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RESEARCH ARTICLE

Growth, nutrient dynamics, and efficiency responses to carbon dioxide and phosphorus nutrition in soybean

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Plant mineral nutrients such as phosphorus may exert major control on crop responses to the rising atmospheric carbon dioxide (CO_2) concentrations. To evaluate the growth, nutrient dynamics, and efficiency responses to CO_2 and phosphorus nutrition, soybean (*Glycine max* (L.) Merr.) was grown in controlled environment growth chambers with sufficient (0.50 mM) and deficient (0.10 and 0.01 mM) phosphate (Pi) supply under ambient and elevated CO_2 (aCO_2, 400 and eCO_2, 800 μmol mol^{-1}, respectively). The $CO_2 \times$ Pi interaction was detected for leaf area, leaf and stem dry weight, and total plant biomass. The severe decrease in plant biomass in Pi-deficient plants (10–76%) was associated with reduced leaf area and photosynthesis (P_{net}). The degree of growth stimulation (0–55% total biomass) by eCO_2 was dependent upon the severity of Pi deficiency and was closely associated with the increased phosphorus utilization efficiency. With the exception of leaf and root biomass, Pi deficiency decreased the biomass partitioning to other plant organs with the maximum decrease observed in seed weight (8–42%) across CO_2 levels. The increased tissue nitrogen (N) concentration in Pi-deficient plants was accredited to the lower biomass and increased nutrient uptake due to the larger root to shoot ratio. The tissue P and N concentration tended to be lower at eCO_2 versus aCO_2 and did not appear to be the main cause of the lack of CO_2 response of growth and P_{net} under severe Pi deficiency. The leaf N/P ratio of >16 was detrimental to soybean growth. The tissue P concentration needed to attain the maximum productivity for biomass and seed yield tended to be higher at eCO_2 versus aCO_2. Therefore, the eCO_2 is likely to increase the leaf critical P concentration for maximum biomass productivity and yield in soybean.

Keywords: critical limit; nitrogen; nutrient utilization and uptake; N/P ratio; photosynthesis

Introduction

Phosphate (Pi) is a major plant nutrient and essential for metabolic processes and plant growth. Phosphate deficiency in soil is a major constraint for crop production worldwide, and natural sources of Pi are limited (Vance et al. 2003; Cordell et al. 2009). The nutrient requirements of crops have increased due to use of modern high-yielding cultivars and are expected to be even higher under rising atmospheric carbon dioxide (CO_2) concentration (Rogers et al. 1993; Lewis et al. 1994). In addition, the critical tissue nutrient concentration of phosphorus is likely to be higher in the CO_2-enriched environment of the future (Conroy 1992; Rogers et al. 1993). The current atmospheric CO_2 of approximately 400 μmol mol^{-1} is projected to double by the end of twenty-first century (IPCC 2007). Moreover, the degree of crop growth enhancement under CO_2-enriched environment is expected to be greatly influenced by the nutrients availability such as Pi (Cure et al. 1988; Lenka & Lal 2012). Since, Pi deficiency and elevated CO_2 (eCO_2) often have opposite effects on crop growth and development, the interaction between these two factors might alter the overall crop response.

Soybean (*Glycine max* [L.] Merr.) is a vital source of protein and vegetable oil, and one of the most versatile and important crops grown globally (Conner et al. 2004).

Phosphate deficiency reduces leaf area, photosynthesis, nitrogen fixation, yield, and seed quality of soybean (Israel & Ruffy 1988; Jaidee et al. 2013). In general, eCO_2 enhances soybean growth mainly through increased leaf area, photosynthesis, and nutrient utilization efficiency (Cure et al. 1988; Prior et al. 1998). However, Pi deficiency exerts major control on the degree of plant response to eCO_2. For example, an increase of over 80% in soybean dry mass observed under eCO_2 was not observed when grown under Pi-deficient condition (Sa & Israel 1998). Similar results have also been observed in other legume crops (Lam et al. 2012). The decrease in growth response at eCO_2 under nutrient deficiency might occur due to alteration in the plant morphology, and acclimation/down regulation of photosynthetic capacity (Qiu & Israel 1992; Sa & Israel 1998; Singh, Badgujar, Reddy, Fleisher & Bunce 2013). Phosphate deficiency and CO_2-enriched environment might coexist under natural growing condition. Therefore, it is imperative to evaluate crop response to the interactive effect of these two factors.

Previous studies suggested that Pi deficiency and eCO_2 may also alter the nutrient dynamics and biomass partitioning in the plant organs and affect the assimilation of other nutrients such as nitrogen (Israel et al. 1990; Rufty et al. 1991; Reddy & Zhao 2005; Fleisher et al. 2012; Singh, Badgujar, Reddy, Fleisher & Timlin 2013).

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Using isotopic N sources (^{15}N), Rufty et al. (1993) reported that Pi deficiency increased the root dry weight and decreased the rate of nitrate uptake while accumulating relatively more ^{15}N in the roots. Singh, Badgujar, Reddy, Fleisher and Timlin (2013) reported increased sensitivity of cotton growth and photosynthesis to Pi deficiency in CO_2-enriched environment. In the same study, Pi deficiency tended to increase plant tissue N concentration whereas eCO_2 exhibited an opposite effect (Singh, Badgujar, Reddy, Fleisher & Timlin 2013). Therefore, the combined effect of these two factors is uncertain on plant growth and development.

Soybean plants are sensitive to Pi availability due to the direct involvement of Pi in carbon and nitrogen metabolism (Lauer, Pallardy, et al. 1989; Rufty et al. 1993). Soybean grown under low nutrient conditions produced fewer flowers, pods, and seeds and exhibited increased flower abortion and pod senescence (Sionit 1983). Phosphate deficiency decreased soybean biomass, photosynthesis, and stomatal conductance and increased leaf thickness in 30-day old plants (Qiu & Israel 1992). Elevated CO_2 increased the soybean seed yield by increasing the number of pods and seeds rather than seed size (Crafts-Brandner 1992b). Soybean responds to the external Pi supply under field conditions by increasing growth, pod number, and seed yield (Jaidee et al. 2013). The magnitude and direction of soybean response to Pi nutrition may be altered when grown under CO_2-enriched environment. In addition, the positive effect of eCO_2 on plant growth may be expected even under nutrient stress condition due to increased carbohydrate supply which may aid the energy demand of the stressed plants (Ahmed et al. 1993; Baker & Allen 1993; Singh, Badgujar, Reddy, Fleisher & Bunce 2013). Prior studies with soybean often have considered Pi nutrition or eCO_2 separately and investigations on the combination of these two factors are limited (Cure et al. 1988; Israel et al. 1990). The objective of this study was to determine the effect of Pi nutrition on soybean growth, allocation of biomass and nutrients, nutrient efficiency, and their relationship with the tissue phosphorus concentration under ambient (aCO_2) and eCO_2 growing conditions.

