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Nutrient remobilization and C:N:P stoichiometry in response to elevated CO_2 and low phosphorus availability in rice cultivars introgressed with and without *Pup1*

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ABSTRACT

The continuously rising atmospheric CO_2 concentration potentially increase plant growth through stimulating C metabolism; however, plant C:N:P stoichiometry in response to elevated CO_2 (eCO_2) under low P stress remains largely unknown. We investigated the combined effect of eCO_2 and low phosphorus on growth, yield, C:N:P stoichiometry, and remobilization in rice cv. Kasalath (*aus* type), IR64 (a mega rice variety), and IR64-Pup1 (*Pup1* QTL introgressed IR64). In response to eCO_2 and low P, the C accumulation increased significantly (particularly at anthesis stage) while N and P concentration decreased leading to higher C:N and C:P ratios in all plant components (leaf, sheath, stem, and grain) than ambient CO_2. The remobilization efficiencies of N and P were also reduced under low P with eCO_2 as compared to control conditions. Among cultivars, the combined effect of eCO_2 and low P was greater in IR64-Pup1 and produced higher biomass and grain yield as compared to IR64. However, IR64-Pup1 exhibited a lower N but higher P concentration than IR64, indicating that the *Pup1* QTL improved P uptake but did not influence N uptake. Our study suggests that the P availability along with eCO_2 would alter the C:N:P ratios due to their differential partitioning, thereby affecting growth and yield.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world (FAO, 2017) and is the primary source of nutrition for more than two-thirds of the world's population, especially some of the low- or middle-income nations have a strong dietary dependence on rice (GRiSP, 2013). In the last five decades, the demand for rice has doubled and is predicted to increase further by 30% towards the end of 2050 (Usui et al., 2016). The rapid increase in atmospheric carbon dioxide (CO_2) concentration over the last few decades, which is a major driver of current climate change, is expected to influence the growth of rice crop in future (Usui et al., 2016). According to modelled projections, atmospheric CO_2 concentration is expected to increase from its current value of 415 μmol mol^{-1} (IPCC, 2021) to 550–650 μmol mol^{-1} by the middle of the century (Smith and Myers, 2018). The anthropogenic acceleration of atmospheric CO_2 has directly altered the morphology and physiology of plants in both positive and negative ways. The increased atmospheric CO_2 stimulates the rate of photosynthesis resulting in higher biomass accumulation and yield as a positive effect. On the other hand, the negative effects of elevated CO_2 (eCO_2) cause reductions in stomatal conductance and rate of transpiration (Xu et al., 2016), and most significantly causes alterations in the stoichiometric ratios of nutrients by changing the chemical composition of tissues (Reich et al., 2006; Reed et al., 2015; Jiang et al., 2020). Earlier reports have demonstrated that C_3 plants generally responded to eCO_2 through increased carbon assimilation (Luo et al., 2006; Sasaki et al., 2007; Wang et al., 2013); however, the magnitude of its effects is determined by several factors such as genotypic variation (Wolfe et al., 1998; Ainsworth and Long, 2005), availability of nutrients (Kim et al., 2003; Leakey et al., 2004; Soumya et al., 2021; Meena et al., 2021a, 2021b; Sharma et al., 2023), and atmospheric temperature (Wang et al., 2012).

Phosphorus (P), next to nitrogen (N), is required in large quantities to

Abbreviations: OTC, open top chamber; *Pup1*, Phosphorus uptake1; aCO_2, ambient carbon di-oxide; eCO_2, elevated carbon di-oxide.

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sustain the optimum growth and productivity. It is well known that P has an essential role in plant growth and development and involved in metabolic, structural, and regulatory functions; however, approximately 70% of the world's agricultural soil is estimated to be low in P availability (López-Arredondo et al., 2014), which could limit plant responses to eCO_2 (Jiang et al., 2020). As an essential macro-nutrient for plants, the application of phosphatic fertilizer remains indispensable practice for sustainable agriculture (Lu and Tian, 2017). Earlier studies have demonstrated that the low P significantly reduces growth and productivity of crop species (Fageria and Baligar, 1997; Wissuwa et al., 2005; Panigrahy et al., 2014; Soumya et al., 2021). The reduction in plant biomass due to low P is primarily associated with a slowdown in photosynthesis. The effect of low P on photosynthesis could be caused by a decrease in ribulose-bisphosphate regeneration and a shift of triose phosphates towards starch synthesis (Marschner and Marschner, 2012).

N and P, along with carbon (C), are the three essential elements for a terrestrial ecosystem, which are strongly coupled in their biological functioning. Ecological stoichiometry is a useful tool for analysing the concentration and distribution of C, N, and P in plants as it focuses on the balance of elements (i.e., the nutritional ratio) from the scale of the individual to the scale of the ecosystem in relation to the available resources (Yang et al., 2018; Bragazza et al., 2021). In plants, the nutrient ratios are influenced by several factors, for example, by eCO_2 in wheat (Wang et al., 2019), by light in tree species (*Elaeocarpus sylvestris* and *Illicium henryi*) (Xie et al., 2018), or semi-arid to arid conditions in coniferous species (Liu et al., 2022). It could be predicted that plants would be exposed to a global nutrient imbalance under eCO_2 with lower availability of P in the soil which can cause increases in the C:N or C:P ratio in plant organs (Yuan and Chen, 2015; Wang et al., 2019). In cereals, the highest proportion of mineral nutrients in grain is contributed from their remobilization by the vegetative organs, of which more than 60% are derived from leaves and stem and the remaining from other organs (Barbottin et al., 2005; Sharma et al., 2023). It was demonstrated that low nutrient concentration in vegetative tissue under eCO_2 at anthesis stage resulted in a reduced nutrient remobilization towards grain (Kimball et al., 2001). On the contrary, a few studies showed no influence of eCO_2 on total plant N remobilization (Tausz et al., 2017; Dier et al., 2019).

