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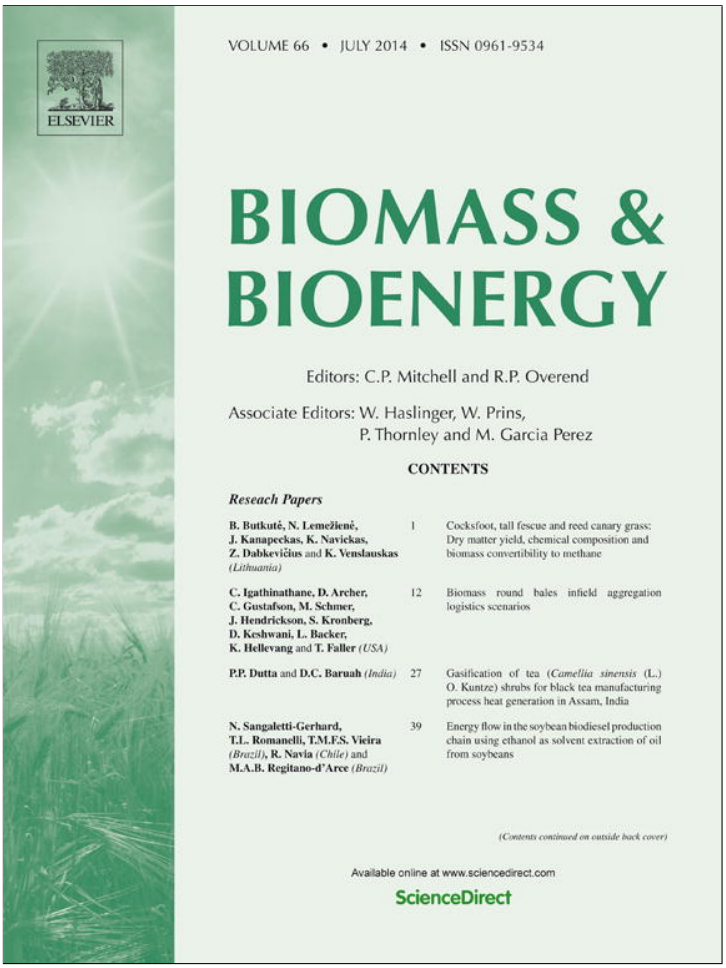

VOLUME 66 • JULY 2014 • ISSN 0961-9534

BIOMASS & BIOENERGY

Editors: C.P. Mitchell and R.P. Overend

Associate Editors: W. Haslinger, W. Prins, P. Thornley and M. Garcia Perez

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BIOMASS AND BIOENERGY 66 (2014) 133–142

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Grasses for biofuels: A low water-use alternative for cold desert agriculture?

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ARTICLE INFO

Article history:
Received 12 September 2013
Received in revised form
21 January 2014
Accepted 31 January 2014
Available online 1 March 2014

Keywords:
Walker lake
Panicum virgatum
Cellulosic biofuel crops
Root diameter
Deficit irrigation
Arid ecosystems

ABSTRACT

In arid regions, reductions in the amount of available agricultural water are fueling interest in alternative, low water-use crops. Perennial grasses have potential as low water-use biofuel crops. However, little is known about which perennial grasses can produce high quantity, high quality yields with low irrigation on formerly high-input agricultural fields in arid regions. We monitored biomass production, weed resistance, rooting depth, and root architecture of nine perennial grasses under multiple irrigation treatments in western Nevada. Under a low irrigation treatment (71 ± 9 cm irrigation water annually), cool-season grasses produced more biomass and were more weed-resistant than warm-season grasses. With additional irrigation (120 ± 12 cm water annually), warm- and cool-season grasses had similar biomass production, but cool-season species remained more weed-resistant. Among species within each grass type, we observed high variability in performance. Two cool-season species (*Elytrigia elongata* and *Leymus cinereus*) and one warm-season species (*Bothriochloa ischaemum*) performed better than the other tested species. Root depth was not correlated with biomass production, but species with deeper roots had fewer weeds. Abundance of fine roots (but not large roots) was correlated with increased biomass and fewer weeds. Both *L. cinereus* and *E. elongata* had deep root systems dominated by fine roots, while *B. ischaemum* had many fine roots in shallow soil but few roots in deeper soil. Cool-season grasses (particularly *E. elongata*, *L. cinereus*, and other species with abundant fine roots) may be worthy of further attention as potential biofuel crops for cold desert agriculture.

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0961-9534/$ – see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.biombioe.2014.01.046

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1. Introduction

In many arid ecosystems, the availability of fresh water for agriculture is declining due to increased drought frequency and intensity [1], as well as competing demands such as urban development [2,3] and aquatic ecosystem restoration [e.g., see Refs. [4,5]]. Over-use of limited water supplies can have strong, negative effects on ecosystems, economies, and human health [e.g., see Refs. [5–8]]. Given the increasing demands for and decreasing supplies of fresh water in arid ecosystems, arid-land agricultural practices will likely shift towards more water-efficient crops [4,9].

In the cold deserts of the western USA, agriculture is currently dominated by alfalfa, a relatively high-input crop [4,9]. Cellulosic biofuel crops could potentially serve as a low water-use alternative for this region. Climate change, increasing oil prices and decreasing oil supply have led to rising interest in biomass as an alternative fuel source [10–12], and efforts are underway to develop cost-effective techniques for transforming complex carbohydrates (e.g., cellulose, hemicellulose) into fuel [10,11]. Studies from more mesic ecosystems suggest that perennial grasses can produce large amounts of biomass while generating less carbon and requiring less water, herbicide, and pesticide than annual crops such as corn [13–16]. However, it is currently unclear whether perennial grasses growing in arid regions and irrigated with relatively small amounts of water (e.g., 0.6–0.8 m^3/m^2, vs. 1.2 m^3/m^2 for alfalfa) can produce enough biomass to be viable as alternative crops. To determine the feasibility of transitioning from traditional crops to low-input biofuel crops, researchers need a better understanding of which perennial grasses are best suited for arid-land biofuel crop development.

