (16.62 NL kgVS⁻¹ day⁻¹); the digestion
255 rate from the beginning of the assay to T_{50} was close to the mean of the harvest times in PHR-2R
256 (18.94 NL kgVS⁻¹ day⁻¹) (Fig. 4). In stems, a similar trend was observed from PHR1 to PHR5,
257 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
258 harvest times (20.28 vs 17.06 NL kgVS⁻¹ day⁻¹) (Fig. 4).

259 The overall biogas production (BBP) was significantly affected by both harvest time and plant part,
260 although the two treatments did not show a significant interaction. In general, BBP was higher in
261 leaves than in stems, although this difference was not significant in PHR4 and PHR-2R. Averaged
262 over harvest times, BBP of leaves and stems was 378.20 NL kgVS⁻¹ and 324.34 NL kgVS⁻¹,
263 respectively. In leaves, biogas potential in PHR1 and PHR2 was significantly higher than in other
264 harvest times, while in stems PHR1 and PHR-2R showed the highest values, although PHR5 only
265 was significantly lower. Analogously, BMP differed significantly according to harvest time and plant
266 part (Table 2). Leaves showed higher values than stems at all the considered harvest times (269.90 vs
267 213.95 NL CH₄ kgVS⁻¹). Contrastingly, the biogas potential of the two organs was similar after crop

268 regrowth. In both leaves and stems, the highest values were observed in PHR1, while the lowest were
269 observed in PHR5 and PHR-2R was intermediate. The methane concentration of biogas (MC) did not
270 vary according to the harvest time, while leaves exhibited consistently higher MC values than stems
271 (71.4% vs 66.0%).

272

273 **3.4 Correlations between biomass quality and biogas**

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

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

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

291

292 **3.5 Methane yields per hectare**

293 Methane yields per hectare increased significantly ($p < 0.001$) with crop maturity from PHR1 (1659
294 $\text{Nm}^3 \text{ha}^{-1}$) to PHR5 (3817 $\text{Nm}^3 \text{ha}^{-1}$) (Fig.7). However, the highest value was observed combining the
295 methane yield of common reed harvested in June (PHR2) with that of its regrowth harvested in
296 September (PHR-2R) (4383 $\text{Nm}^3 \text{ha}^{-1}$), although it did not differ significantly from PHR5. Along the
297 period of observation, the contribution of leaves to the overall methane productions per unit area was
298 about 50% in PHR1 and PHR3, 56% in PHR2 and 43% in PHR4 and PHR5. In PHR-2R, leaves
299 contributed about 56% of the total methane production. Considering the overall double harvest
300 management (PHR2+ PHR-2R), the contribution of the regrown biomass after the first cut was about
301 39%.

302

303 **4. Discussion**

304 The observed pattern in aboveground biomass accumulation along the season (May-September) was
305 similar to that often described in literature, although some differences can be highlighted. For
306 instance, [41] reported an almost continuous increase in aboveground biomass of common reed in
307 Sweden from May to August, when the yield peaked. The same pattern was also observed by [42] in
308 their study conducted in Germany, in which they found the highest yield in August, while [43] in
309 North-Eastern Germany found a biomass yield peak in July. Since the phenology and crop
310 productivity of common reed are highly dependent on temperature [44], the unlimited supply of water
311 provided by the paludiculture conditions, and the high amounts of nutrients due to the eutrophication
312 of the drainage water make possible a longer vegetative season under Mediterranean conditions, thus
313 explaining the biomass peak recorded in September. Positive effects of climate conditions on crop
314 growth can also be inferred looking at the biomass yield values recorded per unit area. In our
315 conditions, the productivity peak of the crop was $19.4 \text{ Mg ha}^{-1} \text{ d.m.}$, whereas at higher latitudes [43]
316 registered $18.7 \text{ Mg ha}^{-1} \text{ d.m.}$, and [41] and [42] reported about $10 \text{ Mg ha}^{-1} \text{ d.m.}$ In autumn and winter,
317 after the yield peak, a lower proportion of green leaves and a markedly higher dry matter
318 concentration were observed, suggesting that inferior characteristics for biogas purposes were

319 reached, while lower dry biomass yields were also recorded (data not shown). Moreover, the moisture
320 concentration of biomass in autumn approached the threshold level for thermochemical conversion
321 (< 25%), while it was further from levels commonly accepted for ensiling (> 50%), what is the most
322 common storage method for biomass addressed to anaerobic digestion [45].

