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The effects of integrated food and bioenergy cropping systems on crop yields, soil health, and biomass quality: The EU and Brazilian experience

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Abstract

Integrated food and bioenergy production is a promising way to ensure regional/national food and energy security, efficient use of soil resources, and enhanced biodiversity, while contributing to the abatement of CO_2 emissions. The objective of this study was to assess alternative crop rotation schemes as the basis for integrating and enhancing the sustainable biomass production within the food-energy agricultural context. Sunn hemp (*Crotalaria* spp.) in rotation with wheat (*Triticum* spp.) in the EU and with sugarcane (*Saccharum* spp.) in Brazil were evaluated. Sunn hemp did not negatively affect crop's productivity and soil fertility; wheat grain yields were maintained around the mean regional production levels (6, 7, 3 and Mg ha^{-1} in Greece, Italy, and Spain, respectively), and the cumulative biomass in the extended rotation (wheat straw+sunn hemp) was between 1.5 and 2.0 times higher than in the conventional rotation. In Brazil, sugarcane stalks yield in clay soils increased by around 15 Mg ha^{-1} year^{-1} under sunn hemp rotation in comparison with bare fallow. Moreover, sunn hemp in the EU rotations did not have negative effects on soil available macronutrients, organic matter, pH, and cation exchange capacity, neither on C and N stocks in Brazil. The qualitative characteristics (mineral, ash, and hemicelluloses contents) of the cumulated biomass were somehow higher (in average +26%, +35%, and +3.4%, respectively) than in the conventional system. In summary, in temperate and tropical climates the integration of dedicated biomass legume crops within conventional systems could lead to enhanced biomass availability, crop diversification, and efficient use (in space and time) of the land resources.

KEYWORDS
advanced biofuels, biomass, legume crops, lignocellulose, quantitative/qualitative traits, SOC, soil fertility, sugarcane

1 | INTRODUCTION

Worldwide projections indicate that between 22% and 30% of transport sector's needs will be covered by the biofuels industry during 2050–2060 (Brown et al., 2020). In the EU, the revised RED-II (2018/2001 EU Directive) and Green Deal have set ambitious targets for advanced biofuels increase share (3.5%) and greenhouse gas (GHG) emission reductions (50%) by 2030, implying that about 100 Mha will have to be dedicated to the production of biofuel feedstocks (Brown & Le Feuvre, 2017; IEA, 2011). In Brazil, the government proposed in the Paris Agreement a reduction of 43% of GHG emissions by 2030 in comparison with those in 2005 (NDC, 2015). To achieve this target, among other strategies, the Brazilian government has implemented the Brazilian National Biofuels Policy known as "RenovaBio" program to boost the share of sustainable liquid biofuels in its energy mix (Ministry of Mines & Energy, 2017). Therefore, the agricultural sector is called to find new solutions capable of guaranteeing large quantities of lignocellulosic biomass for energy production purposes in a rational and sustainable manner without jeopardizing the main role of agriculture to supply food/feed.

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. Lignocellulosic crop residues (i.e., cereal straw, sugarcane bagasse and straw) are a prompt and easy feedstock source but with several competing end uses and sustainability limitations (Bordonal et al., 2018). Therefore, more efficient use of cropped areas and expansion to lower agricultural value lands to avoid conflict with food markets and indirect land use changes (iLUCs) need to be considered taking into account the reduced restrictions set by the EU renewable energy directive and Brazilian policies to produce lignocellulosic feedstocks in agricultural lands (Baltause, 2016; Cherubin et al., 2021).

Satisfying the forecasted increasing feedstock demands for the production of advanced biofuels imply the sustainable intensification of agricultural systems. For that, it is necessary to evaluate the most adequate agronomic strategies and production potential of alternative crops and cropping systems that would allow to integrate the production of food and bioenergy without land competition issues, while complying at the same time with the enhanced soil nutrient management and crop rotations obligations set by the EU reform of the CAP (EU-Commission, 2018). Therefore, it is reasonable to expect that crop rotations including dedicated legume crops will play a fundamental role. Dedicated legume crops have the potential to increase soil quality either when used as a cover crop or through

their root nodules N_2-fixing capacity (Tenelli et al., 2021). Dedicated lignocellulosic legume crops grown on surplus lands could be co-produced with conventional food and bioenergy crops as a means to increase biomass productivity and availability (without land use change effects). This would allow ensuring feedstock supply and a more efficient use of land in time and/or space. Integrating legume crop production within conventional agricultural systems can bring opportunities for increasing agricultural production and productivity, rural development, agricultural diversification, and climate change mitigation (Dauber & Miyake, 2016). Moreover, such system can extend quantitatively and qualitatively the yearly availability of a mixture of feedstocks and reduce the reliance on a single, usually expensive, biomass feedstock source. The mixture of feedstocks (i.e., cereal straw and dedicated biomass crops) will allow using a combination of the least cost feedstocks to meet the conversion process specifications. It has been demonstrated that several pretreatment processes (i.e., ionic liquid, hydrolysis) can handle a mixture of feedstocks with equivalent conversion potential in comparison with single feedstocks (Ray et al., 2017).

In Brazil, soybean (*Glycine max*), maize (*Zea mays*), and sugarcane (*Saccharum officinarum*) are the most cultivated crops (Bordonal et al., 2018). Sugarcane is semi-perennial crop, and in most of the areas, is cultivated in monocropping system without crop rotations in the renovation period (Otto et al., 2016). While across Europe, monocropping cereals are the predominant cultivated crops with wheat (*Triticum* sp.) being by far the most important one. Moreover, in at least two-thirds of the EU's arable land wheat is cultivated in rotation with maize (SmartSOIL, 2013), with a long fallow period in between (up to 9–10 months). Such fallow period may allow, under certain climatic circumstances, the introduction of fast-growing dedicated crops, which could entail besides increased biomass production per hectare, several environmental benefits in terms of soil cover, enhanced biodiversity, carbon (C) neutrality, and farmers' revenue (Feyereisen et al., 2013; Kemp & Lyutse, 2011; Zegada-Lizarazu & Monti, 2011).

Sunn hemp species (i.e., *Crotalaria juncea* and *Crotalaria spectabilis*) are promising leguminous species of tropical origin and rich in fiber that could be easily adapted to temperate climates as a summer annual crop or integrated in sugarcane rotations in tropical climates. Sunn hemp exhibits a fast growth with a relatively short vegetative growing season and able to produce high biomass yields. Understanding its adaptability and productive and environmental performance within crop rotation schemes is fundamental to build sustainable innovative food-energy cropping systems that would be able to provide locally large quantities of suitable biomass feedstock

and/or contribute to increase the soil quality. Therefore, the objective of this study was to assess extended crop rotation schemes that could be the basis for integrating and enhancing sustainable biomass production within the food-energy agricultural contexts through the evaluation of *Crotalaria sp.* in rotation with wheat in the EU and with sugarcane in Brazil.

2 | MATERIALS AND METHODS

### 2.1 | Experimental design and agronomic management in the EU

Conventional and extended crop rotations were established from 2017 to 2020 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′W, 185 m a.s.l.); and (c) Aliartos, Greece (38°22′N, 23°06′, 114 m a.s.l.). At each location, the extended crop rotation (sunn hemp + wheat – sunn hemp + wheat – sunn hemp + wheat) was established and compared with the conventional rotation (maize + fallow – wheat + fallow – maize + fallow) in terms of biomass and grain yield, soil health characteristics, and biomass (straw + sunn hemp) quality for biofuels production. Meteorological characterization of the three locations was performed according to Monti and Venturi (2007), to compare yearly rainfall distribution patterns during the corresponding growing seasons, that is from April to September, from October to June, and from July to September for maize, wheat, and sunn hemp, respectively. Air temperature was also monitored in all cropping seasons and locations. The three sites are located in the Mediterranean area but with evident differences in terms of mean temperature, rainfall amount and evenness of rainfall distribution (Figure 1). Greece and Spain were warmer than Italy for each of the growing seasons. On the other hand, Italy was the wettest. Moreover, Spain showed the most uneven rainfall distribution during maize growing season, whereas Italy and Greece showed during the sunn hemp–growing seasons. One of the main sources of uncertainty in the prediction of the sustainability of the extended crop rotation systems in the EU is undoubtedly the lack of knowledge about the variability of crop yield across locations and time. Therefore, the cumulative productivity (in qualitative and qualitative terms) was compared to provide real data from which optimized systems and prediction outcomes can be developed.

