

Article

Weak Effects of Biochar and Nitrogen Fertilization on Switchgrass Photosynthesis, Biomass, and Soil Respiration

Dafeng Hui ^{1,*} , Chih-Li Yu ¹, Qi Deng ^{1,2}, Priya Saini ³, Kenya Collins ¹ and Jason de Koff ³¹ Department of Biological Sciences, Tennessee State University, Nashville, TN 37209, USA; e390701@gmail.com (C.-L.Y.); dengqi@scbg.ac.cn (Q.D.); kayrose62@gmail.com (K.C.)² Key Laboratory of Vegetation Restoration and Management, South China Botanical Garden, The Chinese Academy of Sciences, Guangzhou 510650, China³ Department of Agricultural and Environmental Sciences, Tennessee State University, Nashville, TN 37209, USA; saini889@gmail.com (P.S.); jdekoff@tnstate.edu (J.d.K.)

* Correspondence: dhui@tnstate.edu; Tel.: +1-615-963-5777

Received: 31 July 2018; Accepted: 13 September 2018; Published: 14 September 2018



Abstract: Application of nitrogen (N) fertilizer plus biochar may increase crop yield, but how biochar will interact with N fertilization to affect bioenergy crop switchgrass physiology, biomass, and soil CO₂ emission (i.e., soil respiration) from switchgrass fields remains unclear. Here, we assessed this issue by conducting a field experiment near Nashville TN with two levels of biochar treatment (a control without biochar addition and biochar addition of 9 Mg ha⁻¹), and four N fertilization levels (0 kg N ha⁻¹, 17 kg N ha⁻¹, 34 kg N ha⁻¹, and 67 kg N ha⁻¹, labeled as ON, LN, MN, and HN, respectively). Results showed that both biochar addition and N fertilization did not influence switchgrass leaf photosynthesis and biomass, but biochar addition enhanced leaf transpiration, and reduced water use efficiency. Soil respiration was reduced by biochar addition, but significantly enhanced by N fertilization. Biochar and N fertilization interactively influenced soil respiration and seasonal variation of soil respiration was mostly controlled by soil temperature. Our results indicated that switchgrass can maintain high productivity without much N input, at least for several years. The findings from this study are useful to optimize N fertilization and biochar addition in the switchgrass fields for maintaining relatively high productive switchgrass biomass while reducing soil CO₂ emission.

Keywords: *Panicum virgatum*; biochar; nitrogen fertilization; productivity; greenhouse gas emissions

1. Introduction

With an increasing world population and improvements in living standards, the demand for energy and food has continued to increase over the past decades [1,2]. To reduce fossil fuel dependence and greenhouse gas emissions, and to meet the mandate of the US Energy Independence and Security Act (EISA) of 2007, the generation of biofuels from bioenergy crop biomass has been promoted [3–5]. One of the most promising bioenergy crops is switchgrass (*Panicum virgatum* L.) [5,6]. Switchgrass is a perennial C₄ grass that is native to North America and widely distributed from Canada to the USA and Mexico [7]. Switchgrass can grow in less productive soils under a broad range of environmental conditions, and requires minimal soil preparation and a relatively small amount of nitrogen (N) [8,9]. Numerous approaches have been proposed to improve switchgrass productivity while reducing environmental impacts [10–14].

Biochar, applied as a soil amendment, has been promoted as a climate change mitigation tool as it has the potential to reduce soil greenhouse gas emissions and increase soil carbon sequestration [14–16].

Thus, it is ideal to combine bioenergy crop cultivation with biochar application [16]. Biochar is a carbon-rich substance produced through pyrolysis, a process of heating a wide variety of biomass in a low-oxygen environment to temperatures above 250 °C [17,18]. Due to its low skeletal density and large surface area, biochar has been explored as an organic amendment to improve soil quality, enhance soil-water holding capacity, and benefit plant growth and crop productivity [4,16,17,19–21]. There is evidence to suggest that biochar addition can improve soil fertility [4,19,22,23] and crop production [2,15], and reduce greenhouse gas emissions [24–26].

Several meta-analyses demonstrated that biochar addition to soil generally increases crop productivity and yield [21,23,27–29]. The reasons could be that biochar improves soil pH, water holding capacity, and nutrient availability [25,30]. For example, biochar addition increased water holding capacity and plant available water, and increased yield of barley by 10% compared to no biochar addition [31]. However, some reports also found that crop yield did not improve after biochar addition [32]. For example, Tammeorg et al. [33] found that a soft wood biochar applied at 5–10 Mg ha^{−1} to a fertile sandy clay loam in a boreal climate did not increase the yield of wheat, turnip, and faba bean. The effects of biochar amendments on the crop yield may vary widely depending on biochar, soil properties, crop species, and climatic and environmental conditions [32]. Some researchers also suggest that biochar should be applied in combination with nutrients [34,35].

The relationship between soil respiration and biochar addition is critical to our understanding of the carbon sequestration potential of biochar-amended soil in cropland ecosystems [4,14,15]. Since soil respiration is one of the largest fluxes of the global terrestrial carbon cycle, soil management strategies such as biochar addition should be carefully evaluated with respect to changes in soil respiration [18]. Biochar has the potential to mitigate climate change by increasing soil carbon sequestration and reducing greenhouse gas emissions from croplands [4,17,18]. Using a meta-analysis, He et al. [36] reported that biochar application significantly increased soil respiration by 22.1% overall but suppressed soil CO₂ emission by 8.6% in N-fertilized soils. Responses of soil respiration to biochar addition may depend on N fertilization levels.

Fertilization is a common agricultural management practice to enhance biomass production and crop yields in croplands [11,12,18]. Biochar has exhibited its beneficial effects in the presence of N fertilization. Using barley as an example, biochar addition plus standard N application increased grain yield by 10% compared to no biochar addition, but biochar addition only decreased grain yield [31]. Grain yields of maize, sunflower, and winter wheat were all reduced with biochar application without N application compared to those with N application. Haider et al. [2] found increased maize biomass yield and N use efficiency with wood chip sieving biochar. However, the yield increase was attributed to soil moisture improvement rather than increased N availability to plants. Biochar and N fertilization may interactively influence leaf physiology, plant productivity, and yield. How biochar addition and N fertilization interactively influence switchgrass productivity and soil respiration has not been well investigated.

