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# Long-Term Yield and Quality Performance of Perennial Energy Grasses (*Agropyron spp.*) on Marginal Land

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**Abstract:** The cultivation of perennial grasses is one of the most desirable alternatives as energy feedstock, but it is difficult to achieve competitive yields under Mediterranean marginal conditions. The aim of this study was to assess the performance of three cool-season grasses (*Agropyron spp.*) from an agronomic and energetic point of view by comparing the dry matter (DM) yields, rain use efficiency, chemical composition, and biomass quality over an eight-year period in Spain under marginal rainfed conditions. The tall wheatgrass (*Agropyron elongatum* (Host) Beauv.) cultivars, Alkar (4.8 Mg DM·ha<sup>-1</sup>) and Jose (4.7 Mg DM·ha<sup>-1</sup>), achieved the highest yields. Productions below 0.5 Mg DM·ha<sup>-1</sup> were obtained when rainfall was lower than 150 mm between March and June. The biomass obtained from the tested grasses showed relatively high contents of ash, silicon, and alkali elements. Net calorific values ranged between 16.7 and 18.5 MJ·kg<sup>-1</sup> db. Differences in the composition among species and cultivars are not likely to affect their combustion behavior from a practical point of view. The ash content, as well as the concentrations of K, S, Na, and Cl, tended to decrease over the years. The results offered would be very useful for the implementation of this type of crop in marginal land.

**Keywords:** cool-season grasses; alternative crops; tall wheatgrass; crested wheatgrass; Siberian wheatgrass; energy crops

## 1. Introduction

The optimism regarding the potential of biofuels as an alternative to fossil fuels was high at the beginning of the 21st century [1]. Benefits, such as greenhouse gases (GHG) emission reduction, heat, electricity de-carbonization, and the development of rural areas, were expected for biofuel use [2]. However, there is speculation regarding the contribution of biofuels to the increase of food prices and the use of freshwater [3,4]. This raised important issues regarding the use of arable lands for growing crops dedicated to biofuel production [5–7] and it created a conflict between both alternatives [8].

In this context, marginal lands have been revealed as an important source of land for growing energy crops [7]. Marginal lands are areas where it is difficult to grow food crops profitably given the conditions that exist at the site, the agricultural policies and available growing methods, as well as the global or national economic situations [9,10]. According to the European Environmental Agency (EEA), marginal land is “a low-quality land whose production barely covers its cultivation costs”. The United States Department of Agriculture (USDA) defines marginal lands as “in farming, poor quality land that is likely to yield a poor term return”. Those lands are therefore characterized by low food and feed productivity associated with socio-economic and biophysical constraints [11,12].

The uses of perennial grasses have been widely reported as the best alternative for producing biomass in marginal lands in agronomic, economic, and environmental terms. Perennial grasses compared to traditional annual crops reduce soil nutrient depletion and soil erosion, require a low input for their production and provide a better profit margin, increase soil quality and carbon sequestration, enhance the agricultural ecosystems' biodiversity by avoiding the mono-cropping and providing protection and feed to wild fauna (mammals, birds and insects), and increase soil water retention [13–29]. The perennial grasses most used for producing biomass in Europe and the US are miscanthus (*Miscanthus giganteus* Greef et Deuter), giant reed (*Arundo donax* L.), switchgrass (*Panicum virgatum* L.), and reed canary grass (*Phalaris arundinacea* L.), but implanting them under marginal conditions is not recommended due to their soil and water requirements [30]. In turn, perennial cool-season grasses can be alternative bioenergy crops for sustainable biomass production on marginal lands generally not suited to grain crop production [31,32]. In particular, tall wheatgrass, crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), and Siberian wheatgrass (*Agropyron sibiricum* (Willd.) P. Beauv.) possess characteristics that can be useful in marginal lands. These species share similar traits regarding their salinity and drought tolerance, winter frost hardiness, and resistance to pests and illnesses [33–35], in addition to the rest of the advantages described above for being perennial grasses. All of them are cool-season grasses that have been traditionally used as pastures for grazing or for obtaining hay [36].

Many studies around the world provide information on yields and the quality of these grasses in terms of forage production for animals [36,37], while others show the production and quality of the biomass of different varieties on fertile arable land or grasslands [38–41]. However, very scarce information is available on the use of these *Agropyron* species as combustion feedstock on arable lands under marginal conditions. The composition and the combustion behavior of grass biomass are strongly influenced by crop management and developmental stage but also depend on the species considered and on site factors, such as soil characteristics and weather conditions [30,42,43]. The literature shows how the composition of cool-season grasses differs greatly within and among different locations [30,44,45], and they can thus have considerably different amounts of certain “anti-quality” minerals like Cl, K, Si, N, or S that cause emissions, slagging, and/or corrosion at the operating temperatures of combustion plants [30,42,43,45,46]. For all these reasons, it is important to clarify from a productive and a quality perspective whether or not cool-season grasses can be a long-term sustainable resource for energy feedstock under marginal conditions.

In the above context, the main aim of this study was to evaluate three species of *Agropyron* spp. from an agronomic and energy perspective, comparing their biomass yield, composition, and quality in a low productive agricultural area under rainfed conditions. One cultivar (cv.) of crested wheatgrass (Hycrest), one cultivar of Siberian wheatgrass (Vavilov II), and three cultivars of tall wheatgrass (Alkar, Jose, and Riparianslopes) were tested for eight years in a marginal location, while another three tall wheatgrass cultivars (Alkar, Bamar, and Szarvasi-1) were tested for five years in a second marginal location. These results are of key importance for determining the commercial future of these crops as a feedstock for energy production when growing under marginal conditions.

## 2. Materials and Methods

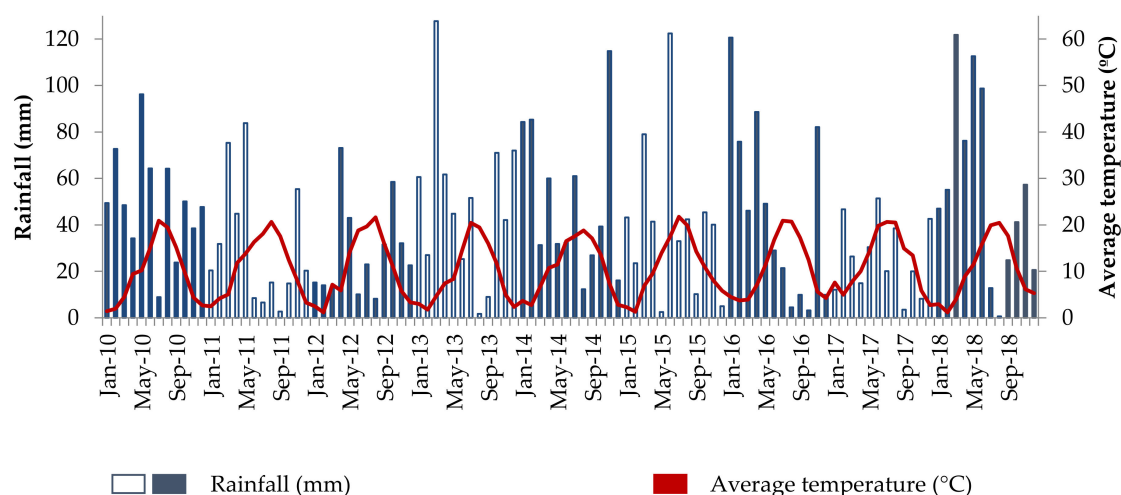
### 2.1. Experimental Sites

The study was carried out in two different locations of Spain's North-Central Plateau, one of the most extended areas of rainfed agriculture in southern Europe. The experimental fields were located in Cubo de la Solana (CS) [41° 36' N and 2° 28' W], 1010 m above sea level, and in Alconaba (AL) [41° 42' N and 2° 22' W], 1000 m above sea level.

Experimental fields were located in areas with severe biophysical limitations [47]. In both places, soils were poor and sandy (81% of sand in the CS fields and 88% of sand in the AL fields), with a content of coarse elements (>2 mm) higher than 15% in soil volume. Soils were characterized by low

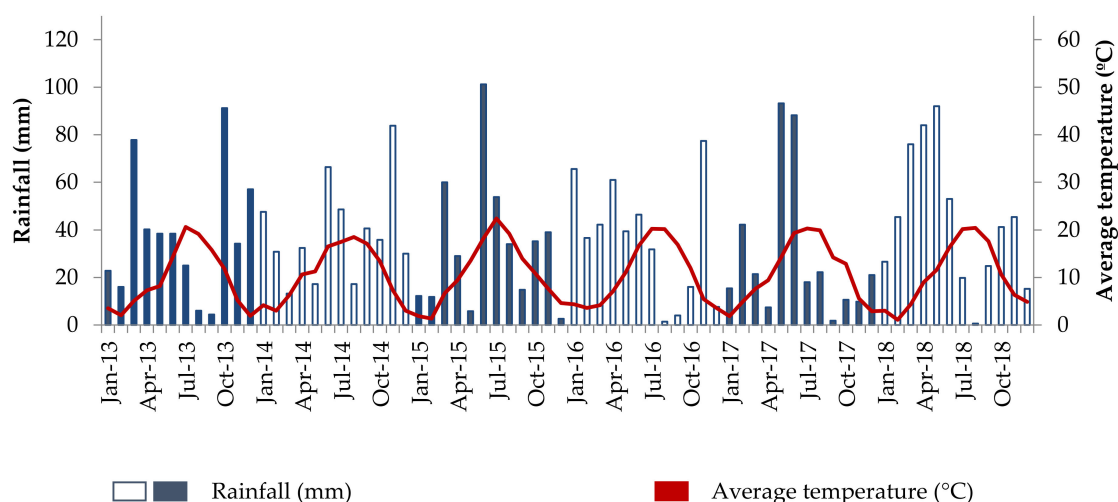
levels of organic matter ( $\leq 1\%$ ), and low water and nutrient retentions, indicating potential high losses by leaching. Soils were pH neutral and did not exhibit salinity problems. Soil nitrogen content was 0.06% in the CS plots and 0.03% in AL; the content in phosphorus ( $P_2O_5$ ) was 10.2 and 10.0  $mg \cdot kg^{-1}$  in CS and AL, respectively; and the content of the soil in potassium ( $K_2O$ ) was 152.5  $mg \cdot kg^{-1}$  in the CS fields and 150  $mg \cdot kg^{-1}$  in the AL plots. Soil samples were taken from the upper layer (0–30 cm) as indicated in ISO 10381-1, and analyzed according to ISO standards for texture (ISO 11277), pH (ISO 10390), electrical conductivity (ISO 11265), organic matter (ISO 10694), phosphorus (ISO 11263), potassium (ISO 11260), and ammonium and nitrates (ISO/TS 14256-1 EX).

Climate conditions were continental Mediterranean with cold winters, warm summers, and low rainfall levels. Climate data were taken from the nearest national grid of meteorological stations. The average annual rainfall during the study period was 502.7 mm in the CS fields, with 2018 being the wettest year (669 mm) and 2017 the driest (315 mm). Harvesting was hardly possible in 2017 due to the extremely low biomass production obtained as a consequence of the low annual precipitation registered at this location, particularly between March and June (123 mm). The annual average temperature was 10.6 °C, with minimum and maximum absolute temperatures of  $-11.9$  °C in January 2011 and 37 °C in August 2012, respectively. The free frost period was from late May to early October, and drought conditions usually appeared during the summer. Figure 1 shows the monthly rainfall and average temperatures during the study period in the CS experimental trials.



**Figure 1.** Rainfall and temperature data for Cubo de la Solana fields during the study years. Hollow and filled bars indicate different consecutive years.

The AL fields registered 437 mm of annual average rainfall, with 2018 being the wettest year (524 mm) and 2017 the driest with 351 mm. The annual average temperature was 10.5 °C, and the minimum and maximum absolute temperatures were  $-10.5$  °C in January 2017 and 36.01 °C in July 2015, respectively. AL exhibited the same frost and drought periods as CS. Figure 2 depicts the monthly rainfall and average temperatures.



**Figure 2.** Rainfall and temperature data for Alconaba fields during the study period. Hollow and filled bars indicate different consecutive years.

## 2.2. Experimental Design and Crop Management

CS field trials were studied for 8 years (October 2010–August 2018). The experiment in the CS fields was designed by sowing three repetitions in a randomized complete block design in 300-m<sup>2</sup> plots (3 m wide × 100 m long) for each experimental unit. As it was commented previously, the varieties sown were tall wheatgrass (cv. Alkar (TWA), cv. Jose (TWJ), cv. Riparianslopes (TWR)), crested wheatgrass (cv. Hycrest) (CWH), and Siberian wheatgrass (cv. Vavilov) (SWV).

In the AL fields, 3 cultivars of tall wheatgrass were sown with 4 repetitions for 5 years (October 2013–August 2018) in plots with a similar surface area, shape, and design as the CS fields. The cultivars sown in the AL experiment were tall wheatgrass (cv. Alkar (TWA), cv. Bamar (TWB), and cv. Szarvasi-1 (TWS)).