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

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

341 Usually plant nitrogen content is positively correlated with methane yields and production rates [27],
342 as well as with methane concentrations in biogas [24]. In this study, a clear role of N concentration
343 was not highlighted. Separated plant parts did not show marked variations in N concentration from
344 May to September, although these differences were statistically significant both in leaves and in

345 stems, possibly because N was not a limiting factor in this environment. Thus, evaluating leaves and
346 stems separately, biomethanation was determined mostly by other factors. The C/N ratio was mainly
347 dependent on these nitrogen variations and was higher in stems than in leaves along the whole season.
348 [31] also found that the nitrogen role in biomethane production from common reed biomass was
349 unclear. In fact, nitrogen can also form plant components that can negatively influence biomethane
350 yields, such as nitrates and lignin-bound proteins, and high concentrations of nitrates have
351 occasionally been found in common reed according to the growing conditions, although they are
352 usually below 100 ppm [17, 31]. However, specific hypotheses at this regard cannot be drawn from
353 this study, while the influence of plant organs was clearer, since higher methane concentrations were
354 observed in leaves.

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

371 component, likely due to a less tight lignification and a higher availability of degradable compounds,
372 and particularly hemicelluloses, as already observed in other studies [18, 19].

373 Considering their experimental BMPs, cellulose and hemicelluloses are recognized as high-potential
374 substrates and their reduced availability is typically acknowledged as the most important limiting
375 factor in anaerobic conversion of biomasses (Triolo et al., 2012; Monlau et al., 2013). In particular,
376 modifications of cellulose crystallinity and physicochemical properties of hemicelluloses have been
377 proposed as factors influencing the digestion of both structural and non-structural carbohydrates [19,
378 51].

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

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

393 The separate anaerobic digestion of different grass organs at different harvest times has already been
394 considered in a previous study regarding reed canary grass [20]. In this case, the specific methane
395 yield decreased with crop maturity in both plant parts, ranging from 384 to 315 NL CH₄ kgVS⁻¹ for
396 leaves and from 412 to 283 NL CH₄ kgVS⁻¹ for stems. Compared with these results, common reed

397 showed overall lower productivity both in leaves and stems. Comparing whole plant data reported in
398 literature from Northern Europe, our results are in line with data from on common reed harvested
399 from mid to late summer. [16] reported specific methane yields of about 180 NL CH₄ kgVS⁻¹, while
400 [55] showed biogas potential values ranging from 400 to 500 NL CH₄ kgDM⁻¹ with a maximum
401 methane content of 55-60%. [56] presented higher BMP values, that approached 250 NL CH₄ kgVS⁻¹
402 ¹, while [19] found lower methane potentials (190-200 NL CH₄ kgVS⁻¹) from biomass harvested in
403 the autumn season. [29] reported higher potentials for green reeds compared with dry reeds, and
404 values higher than 250 NL CH₄ kgVS⁻¹ when green reeds were finely chopped (<5 mm). In
405 substantial agreement with these results, the methane potential of common reed, averaged across all
406 the tested harvest dates, was about 240 NL CH₄ kgVS⁻¹. In detail, the weighted averages for the
407 whole plant ranged from 283 NL CH₄ kgVS⁻¹ in May to 209 NL CH₄ kgVS⁻¹ in September, while
408 the crop regrowth (PHR-2R) achieved 244 NL CH₄ kgVS⁻¹ .

