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Nitrogen fertilisation and harvest time on biomass production and composition of tall wheatgrass in Mediterranean marginal conditions

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ABSTRACT: Among several cool-season grasses, tall wheatgrass (*Elymus elongatus* (Host) Runemark) has been recently identified as a promising energy crop for Mediterranean low productive areas under rainfed conditions. The influence of annual N top dressing applications (0, 30, and 80 kg ha^{-1}) on the production, composition and combustion quality properties of tall wheatgrass biomass was studied for 9 years on marginal land. The effect of harvest time was evaluated from early summer until late winter with three cultivars of tall wheatgrass (`Alkar´, `Jose´, and `Riparianslopes´). Biomass production grew with increasing N input and spring rainfall. The 80 kg N ha^{-1} treatment provided mean dry matter production of 4.1 Mg ha^{-1} yr^{-1} but elevated biomass N levels from 8.5 to 10.0 g kg^{-1}. An autumn harvest in mid-October led to dry matter losses of 16%, and reduced moisture (-48 %), ash (-24%), N (-39%), Cl (-61%), S (-24%)

and ash K_2O (-41%) levels. Delaying harvest time until late winter greatly improved fuel quality, reducing N by 51%, and Cl and K by 80-85%, while increasing deformation and flow temperatures by 370 ºC and 240 ºC, respectively, but led to matter losses of 36-48%. The differences found in the chemical composition of tall wheatgrass across years and N applications up to 80 kg N ha^{-1} yr^{-1} are not expected to be of any practical relevance if this type of biomass is utilised in combustion processes. On the contrary, a harvest delay can improve quality properties for combustion.

Keywords: *Elymus elongatus* (Host) Runemark, cool perennial grasses, top dressing fertiliser, delayed harvest, chemical composition, fuel quality, combustion

1. INTRODUCTION

Solid biofuels are seen as a tool to help meeting the ambitious targets set by the Paris Agreement and the European Directives on the production of energy from renewable sources [1-3]. In the current global scenario, where a balance between food provision, energy production and environmental sustainability is required, the use of marginal lands to grow energy crops has become an interesting research topic as a way to overcome previous controversies regarding land use and food competition [4,5]. The term "marginal land" (poor soils and/or unfavourable climates) can be used to refer to low quality areas with regard to agricultural use, unsuitable for producing food crops due to low or zero economic profitability [6]. It is estimated that, in Europe, 63.7 Mha is marginal land suitable for the production of bioenergy resources [7].

The current demand is for fast-growing non-food energy crops that provide the best possible high energy yields in marginal land for low input (fertiliser, herbicide, water, etc.). Perennial grasses are considered promising energy crops for Europe due to their rapid growth, low demand for nutrient inputs, and ability to tolerate poor growing conditions. Besides, they can actually restore degraded lands by reducing soil erosion and nutrient leaching, while increasing soil organic matter content and providing habitat for wildlife [8-10]. While most dedicated energy perennials studied so far are warm-season grasses, cool-season plants have other qualities that could prove to be very useful for marginal environments. Warm-season grasses are drought-resistant perennials that can be adapted under most unfavourable site conditions, besides having the ability to produce more biomass than warm-season species under low irrigation treatments, or at semi-arid, arid or cold desert sites [11], attributes particularly important under the ensuing climatic change [3,12]. Besides, it was recently demonstrated that growing cool-season grasses in marginal Mediterranean soils does not inflict a higher impact on the environment than cereal farming (the current use) [13].

Among cool-season grasses, tall wheatgrass (*Elymus elongatus* (Host) Runemark, synomyms *Agropyron elongatum* (Host) P. Beauv. and *Thinopyrum ponticum* (Podp.) Barkworth & D. R. Dewey) has been previously identified as a potential energy crop for cultivation in Central Europe [14] and in the European Mediterranean region, particularly for arid and semiarid, low productive areas where traditional crops are not economically viable [11,15-18]. Under those conditions, a recent study demonstrated that tall wheatgrass provided the highest yields among several pure and mixed C_3 perennial grasses [15]. Tall wheatgrass provided better biomass yields and rain use efficiencies than crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) and Siberian wheatgrass (*Agropyron sibiricum* (Willd.) P. Beauv.) under marginal conditions [17]. Promising yields were also obtained in a cold semi-arid Mediterranean area at a high altitude [19]. However, unlike other cool perennial grasses such as giant reed *(Arundo donax* L.) or reed canary grass

(*Phalaris arundinacea* L.) [20-22], the experience with tall wheatgrass for dedicated energy crops is very limited [9,17,23-25], being a lesser-known perennial.

For a plant species to have potential as an energy crop, biomass must be produced with a low level of inputs that themselves require minimal energy for their production and use, so that an improved energy balance is achieved [26]. In order to produce financially viable yields and prevent soil mineral depletion, it is usually necessary to apply N fertilisation in all types of soils. However, such applications should be kept as low as possible as the manufacture of N fertilisers is the main energy input and source of green-house gas emissions during cultivation [9,27].

At the same time, N fertilisation can increase N fuel content, which should also be avoided to minimise NO_x emissions during combustion. Various responses to rising N fertilisation rates (usually between 100 and 300 kg N ha^{-1} yr^{-1}) were observed in the literature for other cool-season grasses [20-22,28,29]; yields and N biomass contents being boosted in some cases, while not being affected in others, probably as a result of the different conditions tested (N rates, climates, soils, number of harvests, etc.).

In the particular case of tall wheatgrass (cv. `Jose´), a significant response to N fertilisation was observed under irrigation in a warm Mediterranean climate in California [9]. However, there is a lack of knowledge about how N fertilisation would affect tall wheatgrass energy crops under the rainfed conditions of Mediterranean marginal areas. Besides, long-term field studies would be extremely useful to determine the optimal fertilisation regime, because the mineral content responses of perennial grasses to N fertilisation may change in relation to crop ageing and soil nutrient availability [20].

When assessing new crops for a particular area, it is important to take into account not only the amount of biomass that can be potentially produced, but also its composition and potential quality as a fuel, as it has a great impact on combustion performance in terms of emissions, corrosion, slagging and deposit formation [8,30-33]. The traditional harvest time for cool-season grasses is during mid-summer, as their yields peak during this time, when daytime is longer. However, summer-harvested grasses are known for having elevated ash contents as well as high levels of certain “anti-quality” elements including Cl, K, Si, N, or S that cause sintering, corrosion and emission related issues at the normal operational temperatures of combustion plants [8,21,30,34-39].

Practices such as leaching during field drying or a harvest delay have been proven effective in improving the combustion quality of grasses through a reduction of the ash content and water soluble elements, resulting in an increase in ash deformation temperatures, but usually at the expense of causing significant dry matter losses [20,22,28,32,34-38]. Current knowledge, however, is being gained mostly from grasslands and fertile lands in North America and Northern Europe, and areas where rain and snow may play an important role in nutrient leaching from the crops to soil during late autumn, winter, and early spring [32,35,37,40-44]. Besides, while delaying harvest appears to be highly effective in removing large amounts of K and Cl from grass biomass, the removal of N and inorganic constituents such as S, Na, P, Mn, Mg, Ca, and Si appeared to be more variable [26,32,35,36,41,42,44,45], mainly depending on the type of grass, harvest date, location and environmental conditions. Scant attention has been paid to the impact of a delayed harvest on cool-season grasses in marginal land under Mediterranean climate conditions, usually characterised by poor soils and semi-arid environments with scarce precipitation and variable rain distributions [20-23].

The objective of this study is to determine the influence of N fertilisation on the production, chemical composition, and combustion quality properties of the biomass of tall wheatgrass and optimise harvest time under Mediterranean marginal conditions. The ultimate aim is to present possible agronomic strategies and provide valuable information to produce the best possible yields and biomass quality for a future implementation of cool perennial grasses as dedicated energy crops on a large scale in low productive marginal areas of the Mediterranean region.

2. MATERIAL AND METHODS

2.1. Experimental location and pedo-climatic conditions

The experiment was conducted over the course of 10 years between 2010 and 2019 at the Research Centre for the Development of Renewable Energies (CEDER-CIEMAT), which is located at 41º 36´ N, 2º 29´ W in the province of Soria, situated in the Spanish Autonomous Region of Castilla y León.

Climate conditions are continental Mediterranean with cold winters, warm summers, and low rainfall levels. According to the Köppen-Geiger classification, the climate is considered to be temperate with a dry season and mild summer (Cfb, [46]). During the study period (Table 1), the mean temperature was 10.6 ºC and the average annual precipitation was 503 mm. Even though historical annual rainfall averages are around 500 mm [47], it is important to note that rainfall can vary considerably over the years (Table 1).

- Insert Table 1 here -

In this region, winter months have average minimum temperatures between -2 ºC and 1 ºC, with absolute minimum temperatures falling below -10 ºC. Summers show average maximum temperatures around 23-28 ºC, and absolute maximum temperatures of 35-37 ºC. The number of days of the year with a minimum absolute temperature lower or equal to 0 ºC is over 100 days with occasional snow (21 days of snow per year). The frost-free period is from mid-May until late September.

Harvest time was studied over a period which followed the typical temperature and rainfall distribution in the region between May 2016 and March 2017 (Figure 1). The annual accumulated pluviometry and mean temperatures registered at the study site (540 mm and 10.7 ºC in 2016) were very close to the 30-year historic means in the region (512 mm and 11.0 ºC) [47].

- Insert Figure 1 here -

The soil at the experimental site has a sandy texture (81% sand, 11% silt, and 8% clay) and organic matter below 1%. N, P, and K levels of 700, 10.5, and 165 mg kg^{-1} were obtained in the upper soil layer at the beginning of the experiment. This is poor, low productive soil with limited water and nutrient retention capacities. For all these reasons, it can be considered marginal soil, not suitable for cultivating traditional crops (cereals) in a sustainable way and under a low-input management.

2.2. Experimental design and agronomical practices

All the experimental plots were distributed in a randomised complete block design, with three replications and covering a surface area of 5400 m^2. Subplots were 300 m^2 (3 m x 100 m) per replicate and condition.

Soil was tilled in June 2010. In November 2010, a fertiliser complex 8-24-8 (N-P_2O_5-K_2O) was applied at a dose of 300 kg ha^{-1}, and the area was cultivated and rolled. Immediately afterwards, tall wheatgrass cv. Riparianslopes (TWR) was sown at 20 kg ha^{-1}. Plots received three different annual top dressing applications with calcium ammonium nitrate (27% N) in late March or early April; none (F0), 30 (F30), or 80 (F80) kg N ha^{-1} yr^{-1}. In March 2015, a fertiliser complex (7-13-10, N-P_2O_5-K_2O) was applied at 400 kg ha^{-1} as a basal dressing. Since grasses were grown under rainfed conditions and pesticides were not required after establishment, these crops can be considered as low input [16].

The crops receiving different N top dressing applications were harvested with a mower conditioner once a year in late July or early August, after the plants had matured, leaving 10 cm of stubble to encourage further regrowth. After 5 to 6 days of field drying (moisture <10 %), biomass was baled in prismatic bales. Annual dry matter yields (DMY) in the period 2011-2019 were calculated by taking into account the total weight of the bales obtained, the moisture content of the biomass before baling and the total surface harvested. Three biomass subsamples per repetition were collected every year just before crop harvest between 2012 and 2019, and mixed to form a combined sample per replication and condition that was further analysed. Every subsample consists of the aerial biomass contained in 0.25 m^2.

The effect of harvest time was evaluated using three tall wheatgrass cultivars; Riparianslopes (TWR), Alkar (TWA), and Jose (TWJ). These cultivars were grown by applying the agricultural practices stated in the fertilisation experiment. Sowing rate was also 20 kg ha^{-1}. These subplots received annual top dressing application of 80 kg N ha^{-1}. Three samples per subplot were taken monthly between May 2006 and March 2007 to estimate the total dry matter produced per hectare. Samples consisted of the aerial biomass contained in 1 m^2. All samples collected between May and November, and in following year´s January and March were analysed for their chemical composition.