The previous crop in both trials was fallow land. It was plowed seven months earlier, cultivated in summer, and sown in October (2010 in the CS fields and 2013 in Al plots). The seeding dose was 20 kg·ha<sup>-1</sup> for tall wheatgrass cultivars and 15 kg·ha<sup>-1</sup> for crested wheatgrass and Siberian wheatgrass, with a seeding drill in rows 13 cm apart. A complex fertilizer NPK 8-24-8 (300 kg·ha<sup>-1</sup>) was applied for base fertilization in the establishment year. Base fertilization was applied two days before sowing and buried with a cultivator. In addition, plots were rolled in mid-March in the establishment year, prior to the application of the top fertilization. Experimental plots were sprayed in late April for controlling broadleaves weeds with 1 L·ha<sup>-1</sup> of 2,4-D acid 34.5% + 2-methyl-4-chlorophenoxyacetic acid 34.5%. Nitrogen fertilizer was applied as top dressing between mid- and late-March every year using nitrogen (27% purity) at 300 kg·ha<sup>-1</sup>·y<sup>-1</sup>. Crops were harvested with a mower conditioner from mid-July to early August depending on the year, always when crops were in the doughy grain stage and leaving 10 cm of stubble to encourage further regrowth. After 5 to 6 days of field drying, biomass was baled in prismatic bales (<10% moisture).

The main limiting factor for plant growth under rainfed conditions is water availability. Biomass production of crops depends on their efficiency in water use. Hence, crops with better efficiency also achieve a better economic margin. The cited efficiency is often expressed as water-use efficiency (WUE) or rain-use efficiency (RUE), and it is measured in terms of crop production per unit of water input. Specifically, RUE = yield (g DM·ha<sup>-1</sup>)/rainfall occurred (l) [48]. Although WUE and RUE have been primarily evaluated in traditional agronomic crops, they can also impact the potential of crops for cellulosic biofuels [49]. The RUE was calculated to assess the differences among cultivars and species. The calculation period occurred in the months with the highest vegetative activity (March to June), which is when rainfall is considered to be of key importance for biomass production in cool-season grasses under rainfed conditions, as other authors reported [48,50].

### 2.3. Experimental Characteristics and Analytical Methods

Sample preparation was performed according to ISO 14780:2017 [51] and test analyses were carried out following the corresponding international standards for solid biofuels (Table 1).

**Table 1.** Analytical techniques and standards followed during testing.

Property	Analytical Technique (Equipment)	Standard
Sample preparation	Subsampling and milling	ISO 14780:2017
Ash	Calcination at 550 °C	ISO 18122:2015
C, H, N	Elemental analysis by IRD and TCD (TruSpec, Leco)	ISO 16948:2015
S and Cl	Combustion bomb + IC (883 Basic IC Plus, Metrohm)	ISO 16994:2016
Calorific value	Calorimetry (C5003, Ika Verke)	ISO 18125:2017
Major elements: Al, Ca, Fe, K, Mg, Mn, Na, P, Si, Ti, Zn	MW digestion (Ethos Pro, Milestone) + ICP-OES (Jarrell Ash, Thermo Fisher)	ISO 16967:2015

IRD: Infrared Detection; TCD: Thermal Conductivity Detection, IC: Ion Chromatography; MW: microwave digestion; ICP-OES: Inductively Coupled Plasma—Optical Emission Spectroscopy.

Bales were weighed with a laboratory scale in the fields. Moisture content was measured by oven-drying subsamples in an oven at 40 °C until constant weight. Dry matter yields were calculated by taking into account the plot area, as well as the weight and moisture content of the bales.

Before baling, a combined sample per replicate plot, cultivar, and year was analyzed to determine the main properties and the chemical composition of the biomass. Every combined sample was formed by manually collecting 3 subsamples from different random locations in the replicate plot and leaving the same stubble as the mower did. Biomass from CS fields was analyzed for 7 years from 2012 until 2018. The biomass from the establishment year was not analyzed. Biomass from the AL plots was analyzed annually between 2014 and 2018.

Major elements were analyzed by digesting the ashes of the biomass obtained at 550 °C (ISO 18122:2015), followed by ICP-OES determination according to ISO 16967:2015. Results were expressed in biomass dry basis (db). Titanium contents were very low and frequently below the method quantification limit (<0.0002%), and thus the results obtained for this element are not shown or treated statistically. Calorific values were analyzed by calorimetry (ISO 16994:2016) and expressed as the gross calorific value at constant volume (GCV<sub>v,o</sub>) and net calorific value at constant pressure (NCV<sub>p,o</sub>), both in db.

A number of fuel predictive indices and ratios between chemical elements were calculated to predict the combustion behavior of the biomass cultivated. These indices were selected from the literature because they were previously used as a measure of the quality of other herbaceous biofuels for combustion.

In this sense, the ratio between the earth alkaline oxides and alkaline oxides (AE/A), the ratio between the content of K and the sum of Ca and Mg [K/(Ca + Mg)], as well as the molar ratio between the sum of Si, P, and K and the sum of Ca and Mg [(Si + P + K)/(Ca + Mg)] were used as indicators of problems related to ash sintering and fusion.

AE/A is calculated by dividing the concentrations of CaO and MgO and the concentrations of K<sub>2</sub>O and Na<sub>2</sub>O in the ash fuel [52]. Solid biofuels with AE/A ratios over 2 have been associated with a lower risk of ash sintering in a 1 MWth bubbling fluidized bed combustion pilot plant [52].

The K/(Ca + Mg) ratio was used as an indicator of slagging for the thermo-chemical processing of grasses [53]. It was estimated that grasses with ratios below 0.5 did not pose issues regarding ash sintering or ash fusion, e.g., in operational gasifiers [53].



A linear correlation between the ash sintering temperature (SST) and the molar ratio  $(\text{Si} + \text{P} + \text{K})/(\text{Ca} + \text{Mg})$  was also empirically demonstrated for P and K-rich fuel ash systems [54,55]. Zeng et al. did find a good correlation between SST and pellets produced from blends of miscanthus, a warm-season grass, with wood and wheat straw [55]. Fuels with lower  $(\text{Si} + \text{P} + \text{K})/(\text{Ca} + \text{Mg})$  molar ratios tend to exhibit higher ash sintering temperatures [54]. Fuels with indices below 1 and 3 are expected to exhibit SSTs equal to or higher than 1200 and 1000 °C, respectively [54].

The sum of  $\text{K} + \text{Na} + \text{Zn} + \text{Pb}$  can be used as an indicator of PM1 emissions [54]. Low PM1 emissions are expected for indices below 1000 mg kg<sup>-1</sup>. In turn, fuels with indices over 10,000 mg kg<sup>-1</sup> tend to cause high PM1 emissions [54]. As trace elements were not determined in this study, a Pb content of 1 mg kg<sup>-1</sup> was used to calculate this index, based on previous experimental trials where the biomass of tall wheatgrass was analyzed in these regions.

The molar Si/K ratio was used to predict aerosol formation during the combustion of grasses, among other fuels [54,56]. A high molar Si/K ratio can lead to a preferred formation of potassium silicates in the bottom ash, resulting in a lower K release and aerosol formation, but gaseous SO<sub>x</sub> and HCl emissions may increase [54].

The molar ratio  $(\text{K} + \text{Na})/[\text{x}(\text{2S} + \text{Cl})]$  was used to predict SO<sub>x</sub> and HCl emission levels [54,55]. In this study, an x factor of 4.9 was used to calculate this index, as this is the value that was experimentally determined by Sommersacher et al. for *Arundo donax*, also a cold-season grass perennial. For fuels with high molar ratios, S and Cl will be preferably embedded in the ashes, forming alkaline sulfates and chlorides, potentially resulting in low SO<sub>x</sub> and HCl emission levels [54,55].

The 2S/Cl molar ratio has been used as an indicator for high-temperature corrosion risks [54]. Fuels with ratios below 2 are expected to pose a severe risk. Minor risks are expected when ratios are >4. In turn, for 2S/Cl ratios <2, severe corrosion risks need to be taken into account, as a result of a high Cl surplus in aerosols [54].

#### 2.4. Statistical Analysis

Statistical analyses were performed using the Statgraphics Centurion XVII (Statpoint Technologies INC, 2017, The Plains, VA, USA) software. The effects of the different species (S), cultivars (C), and years (Y) on biomass production and composition as well as on empirical fuel indices were evaluated by means of the variance analysis (ANOVA). Multifactorial two-way ANOVAs (Type III sums of squares) with a maximum order interaction of 2 were used. All F-ratios were based on the residual mean square error. The normality of the data and the homoscedasticity of variances were verified using the standardized skewness/kurtosis and Levene's test, respectively. No data transformations were considered necessary. In AL fields, C, Y, and the interaction between both factors (C × Y) were considered to be fixed effects. In CS fields, two types of analysis were performed as different species and cultivars were tested. On the one hand, an analysis of variance with S, Y, and S × Y as fixed effects, with the factor species at three levels (tall wheatgrasses—TW, crested wheatgrass—CW, and Siberian wheatgrass—SW). On the other hand, an analysis of variance with species/cultivars (S/C), Y, and their interaction (X/C × Y) as fixed effects, and considering five levels for factor S/C (TWR, TWA, TWJ, CWH, and SWV) was conducted. Block (replicate plots) was considered a random effect at both sites. Mean differences across species, cultivars, and years were assessed using multiple range tests according to the Fisher's least significant difference (LSD) test. Significant effects were revealed at *p*-values ≤0.05 (95% of confidence level). Interactions among factors (species and years or cultivars and years) were depicted by using the means and Fisher's LSD interaction plots. Means, standard deviations (SD), 75% percentiles (P75), as well as minimum and maximum individual values obtained for each property were also included in their corresponding tables.

### 3. Results

#### 3.1. Biomass Yield and Rain Use Efficiency

According to the results obtained (Table 2), significant effects of species, cultivars, and years were found in biomass production at both experimental areas. RUE also had significant effects across species, cultivars, and years at both locations.

**Table 2.** Variance analysis of the fixed effect of species (S), cultivars (C), and year (Y) on dry matter yields and RUE of the biomass produced.

Source	Yield			RUE		
	df	F-Value	p-Value	df	F-Value	p-Value
<b>CS fields</b>						
S	2	4.51	0.0130	2	7.91	0.0006
Y	7	45.61	0.0000	7	28.56	0.0000
S × Y	14	3.65	0.0001	14	3.63	0.0001
<b>AL fields</b>						
C	2	2.70	0.0357	2	5.17	0.0087
Y	4	9.38	0.0000	4	3.53	0.0124
C × Y	8	3.98	0.0012	8	6.45	0.0000

df: degrees of freedom.

Biomass yields from the CS and AL fields are shown in Table 3. In CS fields, the year with the highest production was 2018 (9.8 Mg DM·ha<sup>-1</sup> average value), with tall wheatgrass cv. Jose being the most productive species with 12.6 Mg DM·ha<sup>-1</sup>. It should also be mentioned that the lowest biomass productions were achieved during the previous year (2017), due to the extremely low precipitation levels registered, especially in spring, which even made baling difficult with the technology used in this study. Additionally, low yields were also obtained during the establishment year (1.7 Mg DM·ha<sup>-1</sup>). When taking into account the whole study period (2011–2018), TWA and TWJ provided the highest yields, with annual average productions of 4.8 and 4.7 Mg DM·ha<sup>-1</sup>, respectively. In turn, CWH provided the lowest annual yields (2.8 Mg DM·ha<sup>-1</sup>).

In the AL plots, the best yields were obtained in 2018 (4.9 Mg DM·ha<sup>-1</sup>) and 2015 (4.7 Mg DM·ha<sup>-1</sup>), the latter due to the good performance of TWS (7 Mg DM·ha<sup>-1</sup> in 2015). In general, the lowest yields were achieved during the establishment year (1.9 Mg DM·ha<sup>-1</sup>), which were close to those registered in 2017 (2.8 Mg DM·ha<sup>-1</sup>), the one with the lowest annual rainfall. TWS provided the highest average annual yields (4.3 Mg DM ha<sup>-1</sup>), followed by TWB (3.5 Mg DM ha<sup>-1</sup>) and TWA (3.0 Mg DM ha<sup>-1</sup>). It is also worth mentioning that the TWS cultivar achieved better results during the first years of the experiment while TWA performed better later on.

All tall wheatgrass cultivars tested in CS showed a more efficient rainfall use (around 2.0 g DM·L<sup>-1</sup>) (Table 4) than crested and Siberian wheatgrass, which provided lower biomass yields for the same precipitation. Comparing years, the best rain efficiency was in 2013 and 2018 (2.6 g DM·L<sup>-1</sup> and 2.4 g DM·L<sup>-1</sup>, respectively). Similar rain efficiencies were achieved in 2014 and 2015. In connection with biomass production, 2017 was the year with the lowest rainfall efficiency due to the low precipitation registered during the period with the highest vegetative activity (123 mm). In the AL fields, TWS was the cultivar with the highest RUE (2.2 g DM·L<sup>-1</sup>), differing from TWA (1.4 g DM·L<sup>-1</sup>), the latter being the one with the lowest RUE.

