Soil nutrient removal by four potential bioenergy crops: *Zea mays*, *Panicum virgatum*, *Miscanthus × giganteus*, and prairie

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ABSTRACT

Replacing annual row crops with perennial grasses for bioenergy represents a landscape-level change in species composition, with the potential to impact annual soil nutrient removal on a regional scale. In this study we measured the concentration of ten essential nutrients in harvested material from three potential perennial bioenergy crops: *Panicum virgatum* L., *Miscanthus × giganteus*, and a reestablished prairie to determine annual soil nutrient removals. We compared perennial bioenergy crops to nutrient removals by annual cropping systems of *Zea mays* L. (maize) and *Glycine max* L. (soybean) in Illinois. Crops were grown under management practices typical for the Midwest, US. In addition, we examined geographic variation in nutrient removal of *M. × giganteus* at four US locations. Total removal of N, P, K, Ca, Mg, S, Fe, Mn, Na, and Zn was significantly greater in maize than in any of the perennials. Removal of N, P, and K in *M. × giganteus* was 3.7, 1.8, and 1.8% of the removal in maize, and 49.0, 17.4, and 31.9% of the removal in soybean respectively. At sites in Illinois, Kentucky, Nebraska, and New Jersey we found differences in N and K removal by M. × giganteus that corresponded with differences in biomass. There was no effect of fertilization on *M. × giganteus* biomass, but removal of N, S, and Mg increased and P removal decreased with increasing rates of urea fertilization. Cultivation of *M. × giganteus* and switchgrass on land formerly used for row crops may reduce the need for nutrient additions and potential losses of nutrients to groundwater and the atmosphere.

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1. Introduction

Considerable land area will be required to meet growing demand for bioenergy, particularly with the emergence of second-generation bioenergy crops harvested for ligno-cellulose (Somerville et al., 2010). Annual maize and soybean cropping systems in the United States are expansive, covering ∼70 million hectares (USDA, 2012). Expanding bioenergy crops to marginal lands shows potential, but it seems likely that some acreage currently allocated to grain and bean production will be converted to perennial grasses (Davis et al., 2012) to meet the 60.5 billion liters of bioethanol from cellulosic sources stipulated in the 2007 Department of Energy (DOE) mandate (US Senate, 2007). Direct replacement of fossil fuel

with biomass combustion for electricity also appears plausible (Heaton et al., 2004; Lewandowski and Kicherer, 1997). Should one or both of these energy production systems expand without a change in current crop production, the amount of land currently used for fuel production would undoubtedly increase. Although the ultimate fate, direction, and magnitude of this promising source of energy are unknown, this potential regional scale land-use change raises many ecological questions.

The replacement of annual row crops with perennial grass bioenergy crops is occurring globally in temperate, tropical, and semi arid regions (Somerville et al., 2010). In areas of England and Ireland, annual yields of $20\,Mg\,ha^{-1}$ are common for *Miscanthus × giganteus*, and this crop is now widespread across regions formerly sown with wheat (Lovett et al., 2014). Agroecological zoning created by the Brazilian government has recently identified millions of hectares for sugarcane expansion, a significant portion of which is currently growing maize and soybean (Diário Oficial União Brazil, 2009). Campbell et al. (2008) report 385–472 million

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hectares of abandoned agricultural land globally, much of which is semi arid. Davis et al. (2011, 2014) suggested that *Agave* species currently grown in these regions for fiber or alcoholic beverages could be expanded into biofuel production. Although thought to be beneficial (Anderson-Teixeira et al., 2009), the impact of these expanding land-use changes on the soil is still relatively unknown and will likely vary with climate and soil type.

Without soil amendments, typical row crop agriculture results in a net removal of nutrients from the soil (Heckman et al., 2003; Wilhelm et al., 2004). Harvest removes plant material rich in nutrients to be consumed by animals or humans in another location. This annual removal of nutrients can be compensated for by applications of fertilizers, commonly applied as anhydrous ammonia, urea, urea ammonium nitrate solutions (UAN), diammonium phosphate (DAP), potash, lime, manure, or compost, as well as associated N fixation (Egli, 2008).

Fertilizer manufacturing and application to row crops has several adverse environmental consequences. Production of N fertilizers from the Haber-Bosch process is energy intensive and its production contributes significantly to atmospheric CO_2 (Jensen and Hauggaard-Neilsen, 2003). In addition, denitrification of excess N in the soils used for row crop agriculture is a major contributor of the greenhouse gas nitrous oxide (N_2O) to the atmosphere (IPCC, 2007; Ma et al., 2010). Nitrate leaching from nonpoint sources, including modern agriculture, in the Midwestern United States (McIsaac et al., 2001, 2002) is responsible for 80% of the 1.2 billion kg of N that reach the Gulf of Mexico on average each year, most of it from tile-drained maize and soybean fields (David et al., 2010).

Conversion from traditional row crops to perennial grasses has the potential to reduce many negative environmental consequences associated with N fertilization. For example, it appears that *Miscanthus × giganteus* Greef & Deuter ex Hodkinson & Renvoize, a promising energy crop, requires minimal N fertilization on fertile soils, if any at all. A number of trials have shown no significant growth response of *M. × giganteus* to N-fertilization, even after 10 years (Beale and Long, 1997; Christian et al., 2008; Cadoux et al., 2012), while others have documented variable positive yield responses (Boehmel et al., 2008; Lewandowski and Schmidt 2006). Nitrate leaching under developing perennial grasses drops dramatically as root systems established during the first three years of growth capture mineralized soil N or nitrogenous amendments (Smith et al., 2013; McIsaac et al., 2010). Perennial bioenergy grasses also are highly efficient at retranslocating N, P, and K from foliage to rhizomes during autumn senescence, which can explain vigorous early spring growth as well as extraordinarily low concentrations of these nutrients in senesced tissue (Beale and Long, 1997; Smith et al., 2013). The larger, deeper rooting systems of perennial grasses and reduction of tillage practices in annual agro-ecosystems have the potential to build soil C and N pools (Anderson-Teixeira et al., 2009; Anderson-Teixeira et al., 2013), providing additional ecological benefits in addition to the displacement of fossil fuels.

The focus of early ecological research in potential perennial grass bioenergy systems has been on N, C, and water, but little is known about the dynamics of other nutrients. When thought to be limiting, P, K, and S are commonly applied as fertilizers in agricultural systems to improve yields (Reddy et al., 2015). The concentrations and ratios of Ca and Mg are particularly significant in acidic soils, where binding to soil minerals can lead to plant deficiencies (Baligar et al., 1997). Soils high in Fe have been shown to limit the uptake of Mn, an important nutrient for enzymatic functions (Bansal et al., 1999). Maize and soybean exhibit sensitivity to deficits in Zn (Mengel and Kirkby, 1982), which can be caused by high soil P and high P fertilization (Shuman, 1980), while imbalances of Na can strongly influence plant biomass for all crops (Ghafoor et al., 2004).

