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Intercropping grasses and legumes can contribute to the development of advanced biofuels

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10 **Abstract**

11 Intercropping-dedicated biomass crops can significantly contribute to the sustainable development  
12 of advanced biofuels while improving yield stability. The objective of this study was to quantify the  
13 impact of intercropping of the legume sunn hemp (SH; *Crotalaria juncea*; cv. Ecofix) on the  
14 productivity of pearl millet (PM; *Pennisetum glaucum*; cv. ICMV I707) and biomass sorghum (S;  
15 *Sorghum bicolor* (L.); cv. Triton), with or without nitrogen fertilisation (150 kgNha<sup>-1</sup>). The  
16 intercrops were SxSH and PMxSH. Quantitative and qualitative biomass traits were evaluated for  
17 each cropping system. Land equivalent ratio (LER) and species evenness were used to evaluate the  
18 performance of the intercrops. Across fertilisation levels, average biomass yields in 2018 and 2019  
19 were: 23 and 19 Mg ha<sup>-1</sup> (SxSH), 18 and 17 Mg ha<sup>-1</sup> (PMxSH), 21 and 12 Mg ha<sup>-1</sup> (PM), 24 and 20  
20 Mg ha<sup>-1</sup> (S), and 14 and 13 Mg ha<sup>-1</sup> (SH). Overall, LER showed an increase of 22% in PMxSH and  
21 6% in SxSH over the years. Within the intercrops, S showed a larger competitive effect over SH  
22 than PM did; species evenness ranged between 0.56 and 0.67 in SxSH and between 0.89 and 0.92 in  
23 PMxSH. Moreover, compared to monocropping, intercropping led to improved qualitative  
24 feedstock characteristics for bioenergy applications: intercropped PM showed a higher Si/K ratio  
25 (+32%), while intercropped SH showed increased cellulose content (+17%) and reduced N (-39%),  
26 Mg (-54%), and Na (-15%) contents. Intercropping-dedicated lignocellulosic crops may be feasible  
27 alternatives for providing a mixture of dedicated feedstocks with improved sustainability, yield  
28 stability, and biomass quality.

29 **Keywords:** Bioenergy; Biomass; Land equivalent ratio; Lignocellulose; Quantitative/qualitative  
30 performance; Thermo/biochemical conversions

31

32

## 33 **1. Introduction**

34 Human-induced global warming has several well-documented causes with known repercussions on  
35 the climate. The utilisation of fossil fuels as the predominant energy source has been recognized as  
36 the principal factor (70%) contributing to increased CO<sub>2</sub> emissions and therefore the accelerated  
37 climate change registered in the last few decades [1]. The European Union (EU), among other  
38 governments, has set up long-term energy policies aimed at mitigating CO<sub>2</sub> emissions and,  
39 therefore, the effects of climate change such as RED II, Green Deal, and CETP. One of these  
40 policies is to increase the production of dedicated lignocellulosic feedstocks for advanced biofuels  
41 in diversified crop production systems [2]. Diversified cropping systems with dedicated  
42 lignocellulosic crops offer many economic, environmental, and social advantages over sugar/starch  
43 monocropped feedstocks initially identified for first-generation biofuel production as a source of  
44 renewable energy. Dedicated lignocellulosic crops could result in several benefits: reduction of  
45 greenhouse gas emissions, diversification of feedstock, increased resilience of cropping systems,  
46 and increased biofuel yields while avoiding indirect land use changes (iLUC) effects [3, 4]. The  
47 development of advanced biofuels depends on low-input and sustainable cropping systems that can  
48 efficiently use natural resources without affecting food production. Intercropping could increase  
49 land and resource use efficiency, yield stability, productivity of biomass per unit area, and support  
50 biodiversity.

51 Intercropping is defined as the simultaneous growth of two or more crop species on a single  
52 piece of land during the growing season [5, 6]. Such systems, if properly implemented, could have  
53 particular significance for current and future biomass and bioenergy demands and environmental  
54 concerns. In China, a study on long-term intercropping with food crops revealed that compared to  
55 monocrops, intercrops showed higher levels of carbon sequestration, a 23% higher aboveground  
56 and belowground biomass production, and an increase of 11% in total N soil content [7]. However,  
57 information on the performance (in biological and productivity terms) of intercropping systems that

58 only include dedicated lignocellulosic crops is limited, particularly for legume species. In such a  
59 system, a legume with a high lignocellulosic biomass-yielding potential could not only improve the  
60 yield of a companion grass crop, but also help to maintain a well-balanced soil fertility program and  
61 reduce N fertilisation costs [8, 9]. In addition, intercropping-dedicated lignocellulosic crops (grasses  
62 and legumes) could offer better land use opportunities, such as feedstock production in marginal  
63 lands without competing issues with food crops (low iLUC risks) [10]. Utilising more intensive and  
64 more sustainable crop production systems can increase resource use efficiency, integrate  
65 management practices, and improve the environmental performance of biofuels [11, 12].