2.3. Analysis and calculations

Analyses were performed in the Biomass Laboratory at CIEMAT, according to international ISO standards. The evaluated properties, as well as the analytical techniques and equipment used for testing, are listed in Table 2.

- Insert Table 2 here -

Ashes were obtained in the laboratory by calcination at 550 ºC in accordance with ISO 18122:2015 and ISO 21404:2020 and their composition and properties were analysed by ICP-OES, XRD and optical heating microscopy. XRD analyses were performed using a diffractometer working at 45 kV, 45 mA, with a working range of between 5º and 80º (2θ) and a scanning speed of 0.11º/s. The identification of the mineral phases was made by using the Inorganic Crystal Structure Database (ICSD, Fiz Karlsruhe, Germany). Rietveld analysis was used for semi-quantification.

The fertiliser use efficiency was calculated as the kg of dry matter (DM) produced per kg of N applied, according to [9]. N, P, and K annual offtake at harvest were calculated by taking into account DMY and N, P, and K biomass contents, and they were expressed as the kg of element taken with crop harvest per hectare and year.

2.4. Quality indicators

A number of indicators, including several empirical predictive indices, were considered to assess the influence of the fertilisation and harvest time on the combustion quality properties of the biomass produced (Table 3). They were selected from the literature for being previously used as a measure of the quality of grasses and/or other herbaceous biofuels for combustion [8,17,20,30,41,45,48-52].

- Insert Table 3 here -

2.5. Statistical analysis

Statistical analyses were performed using the Statgraphics Centurion XVII.I (Statpoint Technologies INC, 2017) software.

The effects of different N applications, harvest dates, cultivars and years on biomass production and composition as well as on quality indicators were evaluated by means of the analysis of variance (ANOVA) procedure. A main factor or an interaction effect was considered significant when its significance level or ρ-value was lower than 0.05 at 95.0% confidence level. Interactions between factors were depicted by using the means and Fisher´s least square differences (LSD) interaction plots, where the error bars represent the LSD intervals centred in the mean value. The normality of the data and the homoscedasticity of variances were verified by checking the standardised skewness/kurtosis and Levene´s test.

Mean differences across levels were assessed using multiple range tests according to Fisher´s LSD tests. Different letters were used to denote a statistically significant difference between the pair of means at the 95.0 % confidence level.

The relationship between biomass production and pluviometry was fitted to a linear model using simple regression. A ρ-value < 0.05 for the ANOVA test indicates a significant relationship between both variables. A lack-of-fit (LOF) test was used to check whether the selected model was adequate to describe the observed data. This test compares the variability of the current model residuals to the variability between observations at replicate values of the independent variable. A ρ-value ≥ 0.05 indicates that the selected model was adequate for the observed data at the 95.0 % confidence level.

3. RESULTS AND DISCUSSION

3.1. Response to N fertilisation

3.1.1. Biomass production

This crop starts the annual growth cycle over the course of March. Harvest was performed in late July or early August, after the plants had matured. Biomass productions varied largely (ρ-value <0.01) across years (Figure 2). The highest most productive year, with an accumulated precipitation of 422 mm between March and July, yielded 11.1 Mg ha^{-1} yr^{-1} for the highest fertilised plot (F80) and 8.4 Mg ha^{-1} yr^{-1} for F30 applications. The second most productive year (311 mm during the same period), both F30 and F80 plots yielded 7 Mg ha^{-1} yr^{-1}. Low productions were obtained during establishment (≤ 2.0 Mg ha^{-1} yr^{-1}) and when the spring precipitation was below 250 mm (≤ 4.4 Mg ha^{-1} yr^{-1} regardless of the N application). Biomass production and composition were not determined in 2017, due to the extremely low biomass production obtained as a consequence of the low annual precipitation registered at this location, making baling impossible with the technology used in this study.

- Insert Figure 2 here -

In general, cool perennial grasses tend to provide low yields during the establishment year, as this is when they allocate a large amount of energy for root development and to maximise productivities in the following years [22,53-55]. The low productions obtained after establishment were attributed to the water stress associated to the low precipitations registered those years and the soil limitations at the study site. A significant positive correlation was obtained between biomass production and the precipitation between March and July (ρ-values=0.0000), with the correlation being stronger with increasing fertiliser application. Correlation coefficients of 0.9124 (F80), 0.8206 (F30), and 0.7380 (F0) were obtained for a simple regression, linear model. LOF tests showed ρ-values of 0.1249 (F80), 0.1420 (F30), and 0.0631 (F0).

Biomass production and rainfall was also correlated in a study published by the authors in the same region at two different locations, concluding that wheatgrass species could achieve positive biomass yields in marginal areas under rainfed conditions as long as spring precipitation was higher than 150 mm [17]. Similar correlations were reported in the literature for tall wheatgrass [19] and other cool-season grasses grown under rainfed conditions [56-58], as this is the season when perennial grasses go through their highest vegetative active period. In a recent 3-year study, tall wheatgrass averaged 8.2 and 14.9 Mg ha^{-1} yr^{-1} in a warm and cold semiarid Mediterranean environment, respectively, and reached up to 22 Mg ha^{-1} in the latter, the year when the area received well-distributed rainfall throughout the growing season [19].

Dry matter production increased significantly (ρ-value <0.01) with increasing N input (Figure 2). Across 9 growing seasons, DMY averaged 4.1, 3.3, and 2.3 Mg ha^{-1} yr^{-1} for the F80, F30 and F0 applications, respectively. It is important to highlight that biomass productions were evaluated by taking into account all that produced after harvesting and baling experimental plots of a relatively large surface (300 m^2 per replication and N application). Therefore, the results obtained should be considered more representative than estimations based on the collection of samples in small experimental plots, which tend to overestimate yields.

During the first years of the study, no significant differences were found between the yields provided by the F30 and F80 doses, but they always attained higher yields than control plots (Figure 2). From the 4th year onwards, the plots fertilised with the highest dose provided higher mean yields than F30 plots, and those higher than F0, although differences across treatments were not always statistically significant.

The fertiliser use efficiency was calculated as detailed in section 2.3 and reached its highest peak at 105 kg of dry DM per kg of N applied with the F30 treatment. The fertiliser use efficiency of F80 reaches a maximum of 73 kg of DM per kg of N applied. The values obtained in our study during the most productive years are comparable to those found when tall wheatgrass was grown under irrigation in the Mediterranean climate of California (87 kg of DM per kg of N applied when crops received 100 kg N ha^{-1} yr^{-1}) [9]. Even though C_4 perennials are known for achieving higher fertiliser fuel efficiencies than C_3 cool-season grasses, the values reported for tall wheatgrass in the afore-mentioned study were similar to those reported for warm-season grasses as switchgrass (*Panicum virgatum* L.) and miscanthus [9].

Plant height was significantly greater in the fertilised plots (ρ-value <0.05, data not shown). Plants averaged 110 cm over the study period in F30 and F80 plots, as opposed to the 101 cm achieved by F0 plots.

Unlike other cool-season grasses such as giant reed or reed canary grass, only a few studies deal with tall wheatgrass species dedicated to bioenergy production, particularly under marginal conditions. The yields obtained in our study are in line with those reported in areas with biophysical constraints. We previously reported annual biomass productions between 0.9 and 11.5 Mg ha^{-1} yr^{-1} for different tall wheatgrass cultivars under Mediterranean marginal conditions [17]. Tall wheatgrass yielded 3.1 Mg ha^{-1} yr^{-1} in marginal lands at high elevation in the USA [59], 3-8 Mg ha^{-1} yr^{-1} in the Southern High Plains [60], and 6.6-10.4 Mg ha^{-1} yr^{-1} (cv. Bamar) in low quality land in Poland [61]. Biomass yields between 1.6 and 3.6 Mg ha^{-1} yr^{-1} (cv. Alkar) were also obtained in low productive soil in North Dakota (a loamy fine sand soil), whereas yields of 5.2-10.5 Mg ha^{-1} yr^{-1} were reported in the same region when this cultivar was grown in loamy soils [62].

In marginal land, yields are limited by the restrictions imposed by site conditions. Under unfavourable conditions such as semi-arid regions or in poor soils, cool-season grasses tend to respond positively to N applications. In this sense, several authors reported the dry matter yields of other cool-season grasses to increase at higher N fertilisations in semi-arid Mediterranean environments [22,53,55], where the constraints are low water availability and high temperatures during summer, or at sites with low productive sandy soils [28]. N rates of 100 kg ha^{-1} yr^{-1} considerably increased the DM yield of bulbous canary grass (*Phalaris aquatica* L.) under rainfed conditions and giant reed under irrigation in a Mediterranean environment in Turkey [22]. Additional N rates (150 and 200 kg ha^{-1} yr^{-1}) did not lead to any further significant increases in the DM yields. When giant reed was cultivated under irrigation in the semi-arid Mediterranean environment in a hilly interior area of Sicily, applications of 100 kg N ha^{-1} yr^{-1} resulted in higher yields compared to the 50 kg N ha^{-1} yr^{-1} treatment [55]. Dry matter yields were also enhanced by fertilisation (200 kg N ha^{-1}) when giant reed was cultivated in central Italy, reporting increases of 0.7 kg m^{-2} on average over a period of 6 years [53]. Fertigation at 60 kg ha^{-1} yr^{-1} was also reported to increase biomass yields of giant reed in low fertility soil under a continental Mediterranean climate. In turn, the increase in N fertigation to 120 kg ha^{-1} yr^{-1} N did not affect biomass yield [63].

A yield response was recorded in reed canary grass as well when 50 and 150 kg N ha^{-1} were applied on a low productivity sandy soil in the South East of England [28]. Sandy soils are generally unable to retain any large quantities of water and N, and thus, besides low organic content, they usually exhibit low residual mineral N. Crops grown on this type of soil with low residual mineral N (and low organic content) could be expected to show a strong response to applied N [28].

On the other hand, on more fertile soils, nitrogen fertilisation of perennial grasses is not usually effective, typically obtaining negative or neutral results [36,37]. A detrimental or no yield increase with N application was reported when tall fescue (*Festuca arundinacea* L.), reed canarygrass, and orchardgrass (*Dactylis glomerata* L.) were grown on fertile land under a two-harvest management system for biofuel production [29]. Fertilisation with high N rates affected negatively the number of tall fescue stands, and reduced dry matter yields and the percentage of ground covered with orchardgrass.

The response to N observed in our study suggests that higher yields could have achieved with N fertilisation rates above 80 kg N ha^{-1} yr^{-1}. Therefore, further research would be needed to determine whether N applications of 100 or 200 kg N ha^{-1} yr^{-1} or crop irrigation in years with low spring precipitation would increase biomass production in a sustainable way in order to maximise the economic profitability of these energy crops in marginal Mediterranean areas.

3.1.2. Biomass composition

The composition of the tall wheatgrass biomass cultivated in our study (Tables 4 and 5) are well within the ranges documented in the literature for perennial grasses harvested in summer, which are characterised by relatively high contents of ash, N, Si, and alkali elements such as Cl and K [9,17,20,21,24,30,33,34,36,37,39,64-66].

- Insert Tables 4 and 5 here -

The biomass of tall wheatgrass produced in this study averaged N concentrations of 8.5, 8.7 and 10.2 g kg^{-1} for the 0, 30, and 80 kg N ha^{-1} yr^{-1} treatments, respectively (Table 4 and Figure 2). The application of 80 kg N ha^{-1} yr^{-1} resulted in significantly higher N biomass levels, while the addition of 30 kg N ha^{-1} yr^{-1} did not affect biomass N content statistically. Different authors also reported a positive correlation between the N concentration of the biomass of cool perennial grasses and increasing N fertiliser levels under various N applications (usually above 100 kg N ha^{-1} yr^{-1}) and environmental conditions [9,20,29,36].

N fertilisation has a statistically significant positive effect on Cl contents, decreasing from 2.0 g kg^{-1} for the F0 treatment to 1.5 g kg^{-1} for F80 (Table 4).

