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# Biomass yield and nitrogen use of potential $C_4$ and $C_3$ dedicated energy crops in a Mediterranean climate

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ABSTRACT

Biomass-based fuels from bioenergy crops stand as viable alternatives to fossil fuels. Side-by-side information on yields and N requirements of potential bioenergy crops is, however, lacking. The objectives of our study were to evaluate yields, N removal, and response to N fertilization of perennial $C_4$ and $C_3$ species under different N availability conditions in an irrigated Mediterranean climate. Five $C_4$ species – miscanthus (*Miscanthus* × *giganteus* Greef et Deu ex. Hodkinson et Renvoize), switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii* Vitman), bermudagrass (*Cynodon dactylon* L.), and elephantgrass (*Cenchrus purpureus* (Schumach.) Morrone)-, and two $C_3$ species – tall wheatgrass (*Thinopyrum ponticum* Podp.) and tall fescue (*Festuca arundinacea* Schreb.) – were evaluated under three N fertilization rates (0, 100, and 200 kg N $ha^{-1}$ $yr^{-1}$) from 2009 to 2011 in Davis, CA, USA. Miscanthus, switchgrass, big bluestem, and tall wheatgrass were harvested once per year, tall fescue twice, and bermudagrass four times per year. Elephantgrass was eventually excluded from our study due to winter mortality. The highest N-fertilized and unfertilized dry matter yields were achieved by miscanthus and switchgrass, followed by tall wheatgrass, big bluestem, tall fescue, and bermudagrass, with highest dry matter yields of 33.9, 22.9, 17.2, 16.2, 15.6, and 12.0 Mg $ha^{-1}$ $yr^{-1}$, respectively. Significant responses to N were observed for all crops in all years. Our results suggest that miscanthus and switchgrass have greatest potential as bioenergy crops for several reasons: (1) these crops had the highest yields and required only a single-harvest per year, (2) greatest response to N with yields increasing by 10.9 and 8.4 Mg $ha^{-1}$ in the highest N treatment and fertilizer use efficiency of 69 and 55 kg biomass $kg^{-1}$ N applied, respectively, and (3) lowest biomass N mass fraction of 3.6 and 2.6 g $kg^{-1}$, respectively.

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## 1. Introduction

Interest in alternative sources of energy is growing due to instabilities in the supply and cost of fossil fuels, national security concerns in the USA, and climate change induced by increasing greenhouse gases in the atmosphere (Kering et al., 2012; Solomon et al., 2007). Biomass-based fuels may be a viable alternative energy source (McLaughlin et al., 2002). Potential uses for biomass are electric energy generation through co-firing with coal (Tillman, 2000), gas production by thermo-chemical gasification, and biochemical conversion into liquid fuels such as ethanol (Parrish and Fike, 2005). Ethanol fuel can be used in current internal combustion engines and is the most cost-effective short-term alternative to gasoline (Solomon et al., 2007). In addition, biomass-based ethanol is estimated to achieve higher net energy efficiency and ethanol yield per area (Schmer et al., 2008), while emitting less greenhouse gases than maize-based ethanol (Adler et al., 2007).

Biomass feedstocks are available from agricultural and forestry residues, urban wood wastes, and dedicated energy crops (McLaughlin et al., 2002). Perennial $C_4$ grasses such as switchgrass (*Panicum virgatum* L.) and Miscanthus (*Miscanthus* × *giganteus* Greef et Deu ex. Hodkinson et Renvoize) (Hodkinson and Renvoize, 2011) have strong potential as dedicated energy crops due to high

*Abbreviation:* FUE, fertilizer use efficiency.

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conversion of sunlight into biomass, efficient water and N use, and ability to achieve high biomass yields with limited inputs (Samson et al., 2005). Once established, perennial crop fields can be harvested for several years, reducing the energy input and costs required for annual replanting. In addition, perennial crops have other advantages compared to annual crops, such as reduced soil erosion and nutrient leaching (Samson et al., 2005; Hohenstein and Wright, 1994), increased soil organic matter content (Liebig et al., 2005), and providing habitat for wildlife (Dunn et al., 1993).

Nitrogen management is an important consideration in developing sustainable, energy efficient and environmentally benign cropping systems dedicated for energy production (Guretzki et al., 2011). Fertilizer N is the main energy input and source of greenhouse gases emissions during switchgrass cultivation for biofuel purposes (Schmer et al., 2008; Adler et al., 2007). Furthermore, the response of miscanthus and switchgrass to N fertilizer is not clear and results are conflicting due to variations in soil, crop management, and number of harvests per year (Heaton et al., 2009; Parrish and Fike, 2005). Some studies have reported limited to no yield response to N fertilization (Christian et al., 2001, 2008; Thomason et al., 2005) while others reported significant yield responses (Ercoli et al., 1999; Lemus et al., 2008; Muir et al., 2001; Pedroso et al., 2013; Stroup et al., 2003).

Switchgrass and miscanthus are perennial $C_4$ grasses that have been most extensively studied as dedicated energy crops due to their high yield potential, excellent conservation attributes, adaptability to marginal areas, and high water and N use efficiency (Heaton et al., 2004; McLaughlin et al., 1999; McLaughlin and Kszos, 2005). Switchgrass is native to the North American tall grass prairie, occurring naturally throughout the mainland United States and the Pacific Northwest (USDA, 2006). Reported dry matter yields of switchgrass range from 5.5 to 36 Mg ha$^{-1}$ (Fike et al., 2006; Heaton et al., 2004; McLaughlin et al., 1999; Pedroso et al., 2011; Schmer et al., 2008). *Miscanthus* × *giganteus* is a rhizomatous sterile hybrid of *M. sinensis* and *M. sacchariflorus* (Hodkinson et al., 2002; Swaminathan et al., 2010), and reported dry matter yields range from 8 to 44.1 Mg ha$^{-1}$ yr$^{-1}$ (Heaton et al., 2008; Lewandowski et al., 2000; Maughan et al., 2012; Miguez et al., 2008).

