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Relay-cropping enhances the availability of low iLUC risk lignocellulosic feedstock for advanced biofuels

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ABSTRACT

The relay-cropping (R) of a winter cereal and a high biomass yielding legume for advanced biofuel production can enlarge the legume growing season, thus increase its biomass yield and synchronize nitrogen (N_2)-fixation capacity to uptake. The aim of the present study was to evaluate the biomass and food yield of two sunn hemp (*Crotalaria juncea* L.; cv. Ecofix) – wheat (*Triticum aestivum* L.; cv. Bologna) relay-cropping systems compared to double-cropping, in order to explore the impact on grain and biomass yield of the variable crops overlapping periods. The evaluated relay-cropping systems foreseen an early November wheat sowing into no till bare soil with: i) sunn hemp harvested at full flowering (beginning of November, R1) and ii) sunn hemp harvested at the end of flowering (end of November, R2). In the control (C) double-cropping, sunn hemp was harvested at the beginning of flowering (end of September). Quantitative and qualitative parameters were evaluated for each system across two growing seasons (2019–20 and 2021–22). Sunn hemp biomass and wheat straw residues were determined. The grain qualitative parameters determined for wheat were: grain volume weight (VW), crude protein, wet gluten, starch and Zeleny index (ZI). The R1 and R2 sunn hemp-wheat relay-cropping biomass yield (12.5 Mg d.m. ha^{-1} y^{-1}) was similar to C (11.3 Mg d.m. ha^{-1} y^{-1}). In 2019–20, environmental conditions were more favorable, thus, yields were higher than in 2021–22 by 72 %. Wheat grain yields in 2021–22 were higher in R1 (2.9 Mg d.m. ha^{-1}) than C (2.0 Mg d.m. ha^{-1}), with R2 showing intermediate values. In 2019–20, however, the grain quality was higher in R1 than in C for crude protein (+11 %), wet gluten (+15 %), and ZI (+26 %) eventually due to an increased N_2-fixation by sunn hemp that reached full flowering. The local availability of low iLUC risk lignocellulosic feedstock for advanced biofuel could be increased through crop intensification with relay-cropping, showing the potential to provide additional biomass and enhance food quality thus complying with the additionality measures set out by the Delegated Regulation of REDIII.

1. Introduction

The predominant use of fossil fuels, acknowledged as the primary contributor to heightened CO_2 emissions (Friedlingstein et al., 2022), stands out as the key factor behind the accelerated climate changes witnessed in recent decades (Olivier et al., 2017). Governments, including the European Union (EU), have instituted long-term energy policies, such as RED III, Green Deal, FuelEU Aviation and FuelEU Maritime regulations, CETP, among others to mitigate CO_2 emissions and address the consequences of climate change. The expansion of bioenergy policies within the European Union (EU) has paved the way for an increased role of biomass sources in advanced biofuels, aligning with the net-zero carbon emission target in the Green New Deal (European Commission, 2019). Presently, European transport heavily relies on liquid fossil fuels (European Court of Auditors, 2023), representing a significant opportunity for improvement through the expansion of renewable energy technologies for advanced liquid biofuel production. Despite the recalcitrant dominance of first-generation liquid biofuels (European Parliament, 2018), there is a recognized need to reduce their usage due to concerns about the "food vs fuel" competition and associated indirect Land Use Change (iLUC) issues (Fritsche et al., 2010; Londo et al., 2007; Valin et al., 2015). Advanced bioethanol, produced through the saccharification and fermentation of lignocellulosic biomass, emerges as a crucial alternative for transitioning away from fossil-based transportation fuels.

Diversified cropping systems involving lignocellulosic crops offer

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numerous economic, environmental, and social advantages over sugar/starch monocropped feedstocks initially chosen for first-generation biofuel production as a renewable energy source. The integration of dedicated lignocellulosic crops within food crop systems, indeed, could reduce the greenhouse gas (GHG) emissions, increase of soil organic carbon (SOC) stock), diversification of feedstock, enhance the resilience of cropping systems, and increased biofuel yields (Zegada-Lizarazu et al., 2010; Parenti et al., 2022). The thrive of advanced biofuels hinges on the establishment of low-input and sustainable cropping systems (i.e. relay cropping) capable of efficiently utilizing natural resources without compromising food production. The additional biomass feedstock produced in these low ILUC risk systems could become an incentive for farmers to introduce such practices and increase their incomes and ecosystem services in accordance with the additionality measures set out by the Delegated Regulation of REDII (Art. 2 (5) Delegated Regulation 2019/807). Relay-cropping emerges as a strategy to improve land and resource use efficiency, stabilize yields, boost biomass productivity per unit area, and promote biodiversity (Cecchin et al., 2021).

