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Title:	
INCREASE OF LIGNOCELLULOSIC FEEDSTOCK FROM INTEGRATED CROPPING SYSTEMS	
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1 Introduction

Modern agriculture is now moving towards an agroecological transition aimed at increasing the multifunctionality and sustainability of the primary sector so to link the most suitable feedstocks (quantitatively and qualitatively) to the most resource efficient biofuel technological conversion processes. The introduction of annual lignocellulosic crops within conventional cereal cropping systems, that generally leave the soil uncovered for many months between two main food crops (i.e. wheat and maize), represents an effective alternative to increase the feedstock availability for the production of advanced biofuels in certain environmental/management conditions. Using the land more intensively (i.e. increasing the Land Equivalent Ratio – LER) through growing lignocellulosic crops as intermediate crops would allow to diversify crops, while increasing the annual quantity of feedstock without reducing food crops land. Biomass sorghum, sunn hemp, kenaf and hemp are annual low input and high lignocellulose yielding crops which can be integrated into existing food/feed based cropping system or cultivated in marginal areas with the option to be double cropped to avoid indirect land use changes and direct negative environmental effects. In temperate climates, these crops are amongst the most promising ones considering their potential yield, beneficial effects in the rotation (e.g. sunn hemp is a leguminous species while hemp has allelopathic effect against weeds), and growing season length. Moreover, such system can extend the yearly availability of feedstocks for advanced biofuels production and reduce the reliance on a single, usually expensive, biomass feedstock source. The mixture of feedstock is a promising strategy to reduce supply risks and comply with certain quantitative and qualitative conversion process requirements. In that sense, the composition of the biomass is an important factor for evaluating the suitability of integrated cereals straw and dedicated biomass crops as feedstock to biofuel production. In general biomass sorghum, sunn hemp, kenaf and hemp due to their high cellulose and hemicellulose content and paired with low-medium lignin content, ash and inorganic elements could be considered suitable to the thermochemical and biochemical conversion pathways so to maximize the process efficiency and reduce conversion technology (i.e. slugging, fouling, and corrosion) problems in the production process of advanced biofuels.

The main target of Task 1.2 in the BECOOL project is the establishment of innovative cropping systems so to increase biomass feedstock availability by at least 50% without negatively affecting food production, soil quality, and customary land uses. The proposed new farming systems (double cropping cereals with dedicated lignocellulosic crops in rotation) could also support to enlarge the supply window around the year thus reducing the stored biomass and logistics supply disruption risks. Deliverable 1.4 entitled “Increase of lignocellulosic feedstock from integrated cropping systems” presents a comprehensive summary of the technical feasibility evaluation of such innovative cropping systems carried out in Italy, Spain, and Greece. Moreover, for a more reliable evaluation of the multi-location crop rotation trials, the detailed activities carried out and results achieved in each location (in function the specific pedoclimatic and management characteristics of each location) are extensively presented in the Annex section.

2 Materials and Methods

Crop rotations were established from 2017-2021 in three Southern EU locations: a) Cadriano, Italy (44° 33’ N, 11°26' E, 32 m a.s.l.); b) Guadajira, Spain (38° 51’ N 6°40’ O, 185 m a.s.l.); c) Aliartos, Greece (38° 22’ N, 23° 06’, 114 m a.s.l.). Figure 1 shows the climatic characteristics of each of the three locations. The three sites are located in the Mediterranean area but with evident differences in terms of mean temperature, solar radiation, and rainfall amount. Greece and Spain were warmer and with a higher solar radiation than Italy during the corresponding growing seasons of each crop component of the rotation system. On the other hand, Italy shows the largest precipitation along the corresponding crop rotation cycles.

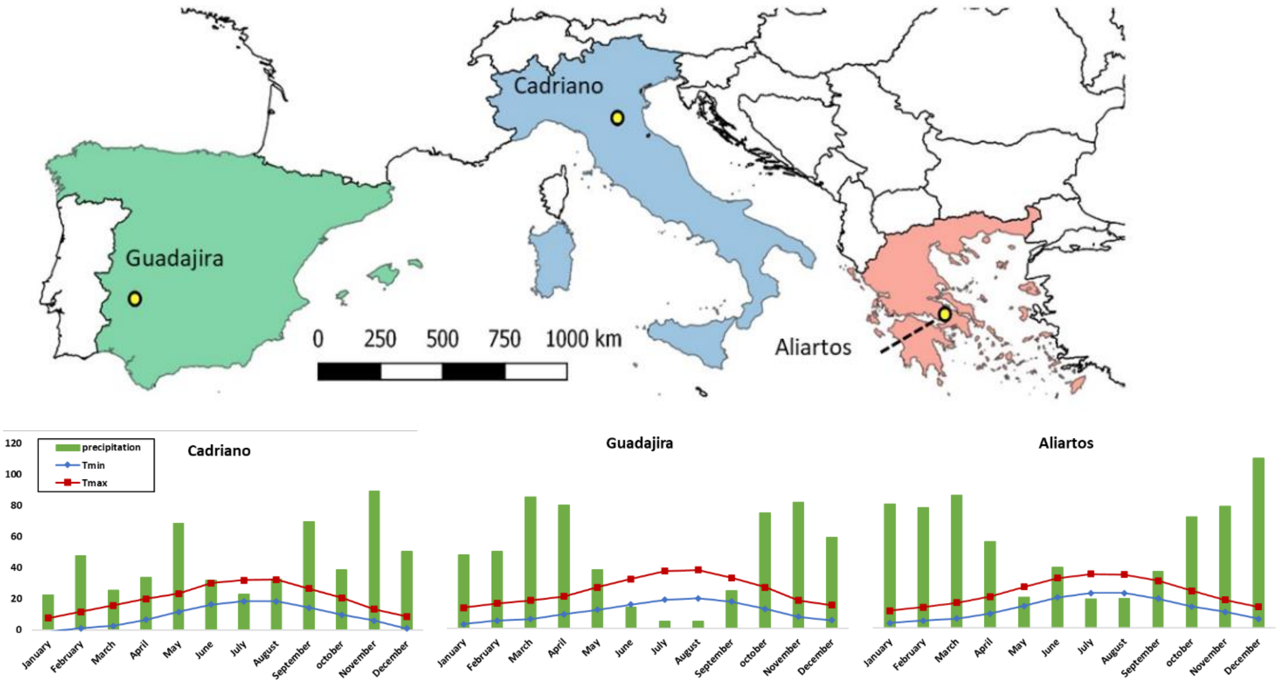


Fig. 1. Experimental sites (Gudajira, Spain; Cadriano, Italy; and Aliartos, Greece) and annual climatic variability in the experimental period (2017-2021).

At each location, five innovative crop rotations and a control one were stablished (Fig. 2). The six rotation systems were designed as follows: i) maize-fallow-wheat (C, control), ii) maize-sunn hemp-wheat+sunn hemp-maize (R1), iii) maize-biomass sorghum-wheat+sunn hemp-maize (R2), iv) maize-kenaf-wheat+sunn hemp-maize (R3), v) maize-industrial hemp-wheat+sunn hemp-maize (R4); vi) wheat+sunn hemp-wheat+sunn hemp (R5).

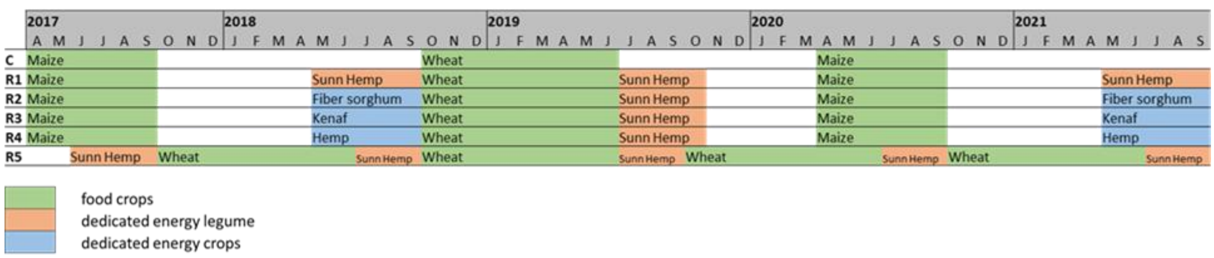


Fig. 2. Crop rotations scheme in Italy, Spain, and Greece

2.1 Experimental design and agronomic management

In all three locations, the same experimental design was followed (randomized block design with four replications), but the agronomic management was somehow different based on the local pedoclimatic conditions and the customary agronomic practices for maize and wheat species included the corresponding rotation schemes. In each location, the most common and widely used varieties of maize and wheat were sown. On the other hand, the same varieties and cultivation protocol, wherever possible, was followed for the dedicated crop species included in the innovative cropping systems (biomass sorghum, sunn hemp, kenaf and hemp). In Italy and Spain, mechanical cultivation was carried out, while in Greece manual cultivation was done. In general, sunn hemp (cv. Ecofix) was cultivated under minimum tillage depending on the time available from the harvest of the preceding crop and sowing of the next one. Sowing was done at an inter-row of 45 cm to arrive to a density of about 33 plants m^{-2}. Sowing was done between the end of May and beginning of June from 2017 to 2021. Being a leguminous crop N fertilization was not necessary, while irrigation was applied whenever needed based on the specific pedoclimatic characteristics of each location. Biomass sorghum (cv. Bulldozer), hemp (cv. Futura 75), and kenaf (cv. H328) were sown on May of 2018 and 2021 at a density of 19, 160, and 25 seeds m^{-2}, respectively. Soil preparation was done following the customary practices of each location. Fertilization rates were variable in function of the soil characteristics and yield potential of each location. Harvest of industrial hemp was usually done at mid-August. Whereas the harvest of sunn hemp, biomass sorghum and kenaf was usually done at the end of September.

2.2 Biomass yield measurements

The aboveground biomass yields were determined at the end of each growing season on a representative randomly selected area per replication. The harvest was carried out manually and then weighted the plants present in the sampling area. Dry biomass was determined by oven drying at 105°C until constant weight following ISO 18134-2:2017.

2.3 Biomass quality measurements

Representative subsamples were taken for basic measurements of cellulose, hemicellulose, lignin and ash content as well as moisture content of each species. The biomass concentrations of the most important minerals in terms of heat exchange reduction in the combustor connected with slagging and fouling processes were also determined in Italy and Spain.

2.4 Soil measurements

A full soil characterization was carried out in Italy and Spain. Soil texture, pH, total $CaCO_3$, available Phosphorus, total N, exchangeable K, C/N were be determined at the begging (2017) and end (2021) of the trial in Italy and Spain to estimate the changes introduced (in the aforementioned parameters) by the innovative cropping systems. More specifically, root biomass, soil organic matter, carbon and nitrogen were determined in winter when the biological activity in the soil was low and coinciding with wheat sowing season.

3 Results and Discussion

3.1 Innovative crop rotation effects on biomass and grain productivity

Figure 3 shows the cumulative grain yield (2017-2021) of the cereal crops in rotation at three southern EU locations. As for maize (conventional rotation), its yields were within the normal ranges at the corresponding locations and growing seasons, that is 15, 24, and 20 Mg ha^{-1} in Italy, Spain and Greece, respectively. In the case of wheat, yields were also within the normal ranges at the corresponding locations. In Italy the cumulated wheat grain yields where 2.5 times higher than in Spain and 1.6 time higher that in Greece. Moreover, the yields of both cereal crops in the conventional rotation were similar to those obtained in the innovative rotations. In fact, even though the cumulative yields of wheat in R5 are higher than in the control and the other rotations (about three times higher) the annual yields were within the ranges of the other rotations. Therefore, the higher yields reported in R5 are the result of the three additional growth cycles. Important to note however that the soil productivity (fertility) was not reduced by the additional cropping cycles (Fig. 7). These results suggests that the introduction of the dedicated crops into the conventional rotation system do not have any negative effects on wheat and maize grain yields. Thus, integration of food and bioenergy crops production seems a possible and promising way to ensure regional/national food and energy security needs, as well as to enhance biodiversity and contribute to the abatement of CO_2 emissions, and provide additional income to the farmers. Wheat and maize grain yield in Italy, Spain and Greece were maintained at the same level in all rotational systems, suggesting that the dedicated energy crops do not depleted the soil resources, while on the other hand they may have contributed to maintain the soil physical and/or chemical characteristics and therefore productivity. In fact, it is well known that alternating crops with contrasting characteristics as in the present case (i.e. rooting properties, nutrient requirements, growth cycles, etc.) it is an important strategy for the successful implementation of crop rotations while maintaining soil productivity and resilience (Zegada-Lizarazu & Monti, 2011).

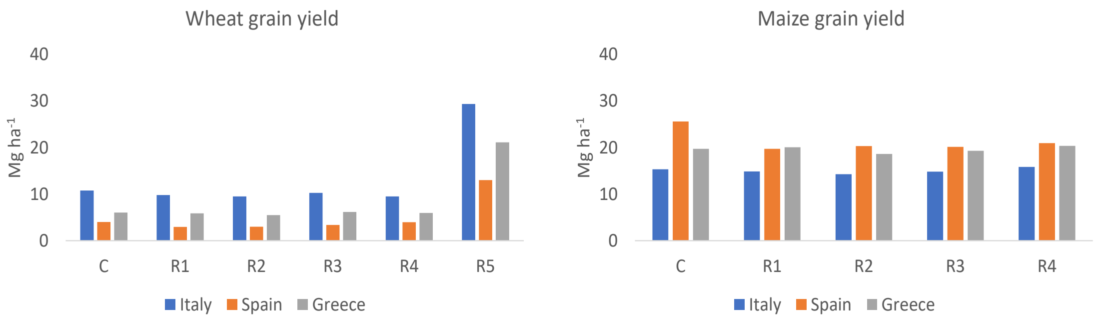


Fig. 3. Cumulative (2017-2021) grain yield (Mg ha^{-1}) of wheat and maize grown under conventional (C) and innovative (R) crop rotation systems at three locations (Italy, Spain, Greece).

Regarding the cumulative (2017-2021) aboveground biomass achieved in the conventional and innovative crop rotation systems (Fig. 4), R2 and R5 were the most productive ones with an average cumulated production (across locations) of 79 and 73 Mg ha^{-1}. On the other hand, the lowest cumulated biomass production was found in the control rotation (27 Mg ha^{-1}), that is 65% less than in the innovative rotations. The largest cumulated biomass was found in Greece and the lowest in Spain, probably because Greek plots were irrigated throughout the growing cycle and also because the growing season available is longer. In R2 the largest contribution to the total biomass produced could be ascribed to sorghum rather than sunn hemp. This is because sorghum is a high yielding crop, well adapted to the pedoclimatic conditions and the available growing season in southern EU, demonstrating that it can be cultivated without problems in a double cropping system with wheat. In fact, sorghum thanks to its low input requirements could be introduced as a good preceding crop to wheat instead of the conventional fallow period. Moreover, this crop sequence offers the possibility of implementing soil conservation practices such as minimum tillage, depending on the management practices of the preceding and following crops in rotation. In fact, the availability of herbicides for the minimum tillage practice could allow wheat to be planted directly into sorghum stubble. Sunn hemp in R2 (and in the other rotation systems as well), although being a relatively new crop in temperate climates, demonstrated a great additional contribution potential to the total biomass accumulation. Moreover, sunn hemp thanks to its N_2 fixing capacity and the complex effects that this crop sequences have on soil physical-chemical and biological properties, could promote increased yields of the following crops in rotation. As for the high amounts of accumulated biomass in R5, this is mostly the result of the three additional growing cycles and the positive effects that this legume could have in the soil fertility, as indicated before.

