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Lignocellulosic Biomass Production and Persistence of Perennial Grass Species Grown in Mediterranean Marginal Lands

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Abstract: Biomass production in marginal lands represents one of the most challenging and promising alternatives to sustainably produce biofuels. Native species seem to be the most adequate option to obtain a profitable output when low-input techniques are applied, and biomass is grown in depleted soils and harsh climatic conditions. In this study, a 5-year field trial in the island of Majorca served to investigate different autochthonous and naturalized Mediterranean perennial grasses as novel candidate lignocellulosic bioenergy crops for the semi-arid Mediterranean area and compare them with commercial ones (both Mediterranean and non-Mediterranean). Species and growing season had a significant effect on biomass production, perennialism and biomass quality. *Arundo donax* (winter crops) and *Piptatherum miliaceum* (autumn crops) performed better than the commercial species tested (*Panicum virgatum* for winter crops and *Festuca arundinacea* for autumn crops) in biomass production and perennialism. In terms of biomass quality, *Panicum virgatum* was the best species, having high structural content (mainly cellulose and hemicellulose), low non-structural content and the lowest ash. However, *Ampelodesmos mauritanicus* and *Arundo donax* rendered similar results, with no significant difference in terms of cellulose production for this latter but with higher lignin content. For the autumn species, *Festuca arundinacea* was the species with the best biomass quality but with the highest ash production for all the species considered. Hence, both for winter or autumn regimes, native or naturalized plants seem to be better suited than the commercial commonly used for biomass production with energy-producing purposes. Further research must be conducted in terms of seed biology and physiology, seedbed preparation methods, sowing time, seedling density and weed control before they can firmly be proposed as adequate alternatives for energy purposes.

Keywords: biomass yield; perennial grasses; survival; Mediterranean region

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1. Introduction

Paris COP21 established a zero balance between the emissions and capture of CO_2 with a maximum rise in temperature of 1.5 °C with respect to the pre-industrial period as the global objective by the end of this century. This objective can only be fulfilled through a complete decarbonization of energy production, along with the establishment of land-use policies that imply a reduction in CO_2 emissions. One of the most solid strategies to follow is the rapid implementation of renewable energies with an increase in carbon capture and sequestration processes [1]. Nowadays, around 19% of the global energy demand is met through renewable sources, out of which, traditional biomass contributes up to 9% and the rest (10%) is fulfilled by modern renewable sources including wind, biofuels, geothermal, solar, etc. [2,3]. This rate is growing by 2.5% per year on a global scale [2].

Hence, in this context, and for the last 20 years, there has been an increasing interest in the production of biomass to generate bioenergy, making it one of the most promising alternatives along with solar, wind and hydropower energy [4]. Nowadays, especially relevant are the denominated second-generation biomass, since this does not compete with food production directly or indirectly [5–7]. The use of marginal lands, fallow agricultural land due to unfavorable crop production conditions [8], to produce cellulosic feedstocks could potentially avoid many problems associated with biofuel production using cropland [9,10] and could be an optimum solution for this second-generation biomass [11]. In fact, many authors have started studying this in different parts of the world [12–14]. It is also relevant to consider that different studies have pointed out the potential positive environmental outcomes the use of marginal lands can have, such as an increase in biodiversity or an improvement in the biogeochemistry of ecosystems [15–17].

However, the use of marginal lands to produce biomass can be further challenging in areas with harsh climatic conditions. The Mediterranean region can be one of these areas, which is characterized by having winter scarce and irregular rains and frequent long drought periods, which limit its capability to grow crops [18]. Furthermore, the Mediterranean region is considered as a "hotspot" for marginal lands [19]. Nowadays, considerable attention has been paid to the effects of regional climate on plant development in order to identify the optimal genotype for a particular location [20]. However, there are many plant species that are still largely unexplored, highly resource-use efficient and well performing in locations with specific constraints. In this sense, the native germplasm of the Mediterranean could be a source of this type of species, which, according to Scordia et al. [21], remain unexplored for bioenergy production and should be further analyzed and assessed due to traits of resistance and phenotypic plasticity to several biophysical constraints [11]. In addition, wild germplasm may be suitable for low-input cultivation techniques while providing sufficient production [21]. Further research regarding their ecology, biology, physiology and agronomy must be conducted before these species can be recommended as potential bioenergy crops.

In addition to the yield, biomass quality is another relevant parameter in bioconversion processes. Biomass productivity, stand longevity and quality of perennial grasses is mainly conditioned by the harvest time, affecting several key aspects such as ash production, cell wall composition or biomass water content, which in turn condition the bioconversion process (post-harvest logistics and bioconversion pathways) [22,23].

Therefore, the present study investigated different autochthonous and naturalized Mediterranean perennial grasses as novel candidates of lignocellulosic bioenergy crops for the semi-arid Mediterranean area and compared them with some commercial ones (both Mediterranean and non-Mediterranean). Six different species were compared in a five-year field trial under low-input agronomic practices with an autumn/winter harvest regime. Considering previous studies, it was hypothesized that some of the assayed species would render positive adequate results, in terms of biomass production and quality, performing better than the commercial species selected.