Relay-cropping is a form of multiple cropping in which a new crop is introduced into an already established one, resulting in an overlapping of the life cycles of the two crops (Berti et al., 2015; Tanveer et al., 2017). Whether effectively implemented, these systems could hold particular importance for present and future needs of biomass for bioenergy. The main advantage is to get two crops, mainly in cold temperate climates where the growing season is too short for double cropping (Berti et al., 2017; Johnson et al., 2017; Tanveer et al., 2017). Other benefits of overlapping two crops are erosion control, nutrient management, soil quality improvement (Cecchin et al., 2021) and higher yields (Gou et al., 2022). Besides that, relay planting offers an opportunity to integrate nitrogen-fixing (N-fixing) legumes into annual cropping systems without compromising grain production (Bergkvist et al., 2011), and with a better synchronization of N_2 release and uptake before it gets completely mineralized as it seems to happen in certain double cropping systems (Stallings and Balkcom, 2015). Research carried out in Ontario on winter wheat-red clover relay-cropping systems showed that a reduced N fertilization leaded to higher economic return from both wheat and red clover (Gaudin et al., 2014). However, for this approach to be successful, careful evaluation and fine-tuned agronomic strategies are essential in selecting the species and management. An important yet understudied aspect of relay-planting is the accurate combinations of sowing time and method to better synchronize N_2 release and uptake, along with the appropriate selection of species and varieties, to prevent issues such as shading, nutrient competition, or inhibition caused by phytotoxic compounds produced during the decomposition of residues from a preceding crop. Moreover, it is often technically more complex to sow in a standing crop, in particular whether the seed dimension requires to be drilled into the soil and not broadcasted. The aforementioned issues can hold back farmers in embracing relay-cropping, although recently, specific modern machineries were developed to successfully drill a relayed crop. In such a setup, incorporating a legume with a substantial potential for lignocellulosic biomass yield serves not only to enhance the yield of a companion cereal crop but also contributes to maintaining a well-balanced soil fertility program and reducing nitrogen fertilization costs (Martin-Guay et al., 2018; Zegada-Lizarazu and Monti, 2011; Zegada-Lizarazu et al., 2022). Adopting more intensive and sustainable crop production systems has the potential to enhance resource use efficiency, integrate effective management practices, and elevate the environmental performance of biofuels (Hill, 2009; Raseduzzaman and Jensen, 2017) without drawbacks on yields (Wallace et al., 1996).

Within promising high yielding lignocellulosic legumes for temperate environment, sunn hemp (*Crotalaria juncea* L.), a legume species of tropical origin, is carving out a niche due to its fast growth, high potential for lignocellulosic biomass production (approximately 10–13 Mg ha^{-1} in about 90 days), and capability to fix 60–80 kg ha^{-1} of N_2 (Mansoer et al., 1997; Schomberg et al., 2007). Traditionally cultivated in its native areas as a non-wood fiber crop, sunn hemp is becoming interesting in temperate areas as low iLUC risk feedstock for advanced biofuel applications (Kamireddy et al., 2013; Parenti et al., 2021, 2019; Paul and Chakraborty, 2018). In a field experiment conducted in Florence, USA, sunn hemp yielded approximately 200 GJ ha^{-1} (Cantrell et al., 2010), whereas according to Heggenstaller et al. (2008) when double cropped, sunn hemp ethanol yield exceeded 2000 L ha^{-1} y^{-1}. Consequently, there is interest in exploring its utilization as an energy crop in relay systems with food crops. However, no available information is available regarding sunn hemp-wheat relay-cropping system for food and lignocellulosic feedstock production purposes in temperate environments. The relay of a winter wheat into sunn hemp is a first-of-a-kind experiment, although some examples of double-cropping between such crop provided encouraging results (Parenti et al., 2022; Zegada-Lizarazu et al., 2022). In fact, in Italy double-cropping systems as a means to enhance the agricultural sustainability of integrated food-energy cropping systems are been widely adopted. Magnolo et al. (2021) indicated that more than 600 farmers are applying double-cropping systems for the production of biogas. The advantage, however of applying relay planting of wheat into sunn hemp compared to double-cropping is to stretch the development of the legume until the full flowering stage, which usually is reached after wheat planting, thus maximize the legume N fixing activity (Mansoer et al., 1997; Schomberg et al., 2007), better synchronize the N_2 release to a subsequent crop, and increase sunn hemp biomass production. In this light, wheat could benefit from some additional N when is most needed, which could increase grain yield and quality. Grain protein, in particular, is positively correlated to soil nitrogen availability (Hoel, 2003; Triboi et al., 2000), but also to wheat selling market price. Thus, it would be an economic and environmental benefit to obtain similar grain protein content with lower mineral N fertilization.

The aim of the present study was to evaluate the biomass and food yield potential of sunn hemp-wheat relay cropping systems compared to double cropping, thus to explore the impact of additional biomass feedstock produced on grain quality by varying the relayed crops overlapping period.

2. Materials and methods

2.1. Study site

The study was carried out at the experimental farm of Bologna University, Italy (44° 33' lat. N, 11° 26' Long. E, 32 m a.s.l.) throughout the 2019–20 and 2021–22 growing seasons. The study location was identified as having fine silty mixed mesic udic ustochrept soil, with average assimilable phosphorus and nitrogen concentrations, and high exchangeable potassium (Table 1). The pH of the soil was neutral, with low organic matter content (1.6 %). The experimental site lies within a temperate humid climate, with hot summers and cold winters (Table 2). Typically, the growth season spans from early spring to the end of summer. Precipitation is unevenly distributed throughout the year, with distinct peaks in spring and autumn. Summers are generally dry, exhibiting the lowest precipitation levels annually.

Air temperature in 2019–20 (Table 2) was generally 0.4 °C warmer than long-term while in 2021–22 temperature was the same as in the long term. As for the sunn hemp growing period (SH), the temperature from July to November 2019 and 2021 was in particular 0.6°C and 0.3°C higher than long-term, respectively. The winter temperatures were close to the historical average. Precipitation was higher (+ 12 mm) than the long-term average in the July - November period of 2019 only, while in 2021 in the same period the precipitation was 35 mm lower. In fact, 2021 was markedly dry in July and August, with cumulated precipitations over two months of only 22 mm and mean daily temperature above 25 °C. As for the November - June periods, the precipitations were 89 mm and 77 mm lower than the long-term in 2019 and 2021, respectively.

Table 1
Main soil physical and chemical properties in 2019–20 and 2021–22.

	Sand	Silt	Clay	pH	Total $CaCO_3$[a]	Active $CaCO_3$[b]	Organic carbon[c]	Organic matter[c]	Total N[d]	P_2O_5[e]	K_2O[f]	C/N
	(%)	(%)	(%)		(%)	(%)	(g kg^{-1})	(%)	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	
19–20	40	40	20	6.9	0.81	0.69	8.3	1.4	0.9	116	163	9.8
21–22	33	40	27	6.7	2.1	0.9	10	1.7	1.1	61	203	9

Determination method: [a]Dietrich-Fruehling, [b]Drouineau, [c]Walkley-Black, [d]Dumas, [e]Olsen, [f]Method XIII.5 defined in Italian ministerial decree 13–9–99.