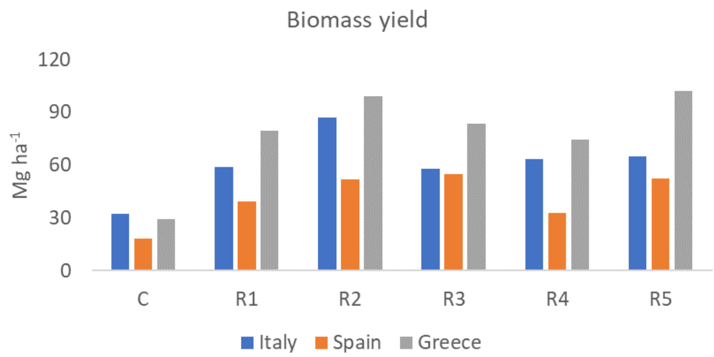


Fig. 4. Cumulative aboveground biomass yield (Mg DM ha^{-1};) in the six rotation systems carried out between 2017 and 2021 at three locations (Greece, Italy, Spain).

In all cases, the contribution of the dedicated energy crops to the total biomass produced was significant (Fig. 5). In average across locations, the contribution of the dedicated energy crops accounted for 54%, 69%, 60%, 52%, and 62% of the total cumulated biomass in R1, R2, R3, R4 and R5, respectively. The second largest contributor to the whole biomass was maize stover (crop residue) with 32%, 21%, 28%, and 32 % in R1, R2, R3, R4, respectively. In R5 wheat straw contributed with 38% of the total. In Italy the most productive rotation was R2 thanks to the high amounts of biomass (58 Mg ha^{-1}) produced by sorghum and sunn hemp (representing 67% of the total biomass cumulated in the rotation system). As indicated before, such large contribution to the total biomass is because sorghum is high yielding species and sunn hemp contributes not only to the total biomass but also to the sustainability of the system. Moreover, sorghum and sunn hemp demonstrated to be well adapted the pedoclimatic conditions of northern Italy. In Spain the most productive rotation was the R3 with 55 Mg ha^{-1}, of which 70% was derived from the energy crops (Kenaf + sunn hemp). Kenaf in rotation with a cereal and a legume, such as wheat and sunn hemp, would provide many advantages in terms of productivity and soil health, in particular thanks to its allelopatic effects against weeds and the control nematodes. Nematodes have been identified as one of the main reasons of yield loses in some legume crops. In the same context, it has been also indicated that Kenaf planted following maize could further help to reduce the incidence of nematodes. Besides R3, in Spain R2 and R5 are amongst the most productive systems. Whereas in Greece, R5 was the most productive rotation system in which sunn hemp contributed with about 73 % of the 102 Mg ha^{-1} of biomass produced in the 2017-2021 period. Important to note, that in this location R2, like in Italy and Spain, was also very productive with close to 99 Mg ha^{-1} (71% coming from sorghum and sunn hemp), suggesting the good adaptability of sorghum and sunn hemp to these environmental conditions too.

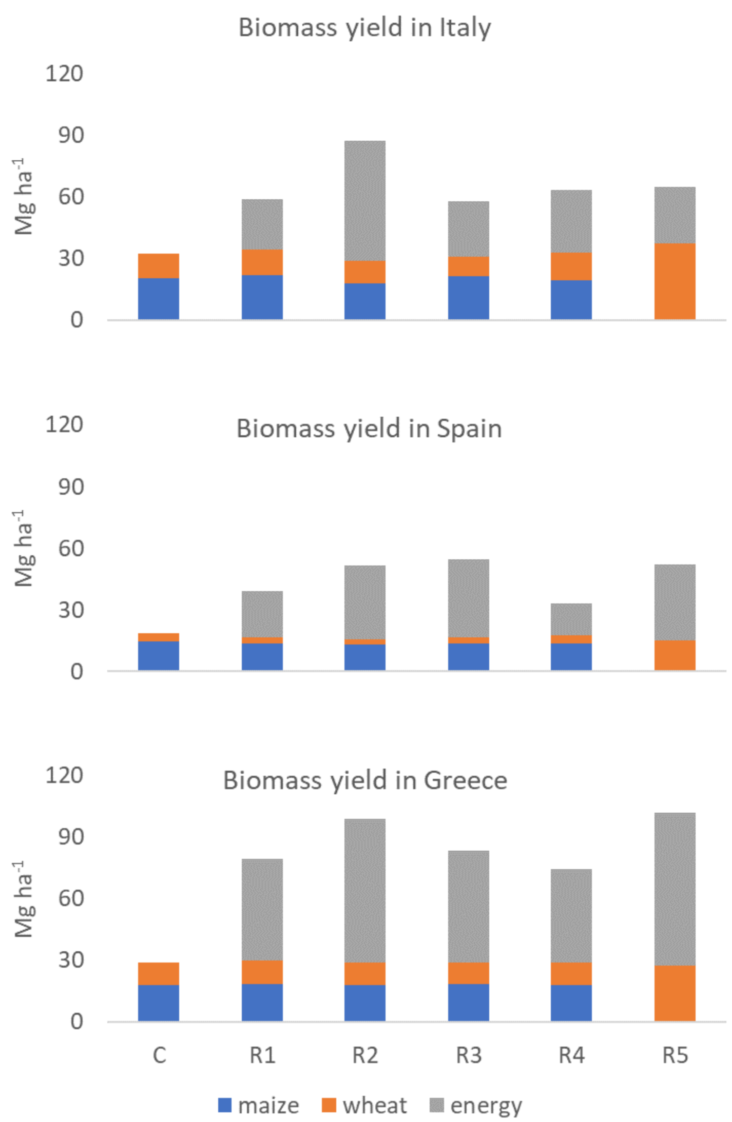


Fig. 5. Aboveground biomass yield (Mg DM ha^{-1}) of maize, wheat, and dedicated energy crops (biomass sorghum, sunn hemp, kenaf and hemp) under conventional and innovative crop rotation systems at three locations (Greece, Italy, and Spain).

3.2 Innovative crop rotation effects on biometric characteristics

Dedicated lignocellulosic crops need to maximize the biomass yields for the production of advanced biofuels; hence the high cellulose and hemicellulose concentration in the fiber of the stems is reflected by the plant's height and stem diameter. Plant height and stem diameter were the most important plant components determining the total biomass yield of all the species in the different rotation systems and locations. At the same time these plant components can also influence the harvest operations and the physical (qualitative) characteristics of the feedstock at the plant gate. In Italy, sorghum was the tallest and with the widest stem diameter (R2, Fig. 6) which explains the highest total biomass accumulated. In fact, the single stem characteristics of the species constitute the largest yielding component in quantitative and qualitative terms. At the same time plant height may determine the low crop resistance to lodging which can hamper harvesting operation and the ash and mineral content of the feedstock. While in Spain, kenaf (R3) was the second tallest species after sorghum, but with the widest stem diameter (Fig. 6), indicating that biomass production is determined primarily by stem diameter and secondarily by plant apical growth. In Greece, the relationships between plant biometric characteristics and biomass production are not that clear as the tallest plants with the widest diameter were kenaf and sorghum (R2 and 3) while the highest biomass producer was sunn hemp. The highest biomass accumulation produced by sunn hemp in R5 could be related to the branching capacity of the species as demonstrated in other studies (Zegada-Lizarazu et al., 2021), and/or due to other undetermined environmental factors.

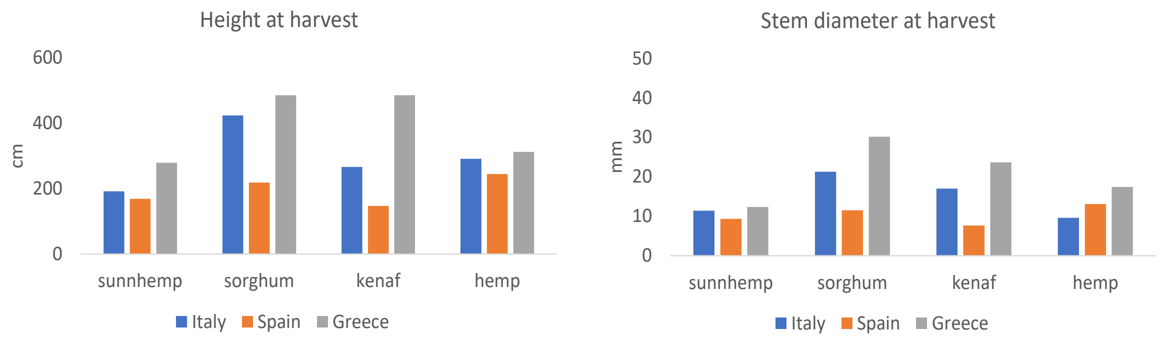


Fig. 6. Plant height and stem diameter of dedicated energy crops (biomass sorghum, sunn hemp, kenaf and hemp) under innovative crop rotation systems at three locations (Greece, Italy, Spain).

3.3 Innovative crop rotation effects on total N and SOC

Figure 7 shows the total soil C and N content in all rotation systems (2021) plus the initial values before the establishment of the trial (2017) in Italy and Spain. At both locations, no large differences were found between the conventional and the innovative crop rotations (R1-R5) in terms of C and N contents. As for the N content, the initial values (2017) were the same in Italy and Spain (about 0.09%), afterwards the N values in Spain increased in a similar way in all rotation systems (by about 2.7 times), probably due to the fertilization levels used there. On the other hand, in Italy the N levels in the rotation system remained more or less similar to the initial ones (about 0.09%) indicating no net increase or decrease of soil fertility due to the introduction of the dedicated energy crops instead of the fallow period. As for the SOC, values were kept more or less similar in both locations and in all rotation systems (about 0.71 %). It is possible that these results are linked to the low organic matter content in the soils, as almost all of the N present was in the organic form regardless the crop rotational systems. Important to mention, however, that the contribution of sunn hemp to sustain the N and C content in the soil may have played a key role. In particular, R1 and R5 include sunn hemp in every growth cycle, thus its contribution to reduce the inorganic nitrogen fertilization requirements for the following crop and therefore to ameliorate the environmental impact of such a practice would be central. In fact, it is reported that sunn hemp species can fix between 60 and 221 kg ha^{-1} of N, thus the species could be utilised as a natural source of N for the subsequent crops and the maintenance of the soil fertility. Moreover, the other dedicated energy crops may have also a role to play on the maintenance of soil fertility but with a different perspective; for example, Kenaf and hemp are leafy species that leave on the soil a large amount of leaf residues, so the re-incorporation of such residues can contribute to increase the soil aggregate stability. Besides that, both species have deep and extensive roots, which could have an important soil structure building effect (a beneficial factor in rotations with shallower rooted crops such as wheat).

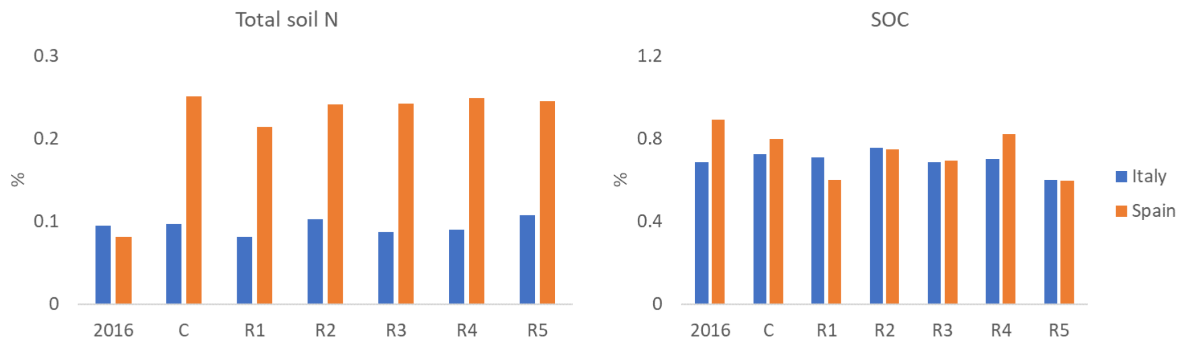


Fig. 7. Soil organic C and available N changes due to different crop rotation schemes in Italy and Spain.

3.4 Innovative crop rotation effects on biomass quality

Table 1 shows the N, C, ash contents and concentration of some minerals in the feedstock produced in the conventional and innovative crop rotations in Italy and Spain. Some of the contents are intrinsic to the species and specific organs included in the rotational systems. But in general terms, within the dedicated lignocellulosic crops, biomass sorghum and hemp have the lowest and highest mineral concentration, respectively, whereas sunn hemp and kenaf showed intermediate values. The high concentration of ash and inorganic elements comes from the leaves, which is an important qualitative issue to tackle in the leafy species such as hemp and kenaf. The ash content in the stems only of sorghum, sunn hemp, kenaf and hemp went down to 4.4, 3.9, 5.8, and 3.4%, respectively. Therefore, a possible solution to reduce high minerals and ash concentrations could be trough the development of harvesting systems able to remove leaves from the final product. Alternatively several pretreatment option are available (i.e. biomass leaching, use of mineral additives, co-combustion with wood, decreasing the combustion temperatures, or even a combination of processes such as pyrolysis followed by combustion), but all these processes are costly and logistically difficult to apply in the field.

Table 1. Ash content, total N and C concentration, and mineral concentration on the main crops characterizing the rotational systems in Italy and Spain.

	Italy							Spain						
	Ash	N	C	Ca	K	Na	P	**Ash**	N	C	Ca	K	Na	P
B. sorghum	4.5	0.8	46.9	2.3	5.1	92	1.1	5.0	0.6	45.5	4.1	6.5	383	3.1
Sunn hemp	5.6	1.7	46.9	7.5	10.5	232	1.5	6.8	1.8	45.9	9.9	6.5	416	1.8
Kenaf	6.0	1.3	45.9	12.6	6.9	391	1.2	6.0	0.94	45.2	10.2	6.4	367	4.9
Hemp	6.6	1.5	46.3	11.2	11.2	212	2.1	12.5	1.97	43.6	22.4	7.8	411	4.2
Straw	7.6	0.4	45.6	2.8	13.2	154	0.6	7.2	0.8	45.3	-	-	-	-
Stover	3.2	1.2	46.2	2.0	7.1	203	1.3	9.4	0.8	44.3	-	-	-	-

Ash, C and N are expressed as a percentage of the total dry matter; the other elements as mg kg^{-1}.

4 Conclusions

The introduction of dedicated energy crops (i.e. sorghum, sunn hemp, kenaf, hemp) in innovative rotation systems did not have a negative impact on cereals (wheat and maize) grain yields while the additional biomass (straw + energy crops biomass) produced by the dedicated crop was substantial. The cultivation of such energy crops leads to an average cumulated biomass of 59, 79, 65, 57, and 73 Mg ha^{-1} from R1 to R5, respectively. From the farmers' point of view, these could be an important source of biomass production, crop diversification, efficient use of the land, and additional income. While from the value chain perspective, the local production of additional feedstocks from dedicated energy crops would facilitate the development of short biomass supply chains, reduce transportation costs, and increase the security of supply for advanced biofuel plants.