In this study, the biomass fertilised with 80 kg N ha^{-1} yr^{-1} showed slightly lower levels of Si and P and higher levels of K (Table 5). The most fertilised biomass showed 330 g kg^{-1} of K_2O, 280 g kg^{-1} of SiO_2 and 59 g kg^{-1} of P_2O_5 ash contents, as opposed to the 290 g kg^{-1} of K_2O, 340 g kg^{-1} of SiO_2, and 66 g kg^{-1} of P_2O_5 found for the F0 treatment. Similarly, N fertilisation had a clear effect on most of the mineral contents of several cool and warm-season grasses in a semi-arid Mediterranean climate in southern Turkey [20]. The significant reduction of Si content with N fertilisation can be attributed to the tendency of plants in unfertilised crops to accumulate this nutrient in order to relieve biotic and abiotic stresses, as was observed in previous studies with giant reed and bulbous canary grass [20,21], wheat straw [67] or rice [68]. Christian et al. [26] also found greater K contents in China reed plots fertilised with 120 kg N ha^{-1} yr^{-1} compared to 60 kg N ha^{-1} yr^{-1} and the control treatments, but this tendency was only observed in the year of establishment. No effects of N fertilisation were found either on the P contents of established crops [26].

The increase in N fertilisation resulted in higher N, P and K offtake at harvest (data not shown), mainly as a result of the improved biomass yields and the higher N and K contents of the biomass. The F80 treatment caused significant (ρ-value <0.01) higher annual N offtakes (47 kg ha^{-1}) than the rest of N applications (34 kg ha^{-1} for F30 and 22 kg ha^{-1} for the zero treatment). P and K annual offtakes were similar for the fertilised plots (5 kg ha^{-1} of P and 46-53 kg ha^{-1} of K) and significantly (ρ-value <0.05) higher than those observed for F0 (3 kg ha^{-1} of P and 39 kg ha^{-1} of K). Other authors also observed higher nutrient removals when harvesting fertilised perennial grasses in comparison with the zero N treatments, derived from the higher biomass productions achieved by those crops in association with elevations in the mass fractions of those elements in the biomass [9,26,69].

N applications did not affect the ash content of the biomass significantly (Table 4). However, slightly lower mean ash contents were generally noticed in the biomass from the highest fertilised plots in comparison with control plots during the second half of the study. Other authors also reported a reduction in the ash content associated with increasing fertilisation rates when studying giant reed [21,53], bulbous canary grass [22] or in grasslands dominated by cool-season grasses [70]. This was attributed to the higher nutrient availability of nutrients in fertilised crops, which may lead to their higher translocation to the rhizomes causing the ash content of the above-ground crop to decrease [21].

Tall wheatgrass biomass averaged 43 g kg^{-1} ash, with individual values ranging from 23 to 59 g kg^{-1}, and with 75% of all the analysed samples $\leq$ 47 g kg^{-1} (Table 4). These contents are well within the typical ash levels reported in the literature for perennial grasses [34]. However, it should be noticed that they are more in line with those reported for warm-season grasses, usually characterised by lower ash contents than cool-season grasses. While reed canary grass and virgin grass generally exhibit typical mean ash contents of 65-70 g kg^{-1} and typical ranges between 25 and 100 g kg^{-1} [34], China reed, a warm-season grass, shows mean ash contents of 40 g kg^{-1} with a typical variation of 10-60 g kg^{-1} [30,34,36]. The relatively low ash contents found in our study might be attributed to the sandy soil of the experimental area and the semi-arid climate of the marginal region considered. It is known that meteorological conditions such as air temperature, water availability and rain distribution play an important role in affecting biomass ash content [37]. In this sense, other authors observed a decrease in the ash content of giant reed grown without irrigation [36], and also after long periods of summer drought and in years with low precipitation in the Mediterranean region [21,37]. In addition, sandy soils have a low water holding capacity, increasing the likelihood of water stress [28]. It has been demonstrated that the ash content of reed canary grass is lower on sandy soils that on clay soils because of the better uptake of silica of the latter [42]. In any case, it should be highlighted that, in our study, plants were collected manually. Higher ash contents are expected when grasses are harvested mechanically, as the risk of picking up soil and stones during collection is introduced [35].

N fertilisation had no significant effect on the calorific value, and on the S, C, H, Ca, Mg, Mn, Na, Al, Fe, and Zn contents of summer-harvested tall wheatgrass biomass (Tables 4 and 5). Our results coincide with other studies performed in the Mediterranean area involving warm-season grasses. In this sense, N fertilisation up to 200 kg N ha^{-1} yr^{-1} had no significant effect on the calorific value of giant reed [53], the Ca, Mg, and S contents of winter-harvested giant reed, or on the autumn-harvested bulbous canary grass [20].

In addition, the biomass of tall wheatgrass cultivated in this study averaged 0.7 g kg^{-1} of Mg (25 g kg^{-1} of MgO in the ashes, Table 5), similar to China reed (0.6 g kg^{-1}), but lower than the typical levels usually reported in the literature for virgin grass in general (1.7 g kg^{-1}) or summer-harvested reed canary grass (1.3 g kg^{-1}) [34]. However, these results are consistent with the Mg levels found in a previous study performed by the authors in two different locations of the same Mediterranean area, where mean Mg levels of 0.6 g kg^{-1} were reported for tall wheatgrass [17]. It is commonly known that low Mg uptakes by plants might be caused by dry soil conditions and high levels of other positive-charged ions, such as K, Ca, and ammonium, which may also compete with this element and reduce its uptake and translocation from the roots to upper plant parts [71,72]. Therefore, the application of calcium ammonium nitrate as fertiliser, together with the low cation exchange capacity of the sandy soil of this marginal semi-arid area might have decreased the availability of Mg and led to reduced uptakes.

Most properties differed significantly across years (Tables 4 and 5). Results suggest an improvement in the biomass quality of the grasses with crop age, confirming our previous findings when studying several wheatgrasses on marginal Mediterranean land [17]. In this sense, the quality of the biomass produced improved from the second growing season onwards by reducing the N, S, K, and Na contents. Cl contents were also found to decrease and Ca and Si levels to increase during the late years.

Other authors also underlined a positive effect of crop age on the chemical characteristics of perennial grasses under different conditions [20,21,38,40,69], which has been related to the different morphological characteristics of the stand crops during ageing, producing a greater amount of thinner stems per unit area with fewer leaves [21,53]. The lowest ash contents and the highest SiO_2/K_2O and CaO/K_2O ratios were found in the 10-year-old stands of giant reed in central Italy, leading to fewer slagging problems [21]. The ash content of giant reed was also documented to decrease with crop age [22]. Nazli and Tansi [20] reported lower mineral contents in subsequent years when compared to the establishment year in several perennial grasses, but higher Ca and Si contents in bulbous canary grass. Silicon is abundant in the walls of grasses, and it is known to contribute to leaf erectness, reducing the susceptibility to lodging [35]. Ash, N, K, and Cl decreases and Si, Ca, and Mg increases have been also documented in old stands of warm-season grasses in south Germany [40,69].

3.1.3. Fuel quality properties

It is very difficult to predict the emission of gaseous pollutants during combustion only from the chemical composition of the solid biofuel, as they result from a complex interaction between fuel and combustion parameters [35]. However, it is widely accepted that the concentration of N, together with other troublesome elements such as Cl, K, and S, should be kept as low as possible in the fuel to maintain an acceptable biomass quality for combustion (Table 3).

According to the literature, emission-related problems and the exceeding of NOx emissions limits could be expected at fuel N concentrations above 6 g kg^{-1} [30] or 10 g kg^{-1} [45], depending on the author. As mentioned, biomass N content in this study averaged 8.5-8.7 g kg^{-1} for the zero and low N application and increased to 10.2 g kg^{-1} under the highest fertiliser regime (Table 4). Medium-N biomass fuels are usually in the range between 4 and 10 g kg^{-1} of N, while the N concentration in high-N fuels typically varies between 10 and 100 g kg^{-1} [8].

Corrosion and HCl emission problems are expected during combustion when Cl contents exceed 1.0-2.0 g kg^{-1} (Table 3). Biomass Cl contents averaged 1.5 g kg^{-1} for the highest N application and 2.0 g kg^{-1} for the control, whereas S was not affected by N fertilisation, and averaged 0.9-1.0 g kg^{-1} (Table 4), close to the guideline (<1.0 g kg^{-1}, Table 3) reported to avoid corrosion risks. At the same time, severe high temperature corrosion risks are expected for 2S/Cl molar ratios below 2 (Table 3). 2S/Cl molar ratios averaged 1.2 for the control and low fertilised plots, and 1.6 for the highest fertilised plot (Table 6).

- Insert Table 6 here –

On the other hand, SO_x, furan or dioxin emissions should not be of significance for this type of biomass (Tables 3 and 4).

The indices considered to predict the combustion behaviour of tall wheatgrass were not influenced by N fertilisation, with the exception of Si/K and 2S/Cl (Table 6). N fertilisation affected positively 2S/Cl, as was previously mentioned, but it has a negative influence on Si/K molar ratios. The highest fertilised plot showed lower Si/K molar ratios (0.7) when compared with control plots (1.0) as a consequence of their lower Si and higher K contents. Our results are in agreement with Nazli et al. [20], who found that other cool-season grasses tended to have the highest Si/K ratios under no or low N inputs in a semi-arid Mediterranean region.

Quality indicators reflected the quality improvement noticed with crop age (Table 6); $(CaO+MgO)/(K_2O+Na_2O)$ and Si/K ratios tended to increase while K/(Ca+Mg) and (Si+P+K)/(Ca+Mg) ratios showed a decreasing tendency. However, in spite of the undeniable improvement of biomass quality as crop aged, it should be underlined that alkaline-earth to alkali oxides ratios were always maintained low (<0.6), and K/(Ca+Mg) and (Si+P+K)/(Ca+Mg) molar ratios were always higher than 2 and 5, respectively, far from the guidelines found in the literature (Table 3) to avoid sintering problems at the conventional combustion temperature [8,41,50]. In addition, Si to K molar ratios were relatively low (generally <1), suggesting the formation of aerosols due to K releases. The sum of K, Na, Pb and Zn varied between 7,600 and 15,500 mg kg^{-1} (Table 6), and thus medium to high aerosol emissions (PM_1) will be expected for this biomass, as typically occurs with grasses and other biofuels such as straw [8].

Therefore, the differences found in the chemical composition of tall wheatgrass among N applications up to 80 kg N ha^{-1} yr^{-1} or different crop ages are not expected to be of any practical relevance if this type of biomass is utilised in combustion processes. Taking into account the chemical composition and the assessment of quality indicators, the tall wheatgrass cultivated in this marginal environment is expected to show the typical combustion behaviour of perennial grasses harvested in the summer.

3.2. Effect of harvest time

3.2.1. Biomass production

Biomass production peaked in mid-summer (mid-late July), when all the cultivars tested (TWR, TWA and TWJ) yielded 8.8 Mg ha^{-1} on average (Figure 3), and plants reached full ripening (hard grains) and their maximum height (130-138 cm). TWR grew slightly taller (124 cm) than TWA (121 cm) and TWJ (119 cm).

- Insert Figure 3 here -

A first dry matter reduction (10%) was observed in August due to grain dehiscence, which usually occurs in mid-summer. Biomass production was then maintained practically constant until mid-autumn, with mean dry matter losses of 16% and yields between 6.8 and 8.3 Mg ha^{-1}, as temperatures in this area are still relatively mild until October (12 ºC).

Considerably yield reductions were observed afterwards, as a consequence of the increasing fragility of plants due to the drastic decrease of mean temperatures to 6 ºC and rainfall (Figure 1). In this sense, dry matter reductions of 36% were observed in mid-November after heavy rain registered before sampling, which resulted in significant biomass losses. Dry matter yields were further reduced in winter harvests, showing reductions of 39-48% between January and March with respect to mid-summer harvest, in connection with the adverse winter meteorological conditions (low temperatures, killing frosts, occasional snows and strong winds) that caused biomass losses. From mid-November onwards, dry matter yields varied between 4.1 and 5.9 Mg ha^{-1} until the end of the winter, depending on the cultivar and the specific harvest date.