**Table 3.** Biomass yields from CS and AL stations. Data provided in Mg of dry matter (DM)·ha<sup>-1</sup>.

Fields	S/C	Years								Mean
		2011	2012	2013	2014	2015	2016	2017	2018	
CS	TWA	2.0	2.6	7.5	3.3	5.3	5.9	0.1	11.5	4.8 ± 3.6 <sup>a</sup>
	TWJ	2.0	2.6	7.6	2.8	4.0	5.9	0.2	12.6	4.7 ± 3.9 <sup>a</sup>
	TWR	2.0	2.1	7.1	3.3	4.6	5.8	0.2	11.5	4.6 ± 3.6 <sup>a,b</sup>
	CWR	1.2	2.6	5.7	1.7	2.8	1.9	0.3	6.5	2.8 ± 2.2 <sup>b</sup>
	SWV	1.3	2.2	6.3	1.9	2.9	2.1	0.3	6.7	2.9 ± 2.3 <sup>a,b</sup>
	<b>mean± SD</b>	<b>1.7 ± 0.4<sup>d</sup></b>	<b>2.4 ± 0.2<sup>d</sup></b>	<b>6.9 ± 0.8<sup>b</sup></b>	<b>2.6 ± 0.8<sup>d</sup></b>	<b>3.9 ± 1.1<sup>c</sup></b>	<b>4.3 ± 2.1<sup>c</sup></b>	<b>0.2 ± 0.1<sup>e</sup></b>	<b>9.8 ± 2.9<sup>a</sup></b>	
AL	TWB	-	-	-	1.3	4.7	3.9	3.2	4.4	3.5 ± 1.5 <sup>a,b</sup>
	TWS	-	-	-	3.5	7.0	3.7	2.3	5.1	4.3 ± 1.8 <sup>a</sup>
	TWA	-	-	-	0.9	2.5	3.7	2.8	5.2	3.0 ± 1.3 <sup>b</sup>
	<b>mean± SD</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>1.9 ± 1.4<sup>c</sup></b>	<b>4.7 ± 2.2<sup>a</sup></b>	<b>3.8 ± 0.1<sup>ab</sup></b>	<b>2.8 ± 0.4<sup>b,c</sup></b>	<b>4.9 ± 0.4<sup>a</sup></b>	

S: species; C: cultivar; SD: standard deviation. Different letters indicate significant different means between cultivars and years for that property at the 95% confidence level according to multiple range tests.

**Table 4.** RUE from CS and AL stations. Data provided in g DM·L<sup>-1</sup>.

Fields	S/C	Years								Mean
		2011	2012	2013	2014	2015	2016	2017	2018	
CS	TWA	0.9	1.9	2.9	2.1	2.2	2.8	0.1	2.8	2.0 ± 1.0 <sup>a</sup>
	TWJ	1.0	1.9	2.9	1.8	1.6	2.8	0.2	3.1	1.9 ± 1.0 <sup>a</sup>
	TWR	1.0	1.5	2.8	2.1	1.9	2.7	0.2	2.8	1.9 ± 0.9 <sup>a</sup>
	CWR	0.6	1.9	2.2	1.1	1.2	0.9	0.2	1.6	1.2 ± 0.7 <sup>b</sup>
	SWV	0.6	1.6	2.4	1.2	1.2	1.0	0.2	1.6	1.2 ± 0.7 <sup>b</sup>
	<b>mean± SD</b>	<b>0.8 ± 0.2<sup>d</sup></b>	<b>1.8 ± 0.2<sup>c</sup></b>	<b>2.6 ± 0.3<sup>a</sup></b>	<b>1.6 ± 0.5<sup>c</sup></b>	<b>1.6 ± 0.4<sup>c</sup></b>	<b>2.0 ± 1.0<sup>b,c</sup></b>	<b>0.2 ± 0.1<sup>e</sup></b>	<b>2.4 ± 0.7<sup>a,b</sup></b>	
AL	TWB	-	-	-	1.0	2.4	2.0	1.8	1.4	1.8 ± 0.5 <sup>a,b</sup>
	TWS	-	-	-	2.7	3.6	2.0	1.1	1.7	2.2 ± 0.9 <sup>a</sup>
	TWA	-	-	-	0.7	1.3	2.0	1.3	1.7	1.4 ± 0.5 <sup>b</sup>
	<b>mean± SD</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>1.5 ± 1.1<sup>b</sup></b>	<b>2.4 ± 1.1<sup>a</sup></b>	<b>2.0 ± 0.1<sup>a,b</sup></b>	<b>1.4 ± 0.4<sup>b</sup></b>	<b>1.6 ± 0.1<sup>b</sup></b>	

S: species; C: cultivar; SD: standard deviation. Different letters indicate significant different means between cultivars and years for that property at the 95% confidence level according to multiple range tests.



### 3.2. Biomass Composition

The statistical significance of the factors considered (species, cultivars, and years) and their corresponding interactions in the biomass properties and chemical composition of the grasses cultivated at both sites (CS and AL) are listed in Table 5. The main properties and chemical composition of the different perennial grasses cultivated in CS during the 2011–2018 harvest period are shown in Tables 6 and 7. As explained in Section 2.1, CWH, SWV, and three tall wheatgrass cultivars (TWA, TWJ, and TWR) were tested. Results for the three tall wheatgrass cultivars tested in the AL experimental trials (TWA, TWB, and TWS, see Section 2.1) from harvest 2013 to harvest 2018 are listed in Tables 8 and 9. These tables include the mean values for three replicate plots per species, cultivar, and year (Table 6, Table 7, Table 8, Table 9).

**Table 5.** Influence of the different species, cultivars, and years on the biomass properties and chemical composition of the biomass cultivated at two locations (CS and AL).

Factor	CS Biomass			AL Biomass		
	S/C	Y	S/C × Y	C	Y	C × Y
df	4	6	24	2	4	8
Ash	**	**	ns	**	**	ns
C	*	**	ns	**	**	ns
H	**	**	ns	*	ns	*
N	ns	**	ns	**	**	ns
S	ns	**	ns	**	**	ns
Cl	ns	**	ns	**	**	**
GCV <sub>v,0</sub>	**	**	ns	**	**	ns
NCV <sub>p,0</sub>	*	**	ns	**	**	ns
Al	ns	**	ns	ns	**	ns
Ca	ns	**	*	ns	**	ns
Fe	ns	**	ns	ns	ns	ns
K	**	**	ns	*	**	ns
Mg	*	**	ns	ns	ns	ns
Mn	ns	**	ns	ns	*	ns
Na	**	*	ns	ns	ns	ns
P	ns	*	ns	ns	**	**
Si	*	**	ns	**	ns	ns
Zn	**	**	**	**	**	ns

S: species; C: cultivar; Y: year; df: degrees of freedom; \*\*  $p$ -value < 0.01; \*  $p$ -value = 0.01–0.05; ns:  $p$ -value > 0.05, according to Fisher's tests (order 2 ANOVA).

As can be seen in Table 5, the species and cultivars tested in the CS fields differed significantly in their calorific value, ash, C, H, K, Mg, Na, Si, and Zn contents. No significant differences were found in their N, S, Cl, Al, Ca, Fe, Mn, and P contents.

For all the properties with  $p$ -values below 0.05 for the S/C factor, the main differences were found across species rather than among cultivars. In this sense, tall wheatgrass showed significant differences with respect to the other wheatgrass species tested (crested and Siberian), while all tall wheatgrass cultivars exhibited similar chemical compositions (Tables 6 and 7).

**Table 6.** Ash content, calorific values, and ultimate analysis of the biomass of different perennial grasses collected between 2012 and 2018 at CS plots. Mean values for three replicate plots per species, cultivar, and year.

S/C	Y	Ash (g·kg <sup>-1</sup> db)	C (g·kg <sup>-1</sup> db)	H (g·kg <sup>-1</sup> db)	N (g·kg <sup>-1</sup> db)	S (g·kg <sup>-1</sup> db)	Cl (g·kg <sup>-1</sup> db)	GCV <sub>v,0</sub> (MJ·kg <sup>-1</sup> db)	NCV <sub>p,0</sub> (MJ·kg <sup>-1</sup> db)
TWA	2012	48	482	60	14	1.6	1.7	19.1	17.8
	2013	44	473	60	10	1.0	1.1	19.0	17.7
	2014	45	459	60	9.5	1.0	1.7	18.6	17.3
	2015	39	473	61	11	1.3	1.9	19.2	17.9
	2016	43	470	61	12	0.7	1.1	18.5	17.2
	2017	47	484	61	15	0.9	2.0	19.1	17.8
	2018	38	472	61	8.4	0.6	1.4	18.6	17.3
	<b>mean ±SD</b>		44 ± 4 <sup>a</sup>	473 ± 8 <sup>b</sup>	6.1 ± 0.5 <sup>b</sup>	11 ± 2.4	1.0 ± 0.4	1.5 ± 0.4	18.9 ± 0.3 <sup>b</sup>
TWJ	2012	50	480	60	13.9	1.6	2.1	19.0	17.7
	2013	45	469	60	9.6	1.1	1.4	18.8	17.5
	2014	36	458	60	8.6	1.0	1.4	18.5	17.2
	2015	37	469	61	8.5	1.2	1.9	19.0	17.6
	2016	44	477	61	10.9	0.8	1.2	18.8	17.5
	2017	48	482	63	14.9	1.3	3.2	19.6	18.3
	2018	41	475	61	7.7	0.7	1.4	18.7	17.3
	<b>mean ±SD</b>		43 ± 5 <sup>a</sup>	473 ± 8 <sup>b</sup>	6.1 ± 1.0 <sup>b</sup>	11 ± 3	1.1 ± 0.3	1.8 ± 0.7	18.9 ± 0.3 <sup>b</sup>
TWR	2012	48	482	60	14.5	1.6	1.5	19.2	17.9
	2013	41	472	60	10.0	1.0	1.0	19.4	18.1
	2014	40	458	60	10.1	1.0	1.8	18.6	17.3
	2015	38	456	62	9.5	1.2	2.1	19.0	17.7
	2016	46	476	61	9.9	0.5	1.4	19.1	17.8
	2017	46	484	63	16.1	1.1	2.5	19.6	18.2
	2018	37	472	61	7.8	0.5	1.2	18.7	17.4
	<b>mean ±SD</b>		42 ± 4 <sup>b</sup>	471 ± 11 <sup>b</sup>	6.1 ± 1.0 <sup>b</sup>	11.1 ± 3.0	1.0 ± 0.4	1.6 ± 0.5	19.1 ± 0.4 <sup>a</sup>

Table 6. Cont.

S/C	Y	Ash (g·kg <sup>-1</sup> db)	C (g·kg <sup>-1</sup> db)	H (g·kg <sup>-1</sup> db)	N (g·kg <sup>-1</sup> db)	S (g·kg <sup>-1</sup> db)	Cl (g·kg <sup>-1</sup> db)	GCV <sub>v,0</sub> (MJ·kg <sup>-1</sup> db)	NCV <sub>p,0</sub> (MJ·kg <sup>-1</sup> db)
CWH	2012	38	487	61	12.4	1.1	1.9	19.2	17.9
	2013	39	462	61	13.5	0.8	1.4	18.9	17.6
	2014	39	460	61	10.9	1.0	1.5	18.9	17.6
	2015	32	475	62	11.5	1.2	2.6	19.5	18.1
	2016	29	465	63	08.1	0.6	0.9	18.9	17.5
	2017	42	486	63	15.6	0.9	2.0	19.7	18.3
	2018	30	474	62	08.1	0.5	0.9	18.6	17.2
	<b>mean ±SD</b>		36 ± 5 <sup>b</sup>	473 ± 11 <sup>b</sup>	62 ± 1.0 <sup>a</sup>	11.4 ± 2.8	0.9 ± 0.2	1.6 ± 0.6	19.1 ± 0.4 <sup>a</sup>
SWV	2012	40	488	61.3	13.1	1.2	1.6	19.4	18.1
	2013	3.5	475	61.0	10.4	1.0	1.3	19.0	17.7
	2014	45	460	61.0	14.7	1.1	1.8	18.8	17.5
	2015	33	481	61.0	12.0	1.3	2.1	19.4	18.0
	2016	36	475	62.3	13.4	1.1	1.6	18.9	17.5
	2017	40	487	63.0	14.4	0.8	1.6	19.5	18.2
	2018	30	479	62.0	09.4	0.5	1.0	18.8	17.4
	<b>mean ±SD</b>		3.7 ± 0.5 <sup>b</sup>	478 ± 9.0 <sup>a</sup>	61.7 ± 0.8 <sup>a</sup>	12.5 ± 2.0	1.0 ± 0.3	1.6 ± 0.4	19.1 ± 0.3 <sup>a</sup>
<b>Global mean ±SD</b>		41 ± 7	476 ± 9	61 ± 1.0	11.7 ± 3.1	1.0 ± 0.4	1.6 ± 0.7	19.0 ± 0.4	17.7 ± 0.4
<b>Min. value</b>		27	456	60	6.2	0.4	0.8	18.3	16.9
<b>Max. value</b>		58	491	64	18.9	1.9	4.6	19.8	18.5
<b>P75</b>		46	484	62	14.0	1.2	1.9	19.3	17.9

Different letters indicate significant different means between species/cultivars for that property at the 95% confidence level according to multiple range tests. Letters were only included when the *p*-value for the cultivar factor was below 0.05.

