Seasonal dynamics of above- and below-ground biomass and nitrogen partitioning in *Miscanthus × giganteus* and *Panicum virgatum* across three growing seasons

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Abstract

The first replicated productivity trials of the C4 perennial grass *Miscanthus × giganteus* in the United States showed this emerging ligno-cellulosic bioenergy feedstock to provide remarkably high annual yields. This covered the 5 years after planting, leaving it uncertain if this high productivity could be maintained in the absence of N fertilization. An expected, but until now unsubstantiated, benefit of both species was investment in roots and perennating rhizomes. This study examines for years 5–7 yields, biomass, C and N in shoots, roots, and rhizomes. The mean peak shoot biomass for *M. × giganteus* in years 5–7 was 46.5 t ha^{-1} in October, declining to 38.1 t ha^{-1} on completion of senescence and at harvest in December, and 20.7 t ha^{-1} declining to 11.3 t ha^{-1} for *Panicum virgatum*. There was no evidence of decline in annual yield with age. Mean rhizome biomass was significantly higher in *M. × giganteus* at 21.5 t ha^{-1} compared to 7.2 t ha^{-1} for *P. virgatum*, whereas root biomass was similar at 5.6–5.9 t ha^{-1}. *M. × giganteus* shoots contained 339 kg ha^{-1} N in August, declining to 193 kg ha^{-1} in December, compared to 168 and 58 kg ha^{-1} for *P. virgatum*. The results suggest substantial remobilization of N to roots and rhizomes, yet still a substantial loss with December harvests. The shoot and rhizome biomass increase of 33.6 t ha^{-1} during the 2-month period between June and August for *M. × giganteus* corresponds to a solar energy conversion of 4.4% of solar energy into biomass, one of the highest recorded and confirming the remarkable productivity potential of this plant.

Keywords: bioenergy, biofuel, C4 photosynthesis, nitrogen use efficiency, photosynthetic efficiency, rhizome, roots, solar energy conversion efficiency

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Introduction

Two promising candidate cellulosic biofuel feedstocks are the C4 perennial grasses *Panicum virgatum* and *Miscanthus × giganteus* Greef et. Deu ex. Hodkinson et Renvoize. Advantages of C4 perennial grasses include their ability to produce large quantities of biomass with minimal agricultural inputs, allowing for an improved carbon footprint compared to annual row crops. Another source of short-term carbon mitigation by perennial crops, such as *M. × giganteus* and *P. virgatum* is the capture and storage of carbon into below-ground biomass.

Both *M. × giganteus* and *P. virgatum*, are herbaceous perennials producing an annual crop of shoots from a perennial rhizome system which stores carbohydrate

and nutrients through the winter, fueling the rapid regrowth of shoots the next spring. Understanding the seasonal dynamics of rhizome biomass will help to determine the effectiveness of carbon and nutrient cycling within these species, and potential for carbon sequestration. A number of European studies which provide estimates of below-ground biomass in *M. × giganteus* at a single point in time (Beuch, 1995; Hansen *et al.*, 2004; Clifton-Brown *et al.*, 2007), and also seasonal measurements of below-ground biomass in the first 2 years following planting (Beale & Long, 1995) and in the third year following planting (Himken *et al.*, 1997). Above- and below-ground biomass and nutrient concentrations of *M. × giganteus* were also determined over successive years in 4–9 year old stands; however, there was only one reported measurement in each season, which could not allow inference of seasonal dynamics (Kahle *et al.*, 2001). Seasonal dynamics of root biomass

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and nutrient content in mature *M.* × *giganteus* have been shown previously, but without analysis of rhizome nutrient dynamics (Neukirchen *et al.*, 1999). Seasonal N-dynamics of *M* × *giganteus* were described in years 3 and 4 after establishment, but without data for older stands to determine how the N-dynamics change as stand age increases (Strullu *et al.*, 2011). Further, above-ground production in these previous trials in Europe were substantially less than reported for *M.* × *giganteus* in central Illinois, the first in the United States (Heaton *et al.*, 2008). Although climate differences between Europe and Illinois certainly play a role in the higher above-ground biomass yields in Illinois, another possible explanation of the higher yields in central Illinois could be a lower allocation of resources to roots and rhizomes. To date, there have been no measurements of seasonal below-ground biomass production in mature stands of *M.* × *giganteus* in North America. There have been studies of *P. virgatum* below-ground biomass across 5 years (McLaughlin & Kszos, 2005), 2–3 years (Bolinder *et al.*, 2002; Frank *et al.*, 2004), and at one point in time in mature stands (Ma *et al.*, 2000; Zan *et al.*, 2001). These showed a wide range of biomass values with substantial below-ground biomass in some cases. However, there have been no previous studies which determine the dynamics, based on sequential sampling through the growing season, of *P. virgatum* root and rhizome mass.

Another major environmental and economic advantage of late-fall or winter harvested *M.* × *giganteus* and *P. virgatum* is annual recycling of nutrients from the above-ground biomass to the rhizome system (Vogel *et al.*, 2002; Heaton *et al.*, 2009). This reduces, and in some cases eliminates, the need to use nitrogen and other fertilizers (Lewandowski *et al.*, 2003b; Christian *et al.*, 2008; Strullu *et al.*, 2011). In Illinois, December harvests have been recommended for *M.* × *giganteus* to provide an optimal balance between maximizing biomass yield while minimizing nutrient offtake and moisture content. The comparative seasonal dynamics of above-ground biomass and nitrogen content of *M.* × *giganteus* and *P. virgatum* have been characterized previously (Heaton *et al.*, 2009). However, this work did not include measurement of below-ground biomass and its nitrogen content which are needed to determine the whole-plant C and N budgets, and their partitioning, nor did it consider older stands (>4 years). In England, Beale & Long (1997) studied seasonal nitrogen, phosphorous, and potassium cycling in *M.* × *giganteus* and the C4 prairie grass *Spartina cynosuroides*. However, that study covered only one growing season in a much colder and less productive climate than that examined here and did not allow for determination of inter-annual variability (Beale & Long, 1997). Furthermore, to date,

there have been no side-by-side comparisons of below-ground biomass and nitrogen dynamics of *M.* × *giganteus* with *P. virgatum* in the peer-reviewed literature.