Under eCO_2 with sub-optimal nutrient (N or P) conditions, the plant growth rate initially increases but it is not sustained at later stages (Pal et al., 2005; Pandey et al., 2015a). This is mainly because the nutrients are utilised more efficiently under eCO_2 than ambient CO_2 (aCO_2) (Imai and Adachi, 1996). Rogers et al. (1993) reported that even under low P conditions, wheat yields were stimulated by eCO_2, while in cotton, yields were restricted by P-limiting conditions in both aCO_2 and eCO_2. Conversely, Seneweera and Conroy (1997) recorded a significant increase in rice yield attributes such as panicle number per plant, grain number per panicle, and grain yield under different P levels, including low P under eCO_2 as compared to aCO_2. However, we found that eCO_2 compensated the negative effects of low P stress in C_3 plants by increasing P use efficiency (Pandey et al., 2015b), but it remains unclear how the eCO_2 with constrained P availability impacts the direction and magnitude of nutrient uptake and their allocation to the various plant organs.

Another way to reduce the adverse effect of limited P availability on rice yield under eCO_2 would be to select P-efficient rice cultivars that possesses higher P use efficiency and produces more yields even under P-limiting conditions. Raviteja et al. (2021) studied the grain nutritional quality of IR64 and IR64-Pup1, and compared it with Kasalath in response to limited P conditions. However, the effect of eCO_2 combined with nutrient stress on the C, N, and P stoichiometry is less deliberated. In the present study, we hypothesised that the increased atmospheric CO_2 level would mitigate the adverse effects of low P stress by altering the C:N:P ratios which might be caused due to their differential remobilization. We tested our hypothesis utilising these three rice cultivars, IR64, IR64-Pup1, and Kasalath to find out whether *Pup1* (*P*hosphorus *up*take1) has any influence on yield, nutrient remobilization, and the C:N:P stoichiometry when grown under eCO_2 with low P condition. Kasalath (*aus*-type), donor of *Pup1*, is a poor-yielder due to lower test weight and grain filling percentage but exhibited improved P uptake (Wissuwa et al., 1998; Ishimaru, 2003). Previous studies reported a potentially high impact of *Pup1* QTL on improving grain yields under drought stress or P deficient conditions (Chin et al., 2011). It is well established that *Pup1* specific protein kinase gene, *PSTOL1* (phosphorus-starvation tolerance1), enhances early root growth leading the plants to acquire more P and other nutrients (Gamuyao et al., 2012). This QTL was introgressed into the mega rice variety, IR64 which is an early-maturing, high-yielding *indica* rice, resulting in improved IR64 called as IR64-Pup1.1 (Chin et al., 2011). The IR64 possesses resistance to blast and bacterial blight disease but was sensitive to abiotic stress, including drought, high temperature, and low P stress (Mackill and Khush, 2018).

2. Material and methods

2.1. Plant material, growth conditions, and treatments

To study the interactive effects of CO_2 and P nutrition on the growth and physiology of rice, an experiment was laid out during Kharif season (July to November) in open top chamber (OTC) facility at the Indian Agriculture Research Institute, New Delhi, India, located between latitude 28°38′23" N, longitude: 77°09′27" E; 228.61 m above mean sea level. The total plant populations were divided into two sets: one set was grown under ambient CO_2 conditions while other set was grown under eCO_2.The dimension of OTC was 1.6 m diameter and 1.8 m in height, with transparent PVC (poly vinyl chloride) lining (120 μm thickness). The CO_2 was supplied by pure CO_2 gas cylinders, and a graduated flow metre was used to regulate the air-CO_2 mixture flow in order to maintain the required CO_2 level (700 ± 50 ppm) for eCO_2. Similar OTC chambers were also used for other sets of plants wherein free air was injected instead of CO_2-air mixture (Pal et al., 2004, 2005). To minimise the errors, two OTC chambers were used for both CO_2 treatments. The CO_2 concentration in the OTC was measured daily using a portable photosynthesis system LI-6800 (Li-Cor, Lincoln NE, USA).

The soil for filling the pots was collected from the site mapped as low P (https://www.iari.res.in/files/IARI_Soil_Fertility_Maps.pdf). The air-dried soil was passed through a 5 mm sieve, followed by soil analysis for nutrients and physicochemical properties (Raviteja et al., 2021). One set of soil was fertilized with single superphosphate at the recommended dose (60 kg P_2O_5 ha^{-1} as "control P"), while the other set was maintained without phosphatic fertilizer ("low P"). The available P (Olsen, 1954) estimated in control P was 22.9 mg P kg^{-1} soil and in low P treatment was 7.96 mg P kg^{-1} soil. In both parts of soil, the recommended amount of N through urea (120 kg N ha^{-1}) was applied in three splits, i.e., half as a basal dose and one fourth at tillering and anthesis stages, whereas potassium was applied as basal dose through muriate of potash (40 kg K_2O ha^{-1}). For raising nursery, seeds were soaked in water for about 10 h and sown in the germination tray containing field soil. After three weeks (last week of July), seedlings were transplanted into the plastic pots (24 cm diameter, 35 cm height) containing 16 kg of soil. After transplanting, pots were shifted to their respective CO_2 treatments in OTCs. Initially, two seedlings were transplanted in each pot and later only one healthy plant was retained, which served as one experimental unit. A total of 12 pots per variety were maintained per treatment. Pots were irrigated on alternate day with tap water. Plants were protected from weeds, pests, and diseases by applying herbicides and pesticides respectively as and when required.