2.1.1 | Italy

At Cadriano, relatively large plots were set up to allow a complete mechanical management so to emulate near-to-practice solutions at a field scale. The plots were 231 m² each (overall rotation area of 924 m²), with 7 m alleys between them allowing all agricultural implements to turn around without machinery wheel traffic overlaps. The soil was classified as fine silty mixed mesic udic ustochrept (9% sand, 34% clay, and 57% silt) with a neutral pH (6.6–7.4), high exchangeable potassium (~163 mg kg^{-1}), average assimilable N and P$_2$O$_5$ contents of approximately 1.1 g kg^{-1} and 82 mg kg^{-1}, respectively, and 1.2% organic matter content.

(i) *Conventional rotation*: in 2017 and 2020 soil was prepared following the traditional practices of the region (winter ploughing, spring disc harrowing, basal fertilization with 115 kg N ha^{-1}, and rotary harrowing). A FAO class 500 maize (*Zea mays* – cv. Pioneer 1028) was sown (22 March 2017 and 7 April 2020) at a density of 9 seeds m^{-2} with a pneumatic planter alongside mineral P$_2$O$_5$ fertilization (80 kg ha^{-1}). In addition, N broadcasting at a rate of 140 kg ha^{-1} together with a mechanical weed control were done at about 8 weeks after sowing. A total of 55 and 95 mm of sprinkler irrigation was split in July 2017 and 2020, respectively. Maize harvest was done at the end of August. Afterwards the soil was tilled before wintertime (one ploughing and two harrowing), and then left fallow until the next autumn so to follow the conventional agronomical practices of the area. In 2018, plots were tilled with a spading machine and a medium-early winter wheat (*Triticum aestivum*, cv. Starpan) was sown on November 19, 2018, at 200 kg seeds ha^{-1}. Nitrogen (N) fertilization was applied in (i) mid-January (69 kg ha^{-1}), (ii) mid-March (100 kg ha^{-1}), and (iii) beginning April (40 kg ha^{-1}). The harvest was carried out on the 2 July 2019.

(ii) *Extended rotation*: Sunn hemp (*C. juncea*; cv. Ecofix) was planted at a density of 33 plants m^{-2} (0.45 m rows apart) with a pneumatic seed drill right after the harvest of wheat, that is on the 5, 2, and 17 July 2017, 2018, and 2019, respectively. Before sowing minimum tillage was performed with a rotary harrow. In each growing season irrigation was necessary for sunn hemp establishment, even though the variable precipitation distribution (Figure 1) lead to very different amounts required (i.e., 200, 16, and 30 mm for 2017, 2018, and 2019, respectively). Harvest was done at full flowering stage (end of September in each growing season) to maximize the biomass yields as determined by Zegada-Lizarazu et al. (2021). A biomass subsample from an area of 8 m² was then weighted before and after oven drying at 105°C for 72 h. The spring wheat cv. Palesio (a high yielding spring cultivar) was used in the extended rotation so to allow enough time for
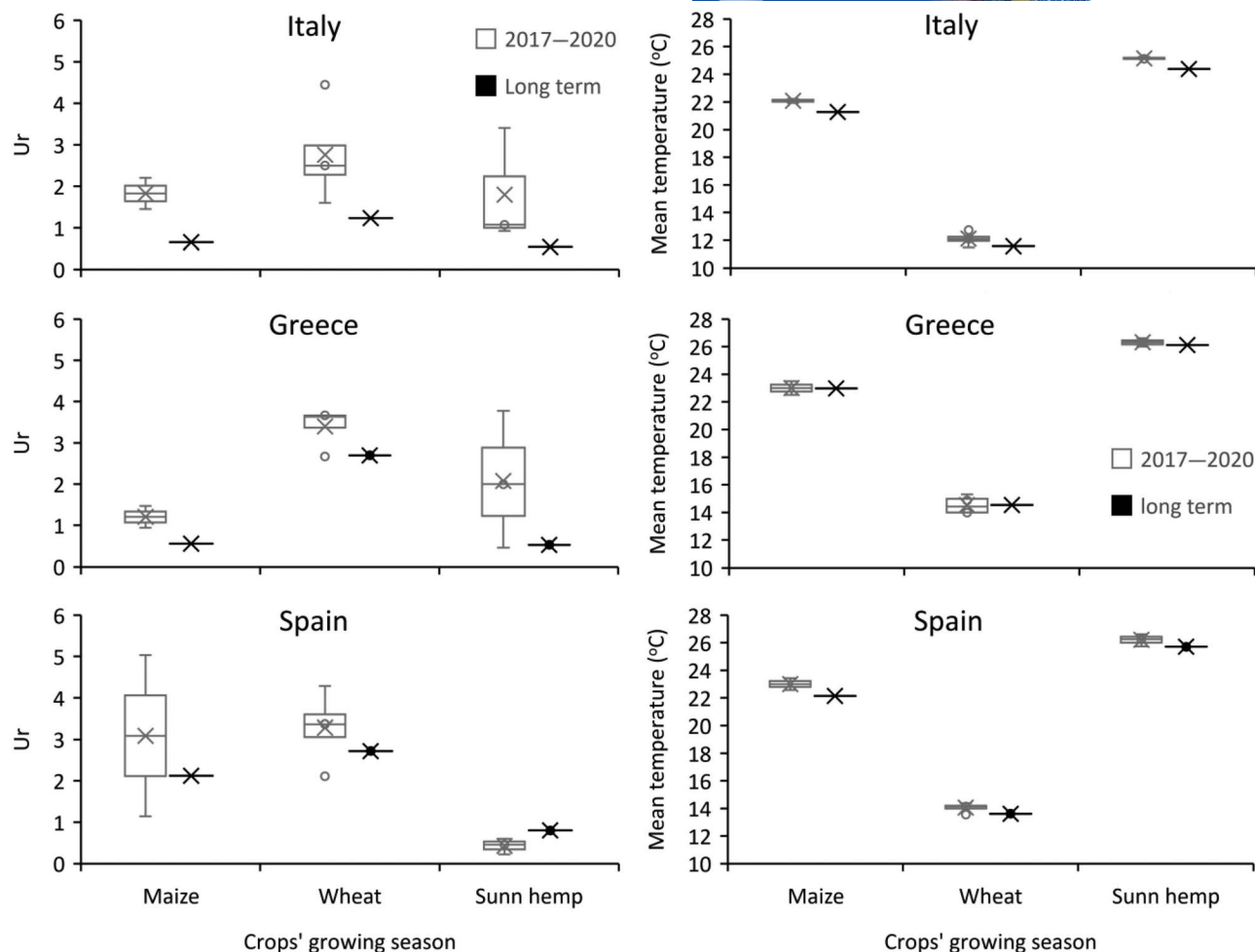
FIGURE 1 Meteorological characterization of the three sites (Greece, Italy, Spain) during the maize, wheat, and sunn hemp growing seasons between 2017 and 2020 in comparison with the long-term period (1999–2020). Ur is the uneven rainfall distribution (the sum of square of the distances of the actual cumulated rainfall points from the respective evenness lines, i.e., daily rainfall). The crops' growing seasons where from April to September, October to June, and July to September for maize, wheat, and sunn hemp, respectively. Boxes represent the proximate 25% and 75% quartiles and maximum and minimum values observed. X indicates the average values, and the solid horizontal line indicates the median

a proper soil preparation for the cereal. Sowing was done at the beginning of January in each growing season. All agronomic management practices (i.e., sowing method, fertilizations, pesticides, and herbicide applications) followed the criteria described above for wheat in the conventional rotation.

2.1.2 | Spain

At Guadajira, the plots were of 120 m^2 with an overall area per rotation of 480 m^2, which also allowed to perform mechanical harvest of crops. As in the Cadriano trial, enough space was provided between plots (~7 m) as well as at the beginning and at the end of the plot to allow the normal traffic of tractors with implements and harvesters without the need to roll over cultivated plots.

The soil in Guadajira was classified as loamy typic haploxeralf (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 54.7 mg kg^{-1}, respectively, and an organic matter content of about 0.8%.