There has been considerable debate over the efficiency of terrestrial plants in converting incident solar energy into biomass energy and the ability of crop systems to even match natural vegetation in energy conversion. Although the theoretical conversion efficiency for C4 photosynthesis is ca. 6% (Zhu *et al.*, 2008, 2010) and realized efficiencies of ca. 3% have been observed (Piedade *et al.*, 1991; Beale & Long, 1995), others have questioned the ability of terrestrial crops to provide even an increase over the average conversion efficiency of all global terrestrial vegetation of just 0.1% (Hoffert *et al.*, 2002; Service, 2005; Pimentel & Patzek, 2006). Previously, *M.* × *giganteus* has been shown to achieve high conversion efficiency into shoot biomass. Since shoot biomass can result both from photosynthesis and translocation from the below-ground storage organs, it is necessary to measure above- and below-ground biomass changes to determine total photosynthetic efficiency.

The study established by Heaton *et al.* (2008) was the first replicated trial of *M.* × *giganteus* in the United States. Here, we extend on this earlier work to address four questions: (1) Are the initial high annual yields of *M.* × *giganteus* relative to *P. virgatum* maintained into the longer term (>5 years)? (2) Does below-ground production and biomass match the high above-ground production, or is it at the expense of below-ground production? (3) Are nitrogen levels in the crop maintained into the longer term, without fertilization and despite significant removal with each annual harvest? (4) What are the total net photosynthetic conversion efficiencies of *M.* × *giganteus* and *P. virgatum*?

Materials and methods

Site description

These field trials were on the South Farms, University of Illinois at Urbana-Champaign (40°03′21.3″N, 88°12′3.4″W, 230 m elevation). The soil is a Drummer-Flanagan soil series (fine-silty, mixed, mesic Typic Endoaquoll) typically very deep and formed from loess and silt parent material deposited on the glacial till and outwash plain. *Miscanthus* × *giganteus* Greef et Deu ex. Hodkinson et Renvoize, 'Illinois' clone and switchgrass (*P. virgatum* L. cv. Cave-in-Rock) were planted in 2002 into plots of 10 × 10 m within a completely randomized design (*n* = 4) as previously described in detail (Heaton *et al.*, 2008). 'Cave-in-Rock' is classified as an intermediate between the 'lowland' and 'upland' ecotypes of *P. virgatum*. It originates from Illinois, and has been a recommended cultivar for planting within the state (Vogel *et al.*, 2002; Heaton *et al.*, 2008). Initial planting density of *M.* × *giganteus* plantlets was

1 plant m^{-2}, and *P. virgatum* was direct seeded at a rate of at least 14 kg live seed ha^{-1}.

Biomass sampling

Above-ground sampling was conducted in the first week of June, August, October, and December 2006–2008 as described previously for sampling from these plots through to 2006 (Heaton *et al.*, 2008). Briefly, two 0.19 m^2 sub-plots were cut at ground-level, from randomly chosen locations within each full plot, weighed immediately to obtain fresh weights, oven-dried at 75°C to a constant mass, then dry weights were recorded and scaled up to predict dry matter per hectare. Litterfall was collected at the same time as above-ground biomass sampling from two litter traps of 0.35 m^2 in each full plot. These were placed in the plots early in the growing season, before being overtopped by the developing shoots. Procedures were, as described previously (Morgan *et al.*, 2005).

On the same day and in the same location within the full plot that above-ground samples were taken, below-ground biomass was sampled after the method of Roberts *et al.* (1993), but modified as follows. Soil cores were removed with a cylindrical metal soil corer of cross-sectional area 20 cm^2 to a depth of 25 cm. Three cores were taken along a transect across each of the two above-ground sampling sub-plots to account for spatial variability, leading to a total of six below-ground subsamples per full plot. For *M. × giganteus*, cores were taken at approximately the center of a plant, the edge of a plant and within the interplant space to ensure representative sampling of the stand. Since the *P. virgatum* was broadcast seeded, a linear transect of the above-ground sampling area was representative of the stand. In addition, a longer (1 m) core of the same cross-sectional area was taken with a motorized corer (Model GSPRS, Giddings Machine Co., Windsor, CO, USA) in the first week of April in 2007 and in 2008, just prior to shoot emergence. This coring was limited to the period following harvest and before regrowth, since at other time the equipment would have caused damage to the crop. These cores were partitioned into a 0–25 cm segment to match the sampling on other dates, and 25–100 cm to determine deep root biomass at this one time point. Cores were stored at 4°C until processed.

Root and rhizome extraction from the soil cores followed the method of Roberts *et al.* (1993). Briefly, cores were placed in root washing columns which were partially filled with water, and air was blown into the columns for 10–15 min to provide agitation which detached the roots and rhizomes from most of the soil. Water was then passed through the column and roots, rhizomes and organic matter allowed to overflow onto a 5 mm^2 mesh sieves. Once the majority of the roots and rhizomes had floated onto the sieve, the remainder of the water was poured through the sieve to capture any further root material. Water was sprayed onto the roots and rhizomes in the sieve to remove the bulk of the remaining soil particles, and rhizomes were then separated from roots. Although rhizomes were distinctive in *M. × giganteus*, in *P. virgatum* careful examination was needed to separate rhizomes from roots. Rhizomes, which are underground stems, were distinguishable from roots

by the presence of nodes. The separated roots and rhizomes were then dried to constant weight at 75°C.

Solar energy conversion efficiency was determined as described previously (Heaton *et al.*, 2008 and Dohleman *et al.*, 2009), but included shoot, root, and rhizome biomass accumulation. It was assumed that the energy content of all dry biomass was 18 MJ kg^{-1}, based on the measurements of Beale & Long (1995).