**Table 7.** Major elements present in the biomass of different grasses collected at CS plots between 2012 and 2018. Mean values for three replicate plots per species and year.

S/C	Y	Al (g·kg <sup>-1</sup> db)	Ca (g·kg <sup>-1</sup> db)	Fe (g·kg <sup>-1</sup> db)	K (g·kg <sup>-1</sup> db)	Mg (g·kg <sup>-1</sup> db)	Mn (g·kg <sup>-1</sup> db)	Na (g·kg <sup>-1</sup> db)	P (g·kg <sup>-1</sup> db)	Si (g·kg <sup>-1</sup> db)	Zn (g·kg <sup>-1</sup> db)
TWA	2012	0.09	2.3	0.033	14.3	0.78	0.069	0.35	1.1	4.3	0.012
	2013	0.03	2.2	0.025	13.8	0.70	0.073	0.12	1.2	4.6	0.013
	2014	0.05	2.1	0.004	11.2	0.55	0.079	0.06	1.0	6.8	0.013
	2015	0.22	1.9	0.098	8.6	0.61	0.071	0.08	0.9	5.8	0.008
	2016	0.06	2.3	0.029	10.8	0.68	0.067	0.07	1.0	6.9	0.008
	2017	0.20	3.1	0.062	11.0	0.85	0.106	0.12	0.9	7.3	0.011
	2018	0.04	1.6	0.011	11.4	0.56	0.052	0.22	1.2	4.9	0.011
	<b>mean ±SD</b>		0.10 ± 0.08	2.2 ± 0.4	0.037 ± 0.032	11.6 ± 1.9 <sup>a</sup>	0.68 ± 0.11 <sup>a</sup>	0.074 ± 0.016	0.15 ± 0.10 <sup>a</sup>	1.0 ± 0.1	5.8 ± 1.2 <sup>a</sup>
TWJ	2012	0.14	2.5	0.049	15.5	0.73	0.095	0.21	1.3	4.3	0.013
	2013	0.01	1.6	0.008	14.5	0.58	0.033	0.14	1.4	4.3	0.011
	2014	0.04	1.6	0.019	10.5	0.45	0.085	0.05	1.0	4.0	0.012
	2015	0.14	2.1	0.083	8.4	0.61	0.093	0.10	0.8	5.7	0.010
	2016	0.08	2.6	0.044	12.1	0.70	0.093	0.07	1.0	5.6	0.011
	2017	0.30	3.6	0.066	11.8	0.92	0.105	0.09	1.0	6.8	0.012
	2018	0.05	1.4	0.022	13.2	0.55	0.089	0.02	1.3	4.6	0.017
	<b>mean ±SD</b>		0.11 ± 0.10	2.2 ± 0.8	0.042 ± 0.027	12.3 ± 2.4 <sup>a</sup>	0.65 ± 0.15 <sup>a,b</sup>	0.085 ± 0.024	0.10 ± 0.06 <sup>a</sup>	1.1 ± 0.2	5.1 ± 1.0 <sup>a,b</sup>
TWR	2012	0.09	2.5	0.033	14.4	0.77	0.076	0.34	1.1	4.3	0.012
	2013	0.05	1.9	0.019	14.0	0.62	0.060	0.14	1.2	4.4	0.014
	2014	0.03	1.9	0.007	11.1	0.52	0.070	0.05	1.0	4.7	0.013
	2015	0.14	2.2	0.071	8.7	0.67	0.066	0.16	0.9	5.3	0.008
	2016	0.04	2.2	0.020	12.9	0.66	0.079	0.07	1.0	6.0	0.011
	2017	0.27	3.2	0.055	11.7	0.90	0.090	0.13	1.0	6.5	0.011
	2018	0.06	1.6	0.034	11.1	0.56	0.111	0.12	1.1	4.3	0.0013
	<b>mean ±SD</b>		0.10 ± 0.08	2.2 ± 0.05	0.034 ± 0.022	12.0 ± 2.0 <sup>a</sup>	0.67 ± 0.13 <sup>a</sup>	0.079 ± 0.017	0.15 ± 0.10 <sup>a</sup>	1.0 ± 0.1	5.1 ± 0.9 <sup>a,b</sup>

Table 7. Cont.

S/C	Y	Al (g·kg <sup>-1</sup> db)	Ca (g·kg <sup>-1</sup> db)	Fe (g·kg <sup>-1</sup> db)	K (g·kg <sup>-1</sup> db)	Mg (g·kg <sup>-1</sup> db)	Mn (g·kg <sup>-1</sup> db)	Na (g·kg <sup>-1</sup> db)	P (g·kg <sup>-1</sup> db)	Si (g·kg <sup>-1</sup> db)	Zn (g·kg <sup>-1</sup> db)
CWH	2012	0.08	1.8	0.033	13.1	0.47	0.049	0.01	1.0	2.6	0.013
	2013	0.02	1.9	0.018	13.2	0.50	0.063	0.04	1.0	3.4	0.018
	2014	0.02	2.8	0.010	08.7	0.52	0.111	0.01	0.8	4.6	0.017
	2015	0.13	2.3	0.061	07.3	0.52	0.064	0.03	0.8	4.8	0.010
	2016	0.05	2.2	0.032	07.5	0.52	0.080	<0.01	0.7	3.0	0.010
	2017	0.24	4.1	0.054	08.9	0.92	0.213	0.01	0.8	5.8	0.022
	2018	0.05	2.1	0.015	08.4	0.53	0.080	<0.01	1.0	3.8	0.014
<b>mean ±SD</b>		0.09 ± 0.08	2.5 ± 0.8	0.032 ± 0.019	9.6 ± 2.5 <sup>b</sup>	0.57 ± 0.16 <sup>b,c</sup>	0.094 ± 0.056	0.02 ± 0.01 <sup>b</sup>	0.9 ± 0.1	4.0 ± 1.1 <sup>b</sup>	0.015 ± 0.004 <sup>b</sup>
SWV	2012	0.15	1.7	0.051	13.3	0.48	0.048	0.02	1.2	2.9	0.013
	2013	0.02	1.8	0.018	11.1	0.43	0.043	0.02	1.1	3.3	0.011
	2014	0.05	4.3	0.026	9.8	0.70	0.147	0.03	1.1	5.9	0.039
	2015	0.16	2.5	0.010	7.4	0.56	0.107	0.04	1.0	4.2	0.012
	2016	0.08	3.1	0.043	9.8	0.57	0.128	0.01	0.7	3.5	0.012
	2017	0.22	3.7	0.064	8.4	0.70	0.191	0.01	0.8	6.3	0.016
	2018	0.05	1.9	0.011	8.6	0.42	0.080	<0.01	0.9	3.1	0.014
<b>mean</b>		0.10 ± 0.07	2.7 ± 1.0	0.045 ± 0.026	9.8 ± 2.0 <sup>b</sup>	0.55 ± 0.12 <sup>c</sup>	0.106 ± 0.054	0.02 ± 0.01 <sup>b</sup>	1.0 ± 0.2	4.2 ± 1.4 <sup>b</sup>	0.017 ± 0.010 <sup>b</sup>
<b>Global mean ±SD</b>		0.11 ± 0.08	2.4 ± 0.8	0.038 ± 0.024	11.2 ± 2.6	0.64 ± 0.17	0.090 ± 0.05	0.09 ± 0.13	1.0 ± 0.2	4.8 ± 1.7	0.013 ± 0.005
<b>Min. value</b>		0.01	1.1	<0.001	6.9	0.39	0.030	<0.01	0.5	1.8	0.007
<b>Max. value</b>		0.42	4.4	0.100	18.4	1.02	0.280	0.62	1.6	9.3	0.039
<b>P75</b>		0.14	2.8	0.053	13.2	0.78	0.100	0.12	1.2	5.9	0.014

Different letters indicate significant different means between species/cultivars for that property at the 95% confidence level according to multiple range tests. Letters were only included when the *p*-value for the cultivar factor was below 0.05.

Tall wheatgrass cultivars showed significantly higher ash contents, with mean values between 42 and 44  $\text{g}\cdot\text{kg}^{-1}$  db, depending on the cultivar, than crested (36  $\text{g}\cdot\text{kg}^{-1}$  db) and Siberian (37  $\text{g}\cdot\text{kg}^{-1}$  db) wheatgrass (Table 6). Accordingly, the calorific values of TWA and TWJ were slightly lower than those found for the rest of the tall wheatgrass cultivars considered, with mean net calorific values of 17.6  $\text{MJ}\cdot\text{kg}^{-1}$  db (TWA and TWJ) as opposed to 17.7–17.8  $\text{MJ}\cdot\text{kg}^{-1}$  db (TWR, CWH, and SWV). With regard to major elements (Table 7), tall wheatgrass cultivars showed higher K and Na (and in general Mg and Si) contents and lower Zn concentrations than the other species (CWH and SWV). TW cultivars averaged 12  $\text{g}\cdot\text{kg}^{-1}$  db K, 0.67  $\text{g}\cdot\text{kg}^{-1}$  db Mg, 5.3  $\text{g}\cdot\text{kg}^{-1}$  db Si, 0.13  $\text{g}\cdot\text{kg}^{-1}$  db Na, and 0.012  $\text{g}\cdot\text{kg}^{-1}$  db Zn, as opposed to CWH and SWV with mean values of 9.7  $\text{g}\cdot\text{kg}^{-1}$  db K, 0.56  $\text{g}\cdot\text{kg}^{-1}$  db Mg, 4.1  $\text{g}\cdot\text{kg}^{-1}$  db Si, 0.02  $\text{g}\cdot\text{kg}^{-1}$  db Na, and 0.016  $\text{g}\cdot\text{kg}^{-1}$  db Zn.

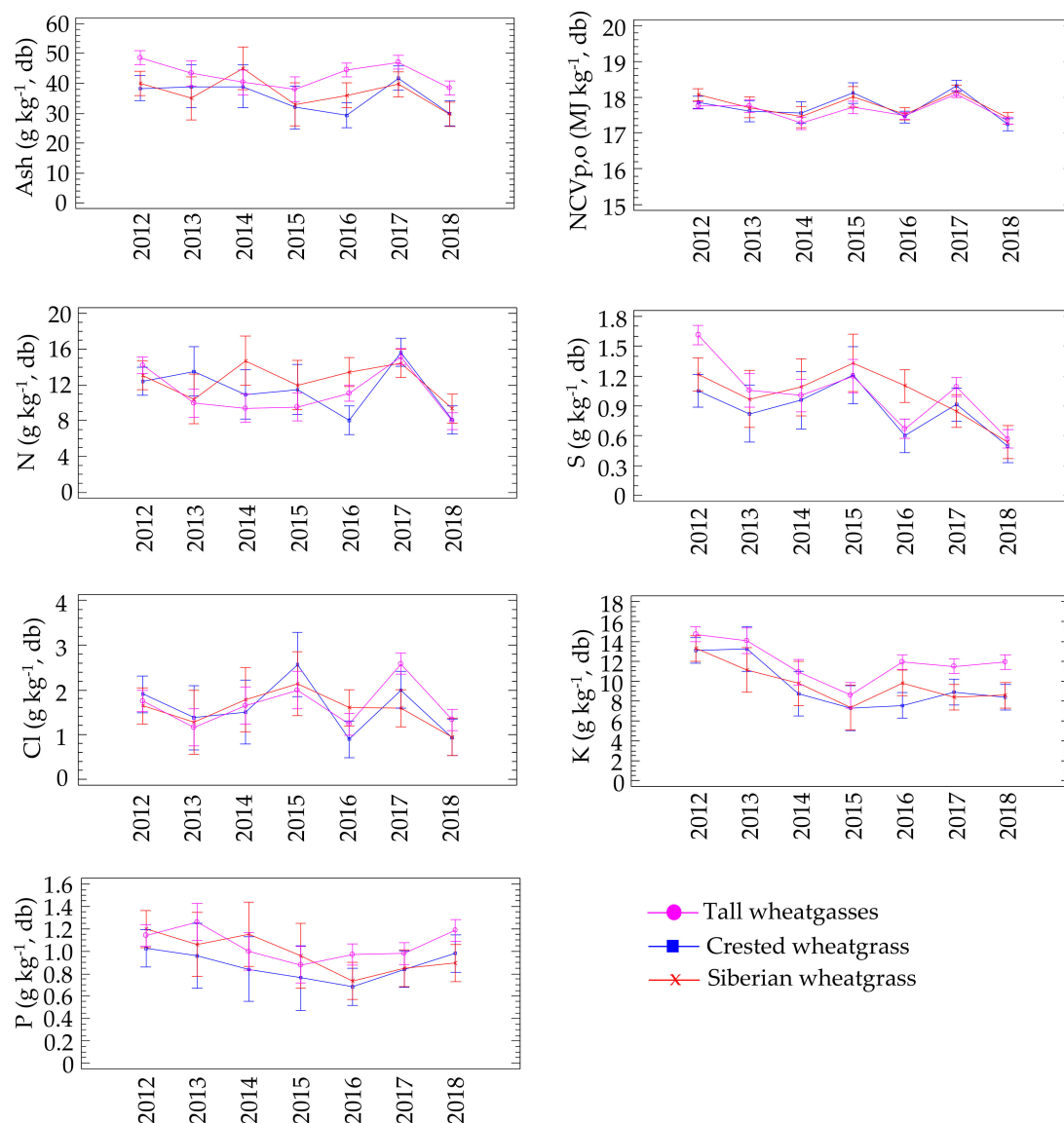
The three tall wheatgrass cultivars grown in the CS fields only differed in their calorific values (Table 6), finding a net calorific value of 17.7  $\text{MJ}\cdot\text{kg}^{-1}$  db for TWR and 17.6  $\text{MJ}\cdot\text{kg}^{-1}$  for TWA and TWJ cultivars, in connection with the slightly lower mean ash contents found in the biomass of the former (not significant).