2.2. Analysis of C, N, and P in plant

For dry matter and nutrient (C, N, and P) analysis, plants were harvested at the ground level at anthesis and physiological maturity stages.

Shoots were separated into leaves, stem, sheath, and grain, and the dry weight (g plant^{-1}) of each organ was recorded after drying in hot-air oven at 65 °C to a constant weight. The C and N% were analysed by the Dumas method using a CHNS analyser (Euro-Vector EA3000, Italy). Briefly, the plant samples were finely ground in a grinder mill (Retsch MM-400, Germany), and 0.9–1.5 mg of fine powder was packed into the 9 × 5 mm tin capsule (Euro-Vector C10-042). The capsules were compressed gently to pack it air-tight and loaded into the autosampler for analysis. The instrument was calibrated according to the manufacturer's instructions and a serial dilution (1–10 mg) of sulphanilamide (Euro-Vector C10-025) was used as a standard for calibration. The certified reference material (Euro-Vector E11031-A) was used for C and N analysis.

For P analysis, tissue samples were digested using a di-acid mixture (nitric acid: perchloric acid; 9:4) in a microwave digestor (Sineo-MDS-15) followed by P% estimation using inductively coupled plasma-optical emission spectrometer (VDV5110 ICP-OES, Agilent Technologies, Singapore). The instrument was calibrated using an ICP-OES wavelength calibration solution (Agilent 6610030-100), and the P analysis was performed in axial viewing mode using a standard curve prepared from a certified P reference solution (Agilent 5190–8428). Stoichiometric ratios of C:N, N:P, and C:P in leaves, stem, sheath, and panicle or grain were estimated at both anthesis and maturity stages using the percentages of nutrients (C, N, and P) present in each shoot component.

2.3. Grain yield, nutrient uptake, and remobilization

At maturity (second week of November), the productive tiller (plant^{-1}) was recorded, followed by harvesting, threshing, and cleaning of grains. Following grain moisture analysis, total grain weight plant^{-1} and test weight (1000 grain weight) as well as grain number panicle^{-1} were recorded. The harvest index (HI) was calculated as the ratio of grain weight to total biomass, while the N and P harvest indices (NHI and PHI) were calculated as the proportion of aboveground nutrients (N and P) in the grain. The N and P remobilization efficiency (NRE and PRE) for each organ (leaves, stem, sheath, and grain) was calculated (Gaju et al., 2014; Sharma et al., 2023).

$$\text{Leaf N remobilisation efficiency} = \frac{\text{Leaf N content at anthesis} - \text{Leaf N content at maturity}}{\text{Leaf N content at anthesis}}$$

$$\text{Leaf P remobilisation efficiency} = \frac{\text{Leaf P content at anthesis} - \text{Leaf P content at maturity}}{\text{Leaf P content at anthesis}}$$

The same formula was used to calculate N and P remobilization efficiency for other organs. The N and P utilization efficiencies (NUtE and PUtE) were computed using the formula:

$$\text{N or P utilisation efficiency} = \frac{\text{Total grain yield}}{\text{Total above ground N or P uptake at maturity}}$$

2.4. Statistical analysis

The experiment was laid out in a completely randomised design (CRD) with three factors, genotypes (G), P levels, and CO_2 (C) treatments. The procedure for basic statistical analysis and ANOVA were carried out in a MS-DOS based statistical software package, AGRES ver. 3.01 (Agres, 1994). To quantify the association between traits, Pearson's correlation coefficient and linear regression were calculated using MS-Excel 2016. Graphs were made using GraphPad Prism version 6.0 (GraphPad Software, La Jolla, CA).

3. Results

We considered the performance of IR64-Pup1 as a reference to which IR64 and Kasalath were compared in response to various treatment conditions. Similarly, for comparing CO_2 effects, aCO_2 was considered as control and compared to eCO_2. Fig. S1 shows the general effect of treatments on the plants.

3.1. Biomass accumulation in response to CO_2 and P nutrition

The accumulation of biomass in leaves, sheath, stem, and panicle or grain at both the growth stages such as anthesis and maturity, was significantly ($P < 0.001$) influenced by all three variables, i.e., CO_2, P, and genotypes, and the interactive effect of G × C (Fig. S2, Table S1). In comparison to aCO_2, the biomass of all plant components, i.e., leaf, sheath, stem, and panicle, increased by >25% under eCO_2 conditions at both growth stages except the grain at maturity. IR64-Pup1 produced significantly higher biomass in vegetative organs, while maximum grain weight was achieved in Kasalath at both growth stages under eCO_2 as compared to aCO_2. The low P resulted in reduced biomass accumulation in all organs at both growth stages as compared to control P and the reduction was higher under aCO_2 than eCO_2.

