Biomass and Bioenergy 107 (2017) 317–325

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: www.elsevier.com/locate/biombioe

Research paper

Nitrogen use efficiency and productivity of first year switchgrass and big bluestem from low to high soil nitrogen

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ARTICLE INFO

Keywords:
Switchgrass
Big bluestem
Establishment year
C_4 photosynthesis
Nitrogen use efficiency
Photosynthetic nitrogen use efficiency

ABSTRACT

Despite an abundance of research on the biomass response to nitrogen fertilizer in established switchgrass fields, there is a dearth of knowledge of the first year response in switchgrass, and nothing yet available for big bluestem. Based on differences in their C_4 photosynthetic subtypes, big bluestem may show greater photosynthetic (PNUE) and above-ground whole plant nitrogen use efficiency (NUE) over switchgrass. Here we report the first year biomass response to nitrogen fertilizer for two cultivars of upland switchgrass and three cultivars of big bluestem. We first performed a greenhouse experiment with four nitrogen treatments ranging from 1.5 dmol m^{-3} to 16 mol m^{-3} aqueous nitrogen solutions. Here we measured PNUE mid-way through the experiment and harvested plants green to measure above-ground whole plant NUE. We also compared these cultivars at two field sites with contrasting soil nitrogen content. At low soil nitrogen, big bluestem "Prairie View" achieved both the highest NUE in the greenhouse and greatest biomass in the field. Switchgrass produced significantly more biomass than big bluestem at the high nitrogen field site, whereas biomass production was similar for both species at the low nitrogen field site. Although both species responded positively to increasing soil nitrogen, switchgrass did increasingly more so compared to big bluestem, with a 20% greater biomass stimulation in the greenhouse from low to high soil nitrogen. Net CO_2 assimilation rates showed a similar response with biomass production to the nitrogen treatments, allowing switchgrass "Sunburst" to achieve a slightly greater PNUE compared to big bluestem "Prairie View".

1. Introduction

Perennial grasses that use C_4 photosynthesis (i.e. warm season) are becoming increasingly popular as dedicated biomass feedstocks for bioenergy and biomaterials. Switchgrass (*Panicum virgatum* L.) has been the most widely used and researched C_4 perennial grass (C_4PG) species in North America and is a model forage and biomass feedstock crop [1,2]. Big bluestem (*Andropogon gerardii* Vitman.) is also used for forage and has yields comparable to switchgrass, however it has only recently been recognized as a candidate biomass feedstock [3–5]. Both switchgrass and big bluestem are important members of the tall and mixed grass prairie ecosystems that once dominated the interior of North America [6,7]. Although less productive in the mid-west than other C_4PGs such as *Miscanthus*, these grasses provide a native compliment to biomass production, increasing feedstock resilience through greater genetic diversity [4,8].

Nitrogen is the most widely applied macro nutrient and often limits growth of biomass crops [9,10]. If the goals of using C_4PGs are to maximize carbon offsets and mitigate greenhouse gas emissions, excess nitrogen fertilization should be avoided to limit N_2O emissions [11]. As both a model forage and biomass crop, there are numerous studies of the biomass response of switchgrass to varying rates of nitrogen fertilizer [12–15]. These studies are conducted several years after crop establishment and show that switchgrass strongly responds to nitrogen fertilizer. The biomass response to nitrogen fertilizer appears to be dependent on latitude, climate, and soil type, with optimal application rates in the US corn belt ranging from 56 kg ha^{-1} in eastern South Dakota to 224 kg ha^{-1} in southern Iowa [16,17] and 150 kg ha^{-1} in Texas [18]. Due to big bluestem's more recent appeal as a bioenergy crop, there are fewer studies of its biomass response to nitrogen fertilization. The studies that exist do compare big bluestem with switchgrass, but again the comparison is made several years after plot establishment. In Iowa, both species respond similarly to nitrogen fertilizer up to 150 kg ha^{-1}; any higher and switchgrass yields continue

Abbreviations: C_4 perennial grass, (C_4PG); nitrogen use efficiency, (NUE); photosynthetic nitrogen use efficiency, (PNUE); nicotinamide adenine dinucleotide phosphate dependent malic enzyme, (NADP-ME); nicotinamide adenine dinucleotide dependent malic enzyme, (NAD-ME); ribulose-1,5-bisphosphate carboxylase/oxygenase, (Rubisco); phosphoenolpyruvate carboxylase, (PEPCase)

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http://dx.doi.org/10.1016/j.biombioe.2017.10.016
Received 26 April 2017; Received in revised form 17 October 2017; Accepted 20 October 2017

to increase whereas big bluestem yields begin to decline [19,20].

