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Impact of Harvest Frequency on Biomass Yield and Nutrient Removal of Elephantgrass, Giant Reed, and Switchgrass

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Abstract Although perennial grasses are being evaluated as a renewable source of biomass for energy production in many countries, no information exits regarding the use of these grasses in Uruguay. In 2008, an 8-year field study was implemented in western Uruguay to determine harvest frequencies for optimal biomass yield and nutrient removal for selected grass species. Elephantgrass (*Pennisetum purpureum* Schum.), giant reed (*Arundo donax* L.), and switchgrass (*Panicum virgatum* L.) were compared using two harvest frequencies: a single harvest after freeze (August) or two harvests (January and August) per year. We evaluated biomass yield, moisture content, nutrient concentration, and nutrient removal of these grasses. This study demonstrated the ability of these grasses to produce high biomass yields. Across years, the double harvest system significantly reduced cumulative biomass yield (~15%) compared to the single harvest of elephantgrass and giant reed; however, switchgrass had 18% more biomass yield (12.70 Mg ha^{-1} year^{-1}) than the other grasses at the summer harvest but no cumulative difference was detected. The single winter harvest of elephantgrass had the highest cumulative biomass yield (140.8 Mg ha^{-1}) and total nutrient removal (563 k N ha^{-1}, 199 kg P ha^{-1}, 2704 kg K ha^{-1}) across a 6-year period among the grasses. Switchgrass may be the grass best suited for dual use systems under Uruguayan conditions because a farmer may utilize initial growth as forage while biomass regrowth is a good direct combustion o biofuel feedstock due to lower moisture content and nutrient removal compared to the other species evaluated.

Keywords Giant reed · Elephantgrass · Switchgrass · Biofuel · Nutrient removal · Harvest frequency

Introduction

Increasing energy demands and worldwide interest in energy independence have motivated scientists to identify alternative energy sources to displace fossil fuel use (coal, oil, and natural gas). One renewable alternative involves perennial plant species (grasses) which are more ecologically suitable than annual crops such as corn [1] since they produce higher biomass, have lower production costs, reduce soil erosion, increase water quality, and enhance wildlife habitat [2, 3]. Perennial grasses such as elephantgrass (*Pennisetum purpureum* Schum.), giant reed (*Arundo donax* L.), and switchgrass (*Panicum virgatum* L.) have been proposed as key bioenergy crops in Europe and the USA based on their low input requirements and high productivity. Switchgrass is a perennial C_4 grass native to North America that has shown excellent potential as a feedstock for producing ethanol by bioconversion techniques or electricity by co-firing [2]. Giant reed is a perennial rhizomatous C_3 grass native to East Asia that is now naturalized throughout Southern Europe and Asia. It is one of the most promising grasses in terms of energy production due to high biomass yield and other advantages such as adaptation to different soil types and weather conditions in Mediterranean environments [4–6]. Elephantgrass is a large C_4 perennial grass native to sub-Saharan Africa that has excellent potential as an energy source since it is capable of producing high biomass yield [7]. However, compared to other energy crop candidates, elephantgrass and giant reed have been less studied.

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Several studies have shown that switchgrass and elephantgrass can be grown for both animal feed and biofuel [8]. Early summer harvests are usually more suitable for animal feed (silage or grazing), and later regrowth can be harvested for biofuel feedstock [9]. This dual purpose can benefit farmers by generating extra income from a diversified use of perennial grasses. However, harvest frequency and timing could influence the amount of biomass produced and its quality for various uses. Multiple harvests per year could reduce persistence and increase leaf and stem nutrient concentration which leads to greater nutrient removal at harvest [10, 11]. One or more harvests each season have been shown to produce optimum yields in most systems [12]. Studies with elephantgrass suggest that a single harvest at the beginning of winter will maximize yield and stand persistence [13]. For giant reed, single and double harvests have been proposed as an alternative management in order to use anaerobic digestion for early harvests and thermochemical processes (low ash and moisture content) for later harvests [14]. Harvest timing not only affects biomass yield, but also impacts moisture content, ash concentration, and nutrient content of switchgrass [15]. Although harvesting before winter (prior to freeze) may be helpful for high biomass yields, high moisture content can increase costs for biomass transportation and storage. Thus, harvesting strategies must be specifically planned to minimize yield losses while optimizing biomass transport [16]. Several studies on switchgrass have shown that delaying harvest until a killing frost reduces N, P, K, and other nutrients in stems and leaves [17–19]. Moreover, when switchgrass is harvested after frost, the translocation of nutrients to stem bases, rhizomes, and roots has already been maximized [20]. Similar results were found with giant reed harvested one to two times per growing period [14, 21].

Biomass energy systems can increase removal of accumulated mineral nutrients, thereby depleting soil nutrient levels over time. Knowledge of nutrient concentrations and removal rates of harvested biomass is needed to determine optimal fertilizer recommendations and to assess the overall economic viability and sustainability of biomass energy cropping systems. Uruguay is a small country which intensively uses land for agriculture and livestock production. Due to Uruguay's location in a sub-tropical region with no severe soil limitations and good rainfall conditions, it may be possible to introduce perennial plant systems for animal feed (silage or grazing) or bioenergy use (biofuel, direct combustion, or biogas among others). No information exists for Uruguay concerning the potential of these grass systems in terms of biomass yield and quality. The objectives of this 8-year study were to determine the feasibility of utilizing these perennial grasses (giant reed, elephantgrass, switchgrass) in western Uruguay and to identify an optimal harvest frequency that maximizes biomass yield while minimizing moisture content and nutrient removal (N, P, K).