3.2. Impact of CO_2 and P nutrition on C, N, and P concentration in various organs

The nutrient concentration (C, N, and P) in different organs (leaves, sheath, stem, and grain) at both anthesis and maturity stages were significantly ($P < 0.05$) influenced by G, C, and P treatments (Table S1). At both anthesis and maturity stages, the C concentration was significantly higher in all organs under eCO_2 as compared to aCO_2. (Table 1). Due to low P stress, the C concentration of all organs reduced at both growth stages as compared to control P, except for the sheath and panicle at anthesis. As compared to control P, the reduction in C concentration at low P was recorded under eCO_2 levels than aCO_2. Among genotypes, mean of CO_2 and P treatments showed increased C concentration in IR64, whereas growth stages had no significant effect on the C concentration of different organs.

In comparison to aCO_2, the N concentration in eCO_2 decreased significantly ($P < 0.01$) in all organs, except stem, at anthesis stage; however, at maturity, the N concentration was higher in all plant components under eCO_2 (Table 2). The grain N concentration decreased significantly under eCO_2 relative to aCO_2. Except for the stem at anthesis, all other organs at both growth stages showed significantly ($P < 0.01$) lower N concentrations under low P compared to control P. Similar to C, the N concentration in different organs was reduced under low P under eCO_2 than aCO_2. In comparison to IR64-Pup1, the N concentration in all organs at both growth stages increased significantly in IR64, while it decreased in Kasalath. When the effects of eCO_2 on each genotype were compared to that of aCO_2, IR64-Pup1 exhibited an increased N concentration. At maturity, N concentration in leaves, stem, and sheath were reduced by >43.6% as compared to anthesis stage, while it was 26.2% higher in grains indicating mobilization of plant N towards grain during grain filling period.

Like N concentration, the concentration of P in various organs was

Table 1
The effect of CO_2 and P nutrition on C concentration in different organs of rice at anthesis and maturity stages. Data corresponds to mean ± SEm (n = 12). *Abbreviation*: aC_CP ambient CO_2 with control P, aC_LP ambient CO_2 with low P, eC_CP elevated CO_2 with control P, eC_LP elevated CO_2 with low P, LSD least significant difference, df degree of freedom.

Treatments	Genotypes	Anthesis (mg C g^{-1} dry wt.)				Maturity (mg C g^{-1} dry wt.)			
		Leaf	Sheath	Stem	Panicle	Leaf	Sheath	Stem	Grain
aC_CP	IR64-Pup1.1	381.6	372.6	362.9	397.2	377.6	362.8	362.8	386.1
	IR64	405.6	381.5	386.5	398.1	385.8	371.2	374.6	385.9
	Kasalath	388.5	366.5	375.0	398.5	381.8	369.6	379.5	393.9
aC_LP	IR64-Pup1.1	372.9	370.9	362.5	387.5	361.1	363.0	351.5	377.0
	IR64	396.5	383.9	381.0	421.9	386.8	379.9	374.7	412.4
	Kasalath	381.8	377.4	377.1	393.0	375.0	367.7	367.1	394.2
eC_CP	IR64-Pup1.1	403.7	385.0	384.8	415.4	398.5	385.7	375.5	411.0
	IR64	415.3	407.0	397.4	420.8	409.5	401.4	389.0	415.6
	Kasalath	409.1	402.9	397.7	413.3	402.0	399.3	390.6	402.5
eC_LP	IR64-Pup1.1	395.4	385.3	386.5	400.3	389.7	374.8	375.6	396.7
	IR64	402.8	398.9	382.7	418.9	398.2	387.2	378.7	409.3
	Kasalath	395.2	383.8	384.6	405.3	385.2	373.7	383.7	400.2
LSD (5%)	Genotypes (G), *df-2*	2.78	2.76	2.88	3.58	2.43	1.89	3.28	2.51
	CO_2 (C), *df-1*	2.27	2.25	2.35	2.93	2.00	1.54	2.68	2.05
	Phosphorus (P), *df-1*	2.27	2.25	2.35	2.93	2.00	1.54	2.68	2.05
	G × C, *df-2*	3.94	3.90	4.07	5.07	3.46	2.67	4.64	3.56
	C × P, *df-1*	3.21	3.18	3.33	4.13	2.83	2.18	3.80	2.90
	G × P, *df-2*	3.94	3.90	4.07	5.07	3.46	2.67	4.64	3.56
	G × C × P, df-2	5.57	5.51	5.76	7.17	4.90	3.79	6.57	5.03

Table 2
The effect of CO_2 and P nutrition on N concentration in different organs of rice at anthesis and maturity stages. Data corresponds to mean ± SEm (n = 12). *Abbreviation*: aC_CP ambient CO_2 with control P, aC_LP ambient CO_2 with low P, eC_CP elevated CO_2 with control P, eC_LP elevated CO_2 with low P, LSD least significant difference, df degree of freedom.