The primary objective of this research was to compare the amount of these ten macro and micronutrients critical to plant growth and development, which are removed via harvest each year, by three potential perennial grass bioenergy treatments in a side-by-side comparison with current row crop annuals. To accurately represent nutrient removals all crops were managed under typical or best-known agricultural management for this region, which varied between crops. Intensive breeding programs and increasing fertilizer application have resulted in greater maize yields and correspondingly elevated removal of soil nutrients (Heckman et al., 2003; Chatterjee, 2013). In this study, we test the paradigm that high yields in agricultural systems necessarily remove large quantities of macro and micronutrients from the soil. We hypothesize that the removal of nutrients by annual row crops, even during instances of lower biomass, will be greater than the removal of nutrients by perennial grass bioenergy crops. To asses nutrient removal from *M. × giganteus* in different soil and climate regimes chosen by the Sun Grant/U.S. Department of Energy Regional Biomass Feedstock Partnership to geographically expand *M. × giganteus* production in the U.S. (Maughan et al., 2012), we also analyze harvested material from four sites in Nebraska, Kentucky, New Jersey, and Illinois. We predict differences in nutrient removal to be site specific, and largely dependent upon the soil fertility and chemistry of the site.

2. Materials and methods

2.1. Site description, University of Illinois Energy Farm

A side-by-side comparison of nutrient removal during harvest of four bioenergy crops was conducted at the University of Illinois Energy Farm located in Urbana, IL USA (40°3′46″N, 88°11′46″W, ~220 m above sea level). Mean annual temperature at this location is 11.1 °C, highly seasonal, and with annual mean precipitation of 1042 mm (Illinois State Water Survey, averaged from 1979 to 2009). Monthly weather data for the duration of this experiment (2008–2010) can be found in Maughan et al. (2012). Soils are Argiudolls, predominately Dana silt loam (fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls), with some Flannagan silt loam (fine smectitic, mesic Aquic Arguidolls) and Blackberry silt loam (fine-silty, mixed, superactive, mesic Oxyaquic Argiudolls). All soil series are deep and formed in loess. Dana and Blackberry are moderately well drained and Flanagan is somewhat poorly drained. Land was planted in annual row crops for more than 100 years prior to planting with bioenergy crops.

Crops were planted in Spring 2008 in a blocked experimental design with four blocks of 0.7 ha plots and a fifth of 3.8 ha plots (Fig. 1). Pre-planting soil data (C, N, pH, and bulk density) were collected for the 0–10 and 10–30 cm depth on all plots (Table 1). One treatment was planted with a maize-maize-soybean rotation (*Zea mays* L., *Glycine max* L.), typical of this region. Three additional treatments were planted with potential bioenergy perennial crops: *M. × giganteus*, a hybrid grass of Asian origin; switchgrass (*Panicum virgatum* L.; 'Cave In Rock'), a grass native to the Midwestern US; and restored prairie, consisting of 28 native species of grasses, forbs, and a sedge including several N fixers (see Zeri et al., (2011) for species composition). The dominant species in this prairie polyculture varied among years but were generally big bluestem (*Andropogon gerardii* Vitman), Indiangrass (*Sorghastrum nutans* L. Nash), yellow coneflower (*Ratibida pinnata* Vent. Barnhart), and sawtooth sunflower (*Helianthus grosseserratus* M. Martens) (Feng and Dietze, 2013). Row spacing of annuals and *M. × giganteus* rhizomes was 75 cm, while prairie and switchgrass treatments were broadcast seeded.

Each of these crops is a current or potentially important agricultural commodity and was planted and managed according
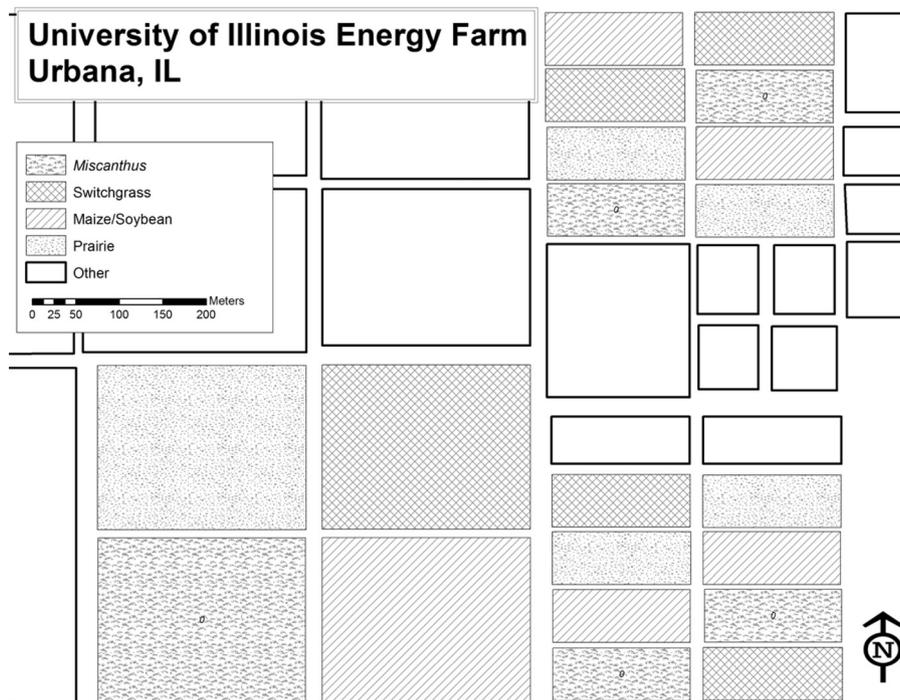
Fig. 1. Map of the University of Illinois Energy Farm. Replicated side-by-side plantings of a maize-maize-soybean rotation and three potential perennial bioenergy crops, $M. \times giganteus$, switchgrass, and a native prairie. Small plots are 0.7 ha, and large plots are 3.8 ha.

to the best-known agricultural practice for this region. This design enabled realistic comparisons among crops as they would actually be farmed, as opposed to using identical plot management, which would give unrealistic estimates of nutrient removal by each crop. Annual row crops were planted in May of each year; maize (DK61-69) was planted in 2008 and 2009, and soybean (Asgrow 3431) in 2010. Switchgrass and prairie establishment were successful, however initial establishment of $M. \times giganteus$ was poor, resulting in lower biomass than previously established neighboring plots (Heaton et al., 2008). Additional $M. \times giganteus$ was planted in 2009 and 2010, depending on previous establishment success described in Smith et al. (2013). Nitrogen fertilizer as 28% UAN was applied to maize at 168 kg N ha^{-1} and 202 kg N ha^{-1} prior to planting in 2008 and 2009, respectively. Switchgrass responds to fertilizer and will likely be managed in the Midwestern United States with annual fertilizer to maximize biomass (Lemus et al., 2008). Granular urea was applied to switchgrass at the Energy Farm at 56 kg N ha^{-1} in 2010. At the time of this experiment trials of $M. \times giganteus$ in Illinois had shown no significant growth response to N fertilization, particularly when soil N is high prior to planting (Maughan et al., 2012; Arundale et al., 2014; Christian et al., 2008; Cadoux et al., 2012), and $M. \times giganteus$ was not fertilized at the Energy Farm. Soybean and prairie also were also not fertilized, due to ongoing N fixation in these treatments.