Table 2
Annual mean air monthly temperature and cumulative precipitation from July to November (sunn hemp (SH) growing season), and from November to June for relayed wheat (WH), in 2019–20 and 2021–22 in comparison to long-term mean of 10 years local precipitation and temperatures.

Mean air monthly temperature (°C)	Long-term	2019–20	2021–22
July-November (SH)	21.4	21.9	21.0
November-June (WH)	10.9	11.4	11.1
Annual mean	14.4	14.8	14.4
Cumulative monthly precipitation (mm)			
July-November (SH)	207	128	171
November-June (WH)	394	397	319
Cumulative annual precipitation	601	525	490

2.2. Treatments and field management

Prior to sowing, the soil was plowed to a depth of 35 cm and subjected to double harrowing to enhance crop establishment in both growing seasons. A basal fertilization with 100 kg of P_2O_5 was applied before the last harrowing. No N fertilization was applied to any of the treatments. Then a glyphosate dosage of 2 kg ha^{-1} was sprayed right before planting to control weeds. The plots were arranged in a randomized complete block design with three replicates in an area of 9 m^2 each. Sunn hemp (*Crotalaria juncea* L.) cultivar 'Ecofix' (largely available in the local market) was planted on the 14th of June 2019 simulating the establishment of a double-cropping after a winter crop. In 2021, the same sunn hemp cultivar was planted after wheat harvest on the 9th of July. The operation was carried out through a pneumatic planter with a row spacing of 0.45 m and a planting rate of 52 seeds m^{-2}. To ensure a favorable emergence rate and seedling establishment, a total of 20 and 55 mm of sprinkled water was applied in 2019 and 2021, respectively. About one month after planting, the plots were mechanically weeded between the rows. In 2019, an attack of *Rhabdomiris striatellus* affected sunn hemp, thus an insecticide treatment with Azadiractin (0.38 L ha^{-1}) was applied at the end of August. Harvests were performed at the onset (C), full (R1), and end of flowering (R2), which corresponded approximately to the end of September, beginning of November, and end of November, respectively (Fig. 1). In no one of the cropping systems there was regrowth after the harvest of the legume crop. The relayed 'Bologna' soft wheat (*Triticum aestivum* L.) cultivar was, thereafter, hand sown between beginning and full flowering stage of sunn hemp, complying with wheat optimal sowing period.

An increased planting density (210 kg of seeds ha^{-1}) by 17 % compared to manufacturer's recommendations was adopted, although a non-conventional interrow distance to place wheat within sunn hemp rows was necessary. There is no mechanical planter available to plant relayed wheat within sunn hemp at parcel level, although it would be feasible at field level with roller crimper. Therefore, a seed furrow was created using a thin hoe and wheat seeds were hand distributed in it, then the furrow was refilled with the soil. The rows distance was set at alternative 15–30 cm to offset the missing row filled by sunn hemp, thus consequently, the planting density on the row was increased compared to conventional. Wheat plots were kept weed free by hand hoeing. No mineral N fertilization was applied in any cropping system.

2.3. Crop measurements

Sunn hemp biomass yield was determined by manually cutting all plants at their bottom on a 3.6 m^2 area, then the dry weight was obtained by oven drying at 105 °C to a constant weight. At the end of November, in the period between wheat planting and sunn hemp R3 harvest, the soil volumetric water content (VWC) was measured at 10 cm depth with a portable TDR probe. Wheat emergence was determined about two months after planting by counting the number of plantlets present on an area of 0.5 m^2 and reported as the ratio to the optimum density of about 400 plants m^{-2} with an average emergence rate of 80 %. SPAD-units for 5 plants per plot were measured on the leaf ear at anthesis (beginning of May) using a Minolta SPAD-502 hand-held absorbance-based dual-wavelength chlorophyll meter. When wheat grain reached physiological maturity, the harvest was carried out by manually cutting the aboveground biomass within a 3.6 m^2 area. The grain was threshed and weighted separately from straw, then the dry weight of both components was determined by oven drying at 105 °C until a constant weight was reached. Harvest index (HI) was calculated as the ratio of grain-yield to the net aboveground biomass at harvest. A sub sample of 1 kg of grain per treatment was collected for further laboratory qualitative analysis using Near Infrared Spectroscopy (NIRS) method (analyser InfratecTM NOVA, FOSS, Hillerød, Denmark). The evaluated parameters included volume weight (VW, kg hL^{-1}), crude protein (% in dry matter), wet gluten (for grain at 14 % moisture), starch (% in dry matter), and Zeleny index (ZI). ZI is carried out through the I.C.C. (International Association for Cereal Science and Technology) method, which entails an indirect assessment of the bread-making value relying on both the quantity and quality of protein, with a specific emphasis on gluten quality. Typically, values below 20 suggest inadequate grain quality for bread making.

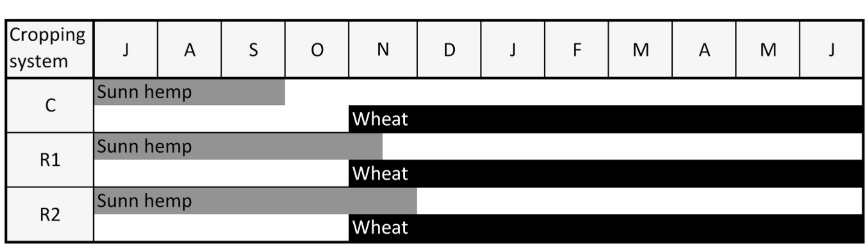

Fig. 1. Cropping systems scheme displaying the sunn hemp (light grey) and wheat (black) cultivation period and the overlapping time. R1 and R2 are the relay cropping systems based on sunn hemp harvest times corresponding to the full and end of flowering, respectively. C indicates the control double cropping with sunn hemp harvested at the beginning of flowering.