Treatments	Genotypes	Anthesis (mg N g^{-1} dry wt.)				Maturity (mg N g^{-1} dry wt.)			
		Leaf	Sheath	Stem	Panicle	Leaf	Sheath	Stem	Grain
aC_CP	IR64-Pup1.1	18.30	15.67	8.50	13.20	7.57	7.07	6.43	17.87
	IR64	24.90	18.60	13.98	15.41	8.10	7.60	7.30	19.10
	Kasalath	17.53	16.20	14.37	14.62	7.53	7.43	6.50	19.53
aC_LP	IR64-Pup1.1	16.93	8.93	7.67	12.57	7.87	7.53	6.20	18.63
	IR64	18.03	17.82	14.57	15.33	8.03	7.50	6.47	18.73
	Kasalath	18.89	15.81	13.05	13.76	7.93	7.67	7.13	19.07
eC_CP	IR64-Pup1.1	24.37	15.77	12.50	14.83	9.40	8.53	7.50	16.07
	IR64	19.50	17.40	12.90	13.61	9.30	8.57	8.10	16.80
	Kasalath	16.60	13.42	12.28	11.17	7.60	6.77	6.67	15.00
eC_LP	IR64-Pup1.1	20.10	12.27	13.69	14.90	8.57	7.97	7.19	15.53
	IR64	17.58	14.07	14.47	11.55	7.93	7.43	7.30	15.97
	Kasalath	14.63	11.57	9.40	12.58	7.10	7.20	7.03	14.33
LSD (5%)	Genotypes (G), *df-2*	0.45	0.55	0.49	0.64	0.26	0.12	0.19	0.37
	CO_2 (C), *df-1*	0.37	0.45	0.40	0.38	0.21	0.10	0.15	0.31
	Phosphorus (P), *df-1*	0.37	0.45	0.401	0.38	0.21	0.10	0.15	0.31
	G × C, *df-2*	0.65	0.78	0.69	0.66	0.37	0.18	0.27	0.53
	C × P, *df-1*	0.52	0.63	0.57	0.54	0.30	0.14	0.22	0.43
	G × P, *df-2*	0.65	0.78	0.69	0.66	0.37	0.18	0.27	0.53
	G × C × P, *df-2*	0.92	1.10	0.98	0.93	0.52	0.25	0.38	0.75

also significantly ($P < 0.01$) reduced under eCO_2 as compared to aCO_2 (Table 3). Under low P, the P concentration in different organs was reduced as compared to control P irrespective of CO_2 levels; however, the reduction was higher under eCO_2 as compared to aCO_2. Both IR64 and Kasalath exhibited significant reduction in organ P concentration as compared to IR64-Pup1. At maturity, P concentration in all vegetative organs was considerably reduced (>37%) as compared to anthesis stage, while in grain, it was increased at maturity stage.

3.2.1. *Alteration in C: N: P stoichiometric ratios by CO_2 and P nutrition*

The C, N, and P ratios in different organs was significantly ($P < 0.05$) influenced by various treatments, genotypes, and growth stages (Table S1). The C:N ratio ranged between 14 and 28 in leaves, 19 to 42 in sheath, 23 to 49 in stem, and 22 to 39 in panicle at anthesis stage, this ratio was altered at maturity ranging from 34 to 56 in leaves, 44–60 in sheath, 46–61 in stem, and 19–30 in grain (Fig. 1). The C:N ratio averaged over P level and genotypes increased significantly in all organs, except stem, at both growth stages under eCO_2 as compared to aCO_2. Under low P, the C:N ratio averaged over CO_2 levels and genotypes increased in sheath (20%), leaves (10%), and panicle (6%) at anthesis stage as compared to control P, while stem C:N ratio was not influenced by P level or growth stages. The leaves C:N ratio varied significantly among different genotypes. It was higher in Kasalath as compared to IR64-Pup1, while it was comparable in IR64 and IR64-Pup1 at both growth stages (Fig. 1A). The C:N ratios in all three rice genotypes were influenced by eCO_2. Specifically, at anthesis, the C:N ratio of sheath decreased in both IR64 and Kasalath compared to IR64-Pup1, but increased at maturity (Fig. 1B). In addition, the C:N ratio of stem and panicle was significantly altered. In IR64, the C:N ratio of both stem and panicle or grain decreased at anthesis and increased at maturity compared to IR64-Pup1 (Fig. 1C and D). However, in Kasalath, the C:N ratio of stem and panicle or grain increased at both anthesis and

Table 3
The effect of CO_2 and P nutrition on P concentration in different organs of rice at anthesis and maturity stages. Data corresponds to mean ± SEm (n = 12). *Abbreviation*: aC_CP ambient CO_2 with control P, aC_LP ambient CO_2 with low P, eC_CP elevated CO_2 with control P, eC_LP elevated CO_2 with low P, LSD least significant difference, df degree of freedom.

Treatments	Genotypes	Anthesis (mg P g^{-1} dry wt.)				Maturity (mg P g^{-1} dry wt.)			
		Leaf	Sheath	Stem	Panicle	Leaf	Sheath	Stem	Grain
aC_CP	IR64-Pup1.1	2.03	1.47	2.40	1.80	1.05	0.97	1.13	2.40
	IR64	1.82	1.42	2.43	1.58	0.80	0.81	1.01	2.23
	Kasalath	1.78	1.65	2.43	1.53	0.87	0.67	0.76	2.30
aC_LP	IR64-Pup1.1	1.24	1.13	1.45	0.83	0.84	0.81	1.04	1.90
	IR64	0.76	0.79	0.78	0.81	0.75	0.83	0.80	1.87
	Kasalath	1.16	0.87	0.71	0.69	0.75	0.86	0.82	1.85
eC_CP	IR64-Pup1.1	1.91	1.45	2.13	1.72	1.00	0.87	1.08	2.25
	IR64	1.79	1.58	2.09	1.62	0.91	0.81	0.99	2.19
	Kasalath	1.77	1.53	1.86	1.62	0.94	0.81	0.96	2.00
eC_LP	IR64-Pup1.1	1.03	0.79	1.56	0.82	0.69	0.60	0.87	1.83
	IR64	0.86	0.72	0.85	0.81	0.54	0.51	0.87	1.87
	Kasalath	0.84	0.79	0.86	0.79	0.51	0.49	0.91	1.77
LSD (5%)	Genotypes (G), *df-2*	0.05	0.05	0.09	0.02	0.03	0.03	0.04	0.07
	CO_2 (C), *df-1*	0.04	0.04	0.07	0.02	0.02	0.03	0.03	0.06
	Phosphorus (P), *df-1*	0.04	0.04	0.07	0.02	0.02	0.03	0.03	0.06
	G × C, *df-2*	0.07	0.07	0.12	0.04	0.04	0.05	0.06	0.10
	C × P, *df-1*	0.06	0.06	0.10	0.03	0.03	0.04	0.05	0.08
	G × P, *df-2*	0.07	0.07	0.12	0.04	0.04	0.05	0.06	0.10
	G × C × P, *df-2*	0.10	0.10	0.17	0.05	0.05	0.07	0.08	0.14