2.2. Site description, Sun Grant/DOE fertilization trials

$M. \times giganteus$ was established in 2008 at DOE-funded research sites in Nebraska (Mead, NE, 41°10′07″ N, 96°28′10″ W), Illinois (Urbana, IL, 40°06′20″ N, 88°19′18″ W), Kentucky (Lexington, KY, 38°07′45″ N, 84°30′08″ W), and New Jersey (Adelphia, NJ, 40°13′31″ N, 74°14′54″ W) to examine geographic variation in yield (referred to hereafter as Sun Grant/DOE plots). Despite relatively similar latitude between sites, precipitation and soils varied widely among sites. Thirty year mean annual precipitation data was 704, 1043, 1166, and 1211 mm for NE, IL, KY, and NJ respectively (Maughan et al., 2012). Surface (0–10 cm) soils ranged

from 1.9% organic matter in Illinois to 5.1% in Nebraska (Maughan et al., 2012; Appendix 1, Table 1). Soil nutrients followed the same pattern: sites in Nebraska and Kentucky were more fertile than Illinois and New Jersey. Additional Sun Grant/DOE site establishment details, soil information, and plot descriptions are available in Maughan et al. (2012) and Appendix 1. At each location, twelve 10 m × 10 m plots of $M. \times giganteus$ were planted and four replicates were fertilized with urea at rates of 0, 60, and 120 kg N ha^{-1} in a randomized complete block design.

2.3. Aboveground biomass

At the Energy Farm aboveground tissues were harvested by hand twice in late summer (August and September) each year near the time of maximum aboveground biomass. We assumed that

Table 1
Pre-planting soil data collected in spring of 2008 at the University of Illinois Energy Farm, Urbana, IL, and $M. \times giganteus$ plots in Nebraska, Illinois, Kentucky, and New Jersey. Energy Farm pH values are from samples taken prior to variable rate liming of all plots to achieve a pH of 6.0.

Location	Crop	Depth (cm)	C (%)	N (%)	pH	Bulk density (g cm^{-3})
Energy Farm	Maize/Maize/Soybean	0–10	1.63	0.14	5.15	1.37
		10–30	1.54	0.13	5.05	1.47
Energy Farm	$M. \times giganteus$	0–10	1.70	0.15	5.25	1.30
		10–30	1.55	0.13	5.16	1.40
Energy Farm	Prairie	0–10	1.78	0.15	5.33	1.34
		10–30	1.72	0.15	5.08	1.48
Energy Farm	Switchgrass	0–10	1.74	0.15	5.44	1.23
		10–30	1.62	0.14	5.07	1.35
Sun Grant/DOE–Nebraska	$M. \times giganteus$	0–10	2.99	0.33	6.1	1.20
		10–30	1.84	0.22	6.7	1.40
Sun Grant/DOE–Illinois	$M. \times giganteus$	0–10	1.14	0.11	5.7	1.44
		10–30	1.10	0.11	5.9	1.63
Sun Grant/DOE–Kentucky	$M. \times giganteus$	0–10	2.49	0.31	5.1	1.38
		10–30	1.36	0.18	5.9	1.57
Sun Grant/DOE–New Jersy	$M. \times giganteus$	0–10	1.23	0.11	5.3	1.27
		10–30	1.19	0.12	5.5	1.54

maximum aboveground biomass occurred near the time of maximum leaf area index, which was measured weekly (Zeri et al., 2011; Appendix 1, Fig. 3). The harvest with the greatest biomass, hereafter referred to as peak biomass, occurred in August for maize, soybean, prairie, and switchgrass, and September for $M. \times giganteus$. Plot level mechanical harvesting of all crops occurred after aboveground tissues had fully senesced but varied among crops based on weather, field logistics, and soil conditions (October for annuals, November for switchgrass and prairie in 2010), in some cases occurring during March of the following spring (e.g. all perennial crops in 2009 and $M. \times giganteus$ in 2010). Additional biomass was sampled by hand at the time of mechanical harvesting to obtain tissue for nutrient analyses, but bale yields were used to calculate biomass and nutrient removal at this time. For all hand harvests, total aboveground biomass was harvested at a height of 10 cm to mimic mechanical harvesting, and sorted into leaf, stem, and reproductive structures. Biomass was harvested from 0.45×0.45 m quadrats for prairie and switchgrass, and from 0.75×0.75 m quadrats for maize, soybean, and $M. \times giganteus$. The larger quadrat size was chosen to ensure sampling of exactly one row in maize, soybean, and $M. \times giganteus$, matching the planted row spacing. Two quadrats were placed randomly in each 0.7 ha plot, and four in each 3.8 ha plot. Tissue for nutrient data was randomly selected from four of five plots ($n = 4$), while biomass data were from all plots ($n = 5$).

Tissue nutrient concentrations and biomass removal for maize and soybean were calculated for grain only, as it was assumed that non-grain biomass would be returned to the soil thereby keeping the nutrients on site. Tissue for nutrient analysis was dried at 60 °C until constant mass and weighed.

Mechanical harvests were conducted to determine grain yields for maize and soybean and biomass removals for the perennial feedstocks. Entire plots of switchgrass, prairie, and $M. \times giganteus$ were harvested with cutting and baling equipment (Case New Holland Global, Burr Ridge IL USA), and maize and soybean were harvested with a combine (Case New Holland Global, Burr Ridge IL USA). Perennial ecosystems were allowed to completely senesce into late fall in order to allow maximum nutrient retranslocation and decrease biomass moisture content. Timing of perennial mechanical harvests varied due to weather, field logistics, and soil conditions. Final yields and values for nutrient removal were calculated using bale weights removed from each plot for the perennial feedstocks and grain yield for maize and soybean.

For the regional fertilizer trials, aboveground biomass from $M. \times giganteus$ was hand harvested as described above using a 0.75×0.75 m placed randomly in each plot. Material was collected at one time point per site in November/December following the 2010 growing season, three years after establishment.

2.4. Nutrient analysis

Dried plant material from all sites was homogenized and ground in a Wiley Mill to pass a 425-μm mesh (Thomas Scientific, Swedesboro New Jersey, USA) and subsequently ground into a powder with a ball mill (Geno Grinder 2010; BT&C Lebanon New Jersey, USA). Ground material was weighed into tin capsules for C and N analysis, and combusted with an elemental analyzer (Costech 4010CHNSO Analyzer, Costech Analytical Technologies Inc. Valencia, California, USA). Acetanalide and apple leaves (National Institute of Science and Technology, Gaithersburg Maryland, USA) were used as standards.

Additional material was weighed into centrifuge tubes and digested in 15 M nitric acid with a microwave system (MARS, CEM Corporation, Matthews North Carolina, USA). Samples were heated at 100 °C for 22 min until plant material was completely digested. Four apple leaf standards were run with each batch of 48 samples

for digestion quality control. Solutions were diluted with deionized water prior to analysis to reduce acidity and increase analytical precision. Solutions were analyzed for Ca, Mg, K, P, S, Fe, Na, Mn, and Zn with an inductively coupled plasma-optical emission spectrometer (PerkinElmer Optima 2000, ICP-OES, PerkinElmer, Waltham Massachusetts, USA). Nutrient concentrations were measured for stems, leaves, and reproductive structures independently, and total aboveground nutrient concentration and content were calculated by summing the values weighted by the mass of each component.

2.5. Statistical analyses

Nutrient removal was calculated as the product of the weighted tissue concentrations and the total biomass at the time of harvest. Since soybean is a known N fixer, and N fixed from the atmosphere was not removed from the soil N pool, this N was discounted in removal calculations. Fixation was assumed to be responsible for 60% of grain N (David et al., 2010; Gentry et al., 2009), and this N was subtracted from all removal calculations (Table 3). Loss of biomass in overwintered material (Hudiburg et al., 2014), foliar leaching (Tonn et al., 2012) and nutrient retranslocation (Beale and Long, 1997; Christian et al., 2006) can be substantial and could not be partitioned in our data set. However, these perennial grasses exhibit efficient soil nutrient cycling and low losses of nutrients to groundwater (Smith et al., 2013), primarily resulting in the retention of these nutrients by the ecosystem. Thus we refer to the difference in nutrient concentration between peak and removed biomass as "percentage retained". The percentage of nutrient retained (Fig. 3) was calculated as the difference of the potential nutrient removal at peak biomass and the nutrient removal at final harvest, multiplied by 100, divided by the potential nutrient removal at peak biomass.