409 Methane yield per hectare was predominantly influenced by biomass production, since the BMP
410 varied only slightly according to the harvest time (coefficient of variation = 12%), while the biomass
411 yields varied more largely (coefficient of variation = 30%). Comparing our results with those of other
412 studied candidate crops for biogas production (e.g. *xFestulolium*, *Phalaris arundinacea*), *Phragmites*
413 *australis* showed lower methane yields, due to generally lower BMPs. In particular, [26] found values
414 exceeding 5000 Nm³ ha⁻¹ in two-cut strategies and 6000 Nm³ ha⁻¹ in three-cut strategies in
415 festulolium, a very digestible species, whose specific methane yields averaged 393 NL CH₄ kgVS⁻¹ .
416 Reed canary grass, which is also tolerant to high water table level, showed higher maximum values
417 under double harvest management (~5500 Nm³ ha⁻¹), while the highest yield observed under single
418 harvest management by [20] was similar to that of PHR5 (~3700 Nm³ ha⁻¹). In literature, values
419 ranging from 5000 to 9000 Nm³ ha⁻¹ are typically reported for maize, which is commonly
420 acknowledged as the reference crop for biogas production. Yields up to 6000 Nm ha⁻¹ have been
421 reported for *Miscanthus* under European continental conditions [22, 23, 24], while at lower latitudes
422 giant reed showed higher potentials (up to 9452 Nm³ ha⁻¹) and a better response to double cutting

423 [21]. However, the attitude of these last species to thrive under paludiculture conditions still has to
424 be fully evaluated [32, 57].

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

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

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

448 cycles [16]. In this perspective, the anaerobic digestion of biomass from *P. australis* could allow
449 farmers to continue their activity on peatland while providing services beneficial to the ecosystem.

450

451 **5. Conclusions**

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

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

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

466

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471

472

473 **Figure captions**

474

475 **Figure 1.** Dry biomass yields (a) and partitioning (b) of common reed harvested at different times; PHR1–
476 PHR5 refer to first cuts, while PHR-2R refer to regrowth from PHR2. For biomass yields, significance level
477 of ANOVA is reported (***, $p < 0.001$); values with the same letter are not significantly different ($p \geq 0.05$).
478 Standard errors are shown as vertical bars.

479

480 **Figure 2.** Seasonal changes in chemical composition of common reed biomass; the secondary axis separates
481 second cut (PHR-2R) from first cuts (PHR1-5). Upper case letters are for comparison between organs within
482 the same date; lower case letters are for comparison among dates within the same organ. Values with the same
483 letter are not significantly different ($p \geq 0.05$). Standard errors are shown as vertical bars.

484

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

489

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

496

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

499

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

502

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

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511 **Compliance with Ethical Standards**

512 The authors declare that they have no potential conflict of interest, since they work for independent,
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517

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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

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

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

695

Harvest time	Date	TS (% of FM)		
		Leaves	Stems	
PHR1	16 May 2014	44.9%	37.6%	696
PHR2	11 June 2014	46.7%	38.3%	697
PHR3	16 July 2014	61.2%	50.3%	698
PHR4	29 August 2014	57.0%	55.9%	699
PHR5	24 September 2014	60.9%	59.2%	700
PHR-R	24 September 2014	55.9%	47.9%	701