Materials and Methods

Site Description

This experiment began in October 2008 and was conducted for 8 years with the main objective of evaluating three perennial crops (giant reed, elephantgrass, switchgrass) under two harvest frequencies (annual harvests in August; biannual harvests in January and August) at the Mario Cassinoni Experiment Station's (EEMAC), Agronomy Faculty of Uruguay (32° 21′ S, 58° 02′ W; 61 m elevation) in western Uruguay. The climate is meso-thermal sub-humid with a mean daily temperature of 25 and 12 °C for summer and winter, respectively. Normally, the last freeze before the start of the growing season is around October and the freeze that ends the growing season is around May. Annual precipitation is 1250 mm distributed (on average) uniformly within the year, but with large intra- and interannual variation. Soil at the site is a fertile Typic Argiudol (Table 1) with a slope of about 1.0%. Between 1940 and 1970, the study site was cultivated with continuous crops (a wheat-fallow rotation) under conventional tillage (inversion tillage plus several secondary operations). From 1970 to 2000, the site was cultivated under a crop-pasture rotation (3 years of pastures followed by three crop years).

Site Management and Experimental Design

The long-term experiment was established in the spring of 2007 following a sod-legume pasture invaded by bermudagrass (*Cynodon dactylon* L.), dallis grass (*Paspalum dilatatum* Poir), and bahiagrass (*Paspalum notatum*). Tillage was conducted in late winter (August) using a disk harrow to a depth of 15–20 cm, followed by a field cultivation to a depth of 10–15 cm. The experimental design was a completely

Table 1 Selected physical and chemical properties of soils at the experimental site (0–20 cm depth) taken at the time of establishment of the long-term experiment at Paysandú, Uruguay

Classification	Typic Argiudol
Texture	Clay loam
Clay (g kg^{-1})	290
Silt (g kg^{-1})	440
Sand (g kg^{-1})	270
Soil organic carbon	30
pH	6.0
P content (mg kg^{-1})	15
K content (cmol kg^{-1})	1.9
Ca content (cmol kg^{-1})	27.7
Mg content (cmol kg^{-1})	2.4
Cation exchange capacity (cmol kg^{-1})	32.7

randomized block with six replications (plots were 17.5 m^2 each). All species were planted in October 2007. For giant reed and elephantgrass, rhizomes with a couple of buds weighing 250–500 g were planted to a 5–10-cm soil depth. These grasses were grown at a population of 20,000 plants ha^{-1}. The lowland switchgrass variety "Alamo" was planted at a pure live seed rate of 5 kg ha^{-1} with a row spacing of 0.50 m using a conventional drill (THD300, Semeato, Brasil). For 2008 and 2009, all species were harvested once per year in May. Since 2010, two harvest treatments were conducted: (1) single harvest (beginning of August after freezing temperatures for biofuel feedstock) and (2) double harvest (first harvest in mid-January for animal feed or biofuel feedstock and later regrowth allowed to mature and harvested in early August for biofuel feedstock). For the double harvest plots, the cumulative annual yield is the sum of the two harvests in the same calendar year. Nitrogen was applied as urea annually in the spring (after August harvest) at 100 kg N ha^{-1}.

Measurements

The three species were hand harvested by cutting 10-cm above ground level (1.0 m × 2.0 m strip down the center of the plot). For each harvest, total fresh weight of the collected biomass samples was recorded. Sub-samples were then chopped, weighed, and oven dried (60 °C for 72 h) to estimate dry matter content. For laboratory analyses, the sub-samples were ground to pass a 1-mm screen. Nutrient concentrations of whole plant sub-samples were determined at the University of Uruguay and the National Institute Investigation Agricultural (INIA). Nitrogen concentration was determined by dry combustion (LECO, TruSpec®Micro, USA). Potassium concentration was determined by atomic absorption spectroscopy and P concentration was obtained by sulfuric digestion followed by ammonium molybdate colorimetric assay. Nitrogen, P, and K removal were determined by multiplying biomass yield (dry basis) of each harvest by N, P, and K concentrations.

Statistical Analysis

Treatment effects were evaluated using a randomized block design with the PROC MIXED procedure of the Statistical Analysis System (SAS) [22]. Replication and its interactions were considered random effects and treatments were considered fixed effects. For the first harvest (January), analyses across years were made for dry matter yield and moisture content at three species, with year treated as a fixed effect to determine interactions involving years. For the second harvest (August), analyses across years were made for dry matter yield and moisture content at two harvest frequencies and three species, with year treated as a fixed effect to determine interactions involving years. For cumulative total harvest, analyses across years were made for dry matter yield at two harvest frequency and three species, with year treated as a fixed effect to determine interactions involving years. For N, P, and K concentration or nutrient removal (data averaged over year 6-year period), statistical analysis was done among harvest frequencies and three species. Least square means comparisons were made using Fisher's protected least significant differences (LSD). A significance level of $P = 0.05$ was established a priori.

Results and Discussion

Weather Conditions

The 8 years of study provided a useful contrast of growing seasons. Most were near the long-term average in terms of temperature and precipitation, one was warmer and drier than average, and one was cooler and wetter than average. This suggests that these data are representative of the likely range of climatic conditions at this location. The long-term average annual air temperature and total precipitation was 18.2 °C and 1240 mm, respectively. Growing season (October–March) incident solar radiation was ~4–5% below the 30-year average in the 2010–2011 and 2014–2015 growing seasons, and ~5% above in 2011–2012 (Table 2). Water deficit occurred in the 2009–2010 grass growing season (Table 2). On the other hand, precipitation was very high for 2010–2011 (1883 mm) and high for 2013–2014 (1236 mm). Regarding the amount of frost per winter, the highest occurrence was in 2008 (34), and the lowest was in 2015 (18).

