GLOBAL CHANGE BIOLOGY
BIOENERGY

GCB Bioenergy (2015), doi: 10.1111/gcbb.12277

Elevated atmospheric [CO_2] stimulates sugar accumulation and cellulose degradation rates of rice straw

CHUNWU ZHU[1], XI XU[1†], DAN WANG[2], JIANGUO ZHU[1], GANG LIU[1] and SAMAN SENEWEERA[3]

[1]*State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, NO.71 East Beijing Road, Nanjing 210008, China,* [2]*International Center for Ecology, Meteorology and Environment, School of Applied Meteorology, Nanjing University of Information Science and Technology, Nanjing 210044, China,* [3]*Centre for Systems Biology, University of Southern Queensland, Toowoomba, Qld 4350, Australia*

Abstract

Rice straw can serve as potential material for bioenergy production. However, the quantitative effects of increasing atmospheric carbon dioxide concentration [CO_2] on rice straw quality and the resulting consequences for bioenergy utilization are largely unknown. In this study, two rice varieties, WYJ and LY, that have been shown previously to have a weak and strong stimulatory response to rising [CO_2], respectively, were grown with and without additional CO_2 at China free-air carbon dioxide enrichment (FACE) platform. Qualitative and quantitative measurements in response to [CO_2] included straw biomass (including leaf, sheath, and stem), the concentration of nonstructural and structural carbohydrates, the syringyl-to-guaiacyl (S/G) ratio of lignin, glucose and xylose release from structural carbohydrate, total sugar release by enzymatic saccharification, and sugar yield and the ratio of cellulose and hemicellulose degradation. Elevated [CO_2] significantly increased straw biomass and nonstructural carbohydrate contents while enhancing the degraded ratio of structural carbohydrates as indicated by the decreased lignin content and increased S/G ratio. Overall, total sugar yield (g m^{-2}) in rice straw significantly increased by 27.1 and 57% for WYJ and LY at elevated [CO_2], respectively. These findings, while preliminary, suggest that rice straw quality and potential biofuel utilization may improve as a function of rising [CO_2].

Keywords: biofuel, elevated [CO_2], rice, saccharification, straw, sugar release

Received 23 December 2014; revised version received 17 April 2015 and accepted 29 April 2015

Introduction

Climate change and energy security have driven renewable energy production to the top of global agendas (Karp & Shield, 2008). Potentially, plant-based sources of bioenergy (e.g., ethanol) could lower CO_2 emissions and help mitigate climate change impacts (Cuevas *et al.*, 2010; Erdei *et al.*, 2010).

Rice is the dominant source of calories for a large portion of the human population (Shimono & Bunce, 2009). It is cultivated globally in about 160 million hectares; in addition to a grain production about 740 million tons, it also produces about ~730 million tons of straw as a by-product (Wang *et al.*, 2011; FAO, 2014). As demand for rice is expected to increase in many countries, the availability of rice straw will also increase (Yoswathana *et al.*, 2010; Lim *et al.*, 2012).

At present, excess rice straw is often subject to open-field burning following harvest (Oanh *et al.*, 2011). Open-field burning wastes energy while resulting in environmental and public health concerns. Alternatively, rice straw represents a potential resource that could be used for biofuel and energy production (Domínguez-Escribá & Porcar, 2010; Lim *et al.*, 2012). For example, it has been estimated that rice straw has the potential to produce 205 billion liters of bioethanol per year, equivalent to 5% of current fossil fuel energy production (Yoswathana *et al.*, 2010).

Rising [CO_2] in addition to its role as a greenhouse gas is the sole source of carbon for photosynthesis, and its increase has been shown to effect rice growth and grain yield (Zhu *et al.*, 2014). However, the impact of rising [CO_2] on quantitative and/or qualitative changes and the subsequent consequences for utilization of rice straw as a source of biofuel have, heretofore, not been investigated.

Higher levels of [CO_2] could alter rice straw quantity by stimulating growth, with the degree of stimulation being cultivar specific (Ziska *et al.*, 1996). Rising CO_2

Correspondence: Chunwu Zhu, tel. +86-25-8688-1120, Fax +86-25-8688-1028, e-mail: cwzhu@issas.ac.cn
†The equal contribution to the first author.

© 2015 The Authors. *Global Change Biology Bioenergy* published by John Wiley & Sons Ltd.
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

could also alter rice straw quality. For example, elevated [CO_2] can increase the ratio of carbon to nitrogen (C : N), alter carbon partitioning between nonstructural and structural carbohydrates (Liu *et al*., 2009; Zhu *et al*., 2012), and alter the amount of sugar release from structural carbohydrates (Studer *et al*., 2011).

A fundamental understanding of how rising [CO_2] affects those biological parameters that, in turn, alter rice straw production and quality will be of obvious interest in determining the future utility of rice straw as a potential biofuel. To identify and quantify these parameters, we grew two rice cultivars that differ in [CO_2] sensitivity at ambient and elevated [CO_2] under free-air carbon dioxide enrichment (FACE) conditions. The objectives of this study were to determine (1) whether, and to what extent, rising [CO_2] affects rice carbohydrate accumulation and sugar release efficiency for biofuel production and (2) whether any observed [CO_2] effect on biomass and biofuel potential differed between rice cultivars.

Materials and methods

Site description

The study was conducted at the FACE facility in Zongcun village (32°35′5″N, 119°42′0″E), Jiangdu city, Jiangsu Province. This facility is situated in the Yangtze River Delta region, where rice is typically grown in a rice–wheat rotation. The region is typical of a north subtropical monsoon climate. Soil is classified as Shajiang Aquic Cambiosol with a sandy loam texture. Soil properties at a depth of 0–15 cm are as follows: bulk density 1.16 g cm^{-3}, soil organic carbon 18.4 g kg^{-1}, total nitrogen 1.45 g kg^{-1}, available phosphorous 10.1 mg kg^{-1}, available potassium 70.5 mg kg^{-1}, and pH 6.8.