This study was designed to determine the effects of biochar, N fertilization, and their interactions on switchgrass leaf physiology, biomass, and soil respiration. The field experiment was set with two levels of biochar treatment (0 Mg ha^{−1} and 9 Mg ha^{−1}) and four levels of N fertilization (0 kg N ha^{−1}, 17 kg N ha^{−1}, 34 kg N ha^{−1}, and 67 kg N ha^{−1}). We hypothesized that (i) biochar addition would increase crop yield and reduce soil respiration overall, particularly in the N fertilized plots, and (ii) N fertilization would enhance crop yield and soil respiration. Specifically, the study addressed the following two research questions: whether there were significant main effects and interactions of biochar and N fertilization on switchgrass leaf physiology and soil respiration? If there were, how did switchgrass leaf photosynthesis and soil respiration vary among different biochar and N fertilization treatments?

2. Materials and Methods

2.1. Experimental Facility and Design

The experiment was conducted at Tennessee State University Agricultural Research and Education Center near Nashville, TN, USA (latitude 36.12' N, longitude 86.89' W, elevation 127.6 m). Soil type at the experimental site was a Lindsides silt loam soil (fine-silty, mixed, active, mesic fluvaquent Eutrudepts, occasionally flooded), slightly acidic (pH = 5.7), and average carbon (11.1 g kg⁻¹), N (1.0 g kg⁻¹), and phosphorus (150 mg kg⁻¹). Annual mean temperature is about 15 °C and total annual precipitation is 1200 mm [37].

Seeds of “Alamo” switchgrass were planted (6.9 kg pure live seed ha⁻¹) in a field plot in spring 2012, and switchgrass stands established in 2014. In 2013, N fertilization of 67 kg N ha⁻¹, the typical recommended application rate, was applied to improve the stand establishment [6]. The treatments of biochar and N fertilization started in 2014.

A completed randomized block design with four replications (blocks) was used in this study with two treatment factors, biochar addition and N fertilization. There were two levels of biochar, a control without biochar application (Control), and a biochar addition (Biochar) with 9 Mg ha⁻¹ biochar added to the plots (3 m × 5 m each). Four N fertilization rates included 0 kg N ha⁻¹, 17 kg N ha⁻¹, 34 kg N ha⁻¹, and 67 kg N ha⁻¹, labeled as ON, LN, MN, and HN, respectively. We set the highest N fertilization rate to the recommended N fertilization, and reduced the N fertilization amounts to see whether reduced N fertilization would suppress the switchgrass growth. Potassium was applied as potash (0-60-0) at a rate of 74 kg ha⁻¹ in 2014 due to low soil test results. It was also reapplied in 2016 at the same rate. Biochar was provided at no cost by CoolTerra (Greenwood Village, CO, USA). It was produced from pine wood at 500 °C for 10–15 min, and contains 63.5% C, 3.3% H, 0.4% N, and 17.4% O with 8.5% moisture, 2.0% ash, 6.1 pH, 1.4 g cm⁻³ bulk density, and 0.6 m³·m⁻³ porosity. Biochar was applied on the surface of soil and held by plant and roots. Nitrogen fertilizer was applied as ammonium nitrate (34-0-0) once in spring each year.

2.2. Field Measurements

Leaf photosynthesis and transpiration rates were measured using a Li-6400 Portable Photosynthesis System (Li-Cor Inc., Lincoln, NE, USA) similar to Hui et al. (2018) [5,37]. Five fully expanded healthy leaves were randomly selected and measured for leaf photosynthesis in each plot. Measurements were conducted six times from April to August 2016, and all measurements were conducted between 10:00 am and 3:00 pm. The photosynthetically active radiation (PAR) was set at 2000 μmol photon m⁻²·s⁻¹, and the CO₂ concentration of the air was set at ambient concentration of 380–400 ppm. Water use efficiency (WUE) was calculated as the ratio of leaf photosynthesis and transpiration. Aboveground biomass was measured after harvesting a 1.12 m swath through the middle of each plot, dried at 60 °C for more than 48 h to constant mass, and weighed. Each year, switchgrass was harvested once to ~15 cm height in December, following the first killing frost.

Soil respiration was measured using the Li-Cor 6400 infrared gas analyzer (Li-COR, Inc., Lincoln, NE, USA) connected to a Li-Cor 6400-09 soil respiration chamber (9.55 cm diameter) (Li-COR, Inc., Lincoln, NE, USA) following Deng et al. (2017) [38,39]. Four PVC soil collars (80 cm² in area and 5 cm in height) were permanently installed about 3 cm deep into the soil in each of the plots of two blocks at least 24 h before the first soil respiration measurements. Soil respiration was measured biweekly between 1:00 pm and 4:00 pm local time. Soil temperature at 5 cm below the soil surface was monitored with a thermocouple sensor attached to the respiration chamber during the soil respiration measurement. Volumetric soil moisture of the top 5 cm soil layer was measured near soil collars using a HydroSense (Campbell Scientific Inc., Logan, UT, USA) connected with a CS620 sensor at the same time that the soil respiration measurements were taken.

2.3. Statistical Analysis

Data analysis was performed using SAS software 9.3 (SAS Inc., Cary, NC, USA) [40]. The effects of biochar, N fertilization, their interaction, measurement time, and block on leaf photosynthesis and transpiration, WUE, soil temperature, soil moisture, and soil respiration were analyzed using repeated measures analysis of variance (ANOVA). Biomass production was analyzed using two-way ANOVA. PROC Mixed was used. When a significant effect at $\alpha = 0.05$ level was detected, least significant difference (LSD) was used for multiple comparisons. Regression analysis was conducted to develop the relationships between soil respiration and soil temperature and soil moisture.

3. Results and Discussion

3.1. Effects of Biochar, Nitrogen Fertilization, and Their Interaction, and Measurement Date on Switchgrass Physiology, Biomass, and Soil Respiration

Results of ANOVA showed that biochar and N fertilization did not influence leaf photosynthesis rate (Table 1). Nitrogen fertilization also did not influence transpiration and water use efficiency, but biochar addition significantly influenced transpiration and WUE (all $p < 0.01$). These results partially support our hypotheses. No significant interaction of biochar and N fertilization was found for leaf photosynthesis, transpiration, and WUE. Biochar addition did not influence soil temperature, but changed soil moisture, and both biochar addition and N fertilization influenced soil temperature and soil respiration (Table 1; Figure 1). Significant interactions were found for soil temperature ($p < 0.05$) and soil respiration ($p < 0.01$). Almost all variables showed significant changes among blocks and measurement dates, indicating that the sources of variation caused by the soil/environmental heterogeneity were effectively separated from the total variations of these variables.

Table 1. Significance of the effects of measurement date, biochar, N fertilization and interaction of biochar and N fertilization on leaf maximum photosynthesis (P_n), transpiration (E), water use efficiency (WUE), biomass, soil respiration (R), soil temperature (T), soil moisture (M) using repeated measures ANOVA. Numbers are F values. Stars indicate the level of significance (* $p < 0.05$, ** $p < 0.01$). – indicated not available.