Biomass Yield and Moisture Content at August Harvest

At the August harvest, the effects of species, harvest frequency, and years and their interactions were significant for biomass yield. In contrast to biomass yield, moisture content was influenced by species, year and harvest frequency, but not by the triple interaction (species × year × harvest frequency) (Table 3). Within single harvest, elephantgrass and giant reed biomass yield was higher than switchgrass averaged over years (23.47 or 22.38 vs. 15.52 Mg ha^{-1}, respectively, $P \leq 0.05$; Table 4). For all species, biomass yield was very poor in the initial year (averaged over species 3.26 Mg ha^{-1}). Afterwards, biomass yield quickly increased in year 2 with elephantgrass (31.77 Mg ha^{-1}) but continued to be low for giant reed and switchgrass (8.05 and 5.59 Mg ha^{-1}, respectively). Averaged over year (regardless of species), double harvest at second cut (August) was always significantly lower than single harvest (7.04 vs. 20.36 Mg ha^{-1}, respectively; $P \leq 0.001$). Biomass yield of switchgrass was about 74% lower with double than single harvest. Similar to switchgrass, giant reed biomass yield was ~70% lower with double than

Table 2 Annual and seasonal climate for the 2008–2015 at the experimental site, Paysandú, Uruguay

Years	Precipitation (mm)		Average temperature (°C)		Frost (N°)	Global radiation (Mj m^2)	
	Year	Season[a]	Year	Season	Year	Year	Season
2007–2008	1380	664	17.6	21.4	34	5495	3932
2008–2009	770	673	18.5	22.4	24	6264	4990
2009–2010	1166	750	18.2	22.9	32	6231	4006
2010–2011	2507	1883	17.8	22.1	29	5886	3894
2011–2012	1334	606	18.2	22.1	21	6312	4232
2012–2013	1805	1079	18.2	22.2	25	6161	4126
2013–2014	1741	1236	17.7	21.7	29	5986	3936
2014–2015	1774	1043	18.3	22.1	18	5827	3860
30-year average	1250	840	18.2	22.2	25	6350	4180

[a] Season is between October 1 and March 31

single harvest. For elephantgrass, biomass yield was 56% lower with double compared to single harvest. For the first year of double harvest (2010), data averaged over species show that biomass yield was 42% lower with double than single harvest (20.40 vs. 11.69 Mg ha^{-1}, respectively). For the following years (2011 to 2013), this decrease in biomass yield when comparing double to single was close to 75% (20.03 vs. 5.04 Mg ha^{-1}, respectively). However, for switchgrass, the highest biomass yield was in the last year (2015) with single harvest. Also, with giant reed, the highest biomass yield was in the last year (2015) with single harvest. On the other hand, the best biomass yield attained by elephantgrass was in the third year (2010), followed by decreasing yields except for 2014.

Species had a strong effect on biomass moisture content at the August harvest (Table 5). Averaged over years and harvest frequency, elephantgrass had the highest moisture content, followed by giant reed and switchgrass (624, 413, and 158 g kg^{-1}, respectively; $P \leq 0.001$). Single harvest had lower moisture content than double harvest (364 vs. 400 g kg^{-1}, respectively). In the January harvest, giant reed had more moisture content than switchgrass for all years and harvest frequencies. For the August harvest (end of winter in Uruguay), moisture content was lower than the January harvest (mid-summer) in all species. However, the decrease in moisture content from January to August varied among species with switchgrass exhibiting the strongest decrease (74%), followed by giant reed (28%), and elephantgrass (15%).

Biomass Yield and Moisture Content at January Harvest

At the January harvest, the effects of species and years and their interactions were significant for dry matter yield and moisture content (Table 3). A significant species by year interaction ($P \leq 0.05$) was observed for biomass yield at the first harvest (January); therefore, means were reported by year (Table 6). Giant reed had the highest biomass yield in 2010 (25.10 Mg ha^{-1}). On the other hand, switchgrass had the lowest biomass yield (19.85 Mg ha^{-1}) in this same year. After 2010, biomass yields strongly declined for all species in subsequent years, where elephantgrass was the most affected (from 21.02 to 4.35 Mg ha^{-1} between 2010 and 2013, respectively). However, in the last year (2015), biomass yield increased for all species, where elephantgrass and switchgrass

Table 3 Effects of species, harvesting frequency (HF), and years on dry matter yield (DMY), and moisture content (MC) at two harvest times (January and August), and cumulative biomass yield at the experimental site, Paysandú, Uruguay

	January harvest		August harvest		Accumulated total harvest
Source of variation	DMY	MC	DMY	MC	DMY
Species	***	***	***	***	***
Year	***	***	***	***	***
HF	NA	NA	***	***	**
Species × year	***	***	***	***	***
Species × HF	NA	NA	**	*	**
Year × HF	NA	NA	***	***	***
Species × year × HF	NA	NA	***	NS[a]	***

DMY dry matter yield, *MC* moisture content, *HF* harvest frequency, *NA* not applicable, *NS* non-significant

*$P < 0.10$; **$P < 0.01$; ***$P < 0.001$

Table 4 Dry matter yield (Mg ha^{-1}) at second harvest (August) among years, species, and harvest frequency (HF) at the experimental site, Paysandú, Uruguay

