

Interactive Effects of Elevated Atmospheric CO₂ and Waterlogging on Vegetative Growth of Soybean (*Glycine max* (L.) Merr.)

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Abstract: Waterlogging is a major predicted agricultural problem for crop production in some areas under current climate change, but no studies are available on the interactive effects of waterlogging and elevated atmospheric CO₂ concentration ([CO₂]). We hypothesized that elevated [CO₂] could alleviate the damage caused by waterlogging, and tested the hypothesis using vegetative growth of soybean (*Glycine max*) in 10 experiments (different sowing time and different soil type) conducted at Morioka and Tsukuba for three years. The 2-week-old plants grown under elevated and ambient [CO₂] were exposed to waterlogging for 2 weeks. Total dry weight at the end of the treatment was higher under elevated [CO₂] than under ambient [CO₂], and it was significantly reduced by waterlogging under both levels of [CO₂], without significant [CO₂] × waterlogging interactions, at both locations. The negative effects of the waterlogging were greater in root dry weight than in top dry weight, and the root exudation per unit root dry weight was also reduced by waterlogging, without a [CO₂] × waterlogging interaction. Therefore, the hypothesis of a [CO₂] × waterlogging interaction can be rejected, and provide an important basis for predicting future damage caused by waterlogging under elevated [CO₂] conditions.

Key words: Atmospheric carbon dioxide concentration, Climate change, Flooding, Nitrogen uptake, Soybean, Stomatal conductance, Waterlogging.

The atmospheric CO₂ concentration ([CO₂]) was less than 280 μmol mol⁻¹ for about 10 000 years ago, but due to human activity since the Industrial Revolution, it increased to 379 μmol mol⁻¹ (2005) in only about 200 years (IPCC, 2007). This rapid increase in [CO₂] and other greenhouse-effect gases in the atmosphere has led to global climate change, including higher temperatures and an increase in extreme weather events such as abnormally heavy precipitation, which can lead to waterlogging and flooding. The impacts of climate change have decreased crop productivity and pose a threat to world food security.

Plant growth can be stimulated by elevated atmospheric [CO₂], through increased photosynthesis and water-use efficiency (e.g., Kimball et al., 2002; Long et al., 2004). A 200 μmol mol⁻¹ increase of [CO₂] from the current level could potentially increase seed yields by ca. 20% in C₃ crops and by ca. 7% in C₄ crops (Long et al., 2004). However, this positive impact of elevated [CO₂] interacts with several environmental stresses, including low temperature (Shimono et al., 2008), high temperature (Matsui et al., 1997; Heinemann et al., 2006), elevated ozone (Ainsworth et al., 2002; Cardoso-Vilhena et al., 2004; Ainsworth, 2008),

salinity (Geissler et al., 2009; Perez-Lopez et al., 2009), N deficits (Kimball et al., 2002), P deficits (Nguyen et al., 2006), and water deficits (Baker et al., 1997; Leakey et al., 2006).

However, the effects of the interaction between elevated [CO₂] and waterlogging (i.e., excess soil water) on plant growth have not been reported. This is an important omission, because waterlogging caused by erratic, unusually high rainfall is a major environmental stress for crops such as wheat (Huang et al., 1997; Musgrave and Ding, 1998), maize (Everard and Drew, 1987), soybean (Sugimoto et al., 1988, 1989; Linkemer et al., 1998; Mochizuki et al., 2000; Boru et al., 2003; Shimamura et al., 2003; Amarante and Sodek, 2006; Shimamura et al., 2006), cotton (Milroy et al., 2009), sunflower (Grassini et al., 2007), peas and barley (Geisler, 1967), and tobacco (Kramer and Jackson, 1954). The frequency and intensity of waterlogging damage is predicted to increase in some areas under high [CO₂] in the future (IPCC, 2007). Accurate estimation of the effects of interactions between atmospheric [CO₂] elevation and waterlogging will therefore be a key factor for predicting future crop production.

