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

Walter Zegada-Lizarazu*, Andrea Parenti, Andrea Monti

Department of Agricultural and Food Sciences, University of Bologna, Viale G. Fanin 44 – 40127, Bologna, Italy.

*Corresponding author: Walter Zegada-Lizarazu: walter.zegadalizarazu@unibo.it; phone: +39 051 2096665

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Abstract

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

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

1. Introduction

Human-induced global warming has several well-documented causes with known repercussions on the climate. The utilisation of fossil fuels as the predominant energy source has been recognized as the principal factor (70%) contributing to increased CO_2 emissions and therefore the accelerated climate change registered in the last few decades [1]. The European Union (EU), among other governments, has set up long-term energy policies aimed at mitigating CO_2 emissions and, therefore, the effects of climate change such as RED II, Green Deal, and CETP. One of these policies is to increase the production of dedicated lignocellulosic feedstocks for advanced biofuels in diversified crop production systems [2]. Diversified cropping systems with dedicated lignocellulosic crops offer many economic, environmental, and social advantages over sugar/starch monocropped feedstocks initially identified for first-generation biofuel production as a source of renewable energy. Dedicated lignocellulosic crops could result in several benefits: reduction of greenhouse gas emissions, diversification of feedstock, increased resilience of cropping systems, and increased biofuel yields while avoiding indirect land use changes (iLUC) effects [3, 4]. The development of advanced biofuels depends on low-input and sustainable cropping systems that can efficiently use natural resources without affecting food production. Intercropping could increase land and resource use efficiency, yield stability, productivity of biomass per unit area, and support biodiversity.

Intercropping is defined as the simultaneous growth of two or more crop species on a single piece of land during the growing season [5, 6]. Such systems, if properly implemented, could have particular significance for current and future biomass and bioenergy demands and environmental concerns. In China, a study on long-term intercropping with food crops revealed that compared to monocrops, intercrops showed higher levels of carbon sequestration, a 23% higher aboveground and belowground biomass production, and an increase of 11% in total N soil content [7]. However, information on the performance (in biological and productivity terms) of intercropping systems that only include dedicated lignocellulosic crops is limited, particularly for legume species. In such a system, a legume with a high lignocellulosic biomass-yielding potential could not only improve the yield of a companion grass crop, but also help to maintain a well-balanced soil fertility program and reduce N fertilisation costs [8, 9]. In addition, intercropping-dedicated lignocellulosic crops (grasses and legumes) could offer better land use opportunities, such as feedstock production in marginal lands without competing issues with food crops (low iLUC risks) [10]. Utilising more intensive and more sustainable crop production systems can increase resource use efficiency, integrate management practices, and improve the environmental performance of biofuels [11, 12].

Among promising lignocellulosic crops, biomass sorghum and pearl millet are interesting multipurpose crops (i.e., grain and straw) options. Biomass sorghum is a drought-tolerant, fast-growing crop with a high dry biomass yield (30 Mg ha^{-1}) already utilised as feedstock for first- and second-generation biofuels [13]. Pearl millet is widely grown for food purposes in many arid and semiarid areas of the world and is more resilient than sorghum under harsh weather conditions such as drought and flood [14-17]. In fact, under harsh environmental conditions it has been shown that pearl millet grain and biomass yields are equal to or higher than those of sorghum, suggesting its potential as an alternative bioenergy crop [18]. Sunn hemp is a fast-growing tropical legume, with a relatively high lignocellulosic biomass production potential (10 -13 Mg ha^{-1} in about three months) that can fix 50 to 60 kg ha^{-1} of N_2 during its life cycle [19, 20]. In its native areas, sunn hemp is traditionally grown as a non-wood fibre crop and is cultivated in rotation with rice (*Oryza sativa*), maize (*Zea mays*), cotton (*Gossypium spp.*), sugarcane (*Saccharum officinarum*), tobacco (*Nicotiana tabacum*) and coffee (*Coffea arabica*) [17, 21, 22]. Therefore, its utilisation as an intercropped energy crop is of interest. However, no available information on sunn hemp intercropped with grass crops such as biomass sorghum and pearl millet for lignocellulosic feedstock production purposes was found; therefore it is important to understand the potential of such intercrops for biomass production (quantitatively and qualitatively), species complementarity, and resource utilisation. In fact, owing to the N_2-fixing capacity of sunn hemp, it could be utilised as a natural source of nitrogen for the companion grass, thus reducing the need for fertilisation. Moreover, the root systems of sorghum, pearl millet, and sunn hemp show contrasting soil exploration layers [23-25] which could lead to significant complimentary resource use due to better root distribution throughout the whole soil profile. Additionally, the sunn hemp-grass systems could be used as bifunctional cropping systems, considering that either biomass sorghum or pearl millet, with the right varietal choice, could produce grain for human or animal feed and straw for biofuel production, further improving the land and resource use efficiency.