		2008	2009	2010	2011	2012	2013	2014	2015	LS means[a]
Species	HF	(Mg ha^{-1}) dry matter								
Giant reed	Single	4.04	8.05	15.01	24.18	21.23	24.36	22.94	26.56	22.38
	Double	NA	NA	12.04	6.12	3.54	5.65	3.63	9.61	6.77
LS means		4.04	8.05	13.53	15.15	12.38	15.01	13.28	18.09	14.49
Elephantgrass	Single	4.49	31.77	34.27	24.21	21.45	17.21	30.30	13.37	23.47
	Double	NA	NA	17.71	5.14	5.68	9.58	22.12	2.02	10.38
LS means		4.49	31.77	25.99	14.67	13.56	13.39	26.21	7.70	16.92
Switchgrass	Single	1.25	5.59	11.91	14.17	15.76	17.74	12.40	21.15	15.52
	Double	NA	NA	5.33	3.72	2.94	3.04	4.32	4.51	3.98
LS means		1.25	5.59	8.61	8.95	9.35	10.39	8.37	12.83	9.75
LS means (cut)	Single	3.22	15.14	20.40	20.85	19.48	19.77	21.88	20.04	20.36
	Double	NA	NA	11.69	4.99	4.05	6.09	10.02	5.38	7.04
LS means (year)		3.26	15.14	16.04	12.92	11.76	12.93	15.95	12.71	
LSD[b] $_{(0.05)}$ (species)										1.02
LSD $_{(0.05)}$ (year)		1.36								
LSD $_{(0.05)}$ (HF)										0.93
LSD $_{(0.05)}$ (species × year)		2.12								
LSD $_{(0.05)}$ (year × HF)				1.78						
LSD $_{(0.05)}$ (species × HF)										1.24
LSD $_{(0.05)}$ (species × year × HF)		2.90								

NA not applicable

[a] LS means averaged from 2010 to 2015

[b] LSD for comparison of dry matter depending on species, year, harvest frequency and its interactions

were higher than giant reed. Averaged over years, switchgrass biomass yield was highest compared to giant reed or elephantgrass (12.70 vs. 11.70 or 9.80 Mg ha^{-1}, respectively; $P \leq 0.01$) at first harvest. Moisture content at the first harvest was affected by species, year, and their interaction. Statistically, the strongest effect was species, where elephantgrass (averaged over years) had the highest moisture content (713 g kg^{-1}), followed by giant reed and switchgrass (557 and 530 g kg^{-1}, respectively) (Table 6). However, between these two species (depending on years), giant reed was sometimes higher than switchgrass (2012, 2014), and in 2011, switchgrass was higher than giant reed (605 vs. 563 g kg^{-1}).

Cumulative Biomass Yield

The harvest frequency × year interaction occurred for all species (Fig. 1a, b, c). Changing harvest frequency from single to double decreased cumulative dry biomass over 6 years ($P \leq 0.01$) from 133.2 to 111.0 Mg ha^{-1} for giant reed (Fig. 1a). In the first year comparison (2010), double harvest produced 147% more cumulative biomass yield than single harvest (Fig. 1a). However, single harvest produced 60% higher cumulative biomass yield than double harvest in the following years (2011–2015).

Similar to giant reed over this 6-year period, elephantgrass with single harvest produced 17% more cumulative biomass yield compared to double harvest (140.9 to 120.8 Mg ha^{-1}, respectively) (Fig. 1b). There was an interaction between harvest frequency and year. There was no difference in cumulative biomass yield due to harvest frequency in 2013, and 2014, but single harvest in 2011 and 2012 produced 164% more cumulative biomass yield than double harvest (Fig. 1b). Double harvest produced statistically more cumulative biomass yield than single harvest in 2010 and 2015 (21.4 vs. 13.4 Mg ha^{-1}, and 38.7 vs. 34.3 Mg ha^{-1} for 2010 and 2015, respectively).

Switchgrass produced similar cumulative biomass yields between these two harvesting frequencies (93.0 and 100.2 Mg ha^{-1} for single and double harvest, respectively). From initiation of harvest frequency treatments (2010) until 2015 (from years 3 to 8), switchgrass biomass yield averaged 16.1 Mg ha^{-1} year^{-1}. There was no difference in cumulative biomass yield due to harvest frequency in 2011, 2012, 2014, and 2015, but in 2010, double harvest produced 112% more cumulative biomass yield than single harvest for switchgrass (Fig. 1c). Single harvest produced more cumulative biomass yield than double harvest only in 2015. The harvest frequency × species occurred for cumulated biomass yield (Fig. 2). As we mentioned before (Table 3), switchgrass biomass yield was

Table 5 Moisture content at second harvest (August) as affected by species and harvest frequency (HF) among years at the experimental site, Paysandú, Uruguay

		2008	2009	2010	2011	2012	2013	2014	2015	LS means[a]
Species	HF	(g kg^{-1}) moisture content								
Giant reed	Single	393	498	433	394	444	259	459	306	398
	Double	NA	NA	460	419	440	266	557	380	427
LS means		393	498	447	406	442	263	508	343	413
Elephantgrass	Single	667	681	692	629	499	493	626	545	603
	Double	NA	NA	727	659	506	615	657	633	644
LS means		667	681	709	644	503	554	641	590	624
Switchgrass	Single	122	314	103	94	186	97	156	131	153
	Double	NA	NA	125	115	180	107	199	155	162
LS means		122	314	114	105	183	102	177	143	158
LS means (cut)	Single	394	498	409	372	376	283	413	327	385
	Double	NA	NA	437	398	375	330	471	390	411
LS means (year)		394	498	423	385	376	306	442	359	
LSD[b] $_{(0.05)}$ (species)										9
LSD $_{(0.05)}$ (year)		14								
LSD $_{(0.05)}$ (HF)										7
LSD $_{(0.05)}$ (species × year)		24								
LSD $_{(0.05)}$ (year × HF)				20						
LSD $_{(0.05)}$ (species × HF)										12
LSD $_{(0.05)}$ (species × year × HF)		NS								