It is known that adverse meteorological conditions during winter, such as killing frosts and heavy snowfall, might cause stem lodging and the break-up of the upper stem parts of perennial grasses [19,22,32,35,37,40,43]. In the conditions of this study, stem lodging was not observed, but the fragility of plants after senescence resulted in leaf losses and the break-up of the upper stem parts and small branches of the grasses, with the subsequent biomass losses.

A recent study also reported significant dry matter reductions in cool-season grasses, including tall wheatgrass, in a semiarid region in Turkey [19], when harvest was delayed from autumn to winter or early spring. Biomass losses ranged 6-47% and 8-71% in cold and warm semiarid environments, respectively.

With regard to other cool-season grasses grown under semi-arid Mediterranean conditions, winter harvest caused similar dry matter yield losses in bulbous canary grass (27%-67%) and between 12% and 18% in giant reed [22]. Losses were attributed to leaf defoliation occurring during winter dormancy in case of giant reed. In turn, bulbous canary grass continued to accumulate biomass during the winter in that region, resulting in younger plants over the course of winter harvests. This young plant availability accounted for the dry matter losses rather than leaf defoliation. In warm Mediterranean areas, it was reported for some cool-season grasses that plant growth can continue with new stems in autumn due to the mild temperatures. Thus, a winter harvest can result in similar yields or even in slight yield increases. In this sense, harvest time did not affect the biomass yield of giant reed in central Italy, averaging 5.6 Mg ha^{-1} and 5.4 Mg ha^{-1} in autumn and winter harvests, respectively [53].

In colder climates, a delayed harvest system for different cool-season grasses frequently resulted in yield reductions of about 10-20%. Reed canary grass yields peaked in the middle of October in Estonia and were reduced by 20% in spring [42]. In Minnesota (USA), where mean annual temperature is 5.8 ºC and mean annual precipitation is 672 mm, big bluestem (*Andropogon gerardii* Bitman) dry matter production was reduced by 10% when harvest was delayed until the following spring [41].

The three cultivars tested in the current study provided similar yields and followed the same trend with regard to how their yields were impacted by different harvest times (Figure 3). However, and even though this study was performed during a period which followed the typical precipitation and temperature distributions of the region, a long-term study would be needed to estimate how the different annual meteorological conditions would impact dry matter productions and biomass composition of this energy crop.

3.2.2. Biomass composition

Harvest time affected the composition of all the cultivars tested similarly (Table 7), and only some significant yet small differences were noticed across cultivars.

- Insert Table 7 here -

Later harvest dates resulted in lower biomass moisture contents (Figure 3), with the exception of punctual biomass moisture increases during rain periods. Mean moisture contents as high as 740 g kg^{-1} were measured in May and averaged 490 g kg^{-1} in mid-summer, when plants reached full maturity and the highest dry matter yields. Moisture contents ranged 260-370 g kg^{-1} in autumn harvests and 110-120 g kg^{-1} in winter harvests. Therefore, a period of field drying after harvest would be required before baling if biomass was harvested between summer and autumn. In turn, biomass could be baled immediately after a winter harvest. It is known that senesced biomass exhibits lower moisture contents, which can avoid field drying after harvest before baling, reducing the costs associated with baling and transportation [9].

Biomass ash content diminished progressively as crop aged (Figure 3), decreasing from an initial mean ash content of 67 g kg^{-1} in late spring to 31-34 g kg^{-1} in winter. Hence, a delayed harvest made possible ash content reductions of 12-24% in autumn and 26-32% in winter with respect to a summer harvest. In this sense, similar ash content reductions (between 23% and 29%) were observed for giant reed when harvests were delayed until winter in a semi-arid Mediterranean environment [22]. In colder climates, ash content reductions of 13% for reed canary grass in Sweden [43] and 55% in Estonia [42] were also noticed in spring with respect to a summer harvest.

A delayed harvest impacted the chemical composition of the three cultivars of tall wheatgrass dramatically (Table 7 and Figures 3-5), which was attributed to a combination of grain and leaf losses, rain lixiviation of water soluble elements (K, Cl, and Na) during the long periods of standing senesced biomass, and to a likely mineral translocation from the shoots to clump bases during senescence (e.g. in case of elements such as N and P) [20,26,73]. All these processes contribute to reducing the mineral concentration of aerial biomass of grasses, which is at its highest during spring and early summer before it declines [26], as was reported when the harvest of natural grasslands and other cool-season grasses was delayed beyond autumn under various environmental conditions [19-22,35,36,41-43,73]. All the cultivars tested followed the same pattern across the studied period, showing only slight differences in their chemical composition.

On the one hand, defoliation improves biomass composition as the levels of ash, N, P, silica and alkali metals in the leaves are much greater in leaf material than in stems [35]. On the other, the water soluble elements remain in ionic water-soluble forms within plant tissues and thus are readily leached from the standing feedstock [41]. In addition, a remobilisation of nutrients occurs within grasses from aboveground tissues to the roots and other storage organs during senescence. This process has been associated with winter survival, so plants can use nutrients during winter for regrowth in the following spring [9,70]. In this sense, allowing the crop to senesce prior to harvesting might decrease the requirement for fertiliser inputs and improve crop sustainability by potentially reducing reliance on N fertilisers [35]. Remobilisation of primary nutrients from leaves and stem to rhizomes was measured for *Miscanthus*, a warm-season grass, and was found to be 21-46% of N, 36-50% of P and 14-30% of K [74].

In our study, N, Cl, and S biomass levels were already largely reduced by the end of the summer (Figure 4). By this time, mean reductions of 36% of N, 61% of Cl and 24% of S were measured.

- Insert Figure 4 here -

Net calorific values slightly increased by 0.5-2.5% from mean values of 17.1-17.5 MJ kg^{-1} (d.b.) in the summer and early autumn until 17.6-17.9 MJ kg^{-1} (d.b.) from mid-autumn onwards (Figure 4), probably in connection with the ash mineral reduction and the slight C and H increases observed as a consequence of delaying harvest (data not shown).

Some of the minor and trace elements (As, Cd, Hg, and V in particular), related to environmental issues [33] and typically present in the biomass at very low concentration levels, increased at later harvest dates (Table 8), probably in connection to the longer period of grasses in the field, which might increase the take up of these elements by plants. As, Cd and Pb levels were also noticed to increase in Sweden when the harvest of reed canary grass was delayed until spring of the following year as opposed to a summer harvest [43]. Compared to the autumn harvest, the concentration of Cr, together with other ash constituents such as Al, Fe, Si and Ti, was also found to increase when the harvest of several warm-season grasses were delayed to spring in Canada [32]. However, regardless of the harvest date, the levels of all the trace elements and heavy metals in the grasses cultivated in this study were extremely low and within the typical ranges for herbaceous biomass [34]. Therefore, this biomass is expected to comply with all the limitations set in the quality international standards for solid biofuels with regard to toxic heavy metals [75,76].

- Insert Table 8 here -

Ash chemical composition varied significantly across collection dates (Figure 5). The levels of K_2O, P_2O_5, and Na_2O (not shown) decreased largely as crops aged, while the contents of SiO_2, CaO, MgO and MnO increased. In this sense, mean reductions of 70% of K_2O, 68% of Cl, 38% of S, 37% of P_2O_5, and 32% of Na_2O were noticed in a mid-autumn harvest in November, with respect to a mid-summer harvest. Therefore, the levels of CaO and SiO_2 in the ashes increased by 96% and 110%, respectively.

- Insert Figure 5 here -

In mid-November, tall wheatgrass cultivars averaged 8.1 g kg^{-1} of N, 0.4 g kg^{-1} of Cl, and 0.4 g kg^{-1} of S, while major ash constituents averaged 570 g kg^{-1} of SiO_2, 130 g kg^{-1} of CaO, 100 g kg^{-1} of K_2O, 35 g kg^{-1} of P_2O_5, 33 g kg^{-1} of MgO and 1.8 g kg^{-1} of Na_2O.

The composition of the three cultivars improved after winter (Figures 3-5), when mean reductions of 85% of K_2O, 80% of Cl, 51% of N, 38% of S, 51% of P_2O_5, and 33% of Na_2O were reached with respect to a mid-summer harvest. Across cultivars, over-wintered biomass averaged 110 g kg^{-1} of moisture, 34 g kg^{-1} of ash, 5.4 g kg^{-1} of N, 0.2 g kg^{-1} of Cl, and 0.4 g kg^{-1} of S. Major ash constituents averaged 590 g kg^{-1} of SiO_2, 130 g kg^{-1} of CaO, 51 g kg^{-1} of K_2O, 27 g kg^{-1} of P_2O_5, 26 g kg^{-1} MgO, and 1.8 g kg^{-1} of Na_2O. The chemical composition of the samples collected in late winter was similar to that of over-wintered China reed, for which low water (160-330 g kg^{-1}) and mineral content (0.3-2.1 g kg^{-1} of Cl, 0.9-3.4 g kg^{-1} of N, and 3.7-11.2 g kg^{-1} of K) were reported [35,36,45]. In a semi-arid Mediterranean climate, reductions of 35% of N, 32% of P, 39% of K, 42% of Ca, 26% of Mg, 18% of S, and 48% of Si were reported when harvesting giant reed after winter [20].

3.2.3. Fuel quality properties

By the end of the summer, the levels of N, Cl, and S were below the recommended guidelines (10 g kg^{-1} for N and 1.0 g kg^{-1} for Cl and S, Table 3) to avoid combustion problems derived from NOx, SOx, HCl, furan and dioxin emissions [30,45,48,49]. In this sense, 2S/Cl molar ratios were over 2 after the summer (Figure 6), suggesting that severe corrosion risks would be avoided with an autumn harvest [8].

- Insert Figure 6 here -

Medium aerosol formation and PM_1 emissions (K+Na+Zn+Pb around 3000 mg kg^{-1} and Si/K molar ratios of 4.5) and medium to high temperature corrosion risks (2S/Cl molar ratios of 2.4) should be expected with a mid-autumn harvest (Figure 6) [8]. In turn, summer-harvested tall wheatgrass exhibited K+Na+Zn+Pb values close to or higher than 10,000 mg kg^{-1}, and Si/K and 2S/Cl molar ratios close to 1 and 2, respectively, which indicates high aerosol formation and PM_1 emissions as well as severe corrosion risks at the high temperatures achieved during combustion, as occurs with cereal straw [8]. Other biomass fuel assortments such as poplar, *Miscanthus*, maize residues, bark, hardwood or waste wood are also most commonly located in the medium to high PM_1 emission range [8].

If over-wintered biomass is used for combustion, corrosion risks at high temperatures as well as aerosol emissions are expected to be of minor importance, as 2S/Cl levels around 4, K+Na+Zn+Pb mean values of 1400 mg kg^{-1} and Si/K molar ratios between 8 and 14 were measured in the samples collected in March (Figure 6). The most restrictive guideline found in the literature to avoid NOx problems (6 g kg^{-1} of N [45,49]) was also met after winter, when N reached its lowest levels.

The indicators for ash melting problems predict significantly better ash fusibility behaviour at later harvest dates (Figure 6), mainly due to the significant K reductions and Ca increases noted as harvest is delayed. It is generally known that Ca and Mg increase ash-melting temperature, while Si in combination with K decrease ash-melting temperature [8,33]. The guidelines to minimise issues related to ash sintering and fusion at the normal operating temperatures of boilers were met in winter, when earth-alkaline to alkali oxides ratios were over 2 and the ratios between K and the sum of Ca and Mg were below 0.5. The (Si+P+K)/(Ca+Mg) index also improved as harvest was delayed, decreasing progressively until winter. Similarly, a delayed harvest was also noticed to improve the fusibility behaviour of other cool and warm-season grasses under different environmental conditions, mainly due to the increased Ca/K and Si/K ratios noticed in winter [20,41,44].