2. Materials and Methods

2.1. Field Experiment Set-Up

The field experiment was developed from autumn 2012 to autumn 2017 at the experimental field of the University of the Balearic Islands (UIB) (39° 38′ N, 2° 38′ E), Majorca (Spain). The main soil characteristics are summarized in Table 1. All the determinations carried out were done following the official laboratory methods of the Spanish Ministry of Agriculture, Fishery and Food [24].

Table 1. Physical and chemical properties of the soil where the assays were carried out.

Soil Property	
Sand(g/kg)	261
Silt (g/kg)	465
Clay (g/kg)	274
Texture	Loam clayey
Organic Carbon(g/kg)	26.1
N (total)(g/kg)	2.24
P (total) (mg/kg)	1010
P (organic) (mg/kg)	332
P (Olsen) (mg/kg)	52.5
CCE (g/kg)	310
Active Lime (g/kg)	60
Electrical Conductivity (1:5; 25 °C)	168
pH H_2O	8.3
Cation Exchange Capacity (cmol/kg)	15.9
Hm −0.3 bar (EG, g/kg)	125
Hm −0.3 bar (g/kg)	194

In a completely randomized block, a control and six species were compared with four replicates (plots) each. The different autochthonous Mediterranean species evaluated were: *Ampelodesmos mauritanicus* L., *Dactylis glomerata* L. and *Piptatherum miliaceum* L.; whilst the commercial ones were *Festuca arundiacea* L. cv Flecha and *Panicum virgatum* L. *cv Alamo.* This latter was the cosmopolitan species tested, due to its relevance in the production of biofuels worldwide. In addition, *Arundo donax* L., a widespread invasive species used as biomass source in several parts of the world, including the Mediterranean region, was also included in the study. Seeds of *Ampelodesmos mauritanicus*, *Dactylis glomerata* and *Piptatherum miliaceum* and cuttings of *Arundo donax* were collected in natural populations in Mallorca, while the seeds of *Festuca arundiacea* cv Jana and *Panicum virgatum cv Alamo* were commercially provided. Control plots were tested with the spontaneous vegetation that was observed in each cycle.

Dactylis glomerata, *Festuca arundiacea* and *Piptatherum miliaceum* (autumn species from now onwards) were evaluated for five cycles that started in autumn (October) and ended at the beginning of summer (July). Four plots (2 × 1 m) were established for each crop, with a planting frame of 25 × 25 cm between seedlings, according to the nature of the species. Previously, these had been obtained by germinating seeds in plates of alveoli, filled with common peat moss, irrigated and kept in a greenhouse. The control was evaluated following the same procedure.

Arundo donax, Ampelodesmos mauritanicus and *Panicum virgatum* (winter species from now onwards) were only evaluated for four cycles that started in winter (December) and ended at the beginning of the next winter (December). All the plants of these three species were pot-grown the first year, using common peat moss, the needed irrigation, and kept in a greenhouse, to ensure establishment and correct development. These species were not cut the first cycle. As for the species previously mentioned, four plots were established for each crop. However, the dimensions of these plots were 2 × 2.25 m and the planting frame 50 × 75 cm between seedlings, according to the nature of the species. Seedlings of *Ampelodesmos mauritanicus* and *Panicum virgatum* were obtained following the same methodology described above. On the other hand, *Arundo donax* plants were obtained from the rooting of axillary buds in a pot.

Prior to the establishment in the field, this and bed preparation followed an autumn ploughing and spring disk-harrowing before transplant. Irrigation was only applied in the first year to ensure plant establishment (150 mm); otherwise, the plants were rainfed. Weeding was performed only during the first cycle to ensure the implementation, and no fertilization was applied. From the second year onwards, no irrigation or other agronomic inputs were provided.

2.2. Determinations

Meteorological parameters were registered by the meteorological station of the University of the Balearic Islands (UIB). Data were collected weekly for the whole period of the assay.

Crop cycle phenology depended on the species: *Arundo donax, Ampelodesmos mauritanicus* and *Panicum virgatum* ended-started cycle in December of each year, while *Dactylis glomerata*, *Festuca arundiacea* and *Piptatherum miliaceum* ended-started cycle in June–September of each year.

At the end of each crop cycle, the fresh and dry aerial biomass produced in each plot was measured, and the result was expressed as biomass production in tons per hectare. Dry biomass production was determined after oven-drying the fresh material at 65 °C for 72 h (until constant weight was achieved).

To assess perennialism, the number of stems alive of three plants per plot was determined at the end of one cycle and at the beginning of the following cycle, along the whole experiment. The percentage of survival plants by crop and plot was also recorded at the end of each cycle and at the beginning of the following.