2.4. Statistical analysis

A repeated measures ANOVA ($P \leq 0.05$) was conducted using Statistix 10.0 (Analytical Software, Tallahassee, FL, USA), with relay-cropping system and year treated as fixed factors and individual plots as random factors. Significant differences were tested using Fisher's LSD to separate means into distinct groups ($P \leq 0.05$). The linear correlation coefficients were calculated as given by Pearson's test.

3. Results

The biomass yield of the evaluated cropping systems among two growing seasons are shown in Fig. 2. No interaction between cropping system and growing season was found. Sunn hemp contribution to the total biomass potentially available for advanced biofuel production is double (8.1 Mg d.m. ha^{-1}) compared to straw (4.0 Mg d.m. ha^{-1}), however no significant difference was found among the three different cropping systems. The cumulated (sunn hemp biomass + wheat straw) biomass amount averaged through the relay systems was 12.5 Mg d.m. ha^{-1} y^{-1} and 11.3 Mg d.m. ha^{-1} y^{-1} in C. In 2019–20 growing season biomass yields were higher than in 2021–22 by 72 % ($P \leq 0.001$). The mentioned difference among growing seasons is due to sunn hemp, which produced 64 % less biomass in 2021–22 compared to 2019–20. An additional recorded difference between relay-cropping systems was found for soil VWC, measured in the period between wheat planting and sunn hemp harvest (R2), which resulted significantly higher in C (53 %) and R1 (54 %) with respect to R2 (44 %) by 22 % (data no shown).

Wheat emergence rate (Fig. 3) highlighted that R1 (6.4 %) was 33 % lower than C (9.9 %) and R2 (9.4 %). Besides, the overall emergence was poor (8.6 % as average), leading to 56 established plant m^{-2} compared to the optimal density of about 400 plants m^{-2} with an average emergence rate of 80 %. In 2019–20 the establishment of the food crop was more solid, accounting for more than double plantlets (84 plants m^{-2}) and an emergence rate of 12.8 % with respect of 35 plants m^{-2} and 5.4 % measured in 2021–22. No interaction between cropping system and growing season was found. Emergence rate turned out to be correlated to crude protein (r = −0.75), wet gluten (r = −0.76), ZI (r = −0.77), and starch (r = 0.70). However, no correlation was found with grain, biomass and volumetric weight (data not shown).

Wheat yields in the cropping systems showed a statistically significant interaction with the growing season (Fig. 4a) both for grain and for straw components. The grain yield was lower by 36 % in C 2021–22 (2 Mg d.m. ha^{-1}) with respect to C 2019–20 (3.3 Mg d.m.ha^{-1}) and to R1 in 2021–22 (2.9 Mg d.m. ha^{-1}), while the other treatment showed intermediate values. In general, R1 and R2 systems resulted as the top grain yielding systems, regardless of growing season, although C in the first

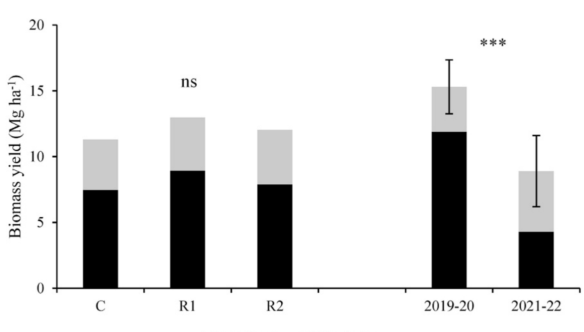

Fig. 2. Biomass yield of the evaluated cropping systems in the two growing seasons. R1 and R2 are the relay cropping systems based on sunn hemp harvest times corresponding to the full and end of flowering, respectively. C indicates the control double cropping with sunn hemp harvested at the beginning of flowering. Black colour refers to sunn hemp biomass while grey to wheat straw. *** indicates statistical differences P ≤ 0.001.

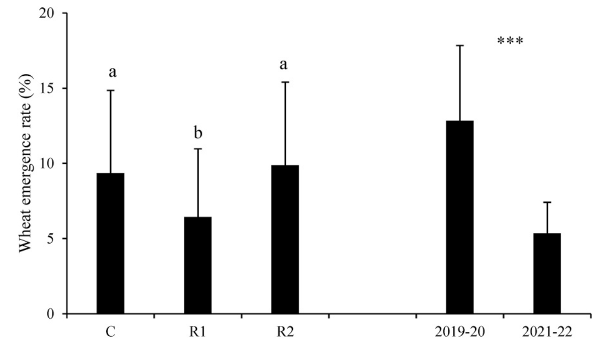

Fig. 3. Wheat emergence of the evaluated cropping systems and in the two growing seasons. R1 and R2 are the relay cropping systems based on sunn hemp harvest times corresponding to the full and end of flowering, respectively. C indicates the control double cropping with sunn hemp harvested at the beginning of flowering. *** indicates statistical differences P ≤ 0.001.