The sustained cereals grain yield in the integrated cropping systems are associated with soil resilience and fertility maintenance and other well-known positive rotational effects (i.e. of weed/pathogens, and improvement of soil quality) that are promoted by the introduction of the dedicated energy crops.

In summary, these results suggest that innovative crop rotations schemes could be effectively and sustainably developed. Such integrated food and dedicated biomass production systems has significantly increased (> 50%) the availability of lignocellulosic feedstock for advanced biofuel plants in the temperate climates of south Europe, without penalizing grain yield of cereals and without reducing food crops land or affecting negatively the soil fertility.

Annex 1. UNIBO report

Dedicated annual lignocellulosic crops in Northern Italy

Author(s): Walter Zegada Lizarazu, Andrea Parenti, Andrea Monti

Introduction

BECOOL project aims at developing advanced biofuel value chain through the scouting of diverse biomass feedstock including perennial and annual dedicated lignocellulosic crops. The introduction of annual lignocellulosic crops to advanced biofuel production represents an effective solution to increase the share of renewable fuels in the transport sector. Biomass sorghum, sunn hemp, kenaf and hemp are annual low input high cellulose yielding crop which can be integrated into existing food/feed based cropping system or cultivated in marginal areas with the option to be double cropped to avoid indirect land use changes and direct negative environmental effects. The effect of such crops in rotation with food crops has been investigated only partially and little information are available on the performance when rotated with cereal crops in temperate climates of Europe. Otherwise, sunn hemp is a legume crop, able to increase nitrogen through biological N fixation suggesting that its effect on a following cereal crop might be positive. Additional benefits from a crop rotation diversification relies in the contribution of the control of pest and disease, amelioration of soil structure and increased yields. Besides, the composition of the biomass is important to identify the potential best suited feedstock to biofuel production. High cellulose and hemicellulose paired with low lignin, ash and inorganic elements are essential in the conversion process.

The objective of this study was to compare at a field scale the biomass/grain productivity and feedstock quality of five innovative cropping schemes in comparison to a conventional control rotation.

Methods and materials

Figure 1: Experimental site position in the Northern part of Italy

The cropping schemes were established in 2017 at the experimental farm of the University of Bologna in Cadriano (32 a.s.l., 44° 33’ N, 11° 21’ E; figure 1) in a loam silty soil, neutral, rich in K_2O, with average N and P_2O_5 (Table 1)

Table 1: Soil characteristics of 0- 20 cm layer in the experimental field

Soil parameter	**Unit**	
Silt	%	45
Sand	%	31
Clay	%	24
pH		6.73
Total $CaCO_3$	%	0.62
Active $CaCO_3$	%	0.60
Organic carbon	g/kg soil	7.19
Organic matter	%	1.24
Total N	g/kg soil	0.95
P_2O_5	mg/kg soil	100
K_2O	mg/kg soil	146
C/N		7.59

[1] *Parenti, A., Cappelli, G., Zegada-Lizarazu, W., Martín, C., Christou, M., Monti, A., & Ginaldi, F. (2021). SunnGro: A new crop model for the simulation of sunn hemp (Crotalaria juncea L.) grown under alternative management practices. Biomass and Bioenergy, 146. https://doi.org/10.1016/j.biombioe.2021.105975*

Five innovative rotations (Figure 2) were established and compared to a control rotation in a randomized block design with four replications. Each plot was settled in order to allow a complete mechanical management for the simulation of near-to-practice solutions at a field scale. The plots were 231 m^2 each, with an overall area per treatment of 924 m^2. The six cropping systems during the period 2017-2021 were designed as follow: i) maize-fallow-wheat-maize (C, control), ii) maize-sunn hemp-wheat+sunn hemp-maize (R1, rotation one), iii) maize-biomass sorghum-wheat+sunn hemp-maize (R2, rotation two), iv) maize-kenaf-wheat+sunn hemp-maize (R3, rotation three), v) maize-industrial hemp-wheat+sunn hemp-maize (R4, rotation four); vi) wheat+sunn hemp-wheat+sunn hemp (R5, rotation five).

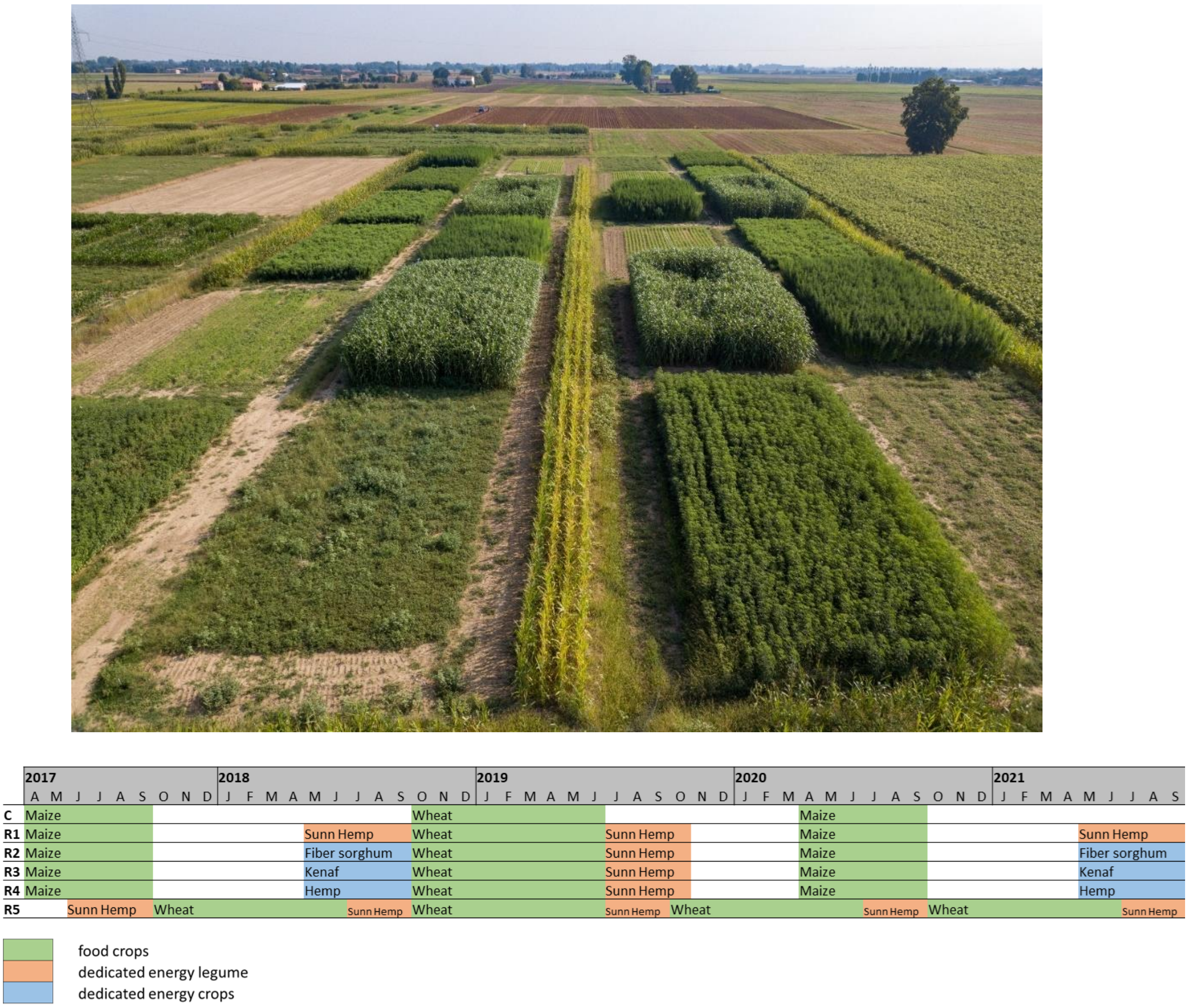


Figure 2: Cadriano's integrated cropping systems in 2018 and experimental layout

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). Along the year, precipitations are fairly distributed, but with two well-defined peaks: one in spring and the other in autumn. Summers are dry and usually with the lowest amount of precipitation registered all over the year (Fig. 3).

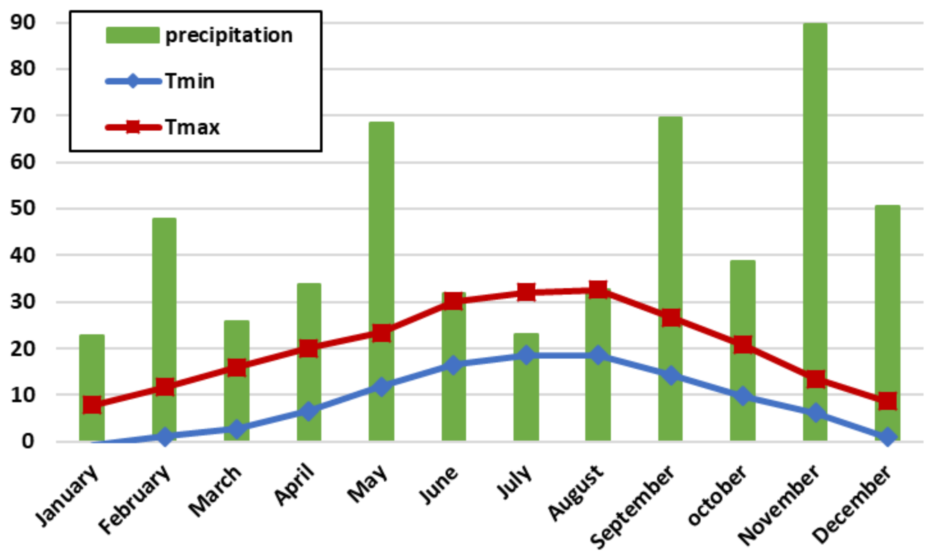


Figure 3: Mean meteorological data (Tmax, Tmin, precipitation) in the trial site in 2017-2021.

Cultural Practices

a) Maize

A winter ploughing, a spring disc harrowing paired with a basal fertilization with 115 kg ha^{-1} of N as urea and a rotary harrowing were performed in order to get a firm seedbed preparation before the rotation's settlement. One chemical weed control was carried out at the end of the winter by spraying 4 kg ha^{-1} of glyphosate and a second one was required straight after maize sowing, which occurred at the end of March by spraying a S-Metolachlor, Atrazine, Mesotrione based pre-emergence herbicide at 4 kg ha^{-1} dose. A FAO class 500 maize (Pioneer 1028) (*Zea mais*) was sown (9 seeds m^{-2}) with a pneumatic planter alongside a granular soil sterilant application lambda-cyhalothrin based 10 kg ha^{-1} and a mineral P_2O_5 fertilizers 16 kg ha^{-1} (with additional 8, 2 and 22 kg ha^{-1} of CaO, MgO and SO_2, respectively). A mechanical weed control was performed about 8 weeks after sowing together with an additional 140 kg ha^{-1} of N broadcasting. An insecticide treatment was required in the first week of July. The harvest was carried out at the end of August. The soil was hence tilled before wintertime, to follow the conventional agronomical practices of the area (one ploughing and two harrowing).

b) Dedicated lignocellulosic crops

Sunn hemp, biomass sorghum and kenaf were sown with a pneumatic planter (varying the settings in order to obtain different sowing densities and depth according to the crop-specific characteristics), whereas industrial hemp with a mechanical one (Figure 4). With exception for industrial hemp, the other crops received a granular soil sterilant application lambda-cyhalothrin based 10 kg ha^{-1} at sowing. A preliminary glyphosate application was carried out at 4 kg ha^{-1} to the whole experimental area, then the 'Futura 75' variety of industrial hemp (*Cannabis sativa* L.) plots were fertilized with about 60 kg ha^{-1} of N and 92 kg ha^{-1} of P_2O_5, harrowed and sown at 157 seeds m^2. The 'Bulldozer' (by KWS) biomass sorghum hybrid (*Sorghum bicolor x Sorghum sudangrass*) was sown at 19 seeds m^{-2}. About one month later the R2 plots were mechanically weeded and fertilized incorporating 120 kg ha^{-1} of N. In R3 plots the 'H328' kenaf (*Hibiscus cannabinus*) variety was coated with an iprodione based fungicide and sown (25 seeds m^2). About one month later kenaf was mechanically weeded and fertilized adding 37 kg ha^{-1} of N. Sunn hemp (*Crotalaria juncea* L.) received an additional 4 kg ha^{-1} glyphosate spraying before sowing occurred, and the same day the 'Ecofix' variety was sown at 52 seeds m^{-2}. Likewise, sunn hemp was mechanically weeded between the rows about one month after sowing, even though it did not receive any further nitrogen input. At mid-August, industrial hemp was manually and mechanically harvested, whereas for hemp and sunn hemp, biomass sorghum and kenaf the harvest occurred at the end of September.

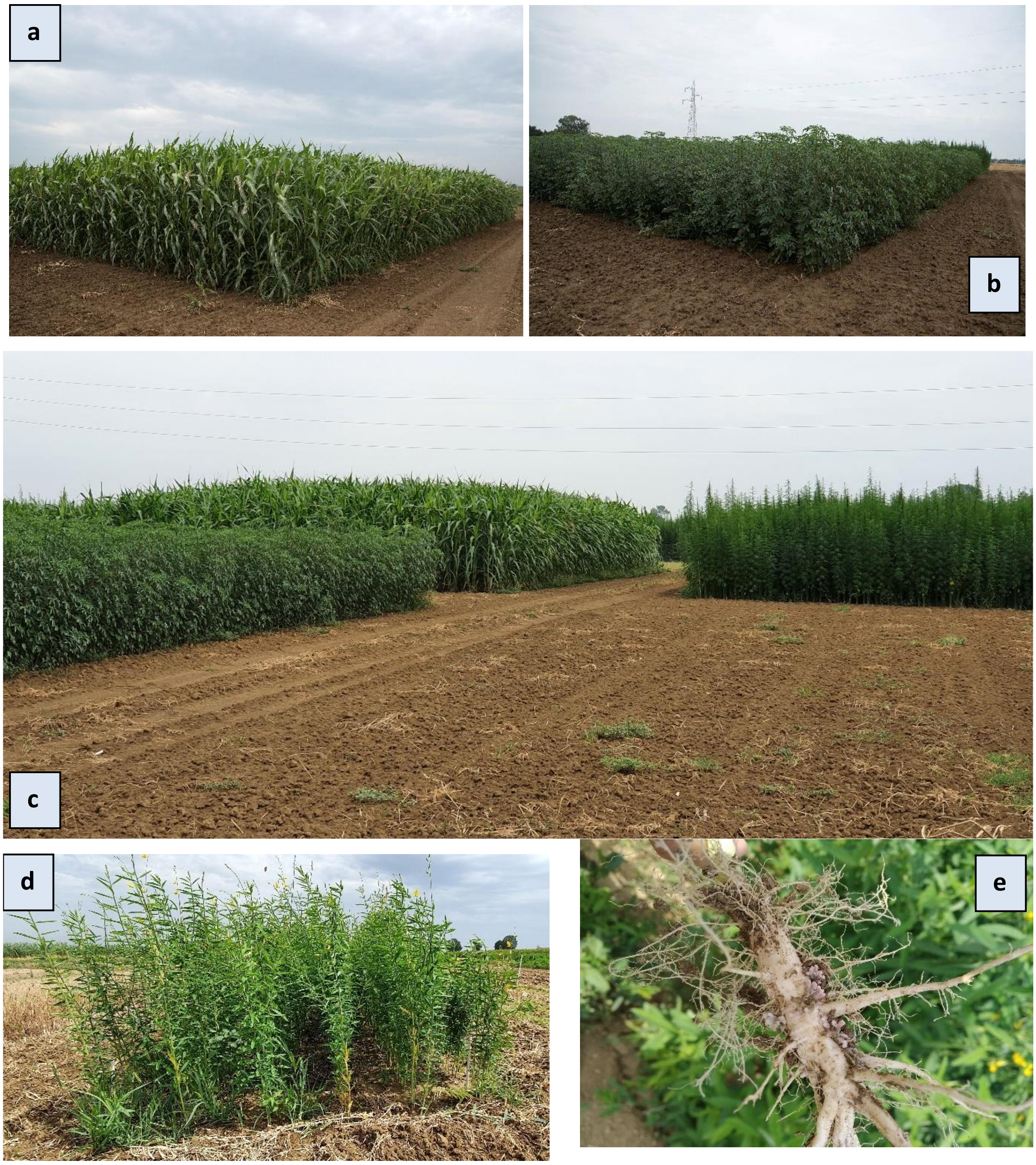


Figure 4: View of biomass sorghum (a), kenaf (b), industrial hemp on the right, biomass sorghum in the middle, kenaf on the left and fallow plots in the close up for the integrated crop rotations in 2021 (c), sunn hemp (d), and sunn hemp taproot close up showing the N-fixing nodules (e).