2.3. Biomass Quality

Oven-dried samples of each crop collected at the end of the second and the fourth cycles were bromatologically analyzed. This analysis included the determination of: dry material (DB), ashes (A), crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF) and protein (P). Cellulose, hemicellulose and lignin content were calculated from these values using the formula proposed by Van Soest et al. [25]. The samples were analyzed in triplicate according to the Van Soest et al. [25] method for structural carbohydrate and ADL by using a raw fiber extractor (FIWE 6, VELP Scientifica Srl, Usmate, Italy), the Kjeldahl method for proteins (Distillation unit B-324, Büchi Italia Srl) and the ASTM E1755-01 standard for ash.

2.4. Statistical Analysis

When variance was homogeneous and data had a normal distribution, factorial ANOVA was performed to establish significant differences and interactions between species, cycle and block for biomass production (fresh and dry), number of stems, survival of plants and biomass composition. Block was not significant for any of the variables studied. The Duncan test was performed to evaluate the statistical differences between means ($p < 0.05$) indicated by the ANOVA analysis. All data analysis was conducted using the IBM SPSS software package (IBM, 2013).

3. Results

3.1. Soil and Meteorological Data

With regards to the soil characteristics, adequate values were obtained for most of the relevant properties influencing biomass production (Table 1). The soil where the assays were carried out can be described as loam clayey. This high clay content, along with also the high organic matter content determined, renders good CEC values, which determines part of the potential chemical activity of soils. Initial nitrogen and phosphorus content were also high and, hence, not being an important drawback in terms of biomass production. However, carbonate content along with active lime content showed relatively high values, which can influence the nutrient dynamics in soil and negatively affect the bioavailability of nutrients, especially micronutrients. This can also be deducted from the high pH value.

Average air temperature and rainfall values throughout the whole assayed period reflected typical southern Mediterranean trends (Figure 1). The mean average temperature for the assayed period was 17.3 °C, and the mean average rainfall was 465 mm per year. The highest mean week temperature was achieved in summer 2015 with a value of 29.8 °C, and the most intense rain episode was registered during autumn 2016 with an accumulated rainfall of 100 mm/week. Drought periods spread from May to October throughout the whole experiment, being particularly relevant during the spring–summer drought in 2016.

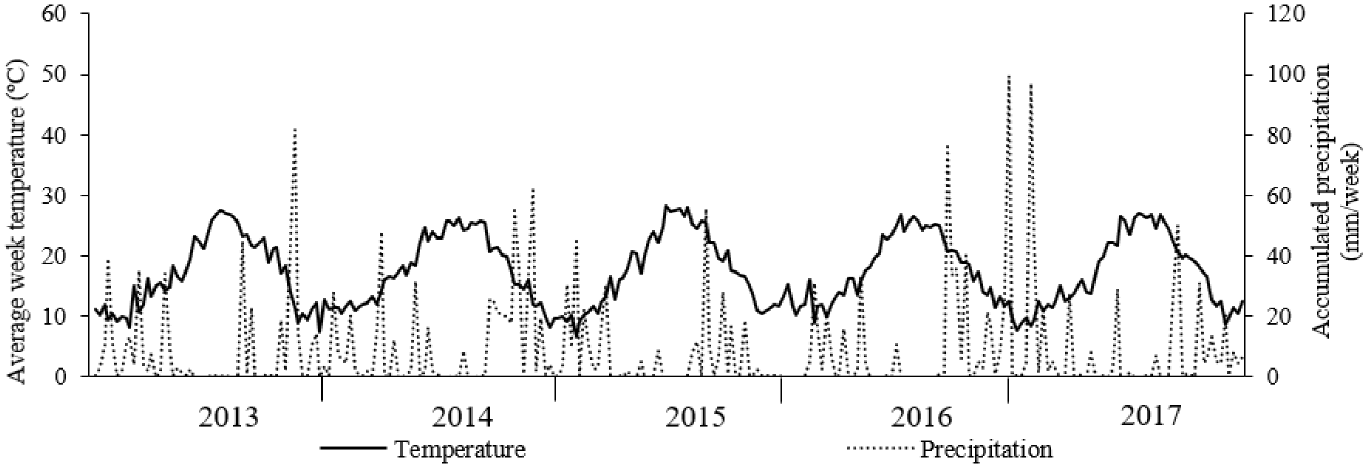

Figure 1. Ombrothermic diagram for the period, showing the average weekly temperature and rainfall.

3.2. Biomass Production

Both species and growing season had a significant effect on biomass production (fresh and dry), and all species produced significantly more than the control (Table 2). It is important to point out that for biomass production, and also for the rest of the variables analyzed (survival and stem production), the statistical analysis indicated an interaction between species and cycle, showing the relevance of both factors, but especially cycle, when analyzing and interpreting the results. This can be clearly observed when analyzing Figure 2, where species present differential behaviors for the different growing seasons analyzed.

Table 2. Fresh and dry biomass of the different perennial grasses assayed throughout the whole experiment and for the different growing seasons considering all the species tested together.