Biomass properties and composition showed significant differences across the years for both experimental sites (Table 5). In CS fields, annual significant variations (2012–2018) were found for all properties (Table 5). Figure 3 shows how the ash content, net calorific value, and N, S, Cl, K, and P concentrations varied across species and years (interaction plots). In general, ash, S, K, Na, and Cl mean contents tended to decrease over time. Mean reductions of 22% ash, 60% S, 27% K, 48% Na, and 35% Cl were found for the biomass collected in 2018 with respect to that collected in 2012, the following growing season after sowing. The ash content of TW cultivars diminished from a mean content of 49  $\text{g}\cdot\text{kg}^{-1}$  db in 2012 to 39  $\text{g}\cdot\text{kg}^{-1}$  db in 2018 (a 21% reduction, Table 8). CW and SW ash contents also decreased over time, from 39  $\text{g}\cdot\text{kg}^{-1}$  db in 2012 to 30  $\text{g}\cdot\text{kg}^{-1}$  db in 2018, a 24% reduction. N, S, and Cl contents in TW cultivars decreased from 14  $\text{g}\cdot\text{kg}^{-1}$  db, 1.6  $\text{g}\cdot\text{kg}^{-1}$  db, and 1.8  $\text{g}\cdot\text{kg}^{-1}$  db in 2012, respectively, to 8.0  $\text{g}\cdot\text{kg}^{-1}$  db, 0.6  $\text{g}\cdot\text{kg}^{-1}$  db, and 1.3  $\text{g}\cdot\text{kg}^{-1}$  db in 2018. CW and SW species showed the same trend, showing 13  $\text{g}\cdot\text{kg}^{-1}$  db N, 1.1  $\text{g}\cdot\text{kg}^{-1}$  db S, and 1.8  $\text{g}\cdot\text{kg}^{-1}$  db Cl in 2012 and 8.7  $\text{g}\cdot\text{kg}^{-1}$  db N, 0.5  $\text{g}\cdot\text{kg}^{-1}$  db S, and 0.9  $\text{g}\cdot\text{kg}^{-1}$  db Cl in 2018, the last year of the study.

Net calorific values ranged from 16.9–18.5  $\text{MJ}\cdot\text{kg}^{-1}$ , and averaged 17.7  $\text{MJ}\cdot\text{kg}^{-1}$  db across species and years (Table 6). When taking into account a particular cultivar or species, the standard deviations across the years for the mean calorific values were maintained in the 0.3–0.4  $\text{MJ}\cdot\text{kg}^{-1}$  range. N and Cl contents averaged 12  $\text{g}\cdot\text{kg}^{-1}$  db and 1.6  $\text{g}\cdot\text{kg}^{-1}$  db across species and years but varied largely across growing seasons. N ranged 6.2–19  $\text{g}\cdot\text{kg}^{-1}$  db and Cl varied between 0.8 and 4.6  $\text{g}\cdot\text{kg}^{-1}$  db, depending on the species and years considered.

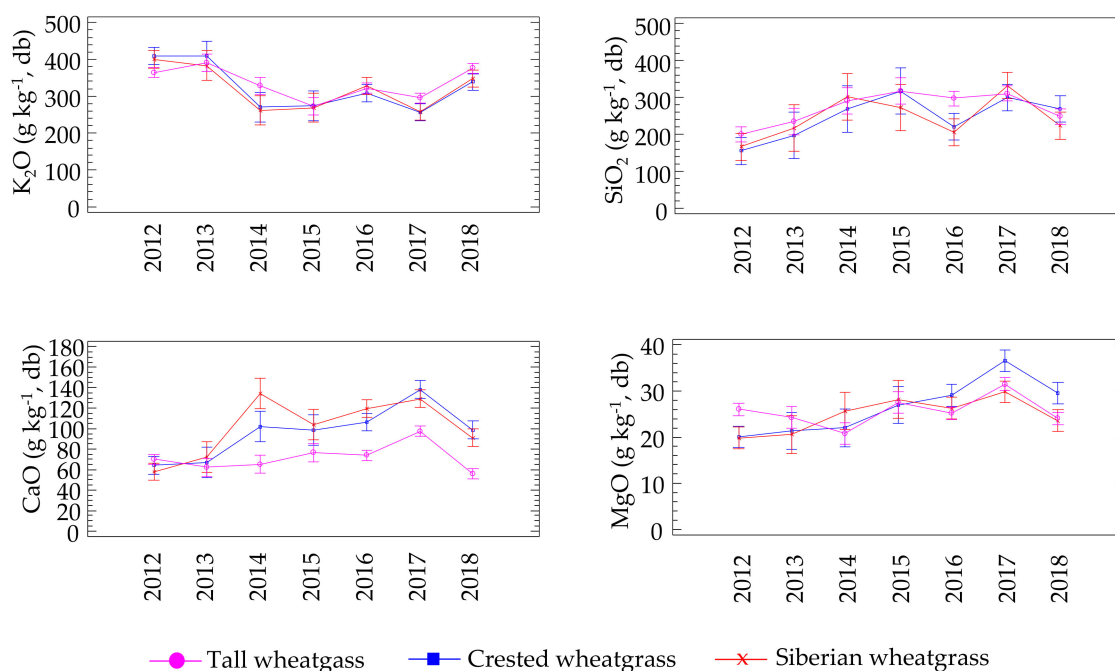
With regard to major ash-forming elements (Table 7), the biomass collected in 2012 showed higher K, Na, Al, and Fe contents than the biomass analyzed in 2018. In this period of time, K decreased from 15  $\text{g}\cdot\text{kg}^{-1}$  db to 12  $\text{g}\cdot\text{kg}^{-1}$  db in TW cultivars (19%), and from 13  $\text{g}\cdot\text{kg}^{-1}$  db to 8.5  $\text{g}\cdot\text{kg}^{-1}$  db in CW and SW species (36%). Na concentrations also decreased from 0.30  $\text{g}\cdot\text{kg}^{-1}$  db in 2012 to 0.12  $\text{g}\cdot\text{kg}^{-1}$  db in 2018 for the TW cultivars (59%). As mentioned earlier, CW and SW showed lower Na contents than TW, averaging 0.016  $\text{g}\cdot\text{kg}^{-1}$  db in 2012, and were below 0.010  $\text{g}\cdot\text{kg}^{-1}$  db in 2018.





**Figure 3.** Net calorific value, ash, N, P, K, S, and Cl contents across species and years for the biomass cultivated in CS fields. Means and Fisher's LSD interaction plots.

The concentrations of  $K_2O$ ,  $SiO_2$ ,  $CaO$ , and  $MgO$  in the ashes of the biomass grown in the CS fields are shown in Figure 4. From 2013 onwards, the contents of  $K_2O$  decreased over time, while  $SiO_2$ ,  $CaO$ , and  $MgO$  concentrations tended to increase as the crop aged. This trend was observed for all the species tested excepting in case of the  $CaO$  content of tall wheatgrass cultivars, which remained practically constant in the period 2012–2016, increased in 2017 (the most arid year), and decreased in 2018. The concentrations of  $CaO$  for CW and SW species averaged  $65 \text{ g}\cdot\text{kg}^{-1} \text{ db}$  in 2012–2013 and almost doubled to  $110 \text{ g}\cdot\text{kg}^{-1} \text{ db}$  from 2014–2018. Averaged across species, a mean  $K_2O$  decrease of 21% (from  $390 \text{ g}\cdot\text{kg}^{-1} \text{ db}$  to  $310 \text{ g}\cdot\text{kg}^{-1} \text{ db}$ ) and mean increases of 14%  $MgO$  (from  $23 \text{ g}\cdot\text{kg}^{-1} \text{ db}$  to  $27 \text{ g}\cdot\text{kg}^{-1} \text{ db}$ ) and 39%  $SiO_2$  (from  $200 \text{ g}\cdot\text{kg}^{-1} \text{ db}$  to  $280 \text{ g}\cdot\text{kg}^{-1} \text{ db}$ ) were found in the ashes of the biomass from 2012–2013 with respect to the rest of the period studied (2014–2018). Significant differences between those periods were also observed for other ash-forming elements (data not shown).  $Na_2O$  and  $P_2O_5$  decreased by 50% and 9%, respectively, while  $ZnO$  increased by 12%.

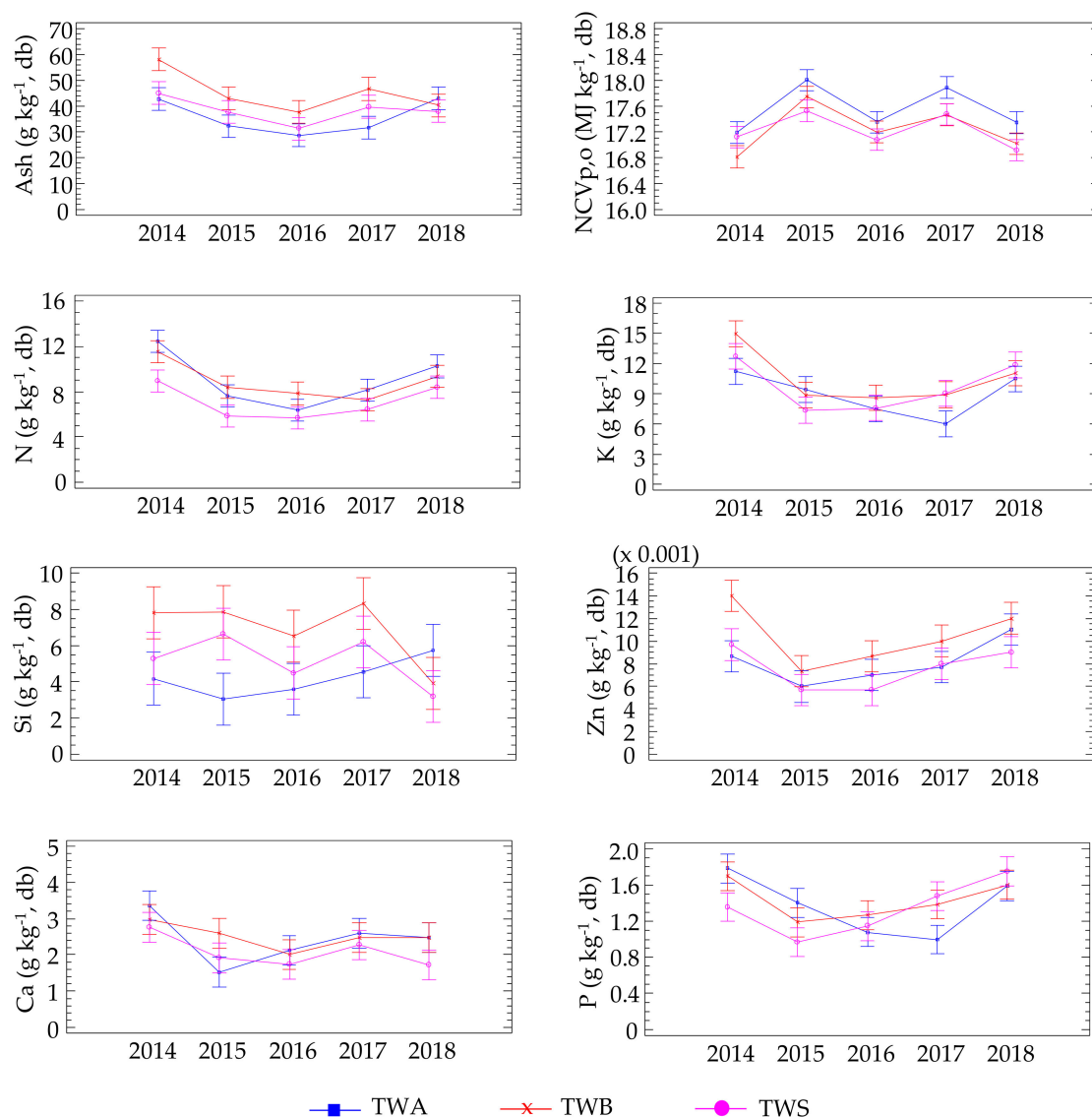


**Figure 4.** Si, K, Ca, and Mg variation across species and years for the biomass cultivated in CS fields (concentrations expressed as their corresponding oxides in the ash). Means and Fisher's LSD interaction plots.