FACE system

Details about the FACE facility have been described previously (Okada *et al*., 2001; Zhu *et al*., 2008). In brief, three rectangular paddy fields were used due to their uniformity in growth and yield. Within each field, a FACE plot was paired with an ambient control, and plot centers were 90 m apart to avoid movement of additional [CO_2] to the ambient plots. Each FACE plot was encircled with an octagonal ring (14 m in diameter) with emission tubes that injected pure CO_2 at 30 cm above the plant canopy. Emission tubes were raised as the canopy grew to maintain the [CO_2] set point at the top of the plant canopy. Ambient control plots did not receive any supplemental CO_2. The CO_2 set point in FACE plots was 200 μmol moL^{-1} above that of ambient control plots. Carbon dioxide release was controlled by a computer program with an algorithm based on wind speed and direction to keep the target CO_2 concentration within the FACE plot. During the 2012 and 2013 seasons, average daytime [CO_2] at canopy height during the experiment was 378 and 374 for the ambient rings, and 571 and 584 μmol moL^{-1} for elevated FACE rings, respectively. The average temperature during the growing stage was ranging from 24.4 °C to 24.8 °C, respectively.

Rice cultivation and sample pretreatment

Two rice (*Oryza sativa* L.) varieties, Wuyunjing21 (WYJ, Japanese inbred) and Liangyou084 (LY, Indica hybrid), were selected. Seeds of each line were sown on May 20, and seedlings were transplanted on June 21 in 2012 and 2013. The spacing of the hills was 16.7 cm × 25 cm (equivalent to 24 hills m^{-2}). The heading dates of LY and WYJ, respectively, were Aug 21 and Aug 25 in 2012, and Aug 20 and Aug 24 in 2013. Both lines were harvested on October 10 and October 17 in 2012 and 2013, respectively. Yield was measured from a 2-m^2 patch (excluding plants in the borders) for each subplot (Yang *et al*., 2009; Zhu *et al*., 2014).

Phosphorus and potassium (9 g m^{-2}) were applied as basal fertilizers before transplanting. Total nitrogen fertilizer was 22.5 g m^{-2}, with 40%, 30%, and 30% of the total amount applied before transplanting, tillering, and heading, respectively. Paddy fields were submerged with water from 13 June to 10 July, drained several times from 11 July to 4 August, and then flooded with intermittent irrigation from 5 August to 10 days before harvest. Herbicide and pesticide were applied as follows for the 2012 and 2013 seasons: prevention of rice stem borer, rice blast and stripe disease using chlorpyrifos, tricyclazole and imidacloprid; prevention of rice sheath blight, rice blast, Cnaphalocrocis medinalis and Chilo suppressalis using Fiponil, chlorpyrifos and sheath blight bane on; prevention of Cnaphalocrocis medinalis, panicle neck disease, rice plant hopper and leaf blight using Armure and Fiponil, validamycin and buprofezin; and prevention of ear disease and rice planthopper using tricyclazole, fenobucarb and chlorpyrifos. As climates between years were similar, and management practices remained the same in 2012 and 2013, samples collected from 2012 to 2013 were combined for analysis.

Nonstructural and structural carbohydrates

Sucrose, free glucose, and fructose were determined using a carbohydrate kit (Sigma-Aldrich, USA) and starch was measured using the starch (HK) assay kit. *β*-1, 3-1, 4-glucan was measured with glucan (mixed linkage) assay kit (Megazyme international, Ireland). Cellulose, hemicellulose, and lignin were measured as previously described (He *et al*., 2008). After enzymatic hydrolysis, the released glucose and xylose were measured using the glucose assay kit and monosaccharides kit (Sigma-Aldrich).

S/G ratio

Syringyl-to-guaiacyl (S/G) ratio of lignin was analyzed as described before (Studer *et al*., 2011). Briefly, ~4 mg of ground straw material was pyrolyzed for 2 min at 500 °C (CDS Pyroprobe 5200, Australia). Pyrolysis vapors were entrained in helium flowing at 2 L min^{-1} to a mass spectrometer (Agilent 5975C, USA). Spectra were read over a mass-to-charge

© 2015 The Authors. *Global Change Biology Bioenergy* published by John Wiley & Sons Ltd., doi: 10.1111/gcbb.12277

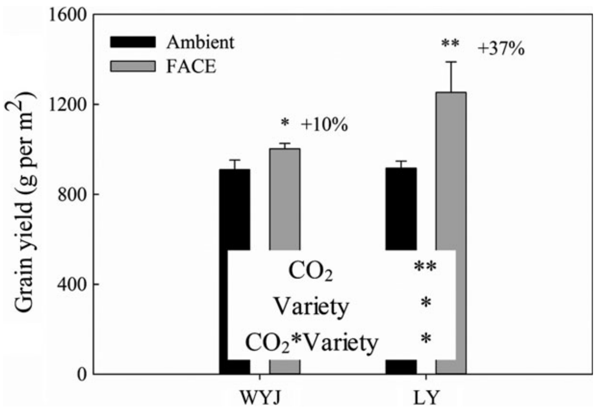

Fig. 1 The average grain yield of 2012 and 2013 seasons for rice cultivars WYJ and LY under ambient and FACE conditions. The mean was the average of 3 replications (n = 3) ± SD. $^{**}P < 0.01$, $^{*}P \leq 0.05$, $^{\dagger}P \leq 0.1$, $^{ns}P > 0.1$.

ratio (m/z) range from 30 to 450 using 22.5-eV electron impact ionization. S/G ratio of lignin was determined by summing up the intensity of the peaks at 154, 167, 168, 182, 194, 208, and 210 and dividing the sum of intensity of guaiacyl peaks at 124, 137, 138, 150, 164, and 178.