In addition, the identification of dedicated intercropping systems with enhanced qualitative feedstock characteristics would represent a significant step forward in producing more sustainable biofuels. In general, traits that define the qualitative characteristics of a feedstock to maximise conversion (biochemical or thermochemical) process efficiencies are moisture content; calorific value; proportions of fixed carbon, volatiles, ash, inorganic elements, and alkali metals; and cell wall composition [26, 27]. In the thermochemical approach, a negative relationship exists between the ash content in the biomass (i.e. Ca, Si, K, P, and Cl content) and the reduction of heat exchange in the combustor connected with slagging and fouling processes. Alkali elements such as K, Na, and Cl are considered the most detrimental elements affecting the process [26]. While in the biochemical pathway cellulose, hemicellulose and lignin contents are useful indicators of the bioethanol yield that could be achieved. Several agronomic factors can affect the biomass composition (i.e. soil type, weather, nitrogen fertilisation, irrigation, harvest time), and among them, intercropping (perennial crops and annual crops such as silage maize and forage sorghum) has been demonstrated to modify the conversion quality of the biomass for some bioenergy applications [28, 29]. However, data on the effects of intercropping on biomass yield and qualitative characteristics of the crop components including dedicated lignocellulosic N_2-fixing species, are lacking. The objective of this study was to quantify the impact of intercropping on the productive potential (in quantitative and qualitative terms) of pearl millet, biomass sorghum and sunn hemp, for energy production, under different nitrogen fertilization conditions.

2. Materials and methods

2.1. Study site

The study was performed at the Cadriano Experimental Farm of Bologna University, Italy (44°33' lat. N, 11° 21' E, 32 m a.s.l.) in 2018 and 2019. The study site was classified as fine silty mixed mesic udic ustochrept soil (9% sand, 34% clay, and 57% silt) with high exchangeable potassium (174 mg kg^{-1}) and average assimilable phosphorus and nitrogen contents (59 mg kg^{-1} of P_2O_5 and 0.14% of total N, respectively). The soil had a neutral pH (6.8) and an organic matter content of 1.3%. The climate of the experimental site is typical of a temperate humid region with cold winters and hot summers. Normally, the growth season lasts from early spring (April) to the end of summer (September). Precipitation occurs throughout the year, but with two well-defined peaks: one in spring and the other in autumn. Summers are dry, usually with the lowest amount of precipitation throughout the year. Mean temperatures recorded from April to September in 2018 and 2019 were 22.2 °C (±4.5) and 20.6 °C (±5.6), corresponding to 0.84 °C higher and 0.72 °C lower than the long-term mean, respectively. During the study period, the cumulative precipitation during the growing season in 2018 was 281 mm and in 2019 was 301 mm, 67 and 47 mm less than the long-term averages, respectively. In May 2019, the long-term average rainfall was exceeded by 100 mm; however, from June to September 2019, the rainfall was similar to that in the same period in 2018, which was between 42% and 48% lower than the long-term average.