66         Among promising lignocellulosic crops, biomass sorghum and pearl millet are interesting  
67 multipurpose crops (i.e., grain and straw) options. Biomass sorghum is a drought-tolerant, fast-  
68 growing crop with a high dry biomass yield (30 Mg ha<sup>-1</sup>) already utilised as feedstock for first- and  
69 second-generation biofuels [13]. Pearl millet is widely grown for food purposes in many arid and  
70 semiarid areas of the world and is more resilient than sorghum under harsh weather conditions such  
71 as drought and flood [14-17]. In fact, under harsh environmental conditions it has been shown that  
72 pearl millet grain and biomass yields are equal to or higher than those of sorghum, suggesting its  
73 potential as an alternative bioenergy crop [18]. Sunn hemp is a fast-growing tropical legume, with a  
74 relatively high lignocellulosic biomass production potential (10 -13 Mg ha<sup>-1</sup> in about three months)  
75 that can fix 50 to 60 kg ha<sup>-1</sup> of N<sub>2</sub> during its life cycle [19, 20]. In its native areas, sunn hemp is  
76 traditionally grown as a non-wood fibre crop and is cultivated in rotation with rice (*Oryza sativa*),  
77 maize (*Zea mays*), cotton (*Gossypium spp.*), sugarcane (*Saccharum officinarum*), tobacco  
78 (*Nicotiana tabacum*) and coffee (*Coffea arabica*) [17, 21, 22]. Therefore, its utilisation as an  
79 intercropped energy crop is of interest. However, no available information on sunn hemp  
80 intercropped with grass crops such as biomass sorghum and pearl millet for lignocellulosic  
81 feedstock production purposes was found; therefore it is important to understand the potential of  
82 such intercrops for biomass production (quantitatively and qualitatively), species complementarity,

83 and resource utilisation. In fact, owing to the N<sub>2</sub>-fixing capacity of sunn hemp, it could be utilised  
84 as a natural source of nitrogen for the companion grass, thus reducing the need for fertilisation.  
85 Moreover, the root systems of sorghum, pearl millet, and sunn hemp show contrasting soil  
86 exploration layers [23-25] which could lead to significant complimentary resource use due to better  
87 root distribution throughout the whole soil profile. Additionally, the sunn hemp-grass systems could  
88 be used as bifunctional cropping systems, considering that either biomass sorghum or pearl millet,  
89 with the right varietal choice, could produce grain for human or animal feed and straw for biofuel  
90 production, further improving the land and resource use efficiency.

91 In addition, the identification of dedicated intercropping systems with enhanced qualitative  
92 feedstock characteristics would represent a significant step forward in producing more sustainable  
93 biofuels. In general, traits that define the qualitative characteristics of a feedstock to maximise  
94 conversion (biochemical or thermochemical) process efficiencies are moisture content; calorific  
95 value; proportions of fixed carbon, volatiles, ash, inorganic elements, and alkali metals; and cell  
96 wall composition [26, 27]. In the thermochemical approach, a negative relationship exists between  
97 the ash content in the biomass (i.e. Ca, Si, K, P, and Cl content) and the reduction of heat exchange  
98 in the combustor connected with slagging and fouling processes. Alkali elements such as K, Na, and  
99 Cl are considered the most detrimental elements affecting the process [26]. While in the  
100 biochemical pathway cellulose, hemicellulose and lignin contents are useful indicators of the  
101 bioethanol yield that could be achieved. Several agronomic factors can affect the biomass  
102 composition (i.e. soil type, weather, nitrogen fertilisation, irrigation, harvest time), and among them,  
103 intercropping (perennial crops and annual crops such as silage maize and forage sorghum) has been  
104 demonstrated to modify the conversion quality of the biomass for some bioenergy applications [28,  
105 29]. However, data on the effects of intercropping on biomass yield and qualitative characteristics  
106 of the crop components including dedicated lignocellulosic N<sub>2</sub>-fixing species, are lacking. The  
107 objective of this study was to quantify the impact of intercropping on the productive potential (in

108 quantitative and qualitative terms) of pearl millet, biomass sorghum and sunn hemp, for energy  
109 production, under different nitrogen fertilization conditions.

## 110 **2. Materials and methods**

### 111 **2.1. Study site**

112 The study was performed at the Cadriano Experimental Farm of Bologna University, Italy (44°33'  
113 lat. N, 11° 21' E, 32 m a.s.l.) in 2018 and 2019. The study site was classified as fine silty mixed  
114 mesic udic ustochrept soil (9% sand, 34% clay, and 57% silt) with high exchangeable potassium  
115 (174 mg kg<sup>-1</sup>) and average assimilable phosphorus and nitrogen contents (59 mg kg<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and  
116 0.14% of total N, respectively). The soil had a neutral pH (6.8) and an organic matter content of  
117 1.3%. The climate of the experimental site is typical of a temperate humid region with cold winters  
118 and hot summers. Normally, the growth season lasts from early spring (April) to the end of summer  
119 (September). Precipitation occurs throughout the year, but with two well-defined peaks: one in  
120 spring and the other in autumn. Summers are dry, usually with the lowest amount of precipitation  
121 throughout the year. Mean temperatures recorded from April to September in 2018 and 2019 were  
122 22.2 °C (±4.5) and 20.6 °C (±5.6), corresponding to 0.84 °C higher and 0.72 °C lower than the  
123 long-term mean, respectively. During the study period, the cumulative precipitation during the  
124 growing season in 2018 was 281 mm and in 2019 was 301 mm, 67 and 47 mm less than the long-  
125 term averages, respectively. In May 2019, the long-term average rainfall was exceeded by 100 mm;  
126 however, from June to September 2019, the rainfall was similar to that in the same period in 2018,  
127 which was between 42% and 48% lower than the long-term average.

