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MINERAL DEFICIENCY STRESS

Effect of Phosphorus Nutrition on Growth and Physiology of Cotton Under Ambient and Elevated Carbon DioxideS. K. Singh^{1,2}, G. B. Badgajar^{2,3}, V. R. Reddy², D. H. Fleisher² & D. J. Timlin²¹ Wye Research and Education Center, University of Maryland Queenstown, MD, USA² Crop Systems and Global Change Laboratory, USDA-ARS Beltsville, MD, USA³ Asian Institute of Technology Pathumthani, Thailand**Keywords**

biomass partitioning; nitrogen; photosynthesis; specific leaf weight; stomatal conductance

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Abstract

Phosphorous deficiency in soil limits crop growth and productivity in the majority of arable lands worldwide and may moderate the growth enhancement effect of rising atmospheric carbon dioxide (CO₂) concentration. To evaluate the interactive effect of these two factors on cotton (*Gossypium hirsutum*) growth and physiology, plants were grown in controlled environment growth chambers with three levels of phosphate (Pi) supply (0.20, 0.05 and 0.01 mM) under ambient and elevated (400 and 800 μmol mol⁻¹, respectively) CO₂. Phosphate stress caused stunted growth and resulted in early leaf senescence with severely decreased leaf area and photosynthesis. Phosphate stress led to over 77 % reduction in total biomass across CO₂ levels. There was a below-ground (roots) shift in biomass partitioning under Pi deficiency. While tissue phosphorus (P) decreased, tissue nitrogen (N) content tended to increase under Pi deficiency. The CO₂ × Pi interactions were significant on leaf area, photosynthesis and biomass accumulation. The stimulatory effect of elevated CO₂ on growth and photosynthesis was reduced or highly depressed suggesting an increased sensitivity of cotton to Pi deficiency under elevated CO₂. Although, tissue P and stomatal conductance were lower at elevated CO₂, these did not appear to be the main causes of cotton unresponsiveness to elevated CO₂ under severe Pi-stress. The alteration in the uptake and utilization of N was suggested due to a consistent reduction (18–21 %) in the cotton plant tissue N content under elevated CO₂.

Introduction

Phosphate (Pi) deficiency in soil is a limiting growth factor in over 30 % of crop lands, and a major production constraint in acidic soils comprising up to 70 % of arable lands worldwide (Vance et al. 2003, Cordell et al. 2009, Lenka and Lal 2012). Phosphate is a component of nucleic acids and cellular membranes, and essential for metabolic processes (Raghothama 1999, Vance et al. 2003). Phosphate deficiency decreases plant growth and photosynthesis and thus biomass accumulation and yield. The crop demand for nutrients such as Pi has increased due to the introduction of high yielding cultivars, and this requirement may be even higher due to increased plant growth under rising atmospheric carbon dioxide concentrations (CO₂) (Rogers et al. 1993, Lewis et al. 1994, Lenka and Lal 2012). The cur-

rent atmospheric CO₂ concentration of approximately 394 μmol mol⁻¹ is projected to be doubled by the end of 21st century (IPCC 2007). In general, elevated CO₂ enhances plant growth, photosynthesis and yield. However, soil nutrients such as Pi exert major control over plant response to elevated CO₂ (Lewis et al. 1994, Campbell and Sage 2006, Lenka and Lal 2012). Studies evaluating the effect of Pi deficiency on cotton growth and physiological processes under current and projected atmospheric CO₂ conditions are limited. Because phosphate deficiency and elevated CO₂ may co-exist under natural environments, which have an opposite effect on plant growth and productivity, it is imperative to evaluate the crop responses to the interaction between these two factors.

Cotton (*Gossypium hirsutum*) is an important fibre crop grown worldwide (FAO 2010). Cotton growth,

photosynthesis and lint yield respond positively to elevated CO₂ and availability of nutrients including Pi (Sawan 1986, Kimball and Mauney 1993, Rogers *et al.* 1993, Singh *et al.* 2006, Girma *et al.* 2007). However, the positive response of elevated CO₂ is often reduced when cotton is grown in combination with low supply of nutrients such as nitrogen (Rogers *et al.* 1993), potassium (Reddy and Zhao 2005) and phosphorus (Rogers *et al.* 1993, Barrett and Gifford 1995). Nutrient deprivation caused imbalance in hydraulic conductance, decreased cell expansion and decreased biosynthesis of photosynthetic pigments and metabolic enzymes, which may inhibit the stimulatory effect of elevated CO₂ on plant growth (Radin and Eidenbock 1984, Rogers and Humphries 2000). Under field conditions, crops are routinely exposed to multiple stress factors; therefore, understanding of the interaction between environmental factors such as CO₂ and Pi nutrition is critical for nutrient management.

The CO₂ enrichment and deficiency of nutrients including Pi have also been reported to affect biomass partitioning and nutrient allocation to plant organs (leaves, stems and roots) (Prior *et al.* 1998, Reddy and Zhao 2005, Fleisher *et al.* 2012). Moreover, the composition of other nutrients such as nitrogen (N) in plant tissues may be altered due to changes in nutrient uptake and utilization under Pi-stress as well as elevated CO₂ leading to more complex interactions between these two factors (Prior *et al.* 1998, Fleisher *et al.* 2012, 2013, Lenka and Lal 2012). Therefore, nutrient availability will play a vital role in determining the magnitude and direction of plant growth and physiological response to CO₂ enriched environment.

Phosphorus deficiency inhibits cotton growth and development by decreasing photosynthetic capacity (Longstreth and Nobel 1980, Radin and Eidenbock 1986, Barrett and Gifford 1995), leaf expansion (Radin and Eidenbock 1984), biomass accumulation (Radin and Eidenbock 1984, Ahmad *et al.* 2001) and yield (Singh *et al.* 2006). Rogers *et al.* (1993) suggested that the critical concentration of Pi required for maximum crop productivity may increase in a CO₂ enriched environment. Thus, plants currently not limited by Pi may become limited due to projected increases in atmospheric CO₂ (Lewis *et al.* 1994, Lenka and Lal 2012). Studies evaluating cotton growth and physiology under varying levels of Pi supply and CO₂ are limited (Gifford *et al.* 2000). Most of the prior studies investigating crop responses under varying nutrient supply have focused on nutrients other than Pi and few have considered major row crops including cotton and their interaction with CO₂ concentrations (Jacob and Lawlor 1991, Reddy and Zhao 2005, Campbell and Sage 2006, Bown *et al.* 2009, Jin *et al.* 2011). We hypothesize that severe Pi deficiency may limit/offset the positive effects of elevated CO₂ on cotton growth and physiological processes due to increased sensitivity.

The objectives of this study were to determine the interactive effects of CO₂ and Pi supply on cotton growth and physiological processes and quantify the allocation of biomass and nutrients in plant organs.

Materials and Methods

Growth conditions

The study consisted of two experiments in 2011, hereafter referred to as Exp. I and Exp. II, which were conducted at USDA-ARS Henry A. Wallace Agricultural Research Center in Beltsville, MD USA. Both experiments used the same six controlled environment growth chambers (EGC Corp., Chagrin Falls, OH, USA) but at different dates and were completed in 112 (Exp. I) and 91 days (Exp. II) from the day after planting (DAP). In addition, these experiments differed from one another with respect to the number and size of pots used, initiation of CO₂ treatments and frequency and timing of the measurements.