2.2 Treatments and field management

Before sowing, to facilitate crop establishment in both growing seasons, the soil was ploughed to a depth of approximately 20 - 25 cm and double-harrowed. During soil preparation, approximately 100 kg of P_2O_5 was applied for basal fertilisation. The trial was set up in a randomised block design with a factorial arrangement with four replicates. The nitrogen levels (N0 and N150) were set as the main plots, and the cropping systems (mono and intercrops) were set as the sub-plots. In the fertilised plots (2018 and 2019), urea (150 kg N ha^{-1}) was broadcasted and incorporated into the soil together with mechanical weeding approximately 30 days after sowing (DAS). Biomass sorghum (S; cv. Triton), pearl millet (PM; cv. ICMV I707), and sunn hemp (SH; cv. Ecofix) were grown as monocrops, while the intercrops were composed of S × SH and PM × SH. Both monocrop and intercrop plots were 5.3 m × 7.5 m and the total planted area covered 1590 m^2. The intercropping layout was a 3:3 replacement strip cropping system. All crops were sown on 8 May 2018 and 24 May 2019 with a pneumatic planter and a distance of 0.45 m between rows. The planting densities within the rows in the monocropped and intercropped systems were 19, 22, and 39 pL m^{-2} for S, PM, and SH, respectively. Due to the low emergence of PM in 2019, re-sowing was done on 18 June. At the time of sowing, a granular soil insecticide (Ercole, 10 kg ha^{-1}) was applied, after which no diseases or pests were detected; therefore, additional pest treatments were not necessary. To ensure a good emergence rate and seedling establishment, a total of 26 mm of water was applied in two supplemental irrigation events within the first 30 DAS in each growing season.

2.3 Crop measurements

The monocropped and intercropped plants were harvested by hand in an area of 5.4 m^2 at the end of the corresponding growing seasons (~DAS 145-150). Biomass sorghum reached full maturity, while SH and PM had reached the beginning of flowering and seed formation, respectively. At harvest time, the biometric parameters plant height, basal stem diameter, number of tillers, and number of branches were recorded for the grass and legume crops. In addition, leaf area was measured with a leaf area meter (LI-3000; LI-COR, Lincoln, Nebraska, USA) in each cropping system. These values were used to calculate the leaf area index (LAI) as the ratio of the total one-sided leaf area per unit ground surface area. Aboveground dry biomass of shoot components (stems and leaves) was determined by oven drying at 105 ^{o}C to a constant weight. To evaluate the effectiveness of intercropping, the land equivalent ratio index (LER) of shoot components and total biomass produced was calculated following Osiru and Willey [30]. The LER is defined as the total land area required under monocropping to produce the same yield as in the intercropping, and is 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 the intercrop yields of the component crops, and S_a and S_b are the monocrop yields. Species evenness was then calculated to assess the relative yield of each species in the intercrop and, therefore, the species dominance. Species evenness is a measure of the relative abundance of species in an intercrop and it is expressed as: Species evenness = $\sum (P_i \ln P_i)/(\ln S)$, where the proportion (P) is the amount of biomass of a species (i) in an intercrop multiplied by the natural log (ln) of that proportion and summed across the species present, and S is the natural log of the number of species in the intercrop [31].