Energy Farm mean values and standard errors were calculated with the Means and Stdev procedure of JMP (V. 4.0.2, SAS Institute). Biomass removal between species and soil nutrient concentrations were compared using a one-way analysis of variance and t-test in JMP. Comparisons between Sun Grant/DOE plots and fertilizer treatments were performed in R using a linear mixed-effects model (Pinheiro et al., 2013), where site and N treatment were fixed effects, and blocks within each site were random variables. Variance was estimated separately for each site; a heteroskedastic model fit the data better than a single-variance model, explaining greater than 80% of total variance for every nutrient. When mean removal differed significantly between sites or treatments, we made *post-hoc* comparisons using pairwise differences between sites or polynomial contrasts between treatments. In pairwise comparisons between sites the p-values were corrected to maintain a familywise error rate of 5% within each nutrient, using a single step adjustment implemented in the R package multcomp (Hothorn et al., 2008). In polynomial contrasts between N treatments the reported p values were not adjusted.

3. Results

3.1. Energy Farm nutrient removal

The amount of biomass varied with year, time of year, and crop type (Fig. 2). Maize yields were greater than all perennials ($p < 0.05$) in 2009 (Fig. 2). Peak biomass for $M. \times giganteus$ occurred later in the growing season than all other crops (Fig. 2). Harvested biomass for prairie and switchgrass in 2009 and 2010 peaked in August and decreased later in the year. There was greater than 49% biomass reduction (Fig. 2) between peak and spring harvests (March 2010 for all perennials, March 2011 for $M. \times giganteus$), and biomass of all perennials was significantly greater at peak than at
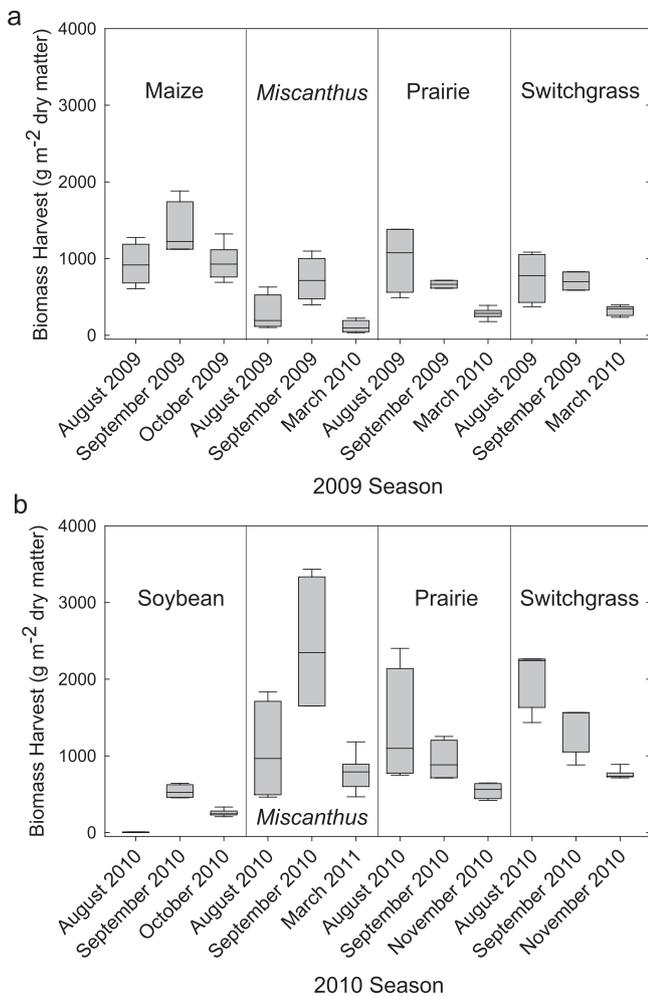
Fig. 2. Box plots of Energy Farm aboveground biomass, taken at three time points in 2009 (a) and 2010 (b). The center horizontal line represents the median, and the bottom and top of the box represent the 25th and 75th percentile. The bottom and top whiskers represent the 10th and 90th percentile, respectively. As only the grain is removed annually for maize and soybean, only grain is shown here. Peak biomass occurred for maize, soybean, prairie, and switchgrass in August while $M. \times giganteus$ biomass peaked in September. August and September biomass was collected by hand, while final harvests (October/November/March) were plot level and mechanized.

final harvest. Grain biomass collected from maize in August and September did not vary from biomass collected at final harvest in October, but was significantly less in soybean (Fig. 2). This reduction in yield and variability of grain biomass was likely due to the difference in harvest techniques. There were no significant differences in final harvest biomass between perennial crops in 2010, and more biomass was removed from all perennial treatments than from soybean ($p < 0.05$). The loss of biomass and exposure to winter precipitation resulting from delayed harvests such as those conducted in March 2010 and 2011 confound direct nutrient removal comparisons between treatments but were unavoidable due to weather and field logistics.

In 2009, nutrient removal per unit area (harvest nutrient concentration • harvest biomass per unit land area, g/m^2) of N, P, K, S, Ca, Mg, Mn, Na, Zn, and Fe was greater for maize than from all perennials ($p < 0.05$; Table 2). Nutrient removal from $M. \times giganteus$ was ≤19% of removal from maize for all nutrients. Prairie and switchgrass removed more N, P, S, Ca, and Zn than $M. \times giganteus$. Additionally, switchgrass removed more Mg and Mn than $M. \times giganteus$. There were no significant differences in nutrient removal between switchgrass and prairie in 2009.

When considering nutrient removal per mass of biomass removed (mg nutrient/gram biomass removed), the same pattern was evident for N, P, K, S, Mg, and Zn, with a decrease in the magnitude of removal between maize and all perennials (Table 4). For example, N removal for $M. \times giganteus$, prairie, and switchgrass was 4%, 10%, and 9%, respectively, than that of maize per unit area, and 44%, 34%, and 39%, respectively, than that of maize per unit biomass. In some cases this change in units removed a significant difference (e.g. prairie: Ca and Fe), and in others it results in higher removal from the perennial (e.g. $M. \times giganteus$: Fe and Mn). The percentage of nutrient retained by the ecosystem (biomass nutrient content at peak—biomass nutrient content at final harvest) × 100/(biomass nutrient content at peak) varied between 36 and 97% in 2009 for N, P, K, Ca, Mg, and S (Fig. 3).

Removal of N, P, and S by perennial crops was 49–85% of soybean removal in 2010 (Table 3). In addition, more K and Mg were removed by soybean than from $M. \times giganteus$ and prairie ($p = 0.05$). Nitrogen, P, K, Ca, and Mg removal was higher in switchgrass than in $M. \times giganteus$, while only P and K removal was higher in switchgrass than in prairie. Calcium removal in prairie was higher than in both switchgrass and $M. \times giganteus$. Iron, Mn, and Na removal was significantly higher in perennial crops than in soybean (Table 3).