Materials and methods

Growth and experimental conditions

The experiment was conducted at USDA-ARS facility in Beltsville, MD, USA using controlled environment growth chambers (EGC Corp., Chagrin Falls, OH, USA). Soybean (cv. Spencer, indeterminate) seeds (five seeds pot^{-1}) were planted in 20 pots (volume 7.6 L) filled with washed concrete sand in each growth chambers. Pots were thinned to one plant per pot at eight days after planting (DAP). Plants were watered with full strength Hoagland's nutrient solution (Hewitt 1952) from emergence (4 DAP) to 12 DAP. Thereafter the treatments were initiated in factorial combination of two levels (ambient, aCO_2 400 µmol mol^{-1} and elevated, eCO_2 800 µmol mol^{-1}) of CO_2 and three levels of phosphate (0.5, 0.10,

and 0.01 mM Pi) in modified Hoagland's nutrient solution. The nutrient solution was applied to flushed (300–350 mL) four to six times during the day. The pots were fully flushed weekly with de-ionized water. At this stage, there were no statistical differences between chambers ($P > 0.90$) for plant height (PH) and number of leaves plant^{-1}. Experiment was replicated twice over time (repeated) using the same six chambers and lasted 110 days each. Pots were rotated periodically within each chamber to minimize effect of within-chamber heterogeneity. The CO_2 treatments were also rotated between the replications for each Pi level to minimize the potential chamber effect across growth CO_2.

The daytime temperature and light was initiated at 6:00 h. The light as photosynthetically active radiation (PAR) of 900 µmol m^{-2} s^{-1} at plant canopy was supplied following seedling emergence during the day period using a combination of metal halide and high-pressure sodium lamps. The PAR at the plant canopy was monitored using a LI-COR Quantum Sensor connected to a LI-1000 data Logger (LI-COR Inc., Lincoln, NE, USA) at multiple locations within each chamber on alternate days and adjusted to the mean value of 900 ± 15 µmol m^{-2} s^{-1} using controllable ballasts (Osram Sylvania, MA, USA). A 28/22°C day/night (12-h/12-h) air temperature was maintained within ±0.15°C in the growth chambers during the experiment. Injection of either CO_2 or CO_2-free air was determined using a TC-2 controller that monitored CO_2 every three seconds measured from an absolute infrared gas analyzer (WMA-2 PP-systems, Haverhill, MA, USA). Relative humidity in growth chambers varied between 50% and 70% during the experiment among the chambers.

Growth and yield measurements

Plants were harvested at 30 (full bloom), 45 (pod development), and 110 (maturity) DAP (six, seven, and seven plants, respectively), and PH and number of main stem nodes (MSNs) were recorded. Plants were separated into roots, stems, leaves, and pods (45 and 110 DAP). The total leaf area (TLA) was measured only on 30-day and 45-day samples and the specific leaf weight (SLW, mg dry weight cm^{-2} leaf area) was determined. Roots were washed in clean water. All plant parts were dried to constant weight at 70°C. The seed and shell mass was determined from pods obtained at maturity after drying at 35°C in forced-ventilation air for 10 days.

Gas exchange measurements

These measurements were made several times between 25 and 65 DAP on the uppermost fully expanded leaves from the stem apex between 9:00 and 13:00 h. The leaf net photosynthesis (P_{net}) was measured using a portable LI-COR 6400 photosynthetic system (LI-COR Inc., Lincoln, NE, USA). The measurements were taken when a steady state (around 4–6 min) was obtained, using a PAR of 1500 µmol m^{-2} s^{-1}, the treatment's CO_2 concentration, and a leaf temperature of 28°C. The relative humidity varied

between 45% and 65%. The values of P_{net}, stomatal conductance (g_s), and intercellular (C_i) CO_2 concentration were automatically computed from the instrument's software (details are available in LI-6400 Instruction Manual, version 5, Li-Cor Inc., Lincoln, NE, USA).

Tissue constituent measurements

The total chlorophyll concentration, tissue phosphorus (hereafter referred to as P to differentiate from phosphate treatments, Pi), and nitrogen (N) concentration were determined from the uppermost fully expanded leaves that were used for gas exchange measurements and in plant organs (leaves, i.e. included all the leaves from main stem and branches, abscised leaves separately, stems, roots, pods, shells, and seeds) at each sampling. The weighted whole-plant P and N concentrations were estimated as the sum of the products of dry mass of plant organs and their nutrient concentration divided by total biomass (TBM). Similarly, the weighted pod P and N concentration at the final harvest were estimated as the sum of the products of dry mass of seeds and shells and their nutrient concentrations divided by pod dry weight. Chlorophyll was extracted by placing two 0.95 cm² leaf disks for each leaf in a vial containing 5 mL of dimethyl sulfoxide and incubating in the dark for 24 h. Thereafter, the absorbance of the supernatant was measured at 664 and 648 nm by using a UV-2101-PC spectrophotometer (Shimadzu Corp., Columbia, MD, USA). The total chlorophyll was estimated by using the equation of Lichtenthaler (1987) and expressed on a leaf area basis ($\mu g\ cm^{-2}$). The dry plant parts were ground using a Wiley Mill (Wiley® Mill, Thomas Scientific, NJ, USA) to pass through a 1 mm screen. The tissue P concentration was measured in the Agriculture Diagnostic Laboratory, University of Arkansas, Fayetteville AR. The N concentration was determined by combustion using a CHN-2000 (Carbon Hydrogen Nitrogen-2000: LECO Corporation, St. Joseph, MI, USA).

The P and N utilization efficiencies for biomass production (PUE and NUE) were estimated by dividing TBM by the whole-plant P or N concentration. The total P or N absorbed (mg plant⁻¹) per unit of root biomass was used as an indicator of P or N uptake efficiency (PUpE or NUpE).

Data analysis

Statistical analyses were performed using SAS (SAS Enterprise Guide, 4.2, SAS Institute Inc., NC, USA). To test for the effect of treatments and their interaction, PROC MIXED with Kenward–Rogers (kr) adjustment of degrees of freedom was used for analysis of variance (ANOVA) using mean data from each repetition of the experiment. Treatments (Pi and CO_2) and their interaction were considered as fixed effects, and repetition as a random effect. The normality assumptions were assessed using SharpioAWilks statistics and log transformation was used, if necessary. Any heterogeneity in the data was corrected using the GROUP option in the REPEATED statement of the PROC MIXED procedure. The treatment comparisons were conducted by least square means (LSMEANS) procedure (at α = 0.05) when a main effect and/or the interaction was significant at $P \le 0.05$ with the letter grouping obtained using pdmix800 macro (Saxton 1998). The relationship of tissue P concentration with other parameters was established using linear or polynomial equations.

Results

Growth and seed parameters

The $CO_2 \times$ Pi interaction was significant ($P \le 0.05$) for TLA and TBM (45 and 110 DAP; Table 1). Phosphate treatment affected all growth and yield parameters ($P \le 0.05$) regardless of the harvest dates except for a few measurements date for PH and number of MSN. The CO_2 treatment also affected these parameters ($P \le 0.05$) especially at the 110 DAP. Compared to the 0.50 mM Pi treatment, means averaged across the two low Pi treatments had decreased TLA (45–56%), TBM (15–53%), pod no. (27–50%), seed no. (47–51%), and seed yield (47–58%) across both CO_2 levels (Tables 1 and 2). Moreover, the degree of stimulation by eCO_2 decreased with the reduction in Pi supply leading to a smaller positive or even a negative change at the lowest Pi supply. For example, at 45 DAP in plants grown under eCO_2, TLA increased by 22% and 15% at 0.50 and 0.10 mM Pi treatments, respectively. However, TLA decreased by 5% at 0.01 mM Pi at eCO_2. Similar observations were also made for TBM and seed yield (Tables 1 and 2). The individual seed weight (g seed⁻¹) was not affected by CO_2.