The biomass response to nitrogen fertilizer is closely coupled to a crop's nitrogen use efficiency (NUE), which is often defined as the unit of oven-dry biomass per unit nitrogen in green tissue. The NUE of total above-ground biomass can relate to photosynthetic nitrogen use efficiency (PNUE, the rate of CO_2 assimilation per unit nitrogen on a leaf area or mass basis) if nitrogen investment strategies are similar between leaves and the rest of the plant tissues [21–23]. The NUE and PNUE of grasses is a combination of their evolutionary lineage and photosynthetic type [24,25]. Both switchgrass and big bluestem use C_4 photosynthesis and therefore should have greater PNUE over ecologically similar grasses that use C_3 photosynthesis [23,26]. However, switchgrass and big bluestem differ in their C_4 subtype with their evolutionary lineages diverging approximately 30 million years ago [27]. Switchgrass (*Panicum*) is of the *Paniceae* tribe and uses the NAD-ME type C_4 pathway. Big bluestem (*Andropogon*), on the other hand, is of the *Andropogoneae* tribe and uses the NADP-ME type C_4 pathway [28–30]. Research comparing their evolutionary lineages and C_4 subtypes suggest that big bluestem may achieve greater NUE and PNUE over switchgrass at both high and low levels of soil nitrogen. Ghannoum et al. [31] were the first to clearly identify greater NUE and PNUE in NADP-ME grasses relative to NAD-ME grasses (including switchgrass) in glasshouse experiments at both high and low soil nitrogen. Recently Pinto et al. [25] again found greater PNUE in NADP-ME over NAD-ME grasses, regardless of evolutionary lineage in fully fertilized plants. Others have found greater PNUE in *Andropogoneae* grasses compared to *Paniceae* and several other lineages, regardless of photosynthetic type [24].

There are few studies of the first year biomass response to nitrogen fertilization in switchgrass, and none yet published for big bluestem. Some agronomists discourage first year fertilization of switchgrass due to weed competition [32,33], or have found no biomass response [34]. Other studies have shown a clear positive response to nitrogen fertilization in first year field trials [35], and in a greenhouse study [36]. Lands to be used in the future for dedicated biomass crops will be varied, and many predict these lands will and should be marginal so as to not compete with food crops [10]. Some of these lands may be converted from annual cropping systems and have high soil nitrogen, whereas other, unmanaged lands may have low soil nitrogen. Farmers need to know how much nitrogen to apply to maximize both first year profits and biomass yields. If soil nitrogen can be taken up and used to enhance first year growth, it will be efficiently recycled by C_4PGs and incorporated into their perennial system. This is especially true when biomass is harvested once per growing season after the above-ground vegetation has fully senesced [14].

Here we hypothesize that switchgrass will be more responsive to nitrogen fertilizer compared to big bluestem and show greater first year biomass in the field and greenhouse at high soil nitrogen. We also hypothesize that big bluestem may show greater NUE and PNUE over switchgrass based on its C_4 subtype and evolutionary lineage at all levels of soil nitrogen in a greenhouse experiment. Our two field sites are agricultural lands that contrast in their prior management and soil nitrogen concentration, located in Manitoba, Canada. We used two cultivars of upland switchgrass ("Dacotah" and "Sunburst") and three cultivars of big bluestem ("Boundary", "Bison", and "Prairie View") that all originate from mid-western North America.