c) Wheat

A winter wheat (*Triticum aestivum*) was planted in November at 200 kg seeds ha^{-1} with a mechanical seeder, straight after the dedicated lignocellulosic crops harvest. The seedbed was prepared with a spading machine then by harrowing twice. Along the vegetative growing season three nitrogen fertilization were applied in i) mid-January (69 kg ha^{-1} of N), ii) during the elongation in mid-March (100 kg ha^{-1} N) and iii) at the inflorescence emergence (40 kg ha^{-1} N). One herbicide treatment was performed in mid-March encompassing both broad and narrow leaf weed control. Two insecticide and two fungicide treatments were applied in mid- and the end of May, following the agronomical practices of the neighbour farmers. The harvest was carried out at the end of June.

Field and lab measurements

The aboveground biomass yields were measured at the end of each growing season on a representative randomly selected area per reps of 8 m^2 for maize, sunn hemp, biomass sorghum, kenaf and industrial hemp, conversely on 1 m^2 on wheat. The harvest was carried out by manually cutting and weighting the plants in the sample area at about 3 cm from the soil surface. Then, the dry biomass was determined by oven-drying the fresh mass at 105°C to constant weight.

Aboveground biomass sub-samples were pooled, oven dried to a constant mass at 60 °C and ground to a diameter of 1 mm. The grinded 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 analyzer, the total N and C contents were determined in four replicates.

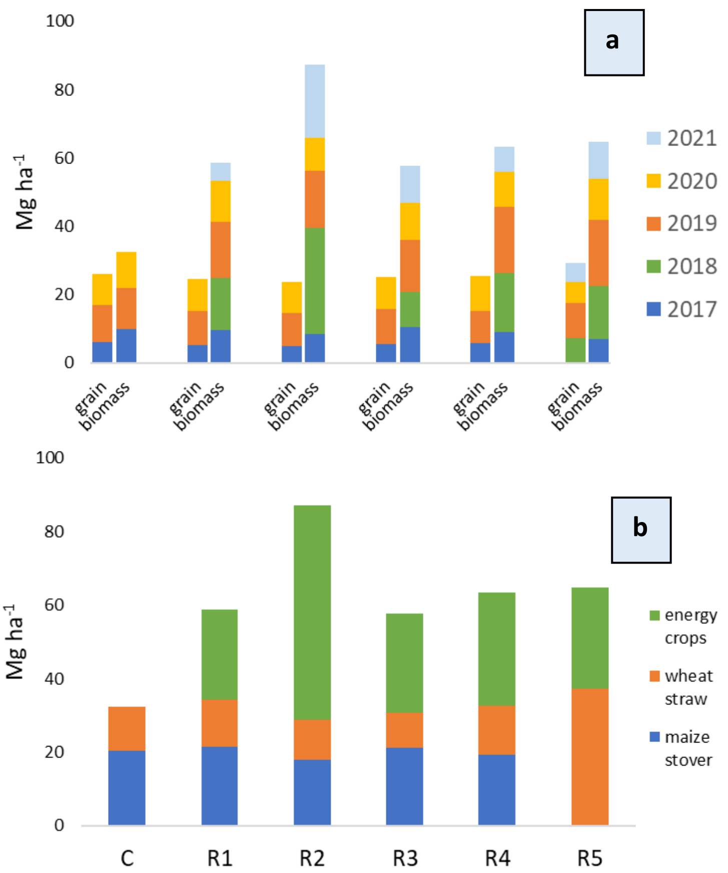
The soil physical and chemical composition was determined at the beginning of the trial in 2016, then following the lignocellulosic crops harvest in October 2018 and 2021.Additional samples were collected separately for bulk density and SOC measurements at 0-40 cm depth interval in 2018 and 2021. The bulk density was measured using a steel cylinder (Ø 48 mm) to collect vertical undisturbed soil cores, which were subsequently oven dried at 105 °C for 72 h to constant weight. The samples for SOC determinations were grinded below 1 mm particle size and subsamples of about 12 mg were pre-treated with HCl to eliminate the carbonates. Soil subsamples were encapsulated with silver and SOC was determined by an elemental analyzer (Flash 200 CHNS/O Analyzer, Thermo Scientific, USA).

Root biomass was determined through soil cores samples taken (70 mm Ø) at 0-25, 25-50 and 50-75 cm depth in between crop rows. Roots were separated from soil by washing and sieving, then weighted before and after oven-drying at 105 °C to constant weight for belowground biomass determination.

Results and discussion

Biomass yields

Figure 5a shows the cumulated yields for grain and biomass for each crop rotation. While the food (grain) production is similar, the biomass yield is highest in R2, which is almost three times more compared to C. R1, R3 and R4 showed similar yields (average 60 Mg ha^{-1} dw). R5, which is wheat-sunn hemp continuous rotation and does not include maize, resulted in slightly higher grain (+16 %) and biomass (+8 %) yields then R1, R3 and R4. The R2 biomass yields is mainly due to the contribution of biomass sorghum (figure 5b in green colour) that averaged 26 Mg ha^{-1} dw. The biomass composition widely differs among R5 and other rotations because of the crops involved in the rotation. In R5 wheat straw and sunn hemp contribute for 57 and 43 % of the total biomass, respectively, whereas in other rotations an average 38 % is attributed to maize stover.



▶ **Figure 5: grain and biomass yield for each cropping system in the period 2017-2021 (a), and biomass composition (b).**

The diverse cropping schemes tested over 5 years did not decrease grain yields compared to C, while they were able to significantly increase the biomass availability. R2 stands out in terms of biomass yields, even though R1, R2, R3, R4 and R5 should be considered in the light of crop diversification and sustainability of the system. In particular, R1 and R5 include sunn hemp in every growth cycle, which as a legume can contribute in reducing the inorganic nitrogen fertilization for the following crop and therefore ameliorate the environmental impact of such a practice.

Morphological parameters

The dedicated lignocellulosic crops need to maximize the biomass yields for advanced biofuel; hence the high cellulose and hemicellulose production which is mainly concentrated in the fiber composition of the stems is reflected by plants height and stem diameter. These parameters can also influence the harvest operations and the physical characteristics of the feedstock at the plant gate. Plant height and stem diameter at harvest are presented in Figure 6. Biomass sorghum reached 420 cm height, then industrial hemp (290 cm), kenaf (270 cm) and sunn hemp (190 cm). The biomass sorghum height might have determined the low crop resistance to lodging which can hamper harvesting operation and the ash and mineral content of the feedstock.

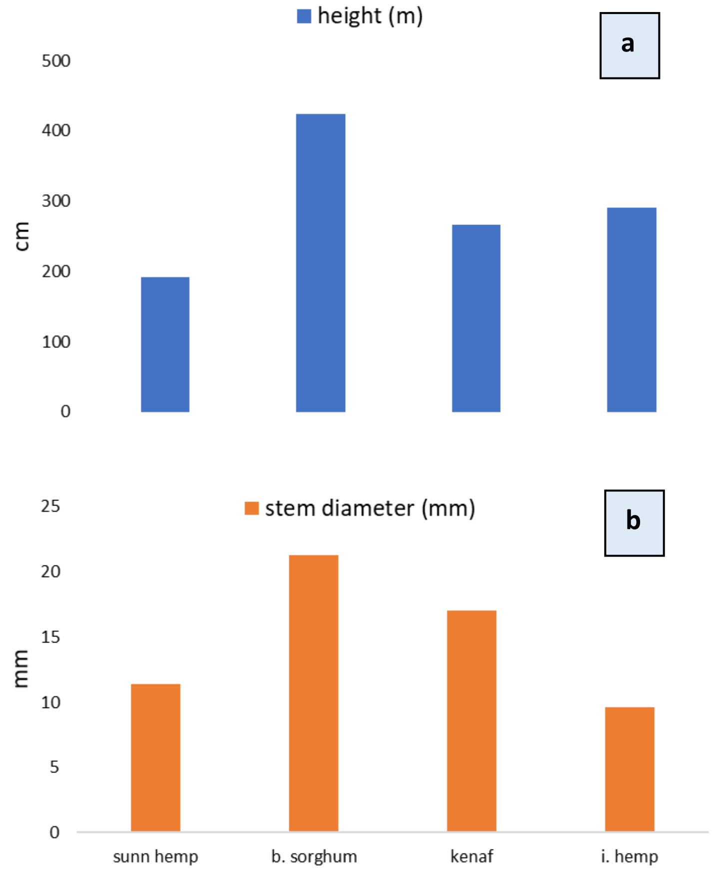


Figure 6: stems height (a) and diameter (b) for the dedicated lignocellulosic crops at harvest.

The stem diameter (Figure 6b) averaged 21 mm for biomass sorghum, which resulted higher by 24 % than kenaf and double compared to sunn hemp and industrial hemp. The presented morphological parameters combined with others such as the plant density and the dry matter content at harvest determines the final aboveground biomass.

Figure 7: root samplings in 2018 after dedicated lignocellulosic crops harvest, performed through soil core samplings

The root weight represents one of the major contributors to soil organic carbon pool with the aboveground biomass residues (Figure 7). In this light, the belowground biomass was determined through soil cores at 75 cm depth. Wheat outperformed maize by producing 64 % more root biomass. Among the dedicated lignocellulosic crops, kenaf resulted with the highest root dry weight, which is double compared to sunn hemp, then 77 and 63 % higher for biomass sorghum and industrial hemp, respectively. In general, food crops (1 mg cm^{-3}) twofold the lignocellulosic crops (0.5 mg cm^{-3}) roots in the top 75 cm soil layer and this might be due to the nature of fiber crops that have deeper roots and high water use efficiency.

Soil characteristics

The total soil nitrogen and organic carbon was measured at the beginning of the experiment, then in 2018 and 2021. The results shown in Figure 8 highlights that the highest soil nitrogen concentration was found in R5 (+ 16 % than in the other rotations mean), and the reason might be due to the annual sunn hemp double cropping plantation and the lack of maize in the rotation. In this light, R1 (which also has sunn hemp but at the same time maize) resulted with the lowest nitrogen concentration (- 14 % than in the other rotations mean). Furthermore, biomass sorghum resulted with high nitrogen in its soil possibly due to the higher nitrogen fertilization that slightly exceeded the crop requirements (150 kg ha^{-1} of N as urea).

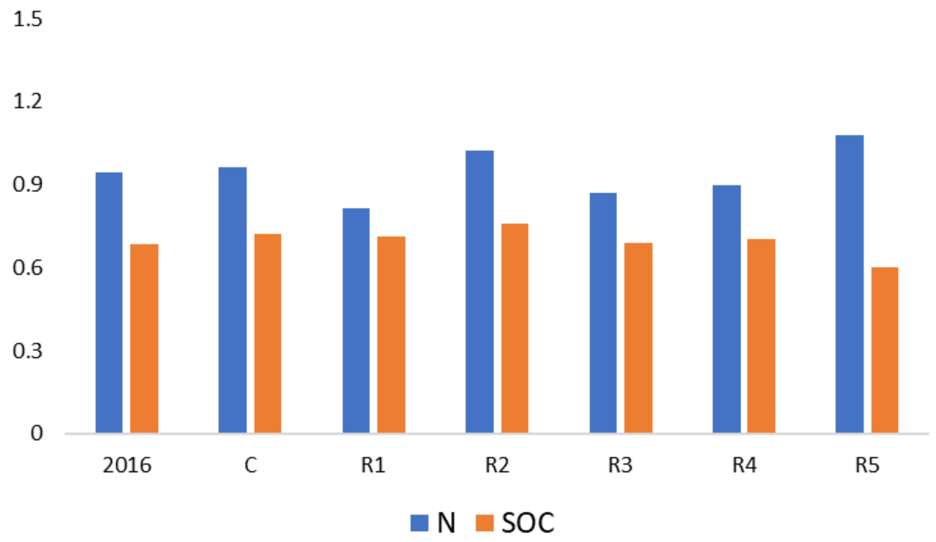


Figure 8: total soil nitrogen (g kg^{-1}) and soil organic carbon (%) at the beginning (2016) of the experiment, then for each rotation at the end of 2021.

The overall SOC concentration (Figure 8) in the tilled layer significantly increased from 2016 to 2021 by 10 %. Biomass sorghum resulted with higher SOC concentration compared to industrial hemp (-13 %) and kenaf (-14 %) but similar to fallow and sunn hemp. However, the generalized increase in SOC over the 5 years rotation highlights the potential of these systems in producing feedstocks for advanced biofuels coupled with SOC maintenance.

Biomass quality

Biomass quality is of utmost importance for bio/thermo-chemical conversion of lignocellulosic materials into ethanol. In particular, low ash and inorganic elements (e.g. alkali) are key parameters in combustion processes as far as they are responsible of slagging, corrosion and fouling. On the other hand, the cell wall composition is the major factor that influences the final ethanol yield. In this regard, biomass sorghum and maize stover showed low value for ashes (Table 2) being herbaceous species. Concentrations of over the 5 % of ash, may render the thermochemical pathway not economically cost-effective and therefore these feedstocks might be more suitable to biochemical conversion. The highest concentration of ash and inorganic elements comes from the leaves, hence a possible solution to reduce them is to develop harvesting systems able to remove leaves from the final product.