702

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

705

706

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	*

707

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

710

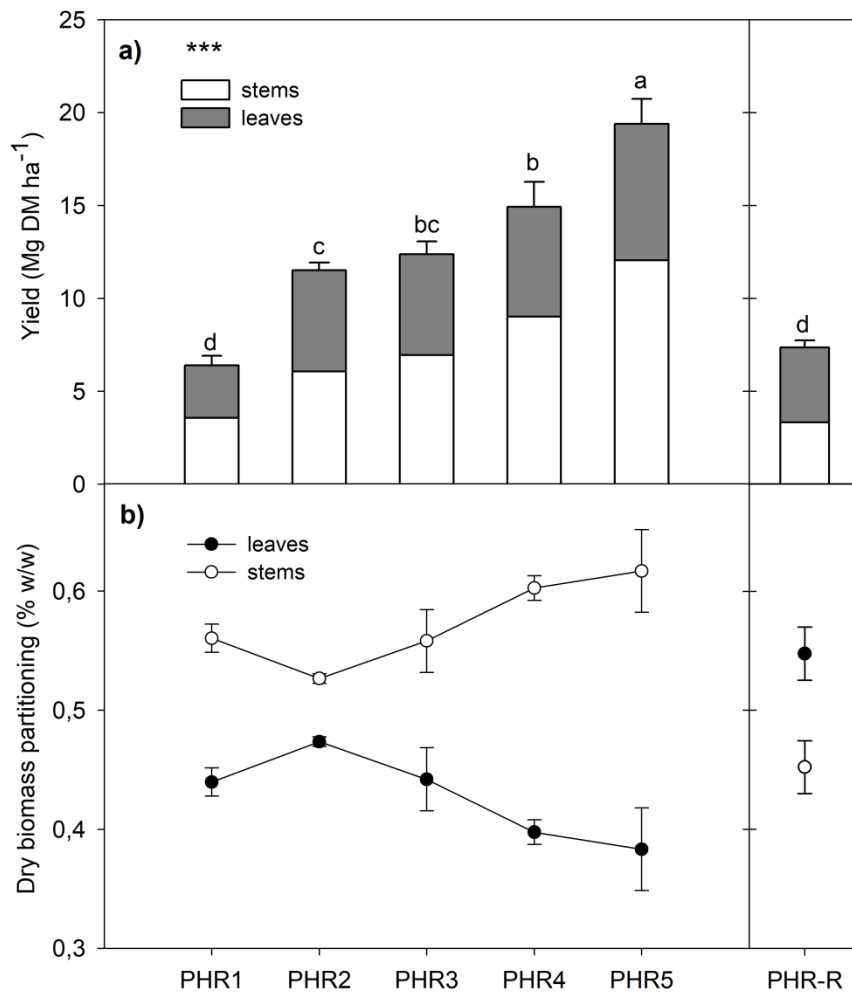


Figure 1. Dry biomass yields (a) and partitioning (b) of common reed harvested at different times; PHR1–PHR5 refer to first cuts, while PHR-R refer to regrowth from PHR2. For biomass yields, significance level of ANOVA is reported (***, $p < 0.001$); values with the same letter are not significantly different ($p \geq 0.05$). Standard errors are shown as vertical bars.