### 128 **2.2 Treatments and field management**

129 Before sowing, to facilitate crop establishment in both growing seasons, the soil was ploughed to a  
130 depth of approximately 20 - 25 cm and double-harrowed. During soil preparation, approximately  
131 100 kg of P<sub>2</sub>O<sub>5</sub> was applied for basal fertilisation. The trial was set up in a randomised block design

132 with a factorial arrangement with four replicates. The nitrogen levels (N0 and N150) were set as the  
133 main plots, and the cropping systems (mono and intercrops) were set as the sub-plots. In the  
134 fertilised plots (2018 and 2019), urea (150 kg N ha<sup>-1</sup>) was broadcasted and incorporated into the soil  
135 together with mechanical weeding approximately 30 days after sowing (DAS). Biomass sorghum  
136 (S; cv. Triton), pearl millet (PM; cv. ICMV I707), and sunn hemp (SH; cv. Ecofix) were grown as  
137 monocrops, while the intercrops were composed of S × SH and PM × SH. Both monocrop and  
138 intercrop plots were 5.3 m × 7.5 m and the total planted area covered 1590 m<sup>2</sup>. The intercropping  
139 layout was a 3:3 replacement strip cropping system. All crops were sown on 8 May 2018 and 24  
140 May 2019 with a pneumatic planter and a distance of 0.45 m between rows. The planting densities  
141 within the rows in the monocropped and intercropped systems were 19, 22, and 39 pL m<sup>-2</sup> for S,  
142 PM, and SH, respectively. Due to the low emergence of PM in 2019, re-sowing was done on 18  
143 June. At the time of sowing, a granular soil insecticide (Ercole, 10 kg ha<sup>-1</sup>) was applied, after which  
144 no diseases or pests were detected; therefore, additional pest treatments were not necessary. To  
145 ensure a good emergence rate and seedling establishment, a total of 26 mm of water was applied in  
146 two supplemental irrigation events within the first 30 DAS in each growing season.

### 147 **2.3 Crop measurements**

148 The monocropped and intercropped plants were harvested by hand in an area of 5.4 m<sup>2</sup> at the end of  
149 the corresponding growing seasons (~DAS 145-150). Biomass sorghum reached full maturity,  
150 while SH and PM had reached the beginning of flowering and seed formation, respectively. At  
151 harvest time, the biometric parameters plant height, basal stem diameter, number of tillers, and  
152 number of branches were recorded for the grass and legume crops. In addition, leaf area was  
153 measured with a leaf area meter (LI-3000; LI-COR, Lincoln, Nebraska, USA) in each cropping  
154 system. These values were used to calculate the leaf area index (LAI) as the ratio of the total one-  
155 sided leaf area per unit ground surface area. Aboveground dry biomass of shoot components (stems  
156 and leaves) was determined by oven drying at 105 °C to a constant weight. To evaluate the



157 effectiveness of intercropping, the land equivalent ratio index (LER) of shoot components and total  
158 biomass produced was calculated following Osiru and Willey [30]. The LER is defined as the total  
159 land area required under monocropping to produce the same yield as in the intercropping, and is  
160 expressed as:  $LER = (Y_a / S_a) + (Y_b / S_b)$ , where Y and S are the yields per unit area,  $Y_a$  and  $Y_b$  are  
161 the intercrop yields of the component crops, and  $S_a$  and  $S_b$  are the monocrop yields. Species  
162 evenness was then calculated to assess the relative yield of each species in the intercrop and,  
163 therefore, the species dominance. Species evenness is a measure of the relative abundance of  
164 species in an intercrop and it is expressed as:  $Species\ evenness = \sum (P_i \ln P_i) / (\ln S)$ , where the  
165 proportion (P) is the amount of biomass of a species (i) in an intercrop multiplied by the natural log  
166 (ln) of that proportion and summed across the species present, and S is the natural log of the number  
167 of species in the intercrop [31].

168 In 2018 separate representative leaf and stem subsamples were pooled, oven dried to a  
169 constant mass at 60 °C and ground to a diameter of 1 mm. The ground biomass was analysed in  
170 four replicates to determine the ash and mineral content. Ash was extracted by incineration of the  
171 dry biomass in a furnace muffle at 550°C for 3 h on a 3 g sub-sample. The concentrations of the  
172 most important minerals (Ca, K, Na, P, S, and Si) in terms of heat exchange reduction in the  
173 combustor connected with slagging and fouling processes were determined through a wet digestion  
174 pre-treatment carried out in a microwave oven by inductively coupled plasma (ICP). The Filter Bag  
175 Technology (FBT, ANKOM technology) was used to determine the cell wall components (i.e.,  
176 lignin, cellulose, and hemicellulose) in four replicates, using the AOAC 991.43 and 985.29  
177 methods. In a CHN combustion analyser, the total N and C contents were determined in four  
178 replicates. These data were used to calculate the amount of nitrogen removed by the crop as the  
179 product of the nutrient concentration and dry biomass yield. The N balance was calculated  
180 following Stoltz and Nadeau [32] as the difference in N inputs (i.e. N content in the soil before  
181 sowing + N fertilisation) minus the N outputs (i.e. the N content in the crop and the residual N in

182 the soil after harvest). The percentage of nitrogen derived from the soil (NDFS%) was calculated as  
183 the atom %  $^{15}\text{N}$  excess in the plant divided by the atom %  $^{15}\text{N}$  excess in the soil (adapted from  
184 Kchaou et al. [33]). The atom %  $^{15}\text{N}$  excess was estimated by subtracting the natural abundance  
185 (0.3663 atom%  $^{15}\text{N}$ ) from the soil and plant samples. The natural abundance of  $^{15}\text{N}$  isotopes were  
186 determined in four replicates of plant (pooled representative subsamples of leaves and stems) and  
187 soil (taken at 20-25 cm depth) materials with the aid of continuous flow–isotope ratio mass  
188 spectrometry (CF–IRMS, Delta V Advantage Thermo Scientific). No distinction between N derived  
189 from the soil and the fertilizer was made at the beginning of the trial (2018) because the N levels  
190 and isotopic signature were similar in the fertilised and non-fertilised plots (2.6 and 2.5 Mg N ha<sup>-1</sup>;  
191 0.3690 and 0.3689 atom%  $^{15}\text{N}$ , respectively). These balanced values are attributed to the preceding  
192 crops and fertilisation management of the whole field in 2017, that is, in the fertilised plots, the  
193 preceding crop was sorghum grown under customary management practices, which include  
194 fertilisation rate of 150 kg N ha<sup>-1</sup>. In the unfertilised plots (N0), the preceding crop was sunn hemp  
195 grown without N fertilisation, thus relying on its own N<sub>2</sub>-fixing capacity. In addition, the subplots  
196 (i.e. cropping systems) within the fertilisation treatments were subsequently rotated to avoid any  
197 potential negative effects of growing the same crop in the same place every year.