(i) *Conventional rotation*: Maize and wheat cultivation was performed according to traditional management practices of farmers of the region. Maize variety P1570 (FAO 700 cycle) was sown on 19 May 2017 and 24 April 2020, respectively, 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. Harvests were done on 15 October 2017 and 9 October 2020. Wheat variety

Cosaco was established at a dose of 180 kg ha^{-1} on 11 December 2018. Soil was prepared by using a semi-chisel, then base fertilization was performed at a dose of 300 kg of NPK 8-15-15, and finally a single pass of cultivator was performed. An eight-row precision seed drill was used for sowing; then top dressing fertilization was applied at a dose of 300 kg of calcium ammonium nitrate 27% of N, and crop was harvested using an auto-propelled harvester for small assays on 28 June 2019.

(ii) *Extended rotation*: Sunn hemp (*C. juncea*) cultivation followed the same cultivation protocol as the one used in Cadriano, Italy. Sowing doses were calculated every year according to seed germination rate to achieve a final plant density of 33 plants m^2. Sowing was done on the 21 June, 17 July and 11 July 2017, 2018, and 2019, respectively. Soil was prepared by using a semi-chisel, then base fertilization was performed at a dose of 400 kg of NPK 8–15–15 (only the first year), 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 (350–400 mm) was applied during each cropping season. Crop was harvested by using a sickle bar mower on 28 October 2017, 11 October 2018 and 1 October 2019. Biomass yields were determined as in the Cadriano trial. Wheat cultivation followed the same criteria described for the wheat in conventional rotation.

2.1.3 | Greece

At Aliartos each plot was 98 m^2 each. The soil was moderately alkaline (pH 7.9–8.4) with organic matter content of about 1.3%. The soil was sandy loam (79% sand, 18% silt and 3% clay), classified as fluvent redoximorphic.

(i) *Conventional rotation*: In the experimental area, maize and durum wheat are important conventional crops and, thus, the farmers' protocol was applied. The sowing of wheat took place on 5 December 2018. Before sowing, a basic fertilization was applied (11-15-15) at a rate of 300 kg ha^{-1}. Row distance was 13 cm and seeding rate was 250 kg seeds ha^{-1} and N fertilization was applied as urea (46-00-00) at a rate of 200 kg ha^{-1}, when plants where around the tillering stage. The final harvest was done by hand on 1 July 2019 (later than usual). Maize was sown on 10 April 2017 and 2020. The same N amount as in durum wheat was applied following the farmers' fertilization protocol. In both years, drip irrigation was applied with an average amount of 360 mm. The distances between the rows were 70 cm

and within the rows 10 cm. The final harvest took place manually in the second part of September (20 September 2017 and 22 September 2020).

(ii) *Extended rotation*: The sowing of sunn hemp (*C. juncea*) was done manually on 20 June 2017 and 2018, and on 1 July 2019. Only in the first year, a basic fertilization (300 kg ha^{-1} of 11-15-15) was applied and thereafter sunn hemp was immediately sown after wheat without additional fertilization. The distance between the rows were 70 cm and at sowing the target was to have at least 30 plants m^2. The crop had to be irrigated (300 mm in total from sowing till harvest; the rainfalls were included). The final harvest was done in October (27 October 2017, 20 October 2018 and 25 October 2019) and biomass determinations followed the same protocol as in Cadriano but in a smaller subsampling area (4 m^2). Durum wheat cultivation was done using the same protocol as in the conventional rotation.

2.1.4 | Soil measurements

In Italy and Spain, soil core samples were taken at the beginning 2017 and 2019 growing seasons, and at end of the 2018 and 2020 (i.e., when the plots were in fallow) in the top 20 cm of the soil profile in each replication for N and C analysis, so to evaluate their potential changes due to the different crop rotation schemes. The air-dried and ground samples were analyzed following the ISO standards in a CHN combustion analyzer to determine the total N and C contents in four replicates of each crop rotational system. Besides that, in Spain only, pH, electrical conductivity, organic matter, phosphorus, potassium, ammonium, and nitrates, and cations were analyzed.

2.1.5 | Biomass quality measurements

In Italy and Spain, 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 analyzed following the corresponding ISO standards in four replicates to determine the cell wall components, 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 subsample. The concentration of the most important minerals (Ca, K, Na, P) in terms of heat exchange reduction in the combustor connected with slagging and fouling processes were determined through a wet digestion pretreatment carried out in a microwave oven by inductively coupled plasma. 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.

2.2 | Experimental design and agronomic management in Brazil

2.2.1 | Experiment description

In Brazil, three field experiments were set up during the renovation (replanting) of sugarcane crop to evaluate the effects of crop rotation with sunn hemp on soil C and N stocks and biomass yield of subsequent sugarcane crop. The experiments were located in commercial sugarcane areas in the municipalities of Quatá (22°14′S, 50°42′W, 540 m a.s.l.), Quirinópolis (18°32′S, 50°26′W, 500 m a.s.l.), and Chapadão do Céu (18°25′S, 42°33′W, 850 m a.s.l.). The soils in these three sites are classified according to Soil Taxonomy as Arenic Kandiudult, Rhodic Eutrudox, and Rhodic Hapludox, and these three areas will, henceforth, be called sandy soil, clay soil 1 and clay soil 2, respectively. More information regarding chemical and physical characterization of these experimental areas can be found in Barbosa et al. (2018) and Tenelli et al. (2019).

Before experiment establishment, previous sugarcane ratoon was desiccated with herbicides, and 2 Mg ha^{-1} of dolomitic lime and 1 Mg ha^{-1} of gypsum were applied in each site. The experiments were initiated in December 2012 with the establishment of the following treatments: (i) cover crop—the plots were planted with sunn hemp (*C. spectabilis*) at rate of 25 kg seed ha^{-1} and (ii) bare fallow—the plots were kept under bare fallow condition. The experimental design was randomized block with four repetitions. It is important to mention that no fertilizers or irrigation were applied during sunn hemp growing season. In March 2013, herbicides were applied to desiccate the cover crop at flowering stage and control weed infestations in the bare fallow treatment. The quantification of sunn hemp biomass was performed by manual harvesting from two central rows of 10 m long. After weighing, an aliquot of biomass was removed to estimate moisture, and the remaining material was spread evenly on the soil surface. It is important to mention that sunn hemp biomass was not harvested for commercial purposes and was maintained in the field as cover crop. The dry biomass yield and C and N content of sunn hemp are presented in Table 1.

After 15 days of sunn hemp desiccation, sugarcane crop (cv. RB96-6928) was planted using a two-row planter with spacing of 1.5-m. Each plot comprised five sugarcane rows with 10-m in length spaced at 1.5-m. The same quantity of fertilizers (in plant cane: 40 kg N ha^{-1}, 125 kg P$_2$O$_5$ ha^{-1},

TABLE 1 Aboveground biomass (in dry basis), carbon and nitrogen content of sunn hemp biomass

Sites	Dry biomass (Mg ha^{-1})	Carbon content (Mg ha^{-1})	Nitrogen content (Mg ha^{-1})
Sandy soil	6.5	3.1	0.17
Clay soil 1	8.1	3.8	0.19
Clay soil 2	8.3	3.9	0.12

Note: Data represent the average of four replicates.

and 125 kg K$_2$O ha^{-1} and ratoons: 120 kg N ha^{-1} and 150 kg K$_2$O ha^{-1}) were applied in both treatments in the three sites. The experimental activities occurred in the period of December 2012 until July 2018 (Figure 2). During the experimental period, applications of fungicides, insecticides and herbicides for sugarcane crop were uniform in all plots and conducted according to the management strategies established by each mill.

2.2.2 | Soil measurements

Soil samples to evaluate the effects of crop rotation with sunn hemp on soil C and N stocks were collected in July 2018 at 0–5, 5–10, 10–20, and 20–40 cm layers. The samples were air-drying at 35°C for 7 days, and after that one portion of the sample (~10 g) was ground and sieved through a 0.150-mm for determination of total soil N and C concentration by dry combustion using the C Analyzer LECO CN628. Undisturbed soil samples were also collected from each corresponding layer using volumetric rings (diameter: 0.05-m; height: 0.05-m) for analysis of bulk density to calculate soil C and N stocks. Because soil samples were collected from fixed layers, the C stock was adjusted for changes in bulk density that occurred after soil management. For this, C stocks were adjusted to an equivalent soil mass using as reference the comparison with the baseline characterization of each experimental site according to methodology of Ellert and Bettany (1995).