In Exp. I, cotton (cv. Deltapine 555) seeds were planted in 12 pots (volume, 16-l) filled with fine sand and vermiculite (3 : 1 volume ratio) in each of the six growth chambers. Two levels (400 and 800 $\mu\text{mol mol}^{-1}$) of CO₂ treatments were initiated each in three chambers from the beginning, and plants were watered with full strength Hoagland's nutrient solution (Hewitt 1952) from the emergence to 34 days after planting (DAP). Thereafter, phosphate treatments (for simplicity referred as Pi) were initiated with the modified Hoagland's nutrient solution by three levels of Pi treatments, 0.20, 0.05 and 0.01 mM each at two levels of CO₂. The Pi treatments were selected based on the previous studies with Pi nutrition in cotton reflecting a range in Pi-stress from 0 % up to 33 % compared with the full nutrient supply (Radin and Eidenbock 1984, 1986, Barrett and Gifford 1995). The selected treatments correspond to the full nutrient (0.20 mM) and Pi-stresses of 25 % (0.05 mM) and 5 % (0.01 mM) of the full. A range in the tissue P content was obtained using the current Pi treatments.

In Exp. II, cotton seeds from the same cultivar were planted outdoor in 120 pots on 27 July 2011 (volume 7.6-l), containing rooting medium similar to the Exp. I. Plants were watered with full strength Hoagland's nutrient solution from emergence to 34 DAP. Thereafter, plants were transferred and randomly assigned to the six growth chambers (20 pots each), and the treatments (two levels of CO₂ and three levels of Pi) were initiated same as in Exp. I. At this stage, there were no statistical differences between chambers ($P > 0.975$) for plant height, leaf area and number of leaves plant⁻¹.

For both experiments, the daytime temperature and light regime were initiated at 6:00 h. The light as

photosynthetically active radiation (PAR) of 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at plant canopy was supplied during day period using a combination of metal halide and high pressure sodium lamps. A 30/22 °C day/night (14-h day and 10-h night) temperature was maintained in the growth chambers during experiments. Injection of either CO₂- or CO₂-free air was determined using a TC-2 controller that monitored CO₂ measured from an absolute infrared gas analyser (WMA-2 PP-systems, Haverhill, MA, USA). Relative humidity varied between 50 and 70 % during the experiment among the chambers.

Growth measurements

For each experiment, plant height (PH) and leaf length on each main stem nodes were measured weekly in six (Exp. I) and eight plants (Exp. II) from 50 (Exp. I) and 34 (Exp. II) DAP to the end of experiments. The leaf lengths were subsequently converted to main stem leaf area (MSLA) using a quadratic equation $y = 0.9569x^2 - 2.0162x + 0.8811$, ($r^2 = 0.96$, $n = 253$, $y = \text{area in cm}^2$ and $x = \text{leaf length in cm}$) established by developing a relationship between the lengths of different leaves and leaf area, measured using a LICOR-3100 leaf area metre (LICOR, Inc., Lincoln, NE, USA) at the time of destructive harvests. Plants were harvested at 85 and 112 DAP (six pots each) in Exp. I, and 67, 81 and 93 DAP (six, six and eight pots, respectively) in Exp. II. Plants were separated into stems, leaves, fruits (includes flowers, squares and bolls) and roots, and plant height (PH), number of main stem nodes (MSN), total leaf area (TLA) and total biomass (TBM) were measured. Roots were washed in clean water. The dry weight of plant materials were determined after drying in an oven at 70 °C until constant weight was obtained.

Gas exchange measurements

In both experiments, leaf photosynthetic rate (P_{net}) and stomatal conductance (g_s) were measured on the upper most fully expanded leaves between 9:00 and 13:00 h using a portable LICOR 6400 Photosynthesis system (LICOR Inc.). This photosynthesis system used blue (475 nm) and red light-emitting diodes (LED) (630 nm) as a light source mounted onto a two cm^2 leaf chamber. The gas exchange measurements were recorded when a steady-state (around 3–6 min) was obtained, at 1500 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR). The leaf cuvette temperature was set to 30 °C. The CO₂ entering in the cuvette was maintained at the treatment CO₂ concentration level of either 400 or 800 $\mu\text{mol mol}^{-1}$ and relative humidity varied between 45 and 60 %. The photosynthetic rate, g_s , and intercellular (C_i) to external (C_a) CO₂ concentration ratio (C_i/C_a) were automatically computed from instru-

ments software (details are available in LI-6400 Instruction Manual, version 5, Li-Cor Inc., Lincoln, Nebraska, USA).

Measurement of tissue constituents

Total chlorophyll concentration, tissue phosphorus (for simplicity and to distinguish from phosphate treatments, referred as P), carbon (C) and nitrogen (N) contents were determined from the upper most fully expanded leaves that were used for gas exchange measurements, and in plant organs (leaves, stems and roots) after each harvest. The individual leaf area and dry weight were also recorded for uppermost fully expanded leaves to determine specific leaf weight (SLW, $\text{mg dry weight cm}^{-2}$ leaf area). The tissue P, C and N contents in uppermost fully expanded leaves were expressed either based on leaf dry mass (mass-based, mg g^{-1}) or leaf area (area-based, mg cm^{-2}). Whole plant tissue P and N contents were estimated as the product of dry mass of plant organs (leaves, stems and roots) and their nutrient concentration (mg g^{-1}). Chlorophyll was extracted by placing two 0.95 cm^2 leaf discs 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 using a UV-2101-PC spectrophotometer (Shimadzu Corp., Columbia, MD, USA). The total chlorophyll was estimated using the equation of Lichtenthaler (1987) and expressed on leaf area basis ($\mu\text{g cm}^{-2}$). The dry tissues were ground using a Wiley Mill (Wiley® Mill, Thomas Scientific, NJ, USA) to pass through 1-mm screen. The tissue P content was quantified in the Soil Testing and Plant Analysis Laboratory, Extension Service, Mississippi State University, MS. The tissue carbon and N contents were determined by combustion using a CHN-2000 (Carbon Hydrogen Nitrogen-2000: LECO Corporation, St. Joseph, MI, USA).

Data analysis

All statistical analyses were performed using SAS procedures (SAS Enterprise Guide, 4.2, SAS Institute Inc., NC, USA). Proc Mixed procedure of SAS was used for regression analysis, to calculate the coefficients and to test for common slope and intercepts. Days after planting were treated as the continuous variable, and CO₂ and Pi treatments as qualitative factors. Proc Mixed was also used to test the treatment differences among the measured variables using individual pots as pseudoreplicates between two levels of CO₂ and three levels of Pi, and chamber as a random effect. The treatment comparisons were conducted by least square means (LSMEANS) procedure with letter grouping obtained using the pdmix800 macro (Saxton 1998). In both experiments, the data were analysed separately for each harvest. Because there were no significant

differences for leaf level measurements between the two experiments, the data measured on uppermost fully expanded leaves were combined.