Source	P_n	E	WUE	Biomass	Soil T	Soil M	Soil R
Block	24.44 **	36.9 **	132.0 **	1.2	564.1 **	0.05	36.7 **
Measurement Date	33.94 **	142.0 **	318.2 **	–	42133 **	191.0 **	527.0 **
Biochar	0.4	6.7 **	9.9 **	0.3	0.05	10.5 **	12.4 **
N fertilization	0.8	0.4	0.7	1.1	5.7 *	0.5	116.5 **
Biochar \times N fertilization	0.6	0.5	2.4	1.7	8.7 **	0.5	22.1 **

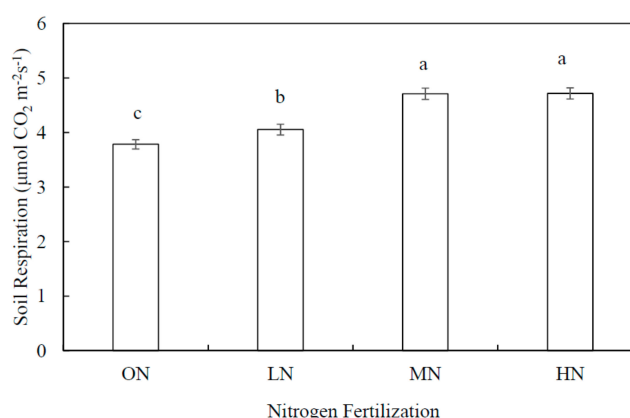


Figure 1. Effects of nitrogen fertilization on soil respiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$). ON: 0 kg N ha⁻¹; LN: 17 kg N ha⁻¹; MN: 34 kg N ha⁻¹; and HN: 67 kg N ha⁻¹. Each value is calculated from 160 pot measurements from two blocks in both the control and biochar treatments. Error bars are standard errors of means. Means with different labels are significantly different at $\alpha = 0.05$ level.

3.2. Multiple Comparisons of Biochar Addition and Nitrogen Fertilization on Switchgrass Physiology, and Soil Respiration

The biochar addition did not influence leaf photosynthesis, but enhanced leaf transpiration of switchgrass by 6.0%, reduced WUE by 3.5%, and did not change biomass compared to no biochar addition (Table 2). There are few studies related to switchgrass physiology change with biochar addition, and plants' responses to biochar addition have been reported to vary considerably [41]. While positive yield increases were found with application of biochar possessing nutrients such as poultry manure biochar [12,14,22,42,43], several studies reported biochar did not improve crop yield and biomass productivity [32,33,44,45], similar to our result. The increases of biomass in those studies was attributed to water retention of the biochar [43]. We did find that biochar enhanced soil moisture and leaf transpiration. Biomass was not enhanced, possibly due to reduced WUE in the biochar treatment. More studies are still needed to further evaluate biochar effects on plant physiology, growth, and biomass productivity.

Table 2. Multiple comparisons of switchgrass leaf transpiration (E, mmol H₂O m⁻²s⁻¹), water use efficiency (WUE, $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$), biomass (Mg ha⁻¹), soil respiration (R, $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), and soil moisture (M, % vol.) for biochar treatments under all nitrogen treatments in 2016. Means \pm standard error followed by the same letter in a column are not significantly different at the $\alpha = 0.05$ level.

Biochar Treatment	E	WUE	Biomass	Soil M	Soil R
Control	4.64 \pm 0.08 a	5.09 \pm 0.06 a	15.32 \pm 0.93 a	22.79 \pm 0.31 a	4.39 \pm 0.07 a
Biochar addition	4.92 \pm 0.09 b	4.91 \pm 0.06 b	16.40 \pm 0.95 a	23.48 \pm 0.31 b	4.24 \pm 0.07 b

Soil temperature was not influenced by the biochar addition, but soil moisture was significantly stimulated by 3.0% and soil respiration was reduced by 3.4% compared to the no biochar addition plots (Table 2). Biochar addition reduced soil temperature under the LN treatment (Figure 2a), but increased soil temperature under the MN, resulting in no change in soil temperature. While most studies found that biochar increases soil water content [46], different responses of soil respiration to biochar addition have been reported [14,16,47]. In this study, the reduction of soil respiration in the biochar addition plots mostly occurred under the LN and ON treatments, while soil respiration was increased under the HN treatment (Figure 2b). A short-term increase in soil respiration is often reported, but an incubation study with an Australian agricultural soil showed that only two out of six biochar additions increased soil respiration in the first 28 days of incubation [47]. Over the long-term, most studies reported reduced soil respiration, similar to our result [14,16,23,48–52]. The reduction of soil respiration in this study was likely due to sorption of labile carbon onto the surface of biochar.

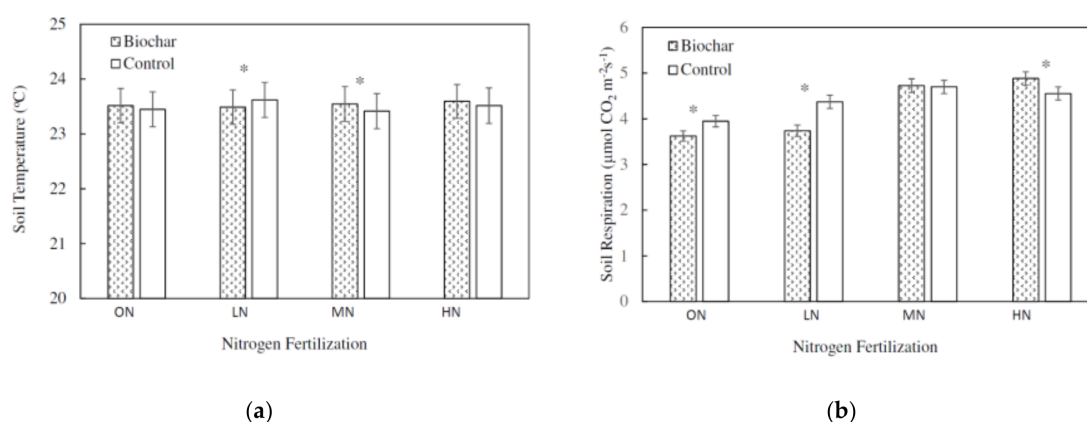


Figure 2. Effects of biochar addition on (a) soil temperature ($^{\circ}\text{C}$) and (b) soil respiration ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) under different nitrogen fertilization levels. ON: 0 kg N ha⁻¹; LN: 17 kg N ha⁻¹; MN: 34 kg N ha⁻¹; and HN: 67 kg N ha⁻¹. Each value is calculated from 80 pot measurements in two blocks. Error bars are standard errors of means. * indicates significant difference between no biochar and biochar addition at $\alpha = 0.05$ level.