Plant phenology may be an important driver of productivity in arid regions, where water stress can preclude growth for large portions of the year. Cool-season species grow during the fall and spring, while warm-season species grow during the summer. In the USA, most existing work on perennial grass biofuels has focused on a few warm-season species (e.g., *Panicum virgatum* and *Miscanthus giganteus*) [11,12,17]. Cool-season species dominate the cold deserts of the western USA (where most precipitation falls during winter), but have received relatively little attention as potential biofuel crops. Recent work from Utah suggests that warm-season species can maintain viable stands in cold deserts [18], but cool-season species may be able to produce more total biomass than warm-season species [19]. These studies did not report on the weed resistance of warm- vs. cool-season species, which may be a critical element of agronomic viability. Moreover, these studies occurred at a relatively wet site (476 mm annual precipitation vs. 129 mm in western Nevada).

Root architecture can also affect plant performance in arid ecosystems [e.g., see Ref. [20]]. In particular, rooting depth and root diameter can impact a plant's ability to acquire water and nutrients from the soil. Coarse roots have longer lifespans and more rapid elongation, whereas fine roots allow more efficient resource acquisition but are shorter-lived [21,22]. While several studies report positive relationships between average root diameter and aboveground biomass production for herbaceous plants [e.g., see Refs. [23–25]], at least one study suggests that under water stress, species able to produce additional fine roots can maintain higher growth rates [20]. Few studies have documented differences in rooting structure among potential cellulosic biofuel crops [but see Ref. [26]], perhaps because most of the research on biofuel crops has occurred in mesic ecosystems.

We monitored the biomass production, weed resistance and root characteristics of five warm-season and four cool-season perennial grasses over four growing seasons and under multiple irrigation regimes in western Nevada. The study addressed two questions:

1. Does grass seasonality or species identity affect biomass production or weed resistance, and does the answer to this question depend on irrigation treatment?
2. Do root depth and density vary based on grass seasonality, species identity or irrigation treatment, and are certain root structures associated with higher biomass production or weed resistance?

At our cold desert sites, we expected cool-season species to produce more biomass and have higher crop water productivity than warm-season species. Because cool-season grasses take up water and nutrients throughout the fall and spring, we expected cool-season species to be more weed-resistant than warm-season species. Finally, we expected root depth, large root abundance and fine root abundance to be positively associated with biomass production and weed resistance.

2. Materials and methods

2.1. Study sites and species

Two study sites were located 11.5 km south of Mason, Nevada USA along the lower reaches of the Walker River (which feeds Walker Lake, one of only eight fresh water terminal lakes >100 km^2 worldwide) [4]. The Valley Vista Ranch (VV) site (38°50′58″N, 119°11′04″W) was used for alfalfa production until the start of the experiment. The 5C Cottonwood Ranch (5C) site (38°50′45″N, 119°11′02″W) was a denuded, formerly grazed pasture. Both sites are located on Malapais (loamy-skeletal, mixed, superactive, mesic Typic Haplocambids) complex soils (dominated by Malapais gravelly sandy loam and Malapais stony sandy loam) [27].

Natural vegetation near the sites is dominated by long-lived desert shrub species. Annual precipitation averages 127 mm but is highly variable across years. Most precipitation falls during the cold winter months, when plants are dormant. Annual precipitation was 68, 165, 177 and 68 mm from 2008 to 2011, respectively.

We planted commercial varieties of nine perennial grasses (see Appendix A for varieties and authorities). Warm-season species were *Andropogon hallii* (sand bluestem, native), *Bothriochloa ischaemum* (old world bluestem, non-native), *Calamovilfa longifolia* (prairie sandreed, native), *P. virgatum* (switchgrass, native), and *Sorghastrum nutans* (indiangrass, native). Cool-season species were *Elytrigia elongata* (tall wheatgrass, non-native), *Festuca arundinacea* (tall fescue, non-

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native), *Leymus cinereus* (basin wildrye, native), and *Leymus racemosus* (mammoth wildrye, non-native). "Native" species are native to North America, with *L. cinereus* the only species native to Nevada. Most species are at least moderately drought-tolerant (Appendix A).

2.2. Experimental design

The experiment was a blocked split-plot design with three 27.4 × 109.7 m blocks at each site. Each block was split into three 9.13 × 109.7 m plots. Plots were randomly assigned to one of three irrigation treatments (low, medium or high irrigation). Within each irrigation plot, nine 9.13 × 7.32 m subplots were assigned to different biofuel crop treatments. In one block per site, the same subplot treatment randomization was inadvertently applied to all three irrigation plots. In the other blocks, subplot treatments were randomized separately within each irrigation plot.

2.3. Management and planting

Mechanical treatments and herbicides were used to prepare the sites for planting and reduce weeds. All plots were ripped, disked, and leveled in September 2007. Glyphosate and dicamba were used to kill alfalfa on VV in 2007, and 2,4-D was applied to all plots in early May 2008.

Cool-season grasses were planted in December 2007. Warm-season grasses were planted in May 2008. All species were sown at recommended seeding rates (Appendix A). Seeds were planted using a Truax drill with seeds placed 1.3 cm deep and followed by press wheels. A cultipacker was used after sowing the cool-season grasses, but not after sowing the warm-season grasses, as the planting surface was sufficiently compacted in May. Post-emergence weed control included spraying Metsulfuron-methyl on warm-season grasses in June 2008, spraying Metsulfuron-methyl + 2,4-D on cool-season grasses in June 2008, and mowing warm-season grasses bi-weekly during the first season of growth to control annual grass weeds. In subsequent years, warm- and cool-season grasses were mowed in May each year and sprayed with 2,4-D during the growing season. All plots were fertilized annually in late April with ammonium sulfate (21-0-0) at 533.7 kg/ha (112 kg/ha N).