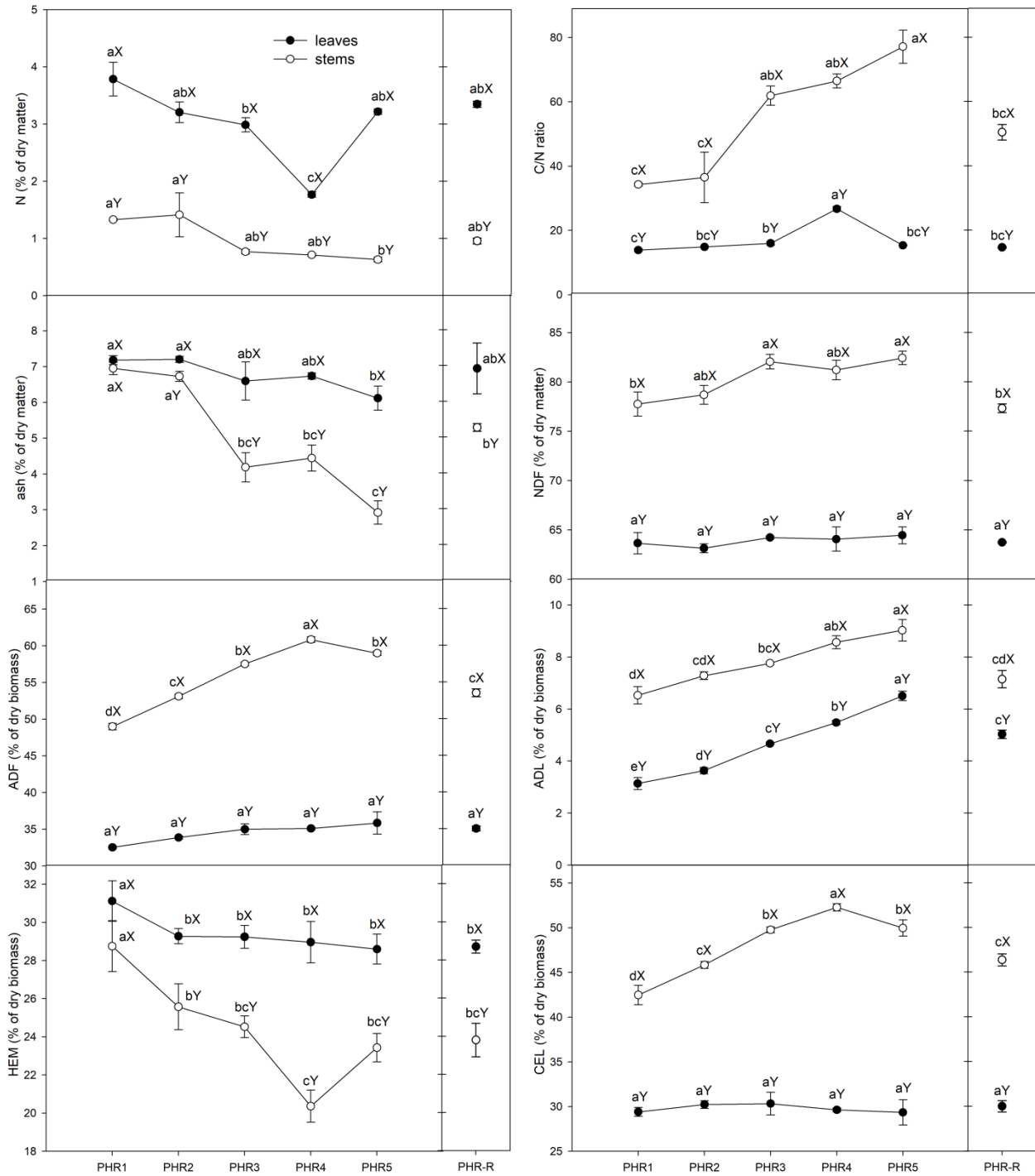


Figure 2. Seasonal changes in chemical composition of common reed biomass; the vertical line separates second cut (PHR-R) from first cuts (PHR1-5). Upper and lower case letters are for comparison within the same date and the same organ, respectively; values with the same letter are not significantly different ($p \geq 0.05$). Standard errors are shown as vertical bars.