It was a little bit surprising that N fertilization did not influence leaf photosynthesis, transpiration, WUE, and biomass in this study (Table 1), as N is a critical and mostly limiting nutrient for production of biomass [6,52]. Indeed, many studies have been conducted with N fertilization on switchgrass and reported positive responses [53–56]. For example, Vogel et al. [12] found that biomass yield responded positively in general to N fertilization rate from 0 to 300 kg ha⁻¹, and optimal biomass yields were obtained when switchgrass was fertilized with 120 kg N ha⁻¹. Muir et al. [11] reported biomass production of Alamo switchgrass generally increased with N rate from 0 to 250 kg ha⁻¹ at two locations in Texas, and application of 168 kg N ha⁻¹ produced biomass yields of 10.7 to 14.5 Mg ha⁻¹ across 3 years to 6 years of research on Alamo, TX, USA. However, some studies did not find a significant influence of N fertilization on switchgrass biomass production, similar to our results [9,10,57,58]. Giannoulis et al. [59] applied N fertilization from 0 kg N ha⁻¹, 80 kg N ha⁻¹, 160 kg N ha⁻¹ to 240 kg N ha⁻¹ to switchgrass at two locations over two years, and found that switchgrass Alamo dry yield was mostly not influenced by N fertilization (except two high N fertilization rates enhanced biomass compared to two low N at one site in one year). Thomason et al. [60] reported that applying 0 N produced almost as much total biomass as 448 kg N ha⁻¹. Makaju et al. [61] showed that switchgrass can grow well without N input for over six years and the 3-yr mean dry matter yield of winter harvests was 5.94 Mg ha⁻¹.

This limited response to N could be explained by a few reasons. (1) Switchgrass is adapted to low N conditions through evolution and can survive in marginal soils [60]. (2) Switchgrass can grow deep roots once the stands are established, and nitrogen in roots and crowns may accumulate [62,63]. (3) Other factors such as antecedent soil N, soil mineralization rates, switchgrass cultivar, and soil types may influence the effects of N on switchgrass biomass. In this study, N that was applied to facilitate the establishment of switchgrass stands may have accumulated in the soil and be used by switchgrass in the following treatment years. Findings from this study support that switchgrass tolerates low soil fertility and grows well without much N input for a short period of time. To sustain biomass production over the long-term, adequate N fertilization such as 67 kg N ha⁻¹ and proper management would be required [6,52,55].

Nitrogen fertilization did not influence soil moisture due to no change in plant biomass, but high N fertilization rate enhanced soil respiration (Table 1; Figure 1). Soil temperature was slightly higher in the LN treatment compared to other N treatments. Nitrogen fertilization enhanced soil respiration at the two high levels (MN and HN) probably due to stimulated microbial activities, and the lowest soil respiration occurred in the control plot (ON) (Figure 1). The enhancement of soil respiration by N fertilization has been reported in grasslands and croplands. For example, using a meta-analysis, Zhou et al. [64] reported N fertilization enhanced soil respiration by 7.8% and 12.4% in grasslands and croplands, respectively. However, the results of N fertilization on soil respiration were not conclusive. Schmer et al. [65] reported that the N fertilization (0 kg N ha⁻¹ and 67 kg N ha⁻¹) applied to switchgrass did not impact soil respiration in the Northern Great Plains, similar to a few other studies [66,67]. Application of different N fertilizers may also influence the soil respiration response. Lee et al. [68] reported that manure applied to switchgrass leads to an increase in soil respiration while N fertilization with NH₄NO₃ did not influence soil respiration, a result that is different from ours.

3.3. Seasonal Variations of Leaf Photosynthesis, Transpiration, WUE, Soil Temperature, Moisture, and Respiration

Leaf photosynthesis varied along time, decreased from 22.9 µmol CO₂ m⁻²s⁻¹ in the control and 23.1 µmol CO₂ m⁻²s⁻¹ in the biochar treatment in early growing season to 20.3 µmol CO₂ m⁻²s⁻¹ in the control and 19.4 µmol CO₂ m⁻²s⁻¹ in the biochar treatment in late June, and increased in July and decreased again until the end of the growing season (Figure 3a). Transpiration reached the highest rate in later July, and reduced after then for both the control and biochar treatments. WUE was relatively higher in the early growing season (6.3 µmol CO₂ mmol⁻¹ H₂O in the control and 5.9 µmol CO₂ mmol⁻¹ H₂O in the biochar treatment in early June), and decreased after July. The patterns and magnitudes of leaf photosynthesis, transpiration, and WUE were similar to

those reported in previous studies. For example, McLaughlin et al. [69] evaluated 25 switchgrass accessions and found that leaf photosynthesis varied from 17.5 to 30.8 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$, transpiration ranged from 6.2 to 13.0 $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$, and WUE ranged from 2.1 to 3.6 $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$. Leaf photosynthesis varied from about 15 to 28 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ among 13 genotypes of switchgrass [70]. Hartman and Nippert [71] reported that leaf photosynthesis varied from 10 to 30 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$, and transpiration varied from 2.6 to 7.4 $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$. Hartman et al. [72] reported that ranges of leaf photosynthesis, transpiration, and WUE were 14–22 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$, 3.4–4.8 $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$, and 3.2–4.0 $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ among different ecotypes of switchgrass.