Waterlogging can restrict plant physiological performance by inhibiting the direct exchange of O₂ between submerged tissues and the atmosphere because O₂ diffusivity in water is approximately 10⁴ times lower than in air (Colmer, 2003). The O₂ deficit created by waterlogging can degrade root functions such as water uptake (Kramer and Jackson, 1954; Bradford and Hsiao, 1982; Grassini et al., 2007) and nutrient uptake (Sugimoto et al., 1989; Bacanamwo and Purcell, 1999; Amarante and Sodek, 2006; Milroy et al., 2009), and can impede hydraulic conductance (Everard and Drew, 1987), leaf expansion (Linkemer et al., 1998), and stomatal conductance and photosynthesis (Sugimoto et al., 1988; Musgrave and Ding, 1998; Sojka et al., 2005). Additionally, in legumes that fix atmospheric N₂ gas by *Rhizobium* in nodules. The formation of nodules and its activity, which has an important role for nitrogen supply and growth for legumes, can be damaged by waterlogging (Linkemer et al., 1998).

However, elevated [CO₂] might alleviate the damage caused by waterlogging by preventing dehydration (Kramer and Jackson, 1954; Bradford and Hsiao, 1982; Grassini et al., 2007), and by increasing photosynthesis that supplies carbohydrates to roots, allowing more efficient production of adenosine triphosphate (ATP). Under O₂-limited conditions, plants can produce ATP by anaerobic respiration (Boamfa et al., 2003; Mustroph et al., 2006); which is less efficient and creates toxic impacts on the plant. In legumes, increased carbohydrate supply by elevated [CO₂] might help maintain the activity of nodules. In addition, elevated [CO₂] can increase root size (Kimball et al., 2002), which might permit an increase in root air space and aerenchyma to supply O₂ from above the ground. Although air space and aerenchyma are critical factors in waterlogging tolerance (Thomson et al., 1990; Boru et al., 2003; Shimamura et al., 2003; Sojka et al., 2005; Irfan et al., 2010), the effects of elevated [CO₂] on root air space and aerenchyma have not been reported.

In the present study, we used young soybean plants, a major C₃ legume crop, to test the hypothesis that elevated [CO₂] could ameliorate the damage caused by waterlogging.

Materials and Methods

1. Crop history and treatment

We used the soybean cultivar ‘Suzukari’, a representative Japanese cultivar and a cultivar in northern Japan (Hashimoto et al., 1987). Seeds were sown in soils with *Rhizobium* (Mamezou for Legumes, Tokachi Nokyoren Co., Tokachi, Japan) added in 4-L pots (159 φ × 190 mm, 1/5000-a Wagner pots; Fujiwara Co., Tokyo, Japan). Three seeds were sown in a pot, leaving one plant per pot at the start of the waterlogging treatment. Table 1 summarizes the treatment and environmental conditions in our study. The plants were grown under ambient and elevated [CO₂] at

Table 1. Experimental conditions used for testing the interaction between elevated [CO₂] and waterlogging with 2-wk-old soybean plants over a 3-yr period.

Study location	Experimental ID	Year	Sowing date	Water treatment		Soil type	Fertilization	Air temperature (°C)	Solar radiation (MJ m ⁻² d ⁻¹)	[CO ₂] (μmol mol ⁻¹)	Replicate of treatment	Plants per replicate	
				Start	End								
Morioka	1	2008	24-Jun	7-Jul	22-Jul	Commercial soil ^A	1.4 g-N, 3.6 g-P ₂ O ₅ , 3.6 g-K ₂ O	27.3	16.3	380	579	1	5
Morioka	2	2008	23-Jul	5-Aug	21-Aug	Commercial soil ^A	1.4 g-N, 3.6 g-P ₂ O ₅ , 3.6 g-K ₂ O	27.8	17.4	381	580	1	5
Morioka	3	2008	23-Jul	5-Aug	21-Aug	Andisol ^C	0.2 g-N, 0.9 g-P ₂ O ₅ , 0.9 g-K ₂ O	27.8	17.4	381	580	1	3
Morioka	4	2009	18-May	8-Jun	22-Jun	Commercial soil ^B	0.8 g-N, 0.7 g-P ₂ O ₅ , 0.4 g-K ₂ O	24.1	17.5	379	579	1	5
Morioka	5	2009	30-Jun	16-Jul	31-Jul	Commercial soil ^B	0.8 g-N, 0.7 g-P ₂ O ₅ , 0.4 g-K ₂ O	27.5	12.3	379	579	1	5
Morioka	6	2009	23-Jul	6-Aug	21-Aug	Commercial soil ^B	0.8 g-N, 0.7 g-P ₂ O ₅ , 0.4 g-K ₂ O	28.6	14.1	377	577	1	5
Morioka	7	2010	28-May	11-Jun	23-Jun	Andisol ^D	0.2 g-N, 0.6 g-P ₂ O ₅ , 0.6 g-K ₂ O	24.9	16.1	397	597	1	4
Morioka	8	2010	28-May	11-Jun	23-Jun	Andisol ^D	0.2 g-N, 0.6 g-P ₂ O ₅ , 0.6 g-K ₂ O	19.9	16.1	397	597	1	4
Morioka	9	2010	28-May	11-Jun	23-Jun	Andisol ^D	1.2 g-N, 0.6 g-P ₂ O ₅ , 0.6 g-K ₂ O	24.9	16.1	397	597	1	4
Tsukuba	10	2010	8-Jun	21-Jun	4-Jul	Gley lowland soil	0.2 g-N, 0.6 g-P ₂ O ₅ , 0.6 g-K ₂ O	22.4	14.3	425	715	4	1