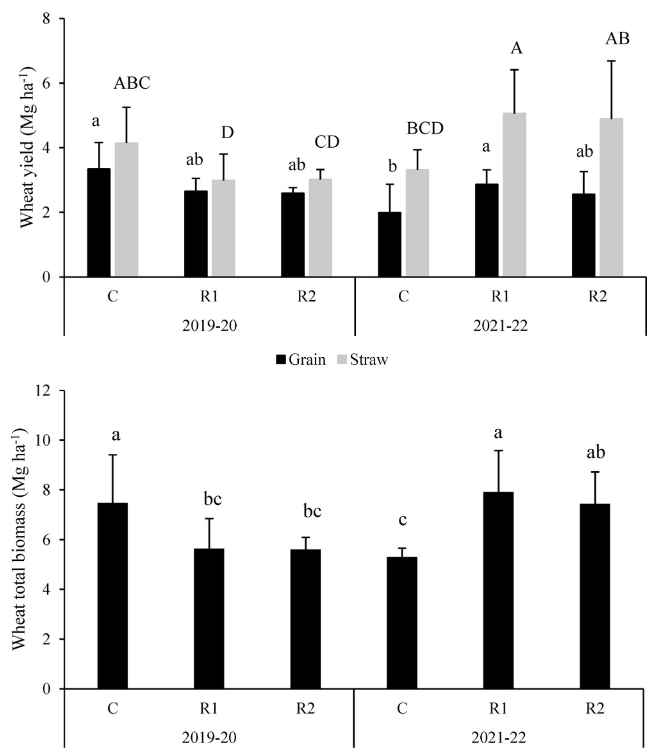

Fig. 4. a) Wheat grain (black) and straw (grey) yields and b) total biomass of the evaluated cropping systems across two growing seasons. R1 and R2 are the relay cropping systems based on sunn hemp harvest times corresponding to the full and end of flowering, respectively. C indicates the control double cropping with sunn hemp harvested at the beginning of flowering.

growing season performed significantly better than in the second (-39 %). Straw mimicked the grain yield trend with R1 in 2021–22 resulting 55 % higher than C in 2021–22, although the behaviour was opposite in 2019–20 where R1 was lower by 27 % than C. The overall average grain yield resulted in 4 Mg d.m. ha^{-1} showing a harvest index of 0.46 and 0.37 (significant with $P \leq 0.01$) in 2019–20 and 2021–22, respectively.

The total wheat biomass (Fig. 4b) resulted highest in C (2019–20), R1 and R2 (2021–22) with 7.6 Mg d.m. ha^{-1} as average, while was lowest in R1, R2 (2019–20), and C (2021–22) with 5.5 Mg d.m. ha^{-1}. Therefore it could be said that C compared with the two relay systems (R1 and R2), among the two growing season the total biomass in C had an opposite outcome by resulting +34 % and −31 % compared to the relay systems, for 2019–20 and 2021–22, respectively.

Table 3 shows how the cropping system and growing season influenced some wheat grain qualitative parameters commonly measured to determine the end use and the price of grain. Volume weight (VW) was 4 % higher in 2019–20, besides that the relay systems (R1 and R2) recorded an increase by 2 % with respect to C. No interaction between cropping system and growing season emerged for volume weight, whereas interactions were found for crude protein, wet gluten and ZI. Crude protein content was higher in 2021–22 by 50 %, thus differences arose between cropping systems with +11 % in C compared to R1, while R2 was intermediate. A similar behavior was observed for wet gluten and ZI, in which the values were double in 2021–22 compared to 2019–20. Moreover, the trend was similar to crude protein for the differences emerged in cropping systems for wet gluten and ZI. In particular, in 2019–20, C was 13 and 31 % lower than R1 for wet gluten and ZI, respectively, while R2 was intermediate. Conversely, starch content was higher in 2019–20 with respect to 2021–22, by 7 %.

A PCA analysis was performed to obtain an overview of the effects of the relay-cropping and double-cropping systems on the wheat grain quality factors across years (Fig. 5). PC1 explained 57.6 % of the total observed variation in the qualitative wheat grain characteristics as affected by the harvest times of sunn hemp in the different cropping systems (i.e. biomass yield) along the two years of the trial. It was found that the variables wheat starch content, wheat volume weight, wheat planting density and sunn hemp yield (in lieu of its potential N_2 contribution to the system) are positively correlated with PC1. In particular, the sunn hemp biomass was crucial to drive these variables as it almost completely overlapped the PC1 with the highest relative contribution (close to 1). The wheat planting density was also positively correlated with PC1, but with a minor contribution to PC1. On the other hand, the variables wheat protein, gluten, ZI index and wheat SPAD are negatively correlated with PC1 and in the opposite quadrant to sunn hemp biomass and wheat plant density, stating that the former crop harvest time in the different cropping systems had a direct effect (on both directions) on the qualitative yield of wheat. Whereas the quantitative biomass yield of wheat (i.e. grains and straw) is correlated to other factors (PC2), most probably to water availability. The year was taken as a supplementary factor indicating a close but inverse correlation with PC1.

Chlorophyll content (Fig. 6) was measured at wheat anthesis and results ($P \leq 0.001$) showed a concentration of 37.9 in 2019–20 and 43.4 in 2021–22. Neither interaction among cropping systems and growing season, nor individually differences for cropping system emerged.

Fig. 7 plots the relationships between wheat chlorophyll and sunn hemp yield (Fig. 7a), then wheat chlorophyll and crude protein (Fig. 7b). A significant ($P \leq 0.01$) negative ($r = -0.68$) and positive ($r = 0.65$) correlation was found, respectively. Sunn hemp yield was also found to be in relation to wheat crude protein ($r = -0.91$), volume weight ($r = 0.78$), starch ($r = 0.94$), wet gluten ($r = -0.92$), and ZI ($r = 0.91$).