Biomass partitioning

Among the plant parts, the $CO_2 \times$ Pi interactions were only significant ($P \le 0.05$) for leaf and stem biomass especially at 45 and 110 DAP (Table 2). At 110 DAP, the main effects of Pi and CO_2 were significant ($P \le 0.05$) for most of the plant organs. At 110 DAP, averaged two lower Pi treatments caused >30% decrease in biomass accumulation for all organs, except roots, as compared to the 0.5 mM Pi treatment for each CO_2 level. When averaged across all Pi treatments, eCO_2 increased biomass accumulation by more than 24% for all plant organs compared to aCO_2; however, these increments were minimal at 0.01 mM Pi. Pi treatment did not affect ($P > 0.05$) the biomass partitioning to the leaves at 30 and 45 DAP; but it was significantly higher by 36–50% for aCO_2 and by 6–21% for eCO_2 at 110 DAP in the Pi-deficient plants. The changes in the fraction of stem biomass to the TBM depended upon the treatments and tended to either decrease with lower Pi supply or increase with eCO_2. Regardless of the harvest dates, root fractions consistently increased under Pi deficiency across growth CO_2. Averaged across CO_2, the fractions of pod and seed weight to the TBM were 22% and 29% lower, respectively, at 0.01 mM versus 0.50 mM

Table 1. Effect of CO_2 levels (μmol mol^{-1}) and phosphate (Pi, mM) supply on PH (cm), number of MSNs plant^{-1}, TLA (cm^2 plant^{-1}), TBM (g plant^{-1}), number of pod plant^{-1} (Pod no.) and seed plant^{-1} (Seed no.), and individual seed weight (g seed^{-1}) of soybean at three destructive harvests at 30, 45, and 110 (maturity) DAP.

CO_2	Pi	30 DAP				45 DAP					110 DAP					
		PH	MSN	TLA	TBM	PH	MSN	TLA	TBM	Pod no.	PH	MSN	TBM	Pod no.	Seed no.	g Seed^{-1}
400	0.50	64.0^a	11.4	2191^b	14.4^{bc}	139	16.5^{ab}	3981^b	50.0^{bc}	79.0^a	146^{ab}	18.7^{abc}	138.9^b	126^b	287^b	0.247^{ab}
	0.10	64.8^a	11.3	1521^c	13.2^{bcd}	144	16.1^{abc}	2728^d	45.3^c	74.0^a	147^{ab}	18.5^{abc}	124.1^b	88^c	202^c	0.282^a
	0.01	57.3^b	10.0	908^d	11.3^d	138	14.3^c	1354^e	27.5^d	37.0^b	128^b	15.3^c	50.3^c	42^d	98^c	0.191^{bc}
800	0.50	64.8^a	12.0	2784^a	19.2^a	151	18.2^a	4857^a	62.5^a	78.6^a	174^a	20.6^{ab}	215.1^a	159^a	386^a	0.265^a
	0.10	62.4^{ab}	11.5	1536^c	15.7^b	151	17.9^a	3150^c	53.3^b	79.9^a	161^a	21.1^a	152.7^b	113^{bc}	269^b	0.246^{ab}
	0.01	58.8^{ab}	10.9	922^d	11.9^{cd}	138	15.1^{bc}	1294^e	23.5^d	28.7^b	160^a	17.5^{bc}	50.3^c	47^d	106^c	0.170^c
ANOVA†	CO_2	0.9612	0.1629	0.0349	0.0135	0.1206	0.0321	0.0056	0.022	0.7624	0.015	0.0352	0.0130	0.0336	0.0483	0.4752
	Pi	0.0312	0.1326	<0.0001	0.0047	0.1583	0.0098	<0.0001	<0.0001	<0.0001	0.2485	0.0255	0.0003	0.0003	0.0011	0.0104
	$CO_2 \times$ Pi	0.5565	0.7069	0.0345	0.1347	0.4263	0.7025	0.0209	0.0237	0.2209	0.5365	0.9407	0.0491	0.3717	0.3295	0.4292

†The significance test (*P*-values) of the ANOVA between Pi and CO_2. Within columns means followed by same letters are not significantly different at $\alpha = 0.05$.
Note: The data are the mean of 12 (30 DAP) and 14 (45 and 110 DAP) plants.

Pi-treated plants. Averaged across all treatments, the highest contribution to the TBM at the final harvest (110 DAP) was accredited to the pods (59.2% where seeds 43.1% and shells 16.1%) followed by leaves (19%).

Gas exchange

Rate of photosynthesis decreased as plant aged and was consistently reduced in the Pi-deficient plants and increased under eCO_2, especially in the higher Pi treatments (Figure 1A). Both Pi deficiency and eCO_2 showed lower g_s which also tended to decrease as plants aged (Figure 1B). The C_i was either lower (eCO_2) or slightly affected (aCO_2) under Pi deficiency especially at early measurement dates but increased in the lowest Pi-treated plants at the later (58 and 65 DAP) measurement dates (Figure 1C). When averaged over measurement dates, Pi deficiency significantly ($P < 0.05$) decreased P_{net} across CO_2 levels, whereas a significant ($P < 0.05$) increase in P_{net} (25.5%) at eCO_2 versus aCO_2 was observed only at 0.50 mM Pi treatment (data not shown). In addition, eCO_2 caused 21–56% reductions in g_s across Pi treatments and the effect of Pi treatment was not significant ($P \geq 0.1$) for C_i.

Tissue constituents of uppermost fully expanded leaves

Chlorophyll concentration exhibited a curvilinear response as plant aged and tended to be lower in the lowest Pi treatment and at the eCO_2 (Figure 1D). Tissue P concentration was also consistently lower at eCO_2 and decreased as plant aged (Figure 1E). Averaged over measurement dates, both treatments affected the tissue N and P concentration ($P \leq 0.05$, data not shown). Averaged across CO_2 treatments, leaf P concentration in 65 DAP decreased by 2.68, 1.71, and 0.60 mg g^{-1} for 0.50, 0.10, and 0.01 mM Pi treatments, respectively, as compared to those for 26 DAP (Figure 1E). The N concentration also tended to be lower in eCO_2-treated plants and decreased as plant aged except for the lowest Pi treatment which increased for the last two measurements (58 and 65 DAP; Figure 1F). The N:P ratio was higher in Pi-deficient plants and increased as plant aged for all treatment combinations with the highest rate observed in the lowest Pi-treated plants (Figure 1G).

Tissue constituents of plant organs

The $CO_2 \times$ Pi interactions were not significant ($P > 0.05$), but Pi deficiency decreased ($P \leq 0.05$) tissue P concentration of whole-plant and organs regardless of the harvest dates (Table 3). Although the effect of CO_2 was mostly significant ($P \leq 0.05$) only at 110 DAP, all the plant organs often exhibited lower P concentration under eCO_2. Except for stem and roots, Pi treatment significantly ($P \leq 0.05$) affected tissue N concentration at the final harvest (Table 3). The tissue N concentration of whole-plant leaves decreased as plant aged across CO_2 levels. Although, not always significant, eCO_2 often decreased N concentration of whole-plant and

Table 2. Effect of CO_2 levels (μmol mol^{-1}) and phosphate (Pi, mM) supply on biomass partitioning of soybean plants at 30, 45, and 110 (maturity) DAP.