Despite the fact that the variations for most properties was small, the three cultivars tested in the AL (TWA, TWB, and TWS) fields differed significantly in their calorific value, ash, C, H, N, S, Cl, K, Si, and Zn contents (Table 5). No significant differences were found across cultivars regarding their Al, Ca, Fe, Mg, Mn, Na, and P contents.

NCV averaged 17.4 MJ $\cdot$ kg $^{-1}$  db across cultivars and years and ranged from 16.7–18.1 MJ $\cdot$ kg $^{-1}$  db (Table 10). The TWA cultivar showed slightly higher net calorific values (17.6 MJ $\cdot$ kg $^{-1}$  db) than TWB and TWS (17.3 MJ $\cdot$ kg $^{-1}$  db), in accordance with its lower mean ash content (3.6%) and higher C content (47.3%). The TWB cultivar exhibited slightly higher ash (46 g $\cdot$ kg $^{-1}$  db), K (10 g $\cdot$ kg $^{-1}$  db), Si (7.8 g $\cdot$ kg $^{-1}$  db), and Zn (0.010 g $\cdot$ kg $^{-1}$  db) contents than the rest of the cultivars, which averaged 37 g $\cdot$ kg $^{-1}$  db ash, 8.9 g $\cdot$ kg $^{-1}$  db K, 4.9 g $\cdot$ kg $^{-1}$  db Si, and 0.008 g $\cdot$ kg $^{-1}$  db Zn (Table 9). TWA showed the lowest Cl contents (averaging 2.0 g $\cdot$ kg $^{-1}$  db across years), while TWS exhibited the lowest N (6.6 g $\cdot$ kg $^{-1}$  db) and S (0.9 g $\cdot$ kg $^{-1}$  db) contents (Table 8).

Annual significant variations were found for all properties in the study period (2014–2018), except for H, Fe, Mg, Na, and Si contents (Table 5). Similarly, for the biomass cultivated in the CS fields, ash, S, and K contents tended to decrease over time (Figure 5). The mean contents of other elements, such as N, Ca, P, and Mn, also decreased across the years (Tables 8 and 9), finding significant differences particularly between the biomass collected the first year after establishment (2014) with respect to that collected the following years (2015–2018). Ash contents were always maintained below 63 g $\cdot$ kg $^{-1}$  db, and averaged 48 g $\cdot$ kg $^{-1}$  db in 2014, with mean contents varying between 43 g $\cdot$ kg $^{-1}$  db and 58 g $\cdot$ kg $^{-1}$  db depending on the cultivar, and 38 g $\cdot$ kg $^{-1}$  db between 2015 and 2018 (29–47 g $\cdot$ kg $^{-1}$  db). K, N, and S averaged 12 g $\cdot$ kg $^{-1}$  db, 9.6 g $\cdot$ kg $^{-1}$  db, and 1.2 g $\cdot$ kg $^{-1}$  db in 2014, decreasing the following years to mean contents of 8.4 g $\cdot$ kg $^{-1}$  db, 7.3 g $\cdot$ kg $^{-1}$  db, and 0.9 g $\cdot$ kg $^{-1}$  db, respectively. However, although significant differences were found across the years, no clear trend was found for other elements like Cl and Al.



**Figure 5.** Net calorific value, ash, N, P, K, Ca, Si, and Zn contents across cultivars and years for the biomass cultivated in AL fields. Means and Fisher's LSD interaction plots.

The Na and Cl Concentration levels of tall wheatgrass varieties cultivated in the AL fields (Tables 8 and 9) were higher than those of the biomass grown in CS (Tables 6 and 7). TWA, the only cultivar that was considered at both sites, averaged  $1.5 \text{ g}\cdot\text{kg}^{-1}$  db of Cl and  $0.15 \text{ g}\cdot\text{kg}^{-1}$  db of Na across the years (2012–2018) in the CS fields. When the same cultivar was cultivated in AL (2014–2018), it showed  $2.0 \text{ g}\cdot\text{kg}^{-1}$  db and  $0.31 \text{ g}\cdot\text{kg}^{-1}$  db of Cl and Na, respectively. In turn, calorific values, N, K, Mn, and Zn were higher in CS than in AL. The rest of the properties were found to be very similar between both locations. However, as the study periods are different for CS and AL, it is difficult to separate the effect of weather conditions and crop age when comparing the chemical composition between both locations.

**Table 8.** Ash content, calorific values, and ultimate analysis of the tall wheatgrass cultivars collected at AL plots from 2014–2018. Mean values for three replicate plots per cultivar and year.

C	Y	Ash	C	H	N	S	Cl	GCV <sub>v,0</sub>	NCV <sub>p,0</sub>
		(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(MJ·kg <sup>-1</sup> db)	(MJ·kg <sup>-1</sup> db)
TWA	2014	43	460	62	12.4	1.4	2.4	18.5	17.2
	2015	32	478	61	7.6	1.3	1.8	19.3	18.0
	2016	29	468	61	6.4	0.7	1.4	18.7	17.4
	2017	32	481	61	8.1	1.2	1.8	19.2	17.9
	2018	43	480	60	10.2	1.1	2.5	18.7	17.3
<b>mean ±SD</b>		36 ± 7 <sup>b</sup>	473 ± 9 <sup>a</sup>	61.1 ± 0.7 <sup>a</sup>	9.0 ± 2.4 <sup>a</sup>	1.1 ± 0.3 <sup>a</sup>	2.0 ± 0.5 <sup>b</sup>	18.9 ± 0.4 <sup>a</sup>	17.6 ± 0.4 <sup>a</sup>
TWB	2014	58	452	60	11.5	1.4	3.4	18.1	16.8
	2015	43	471	61	8.4	1.2	3.9	19.1	17.7
	2016	38	460	61	7.8	0.6	2.7	18.5	17.2
	2017	47	467	60	7.3	1.0	5.2	18.8	17.5
	2018	46	464	60	8.3	1.0	3.9	18.7	17.4
<b>mean ±SD</b>		46 ± 7 <sup>a</sup>	463 ± 7 <sup>b</sup>	61 ± 0.4 <sup>b</sup>	8.7 ± 1.6 <sup>a</sup>	1.0 ± 0.3 <sup>a</sup>	3.8 ± 0.9 <sup>a</sup>	18.6 ± 0.4 <sup>b</sup>	17.3 ± 0.4 <sup>b</sup>
TWS	2014	45	460	61	9.0	1.1	5.3	18.5	17.1
	2015	38	468	61	5.8	0.9	3.7	18.9	17.5
	2016	31	460	62	5.7	0.5	1.3	18.4	17.1
	2017	40	470	61	6.4	1.0	3.6	18.8	17.5
	2018	36	466	61	6.0	0.8	2.9	18.7	17.4
<b>mean ±SD</b>		38 ± 5 <sup>b</sup>	464 ± 5 <sup>b</sup>	61 ± 0.4 <sup>a</sup>	6.6 ± 1.4 <sup>b</sup>	0.9 ± 0.2 <sup>b</sup>	3.3 ± 1.4 <sup>a</sup>	18.6 ± 0.2 <sup>b</sup>	17.3 ± 0.2 <sup>b</sup>
<b>Global mean ±SD</b>		40 ± 8	468 ± 10	61.0 ± 0.7	8.3 ± 2.2	0.10 ± 0.03	0.28 ± 0.15	18.7 ± 0.4	17.3 ± 0.4
<b>Min. value</b>		27	448	59.0	5.1	0.05	0.08	18.0	16.7
<b>Max. value</b>		63	487	62.0	14.4	0.16	0.71	19.4	18.1
<b>P75</b>		46	476	61.0	9.8	0.13	0.40	19.0	17.6

Different letters indicate significant different means between cultivars for that property at the 95% confidence level according to multiple range tests. Letters were only included when the *p*-value for the cultivar factor was below 0.05.

**Table 9.** Major elements present in the biomass of the tall wheatgrass cultivars collected at AL plots from 2014–2018. Mean values for three replicate plots per cultivar and year.

C	Y	Al	Ca	Fe	K	Mg	Mn	Na	P	Si	Zn
		(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)	(g·kg <sup>-1</sup> db)
TWA	2014	0.16	3.4	0.075	11.2	0.76	0.031	0.31	1.8	4.2	0.009
	2015	0.05	1.5	0.021	9.4	0.50	0.021	0.15	1.4	3.0	0.006
	2016	0.13	2.1	0.101	7.5	0.56	0.028	0.17	1.1	3.6	0.007
	2017	0.11	2.6	0.041	6.0	0.66	0.018	0.49	1.0	4.6	0.008
	2018	0.09	2.5	0.136	10.5	0.71	0.024	0.42	1.6	5.7	0.011
<b>mean ± SD</b>		0.11 ± 0.04	2.4 ± 0.7	0.075 ± 0.046	8.9 ± 2.2 <sup>b</sup>	0.64 ± 0.11	0.024 ± 0.005	0.31 ± 0.15	1.4 ± 0.3	4.2 ± 1.0 <sup>b</sup>	0.008 ± 0.002 <sup>b</sup>
TWB	2014	0.12	3.0	0.046	15.0	0.65	0.032	0.41	1.7	7.8	0.014
	2015	0.06	2.6	0.020	8.9	0.59	0.020	0.69	1.2	7.9	0.007
	2016	0.08	2.0	0.041	8.6	0.49	0.020	0.33	1.3	6.5	0.008
	2017	0.17	2.5	0.030	8.9	0.56	0.018	0.70	1.4	8.3	0.010
	2018	0.12	2.7	0.030	8.9	0.55	0.019	0.57	1.3	8.6	0.009
<b>mean ± SD</b>		0.11 ± 0.04	2.5 ± 0.35	0.034 ± 0.010	10.1 ± 2.7 <sup>a</sup>	0.57 ± 0.06	0.022 ± 0.01	0.54 ± 0.16	1.4 ± 0.20	7.8 ± 0.79 <sup>a</sup>	0.010 ± 0.003 <sup>a</sup>
TWS	2014	0.06	2.8	0.027	12.7	0.56	0.025	0.38	1.4	5.3	0.010
	2015	0.06	1.9	0.016	7.3	0.50	0.016	0.54	1.0	6.6	0.006
	2016	0.07	1.7	0.051	7.6	0.50	0.022	0.25	1.1	4.5	0.006
	2017	0.14	2.3	0.035	9.0	0.52	0.014	0.40	1.5	6.2	0.008
	2018	0.09	2.0	0.034	8.0	0.51	0.017	0.40	1.2	5.8	0.006
<b>mean ± SD</b>		0.09 ± 0.03	2.1 ± 0.4	0.033 ± 0.013	8.9 ± 2.2 <sup>b</sup>	0.52 ± 0.02	0.019 ± 0.005	0.40 ± 0.10	1.2 ± 0.2	5.7 ± 0.8 <sup>b</sup>	0.007 ± 0.002 <sup>b</sup>
<b>Global mean ± SD</b>		0.10 ± 0.06	2.3 ± 0.6	0.050 ± 0.051	9.7 ± 2.6	0.59 ± 0.014	0.025 ± 0.015	0.36 ± 0.029	1.4 ± 0.3	5.4 ± 2.2	0.009 ± 0.03
<b>Min. value</b>		0.02	1.3	0.014	5.5	0.38	0.011	0.00	0.9	2.3	0.005
<b>Max. value</b>		0.28	3.8	0.327	16.8	1.14	0.108	1.27	2.0	10.4	0.017
<b>P75</b>		0.13	2.8	0.055	11.4	0.66	0.028	0.54	1.6	7.1	0.010

Different letters indicate significant different means between cultivars for that property at the 95% confidence level according to multiple range tests. Letters were only included when the *p*-value for the cultivar factor was below 0.05.

### 3.3. Fuel Predictive Indices

Fuel predictive indices were calculated for the biomass cultivated in both the CS and AL fields (see Section 2.4). Table 10 shows the results for the different species tested in CS and the three cultivars of tall wheatgrass grown in the AL fields, averaged over the years. The interaction plots across species and years are graphically depicted in Figure 6 for the AE/A, Si/K, (Si + P + K)/(Ca + Mg), and K + Na + Pb + Zn fuel indices.

**Table 10.** Empirical fuel predictive indices for the biomass cultivated at both locations (CS and AL) and significance levels for the factors considered. Values averaged across the years.