In 2018 separate representative leaf and stem subsamples were pooled, oven dried to a constant mass at 60 °C and ground to a diameter of 1 mm. The ground biomass was analysed in four replicates to determine the ash and mineral content. Ash was extracted by incineration of the dry biomass in a furnace muffle at 550°C for 3 h on a 3 g sub-sample. The concentrations of the most important minerals (Ca, K, Na, P, S, and Si) in terms of heat exchange reduction in the combustor connected with slagging and fouling processes were determined through a wet digestion pre-treatment carried out in a microwave oven by inductively coupled plasma (ICP). The Filter Bag Technology (FBT, ANKOM technology) was used to determine the cell wall components (i.e., lignin, cellulose, and hemicellulose) in four replicates, using the AOAC 991.43 and 985.29 methods. In a CHN combustion analyser, the total N and C contents were determined in four replicates. These data were used to calculate the amount of nitrogen removed by the crop as the product of the nutrient concentration and dry biomass yield. The N balance was calculated following Stoltz and Nadeau [32] as the difference in N inputs (i.e. N content in the soil before sowing + N fertilisation) minus the N outputs (i.e. the N content in the crop and the residual N in the soil after harvest). The percentage of nitrogen derived from the soil (NDFS%) was calculated as the atom % ^{15}N excess in the plant divided by the atom % ^{15}N excess in the soil (adapted from Kchaou et al. [33]). The atom % ^{15}N excess was estimated by subtracting the natural abundance (0.3663 atom% ^{15}N) from the soil and plant samples. The natural abundance of ^{15}N isotopes were determined in four replicates of plant (pooled representative subsamples of leaves and stems) and soil (taken at 20-25 cm depth) materials with the aid of continuous flow–isotope ratio mass spectrometry (CF–IRMS, Delta V Advantage Thermo Scientific). No distinction between N derived from the soil and the fertilizer was made at the beginning of the trial (2018) because the N levels and isotopic signature were similar in the fertilised and non-fertilised plots (2.6 and 2.5 Mg N ha^{-1}; 0.3690 and 0.3689 atom% ^{15}N, respectively). These balanced values are attributed to the preceding crops and fertilisation management of the whole field in 2017, that is, in the fertilised plots, the preceding crop was sorghum grown under customary management practices, which include fertilisation rate of 150 kg N ha^{-1}. In the unfertilised plots (N0), the preceding crop was sunn hemp grown without N fertilisation, thus relying on its own N_2-fixing capacity. In addition, the subplots (i.e. cropping systems) within the fertilisation treatments were subsequently rotated to avoid any potential negative effects of growing the same crop in the same place every year.

2.4 Statistical analysis

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

3. Results

3.1 Biometric, productive, and nitrogen uptake parameters

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

Figure 1 shows the effects of intercropping and N fertilisation on the biomass production of grasses and legume crops. N fertilisation did not have significant effects, whereas the cropping system significantly changed the biomass yield. In both years, the intercropping systems (PM × SH and S × SH) showed similar biomass yields (on average of both years 18 and 20 Mg ha^{-1}, respectively) although the dominant components had variable yields; in 2018 both grasses were dominant over SH, while in 2019 SH overtook PM due to the re-sowing of PM three weeks later. In all cases the intercropped yields were similar to grasses production potential under monocropping conditions, with S (either monocropped or intercropped) showing significantly higher ($P \leq 0.05$) values than the monocropped legume. SH showed a considerable yield reduction when intercropped with S (-78% and -75% in 2018 and 2019, respectively) and when SH was intercropped with PM the yield reduction ranged between -55% and -38% in 2018 and 2019, respectively. This is supported by the species evenness indicator (Fig. 2), which was 0.9 and 0.6 in PM × SH and S × SH intercrops, respectively. These results reveal a higher competitive effect of S than PM over SH. Figure 3 shows the productivity of the intercrop systems and the competitive interactions between the intercropped species. Statistically, no significant differences were observed between the different intercropping systems. The average LER across year and cropping systems was 1.14, indicating a 14% increase in productivity compared to monocropping. In both years, the LER of PM × SH was slightly higher (1.10 and 1.35) than that of S × SH (1.07 and 1.04). This could be related to the more complementary responses observed between pearl millet and sunn hemp, where the partial LERs were 0.59 and 0.63, respectively. Conversely, the average partial LER of sorghum and sunn hemp were 0.82 and 0.24, respectively. Across fertilisation levels and years, the total LER of intercropping sunn hemp with pearl millet showed an increase of 22%, while that of S × SH was only 6%. All these productive and competitive patterns are also clearly reflected in the leaf area index LER's (Fig. 3).