Results

Plant growth

In both the experiments regardless of the harvest dates, low Pi treatments (0.05 and 0.01 mM) significantly ($P < 0.05$) decreased almost all the growth parameters, whereas elevated CO₂ caused significant ($P < 0.05$) increases in growth, mostly in high Pi-treated plants (Table 1). Plant height, MSN and MSLA followed asymptotically linear growth patterns during crop development, which were more obvious in the plants grown at 0.20 mM Pi (Fig. 1a–f). However, under Pi-stress treatments, MSLA started to decline 75 DAP in both experiments due to leaf senescence (Fig. 1b,e). At the final harvest, compared with the 0.20 mM Pi treatment, Pi-stress treatments (averaged 0.05 and 0.01 mM) caused approximately 52, 25 and 85 % decline in PH, MSN and TLA, respectively, across experiments, whereas TBM decreased by 89.4 % (Exp. I) and

77.7 % (Exp. II). Averaged across Pi treatments, cotton grown at elevated CO₂ had significantly ($P < 0.05$) taller plants (18.5–23.0 %), higher TLA (10–28 %) and TBM (31–33 %) at the final harvest across experiments (Table 1). The CO₂ treatment did not affect MSN of cotton plants. The averaged specific leaf weight (calculated using all leaves) tended to be slightly higher under Pi-stress and at elevated CO₂.

Biomass partitioning

At final harvest in both experiments, Pi treatment had significant ($P < 0.001$) effect on dry matter partitioning between plant organs (leaves, stems, fruits and roots), and when averaged across CO₂ levels, Pi-stress treatments (averaged 0.05 and 0.01 mM Pi) exhibited >80 % decreases in biomass accumulation in each of the components as compared with 0.20 mM Pi treatment (Table 2). The CO₂ × Pi interactions were significant ($P < 0.01$) for leaves and stems in both experiments. Averaged across Pi treatments, elevated CO₂ significantly ($P < 0.01$) increased leaf and stem biomass; however, this increment was not significant ($P > 0.05$) for fruit and root biomass. The

Table 1 Effect of CO₂ levels ($\mu\text{mol mol}^{-1}$) and phosphate (Pi, mM) supply on plant height (PH, cm), number of main stem nodes (MSN plant⁻¹), total leaf area (TLA, cm² plant⁻¹), total biomass (TBM, g plant⁻¹) and specific leaf weight (SLW, mg cm⁻² of whole plant leaves) of cotton, in Exp. I (84 and 112 days after planting, DAP) and Exp. II (67 and 91 DAP). Analysis of variance (ANOVA) between Pi and CO₂ is given. The data are the mean of six-eight individual plants

CO ₂	Pi	PH	MSN	TLA	TBM	SLW	PH	MSN	TLA	TBM	SLW
Experiment I											
				84 DAP				112 DAP			
400	0.20	47.51b	21.5b	1993b	61.98b	10.71b	57.67b	27a	3177b	115.66b	9.40c
	0.05	23.43d	18.0cd	368cd	10.02d	11.33b	31.17d	21bc	985c	29.38c	12.15ab
	0.01	20.00de	16.6de	244cd	8.38d	12.14ab	23.42e	21bc	92e	5.63d	9.23c
800	0.20	64.66a	24.6a	3714a	88.08a	10.75b	73.00a	29a	4112a	173.42a	11.71abc
	0.05	36.00c	19.0bc	503c	21.94c	13.50a	39.28c	23b	516d	22.95c	12.25a
	0.01	24.00e	16.0e	89d	7.63d	12.40ab	25.70e	19c	67e	3.34d	9.55bc
ANOVA	CO ₂	***	ns	***	***	ns	***	ns	*	***	ns
	Pi	***	***	***	***	*	***	***	***	***	*
	CO ₂ × Pi	***	*	***	***	ns	***	ns	***	***	ns
Experiment II											
				67 DAP				91 DAP			
400	0.20	35.40a	15.6a	971b	16.49b	6.64c	60.65b	21a	2286b	62.45b	7.82d
	0.05	25.13bc	14.1b	400c	10.12c	9.39ab	30.84d	16b	444c	15.41c	9.20 cd
	0.01	21.90c	12.4c	274c	7.86c	9.67ab	28.59d	16b	435c	14.73c	9.67bc
800	0.20	39.42a	16.3a	1208a	24.21a	8.46bc	72.03a	23a	3203a	85.16a	8.76 cd
	0.05	28.66b	14.0b	388c	10.86c	10.78a	38.95c	18b	444c	19.13c	11.20a
	0.01	23.65c	12.1c	221c	6.60c	11.31a	31.36d	16b	416c	17.41c	10.67ab
ANOVA	CO ₂	*	ns	ns	*	*	***	*	*	**	**
	Pi	***	***	***	***	***	***	***	***	***	***
	CO ₂ × Pi	ns	ns	ns	*	ns	ns	ns	*	*	ns

Significant at * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; and ns, non-significant ($P > 0.05$). Within columns for each experiment, means followed by same letters are not significantly different at $\alpha = 0.05$.

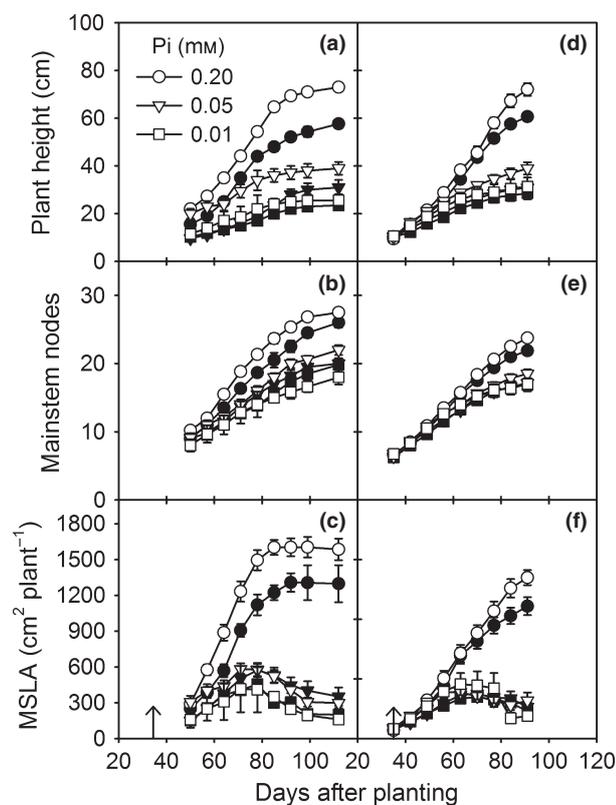


Fig. 1 Changes in plant height, number of main stem node (plant^{-1}) and main stem leaf area (MSLA) for different phosphate-Pi treated cotton plants grown at either ambient (filled symbols, $400 \mu\text{mol mol}^{-1}$) or elevated (unfilled symbols, $800 \mu\text{mol mol}^{-1}$) CO₂ for Experiments I (a–c) and II (d–f). Treatments were initiated at zero (CO₂) and 34 (Pi, arrow) days after planting (DAP) in Exp. I, and both treatments were initiated at 34 DAP in Exp. II.

fraction of leaf, stem and fruit biomass to the total biomass tended to be lower under Pi-stress treatments (0.05 and 0.01 mM Pi), whereas fraction of root biomass to the total biomass increased up to threefold consistently across experiments (Table 2). Elevated CO₂ significantly ($P < 0.01$) increased the fraction of stem biomass to the total biomass in both experiments.