Air temperature and solar radiation values represent daily averages, [CO₂] values represent the daytime average from 06:00 to 18:00, except in 2008 (from 04:00 to 19:00).

^A Commercial soil type: Hokuhoku-baid (Kanuma-Sangyo Co., Kanuma, Japan). ^B Commercial soil: mixed soils 1:2 v/v of Koganebaido and Muhiyobaido (Aikei Co., Akita, Japan), ^C Andisol: soil from the field at the National Agricultural Research Center for Tohoku region, ^D Andisol: soil from the field at Iwate University.

two locations, Morioka, Iwate Prefecture (39°42'N, 141°08'E) and Tsukuba, Ibaraki Prefecture (36°01'N, 140°07'E). We exposed 2-wk-old plants (at the first trifoliolate stage) to two water regimes (control, with groundwater 20 cm below the soil surface, and waterlogging, with 1 cm of water above the soil surface using tap water) for 2 weeks. We used four combinations of treatments (2 [CO₂] × 2 water regimes), with 3 to 5 plants per treatment. At Morioka, nine experiments were conducted using two sun-lit temperature-gradient chambers (Okada et al., 2000) at the National Agricultural Research Center for Tohoku Region under various growth conditions; these experiments were conducted over a 3-yr period (2008 to 2010) under ambient and elevated (ambient +200 μmol mol⁻¹) [CO₂] conditions. At Tsukuba, one experiment with four replications was conducted in 2010 (ambient and elevated (ambient +300 μmol mol⁻¹) [CO₂]) using two sun-lit chambers (Sakai et al., 2001) at the National Institute for Agro-Environmental Sciences.

Air temperature and [CO₂] were measured inside the chambers, and solar radiation outside the chambers was obtained from the Meteorological Agency of Japan monitoring station near each location. A wide range of environmental conditions was obtained in the 10 experiments at the two locations and during the 3 years; average air temperature during the waterlogging treatments ranged from 20 to 29°C, average solar radiation ranged from 12 to 18 MJ m⁻² d⁻¹, and N fertilization ranged from 0.2 to 1.4 g N per pot (Table 1).

2. Measurements

At the end of the waterlogging treatment, we measured the plant height and the number of fully expanded leaves on the main stem, and we examined the SPAD reading on the uppermost fully expanded leaves to represent the chlorophyll content (SPAD-502, Konica-Minolta, Tokyo, Japan). We measured the green leaf area in all experiments, then destructively sampled whole plants (including the roots), and determined the dry weight of the aboveground parts (leaves and stems) and roots separately after oven-drying at 80°C for 72 h. Before sampling, we counted the number of root nodules and measured the root exudation rate in four experiments (ID 7 to 10, Table 1); root exudation was collected for 1 hour at 6 cm above the ground level and was then weighed following the method of Shimono et al. (2004). Plant N concentrations (mg N g⁻¹ DW) in the dried tissues was measured by the Kjeldahl method in three experiments (ID 4 to 6). Leaf stomatal conductance of the uppermost fully expanded leaves was measured four to nine times per day from 09:00 to 17:00 in three plants per treatment (one uppermost leaf per plant) using an SC-1 leaf porometer (Decagon Devices Inc., Pullman, WA, USA) in three experiments (ID 4 to 6), and the daily mean value was used for analysis.