## 198 **2.4 Statistical analysis**

199 The Bartlett test was used to determine homogeneity of variance across growing seasons.  
200 Homogeneity was not detected for all the parameters evaluated; therefore, the analyses were  
201 performed separately for each parameter. All parameters were then subjected to analysis of variance  
202 (ANOVA), and when significant differences ( $P < 0.05$ ) were detected, Fisher's LSD test for  
203 comparison of means was performed.

## 204 **3. Results**

### 205 **3.1 Biometric, productive, and nitrogen uptake parameters**

206 The biometric parameters of monocropped and intercropped PM, S and SH in 2018 and 2019 are  
207 shown in Table 1. No interactions between fertilisation level and cropping systems were found for  
208 any of the parameters. Monocropping resulted in statistically significant lower values than  
209 intercropping: intercropped PM showed lower plant height in 2019 and lower stem diameter in  
210 2018; S showed lower plant height in 2018 only; and SH showed smaller stem diameter in 2018 and  
211 reduced branching capacity especially when intercropped with S in both growing seasons. The  
212 effect of fertilisation was only significant on the stem diameter of the intercropped PM and SH.

213 Figure 1 shows the effects of intercropping and N fertilisation on the biomass production of  
214 grasses and legume crops. N fertilisation did not have significant effects, whereas the cropping  
215 system significantly changed the biomass yield. In both years, the intercropping systems (PM × SH  
216 and S × SH) showed similar biomass yields (on average of both years 18 and 20 Mg ha<sup>-1</sup>,  
217 respectively) although the dominant components had variable yields; in 2018 both grasses were  
218 dominant over SH, while in 2019 SH overtook PM due to the re-sowing of PM three weeks later. In  
219 all cases the intercropped yields were similar to grasses production potential under monocropping  
220 conditions, with S (either monocropped or intercropped) showing significantly higher ( $P \leq 0.05$ )  
221 values than the monocropped legume. SH showed a considerable yield reduction when intercropped  
222 with S (-78% and -75% in 2018 and 2019, respectively) and when SH was intercropped with PM  
223 the yield reduction ranged between -55% and -38% in 2018 and 2019, respectively. This is  
224 supported by the species evenness indicator (Fig. 2), which was 0.9 and 0.6 in PM × SH and S × SH  
225 intercrops, respectively. These results reveal a higher competitive effect of S than PM over SH.  
226 Figure 3 shows the productivity of the intercrop systems and the competitive interactions between  
227 the intercropped species. Statistically, no significant differences were observed between the  
228 different intercropping systems. The average LER across year and cropping systems was 1.14,  
229 indicating a 14% increase in productivity compared to monocropping. In both years, the LER of PM  
230 × SH was slightly higher (1.10 and 1.35) than that of S × SH (1.07 and 1.04). This could be related

231 to the more complementary responses observed between pearl millet and sunn hemp, where the  
232 partial LERs were 0.59 and 0.63, respectively. Conversely, the average partial LER of sorghum and  
233 sunn hemp were 0.82 and 0.24, respectively. Across fertilisation levels and years, the total LER of  
234 intercropping sunn hemp with pearl millet showed an increase of 22%, while that of S × SH was  
235 only 6%. All these productive and competitive patterns are also clearly reflected in the leaf area  
236 index LER's (Fig. 3).

237 There were generally no significant interactions between cropping systems and N  
238 application in terms of N uptake and N balance. As for N uptake, the fertilized monocropped and  
239 intercropped species used 17% more soil N than the unfertilized crops (Fig. 4). Among the crops,  
240 sunn hemp used approximately 1.7 times more N than the grasses. However, under intercropping  
241 conditions, the intercropped PM × SH used 1.5% more N than the S × SH intercrop. These N uptake  
242 patterns were mirrored by the calculated N balance at both fertilisation levels but not in the  
243 cropping systems, that is, in the fertilised plots, the N balance was lower than in N0 due to a higher  
244 N uptake (Fig. 5). The N balances among the monocropped and intercropped systems were  
245 statistically similar.

246 N recovery from soils (NDFS) is shown in Figure 6. The major fraction of N absorbed by  
247 monocropped grasses originated from soils. Furthermore, the fraction of N absorbed from the soil  
248 was on average 1.1 times higher in the unfertilised plots than in the fertilised plots. Among the  
249 monocrops, sunn hemp recovered the lowest fraction of N from the soil. In contrast to the  
250 monocropped grasses, SH recovered more N from the soil under fertilised than unfertilised  
251 conditions because of the well-know reduced symbiotic N<sub>2</sub>-fixing capacity under such conditions.  
252 As for the intercrops (PM × SH and S × SH) the N recovery from the soil followed the dominant  
253 trend of the monocropped grasses but with significantly lower values in each case; the average  
254 reduction recovery potential was 17% and 23% for the PM × SH and S × SH systems, respectively.

255 Moreover, the NDFS of intercropped S × SH was significantly lower than that of PM × SH,  
256 probably because of the enhanced competition between S and SH.