Gas exchange measurements

The CO₂ × Pi interactions were significant ($P < 0.05$) for P_{net} and g_s , when averaged over measurement dates (Table 3). Plants grown under Pi-stress treatments (0.05 and 0.01 mM) consistently exhibited lower P_{net} for all measurement dates irrespective of CO₂ treatments (Fig. 2a). Averaged across measurement dates, the severe Pi-stress treatment (0.01 mM) caused 68 and 77 % reduction in P_{net} at ambient and elevated CO₂, respectively, as compared to 0.20 mM Pi treatment (Table 3). Elevated CO₂ caused

>18 % higher P_{net} at 0.20 and 0.05 mM Pi, relative to the $400 \mu\text{mol mol}^{-1}$ CO₂ treatment, whereas P_{net} was 17 % lower under elevated CO₂ in the 0.01 mM Pi-treated plants. Averaged across Pi and CO₂ treatments, g_s decreased by 33 % at elevated CO₂ and by 60 % under Pi-stress. There was significant ($P < 0.05$) CO₂ × Pi interaction for C_i/C_a leading to relatively lower C_i/C_a at 0.01 mM Pi only at elevated CO₂. The main effect of Pi was not significant ($P > 0.05$) for C_i/C_a .

Tissue constituents

There was significant CO₂ × Pi interaction for chlorophyll concentration (Table 3). Irrespective of CO₂ levels, chlorophyll concentration decreased significantly ($P < 0.001$) under Pi-stress treatments. The P, C and N contents in uppermost fully expanded leaves were assessed on both leaf mass (mg g^{-1}) and leaf area basis (mg cm^{-2}). The P content (mass-based) in uppermost fully expanded leaves tended to be lower at most of the measurement dates in plants grown at either elevated CO₂ or under Pi-stress treatments (Fig. 2b,c). When averaged across measurement dates, leaf P content of uppermost fully expanded leaves significantly ($P < 0.05$) decreased under Pi-stress treatments irrespective of the calculation methods (leaf mass- or leaf area-based) (Table 3). However, elevated CO₂ significantly ($P < 0.05$) decreased leaf mass-based P content but not leaf area-based P content (Table 3). Elevated CO₂ significantly ($P < 0.05$) affected N content leading to lower tissue N, whereas effect of Pi was not significant ($P > 0.05$). Irrespective of Pi treatments, C content (area basis) and C:N ratio were significantly ($P < 0.05$) higher at elevated CO₂ in uppermost fully expanded leaves.

The overall nutrient composition in the cotton plant organs (leaves i.e. total from main stem and branches, stems and roots) for the final harvest in both experiments is shown in Table 4. The CO₂ × Pi interactions for P contents were not significant ($P > 0.05$) except for the stems in Exp. I. Pi-stress significantly ($P < 0.05$) decreased P content in all plant organs, whereas the effect of CO₂ was not consistent. On average, tissue P and N tended to be either slightly higher (leaf and stem) or lower (roots) in Exp. I vs. Exp. II. Stems appeared to have the lowest P and N contents in both experiments. The N content did not show significant ($P > 0.05$) CO₂ × Pi interactions except in roots (Table 4). In general, the tissue N tended to be higher under Pi-stress and lower at elevated CO₂ across plant organs.

Relationship of tissue P with other parameters

Regressions analysis revealed significant relationships between tissue P contents and all other parameters

Table 2 Effect of CO₂ levels ($\mu\text{mol mol}^{-1}$) and phosphate (Pi, mM) supply on biomass partitioning of cotton plants at final harvests of Exp. I (112 DAP) and Exp. II (91 DAP). Analysis of variance (ANOVA) between Pi and CO₂ is given. The data are the mean of six-eight individual plants

CO ₂	Pi	Tissue biomass (g plant ⁻¹)				Fraction of total biomass (%)			
		Leaves	Stems	Fruits	Roots	Leaves	Stems	Fruits	Roots
Experiment I									
400	0.20	29.83b	29.46b	34.30a	22.07b	26.02bc	25.64bc	29.22a	19.12d
	0.05	12.06c	7.29c	1.07b	8.95c	41.70a	24.59c	3.38b	30.33c
	0.01	0.91e	1.40d	0.28b	3.31de	15.25d	24.30c	0.27b	60.19a
800	0.20	47.81a	55.86a	41.27a	28.48a	27.77b	32.33ab	23.50a	16.41d
	0.05	6.36d	5.39cd	5.91b	5.28d	28.81b	23.61c	24.84a	22.75d
	0.01	0.81e	1.07d	0.06b	1.47e	18.38cd	35.70a	0.12b	45.92b
ANOVA	CO ₂	***	***	ns	ns	ns	**	ns	***
	Pi	***	***	***	***	***	ns	***	***
	CO ₂ × Pi	***	***	ns	***	*	ns	***	ns
Experiment II									
400	0.20	17.75b	19.60b	15.26a	9.84a	27.77b	30.54bc	24.12a	15.15d
	0.05	4.08c	4.08c	2.64b	4.61b	25.94bc	26.47cd	16.63b	30.96bc
	0.01	3.29c	3.75c	3.02b	4.67b	22.38c	25.42d	15.46b	36.74ab
800	0.20	28.02a	30.88a	14.26a	12.01a	32.98a	36.39a	16.53b	14.10d
	0.05	4.99c	5.37c	3.80b	4.98b	24.65bc	28.48bcd	19.81ab	27.06c
	0.01	4.27c	5.83c	1.17b	6.13b	22.97c	31.41b	3.47c	42.15a
ANOVA	CO ₂	**	***	ns	ns	ns	***	**	ns
	Pi	***	***	***	***	***	***	***	***
	CO ₂ × Pi	**	**	ns	ns	ns	ns	**	ns

Significant at * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; and ns = non-significant ($P > 0.05$). Within columns for each experiment, means followed by same letters are not significantly different at $\alpha = 0.05$.

Table 3 Effect of CO₂ levels ($\mu\text{mol mol}^{-1}$) and phosphate (Pi, mM) supply on the rate of photosynthesis (P_{net} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$), internal (C_i) to external (C_a) CO₂ ratio (C_i/C_a), specific leaf weight (SLW, mg cm^{-2} of single leaf), total chlorophyll concentration (Chl, $\mu\text{g cm}^{-2}$), carbon (C) and nitrogen (N) ratio (C/N), tissue phosphorus (P), C, and N contents based on leaf mass (mg g^{-1} dry weight) and area (mg cm^{-2} area) of upper most fully expanded leaves of cotton. Analysis of variance (ANOVA) between Pi and CO₂ is given. The data are mean of 13-day measurements between 57 and 112 days after planting across two experiments

CO ₂	Pi	P_{net}	g_s	C _i /C _a	SLW	Chl	C/N	mg g ⁻¹ leaf dry weight			mg cm ⁻² leaf area		
								P	C	N	P	C	N
400	0.20	23.35b	0.35a	0.63c	9.79c	25.7a	13.4c	1.19a	428a	33.2a	0.0114a	4.17d	0.324abc
	0.05	11.07d	0.16c	0.64bc	10.29bc	10.0c	13.7c	0.61c	438a	32.4a	0.0063b	4.51d	0.334ab
	0.01	7.73e	0.14c	0.69ab	10.65b	8.6c	13.8c	0.54cd	433a	32.0a	0.0057b	4.64cd	0.340a
800	0.20	28.3a	0.25b	0.69ab	12.18a	29.5b	17.8b	0.92b	422a	24.2bc	0.0111a	5.19b	0.297bc
	0.05	13.12c	0.14c	0.72a	11.97a	12.9cd	16.7b	0.51cd	424a	26.0b	0.0061b	5.06bc	0.307abc
	0.01	6.37e	0.04d	0.67abc	12.48a	7.2d	20.1a	0.49d	442a	22.3c	0.006b	5.71a	0.287c
ANOVA	CO ₂	***	***	*	***	***	***	***	ns	***	ns	***	*
	Pi	***	***	ns	ns	***	**	***	ns	ns	***	*	ns
	CO ₂ × Pi	***	*	*	ns	*	*	*	ns	ns	ns	ns	ns

Significant at * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; and ns = non-significant ($P > 0.05$). Within columns, means followed by same letters are not significantly different at $\alpha = 0.05$.