3. Statistical analysis

Statistical analysis was conducted using two-way analysis of variance (ANOVA). At Tsukuba, the plants in the two chambers for ambient and elevated [CO₂] treatments were moved to the other chamber every three days to remove any chamber effects, so the plant *per se* was treated as the replicate (n=4). At Morioka, we did not move the plants, so the mean value for the plants in each experiment was used for the analysis (n=9, except for plant N concentration, nodule number, root exudation rate, and stomatal conductance, for which n=3). Statistic model was used as Experiment+CO₂+Water+CO₂×Water.

Results

1. Morphology

Plant height at the end of the treatment was increased by elevated [CO₂] at Morioka, but the difference was not significant ($P=0.12$) and was significant only at Tsukuba ($P<0.01$). Height was significantly reduced (by 23 to 30%) by the waterlogging treatment at both locations and both [CO₂] levels ($P<0.01$), without a significant interaction between waterlogging treatment and [CO₂] level (Table 2). Leaf number on the main stem showed a similar trend.

2. Dry weight

The total dry weight of the plants at Tsukuba was significantly heavier (by 19 to 23%) under elevated [CO₂] than ambient [CO₂] conditions ($P<0.01$); total dry weight at Morioka tended to be 19 to 24% heavier but the difference was not significant ($P=0.18$) (Fig. 1). Under both [CO₂] levels, the waterlogging treatment reduced the total dry weight by 54% at ambient [CO₂] and by 52% at elevated [CO₂] at Morioka ($P<0.001$). Note that there was a significant difference among nine experiments ($P<0.001$). At Tsukuba, waterlogging reduced total dry weight by 12% at ambient [CO₂] and by 10% at elevated [CO₂], although the effect of water regimes was marginally significant ($P<0.1$). No significant interaction between [CO₂] and water regime was observed at either site although the reduction of dry weight by waterlogging under elevated [CO₂] condition was slightly less than that under an ambient [CO₂] condition. Fig. 2 shows the relative total dry weight (the value under waterlogging relative to the control value) under elevated [CO₂] to that under the ambient [CO₂] condition pooled over the 10 experiments. This shows that the values were mostly on a 1:1 line especially at lower relative values under ambient [CO₂]. However, at higher relative values under ambient [CO₂], the values under elevated [CO₂] showed higher than those under ambient [CO₂], suggesting that under the condition that waterlogging damage was relatively small, elevated [CO₂] might alleviate the waterlogging damage although in the present experiment, we could not

Table 2. The effects of elevated [CO₂] and waterlogging (WL) on soybean growth during the early growth stage (2-wk-old plants subjected to waterlogging for 2 wks).