When considering nutrient removal per mass of biomass removed, the same pattern was observed for P, K, S, Fe, and Mn with an increase in the magnitude of the difference between soybean and the perennials (Table 5). For example, P removal for $M. \times giganteus$, prairie, and switchgrass was 17%, 35%, and 66%, respectively, than that of soybean per unit area, and 7%, 19%, and 25%, respectively, than that of soybean per unit biomass. In some cases this change in units removes a significant difference (e.g. $M. \times giganteus$: Fe), and in others removal by soybean is significantly increased (e.g. $M. \times giganteus$: Ca and Zn; switchgrass: Mg, Na and Zn; prairie: Zn). Nitrogen removal considered per unit biomass did not change differences between soybean and perennials, but did decrease the magnitude of the difference between prairie and soybean. The percentage of nutrient retained by perennials varied between 42 and 91% in 2010 for N, P, K, Ca, Mg, and S (Fig. 3).

3.2. Effect of location and fertilizer on nutrient removal

Dry-matter yield for $M. \times giganteus$ varied significantly among these four regions of the US (Table 6, $p < 0.05$). Across all sites, N and K removals were directly proportional to biomass harvested, with the exception of a higher tissue N concentration in Kentucky resulting in no significant difference between total N removal there and at the Nebraska site. Significantly more P, Mg, and Mn were removed from the Kentucky site than all others, while less P, Mg, and Mn was removed from the New Jersey site ($p < 0.05$). More Ca was removed from the Kentucky and Nebraska sites than from the Illinois and New Jersey sites. More Fe was removed from the Nebraska site than from New Jersey and Illinois sites. There were no significant differences in Na and Zn removed among sites, despite differences in biomass. Significantly less S was removed at the Illinois site than at all others (Table 6).

No effect of N fertilization (0, 60, and 120 kg ha^{-1}) on $M. \times giganteus$ biomass yield was resolved at any of the four Sun Grant/DOE sites in this study in 2010. When considering all sites in the linear mixed-effects model, removal of N, S, Mg, and Mn increased with higher rates of fertilization, while removal of P decreased with fertilization (Table 7). The S, P, and Mn effects differed between sites: *Post-hoc* comparisons of fertilization effects within each site showed that S removal in New Jersey tended to decline with increasing fertilization instead of tending to increase as in the other sites, P removal in Nebraska was nearly unchanged with increasing fertilization instead of lower as in the other sites,
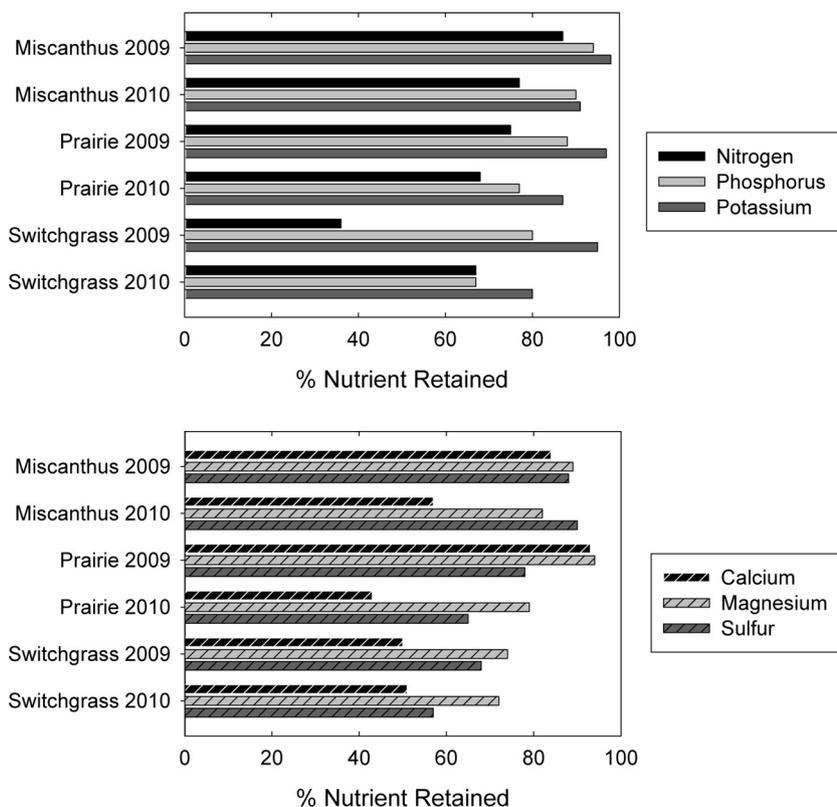
Fig. 3. Percentage of nutrients retained by the ecosystem between peak biomass and total aboveground harvest for perennial bioenergy crops. This calculation does not partition the fluxes of nutrients caused by loss of overwintered biomass, retranslocation, and foliar leaching, but instead assumes they will be retained within the ecosystem by plants with large root systems, long growing seasons, and low nutrient leaching. Calculated as (biomass nutrient content at peak−biomass nutrient content at final harvest) × 100/(biomass nutrient content at peak).

Table 2
Mean nutrient removal in final harvest of maize (grain only), $M. \times giganteus$, prairie and switchgrass at the University of Illinois Energy Farm in 2009 (harvest occurred in Mar 2010 for all perennial crops). Numbers in parentheses represent percentage difference in removal relative to maize. Shared letters across a row are not significantly different, $p < 0.05$.

Nutrient	Maize	$M. \times giganteus$	Prairie	Switchgrass
	g m^{-2} (% relative to maize)			
Biomass	1362.3a	112.1c	284.8b	321.8b
N	21.84a	0.800 (4%)c	2.080 (10%)b	2.022 (9%)b
P	2.166a	0.039 (2%)c	0.152 (7%)b	0.173 (8%)b
K	20.388a	0.155 (2%)b	0.293 (1%)b	0.243 (1%)b
S	2.269a	0.085 (4%)c	0.187 (8%)b	0.182 (8%)b
Ca	3.175a	0.226 (1%)c	0.675 (21%)b	0.640 (20%)b
Fe	0.176a	0.034 (19%)b	0.070 (40%)b	0.069 (39%)b
Mg	2.389a	0.067 (3%)c	0.121 (5%)bc	0.178 (8%)b
Mn	0.037a	0.007 (19%)c	0.009 (24%)bc	0.016 (43%)b
Na	0.058a	0.007 (12%)c	0.011 (19%)b	0.011 (19%)b
Zn	0.033a	0.001 (7%)c	0.004 (12%)b	0.005 (15%)b

and Mn removal increased with fertilization in Nebraska but was nearly unchanged at all other sites. Significant post hoc comparisons were observed within some sites for N, P, Mg, and Mn, but not S (Table 7). Non-linear responses to fertilizer were not observed for any nutrient, and fertilization had no detectable effect on removal of K, Ca, Fe, Na, and Zn at any site.

4. Discussion

As hypothesized, annual nutrient removal from perennial grass bioenergy crops was significantly less than from annual row crops such as maize and soybean (Tables 2 and 3). The difference in nutrient load present in the aboveground biomass between growing season peak and harvested biomass (Fig. 3) was the result of a decrease in nutrient concentration and lost biomass. All perennials demonstrated high ecosystem nutrient retention in both years. The combination of sizeable biomass and high tissue nutrient concentrations of N, P, K, Ca, Mg, S, Fe, Mn, Na, Zn removed annually from maize crops resulted in extreme differences in total nutrient removal when compared to establishing perennials per unit area (Table 2). Despite lower than expected yields during establishment, nutrient removal considered per unit of mass removed was still lower for most nutrients than from maize (Table 4). Conversely, comparisons of removal by unit mass increased the magnitude of difference between perennials and soybean (Table 5), largely due to the smaller soybean biomass removed. Total removal data from soybean further support our hypothesis, following the same trend for N, P, K, S, and Mg as in maize despite its lower biomass (Table 3). It has been estimated that soybean incorporates between 78 and 110 kg N ha^{-1} yr^{-1} of its N through atmospheric N fixation (David et al., 2010; Gentry et al., 2009). Discounting the N incorporated via fixation, soybean still removed more N than prairie and $M. \times giganteus$ from the soil. If cellulosic biofuel production increases, this difference in nutrient removal between annual and perennial cropping systems could translate into a significant benefit to soil nutrient sustainability on a regional scale.