Pretreatment and enzymatic hydrolysis

For quantification of nonstructural carbohydrates, samples of rice straw (300 mg) were placed in plastic tubes and 3 mL distilled water added. The tubes were heated to 100 °C for 10 min while agitating every 2 min with a vortex mixer. Sodium acetate buffer (3 mL, pH 4.8) with amyloglucosidase (1 mg, 60 units mg^{-1}, Sigma, USA) and β-glucosidase (0.5 mg, 30 units mg^{-1}, Solarbio, China) was then added and the tube incubated on a shaker at 50 °C for 50 rpm. After 4 h of digestion, the tubes were centrifuged at 12 000 g for 10 min and the liberated glucose was measured. The total recovery of glucose after enzymatic hydrolysis was estimated as the amount of liberated glucose plus the amounts of free glucose, fructose, and sucrose.

After the remaining supernatant was removed, 4 mL pure water was added twice to wash the remaining soluble sugars. Then, sodium acetate buffer (5 mL, pH 4.8) with cellulose (60 mg, 0.93 U mg^{-1}, Sigma) and hemicellulose (40 mg, 2.50 U mg^{-1}, Sigma) was added. Tubes were agitated for 5 min with a vortex mixer, then incubated in a shaker at 50 °C for 48 h at 50 rpm, and then centrifuged at 12 000 g for 10 min (Park *et al.*, 2010). Supernatant was used to determine glucose and xylose concentration in the sample. Each sample was measured twice.

During the saccharifying progress, cellulose ($C_6H_{10}O_5$, molecular weight: 162) was hydrolyzed to glucose ($C_6H_{12}O_6$, molecular weight: 180). The hemicellulose ($C_5H_8O_4$, molecular weight: 132) was hydrolyzed into the xylose ($C_5H_{10}O_5$, molecular weight: 150). The degradation ratio of structural carbohydrates was calculated according to equations (1) and (2) as described (Poornejad *et al.*, 2013).

Table 1 Component (each component per dry weight) of rice straw at mature stage of WYJ and LY under ambient (AMB) and FACE conditions. Mean was the average of replications (n = 3)

		Nonstructural carbohydrates								
CO_2	Variety	Starch (%)	Sucrose (%)	Free glucose (%)	Free fructose (%)	β-1, 3-1, 4-glucan (%)	Sum‡ (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
AMB	WYJ	4.03 ± 0.40	4.39 ± 0.16	0.29 ± 0.04	0.30 ± 0.03	0.26 ± 0.01	9.27 ± 0.36	27.97 ± 0.95	23.71 ± 1.61	11.41 ± 0.52
FACE	WYJ	4.89 ± 0.24	5.43 ± 0.49	0.37 ± 0.04	0.38 ± 0.05	0.33 ± 0.04	11.40 ± 0.63	23.25 ± 1.46	23.19 ± 1.45	10.53 ± 0.47
AMB	LY	1.73 ± 0.40	6.39 ± 0.29	0.36 ± 0.03	0.36 ± 0.03	0.24 ± 0.04	8.84 ± 0.33	27.02 ± 1.25	20.59 ± 0.67	12.91 ± 0.61
FACE	LY	2.25 ± 0.40	7.51 ± 0.30	0.43 ± 0.03	0.44 ± 0.03	0.31 ± 0.02	10.92 ± 0.35	24.65 ± 1.25	20.62 ± 1.74	10.63 ± 0.44
CO_2		**	**	**	**	**	**	**	ns	**
Variety		**	**	*	*	ns	ns	ns	**	*
CO_2* Variety		ns	ns	ns	ns	ns	ns	ns	ns	†

$^{**}P < 0.01$; $^{*}P \leq 0.05$; $^{\dagger}P \leq 0.1$, $^{ns}P > 0.1$.

‡The amount of soft carbohydrates was calculated as the sum of starch, sucrose, free glucose, free fructose, and β-1,3-1,4-glucan.

© 2015 The Authors. *Global Change Biology Bioenergy* published by John Wiley & Sons Ltd., doi: 10.1111/gcbb.12277

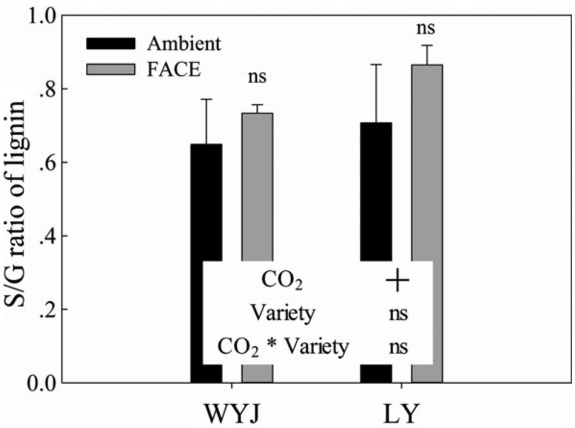

Fig. 2 Syringyl-to-guaiacyl (S/G) ratio of lignin within rice straw for rice cultivars WYJ and LY at maturity under ambient and FACE conditions for the 2012 and 2013 seasons. The mean was the average of 3 replications (n = 3) ± SD. $^{**}P < 0.01$, $^{*}P \leq 0.05$, $^{\dagger}P \leq 0.1$, $^{ns}P > 0.1$.

Ratio of cellulose degradation (%)

$$= \text{Glucose produced} / (\text{Cellulose in sample} \times 1.111) \times 100\% \quad (1)$$

Ratio of hemicellulose degradation (%)

$$= \text{Xylose produced} / (\text{Hemicellulose in sample} \times 1.136) \times 100\% \quad (2)$$

Total sugar release was determined as follows:

$$\text{Total sugar release (g per g straw)} = \text{Sugars release from nonstructural cabohydrate} + \text{cellulose} + \text{hemicellulose(g per g straw)} \quad (3)$$

Sugar yield of straw was determined as follows:

$$\text{Total Sugar yield (g per m2)} = \text{Rice straw biomass (g per m2)} + \text{Total sugar release (g per g straw)} \quad (4)$$