	FB [1] (t/ha)	DB [2] (t/ha)
Control	6.60 ± 1.457 [a,3]	2.95 ± 0.717 [a]
Ampelodesmos mauritanicus	16.34 ± 1.629 [bc]	8.03 ± 0.802 [bc]
Arundo donax	45.11 ± 1.629 [d]	24.59 ± 0.802 [d]
Dactylis glomerata	13.47 ± 0.841 [b]	6.25 ± 0.414 [b]
Festuca arundinacea	16.64 ± 1.457 [bc]	6.37 ± 0.717 [b]
Piptatherum miliaceum	18.11 ± 1.030 [c]	8.42 ± 0.507 [c]
Panicum virgatum	16.64 ± 1.629 [bc]	8.77 ± 0.802 [c]
1	3.16 ± 1.371 [a]	2.04 ± 0.675 [a]
2	18.01 ± 1.124 [c]	7.81 ± 0.553 [b]
3	25.84 ± 1.124 [d]	10.96 ± 0.553 [d]
4	22.44 ± 1.124 [c]	11.96 ± 0.553 [c]
5	15.70 ± 1.124 [b]	8.90 ± 0.553 [b]
R^2	0.852	0.839
P-value (Species)	0.000	0.000
P-value (Cycle)	0.000	0.000
P-value (Species × Cycle)	0.000	0.000

[1] FB = fresh biomass. [2] DB = dry biomass. [3] Mean ± standard error of 4 replicates. Different letters indicate significant differences between species or growing cycle.

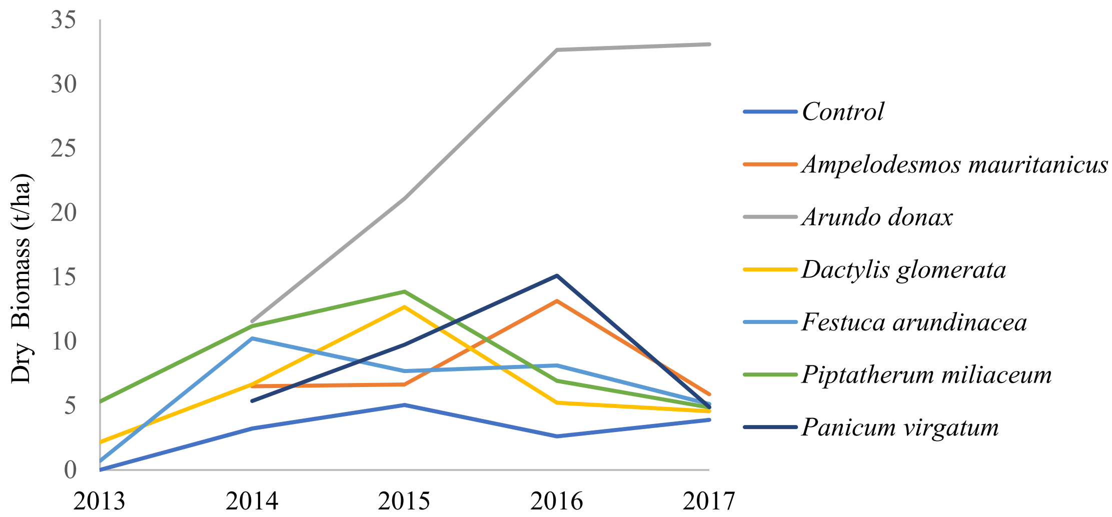

Figure 2. Annual evolution of dry biomass yield in five consecutive growing seasons of the different perennial grasses tested.

Across species, *Arundo donax* was the species that performed best, producing 3 to 4 times more than the rest of the species. Furthermore, it was the species with the highest DB/FB ratio (Table 2). When analyzing dry biomass of winter species, again, *Arundo donax* was the best performer, producing three times more than the two other winter species, *Ampelodesmos mauritanicus* being the worst. However, in this group, it is important to notice that, in spite of the lowest value for *Ampelosdesmos mauritanicus*, no significant difference was observed between *Ampelodesmos mauritanicus* and *Panicum virgatum*, and hence, it can be considered that both rendered similar results. When analyzing dry biomass of autumn species, *Piptatherum miliaceum* performed best, having a significantly higher biomass than *Festuca arundinaceae* Flecha (the commercial variety). The lowest biomass production was obtained by *Dactylis glomerata*, with similar values to those obtained by *Festuca.*

The analysis of the growing seasons indicated that, across cycles, all species performed better in the third cycle (Table 2). However, when analyzing species independently, other trends can be observed (Figure 2, Table S1). Autumn species did better in the second–third cycle, while winter species did better in the fourth. Considering that winter species were pot-grown the first year and they received no cut, the results indicated that most of the tested species reduced significantly their biomass production after the third growing season. *Arundo donax* is the only species that did not undergo this severe reduction, although it can be observed that its production did not continue increasing but it kept stable.

3.3. Perennialism

As for biomass production, both species and growing season had a significant effect on the survival and the production of stems, which in turn indicate the perennialism of the species selected (Tables 3 and 4).