### 257 **3.2. Intercrop yield quality for advanced biofuels**

258 Table 2 shows the cell wall components and total nitrogen, total carbon, and ash content of the  
259 different crops under monocropped and intercropped conditions. Some of the reported differences  
260 described below are intrinsic to the species used regardless of the cropping system. For example,  
261 pearl millet and sorghum showed about twice as much hemicellulose content as sunn hemp,  
262 whereas sunn hemp showed the highest cellulose and lignin content. The ash content was similar  
263 among the three crop species (approximately 5%). However, no interaction between cropping  
264 system and fertilisation was observed, and neither fertilisation level had a significant effect on any  
265 of the parameters evaluated. A difference in intercropped and monocropped pearl millet was  
266 recorded only in the cellulose content, which was 9% lower in the PM × SH compared to PM alone,  
267 whereas similar values were observed for other parameters in both cropping systems. No  
268 differences were found between monocropped and intercropped sorghum. As for sunn hemp, the  
269 SH × S intercrop showed a 12% and 17% increase in the hemicellulose and cellulose content,  
270 respectively, and a reduction in total N (-28%) and C (-10%) compared to monocropped SH.

271 The mineral concentration (Table 3) did not vary between the monocropped and  
272 intercropped grasses, except for the PM × SH where the Si/K ratio of the intercropped PM was 32%  
273 higher than that in the monocropped scenario. Conversely, sunn hemp showed marked differences  
274 in mineral concentrations depending on the intercropping system. In particular, Al content was 2.6  
275 times higher in the SH × PM intercrop than in the monocrop scenario, and Mg and Na content  
276 decreased by 35% and 13%, respectively, in SH × S compared to monocropped SH. The Si/K ratio  
277 showed the following trend: SH × S > SH × PM > SH, whereas the Ca/K ratio was as follows: SH ×  
278 PM > SH × S = SH. Nitrogen fertilisation only affected the Na content in the biomass, resulting in a  
279 33% higher concentration in the fertilised plots than in the unfertilised plots.

280           Among the three species, the mineral concentration was generally lowest in sorghum,  
281 whereas pearl millet and sunn hemp showed alternating peaks. Compared to sunn hemp, pearl millet  
282 presented higher concentrations in two of the 17 parameters evaluated (higher K and P by 42% and  
283 28%, respectively). Conversely, sunn hemp showed higher concentrations of N, Ca, and Mg  
284 compared to pearl millet (3.0, 3.6, and 1.6 times, respectively). Si/K and Ca/K ratios were highest  
285 for sorghum and sunn hemp, respectively with pearl millet showing intermediate values in both  
286 cases.

## 287 **4. Discussion**

### 288 **4.1 Biomass yields, nitrogen use, and competition between intercrops**

289 Intercropping-dedicated lignocellulosic crops, particularly if legumes are included, are a promising  
290 solution to the development of advanced biofuels and to enhance the sustainability and risk  
291 minimisation (i.e. soil degradation and stable production) of low iLUC energy cropping systems.  
292 However, information on biomass potentials (quantitatively and qualitatively), crop  
293 complementarities, and resource use of such cropping systems is limited. This study focused on a  
294 new leguminous species, sunn hemp, intercropped with sorghum (S × SH) or pearl millet (PM ×  
295 SH). The results showed that PM × SH and S × SH cropping systems had statistically similar  
296 biomass yields, either with or without N fertilisation (Fig. 1). The lack of response to N fertilisation  
297 may be attributed to the preceding crop on the unfertilised plots being sunn hemp, which may have  
298 fixed enough N<sub>2</sub> to a similar level as the fertilised plots on which the preceding crop was sorghum.  
299 A possible advantage of intercropping a leguminous crop is that some of the N<sub>2</sub> fixed by the legume  
300 can be transferred to the grass. Chu et al. [34], for example, found that N<sub>2</sub> fixed by a peanut  
301 (*Arachis hypogaea*) crop was transferred to the intercropped rice at decreasing rates (from 12% to  
302 6%) when mineral N fertilisation was increased. Moreover, the beneficial effect of fixed N<sub>2</sub> on the  
303 subsequent crop, more than on the companion crop, is a well-known phenomenon already observed  
304 in many legume-grass mixtures [9, 35-37]. In a companion crop, the beneficial effects could be

305 related to enhanced root growth and complementary functioning, leading to a greater yield stability  
306 [12, 38].

307 Our results show that biomass yield for both intercrops was within the productive range of  
308 monocropped grasses [13, 20, 39], but higher than that of monocropped sunn hemp. Within the  
309 intercrops, sorghum showed a significantly greater suppressive effect on sunn hemp than pearl  
310 millet in terms of biomass yield, stem diameter, branching capacity, and biomass and LAI partial  
311 LERs (Table 1, Fig. 3). Biomass sorghum reached full canopy development earlier than pearl millet,  
312 resulting in a greater shading effect on sunn hemp. Similar effects of competition for light in other  
313 grass-legume intercrops have been reported in the literature [40, 41]. Additionally, biomass  
314 sorghum produces root exudates such as sorgoleone (a potent PSII inhibitor), that disrupts the  
315 biosynthesis of carotenoids, and introduces anatomical changes in the stems of legumes and other  
316 broadleaf species [42]. Moreover, shading of the intercropped legume might have affected its  
317 photosynthetic capacity and nodule vitality and therefore N<sub>2</sub> fixation capacity [43, 44]. Given the  
318 much higher early growth rate of sorghum than pearl millet [40], it is possible that postponing  
319 sorghum sowing by two or three weeks, as in the case of pearl millet in 2019, could result in a better  
320 balance when sorghum is intercropped with sunn hemp. However, the evenness values (near 1) of  
321 the PM × SH intercrop (Fig. 2) suggest that this system is more suitable for yield stability and  
322 diversification of feedstock production for biofuels, because the proportion of grass and legume  
323 crops would be better balanced. The better suitability of pearl millet over sorghum needs to be  
324 confirmed in future studies where the effects of delayed sowing of sorghum are evaluated.