		Tissue biomass (g plant^{-1})						Fraction of TBM (%)					
CO_2	PI	Leaves	Stems	Roots	Pods	Shells	Seeds	Leaves	Stems	Roots	Pods	Shells	Seeds
						30 DAP							
400	0.50	7.37[b]	4.69[bc]	2.37[b]				51.0	32.5[ab]	16.5[cd]			
	0.10	6.42[b]	4.14[cd]	2.60[ab]				48.9	31.4[b]	19.7[bcd]			
	0.01	5.69[b]	3.09[e]	2.54[b]				49.3	27.4[c]	23.3[ab]			
800	0.50	9.74[a]	6.58[a]	2.85[ab]				50.7	34.3[a]	15.0[d]			
	0.10	7.32[b]	5.10[b]	3.24[a]				46.8	32.6[ab]	20.7[abc]			
	0.01	5.54[b]	3.42[de]	2.90[ab]				46.9	28.5[c]	24.6[a]			
ANOVA	CO_2	0.0704	0.0029	0.0220				0.2352	0.0602	0.7935			
	Pi	0.0088	0.0005	0.3202				0.1649	0.0012	0.0041			
	$CO_2 \times$ Pi	0.1676	0.0458	0.7810				0.7738	0.8290	0.5412			
						45 DAP							
400	0.50	18.3[bc]	18.0[b]	6.76	6.90[a]			36.7	36.2[b]	13.4[c]	13.8		
	0.10	17.3[c]	13.7[c]	7.49	6.75[a]			38.1	30.3[cd]	16.9[abc]	14.7		
	0.01	9.9[d]	7.5[d]	4.94	5.16[ab]			35.9	27.2[d]	18.1[a]	18.9		
800	0.50	24.4[a]	25.1[a]	6.14	6.87[a]			39.0	40.4[a]	9.8[c]	10.8		
	0.10	21.5[ab]	17.4[b]	6.50	7.84[a]			40.3	32.8[c]	12.2[bc]	14.7		
	0.01	8.3[d]	7.6[d]	4.81	2.81[b]			35.5	32.0[c]	21.0[a]	11.5		
ANOVA	CO_2	0.0124	0.0004	0.4959	0.6373			0.2622	0.0026	0.3524	0.0658		
	Pi	<0.0001	<0.0001	0.1693	0.0483			0.106	0.0002	0.0337	0.3219		
	$CO_2 \times$ Pi	0.0194	0.0040	0.9104	0.3301			0.5397	0.4745	0.2468	0.2186		
						110 DAP							
400	0.50	21.3[b]	16.4[bc]	5.2[c]	96.0[b]	24.8[b]	71.2[b]	15.3[c]	11.9	3.73[de]	69.0[a]	17.8[a]	51.2[a]
	0.10	26.1[b]	15.5[bc]	7.5[b]	75.0[b]	18.3[b]	56.7[c]	20.9[ab]	12.1	6.17[cd]	60.8[ab]	14.8[b]	46.0[a]
	0.01	11.6[c]	7.7[c]	4.7[c]	26.3[c]	8.1[c]	18.2[d]	22.9[a]	15.6	9.49[ab]	52.1[b]	16.2[ab]	35.8[bc]
800	0.50	36.4[a]	38.1[a]	6.3[bc]	134.3[a]	32.4[a]	101.8[a]	16.8[c]	17.7	2.92[e]	62.6[a]	15.1[b]	47.4[a]
	0.10	26.9[b]	23.2[b]	10.3[a]	92.2[b]	24.1[b]	68.1[c]	17.8[bc]	15.2	7.01[bc]	59.9[ab]	15.8[ab]	44.2[ab]
	0.01	10.2[c]	9.6[c]	5.1[c]	25.4[c]	8.3[c]	17.2[d]	20.3[ab]	19.0	10.1[a]	50.6[b]	16.4[ab]	34.2[c]
ANOVA†	CO_2	0.0673	0.0075	0.0104	0.0635	0.0374	0.0506	0.1319	0.0852	0.7612	0.2737	0.3952	0.3482
	Pi	0.0017	0.0041	0.0003	0.0006	0.0002	0.0006	0.0059	0.3878	0.0015	0.0079	0.2886	0.0065
	$CO_2 \times$ Pi	0.0443	0.0468	0.2615	0.2098	0.2602	0.1848	0.1169	0.8392	0.5889	0.6121	0.1000	0.9224

†The significance test (*P*-values) of the ANOVA between Pi and CO_2. Within columns means followed by same letters are not significantly different at $\alpha = 0.05$.
Note: The data are the mean of 12 (30 DAP) and 14 (45 and 110 DAP) plants.

organs. At the final harvest, seeds had the highest P (4.81 mg g^{-1}) and N (65.9 mg g^{-1}) concentration followed by pods then leaves when averaged across treatments.

Leaf abscission

Soybean leaves started to abscise at approximately 50 DAP and up to 80% of the leaves were abscised before the final harvest depending upon treatments (Figure 2A and 2B). Elevated CO_2 did not appear to have a major influence on the leaf abscission. Regardless of the CO_2 treatment, the tissue P concentration was lower whereas N concentration was higher in the abscised leaves of Pi-deficient plants as compared to 0.50 mM Pi-treated plants (Figure 2C–F). When averaged across all measurements and treatments, the P and N concentrations of abscised leaves were 50% and 18% lower, respectively, as compared to the P and N concentrations of whole-plant leaves at 110 DAP.

Phosphorus and nitrogen utilization and uptake efficiencies

The $CO_2 \times$ Pi interaction was not significant ($P > 0.05$) for these parameters except the PUE (110 DAP) and NUE (45 and 110 DAP; Table 4). Pi deficiency increased PUE and decreased NUE ($P \leq 0.05$) regardless of the CO_2 treatments. Elevated CO_2 tended to increase both PUE (19–81%) and NUE (7–60%) depending upon the time of the harvests and the Pi treatments. Irrespective of the CO_2 treatments, the P and N uptake efficiencies were significantly ($P \leq 0.05$) lower in Pi-deficient plants.

Relationship of tissue P with other parameters

The response of the leaf N concentration showed an increasing (110 DAP) pattern especially at the lowest Pi treatment as the leaf P concentration decreased (Figure 3A). The SLW also increased as leaf P concentration decreased but did not differ between CO_2 levels (Figure 3B). The individual seed weight and seed
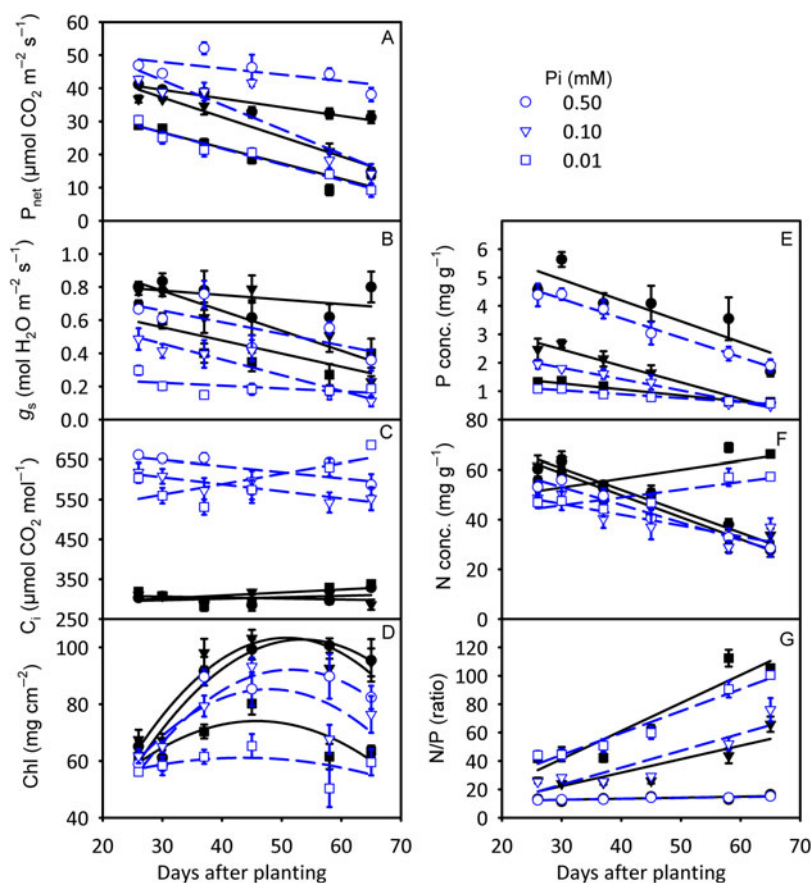
Figure 1. Photosynthetic rate (P_{net}) (A), stomatal conductance (g_s) (B), internal CO_2 concentration (C_i) (C), chlorophyll concentration (Chl) (D), phosphorus (P) (E), and nitrogen (N) (F) concentrations and N/P ratio (G) of uppermost fully expanded soybean leaves during experiment grown at either ambient (filled symbols and solid lines, 400 µmol mol^{-1}) or elevated (unfilled symbols and dashed lines, 800 µmol mol^{-1}) CO_2 under different phosphate (Pi) treatments. The error bars represent the standard error of 6–9 individual plants. The lines represent the fit of linear ($y = bx + c$) or polynomial second order (C; $y = ax^2 + bx + c$).