Table 2: Cell wall composition, ash content, total N and C concentration, and mineral concentration of each crop

	B. sorghum	Sunn hemp R1	Sunn hemp R5	Kenaf	Hemp	Straw	Stover
Ash (%)	**4.5**	**5.6**	**7.5**	**6.0**	**6.6**	**7.6**	**3.2**

N (%)	**0.8**	**1.7**	**2.3**	**1.3**	**1.5**	**0.4**	**1.2**
C (%)	**46.9**	**46.9**	**43.5**	**45.9**	**46.3**	**45.6**	**46.2**

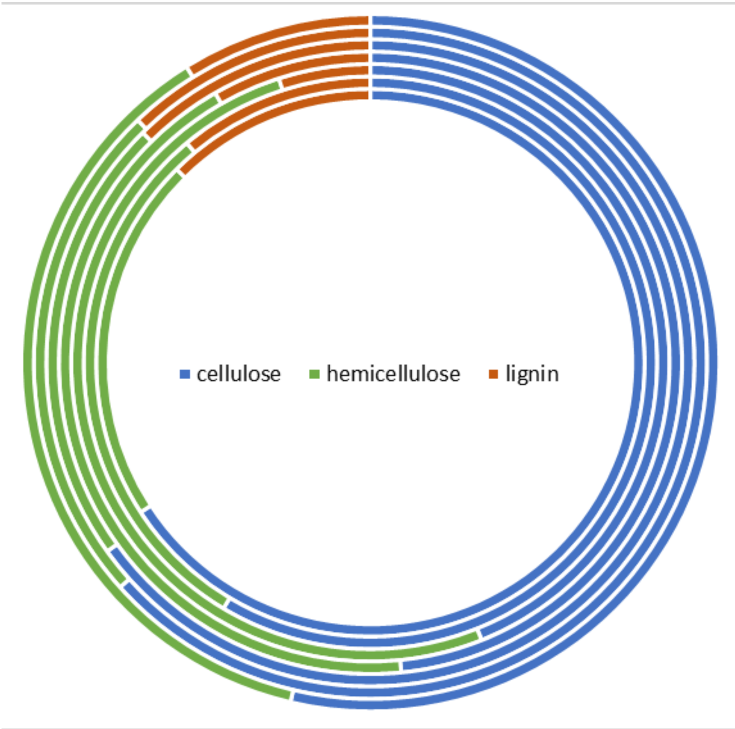


Figure 9: fiber content in aboveground biomass, from the inside of the circle hemp, kenaf, stover, sorghum, sunn hemp as main crop, sunn hemp double cropped and straw

In general terms, within the dedicated lignocellulosic crops, biomass sorghum and hemp have the general lowest and highest mineral concentration, respectively, whereas sunn hemp and kenaf showed intermediate values. Biomass sorghum (Fig. 9) is a good candidate as feedstock for advanced biofuel for the highest cellulose and hemicellulose content (58 % of the total dry matter) within lignocellulosic crops. Conversely, sunn hemp when double cropped (R5) showed the lowest content (46 %). Opposite characteristics were shown by wheat straw and maize stover. In particular, straw has a relatively high ash and mineral concentration with high cellulose and hemicellulose, whereas maize stover showed low ash and minerals but low fiber content.

Conclusions

In summary, the evaluated systems increased the biomass feedstocks for advanced biofuels without affecting the food production. Biomass sorghum demonstrated to be the most promising feedstock for the development of both thermo/biochemical-based advanced biofuels for its high yields, favorable biomass characteristics and wide seeds and variety availability for temperate climate. Conversely, sunn hemp, hemp and kenaf can represent a valuable feedstock alternative mainly for biochemical conversion, which can handle with a greater ease their higher ash and mineral concentration, compared to combustion processes. In addition, the systems did not deplete soil fertility but conversely witnessed a slight increase in soil organic carbon concentration over the 5 years rotation. Another full rotation cycle of food and non-food crops would be recomendable to confirm these results.

Annex 2. CIEMAT report

INCREASE OF LIGNOCELLULOSIC FEEDSTOCK FROM INTEGRATED CROPPING SYSTEMS IN SPAIN

Authors: Carlos Martín Sastre, Ruth Barro Piñeiro, Miguel Fernández Llorente y Pilar Ciria Ciria

Introduction

Biomass production for energy could expand the biomass markets creating a potential displacement of traditional food crops from agricultural land and threatening the food security. The introduction of annual lignocellulosic crops within conventional cropping systems, between two main food crops, is an effective alternative to increase the feedstock availability for the production of biofuels without reducing food production. This cropping system would allow diversifying crops, while increasing the annual quantity of lignocellulosic feedstock without reducing food crops land. Biomass sorghum, sunn hemp, kenaf and hemp are annual low input high lignocellulose yielding crops which can be integrated into existing food/feed based cropping system. In this context, the objectives of the Task 1.2 in the Becool project are based.

The main target is the establishment of innovative cropping systems so as to increase biomass feedstock availability by at least 50% without negatively impacting food production, soil quality, and customary land uses. A reliable evaluation of crop rotations needs long time or multi-location trials, so comparison between innovative and conventional cropping systems were carried out in Spain following a protocol that allows the results to be compared with those obtained in other countries within the BeCool project.

Materials and Methods

Experimental design and crop management

Five innovative crop rotations and the control rotation were stablished (Figure 2). The six rotation systems were designed as follow: i) maize-fallow-wheat-maize (C, control), ii) maize-sunn hemp-wheat+sunn hemp-maize (R1), iii) maize-biomass sorghum-wheat+sunn hemp-maize (R2), iv) maize-kenaf-wheat+sunn hemp-maize (R3), v) maize-industrial hemp-wheat+sunn hemp-maize (R4); vi) wheat+sunn hemp-wheat+sunn hemp (R5). Due to extension of the project crop rotations were able to continue until autumn 2022 ending with the harvest of the energy crops.

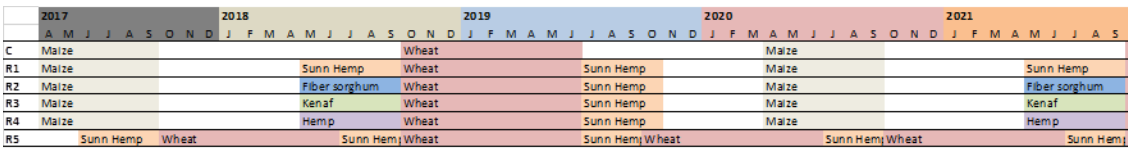


Figure 1 Becool crops rotations schemes for the increase of biomass feedstock avaibility through intensification

The plot chosen for Spanish rotation cultivation between spring of 2017 and autumn 2021 was in the municipality of Guadajira belonging to the Spanish region of Badajoz (South-Western Spain) its coordinates are 38 º 51’ 48´´ North and 6 º 40’22’’ West with and altitude of 185 m ASL. It has a cultivated area of about 0.8 ha which included 24 plots of 120 m^2 and the spaces needed to allow the works of the agricultural machinery. The satellite photograph of the plot with the crops under development could be seen in Figure 2

Figure 2 Satellite photograph of the Spanish' crop rotation being cultivated in the municipality of Guadajira belonging to the Spanish region of Extremadura in 2017.

The soil in Guadajira was classified as loamy typic haploploxeralf (45% sand, 25% clay, and 30% silt), slightly acidic (pH 6.2) with N, P, and K content of 0.7 g kg^{-1}, 14 mg kg^{-1} and 93 mg kg^{-1}, respectively, and an organic matter content of about 0.8%

The climate corresponds to hot summer Mediterranean with low rainfall. The Badajoz's fields registered 362 mm of annual average rainfall, with 2018 being the wettest year with 480 mm and 2017 the driest with 284 mm. The average temperature was 16.8 ºC with 44.0 ºC as the highest temperature registered in September 2021 over all the experimental period and -5.0 ºC as the lowest in January 2017. Complete information about the meteorological conditions registered during the lifetime of the rotations can be seen in Figure 3.

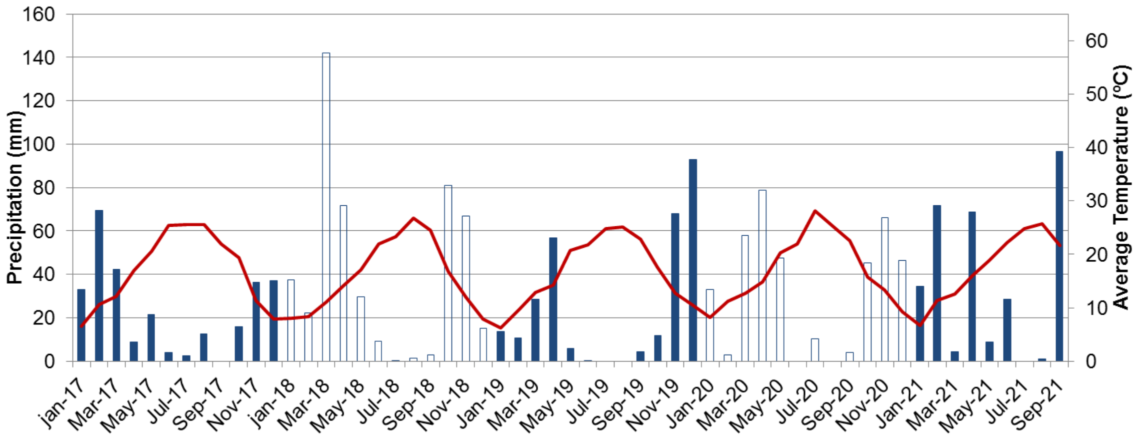


Figure 3 Ombrothermic diagram corresponding to the nearest weather station to Guadajira's plot (Badajoz) during the years of cultivation of the crop rotations.

a) Conventional crops (Maize & Wheat)

Maize cultivation was performed twice in 2017 and 2020 according to traditional management practices of farmers of the region. Maize variety P1570 (FAO 700 cycle) was sown between the end of April and May at a density of 9.5 seeds m^2 after soil preparation and application of a base fertilization (700 kg ha^{-1} of NPK 8 15-15). Top dressing fertilization was applied at dose of 400 kg ha^{-1} of urea 46% N. Drip irrigation of approximately 600 mm was applied each season. Mechanical harvests were performed between the end of September and the first half of October.

As for wheat (*Triticum aestivum*), soil was prepared each season from 2017 until 2020 starting with a semi-chisel pass, then base fertilizing at a dose of 300 kg ha^{-1} of NPK 8-15-15, and finalizing with a single pass of cultivator. An eight-row precision seed drill was used for sowing Cosaco variety at a dose of 180 kg ha^{-1} from the second half of November to the first half of December. Top dressing fertilization was applied at a dose of 300 kg ha^{-1} of calcium ammonium nitrate (27% N), and crop was harvested using an auto-propelled harvester from June to the first days of July.

The Figure 4 shows a photograph of maize at the moment of harvest and of wheat in development.

Figure 4 Maize in Guadajira the 17th of October of 2017 (above photo) and wheat the 3rd of April of 2018 (below)

b) Dedicated lignocellulosic crops

Sunn hemp cultivation was performed every year between 2017 and 2021 (see Figure 1). Sowing doses were calculated every season according to seed germination rate to achieve a final plant density of 33 plants m^2. Soil was prepared by using a disk harrow and a semi-chisel, then base fertilization was performed at a dose of 400 kg ha^{-1} of NPK 8–15–15 (only the first year in R5), after that a pre-emergence treatment with glyphosate at 2L ha^{-1} was applied and finally a single pass of rotary tiller was performed. A pneumatic seeder was used for sowing. Supplemental drip irrigation (250–400 mm) was applied during each cropping season. Crop was harvested by using a sickle bar mower.

Fiber sorghum cultivation was performed in 2018 and in 2021. Sowing doses of Amigo variety were calculated both seasons according to seed germination rate to achieve a final plant density of 15 plants m^2. Soil was prepared by using a disk harrow, a semi-chisel and a rotatory tiller, then base fertilization was performed at a dose of 400 kg ha^{-1} of NPK 8–15–15, after that a pre-emergence treatment with glyphosate at 3 L ha^{-1}. A pneumatic seeder was used for sowing between the end of April and the beginning of May. 260 kg ha^{-1} of Calcium Ammonium Nitrate was applied for top fertilization. Supplemental drip irrigation (≈300 mm) was applied during each cropping season. Crop was harvested by using a forage harvester in August.

Kenaf cultivation was performed in 2018 and in 2021. Sowing doses of H328 variety were calculated both seasons according to seed germination rate to achieve a final plant density of 20 plants m^2. Soil was prepared by using a disk harrow, a semi-chisel and a rotatory tiller, then base fertilization was performed at a dose of 374-400 kg ha^{-1} of NPK 8–15–15, after that a pre-emergence treatment with glyphosate at 3 L ha^{-1}. A pneumatic seeder was used for sowing between the end of April and the first day of June, 3 L ha^{-1} of Pedimentalin 33% were applied as well. 260 kg ha^{-1} of Calcium Ammonium Nitrate was applied for top fertilization. Supplemental drip irrigation (300-500 mm) was applied during each cropping season. Crop was harvested by using a forage harvester between August and September

Hemp cultivation was performed in 2018 and in 2021. Sowing doses of Future 75 variety were calculated both seasons according to seed germination rate to achieve a final plant density of 120 plants m^2. Soil was prepared by using a disk harrow, a semi-chisel and a rotatory tiller, then base fertilization was performed at a dose of 333 kg ha^{-1} of Superphosphate (18% P) and Urea 217 kg ha^{-1} (46%N). A pneumatic seeder was used for sowing. Supplemental drip irrigation (100-200 mm) was applied during each cropping season. Crop was harvested by using a forage harvester.

The Figure 5 shows a photograph of the development each of these lignocellulosic crops at the moment of its harvest.

Figure 5 Sunn Hemp in Guadajira the 09 of October of 2018 (above-left photo), fiber sorghum the 28th of August of 2018 (above-right), Kenaf the 18 of September of 2018 (bellow-left) and hemp the 19 of September of 2018 (below-right).

Biomass yield measurements

The aboveground biomass yields were measured at the end of each growing season by mechanically harvesting 60 m2 of the central part of the plot for maize, sunn hemp, biomass sorghum, kenaf and industrial hemp and wheat. For maize and wheat, the grains and the residues (stover and straw) were measured separately. Average heights of lignocellulosic crops were measured at the moment of harvest. Dry biomass was determined by measuring moisture content by oven-drying at 105 °C until constant weight, following ISO 18134-2:2017.

Soil measurements

The composition of the soil was determined at the beginning of the trial in 2017 and at the end in 2020-2021. Soil samples were taken as indicated in ISO 10381-1, and analyzed according to ISO standards for texture (ISO 11277), pH (ISO 10390), organic matter (ISO 10694), phosphorus (ISO 11263), potassium (ISO 11260), and ammonium and nitrates (ISO/TS 14256-1 EX)

Biomass quality measurements

Sample analyses were performed in the "Laboratory of Biomass Characterization" at CIEMAT, according to the current international ISO standards for solid biofuels. The evaluated properties, as well as the analytical techniques and equipment used for testing are listed in Table 1.

Table 1 Analytical techniques, equipment and international standards used.