Fusibility tests corroborated index predictions (Table 9). In this sense, ash characteristic temperatures were lower in mid-summer and increased as the harvest was delayed. Deformation temperatures of 1180 ºC and flow temperatures of 1310 ºC were measured by the end of winter, increasing by 370 ºC and 240 ºC, respectively, with respect to a mid-summer harvest.

- Insert Table 9 here -

The improvement in the ash fusibility behaviour noticed at later harvest dates once that plants reached full maturity was associated to the significant changes observed in the chemical composition of the cultivated grasses, and confirmed by XRD analysis, which also detected significant differences in the major ash-forming mineral compounds (Figure 7).

- Insert Figure 7 here -

The ash mineral composition of summer-harvested tall wheatgrass was dominated by alkali compounds, mainly potassium sulphates (≈31% of arcanite), carbonates (≈35% fairchildite, among others) and chlorides (≈12% of sylvine). Potassium chlorides and potassium carbonates, with melting points between 770 ºC and 890 ºC, melt at the normal operating temperatures of boilers. According Rietveld semi-quantification, calcium carbonates (calcite) were only present at levels of ≈7% (melting point of 1339 ºC).

Delayed harvest reduced alkali compounds, which are known to decrease ash-sintering temperatures, and increased the presence of earth-alkaline compounds, which improve ash fusibility behaviour due to their higher melting points. By the end of the winter, ashes were formed by ≈72% of calcium carbonates and sulphates (calcite and anhydrite), ≈18% of silicon dioxide (quartz), all with melting points above 1300 ºC, and only ≈10% of potassium sulphates (arcanite), with a melting point of 1069 ºC. However, it should be noticed that these tests were carried out on a laboratory scale, and thus combustion tests with tall wheatgrass harvested at different dates between mid-summer and the end of the winter would very useful in order to compare the composition and fusibility behaviour of the different type of ashes produced during the thermal treatment at the operational combustion conditions in a real boiler.

The reduction in the expected emissions, and slagging, fouling, and corrosion potentials, together with the reduction of mineral nutrients removed from the field derived from rain leaching and translocation to the roots (which might decrease the requirement for fertiliser inputs at the same time), without a dramatic loss in biomass or caloric content provide motivation for delaying harvest at least until mid-autumn.

Further research should address other practical challenges associated with adopting such a practice, as the risk of incurring into additional dry matter losses when the fragile senesced biomass is harvested mechanically, the possibility of biomass contamination depending on the soil conditions at harvest, or the risk of compromising next year yields if the harvest is delayed until late winter, when new shoots can already appear. A long-term field study under Mediterranean marginal conditions would be essential to identify the ideal harvest time since the responses of cool-season grasses and their mineral composition are expected to change in relation to meteorological conditions, crop ageing, and soil nutrient availability for the following years. The results of this study are applicable to more than 10 Mha categorised as marginal land in Europe [17,77], where food crops fail to provide financially viable yields on inherently low productivity sites, and where tall wheatgrass could provide satisfactory biomass yields under rainfed conditions and minimum inputs, as long as spring rainfall is maintained at acceptable levels. It provides valuable information for a potential implementation of cool perennial grasses for dedicated energy crops in low input agriculture in marginal Mediterranean areas.

5. CONCLUSIONS

This study shows how the production, composition and combustion quality properties of tall wheatgrass biomass are affected by N fertilisation and harvest time under Mediterranean marginal conditions.

Biomass production grew with increasing N input and spring rainfall. The application of 80 kg N ha^{-1} yr^{-1} resulted in higher N biomass contents with respect to the 30 and 0 kg N ha^{-1} yr^{-1} treatments. The rest of the chemical properties were not affected from a practical point of view. Therefore, taking into account the slight differences detected and that NOx emissions can be controlled, at least to a certain extent, during the thermal treatment of the biomass by using the appropriate technologies and operational conditions, a minimum N application of 80 kg N ha^{-1} yr^{-1} would be recommended in low productive soils under marginal Mediterranean conditions in order to maximise biomass production without significantly affecting quality properties.

As expected, summer-harvested tall wheatgrass biomass is characterised by relatively high contents of ash, N, Si, and alkali elements such as Cl and K. Therefore, low sintering temperatures, severe corrosion risks, and high aerosol emissions should be expected during combustion if biomass is collected at this time. The choice of a delayed harvest greatly improved the quality properties of `Alkar´, `Jose´, and `Riparianslopes´ tall wheatgrass cultivars for combustion purposes under the marginal Mediterranean conditions of this study. An autumn harvest greatly reduces moisture, ash, N, Cl, Na, K, and S levels. Over-wintered biomass shows even better combustion quality properties as it causes even greater K, Cl, and N reductions.

All quality indicators clearly predict a positive impact of adopting a delayed harvest system on the fuel properties of tall wheatgrass biomass for thermal conversion processes. In this sense, an autumn harvest is expected to reduce NOx, SOx and HCl emissions, decrease aerosol and formation and PM_1 emissions to medium levels, and avoid severe corrosion risks at high temperatures. However, these risks would not be minimised unless biomass is harvested after winter.

Delaying harvest time improves the fusibility behaviour of tall wheatgrass ashes by increasing deformation and flow temperatures. Deformation temperatures of 1180 ºC were measured by the end of the winter, increasing by 370 ºC with respect to a mid-summer harvest. Adopting such a system reduces alkali compounds, which are known to decrease ash-sintering temperatures, and increases the presence of earth-alkaline compounds, which improve ash fusibility behaviour due to their high melting points. In this sense, while the ash mineral composition of summer-harvested tall wheatgrass is dominated by potassium sulphates, carbonates and chlorides, the major mineral phases detected in samples collected after winter are calcium carbonates and sulphates and silicon dioxide.

Dry matter losses can be maintained at acceptable levels (16%) until early or mid-autumn, as temperatures in Mediterranean areas are still relatively mild until this time.

However, considerable yield reductions should be expected (36-48%) at later harvest dates in this type of temperate environments with cold winters, as the adverse climatological conditions during late autumn and winter can cause significant biomass losses derived from the fragility of senesced biomass.

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FIGURE CAPTIONS:

Figure 1. Monthly rainfall distribution and mean temperature at the experimental site during the harvest time study.

Figure 2. Biomass production and N contents across annual N top dressing applications (0, 30, and 80 kg ha^{-1}) and years for the biomass of tall wheatgrass (summer harvest). Fisher´s LSD plots.

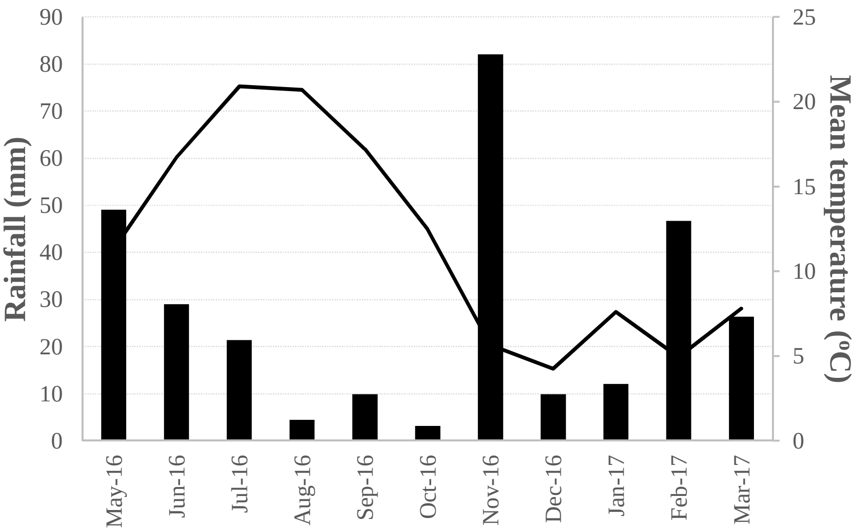
Figure 3. Effect of harvest time on biomass production, plant height, moisture and ash content of different tall wheatgrass cultivars. Fisher´s LSD plots.

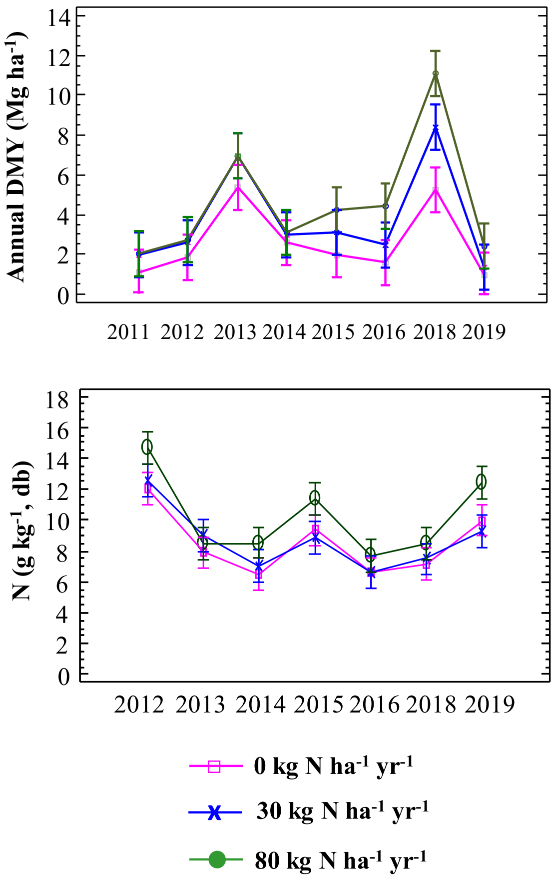
Figure 4. Effect of harvest time on the calorific value, and the N, Cl, and S contents of different tall wheatgrass cultivars. Fisher´s LSD plots.

Figure 5. Effect of harvest time on the ash chemical composition of different tall wheatgrass cultivars. Fisher´s LSD plots.

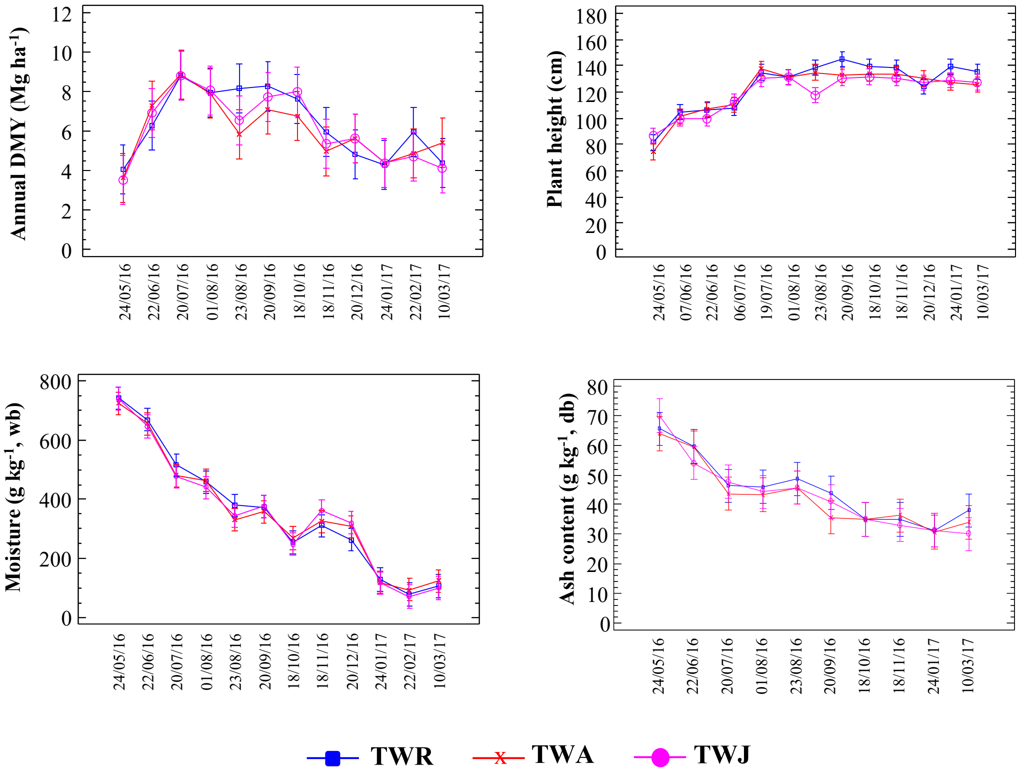
Figure 6. Effect of harvest time on the fuel combustion indices of different tall wheatgrass cultivars. Fisher´s LSD plots.