325 Intercropped grasses and legumes also compete for soils resources, especially at early  
326 growth stages, when root systems are not specialised and are distributed in different soil layers. In  
327 the present study, both intercrops removed less N than the monocropped legume (Figs. 4 and 5),  
328 mainly because the N<sub>2</sub> fixed by the intercropped sunn hemp was reduced by 55% and 84% in the  
329 PM × SH and S × SH intercrops, respectively. The largest reduction in S × SH indicates that

330 biomass sorghum has a greater competitive ability for mineral N than pearl millet. Moreover, the  
331 intraspecific competition within the grass species might have been limited by intercropping, thus  
332 further reducing the competitiveness of sunn hemp; consequently, its N<sub>2</sub> fixation capacity might  
333 have been promoted, although the overall N<sub>2</sub> fixed was reduced due to the lowered biomass  
334 production [40]. However, N fertilization had an effect on the N amount taken up by the crops,  
335 leading to a 20% increase in N removal in comparison with the unfertilised plot, where the main  
336 source of N could be associated with the fixed N<sub>2</sub> by the preceding leguminous crop (sunn hemp).  
337 In fact, several studies on leguminous crops with high levels of N fertilisation have shown that N is  
338 mostly derived from soil and little from N<sub>2</sub> fixation, as in our trial (Fig. 6) [43, 44]. Although the N  
339 levels at the beginning of the trial (2018) were similar in the fertilised and unfertilised plots (2.6 and  
340 2.5 Mg N ha<sup>-1</sup>), these results could have been influenced by the preceding leguminous crop (sunn  
341 hemp in 2017) and the consequent N mineralisation.

#### 342 **4.2. Intercrop yield quality as advanced biofuels feedstock**

343 Cell wall components determine the final fuel yield, in particular high hemicellulose and cellulose  
344 contents are desirable for maximising ethanol production. Moreover, low ash (<5%) and mineral  
345 concentration (i.e., Na, K, Ca, S, Si, and the combination of alkali metals with silica) reduce  
346 fouling, slagging, and corrosion during combustion [26, 27, 45, 46], which is essential for efficient  
347 thermochemical biomass processing. In our study, the ash content in all crops and cropping systems  
348 was around 5% threshold (Table 2; [27]); however, most of the minerals and ashes were  
349 concentrated in the leaf fraction (data not shown), indicating that with optimised species  
350 combinations and proportions, selected cultivars, agronomic practices, and postharvest logistics  
351 could be reduced/eliminated, thereby improving biomass quality but at the expense of biomass  
352 quantity. The leaf fraction, however, can be either left or incorporated into the soil to preserve its  
353 fertility, but careful management is required as high N rates might lead to an increase in ash content  
354 of the harvested biomass and consequently slagging problems and potential NO<sub>x</sub> emissions from  
355 combustion processes. In our study, however, the similar cell wall components as well as ash and



356 mineral contents (Tables 2 and 3) of the biomass in the fertilised and unfertilised plots could be  
357 related to the preceding crop (sunn hemp) in the unfertilised plot that was able to fix N<sub>2</sub> to an  
358 adequate level, similar to that of the fertilised plots.

359 Monocropped and intercropped sorghum showed the most favourable characteristics for  
360 both thermo- and biochemical conversions (Tables 2 and 3) because of the high hemicellulose and  
361 cellulose content, and low lignin and mineral concentrations compared to the other cropping  
362 systems. Biomass sorghum in either cropping system was harvested at the full ripening stage, which  
363 may have contributed to the higher cellulose content, hemicellulose deposition, and lower mineral  
364 concentration. Thus, the difference between pearl millet and sunn hemp could be explained by the  
365 maturity stage at which both species were harvested; both were harvested at the beginning of the  
366 reproductive stage when the plants were still green and nutrients had not yet been mobilised back to  
367 the soil. Moreover, the better suitability of biomass sorghum for thermochemical conversion, in  
368 comparison with pearl millet and sunn hemp, is indicated by the high Si/K ratio (Table 3), which  
369 can help in lowering the slagging tendency of the boilers. This higher Si/K ratio could be due to the  
370 higher efficiency of biomass sorghum in utilising K [28], one of the most important alkali metals  
371 (together with Ca, Si, and Cl) to affect thermochemical processes [26, 27].

372 Compared to sorghum, pearl millet showed a high cellulosic fraction; hence, it might have  
373 good potential as feedstock for advanced biofuel, even though some suboptimal mineral  
374 concentration may cause issues in managing a thermochemical conversion plant with this type of  
375 feedstock. In particular, K, Na, P, and S were almost two-fold more concentrated in pearl millet  
376 than in sorghum (Tables 2 and 3) probably due to harvesting of pearl millet in the early stage of  
377 maturity. However the quality of the intercropped pearl millet improved in terms of Si/K (+32%) as  
378 a result of reduced K uptake in the acidified rhizosphere created by the protons released by the  
379 legume roots [47]. Moreover, the slight decrease in cellulose content (-9%) compared to the sole  
380 pearl millet suggests that intercropped PM, irrespective of yield level, might be better suited to

381 thermochemical conversion. The lowered cellulose content could be a stress response to  
382 competition with sunn hemp for the most limiting resources (i.e. temperature, soil moisture, light,  
383 nutrients), as demonstrated in perennial grasses facing stress. Significant changes in the cell wall  
384 structure, biomass recalcitrance, and sugar release for ethanol production in *Miscanthus* were  
385 observed under drought and nutrient deficiencies [48].