Plots were watered using deficit irrigation, in which water is applied at rates below the full crop water requirement [9]. This form of irrigation is common in the western USA and generally results in water application from spring until water is unavailable (in late summer, or earlier during a drought). In our experiment, irrigation began in late April or early May, as needed, and continued until August (2008 and 2009) or October (2010 and 2011). Irrigation amounts and duration of water application varied by year due to differences in precipitation and water availability, and lack of available irrigation water at the end of the growing season resulted in little difference between the medium and high water treatments in most years (Appendix A). Across years, the low water treatment received ~60% and the medium water treatment received ~90% as much water as the high water treatment (Appendix A). Due to the minimal difference in methods between the medium and high water treatments, results were similar across these two treatments (e.g., *S. nutans* and *L. cinereus* produced intermediate biomass in the medium water treatment, but for other species biomass production did not differ significantly between the medium and high water treatments; data not shown). Therefore, our analyses focused on differences between the high and low water treatments and excluded the medium water treatment. Irrigation was applied using sprinklers (set on a 9.1 × 9.1 m pattern) that delivered approximately 7.6 L/min/sprinkler.

Cattle grazing occurred during plant dormancy at the VV site in 2009, 2010, and 2011. This unplanned treatment was the result of animals entering fields during cold winter months in spite of electric fencing. Livestock grazing did not occur at the 5C site.

2.4. Data collection

2.4.1. Biomass and weeds

In September or October of 2009, 2010 and 2011, biomass was harvested and weighed from a 0.9 m × 7.32 m swath running through the center of each subplot, using a Carter flail-type forage harvester. For each subplot, a subsample of harvested material was used to determine moisture content. Subsamples were weighed, then oven-dried at 40 °C until they achieved a constant weight. Wet and dry weights were used to convert large-scale biomass weights to dry mass.

To estimate weed contamination, biomass was harvested in three 1 × 1 m quadrats per subplot in August of 2009, 2010, and 2011. For each quadrat, weeds and crops were weighed separately, and a subsample was used to calculate dry mass as described above. Final crop biomass values were adjusted by multiplying the average percent crop from small-scale sampling by the total biomass from large-scale sampling.

2.4.2. Roots

Roots of planted species were sampled in October 2010 and 2011. At each sampling period, one plant within each subplot was selected randomly for measurement (resulting in six sampled plants per treatment combination per year). *C. longifolia* subplots were excluded due to low plant abundance. Selected plants were removed at the soil surface and soil directly under the plant was sampled using a 7.62 cm bucket auger. Because samples were taken directly under a large perennial plant, we assumed that roots in this soil were almost exclusively roots of the target species, though there could have been minor contamination from neighboring weeds. Soil was sampled down to 90 cm in 15 cm increments. Each sample was homogenized in a bucket, and a blind subsample of 240 mg was taken. In the laboratory, a categorical ranking system (0 = no roots to 5 = abundant roots) was used to assess large and fine root content (Appendix A). To assess the accuracy of this qualitative ranking system, oven-dried root weights were compared to qualitative rankings for a subset of root samples. Qualitative rankings were significantly correlated with measured root weights (Appendix A). For each subplot, the maximum depth at which roots were present was defined as the "root depth" (given sampling limitations, maximum possible depth = 91 cm).

2.5. Data analysis

Data were analyzed using generalized linear mixed models. Aboveground response variables were crop biomass, crop

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water productivity (determined by dividing total biomass values for each subplot and year by meters of irrigation water applied to that subplot during that year's growing season), and percent weed contamination (averaged at the subplot level). Root response variables were root depth, large root abundance ranking, and fine root abundance ranking.

For aboveground models, random factors included site, block nested within site, irrigation plot nested within block and site, and crop subplot nested within plot, block and site. An autoregressive covariance structure was used to account for temporal autocorrelation between years. Fixed factors included irrigation treatment (low vs. high water), grass type (warm- vs. cool-season), and the irrigation*type interaction. For root models, random factors included factors listed above and in addition, year nested within crop subplot, plot, block and site. Fixed factors included irrigation treatment, grass type, depth, and all interactions. For all response variables, in order to determine which species were driving results, we ran parallel models in which grass type was replaced by grass species.

If interaction terms were significant ($P < 0.05$), simple effects tests were performed. Interaction terms were removed from models in which they had P-values >0.05. In all cases, Tukey's Honestly Significant Difference (HSD) tests ($\alpha = 0.05$) were used for post-hoc means comparisons. Variance-weighting was used when variances were not homogenous, and response values were transformed when necessary. To investigate associations among different response variables, we calculated pair-wise Pearson correlation coefficients. Analyses were run in R 2.12.2 [packages nlme and Hmisc, [28, 29]]. Results are reported as means ± 1 standard error.

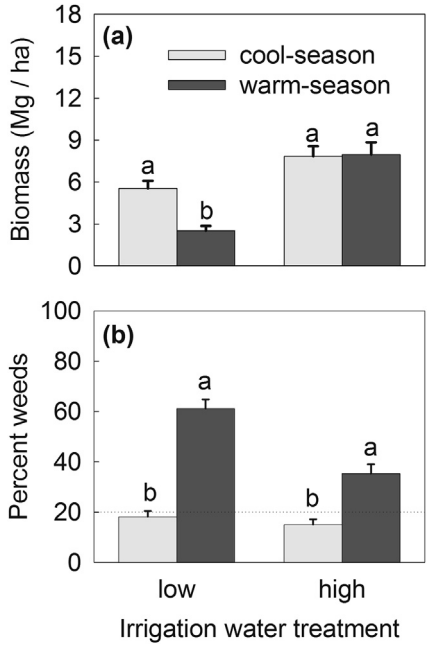

Fig. 1 – Crop biomass production (a) and weed contamination (b) by grass type (warm- or cool-season) and water treatment. Within each water treatment, grass types sharing the same letter do not differ (Tukey's HSD for simple effects). Dotted line indicates 20% weed contamination. Analyses were performed on log-transformed data. Values shown are averaged across sites, blocks and growing seasons.

3. Results

3.1. *Crop biomass*

Biomass production was highest in 2010 (>15 Mg/ha for six species in the high water treatment) and lowest in 2011 (<5 Mg/ha for most species and water treatments; see Appendix B for results separated by year). Though limited cattle grazing occurred at VV during plant dormancy, biomass production was similar between sites (averaged across all subplots and years, production = 5.25 ± 0.42 Mg/ha at 5C and 6.53 ± 0.56 Mg/ha at VV).