NA not applicable, *NS* non-significant

[a] LS means averaged from 2010 to 2015

[b] LSD for comparison of moisture content depending on species, year, harvest frequency and its interactions

the highest compared to giant reed or elephantgrass (averaged over years) at first harvest (January). On the other hand, averaged over years for second harvest (August), elephantgrass and giant reed biomass yield with single harvest was the highest compared to the other species and harvest frequency combinations (Table 4, Fig. 2). Moreover, cumulative biomass

Table 6 Dry matter yield and moisture content at first harvest (January) as affected by species among years at the experimental site

	2010	2011	2012	2013	2014	2015	LS means
Species	(Mg ha^{-1}) dry matter						
Giant reed	25.10	11.35	9.30	8.08	5.13	11.32	11.70
Elephantgrass	21.02	1.97	4.53	4.35	7.42	19.35	9.80
Switchgrass	19.85	7.75	11.77	9.14	9.82	17.86	12.70
LS means	21.99	7.02	8.53	7.19	7.46	16.18	
LSD[a] $_{(0.05)}$ (year)	0.93						
LSD $_{(0.05)}$ (species)							0.66
LSD $_{(0.05)}$ (year × species)	1.66						
Species	(g kg^{-1}) moisture content						
Giant reed	568	563	580	594	568	471	557
Elephantgrass	742	748	692	755	679	660	713
Switchgrass	550	605	490	598	457	481	530
LS means	620	639	587	649	568	537	
LSD[a] $_{(0.05)}$ (year)	16						
LSD $_{(0.05)}$ (species)							11
LSD $_{(0.05)}$ (year × species)	27						

[a] LSD for comparison of dry matter depending on species, year, harvest frequency and its interactions

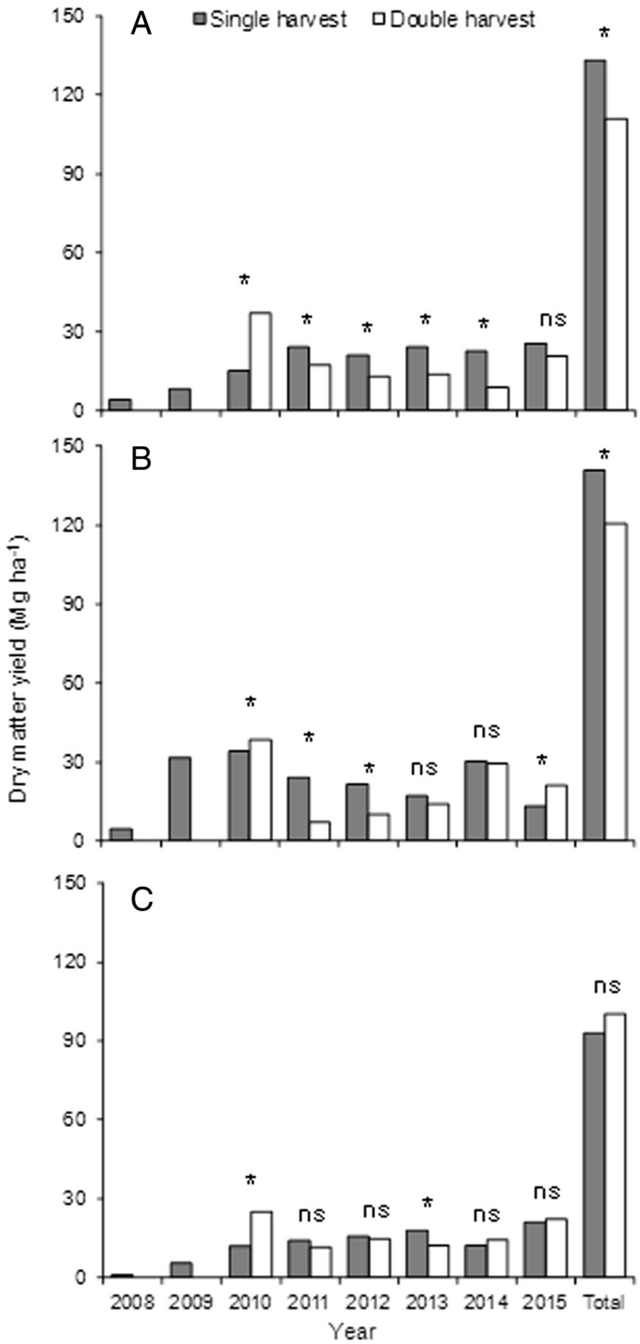

Fig. 1 Single and double harvest per year system productivity (Mg ha^{-1}) of giant reed (**a**), elephantgrass (**b**), and switchgrass (**c**) for 2008–2015. Total cumulative biomass is from 2010 to 2015. White histograms represent cumulative biomass of double harvest per year (January and August), while black histograms are single harvest (August). * refer to statistically different ($P \leq 0.05$) means between single and double harvest, for the same species and year and for cumulative biomass yield

yields (January + August harvest) were the highest using elephantgrass and giant reed with single harvest followed by the same species with double harvest. The lowest cumulative biomass yields were obtained with switchgrass in both harvest frequencies.