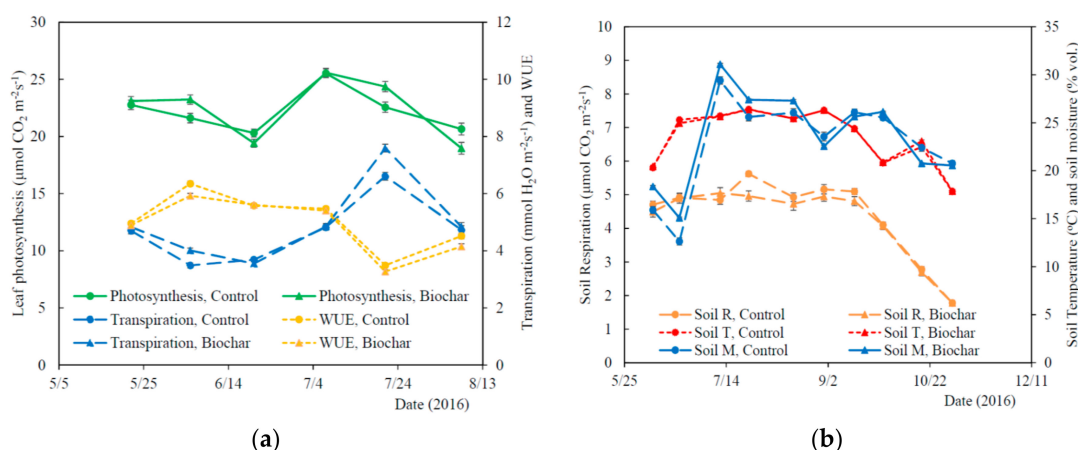


Figure 3. Seasonal variations of (a) leaf photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), transpiration ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$), and water use efficiency (WUE, $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$), (b) seasonal variations of soil temperature (T, $^{\circ}\text{C}$), soil moisture (M, % vol.), and soil respiration (R, $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$). Data points are means of the control or biochar treatment. Each data point is calculated from 60 leaf to 75 leaf measurements for leaf photosynthesis, transpiration, and WUE in four blocks, and 32 pot measurements for soil temperature, moisture, and respiration in two blocks. Error bars are standard errors of means.

Soil temperature showed a seasonal variation, with the highest values between early June and late August, which then decreased after September for both the control and biochar treatment (Figure 3b). Soil moisture had a larger fluctuation than soil temperature, and the pattern was similar to soil temperature, except in the early growing season. Soil moisture in the biochar treatment was higher than that in the control in the early season. The seasonal pattern of soil respiration was very similar to soil temperature (Figure 3b). Seasonal variation of soil respiration and soil temperature are a common phenomenon [14,38]. Soil moisture often varied more than soil temperature, as precipitation pattern and soil type had significant influences on soil moisture [38].

3.4. Relationships Between Soil Respiration and Soil Temperature, Soil Moisture

Soil respiration increased with soil temperature following an exponential model (Figure 4). Soil temperature sensitivity of respiration ($Q_{10} = \exp(10 \times b)$) was 2.53. Soil respiration tended to respond to soil moisture following a quadratic polynomial model (Figure 4). Soil respiration was the lowest when soil moisture was 20% and increased with increasing soil moisture. Our results were supported by a recent study that found seasonal soil respiration in a semiarid farmland of switchgrass was strongly dependent on soil temperature, rather than soil moisture [14]. Lee et al. [68] also found that soil temperature was the most significant factor controlling soil respiration, and soil moisture was not a limiting factor. The exponential relationship between soil respiration and soil temperature accounted for 60% of soil respiration variation. The Q_{10} value of 2.7 was close to the Q_{10} value in this study.

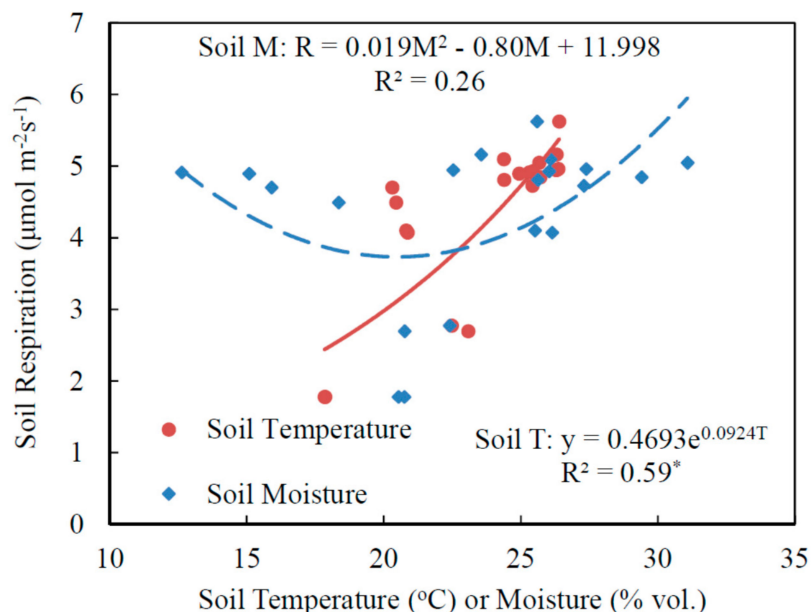


Figure 4. Relationships between soil respiration ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) and soil temperature (T , $^{\circ}\text{C}$), and soil moisture (M , % vol.). Data points are means of treatments. Each data point is calculated from 32 pot measurements in two blocks. * indicates significant at $\alpha = 0.05$ level.

4. Conclusions

To conclude, we found that biochar addition did not influence leaf physiology and biomass productivity of switchgrass, but reduced soil respiration. While N fertilization did not influence switchgrass leaf photosynthesis and biomass, it enhanced soil respiration. These findings partially support our hypotheses. The biochar treatment did not enhance soil water retention in this study, and did not improve biomass. The limited responses of switchgrass to N application could be that switchgrass is adapted to low N conditions through evolution. The reduced soil respiration in the biochar treatment might be caused by sorption of labile carbon onto the surface of biochar while the enhanced soil respiration in the N application was probably due to enhanced soil microbial activities. Our results indicated that switchgrass tolerates low soil fertility and grows normally under lower or no N fertilization, at least for several years. To maintain high productivity and low soil respiration over a long term, a relatively lower N fertilization rate plus biochar addition is recommended.

Author Contributions: Conceptualization, D.H. and J.d.K.; Data curation, C.-L.Y., Q.D. and K.C.; Formal analysis, D.H., C.-L.Y., Q.D. and K.C.; Funding acquisition, D.H. and J.d.K.; Investigation, C.-L.Y. and Q.D.; Methodology, D.H., P.S. and J.d.K.; Resources, P.S.; Writing—original draft, D.H.; Writing—review & editing, J.d.K.

Funding: This research was supported by the USDA Evans-Allen and Capacity Building Grant, and NSF TIP project. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the experimental design, data collection and analyses, interpretation of results, the writing of the manuscript, and the decision to publish the results.