Table 3. Percentage of survival of the different perennial grasses assayed throughout the whole experiment and for the different growing seasons considering all the species tested together.

	Survival (%)
Ampelodesmos mauritanicus	98.8 ± 0.56 [1]
Arundo donax	100.0 ± 0.00
Dactylis glomerata	85.1 ± 2.32
Festuca arundinacea	81.1 ± 5.33
Piptatherum miliaceum	82.7 ± 3.14
Panicum virgatum	95.0 ± 2.66
1	98.0 ± 0.90 [2]
2	97.9 ± 0.90
3	94.4 ± 1.49
4	86.2 ± 1.90
5	66.2 ± 4.10

[1] Mean ± standard error of 4 replicates. [2] Mean ± standard error of 24 replicates.

Table 4. Number of stems of the different perennial grasses assayed throughout the whole experiment and for the different growing seasons considering all the species tested together.

	Stems (nº of Stems)
Ampelodesmos mauritanicus	87.35 ± 2.886 [e,1]
Arundo donax	10.65 ± 2.886 [a]
Dactylis glomerata	38.19 ± 1.700 [b]
Festuca arundinacea	50.29 ± 2.707 [c]
Piptatherum miliaceum	37.29 ± 2.220 [b]
Panicum virgatum	77.88 ± 2.886 [d]
1	22.23 ± 2.605 [a]
2	45.99 ± 2.115 [c]
3	44.21 ± 2.306 [b]
4	45.44 ± 2.115 [b]
5	76.26 ± 2.537 [d]
R^2	0.760
P (Species)	0.000
P (Cycle)	0.000
P (Species × Cycle)	0.000

[1] Mean ± standard error of 12 replicates. Different letters indicate significant differences between species or growing cycle.

Across species, *Arundo donax* was the species that achieved the highest survival while *Festuca arundinaceae* plants the lowest. When analyzing winter species, *Arundo donax* is the best performer in terms of survival but the worst in stem production. It is important to notice that *Ampelosdesmos mauritanicus* is the second-best survivor in this group, which indicates that both *Arundo* and *Ampelosdesmos* are a better option than the commercial variety *Panicum virgatum* cv *Alamo.* When analyzing autumn species, one can observe that compared to the winter ones, their survival was 15–20% less and that *Dactylis glomerata* was the species that survived the most. Again, the species that performed worst was the commercial one, *Festuca arundinaceae* cv *Flecha*.

Across growing seasons, the general trend showed that all species underwent a small decrease with time, with a drastic reduction in survival after the fourth growing season.

With regards to stem production, the results differed significantly to those of survival.

Across species, *Ampelosdesmos mauritanicus* was the species that produces more stems over time while *Arundo donax* produced the least. When analyzing winter species, *Ampelosdesmos mauritanicus* is the best performer in terms of stems production, but this time, the second-best was *Panicum virgatum*. When analyzing autumn species, these produced around two times less than the other group of species, and *Festuca arundinaceae* was the species that produced more stems.

Across growing seasons, the general trend showed that all species underwent a significant increase year after year. This increase was especially high in the last growing cycle, when the plants produced nearly twice the stems produced in the previous cycle.

3.4. Biomass Quality

Cultivation year and species had significant effects on all the bromatology parameters determined. Significant interactions were also detected for all the parameters analyzed (Table 5).

Table 5. Bromatology analysis of the different perennial grasses assayed.

Species	DB [1]	A [2] (g/100 g DB)	CF [3] (g/100 g DB)	ADF [4] (g/100 g DB)	NDF [5] (g/100 g DB)	P [6] (g/100 g DB)	Cellulose (g/100 g DB)	Hemicellulose (g/100 g DB)	Lignin (g/100 g DB)
Control	92.03 ± 0.198 [d.7]	8.76 ± 0.118 [f]	37.40 ± 0.563 [a]	49.34 ± 0.502 [b]	65.49 ± 0.492 [a]	5.40 ± 0.202 [c]	43.36 ± 0.438 [e]	16.14 ± 0.696 [a]	5.98 [b] ± 0.289
Ampelodesmos mauritanicus	93.42 ± 0.140 [f]	5.78 ± 0.083 [b]	39.36 ± 0.398 [b]	46.56 ± 0.355 [a]	74.81 ± 0.348 [d]	8.08 ± 0.143 [e]	40.48 ± 0.309 [bc]	28.25 ± 0.492 [de]	6.08 [b] ± 0.204
Arundo donax	93.77 ± 0.140 [f]	6.21 ± 0.083 [c]	43.70 ± 0.398 [d]	51.60 ± 0.355 [c]	72.90 ± 0.348 [c]	4.64 ± 0.143 [b]	42.38 ± 0.309 [d]	21.31 ± 0.492 [b]	9.21 [d] ± 0.204
Dactylis glomerata	90.92 ± 0.081 [c]	6.96 ± 0.048 [d]	37.67 ± 0.230 [a]	45.70 ± 0.205 [a]	69.33 ± 0.201 [b]	6.10 ± 0.083 [d]	39.70 ± 0.179 [b]	23.63 ± 0.284 [c]	6.00 [b] ± 0.118
Festuca arundinacea	89.72 ± 0.140 [a]	7.36 ± 0.083 [e]	37.43 ± 0.398 [a]	45.80 ± 0.355 [a]	70.23 ± 0.348 [b]	3.63 ± 0.143 [a]	41.33 ± 0.309 [c]	24.43 ± 0.492 [c]	4.48 [a] ± 0.204
Piptatherum miliaceum	90.27 ± 0.099 [b]	5.62 ± 0.059 [b]	38.87 ± 0.281 [b]	46.60 ± 0.251 [a]	74.25 ± 0.246 [d]	5.67 ± 0.101 [c]	37.96 ± 0.219 [a]	27.65 ± 0.348 [d]	8.64 [d] ± 0.144
Panicum virgatum	92.96 ± 0.140 [e]	5.21 ± 0.083 [a]	41.26 ± 0.398 [c]	49.84 ± 0.355 [b]	79.09 ± 0.348 [e]	4.02 ± 0.143 [a]	42.54 ± 0.309 [de]	29.25 ± 0.492 [e]	7.30 [c] ± 0.204
R^2	0.983	0.988	0.950	0.967	0.982	0.973	0.958	0.911	0.946
P (Species)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
P (Cycle)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000
P (Species × Cycle)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