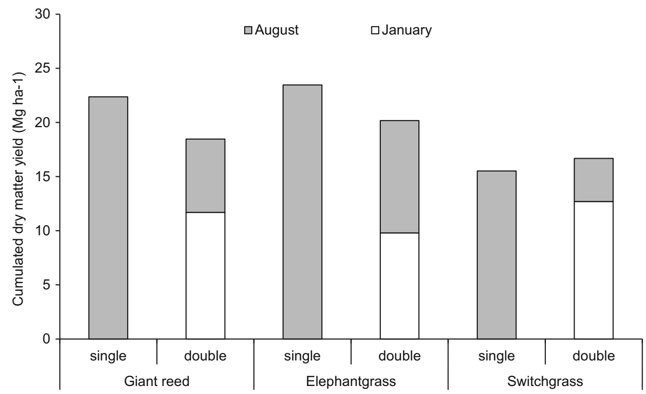

Fig. 2 Cumulative biomass yield across years (2010–2015) as affected by species and harvest frequency at first (January) and second (August) harvests at Paysandú, Uruguay. Within harvest time (January or August), means with the same *small letter* are not significantly different ($P \leq 0.05$). Cumulative biomass yield with the same *capital letter* are not significantly different ($P \leq 0.05$)

Nutrient Concentration

Year and its interactions were not significant for nutrient concentration; however, species at the January harvest and species and harvest frequency × species interaction at the August harvest were significant for nutrient concentrations in all periods. Therefore, mean nutrient concentrations were reported by species averaged over years for the January harvest, and for species and harvest frequency × species interaction, we report averages over years for the August harvest (Table 7). At the January harvest, giant reed had the lowest N concentration compared to elephantgrass and switchgrass (9.1 vs. 11.2 and 11.1 g kg^{-1}, respectively; Table 7). However, elephantgrass had higher P and K concentrations (1.53 and 26.9 g kg^{-1}, respectively) compared to giant reed (1.10 and 12.7 g kg^{-1}, respectively) and switchgrass (0.90 and 7.7 g kg^{-1}, respectively). At the August harvest, elephantgrass had the highest N, P, and K concentrations (5.0, 1.46, and 19.5 g kg^{-1}, respectively) (Table 7). On the other hand, switchgrass had the lowest N, P, and K concentrations (3.1, 0.42, and 1.5 g kg^{-1}, respectively). In double harvest treatments, N concentration increased compared to single harvest (5.9 vs. 4.0 g kg^{-1}, respectively) in elephantgrass. There was no effect of harvest frequency for giant reed or switchgrass N, P, and K concentrations at the second harvest. Nutrient concentrations decreased between the January and August harvest periods in all species. However, the decrease in N, P, and K concentrations was species dependent. The highest reduction was in switchgrass (72, 53, and 81% averaged over harvest frequency for N, P and K, respectively) followed by giant reed which dropped by 52, 45, and 67%. Whole N concentrations for elephantgrass displayed a reduction (64%) similar to the other species. However, whole plant P and K concentrations only decreased by 3 and 28%, respectively. In contrast to the response pattern of the present study across harvests, Na et al. (2015) reported a

Table 7 Whole plant N, P, and K concentration (averaged over years) as affected by species and harvest frequency (HF) at first (January) and second (August) harvest at Paysandú, Uruguay

Species		January harvest			August harvest		
		N	P	K	N	P	K
	HF	($g\ kg^{-1}$) concentration					
Giant reed	Single	NA	NA	NA	4.5	0.61	4.1
	Double	9.1	1.10	12.7	4.3	0.58	4.2
LS means					4.4	0.60	4.2
Elephantgrass	Single	NA	NA	NA	4.0	1.41	19.2
	Double	11.2	1.53	26.9	5.9	1.52	19.8
LS means					5.0	1.46	19.5
Switchgrass	Single	NA	NA	NA	3.3	0.42	1.5
	Double	11.1	0.90	7.7	2.9	0.41	1.5
LS means					3.1	0.42	1.5
LSD[a] $_{(0.05)}$ (species)		2.1	0.21	4.4	0.3	0.16	1.9
LSD $_{(0.05)}$ (HF)		NA	NA	NA	NS	NS	NS
LSD $_{(0.05)}$(species × HF)		NA	NA	NA	0.6	NS	NS

NA not applicable, *NS* non-significant

[a] LSD for comparison of concentration depending on species, harvest frequency, and its interaction

large reduction in P and K concentrations (36 and 47%, respectively) between early and late cuts.