Figure 7. Effect of harvest time on the ash mineral composition of the crops (tall wheatgrass, cv. Alkar) (a) full XRD diffractograms and (b) detailed diffractograms between 22^{o} and 36^{o} (2θ).

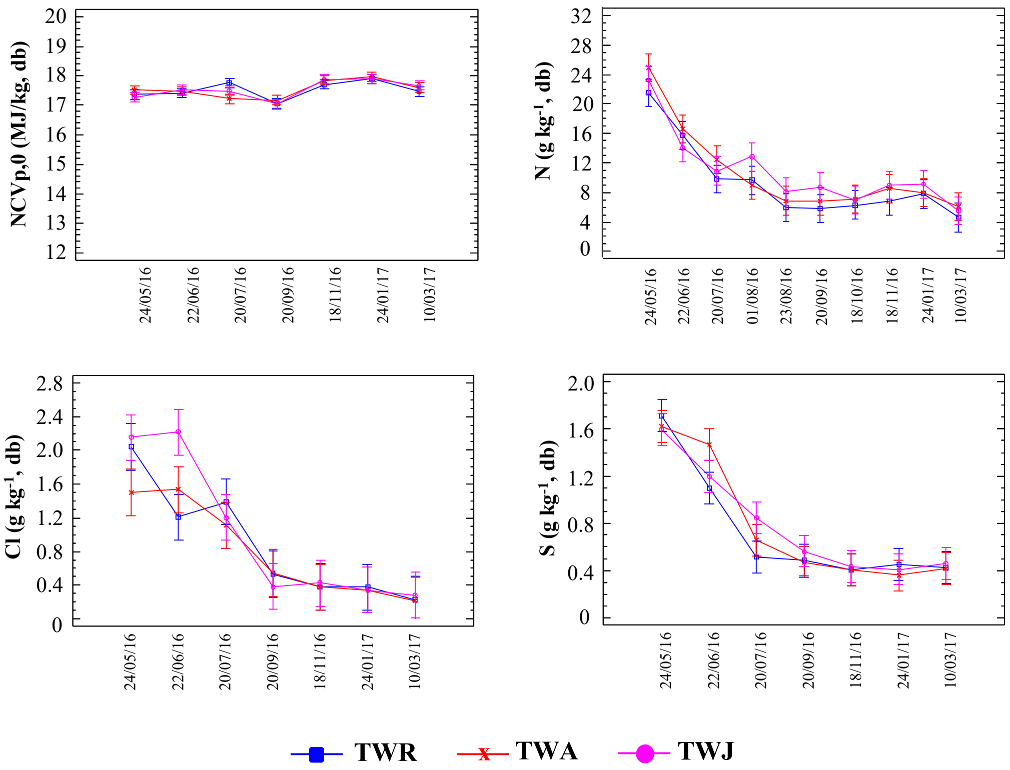

Rainfall (mm)
Mean temperature (°C)
0
10
20
30
40
50
60
70
80
90
0
5
10
15
20
25
May-16
Jun-16
Jul-16
Aug-16
Sep-16
Oct-16
Nov-16
Dec-16
Jan-17
Feb-17
Mar-17



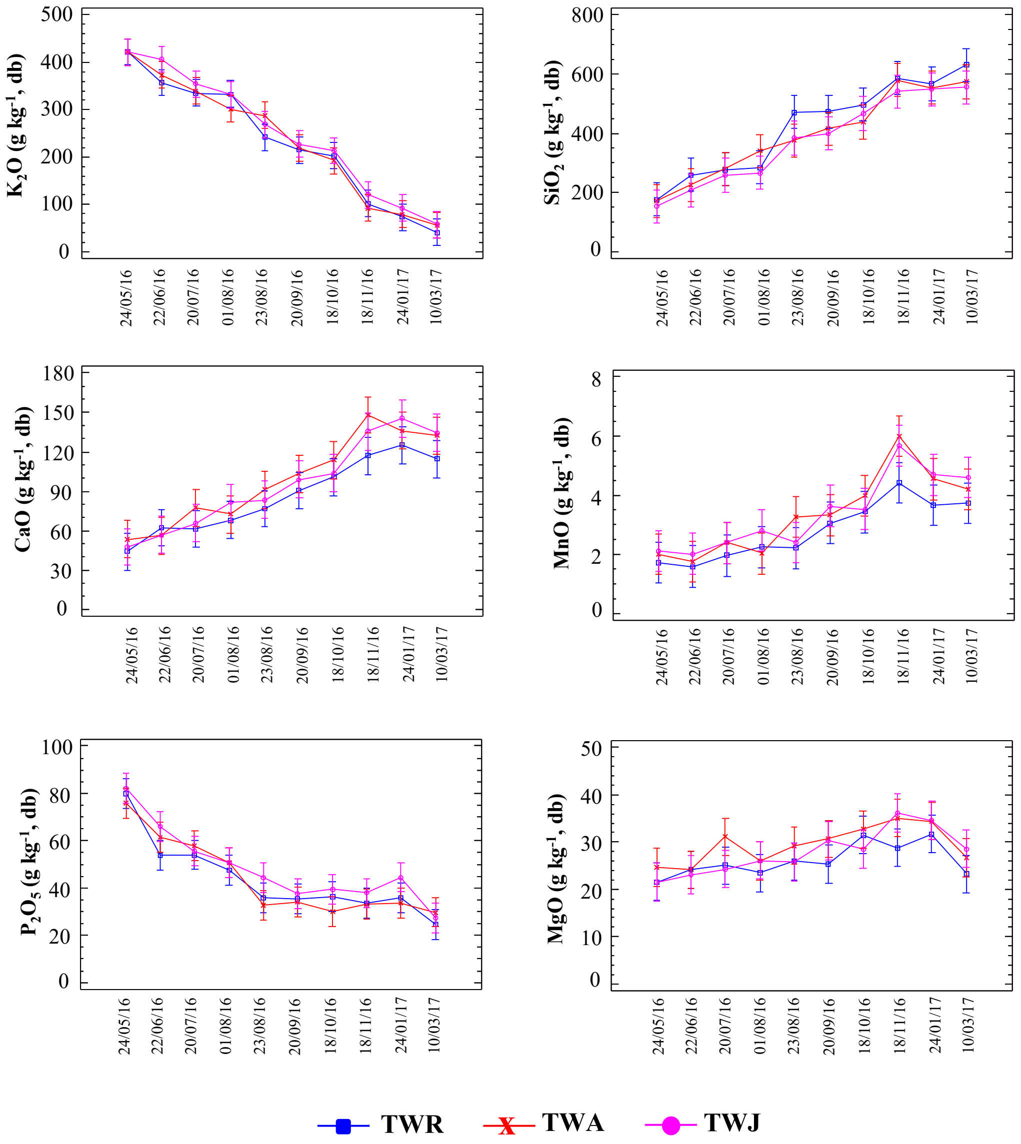
db: dry basis; DMY: dry matter yields



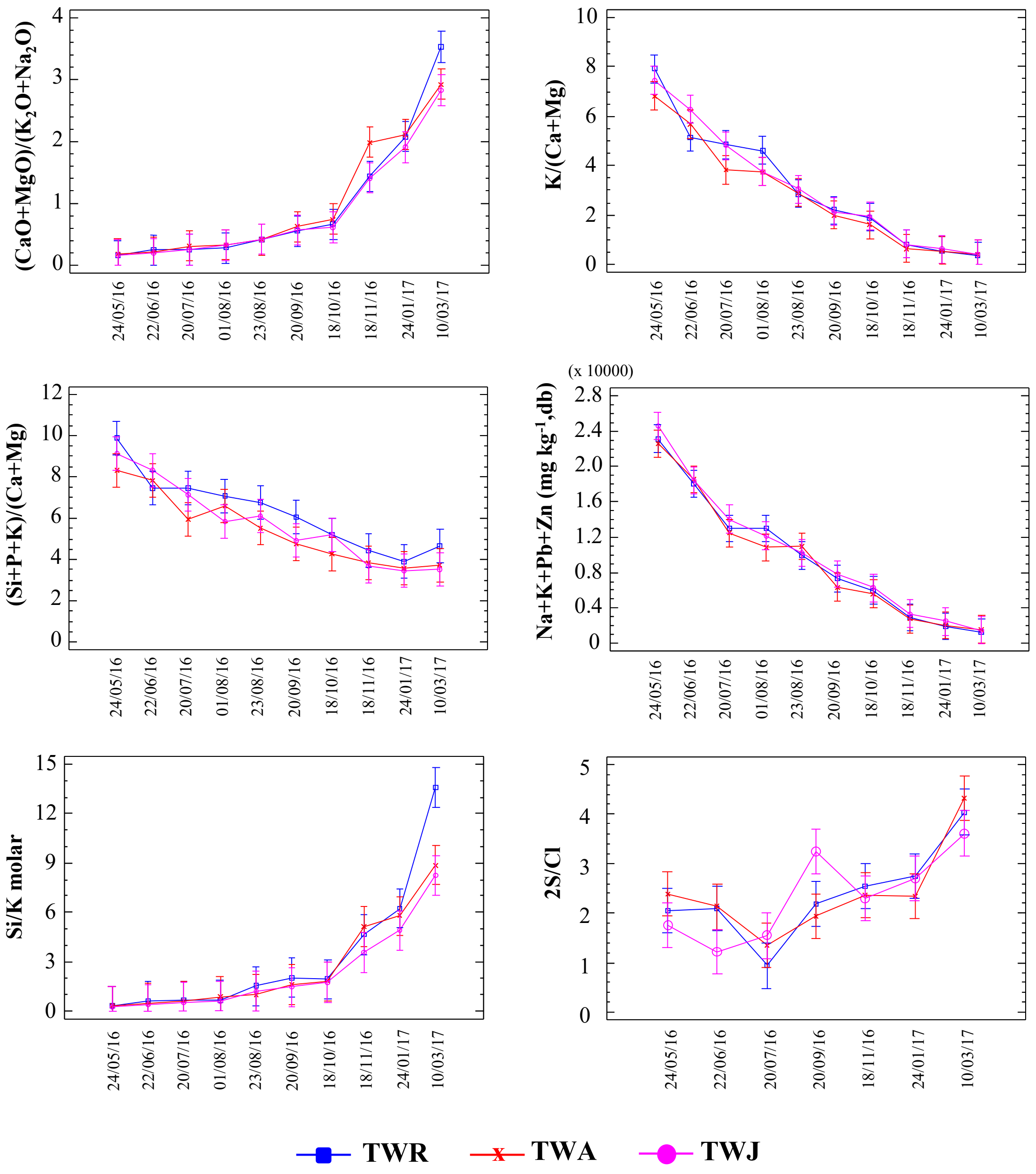
Tall wheatgrass cv. Riparianslopes (TWR), cv. Alkar (TWA), and cv. Jose (TWJ); db: dry basis; DMY: dry matter yields.



Tall wheatgrass cv. Riparianslopes (TWR), cv. Alkar (TWA), and cv. Jose (TWJ); db: dry basis; NCVp,o: Net calorific value at constant pressure in db.