References

1. Gelfand, I.; Sahajpal, R.; Zhang, X.; Izaurralde, R.C.; Gross, K.L.; Robertson, G.P. Sustainable bioenergy production from marginal lands in the US midwest. *Nature* **2013**, *493*, 514–517. [[CrossRef](#)] [[PubMed](#)]
2. Haider, G.; Steffens, D.; Müller, C.; Kammann, C.I. Standard extraction methods may underestimate nitrate stocks captured by field-aged biochar. *J. Environ. Qual.* **2016**, *45*, 1196–1204. [[CrossRef](#)] [[PubMed](#)]
3. Goldemberg, J. Ethanol for a sustainable energy future. *Science* **2007**, *315*, 808–810. [[CrossRef](#)] [[PubMed](#)]

4. Zhang, Y.; Lin, F.; Wang, X.; Zou, J.; Liu, S. Annual accounting of net greenhouse gas balance response to biochar addition in a coastal saline bioenergy cropping system in China. *Soil Tillage Res.* **2016**, *158*, 39–48. [[CrossRef](#)]
5. Hui, D.; Yu, C.-L.; Deng, Q.; Dzantor, E.K.; Zhou, S.; Dennis, S.; Sauve, R.; Johnson, T.L.; Fay, P.A.; Shen, W.; et al. Effects of precipitation changes on switchgrass photosynthesis, growth, and biomass: A mesocosm experiment. *PLoS ONE* **2018**, *13*, e0192555. [[CrossRef](#)] [[PubMed](#)]
6. McLaughlin, S.B.; Adams Kszos, L. Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. *Biomass Bioenergy* **2005**, *28*, 515–535. [[CrossRef](#)]
7. Sanderson, M.A.; Adler, P.R.; Boateng, A.A.; Casler, M.D.; Sarath, G. Switchgrass as a biofuels feedstock in the USA. *Can. J. Plant Sci.* **2006**, *86*, 1315–1325. [[CrossRef](#)]
8. Lewandowski, I.; Scurlock, J.M.O.; Lindvall, E.; Christou, M. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass Bioenergy* **2003**, *25*, 335–361. [[CrossRef](#)]
9. Owens, V.N.; Viands, D.R.; Mayton, H.S.; Fike, J.H.; Farris, R.; Heaton, E.; Bransby, D.I.; Hong, C.O. Nitrogen use in switchgrass grown for bioenergy across the USA. *Biomass Bioenergy* **2013**, *58*, 286–293. [[CrossRef](#)]
10. Sanderson, M.A.; Reed, R.L. Switchgrass growth and development: Water, nitrogen, and plant density effects. *J. Range Manag.* **2000**, *53*, 221–227. [[CrossRef](#)]
11. Muir, J.P.; Sanderson, M.A.; Ocumpaugh, W.R.; Jones, R.M.; Reed, R.L. Biomass production of ‘Alamo’ switchgrass in response to nitrogen, phosphorus, and row spacing. *Agron. J.* **2001**, *93*, 896–901. [[CrossRef](#)]
12. Vogel, K.P.; Brejda, J.J.; Walters, D.T.; Buxton, D.R. Switchgrass biomass production in the Midwest USA. *Agron. J.* **2002**, *94*, 413–420. [[CrossRef](#)]
13. Filiberto, D.; Gaunt, J. Practicality of biochar additions to enhance soil and crop productivity. *Agriculture* **2013**, *3*, 715–725. [[CrossRef](#)]
14. Shen, Y.; Zhu, L.; Cheng, H.; Yue, S.; Li, S. Effects of biochar application on CO₂ emissions from a cultivated soil under semiarid climate conditions in northwest China. *Sustainability* **2017**, *9*, 1482. [[CrossRef](#)]
15. Woolf, D.; Amonette, J.E.; Street-Perrott, F.A.; Lehmann, J.; Joseph, S. Sustainable biochar to mitigate global climate change. *Nat. Commun.* **2010**, *1*, 56. [[CrossRef](#)] [[PubMed](#)]
16. Case, S.D.C.; McNamara, N.P.; Reay, D.S.; Whitaker, J. The effect of biochar addition on N₂O and CO₂ emissions from a sandy loam soil—The role of soil aeration. *Soil Biol. Biochem.* **2012**, *51*, 125–134. [[CrossRef](#)]
17. Lehmann, J.; Rillig, M.C.; Thies, J.; Masiello, C.A.; Hockaday, W.C.; Crowley, D. Biochar effects on soil biota—A review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836. [[CrossRef](#)]
18. Hawthorne, I.; Johnson, M.S.; Jassal, R.S.; Black, T.A.; Grant, N.J.; Smukler, S.M. Application of biochar and nitrogen influences fluxes of CO₂, CH₄ and N₂O in a forest soil. *J. Environ. Manag.* **2017**, *192*, 203–214. [[CrossRef](#)] [[PubMed](#)]
19. Glaser, B.; Lehmann, J.; Zech, W. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—A review. *Biol. Fertil. Soils* **2002**, *35*, 219–230. [[CrossRef](#)]
20. Chan, K.Y.; Van Zwieten, L.; Meszaros, I.; Downie, A.; Joseph, S. Agronomic values of greenwaste biochar as a soil amendment. *Aust. J. Soil Res.* **2007**, *45*, 629–634. [[CrossRef](#)]
21. Hol, W.H.G.; Vestergård, M.; ten Hooven, F.; Duyts, H.; van de Voorde, T.F.J.; Bezemer, T.M. Transient negative biochar effects on plant growth are strongest after microbial species loss. *Soil Biol. Biochem.* **2017**, *115*, 442–451. [[CrossRef](#)]
22. Spokas, K.A.; Koskinen, W.C.; Baker, J.M.; Reicosky, D.C. Impacts of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. *Chemosphere* **2009**, *77*, 574–581. [[CrossRef](#)] [[PubMed](#)]
23. Liu, Y.; Yang, M.; Wu, Y.; Wang, H.; Chen, Y.; Wu, W. Reducing CH₄ and CO₂ emissions from waterlogged paddy soil with biochar. *J. Soils Sediments* **2011**, *11*, 930–939. [[CrossRef](#)]
24. Kammann, C.; Ratering, S.; Eckhard, C.; Müller, C. Biochar and hydrochar effects on greenhouse gas (carbon dioxide, nitrous oxide, and methane) fluxes from soils. *J. Environ. Qual.* **2012**, *41*, 1052–1066. [[CrossRef](#)] [[PubMed](#)]
25. Cayuela, M.L.; van Zwieten, L.; Singh, B.P.; Jeffery, S.; Roig, A.; Sánchez-Monedero, M.A. Biochar’s role in mitigating soil nitrous oxide emissions: A review and meta-analysis. *Agric. Ecosyst. Environ.* **2014**, *191*, 5–16. [[CrossRef](#)]