Across years, the relative performance of different crops varied based on irrigation treatment (Fig. 1(a); crop*water $F_{1,94} = 5.06$, $P = 0.03$). In low water plots (receiving 71 ± 9 cm irrigation water annually), cool-season species produced more than twice as much biomass as warm-season species (Fig. 1(a); $F_{1,47} = 20.5$, $P < 0.0001$). In high water plots (120 ± 15 cm water annually), cool- and warm-season species produced similar biomass (Fig. 1(a); $F_{1,47} = 0.70$, $P = 0.4$). For both grass types, high water plots produced more biomass than low water plots, but this pattern was much stronger for warm-season species (Fig. 1(a); cool: $F_{1,5} = 9.95$, $P = 0.03$, warm: $F_{1,5} = 20.1$, $P = 0.007$). Cool-season species had 45% higher crop water productivity than warm-season species (6.93 ± 0.47 vs. 4.76 ± 0.41 Mg/ha/meter irrigation water applied, $F_{1,95} = 16.4$, $P = 0.0001$). Differences between warm- and cool-season grass types were most apparent in the highest productivity year (2010), and differences were minimal in 2011, the lowest productivity year (Appendix B).

When we compared biomass among species, we found that three species (one warm-season and two cool-season) outperformed the rest: *B. ischaemum*, *E. elongata* and *L. cinereus*. The relative performance of different crops varied based on irrigation treatment (Fig. 2(a), Table 1; crop*water $F_{8,80} = 14.0$, $P < 0.0001$). In low water plots, *E. elongata* produced more than 2.5 times as much biomass as *P. virgatum*, *S. nutans* or *C. longifolia* (Fig. 2(a), Table 1; $F_{8,40} = 44.3$, $P < 0.0001$), while other species produced intermediate biomass. In high water plots, *B. ischaemum* and *E. elongata* produced significantly more biomass than *L. racemosus*, *F. arundinacea* or *C. longifolia* (Fig. 2(a), Table 1; $F_{8,40} = 129$, $P < 0.0001$), while other species produced intermediate biomass.

For all warm-season species except *C. longifolia* (which produced almost no biomass), the high water treatment produced significantly more biomass than the low water treatment (Fig. 2(a)); these results were most apparent in the highest productivity year (Appendix B). In contrast, biomass production did not differ significantly between water treatments for any of the cool-season species (Fig. 2(a)).

E. elongata had significantly higher crop water productivity than *F. arundinacea*, *P. virgatum*, *S. nutans* or *C. longifolia* (Fig. 3; $F_{8,88} = 157$, $P < 0.0001$). *B. ischaemum* and *L. cinereus* had significantly higher crop water productivity than *S. nutans* or *C. longifolia* (Fig. 3).

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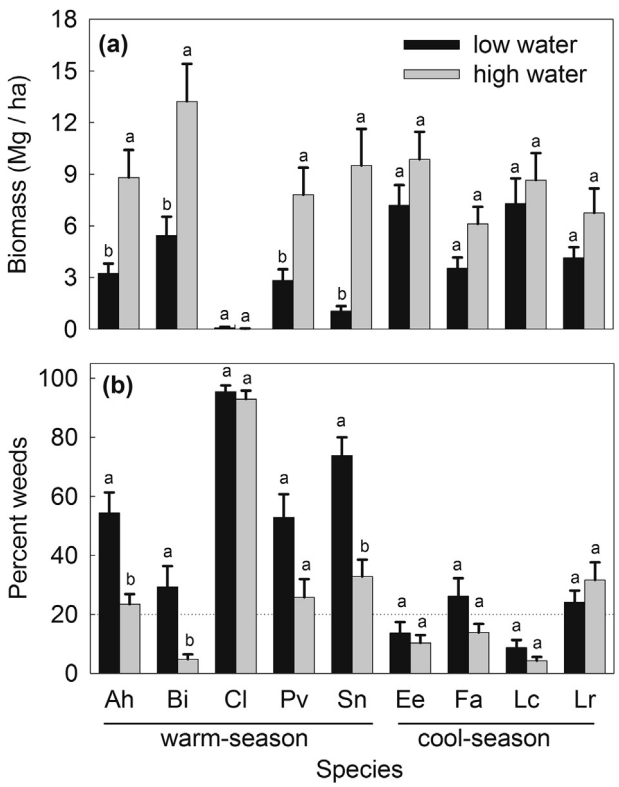

Fig. 2 – Biomass production (a) and weed contamination (b) by species and water treatment. Within each species, water treatments sharing the same letter do not differ (Tukey's HSD for simple effects). Dotted line indicates 20% weed contamination. Analyses were performed on log-transformed data. Values shown are averaged across sites, blocks and growing seasons. Warm-season spp.: Ah = *Andropogon hallii*, Bi = *Bothriochloa ischaemum*, Cl = *Calamovilfa longifolia*, Pv = *Panicum virgatum*, Sn = *Sorghastrum nutans*. Cool-season spp: Ee = *Elytrigia elongata*, Fa = *Festuca arundinacea*, Lc = *Leymus cinereus*, Lr = *Leymus racemosus*.

3.2. *Weed contamination*

Weed contamination was highest in 2009 and lowest in 2011. Differences among grass and water treatments were most pronounced in 2010 (high productivity year) and least pronounced in 2011 (low productivity year, Appendix B).

Across years, weeds growing with different grass types responded differently to water treatments (Fig. 1(b); season*water $F_{1,94} = 6.00$, $P = 0.02$). Cool-season grasses had significantly less weed contamination than warm-season grasses, but the difference between grass types became smaller as water application increased (Fig. 1(b); low water $F_{1,47} = 57.3$, $P < 0.0001$, high water $F_{1,47} = 14.9$, $P = 0.0003$). For cool-season grasses, weed contamination levels were <20% across all water treatments, and water treatment did not significantly affect weed contamination (Fig. 1(b); $F_{1,5} = 0.60$, $P = 0.5$). For warm-season grasses, weed contamination in low water plots was 73% higher than weed contamination in high water plots (Fig. 1(b); $F_{1,5} = 7.47$, $P = 0.04$).