Nitrogen and carbon analysis

Above-ground sub-samples of the dried biomass were milled with a 2 mm sieve (Model 4 Wiley Mill; Thomas Scientific, Swedesboro, NJ, USA), and then a subsample of ~10 cm^3 was ground to a fine powder using a stainless steel pulverizer (Kleco Pulverizer; Kinetic Laboratory Equipment Company, Visalia, CA, USA). Below-ground samples were directly ground using the stainless steel pulverizer. C and N contents were determined in the resulting powder with a combustive elemental analyzer (Costech Analytical Technologies, Valencia, CA, USA), calibrated with an acetanilide standard.

Statistical analysis

In all cases, the replicated full plots were identified as the experimental unit, with this value being the mean of the two above-ground subsamples or six below-ground subsamples within a plot at a single timepoint, to avoid pseudoreplication. That is, the mean of these subsamples was treated as a single plot value for statistical analysis ($n = 4$). All biomass measurements were analyzed using a mixed-model repeated measures analysis of variance (PROC MIXED, SAS v9.1; SAS Institute, Cary, NC, USA), with date, species, and the date by species interaction as fixed effects, and year as a random effect. The best-fit covariance matrices were chosen for each variable using Akaike's information criterion to correct for inequality of variance between sampling dates (Keselman *et al.*, 1998; Littell *et al.*, 1998, 2000). To account for the inherent variability and minimize the risk of a type two error given the low sample size, statistical significance is reported at $\alpha = 0.1$. Least squared means are reported ±1 SE. To assess significance of variables between dates, pairwise comparisons were made using a mixed-model repeated measures ANOVA with date as a fixed effect and year as a random effect by species and using the PDIFF application of the least squared means (PROC MIXED; SAS Institute). To correct for the experiment-wide error rate associated with multiple pairwise comparisons, a Bonferroni correction was used in all cases.

Results

Climate conditions

The 2006 growing season was about the same temperature as, and 4% drier than, the 30-year average, 2007 was about 1°C warmer and 16% drier, and 2008 about 1°C cooler and 34% wetter (Fig. 1). Growing season
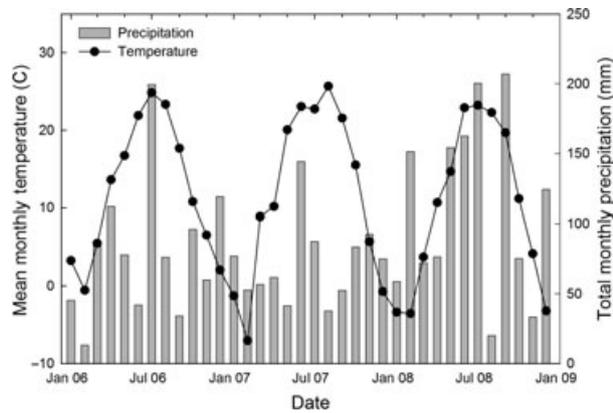
Fig. 1 Annual course of total precipitation and mean daily temperature over the 2006, 2007, and 2008 growing seasons. Data were collected daily from the monitoring stations of the Illinois Climate Network located at Urbana, IL as reported previously (Dohleman & Long, 2009; Dohleman *et al.*, 2009).

(April–October) incident solar radiation was about 4% above the 20-year average in 2006, 6% above in 2007, and 3% above in 2008 (Fig. 1; Table 1). The 3 years provided a useful contrast with one growing season that was near the long-term average in terms of temperature and precipitation, one that was warmer and drier than average and one that was wetter and cooler than average, suggesting that these data are representative of the likely range of climatic conditions at this location.

Table 1 Mean annual and April–October (growing season) climate data collected daily from the monitoring stations of the Illinois Climate Network located at Urbana, Illinois

Year	Solar radiation (MJ m^{-2})	Mean annual temperature (°C)	Precipitation (mm)
Annual			
2006	5215	12.2	962.2
2007	5696	12.1	862.1
2008	5512	10.2	1335.5
20/30-year average	5368[A]	11.1	1022.6
Growing season (April–October)			
2006	4266	18.4	637.3
2007	4341	19.8	508.3
2008	4199.2	17.8	895.9
20/30-year average	4083	18.4	667.3

30 years of precipitation and temperature (1979–2008) were used, but only 20 years of solar radiation was available (1989–2008) as reported previously in Dohleman & Long (2009) and Dohleman *et al.* (2009).

Above-ground, rhizome, and root biomass

On all dates, above-ground biomass of *M. × giganteus* was higher than that of *P. virgatum*, with a 3-year mean peak of 46.5 t ha^{-1} that declined to 38.1 t ha^{-1} by the completion of dry-down in December compared to the *P. virgatum* 3-year mean peak of 20.7 t ha^{-1} in October, when dry-down was complete for this crop (Fig. 2A) On average, across the 3 years, above-ground biomass of both species peaked in October. December figures are also given in parenthesis, since this is when crop dry down is complete for *M. × giganteus* and therefore when harvest is likely to occur. Mean above-ground biomass values were 39.0 (44.1), 52.2 (38.2), and 52.2 (36.3) t ha^{-1} in 2006, 2007, and 2008, respectively, in *M. × giganteus*. These were all significantly higher ($P < 0.0001$) than the respective October (December) values for *P. virgatum* of 15.6 (14.7), 21.8 (13.2), and 25.9 (10.5) t ha^{-1} for *P. virgatum*, in 2006, 2007, and 2008, respectively (Fig. 2A). There was no significant change in the above-ground biomass between years, for either species.

Rhizome biomass was also significantly higher in *M. × giganteus* than in *P. virgatum* across all dates (Fig. 2B; $P < 0.0001$). Averaged over all months and all years in this study, *M. × giganteus* rhizome biomass was 21.5 and 7.2 t ha^{-1} for *P. virgatum*, with an average [C] of 45.7% for both species. When averaged over the 3 years, *M. × giganteus* rhizome biomass dropped significantly from 24.1 t ha^{-1} in April to 11.8 t ha^{-1} in June, but then recovered back to 23.8 t ha^{-1} by August and maintained this mass of rhizome over the remainder of the year. *P. virgatum* rhizome biomass declined from 9.4 t ha^{-1} in April to 6.0 t ha^{-1} in June and recovered slightly up to 6.7 t ha^{-1} by August and 7.8 t ha^{-1} in October; however, none of these changes in *P. virgatum* were significant (Fig. 2B). There was no significant difference in rhizome biomass between years in either species.