Although switchgrass and miscanthus have received most of the attention, regional and local environmental characteristics may favor the use of different crops (Wright, 2007). Mediterranean climates exhibit high solar radiation and longer growing season, which allows for a higher yield potential. Elephantgrass (*Cenchrus purpureus* (Schumach.) Morrone), big bluestem (*Andropogon gerardii* Vitman), and bermudagrass (*Cynodon dactylon* L.) may be well suitable for energy production in such climates, especially under conditions of low soil N. Elephantgrass is a high yielding grass native to tropical Africa (Knoll et al., 2012) that has traditionally been used for dairy cattle feed due to its high yield and protein content (de Morais et al., 2009). Reported dry matter yields range from 20 to 70 Mg ha$^{-1}$ yr$^{-1}$ under high N fertilization rates and multiple harvests per year (de Morais et al., 2009; Valencia-Gica et al., 2012; Woodard and Prine, 1993). Big bluestem is native to the American tall grass prairie (Price et al., 2012) and has traditionally been used as high quality forage. Big bluestem could be used for biofuel purposes due to its high fermentability (Weimer and Springer, 2007). Reported big bluestem dry matter yields range from 2.5 to 9.2 Mg ha$^{-1}$ yr$^{-1}$ (Propheter et al., 2010; Robins, 2010). Bermudagrass occurs naturally in North Africa, Asia, Australia and Southern Europe (Xu et al., 2011). It has been extensively used as a ruminant feed and is well adapted to soils with salinity and drought issues (Burton et al., 1957; Stone et al., 2012). Yields up to 24.2 Mg ha$^{-1}$ yr$^{-1}$ in irrigated multi-harvest systems have been reported (Brink et al., 2003).

While most dedicated energy crops studied so far are $C_4$ plants, perennial $C_3$ crops may be well suited in regions where winter rainfall is available, such as in the warm Mediterranean climate of California. Perennial $C_3$ crops, like tall wheatgrass (*Thinopyrum ponticum* Podp.) and tall fescue (*Festuca arundinacea* Schreb.), may require less irrigation in Mediterranean climates because of their ability to grow during the wet cooler months. In addition, agricultural production systems in Mediterranean climates are often irrigated, and $C_3$ cool-season grasses have been reported to outperform $C_4$ warm-season grasses in irrigated cropping systems (Robins, 2010). Tall wheatgrass is native to Turkey, Asia Minor and Russia (USDA, 2008). 'Jose' tall wheatgrass has shown high saline tolerance (Suyama et al., 2007) and dry matter yields up to 8.0 Mg ha$^{-1}$ yr$^{-1}$ under saline drainage water irrigation (Robinson et al., 2004). Tall fescue is native to Europe (Gibson and Newman, 2001) and is popular as forage in the transition zone between the adaptive areas of cool-season and warm-season grasses (Asay et al., 2001). Tall fescue yields up to 23.1 Mg ha$^{-1}$ yr$^{-1}$ have been reported from multi-harvest systems under irrigation (Robins, 2010).

**Table 1**
Soil properties (0–10 cm) at the location used to evaluate different dedicated energy crops.

| Climatic characteristics | Davis |
|---|---|
| Soil name | Brentwood silty clay loam |
| Clay (g kg$^{-1}$) | 280 |
| Silt (g kg$^{-1}$) | 480 |
| Sand (g kg$^{-1}$) | 240 |
| pH (saturated paste extract) | 7.2 |
| CEC (cmol$_c$ kg$^{-1}$) | 35.4 |
| Olsen-P (mg kg$^{-1}$) | 13.6 |
| $NO_3$-N (mg kg$^{-1}$) | 9.9 |
| Extractable K (mg kg$^{-1}$) | 375 |
| Organic matter (Walkley-Black) (g kg$^{-1}$) | 19.1 |
| Total N (combustion) (g kg$^{-1}$) | 1.3 |

When assessing new biofuel crops for a region, it is ideal to initially evaluate a broad range of potential species. Moreover, the species response to N may play a major role in the selection and performance of dedicated energy crops. However, side by side comparisons of different dedicated energy crops are limited for Mediterranean climates. California's Mediterranean climate has a high yield potential but may also require higher N inputs than in the traditional Mid-Western regions where energy crops have been studied. The objective of this study was to test potential $C_4$ and $C_3$ species as dedicated energy crops under irrigation in a warm Mediterranean climate by evaluating biomass yields, crop N removal, and fertilizer N use efficiency under conditions of low, moderate, and high N availability.

## 2. Materials and methods

### 2.1. Site and species description

The experiment was conducted at the UC Davis Agronomy Field Headquarters in Davis (38.5° N; 121.7° W), situated in the Central Valley of California. Davis is located in a warm Mediterranean climate (CSa) (Peel et al., 2007), exhibiting warm and dry summers and cool and wet winters, with average annual precipitation of 445 mm during late fall, winter, and early spring. The soil is classified as a very deep well drained Brentwood silty clay loam (fine, smectitic, thermic Typic Haploxerepts) with a water table at more than 200 cm. Soil samples were taken prior to establishment in 2007 and averaged over 10 cm deep. Samples were air dried, ground through a 2 mm mesh screen, and analyzed for pH (saturated paste extract), $NO_3$-N, Olsen-P, extractable-K, CEC, organic matter (Walkley-Black), particle size, and total N (Table 1). Temperature and precipitation data for the duration of the experiment were obtained from the Western Regional Climate Center (Table 2).