The three species with the highest crop water productivity (*E. elongata*, *B. ischaemum* and *L. cinereus*) also had the lowest levels of weed contamination. The degree to which different crops experienced weed contamination depended upon irrigation treatment (crop*water $F_{8,80} = 3.32$, $P = 0.003$). In the low water treatment, *E. elongata* and *L. cinereus* had <15% weed contamination, while *A. hallii*, *P. virgatum*, *S. nutans* and *C. longifolia* had >50% (Fig. 2(b), Table 1; $F_{8,40} = 28.3$, $P < 0.0001$). In the high water treatment, *L. cinereus*, *B. ischaemum* and *E. elongata* all had <11% weed contamination, while *S. nutans* and *C. longifolia* had >32% (Fig. 2(b), Table 1; $F_{8,40} = 27.3$, $P < 0.0001$).

For three warm-season species (*A. hallii*, *B. ischaemum*, and *S. nutans*), subplots in the low water treatment had significantly more weed contamination than subplots in the high water treatment (Fig. 2(b)). Only *E. elongata* and *L. cinereus* subplots had <20% weed contamination across both water treatments (Fig. 2(b)). The only warm-season grass to achieve <20% weed contamination was *B. ischaemum* in high water plots (Fig. 2(b)).

Across treatments and years, weed contamination was negatively correlated with crop biomass production ($r = -0.55$, $n = 321$, $P < 0.0001$).

Table 1 – Biomass production and weed contamination by species and water treatment. Within each water treatment, species sharing letters did not differ in biomass production or weed contamination. Analyses were performed on log-transformed data.

	Biomass production (Mg/ha)				Weed contamination (percent)			
	Low water		High water		Low water		High water	
Warm-season spp.								
Andropogon hallii	3.2 ± 0.57	AB	8.8 ± 1.6	BC	54 ± 6.9	BC	23 ± 3.5	BC
Bothriochloa ischaemum	5.4 ± 1.1	AB	13 ± 2.2	A	29 ± 7.0	C	4.8 ± 1.6	E
Calamovilfa longifolia	0.07 ± 0.06	D	0.02 ± 0.01	D	95 ± 2.2	A	93 ± 2.9	A
Panicum virgatum	2.8 ± 0.65	BC	7.8 ± 1.6	BC	53 ± 7.9	ABC	26 ± 6.2	BCD
Sorghastrum nutans	1.0 ± 0.29	C	9.5 ± 2.1	ABC	74 ± 6.2	AB	33 ± 5.8	B
Cool-season spp.								
Elytrigia elongata	7.3 ± 1.5	A	9.9 ± 1.6	AB	14 ± 3.7	C	10 ± 2.7	DE
Festuca arundinacea	3.5 ± 0.63	AB	6.1 ± 0.99	C	26 ± 6.1	C	14 ± 2.9	BCDE
Leymus cinereus	7.2 ± 1.2	AB	8.7 ± 1.2	BC	8.8 ± 2.6	C	4.3 ± 1.3	E
Leymus racemosus	4.1 ± 0.61	AB	6.7 ± 1.4	C	24 ± 4.0	C	32 ± 6.0	BCD

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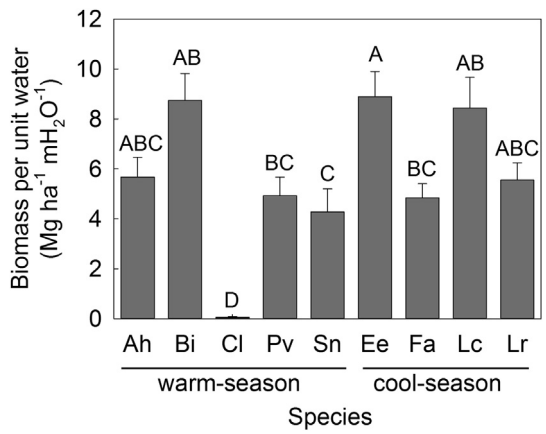

Fig. 3 – Crop water productivity (total biomass divided by irrigation water applied during each growing season; values shown are averaged across sites, blocks and growing seasons). Species sharing the same letter do not differ (Tukey's HSD). Analysis was performed on log-transformed data. Refer to Fig. 2 for species codes.

3.3. *Root depth and abundance*

3.3.1. *Depth*

The relationship between root depth and grass type varied by watering treatment (season*water $F_{1,66} = 5.11$, $P = 0.03$). In low water plots, cool-season grasses had deeper roots than warm-season grasses (77.8 ± 1.9 cm vs. 62.6 ± 2.1 cm, $F_{1,32} = 5.01$, $P = 0.03$), but in high water plots root depth did not differ between grass types (cool: 80.4 ± 1.5 cm, warm: 84.0 ± 1.1 cm, $F_{1,34} = 0.59$, $P = 0.4$). For cool-season grasses, root depth did not vary between water treatments ($F_{1,4} = 0.14$, $P = 0.7$), but for warm-season grasses, roots were deeper in high vs. low water plots ($F_{1,5} = 13.3$, $P = 0.01$).

When we compared root depths among grass species, species identity and water treatment had significant main effects on root depth (crop: $F_{7,61} = 2.14$, $P = 0.05$, water: $F_{1,5} = 9.78$, $P = 0.03$). Overall, high water plots had 19% deeper roots than low water plots (82.3 ± 0.9 cm vs. 69.4 ± 1.5 cm). *E. elongata* subplots had 41% deeper roots than *B. ischaemum* subplots, while other species had intermediate root depths (Table 2).

Across treatments and years, root depth was not correlated with crop biomass ($r = 0.11$, $n = 135$, $P = 0.2$), but subplots with deeper roots had less weed contamination ($r = -0.30$, $n = 135$, $P = 0.0005$).

3.3.2. *Large roots*

Warm-season grasses had 81% more abundant large roots than cool-season grasses (Fig. 4, rankings: 0.88 ± 0.06 vs. 0.48 ± 0.04, $F_{1,83} = 8.88$, $P = 0.004$). At the level of grass type, water treatment did not affect large root abundance (Fig. 4, high ranking: 0.71 ± 0.05, low ranking: 0.66 ± 0.05, $F_{1,5} = 0.07$, $P = 0.8$).