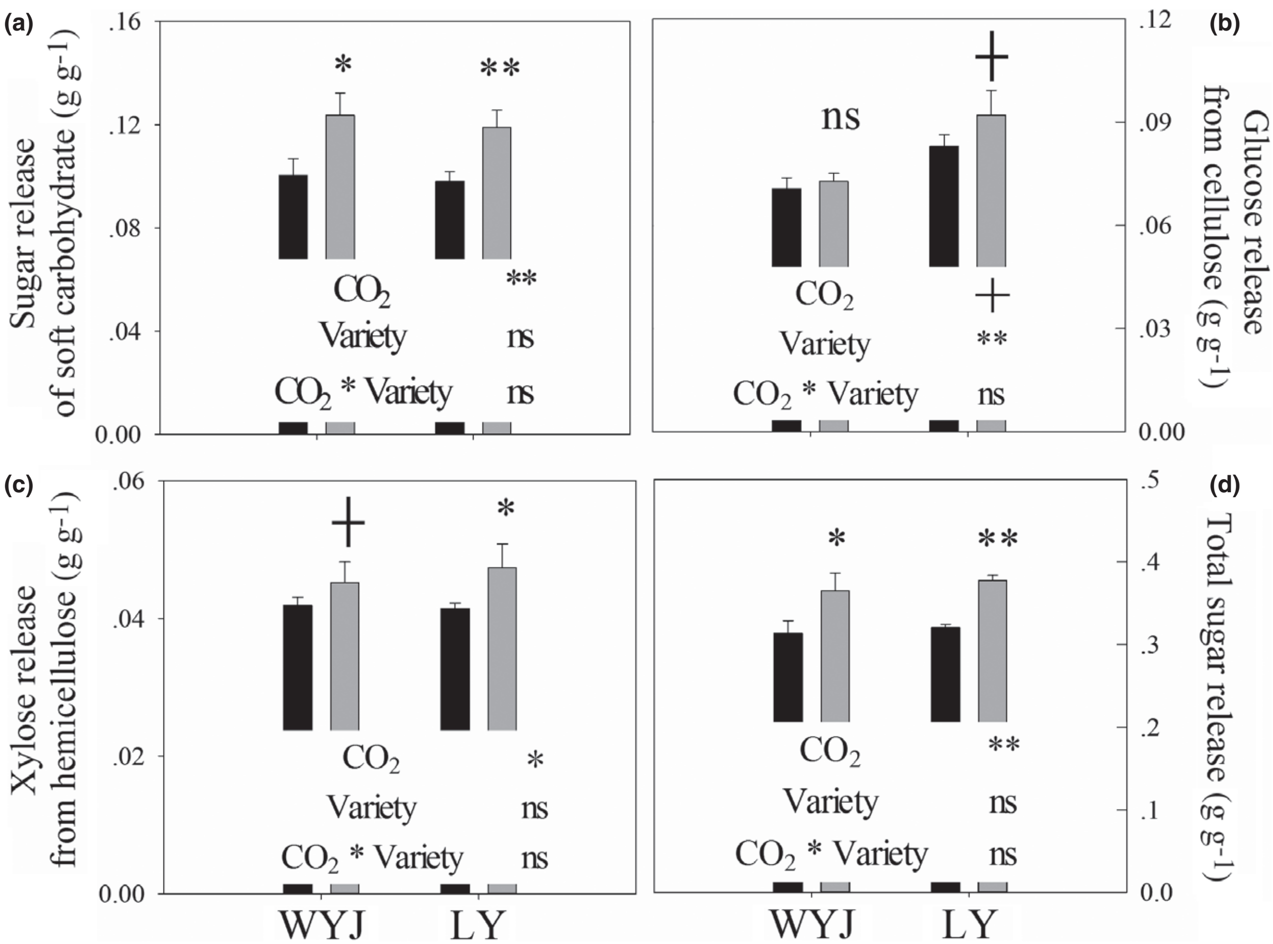

Fig. 3 Sugar release from nonstructural carbon (NSC) (a), glucose release from cellulose (b), xylose release from hemicellulose (c), and total sugar release from nonstructural and structural carbon (d) within rice straw for cultivars WYJ and LY 10 8 at maturity for ambient and FACE conditions for both 2012 and 2013 seasons. The mean was the average of 3 replications (n = 3) ± SD. $^{**}P < 0.01$, $^{*}P \leq 0.05$, $^{\dagger}P \leq 0.1$, $^{ns}P > 0.1$.

© 2015 The Authors. *Global Change Biology Bioenergy* published by John Wiley & Sons Ltd., doi: 10.1111/gcbb.12277

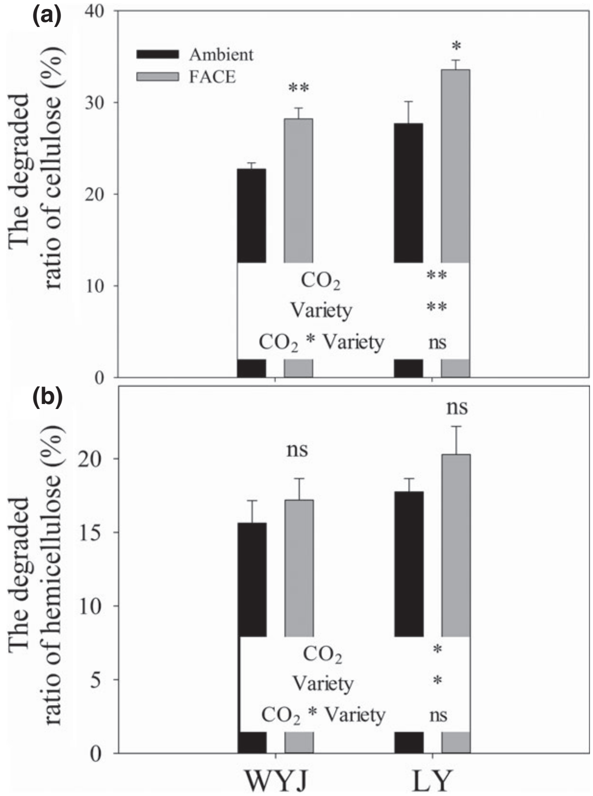

Fig. 4 Changes in the ratio of cellulose (a) and hemicellulose (b) degradation for rice straw biomass for rice cultivars WYJ and LY under ambient and FACE conditions for both 2012 and 2013 seasons. The mean was the average of replications (n = 3) ± SD. **P < 0.01, *$P \leq 0.05$, $^{\dagger}P \leq 0.1$, $^{ns}P > 0.1$.