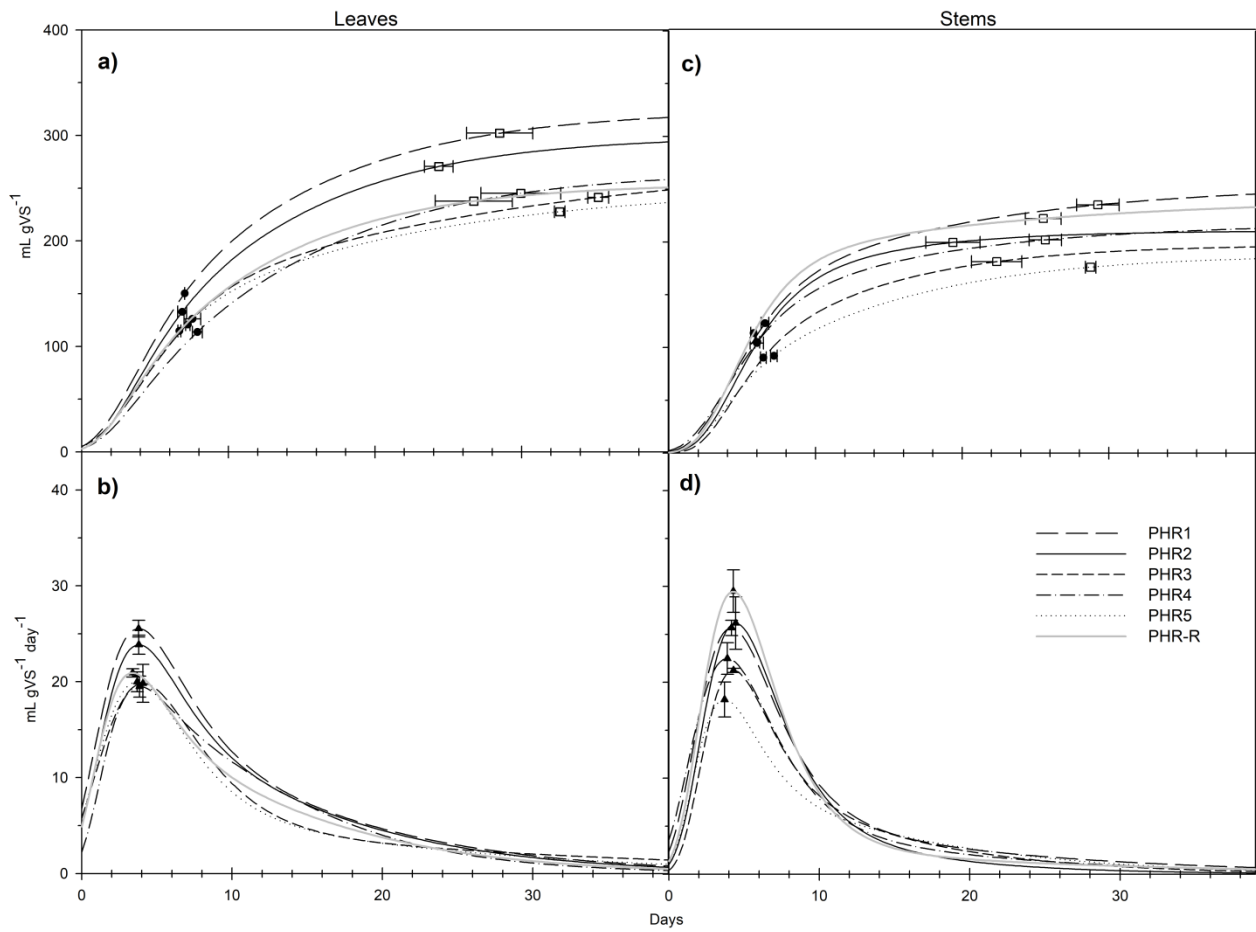


Figure 3. Kinetics of fermentation of common reed harvested at different times; PHR1–PHR5 refer to first cuts, while PHR-R refer to regrowth from PHR2. Cumulative methane production of leaves (a) and stems (c), daily methane production rates of leaves (b) and stems (d) estimated as the first derivative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