26. Van Zwieten, L.; Kimber, S.; Morris, S.; Chan, K.Y.; Downie, A.; Rust, J.; Joseph, S.; Cowie, A. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant Soil* **2009**, *327*, 235–246. [[CrossRef](#)]
27. Jeffery, S.; Verheijen, F.G.A.; van der Velde, M.; Bastos, A.C. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agric. Ecosyst. Environ.* **2011**, *144*, 175–187. [[CrossRef](#)]
28. Novotny, E.H.; Maia, C.M.B.D.F.; Carvalho, M.T.D.M.; Madari, B.E. Biochar: Pyrogenic carbon for agricultural use—A critical review. *Rev. Bras. Ciênc. Solo* **2015**, *39*, 321–344. [[CrossRef](#)]
29. Cao, C.T.N.; Farrell, C.; Kristiansen, P.E.; Rayner, J.P. Biochar makes green roof substrates lighter and improves water supply to plants. *Ecol. Eng.* **2014**, *71*, 368–374. [[CrossRef](#)]
30. De Voorde, T.F.J.V.; Bezemer, T.M.; Van Groenigen, J.W.; Jeffery, S.; Mommer, L. Soil biochar amendment in a nature restoration area: Effects on plant productivity and community composition. *Ecol. Appl.* **2014**, *24*, 1167–1177. [[CrossRef](#)]
31. Karer, J.; Wimmer, B.; Zehetner, F.; Kloss, S.; Soja, G. Biochar application to temperate soils: Effects on nutrient uptake and crop yield under field conditions. *Agric. Food Sci.* **2013**, *22*, 390–403. [[CrossRef](#)]
32. Haider, G.; Steffens, D.; Moser, G.; Müller, C.; Kammann, C.I. Biochar reduced nitrate leaching and improved soil moisture content without yield improvements in a four-year field study. *Agric. Ecosyst. Environ.* **2017**, *237*, 80–94. [[CrossRef](#)]
33. Tammgeorg, P.; Simojoki, A.; Mäkelä, P.; Stoddard, F.L.; Alakukku, L.; Helenius, J. Biochar application to a fertile sandy clay loam in boreal conditions: Effects on soil properties and yield formation of wheat, turnip rape and faba bean. *Plant Soil* **2013**, *374*, 89–107. [[CrossRef](#)]
34. Glaser, B.; Wiedner, K.; Seelig, S.; Schmidt, H.-P.P.; Gerber, H. Biochar organic fertilizers from natural resources as substitute for mineral fertilizers. *Agron. Sustain. Dev.* **2015**, *35*, 667–678. [[CrossRef](#)]
35. Liu, J.; Schulz, H.; Brandl, S.; Miehtke, H.; Huwe, B.; Glaser, B. Short-term effect of biochar and compost on soil fertility and water status of a Dystric Cambisol in NE Germany under field conditions. *J. Plant Nutr. Soil Sci.* **2012**, *175*, 698–707. [[CrossRef](#)]
36. He, Y.; Zhou, X.; Jiang, L.; Li, M.; Du, Z.; Zhou, G.; Shao, J.; Wang, X.; Xu, Z.; Hosseini Bai, S.; et al. Effects of biochar application on soil greenhouse gas fluxes: A meta-analysis. *GCB Bioenergy* **2016**, *9*, 743–755. [[CrossRef](#)]
37. Deng, Q.; Aras, S.; Yu, C.-L.; Dzantor, E.K.; Fay, P.A.; Luo, Y.; Shen, W.; Hui, D. Effects of precipitation changes on aboveground net primary production and soil respiration in a switchgrass field. *Agric. Ecosyst. Environ.* **2017**, *248*, 29–37. [[CrossRef](#)]
38. Deng, Q.; Hui, D.; Zhang, D.; Zhou, G.; Liu, J.; Liu, S.; Chu, G.; Li, J. Effects of precipitation increase on soil respiration: A three-year field experiment in subtropical forests in China. *PLoS ONE* **2012**, *7*, e41493. [[CrossRef](#)]
39. Yu, C.-L.; Hui, D.; Deng, Q.; Dzantor, E.K.; Fay, P.A.; Shen, W.; Luo, Y. Responses of switchgrass soil respiration and its components to precipitation gradient in a mesocosm study. *Plant Soil* **2017**, *420*, 105–117. [[CrossRef](#)]
40. Hui, D.; Jiang, C. *Practical SAS Usage*; Beijing University of Aeronautics & Astronautics Press: Beijing, China, 1996.
41. Clough, T.; Condon, L.; Kammann, C.; Müller, C. A review of biochar and soil nitrogen dynamics. *Agronomy* **2013**, *3*, 275–293. [[CrossRef](#)]
42. Uzoma, K.C.; Inoue, M.; Andry, H.; Fujimaki, H.; Zahoor, A.; Nishihara, E. Effect of cow manure biochar on maize productivity under sandy soil condition. *Soil Use Manag.* **2011**, *27*, 205–212. [[CrossRef](#)]
43. Allaire, S.E.; Baril, B.; Vanasse, A.; Lange, S.F.; MacKay, J.; Smith, D.L. Carbon dynamics in a biochar-amended loamy soil under switchgrass. *Can. J. Soil Sci.* **2015**, *95*, 1–13. [[CrossRef](#)]
44. Prendergast-Miller, M.T.; Duvall, M.; Sohi, S.P. Localisation of nitrate in the rhizosphere of biochar-amended soils. *Soil Biol. Biochem.* **2011**, *43*, 2243–2246. [[CrossRef](#)]
45. Unger, R.; Killorn, R. Effect of the application of biochar on selected soil chemical properties, corn grain, and biomass yields in Iowa. *Commun. Soil Sci. Plant Anal.* **2011**, *42*, 2441–2451. [[CrossRef](#)]
46. Sohi, S.P.; Krull, E.; Lopez-Capel, E.; Bol, R. A review of biochar and its use and function in soil. In *Advances in Agronomy*; Elsevier: New York, NY, USA, 2010; pp. 47–82.
47. Van Zwieten, L.; Kimber, S.; Morris, S.; Downie, A.; Berger, E.; Rust, J.; Scheer, C. Influence of biochars on flux of N₂O and CO₂ from ferrosol. *Aust. J. Soil Res.* **2010**, *48*, 555–568. [[CrossRef](#)]