When data were analyzed by grass species, large root abundance patterns differed between water treatments (Fig. 4, Table 2; crop*water $F_{7,70} = 2.36$, $P = 0.03$). In low water plots, *P. virgatum* had more large roots than all other species except *L. racemosus* (Fig. 4(a), Table 2; $F_{7,35} = 7.16$, $P < 0.0001$). In the high water treatment, *P. virgatum* had more large roots than all other species, and *L. racemosus* and *S. nutans* had more large roots than *F. arundinacea* or *E. elongata* (Fig. 4(b), Table 2; $F_{7,35} = 30.0$, $P < 0.0001$). Within each grass species, high and low water plots had similar large root abundance (all simple effects *P*-values >0.05).

Large root abundance declined with depth, and the strength of this decline varied between grass types (depth*-season $F_{1,788} = 17.7$, $P < 0.0001$) and among grass species (depth*crop $F_{7,782} = 6.86$, $P < 0.0001$). The decline in large root abundance with depth was more pronounced for warm- than for cool-season grasses (Fig. 4, depth coefficient for log-transformed large root abundance was −0.027 ± 0.002 for warm-season, −0.017 ± 0.002 for cool-season). Among grass species, the decline was strongest for *P. virgatum* and weakest for *E. elongata* (Fig. 4, Table 2).

Across treatments and years, large root abundance was not significantly correlated with aboveground biomass production,

Table 2 – Root depth, large and fine root abundance (averaged across depths), and depth coefficients (change in root abundance with depth). Large and fine root abundances were quantified using rankings from 0 (no roots present) to 5 (roots most abundant). For large root abundance, analyses were performed on log-transformed data.

	Root depth (cm)	Large root abundance ranking		Fine root abundance ranking		Large root depth coefficient[a]	Fine root depth coefficient[a]
		Low water	High water	Low water	High water		
Warm-season spp.							
Andropogon hallii	73.8 ± 7.1	0.73 ± 0.18	0.64 ± 0.14	2.0 ± 0.17	2.5 ± 0.11	−0.027	−0.042
Bothriochloa ischaemum	61.9 ± 6.6	0.43 ± 0.12	0.30 ± 0.08	2.1 ± 0.19	2.5 ± 0.15	−0.020	−0.083
Panicum virgatum	81.0 ± 5.0	1.6 ± 0.19	2.2 ± 0.18	1.8 ± 0.17	2.3 ± 0.13	−0.037	−0.045
Sorghastrum nutans	77.7 ± 4.9	0.41 ± 0.09	0.69 ± 0.12	2.1 ± 0.12	2.8 ± 0.12	−0.025	−0.034
Cool-season spp.							
Elytrigia elongata	87.1 ± 2.5	0.35 ± 0.10	0.08 ± 0.04	3.0 ± 0.13	2.8 ± 0.12	−0.010	−0.060
Festuca arundinacea	80.9 ± 7.2	0.30 ± 0.11	0.30 ± 0.09	2.7 ± 0.11	2.6 ± 0.17	−0.020	−0.040
Leymus cinereus	83.8 ± 3.1	0.61 ± 0.16	0.41 ± 0.09	2.8 ± 0.16	2.9 ± 0.13	−0.021	−0.064
Leymus racemosus	67.7 ± 7.5	0.88 ± 0.16	0.98 ± 0.16	1.7 ± 0.15	2.6 ± 0.16	−0.018	−0.052

[a] Change in root abundance ranking per inch of soil depth.

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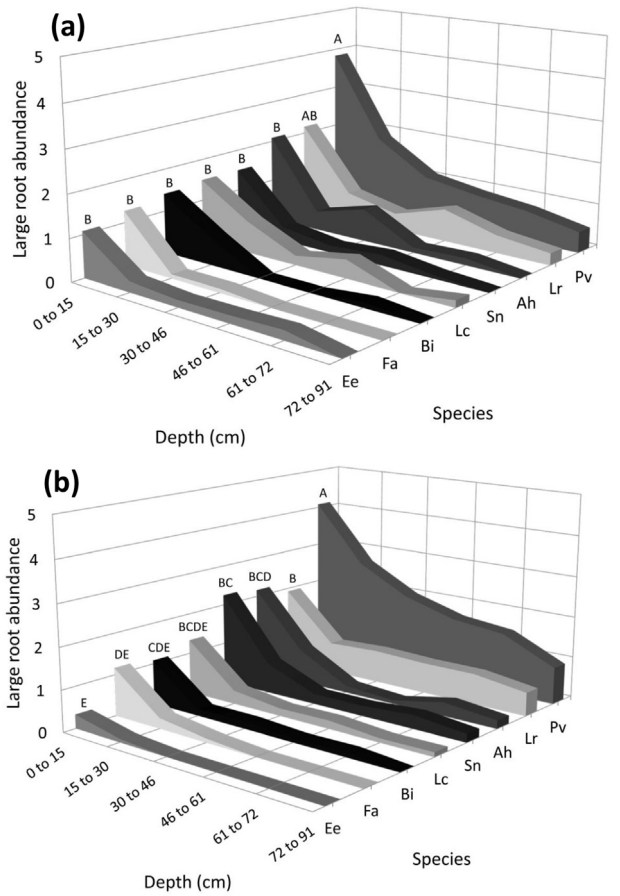

Fig. 4 – Large root abundance by species and depth for low water (a) and high water (b) treatments. Within each water treatment, species sharing the same letter do not differ (Tukey's HSD for simple effects). Analyses were performed on log-transformed data. Refer to Fig. 2 for species codes. Lighter gray = cool-season species; darker gray = warm-season species.