P concentrations increased, while seed N concentration and root:shoot ratio decreased as leaf P concentration increased, and did not differ between CO_2 levels (Figure 4A–D). TBM and seed yield showed increasing trend and the response to the eCO_2 increased as leaf P concentration increased (Figure 4E and 4F).

Discussion

Growth and biomass partitioning

Phosphate deficiency decreased soybean growth primarily by reducing leaf area and P_{net}, thus causing severe reduction in canopy photosynthetic capacity leading to over 68% and 74% lower biomass and seed yield, respectively, in the lowest Pi treatment. Decreased leaf cell expansion due to reduced hydraulic conductance inside the plants has been reported to be the primary cause of smaller leaf area in Pi-deficient soybean and cotton (Radin & Eidenbock 1984; Fredeen et al. 1989). Pi deficiency reduced the P_{net} concomitantly with g_s which was in accordance with the previous study on soybean (Lauer, Pallardy, et al. 1989). The small effect of Pi deficiency on C_i suggested a minor role of g_s to the photosynthetic limitations under Pi deficiency. Moreover, the increased C_i at the later growth stage in Pi-

deficient plants indicated that mesophyll limitations to photosynthesis might have occurred, as suggested by other studies (Brodribb 1996; Singh & Reddy 2011). Therefore, major limitations to P_{net} under Pi deficiency might still be attributed to the factors other than g_s such as mesophyll and inhibition of photo-biochemical process (Lauer, Pallardy, et al. 1989; Jacob & Lawlor 1991; Singh, Badgujar, Reddy, Fleisher & Bunce 2013).

Similar to this study, the increased biomass partitioning toward roots under Pi deficiency has also been reported previously and attributed to the ability of plant to adjust the above- and below-ground growth in an effort to increase nutrient uptake (Lenka & Lal 2012). Under stress conditions plants allocate the biomass toward the organ associated with acquiring the limited resources, such as Pi in this case (Bazzaz 1997). The lower individual seed weight at the lowest Pi treatment was in accordance with previous research (Crafts-Brandner 1992b). Seed size tends to be fairly stable across Pi-nutrition and CO_2 except at very low Pi supply (Cassman et al. 1981; Bharati et al. 1986; Cure et al. 1988). The biomass partitioning to seeds reflects the harvest index and has not been found to be affected by eCO_2 across Pi nutrition in soybean (Cure et al. 1988). Increase in the number of pods and seeds rather than the

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Table 3. Effect of CO_2 levels (μmol mol^{-1}) and phosphate (Pi, mM) supply on phosphorus (P) and nitrogen (N) concentration in tissues of soybean plant and its organs 30, 45, and 110 DAP.

CO$_2$	Pi	P (mg g^{-1})							N (mg g^{-1})						
		Plant	Leaves	Stems	Roots	Pods	Shells	Seeds	Plant	Leaves	Stems	Roots	Pods	Shells	Seeds
					30 DAP										
400	0.50	5.66ᵃ	5.44ᵃ	5.5ᵃ	6.59ᵃ				49.4ᵃ	63.6ᵃ	30.6	42.4			
	0.10	2.54ᵇ	3.02ᵇ	1.88ᵇ	2.38ᵇ				49.3ᵃ	60.8ᵃ	32.6	47.4			
	0.01	1.43ᵇᶜ	1.49ᶜ	1.26ᵇ	1.47ᵇ				46.1ᵇᶜ	54.1ᵇ	30.9	47.5			
800	0.50	5.10ᵃ	5.10ᵃ	4.95ᵃ	5.36ᵃ				47.2ᵃᵇ	60.7ᵃ	30.2	41.0			
	0.10	2.03ᵇᶜ	2.23ᵇᶜ	1.75ᵇ	1.99ᵇ				44.3ᵇᶜ	55.1ᵇ	30.6	41.3			
	0.01	1.28ᶜ	1.19ᶜ	1.20ᵇ	1.51ᵇ				42.9ᶜ	49.6ᶜ	30.4	45.2			
ANOVA†	CO$_2$	0.2052	0.1183	0.2572	0.414				0.0047	0.0022	0.2910	0.1696			
	Pi	0.0002	0.0001	<0.0001	0.0032				0.0196	0.0003	0.5031	0.2611			
	CO$_2$ × Pi	0.8116	0.6980	0.5737	0.695				0.3412	0.4036	0.6860	0.6426			
					45 DAP										
400	0.50	4.59ᵃ	4.19ᵃ	4.4ᵃ	4.68ᵃ	5.91ᵃ			37.7ᵇᶜ	48.7ᵃᵇ	24.5ᵃᵇ	42.8	38.5ᵃᵇ		
	0.10	1.71ᵇ	1.74ᵇ	1.02ᵇ	1.62ᵇ	3.23ᵇ			38.0ᵃᵇᶜ	46.0ᵇᶜ	25.9ᵃᵇ	47.4	32.8ᶜ		
	0.01	1.11ᵇ	0.82ᵇ	0.67ᵇ	1.29ᵇ	2.12ᵈ			42.2ᵃᵇ	51.7ᵃ	30.1ᵃ	52.3	31.5ᶜ		
800	0.50	4.16ᵃ	3.76ᵃ	4.03ᵃ	4.34ᵃ	5.86ᵃ			36.1ᶜ	48.6ᵃᵇ	22.1ᵇ	42.8	38.8ᵃ		
	0.10	1.57ᵇ	1.51ᵇ	1.01ᵇ	1.61ᵇ	2.98ᵇᶜ			34.2ᶜ	41.8ᶜ	20.9ᵇ	45.9	33.5ᵇᶜ		
	0.01	1.01ᵇ	0.75ᵇ	0.64ᵇ	1.31ᵇ	2.25ᶜᵈ			42.4ᵃ	52.2ᵃ	30.6ᵃ	49.2	33.3ᵇᶜ		
ANOVA†	CO$_2$	0.4255	0.3256	0.6305	0.8514	0.7650			0.1606	0.3162	0.2415	0.5320	0.4703		
	Pi	0.0003	0.0002	0.0002	0.0104	<0.0001			0.0085	0.0067	0.0336	0.1151	0.0151		
	CO$_2$ × Pi	0.8544	0.8161	0.8346	0.9594	0.7098			0.3508	0.2828	0.4928	0.8660	0.8837		
					110 DAP										
400	0.50	5.96ᵃ	5.21ᵃ	5.20ᵃ	3.37ᵃ	6.71ᵃ	2.68ᵃ	8.11ᵃ	41.1ᵃᵇᶜ	31.0ᵇ	20.7	27.5	48.3ᵈ	6.15ᵇᶜ	63.0ᶜ
	0.10	2.41ᵇ	1.76ᶜ	1.32ᵇ	1.58ᵇ	3.14ᵇ	0.4ᵇ	4.02ᶜ	43.5ᵃᵇ	32.4ᵇ	29.4	33.0	51.9ᵃ	6.03ᵇᶜ	66.7ᵇ
	0.01	1.49ᵇᶜ	0.90ᵈ	0.77ᵇ	1.07ᵇ	2.13ᶜ	0.4ᵇ	2.87ᵈ	44.4ᵃ	49.7ᵃ	25.7	29.0	51.5ᵃᵇ	8.85ᵃ	69.5ᵃ
800	0.50	5.03ᵃ	3.98ᵇ	3.67ᵃᵇ	4.47ᵃ	6.13ᵃ	1.86ᵃᵇ	7.49ᵇ	39.5ᶜ	25.3ᵇ	17.4	34.5	49.1ᶜᵈ	5.35ᶜ	63.0ᶜ
	0.10	2.02ᵇᶜ	1.23ᶜ	0.75ᵇ	1.24ᵇ	2.81ᵇ	0.37ᵇ	3.66ᶜ	40.4ᵇᶜ	23.4ᵇ	19.2	30.9	50.1ᵇᶜ	5.50ᶜ	65.8ᵇ
	0.01	1.25ᶜ	0.53ᵈ	0.50ᵇ	1.03ᵇ	1.90ᶜ	0.26ᵇ	2.68ᵈ	42.5ᵃᵇᶜ	48.2ᵃ	26.6	22.7	50.6ᵃᵇᶜ	7.14ᵇ	67.6ᵃᵇ
ANOVA†	CO$_2$	0.0403	0.0405	0.3836	0.5700	0.0363	0.4686	0.0049	0.0352	0.0680	0.1121	0.8224	0.1185	0.0305	0.1123
	Pi	<0.0001	0.0018	0.0249	0.0039	<0.0001	0.021	<0.0001	0.0509	0.0012	0.1207	0.0905	0.0045	0.0044	0.0004
	CO$_2$ × Pi	0.4355	0.5169	0.8166	0.3705	0.5826	0.7243	0.1858	0.7331	0.2763	0.2130	0.0894	0.0708	0.3956	0.3722