[1] DB: dry biomass; [2] A: ash; [3] CF: crude fiber; [4] ADF: acid detergent fiber; [5] NDF: neutral detergent fiber; [6] P: protein. Different letters indicate significant differences between species.

Across species and harvest regimes, *Panicum virgatum* was the species with the highest structural content, whilst *Dactylis glomerata* was the one with the lowest content. Winter species had generally higher structural content, while the autumn groups had generally higher non-structural content. *Piptatherum miliaceum* and *Panicum virgatum* were the autumn and winter species, respectively, with the highest structural content, while *Arundo donax* and *Dactylis glomerata* were the winter and autumn species with the lowest structural content. *Ampelosdemos mauritanicus* was the species with the highest non-structural content and *Festuca arundinaceae* the one with the lowest. The highest ash production corresponded to *Festuca arundinaceae* and the lowest to *Panicum virgatum.*

In terms of bioconversion adequacy, it is important to consider that species with high lignin content are more suited to thermochemical bioconversion (torrefaction, pyrolysis, combustion, gasification, etc.) provided that the ash amount is not too high. On the other hand, species with high structural polysaccharides (cellulose and hemicellulose) are better suited to biochemical bioconversion (hydrolysis and fermentation to bioethanol and anaerobic digestion to biomethane production) [26].

Considering this, *Panicum virgatum* was the species with the highest cellulose content followed closely by *Arundo donax* and *Festuca arundinaceae.* Therefore, these species are the best suited for biochemical conversion.

However, *Arundo donax* also had the highest lignin content, with *Piptatherum miliaceum* rendering similar results (lower value but no statistical differences). Hence, these species are the best suited to conduct thermochemical bioconversion This distribution of matter is of great interest when considering biomass for energy production.

4. Discussion

4.1. Biomass Production and Perennialism

With regards to biomass production, all species rendered the lowest biomass production in the first harvest, which is an important drawback for perennial grasses [27]. From then onwards, all species gradually increased their biomass production. Special mention has to be made to *Arundo donax*, with a very significant increase year after year, producing

3–4 times more than the rest of the species every year. This was an expected result, as pointed out by Webster et al. [28].

Biomass increased every growing season until the third cut (third cycle for autumn species and fourth cycle for winter species) (Table S1). The fact that this happened in all species independently of their harvest regime indicates that perennial grasses and other potential Mediterranean species behave similarly with regards to biomass production, reaching the same physiological status after the third cut. This was further reinforced by the survival data, which showed that there was a significant decrease after the third growing season, further pushed after the fourth cycle. Except for *Arundo donax*, the decrease in the last growing season was even more acute than the year before, and this can be explained when analyzing the meteorological data. Spring and summer of 2016 were especially dry, with a very significant dry period that spanned from May to September. Only 11 mm of rainfall were registered over 5 months. Hence, this fact powered the harshness of this period, which had a significant impact both in biomass production and survival. Similar results have been obtained by other authors [21,29–31].

Stem production data also supported the trends observed for biomass production and survival. After the first cycle, stem production increased, indicating the fitness of plants, and in the fifth growing season, stem production increased drastically. This indicated that, most probably, the plants that survived the harsh spring–summer season of 2016 were those that had produced more stems, and hence, they were the only ones that were alive. That is presumably the reason why this latter value is the highest of the growing seasons.