Root biomass to a depth of 25 cm was not different between species over the duration of this experiment ($P = 0.28$), averaging 5.6 t ha^{-1} for *M. × giganteus* and 5.9 t ha^{-1} for *P. virgatum* (Fig 2C). Pairwise comparisons revealed that the root biomass did not change over time within or between years for either species. Average carbon concentrations of *M. × giganteus* and *P. virgatum* roots were 41% and 44%, respectively. In April of the 2007 and 2008 growing seasons, root biomass was collected to 1 m. The deep root biomass (25–100 cm) was also not significantly different between species when averaged across both years ($P = 0.21$), with an average of 3.8 t ha^{-1} for *M. × giganteus* and 4.5 t ha^{-1} for *P. virgatum*.

When averaged over the duration of the study, *P. virgatum* (root + rhizome) : shoot ratio was significantly higher than in *M. × giganteus* ($P < 0.0001$), but
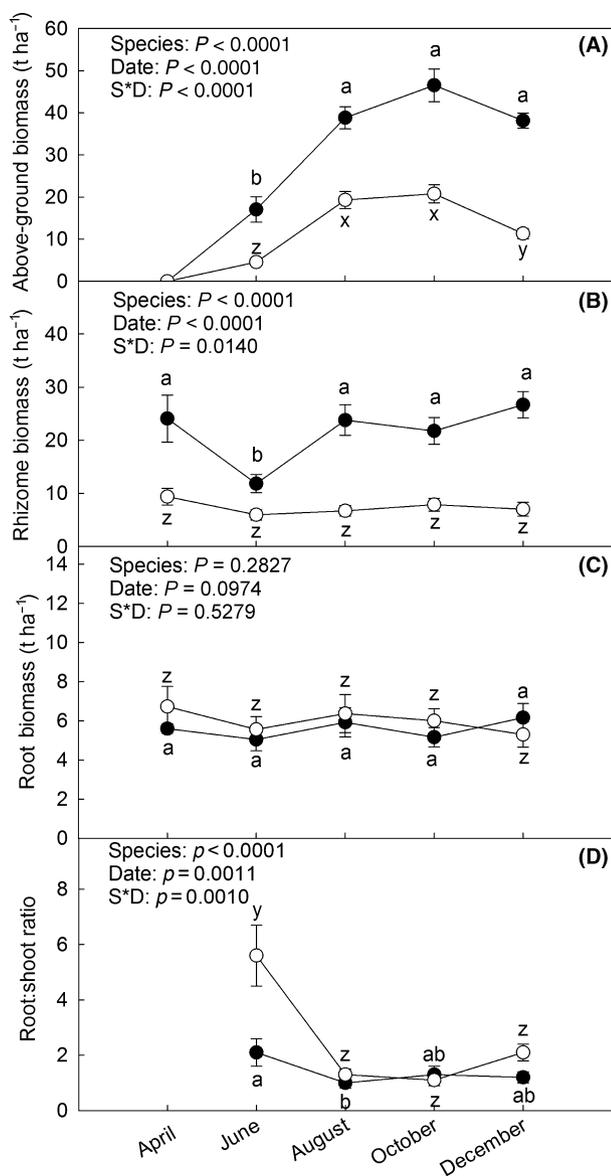
Nitrogen concentration and total plant nitrogen

Miscanthus × giganteus and *P. virgatum* did not differ in their above-ground [N] (*P* = 0.74). Both species showed a significant and similar decline over time (*P* < 0.0001) as shown by the insignificant species by time interaction (*P* = 0.62; Fig. 3A). *Miscanthus × giganteus* and *P. virgatum* [N] averaged 1.4% and 1.6%, respectively, in June, and both declined to 0.5% by December. Therefore, given the much larger quantity of biomass per unit land area for *M. × giganteus*, the absolute quantity of N would be similarly higher than in *P. virgatum* over the 3 years (*P* < 0.0001; Fig. 3B), with an average peak N content across the years of 339 kg ha^{-1} in August, declining to 193 kg ha^{-1} in December, 17 kg ha^{-1} of which was lost as leaf litter over that time. *P. virgatum* had a peak above-ground N content 168 kg ha^{-1} in August, declining to 58 kg ha^{-1} in December, 9 kg ha^{-1} of which was lost as leaf litter in that time. There was no discernable difference between years in above-ground [N] or shoot N per unit land area.
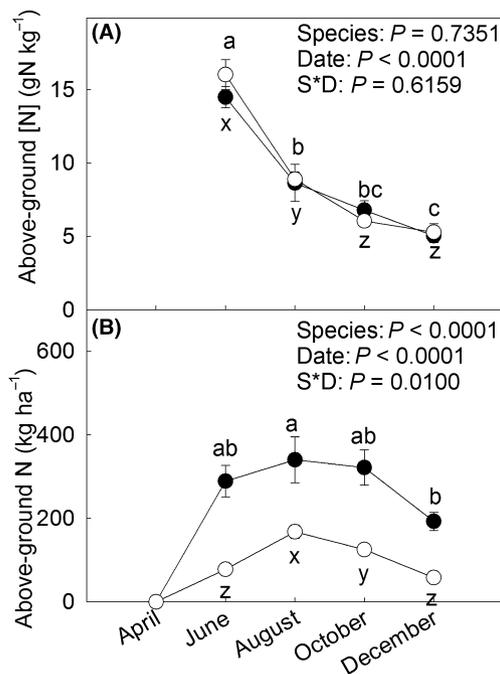
Fig. 2 (A) Above-ground, (B) rhizome, (C) root biomass, and (D) root : shoot biomass ratio averaged over the 2006–2008 growing seasons for *Miscanthus × giganteus* (●) and *Panicum virgatum* cv. Cave-in-Rock (○). Above-ground data for 2006 were presented previously (Heaton *et al.*, 2008), and were also included in this analysis for comparison with below-ground data. Points represent least squared means ± 1SE and *P*-values represent results from mixed-model repeated measures ANOVA with year as a random effect. Letters represent Bonferroni corrected pairwise comparisons across time separately within each species (a ,b for *M. × giganteus*, x, y, z for *P. virgatum*).

that was mostly due to the higher ratio in June for *P. virgatum* (Fig. 2D). *A posteriori* pairwise comparisons revealed that there was no difference between species in (root + rhizome) : shoot ratio in August or October (*P* = 0.68 and 0.7969, respectively).