†The significance test (*P*-values) of the ANOVA between Pi and CO$_2$. Within columns means followed by same letters are not significantly different at α = 0.05.
Note: The data are the mean of 12 (30 DAP) and 14 (45 and 110 DAP) plants.
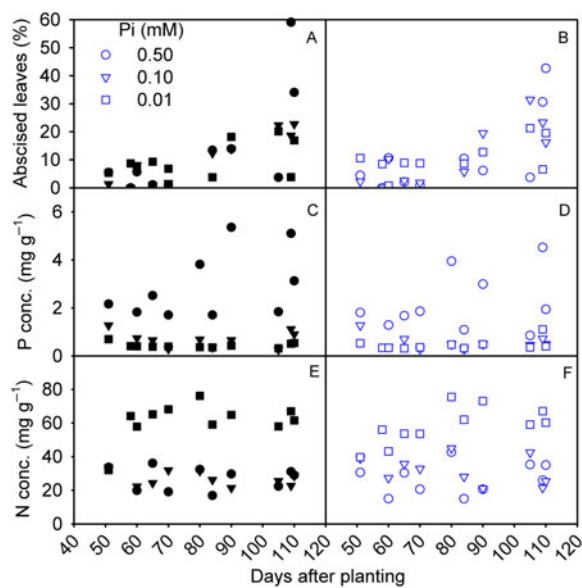
Figure 2. The abscised leaf (% of the total leaf dry weight plant^{-1}; (A, B) and their P (C, D) and N (E, F) concentration during the experimental period for soybean grown at either ambient (left panel, filled symbols, 400 µmol mol^{-1}) or elevated (right panel, unfilled symbols, 800 µmol mol^{-1}) CO_2 under different phosphate (Pi) treatments.

seed size contributed to higher seed yield under eCO_2 conditions. Prior and Rogers (1995) also reported that number of seed per plant increased at eCO_2 regardless of water regime.

The PUE was always higher but the NUE decreased in Pi-deficient plants across CO_2 treatments. However, this increase in the PUE under Pi-deficiency was influenced by the time of the harvest. The highest PUE was observed in 0.01 mM Pi at 30 DAP whereas PUE was the highest in 0.1 mM Pi treatment at the 45 and 110 DAP. Under severe Pi-deficiency, soybean growth is highly suppressed leading to lower PUE as also observed in other study (Cure et al. 1988). Nonetheless, in the current study, the increase in the PUE occurred despite the reduction in the PUpE. An increased root to shoot ratio in the plants grown under nutrient deficient or eCO_2 as observed in this and other studies might increase the nutrient acquisition, thus providing more root surface for nutrient absorption and enhanced nutrient uptake to support the above-ground biomass (Prior et al. 1998; Singh, Badgujar, Reddy, Fleisher & Timlin 2013). The PUE increases even under declined nutrient uptake efficiency due to favored root growth and overall increased in the total amount of nutrient uptake (Cure et al. 1988; Rogers et al. 1994; Singh, Badgujar, Reddy, Fleisher & Timlin 2013).

The Pi supply exerted major control upon the degree of stimulation by eCO_2 on plant growth, P_{net}, and seed yield. For example, eCO_2-mediated increase in these parameters was the greatest at 0.50 mM Pi and was insignificant or none in the low Pi treatments. Increased growth at eCO_2 was mainly associated with increased leaf area, P_{net}, and PUE of biomass production. Previous studies also reported that increase in canopy

Table 4. Effect of CO_2 levels (µmol mol^{-1}) and phosphate (Pi, mM) supply on phosphorus and nitrogen utilization efficiency (PUE and NUE; g^2 dry weight mg^{-1} P) and phosphorus and nitrogen uptake efficiency of soybean plants (PUpE and NUpE; mg P g^{-1} root dry weight) at the three destructive harvests 30, 45, and 110 DAP.