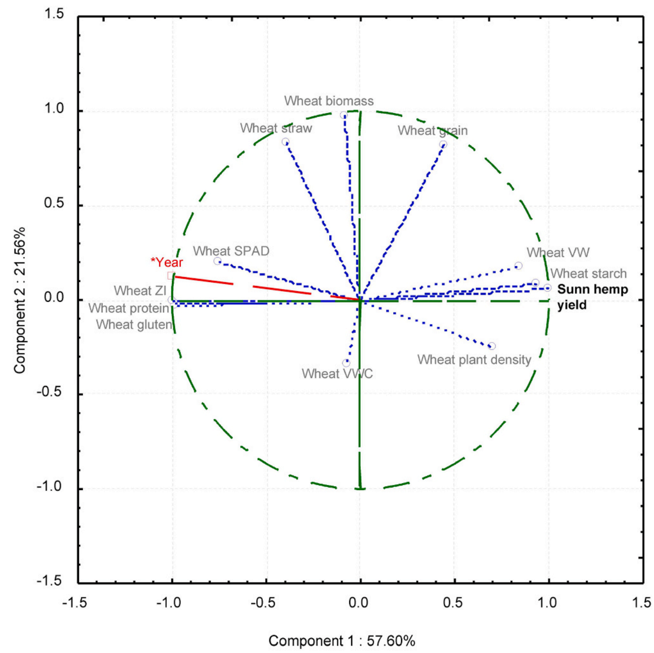

Fig. 5. Principal Component Analysis. The variables used to compute PCs and their correlation and contribution with the firsts two PCs are shown. The length of the lines of each factor shows how much variability each principal component was able to explain. Wheat volume weight (VW), wet gluten, Zeleni index (ZI), protein, starch were measured in 2019–20 and 2021–22. Wheat VWC, volumetric water content (VWC) and plant density measured at the beginning of the season.

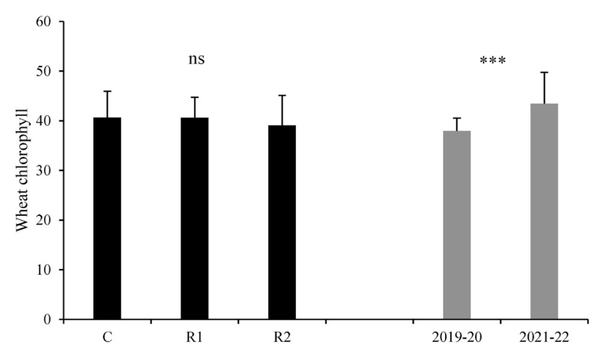

Fig. 6. Wheat chlorophyll at anthesis of each evaluated cropping systems in two growing seasons. R1 and R2 are the relay cropping systems based on sunn hemp harvest times corresponding to the full and end of flowering, respectively. C indicates the control double cropping with sunn hemp harvested at the beginning of flowering. *** indicates statistical differences $P \leq 0.001$.

Table 3
Grain qualitative parameters of each cropping system across two growing seasons. R1 and R2 are the relay cropping systems based on sunn hemp harvest times corresponding to the full and end of flowering, respectively. C indicates the control double cropping with sunn hemp harvested at the beginning of flowering.

			Volume weight(kg hl^{-1})		Crude protein(%)		Starch(%)		Wet gluten(%)		ZI(ml)	
			19-20	21-22	19-20	21-22	19-20	21-22	19-20	21-22	19-20	21-22
Year	Y		79.1 a	76.2 b			61.8 a	57.6 b				
Cropping system	CS	C	76.4 b									
		R1	78.0 a				ns					
		R2	77.7 a									
	CS*Y	C			9.2 c	15 a			16.4 c	32.5 a	18.2 c	53.3 a
		R1	ns		10.2 b	14.2 a	ns		18.8 b	31.1 a	26.4 b	51.4 a
		R2			9.5 bc	14.2 a			17.1 bc	31 a	23.5 bc	51.3 a

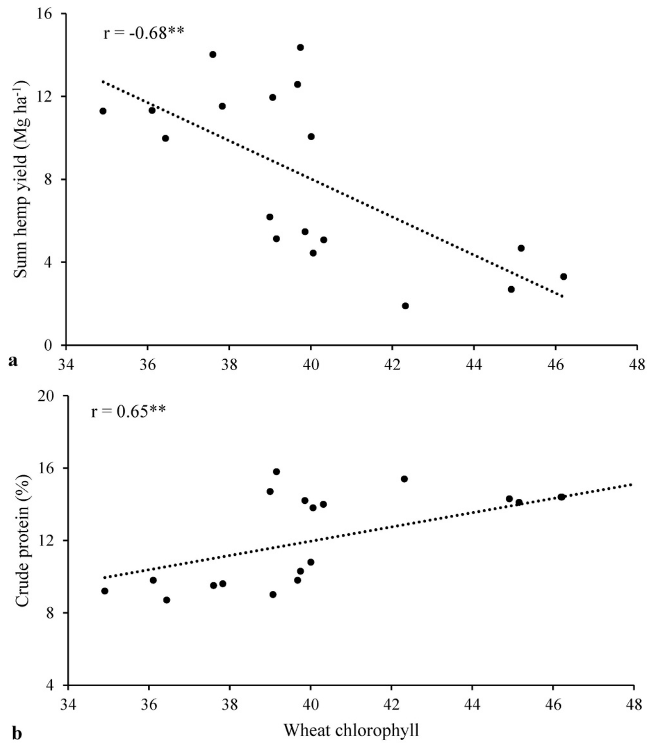

Fig. 7. Relationships between wheat chlorophyll and the a) sunn hemp yield and b) grain crude protein measured in the relayed wheat across 2019–20 and 2021–22 growing seasons. ** indicates statistical differences $P \leq 0.01$.