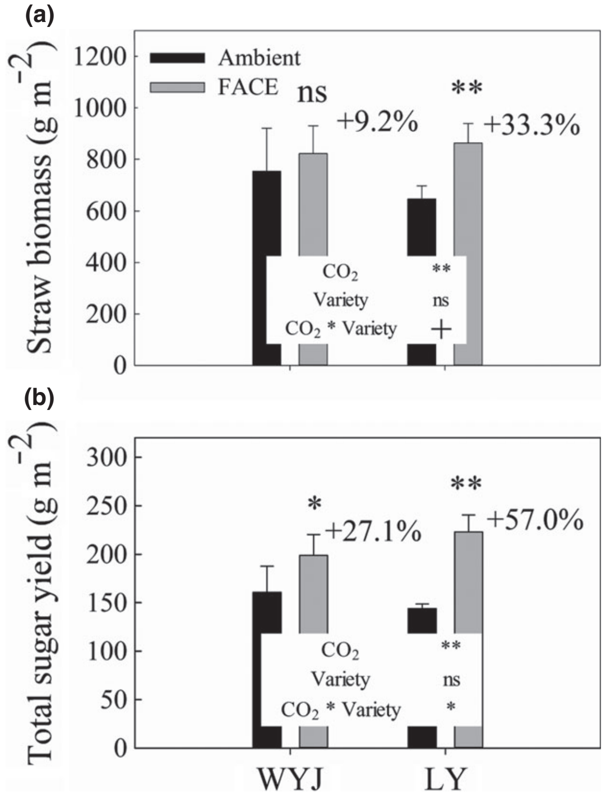

Fig. 5 Average rice straw biomass (a) and total sugar yield [straw biomass (g m^{-2}) × total sugar release (g g^{-1})] by saccharification (b) for rice cultivars WYJ and LY under ambient and FACE conditions for both 2012 and 2013 seasons. n = 3 ± SD. **P < 0.01, *$P \leq 0.05$, $^{\dagger}P \leq 0.1$, $^{ns}P > 0.1$.

Statistical analyses

The experiment design was a split-plot factor arranged within a randomized complete block design with 3 replications (three rectangular paddy fields) to test for the effect of [CO_2] on the rice carbohydrate accumulation and sugar release efficiency for biofuel productions. Subplots were blocked by variety levels to test the difference between the two varieties. For statistical analysis, [CO_2] was treated as the fixed-effect whole-plot factor, variety as the split-plot factor, and block as the random effect factor. The statistics were derived using a mixed linear model procedure (SPSS statistical software 19.0, SPSS Inc., USA) to test the effect of [CO_2], variety, and their interactions. The linear relationship between sugar releases, the ratio of cellulose and hemicellulose degradation to lignin content, and S/G ratio of lignin was determined using the linear regression model.

Results

Yield

Elevated [CO_2] significantly enhanced the grain yield for both varieties (Fig. 1). Consistent with previous studies, the stimulation of yield was larger for LY (37%) than WYJ (10%). A significant [CO_2] × cultivar interaction was observed.

Nonstructural and structural carbohydrates and S/G ratio

As shown in Table 1, a large amount of nonstructural carbohydrates are represented by straw, with starch and sucrose as the major components in both varieties. At ambient [CO_2], WYJ had similar starch and sucrose contents, whereas LY had more sucrose relative to starch (Table 1). At the elevated [CO_2] treatment, starch, sucrose, free glucose, fructose, and β-1, 3-1, 4-glucan contents were significantly increased, and the total nonstructural carbohydrate content was increased from 9.27% to 11.40% for WYJ and from 8.4% to 10.92% for LY in response (Table 1). Conversely, cellulose and lignin were significantly reduced at elevated [CO_2] for both varieties, while hemicellulose content did not change. There was a marginally significant interactive effect of CO_2 and variety on lignin content (Table 1). S/G ratio of lignin was reduced at elevated [CO_2] (Fig. 2).

© 2015 The Authors. *Global Change Biology Bioenergy* published by John Wiley & Sons Ltd., doi: 10.1111/gcbb.12277