Study location	[CO ₂]	Water	Plant height (cm)	Leaf number on the main stem	Green leaf area (cm ² per plant)	Top dry weight (g per plant)	Root dry weight (g per plant)	Top/Root ratio	Root exudation			SPAD readings	Total dry weight / Green leaf area (mg cm ⁻²)	
									Plant N concentration (mg g ⁻¹ DW)	Root nodule number (pl ⁻¹)	Plant N concentration (mg g ⁻¹ DW)			rate per root dry weight (g g ⁻¹ h ⁻¹)
Morioka	Ambient	Control	43 ± 2	5.2 ± 0.2	608 ± 77	2.90 ± 0.29	1.23 ± 0.08	2.34 ± 0.18	0.60 ± 0.10	55.3 ± 0.45	90.4 ± 75.3	30.2 ± 1.8	8.7 ± 0.8	
		WL	30 ± 1	3.7 ± 0.2	215 ± 28	1.46 ± 0.13	0.51 ± 0.09	4.29 ± 1.59	0.26 ± 0.16	43.7 ± 0.78	0.4 ± 0.4	25.2 ± 1.6	11.7 ± 1.1	
	Elevated	Control	44 ± 2	5.3 ± 0.3	634 ± 125	3.45 ± 0.57	1.46 ± 0.21	2.31 ± 0.17	0.58 ± 0.04	60.5 ± 2.41	175.7 ± 117.8	30.0 ± 1.3	10.1 ± 0.9	
		WL	33 ± 1	3.7 ± 0.2	241 ± 27	1.72 ± 0.12	0.60 ± 0.07	3.33 ± 0.58	0.12 ± 0.10	44.9 ± 1.17	8.3 ± 6.4	26.5 ± 1.8	12.6 ± 1.6	
	Ratio	<i>Ambient</i>	0.70	0.71	0.35	0.51	0.41	1.83	0.44	0.79	0.00	0.84	1.34	
		<i>Elevated</i>	0.76	0.70	0.38	0.50	0.41	1.45	0.21	0.74	0.05	0.88	1.25	
	ANOVA	<i>Experiment</i>		**	***	*	*	ns	*	*	ns	+	ns	***
			<i>CO₂ (A)</i>	ns	ns	ns	ns	ns	ns	ns	+	ns	ns	+
			<i>Water (B)</i>	***	***	***	***	***	***	*	***	***	*	***
		<i>A × B</i>		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Control			37 ± 1	3.4 ± 0.1	375 ± 15	1.94 ± 0.10	0.76 ± 0.05	2.56 ± 0.14	0.48 ± 0.05	-	27.8 ± 4.8	-	7.2 ± 0.3	
WL			28 ± 1	2.9 ± 0.2	268 ± 9	1.94 ± 0.09	0.43 ± 0.02	4.49 ± 0.20	0.13 ± 0.03	-	22.8 ± 3.3	-	8.8 ± 0.1	
Elevated	Control	39 ± 0	4.2 ± 0.0	422 ± 28	2.42 ± 0.14	0.93 ± 0.02	1.94 ± 0.66	0.48 ± 0.08	-	27.5 ± 5.7	-	7.7 ± 0.3		
	WL	30 ± 1	3.2 ± 0.0	305 ± 10	2.39 ± 0.15	0.52 ± 0.04	4.62 ± 0.20	0.25 ± 0.05	-	22.0 ± 3.0	-	9.5 ± 0.5		
Ratio	<i>Ambient</i>	0.77	0.85	0.71	1.00	0.57	1.76	0.26	-	0.82	-	1.23		
	<i>Elevated</i>	0.76	0.76	0.72	0.99	0.56	2.39	0.52	-	0.80	-	1.24		
ANOVA	<i>CO₂ (A)</i>	**	**	*	**	**	ns	ns	-	ns	-	ns		
	<i>Water (B)</i>	**	**	**	ns	**	**	**	-	ns	-	**		
	<i>A × B</i>	ns	*	ns	ns	ns	ns	ns	-	ns	-	ns		

Values are means ± standard error; n = 9 at Morioka (except for the nodule number and root exudation rate, for which n = 3 (experiment IDs 7, 8, and 9) and except for the plant N concentration, for which n = 3 (experiment IDs 4, 5, and 6), and n = 4 at Tsukuba). *** P < 0.001, ** P < 0.01, * P < 0.05, + P < 0.1, ns not significant.

detect a significant interaction between [CO₂] and water regime in ANOVA analysis.

The reduction of total dry weight caused by waterlogging was greater in roots than in the aboveground parts under both [CO₂] conditions (Table 2). The ratio of the aboveground weight to root weight was significantly increased (by more than 70%) by waterlogging at both

sites ($P < 0.05$). The effects of elevated [CO₂] and interactions between [CO₂] and waterlogging were not significant at either site.

Dry matter production is determined by the amount of radiation absorption in the canopy and by the photosynthetic activity of the leaves (Monteith, 1977); as indicators of these factors, we used green leaf area and the ratio of total dry weight per unit green leaf area, respectively. The negative impact of waterlogging on dry weight was caused mainly by the large decrease in green leaf area as a result of waterlogging rather than by the changes in the ratio of weight to leaf area (Table 2). On the other hand, the increased dry weight caused by