Fig. 3 (A) N-concentration and (B) total above-ground N near Urbana, IL averaged over the 2006–2008 growing seasons for *Miscanthus × giganteus* (●) and *Panicum virgatum* cv. Cave-in-Rock (○). Points represent arithmetic means ± 1 SE and *P*-values represent results from mixed-model repeated measures ANOVA with species, date and species by date interaction as fixed effects and year as a random effect. Letters represent Bonferroni corrected pairwise comparisons across time separately within each species (a, b, c for *M. × giganteus*, x, y, z for *P. virgatum*).
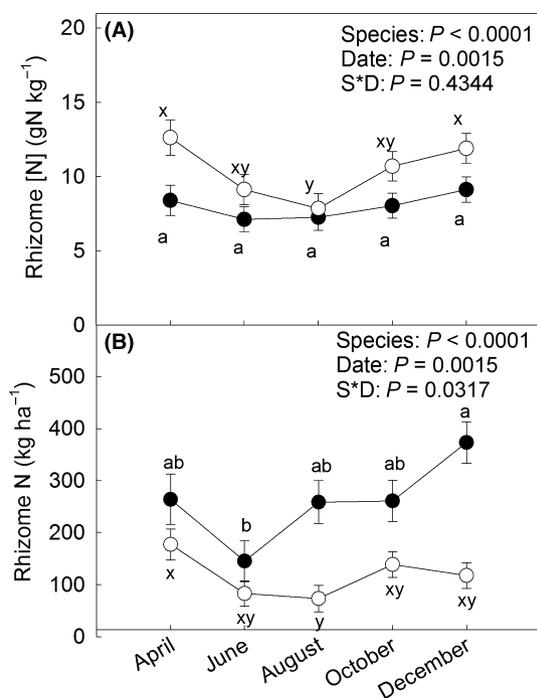
Fig. 4 Rhizome (A) N-concentration and (B) total N content at Urbana, IL averaged over the 2006–2008 growing seasons for *Miscanthus × giganteus* (●) and *Panicum virgatum* cv. Cave-in-Rock (○). Points represent arithmetic means ± 1 SE and *P*-values represent results from mixed-model repeated measures ANOVA with year as a random effect ($n = 4$). Letters represent Bonferroni corrected pairwise comparisons across time separately within each species (a, b for *M. × giganteus*, x, y for *P. virgatum*).

Rhizome [N] was significantly higher in *P. virgatum* compared to *M. × giganteus* over the duration of the experiment ($P < 0.0001$, Fig. 4A) and there was an effect of time of year ($P = 0.002$). *A posteriori* pairwise comparisons of rhizome [N] showed no change in *M. × giganteus* rhizome [N] with time of year ($P = 0.42$), but a change in *P. virgatum* rhizome [N] over time ($P = 0.01$). Average rhizome [N] for *M. × giganteus* was 0.79% compared to 1.03% for *P. virgatum*. Despite the lower [N], the much higher rhizome biomass resulted in the average total N stored in rhizome per unit land area being higher in *M. × giganteus* at 260 kg ha^{-1} compared to 114 kg ha^{-1} in *P. virgatum* ($P < 0.0001$; Fig. 4B). The amount of N in rhizomes per unit land area, paralleling changes in biomass, dropped from 264 kg ha^{-1} in April to 145 kg ha^{-1} in June, then increased to 373 kg ha^{-1} by December in *M. × giganteus*. The amount of N in *P. virgatum* rhizomes showed a slightly but nevertheless significantly different pattern ($P = 0.03$) with 177 kg ha^{-1} in April, dropping to 73 kg ha^{-1} by August, then rising back up to 139 kg ha^{-1} by October (Fig. 4B). The patterns shown over the average of all 3 years for

rhizome [N] and total N content did not appear to vary within any of the individual years.

Total plant biomass (root + rhizome + shoot) was significantly higher in *M. × giganteus* than *P. virgatum* over the duration of the experiment ($P < 0.0001$; Fig. 5A). When averaged over all three growing seasons, total plant biomass for *M. × giganteus* remained steady between April and June, but then increased dramatically from 43.9 t ha^{-1} in June to 72.1 t ha^{-1} in August and up again to 90.8 t ha^{-1} in October before declining to 81.4 t ha^{-1} in December. For *P. virgatum* a significantly different pattern occurred, with similar values between April and June, then increasing from 24.8 in June to 44.1 t ha^{-1} in August and remaining steady through October before declining slightly to 34.8 t ha^{-1} in December. Total plant N per unit land area was significantly higher in *M. × giganteus* compared to
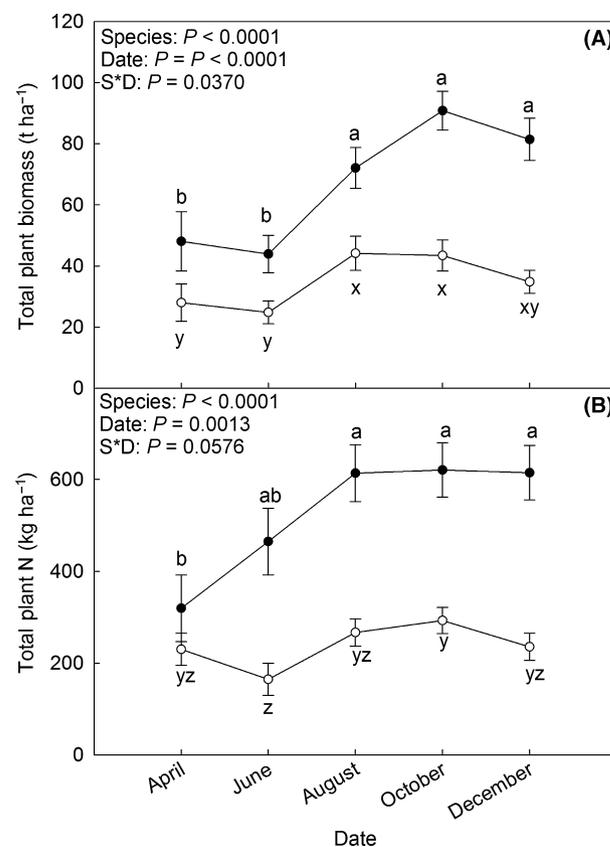