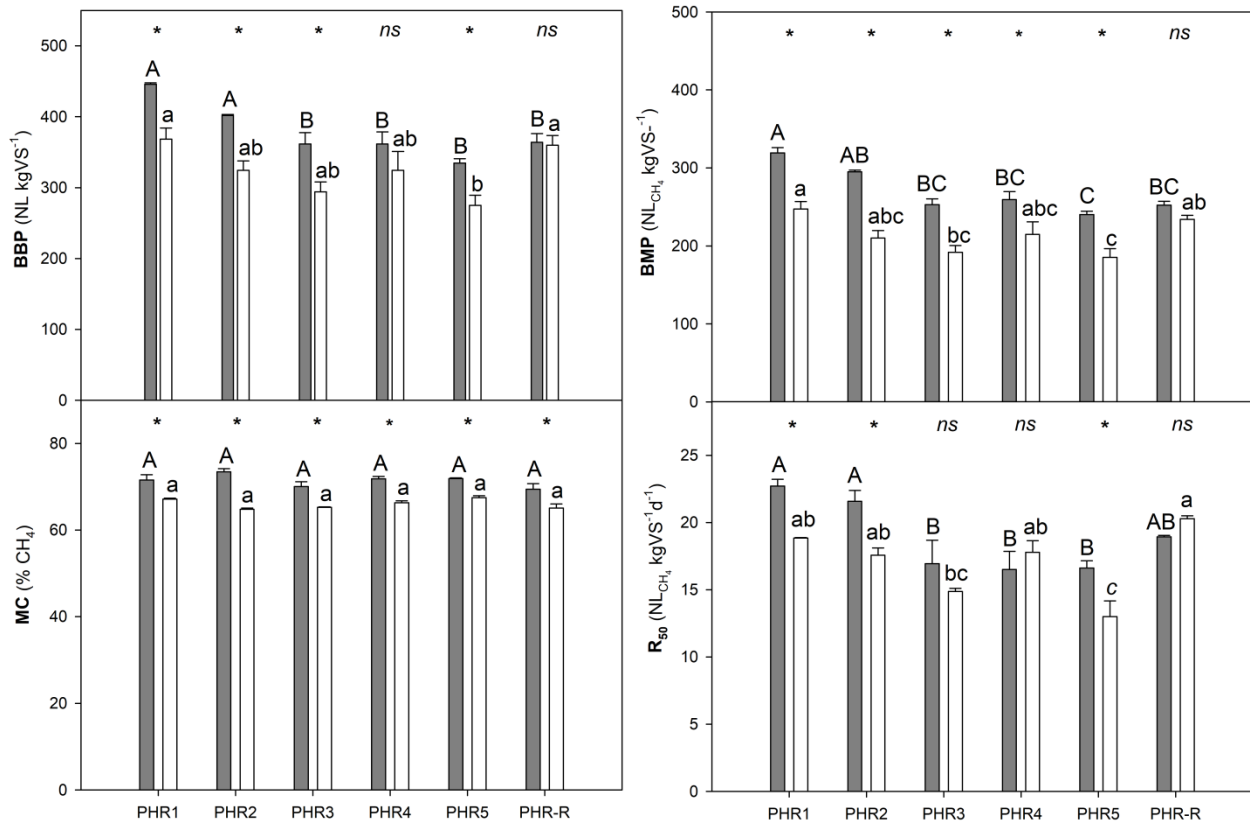


Figure 4. BBP, BMP, average MC, and R50 of the investigated substrates. Upper case letters are for comparisons between leaves (grey bars) harvested at different times, while lower case letters are for comparisons between stems (white bars); values with the same letter are not significantly different. 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.

a)

	BBP						
BBP	1						
BMP	0,99	1					
MC	0,30	0,46	1				
T50	-0,35	-0,32	0,04	1			
T95	-0,50	-0,54	-0,41	0,65	1		
Rmax	0,89	0,90	0,41	-0,68	-0,73	1	
R50	0,92	0,93	0,35	-0,66	-0,70	0,99	1

b)

	BBP						
BBP	1						
BMP	0,99	1					
MC	-0,15	0,00	1				
T50	-0,60	-0,48	0,78	1			
T95	0,15	0,28	0,88	0,54	1		
Rmax	0,88	0,80	-0,56	-0,81	-0,23	1	
R50	0,95	0,91	-0,37	-0,80	0,00	0,94	1

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

a)

	BBP	BMP	MC	T50	T95	Rmax	R50	N	C/N	NDF	ADF	ADL	EMI	CEL
N	0,48	0,43	-0,10	-0,80	-0,21	0,64	0,65	1						
C/N	-0,31	-0,27	0,12	0,80	0,15	-0,55	-0,54	-0,97	1					
NDF	-0,70	-0,73	-0,42	0,61	0,90	-0,87	-0,84	-0,31	0,28	1				
ADF	-1,00	-0,98	-0,29	0,34	0,47	-0,89	-0,92	-0,49	0,33	0,68	1			
ADL	-0,95	-0,92	-0,20	0,39	0,46	-0,88	-0,90	-0,50	0,41	0,75	0,96	1		
HEM	0,92	0,88	0,16	-0,13	-0,15	0,69	0,75	0,47	0,28	0,36	-0,93	-0,84	1	
CEL	-0,13	-0,17	-0,30	-0,16	0,05	-0,01	-0,05	0,07	-0,27	-0,23	0,12	-0,18	-0,27	1

b)