2.2.3 | Biomass measurements

The sugarcane stalk yield was measured after approximately 16 months after planting (in plant-cane cycle) and 12 months in successive ratoon cycles. At the harvest period, each plot was harvested by means of a mechanical harvester and stalk yields (expressed in Mg ha^{-1}) were computed for the three central rows through an instrumented truck equipped with a loading cell (used exclusively for experiments).
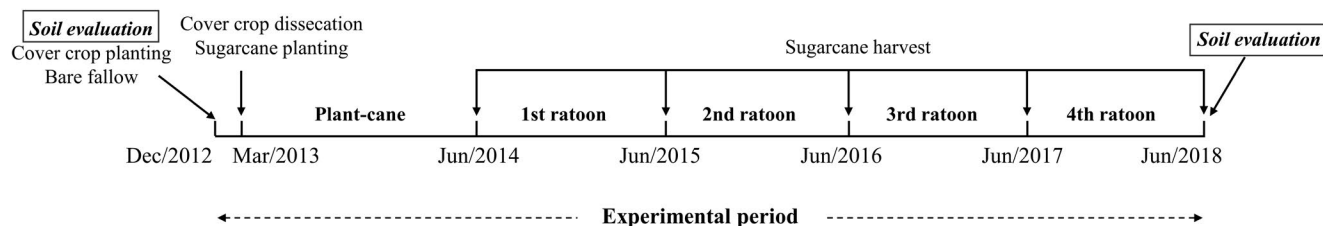
FIGURE 2 Timeline highlighting the main management practices and experimental period

2.3 | Statistical analysis

In all trials, all parameters were subjected to analysis of variance (ANOVA), and when significant differences ($p < 0.05$) were detected, Tukey tests for comparison of means were performed. Homoscedasticity of data was checked by the Bartlett's test prior the ANOVA tests. The biomass quality parameters were evaluated using a two-way ANOVA. As for the other production parameters, a one-way ANOVA was used at each crop rotation scheme. Soil parameters were evaluated by one-way ANOVA and by descriptive statistics.

3 | RESULTS

3.1 | EU rotations

3.1.1 | Extended crop rotation effects on biomass and grain productivity

Figure 3 shows the cumulative (2017–2020) aboveground biomass (vegetative components only) achieved in the
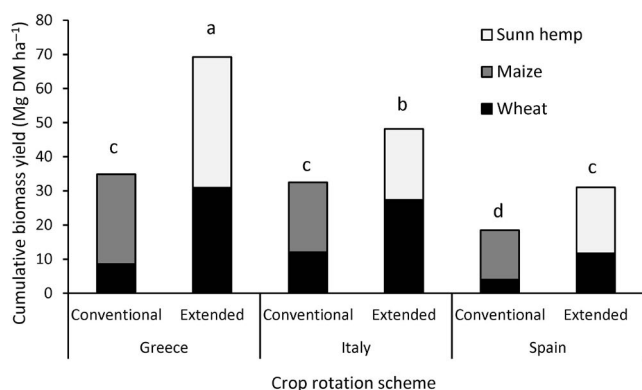

FIGURE 3 Cumulative aboveground biomass yield (Mg DM ha^{-1}; vegetative components only) of maize, wheat, and sunn hemp grown between 2017 and 2020 under conventional and extended crop rotation systems at three locations (Greece, Italy, Spain). The conventional rotation was maize–fallow–wheat–fallow–maize. The extended rotation was: sunn hemp–wheat–sunn hemp–wheat–sunn hemp–wheat. Different letters indicate significant difference between crop rotation schemes and locations
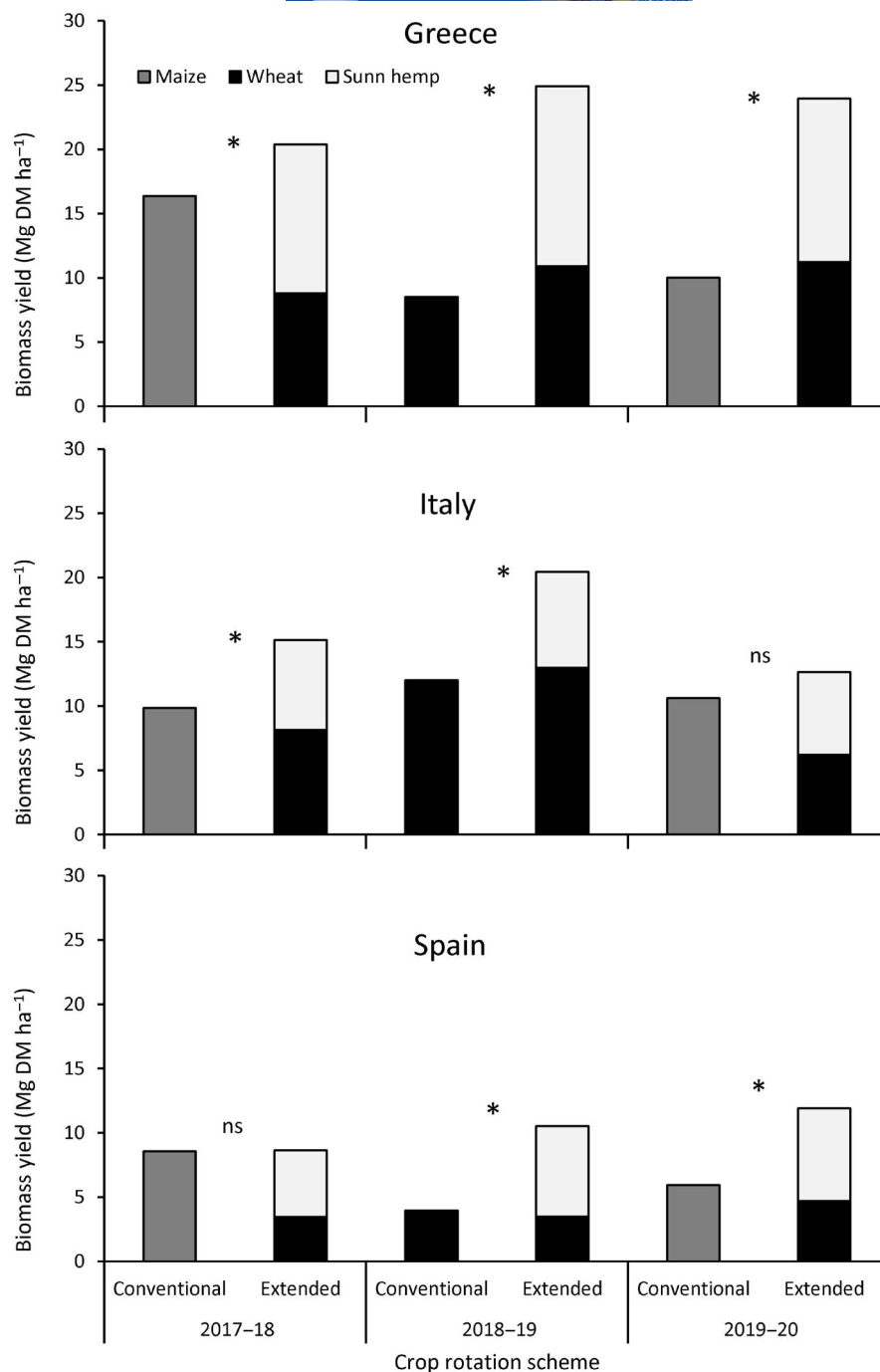
conventional and extended crop rotation systems at three southern EU locations. In general terms, cumulative biomass in the extended rotation was between 1.5 and 2.0 times higher than in the conventional rotation. The largest cumulative biomass was found in Greece and the lowest in Spain. In all cases, the contribution of sunn hemp to the total biomass was significant, accounting for 43%, 55%, and 62% of the total in Italy (48 Mg ha^{-1}), Greece (69 Mg ha^{-1}), and Spain (31 Mg ha^{-1}), respectively. In fact, Figure 4 (vegetative components only) shows in detail the large contribution of sunn hemp per growing season in all three locations, whereas the straw produced by wheat under the extended crop rotation system was maintained within the ranges produced in the conventional rotation, except for the 2019–2020 growing cycle in Italy where a slight reduction was evident. The average wheat straw production in the conventional rotation systems was 12.0, 8.5, and 4.0 Mg ha^{-1} in Italy, Greece, and Spain, respectively. In the extended rotation the average wheat straw production was 9.1, 10.3, and 3.9 Mg ha^{-1} in Italy, Greece, and Spain, respectively.