Property	Analytical technique	Equipment	Standard
Sample preparation	Subsampling and milling		ISO 14780:2017
Ash	Calcination at 550 °C		ISO 18122:2015
C, H, N	Elemental analysis: CC+IR+TCD	TruSpec (Leco Instruments)	ISO 16948:2015
S and Cl	Combustion bomb + IC	883 Basic IC Plus (Metrohm)	ISO 16994:2016
O	Calculation by difference		ISO 16993:2017
Calorific value	Calorimetry	6400 (Parr)	ISO 18125:2017

CC: flash catalytic combustion; IR: infrared detection; TCD: thermal conductivity detection; IC: ion chromatography

Results and Discussion

Biomass production

The Figure 6 shows the mechanical harvest yield (wet and dry) and the height for each time that an energy crop was grown during the rotation (see Figure 1). Sunn hemp at the moment of harvest had a humidity that ranged from 65 to 82 % with a height that went from 1.7 to 2.3m. The dry matter yields obtained ranged from 5 to 10 Mg ha^{-1}. The yields in the low range from 5 to 7 Mg ha^{-1} occurred when the crop had some problems, for instance plagues while we consider that yield in the range from 8 to 10 correspond to an optimum development of the crop for the conditions of the experiment. Fiber sorghum had a humidity of about 65% with a height between 3.3 and 3.6 m. The yield obtained in 2018 was low for the expectations while the yield obtained in 2021 (> 17 Mg D.M. ha^{-1}) was more in line with them for the sowing density used. Kenaf had a humidity at the moment of harvest about 67% with a height between 2.9 and 3.2m. The yield obtained were from 14 to little more than 16 Mg D.M. ha^{-1}, we consider that the crop had a good development both years. Hemp, at harvest time, had a humidity of 60% the first year an of 75% the second while the heights were of about 1.6 m. The crop did not have a good development due to bad nascence and the yields obtained were low (≈4.5 Mg D.M. ha^{-1}).

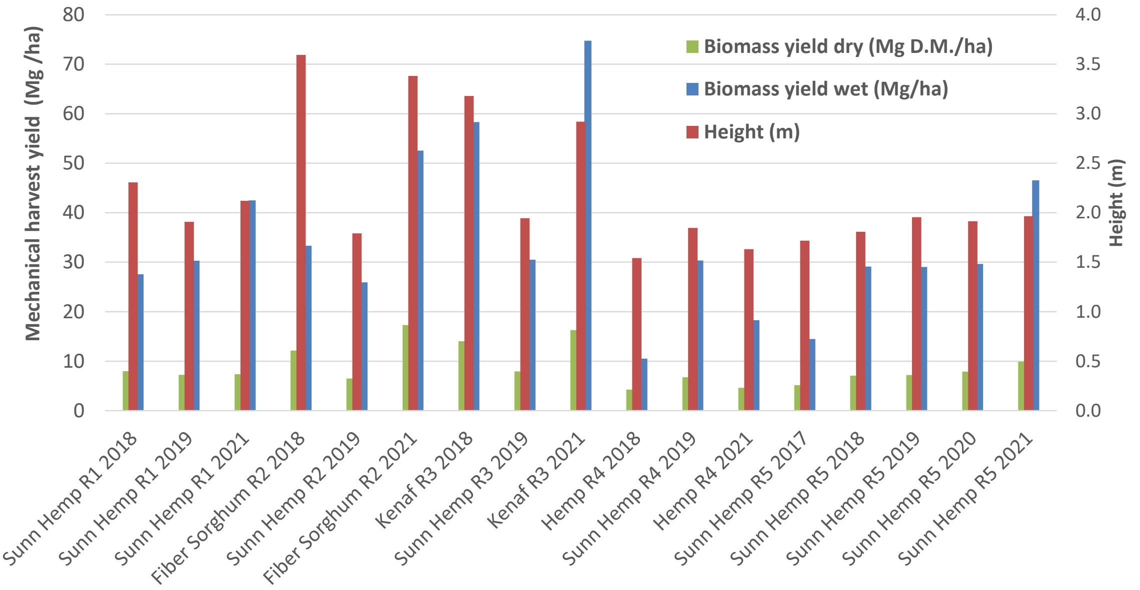


Figure 6 Biomass yields dry and wet (humidity at harvest time) and height of the biomass fiber crops in the rotation

In the Figure 7 the grain and the biomass yield (stover for corn and straw for wheat) of the food crops of the rotations are shown. The humidity of maize stover at the moment of harvest went from 30 to 50% with a yield that went from 5 to 8.5 Mg D.M. ha-1, grain yield went from ≈8.5 to 12 Mg D.M. ha-1. The first year the maize was cultivated performed better with grain yields between 11 to 12 Mg D.M. ha-1 than the second (8.5 to 10 Mg D.M. ha-1). The yields obtained for the control were very similar for the control and the rest of the rotations in both years of maize cultivation. The humidity of wheat straw at harvest was low (8-215%), this straw yielded between 3.5 and 5.0 Mg D.M. ha^{-1} while the grain yields went from 2.5 to something more than 4 Mg D.M. ha^{-1}. The crop performed better the first year of R5 with >4 Mg D.M. ha^{-1} of grain while for the rest of the years and rotation yield were < 4 Mg D.M. ha^{-1} with the exception of the control(C) in 2019. The grain yield of the control (C) was slightly better than the ones for R1-R3 while for R4 and most of R5 wheat cultivation seasons yields were very similar.

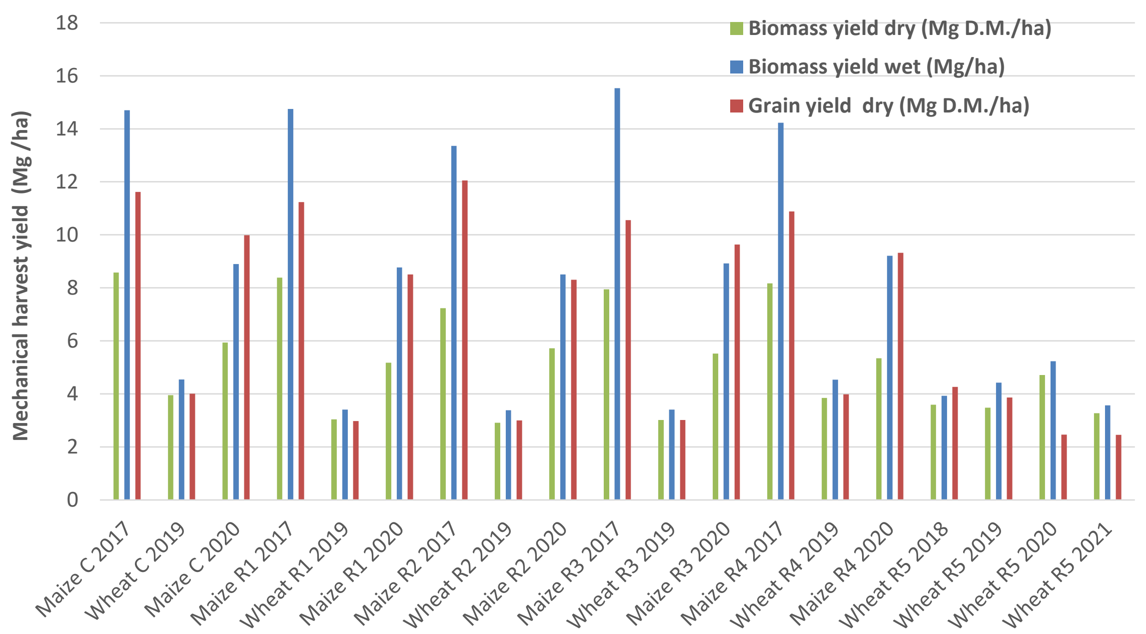


Figure 7 Dry grain yield and biomass yield dry and wet (humidity at harvest time) of the food crops in the rotation

The Figure 8 shows the accumulated grain yield of wheat and maize as well as the cumulated biomass yield for all the rotations. It can be observed that for R5 the accumulated wheat grain yields were much higher, this is due to the higher number of wheat cultivation cycles in this rotation. Accumulated maize grain yield was very similar for all the rotations (≈ 20 Mg D.M. ha^{-1}). The accumulated biomass generated in the intensified rotations was between 1.8 and 3.0 times higher than the one generated in the control(C) that was 18.5 Mg D.M. ha^{-1}. The best rotation in terms of biomass production was the one of kenaf (R3) with ≈55 Mg D.M. ha^{-1} closely followed by the one of sorghum (R2) and the one alternating wheat and sunn hemp (R5) that both yielded ≈52 Mg D.M. ha^{-1}.

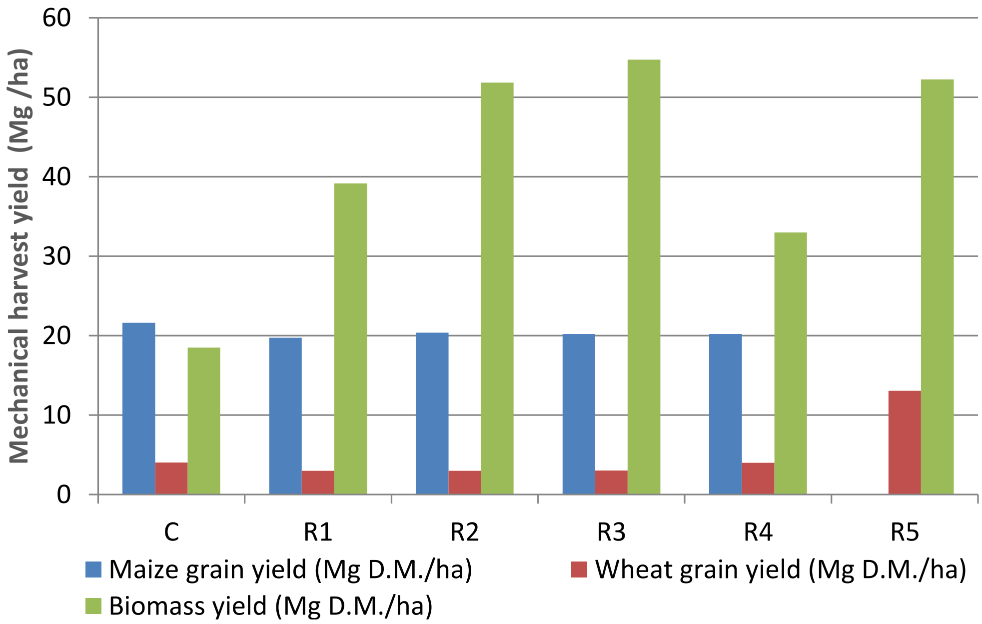


Figure 8 Total biomass and grain yields produced in each rotation

The Figure 9 shows the accumulated biomass for each rotation by type: energy crops, wheat straw and maize stover. It can be observed the significant contribution of the energy crops for biomass production in all the intensified rotations. When the control (C) and the rotations under study are observed (R1-R4), the significant contribution of the maize stover over the wheat straw is evident with between 3.5 and 4.5 more times of biomass generated. In the rotation R5 sunn hemp generated 2.5 times more biomass than the wheat straw.

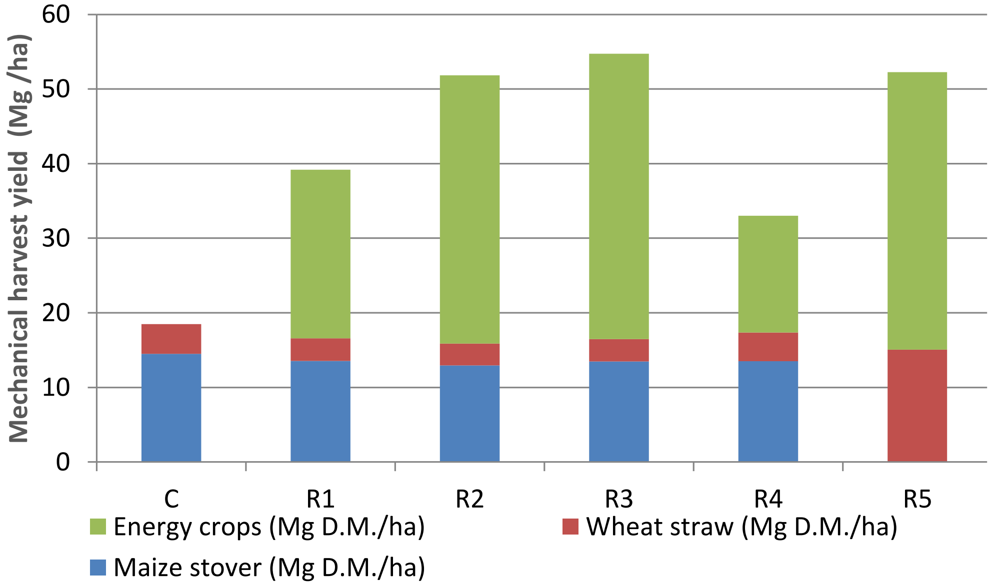


Figure 9 Total biomass produced in each rotation by origin type

Considering all the previous results shown it has been demonstrated that for these experimental conditions all the intensified rotations tested were able to produce much more biomass than the control without remarkably affecting grain yields.

Soil evolution

The Figure 10 shows the evolution of the soil parameters (Organic matter, N, P and K) with respect to the initial status of the parcel were the BeCool's crop rotations were stablished and allow the comparisons among each rotation. With respect to the organic matter, it can be seen that no relevant changes were produced with respect to the initial status. It is important to remark that when the control "C" is compared with respect to the energy crops rotations the changes are even less. We would expect that the intensification of the energy crops rotations (R1-R5) with more tillage operations (more organic matter respiration) and much less fallow (can slightly increase organic matter) than the control could have let to relevant decrease of the organic matter. As this has not be the case, we can say that this possible drawback of the intensification of food crop lands with energy crops to produce biomass has been dismissed, at least in our experiment. The increase of the total nitrogen with respect to the initial situation could be due to the nitrogen fertilizers used. There are changes in the P and K contents of the soil, apparently P has increased and K has slightly decreased with respect to the initial situation. There are also changes among the rotations and with respect to the control. We believe that these changes could be due to P and K fertilization and the different use of this nutrients made by the crops. However, we tend to think that these changes are not of importance since P and K fertility is maintained.

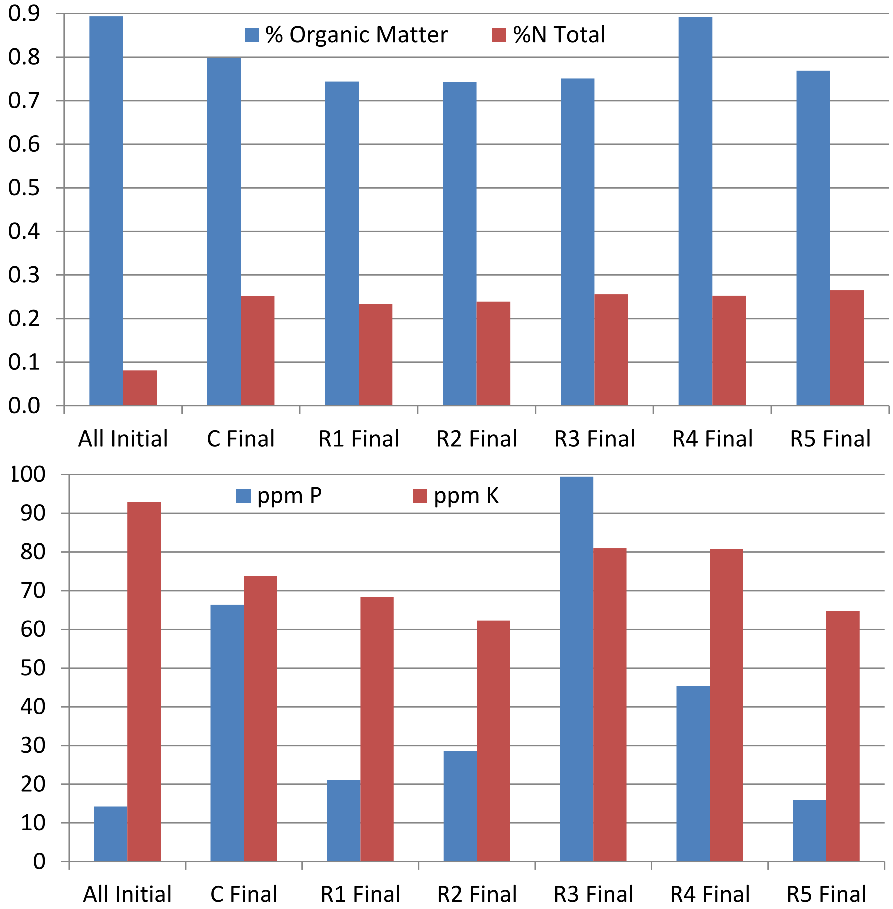


Figure 10 Initial soil analysis for the whole parcel (all rotations) and final for each rotation, organic matter and total nitrogen (above) and phosphorus and potassium (below).