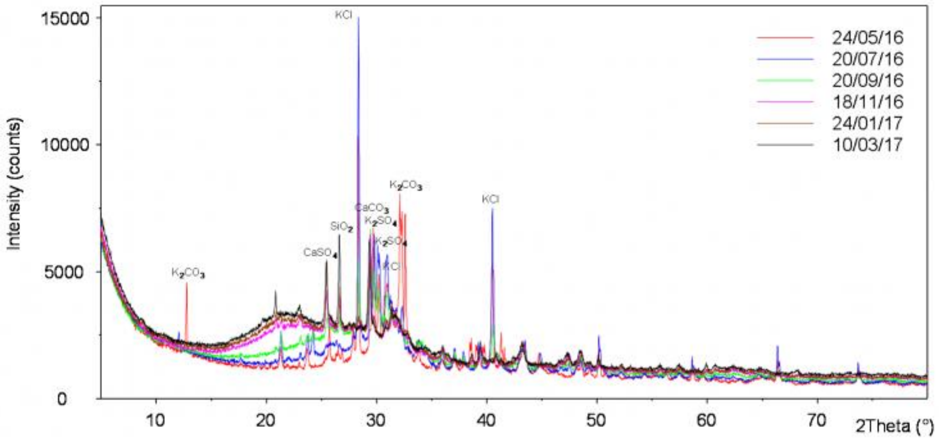


Tall wheatgrass cv. Riparianslopes (TWR), cv. Alkar (TWA), and cv. Jose (TWJ); db: dry basis.



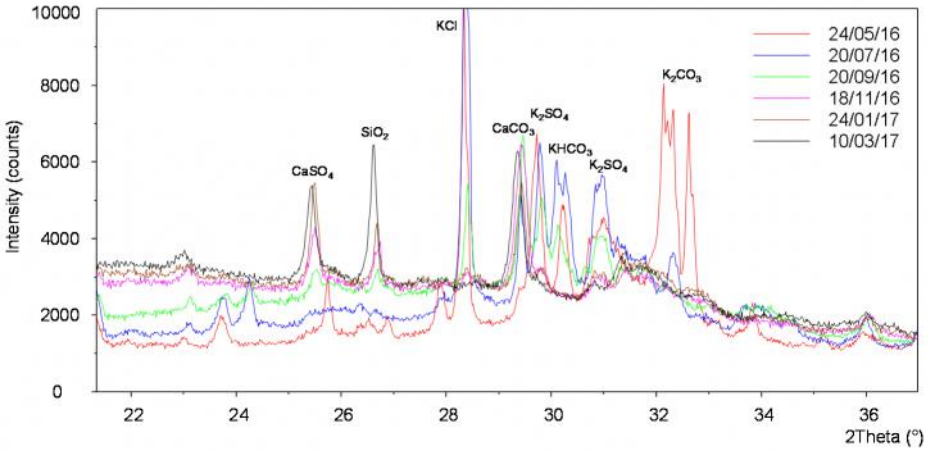
Tall wheatgrass cv. Riparianslopes (TWR), cv. Alkar (TWA), and cv. Jose (TWJ); db: dry basis.

a)



Intensity (counts)
15000
10000
5000
0
10
20
30
40
50
60
70
2Theta (°)
KCl
K_2CO_3
$CaCO_3$
K_2SO_4
SiO_2
$CaSO_4$
K_2SO_4
KCl
KCl
K_2CO_3
24/05/16
20/07/16
20/09/16
18/11/16
24/01/17
10/03/17

b)



Intensity (counts)
10000
8000
6000
4000
2000
0
22
24
26
28
30
32
34
36
2Theta (°)
KCl
K_2CO_3
$CaCO_3$
K_2SO_4
SiO_2
KHCO$_3$
$CaSO_4$
K_2SO_4
24/05/16
20/07/16
20/09/16
18/11/16
24/01/17
10/03/17

Table 1. Weather conditions at the experimental site throughout the study period (2010-2019).

Year	**AMT (ºC)**	**AmT (ºC)**	**MMT (ºC)**	**MmT (ºC)**	**MT (ºC)**	**AP (mm)**	**SP (mm)**	**SR (Wh m^{-2})**	**RH (%)**
2010	33.4	-11.0	15.6	4.2	9.6	599	252	125667	68.0
2011	35.7	-11.9	17.9	4.9	11.1	380	219	135767	66.5
2012	37.0	-10.3	17.7	4.5	10.6	344	162	138297	62.9
2013	33.8	-8.8	16.5	3.2	9.5	595	311	128987	70.4
2014	33.3	-6.7	18.0	3.6	10.7	596	217	133304	68.7
2015	36.0	-9.5	18.1	4.6	11.0	488	278	133817	67.1
2016	34.7	-7.5	17.3	4.8	10.7	540	234	117042	69.5
2017	35.3	-11.7	18.5	4.8	11.9	315	143	137881	63.0
2018	35.3	-8.3	16.6	4.9	10.4	669	422	107875	73.4
2019	37.6	-6.8	18.0	4.8	11.1	529	216	141525	64.7
Mean	35.2	-9.3	17.4	4.4	10.7	505	245	130016	67.4

T: temperature, AMT: Absolute maximum T; AmT: Absolute minimum T; MMT: Mean maximum T; MmT: Mean minimum T; MT: Mean T; AP: Annual accumulated precipitation; SP: Accumulated precipitation between March and July; SR: Solar radiation; RH: Relative humidity

Table 2. Analytical techniques, equipment and international standards used.

Property	**Analytical technique**	**Equipment**	**Standard**
Biomass analysis			
Sample preparation	Subsampling and milling		ISO 14780:2017
Moisture	Oven drying at 105 ºC		ISO 18134-2:2017
Ash	Calcination at 550 ºC		ISO 18122:2015
C, H, N	Elemental analysis: CC+IR+TCD	TruSpec (Leco Instruments)	ISO 16948:2015
S and Cl	Combustion bomb + IC	883 Basic IC Plus (Metrohm)	ISO 16994:2016
Calorific value	Calorimetry	C5003 (Ika Verke)	ISO 18125:2017
Minor and trace elements: As, Cd, Cr, Cu, Ni, Pb, V, Zn	MWD + ICP-MS	Ethos Pro (Milestone) + iCAP Q (Thermo Fisher)	ISO 16968:2015
Hg	TD + Gold amalgamation + AAS	DMA-80 (Milestone)	ISO 16968:2015
Ash analysis			
Ash major elements: Al, Ca, Fe, K, Mg, Mn, Na, P, Si, Ti	MWD + ICP-OES	Ethos Pro (Milestone) + Jarrell Ash (Thermo Fisher)	ISO 16967:2015
Ash fusibility	Optical heating microscopy	Ash fusion determinator (Hesse)	ISO 21404:2020
Ash mineral phases	XRD	X´Pert Pro (Panalytical)	n.s.

CC: flash catalytic combustion; IR: Infrared detection; TCD: Thermal conductivity detection; IC: Ion chromatography; MWD: microwave assisted digestion; ICP-OES: Inductively coupled plasma – Optical Emission Spectroscopy; ICP-MS: Inductively coupled plasma - Mass spectrometry; TD: catalytic thermal desorption; AAS: Atomic absorption spectroscopy; XRD: X-ray diffraction; n.s.: not standardized

Table 3. Guideline to avoid problems during the thermal treatment of solid biofuels.

Property / Index	**Guideline**	**Reference**	**Limiting parameter**
N	< 6-10 g kg^{-1}	[30,45,48,49]	NOx emissions
S	< 1 g kg^{-1}	[48]	Corrosion
	< 2 g kg^{-1}	[30,49]	SOx emissions
Cl	< 1-2 g kg^{-1}	[30,45,48,49]	Corrosion, HCl emissions
	< 2-3 g kg^{-1}	[30,45]	Dioxins and furans emissions
$(CaO+MgO)/(K_2O+Na_2O)$	> 2	[50]	Sintering
K/(Ca+Mg)	< 0.5	[41]	Sintering and fusion
(Si+P+K)/(Ca+Mg) molar ratio	≤ 1 (sintering T ≥ 1200 ºC) < 3 (sintering T ≥ 1000 ºC)	[8]	Sintering
K+Na+Zn+Pb	< 1000 mg kg^{-1} (low risk) 1000-10000 mg kg^{-1} (medium risk) > 10000 mg kg^{-1} (high risk)	[8]	Aerosol emissions (PM_1) and deposit buildup
Si/K molar ratio	as high as possible	[8]	K release; aerosol formation
2S/Cl molar ratio	< 2 severe risk > 4 minor risk ≥ 8 no risk	[8]	High temperature corrosion

Table 4. Effect of different annual N top dressing application (0, 30, and 80 kg ha^{-1}) on the main properties of tall wheatgrass biomass across years (summer harvest).

N application	**Year**	**Ash** (g kg^{-1}, db)	**C** (g kg^{-1}, db)	**H** (g kg^{-1}, db)	**N** (g kg^{-1}, db)	**Cl** (g kg^{-1}, db)	**S** (g kg^{-1}, db)	**GCVv,o** (MJ kg^{-1} db)	**NCVp,o** (MJ kg^{-1} db)
0 kg ha^{-1}	2012	41	474	60	12	2.0	1.5	19.00	17.69
	2013	35	470	60	7.9	1.7	0.8	18.87	17.55
	2014	47	465	60	6.6	2.1	0.8	18.49	17.19
	2015	45	466	60	9.3	2.5	1.0	18.70	17.38
	2016	45	470	60	6.6	2.0	0.8	18.67	17.34
	2018	54	456	60	7.1	2.5	0.8	18.28	16.96
	2019	43	469	60	10	1.2	1.0	19.02	17.70
	mean ± SD	44 ± 6 ^{a}	467 ± 6 ^{a}	60 ± <1 ^{a}	8.5 ± 2.0 ^{a}	2.0 ± 0.5 ^{b}	1.0 ± 0.2 ^{a}	18.7 ± 0.3 ^{a}	17.4 ± 0.3 ^{a}
30 kg ha^{-1}	2012	49	474	60	13	1.6	1.4	19.00	17.68
	2013	35	473	61	9.0	1.6	0.8	18.89	17.56
	2014	44	466	60	7.0	2.2	0.9	18.67	17.36
	2015	40	467	60	8.8	2.5	1.1	18.89	17.56
	2016	46	466	61	6.7	2.3	0.7	18.51	17.17
	2018	47	470	61	7.5	1.3	0.6	18.75	17.43
	2019	42	471	60	9.3	1.2	0.8	18.99	17.67
	mean ± SD	43 ± 5 ^{a}	469 ± 3 ^{a}	60 ± <1 ^{a}	8.7 ± 2.0 ^{a}	1.8 ± 0.5 ^{b}	0.9 ± 0.3 ^{a}	18.8 ± 0.2 ^{a}	17.5 ± 0.2 ^{a}
80 kg ha^{-1}	2012	51	476	60	15	1.5	1.5	19.07	17.76
	2013	37	472	61	8.5	1.4	0.8	18.72	17.39
	2014	44	467	60	8.5	1.8	1.0	18.74	17.43
	2015	37	471	61	11	2.2	1.2	19.01	17.69
	2016	40	473	61	7.7	2.1	0.9	18.76	17.41
	2018	34	473	60	8.5	1.2	0.6	18.85	17.53
	2019	40	466	61	12	0.5	0.8	18.78	17.47
	mean ± SD	40 ± 6 ^{a}	471 ± 4 ^{a}	60 ± 1 ^{a}	10.2 ± 2.6 ^{b}	1.5 ± 0.6 ^{a}	1.0 ± 0.3 ^{a}	18.8 ± 0.1 ^{a}	17.5 ± 0.1 ^{a}
	global mean ± SD	43 ± 8	469 ± 6	60 ± <1	9.1 ± 2.3	1.8 ± 0.5	0.9 ± 0.3	18.8 ± 0.2	17.5 ± 0.2
	P75	47	474	61	11	2.3	1.0	19.00	17.67
		ρ-values							
	N application (F)	n.s.	n.s.	n.s.	**	**	n.s.	n.s.	n.s.
	Year (Y)	n.s.	*	n.s.	**	**	**	**	**
	F*Y	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

db: dry basis; GCVv,o: gross calorific value at constant volume in db; NCVp,o: net calorific value at constant pressure in db; SD: standard deviation; P75: percentile 75%. Factors: Annual N top dressing applications (F), year (Y) and their interaction (F*Y). ρ-value <0.01 (**), >0.05 (n.s.) and 0.01-0.05 (*). Different letters denote significant different means among N applications at the 95% confidence level

Table 5. Effect of different annual N top dressing application (0, 30, and 80 kg ha^{-1}) on the ash chemical composition of tall wheatgrass biomass across years (summer harvest).