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

N recovery from soils (NDFS) is shown in Figure 6. The major fraction of N absorbed by monocropped grasses originated from soils. Furthermore, the fraction of N absorbed from the soil was on average 1.1 times higher in the unfertilised plots than in the fertilised plots. Among the monocrops, sunn hemp recovered the lowest fraction of N from the soil. In contrast to the monocropped grasses, SH recovered more N from the soil under fertilised than unfertilised conditions because of the well-know reduced symbiotic N_2-fixing capacity under such conditions. As for the intercrops (PM × SH and S × SH) the N recovery from the soil followed the dominant trend of the monocropped grasses but with significantly lower values in each case; the average reduction recovery potential was 17% and 23% for the PM × SH and S × SH systems, respectively.

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

3.2. Intercrop yield quality for advanced biofuels

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

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

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

4. Discussion

4.1 Biomass yields, nitrogen use, and competition between intercrops

Intercropping-dedicated lignocellulosic crops, particularly if legumes are included, are a promising solution to the development of advanced biofuels and to enhance the sustainability and risk minimisation (i.e. soil degradation and stable production) of low iLUC energy cropping systems. However, information on biomass potentials (quantitatively and qualitatively), crop complementarities, and resource use of such cropping systems is limited. This study focused on a new leguminous species, sunn hemp, intercropped with sorghum (S × SH) or pearl millet (PM × SH). The results showed that PM × SH and S × SH cropping systems had statistically similar biomass yields, either with or without N fertilisation (Fig. 1). The lack of response to N fertilisation may be attributed to the preceding crop on the unfertilised plots being sunn hemp, which may have fixed enough N_2 to a similar level as the fertilised plots on which the preceding crop was sorghum. A possible advantage of intercropping a leguminous crop is that some of the N_2 fixed by the legume can be transferred to the grass. Chu et al. [34], for example, found that N_2 fixed by a peanut (*Arachis hypogaea*) crop was transferred to the intercropped rice at decreasing rates (from 12% to 6%) when mineral N fertilisation was increased. Moreover, the beneficial effect of fixed N_2 on the subsequent crop, more than on the companion crop, is a well-known phenomenon already observed in many legume-grass mixtures [9, 35-37]. In a companion crop, the beneficial effects could be related to enhanced root growth and complementary functioning, leading to a greater yield stability [12, 38].

Our results show that biomass yield for both intercrops was within the productive range of monocropped grasses [13, 20, 39], but higher than that of monocropped sunn hemp. Within the intercrops, sorghum showed a significantly greater suppressive effect on sunn hemp than pearl millet in terms of biomass yield, stem diameter, branching capacity, and biomass and LAI partial LERs (Table 1, Fig. 3). Biomass sorghum reached full canopy development earlier than pearl millet, resulting in a greater shading effect on sunn hemp. Similar effects of competition for light in other grass-legume intercrops have been reported in the literature [40, 41]. Additionally, biomass sorghum produces root exudates such as sorgoleone (a potent PSII inhibitor), that disrupts the biosynthesis of carotenoids, and introduces anatomical changes in the stems of legumes and other broadleaf species [42]. Moreover, shading of the intercropped legume might have affected its photosynthetic capacity and nodule vitality and therefore N_2 fixation capacity [43, 44]. Given the much higher early growth rate of sorghum than pearl millet [40], it is possible that postponing sorghum sowing by two or three weeks, as in the case of pearl millet in 2019, could result in a better balance when sorghum is intercropped with sunn hemp. However, the evenness values (near 1) of the PM × SH intercrop (Fig. 2) suggest that this system is more suitable for yield stability and diversification of feedstock production for biofuels, because the proportion of grass and legume crops would be better balanced. The better suitability of pearl millet over sorghum needs to be confirmed in future studies where the effects of delayed sowing of sorghum are evaluated.