($P < 0.05$) (Figs 3–6), except tissue N content of the uppermost fully expanded leaves (Fig. 6b). The slope of the linear regression between CO₂ levels differed significantly ($P < 0.05$) for whole plant tissue P content vs. TBM measured at the final harvest across experiments (Fig. 3a). Elevated CO₂ exhibited more biomass accumulation and the

differences between elevated vs. ambient CO₂ tended to increase with increase in tissue P content. The relationship between whole plant tissue P content and root:shoot ratio was nonlinear and did not differ significantly ($P > 0.05$) between CO₂ treatments (Fig. 3b). The root:shoot ratio increased with decrease in tissue P content. A curvilinear

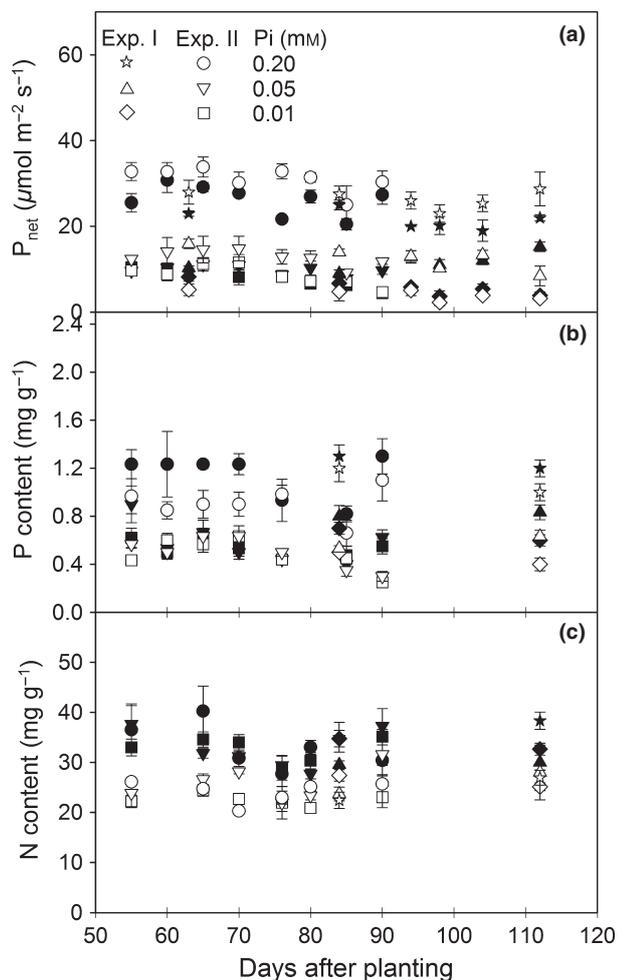


Fig. 2 Photosynthetic rate (P_{net}), phosphorus (P) and nitrogen (N) contents of dry tissue for upper most fully expanded cotton leaves during experiment grown at either ambient (filled symbols, $400 \mu\text{mol mol}^{-1}$) or elevated (unfilled symbols, $800 \mu\text{mol mol}^{-1}$) CO₂ under different phosphate (Pi) treatments. The data are from two experiments (Exps I and II). The error bars represent the standard error of 3–5 individual plants.

(polynomial second order) relationship was observed between mass- and area-based tissue P content in uppermost fully expanded leaves (Fig. 4). The linear term between CO₂ levels differed ($P < 0.05$) for mass- vs. area-based P curve. For the two levels of CO₂ treatments, the slope of the regression between tissue P content (both mass- and area-based) and P_{net} of uppermost fully expanded leaves differed significantly ($P < 0.05$) (Fig. 5a, b). The g_s response to leaf P content between CO₂ levels differed only when tissue P content was expressed on leaf area basis (Fig. 5c,d). Chlorophyll concentration also increased with leaf P content (mg g^{-1}) and did not differ significantly ($P > 0.05$) between CO₂ treatments (Fig. 6a). A significant relationship between leaf P and N contents

was not established; however, N tended to be higher at ambient vs. elevated CO₂ at a given leaf P content (Fig. 6b).

Discussion

Growth and development

The patterns of cotton growth responses to the treatments (Pi and CO₂) were similar in both experiments. However, they differed in magnitude possibly due to the differences in initiation of CO₂ treatment and time of harvests between Exp. I and Exp. II. Despite some interaction between treatments mostly in Exp. I, the growth and development responses to Pi supply were similar across both CO₂ levels. The observed stunted plants with fewer main stem nodes and decreased leaf area under Pi-stress treatments were consistent with the observations made in other nutrient studies (Reddy and Zhao 2005, Fleisher et al. 2012). Smaller plant stature under Pi-stress resulted in over 77 % lower biomass accumulation across CO₂ treatments. Radin and Eidenbock (1984) reported that phosphate deficiency limited cell expansion by reducing hydraulic conductance inside plants, which may lead to reduced plant size and leaf area expansion. This was supported by severely decreased leaf area under Pi-stress treatments in the current study. In fact, main stem leaf area began to decline under Pi-stress roughly 75 DAP in both experiments due to leaf senescence. Phosphate deficiency-induced early leaf senescence has also been suggested in potato (Fleisher et al. 2013). The plant dry weight and total leaf area followed similar trend across the treatments at the first harvest in both the experiments; however, it varied in the final harvest particularly under Pi-stress due to leaf senescence. Elevated CO₂ significantly stimulated stem elongation, leaf area expansion and total biomass at 0.20 mM Pi treatments; however, this stimulation was rarely significant for 0.05 mM Pi and none for 0.01 mM Pi treatments suggesting unresponsiveness of these traits to CO₂ enrichment under Pi-stress treatments.

Biomass partitioning

The observed decrease (Pi-stress treatments) or increase (elevated CO₂, especially at higher Pi supply) in biomass accumulation of plant organs was in accordance with other studies and attributed to the adjustment of plant growth under nutrient deficiency and CO₂ enrichment (Kimball and Mauney 1993, Reddy and Zhao 2005, Fleisher et al. 2012, Lenka and Lal 2012). In spite of an increased fruit and root biomass at elevated CO₂ under full nutrient supply (0.20 mM Pi), the main effect of elevated CO₂ on fruit and root biomass was not significant. The fruit abscission was affected more under elevated CO₂ than under ambient

Table 4 Effect of CO₂ levels ($\mu\text{mol mol}^{-1}$) and phosphate (Pi, mM) supply on phosphorus (P) and nitrogen (N) content in tissues of cotton plant and its organs (leaves, stems and roots). Analysis of variance (ANOVA) between Pi and CO₂ is given. Data are from six-eight individual plants at the final harvests of the two experiments