Fig. 5 (A) Total plant biomass (above-ground + rhizome + root) and (B) total plant N at Urbana, IL averaged over the 2006–2008 growing seasons for *Miscanthus × giganteus* (●) and *Panicum virgatum* cv. Cave-in-Rock (○). Points represent least squared means ± 1 SE and *P*-values represent results from mixed-model repeated measures ANOVA with year as a random effect. Letters represent Bonferroni corrected pairwise comparisons across time separately within each species (a, b for *M. × giganteus*, x, y for *P. virgatum*).

P. virgatum over the duration of the experiment ($P < 0.0001$; Fig. 5B). *Miscanthus* × *giganteus* total N content increased steadily from 320 kg ha^{-1} in April, all stored in roots and rhizomes, to 614 kg ha^{-1} in August, and did not change significantly after that date. *Panicum virgatum* total plant N content was lowest in June at 165 kg ha^{-1}; however, this was not significantly different from April, increasing to 293 kg ha^{-1} by October.

Solar energy conversion efficiency

Averaged over the 3 years, the ratio of annual peak biomass to the integral of solar radiation (Table 1) yielded an efficiency of 1.5%, rising to 2.0% if based on solar radiation receipt for the growing season alone in *M.* × *giganteus*. The equivalent numbers for *P. virgatum* were 0.7% and 0.9%. During the 2-month period from June-August, the increase in shoot and rhizome biomass of 33.6 t ha^{-1} for *M.* × *giganteus* highlights the remarkable productivity potential of this plant, corresponding to an average solar energy conversion of 4.4% of full spectrum solar radiation into biomass, or in terms of dry mass, 2.4 g MJ^{-1} of full spectrum radiation.

Discussion

The objectives of this study were to address four questions, these are now discussed in turn.

(1) *Are the initial high annual yields of M. × giganteus relative to P. virgatum maintained into the longer term?*

Heaton *et al.* (2008) reported above-ground end-of-season dry biomass recorded for this site as 25.1, 31.1, and 44.1 t ha^{-1} for 2004–2006 for *M.* × *giganteus*. In this study, these high yields are shown to continue at 38.2 and 36.3 t ha^{-1} for 2007–2008, with no significant difference between 2006, 2007, or 2008. These yields continue to be at the upper end of the 10–40 t ha^{-1} range of European studies (Lewandowski *et al.*, 2003b; Miguez *et al.*, 2009), likely due to the longer, wetter, and warmer growing season than at most European sites. Yields also remained around double those of *P. virgatum* (Fig. 2A). Mean October *P. virgatum* biomass production of 20.8 t ha^{-1} exceeded the range of 11–16 t ha^{-1} observed for the same location in earlier years (Heaton *et al.*, 2008). The cultivar used, cave-in-rock, is an upland-lowland intermediate which is native to Illinois and was selected for this trial due to its local adaptation (Heaton *et al.*, 2008). Biomass productivities reported here are at the high end of the range of 6–22 t ha^{-1} for varieties presented by McLaughlin & Kszos (2005), where many of the trials were of lowland varieties growing in the longer growing seasons of the southern United States. The yields are also higher than the range of 7–13 t ha^{-1} reported across 20 cultivars under similar climate conditions in the Midwestern United States (Lemus *et al.*, 2002) and are above the range of 3–18 t ha^{-1} reported for the upper Midwest (Casler & Boe, 2003). Despite the high *P. virgatum* yields in this study, *M.* × *giganteus* were still two to three times higher. Kiniry *et al.* (2011) found that yields of *M.* × *giganteus* were substantially higher than three *P. virgatum* cultivars, including Cave-in-Rock, in the Midwestern United States. However, in the hotter, drier climate of central Texas, USA, the *P. virgatum* cultivar 'Alamo' was more productive than *M.* × *giganteus*, particularly under nonirrigated conditions. Within this study, there was no discernable decline in annual yield for either species in these later years, relative to the yields reported by Heaton *et al.* (2008), however, continued measurements are necessary to determine whether there will be a decline in productivity at some stage in the longer term.

(2) *Does below-ground production and biomass match the high above-ground production?*

The average total below-ground biomass of 27.1 t ha^{-1} for *M.* × *giganteus* between 0 and 25 cm in depth was shown to be more than double the 13.1 t ha^{-1} of *P. virgatum* for the top 25 cm of soil, with the entirety of that difference being due to the higher rhizome biomass in *M.* × *giganteus* as there was no significant difference in root biomass between species. *Miscanthus* × *giganteus* below-ground biomass reported here (Figs 2B,C and 3) is higher than all previous studies of mature stands in European trials (Table 2). The higher below-ground biomass at this location parallels the higher above-ground biomass, consistently peaking over 40 t ha^{-1} at this location (Fig. 2A), compared to those in the European studies. Since 2006 was the fifth year after planting, root, and rhizome production, must have averaged a minimum of 10 t ha^{-1} yr^{-1} simply to reach this mass. There was no significant change over the 3 years of this study suggesting that death of old roots and rhizomes must have offset production of new.