**Table 2**
Monthly average temperature, frost free days, growing degree days, and precipitation from 2009 to 2011 at Davis, CA.

| Month | Temperature (°C) | | | Precipitation (mm) | | |
|---|---|---|---|---|---|---|
| | 2009 | 2010 | 2011 | 2009 | 2010 | 2011 |
| January | 8.3 | 8.6 | 7.4 | 37 | 168 | 48 |
| February | 9.9 | 11.0 | 8.9 | 158 | 73 | 88 |
| March | 12.2 | 12.2 | 11.5 | 41 | 0 | 161 |
| April | 15.2 | 12.9 | 15.0 | 16 | 82 | 2 |
| May | 20.1 | 16.4 | 16.6 | 19 | 15 | 24 |
| June | 21.4 | 22.4 | 20.6 | 12 | 0 | 44 |
| July | 23.6 | 22.9 | 23.7 | 0 | 0 | 0 |
| August | 23.3 | 21.8 | 22.8 | 0 | 0 | 0 |
| September. | 23.5 | 22.4 | 24.1 | 5 | 5 | 0 |
| October | 16.6 | 18.2 | 18.3 | 104 | 23 | 30 |
| November | 12.1 | 12.3 | 10.7 | 8 | 54 | 31 |
| December | 7.1 | 10.1 | 7.7 | 57 | 139 | 10 |
| Mean/total | 16.1 | 15.9 | 15.6 | 458 | 560 | 439 |
| Frost free days | 350 | 363 | 344 | – | – | – |
| GDD† (base temp. 10 °C) | 2442 | 2305 | 2302 | – | – | – |

*Source*: Western Regional Climate Center.
† GDD, growing degree days.

Seven potential crops for bioenergy production were identified. The $C_4$ warm-season grasses were miscanthus ('Hybrid Giganteus' variety), switchgrass ('Alamo' variety), elephantgrass ('Promor A' variety), big bluestem ('Big Kaw' variety), and bermudagrass ('Giant NK 37' variety) and the $C_3$ cool-season grasses were tall wheatgrass ('Jose' variety) and tall fescue ('Fawn' variety).

### 2.2. Crop establishment and management

The experimental design was a split-plot randomized complete block design with four replications. The main plots treatments were seven perennial grass species with N rate as the sub-plots treatments. The size of the main plots was 5 by 15 m. The crops were established in the main plots during 2007 and 2008 (Table 3). Prior to planting any of the crops the field was uniformly disked and a seedbed prepared to ensure a firm and fine soil surface. Tall wheatgrass and tall fescue were drill seeded at a depth of 0.5 cm and 25 cm between rows and bermudagrass was broadcast seeded in September of 2007. Seeding rates were 17, 18, and 9 kg ha$^{-1}$ of pure live seeds for tall wheatgrass, tall fescue and bermudagrass, respectively. Due to the low temperatures in fall 2007, the seeding of switchgrass and bigbluestem were postponed until the following summer. In August of 2008, switchgrass and big bluestem were broadcast seeded at a seeding rate of 7.2 and 9 kg ha$^{-1}$ of pure live seeds, respectively. Miscanthus rhizomes were obtained from Mendel Biotechnology Inc. (Hayward, CA, USA) in October of 2007. Rhizomes of approximately 25 g were transplanted into 12 cm square pots containing a potting mix and grown during the 2007/2008 winter in a greenhouse in Davis, CA. In July of 2008, the above ground portion of the miscanthus plants was trimmed and the potted plants were transplanted at a uniform spacing of 75 cm × 75 cm. Approximately 10% of the plants died and were replanted in spring of 2009. By the fall of 2009 the area between plants had largely filled in as the miscanthus spread. Elephantgrass was obtained from the USDA Research Station in Brawley, California. Plant stems were harvested and cut into billets containing 2 nodes in September of 2007. The billets were transplanted in the same month, by placing a continuous row of billets into furrows 75 cm apart and covering with 5 cm of soil. The billets germinated and grew approximately 1 m tall before the onset of winter. However, the plants did not survive the 2007/2008 winter and the crop had to be replanted. Newly harvested billets from the same source were transplanted in August of 2008. Despite the longer establishment period, the elephantgrass plants once again did not survive the 2008/2009 winter. Elephantgrass was therefore excluded from the experiment due to high winter mortality.

The experimental field was kept under sprinkler irrigation during the 2007 and 2008 establishment years. Invasive plants were controlled by hand weeding as necessary in 2007 and 2008 and no fertilizers were applied in 2007. In July of 2008, 20 kg N ha$^{-1}$ was applied as urea ($CH_4N_2O$) to all plots.

Fertilizer N treatments (0, 100, 200 kg N ha$^{-1}$ yr$^{-1}$) were imposed in 2009 (subplots of 5 by 5 m) and maintained through 2011. Each individual experimental sub-plot received the same N rate as a single application of urea for the duration of the experiment. In addition, phosphorus, potassium and sulfur were applied annually to all plots at a rate of 100 kg ha$^{-1}$ of $P_2O_5$ and $K_2O$ and 34 kg ha$^{-1}$ of S to ensure these nutrients were not limiting. From 2009 to 2011 the irrigation was converted to a flood irrigation system and flooded every two to three weeks during the growing season. The main field operations and their timing are shown in Table 3.

Above ground biomass yields were determined in 2009–2011. Due to differences in growth patterns among crops, the timing and number of harvests varied by crop. Miscanthus, switchgrass, and big bluestem were harvested once per year after flowering and senescence in mid-fall. Tall wheatgrass was harvested once per year after flowering in mid-summer. Tall fescue was harvested twice per year, in early-spring and mid-fall. Bermudagrass was harvested four times per year, with the first harvest being performed in late-spring and the last one in mid-fall. With the exception of miscanthus, all crops were harvested using a walk-behind tractor (BCS 750 model, BCS America, Portland, OR, USA) equipped with a 76 cm wide sickle bar mower set at a cutting height of 10 cm. The borders were cleaned and the area harvested from each experimental unit was 5 m$^2$. Miscanthus was harvested using a chainsaw to cut plants 15 cm above ground level from a 9 m$^2$ area in the middle of each subplot. For all harvests the biomass was weighed and a subsample taken for dry matter determination (60 °C). Biomass yields are expressed on a dry matter basis throughout the manuscript. The subsamples were coarsely ground in a Wiley mill, finely ground in a ball mill, and analyzed for N mass fraction by combustion at the Stable Isotope Facility at UC Davis.