CS Biomass		Species			p-Values		
Fuel index	Units	TW	CW	SW	S	Y	S × Y
AE/A		0.30 <sup>b</sup>	0.41 <sup>a</sup>	0.42 <sup>a</sup>	**	**	**
K/(Ca + Mg)		4.4 <sup>a</sup>	3.5 <sup>b</sup>	3.4 <sup>b</sup>	**	**	**
(Si + P + K)/(Ca + Mg)	molar ratio	7.5 <sup>a</sup>	5.8 <sup>b</sup>	5.7 <sup>b</sup>	**	**	**
K + Na + Pb + Zn	mg kg <sup>-1</sup> db	12100 <sup>a</sup>	9600 <sup>b</sup>	9800 <sup>b</sup>	**	**	ns
Si/K	molar ratio	0.65 <sup>a</sup>	0.63 <sup>a</sup>	0.63 <sup>a</sup>	ns	**	ns
2S/Cl	molar ratio	1.5 <sup>a</sup>	1.3 <sup>a</sup>	1.4 <sup>a</sup>	ns	**	ns
(K + Na)/4.9*(2S + Cl)	molar ratio	1.9 <sup>a</sup>	1.5 <sup>a</sup>	1.5 <sup>a</sup>	ns	**	ns
AL biomass		Cultivars			p-values		
Fuel index	Units	TWA	TWB	TWS	C	Y	C × Y
AE/A		0.47 <sup>a</sup>	0.37 <sup>a,b</sup>	0.34 <sup>b</sup>	*	*	*
K/(Ca + Mg)		2.8 <sup>a</sup>	3.1 <sup>a</sup>	3.3 <sup>a</sup>	ns	*	*
(Si + P + K)/(Ca + Mg)	molar ratio	5.1 <sup>b</sup>	7.3 <sup>a</sup>	6.9 <sup>a</sup>	**	ns	ns
K + Na + Pb + Zn	mg kg <sup>-1</sup> db	8400 <sup>b</sup>	10700 <sup>a</sup>	9500 <sup>a,b</sup>	*	**	ns
Si/K	molar ratio	0.75 <sup>a</sup>	1.20 <sup>a</sup>	0.91 <sup>a</sup>	ns	**	ns
2S/Cl	molar ratio	1.38 <sup>a</sup>	0.60 <sup>b</sup>	0.63 <sup>b</sup>	**	ns	**
(K + Na)/4.9*(2S + Cl)	molar ratio	1.0 <sup>a</sup>	0.6 <sup>a</sup>	0.7 <sup>a</sup>	ns	**	*

S: species; C: cultivar; Y: year; df: degrees of freedom; \*\*  $p$ -value < 0.01; \*  $p$ -value = 0.01–0.05; ns:  $p$ -value > 0.05, according to Fisher's tests (order 2 ANOVA). Different letters indicate significant different means between those species or cultivars for that index at the 95% confidence level according to multiple range tests.

The biomass of tall wheatgrass cultivars showed significantly lower AE/A ratios and higher K/(Ca + Mg), (Si + P + K)/(Ca + Mg), and K + Na + Pb + Zn indices than the biomass of crested and Siberian wheatgrass (Table 10), in connection with their higher contents of K, Na, and Si (Table 9). Even though significant differences were detected among species (Table 7), it should be noted that the biomass produced was characterized by similar fuel indices (Table 10). TW cultivars showed an AE/A index of 0.30, a K/(Ca + Mg) index of 4.4, and a (Si + P + K)/(Ca + Mg) molar ratio of 7.5, as opposed to CW and SW with indices of 0.41–0.42, 3.4–3.5, and 5.7–5.8, respectively. The K + Na + Pb + Zn fuel index averaged 12,100 mg kg<sup>-1</sup> db for TW species and 9600–9800 mg kg<sup>-1</sup> db for CWH and SWV. No statistically significant differences were found among the different species cultivated with regard to the Si/K, 2S/Cl, and (K + Na)/4.9\*(2S + Cl) molar ratios (Table 10).

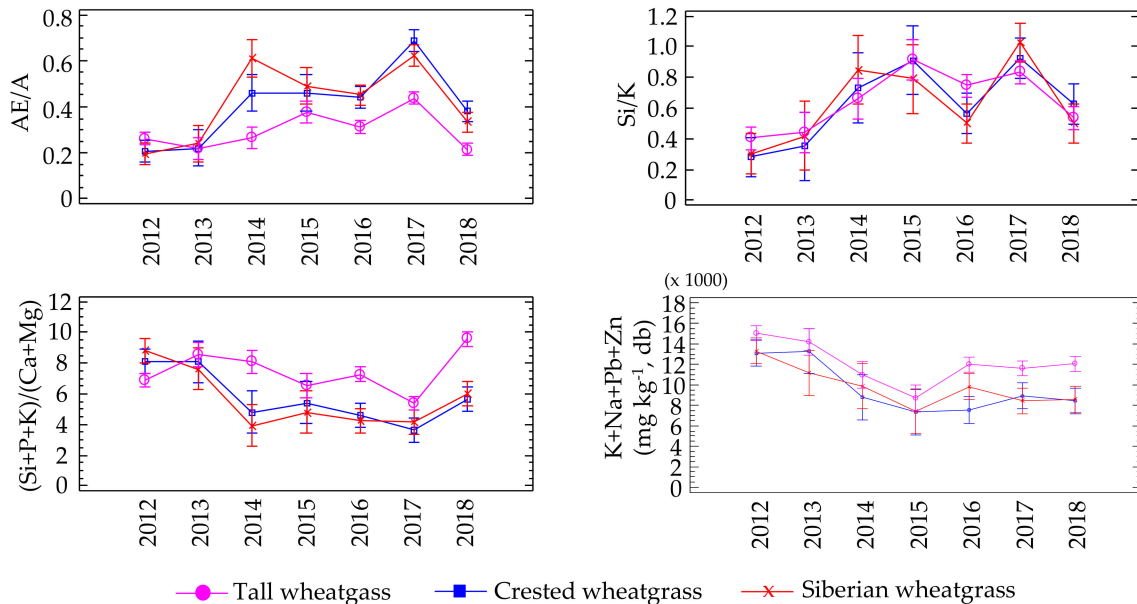
The AE/A and Si/K indices increased after the two first years of the study for all species considered while K/(Ca + Mg), (Si + P + K)/(Ca + Mg), and K + Na + Pb + Zn tended to decrease over time, with the exception of the molar (Si + P + K)/(Ca + Mg) ratio of TW cultivars (Figure 6). The most important differences among indices seem to be mainly connected to the K decrease observed across years (Figures 3 and 4). Mean reductions of 30% for K/(Ca + Mg), 24% for K + Na + Pb + Zn, and 6% for (Si + P + K)/(Ca + Mg), and increases of 33% for AE/A and 73% for Si/K were found for the biomass collected in later years (2014–2018) with respect to that grown in the first years (2012–2013). No clear tendency was found across the years for the rest of the calculated indices.

The biomass of the three TW cultivars grown in Alconaba (TWA, TWB, and TWS) differed statistically in its AE/A, (Si + P + K)/(Ca + Mg), K + Na + Pb + Zn, and 2S/Cl, but again showing very close values among cultivars for these indices (Table 9). AE/A varied across cultivars in the range



0.34–0.47, and  $(\text{Si} + \text{P} + \text{K})/(\text{Ca} + \text{Mg})$  was between 5.1 and 7.3. TWA showed higher 2S/Cl molar ratios (1.38) than TWB and TWS (0.6) but still these values were maintained low and always  $<2$ .

Results also showed comparable fuel indices between the grasses cultivated in CS and the tall wheatgrass cultivars grown in AL (Table 10).



**Figure 6.** Fuel indices across species and years for the biomass cultivated in the CS fields. Means and Fisher's LSD interaction plots.

#### 4. Discussion

As normally occurs in all perennial grasses [57], the lowest yields were obtained during the first annual harvest, no matter the species, cultivar, or location considered, with the exception of 2017. The highest yield among all the species and cultivars tested at this site (TWA, TWJ, TWR, CWH, and SWV) was achieved by TWA ( $4.8 \text{ Mg DM}\cdot\text{ha}^{-1}$ ) and TWJ ( $4.7 \text{ Mg DM}\cdot\text{ha}^{-1}$ ). In turn, TWA was the cultivar that provided the lowest yield in the AL plots ( $3.0 \text{ Mg DM}\cdot\text{ha}^{-1}$ ), where another two tall wheatgrass cultivars were also tested (TWB and TWS).

Only a few studies deal with perennial grasses in marginal land, particularly when it comes to *Agropyron* spp. species dedicated to bioenergy production. In turn, biomass yields are commonly reported in grasslands and fertile land. In this study, tall wheatgrass cultivars performed better in terms of biomass production than they did in another study that was carried out under marginal conditions in the USA, where yields of  $3.1 \text{ Mg DM}\cdot\text{ha}^{-1}$  were reported [58]. However, studies under irrigation or located in areas without biophysical constraints provided better yields for tall wheatgrass cultivars, with biomass productions over  $10 \text{ Mg DM}\cdot\text{ha}^{-1}$  in North America [37,59] and of  $17.2 \text{ Mg DM}\cdot\text{ha}^{-1}$  in Germany for biogas production [23].

As it was commented before, TWA averaged  $4.8$  and  $3.0 \text{ Mg DM}\cdot\text{ha}^{-1}$  in the CS and AL fields, respectively, lower than the yields of  $8.2 \text{ Mg DM}\cdot\text{ha}^{-1}$  reported in a 3-year study in fertile land in the USA [39].

TWJ yielded  $12.6 \text{ Mg DM}\cdot\text{ha}^{-1}$  in the current study. Lower yields ( $8 \text{ Mg DM}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$ ) were reported in irrigated areas of the Mediterranean area suffering from salinity problems [60]. This cultivar achieved yields of  $5.9$ – $8.3 \text{ Mg DM}\cdot\text{ha}^{-1}$  (average production during a 2-year period) under irrigation in marginal lands for forage production [61].

TWS yielded up to  $25 \text{ Mg DM}\cdot\text{ha}^{-1}$  in fertile land, obtaining annual biomass productions of  $5 \text{ Mg DM}\cdot\text{ha}^{-1}$  in areas with biophysical constraints [62], close to those obtained in the AL station ( $4.3 \text{ Mg DM}\cdot\text{ha}^{-1}$ ).

In a 3-year study carried out in Poland [63], TWB provided yields in the range 6.6–10.4 Mg DM·ha<sup>-1</sup>. These results were also obtained in low-quality land although in an area characterized by higher precipitation than the one in this study.

Biomass production obtained in this study for CWH (2.8 Mg DM·ha<sup>-1</sup>) was similar to that reported in North America (2.9 to 3.6 Mg DM·ha<sup>-1</sup>), where this cultivar was monitored during two years under rainfed conditions for forage production [64]. Higher biomass productions (5.55–8.05 Mg DM·ha<sup>-1</sup>) were reported in irrigated lands in the U.S. [65].

No matter the species or the cultivar, biomass production increased with annual rainfall. However, the extent to which precipitation affected yields depended on the species considered [28]. Generally, the RUE of warm-season grasses, such as switchgrass, is higher than cool-season grasses due to their different metabolism [49]. It is also worth mentioning that biomass production was found to increase in connection with spring rainfall, as this is the season when perennial grasses go through their highest active vegetative period. Other authors also noted a similar correlation [49,66,67]. According to our results, the *Agropyron* spp. considered in this study achieved higher biomass yields when the precipitation registered between March and June was over 240 mm. Poor biomass yields were obtained when precipitation during the aforementioned period was around 150 mm. Rainfall levels below 120 mm resulted in a great difficulty in harvesting the crop, as other authors also pointed out [13].

The *Agropyron* spp. cultivated in both marginal locations exhibited the typical chemical composition of perennial grasses harvested in summer, which are characterized by high contents of ash, silicon, and alkali elements [30,42,43,46,68].

The perennial grasses cultivated in both locations (CS and AL) averaged 40 g·kg<sup>-1</sup> db ash and ranged from 27–63 g·kg<sup>-1</sup> db, and 75% of all the analyzed samples was ≤ 46 g·kg<sup>-1</sup> db no matter the site. This was well within the typical ash mean values and ranges specified in ISO 17225-1:2014 [68] for virgin grass in general (hay, 10–100 g·kg<sup>-1</sup> db) and other cool-season grasses, such as reed canary grass (summer harvest: 25–100 g·kg<sup>-1</sup> db). However, the ash content of the grasses cultivated in this study is more comparable to that of miscanthus, a C4 grass that is usually characterized by lower ash contents than C3 grasses, showing in the literature mean ash contents of 40 g·kg<sup>-1</sup> db [30,46,68] and a typical variation from 10–60 g·kg<sup>-1</sup> db [68]. Martyniak et al. [41] studied TWS and four wild populations of tall wheatgrass in Poland, reporting elevated ash contents for these grasses (78 g·kg<sup>-1</sup> db for TWS and 100–170 g·kg<sup>-1</sup> db for the wild populations). Nazli et al. reported ash contents between 51 and 96 g·kg<sup>-1</sup> db for other C3 grasses (giant reed and bulbous canary grass) and in the range of 31–60 g·kg<sup>-1</sup> db for C4 grasses (miscanthus and switchgrass) in marginal land in Turkey [31]. It has been reported that air temperature, water availability, and rain distribution play an important role in affecting the biomass ash content [42]. A decrease in ash content has been observed in giant reed grown without irrigation [30] and in central Italy in years with low precipitation and after long periods of summer drought [42]. Besides meteorological conditions, sandy soils like the ones in the study have a low water-holding capacity, increasing the likelihood of water stress [69]. Therefore, the relatively low ash contents found in our study could be due to the sandy soils and the semi-arid climate of the marginal regions considered. Nevertheless, it should be pointed out that ash contents higher than those shown in our study, where grasses were collected manually, should be expected when biomass is harvested mechanically, which could result in the inclusion of mineral impurities from soil.