Figure 5 depicts the grain production of the cereal crops in the corresponding rotational systems. As for maize (conventional rotation), its yields were within the normal ranges at the corresponding locations and growing seasons. The same was for wheat, which in the conventional rotation yielded 2.5, 5.5, and 6.9 Mg ha^{-1} of grain in Spain, Greece, and Italy, respectively. Under the extended rotation, the mean grain yields averaged over the three growing cycles was in line with the yields obtained in the conventional system (i.e., 2.9, 5.6, and 6.4 Mg ha^{-1} of grain in Spain, Greece, and Italy, respectively), suggesting that the introduction of sunn hemp into the system do not have any negative effect on wheat grain yields.

3.1.2 | Extended crop rotation effects on soil fertility

Figure 6 shows the total C and N content in both rotational systems in Italy and Spain. At both locations, no significant differences were found between the conventional and extended crop rotations. The higher content in the Italian rotations could be due to the higher organic matter content in the soils compared with the Spanish soils (1.2

FIGURE 4 Aboveground biomass yield (Mg DM ha^{-1}; vegetative components only) of maize, wheat, and sunn hemp grown under conventional and extended crop rotation systems at three locations (Greece, Italy, Spain). The conventional rotation was as follows: maize–fallow–wheat–fallow–maize. The extended rotation was as follows: sunn hemp–wheat–sunn hemp–wheat–sunn hemp–wheat. * indicate significant differences between crop rotation schemes

vs. 0.7, in Italy and Spain, respectively). As for the N content, the analysis showed medium-low content regardless the rotational systems in either location.

A more detailed analysis of the effect of the extended crop rotation system on the main soil fertility indicators was carried out in Spain. Figure 7a shows the box plots for macronutrient content in the soil. In general terms, N, P, and K contents in both rotational systems were the similar, although the variability was larger in the extended system, suggesting that the legume crop and fertilization management of the plots may have contributed to build up soil fertility in the extended crop rotation. In fact, the cation exchange

capacity (especially Ca and Mg; Figure 7c) and organic matter (Figure 7b) were more variable in the extended rotation than in the conventional one; while the pH (Figure 7d) was keep stable around neutrality, helping to maintain the soil fertility at the level of the conventional rotation.

3.1.3 | Extended crop rotation effects on feedstock (biomass) quality

Table 2 shows the cumulative cell wall components and N, C, and ash contents of the conventional and extended
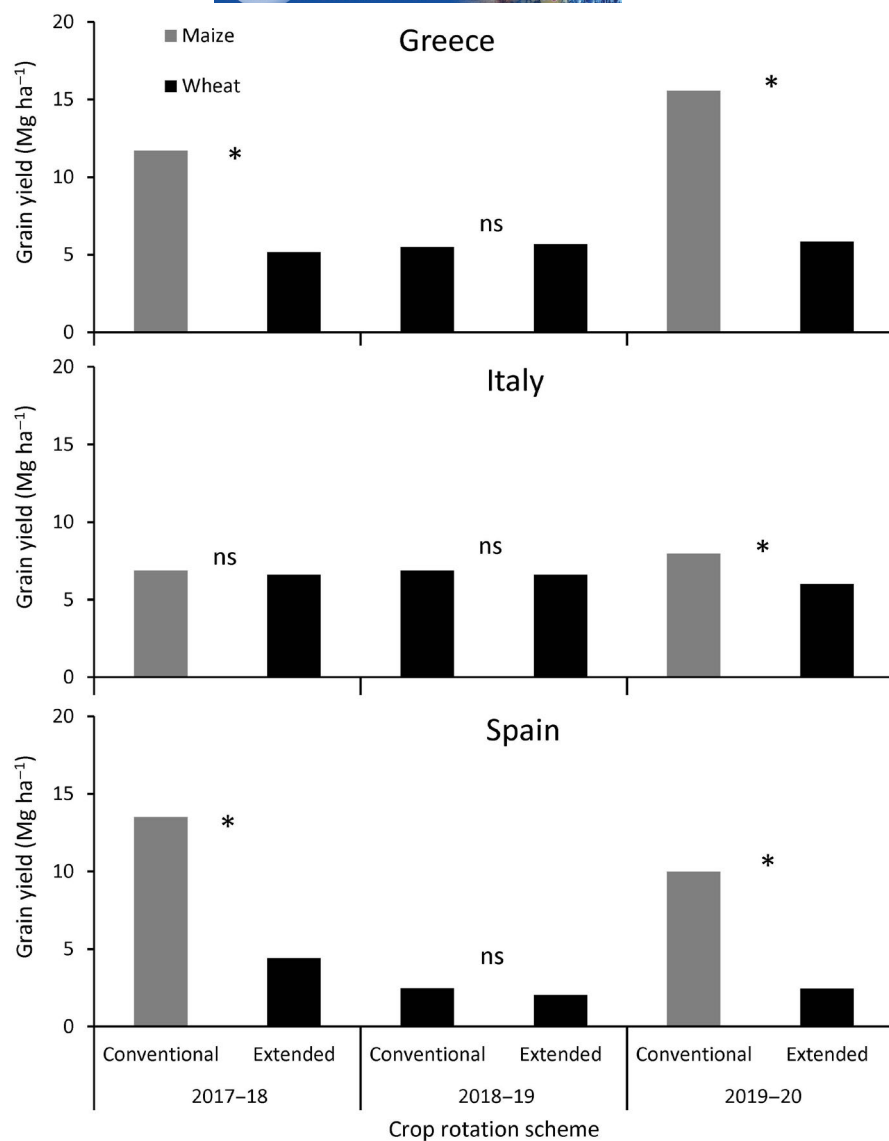
FIGURE 5 Grain yield (Mg ha^{-1}) of maize and wheat grown under conventional and extended crop rotation systems at three locations (Greece, Italy, Spain). The conventional rotation was as follows: maize–fallow–wheat–fallow–maize. The extended rotation was as follows: sunn hemp–wheat–sunn hemp–wheat–sunn hemp–wheat. * indicate significant differences between crop rotation schemes

crop rotations in Italy and Spain. Some of the differences described below are intrinsic to the species included in the rotational systems. For example, the hemicellulose content was between 11% and 15% lower in the extended crop rotation than in the conventional one, while the N content was 1.3 and 4.6 times higher in the extended crop rotations in Italy and Spain, respectively. These findings are most probably because the legume crop represented a large proportion the cumulative biomass. Ash content also increased in close relation with the large amounts of cumulative biomass in the extended rotations in either location. Cellulose changes were evident in Italy only, with a 39% increase in the extended rotations. Although in Spain only, the C content was lowest in the conventional rotation.

The mineral concentration (Table 3) was significantly higher in the extended rotations than in the conventional rotation, with the exception of Na and Si in Italy and K in

Spain. In Italy, the contents of P, Ca, K, and the ratio of Ca/K increased by 1.3, 2.6, 1.5, and 1.8 times, respectively, while the Na content was similar in both rotational systems, and the Si and Si/K ratio decrease in the extended rotation. In Spain, the contents of Na, P, Ca, and Ca/K increased by 4.7, 4.9, 2.6, 3.9 times, respectively, while K was reduced.

3.2 | Brazilian rotations

3.2.1 | Inclusion of sunn hemp in sugarcane rotation system: Effects on soil C and N stocks

Table 4 shows the soil C and N stocks in sugarcane areas under sunn hemp and bare fallow rotation systems in Brazil. The cultivation of sunn hemp during sugarcane replanting period did not significantly affect soil C stocks
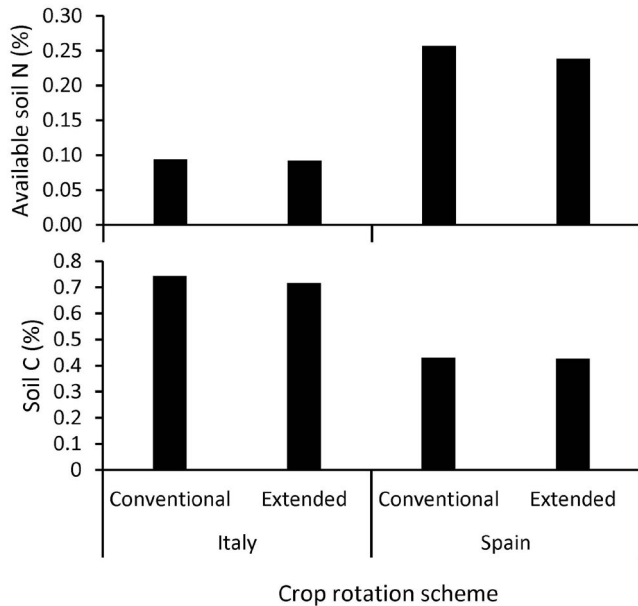
FIGURE 6 Soil C and available N changes due to different crop rotation schemes in Italy and Spain. The conventional rotation was maize–fallow–wheat–fallow–maize. The extended rotation was sunn hemp–wheat–sunn hemp–wheat–sunn hemp–wheat. No significant differences were found between the crop rotation schemes in both locations

compared with bare fallow, but increased N stock in the 0–5 cm depth at all soil types, and in 0–40 cm in sandy soil. Considering the average of the three soil types, the N stocks tended to be higher under sunn hemp rotation compared with bare fallow.