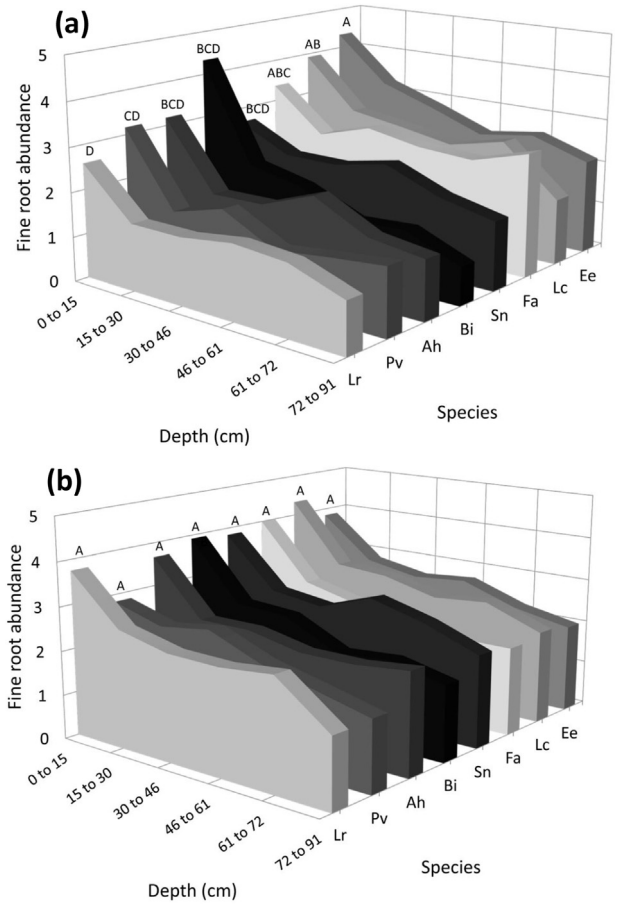

Fig. 5 – Fine root abundance by species and depth for low water (a) and high water (b) treatments. Within each water treatment, species sharing the same letter do not differ (Tukey's HSD for simple effects). Refer to Fig. 2 for species codes. Lighter gray = cool-season species; darker gray = warm-season species.

weed contamination, or root depth (r-values < 0.15, P-values > 0.10).

3.3.3. *Fine roots*

When data were analyzed by grass type, high water plots had 16% more abundant fine roots than low water plots (Fig. 5, rankings: 2.64 ± 0.05 vs. 2.28 ± 0.06, $F_{1,5} = 11.8$, $P = 0.02$), and cool-season grasses had 16% more abundant fine roots than warm-season grasses (Fig. 5, rankings: 2.66 ± 0.05 vs. 2.30 ± 0.05, $F_{1,83} = 11.9$, $P = 0.0009$). Fine root abundance declined with depth, and the strength of this decline was similar across grass types (depth coefficient: -0.052 ± 0.003, $F_{1,789} = 351$, $P < 0.0001$).

When data were analyzed by grass species, differences in fine root abundance varied based on water treatment (crop*water $F_{7,70} = 2.16$, $P = 0.05$). In low water plots, *E. elongata* and *L. cinereus* had more fine roots than *P. virgatum* or *L. racemosus* (Fig. 5(a), Table 2; $F_{7,35} = 6.31$, $P = 0.0001$). In high water plots, all grass species had similar fine root abundance (Fig. 5(b), Table 2; $F_{7,35} = 1.41$, $P = 0.2$). For all species except *L. racemosus*, high and low water plots had similar amounts of fine roots (simple effects P-values >0.05). For *L. racemosus*, high water plots had 54% more fine roots than low water plots (Fig. 5; $F_{1,5} = 13.0$, $P = 0.02$). The decline in fine root abundance with depth varied among grass species (depth*crop $F_{7,782} = 3.85$, $P = 0.0004$). The decline was strongest for *B. ischaemum* and weakest for *S. nutans* (Fig. 5, Table 2).

Across treatments and years, fine root abundance was positively correlated with biomass production ($r = 0.23$, $n = 189$, $P = 0.001$) and negatively correlated with weed contamination ($r = -0.33$, $n = 189$, $P < 0.0001$). Fine root abundance was positively correlated with root depth, ($r = 0.67$, $n = 135$, $P < 0.0001$) and uncorrelated with large root abundance ($r = -0.01$, $n = 190$, $P = 0.9$).

4. Discussion

Cellulosic biofuels could potentially be valuable as a low water-use alternative crop for arid ecosystems, but relatively

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little is known about which perennial grasses will perform best in cold deserts with low irrigation [but see Refs. [18,19,30]]. This study builds on previous work by measuring biomass production and crop water productivity at an especially dry site, reporting on weed resistance, and providing data on root characteristics.

Under a low irrigation treatment, cool-season species produced more biomass than warm-season species. However, with additional water, this advantage dissipated because additional irrigation increased biomass production for warm-season species but did not change biomass production for cool-season species (Figs. 1 and 2). Differences in plant phenology between warm- and cool-season species are likely responsible for the different responses to watering treatments. Additional water was applied during the summer months, when cool-season species had begun to go dormant. In contrast, warm-season species were actively growing while additional irrigation was applied, and were likely more responsive to additional water. Poor performance of warm-season species in the low water treatment could also be a result of poor establishment. In 2008 (the establishment year), lack of water in the late summer could have disproportionately hindered warm-season plant establishment, with lasting consequences for biomass production in subsequent years. Under the form of deficit irrigation practiced in the western US, water supplies often run out during the summer months, especially during droughts. Given these constraints, cool-season crops may be more appropriate than warm-season crops for arid-land agriculture that relies on deficit irrigation. Irrigation of cool-season species may be more effective at increasing biomass if conducted in the late winter or early spring, rather than summer.

Phenology may also help to explain differences in weed resistance between warm- and cool-season species. Regardless of irrigation treatment, cool-season species were better able to resist weeds than warm-season species (Figs. 1 and 2). Many weeds in this ecosystem grow during the fall and early spring, when cool-season grasses are also actively growing. By the time warm-season grasses begin to take up water and nutrients in the late spring, many cool-season weed species are already setting seed. This makes it difficult for warm-season grasses to suppress cool-season weeds. Moreover, because of their earlier phenology, cool-season grasses may also be better able to usurp resources and suppress warm-season weeds. Weed contamination is particularly important from an agricultural perspective because weeds lower crop value and weed control is costly (e.g., costs of herbicides, fuel, labor).

In addition to overall differences between warm- and cool-season grasses, there were significant performance differences among species. Two cool-season species (*E. elongata* and *L. cinereus*) had higher biomass production, lower weed contamination, and higher crop water productivity than the other cool-season species we tested. Similarly, one warm-season species (*B. ischaemum*) performed substantially better than all the other warm-season species (and some cool-season species), especially under low water conditions. Response values rarely differed significantly among *E. elongata*, *L. cinereus* and *B. ischaemum*.