This study was conducted during the second and third years of establishment of these perennial crops, and the resulting biomass did not necessarily reflect what would be expected of mature crops, particularly for $M. \times giganteus$. Switchgrass and prairie yields measured in 2011 (Anderson-Teixeira et al., 2013) were no greater than the values presented here in 2010 (Fig. 2) indicating these crops were near their maximum in 2010. Yields from our third year

Table 3
Mean nutrient removal in final harvest of soybean (grain only), *M. × giganteus*, prairie and switchgrass at the University of Illinois Energy Farm in 2010 (harvest occurred in Mar 2011 for *M. × giganteus*, and November 2010 for switchgrass and prairie). Numbers in parentheses represent percentage difference in removal relative to soybean. Shared letters across a row are not significantly different, $p < 0.05$. Nitrogen removed by atmospheric N fixation, which would not be removed from the soil N pool, was assumed to be 60% of grain N and was subtracted from this total for soybean (*).

Nutrient	Soybean	*M. × giganteus*	Prairie	Switchgrass
	g m^{-2} (% relative to soybean)			
Biomass	291.6b	775.7a	536.4a	761.0a
N	5.400a*	2.64 (49%)b	3.237 (60%)b	4.578 (85%)a
P	1.118a	0.195 (17%)d	0.393 (35%)c	0.740 (66%)b
K	4.381a	1.398 (32%)b	1.841 (42%)b	4.197 (96%)a
S	0.875a	0.204 (23%)b	0.172 (20%)b	0.244 (28%)b
Ca	0.837c	0.710 (81%)c	2.050(+145%)a	1.268 (+45%)b
Fe	0.027c	0.106 (+294%)b	0.127 (+370%)ab	0.157 (+481%)a
Mg	0.559a	0.212 (38%)b	0.339 (61%)b	0.529 (95.0%)a
Mn	0.005c	0.061 (+1120%)a	0.024 (+380%)bc	0.036 (+620%)b
Na	0.004c	0.060 (+1500%)a	0.012 (+200%)c	0.022 (+450%)b
Zn	0.010ab	0.006 (60%)b	0.012 (+20%)a	0.004 (40.0%)b

Table 4
Mean nutrient removal as a fraction of removed biomass in final harvest of maize (grain only), *M. × giganteus*, prairie and switchgrass at the University of Illinois Energy Farm in 2009 (harvest occurred on 15 Mar 2010 for all perennials). Numbers in parentheses represent percentage difference in removal relative to maize. Shared letters across a row are not significantly different, $p < 0.05$.

Nutrient	Maize	*M. × giganteus*	Prairie	Switchgrass
	mg nutrient/g biomass removed (% relative to maize)			
N	16.03a	7.14 (44%)b	7.30(34%)b	6.28 (39%)b
P	1.59a	0.35 (22%)c	0.53 (33%)b	0.54 (34%)b
K	14.97a	1.38 (9%)b	1.02 (7%)b	0.76 (5%)c
S	1.67a	0.76 (46%)b	0.66 (39%)c	0.57 (34%)c
Ca	2.33a	2.01 (87%)b	2.37(+1%)a	1.99 (85%)b
Fe	0.13c	0.30 (+135%)a	0.25 (+93%)ab	0.21 (+66%)b
Mg	1.75a	0.60 (34%)b	0.42 (24%)b	0.55 (32%)b
Mn	0.03b	0.06 (+130%)a	0.03 (+16%)b	0.04 (+44%)b
Na	0.04ab	0.06 (+47%)a	0.04 (90%)b	0.03 (80%)b
Zn	0.03a	0.01 (37%)b	0.01 (58%)b	0.02 (64%)b

Table 5
Mean nutrient removal as a fraction of removed biomass in final harvest of soybean, *M. × giganteus*, prairie and switchgrass at the University of Illinois Energy Farm in 2010 (harvest occurred in Mar 2011 for *M. × giganteus*, and October/November 2010 for switchgrass and prairie). Numbers in parentheses represent percentage difference in removal relative to soybean. Shared letters across a row are not significantly different, $p < 0.05$. Nitrogen removed in soybean biomass that is attributable to atmospheric N fixation, which would not be removed from the soil N pool, was assumed to be 60% of grain N and was subtracted from this total (*).

Nutrient	Soy	*M. × giganteus*	Prairie	Switchgrass
	mg nutrient/g biomass removed (% relative to soybean)			
N	7.41a*	3.4 (46%)c	6.03 (81%)b	6.02 (81%)b
P	3.83a	0.25 (7%)d	0.73 (19%)c	0.97 (25%)b
K	15.02a	1.80 (12%)d	3.43 (23%)c	5.15 (37%)b
S	3.00a	0.26 (9%)b	0.32 (11%)b	0.32 (11%)b
Ca	2.87b	0.92 (32%)d	3.82 (+33%)a	1.67 (58%)c
Fe	0.09b	0.14 (+48%)b	0.24 (+156%)a	0.21 (+123%)a
Mg	1.92a	0.27 (14%)c	0.63 (33%)b	0.70 (36%)b
Mn	0.02c	0.08 (+359%)a	0.04 (+161%)b	0.05 (+176%)b
Na	0.01b	0.08 (+4564%)a	0.02 (+63%)b	0.03 (+111%)b
Zn	0.03a	0.01 (23%)b	0.02 (65%)b	0.01 (15%)b

M. × giganteus (Fig. 2) were much lower than the Illinois average of 23.4 Mg ha^{-1} for a mature crop (Arundale et al., 2014). Poor establishment caused this discrepancy and we expect our system to reach the totals observed by Arundale et al. (2014) as it continues to mature. Biomass removed from *M. × giganteus*

exceeded maize grain removed in 2011, while removed biomass from switchgrass and maize was not significantly different (Smith et al., 2013). As aboveground biomass production increases with maturation, total nutrient content removed will increase as well. However, the concentration of nutrients in harvested *M. × giganteus* tissue was so low relative to maize grain (Table 4) that the difference in nutrient removal between these treatments would be considerable even at the high yields predicted for mature *M. × giganteus*. For example, assuming tissue concentrations remain the same as yield increases, *M. × giganteus* yields of 20 Mg ha^{-1} would remove 60–71% less N, P, and K than is removed by maize yields at 15 Mg ha^{-1}, which is the maximum grain yield observed on site in 2008 (Anderson-Teixeira et al., 2013). Christian et al. (2008) reported small annual variation in *M. × giganteus* tissue concentration of N, P, and K over a ten-year time period, so we expect these differences would continue in future years. As the biomass produced per unit area increases for perennial crops as they mature, the nutrient removal per unit of biomass will not. Both unit comparisons indicate long-term removal will be significantly less in all three perennial ecosystems for N, P, K, S, Mg, and Zn than that from maize crops (Tables 2 and 4).