Biomass quality

Table 2 shows the ash content, calorific value and ultimate analysis of the biomass from the six different species included in this project. For each type of biomass, the mean content, averaged across years and rotations, as well as the standard deviation and the number of samples analyzed are included.

Table 2 Main properties of the different types of biomass (mechanical harvest).

Species	Part of the plant		Ash %, d.b.	C %, d.b.	H %, d.b.	N %, d.b.	S %, d.b.	Cl %, d.b.	O %, d.b.	$GCV_{v,0}$ MJ/kg, d.b.	$NCV_{p,0}$ MJ/kg, d.b.
Hemp	**Aerial biomass**	**mean**	12.5	43.6	5.8	1.97	0.17	0.43	35.6	17.57	16.31
		SD	2.0	2.2	0.3	0.40	0.04	0.11	1.3	1.19	1.14
		n	6	6	6	6	6	6	6	6	6
Sunn hemp	**Aerial biomass**	**mean**	6.8	45.9	5.9	1.80	0.22	0.97	38.5	18.52	17.22
		SD	1.0	0.6	0.1	0.36	0.07	0.19	0.9	0.26	0.26
		n	30	30	30	30	30	30	30	30	30
Kenaf	**Aerial biomass**	**mean**	6.0	45.2	5.9	0.94	0.19	0.88	41.0	17.98	16.69
		SD	0.8	0.3	0.1	0.26	0.02	0.08	0.6	0.08	0.07
		n	7	7	7	7	6	6	6	6	6
Sorghum	**Aerial biomass**	**mean**	5.0	45.5	6.0	0.57	0.07	0.55	42.1	18.07	16.76
		SD	0.5	0.6	0.1	0.22	0.01	0.08	1.2	0.09	0.08
		n	7	7	7	7	6	6	6	6	6
Maize	**Straw**	**mean**	9.5	44.3	5.6	0.77	0.09	0.65	39.1	17.55	16.32
		SD	2.8	1.0	0.1	0.10	0.01	0.09	1.9	0.41	0.39
		n	15	15	15	15	15	15	15	15	15
	Grain	**mean**	1.7	45.0	6.4	1.32	0.09	0.05	45.2	18.23	16.84
		SD	0.3	0.7	0.2	0.10	0.01	0.01	0.4	0.26	0.25
		n	15	20	20	20	15	15	15	15	15
Wheat	**Straw**	**mean**	7.2	45.3	5.8	0.80	0.17	0.80	39.9	18.14	16.87
		SD	0.9	0.5	0.1	0.16	0.06	0.29	1.1	0.32	0.33
		n	27	27	27	27	27	27	27	27	27
	Grain	**mean**	2.2	45.2	6.4	2.47	0.09	0.08	43.5	18.41	17.02
		SD	0.1	0.2	0.0	0.32	0.1	0.0	0.6	0.36	0.35
		n	27	27	27	27	27	27	27	27	27

d.b.: dry basis; SD: standard deviation; n: number of samples; $GCV_{v,0}$: gross calorific value at constant volume and d.b.; $NCV_{p,0}$: net calorific value at constant pressure and d.b.

The composition of the crops (Table 2) is well within the typical levels reported in the literature for wheat and maize straw [1-8], as well as for sorghum [3,6,9,10], kenaf [4,5,11,12], hemp [4] and sunn hemp [9,13] aboveground biomass.

The biomass of sorghum and hemp showed the lowest and highest ash content, averaging 5.0% and 12.5%, respectively. It should also be highlighted that the crops were harvested mechanically, which tends to increase ash contents by introducing mineral contamination from soil particles. Lower ash contents are typically expected when plants are collected manually. Therefore, more attention should be paid in developing harvesting systems that prevent biomass contamination by avoiding the inclusion of mineral particles.

Maize and wheat grains averaged ash contents of 2 %; the typical value for virgin cereal grain materials [1,3]. When compared with straw, wheat and maize grains showed higher N contents and lower Cl levels. Cereal grains typically exhibit N contents of 2%, and Cl contents of 0.11%, with a typical range between 0.05 % and 0.5 % for Cl [1].

Cereal straws exhibited the lowest N contents (0.8 %), followed by kenaf (0.9 %). In turn, the biomass of hemp and sunn hemp showed high N levels, averaging 2 % of N, and clearly surpassing e.g. the recommended guidelines to avoid NO_x emission problems in combustion (<0.6 % or <1 %, depending on the author) [3,14-16]. Previous studies also shown elevated N contents for this type of biomass [9,13].

Gross calorific values averaged 18.5 MJ/kg (d.b.) for sunn hemp, 18.1 MJ/kg for sorghum and wheat, and 18.0 MJ/kg for kenaf samples. Calorific values were lower for hemp and maize biomass (17.5 MJ/kg), as they exhibited higher ash contents and therefore lower C contents.

The quality requirements of non-woody pellets regarding ash, N, Cl, S, trace elements and other properties have been lately established in the international standard ISO 17225-6:2021 [17]. If this biomass was used to produce pellets, the high Cl contents already present in all the raw materials analysed indicate that the pellets produced would not be able to comply with the requirements set in the afore-mentioned standard for either class A (≤ 0.10 % of Cl) or B (≤ 0.40 % of Cl). In addition, hemp pellets would also exceed the maximum ash content allowed in this standard (≤ 6 % for class A and ≤ 10 % for class B).

From a combustion point of view, corrosion and HCl emission problems are expected when Cl contents exceed 0.10-0.20 % [3,14-16]. Besides, Cl levels should be maintained below 0.2-0.3% to avoid dioxins and furans emissions [3,14]. Biomass Cl contents always exceeded these guidelines, averaging from 0.43% (hemp) to 0.97 % (sunn hemp), limiting the use of this raw materials in combustion unless additional measures are taken.

In this respect, several solutions have been proposed in the literature to reduce corrosion, sintering and emission problems during the thermo-chemical conversion processes of herbaceous biomass. Among them, biomass leaching is very effective in modifying the composition of herbaceous fuels by removing water-soluble compounds such as Cl, K and Na. In this sense, several approaches can be taken, such as washing the raw material [18,19] or the bales [20] with water as a feedstock pre-treatment, an exposure to natural rainfall in the field before crop harvesting [2], natural leaching by rainfall during the period between biomass harvest and collection [21], or a delayed harvest after senescence to allow, besides the natural wash-out effect by precipitation, a loss of nutrients by defoliation and their translocation to the root system [22,23]. Other solutions include the use of mineral additives [24], co-combustion with wood fuels [25], decreasing the combustion temperatures [26], or even a combination of processes such as pyrolysis followed by combustion [27].

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Annex 3. CRES report

Increase of lignocellulosic feedstock from integrated cropping systems including annual specialist lignocellulosic in Greece

Authors: Efthymia Alexopoulou, Myrsini Christou

Introduction

Conventional cropping systems generally leave soil uncovered for many months between two main food crops. Using soil more intensively (i.e. increasing the Land Equivalent Ratio – LER) through growing lignocellulosic crops as intermediate crops would allow to diversify crops, while increasing the annual quantity of lignocellulosic feedstock without reducing food crops land. The performance of such innovative cropping systems had been quantitatively and qualitatively evaluated in terms of annual productivity of specialist (dedicated) lignocellulosic crops (sunn hemp, biomass (fibre) sorghum, kenaf and hemp). These crops are most promising taking into account their potential yield, beneficial effects in the rotation (e.g. sunn hemp is a leguminous species), and growing season length. The integrated cropping systems in BECOOL included food and lignocellulosic crops. The selected crops are of tropical origin so as to be able to grow also in Brazil. As such, crops and rotation schemes can be applied in South Europe, thus it was carried out trials in Greece, Italy and Spain. A reliable evaluation of crop rotations needs long time or multi-location trials, so in BECOOL the experiment replicated in three locations of South Europe.

Methods and materials

A field trial to evaluate the proposed rotation scheme was established in Central Greece (Aliartos; 38° 89′ N, 23° 12′ E) in May 201 (Figure 1).

Figure 1:View of rotation field trial

In figure 2 presented the layout with the six rotation schemes (five innovative compared with a conventional one) compared in four blocks. In figure 3 presented the layout per growing period. In the 4th growing period the layout was the same with the 1st and in the 5th was the same with the 2nd.

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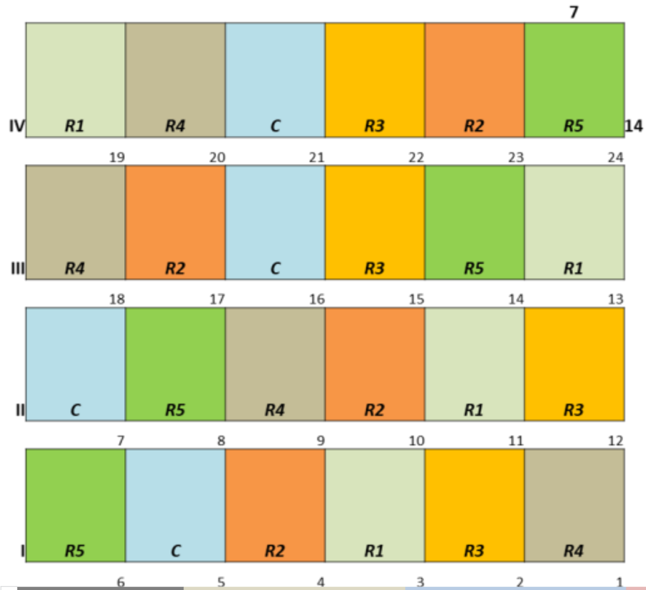
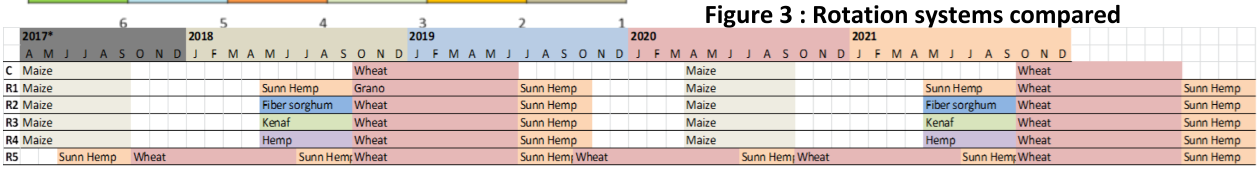


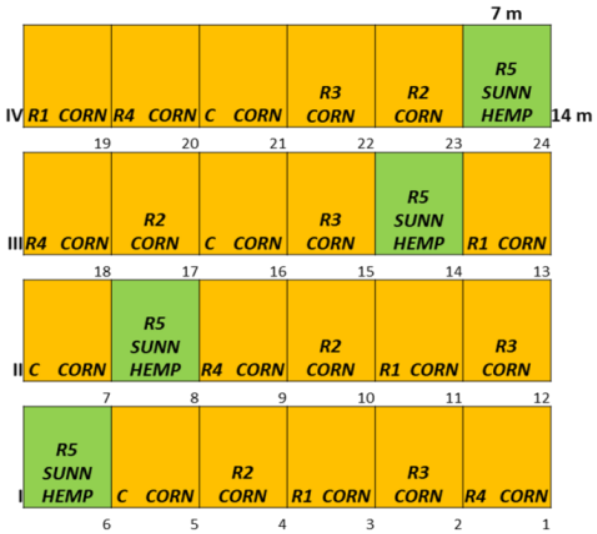
Six rotation systems compared in four blocks

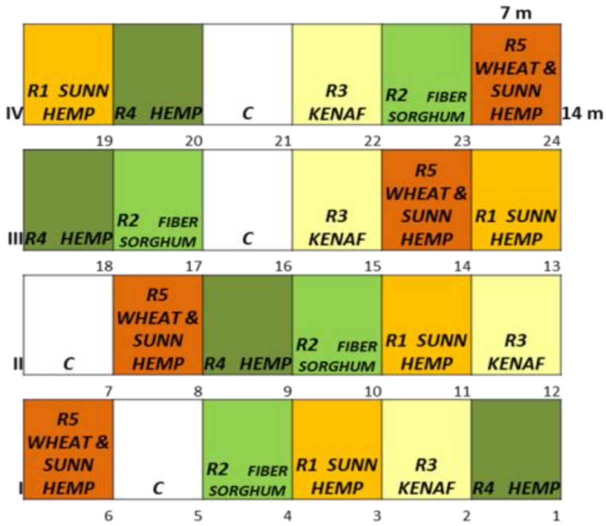
C	Maize /Wheat
R1	Maize /Sunn hemp/ Wheat / Sunn hemp
R2	Maize / Fiber sorghum/ Wheat / Sunn hemp
R3	Maize/kenaf /Wheat/ Sunn hemp
R4	Maize/Hemp /Wheat/ Sunn hemp
R5	Sunn hemp/Wheat

- The size of each plot was 7x14 ($98m^2$).
- Before the establishment a basic fertilization was applied (11-15-15).
- A top N fertilization was applied 30-40

Figure 3 : Rotation systems compared







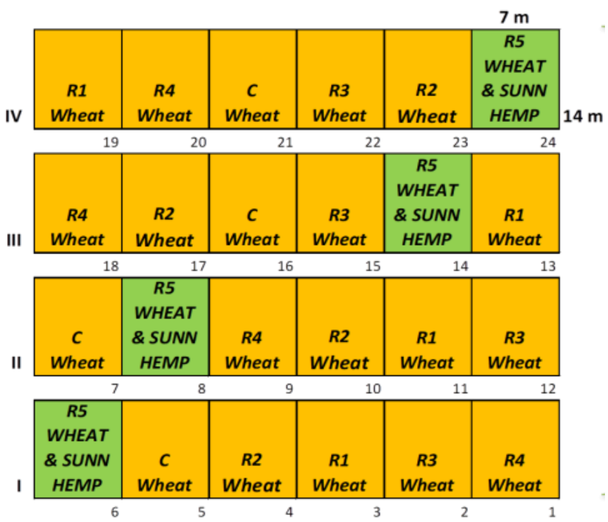


Figure 4 : Experimental layout from the 1st till the 3rd growing period

The experimental per growing period is presented in figure 4 (2017-2019). During the project lifetime time fifth growing periods had been completed. By autumn 2022 the compared rotation schemes would have been complicated twice.