The pattern of below-ground biomass accumulation in *M.* × *giganteus*, with low biomass in the mid-summer and increasing as senescence occurs is also similar to that reported in southern England (Beale & Long, 1995). They assumed that this pattern reflected a mobilization of resources from the rhizomes to the emerging shoot, and subsequent replenishment of the rhizome in the late summer and fall. Averaged over 2006–2008, rhizome biomass declined by 12 t ha^{-1} between April and June, and shoot biomass increased by 17 t ha^{-1}, suggesting that much of this early growth could be the result of remobilization from the rhizomes, but not roots; given no significant change in root biomass. Beyond June, the rhizome biomass lost in early growth is recovered and shoot biomass also increased by 22 t ha^{-1}. The June–

Table 2 Below-ground dry biomass reported in the peer-reviewed literature, together with latitude and citation for (a) *Miscanthus × giganteus* and (b) *Panicum virgatum*

Latitude (°N)	Below-ground biomass (t ha^{-1})	References
(a)		
40	27.1	Present study
41–45	7.6[r]	Monti & Zatta (2009)
46–50	16.1–21.0	Strullu *et al.* (2011), Amougou *et al.* (2010), Kahle *et al.* (2001)
51–55	12.0[r]–27.0	Clifton-Brown *et al.* (2007), Himken *et al.* (1997), Neukirchen *et al.* (1999), Kahle *et al.* (2001)
56–60	14.1–16.3	Hansen *et al.* (2004)
(b)		
40	13.1	Present study
30–35	10.9–17.8	McLaughlin & Kszos (2005), Ma *et al.* (2000), Garten *et al.* (2011, 2010)
36–40	1.57[r]–11.9	McLaughlin & Kszos (2005), Xu *et al.* (2010)
41–45	2.5–17.1[r]	Bolinder *et al.* (2002), Zan *et al.* (2001), Heggenstaller *et al.* (2009), Monti & Zatta (2009), Collins *et al.* (2010)
46–50	6.7[r]	Frank *et al.* (2004)

[r]Only roots were sampled.
Biomass values have varying sample depths.

August increase in rhizome biomass of 11.8 t ha^{-1}, coincided with a 21.8 t ha^{-1} increase in shoot mass (Fig. 2), a combined increase of 33.6 t ha^{-1} over just 2 months.

A similar early season decline in rhizome biomass followed by a recovery in the summer was indicated for *P. virgatum*, however, the changes were not significant (Fig. 2B). In parallel with the above-ground biomass, mean *P. virgatum* below-ground biomass is near the top of the range reported previously in the literature (Table 2). The root–shoot ratios shown in Fig. 2D suggest that *M. × giganteus* is more effective at using its below-ground energy stores to promote above-ground growth early in the spring, however, at peak biomass, the root : shoot ratios are not different between species suggesting that *M. × giganteus* and *P. virgatum* both partition assimilate between above- and below-ground organs in similar proportions, overall. Furthermore, this indicates that both species could provide substantial stores of carbon within the below-ground biomass. At peak biomass, the present analysis only considers roots and rhizomes at 25 cm depth; however, root biomass to 100 cm at the beginning of each growing season was not significantly different between species suggesting that root : shoot ratios would not change when deep roots are included. The biomass between 0 and 25 cm represented 88% of the biomass in the top 100 cm of soil for *M. × giganteus* and 74% in *P. virgatum*. No rhizomes of either species were found below 25 cm.

(3) *Are nitrogen levels in the crop maintained into the longer term, without fertilization and despite significant removal with each annual harvest?*

The end-of-season above-ground [N] of 0.5% reported here for *M. × giganteus* is very similar to that reported in southern England (Beale & Long, 1997), and within the range of 0.19–0.67% reviewed by Lewandowski *et al.* (2003b) but higher than those in other studies (Lewandowski *et al.*, 2003a; Heaton *et al.*, 2009). The *P. virgatum* [N] of 0.5% is also similar to the values reported by Lemus *et al.* (2002) and slightly lower than the range of 0.7–1.4% reviewed by Lewandowski *et al.* (2003b).

If the crop was harvested in December then, based on Fig. 3, it would result in a very substantial N removal of 193 kg ha^{-1} for *M. × giganteus* compared to 58 kg ha^{-1} for *P. virgatum*. This is very much less than the amount that would be removed at the peak above-ground N content of 340 kg ha^{-1} in *M. × giganteus*. This decline of 147 kg ha^{-1} in above-ground N between October and December is matched closely by the sum of the 113 kg ha^{-1} increase in N in rhizomes N and 17 kg [N] ha^{-1} in leaf litter, suggesting that the majority of N lost from the above-ground biomass is directly translocated to the rhizome system at senescence. Beale & Long (1997) reported a drop of 156 kg [N] ha^{-1} in *M. × giganteus* from 253 kg [N] ha^{-1} in above-ground biomass at peak to 97 kg [N] ha^{-1} in February, with N concentrations quite similar to those reported in this study, and lower above-ground biomass. Heaton *et al.* (2009), however, reported a more substantial drop in both biomass and [N] between mid-summer and end-of-season at the same location, suggesting that *M. × giganteus* has an average translocation of 264 kg [N] ha^{-1}, and an average N-removal rate of only 69 kg [N] ha^{-1} over the 2004 and 2005 growing seasons in December.

The 60% biomass drop between peak biomass and December of 2004 in the Heaton *et al.* (2008) study is more than double the expected drop from many other studies as reviewed by Lewandowski *et al.* (2003b), and average end-of-season N concentrations of *M. × giganteus* biomass of 0.25% in Heaton *et al.* (2009) is half of that reported here and at the low end of the range as reviewed by Lewandowski *et al.* (2003b). The lower value of the Heaton *et al.* (2009) study possibly reflects the early stage of the stands examined. Here, in years 5–7 after planting, total N in the plant is over 600 kg ha^{-1}, despite the fact no fertilizer was applied. This is all the more remarkable, given that the December contents suggest that almost 200 kg [N] ha^{-1} could be lost from these stands each winter in the harvest.

In *P. virgatum*, the peak N content was 168 kg [N] ha^{-1}, suggesting that 110 kg [N] ha^{-1} is remobilized between peak biomass and senescence, 9 kg [N] ha^{-1} of which was accounted for as litterfall. The average N removal of 58 kg [N] ha^{-1} falls within the range of 31–63 kg [N] ha^{-1} reviewed by Lewandowski *et al.* (2003b), and is similar to the average of 48 kg [N] ha^{-1} reported in 2004 and 2005 at the same location as this study (Heaton *et al.*, 2009).