Nutrient Removal

Species for the January harvest and species and harvest frequency at the August harvest were significant for cumulative N, P, and K removal in all periods (Fig. 3). In addition, year and some interactions were significant; nutrient removal was reported by species averaged over years at the January harvest, and by harvest frequency × species interaction averaged over years at the August harvest (Fig. 3). At the January harvest, switchgrass had the highest N removal compared to elephantgrass and giant reed (846 vs. 656 and 640 kg ha^{-1}, respectively). However, elephantgrass had more K removal (1577 kg ha^{-1}) compared to giant reed and switchgrass (893 and 587 kg ha^{-1}, respectively). At the August harvest, species and harvest frequency had a large effect on nutrient removal. Elephantgrass had the highest cumulated N, P, and K removal (averaged over harvest frequency) over 6 years (465, 147, and 1968 kg ha^{-1}, respectively) while switchgrass had the lowest N, P, and K removal (188, 24, and 88 kg ha^{-1}, respectively) averaged harvest frequency. Due to biomass yield differences among harvest frequency, single harvest (averaged over species) removed more N, P, and K (492, 107, and 1131 kg ha^{-1}, respectively) compared to double harvest (204, 43, and 480 kg ha^{-1}, respectively). However, the effect of harvest frequency on nutrient removal was higher in switchgrass and giant reed compared to elephantgrass. Changing from double to single harvest frequency in switchgrass at August harvest resulted in total N, P, and K removal increase of 344, 290, and 289%, respectively. Giant reed showed a similar tendency (246, 242, and 224% for total N, P, and K removal, respectively. On the other hand, elephantgrass resulted in lowest total N, P, and K increase of 53,109, and 119%, respectively. For the three species under study, this was as a result of the higher yield from single harvest compared to the double harvest as previously mentioned (20.40 vs. 7.04 Mg ha^{-1}, respectively). Analyzing cumulative N removal (1st and 2nd cuts) in these 6 years (2010 to 2015), the double harvest was higher than single harvest for all species. The largest impact on N removal was seen in switchgrass (915 to 307 kg ha^{-1}, respectively), followed by elephantgrass (1141 to 563 kg ha^{-1}, respectively) and giant reed (814 to 604 kg ha^{-1}, respectively). Cumulative P removal was not significantly different among harvest frequency for giant reed and elephantgrass. Switchgrass removed significantly more P with double harvest than single harvest (78 vs. 39 kg ha^{-1}). Elephantgrass consistently removed more K compared to giant reed or switchgrass, and there was no difference between harvest frequency (2704 and 2810 kg ha^{-1} for single to double harvest, respectively). For switchgrass and giant reed, there was a large difference between harvest frequencies. Switchgrass had 345% and giant reed 93% greater cumulative K removal with double harvest than single harvest.

Discussion

Determining which perennial grasses are best suited for animal feed (silage or grazing) or bioenergy use in western Uruguay is a high priority. Therefore, it is critical to identify best harvest management practices that maximize long-term biomass yields and quality (i.e., low moisture and nutrient concentration) for bioenergy feedstock. Giant reed,

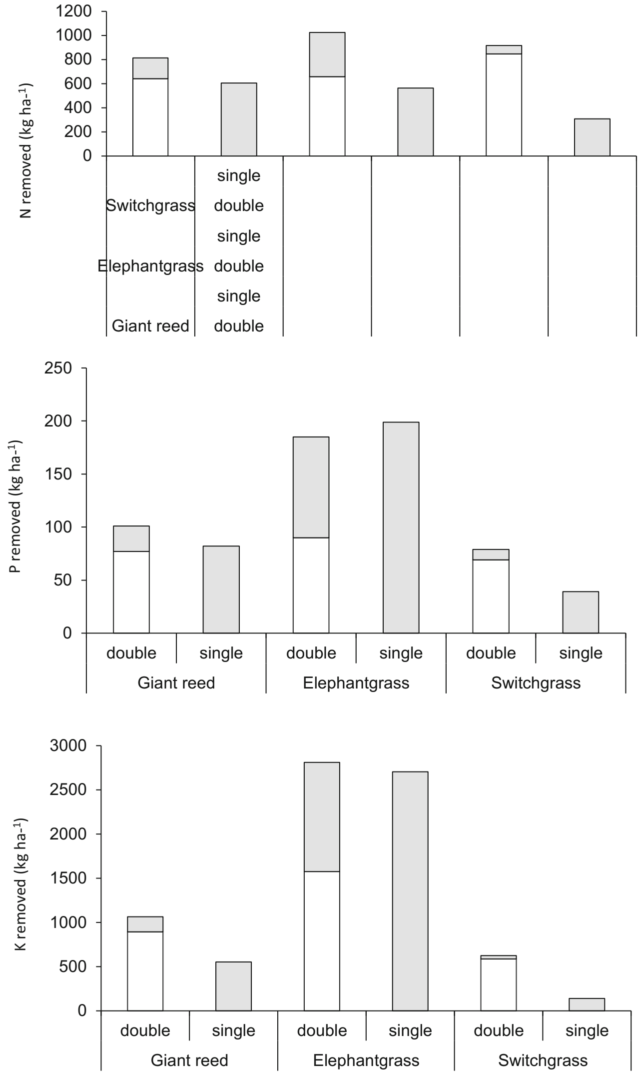

Fig. 3 Cumulative N, P, and K removal across years (2010–2015) as affected by species and harvest frequency at first (January) and second (August) harvests at Paysandú, Uruguay. Within harvest time (January or August), means with the same *small letter* are not significantly different ($P \leq 0.05$). Cumulative nutrient removals with the same *capital letter* are not significantly different ($P \leq 0.05$)

elephantgrass, and switchgrass exhibit great potential for biomass production under Uruguay's climatic conditions. It is well recognized that poor yields during the establishment of rhizomatous perennial grasses are initial major constraints in bioenergy production systems. Elephantgrass biomass yield increased quickly in the second year, while yields remained low for giant reed and switchgrass. These results are consistent with switchgrass establishment where resources are allocated to the formation of an extensive root system during the first and second year, thereby not reaching its full yield potential until the third year [17]. However, Angelini et al. [23] reported giant reed yields above 51 Mg ha^{-1} in central Italy by the second year under dry conditions. Causes behind the low biomass yields of giant reed in our experiment in 2009 (second cut at August) are unclear, but the very dry summer conditions could have been a contributing factor (Table 2). For giant reed and elephantgrass, single harvests produced generally higher cumulative biomass yields than double harvests. Similar results were reported by Woodard and Prine [24], where elephantgrass biomass yield in a double harvest system was reduced by 19% compared to single harvests. It appears that management decisions that maximize productivity in the short-term (by repeated cutting) may deplete belowground reserves and reduce the life spans of giant reed and elephantgrass systems [14, 24]. Cumulative biomass yields for switchgrass were similar for both harvest frequencies; however, biomass yield at August was lower for the double than for the single harvest. Mohammed et al. [8] reported similar results for switchgrass when comparing double harvest (June and November) to single harvest (November) in cases where switchgrass used for animal feed (June) reduced the harvest for biofuel feedstock by 71% (November). Switchgrass findings of Monti et al. [25] confirmed our results where accumulated biomass yield in the second year of the experiment was higher under the double harvest system than the single harvest system. However, the two-cut system decreased total biomass by 25% compared to the single harvest in the following years; the first cut contributed 65% toward the cumulative season yield. This could be due in part to a loss of rhizomes caused by early interruption of the biological cycle, and a consequent decrease of nutrients and reserve translocation to roots [25]. For our study, switchgrass was fertilized with 100 kg N ha^{-1} after the second cut (August). Thus, it appears that the grass systems with a double harvest frequency may require an N application following the first cut (January) to insure better regrowth and higher productivity. All grasses harvested in January belong to double harvest systems; therefore, the strong decline in biomass yields could be attributed to shoot N removal (along with other nutrients) at the first harvest that would otherwise have been translocated to roots and crowns for successful regrowth in the following year [15, 26].