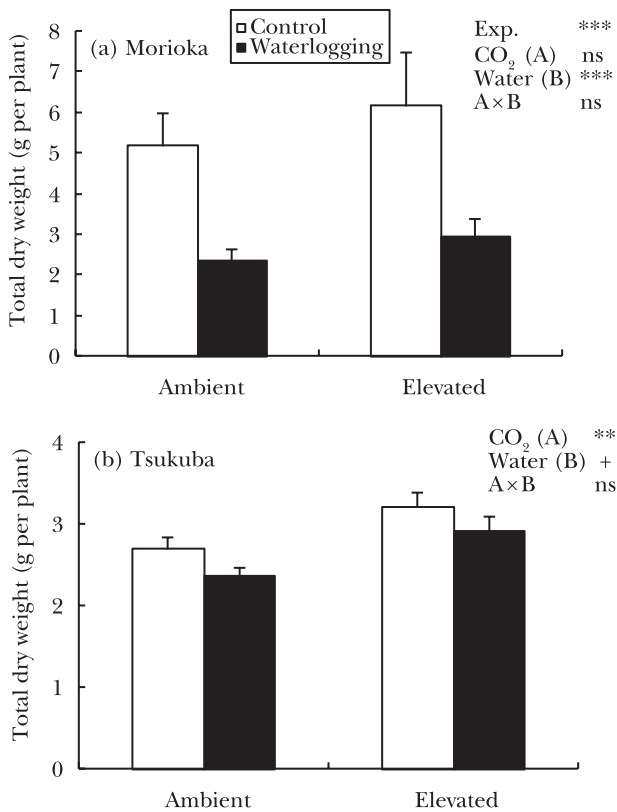


Fig. 1. Dry weight of soybean plants under ambient and elevated [CO₂] conditions with and without waterlogging at (a) Morioka and (b) Tsukuba. *** $P < 0.001$, ** $P < 0.01$, + $P < 0.1$, ns, not significant. Values represent means and standard errors (n=9 at Morioka, n=4 at Tsukuba).

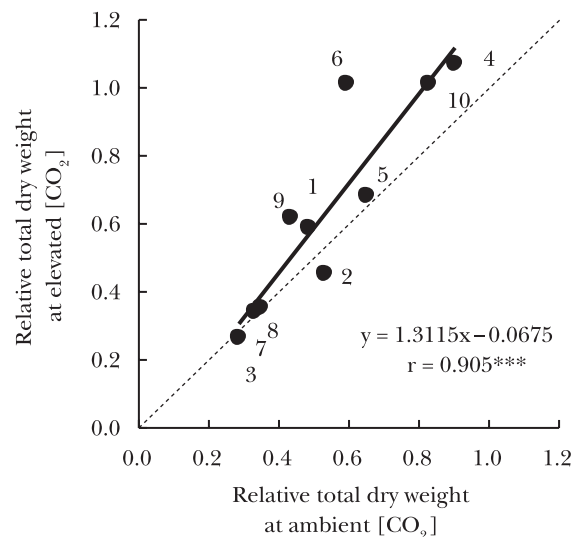


Fig. 2. Relationship between relative total dry weight under waterlogging the control value of soybean plants under elevated and ambient [CO₂] conditions in each experiment shown in Table 1. *** $P < 0.001$, ** $P < 0.01$, + $P < 0.1$, ns, not significant. Numbers beside the data points represent the experimental IDs (Table 1). Values represent means and standard errors (n=9 at Morioka, n=4 at Tsukuba).

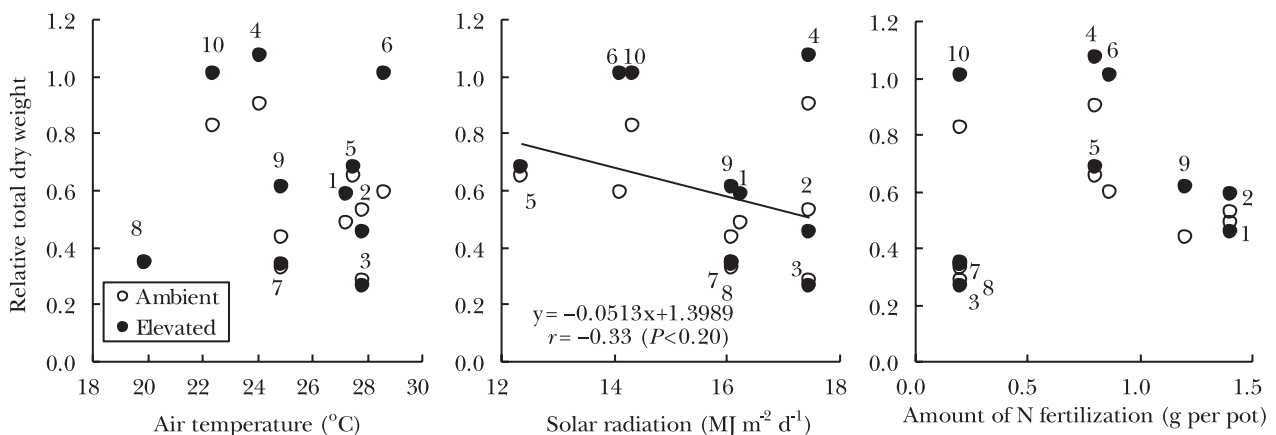


Fig. 3. Correlation of relative dry weight (the value under waterlogging relative to the control value) with air temperature, solar radiation, and N fertilization under ambient and elevated [CO₂] conditions. Numbers beside the data points represent the experimental IDs (Table 1).

elevated [CO₂] resulted from both the increased green leaf area and the increased ratio, as expected, although the effects were mostly not significant.