How is it possible for these crops continue to produce high yields with this amount of nitrogen removed from the system annually? At the end of this study, these plots had gone through seven complete growing seasons with no addition of fertilizer after the establishment year (Heaton *et al.*, 2008) and have shown no decline in above-ground biomass yield over time. *Miscanthus × giganteus* productivity is not expected to respond to increasing N fertilization (Himken *et al.*, 1997; Lewandowski *et al.*, 2003b; Heaton *et al.*, 2004; Christian *et al.*, 2008). However, in the 14-year study of this crop at Rothamsted, England, the maximum amount of N removed in any annual harvest was 100 kg ha^{-1} (Christian *et al.*, 2008), about half the value observed here. Total atmospheric N deposition ($NH_4 + NO_3$ + inorganic N) as recorded ~10 km from the field plots used here is on average 22 kg [N] ha^{-1} yr^{-1} (Illinois State Water Survey, 2009), which could replace, on average, 38% of the N removed in harvesting *P. virgatum* at this site and 11% of the N removed in harvesting *M. × giganteus*. The data presented here combined with the Heaton *et al.* (2009) study suggests that a total of 714 kg [N] ha^{-1} have been removed in *M. × giganteus* harvests and 270 kg [N]ha^{-1} have been removed in *P. virgatum* harvests over the duration of the field trial. Assuming that 154 kg [N] ha^{-1} has been added via atmospheric deposition over this period, then by difference *M. × giganteus* would have had to assimilate 560 kg [N] ha^{-1} from the soil and *P. virgatum* 124 kg [N] ha^{-1}. Soil N-mineralization

rates on unfertilized *Zea mays* near the location of this study have previously been shown to average 86 kg [N] ha^{-1} yr^{-1} (Gentry *et al.*, 2001). If this rate was maintained over 7 years, it would be sufficient to meet the deficit. However, since this rate is for a single year after conventional cropping it seems unlikely that it could be maintained for 7 years. Free-living nitrogen fixers have been shown previously in various *Miscanthus* species (Kirchhof *et al.*, 1997, 2001; Eckert *et al.*, 2001) and have recently been characterized and appeared to be functioning in the *M. × giganteus* clone used in trials at this location (Davis *et al.*,2010). The data provided here suggest a missing source of N into this *M. × giganteus* cropping system which might, at least partially, be explained by N-fixing associations in the *M. × giganteus* rhizosphere.

(4) *Determine the total net photosynthetic conversion efficiency of these crops*

Based on the records of solar energy receipt and accumulation of biomass, energy conversion efficiencies may be calculated. These assume a biomass-energy content of 18 GJ t^{-1} (Beale & Long, 1995). Averaged over the 3 years, the ratio of annual peak biomass to the integral of solar radiation (Table 1) yields an efficiency of 1.5%, rising to 2.0% if based on solar radiation receipt for the growing season alone in *M. × giganteus*. The equivalent numbers for *P. virgatum* are 0.7% and 0.9%. Between April and June, increase in shoot biomass of *M × giganteus* is offset by a similar decrease in rhizome biomass, suggesting that during this initial phase the shoots are growing primarily as a result of exports from the rhizomes. However, between June and August both rhizome and shoot mass increase greatly. When these are summed, it shows an efficiency of conversion of incident solar radiation into biomass of 4.4% or 8.8% in terms of photosynthetically active radiation (PAR), assuming 50% of incoming radiation is PAR. This is almost three-quarters of the theoretical maximum conversion efficiency of C4 photosynthesis (Zhu *et al.*, 2010). This not only shows the exceptional capacity of *M. × giganteus* as a bioenergy crop, but also shows the need to understand how this plant achieves these high efficiencies so that these traits might be selected for and transferred to other crops (Wang *et al.*, 2008; Dohleman & Long, 2009).

Conclusion

In total, this study shows that the high initial annual yields of these first replicated trials of *M. × giganteus* in the United States are sustained beyond 5 years, providing over 35 t ha^{-1} in each of years 5–7. Analysis of below-ground biomass dynamics suggests that while shoot production is at the expense of rhizome mass in

the spring, this is recovered by late summer. Between June and August, the crop converts the available solar radiation into biomass energy at an efficiency of 4.4%, one of the highest reported for terrestrial plants and three-quarters of the theoretical conversion efficiency for C4 photosynthesis. The Midwestern US climate appears to be near optimal for *M. × giganteus* productivity, and lower productivities have been shown at higher latitudes and under water-limited conditions. This study also provides the first side-by-side comparison of below-ground biomass dynamics in mature stands of *M. × giganteus* and *P. virgatum* and shows *M. × giganteus* stores more than double the amount of carbon in its below-ground organs compared to *P. virgatum* when averaged over three growing seasons, indicating that *M × giganteus* could provide a large sink for CO_2 if planted at large scale. Once *M. × giganteus* and *P. virgatum* stands reach maturity, there does not appear to be a trend toward increasing below-ground biomass in either species. Remarkably, even after the removal of 714 kg [N] ha^{-1} over the lifetime of these *M. × giganteus* stands, there is no decline in above-ground productivity despite the fact no fertilizer has been added since the establishment year.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (a) Above-ground, (b) rhizome, and (c) root biomass over 2006–2008 growing seasons for *Miscanthus × giganteus* (●) and *Panicum virgatum* cv. Cave-in-Rock (○). Above-ground data for 2006 was presented previously (Heaton *et al*., 2008), and was also included in this analysis for comparison with below-ground data. Points represent least squared means ± 1 SE and p-values represent results from mixed model repeated measures ANOVA with year as a random effect.

Figure S2. (a) N-concentration and (b) total above-ground N near Urbana, IL over the 2006–2008 growing seasons for *Miscanthus × giganteus* (●) and *Panicum virgatum* cv. Cave-in-Rock (○). Points represent arithmetic means ± 1 SE.

Figure S3. Rhizome (a) N-concentration and (b) total N content at Urbana, IL over the 2006–2008 growing seasons for *Miscanthus × giganteus* (●) and *Panicum virgatum* cv. Cave-in-Rock (○). Points represent arithmetic means ± 1SE.

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