Normally, late harvests decrease biomass moisture content which reduces costs related to transportation and further biomass drying [14, 15, 28]. Higher moisture content was observed for elephantgrass and giant reed. These values were similar to a previous study comparing single to double harvest frequency of giant reed [14], and to elephantgrass findings reported by others [27]. Considering direct combustion or ethanol production in Uruguay, high moisture content presents some challenges during transportation of harvested biomass and further drying before use. Moisture content should not be higher than 250 g kg^{-1} for biomass supplied for direct combustion usage [28]. Our results showed that elephantgrass and giant reed exceeded 250 g kg^{-1}; therefore, these two species may not be suitable for combustion processes. However, these energy grasses could be used for anaerobic digestion; however, no information exists for Uruguay concerning this renewable resource. On the other hand, switchgrass produced a high-quality biomass with low moisture content that could reduce transportation and storage costs.

The high nutrient concentrations early in the season (all species) were attributed to the predominance of young vegetative tissue. Numerous warm-season perennial grass studies have shown that whole plant N, P, and K concentrations decrease with increasing maturity [10, 29]. Similar N, P, and K concentrations have been reported with delayed harvest [7, 27, 30] as observed in the current study (after frost). Switchgrass had the lowest N, P, and K concentrations. Heaton et al. [31] reported that switchgrass evaluated at different temperate locations could potentially remove as much as 187 kg N ha^{-1} if harvested in early summer, and as little as 5 kg N ha^{-1} if harvested in late winter. Findings by Dragoni et al. [21] on giant reed showed a high reduction in N, P, and K concentrations from the first cut (July) to the second cut (January) as observed in our experiment. Our results indicated that N concentrations for elephantgrass showed reductions similar to giant reed and switchgrass, but P and K concentrations were only reduced by 3 and 28%, respectively. In contrast to P and K response patterns between our two harvests, Na et al. [27] reported a large reduction in P and K concentrations (36 and 47%, respectively) between early and late cut. The difference between early and late cut in our study could be due to our mild winters (average July minimal air temperature of 8.0 °C) relative to other nearby regions which are lower. Elephantgrass consistently removed more K compared to giant reed or switchgrass. This large amount of K in elephantgrass biomass was comparable to that reported by [7] in the Southeastern US. These authors found a K removal of 484 kg ha^{-1} per year for single harvest elephantgrass. Na et al. [27] reported that N removal by elephantgrass from double harvest (summer and winter) was 112% greater than for a single harvest. Due to the considerable amount of N, P, and K that are removed by biomass harvests, periodic soil analyses should be conducted to avoid nutrient deficiencies, especially in elephantgrass or giant reed or switchgrass based on double harvest system.

Conclusion

Giant reed, elephantgrass, and switchgrass displayed great potential for biomass production under the climatic conditions of western Uruguay. For early harvests in dual use systems, switchgrass (12.7 Mg ha^{-1}) had 18% more biomass yield than the average of the other grass species. For later harvests (after frost), elephantgrass with a single harvest displayed the highest biomass yield (23.47 Mg ha^{-1}), followed by giant red (22.38 Mg ha^{-1}). While multiple harvests produced more biomass yield than single harvest for all species in the early years of our systems, later results indicate that a decline in productivity can occur due to intensive harvest management. Averaged over years, elephantgrass and giant reed with a single harvest had higher biomass yield compared to a double harvest frequency (20 and 17% increase, respectively), but switchgrass was similar under both harvest frequencies. Elephantgrass had higher moisture content in both harvest periods (713 and 624 g kg^{-1} for January and August, respectively), followed by giant reed (557 and 413 g kg^{-1}). Biomass nutrient concentration of perennial grasses declined from early to late cuts. The highest decline between these cuts was for switchgrass (70, 53, and 81% for N, P, and K, respectively). Double harvesting of the three grasses led to increased nutrient removal compared to single harvests because the early harvest had higher tissue nutrient concentrations. The high nutrient removal rates from double harvest management suggest that it may be unsustainable without a good nutrient management plan. Elephantgrass removed 624 and 242% more K compared to switchgrass and giant reed, respectively. Switchgrass had less total nutrient removal and lower moisture content compared to the other grasses evaluated. Results of this study suggest that switchgrass may be the best suited perennial grass for Uruguayan conditions due to high biomass yield in summers (silage or grazing) and good winter harvest characteristics (low moisture content and nutrient concentration) required for thermochemical uses compared to the other species evaluated.

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