maturity stages compared to IR64-Pup1. When comparing the anthesis and maturity stages, across all treatments and genotypes, it was observed that the C:N ratio of the leaves, sheath, and stem increased by 2.3, 2.0, and 1.3-folds, respectively. On the other hand, the C:N ratio of the panicle or grain decreased by 1.3-folds at maturity compared to the anthesis stage.

The C:P ratio exhibited variations in different plant organs and at different growth stages. At anthesis stage, the C:P ratio ranged from 172 to 527 in the leaves, 206 to 565 in the sheath, 137 to 540 in the stem, and 205 to 582 in the panicle. At maturity, the C:P ratio ranged from 353 to 777 in the leaves, 354–781 in the sheath, 311–551 in the stem, and 154–236 in the grain (Fig. 2A–D). Comparing different treatments, it was observed that under eCO_2, the C:P ratio in leaves and sheath organs significantly increased (>12.5%) compared to aCO_2at both growth stages. The effects of eCO_2 on the stem and grain C:P ratios were not significant, except for the grain at maturity stage. When compared to control P levels, the C:P ratio increased by more than 89% in all organs at anthesis under low P conditions. At maturity, the maximum increase in C:P ratio was found in the leaves (39.2%), followed by sheath (21.6%) and grain (18.1%). Among genotypes, the C:P ratio was significantly higher in both IR64 and Kasalath compared to IR64-Pup1 in all organs and the influence of eCO_2 was observed in all three genotypes. Similar to the C:N ratio, the C:P ratio also increased in leaves, sheath, and stem (>1.4-fold) but decreased in grain (by 1.9-fold) at maturity compared to anthesis stage.

The N:P ratio in all plant organs was significantly ($P < 0.05$) influenced by both CO_2 and P treatments at both anthesis and maturity stages in all genotypes (Table S1). At anthesis stage, the N:P ratio ranged between 9 and 32 in leaves, 8 to 25 in sheath, 3 to 22 in stem, and 7 to 20 in panicle. Whereas at the maturity stage, the N:P ratio was altered which ranged between 7 and 22 in leaves, 7 to 17 in sheath, 5 to 9 in stem, and 7 to 11 in grain (Fig. 3A–D). The N:P ratios of different plant organs showed a wide variation compared to the C:N and C:P ratios. At anthesis stage, eCO_2 exposure resulted in a significant decrease in N:P ratios in all plant organs, except leaves as compared to aCO_2, while at maturity, this ratio increased (by 4.3–29.3%) in all organs. When compared to control P, the N:P ratio increased by more than 68% in all organs at anthesis under low P conditions. At maturity, the maximum increase in N:P ratio was found in the leaves (34.5%), followed by sheath (21.3%), and grain (11.9%). When compared between genotypes, the N:P ratio was significantly higher in both IR64 and Kasalath relative to IR64-Pup1 in all organs, with a maximum increase (>81.3%) in the stem at maturity for both genotypes. In contrast to C:N and C:P ratios, the N:P ratio decreased by more than 1.3-fold at maturity compared to anthesis stage in all organs.

3.2.2. *Influence of CO_2 and P nutrition on N and P remobilization and utilization efficiency*

The analysis of variance for the amount of N and P remobilized (NRE and PRE) from each organ (leaves, stem, and sheath) indicated that genotypes had a highly significant ($P < 0.001$) effect on N and P remobilization traits (Table S2). Furthermore, the effect of genotype was dependent on the CO_2 and P levels, as evidenced by the significant ($P < 0.05$) genotype × CO_2 × P level interactions, except for NRE_{ST} and PRE_{ST} (Table 4). Specifically, N remobilization from leaves (NRE_L), sheath (NRE_{SH}), and stem (NRE_{ST}), and total shoot (NRE_T) was significantly reduced (by 4.2–12.7%) under both eCO_2 as compared to aCO_2. Additionally, a significant ($P < 0.05$) decrease in sheath and stem NRE was observed under low P compared to control P treatment, while no significant alteration was observed in NRE_L. When compared among genotypes, significant ($P < 0.05$) increases were recorded in NRE_{SH} and NRE_{ST} in IR64 and Kasalath with respect to IR64-Pup-1. In contrast, NRE_L showed an opposite trend and was reduced in IR64 and Kasalath. However, no significant effect of genotypes and P levels was found on NRE_G.