### 3.2.2 | Inclusion of sunn hemp in sugarcane rotation system: Effects on sugarcane yield

Sugarcane stalk yield were much higher in the clay soils than in sandy soil over the crop cycles, which ranged from 28 to 91 Mg ha^{-1} in sandy soil, 71 to 168 Mg ha^{-1} in clay soil 1 and 119 to 157 Mg ha^{-1} in clay soil 2 (Figure 8). Overall, sugarcane yield declined with aging at all soil types. In sandy soil, there was positive response of sugarcane yield to sunn hemp rotation in the plant-cane and third ratoon cycle relative to bare fallow system. In clay soil 1, higher yields under sunn hemp treatment were observed in plant-cane, first and third ratoon cycles. In clay soil 2, sunn hemp increased sugarcane yield only in plant-cane cycle. Considering the average yield along the experimental period, the adoption of crop rotation with sunn hemp significantly increased sugarcane yield by 11% (15 Mg ha^{-1}) in clay soil 1 and 9% (14 Mg ha^{-1}) in clay soil 2 compared with bare fallow, while no significant difference was observed in sandy soil.

4 | DISCUSSION

4.1 | Crop rotation effects on biomass and grain productivity

The integration of food and bioenergy crops production is a promising way to ensure regional/national food and energy security, as well as to enhance biodiversity and

FIGURE 7 Variability of (a) macronutrients (N, P, K); (b) organic matter; (c) exchangeable Ca, Mg, and Na; and (d) pH in maize, wheat, and sunn hemp grown under conventional and extended crop rotation systems in Spain during the 2019–2020 growing season. The conventional rotation was maize–fallow–wheat–fallow–maize. The extended rotation was sunn hemp–wheat–sunn hemp–wheat–sunn hemp–wheat. Boxes represent the proximate 25% and 75% quartiles and maximum and minimum values observed. X indicates the average values, and the solid horizontal line indicates the median
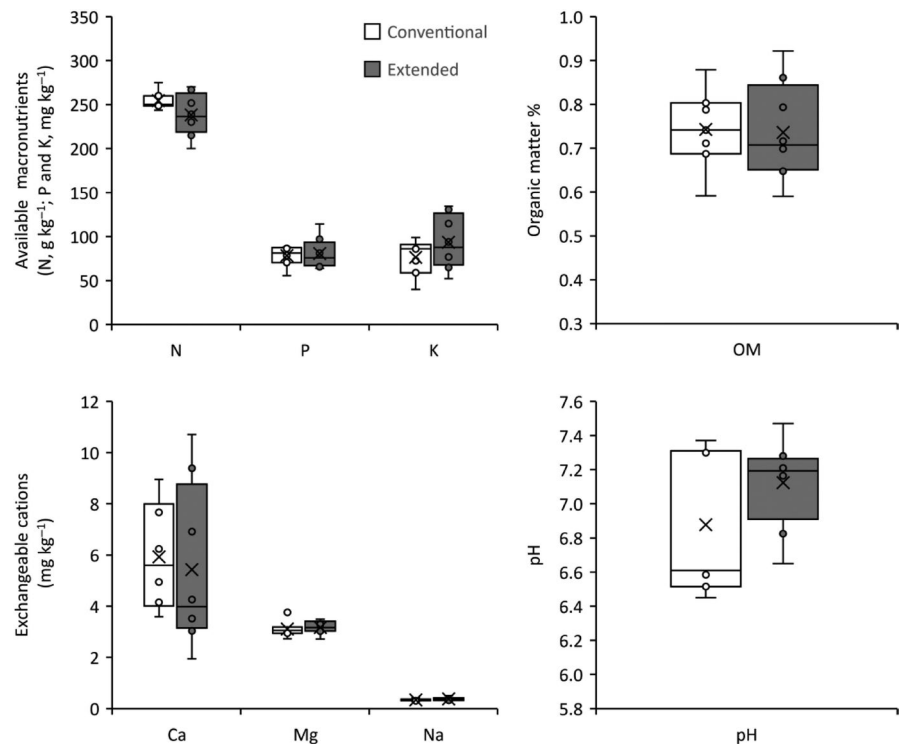
	Italy		Spain	
	Conventional (%)	Extended (%)	Conventional (%)	Extended (%)
Hemicellulose	24.2 a	21.6 b	23.5 a	20.0 c
Cellulose	26.8 c	37.2 a	32.0 b	32.3 b
Ash	5.0 d	7.8 a	5.7 c	6.6 b
N	0.9 c	1.2 b	0.3 d	1.4 a
C	46.0 a	44.6 a	42.8 b	44.8 a

TABLE 2 Cumulative cell wall components, ash content, and total N and C concentration under conventional and extended crop rotation schemes in two Mediterranean countries

Note: Different letters within a row indicate statistically significant differences between cropping systems and locations. Conventional rotation: maize–fallow–wheat–fallow–maize. Extended rotation: sunn hemp–wheat–sunn hemp–wheat–sunn hemp–wheat.

	Italy		Spain	
	Conventional (%)	Extended (%)	Conventional (%)	Extended (%)
Na	0.02 b	0.02 b	0.01 c	0.05 a
P	0.11 c	0.14 b	0.04 d	0.22 a
Ca	0.25 d	0.66 b	0.43 c	1.14 a
K	1.01 b	1.51 a	1.42 a	0.97 b
Si	0.04 a	0.03 b	—	—
Si/K	0.04 a	0.02 b	—	—
Ca/K	0.25 c	0.44 b	0.30 c	1.17 a

TABLE 3 Cumulated mineral concentration under conventional and extended crop rotation schemes in two Mediterranean countries

Note: Different letters within a row indicate statistically significant differences between cropping systems and locations. Conventional rotation: maize–fallow–wheat–fallow–maize. Extended rotation: sunn hemp–wheat–sunn hemp–wheat–sunn hemp–wheat.

contribute to the abatement of CO_2 emissions. Farming systems that combine the food and bioenergy crop species besides a more efficient use of soil resources, allow an integral use of biomasses for alternative purposes (i.e., straw residues + dedicated lignocellulosic feedstocks to produce bioenergy), and therefore additional incomes to the farmers (Bogdanski et al., 2011; Bordonal et al., 2018). In the present study, it was evidenced that the introduction of sunn hemp in rotation systems did not have a negative impact on the wheat yield (Figures 3–6) in the EU while increased sugarcane stalk yield in Brazil (Figure 8).

Wheat grain yields in the three studied regions in the EU were maintained at the same level in both rotation systems (Figure 5), suggesting that sunn hemp through its N_2 fixing capacity may have contributed to maintain the soil fertility and/or health and, therefore, productivity. In fact, it is well known that alternating crops with contrasting characteristics (i.e., cereals, bioenergy and legumes) is an important strategy for the successful implementation of crop rotations and maintaining (increasing) productivity and soil resilience (Zegada-Lizarazu & Monti, 2011). Moreover, besides the N_2 fixation by the legume crop, the increased/sustained cereal yields could be ascribed to the complex effects that crop sequences have on soil

physical-chemical and biological properties (Kaye et al., 2007; Tenelli et al., 2021; Zegada-Lizarazu & Monti, 2011).