Across species, *Arundo donax* was the species that best performed during the assay both in terms of biomass production and survival, producing 3 to 4 times more than the rest of the species. This includes *Panicum virgatum cv Alamo*, the cosmopolitan species tested and one of the most financially supported species, indicating that this species may not be suitable in many Mediterranean marginal lands where soil characteristics and the long summer drought to a great extent limit the biomass production of this C4 species. In fact, *Panicum* is the only C4 species among those included in the study, and this physiological trait may explain its general low performance, since most of the native Mediterranean species C3 and C4 are only significantly found in salty environments or in areas with a shallow water table [32]. The other species included in the winter crops, *Ampelodesmus mauritanicus,* also performed better than *Panicum virgatum,* pointing out that both the native and the naturalized Mediterranean perennial grasses selected in this group may be better options than *Panicum* to produce biomass with energy production purposes. For the autumn species compared, again a native Mediterranean perennial grass (*Piptatherum miliaceum*) performed better than the commercial species selected (*Festuca arundinacea*) both in terms of biomass production and survival.

Hence, both for winter or autumn regimes, native or naturalized plants seem to be better suited to produce and survive more than the commercial commonly used for biomass production with energy-producing purposes.

Although most of the non-commercial species tested were able to produce viable seed in this environment, further research must be conducted with regards to seed biology and physiology, seedbed preparation methods, sowing time, seedling density and weed control. There is still plenty of unexplored knowledge for many of the species tested before they can be considered adequate to exploit at farm-scale level and to deliver ideotype varieties tailored to the different European environmental conditions [33].

4.2. Biomass Quality and Potential Energy Production

According to Scarlat et al. [34], lignocellulosic biomass is the most abundant and low-cost raw material on earth suitable to develop a competitive, resource-efficient and low-carbon economy in Europe. Considering this assertion, for the autumn regime, native plants seem to be better suited than the commercial commonly used for biomass production with energy-producing purposes.

Species and growing season main effects differed significantly (Table 5) and across species, *Panicum virgatum* was the best species, having high structural content (mainly lignin) and low non-structural content and ash. On the contrary, *Dactylis glomerata* was the species with the lowest fiber content, and the second highest was ash content. When comparing winter species, it is important to notice that *Ampelodesmos mauritanicus* and *Arundo donax* rendered similar results to *Panicum virgatum*, with no significant difference in terms of cellulose production for *Arundo*. Especially important is this last aspect, since *Arundo* was the species with the highest DB/FB ratio, hence increasing the efficiency and making it more profitable than any other species (Table 2). Amongst the autumn species, *Piptatherum miliaceum* showed the best biomass quality and had the highest biomass production. Moreover, the ash production was the lowest of the three autumn species.

When comparing winter against autumn species, it can be observed that, generally, winter species had a higher structural content and both groups had similar content in nonstructural molecules. However, autumn species tended to have a higher ash production, which can condition bioconversion processes. In perennial grasses, seasonal dynamics of nutrient accumulation and partitioning has been indicated as the main determinant of biomass quality for thermal conversions, since lower moisture, ash and inorganic elements avoids slagging, fouling and corrosion of the combustion equipment [22,35].

A high structural content is desirable in terms of energy production, as these fractions are the ones with the highest energy values [26,36]. In particular, species with high lignin content are more suited to thermochemical bioconversion (torrefaction, pyrolysis, combustion, gasification, etc.) provided that the ash amount is not too high; whilst species with high structural polysaccharides (cellulose and hemicellulose) are better suited to biochemical bioconversion (hydrolysis and fermentation to bioethanol and anaerobic digestion to biomethane production) [26]. In this sense, again *Panicum virgatum* is the species that obtained the best results (values) for biochemical bioconversion, but not being significantly better than the values of *Arundo donax*. However, this latter also had the highest lignin content (when comparing all species), which is a desirable attribute when applying thermochemical bioconversion processes.

Another important aspect to consider and analyze is how stable this content is with time, since a stable biomass composition delivered at the bioconversion site avoids continual modifications to processing operations [36]. As it can be observed in Figure 3 and Tables S2 and S3, the content of the different species did not vary from cycle 2 and cycle 4.

As commented previously, and in terms of energy production through thermochemical bioconversion, a high lignin content is more desirable, as combustion/pyrolysis parameters are much better [26]. Hence, it is important to take into account that when using the species assayed bioconversion processes need to be adapted to the different composition of the cuts. However, this is not desirable in terms of bioenergy production to avoid constant modifications.