	BBP	BMP	MC	T50	T95	Rmax	R50	N	C/N	NDF	ADF	ADL	EMI	CEL
N	0,64	0,61	-0,27	-0,34	-0,30	0,64	0,56	1						
C/N	-0,77	-0,73	0,34	0,46	0,27	-0,76	-0,69	-0,97	1					
NDF	-0,92	-0,88	0,30	0,58	0,02	-0,94	-0,89	-0,78	0,85	1				
ADF	-0,74	-0,74	0,09	0,18	-0,05	-0,68	-0,58	-0,87	0,91	0,85	1			
ADL	-0,85	-0,81	0,36	0,51	0,18	-0,83	-0,77	-0,85	0,95	0,87	0,91	1		
HEM	0,43	0,46	0,10	0,19	0,10	0,31	0,20	0,75	-0,75	-0,54	-0,90	-0,73	1	
CEL	-0,69	-0,70	0,06	0,09	-0,10	-0,62	-0,52	-0,85	0,87	0,82	0,99	0,86	-0,92	1

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 ($p < 0.05$).

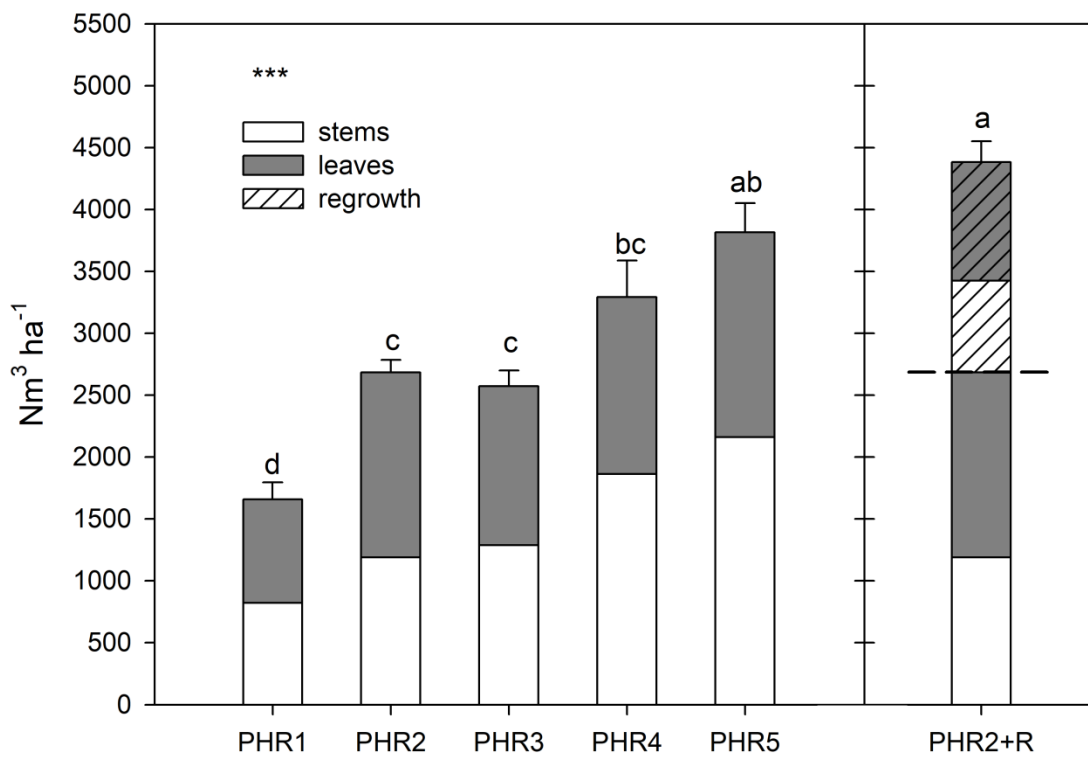


Figure 7 Methane yields per hectare obtained at different harvest times from May to September (PHR1–PHR5) and combining a first harvest in June with a second harvest in September (PHR2+R). Standard errors and significance level of ANOVA are reported (***, $p < 0.001$). Values with the same letter are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