The maize-maize-soybean rotation in this study is typical of this region but does not allow comparisons of each annual crop in the same year, and yield differences between years make cumulative scaling of this treatment difficult. Inter-annual variation in nutrient removal can be significant in maize and soybean, but is largely dependent on yield and not nutrient concentration (Karlen et al., 1987; Anthony et al., 2012). However, it is evident from these data (Table 2) the magnitude of removal from maize greatly exceeds that of all perennials as well as soybean. For example, three years of N removal at the rate measured in 2010 for all perennials was 16%, 20%, and 28% for *M. × giganteus*, prairie, and switchgrass, respectively, than that of the maize-maize-soybean rotation (Tables 2 and 3). As stated previously, increases in *M. × giganteus* biomass with crop maturation would undoubtedly decrease the magnitude of this difference, but will not approach the levels of removal from an annual rotation.

Best agronomic practices vary among bioenergy crops in the Midwest. Typically, switchgrass receives nominal N additions, where prairie and *M. × giganteus* are not fertilized (Davis et al., 2012). Changes in fertilization regime, climate, and soil type would likely influence nutrient removal as well as potential yield responses. Nitrate leaching and nitrous oxide emission would also be influenced by changes in N management. Fertilization (0–120 kg N ha^{-1}) in the Sun Grant/DOE plots increased N removal in *M. × giganteus* by ~19–30% (Table 7) across sites, and in some cases also influenced removal of P, Mg, and Mn. While a 30% increase in N removal with fertilization is substantial for *M. × giganteus*, total N removed would still be considerably lower than removal by maize. The difference in nutrient removal between maize and these perennials would increase even further if any maize residues were removed and used for energy production (Sheehan et al., 2003).

The decline in aboveground nutrient load between peak biomass and the material removed at harvest (Fig. 3) can be explained by a combination of foliar leaching, retranslocation, and the loss of biomass over the winter season. Tukey (1970) reported that of the soluble nutrients, K, Ca, Mg, and Mn are usually leached in the greatest quantities from plant tissues. Annual plants concentrate nutrients from other tissues into seeds (grain) at the end of the growing season, and retranslocation to roots is minimal (Chapin et al., 1990). Harvest delay of senesced perennial biomass until winter or spring would expose tissue to further leaching, returning these nutrients to the soil to potentially be reabsorbed by plant roots. In addition, spring harvest can result in significant biomass losses over the winter season (Larsen et al., 2014; Iqbal and Lewandowski, 2014). Spring harvests of perennials

Table 6

Miscanthus × giganteus biomass and nutrient removal at Sun Grant/DOE research sites in November/December 2010. All samples were collected by hand. Values are mean ± standard deviation across all N treatments (0, 60, 120 kg N ha^{-1}) for each site (n = 12). Shared letters across a row are not significantly different, (p < 0.05).

	Nebraska	Illinois	Kentucky	New Jersey
	g m^{-2}			
Biomass	2771.1 ± 317.3a	1561.9 ± 252.5c	1904.7 ± 276.7b	956.2 ± 188.1d
N	11.61 ± 1.75a	5.42 ± 1.22b	11.27 ± 3.13a	2.71 ± 0.94c
P	0.79 ± 0.38b	0.34 ± 0.13c	1.44 ± 0.26a	0.43 ± 0.30c
K	16.53 ± 2.22a	3.96 ± 0.75c	7.45 ± 2.73b	3.26 ± 0.94c
S	1.43 ± 0.42a	0.40 ± 0.18b	1.49 ± 0.44a	0.96 ± 0.32a
Ca	1.98 ± 0.37a	1.31 ± 0.27b	2.84 ± 1.50a	1.04 ± 0.24b
Fe	0.064 ± 0.033a	0.029 ± 0.007b	0.053 ± 0.037ab	0.021 ± 0.011b
Mg	0.92 ± 0.21b	0.73 ± 0.28b	2.60 ± 0.80a	0.52 ± 0.11c
Mn	0.14 ± 0.05b	0.11 ± 0.02b	0.30 ± 0.09a	0.04 ± 0.02c
Na	0.029 ± 0.052a	0.016 ± 0.007a	0.041 ± 0.034a	0.014 ± 0.003a
Zn	0.018 ± 0.005a	0.009 ± 0.003b	0.011 ± 0.004b	0.020 ± 0.022ab

Table 7

Miscanthus x *giganteus* nutrient removal at Sun Grant/DOE research sites in 2010 for nutrients affected by urea fertilization with 0, 60, and 120 kg of N per hectare. The p values for contrasts between fertilization treatments within each site, and means ± s.d. of removal from individual treatments, are shown. Boldfaced values differed linearly (p < 0.05) with increasing fertilization within a site. Nonlinear fertilization responses were tested for, but none were observed (p > 0.2 for all quadratic terms). There was no effect of fertilization on nutrient removal for K, Ca, Fe, Na, Zn (p > 0.05 for the all-sites linear mixed-effects model.)

	Nebraska	Illinois	Kentucky	New Jersey
	g m^{-2}			
N	p = 0.1354	p = 0.0586	p = 0.8593	**p = 0.0027**
0	10.38 ± 1.45	4.48 ± 0.90	10.54 ± 3.79	**2.31 ± 1.09**
60	11.55 ± 1.72	5.50 ± 1.04	10.13 ± 0.99	**2.52 ± 0.83**
120	12.90 ± 1.36	6.28 ± 1.19	13.14 ± 3.66	**3.31 ± 0.78**
P	p = 0.9999	**p = 0.0397**	p = 0.8659	p = 0.0001
0	0.69 ± 0.10	**0.48 ± 0.13**	1.51 ± 0.17	0.78 ± 0.25
60	1.04 ± 0.60	**0.29 ± 0.07**	1.51 ± 0.28	0.34 ± 0.11
120	0.63 ± 0.17	**0.27 ± 0.09**	1.30 ± 0.30	0.19 ± 0.04
S	p = 0.0576	p = 0.3411	p = 0.6999	p = 0.9508
0	1.09 ± 0.16	0.34 ± 0.18	1.21 ± 0.59	1.06 ± 0.37
60	1.56 ± 0.33	0.35 ± 0.21	1.60 ± 0.25	0.92 ± 0.21
120	1.65 ± 0.53	0.51 ± 0.14	1.65 ± 0.35	0.89 ± 0.41
Mg	p = 0.4036	**p = 0.0028**	p = 0.7688	p = 0.0539
0	0.76 ± 0.16	**0.45 ± 0.14**	2.33 ± 0.48	0.43 ± 0.12
60	0.99 ± 0.02	**0.76 ± 0.17**	2.39 ± 0.82	0.53 ± 0.09
120	1.01 ± 0.28	**0.98 ± 0.23**	3.09 ± 0.97	0.61 ± 0.05
Mn	**p < 0.0001**	p = 0.9866	p = 0.7880	p = 0.5760
10	**0.08 ± 0.02**	0.11 ± 0.03	0.25 ± 0.08	0.06 ± 0.02
160	**0.15 ± 0.03**	0.11 ± 0.03	0.32 ± 0.07	0.03 ± 0.01
1120	**0.20 ± 0.02**	0.10 ± 0.03	0.34 ± 0.12	0.04 ± 0.02

(Christian et al., 2006). The differences in nutrient content we observed at the Energy Farm between peak biomass and harvest exceed these literature totals, likely due to significant losses in biomass from overwintering as well as foliar leaching. We observed declines in total aboveground nutrient content of N, Ca, Mg, K, P, S, Fe, Mn, and Zn between 61.1 and 97.7% in 2009, and 56.8 and 90.0% in 2010 for *M. × giganteus* (Fig. 3). We found several instances of no decline in nutrient content between peak and removal for metallic nutrients Fe and Zn in switchgrass and prairie and for Na in *M. × giganteus*. However, these concentrations were low and the associated measurement error was large.