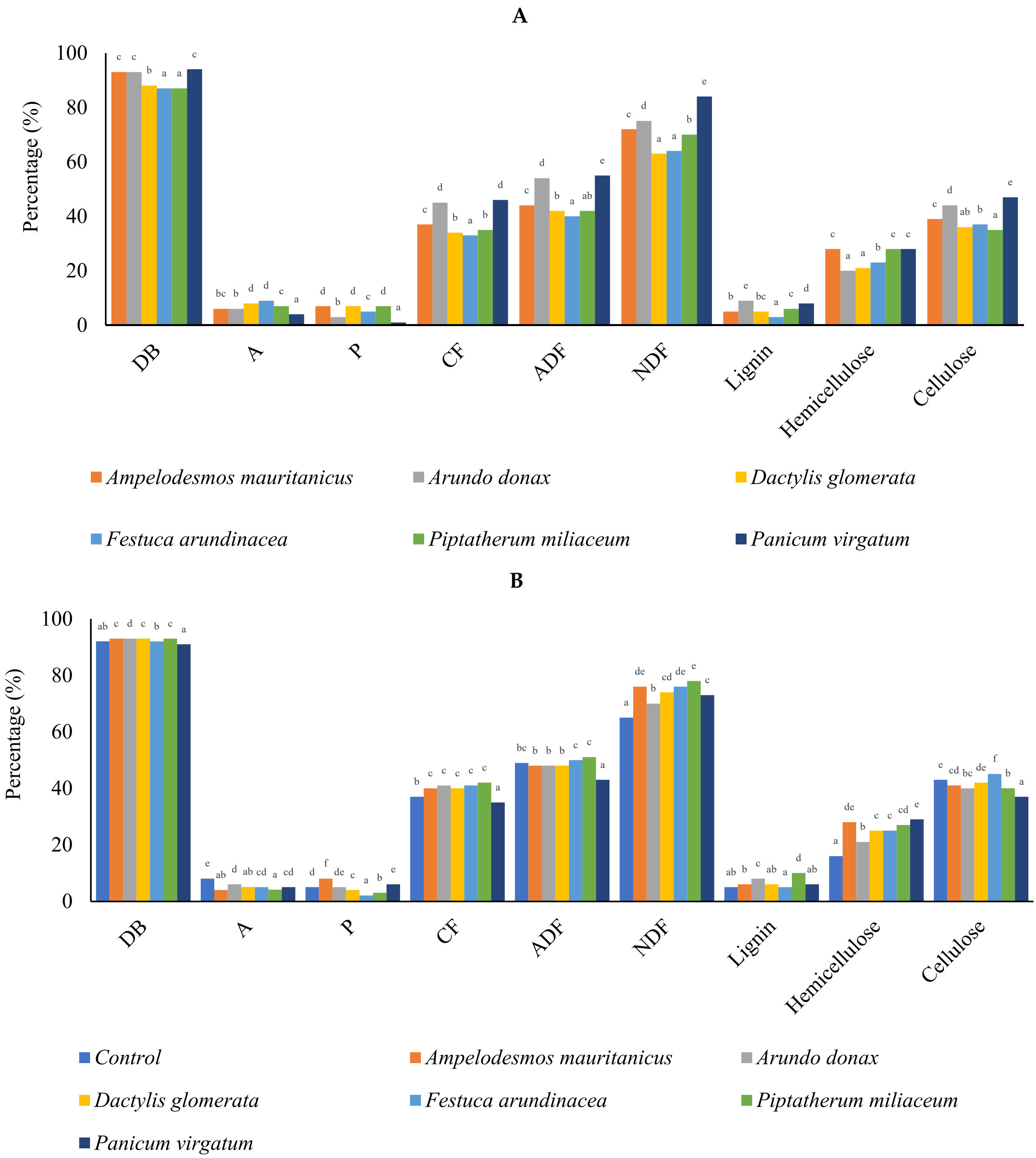

Figure 3. Bromatology results of the two cuts (2014—(**A**) and 2016—(**B**)) analyzed. (DB: dry biomass; A: ash; CF: crude; ADF: acid detergent fiber; NDF: neutral detergent fiber; P: protein). Different letters indicate significant differences between species or growing cycle.

5. Conclusions

The results reported here show that biomass production in Mediterranean marginal lands cannot be faced by using species and cultivars selected for other climatic areas or, even, for very particular Mediterranean areas, such as those having a shallow water table. In this sense, long summer drought periods and relatively low winter temperatures limit to a great extent the biomass production of C4 species such as *Panicum virgatum*, a widely used species in several summer–rain climatic areas where it has shown to be able to produce much more biomass that it did in this study.

On the other hand, the Mediterranean perennial grasses included in this study showed a significantly lower biomass production along a 5-year period than the naturalized *Arundo donax*. Despite the high biomass production capacity of this species, it presents several issues, which reduces its interest as a biomass source. Firstly, *Arundo donax* has been included in the list of invasive species in different countries, which limits its use with commercial purposes; secondly, it cannot be reproduced by seeds, which increases the cost of plantlet production and crop establishment. The use of arbuscular mycorrhiza inoculum of giant reed plantlets can be very useful in improving plant tolerance to marginal lands, in terms of plant establishment and harvest yield [37].

Although most of the non-commercial species tested were able to produce viable seed in this environment, further research must be conducted with regards to seed biology and physiology, seedbed preparation methods, sowing time, seedling density and weed control [33].

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11102060/s1. Table S1. Annual dry biomass yield in five consecutive growing seasons of the different perennial grasses tested. Table S2. Bromatology analysis of the different perennial grasses assayed in the second cut (2014). Table S3. Bromatology analysis of the different perennial grasses assayed in the second cut (2017).

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