CO_2	Pi	PUE			NUE			PUpE			NUpE		
		30 DAP	45 DAP	110 DAP	30 DAP	45 DAP	110 DAP	30 DAP	45 DAP	110 DAP	30 DAP	45 DAP	110 DAP
400	0.50	2.64[c]	11.46[d]	23.84[d]	0.295[bc]	1.34[bc]	3.38[bc]	34.20[a]	34.26[b]	159.3[a]	299.7[a]	291.0[ab]	1103.3[a]
	0.10	5.46[bc]	28.32[ab]	51.48[b]	0.270[c]	1.22[c]	2.85[c]	13.01[b]	10.30[cd]	40.9[b]	251.7[b]	236.1[b]	724.5[b]
	0.01	7.93[ab]	25.95[ab]	34.72[cd]	0.252[c]	0.66[d]	1.13[d]	6.23[c]	6.17[cd]	16.5[b]	199.3[c]	234.5[b]	481.2[bc]
800	0.50	3.87[c]	15.29[cd]	43.21[bc]	0.409[a]	1.74[a]	5.43[a]	33.88[bc]	42.26[a]	171.8[a]	315.0[a]	368.1[a]	1351.4[a]
	0.10	7.86[ab]	35.56[a]	75.99[a]	0.355[ab]	1.59[ab]	3.77[b]	9.83[c]	13.01[c]	32.1[b]	214.4[bc]	281.5[ab]	618.1[bc]
	0.01	9.49[a]	24.22[bc]	42.01[bc]	0.282[c]	0.56[d]	1.18[d]	5.18[c]	4.88[d]	12.3[b]	173.9[c]	206.7[c]	420.6[c]
ANOVA†	CO_2	0.0494	0.2285	0.0011	0.005	0.0106	0.0034	0.3114	0.119	0.9819	0.1968	0.2920	0.6912
	Pi	0.0032	0.0031	0.0004	0.020	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	0.0446	0.0005
	$CO_2 \times Pi$	0.7731	0.3440	0.0909	0.1887	0.0260	0.0222	0.6873	0.1693	0.4788	0.1996	0.3409	0.1387

†The significance test (P-values) of the ANOVA between Pi and CO_2. Within columns means followed by same letters are not significantly different at α = 0.05.
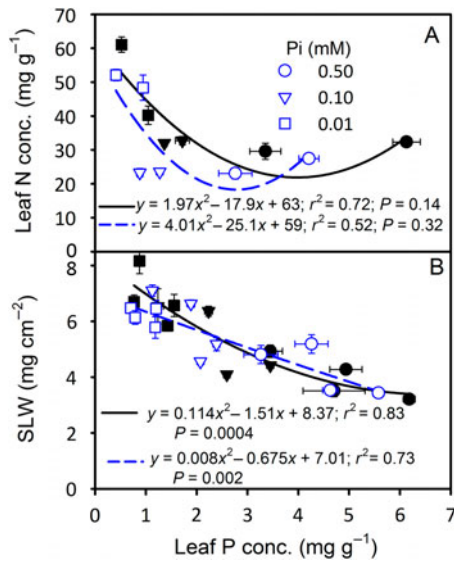Note: The data are the mean of 12 (30 DAP) and 14 (45 and 110 DAP) individual plants.

Figure 3. The tissue phosphorus (P) versus nitrogen (N); (A) concentration and SLW; (B) of soybean whole-plant leaves grown at either ambient (filled symbols and solid lines, 400 μmol mol^{-1}) or elevated (unfilled symbols and dashed lines, 800 μmol mol^{-1}) CO_2 under different phosphate (Pi) treatments. Data are from destructive harvests either 110 (A) or combined 30 and 45 (B) DAP. Error bars for the *x*- and *y*-axis represent standard errors of seven individuals.

photosynthesis due to increased leaf area and P_{net} at eCO_2 contributed the most to increased growth under Pi nutrition (Cure et al. 1988; Singh, Badgujar, Reddy,

Fleisher & Timlin 2013). Although none of plant organs except the stems showed appreciable increases in biomass partitioning at eCO_2, the biomass accumulation for all plant organs was higher across Pi nutrition which led to the observed growth stimulations and higher P and N utilization efficiencies. Responses of TBM and P_{net} at eCO_2 were maximized due to increased P and N uptake on per plant basis and were closely associated with the P and N utilization efficiencies. For instance, the PUE and NUE increased with TBM and decreased when TBM was low at eCO_2 (e.g. 45 DAP at 0.01 mM Pi). This clearly indicated that, despite the lower tissue P or N concentration, growth enhancement occurred at eCO_2. Moreover, the higher growth at eCO_2 occurred without a significant increase in the P and N uptake efficiencies, especially in Pi-deficient plants. The higher root mass at eCO_2 may also contribute to increased uptake due to increased root surface area (Cure et al. 1988).

Tissue constituents

The observed decrease in tissue P and N concentration as the plant aged might be attributed to the remobilization of nutrients to new growth and seeds (Hanway & Weber 1971a, 1971b; Jones et al. 1977; Lauer, Blevins, et al. 1989). Hanway and Weber (1971a) reported mobilization of approximately half of nutrients (N and P) from vegetative parts to seeds. Tissue P and N concentration followed nearly a similar trend for both the uppermost fully expanded leaves and in the whole-plant or
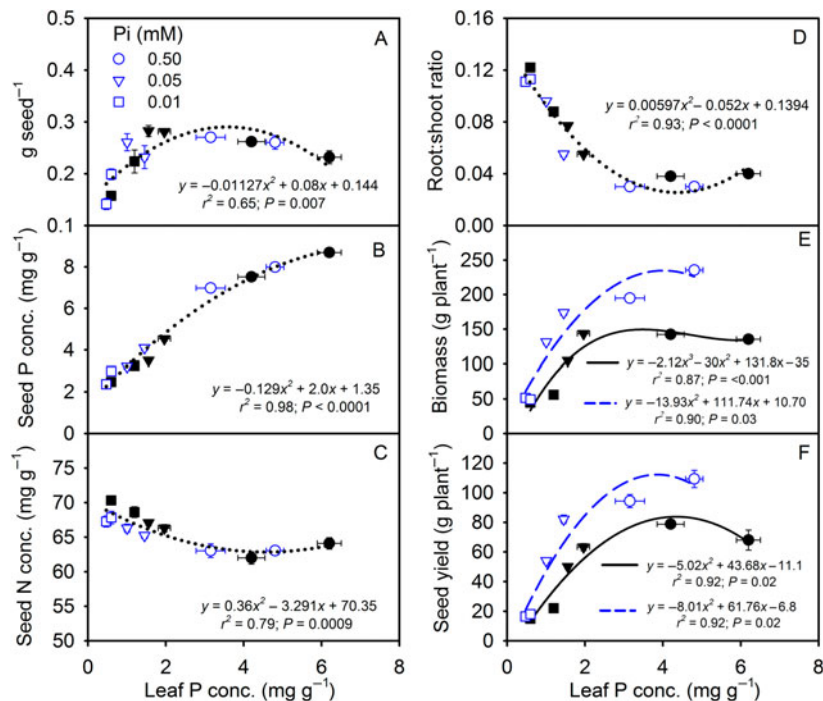

Figure 4. Relationship between leaf tissue phosphorus (P) concentration with individual seed weight (A), seed P (B), or N (C) concentration, root: shoot ratio (D), TBM (E), and seed yield (F) of soybean grown at either ambient (filled symbols and solid lines, 400 μmol mol^{-1}) or elevated (unfilled symbols and dashed lines, 800 μmol mol^{-1}) CO_2 under different phosphate (Pi) treatments. Data are the mean of seven individual plants measured at 110 DAP. Error bars for the *x*- or *y*-axis represent standard errors of seven individuals. The lines are the fit of polynomial second-order equation. Dotted lines represent fit across CO_2. Since tissue P versus biomass regression fit of polynomial second order at aCO_2 was not significant ($y = -8.44x^2 + 72.46x + 4.94$; $r^2 = 0.81$; $P = 0.06$), a polynomial third-order equation was fitted (E).

whole-plant leaves. The observed decrease (P) or increase (N) in the tissue nutrient concentration under Pi deficiency has also been reported in other studies (Fleisher et al. 2012; Singh, Badgujar, Reddy, Fleisher & Timlin 2013). Apparently, due to Pi deficiency the tissue P concentration decreased in greater proportion than the plant biomass leading to markedly increased PUE. However, at the high Pi treatment, the decline in the PUE might have been associated with continued increase in plant P concentration even after the maximum growth was attained (Cure et al. 1988). This was also supported by higher PUpE in the Pi sufficient versus Pi-deficient plants. The nutrient leakage and lower energy balance in Pi-deficient roots might have also contributed to lower P uptake leading to the greater reduction of tissue P concentration than biomass production under Pi deficiency (Ratnayake et al. 1978; Rufty et al. 1993).