Fertilizer use efficiency (FUE) represents the increase in biomass per unit of fertilizer N applied, and was calculated as follows where yields and N fertilization rate are expressed in kg ha$^{-1}$:

$$\text{FUE} = \frac{(\text{Yield N fertilized plot} - \text{yield unfertilized plot})}{\text{N fertilization rate}}$$

**Table 3**
Primary management practices from 2007 to 2011.

| Year | Management | Miscanthus | Switchgrass | Bermudagrass | Big bluestem | Tall wheatgrass | Tall fescue |
|---|---|---|---|---|---|---|---|
| 2007 | Planting | – | – | September 5 | – | September 4 | September 4 |
| 2008 | Planting | July 9 | August 8 | – | August 8 | – | – |
| | Fertilization | August 22 | August 22 | August 22 | August 22 | August 22 | August 22 |
| | Harvest | – | – | July 9; November 20 | – | July 9 | July 9; November 20 |
| 2009 | Fertilization | April 10 | April 10 | April 10 | April 10 | April 10 | April 10 |
| | Harvest | November 3 | November 3 | May 12; June 24; August 17; November 3 | November 3 | June 24 | May 12; November 3 |
| 2010 | Fertilization | April 7 | April 7 | April 7 | April 7 | April 7 | April 7 |
| | Harvest | October 19 | October 19 | June 1; July 14; August 7; October 19 | October 19 | August 6 | May 5; September 16 |
| 2011 | Fertilization | April 8 | April 8 | April 8 | April 8 | April 8 | April 8 |
| | Harvest | October 12 | October 12 | April 10; June 21; August 1; October 12 | October 12 | August 1 | April 10; October 12 |

### 2.3. Statistical analysis

Data were analyzed for normality and constant variance of errors, and data transformations were performed when assumptions were violated. Analyses of variance on biomass yields, biomass N mass fraction, crop N removal, and FUE were performed using the Mixed Procedure in SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Species, year, N treatment, and the interactions between species × year, species × N treatment, year × N treatment, and species × year × N treatment were considered to be fixed effects, while block was considered a random effect.

Due to significant interactions (Table 4), analysis of variance on biomass yield data was performed by crop, with N treatment, year and the interaction between N treatment × year as fixed effects, and block as random effect. When no significant interaction was detected between N treatment × year within the same species, the effect of N fertilization was analyzed across years. When the interaction between N treatment × year was significant, the effect of N fertilization was analyzed for each year. Biomass N mass fraction, crop N removal, and FUE data were analyzed by crop at each year and N fertilization rate. Differences were considered to be significant at the 5% probability level.

## 3. Results and discussion

### 3.1. Biomass yields, response to N fertilization, and FUE

Due to the significance of the fixed effects species, N treatment, and year, and the interactions between species × N treatment and species × year (Table 4), biomass yields, response to N fertilization, and FUE data are presented for each crop and year.

Averaged across N treatments and years, miscanthus had the highest dry matter yields at 20.3 Mg ha$^{-1}$ yr$^{-1}$, followed by switchgrass (16.1 Mg ha$^{-1}$ yr$^{-1}$), big bluestem, tall wheatgrass, and tall fescue (9.8, 10.5, and 8.6 Mg ha$^{-1}$ yr$^{-1}$, respectively), and lastly by bermudagrass at 7.2 Mg ha$^{-1}$ yr$^{-1}$ (Fig. 1). In the first year, tall wheatgrass, miscanthus, and switchgrass were the highest yielding crops with average yields across N rates of 15.3, 14.7, and 12.7 Mg ha$^{-1}$ yr$^{-1}$, respectively. In the second year, switchgrass (18.7 Mg ha$^{-1}$ yr$^{-1}$) and miscanthus (17.8 Mg ha$^{-1}$ yr$^{-1}$) had the highest yields, followed by big bluestem, tall wheatgrass, tall fescue, and bermudagrass. Similarly in the third year, miscanthus was the highest yielding crop at 28.3 Mg ha$^{-1}$ yr$^{-1}$, followed by switchgrass (17.0 Mg ha$^{-1}$ yr$^{-1}$). A significant effect of year on yields was observed for all crops (Table 4). Yields of some species increased from the first to the third year ($C_4$ species miscanthus, switchgrass, and big bluestem), while other decreased over the same time period (bermudagrass and tall wheatgrass).

When no N was applied, miscanthus and switchgrass had the highest yields (13.8 and 11.1 Mg ha$^{-1}$ yr$^{-1}$, respectively) and bermudagrass and tall fescue the lowest (4.2 and 4.3 Mg ha$^{-1}$ yr$^{-1}$, respectively) (Fig. 1). In addition, miscanthus and switchgrass were the highest yielding crops when fertilized with 100 and 200 kg N ha$^{-1}$ yr$^{-1}$, while bermudagrass had the lowest yields at these N fertilization rates. Average yields across years of the treatments receiving 200 kg N ha$^{-1}$ yr$^{-1}$ were 24.7, 19.4, 13.6, 12.8, 12.5, and 10.0 Mg ha$^{-1}$ yr$^{-1}$ for miscanthus, switchgrass, tall wheatgrass, tall fescue, big bluestem, and bermudagrass, respectively. The highest single year yield achieved by the different species were 33.9, 22.9, 17.2, 16.2, 15.6, and 12.0 Mg ha$^{-1}$ yr$^{-1}$ for miscanthus, switchgrass, tall wheatgrass, big bluestem, tall fescue, and bermudagrass, respectively, with N fertilization rates of 200 kg N ha$^{-1}$ yr$^{-1}$ (Fig. 1).