		Al_2O_3	**CaO**	**Fe_2O_3**	**MgO**	**MnO**	**Na_2O**	**P_2O_5**	**K_2O**	**SiO_2**	**ZnO**
N application	**Year**	(g kg^{-1}, db)	(g kg^{-1}, db)	(g kg^{-1}, db)	(g kg^{-1}, db)	(g kg^{-1}, db)	(g kg^{-1}, db)	(g kg^{-1}, db)	(g kg^{-1}, db)	(g kg^{-1}, db)	(mg kg^{-1} db)
0 kg ha^{-1}	2012	5.0	71	0.95	28	2.5	5.4	62	373	199	29
	2013	3.4	78	0.88	26	2.8	2.4	75	291	365	37
	2014	1.6	55	0.75	18	2.1	0.8	68	279	371	29
	2015	7.2	78	2.8	24	2.9	0.8	65	206	379	24
	2016	2.9	66	0.48	21	2.0	0.3	60	281	364	27
	2018	4.2	58	0.74	26	2.8	0.7	61	303	376	31
	2019	4.1	110	1.0	30	2.7	1.2	74	270	328	36
	mean ± SD	4 ± 2 ^{a}	70 ± 20 ^{a}	1.1 ± 0.8 ^{a}	25 ± 4 ^{a}	2.6 ± 0.3 ^{a}	2 ± 2 ^{a}	66 ± 6 ^{b}	290 ± 50 ^{a}	340 ± 60 ^{b}	30 ± 5 ^{a}
30 kg ha^{-1}	2012	4.0	68	0.89	29	2.7	6.2	68	373	208	32
	2013	2.5	72	1.0	29	3.3	5.3	80	343	283	39
	2014	0.9	58	0.55	19	2.3	1.7	64	301	336	31
	2015	7.7	82	3.1	29	3.8	2.1	69	248	368	30
	2016	2.6	67	0.23	20	1.9	0.2	53	260	396	26
	2018	2.9	52	1.1	26	6.0	0.5	59	365	237	45
	2019	3.5	82	0.61	26	2.1	0.6	79	287	334	32
	mean ± SD	3 ± 2 ^{a}	70 ± 10 ^{a}	1.1 ± 0.9 ^{a}	25 ± 4 ^{a}	3.2 ± 1.4 ^{a}	2 ± 2 ^{a}	67 ± 10 ^{b}	310 ± 50 ^{ab}	310 ± 70 ^{ab}	33 ± 7 ^{a}
80 kg ha^{-1}	2012	4.2	73	1.2	28	2.5	6.3	59	362	201	33
	2013	4.1	83	1.0	25	3.3	2.0	69	308	331	37
	2014	1.5	68	0.53	25	2.7	1.7	49	314	306	32
	2015	7.7	81	2.8	29	3.0	2.7	54	281	307	30
	2016	4.1	63	0.52	23	2.1	2.1	51	320	314	32
	2018	1.8	58	0.88	23	2.9	0.6	63	398	215	40
	2019	5.9	100	1.1	30	2.5	3.1	71	293	284	33
	mean ± SD	4 ± 2 ^{a}	80 ± 10 ^{a}	1.2 ± 0.8 ^{a}	26 ± 3 ^{a}	2.7 ± 0.4 ^{a}	3 ± 2 ^{a}	59 ± 9 ^{a}	330 ± 40 ^{b}	280 ± 50 ^{a}	34 ± 3 ^{a}
	global mean ± SD	4 ± 2	70 ± 10	1.1 ± 0.8	25 ± 4	2.8 ± 0.9	2 ± 2	64 ± 9	370 ± 50	310 ± 60	33 ± 5
	P75	5.1	81	1.3	29	3.4	3.0	73	360	370	35
		ρ-values									
	N application (F)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*	n.s.
	Year (Y)	**	**	**	**	n.s.	**	**	**	**	**
	F*Y	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

db: dry basis; SD: standard deviation; P75: percentile 75%. Annual N top dressing applications (F), year (Y) and their interaction (F*Y). ρ-value <0.01 (**), >0.05 (n.s.) and 0.01-0.05 (*). Different letters denote significant different means among N applications at the 95% confidence level

Table 6. Effect of different annual N top dressing application (0, 30, and 80 kg ha^{-1}) on the quality indicators of tall wheatgrass biomass across years (summer harvest).

N application	**Year**	**(CaO+MgO) / (K_2O+Na_2O)**	**K / (Ca+Mg)**	**(Si+P+K) / (Ca+Mg)** (molar)	**K + Na + Zn + Pb** (mg kg^{-1}, db)	**Si/K** (molar)	**2S/Cl** (molar)
0 kg ha^{-1}	2012	0.26	4.6	6.9	13000	0.39	1.7
	2013	0.36	3.4	7.1	8400	0.97	1.0
	2014	0.26	4.7	10	10900	0.98	0.9
	2015	0.49	2.5	6.5	7700	1.4	0.9
	2016	0.31	4.2	8.9	10400	1.03	0.8
	2018	0.27	4.6	9.7	13500	0.98	0.7
	2019	0.53	2.4	5.1	9400	1.0	1.9
	mean ± SD	0.36 ± 0.11 ^{a}	4 ± 1 ^{a}	8 ± 2 ^{a}	11000 ± 2000 ^{a}	1.0 ± 0.3 ^{b}	1.2 ± 0.5 ^{a}
30 kg ha^{-1}	2012	0.26	4.7	7.1	15300	0.41	2.1
	2013	0.30	4.2	7.3	10300	0.63	1.2
	2014	0.25	4.8	9.4	11000	0.82	0.9
	2015	0.47	2.8	6.4	7600	1.2	0.9
	2016	0.34	3.6	8.4	9600	1.2	0.7
	2018	0.21	5.8	9.4	14500	0.52	1.1
	2019	0.39	3.2	6.7	9900	0.98	1.6
	mean ± SD	0.32 ± 0.09 ^{a}	4 ± 1 ^{a}	8 ± 1 ^{a}	11000 ± 3000 ^{a}	0.8 ± 0.3 ^{b}	1.2 ± 0.5 ^{a}
80 kg ha^{-1}	2012	0.27	4.4	6.7	15500	0.41	2.2
	2013	0.37	3.5	6.7	9600	0.88	1.2
	2014	0.30	4.1	7.5	11200	0.75	1.2
	2015	0.40	3.1	6.1	9300	0.88	1.2
	2016	0.27	4.5	8.4	10600	0.78	1.0
	2018	0.20	6.0	9.1	11200	0.43	1.2
	2019	0.44	2.7	5.2	9700	0.80	3.5
	mean ± SD	0.32 ± 0.08 ^{a}	4 ± 1 ^{a}	7 ± 1 ^{a}	11000 ± 2000 ^{a}	0.7 ± 0.2 ^{a}	1.6 ± 0.9 ^{b}
global mean ± SD		0.33 ± 0.09	4 ± 1	8 ± 2	1100 ± 2000	0.8 ± 0.3	1.3 ± 0.7
P75		0.39	4.9	8.5	12800	1.1	1.5
				ρ-values			
N application (F)		n.s.	n.s.	n.s.	n.s.	*	**
Year (Y)		**	**	**	**	**	**
F*Y		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

db: dry basis; SD: standard deviation; P75: percentile 75%. Annual N top dressing applications (F), year (Y) and their interaction (F*Y). ρ-value <0.01 (**), >0.05 (n.s.) and 0.01-0.05 (*). Different letters denote significant different means among N applications at the 95% confidence level

Table 7. Statistical significance (ρ-values) of harvest time and type of cultivar on the production, chemical composition and quality properties of tall wheatgrass biomass.

	Factors		
Property	**Harvest time (HT)**	**Cultivar (C)**	**HT*C**
DMY	**	n.s.	n.s.
Plant height	**	**	n.s.
Moisture	**	n.s.	n.s.
Ash	**	n.s.	n.s.
GCVv,o	**	n.s.	n.s.
NCVp,o	**	n.s.	n.s.
C	**	n.s.	n.s.
Cl	**	n.s.	n.s.
H	**	n.s.	n.s.
K	**	n.s.	n.s.
N	**	*	n.s.
P	**	n.s.	n.s.
S	**	n.s.	n.s.
Ash chemical composition			
Al_2O_3	**	n.s.	*
CaO	**	*	n.s.
Fe_2O_3	**	n.s.	n.s.
K_2O	**	n.s.	n.s.
MgO	**	*	n.s.
MnO	**	*	n.s.
Na_2O	**	n.s.	n.s.
P_2O_5	**	*	n.s.
SiO_2	**	n.s.	n.s.
ZnO	**	n.s.	n.s.
Fuel indices			
AE/A	**	n.s.	n.s.
K/(Ca+Mg)	**	n.s.	n.s.
(Si+P+K)/(Ca+Mg)	**	**	n.s.
K+Na+Zn+Pb	**	n.s.	n.s.
Si/K	**	n.s.	n.s.
2S/Cl	**	n.s.	n.s.

ρ-values: <0.01 (**), >0.05 (n.s.) and 0.01-0.05 (*)

Table 8. Effect of harvest time on the minor and trace elements of tall wheatgrass averaged across cultivars.

Harvest date	**As**	**Cd**	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**V**	**Zn**
	Mean (mg kg^{-1}, db)								
24/05/2016	<0.010 ^{a}	0.016 ^{bc}	0.58	8.8	0.004 ^{a}	2.0	0.28	<0.10 ^{a}	20 ^{c}
20/07/2016	<0.010 ^{a}	0.011 ^{ab}	0.61	11	0.005 ^{b}	1.0	0.41	<0.10 ^{ab}	16 ^{b}
20/09/2016	<0.010 ^{a}	0.008 ^{a}	1.0	6.8	0.006 ^{bc}	1.3	0.28	0.13 ^{abc}	13 ^{a}
18/11/2016	<0.010 ^{a}	0.021 ^{c}	0.70	7.8	0.009 ^{d}	2.1	0.38	0.17 ^{bc}	13 ^{a}
24/01/2017	<0.010 ^{a}	0.018 ^{c}	0.56	7.7	0.008 ^{cd}	1.8	0.54	0.22 ^{cd}	0.458
10/03/2017	0.028 ^{b}	0.019 ^{c}	0.91	6.9	0.008 ^{cd}	1.4	0.77	0.30 ^{d}	0.000
mean	<0.010	0.015	0.73	8.2	0.007	1.59	0.44	0.16	14
SD	0.013	0.005	0.34	2.0	0.002	0.92	0.28	0.11	3.4
Minimum value	<0.010	<0.010	0.15	5.1	0.003	<1.0	<1.0	<0.10	9.4
Maximum value	0.047	0.023	1.5	13	0.009	4.0	1.4	0.41	21
P75	<0.010	0.019	0.83	9.7	0.008	1.8	0.53	0.18	17
	ρ-values								
Harvest time	*	**	n.s.	n.s.	**	n.s.	n.s.	**	**
Cultivar	ns	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

ρ-values <0.01 (**), >0.05 (n.s.) and 0.01-0.05 (*); db: dry basis; SD: standard deviation; P75: percentile 75%. Different letters denote significant different means among harvest dates at the 95% confidence level

Table 9. Effect of harvest time on the ash fusibility of the crops (tall wheatgrass, var. Alkar).

Harvest date	**DT**	**ST**	**HT**	**FT**
		(ºC)		
24/05/2016	1130	n.d.	1250	1290
20/07/2016	810	860	910	1070
20/09/2016	820	910	1060	1120
18/11/2016	1090	1170	1200	1240
24/01/2017	1120	1170	1210	1250
10/03/2017	1180	n.d.	1240	1310

Deformation (DT), sphere (ST), hemisphere (HT) and fluid (FT) temperatures; n.d.: not detected