The yearly wheat straw production was also similar in both rotational systems at each study location, except for the 2019–2020 season in Italy, in which the straw yield was somehow lower in the extended rotation than in the conventional one (Figure 4). This lower yield could be related to the uneven rainfall distribution, especially at early vegetative growing stages with extreme low values in January and February (Figure 1). Guo et al. (2012) found that wheat yields are highly influenced by autumn precipitation, but not by the total amount received during the entire growing season. Moreover, compared with Spain and Greece, Italy showed the largest uneven rainfall distribution (Figure 1). Apart from this, the mean biomass production levels were within the normal ranges of each location. It is important to note that the additional biomass contributed by the dedicated leguminous crop in the extended rotational system was substantial. In general, the cumulative biomass yields (2017–2020) were higher in the extended rotation than in the conventional one (Figure 3), and in line with the yearly productivity trends (Figure 4). From the farmer's point of view, this could be an important source of additional income. From

TABLE 4 Soil C and N stocks (Mg ha^{-1}) under sunn hemp rotation and bare fallow systems in the three locations in Brazil

| Soil depth (cm)	Soil C stocks (Mg ha^{-1})		Soil N stocks (Mg ha^{-1})	
	Sunn hemp	Bare fallow	Sunn hemp	Bare fallow
	Sandy soil			
0–5	6.9	6.5	0.5 a	0.4 b
5–10	3.7	4.0	0.3	0.2
10–20	5.1	4.0	0.6 a	0.3 b
20–40	7.4	7.5	0.7	0.6
0–40	23.1	21.9	2.1 a	1.6 b
	Clay soil 1			
0–5	20.1	19.1	1.8 a	1.2 b
5–10	16.7	16.0	1.4	1.1
10–20	26.9	26.8	2.1	2.0
20–40	38.5	40.0	2.9	2.7
0–40	102.2	101.9	8.2	7.1
	Clay soil 2			
0–5	18.2	16.8	1.1 a	0.9 b
5–10	14.7	14.8	0.8	0.8
10–20	24.8 b	27.3 a	1.2	1.3
20–40	41.4	42.9	1.8 b	2.0 a
0–40	99.2	101.8	4.9	5.0

Note: Data represent the average of four replicates. Means followed by same letter do not indicate significant difference by Tukey test ($p < 0.05$).

a basic economic balance analysis taking into account an average price of 55 and 245 € Mg^{-1} for the biomass (straw and sunn hemp biomass) and the cereals grains, respectively, and considering a mean cumulative productivity across locations of 29 and 50 Mg ha^{-1} of biomass and 27 and 15 Mg ha^{-1} of cereal grains for the conventional and extended rotations, respectively (after deducting the corresponding production cost), the revenues were 1.5 times higher in the extended rotation than in the conventional

one (2469 vs. 3681 € ha^{-1}, respectively). It is worth noting that all these crops in the three locations were grown using customary standard cultivation techniques (i.e., planting density, fertilization, supplemental irrigation); therefore, the higher cumulative yields in Greece, due to the better performance of sunn hemp could be due to the closer proximity to the latitude of origin of the species compared with Italy and Spain. Moreover, in Spain sum hemp was affected by pests attack (Agriotes spp. and Spodoptera spp.) at early growing stages resulting in lower yields in 2017–2018 growing season, although the low straw productivity of wheat was in line with the regional averages determined by the typical precipitation rates of the region.

In Brazil, the cultivation of sunn hemp in sugarcane areas occurs only in a short period (four months in each 5-year period) in the replanting period. Sugarcane is a semi-perennial crop that is harvested on annual cycle and cultivated in a monoculture system for around 5 years. In the present study, the adoption of crop rotation with sunn hemp significantly increased the average annual sugarcane yield by 15 and 14 Mg ha^{-1} for clay soil 1 and clay soil 2, respectively (Figure 8). These increases in sugarcane yield along the crop cycles (ranging from 9% to 11%) in clay soils are consistent with those reported previously for other rotation experiments, which the sugarcane yield gains varied from 15% to 25% over 4 years in Australia (Garside et al., 2002) and up to 30% over 5 years after cultivation with *C. junceae* in Brazil (Ambrosano et al., 2011). It is important to highlight that the cultivation of sunn hemp also increases sugarcane straw yields (data not shown) that can be used as feedstock for bioenergy production. The sugarcane yield gains promoted by sunn hemp are associated, as indicated before, with atmospheric N$_2$ fixation from the symbiotic association with bacteria (rhizobia) and, non-related N effects that are associated to the improvement of production environment for subsequent sugarcane cultivation (Ambrosano et al., 2011; Garside & Bell, 2001). In a
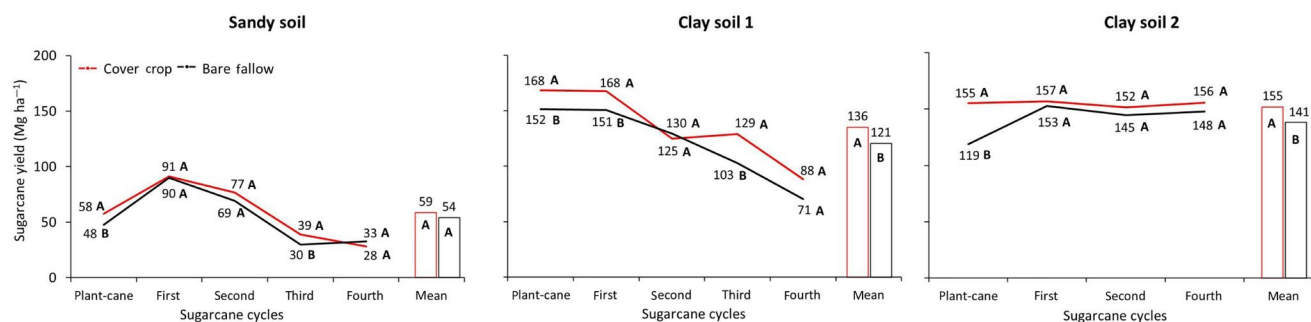
FIGURE 8 Sugarcane yield (Mg ha^{-1}) at each crop cycle under cover crop with sunn hemp (red color) and bare fallow (black color) at the three locations in Brazil. The mean values represent the average stalk yield over the crop cycles. Means followed by same letter do not indicate significant difference by Tukey test ($p < 0.10$). The sugarcane yield data for third ratoon in clay soil 2 is not available because the area was accidentally harvested by sugarcane mill

recent study, Tenelli et al. (2021) isolated the N and non-N effects of sunn hemp on sugarcane yields and observed that most of the yield gains were related to non-related N effects. These non-N benefits associated sunn hemp cultivation include the less incidence of weed/pathogens, and improvement of soil quality (Bordonal et al., 2018; Fernandes et al., 2012; Perin et al., 2004). In addition to the potential of increasing sugarcane yields, Otto et al. (2020) found that sunn hemp reduced the need for N fertilization in sugarcane crop. Similarly, in Australian conditions Park et al. (2010) observed that adoption of legumes in replanting period could substantially reduce the need for N fertilization along the crop cycle. Using life-cycle assessment approach, Chagas et al. (2016) observed that adoption of rotation with sunn hemp reduced GHG emissions and improves the sustainability of sugarcane biomass. It follows that comprehensive real field yield data, as the one presented here for the EU and Brazil, is of outmost importance for providing key information to farmers and entrepreneurs willing to put forward cost-effective plans, such as dimensioned and tailor-designed processing plants.

4.2 | Extended crop rotation effects on soil fertility

This study was focused on the hypothesis that the inclusion of sunn hemp in rotation systems do not have negative effects on the soil fertility and health in temperate and tropical climates. In the EU, the total soil N and C were maintained at similar levels when comparing both rotational systems either in Italy and Spain (Figure 6). 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 system. Important to mention, however, that in the extended rotation the soil was able to support six-crop growth cycles compared with the three cycles in the conventional rotation intermingled with long fallow periods. The contribution of sunn hemp to sustain the N and C content in the soil may have played an important role on the soil resilience and health. It has been shown that several sunn hemp species can fix between 60 and 221 kg ha^{-1} of N (Ambrosano et al., 2011; Mansoer et al., 1997; Samba et al., 2002; Schomberg et al., 2007); thus, the species could be used as a natural source of N for the subsequent crops and the maintenance of the soil fertility. In fact, the beneficial effect of fixed N_2 on soils and subsequent crops is a well-known phenomenon already observed in many legume–grass mixtures (Ambrosano et al., 2011; Chapagain & Riseman, 2014, 2015; Martin-Guay et al., 2018; Thilakarathna et al., 2012).