Significant responses to N fertilization were observed for all crops in all three growing seasons (Fig. 1). Miscanthus, switchgrass, tall wheatgrass, and tall fescue exhibited greater responses to fertilizer N than big bluestem and bermudagrass (Fig. 1). On average across years, yields of the 200 kg N ha$^{-1}$ yr$^{-1}$ treatment compared to the zero N treatment increased by 10.8, 8.2, 6.0, 5.8, 7.5, and 8.5 Mg ha$^{-1}$ yr$^{-1}$ for miscanthus, switchgrass, big bluestem, bermudagrass, tall wheatgrass, and tall fescue, respectively. Miscanthus achieved the highest yield response to N fertilization observed in our study, with yields increasing by 14.4 Mg ha$^{-1}$ yr$^{-1}$ in the highest N treatment compared to the zero N treatment in the third growing season (Fig. 1). In contrast, bermudagrass exhibited the lowest yield response to N fertilization, with yields increasing by 3.9 Mg ha$^{-1}$ yr$^{-1}$ in the highest N treatment compared to the zero N treatment.

The effect of N fertilization on yields differed among years for miscanthus, switchgrass, tall wheatgrass, and tall fescue (represented by the different slopes in Fig. 1). Miscanthus and switchgrass exhibited increasing yield responses to N fertilization over time mainly due to yield increases of the fertilized plots, whereas tall wheatgrass and tall fescue showed increasing response to N mainly due to a decrease in yields of the zero N treatment. Although big bluestem and bermudagrass showed the same yield response to N in all years, big bluestem showed an increase in yields of all N treatments over time, whereas bermudagrass showed a decrease in yields of all N treatments over time (Fig. 1).

Fertilizer use efficiency is an important criterion in the selection of dedicated energy crops. In the first and second years when fertilized with 100 kg N ha$^{-1}$ yr$^{-1}$, miscanthus, switchgrass, and tall wheatgrass had the highest FUE values at 65, 81 and 87 kg biomass kg$^{-1}$ N applied, respectively (Fig. 2). In the third year, miscanthus exhibited the highest FUE value of our study, at 120 kg biomass kg$^{-1}$ N applied, followed by switchgrass, big bluestem, and tall fescue (58, 55, and 56 kg biomass kg$^{-1}$ N applied, respectively). When fertilized with 200 kg N ha$^{-1}$ yr$^{-1}$ the differences in FUE values among species were smaller to non-significant

**Table 4**
Summary statistics with significance of fixed effects on biomass yield, biomass N mass fraction, crop N removal, and FUE. *P*-values lower than 0.05 are considered statistically significant.

| Fixed effects | df | Biomass yield | | N mass fraction | | Crop N removal | | FUE | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *F* value | *P*-value | *F* value | *P*-value | *F* value | *P*-value | df | *F* value | *P*-value |
| Species | 5 | 98.11 | <0.0001 | 535.83 | <0.0001 | 51.28 | <0.0001 | 5 | 11.58 | <0.0001 |
| N treatment | 2 | 213.33 | <0.0001 | 66.05 | <0.0001 | 205.34 | <0.0001 | 1 | 30.24 | <0.0001 |
| Year | 2 | 4.07 | 0.0189 | 3.86 | 0.0231 | 4.70 | 0.0104 | 2 | 5.53 | 0.0053 |
| Species × N treatment | 10 | 2.64 | 0.0053 | 1.5 | 0.1446 | 2.73 | <0.0040 | 5 | 2.91 | 0.0151 |
| N treatment × year | 4 | 2.37 | 0.0549 | 0.86 | 0.4883 | 1.98 | 0.1002 | 2 | 0.11 | 0.8923 |
| Species × year | 10 | 22.41 | <0.0001 | 8.67 | <0.0001 | 23.26 | <0.0001 | 10 | 4.93 | <0.0001 |
| Species × N treat. × year | 20 | 2.36 | 0.0017 | 3.52 | <0.0001 | 1.03 | 0.4316 | 10 | 1.34 | 0.2218 |

due to a decrease in FUE observed with miscanthus, switchgrass, and tall wheatgrass (Fig. 2). On average across years, FUE values of miscanthus, switchgrass, and tall wheatgrass decreased by 29, 31, and 34 kg biomass kg$^{-1}$ N applied, respectively, whereas tall fescue and bermudagrass had equal FUE values between both N-fertilized treatments.

Except for bermudagrass, the yields achieved by the different species in our study are within the higher range of reported yields. Big bluestem yields of 16.2 Mg ha$^{-1}$ yr$^{-1}$ from our study is substantially greater than yields of 9.2 Mg ha$^{-1}$ yr$^{-1}$ reported from a multi-harvest system in Utah (Robins, 2010) and 7.7 Mg ha$^{-1}$ yr$^{-1}$ in Pennsylvania (Sanderson et al., 2004). The greater yields achieved in our study may be due to the longer growing season. Big bluestem is photoperiod sensitive (USDA, 2012). Early dormancy break due to warm temperatures in early spring allows for a longer vegetative growth, potentially resulting in greater yields. Similarly, the highest tall fescue and tall wheatgrass yields (15.6 and 17.2 Mg ha$^{-1}$ yr$^{-1}$, respectively) are greater than the yields of 9.5 (tall fescue) and 7.7 (tall wheatgrass) Mg ha$^{-1}$ yr$^{-1}$ reported in New Mexico (Lauriault et al., 2005; Smeal et al., 2005). Suyama et al. (2007) reported tall wheatgrass yields of 8.3 Mg ha$^{-1}$ yr$^{-1}$ in the San Joaquin Valley of California with drainage water irrigation. Tall wheatgrass yields declined over time in our study (Fig. 1). While the cause of this decline is uncertain, it may be due to high N removal in the first year (Fig. 3) and a subsequent depletion of soil N reserves. Despite the relatively high yields exhibited by big bluestem, tall fescue, and tall wheatgrass, all species had a linear response to N up to 200 kg N ha$^{-1}$ yr$^{-1}$ (Fig. 1), suggesting that higher yields could have been achieved with higher N fertilization rates.