386         The biomass quality of sunn hemp was higher when intercropped with sorghum, compared  
387 to that when monocropped, in terms of the increased hemicellulose and cellulose contents and a  
388 drastic reduction in N and Na concentrations (Tables 2 and 3). Changes in cell wall structure and  
389 the whole plant architecture occur due to shading, as indicated previously. For example, shading has  
390 been shown to lead to changes in tissue proportions, cell wall concentration, and composition in  
391 alfalfa [49]. However, in our study, cell wall loosening, which explains the larger proportions of  
392 cellulose and hemicellulose, appears more related to a suppressed or delayed development of sunn  
393 hemp rather than to changes in the cell wall lignification patterns (Table 3; [50]). Similarly, the  
394 reduced mineral (N, Na) of intercropped sunn hemp could be attributed to shading rather than to  
395 competition at the root level. In fact, compared to monocropping, shading was found to impair  
396 mineral contents of intercropped legumes mainly due to reduced photosynthetic capacity, modified  
397 canopy structure, and reduced biomass accumulation [51]. These findings highlight that the  
398 biochemical pathway fits well sunn hemp characteristics, whereas, the feedstock blend from an  
399 intercrop with sorghum could be suitable for thermochemical conversion as well, even though this  
400 option needs to be further investigated. Nevertheless, the mixture of feedstock harvested at the same  
401 time could be a valid alternative to overcome rigid conversion technologies that were optimised for  
402 a single feedstock. Flexible fractionation technologies are considered the most cost-effective  
403 processing technologies to produce lignin and C6/C5 fractions; therefore, the proportions of the  
404 most desirable feedstock fractions could be pre-defined at the crop production stage. In summary,  
405 the improvement of biomass quality in the considered systems is agronomically feasible by

406 delaying the harvest time until complete senescence of the crops when most leaves have fallen off.  
407 Leaves are known to have high ash and mineral contents, which worsen the overall biomass  
408 composition of the feedstock. This scenario can significantly improve feedstock quality, although it  
409 can lead to some agronomical drawbacks such as the increased risk of wet soil conditions at  
410 harvesting, which in turn can cause: i) excessive soil compaction; ii) yield reduction for subsequent  
411 crops; iii) delay or impossibility of planting a winter grass in a crop rotation framework; and iv)  
412 reduced harvest options (only self-propelled forage harvester) to avoid the field drying phase.

## 413 **5. Conclusion**

414 Intercropping had a direct and positive impact on biomass production and stability and on the  
415 qualitative characteristics of the dedicated species as advanced biofuel feedstocks. Biomass  
416 sorghum, rather than pearl millet, seems to have a competitive advantage over sunn hemp. The PM  
417 × SH intercrop appears better balanced and synchronised due to reduced species competition and/or  
418 increased complementarity. However, the delayed sowing date of pearl millet in 2019 may have  
419 influenced its competitiveness.

420 Biomass sorghum, whether monocropped or intercropped, produced the highest biomass  
421 yields (22 and 17 Mg ha<sup>-1</sup>, respectively). Moreover, the hemicellulose, cellulose, and lignin contents  
422 were within the optimal ranges for ethanol production through the biochemical conversion pathway.  
423 The mineral and ash contents were proximate to the generally recommended thresholds to ensure  
424 efficient thermal conversion.

425 The present study has shown that intercropping not only maintains the overall biomass  
426 production close to that of the monocropped grasses (LER increase of 22% and 6% in the PM × SH  
427 and S × SH, respectively), but can also lead to improved feedstock characteristics for determined  
428 bioenergy applications: intercropped pearl millet resulted in improved mineral composition in terms  
429 of increased Si/K ratio (+32%; increased Si and decreased K content) and therefore limited slagging  
430 problems in the boilers. In addition, intercropped sunn hemp (especially with sorghum) showed

431 increased cellulose content and a drastic reduction in mineral content, resulting in improved cell  
432 wall polysaccharide availability for biochemical conversion processes. Intercropping-dedicated  
433 lignocellulosic crops seem to be a feasible alternative for providing a mixture of feedstocks with  
434 improved biomass quality, however, significant developments are still needed in terms of the  
435 quantitative and qualitative suitability of the feedstocks as a function of the species and variety  
436 choice/combinations and their agronomic management (i.e. sowing times, fertilisation practices, and  
437 harvesting operations). It is important to note that the inclusion of a legume in an intercropping  
438 system can enhance the quantitative and qualitative biomass availability and provide valuable co-  
439 products such as food proteins.

440

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444 pearl millet material (PM; cv. ICMV I707), respectively.

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578

Table 1. Biometric parameters of each cropping system and N levels in two consecutive growing seasons. \* indicates the statistical differences of each species among monocropped and intercropped systems and fertilisation levels. ns, no significant difference. CS, cropping system; PM, pearl millet; S biomass sorghum; SH, sunn hemp.

		Height (cm)		Stem diameter (mm)		Branch/tiller <sup>†</sup> (No. m <sup>-2</sup> )	
		2018	2019	2018	2019	2018	2019
Cropping systems (CS)	<b>Pearl Millet (PM)</b>						
	Monocrop (PM)	244	256	12.9	15.5	3.76	3.87
	Intercrop (PM× SH)	243 ns	220 *	14.2 *	14.3 ns	3.72 ns	3.61 ns
	<b>Sorghum (S)</b>						
	Monocrop (S)	375	321	22.1	23.8	---	---
	Intercrop (S× SH)	345 *	289 ns	23.1 ns	23.9 ns	---	---
	<b>Sunnhemp (SH)</b>						
	Monocrop (SH)	248	254	12.9	13.3	6.00	5.34
	Intercrop (PM ×SH)	237 ns	243 ns	11.1 *	14.7 *	5.87 *	5.26 ns
Intercrop (S ×SH)	247 ns	270 ns	9.1 *	13.2 ns	4.76 *	4.68 *	
N level (N)	N0	277	267	14.7	16.2	4.25	4.61
	N150	276 ns	265 ns	15.4 *	17.7 *	4.22 ns	4.50 ns
	CS x N	ns	ns	ns	ns	ns	ns

<sup>†</sup>LN transformed values

Table 2. Cell wall composition, ash content, and total N and C concentration of each cropping system and N level. \* indicates the statistically significant differences for each species among monocropped and intercropped systems and fertilisation levels. ns, no significant difference. CS, cropping system; PM, pearl millet; S biomass sorghum; SH, sunn hemp.