The Spanish soils are loamy soils but with low organic matter content and low cation exchange capacity (CEC; Figure 7). Even in the case of the extended rotations, where the integrated legume species could add organic matter and N to the soils, the available macronutrients and the CEC (fundamental for sustaining the soil fertility) were kept at the same level than in the conventional rotation (Figure 7). These results confirm the fact that improving the CEC and therefore the soil fertility, requires years if not decades (Chowdhury et al., 2021). In fact, an 11-year maize-winter wheat-summer soybean rotation study found an increase in total N after the fifth year onward (Li et al., 2018), and other study suggests that a CEC value above 10 cmol kg^{-1} soil is ideal for optimal plant growth (Chowdhury et al., 2021). In our case, the observed unaffected values of macronutrients availability and CEC can be due to the shorter duration of the trial (2017–2020). Moreover, the presumable larger root biomass accumulation by the different species included in the repeated growth cycles may have somehow contributed to the steady soil fertility, regardless the fact that all the aboveground biomass was removed for bioenergy production purposes. Other factors that may have influenced the steady macronutrients content and CEC would be the soil properties, the low organic matter content, and climatic conditions (Figures 1 and 7). In this study, the pH did not significantly change, which is in line with the soil capacity to maintain exchangeable cations. The pH was 6.9 and 7.1 in the conventional and extended systems, respectively (Figure 7), which is within the ideal range for optimum soil productivity and plant growth. Therefore, these results suggest that meaningful soil fertility enhancements would require longer implementation times of the rotations. However, a 3-year study with a rotation of sweet sorghum–grazing vetch–sweet sorghum found that the CEC was significantly increased when 30% of the crop residues was left in the soil in combination with no tillage (Malobane et al., 2020). Even though our trial also lasted for three crop growth cycles, the CEC remained unvaried suggesting that part of the aboveground biomass produced in the extended systems should remain in the soil (especially that coming from the legume species) to enhance the soil fertility and productivity, as was the case in the Brazilian soils (Figure 8). In addition, it is well known that changes in the CEC is site specific and depend on the cropping system, soil type, fertilization and other agronomic practices, and crop residue quality (Hubbard & Jordan, 1996; Rahman et al., 2008).

The inclusion of sunn hemp in rotation system did not affect soil C stocks in the three areas under sugarcane production in Brazil (Table 4). Overall, the possible effect of sunn hemp on soil C stocks can be attributed to the C input from sunn hemp above- and belowground crop

residues, and indirectly by the C input from the increase in sugarcane biomass production (Ambrosano et al., 2011; Garside & Bell, 2001; Tenelli et al., 2021). In this study, the sum of the direct (ranging from 3.1 to 3.9 Mg ha^{-1} – Table 1) and indirect inputs of C resulted from a single sunn hemp was not enough to change SOC stocks after 5 years. It is important to highlight that the inclusion of sunn hemp within sugarcane system occurs only once every 5 years, which may take several years to effectively change soil C stocks (Cha-un et al., 2017). Our data suggest that sunn hemp supplies between 120 and 190 kg ha^{-1} of N to the system and that this could be persistent throughout the sugarcane ratoons cycles, especially in upper soil layers (Table 4). Little changes in soil N stock (0–40 cm depth) could be attributed to the relative low N amount added in the system via sunn hemp residues compared with the very large amount of total N stored in soil (Table 4), which may have been insufficient to affect C/N ratio, and thus change soil C and N stocks. In any case, either in temperate or tropical soils the inclusion of sunn hemp in the rotation has positive effects on the soil resilience and productivity.

4.3 | Crop rotation effects on feedstock (biomass) quality

The use of reliable, economical, and suitable quality feedstocks for the production of advanced biofuels is crucial for the successful development of innovative integrated cropping systems. In the past, this has been overlooked in systems aiming at being rapidly scaled up as for example happened in some large-scale biogas programs (Bogdanski et al., 2011). Low biomass quality can drastically lower the net energy output either by limiting the cost-effectiveness of the conversion process and/or by decreasing the feedstock heating value (Zegada-Lizarazu et al., 2010). Cell wall components, in terms of lignocellulosic materials, minerals, and ash contents determine the final biofuels yield. Therefore, evaluating the lignocellulosic and mineral composition of blended feedstocks coming from integrated-innovative cropping systems is highly needed to understand their suitability to different conversion process. Blending makes possible the use of low-cost feedstock avoiding the reliance on high-cost single feedstock; and through the manipulation of feedstock proportions, it would be possible to meet specific qualitative requirements for a given biofuel. In the present study, ash and N contents were higher in the extended system compared with the conventional one (Table 2). The increased N content could be related to the legume component of the mix that contributed with 54% to the total cumulative biomass produced (Figure 3) and due to its capacity to fix large quantities of N$_2$. Moreover, sunn

hemp was harvested at beginning of flowering stage, when the plants are still green and at its maximum capacity to fix N$_2$; therefore most nutrients remain in the shoots (mostly in the leaves; Zegada-Lizarazu et al., 2021) as they had not yet been mobilized back to the soil. It follows that the ash (above recommended thresholds of 5% to ensure cost effective conversion process), P and Ca contents were also higher in the extended systems (Tables 2 and 3). High P values can contribute to the slagging potential of deposits while Ca may increase the melting point, but thanks to the higher ratio between K and Ca, due to the relatively low K (Table 3), the blend is still considered suitable for energy production. Therefore, besides formulating the proportion of the legume to be included in the blend to bring it closer to the specifications required to produce a determined type of biofuel, agronomic management practices such defoliation (i.e., chemical defoliation) or delayed harvest could significantly contribute to improve the feedstock quality. Moreover, recent technological developments have demonstrated that new conversion technologies (i.e., ionic liquid, dilute acid, soaking in aqueous ammonia, enzymatic hydrolysis pretreatments) can process blended biomass feedstocks with minimal negative impact in terms of the overall performance (Ray et al., 2017). As for the cell wall components, Na and C contents (Tables 2 and 3), the Spanish and Italian trials presented contrasting patterns; therefore, no clear explanations can be given about the inherent or environmental effects the extended systems may have had on these results. In fact, high variability in ash and mineral content among dedicated energy crops is reported in the literature and related to genetic and environmental factors as well as physiological and morphological characteristics of the species (Casler & Boe, 2003; Lewandowski & Schmidt, 2006; Zegada-Lizarazu, Parenti, & Monti, 2021). In any case, low biomass quality is more related to an attitude of the producer and the user, rather than a question of technology development and knowledge (Ray et al., 2017).

5 | CONCLUSIONS

It was evidenced that the introduction of sunn hemp in rotation systems did not have a negative impact on the wheat yield (about 6, 7, 3 Mg ha^{-1}, in Greece, Italy, and Spain, respectively) while the additional biomass (straw + sunn hem biomass) produced by the dedicated leguminous crop was substantial (cumulative biomass between 1.5 and 2.0 times higher than in the conventional rotation). The cultivation of sunn hemp in sugarcane replanting time, increased stalk yields in clay soils at rates ranging from 14 to 15 Mg ha^{-1} year^{-1}. From the EU and Brazilian farmers' point of view, these could be an important source

of biomass production, crop diversification and additional income.

The sustained grain (wheat) and increased biomass yields (cereal straw, sugarcane) promoted by sunn hemp are associated with atmospheric N_2 fixation from the symbiotic association with bacteria (rhizobia) and, other well-known positive rotational effects (i.e., of weed/pathogens, and improvement of soil quality) associated to the soil resilience and fertility. Sunn hemp has contributed to sustain the available macronutrients, organic matter and CEC in the EU trials and in addition the soil N stocks in Brazil. Therefore, dedicated legume crops such as sunn hemp can significantly contribute to the soil quality and resilience in either temperate or tropical soils.

In summary, in both temperate and tropical climates the integration of dedicated biomass legume crops within conventional systems could lead to enhanced biomass availability, crop diversification, and efficient use (in space and time) of the land resources; however, a further life cycle analysis could help to better determine the feasibility and sustainability of the systems at large-scale levels.

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CONFLICT OF INTEREST

The authors declare that they do not have competing financial interests or personal relationships that could have influence on the present work.

AUTHOR CONTRIBUTIONS

Walter Zegada-Lizarazu: experiment design and performed the field experiment, analysis of data, and drafting of the manuscript, project administration; João L. N. Carvalho: design the field experiment, analysis of data, and manuscript drafting and editing; Andrea Parenti: experimental management, sampling, data analysis, and discussion; Sarah Tenelli: experimental management, sampling and analysis, drafting of the manuscript; Carlos Martín Sastre: experimental management, sampling and analysis, drafting of the manuscript; Pilar Ciria: data analysis and discussion; Myrsini Christou: data analysis and discussion; Alexopoulou Efthymia: experimental management, data analysis and discussion; Antonio Bonomi: project administration, funding acquisition, experiment design; Andrea Monti: coordination and intellectual input, experiment design, project administration, and funding acquisition.

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