CO ₂	Pi	P (mg g ⁻¹)				N (mg g ⁻¹)			
		Plant	Leaves	Stems	Roots	Plant	Leaves	Stems	Roots
Experiment I									
400	0.20	1.05a	1.15a	0.83a	1.07a	27.5b	45.0b	20.6b	20.2a
	0.05	0.88b	0.80b	0.97a	0.92ab	34.1a	52.3a	20.5b	20.2a
	0.01	0.54c	0.87b	0.53b	0.62c	29.7cd	53.0a	22.0a	18.9ab
800	0.20	0.78b	0.82b	0.58b	0.92ab	22.6de	34.0c	15.7c	16.7b
	0.05	0.65c	0.60c	0.62b	0.78bc	28.2bc	45.6b	17.9bc	18.4ab
	0.01	0.60c	0.68bc	0.50b	0.60c	24.0e	45.0b	20.6b	20.2a
ANOVA	CO ₂	***	***	***	ns	***	***	***	ns
	Pi	***	***	***	***	***	***	ns	ns
	CO ₂ × Pi	ns	ns	*	ns	ns	ns	ns	*
Experiment II									
400	0.20	0.84a	1.01a	0.50b	1.13b	25.4bc	35.2ab	12.5b	25.8bc
	0.05	0.61b	0.60b	0.31d	0.88bc	30.4a	38.6a	16.7a	35.8a
	0.01	0.59b	0.63b	0.38cd	0.73cd	28.4ab	38.0a	17.7a	30.2ab
800	0.20	0.94a	1.00a	0.61a	1.23a	21.5d	28.2c	14.9ab	33.0ab
	0.05	0.61b	0.60b	0.39cd	0.86c	23.0cd	32.1bc	18.6a	21.9cd
	0.01	0.56b	0.59b	0.45bc	0.63d	22.0d	28.0c	17.3a	17.7d
ANOVA	CO ₂	ns	ns	**	ns	***	***	ns	**
	Pi	***	***	***	***	*	ns	**	*
	CO ₂ × Pi	ns	ns	ns	ns	ns	ns	ns	***

Significant at * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; and ns = non-significant ($P > 0.05$). Within columns for each experiment, means followed by same letters are not significantly different at $\alpha = 0.05$.

due to Pi-stress, reversing the positive effect of CO₂ enrichment observed under full nutrient supply. This might have caused a non-significant effect of elevated CO₂ on fruit. Similarly, at elevated CO₂, the root biomass was either reduced (Exp. I) or showed minor increase (Exp. 2) in Pi-stressed plants resulting in a lack of overall significant effect of elevated CO₂ on root biomass. Fleisher et al. (2013) also reported non-significant effect of elevated CO₂ on potato root biomass. Under Pi-stress, the increased biomass partitioning to the roots was similar to trends previously observed in cotton and potato (Radin and Eidenbock 1984, Fleisher et al. 2012). Increased biomass partitioning to the roots under Pi-stress may be a mechanism to exploit below-ground resources in an effort to supply plant demand for nutrients such as phosphorus. Similarly, under elevated CO₂, increased above-ground growth is often associated with increased root biomass thus, indicating adjustment between above- and below-ground plant growths (Lenka and Lal 2012). Severe fruit abscission under Pi-stress may also reduce the fraction of above-ground biomass and increase the fraction of root biomass contributing to total biomass. In field studies with cotton, Mullins and Burmester (1990) found highest biomass partitioning towards the fruiting structures, followed by leaves and then stem. Because the experiments were terminated well before

plant maturity, the contribution of fruits to total biomass was lower under the highest Pi supply except for ambient CO₂ in Exp. I.

Gas exchange measurements

Significant reductions in P_{net} under Pi-stress have been reported in other studies (Barrett and Gifford 1995, Fleisher et al. 2012). Although, g_s reduced more than P_{net} under low Pi, it did not appear to be the main cause of photosynthetic limitation as deduced from an insignificant effect of Pi on C_i/C_a . Similar results have also been reported in other nutrient studies (Zhao et al. 2001, Jin et al. 2011). However, the minor decrease in P_{net} at elevated vs. ambient CO₂ under lowest Pi treatment (0.01 mM) might also suggest critical role of stomatal limitation to photosynthesis. The extremely low g_s ($0.04 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) under this particular situation might have reduced C_i leading to lower C_i/C_a ratio, thus the observed CO₂ × Pi interaction. An increase in C_i (thus C_i/C_a ratio, as observed under Pi-stress across both CO₂ except the 0.01 mM Pi under elevated CO₂) has often been associated with the onset of mesophyll/biochemical limitation to photosynthesis that highly depends on the severity of the stress (Brodribb 1996, Singh and

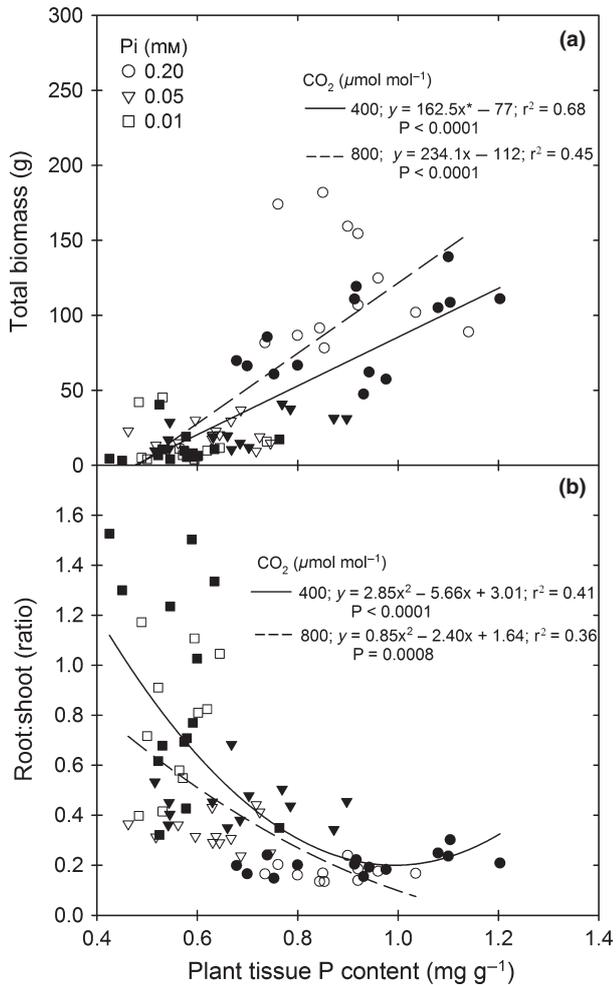


Fig. 3 Relationship between plant phosphorus (P) content and (a) total biomass and (b) root:shoot ratio of cotton grown at either ambient (filled symbols, 400 μmol mol⁻¹) or elevated (unfilled symbols, 800 μmol mol⁻¹) CO₂ under different phosphate (Pi) treatments. The slope (a) between CO₂ differed significantly ($P < 0.05$) as designated by an asterisk (*). Data are from individual plants at the final harvest of both experiments.