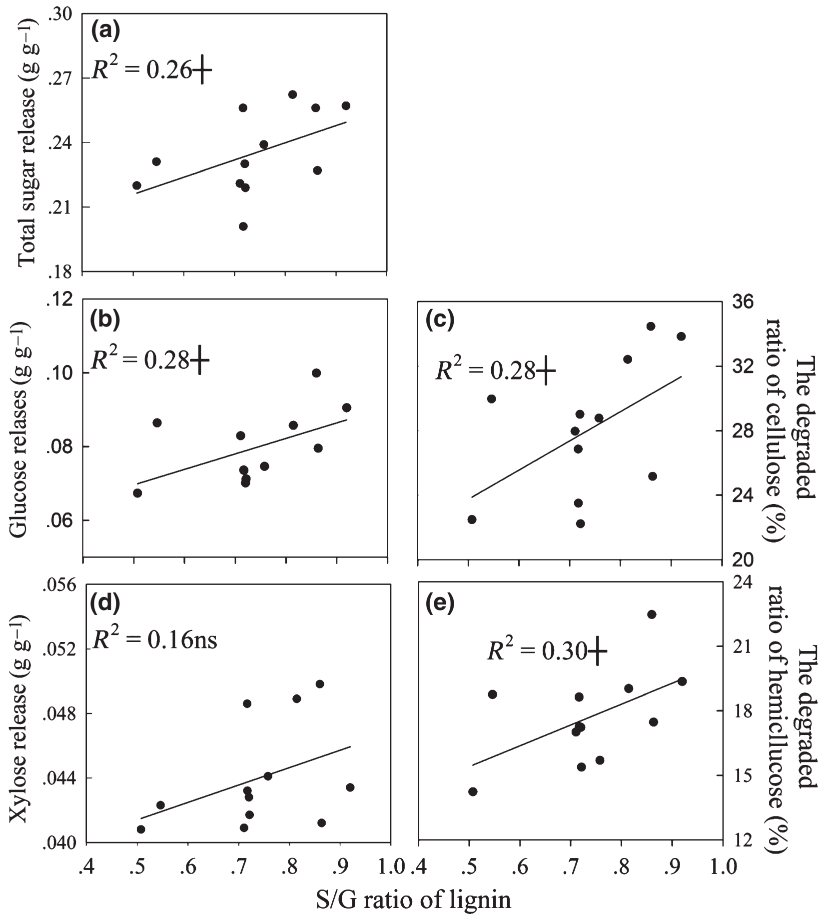

Fig. 6 Total sugar release (a), glucose release from cellulose (b), degraded cellulose ratio (c), xylose release from hemicelluloses (d), and degraded hemicellulose ratio (e), and their relationship with S/G ratio of lignin within rice straw for rice cultivars WYJ and LY under ambient and FACE conditions for both 2012 and 2013 seasons. n = 12 [2 CO_2 × 2 varieties × 3 replicates], $^*P \le 0.05$, $^{\dagger}P \le 0.1$, $^{ns}P > 0.1$.

Sugar release from nonstructural and structural carbohydrates

Elevated [CO_2] significantly increased the total sugar release from nonstructural and structural carbohydrates for both varieties (Fig. 3). Elevated [CO_2] tended to increase the glucose release from cellulose, but there was only a marginal increase in sugar release for LY. Elevated [CO_2] also increased the amount of xylose release from hemicellulose for both varieties. Cellulose degradation was significantly increased for both varieties; however, the degradation of hemicellulose was only slightly enhanced under elevated [CO_2] (Fig. 4).

Straw biomass and total sugar yield from straw

Elevated [CO_2] increased straw biomass by 9.2% and 33.3% for WYJ and LY, respectively (Fig. 5). Similar cultivar-specific increases in total sugar yield were also observed (27.1% and 57.0% for WYJ and LY, respectively).

The relationship between S/G and lignin content to sugar release

Total sugar release, glucose release from cellulose, and the ratio of cellulose and hemicellulose degradation were marginally ($P < 0.10$) positively correlated with S/G ratio (Fig. 6a,b,c,e). Total sugar release was marginally ($P < 0.10$) negatively correlated with increasing lignin; however, a significant correlation was observed for xylose release as lignin content increased (Fig. 7a,e).

Discussion

The current study indicates that elevated [CO_2], by stimulating vegetative biomass, could enhance the

© 2015 The Authors. *Global Change Biology Bioenergy* published by John Wiley & Sons Ltd., doi: 10.1111/gcbb.12277

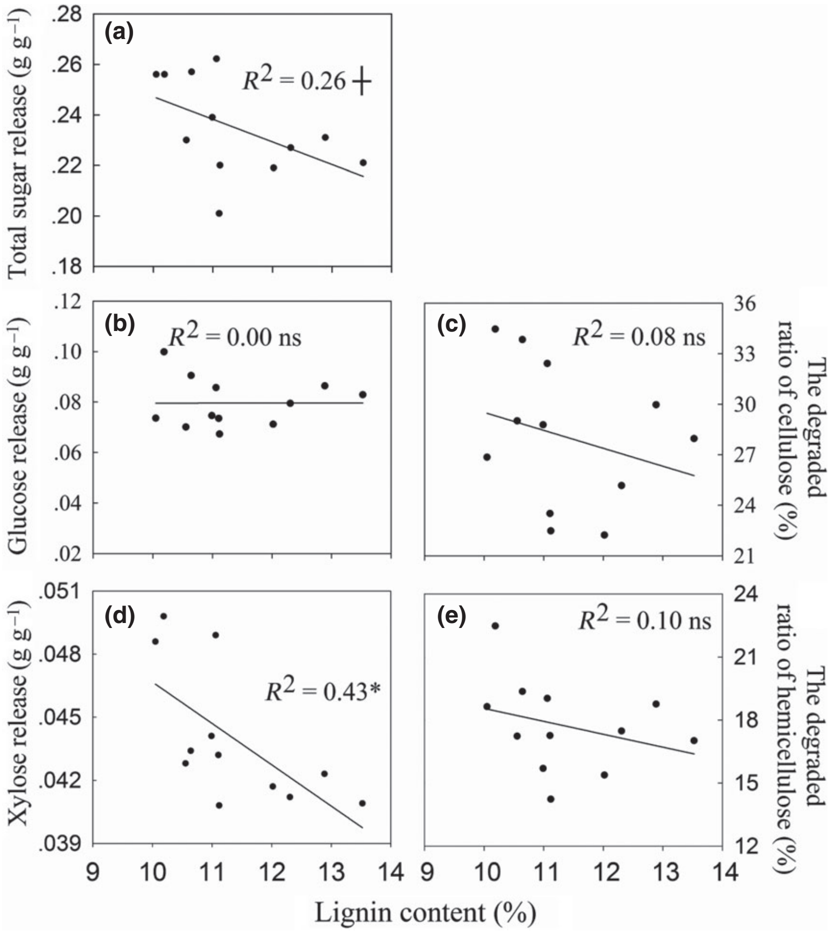

Fig. 7 Total sugar release (a), glucose release from cellulose (b), degraded cellulose ratio (c), xylose release from hemicellulose (d), and degraded hemicellulose ratio (e), and their relationship with lignin content within rice straw of WYJ and LY at mature stage under ambient and FACE conditions for both 2012 and 2013 seasons. n = 12 [2 CO_2 × 2 varieties × 3 replicates], $^*P \leq 0.05$, $^{\dagger}P \leq 0.1$, $^{ns}P > 0.1$.

potential of rice straw as a bioethanol source in the future. In addition, the degree of stimulation by elevated [CO_2] appeared to be cultivar specific. For example, in the current study, the hybrid LY showed a higher shoot biomass response (+33.3%) than the conventional WYJ variety (+9.2%). Such variation is consistent with other studies that have shown that [CO_2] could enhance shoot biomass from 5% to 39% among rice lines (Yang *et al.*, 2006; Liu *et al.*, 2008; Shimono *et al.*, 2009). This variation, in turn, could be considered in selecting rice lines that could show a stronger vegetative response to elevated [CO_2] (Shimono *et al.*, 2009).