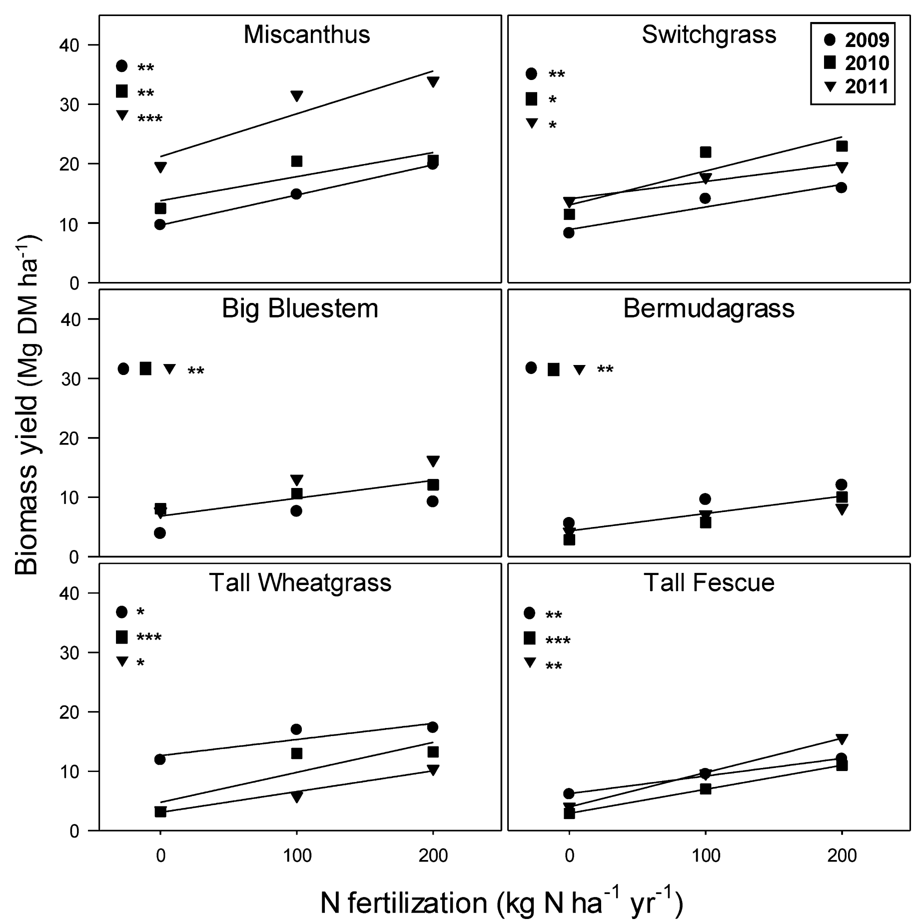


**Fig. 1.** Biomass yield response to N fertilization by crop and year. The effect of N fertilization on biomass yields was analyzed for each year when the interaction between N treatment × year was significant, and across years when the interaction was not significant. The symbols *, **, *** indicate that N fertilization effect on biomass yield is significant at $P < 0.05$, <0.001 and <0.0001, respectively.

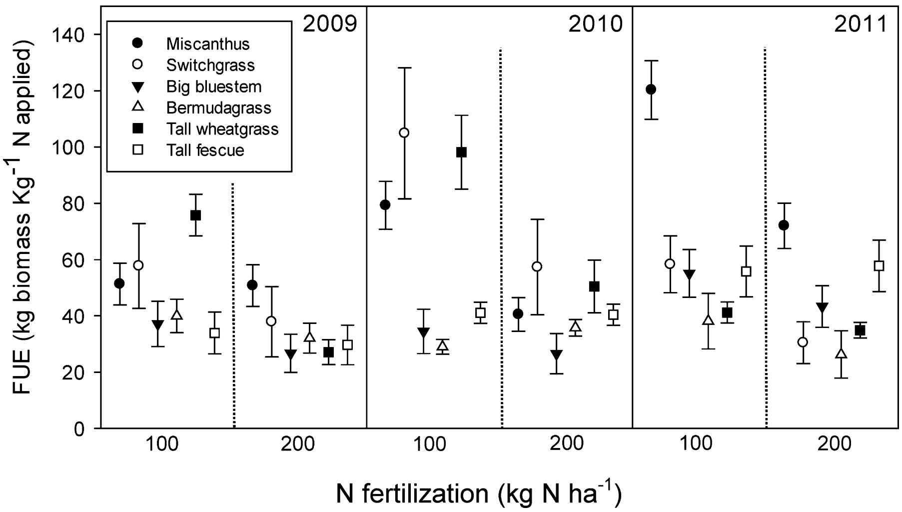


**Fig. 2.** Fertilizer use efficiency (FUE) by species, year, and N fertilization rate. Species mean separations were performed within years at each N fertilization rate. Error bars represent confidence intervals. Confidence intervals within the same year and N rate that do not overlap indicate that FUE values are statistically different ($P < 0.05$).

Bermudagrass, on the other hand, achieved maximum yield of 12 Mg ha$^{-1}$ yr$^{-1}$ in our study, which is within the lower range of reported yields of 6–27 Mg ha$^{-1}$ yr$^{-1}$ (Xu et al., 2011). The relatively low yields achieved in our study by bermudagrass are likely due to the N fertilizer management, in which the entire N fertilizer was applied in a single application in early spring (Table 3). Although increasing and splitting the N fertilization rates may result in higher yields (Brink et al., 2003; Xu et al., 2011), the costs and energy input associated with this management needs to be taken into consideration if bermudagrass is considered a potential dedicated energy crop.