Reddy 2011). Therefore, the major influence of Pi on P_{net} can still be attributed to factors other than g_s such as mesophyll resistance and inhibition of photo-biochemical processes as suggested in other studies (Longstreth and Nobel 1980, Jacob and Lawlor 1991, Fleisher et al. 2012). Jacob and Lawlor (1991) also found that relative stomatal limitation did not vary due to phosphorus nutrition but mesophyll limitation was greatly enhanced under Pi-stress across species. The severe reduction in chlorophyll concentration in the current study also suggested that metabolic inhibition of photosynthesis had occurred under Pi-stress. Enhancement of P_{net} by elevated CO₂ was greater at 0.20 mM vs. 0.05 mM Pi and none for severe Pi-stress (0.01 mM) indicating reduction or lack in

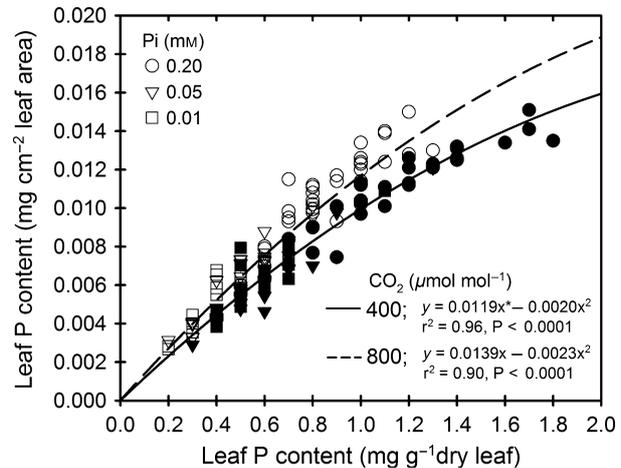


Fig. 4 Relationship between mass (mg g⁻¹ leaf dry weight) and area bases (mg cm⁻² leaf area) tissue P content in uppermost fully expanded cotton leaves grown at either ambient (filled symbols, 400 μmol mol⁻¹) or elevated (unfilled symbols, 800 μmol mol⁻¹) CO₂ under different phosphate (Pi) treatments. Data are from individual plants measured between 57 and 112 days after planting across two experiments. A polynomial second order equation was used, and intercept was forced to pass through origin (zero). The asterisk (*) indicated significant ($P < 0.05$) difference in the linear term of the equations between two CO₂ levels.

the stimulatory effect of elevated CO₂ on photosynthetic response of plants as Pi-stress increased. Although tissue P content (mg g⁻¹) of the uppermost fully expanded leaves was significantly lower at elevated vs. ambient CO₂, this study suggested that it was not associated with downregulation of P_{net} under elevated CO₂ in Pi-stressed plants (Barrett and Gifford 1995). This might be due to the fact that the P content on a leaf area basis (mg cm⁻²) did not differ between CO₂ treatments. Plant leaf nutrient status may provide a stable estimate based on leaf area as suggested in other studies (Bélanger et al. 2011). The increased C content when estimated based on leaf area (mg cm⁻²) may be caused by restricted carbon export under Pi-stress, and increased carbon accumulation under elevated CO₂ as also suggested by Radin and Eidenbock (1986).

Tissue constituents

In the uppermost fully expanded leaves, the observed reduction in the tissue P content without a significant effect on N content under Pi-stress treatments was comparable to the results in Pi deficient potato (Fleisher et al. 2012). Additionally, reduction in leaf tissue P and N contents under elevated CO₂ is often reported similar to the observation made in this study (Rogers et al. 1993, Taub and Wang 2008, Lenka and Lal 2012). However, P content did

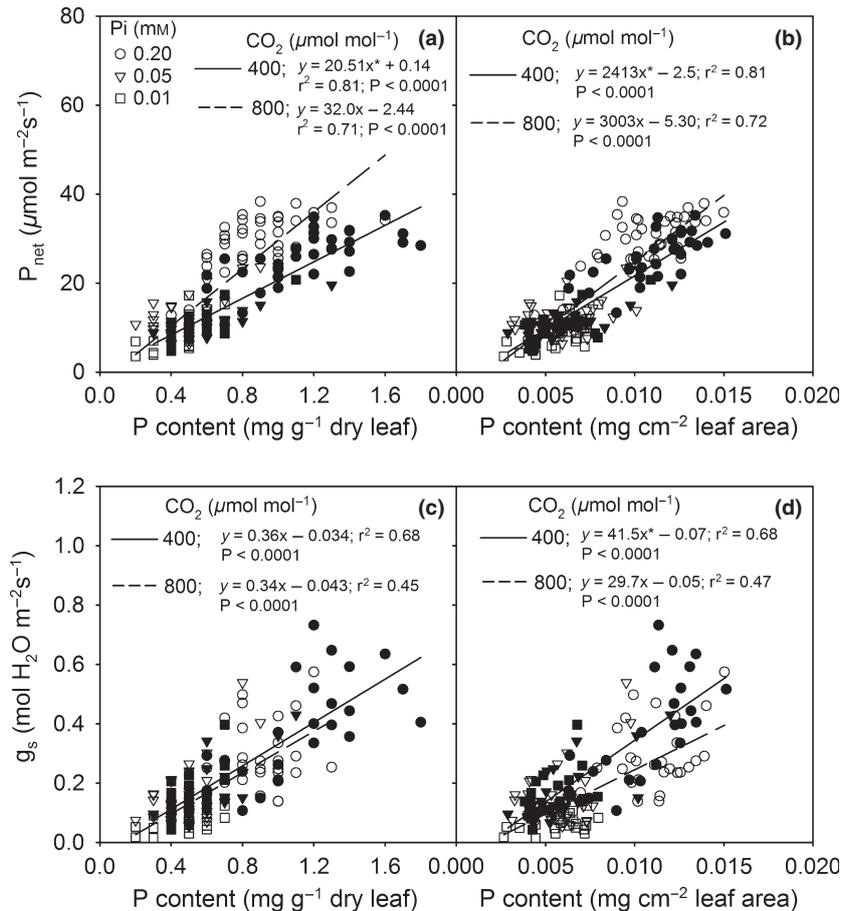


Fig. 5 Relationship of phosphorus (P) content with photosynthetic rate (P_{net}) (a,b) and stomatal conductance (g_s) (c,d) for uppermost fully expanded cotton leaves grown at either ambient (filled symbols, $400 \mu\text{mol mol}^{-1}$) or elevated (unfilled symbols, $800 \mu\text{mol mol}^{-1}$) CO₂ under different phosphate (Pi) treatments. Data are from individual plants measured between 57 and 112 days after planting across two experiments. The asterisk (*) indicated significant ($P < 0.05$) difference in the slope between two CO₂ levels.

not differ between CO₂ levels in uppermost fully expanded leaves when expressed on leaf area basis (mg cm^{-2}) indicating the dilution of the nutrients (e.g. P and N) due to increased leaf thickness (e.g. SLW) and carbon accumulation under elevated CO₂ (Taub and Wang 2008, Lenka and Lal 2012). This was also supported by increased C:N ratio of cotton leaves. Specific leaf weight signifies leaf thickness and similar to the current study has also been reported to increase in response to elevated CO₂ in a wide range of species (Gifford et al. 2000).