4. Discussion

Growing dedicated lignocellulosic crops, especially when incorporating legumes, holds significant promise for advanced biofuel production and ecosystem services. Nevertheless, there is a scarcity of information regarding the feasibility and the effect that these new systems may have on a following food crop and total biomass productivity. This investigation specifically examines the relay of winter wheat with a novel lignocellulosic leguminous species, sunn hemp in comparisons to double-cropping. The advantage of relay planting would be to stretch the development of the legume until the full flowering stage, thus maximize the legume N fixing activity (Mansoer et al., 1997; Schomberg et al., 2007), better synchronize the N_2 release with the rapid uptake of wheat at early development stages, and increase sunn hemp biomass production. The results showed (Fig. 2) that even though high variability across years (sunn hemp produced twice as much biomass in 2019–20 than in 2021–22, due to environmental constrains, soil characteristics, and variable sunn hemp growing cycle length) (Table 2) the average productivity in the relay-cropping systems (R1, R2) was about 12.5 Mg d.m. ha^{-1} compared to 11.3 Mg d.m. ha^{-1} in the C. This is comparable to the productivity of some high yielding perennial grasses (i.e. switchgrass, miscanthus, etc.) but with the advantage that these biomass levels can be achieved in annual basis, so the land is not subjected to long-term cycles of preclusion to other more economically attractive production systems that the market trends would determine. Besides that, integrating a dedicated legume in a relay planting system allows more efficient use of water and nutrients (i.e. synchronized N_2 release), resulting in reduced degradation of soil and water quality within a single growing season. These results may help to pave the way for a significant contribution from existing low iLUC risk food-bioenergy cropping systems to the development of sustainable and integrated advanced biofuel value chains within the EU regulations and directives (i.e. REDIII, European Green Deal, etc.). If carefully planned and managed, the proposed innovative relay-cropping systems could not only increase the system productivity, but also significantly improve the local feedstock availability, complying with the additionality measures set out by the Delegated Regulation of REDIII.

Moreover, the present results show the suitability of sunn hemp (regardless its tropical origin) as a new crop in relay-cropping systems in temperate climates where the available growing season is limiting for the cultivation of two crops in sequence during the same growing season. In that sense, sunn hemp establishment in a temperate climate, planted after a winter cereal, may occur either in late spring or beginning of summer (Parenti et al., 2021), facing however the risk of a reduced available growing season and low germination rates due to low precipitations. In this regard, during sunn hemp establishment, 2021 was particularly dry with respect to 2019 (-24 mm), and even more if compared to the long-term mean (-82 mm). To cope with the limited resources and available growing season, relay planting with wheat and harvesting sunn hemp at different growths stages (from full to the end of flowering) may have rendered possible to enlarge as much as possible the growing season and therefore bring sunn hemp to full, or close to full, maturity with the beneficial effect on increased/maximized N_2 contribution to the system and increased biomass with reduced moisture and minerals in the lignocellulosic feedstock. In fact, biomass yield of sunn hemp is strongly related to the length of the growing period, and to high temperatures coupled with abundance of precipitations, as assessed in a multi-year and multi-location yield simulation study. In this light, Parenti et al. (2021) estimated that sunn hemp planted at DOY 190 in Central Italy (summer planting), which corresponds to what was carried out in 2021–22 growing season, would arrive to a biomass yield of about 5 Mg d.m. ha^{-1}. Such estimation is in line with the present experiment findings of 4.3 Mg d.m. ha^{-1} obtained when sunn hemp was harvested between the full and end of flowering (R1 and R2). Such low sunn hemp biomass yield in 2021–22 was crucial in determining the overall system production (Fig. 2) since wheat straw yield was kept constant across the two growing seasons and close to the potential production of the area. Wheat straw yield (4 Mg d.m. ha^{-1} y^{-1}, Fig. 4a) is within the range of 3–7 Mg d.m. ha^{-1} y^{-1} as assessed by Schils et al. (2018) in a comprehensive productivity study for European environments. It is worth mentioning, however, that the present results were obtained without mineral N fertilization, thus the entire N required by wheat may have come from the N_2 fixed by the legume species. Therefore, delaying as much as possible the harvest of sunn hemp could be beneficial in this regard (i.e. between R1 and R2). In fact, as part of a larger crop rotation study set up between 2017 and 2020 in an adjacent plot to the present study where a conventional rotation (wheat - fallow - maize - wheat) was compared with an innovative one (wheat - sunn hemp - wheat) it was determined that at the full flowering stage sunn hemp fixed 54 Kg N_2 ha^{-1} (own unpublished data). Such value is in line with the 60–80 kg N_2 ha^{-1} reported elsewhere in the literature (Mansoer et al., 1997; Schomberg et al., 2007; Balkcom and Reeves, 2005), constituting, probably, a significant N_2 contribution to the following crop in rotation; thus enhancing the sustainability of the systems and the farmers' revenue. Therefore, harvesting sunn hemp at the right time is very important not only to ensure the highest yield and the largest N_2contribution possible to the subsequent crops, but also to ensure increased soil organic matter and soil stability (Parenti et al., 2022) thus the sustainability of the system. Stallings and Balkcom (2015) indicated that sunn hemp N concentration decreased with plant age but N content increased with biomass accumulation.

Relay planting had contrasting effects on wheat performance depending on the weather trends of the growing season and harvest time of sunn hemp. Whereas in 2021–22, wheat performance was clearly influenced by a general poor emergence rate (Fig. 3). The low emergence rate (8.2 %) in the relay plots was most probably due to the presence of sunn hemp stalks and roots either as residues or as standing crop that somehow burdensome the hand sowing operations under no tillage conditions. The number of plants per square meter resulted one-seventh of the regional optimal (400 plants m^{-2}), although the grain yield (Fig. 4a) was only halved. The grain yield in an adjacent crop rotation study (Zegada-Lizarazu et al., 2022) was 6.9 Mg d.m. ha^{-1}. Such limited effect on grain yield might be related to an increased number of ear-bearing tillers (Li et al., 2020). On the other hand, in 2021–22 besides the presence of sunn hemp plants, the lower wheat emergence can be related to the higher clay content of the soil (Table 1) combined with a relatively high soil water content (50 % as average; data not shown). Such pedo-climatic combination may have determined sub-optimal planting conditions, especially under no-tillage. A tilled soil with a refined bed for planting can, indeed, drain the excess water faster than no till, which is characterized by higher moisture content (De Vita et al., 2007; Fabrizzi et al., 2005; Król et al., 2018; Mujdeci et al., 2010). R2 showed a lower soil VWC compared to C and R1 (data not shown) at wheat emergence because it was the only system taking advantage of sunn hemp coverage that was able to use part of the superficial soil water and dry the top layer at a greater extent (Blackshaw et al., 2010; Munawar et al., 1990). In R1, the wheat emergence rate (Fig. 3) was 33 % lower than C and R2, but without any negative effect on yields (Fig. 4a). Besides that, wheat emergence rate was related neither to grain nor to biomass yield, however it was negatively correlated to crude protein, wet gluten, and ZI, whereas positively with starch (Fig. 5). These results are in agreement with some studies showing that higher seeding (emergence) rates may decrease protein levels due to intensified intraspecific competition for soil nitrogen (Arduini et al., 2006; Gooding et al., 2002), especially when limited nitrogen availability conditions occurs, as in the present case where most of the available N was fixed by sunn hemp (about 54 Kg N_2 ha^{-1} as indicated before).