Net calorific values in db ranged from 16.7–18.5 MJ·kg<sup>-1</sup>, and averaged 17.7 MJ·kg<sup>-1</sup> for the biomass cultivated in CS and 17.3 MJ·kg<sup>-1</sup> in AL. They are within the typical ranges listed in ISO 17225-1 [58] for perennial grasses (16–19 MJ·kg<sup>-1</sup>). This international standard [68] also lists the mean typical net calorific values for virgin grass (17.1 MJ·kg<sup>-1</sup>), reed canary grass (16.6 MJ·kg<sup>-1</sup>), and miscanthus (17.7 MJ·kg<sup>-1</sup>). Again, the tall wheatgrass cultivars considered in our study showed mean calorific values closer to miscanthus, a warm-season grass, than to other cool-season grasses. As calorific values are known to be negatively correlated to ash content [43,70], the higher heating values found in our study are in line with the relatively lower ash contents that characterized this biomass. Our net calorific values are very close to those found for giant reed harvested in the fall

(17.4 MJ·kg<sup>-1</sup>) and bulbous canary grass (17.2 MJ·kg<sup>-1</sup>), the latter in marginal land in a semi-arid Mediterranean environment in Turkey [31]. Lower net calorific values were found for wild populations of tall wheatgrass in Poland (15.8–17.0 MJ·kg<sup>-1</sup>), in connection with the elevated ash contents obtained for the biomass (78–170 g·kg<sup>-1</sup>) [41].

The chemical composition of the perennial grasses cultivated in our study are well within the ranges documented in the literature for cool perennial grasses [30,41–46,56,60,68]. *Agropyron* spp. averaged 12 g·kg<sup>-1</sup> db and 8.3 g·kg<sup>-1</sup> db N in CS and AL, respectively, 1.6 g·kg<sup>-1</sup> db Cl in CS, 2.8 g·kg<sup>-1</sup> db Cl in AL, and 0.10% S at both sites. ISO 17225-1 specifies typical values of 1.3% N, 0.1–0.2% S, and 0.5–0.7% for virgin grasses and reed canary grass harvested in summer, and 0.7% of N, and 0.2% of S and Cl for miscanthus [68]. Sulfur contents between 0.9 and 1.0 g·kg<sup>-1</sup> db and chlorine contents from 0.13–0.25% were reported for TWS and wild populations of tall wheatgrass in Poland [41]. Tall wheatgrass ranged from 6–11 g·kg<sup>-1</sup> db N, depending on the N fertilization rate in the Mediterranean climate in California [60]. In marginal land, giant reed and bulbous canary grasses harvested in the fall, two different C3 species, ranged from 5.4–16 g·kg<sup>-1</sup> db N and 1.1–3.0 g·kg<sup>-1</sup> db S across different N fertilization rates and years [56]. Perennial pastures dominated by cool-season grass species in the U.S. ranged from 6–18 g·kg<sup>-1</sup> db N, 1.0–2.5% S, and 1.0–5.0 g·kg<sup>-1</sup> db Cl [44]. Similar concentrations were also reported for native temperate grasses in the maturity stage in the Western U.S. [45], obtaining 0.3–1.8 g·kg<sup>-1</sup> db of S and 0.5–4.0 g·kg<sup>-1</sup> db of Cl.

When a fuel N concentration exceeds 6 g·kg<sup>-1</sup> db, the NO<sub>x</sub> emission limits during combustion could be exceeded [46]. Corrosion and HCl emission problems are to be expected as well at Cl concentrations above 1 g·kg<sup>-1</sup> db and S concentrations over 1 g·kg<sup>-1</sup> db, while emissions of dioxins and furans could be of relevance when the concentration of Cl exceeds 0.3% [46]. Significant SO<sub>x</sub> emissions should also be considered when the concentration of S in the fuel is >20 g·kg<sup>-1</sup> db [46]. When using this type of biomass for combustion purposes, severe high corrosion risks should be taken into account. The biomass grown in this study always exhibited 2S/Cl molar ratios below 2, the guideline specified in [54] to avoid this issue.

Other important elements from a quality point of view, such as K, Si, Ca, Mg, P, and Na were also well within the typical values gathered in the literature for other cool perennial grasses cultivated under different conditions [30,43,46,68]. Besides the developmental stage, the content of major elements in grasses is strongly influenced by site factors and environmental conditions, such as soil type and weather conditions, and therefore differences in the mineral concentrations of these grasses are expected between different locations [30,42,45]. Depending on the location, our grasses averaged 2.3–2.4 g·kg<sup>-1</sup> db of Ca, 9.7–11 g·kg<sup>-1</sup> db of K, 0.09–0.36 g·kg<sup>-1</sup> db of Na, 4.8–5.4 g·kg<sup>-1</sup> db of Si, 1.0–1.4 g·kg<sup>-1</sup> db of P, and 0.59–0.64 g·kg<sup>-1</sup> db Mg. For grasses harvested in summer or in the fall, other authors reported values ranging from 0.6–8.1 g·kg<sup>-1</sup> db K, 5.6–37 g·kg<sup>-1</sup> db Si, and 0.8–3.2 g·kg<sup>-1</sup> db Ca for reed canary grass [30], or 2.2–23 g·kg<sup>-1</sup> db Si, 0.9–3.0 g·kg<sup>-1</sup> db P, and 9.6–22 g·kg<sup>-1</sup> db K for native temperate grasses in the western US [45]. Similar mineral concentrations were obtained in marginal land for giant reed and bulbous canary grass (*Phalaris aquatica* L.), with 7.0–21 g·kg<sup>-1</sup> db K, 4.4–16 g·kg<sup>-1</sup> db P, 1.6–3.7 g·kg<sup>-1</sup> db Ca, 12–21 g·kg<sup>-1</sup> db Si, and 0.9–1.4 g·kg<sup>-1</sup> db Mg [56].

When grown in the same location (CS), the main differences were found across species rather than among cultivars. In this sense, the chemical composition of tall wheatgrass cultivars differed from crested and Siberian wheatgrass, finding higher ash, K, Na, and Si contents for the former, which resulted in lower AE/A ratios and higher K/(Ca + Mg), (Si + P + K)/(Ca + Mg), and K + Na + Pb + Zn indices. Therefore, fuel indices predict a slightly better combustion behavior of crested and Siberian wheatgrass with regard to ash sintering and aerosol emissions.

When different tall wheatgrass cultivars were grown in the same environment (TWA, TWJ, and TWR in CS and TWA, TWB, and TWS in AL), most of their properties varied in a relatively narrow range. Genotypic differences in the accumulation of minerals and the fuel quality of other grass species have been previously reported [30,45,71], and they can only be quantified when the grasses are grown at the same location.

However, it should be highlighted that the slight differences found among all the species and cultivars tested are not expected to be of any practical relevance if these grasses are utilized in combustion processes. No matter the species or year considered, AE/A ratios were always maintained low ( $<0.8$ ), and  $K/(Ca + Mg)$  ratios were  $>2$ , far from the guidelines to avoid sintering problems ( $AE/A >2$  [52], and  $K/(Ca + Mg) <0.5$  [53]). In addition,  $(Si + P + K)/(Ca + Mg)$  molar ratios were always from 3–10. Therefore, low sintering temperatures are expected for the cultivated grasses [52–55].  $K + Na + Pb + Zn$  were always between 8000 and 16,000  $mg\ kg^{-1}$  (Figure 5), suggesting medium aerosol emissions ( $PM_{10}$ ) for crested and Siberian wheatgrass to high aerosol emissions in the case of TW cultivars [54]. Si/K molar ratios were low ( $<1.2$ , Figure 5), also suggesting a high K release and aerosol formation.

Our results suggest an improvement in the biomass quality of the grasses with crop age. In this sense, the ash content, as well as the concentrations of K, S, Na, and Cl, tended to decrease over the years, while the content of CaO, MgO, and  $SiO_2$  in the ashes tended to increase. As a result of the changes noted in the chemical composition, the biomass collected during the first years exhibited lower AE/A and Si/K ratios, and higher  $(Si + P + K)/(Ca + Mg)$  and  $K + Na + Pb + Zn$  indices. Therefore, the results obtained suggest a better ash fusibility behavior for the biomass harvested during the later years of the study, as well as lower gaseous and aerosol emissions. The positive effect of crop age on the chemical characteristics of other grasses was previously documented [31,42,45,56,71]. Similar to our results, ash content reductions and higher  $SiO_2/K_2O$  and  $CaO/K_2O$  ratios were found in 10-year-old giant reed crops in central Italy [42]. The mineral concentration decline with plant maturation has been previously related to the different morphological characteristics of the stand crops during ageing, which produces a greater amount of thinner stems per unit area with fewer leaves [42,72]. However, as suggested by Di Nasso [42], it is very difficult to separate the effect of crop age from the effect of different annual meteorological conditions, particularly the rainfall distribution during the growing seasons.

Based on the chemical composition and the assessment of the calculated fuel combustion indices, the grasses cultivated in these marginal regions are expected to show the typical combustion behavior of cold perennial grasses harvested in summer. When assessing the fuel indices, results suggest that the differences found among wheatgrass species and cultivars for some of their combustion properties and chemical characteristics are not likely to affect their combustion behavior from a practical point of view. If these grasses are going to be used for producing bioenergy in a thermochemical conversion process, technological measures in power plants should be implemented to avoid sintering, corrosion, and emission-related problems [30,45,46]. Another possibility worth considering would be to try reducing the content of troublesome elements directly in the fuel. Practices, such as leaching during field drying or a harvest delay, have been proven effective for grasses [30,31,43,56,68,71], due to the reduction in ash content and water-soluble elements in the fuel, as well as an increase in ash deformation temperatures. The impact of leaching and/or a delayed harvest on the chemical composition and fuel quality parameters of these grasses should be explored in marginal land and semi-arid environments with scarce precipitation and variable rain distribution. In addition, an economic assessment would also be useful to determine whether these alternatives would be viable in low-productive soils, as the improvement in the quality properties of the fuel is usually accompanied by significant dry matter losses and the risk of displacing the baling to periods with unfavorable weather conditions [43,71]. The biomass cultivated in this study could also be used to produce herbaceous pellets, class B [73]. The limitations established in ISO 17225-6 for herbaceous pellets, class A, would not be met, as the Cl content of the cultivated perennial grasses surpassed the limitation of  $1.0\ g\cdot kg^{-1}\ db$  at both locations, but especially in AL, where higher levels of Na and Cl were found.

## 5. Conclusions

Tall wheatgrass can be an effective alternative for growing energy feedstock under marginal conditions as it provided better biomass yields and rain use efficiencies than crested and Siberian wheatgrass. Among all the tall wheatgrass cultivars tested, both Alkar and Szarvasi-1 provided the

best biomass production. *Agropyron* spp. can achieve positive biomass yields in areas with biophysical constraints under rainfed conditions as long as the precipitation between March and June is higher than 150 mm.

The major differences in biomass composition were across species rather than among cultivars. Tall wheatgrass exhibited the highest ash content, K, Na, and Si concentrations. The net calorific value was higher in crested, Siberian, and tall wheatgrass cv. Riparianslopes than the rest of the tall wheatgrass cultivars. All species and cultivars showed similar fuel indices, being slightly better for crested and Siberian wheatgrass. In this sense, the differences found among species and cultivars regarding their main properties and chemical composition are not likely to be of any practical relevance if these grasses are utilized in combustion processes. The ash content, as well as the concentrations of K, S, Na, and Cl, tended to decrease over the years, while the content of other ash-forming elements, such as Ca, Mg, and Si, tended to increase, making the biomass collected during later years better in quality.

A genetic improvement of these grasses could be helpful to improve biomass productions for bioenergy in low-input agriculture. Later harvest times under marginal conditions should also be explored in order to improve the combustion quality of this type of biofuels.

This experiment could be replicated in more than 10 million ha located in Europe that have been categorized as marginal land [13], and where *Agropyron* spp., tall wheatgrass in particular, could perform well in terms of biomass production and water use efficiency. In addition, and according to FAOSTAT [74], there are more than five million hectares around the world that are actually sown with rye (*Secale cereale* L.), offering a doubtful positive economic margin and environmental balance [16], and which could be used to cultivate *Agropyron* species for bioenergy purposes. Further research should be carried out to assess whether later harvest dates, with the aim of improving the quality of the biomass produced, would be feasible under marginal conditions. Additionally, an assessment on the applicability of these grasses as feedstock for second-generation biofuels would be useful.

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