The higher tissue N/P ratio observed in Pi-deficient plant, regardless of the CO_2 treatments, is also an indicator of Pi-stress (Güsewell 2004). The mean N/P ratio in the Pi sufficient plants varied between 11 and 16 whereas it was >20 (up to 105) under Pi-deficiency exhibiting increased Pi limitation as the deficiency increased similar to the earlier findings (Koerselman & Meuleman 1996; Güsewell 2004). Therefore, the critical N/P ratio in soybean appeared to be <16 for optimal growth across CO_2 condition.

Increased tissue N in Pi-deficient plants clearly indicated alteration in the N assimilation and reduced mobility inside the plant (Rufty et al. 1993). The markedly increased fraction of root biomass in Pi-deficient plant might have also assisted increased N uptake because N was not limited in the rooting medium (Singh, Badgujar, Reddy, Fleisher & Timlin 2013). When, other nutrients such as Pi is limited, the excess N in the leaf tissue is often stored as the non-functional protein at the expense of other essential cell components such as enzymes and chlorophyll (Israel & Ruffy 1988; Staswick et al. 1991). This was also consistent with the markedly reduced chlorophyll concentration in the lowest Pi-treated plants in the current study.

The leaf abscission started to occur approximately 50 DAP in all treatments which was similar to the observation made elsewhere (Hanway & Weber 1971a). Generally, at this early growth stage, plant leaves act as a sink for mineral nutrients while later becoming nutrient source for the new growth and the development of seeds (Himelblau & Amasino 2001). Remobilization of nutrients from the leaves was apparent from the overall lower P and N concentration of abscised versus attached leaves. However, a marked increase in P (> 80 DAP at 0.50 mM Pi) or N concentration (> 58 DAP at 0.01 mM Pi) in the abscised leaves also suggested the lack of complete utilization of these nutrients during the senescence (Figure 2). Since the seed N concentration was not reduced in the Pi-deficient plants indicating low demand, the N might have been accumulated in excess in the plant tissue. Therefore, the remobilization of the nutrient may be complex in nature, at least when Pi is deficient, and might not exert a primary regulatory control in the

process of leaf senescence in soybean (Crafts-Brandner 1992a, 1992b; Mauk & Noodén 1992).

The lower nutrient concentrations at eCO_2 have often been reported and might be attributed to reduced demand, lower transpiration, adjustment in the nutrient uptake and utilization, and increased growth and leaf thickness (which act as dilution effects; Cure et al. 1988; Taub & Wang 2008; Singh, Badgujar, Reddy, Fleisher & Timlin 2013). Increased growth and lower transpiration due to reduced g_s at eCO_2 were also observed in the current study. However, the SLW, signifying leaf thickness, of both uppermost fully expanded leaves (data not shown) and whole-plant leaves were not affected by eCO_2, contrary to the earlier results in cotton (Singh, Badgujar, Reddy, Fleisher & Timlin 2013). The alteration in the N metabolism may influence nitrate assimilation and biosynthesis of organic compounds, thus affecting the overall response of plants to eCO_2 (Bloom et al. 2010; Singh, Badgujar, Reddy, Fleisher & Timlin 2013).

Tissue P in relation with plant parameters

The observed curvilinear response of growth and seed yield with the leaf P concentration suggested that the highest Pi treatments reflected sufficient Pi supply in the current study. The plant tissue and seed P concentration in 0.50 mM Pi treatment was also comparable to observations made in other studies (Cassman et al. 1981; Walker et al. 1985). The individual seed weight increased with leaf P concentration but did not differ between CO_2 levels which was in accordance with the observed non-significant effect of the CO_2 on seed size in the current study and elsewhere (Prior & Rogers 1995). Although seed P concentration was significantly lower at eCO_2 across Pi treatments, the seed P response to leaf tissue P concentration did not differ between CO_2 levels. The inverse relationship observed between seed P and N concentration was in agreement with the earlier findings (Crafts-Brandner 1992b) and likewise, suggested that N remobilization to the seeds was not restricted and the net seed N demand for the plant was lower due to decreased seed yield in the Pi-deficient plants.

TBM and seed yield showed an increasing response to tissue P concentration and differed between CO_2 levels. Previous studies indicated that eCO_2 increases the overall nutrient demands and higher concentration of leaf P may be required for maximum plant productivity (Conroy 1992; Rogers et al. 1993). Therefore, the critical leaf P concentration is likely to be higher in plants grown under eCO_2. In fact, in the current study, the leaf P concentration (mg g^{-1}) at maximum biomass and seed yield was higher at eCO_2 (4.8 mg) versus aCO_2 (< 4.2 mg) indicating higher critical foliar concentration (the data points in the Figure 4E and 4F). This is in contrast to the lower critical tissue N concentration at eCO_2 for wheat and cotton as reported previously (Rogers et al. 1993). However, similar to our study, an increased critical tissue P and K (potassium) concentration has been suggested in other crops (Conroy 1992; Rogers et al. 1993; Reddy & Zhao 2005). This clearly suggested

that it is not only the nutrient demand but also the sensitivity of crop may be increased especially for P and K nutrition under CO_2-enriched atmosphere of the future. The lower demand of N but higher requirement of P or K under eCO_2 might be one of the major causes for the observed differences of the critical nutrient concentration among N, P, and K. Therefore, the current understanding that relates to the requirement of the tissue nutrient concentration to achieve optimum crop growth and yield under ambient CO_2 is likely to change in to the CO_2-enriched atmosphere.

Conclusions

The marked decrease in the soybean biomass and seed yield under Pi deficiency was primarily attributed to severely decreased leaf area and photosynthesis. Stomatal conductance did not appear to be the main cause of photosynthetic inhibition in Pi-deficient plants. The greater PUE and lower P uptake efficiency in Pi-deficient plants were attributed to the alteration in biomass partitioning and the dynamics in the nutrient allocation in plant organs. In the Pi-deficient plants, the alteration in N uptake and utilization was apparent due to increased tissue N concentration in both intact and abscised leaves. A tissue N/P ratio of >16 appeared to exhibit phosphorus limitation for soybean growth and development. The higher root:shoot ratio might have also assisted in greater N uptake resulting in the higher tissue N concentration in Pi-deficient plant. The growth stimulation at eCO_2 was closely associated with increased P utilization efficiency. However, the degree of soybean growth, yield, and photosynthetic response to eCO_2 were depended upon the Pi nutrition. The tissue P concentration required for maximum productivity for biomass and seed yield tended to be higher at eCO_2 versus aCO_2. The results suggested that eCO_2 is likely to increase the leaf critical P concentration for biomass productivity and yield in soybean. Therefore, the optimum soil phosphorus availability will be vital to gain maximum benefit from the CO_2 enriched environment.

Acknowledgments

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