The highest wheat grain yields (Fig. 4a) were obtained when sunn hemp was harvested between full and the end of flowering (R1 and R2) in 2021–22 and no significant differences were observed between the relay systems and the C in 2019–20, probably thanks to the synchronous N_2 release and rapid uptake of wheat at early growth stages. In fact, it was determined that the mineralization of sunn hemp N residues occurs fast (in about 2 weeks; Stallings and Balkcom, 2015). This could be the case of the C treatment where wheat was sown about one month after sunn hemp harvest. Whereas in the relay systems (R1 and R2) the species overlapped between 2 and 4 weeks, which may have favored the uptake by the succeeding cereal of the fixed N_2. Some studies report that legumes root nodule formation reaches a maximum activity between full flowering and pod-filling stage, with the nodule number and weight representing a predictor of biological nitrogen fixation capacity of the species (Pitumpe Arachchige et al., 2020). In fact, the highest number of sunn hemp nodules were observed in this period (data not shown, personal observation). Hence, the nitrogen fixed into the soil by sunn hemp may have reached similar values found (about 54 Kg N_2 ha^{-1}) in the adjacent crop rotation study (own unpublished data) between R1 and R2 stages, thus favoring its availability for the following wheat crop. In soybean it has been also observed that the maximum N_2 fixation rate occurs around the end of flowering / beginning of pod formation (Ciampitti et al., 2021). In the present study, the lack of additional mineral N fertilization on wheat might have emphasized the effect of the relay planting and the overall cropping systems performance. In addition, keeping the traditional sowing period of wheat at the end of October/beginning of November is an advantage in practical terms because in temperate humid climates as in the present study, autumn rains in mid-late November get the soil wet and impracticable, therefore the risk of soil sealing in late sowing increases significantly.

The aforementioned hypothesis is supported by the qualitative grain parameters presented in Table 3 and Fig. 5, which are deemed to be strictly dependent on nitrogen availability (Hoel, 2003; Pan et al., 2006; Triboi et al., 2000). Our measurements in a parallel trial shows (as indicated before) that sunn hemp is able to fix about 54 Kg N_2 ha^{-1} (own unpublished data). In this light, the PCA indicates that wheat qualitative traits were closely related to the harvest time and productivity of the precedent crop while wheat-planting density had a relatively lesser effect (Fig. 5). In fact, Stallings and Balkcom (2015) found that sunn hemp N concentration decreased with plant maturity but N content increased with biomass accumulation. Whereas the grains and straw yield of wheat was driven by the water availability (Fig. 5). Crude protein, wet gluten and ZI in 2019–20 were highest when sunn hemp was harvest at full flowering stage (R1) compared to C. The correlation between crude protein and wheat chlorophyll was significant and positive (Fig. 7b; $r = 0.65$), as well as all the other flour qualitative parameters suggesting that at this stage the legume crop reached its maximum capacity to fix N_2, and in synchrony with cereal crop uptake demands. Nitrogen availability in soil is also known to determine high chlorophyll content in leaves (Walsh et al., 2020), which in turn lead to increased protein content in grain (Hoel, 2003). In the present study a clear higher chlorophyll content emerged only in 2021–22 (Fig. 7), thus reflecting the general higher grain quality in this growing season (Table 3).

5. Conclusion

The present study has shown that relay-cropping not only maintains stable the overall biomass production of the system, but can also lead to improved grain quality for bread-making wheat (i.e. crude protein, wet gluten and ZI). The sunn hemp - wheat relay-cropping lignocellulosic biomass yield in R1 and R2 did not decrease with respect to C. In this light, postponing the harvest of sunn hemp until the full flowering stage (R1, November) maximized the grain quality with respect to sunn hemp harvested at early flowering stage (C, September), eventually from an enhanced availability of fixed N_2. Therefore, the implementation of relay systems in temperate environment appears to be a viable option to stretch the growing season of high yielding lignocellulosic crops of tropical origin such as sunn hemp. Nevertheless, substantial improvements are still required in for example species and variety selection, as well as agronomic management practices such as sowing times, methods, and harvesting operations. It's noteworthy that incorporating a lignocellulosic legume into a cereal based cropping system has the potential to improve the yield quantity and quality, while also provide valuable co-products like food proteins. Overall, the local availability of dedicated lignocellulosic feedstock could be greatly enhanced (over 10 Mg d.m. ha^{-1} y^{-1}) with a relay-cropping system that do not require mineral N fertilization and do not compete with food production, thus helping to reduce the gap in the transition towards carbon neutrality and complying with the additionality measures to produce bioenergy feedstocks set out by the Delegated Regulation of REDIII.

CRediT authorship contribution statement

Walter Zegada-Lizarazu: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Andrea Monti:** Writing – review & editing, Validation, Project administration, Funding acquisition. **Andrea Parenti:** Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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