Contrary to the trends observed for NRE, which decreased under eCO_2 compared to aCO_2, the P remobilization efficiency from leaves (PRE_L), sheath (PRE_{SH}), and stem (PRE_{ST}) was significantly higher while PRE_T did not exhibit any significant ($P < 0.001$) variation under eCO_2. The results also demonstrated a significant reduction (by >15%) in PRE_G and P remobilization from all plant components, including leaves, sheath, and stem, under low P compared to control P treatments. In terms of genotype differences, PRE_{SH} and PRE_{ST} exhibited significant increases in IR64 and Kasalath compared to IR64-Pup-1, similar to the trends observed for NRE_{SH} and NRE_{ST}. On the other hand, NRE_L was comparable between all three genotypes.

It was found that the genotypes, P treatments, CO_2 levels, and their interactions had significant ($P < 0.001$) effects on N utilization efficiency (NUtE), while only genotype and P level exhibited a significant effect on P utilization efficiency (PUtE) (Table S2). The NUtE increased significantly under eCO_2 in comparison to aCO_2. Both PUtE and NUtE increased under low P by 23% and 7.7%, respectively, compared to

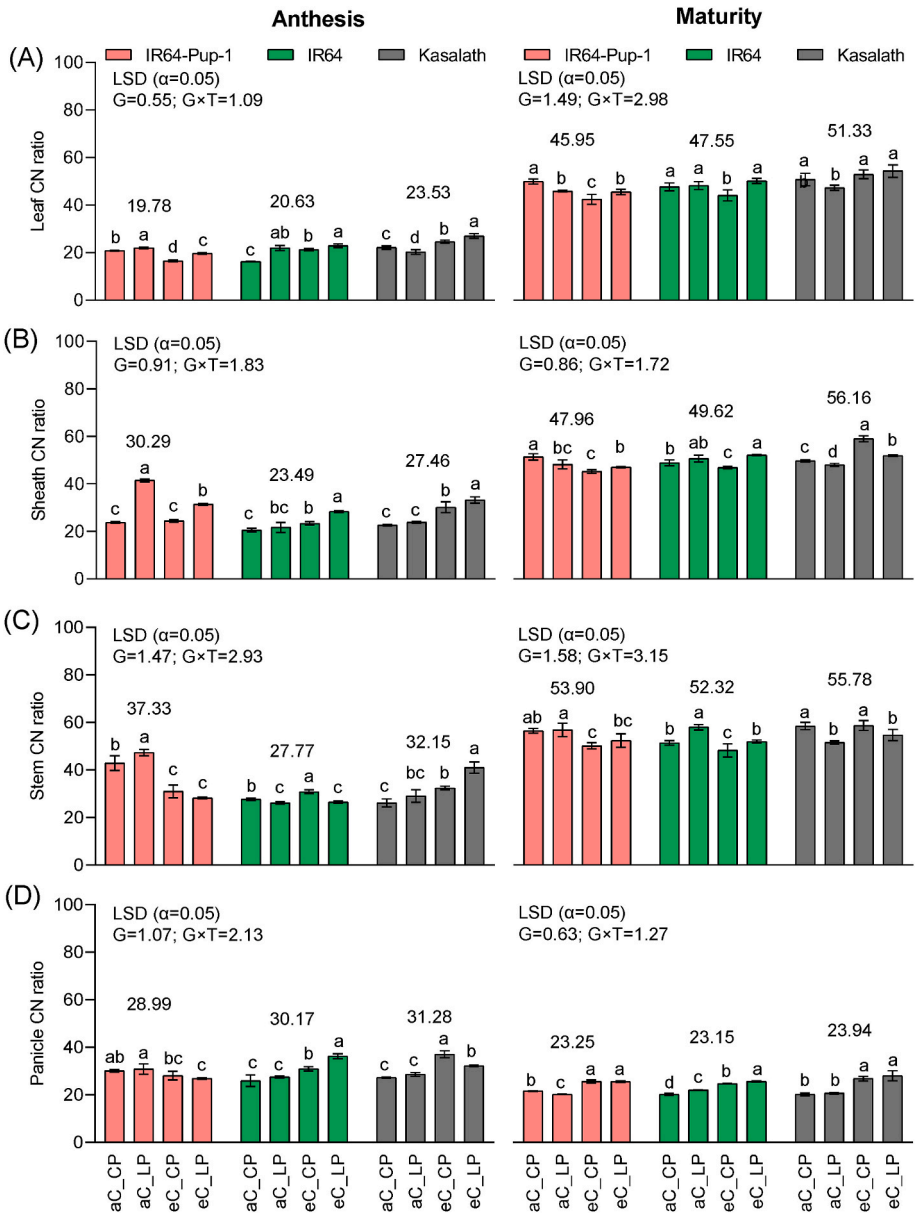

Fig. 1. Effect of CO_2 and P nutrition on the C:N ratio of (A) leaf, (B) sheath, (C) stem, and (D) panicle or grain of rice plants grown in soil with sufficient and low P in the OTC and natural ambient conditions. The data correspond to a mean ± SEm (n = 12). Data analysis was carried out using one-way ANOVA separately for different treatments for each genotype at both growth stages, and the least significant difference was calculated. Mean with the same letter are not significantly different at $P < 0.05$. The values on the bars represent mean of all treatments for each genotype, and the LSD ($P < 0.05$) value is presented for comparison between different genotypes. Abbreviations: aC_CP ambient CO_2 with control P, aC_LP ambient CO_2 with low P, eC_CP elevated CO_2 with control P, and eC_ elevated CO_2 with low P.

control P treatment. Among genotypes, maximal NUtE was observed in Kasalath indicating a more efficient utilization of N. Conversely, PUtE was higher in IR64 as compared to IR64-Pup1, suggesting a better P utilization efficiency in IR64.