Although all of the macro- and micronutrients measured in this study are necessary for plant growth, increased ecosystem nutrient retention for N, P, K, S, Ca, and Mg relative to traditional row crops are particularly interesting from both an agronomic and energy production point of view. These nutrients often are limiting to plant growth in terrestrial systems, and farmers apply fertilizers such as UAN, DAP, potash, and lime as needed (Egli, 2008). These amendments provide additional nutrients for direct uptake, or increase soil pH and cation exchange capacity to facilitate increased uptake. A reduction in annual removal of these nutrients could provide ecological and economic benefits. The carbon footprint of fertilizer production is high, in addition to the environmental cost of nitrate leaching, soil compaction, and erosion associated with application via commercial agricultural equipment (Jensen and Hauggaard-Nielsen, 2003). At the Energy Farm, nitrate leaching from maize was 40–60 kg N ha^{-1} yr^{-1} between 2008 and 2012 while leaching under the perennial systems was <5 kg N ha^{-1} yr^{-1} over the same period (Smith et al., 2013). It is estimated that maize production at the level needed to produce 56–136 billion liters of ethanol could increase dissolved inorganic N flux to the Mississippi river by 10–34%, further contributing to hypoxia in the Gulf of Mexico (Donner and Kucharik, 2008). High concentrations of macro- and micronutrients in plant tissue destined for energy production also can complicate the associated chemical reactions. Minerals such as K, Mg, Ca, and Na cause slagging and chemical erosion of boilers during combustion or gasification, interfere with efficiency of conversion, and contribute to the production of NO_x and SO_x released in exhaust gases exacerbating pollution (Smith and Slater, 2011).

The data reported in this study support the hypothesis that differences in climate and soil properties would contribute to regional differences in nutrient removal (Table 6). We observed luxury uptake (apparent acquisition of the nutrient in excess of need for growth) of K by *M. × giganteus*. The concentration of extractable K in the soil (0–100 cm) at the Nebraska site was three times higher than at the Kentucky site, and six times higher than at the Illinois and New Jersey sites, which were similar (Maughan et al., 2012 Appendix 1, Table 1). It is evident from the nutrient removal at these four sites (Table 6), which followed the same pattern as extractable soil K, that *M. × giganteus* will take up K in proportion to its availability. However, it is unclear from these data if K is limiting biomass yield at the sites with lower soil availability. Soil P was high at the Kentucky site, and more P was removed in the biomass at this site than all others, suggesting P luxury uptake as well. Beale and Long (1997) suggest poor K retranslocation efficiency relative to other nutrients and observed concentrations of luxury K in removed biomass at our Nebraska and Kentucky sites support this suggestion. Tonn et al. (2012) reported high losses of K due to leaching in simulated rainfall, returning much of this K to the soil. Further research using K and P fertilization trials is needed to demonstrate the impact of these nutrients on yield.

The same general pattern in soil chemistry was evident among these sites for Ca and Mg, with slight differences in magnitude (Maughan et al., 2012 Appendix 1, Table 1). Despite this difference

in this study (Fig. 2) complicated direct nutrient removal comparisons with annual row crops not exposed to foliar leaching and biomass fractionation, and the magnitude of some of the differences presented in this study would likely decline with early fall harvests.

Although not directly measured in this study, nutrient retranslocation to the root system by perennials during senescence of aboveground tissues may explain the decline in nutrient concentrations between peak biomass and final harvest (Fig. 3). Comprehensive nutrient budgets for *M. × giganteus* found that 18–46%, 33–55%, and 15–30% of the N, P, and K, respectively, present in peak aboveground biomass was retranslocated for use in following growing seasons. Other studies have traced N in rhizomatous grasses using isotopes, reporting a similar range of retranslocation

in soil nutrient availability, the differences in Ca removed as aboveground biomass between Kentucky and Nebraska sites were not significant. We did not measure growing-season nutrient concentrations in aboveground tissue at the Sun Grant/DOE sites, but this could suggest active retranslocation and tight cycling of Ca at these sites. Even more surprising, nutrient removal at the Kentucky site was significantly higher for Mg than at the Nebraska site (Table 6), despite three times more available Mg in the soil (0–100 cm) in Nebraska (Appendix 1, Table 1). This contrasts with previous findings that plants will respond to excess Mg with luxury uptake (Christenson et al., 1972). However, Mg uptake is known to be affected by the presence of Na, Ca, and K, as well as soil pH (Fageria, 2001), and the Ca:Mg ratio in the Nebraska soil was considerably higher than the Kentucky soil.

Biomass yield at these sites did not increase with additions of 60 or 120 kg ha^{-1} of N fertilizer (Maughan et al., 2012). However, when all sites were considered, there was an increase in the nutrient removal of N, S, Mg, and Mn in the aboveground biomass (Table 7). The increasing concentration of these nutrients in the tissue suggests either an increase in uptake or a decrease in retranslocation. The significant decline in P removal with increasing N fertilization could indicate an increase in retranslocation efficiency facilitated by more available N. If P is limiting to *M.* × *giganteus* growth, it seems likely these systems will eventually respond to these levels of fertilization with increases in yield made possible by conserved P from previous years. Conversely, it is possible repeated N fertilization lowered soil pH and decreased P availability. Ammonium based fertilizers such as the urea used in these plots have the potential to acidify soil when rainfall is high enough to leach nitrate and lead to net production of H$^+$ ions (Smiley and Cook, 1973). Decreasing availability of P would eventually decrease the yields in these plots, with actual differences likely to be site dependent. Regardless of the mechanism behind the decline in P removal, increasing concentrations of of N, S, Mg, or Mn in removed biomass would indicate these nutrients did not limit growth of *M.* × *giganteus* in the Sun Grant/DOE plots. Inability to resolve within site post hoc comparisons for some nutrients, despite a change in the absolute value, is likely the result of small sample size and could potentially be resolved with more intensive sampling.

5. Conclusions

The efficient N cycling in three potential bioenergy crops demonstrated by Smith et al. (2013) extends to other nutrients as well (P, K, S, Ca, Mg, Mn, Na, Zn, and Fe). Conventional harvest of a maize-maize-soybean rotation removed more of each of these nutrients from the soil than an aboveground harvest of *M.* × *giganteus*, switchgrass, or a native prairie mix during their establishment period. This difference in nutrient removal, plus the ability of these perennials to retranslocate nutrients for future use could potentially translate into longer periods of productive soil fertility and biomass production. This assumes that perennial crops would be harvested after senescence. Our study demonstrates differences in *M.* × *giganteus* uptake of macro- and micronutrients, and their resulting removal at harvest, among sites with differing soil nutrient availability. In addition, the magnitude of difference in nutrient removal and the uncertainty of response to fertilizer additions in *M.* × *giganteus* suggests that a reduction in environmentally and economically costly soil amendments could be implemented in these systems. Given the large area devoted to row crop agriculture in the US, of which 38% of current maize grain is allocated to ethanol production (USDA, 2015), conversion to perennial grass bioenergy crops has the potential to significantly alter regional nutrient cycles.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2015.09.016.

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