Elevated [CO_2] can not only stimulate the amount of biomass, but also the quality of the biomass produced. For example, Henning *et al.* (1996) and Booker *et al.* (2005) reported that elevated [CO_2] increased the lignin concentration in sorghum stems; Billings *et al.* (2003) showed that elevated [CO_2] had no effect on cellulose and lignin concentration for soybean and four shrub species; Hall *et al.* (2005) found that rising [CO_2] did not change the content of cellulose, hemicellulose, and lignin across plant species within a scrub oak community. Alternatively, Newman *et al.* (2003) found that rising [CO_2] decreased lignin concentrations of tall fescue. It has been previously documented that elevated [CO_2] can increase nonstructural carbohydrate accumulation in stems and leaf blades of C_3 crops, including rice (Seneweera *et al.*, 2002; Ainsworth *et al.*, 2004). It has also been demonstrated that elevated [CO_2] significantly increased the content of starch through increasing the size and number of starch in C_3 tissues (Teng *et al.*, 2006).

For this study, elevated [CO_2] also affected straw quality. Nonstructural carbohydrate was significantly increased, but structural carbon content was significantly reduced, with a subsequent decline in cellulose

© 2015 The Authors. *Global Change Biology Bioenergy* published by John Wiley & Sons Ltd., doi: 10.1111/gcbb.12277

and lignin. Under elevated [CO_2], the cellulose content was reduced, but the degradation of cellulose was increased as was the glucose release from cellulose, especially for LY. Interestingly, the hemicellulose content was unaffected by [CO_2], but the ratio of degradation of hemicelluloses was enhanced. The amount of xylose release from hemicellulose was also significantly enhanced with elevated [CO_2]. Previous studies have demonstrated the possible mechanism of cell wall traits, anatomy, and biochemistry associated with resistance to carbohydrate degradation. A common assumption was that high lignin content adversely affected enzymatic hydrolysis (Chang & Holtzapple, 2000; Dien *et al*., 2006). Furthermore, S-rich lignin is more reactive to be degraded (Stewart *et al*., 2009; Sannigrahi *et al*., 2010). In this study, decreased lignin content and increased S/G ratio under elevated [CO_2] could have also altered the rate of straw degradation. However, the differential response of hemicellulose content relative to cellulose and lignin content within rice straw in response to elevated [CO_2] requires further study.

Overall, in contrast to biomass and straw production, similar enhancement of total sugar release from straw was observed for both lines (e.g., 16.4% for WYJ and 17.8% for LY) in response to [CO_2]. Selection for total sugar release may be possible in response to elevated [CO_2], but a larger range of cultivars will need to be evaluated.

To the best of our knowledge, this is the first study that has quantified [CO_2]-induced changes in rice straw in the context of its utility as a biofuel source. Although there is merited interest in the impact of rising [CO_2] on grain yield, less is known with respect to how [CO_2] can alter bioethanol production. Yet, given the importance of rice cultivation globally, rice straw could, potentially, represent a large, potential energy source. In this context, we would argue that it is important to evaluate how rising [CO_2] could influence growth of other major crops and the subsequent potential of those crops for biofuel. We would suggest that other crop FACE studies (soybean and maize-FACE of USA; barley, wheat, and maize-FACE of Germany, wheat-FACE of Australia, rice and wheat-FACE of China, and rice-FACE of Japan) could be used to accumulate a database on how elevated [CO_2] alters biofuel production of major crops globally.

Acknowledgements

This work was supported by the National Basic Research Program (973 Program, 2014CB954500), the National Natural Science Foundation of China (Grant No. 31370457, 41301209, 31261140364, 31201126, 41101232), and Natural Science Foundation of Jiangsu Province in China (Grant No. BK20131051, BK20140063). The FACE system instruments were supplied by the National Institute of Agro-Environmental Sciences and the Agricultural Research Center of Tohoku Region (Japan). We also thank Dr. Lewis H. Ziska of USDA-ARS for his review and suggestions to the manuscript.

Reference

Ainsworth EA, Rogers A, Nelson R *et al*. (2004) Testing the "source-sink" hypothesis of down-regulation of photosynthesis in elevated CO_2 in the field with single gene substitutions in Glycine max. *Agricultural and Forest Meteorology*, **122**, 85–94.

Billings SA, Zitzer SF, Weatherly H *et al*. (2003) Effects of elevated carbon dioxide on green leaf tissue and leaf litter quality in an intact Mojave Desert ecosystem. *Global Change Biology*, **9**, 729–735.

Booker FL, Prior SA, Torbert HA *et al*. (2005) Decomposition of soybean grown under elevated concentrations of CO_2 and O_3. *Global Change Biology*, **11**, 685–698.

Chang VS, Holtzapple MT (2000) Fundamental factors affecting biomass enzymatic reactivity. *Applied Biochemistry and Biotechnology*, **84–86**, 5–37.

Cuevas M, Sanchez S, Bravo V *et al*. (2010) Determination of optimal pre-treatment conditions for ethanol production from olive-pruning debris by simultaneous saccharification and fermentation. *Fuel*, **89**, 2891–2896.

Dien BS, Jung HJG, Vogel KP *et al*. (2006) Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. *Biomass and Bioenergy*, **30**, 880–891.

Domínguez-Escribá L, Porcar M (2010) Rice straw management: the big waste. *Biofuels, Bioproducts and Biorefining*, **4**, 154–159.

Erdei B, Barta Z, Sipos B *et al*. (2010) Ethanol production from mixtures of wheat straw and wheat meal. *Biotechnology for Biofuels*, **3**, 16.

FAO (2014) FAOSTAT: Production-Crops, 2012 data.