Intercropped grasses and legumes also compete for soils resources, especially at early growth stages, when root systems are not specialised and are distributed in different soil layers. In the present study, both intercrops removed less N than the monocropped legume (Figs. 4 and 5), mainly because the N_2 fixed by the intercropped sunn hemp was reduced by 55% and 84% in the PM × SH and S × SH intercrops, respectively. The largest reduction in S × SH indicates that biomass sorghum has a greater competitive ability for mineral N than pearl millet. Moreover, the intraspecific competition within the grass species might have been limited by intercropping, thus further reducing the competitiveness of sunn hemp; consequently, its N_2 fixation capacity might have been promoted, although the overall N_2 fixed was reduced due to the lowered biomass production [40]. However, N fertilization had an effect on the N amount taken up by the crops, leading to a 20% increase in N removal in comparison with the unfertilised plot, where the main source of N could be associated with the fixed N_2 by the preceding leguminous crop (sunn hemp). In fact, several studies on leguminous crops with high levels of N fertilisation have shown that N is mostly derived from soil and little from N_2 fixation, as in our trial (Fig. 6) [43, 44]. Although the N levels at the beginning of the trial (2018) were similar in the fertilised and unfertilised plots (2.6 and 2.5 Mg N ha^{-1}), these results could have been influenced by the preceding leguminous crop (sunn hemp in 2017) and the consequent N mineralisation.

4.2. Intercrop yield quality as advanced biofuels feedstock

Cell wall components determine the final fuel yield, in particular high hemicellulose and cellulose contents are desirable for maximising ethanol production. Moreover, low ash (<5%) and mineral concentration (i.e., Na, K, Ca, S, Si, and the combination of alkali metals with silica) reduce fouling, slagging, and corrosion during combustion [26, 27, 45, 46], which is essential for efficient thermochemical biomass processing. In our study, the ash content in all crops and cropping systems was arround 5% threshold (Table 2; [27]); however, most of the minerals and ashes were concentrated in the leaf fraction (data not shown), indicating that with optimised species combinations and proportions, selected cultivars, agronomic practices, and postharvest logistics could be reduced/eliminated, thereby improving biomass quality but at the expense of biomass quantity. The leaf fraction, however, can be either left or incorporated into the soil to preserve its fertility, but careful management is required as high N rates might lead to an increase in ash content of the harvested biomass and consequently slagging problems and potential NO_x emissions from combustion processes. In our study, however, the similar cell wall components as well as ash and mineral contents (Tables 2 and 3) of the biomass in the fertilised and unfertilised plots could be related to the preceding crop (sunn hemp) in the unfertilised plot that was able to fix N_2 to an adequate level, similar to that of the fertilised plots.

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

Compared to sorghum, pearl millet showed a high cellulosic fraction; hence, it might have good potential as feedstock for advanced biofuel, even though some suboptimal mineral concentration may cause issues in managing a thermochemical conversion plant with this type of feedstock. In particular, K, Na, P, and S were almost two-fold more concentrated in pearl millet than in sorghum (Tables 2 and 3) probably due to harvesting of pearl millet in the early stage of maturity. However the quality of the intercropped pearl millet improved in terms of Si/K (+32%) as a result of reduced K uptake in the acidified rhizosphere created by the protons released by the legume roots [47]. Moreover, the slight decrease in cellulose content (-9%) compared to the sole pearl millet suggests that intercropped PM, irrespective of yield level, might be better suited to thermochemical conversion. The lowered cellulose content could be a stress response to competition with sunn hemp for the most limiting resources (i.e. temperature, soil moisture, light, nutrients), as demonstrated in perennial grasses facing stress. Significant changes in the cell wall structure, biomass recalcitrance, and sugar release for ethanol production in *Miscanthus* were observed under drought and nutrient deficiencies [48].