		Hemicellulose	Cellulose	Lignin	Ash	N	C
Cropping systems (CS)	<b>Pearl Millet (PM)</b>						
	Monocrop (PM)	27.1	33.8	4.9	5.7	0.63	45.0
	Intercrop (PM ×SH)	26.4 ns	30.7 *	5.3 ns	5.2 ns	0.72 ns	44.9 ns
	<b>Sorghum (S)</b>						
	Monocrop (S)	25.7	29.8	5.0	4.9	0.62	40.5
	Intercrop (S ×SH)	25.8 ns	29.3 ns	5.4 ns	4.9 ns	0.49 ns	40.4 ns
	<b>Sunnhemp (SH)</b>						
	Monocrop (SH)	14.2	35.1	7.9	5.1	1.81	45.3
	Intercrop (SH ×PM)	14.5 ns	36.5 ns	7.6 ns	5.3 ns	1.74 ns	45.4 ns
Intercrop (SH ×S)	15.9 *	41.2 *	8.0 ns	4.9 ns	1.30 *	40.8 *	
N level (N)	N0	21.3	33.4	6.6	5.1	1.02	43.5
	N150	21.5 ns	34.2 ns	5.9 ns	5.2 ns	1.06 ns	42.9 ns
CS x N		ns	ns	ns	ns	ns	ns

Hemicellulose, cellulose, lignin, ash, N, and C are expressed as %, and the other elements are expressed as mg kg<sup>-1</sup>.

Table 3. Mineral concentration of each cropping system and N level. \* indicates statistically significant differences for each species among monocropped and intercropped systems and fertilisation levels. ns, no significant difference. CS, cropping system; PM, pearl millet; S biomass sorghum; SH, sunn hemp.

		Al	Ca	Fe	K	Mg	Na	P	S	Si	Si/K	Ca/K
Cropping systems (CS)	<b>Pearl Millet (PM)</b>											
	Monocrop (PM)	17	2388	60	15540	2176	237	1937	1554	355	0.0234	0.1596
	Intercrop (PM × SH)	36 ns	2452 ns	87 ns	13196 ns	2432 ns	296 ns	1678 ns	1542 ns	393 ns	0.0309 *	0.1895 ns
	<b>Sorghum (S)</b>											
	Monocrop (S)	13	1889	30	7729	1330	138	1021	738	378	0.0490	0.2461
	Intercrop (S × SH)	29 ns	2006 ns	37 ns	8073 ns	1403 ns	150 ns	808 ns	679 ns	406 ns	0.0510 ns	0.2497 ns
	<b>Sunn hemp (SH)</b>											
	Monocrop (SH)	19	8547	58	10944	3390	229	1511	1548	270	0.0250	0.7894
	Intercrop (SH × PM)	49 *	9627 ns	76 ns	10282 ns	3433 ns	334 ns	1495 ns	1519 ns	311 ns	0.0316 *	0.9560 *
Intercrop (SH × S)	41 ns	7492 ns	54 ns	9009 ns	2201 *	199 *	1146 ns	1204 ns	354 ns	0.0401 *	0.8401 ns	
N level (N)	N0	28	4793	56	10927	2389	197	1436	1289	361	0.0363	0.4752
	N150	31 ns	5022 ns	60 ns	10522 ns	2288 ns	261 *	1309 ns	1227 ns	346 ns	0.0353 ns	0.5040 ns
CS x N		ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns

Mineral concentration is expressed as mg kg<sup>-1</sup>

## 1 **Caption of Figures**

2 Fig. 1. Effects of intercropping and N fertilisation on biomass production of grass and legume crops  
3 in two consecutive growing seasons. Different lowercase letters indicate significant differences  
4 between crops and cropping systems. Different uppercase letters indicate significant differences  
5 between N fertilisation levels. PM, pearl millet; S biomass sorghum; SH, sunn hemp.

6 Fig. 2. Species evenness in different cropping system treatments. Different letters indicate  
7 significant differences between intercropping systems. PM, pearl millet; S biomass sorghum; SH,  
8 sunn hemp.

9 Fig. 3. Comparison of land equivalent ratio (LER) of pearl millet (PM) × sunn hemp (SH) and  
10 biomass sorghum (S) × sunn hemp (SH) intercropping systems in two consecutive growing seasons.  
11 LER was determined as a function of the total biomass produced and the total leaf area per land  
12 area (LAI). No significant differences between cropping systems were found.

13 Fig. 4. Nitrogen removal for each cropping system and N level. Different lowercase letters indicate  
14 significant differences between crops and cropping systems. Different uppercase letters indicate  
15 significant differences between N fertilisation levels. PM, pearl millet; S biomass sorghum; SH,  
16 sunn hemp.

17 Fig. 5. Nitrogen balance for each cropping system and N level. No letters indicate non-significant  
18 differences between crops and cropping systems. Different uppercase letters indicate significant  
19 differences between N fertilisation levels. PM, pearl millet; S biomass sorghum; SH, sunn hemp.

20 Fig. 6. Percent nitrogen derived from soil (NDFS) in whole plants in each cropping system.  
21 Different letters indicate significant differences between crops and cropping systems. PM, pearl  
22 millet; S biomass sorghum; SH, sunn hemp.

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