Fig. 3 illustrates the relationship between the relative values of total dry weight (i.e., the value in the waterlogging treatment divided by the control value) and the following factors: air temperature during the treatments, solar radiation during the treatments, and the amount of N fertilization before the treatments began. There was no significant relationship between relative dry weight and either air temperature or the amount of N fertilization at either [CO₂] level, and there was a slight but not significant negative relationship ($r=-0.33$, $P<0.20$) with solar radiation.

3. Root exudation rate, N status, and stomatal conductance

The root exudation per unit root dry weight was not affected by [CO₂] at either site, but was significantly reduced (by 48 to 79%) by waterlogging at both sites ($P<0.01$), without a significant [CO₂] × water interaction at either site (Table 2). Plant N concentration and the SPAD reading at the end of the treatment were significantly reduced by waterlogging ($P<0.05$), and slightly but not significantly increased by elevated [CO₂]. Root nodule number tended to be higher under elevated [CO₂] than under ambient [CO₂], but the difference was not significant, and was significantly reduced by waterlogging at Morioka ($P<0.05$) but not at Tsukuba. Stomatal conductance of the uppermost leaves was significantly decreased by elevated [CO₂] during the treatment period (Fig. 4). Although the waterlogging also tended to decrease the stomatal conductance, the decrease was not statistically significant. There were no significant interaction effects.

Discussion

Our original hypothesis that elevated [CO₂] alleviated waterlogging damage was statistically rejected: there was no significant interaction between [CO₂] and waterlogging (Fig. 1) although in our preliminary experiment waterlogging damage was partially alleviated by elevated [CO₂] under the moderate waterlogging stress conditions (Fig. 2). Further experiments with controlled waterlogging stress are necessary to test this hypothesis. On the other hand, in the present experiments without controlling waterlogging stress strength, elevated [CO₂] and waterlogging had positive and negative effects, respectively, on dry matter production (Fig. 1). To the best of our knowledge, this is the first study on the effects of the interaction between elevated [CO₂] and waterlogging on plant growth.

In the present study, elevated [CO₂] did not alleviate the waterlogging damage especially under severe waterlogging conditions. It is known that decreasing water flow in plants as a result of low stomatal conductance under an elevated [CO₂] condition can alleviate certain environmental stresses, such as water deficits (Baker et al., 1997; Leakey et

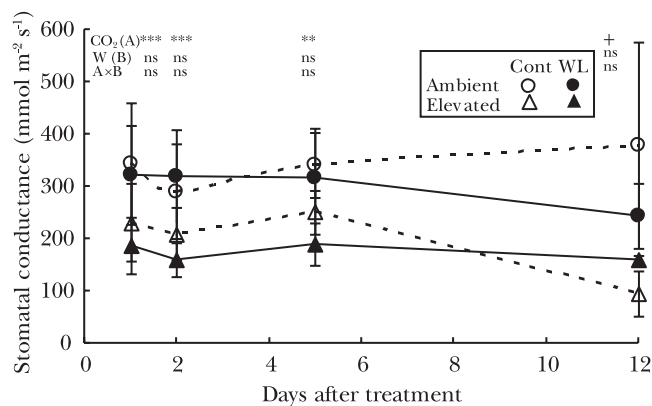


Fig. 4. Stomatal conductance of the uppermost leaves as a function of [CO₂] level (ambient or elevated) and water treatment (control vs. waterlogging (WL)) in soybean plants in the experiments at Morioka. The values represent the daily mean of four to nine measurements between 09:00 and 17:00. *** $P<0.001$, ** $P<0.01$, + $P<0.1$, ns not significant. Values represent means and standard errors ($n=3$).

al., 2006), ozone exposure (Ainsworth et al., 2002; Cardoso-Vilhena et al., 2004; Ainsworth, 2008), and salinity (Perez-Lopez et al., 2009; Geissler et al., 2009), resulting in a significant interaction between the stress response and elevated [CO₂]. In the present study, the root exudation rate per unit root weight, which represents the level of root activity, was significantly reduced by waterlogging at both sites (Table 2), but stomatal conductance was not significantly decreased by waterlogging even under the ambient [CO₂] condition (Fig. 4). Water stress thus might not be the key factor responsible for waterlogging damage in the present study.