2. Materials and methods

2.1. Plant material and initial growth conditions

Seeds of big bluestem "Bison" and switchgrass "Dacotah" and "Sunburst" were purchased from Millborn Seeds (http://www.millbornseeds.com). Seeds of big bluestem "Prairie View" were purchased from Ernst Seeds (http://www.ernstseed.com) and big bluestem "Boundary" were generously donated by Native Plant Solutions (http://www.nativeplantsolutions.ca). All cultivars except big bluestem ecovar "Boundary" and switchgrass "Sunburst" [37] were bred by the United States Department of Agriculture (USDA) and originate from native populations spanning a latitudinal gradient across the midwest USA [38]. Switchgrass "Dacotah" originates near Breien in south-central North Dakota, and "Sunburst" originates near Yankton in southeast South Dakota, USA. Big bluestem "Boundary" was bred by Native Plant Solutions and originates from southern Manitoba and southeastern Saskatchewan, Canada. Big bluestem "Bison" originates near Price in south-central North Dakota, and "Prairie View" originates from populations across Indiana, USA [38].

Seeds were first washed with a 0.5% bleach aqueous solution before germination on petri plates on May 2nd – 4th, 2016. Seeds of switchgrass were germinated on filter paper wetted with 2.5 cm^3 of a 0.2% KNO_3 aqueous solution and put in a fridge to cold stratify for two weeks. Seeds of big bluestem were germinated on filter paper and covered with washed quartz and wetted with 10 cm^3 of tap water. Germinating seedlings were transferred into a peat based soilless potting mix (Sunshine #4, http://www.sungro.com) in plug trays (245 cm^3 cell volume) in a temperature controlled greenhouse between May 16th – 20th. Seedlings were initially soaked with a 0:10:10 (N:P:K, 1.32 $dm^3\ m^{-3}$) aqueous fertilizer solution to stimulate root growth and thereafter watered as needed. Beginning the week of June 6th, plantlets were soaked twice weekly with a 20:20:20 (N:P:K, 4 $dm^3\ m^{-3}$) aqueous fertilizer solution until transplanted into the field or once weekly starting the week of June 27th before being potted up for the greenhouse experiment. Plants were moved outside from the greenhouse on June 13th.

2.2. Field trials

Plants were transplanted at two field sites, known to contrast in their soil nitrogen concentration. The first is a low soil nitrogen field site at "The Point" field research laboratory in Winnipeg, agricultural land on an oxbow of the Red River at the University of Manitoba, Fort Garry Campus in Winnipeg, Manitoba, Canada (hereafter Winnipeg) (49°48′49.06″N, 97°07′08.41″W). The soil at Winnipeg is Red River clay, classified as a Riverdale clay [39]. At Winnipeg, winter wheat (*Triticum aestivum* L.) was grown from 2011 – 2012 and thereafter the land was left fallow until our planting, with some tilling and weed management but no fertilizer added. The second is a high soil nitrogen field site at the Ian N. Morrison Research Farm, just outside Carman, Manitoba, Canada (hereafter Carman) (49°29′46.70″N, 98°2′43.64″W). The soil at Carman is a textured sandy loam classified as Orthic Black Chernozem [40]. In Carman, wheat (*T. aestivum*) was planted in 2012 and 2014, with 70:20:0 and 63:23:0 (N:P:K, kg ha^{-1}) fertilizers applied, respectively. Soybeans (*Glycine* max L. Merr.) were planted in 2013 and oats (*Avena sativa* L.) were planted in 2015 with a 42:15:0 fertilizer applied (N:P:K, kg ha^{-1}).

On June 22nd, 36 plants of each cultivar were hand planted in square 6 × 6 plant plots at Winnipeg after tilling. Plots were randomly placed in a grid with 2 m rows between plots. On June 24th and 25th, 6 × 6 plant plots using the same planting design were hand planted at Carman after tilling. At Carman, each 6 × 6 plant plot was replicated three times, as part of a larger scale trial. Plots at both field sites were 2 × 2 m with 4 dm spacing between plants and hand weeded throughout the growing season as necessary. Individual plants were harvested for biomass after all above-ground vegetation had fully senesced at the end of November, 2016. The internal 16 plants from each plot were hand-harvested 7.5 cm from the ground and put immediately into drying ovens at 65 °C for at least one week before weighing each plant individually. In October 2016, soil was sampled to a depth of 61 cm across each field site and analyzed for nitrate, phosphorus, potassium, sulfur, chloride, and copper concentrations (Agvise Laboratories, www.agvise.com).