Only one of the three highest-performing species was native to the United States. Non-native species may outperform natives due to enemy release (i.e. an absence of predators in the introduced ecosystem), superior competitive ability, and other advantages [31]. However, some sources indicate that it may be safest to use native species for biofuel crop development in order to avoid the accidental introduction of a problematic invader [32]. The only Nevada native included in our study (*L. cinereus*) was also the only North America native that performed well. Many species show evidence of adaptation to local climates (Leimu & Fischer 2008), and *L. cinereus* is more drought-tolerant than any other species used in this study (Appendix A). Although *L. cinereus* was the most weed-resistant species across both water treatments, *E. elongata* and *B. ischaemum* (non-natives) were capable of producing more biomass than *L. cinereus* (especially in the high water treatment, Fig. 2).

One of the most well-studied potential biofuel grasses, *P. virgatum*, did not perform well in our experiment. Compared to other species, *P. virgatum* had relatively low biomass production, high weed contamination, and low crop water productivity (Figs. 2 and 3). Aside from recent work in Utah [18,19,30], little research has investigated *P. virgatum* performance in the cold deserts of the western USA [17,33]. For example, none of the USA Department of Energy Biofuels Feedstock Development Program testing sites were located west of North Dakota [17]. We used a lowland variety of *P. virgatum* from Nebraska (Nebraska 28). Our results indicate that this cultivar does not perform well at arid, cold desert sites. Previous studies suggest that other varieties (e.g., Blackwell) might perform better [18,19], and efforts are also underway to develop novel cultivars designed specifically for semi-arid environments [30]. As climate change increases the likelihood of drought, it will be important to test new cultivars across a wide range of climatic conditions.

Our data suggest that differences in biomass production may be related to differences in root architecture. Fine root abundance was positively correlated with biomass production and weed resistance, while large root abundance was not significantly associated with either. Although several studies from wetter ecosystems have reported a negative relationship between fine root abundance (or specific root length) and aboveground biomass production [e.g., see Refs. [23–25,34]], greater allocation to fine roots may be associated with higher growth rates under drought conditions [20], and fine root abundance has been associated with drought tolerance in other arid ecosystems [35]. Two of the best-performing species in our experiment (*E. elongata* and *L. cinereus*) had deep root systems dominated by fine roots (Table 2, Fig. 5). The third species (*B. ischaemum*) had many fine roots in the surface soil, but few roots in deeper soil layers (Table 2, Fig. 5). Differences in root architecture may also help to explain the mediocre performance of *P. virgatum*, which had many large roots but relatively few fine roots (Figs. 4 and 5). Without further research, it is impossible to tell whether root characteristics were driving or responding to aboveground biomass production. However, our results suggest that root architecture may be important to consider when evaluating potential biofuel species for use in arid regions.

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In this study, we monitored biomass production over four growing seasons (three production seasons). Differences in biomass production between cool-season and warm-season species were most pronounced in 2009 (when plants were young) and least pronounced in 2011 (when plants were older, Appendix B). The biomass production of several warm-season species (*A. hallii*, *P. virgatum* and *S. nutans*) increased (relative to other tested species) over the course of our study, but similar trends were absent for cool-season species. This suggests that cool-season species may achieve mature production levels more quickly than warm-season species. In a recent review, Wright and Turhollow [17] concluded that *P. virgatum* stands require 2–3 growing seasons to achieve mature yields. A complicating factor in our study was variation in water availability over time; in particular, the lack of difference between grass types in 2011 may have been related to a regional drought. A highly variable water supply is common in cold desert agriculture, but as a result, we were not able to independently manipulate water and plant age. Longer-term studies, and studies conducted within a variety of water and climate conditions, are needed to determine whether warm-season yields could eventually outpace cool-season yields, even in arid environments.

In the western US, the viability of any new field crop depends on its economic performance relative to alfalfa hay, which currently dominates agriculture in arid regions [4,9,36]. Our results suggest that perennial grasses may be able to produce as much biomass per unit area as alfalfa, especially under low water conditions [4,9]. Aside from water, production costs are similar between alfalfa and perennial grasses. Although alfalfa requires more harvesting, perennial grasses may require more fertilizer, and warm-season perennial grasses require at least two growing seasons to reach maturity [4,37,38]. Moreover, in most locations, prices received for alfalfa are often more than three times as high as prices for perennial grass biomass, and alfalfa has the additional advantage of readily accessible markets and processing facilities [37,38]. Due to these price and market differences, alfalfa still remains substantially more profitable than cellulosic biofuels [e.g., see Refs. [4,37,38]], even under low water conditions. However, as technologies and markets mature, low water-use biofuel production could eventually become economically viable.

5. Conclusions

Our findings support previous research [19] suggesting that it may be worthwhile to think outside the 'warm-season grass' box when it comes to biofuel crop development for cold desert agriculture. Notwithstanding current efforts to develop *P. virgatum* cultivars that perform better in the western USA [30], our results point to two cool-season species, *E. elongata* and *L. cinereus*, that may be worthy of further attention. These species can (1) produce large amounts of biomass with about 40% less irrigation water than farmers currently use for alfalfa, (2) produce harvestable biomass in the first or second growing season, and (3) minimize production costs and increase crop value by successfully resisting weeds. Both *E. elongata* and *L. cinereus* have root systems dominated by fine roots, which may help them acquire water and nutrients more efficiently. More generally, our results emphasize that phenology and physiological traits (e.g. root architecture) can be strongly linked to the success of potential biofuel crops in arid ecosystems.

Acknowledgments

We thank Kevin Badik, Owen Baughman, Earl Creech, Colt Daniels, Cynthia Downs, Taraneh Emam, Akiko Endo, Michael Laca, Sandra Li, Allison Phillips, Anthony Regalia, Chris Simons, Cub Wolfe, and Hiro Zamma for field assistance. We are grateful to Michael Collopy for coordinating this project. We thank the VV and 5C Ranches for the opportunity to conduct research on their properties. This project was funded by a grant under Public Law 109-103, Section 208(a), through the US Bureau of Reclamation (Cooperative Agreement 06FC204044).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biombioe.2014.01.046.

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