48. Spokas, K.A.; Cantrell, K.B.; Novak, J.M.; Archer, D.W.; Ippolito, J.A.; Collins, H.P.; Boateng, A.A.; Lima, I.M.; Lamb, M.C.; McAloon, A.J.; et al. Biochar: A synthesis of its agronomic impact beyond carbon sequestration. *J. Environ. Qual.* **2012**, *41*, 973–989. [[CrossRef](#)] [[PubMed](#)]
49. Pedroso, G.M.; van Kessel, C.; Six, J.; Putnam, D.H.; Linquist, B.A. Productivity, ¹⁵N dynamics and water use efficiency in low- and high-input switchgrass systems. *GCB Bioenergy* **2014**, *6*, 704–716. [[CrossRef](#)]
50. Bailey, V.L.; Fansler, S.J.; Smith, J.L.; Bolton, H. Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. *Soil Biol. Biochem.* **2011**, *43*, 296–301. [[CrossRef](#)]
51. Fernández, J.M.; Nieto, M.A.; López-de-Sá, E.G.; Gascó, G.; Méndez, A.; Plaza, C. Carbon dioxide emissions from semi-arid soils amended with biochar alone or combined with mineral and organic fertilizers. *Sci. Total Environ.* **2014**, *482–483*, 1–7. [[CrossRef](#)] [[PubMed](#)]
52. Sadeghpour, A.; Hashemi, M.; Jahanzad, E.; Herbert, S.J. Switchgrass stand density and yield as influenced by seedbed preparation methods in a sandy loam soil. *BioEnergy Res.* **2015**, *8*, 1840–1846. [[CrossRef](#)]
53. Lemus, R.; Parrish, D.J.; Wolf, D.D. Switchgrass cultivar/ecotype selection and management for biofuels in the upper southeast USA. *Sci. World J.* **2014**, *2014*, 1–10. [[CrossRef](#)] [[PubMed](#)]
54. Guretzky, J.A.; Biermacher, J.T.; Cook, B.J.; Kering, M.K.; Mosali, J. Switchgrass for forage and bioenergy: Harvest and nitrogen rate effects on biomass yields and nutrient composition. *Plant Soil* **2010**, *339*, 69–81. [[CrossRef](#)]
55. Prayogo, C.; Jones, J.E.; Baeyens, J.; Bending, G.D. Impact of biochar on mineralisation of C and N from soil and willow litter and its relationship with microbial community biomass and structure. *Biol. Fertil. Soils* **2013**, *50*, 695–702. [[CrossRef](#)]
56. Waramit, N.; Moore, K.J.; Heaton, E. Nitrogen and harvest date affect developmental morphology and biomass yield of warm-season grasses. *GCB Bioenergy* **2013**, *6*, 534–543. [[CrossRef](#)]
57. Hartnett, D.C. Regulation of clonal growth and dynamics of *Panicum virgatum* (Poaceae) in tallgrass prairie: Effects of neighbor removal and nutrient addition. *Am. J. Bot.* **1993**, *80*, 1114–1120. [[CrossRef](#)]
58. Aliero, A.A.; Abdullahi, A.A.; Aliero, B.L.; Zuru, A.A. Effects of irrigation regime, organic and inorganic mineral source on growth and yield components of switchgrass (*Panicum virgatum* L.) in upland and lowland conditions in Sokoto, Nigeria. *Pak. J. Biol. Sci.* **2013**, *16*, 51–58. [[CrossRef](#)]
59. Giannoulis, K.D.; Karyotis, T.; Sakellariou-Makrantonaki, M.; Bastiaans, L.; Struik, P.C.; Danalatos, N.G. Switchgrass biomass partitioning and growth characteristics under different management practices. *NJAS Wagening. J. Life Sci.* **2016**, *78*, 61–67. [[CrossRef](#)]
60. Thomason, W.E.; Raun, W.R.; Johnson, G.V.; Taliaferro, C.M.; Freeman, K.W.; Wynn, K.J.; Mullen, R.W. Switchgrass response to harvest frequency and time and rate of applied nitrogen. *J. Plant Nutr.* **2005**, *27*, 1199–1226. [[CrossRef](#)]
61. Makaju, S.O.; Wu, Y.Q.; Zhang, H.; Kakani, V.G.; Taliaferro, C.M.; Anderson, M.P. Switchgrass winter yield, year-round elemental concentrations, and associated soil nutrients in a zero input environment. *Agron. J.* **2013**, *105*, 463–470. [[CrossRef](#)]
62. Parrish, D.J.; Fike, J.H. The biology and agronomy of switchgrass for biofuels. *Crit. Rev. Plant Sci.* **2005**, *24*, 423–459. [[CrossRef](#)]
63. Barney, J.N.; Mann, J.J.; Kyser, G.B.; Blumwald, E.; Van Deynze, A.; DiTomaso, J.M. Tolerance of switchgrass to extreme soil moisture stress: Ecological implications. *Plant Sci.* **2009**, *177*, 724–732. [[CrossRef](#)]
64. Zhou, L.; Zhou, X.; Zhang, B.; Lu, M.; Luo, Y.; Liu, L.; Li, B. Different responses of soil respiration and its components to nitrogen addition among biomes: A meta-analysis. *Glob. Chang. Biol.* **2014**, *20*, 2332–2343. [[CrossRef](#)] [[PubMed](#)]
65. Schmer, M.R.; Vogel, K.P.; Mitchell, R.B.; Perrin, R.K. Net energy of cellulosic ethanol from switchgrass. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 464–469. [[CrossRef](#)] [[PubMed](#)]
66. Gregorich, E.G.; Rochette, P.; McGuire, S.; Liang, B.C.; Lessard, R. Soluble organic carbon and carbon dioxide fluxes in maize fields receiving spring-applied manure. *J. Environ. Qual.* **1998**, *27*, 209–214. [[CrossRef](#)]
67. Mbonimpa, E.G.; Hong, C.O.; Owens, V.N.; Lehman, R.M.; Osborne, S.L.; Schumacher, T.E.; Clay, D.E.; Kumar, S. Nitrogen fertilizer and landscape position impacts on CO₂ and CH₄ fluxes from a landscape seeded to switchgrass. *GCB Bioenergy* **2014**, *7*, 836–849. [[CrossRef](#)]
68. Lee, D.K.; Doolittle, J.J.; Owens, V.N. Soil carbon dioxide fluxes in established switchgrass land managed for biomass production. *Soil Biol. Biochem.* **2007**, *39*, 178–186. [[CrossRef](#)]

69. McLaughlin, S.B.; Kiniry, J.R.; Taliaferro, C.M.; De La Torre Ugarte, D. Projecting yield and utilization potential of switchgrass as an energy crop. In *Advances in Agronomy*; Elsevier: New York, NY, USA, 2006; pp. 267–297.
70. Cordero, Á.; Osborne, B.A. Variation in leaf-level photosynthesis among switchgrass genotypes exposed to low temperatures does not scale with final biomass yield. *GCB Bioenergy* **2016**, *9*, 144–152. [[CrossRef](#)]
71. Hartman, J.C.; Nippert, J.B. Physiological and growth responses of switchgrass (*Panicum virgatum* L.) in native stands under passive air temperature manipulation. *GCB Bioenergy* **2012**, *5*, 683–692. [[CrossRef](#)]
72. Hartman, J.C.; Nippert, J.B.; Springer, C.J. Ecotypic responses of switchgrass to altered precipitation. *Funct. Plant Biol.* **2012**, *39*, 126–136. [[CrossRef](#)]



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).