Others have reported that miscanthus and switchgrass require two to five years to mature and reach peak yield levels (Heaton et al., 2004; Miguez et al., 2008). In our study, switchgrass yields

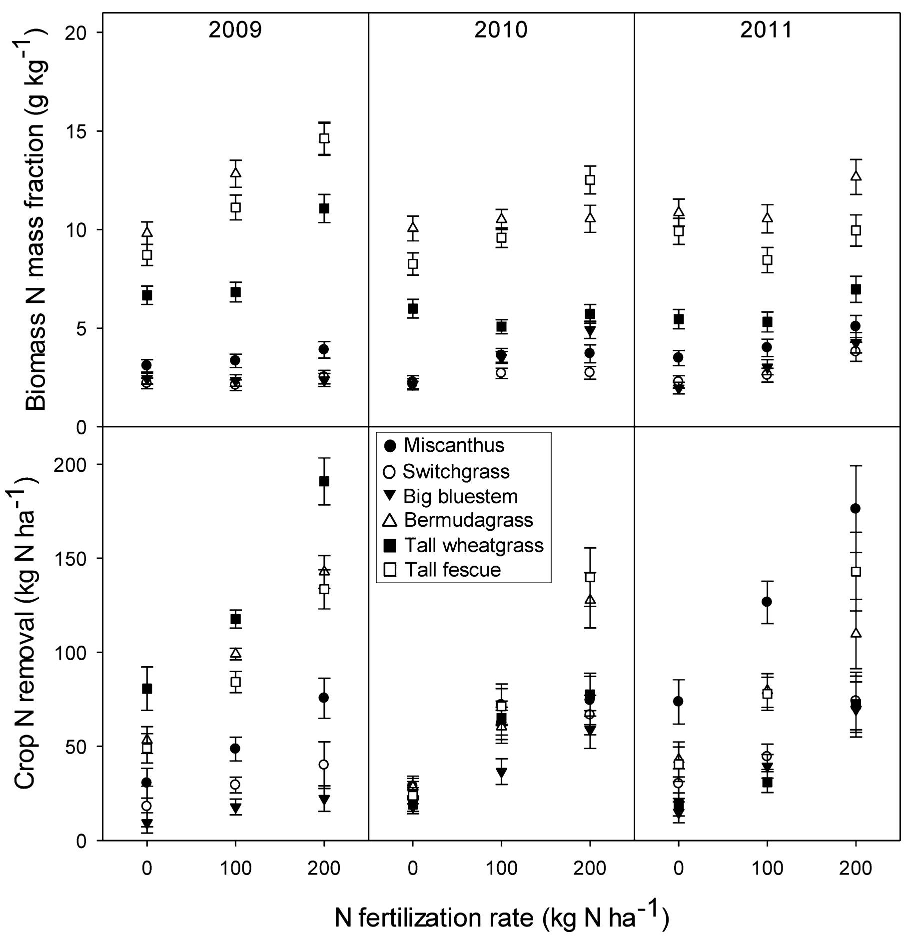


**Fig. 3.** Biomass N mass fraction and crop N removal by species, year and N fertilization rate. Error bars represent confidence intervals. Confidence intervals within the same year and N fertilization rate that do not overlap indicate that biomass N mass fraction and crop N removal are statistically different ($P < 0.05$).

peaked in 2010, which was the second full production year after establishment (Fig. 1). On the other hand, miscanthus had yields which increased in every year of the experiment thus it is not possible to conclude whether miscanthus reached its maximum yield potential. Nevertheless, average miscanthus and switchgrass yields are significantly higher than the average yields reported by (Heaton et al., 2004) of 22 Mg $ha^{-1}$ $yr^{-1}$ (miscanthus) and 10.3 Mg $ha^{-1}$ $yr^{-1}$ (switchgrass) when averaged across N rates and precipitation levels.

The highest yields achieved by miscanthus and switchgrass in our experiment were 33.9 and 22.9 Mg $ha^{-1}$ $yr^{-1}$, respectively; however higher yields have been reported elsewhere. For example, Heaton et al. (2008) reported miscanthus yields up to 44.1 Mg $ha^{-1}$ $yr^{-1}$ in Illinois. Fike et al. (2006) reported switchgrass yields up to 28 Mg $ha^{-1}$ $yr^{-1}$ in a single-harvests system in Virginia. However, the single-harvest system may have limited switchgrass yields in our study. Thomason et al. (2005) reported that switchgrass yields were 14 and 26% higher when switchgrass was harvested two and three times per year, respectively, in comparison to single-harvest systems. Also, Pedroso et al. (2011) reported switchgrass yields of 38 Mg $ha^{-1}$ $yr^{-1}$ in a two-harvest system in the Central Valley of California.

The higher yield achieved by miscanthus relative to switchgrass is in agreement with other side-by-side studies (Dohleman et al., 2012; Propheter et al., 2010), where switchgrass produced on average 53% less biomass per year than miscanthus. The yield differences between miscanthus and switchgrass observed in our study would likely have been reduced if switchgrass had been managed in a two-harvest system. However, the use of multi-harvest systems for bioenergy production is a topic requiring further study, because the higher energy input and crop N removal associated with multiple harvests may offset any gains in biomass yield.

Miscanthus, switchgrass, and tall wheatgrass were more efficient in the use of fertilizer N than the other species (Fig. 2), achieving the highest FUE when fertilized with 100 and 200 kg N $ha^{-1}$ $yr^{-1}$. In addition, miscanthus and switchgrass exhibited a decrease in FUE when fertilized with 200 kg N $ha^{-1}$ $yr^{-1}$, indicating that the maximum FUE was reached at 100 kg N $ha^{-1}$ $yr^{-1}$. In contrast, big bluestem, bermudagrass, and tall fescue had smaller to non-significant decreases in FUE when fertilized with 200 kg N $ha^{-1}$ $yr^{-1}$, which suggests that maximum FUE may have not been achieved in our study. The maximum FUE at lower N fertilization rates observed with miscanthus, switchgrass, and tall wheatgrass are an additional advantage over the other species, because it indicates low N fertilization requirements to achieve maximum return of biomass per unit of N applied. Although $C_3$ cool-season grasses are known to achieve lower FUE values than $C_4$ warm-season grasses, tall wheatgrass and tall fescue had FUE values similar to switchgrass in all years.