Hall MC, Stiling P, Moon DC *et al*. (2005) Effects of elevated CO_2 on foliar quality and herbivore damage in a scrub oak ecosystem. *Journal of Chemical Ecology*, **31**, 267–286.

He YF, Pang YZ, Liu YP *et al*. (2008) Physicochemical characterization of rice straw pretreated with sodium hydroxide in the solid state for enhancing biogas production. *Energy & Fuels*, **22**, 2775–2781.

Henning FP, Wood CW, Rogers HH *et al*. (1996) Composition and decomposition of soybean and sorghum tissues grown under elevated atmospheric carbon dioxide. *Journal of Environmental Quality*, **25**, 822–827.

Karp A, Shield I (2008) Bioenergy from plants and the sustainable yield challenge. *New Phytologist*, **179**, 15–32.

Lim JS, Manan ZA, Alwi SRW *et al*. (2012) A review on utilisation of biomass from rice industry as a source of renewable energy. *Renewable and Sustainable Energy Reviews*, **16**, 3084–3094.

Liu HJ, Yang LX, Wang YL *et al*. (2008) Yield formation of CO_2-enriched hybrid rice cultivar Shanyou 63 under fully open-air field conditions. *Field Crops Research*, **108**, 93–100.

Liu J, Han Y, Cai ZC (2009) Decomposition and products of wheat and rice straw from a FACE experiment under flooded conditions. *Pedosphere*, **19**, 389–397.

Newman JA, Abner ML, Dado RG *et al*. (2003) Effects of elevated CO_2, nitrogen and fungal endophyte-infection on tall fescue: growth, photosynthesis, chemical composition and digestibility. *Global Change Biology*, **9**, 425–437.

Oanh NTK, Ly BT, Tipayarom D *et al*. (2011) Characterization of particulate matter emission from open burning of rice straw. *Atmospheric Environment*, **45**, 493–502.

Okada M, Lieffering M, Nakamura H *et al*. (2001) Free-air CO_2 enrichment (FACE) using pure CO_2 injection: system description. *New Phytologist*, **150**, 251–260.

Park JY, Arakane M, Shiroma R *et al*. (2010) Culm in rice straw as a new source for sugar recovery via enzymatic saccharification. *Bioscience Biotechnology and Biochemistry*, **74**, 50–55.

Poornejad N, Karimi K, Behzad T *et al*. (2013) Improvement of saccharification and ethanol production from rice straw by NMMO and BMIM OAc pretreatments. *Industrial Crops and Products*, **41**, 408–413.

Sannigrahi P, Ragauskas AJ, Tuskan GA (2010) Poplar as a feedstock for biofuels: a review of compositional characteristics. *Biofuels Bioproducts & Biorefining*, **4**, 209–226.

Seneweera SP, Conroy JP, Ishimaru K *et al*. (2002) Changes in source-sink relations during development influence photosynthetic acclimation of rice to free air CO_2 enrichment (FACE). *Functional Plant Biology*, **29**, 945–953.

Shimono H, Bunce JA (2009) Acclimation of nitrogen uptake capacity of rice to elevated atmospheric CO_2 concentration. *Annals of Botany*, **103**, 87–94.

Shimono H, Okada M, Yamakawa Y *et al.* (2009) Genotypic variation in rice yield enhancement by elevated CO_2 relates to growth before heading, and not to maturity group. *Journal of Experimental Botany*, **60**, 523–532.

Stewart JJ, Akiyama T, Chapple C *et al.* (2009) The effects on lignin structure of overexpression of ferulate 5-hydroxylase in hybrid poplar. *Plant Physiology*, **150**, 621–635.

Studer MH, DeMartini JD, Davis MF *et al.* (2011) Lignin content in natural Populus variants affects sugar release. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 6300–6305.

Teng N, Wang J, Chen T *et al.* (2006) Elevated CO_2 induces physiological, biochemical and structural changes in leaves of Arabidopsis thaliana. *New Phytologist*, **172**, 92–103.

Wang F, Hu GH, Xiao JB *et al.* (2011) Improvement in the productivity of xylooligosaccharides from rice straw. *Archives of Biological Science Belgrade*, **63**, 161–166.

Yang LX, Huang JY, Yang HJ *et al.* (2006) Seasonal changes in the effects of free-air CO_2 enrichment (FACE) on dry matter production and distribution of rice (Oryza sativa L.). *Field Crops Research*, **98**, 12–19.

Yang LX, Liu HJ, Wang YX *et al.* (2009) Yield formation of CO_2-enriched intersubspecific hybrid rice cultivar Liangyoupeijiu under fully open-air field condition in a warm sub-tropical climate. *Agriculture Ecosystems and Environment*, **129**, 193–200.

Yoswathana N, Phuriphipat P, Treyawutthiwat P *et al.* (2010) Bioethanol production for rice straw. *Energy & Fuels*, **1**, 26–31.

Zhu CW, Zeng Q, Ziska L *et al.* (2008) Effect of nitrogen supply on carbon dioxide–induced changes in Competition between Rice and Barnyardgrass (Echinochloa crus-galli). *Weed Science*, **56**, 66–71.

Zhu CW, Ziska L, Zhu JG *et al.* (2012) The temporal and species dynamics of photosynthetic acclimation in flag leaves of rice (Oryza sativa) and wheat (Triticum aestivum) under elevated carbon dioxide. *Physiologia Plantarum*, **145**, 395–405.

Zhu CW, Zhu JG, Cao J *et al.* (2014) Biochemical and molecular characteristics of leaf photosynthesis and relative seed yield of two contrasting rice cultivars in response to elevated [CO_2]. *Journal of Experimental Botany*, **65**, 6049–6056.

Ziska LH, Manalo PA, Ordonez RA (1996) Intraspecific variation in the response of rice (Oryza sativa L.) to increased CO_2 and temperature: growth and yield response of 17 cultivars. *Journal of Experimental Botany*, **47**, 1353–1359.

© 2015 The Authors. *Global Change Biology Bioenergy* published by John Wiley & Sons Ltd., doi: 10.1111/gcbb.12277