The biomass quality of sunn hemp was higher when intercropped with sorghum, compared to that when monocropped, in terms of the increased hemicellulose and cellulose contents and a drastic reduction in N and Na concentrations (Tables 2 and 3). Changes in cell wall structure and the whole plant architecture occur due to shading, as indicated previously. For example, shading has been shown to lead to changes in tissue proportions, cell wall concentration, and composition in alfalfa [49]. However, in our study, cell wall loosening, which explains the larger proportions of cellulose and hemicellulose, appears more related to a suppressed or delayed development of sunn hemp rather than to changes in the cell wall lignification patterns (Table 3; [50]). Similarly, the reduced mineral (N, Na) of intercropped sunn hemp could be attributed to shading rather than to competition at the root level. In fact, compared to monocropping, shading was found to impair mineral contents of intercropped legumes mainly due to reduced photosynthetic capacity, modified canopy structure, and reduced biomass accumulation [51]. These findings highlight that the biochemical pathway fits well sunn hemp characteristics, whereas, the feedstock blend from an intercrop with sorghum could be suitable for thermochemical conversion as well, even though this option needs to be further investigated. Nevertheless, the mixture of feedstock harvested at the same time could be a valid alternative to overcome rigid conversion technologies that were optimised for a single feedstock. Flexible fractionation technologies are considered the most cost-effective processing technologies to produce lignin and C6/C5 fractions; therefore, the proportions of the most desirable feedstock fractions could be pre-defined at the crop production stage. In summary, the improvement of biomass quality in the considered systems is agronomically feasible by delaying the harvest time until complete senescence of the crops when most leaves have fallen off. Leaves are known to have high ash and mineral contents, which worsen the overall biomass composition of the feedstock. This scenario can significantly improve feedstock quality, although it can lead to some agronomical drawbacks such as the increased risk of wet soil conditions at harvesting, which in turn can cause: i) excessive soil compaction; ii) yield reduction for subsequent crops; iii) delay or impossibility of planting a winter grass in a crop rotation framework; and iv) reduced harvest options (only self-propelled forage harvester) to avoid the field drying phase.

5. Conclusion

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

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

The present study has shown that intercropping not only maintains the overall biomass production close to that of the monocropped grasses (LER increase of 22% and 6% in the PM × SH and S × SH, respectively), but can also lead to improved feedstock characteristics for determined bioenergy applications: intercropped pearl millet resulted in improved mineral composition in terms of increased Si/K ratio (+32%; increased Si and decreased K content) and therefore limited slagging problems in the boilers. In addition, intercropped sunn hemp (especially with sorghum) showed increased cellulose content and a drastic reduction in mineral content, resulting in improved cell wall polysaccharide availability for biochemical conversion processes. Intercropping-dedicated lignocellulosic crops seem to be a feasible alternative for providing a mixture of feedstocks with improved biomass quality, however, significant developments are still needed in terms of the quantitative and qualitative suitability of the feedstocks as a function of the species and variety choice/combinations and their agronomic management (i.e. sowing times, fertilisation practices, and harvesting operations). It is important to note that the inclusion of a legume in an intercropping system can enhance the quantitative and qualitative biomass availability and provide valuable co-products such as food proteins.

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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† (No. m^{-2})	
		2018	2019	2018	2019	2018	2019
Cropping systems (CS)	**Pearl Millet (PM)**						
	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
	Sorghum (S)						
	Monocrop (S)	375	321	22.1	23.8	---	---
	Intercrop (S× SH)	345 *	289 ns	23.1 ns	23.9 ns	---	---
	Sunnhemp (SH)						
	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

† 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)	**Pearl Millet (PM)**						
	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
	Sorghum (S)						
	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
	Sunnhemp (SH)						
	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^{-1}.

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)	**Pearl Millet (PM)**											
	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
	Sorghum (S)											
	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
	Sunnhemp (SH)											
	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^{-1}

Caption of Figures

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

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

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

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

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

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