Our results indicate that miscanthus and switchgrass are better adapted as dedicated energy crops under irrigation in Mediterranean climates, achieving greater yields than the other crops in conditions of low, moderate, and high N availability. Miscanthus and switchgrass were more efficient in the use of available soil-N, achieving the highest yield when not fertilized with N. The highest N-fertilized yields were also achieved by miscanthus and switchgrass, followed by tall wheatgrass, and tall fescue. In addition, the highest increases in yield per unit of fertilizer N (i.e. FUE) were also achieved by miscanthus and switchgrass, indicating that those species were more efficient in the use of fertilizer N.

### *3.2. Biomass N mass fraction and crop N removal*

Due to the significant three-way interaction (biomass N mass fraction) and significant interactions between species × N treatment and species × year (crop N removal) (Table 4), biomass N mass fraction and crop N removal data are presented by species, year, and N fertilization rate.

At all N fertilization rates and years, biomass N mass fraction at harvest was lowest for miscanthus, switchgrass, and big bluestem, and highest for bermudagrass and tall fescue (Fig. 3). Average biomass N mass fraction across N rates and years were 2.6, 3.0, 3.6, 6.5, 10.3, and 11.2 g $kg^{-1}$ for switchgrass, big bluestem, miscanthus, tall wheatgrass, tall fescue, and bermudagrass, respectively (Fig. 3). Overall across crops and years, the addition of 100 kg N $ha^{-1}$ $yr^{-1}$ did not significantly increase biomass N mass fraction in relation to the zero N treatments; however, the application of 200 kg N $ha^{-1}$ $yr^{-1}$ resulted in significantly greater biomass N mass fraction compared to the zero and 100 kg N $ha^{-1}$ $yr^{-1}$ treatments.

The low biomass N mass fraction exhibited by miscanthus, switchgrass, and big bluestem presents an advantage over the other species. Low biomass N mass fraction feedstocks are desirable for bioenergy production, because high mineral concentration in the biomass feedstock can cause char formation (Rejai et al., 1993), corrosion and increased $NO_x$ emissions (Jorgensen, 1997) during the thermochemical conversion of biomass. In addition, senesced biomass exhibits lower moisture content, which can reduce the costs with bailing and transportation.

Crop N removal is a concern when managing dedicated energy crops. High rates of crop N removal indicate high N fertilization requirements to replenish soil and plant N reserves to sustain high yields. Miscanthus, bermudagrass, tall wheatgrass, and tall fescue had the greatest rates of crop N removal at all N fertilization rates, including the zero N treatments (Fig. 3). Accumulated crop N removal at the end of the experiment from the zero N treatments was 133, 124, 119, and 113 kg N $ha^{-1}$ $yr^{-1}$, for miscanthus, bermudagrass, tall wheatgrass, and tall fescue, respectively. When averaged across years and N rates, tall fescue, bermudagrass, miscanthus, and tall wheatgrass removed on average 85, 82, 78, and 75 kg N $ha^{-1}$ $yr^{-1}$, respectively (Fig. 3). In contrast, switchgrass and big bluestem had the lowest crop N removal, at 43 and 32 kg N $ha^{-1}$ $yr^{-1}$. The high crop N removal observed with miscanthus was caused by the high yields achieved by this crop (Fig. 1), as indicated by the low biomass N mass fraction (Fig. 3). In contrast, bermudagrass, tall wheatgrass, and tall fescue removed as much N as miscanthus mostly because of high biomass N mass fraction. Crop N removal increased significantly with N fertilization rates at all years and for all crops. On average across years and species, the application of 100 and 200 kg N $ha^{-1}$ $yr^{-1}$ increased crop N removal by 30.9 and 66 kg N $ha^{-1}$ $yr^{-1}$, respectively.

The low N mass fraction in the biomass of miscanthus, switchgrass, and big bluestem is likely a result of the single-harvest management system. Those crops were harvested once a year in mid-fall after going through the senescence process. It is known that warm-season grasses translocate N during senescence from above- to belowground tissues, for use in overwintering and regrowth in the following spring (Clark, 1977). Therefore, the single-harvest management allowed for the translocation of N from above- to below-ground biomass, lowering the biomass N mass fraction prior to harvest and consequently lowering crop N removal per unit of biomass produced (Guretzki et al., 2011; Parrish and Fike, 2005; Sanderson et al., 1999; Vogel et al., 2002). In addition to potentially reducing long-term N fertilization requirements due to lower crop N removal, the N stored in below-ground biomass may be re-allocated to above-ground the following growing season, which may further decrease fertilizer N requirements and increase long-term system sustainability.

## 4. Conclusions

Results from our study suggest that miscanthus and switchgrass have the greatest potential as dedicated energy crops in California's Mediterranean climate. Miscanthus and switchgrass achieved the highest yields in the Mediterranean climate of California, while bermudagrass ($C_4$) and tall fescue ($C_3$) produced the lowest average yields. In addition, miscanthus and switchgrass achieved the highest yields with and without the application of N fertilizer. Big bluestem and tall wheatgrass performed relatively well when fertilized with 200 kg N $ha^{-1}$ $yr^{-1}$. Tall fescue and bermudagrass were able to achieve comparable yields to big bluestem under the highest N fertilization treatment. However, those crops showed the highest rates of crop N removal and were harvested two and four times per year, respectively, which increases the energy input and costs associated with multiple harvests and may increase long-term N requirements. Significant yield responses to fertilizer N indicate that perennial dedicated energy crops require moderate to heavy N fertilization to achieve high yields in Mediterranean climates. Miscanthus and switchgrass showed the highest yield response to N fertilization and FUE values, while bermudagrass had the lowest response and FUE values. Furthermore, miscanthus and switchgrass exhibited the lowest biomass N mass fraction of our study. The results indicate that miscanthus and switchgrass are better adapted as dedicated energy crops for Mediterranean climates, due to high fertilized and unfertilized yields and high use efficiency of both native soil N and fertilizer N. In addition, N translocation during senescence from above- to belowground biomass may have been responsible for the low biomass N mass fraction exhibited by miscanthus and switchgrass, suggesting high conservation of N within the plant-soil system. The storage and re-cycling of N within perennial bioenergy crops is a topic that requires further studies.

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