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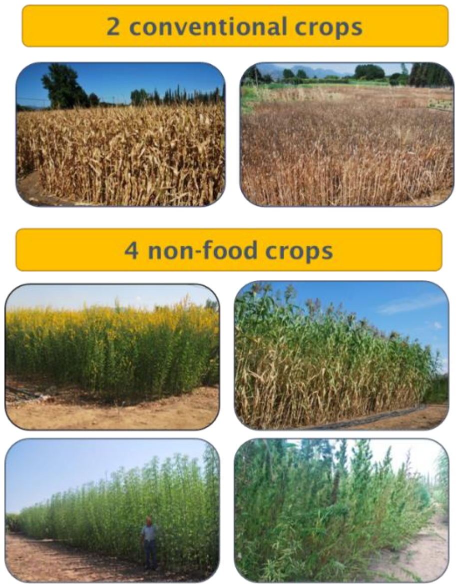
In Becool rotation schemes a total number of six crops have been selected; two conventional (corn and wheat) and 4 non-food crops (sunn hemp, fiber sorghum, kenaf and hemp) (aside graph).



A basic fertilization was applied annually at a rate of 200 kg/ha of fertilizer 11-15-15. In each crop a nitrogen fertilization (34.5-0-0) was applied a month from emergence. Drip irrigation was applied in the field that was also used for the nitrogen fertilization. All activities took place by hand. A common protocol had been agreed in the beginning of the project and it has been followed by CRES with small adjunctions based from the specific climatic data of the trial site. In the table below is presented the sowing dates per crop and year. The rotation trial started in spring 2017 and it is still on going.

Crops and sowing dates	**1^{st} year**	**2^{nd} year**	**3^{rd} year**	**4^{th} year**	**5^{th} year**
Corn	**10/4/17**	**-**	**-**	**15/4/20**	**-**
Wheat	**-**	**19/12/17**	**5/12/18**	**6/12/19**	**8/12/20**
Sunn hemp	**-**	**24/4/18**	**-**	**-**	**28/4/21**
Fiber sorghum	**-**	**10/5/18**	**-**	**-**	**28/4/21**
Kenaf	**-**	**10/5/18**	**-**	**-**	**29/4/21**
Hemp	**-**	**24/4/18**	**-**	**-**	**29/4/21**
Sunn hemp (after wheat)	**20/6/17**	**14/6/18**	**5/7/19**	**30/6/20**	**29/6/21**

The mean meteorological data (Tmax, Tmin, precipitation) for the period of the trial is presented in the figure below. During the hot summer months the precipitation was quite low and was around 20 mm for June and July and 12.5 mm in August. It should be pointed out that the precipitation was quite low In September and October (slighter higher than 20 mm). At the same time the Tmax was higher than 30^{0}C during the summertime.

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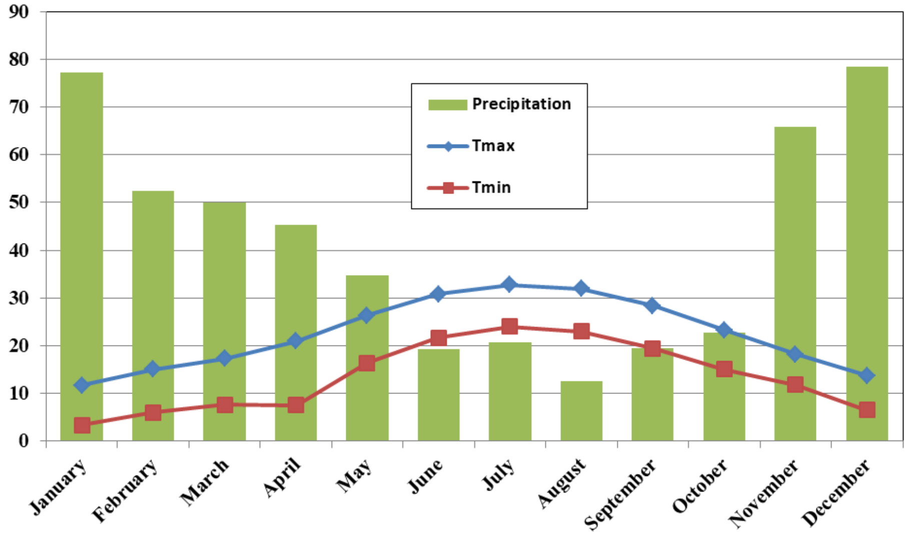

Figure 5: Mean meteorological data (Tmax, Tmin, precipitation) for the period of the trial (2017-2021).

In figure 6 the soil layers in the field trial is presented. In total six soil layers have been detected (Table 1). The soil type was SL and with pH>7. The electrical conductivity was 53.9 mS/m vertical and 34.9 mS/m horizontal.

Table 1: Soil layers

Layers	Soil type	pH	$CaCO_3$
0-18	SL	7.41	14.2
18-35	SL	7.36	15.0
35-50	SL	7.70	17.7
50-70	SCL	7.44	15.0
70-120	SL	7.51	19.6
120+	SL	7.69	20.3

During the trial duration a number of phenological observations and measurements had been collected. At the end of each growing period and crop the measurements carried out where: canopy height, tiller density and stem diameter, while a final harvest was carried out in a marked area and the harvested biomass was weighted and then separated into the plant fractions. Samples for all plant fractions had been taken for

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moisture content determination and biomass characterization analyses. In the figure presented the rotation trial from the 1st till the 3rd growing period.

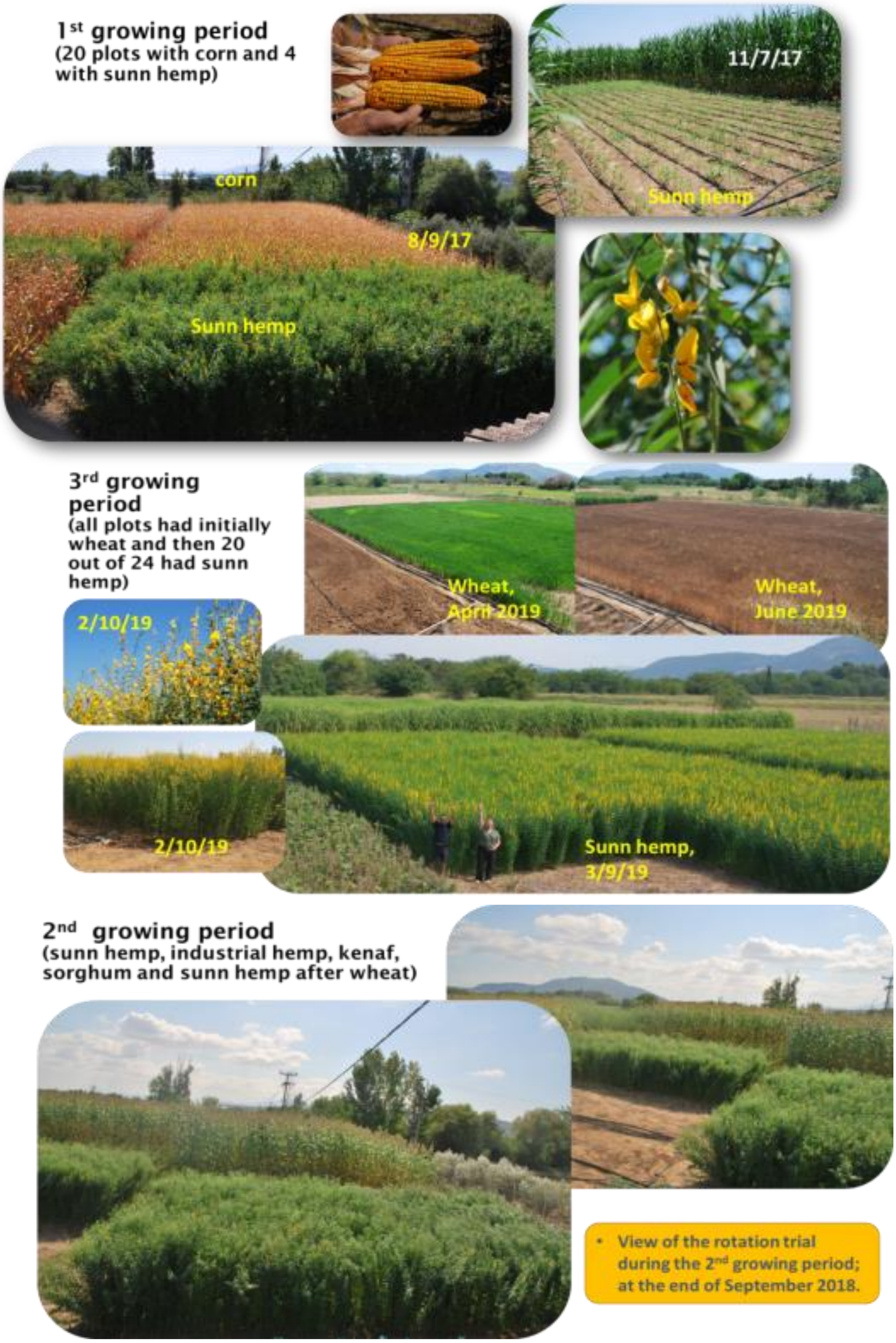

Figure 7 : View of the rotation trial from the 1st till the 3rd growing period.

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Figure 8: View of the trial in the 4th growing period

Results and discussion

Figures 9-11 outlines the yields per crop, rotation type and year.

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Figure 9: Schematic view of all rotation schemes compared; per year and crop yields are being provided.

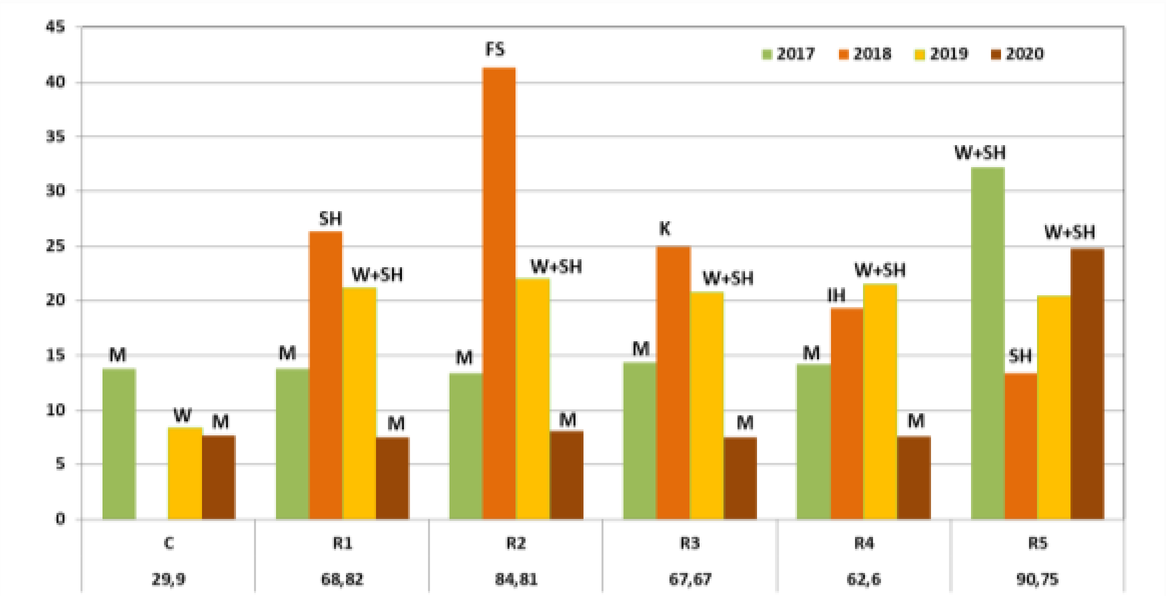

Figure 10: Biomass yields (oven dried, t/ha) per rotation scheme for the period 2017-20.

The highest biomass production was recorded in R5 (90.75 t/ha), followed by R3 (84.82 t/ha), where in the 2nd growing period the crop was biomass sorghum. Then, R1 and R3 gave quite comparable yields; 68.82 t/ha for R1 and 67.67 t/ha for R3. The lowest biomass yields (t/ha) had been recorded by C rotation scheme where maize is being rotated with corn.

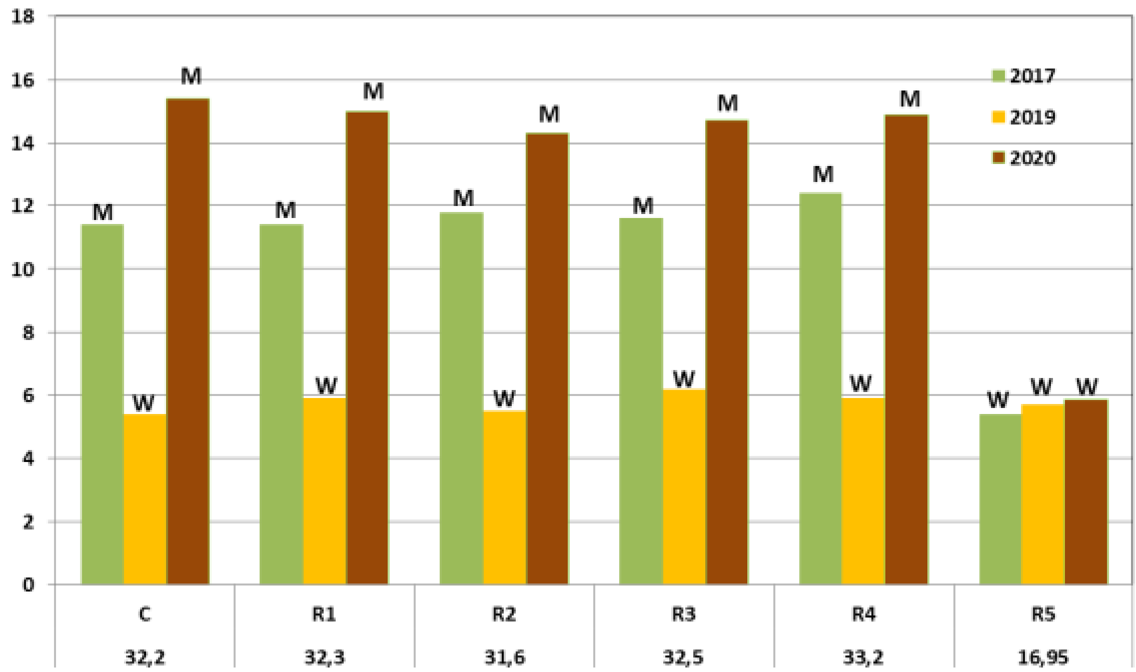

Figure 11: Grain yields (t/ha) per rotation scheme for the period 2017-20.

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In terms of grain production, the highest grain production was recorded in R3 (33.5t/ha), where in the 2nd growing period industrial hemp was cultivated. The lowest grain yields had been recorded by R5 that wheat was rotated with sunn hemp. It should be pointed out that the grain yields among the other five compared rotation schemes were quite close and varied from 31.6 t/ha (R2) to 33.5 t/ha (R3).

Conclusions

The data collected from the rotation trial showed that the biomass and grain yields dint not affected negatively by the insertion of non-food crops on the conventional rotation system that corn had been rotated with wheat. It would be very important for the validation of our results if the trial could be continued in the following years in order two full set data of data for the proposed cropping systems to be completed. In this way we can see if the positive results recorded within the period 2017-2020 will be repeated in the 2nd rotation cycle.

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