Another important factor is the N status. N uptake from roots was significantly reduced by waterlogging (Table 2), and this decrease was greater than the decrease in dry matter production, resulting in reduced plant N concentrations and SPAD readings under both [CO₂] conditions, without a significant [CO₂] × waterlogging interaction. Root nodule number was reduced by waterlogging without [CO₂] × waterlogging interaction (Table 2). Sugimoto et al. (1989) and Shimamura et al. (2006) examined the effects of foliar N application on waterlogging damage in soybean, and found that high foliar N application alleviated waterlogging damage. In the present study, over a range of N fertilization conditions (0.2 to 1.4 g of N per pot) a large amount of N input did not alleviate the waterlogging damage, and there was no significant relationship between the effects of waterlogging and N fertilization (Fig. 3). In addition, elevated [CO₂] increased the N concentration in the plants, but not significantly (Table 2), and there was no significant [CO₂] × waterlogging interaction for total dry weight (Fig. 1). This suggests that the N supply *per se* also would not be the key factor for alleviating waterlogging damage.

The O₂ supply to the roots might directly affect the response to waterlogging, irrespective of [CO₂]. Decreased O₂ supply to the roots under waterlogging can inhibit all plant metabolic functions (Colmer, 2003; Sojka et al., 2005; Irfan et al., 2010). Sojka et al. (2005) reviewed the interactions between stomatal conductance and both soil temperature or soil O₂ concentration, and showed that the relationship between the conductance and soil temperature was diminished at low O₂ concentrations. In our study, we did not measure the soil O₂ concentration, but decreased O₂ as a result of waterlogging might have concealed the effect of elevated [CO₂].

Under waterlogging, the O₂ is supplied mostly from aerenchyma and root air spaces (Shimamura et al., 2003). There is genotypic variation in aerenchyma formation in soybean cultivars; for example, Mochizuki et al. (2000) found that the soybean cultivar 'Asoagari' had a large aerenchyma under waterlogging and had a high tolerance to waterlogging. In the present study, we used a local cultivar, 'Suzukari', but if a cultivar with a large aerenchyma formation, such as 'Asoagari', had been used, the direct effects of an O₂ deficit might have been alleviated by elevated [CO₂]. In wetland species such as paddy rice that have enough aerenchyma to supply O₂ to the roots, elevated [CO₂] significantly promoted dry matter production under submerged conditions (Ainsworth, 2008; Shimono et al., 2008). Our hypothesis that elevated [CO₂] can mitigate the effects of waterlogging should therefore be tested in future studies with cultivars that differ in their ability to form aerenchyma.

The interactions between waterlogging and other environmental conditions, such as salinity (Barrett-Lennard, 2003), solar radiation (Hwang et al., 1999; Barta and Sulc, 2002), and soil temperature (Przywara and Stepniewski, 1999; Sojka et al., 2005) have been reported. The present results showed that [CO₂], air temperature, and soil N input did not significantly affect the damage caused by waterlogging (Fig. 1, 3). On the other hand, lower solar radiation tended to decrease the impact of waterlogging (Fig. 3), although the decrease was not significant. This is consistent with previous reports (Hwang et al., 1999; Barta and Sulc, 2002), although the range of solar radiation levels examined in the present study was high (>12 MJ m⁻² d⁻¹).

The results of our 10 experiments, conducted at Morioka and Tsukuba for 3 years, demonstrated that elevated [CO₂] did not alleviate waterlogging damage. The results therefore provide an important basis for predicting the future damage to soybean crops under waterlogging induced by a climate change. However, it should be noted that the present experiment was focused on the vegetative growth alone, and the effects on the recovery from waterlogging damage under different [CO₂] were not examined. Also, our results partially suggested that waterlogging damage can be alleviated by elevated [CO₂]

under moderate waterlogging stress conditions (Fig. 2). To fully understand interactive effects of [CO₂] and waterlogging on crop production, further studies under different treatment periods until maturity, and also the different strength of waterlogging damage are necessary.

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