Tissue P content of plant organs decreased under Pi-stress, but the differences between 0.05 and 0.01 mM Pi treatments were mostly not significant. Compared with other major nutrients, plants require phosphorus in smaller amounts and the low Pi treatments may not have shown large differences in the tissue P content, yet plant growth parameters largely differed. The observed lower nutrient content of stems compared with other plant organs may be explained by the high mobility of N and P (Prior et al. 1998). Mullins and Burmester (1990) also reported lower shoot N and P contents as compared with leaves and fruiting structures of cotton. The N content often increased under Pi-stress across plant organs

indicating greater N uptake, as also suggested in earlier studies (Almeida et al. 2000, Fleisher et al. 2012). In the current study, the increased root:shoot ratio was due to increased biomass partitioning to the roots under Pi-stress suggesting relatively higher root volume might have assisted in the uptake of other nutrients such as N. Under Pi-stress, the trend in tissue P content of plant organs was consistent with the uppermost fully expanded leaves; however, the variable results with respect to CO₂ treatments were similar to the inconsistencies reported in other studies (Prior et al. 1998, 2003, Gifford et al. 2000). Under CO₂ treatments, the variability in tissue P content between upper most fully expanded leaves and whole plant leaves might have partly been due to the fact that later consisted all the leaves from main stem and branches. The reductions in plant tissue P and N under elevated CO₂ across Pi treatments have been reported by others; however, large variability also existed (Prior et al. 1998, 2003, Gifford et al. 2000). Elevated CO₂ also exhibited a relatively consistent response by decreasing N content across plant components, except the roots in both experiments. This was also supported by the lack of CO₂ × Pi interaction for tissue N content. The lack of CO₂ × Pi

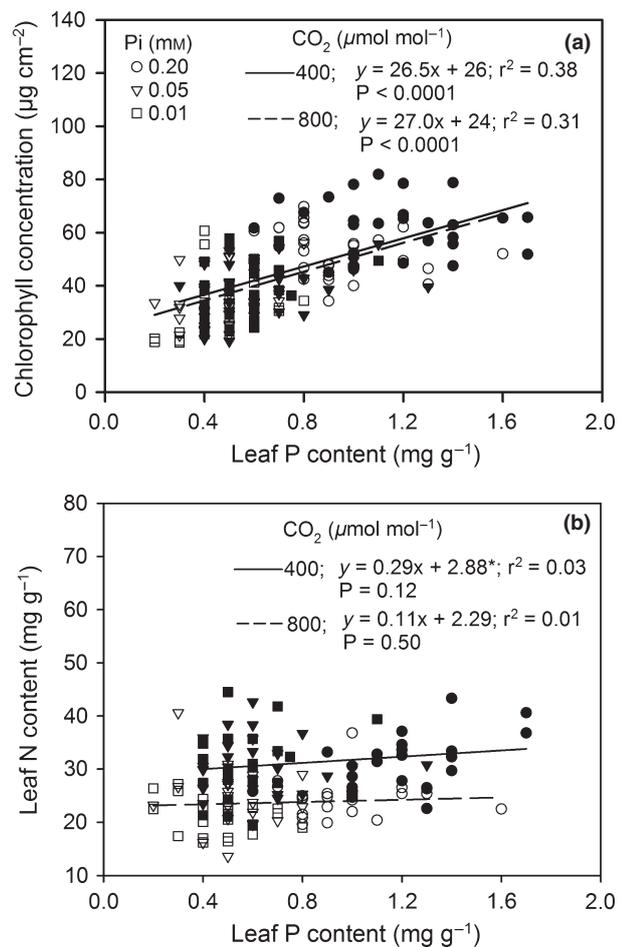


Fig. 6 Relationships of phosphorus (P) content with chlorophyll concentration (a) and nitrogen (N) content (b) for uppermost fully expanded cotton leaves grown at either ambient (filled symbols, 400 $\mu\text{mol mol}^{-1}$) or elevated (unfilled symbols, 800 $\mu\text{mol mol}^{-1}$) CO₂ under different phosphate (Pi) treatments. Data are from individual plants measured between 57 and 112 days after planting across two experiments. The asterisk (*) indicated significant ($P < 0.05$) difference in the intercept between two CO₂ levels in (b).

interaction has also been reported in several potato organs (Fleisher et al. 2013). Although not clearly understood, factors such as dilution of N due to increased carbon assimilation, decreased N demand and restricted uptake and lower transpiration due to decrease in g_s may contribute to the lower tissue N at elevated CO₂ (Gifford et al. 2000, Taub and Wang 2008). The current study also suggested the existence of a dilution effect and lower transpiration as deduced from higher leaf thickness (SLW) and decreased g_s under elevated CO₂. This clearly indicated alteration in the uptake and utilization of N, and lower N content under elevated CO₂ might influence nitrate-assimilation and biosynthesis of organic compounds (Bloom et al. 2010, Kawakami et al. 2012).

Relationships between plant parameters and tissue P content

Regardless of the CO₂ levels, a linear increase in TBM, P_{net} , g_s and chlorophyll concentration with tissue P content suggested that the highest Pi treatments of the current study did not reflect excessive Pi supply. The magnitude of the response of total biomass and P_{net} to the tissue P (mg g^{-1}) differed between the two levels of CO₂, which was in accordance with the significant CO₂ effect observed for these parameters across Pi nutrition. Moreover, elevated CO₂ increased P_{net} response to leaf P content regardless of P calculation methods (mass vs. area). Studies reporting similar relationships between a range of leaf tissue P content (mass- vs. area-based) or with biomass and photosynthetic processes are limited. Reddy and Zhao (2005) found that the critical leaf potassium content varied between CO₂ treatments for canopy photosynthesis and biomass production. The curvilinear relationships between mass- and area-based P contents across CO₂ levels for uppermost fully expanded leaves delineated the subtle differences between expressions of nutrient content in leaves. For example, the difference in the coefficient of the linear regression (slope) of P_{net} vs. leaf P content was smaller between ambient and elevated CO₂ when P was expressed on leaf area basis than on a leaf mass basis (Fig. 5b). Similarly, compared with ambient CO₂, the significantly lower regression slope between g_s and leaf P under elevated CO₂ was only observed when P was expressed on an area basis (mg cm^{-2}). Reduction in g_s in response to elevated CO₂ is a well-known phenomenon, which was also observed in the current study across Pi supply (Ainsworth and Rogers 2007). Because the overall P_{net} , g_s or light interception are based on leaf area, it may be suitable to express leaf nutrient content on area basis for determining their relationships. The parallel response of chlorophyll concentration to tissue P for the two level of CO₂ was consistent with the potassium nutrition study in cotton by Reddy and Zhao (2005). Although, there was not a clear relationship between leaf tissue N and P contents, N tended to be lower at elevated CO₂ across the range of tissue P content. The nutrient dilution in plant tissue due to enhanced growth and decreased nutrient uptake may contribute to the lower tissue N at elevated CO₂. However, more studies are needed to confirm the underlying physiological mechanisms (Taub and Wang 2008).

In summary, a substantial decrease in plant biomass under Pi deficiency was attributed to stunted growth primarily due to decreased leaf area accompanied with early leaf senescence and reduced photosynthesis. Consequently, Pi deficiency significantly shifted biomass partitioning by decreasing leaf and fruit production and increasing the root

fraction of total biomass. Cotton growth and photosynthesis increased with tissue P content. Furthermore, the plant tissue N content tended to increase under Pi-stress. The increased fraction of root biomass under Pi-stress might have enhanced N uptake leading to higher tissue N content. The CO₂ × Pi interactions were dependent on plant organs and type of growth processes measured. The growth enhancement effects of elevated CO₂ were either highly reduced or trivial under the studied range of Pi-stress treatments. As a consequence, several processes such as number of main stem nodes and leaf area development, fruit set, photosynthesis, chlorophyll concentration were negatively affected by elevated CO₂ under severe Pi-stress (0.01 mM). Despite the lower leaf P and stomatal conductance at elevated CO₂, the contribution of these factors to the inhibition of growth and photosynthetic response at elevated CO₂ under severe Pi-stress treatments were insignificant. A significant reduction in tissue N content (up to 21 % in plant, averaged across Pi supply) under elevated CO₂ appears to be critical and may need more attention to understand its influence on the overall effect of CO₂ enrichment on plants.

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