3.2.3. *Influence of CO_2 and P nutrition on yield attributes and harvest indices*

The yield attributes, including grain yield, number of productive tillers, and number of grains per panicle exhibited significant ($P < 0.01$) differences due to genotypes, P treatment and CO_2 level, except for the test-weight, which showed significant ($P < 0.05$) differences only between genotype and P treatment. No significant interaction was observed between the variables (genotypes, P treatment, and CO_2 levels) for the yield attributes, indicating that one variable did not influence the other (Table S1). Productive tiller number, grain yield, and grains per panicle were increased significantly (by 5.5%, 9.53%, and 14.6%, respectively) under eCO_2 as compared to aCO_2 (Figs. S3A–D). Among genotypes, all yield attributes except grains per panicle, were significantly reduced in IR64 under eCO_2 in comparison to aCO_2. On the other hand, a significant increase was recorded in IR64-Pup1 and Kasalath for most of the yield traits in response to eCO_2. Under low P, a significant ($P < 0.05$) reduction was observed in all yield attributes in comparison to control P treatment. However, the interaction between CO_2 and P levels indicated that the reduction in grain yield was greater when low P stress was combined with eCO_2. Among genotypes, averaged over CO_2 and P levels, the highest grain yield was obtained from Kasalath but the number of productive tillers was highest in IR64 (>60%) compared to the other two genotypes. The lower grain yield of IR64 could be attributed to the higher number of unfilled grains per panicle as compared to IR64-Pup1 and Kasalath. The test-weight was significantly

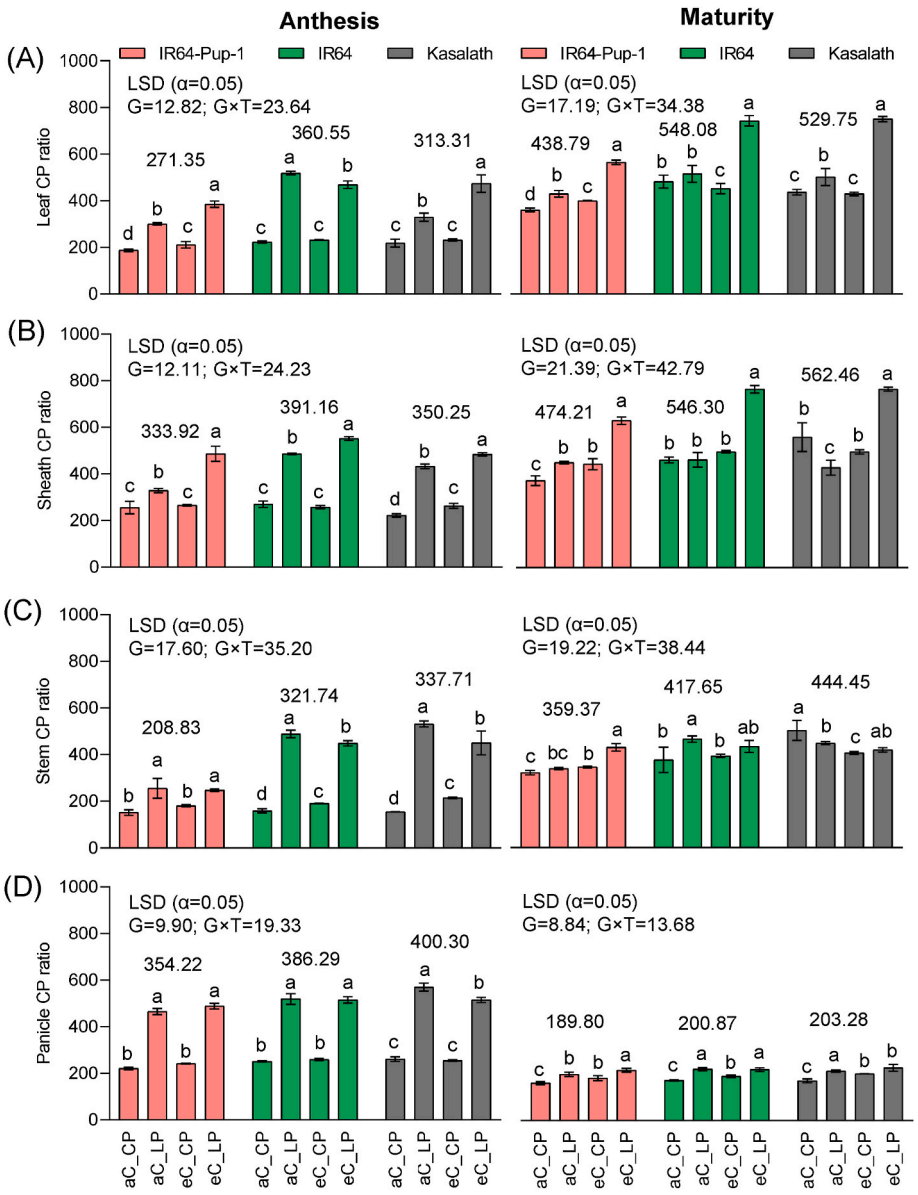

Fig. 2. Effect of CO_2 and P nutrition on the C:P ratio of (A) leaf, (B) sheath, (C) stem, and (D) panicle or grain of rice plants grown in soil with sufficient and low P in the OTC and natural ambient conditions. The data correspond to a mean ± SEm (n = 12). Data analysis was carried out using one-way ANOVA separately for different treatments for each genotype at both growth stages, and the least significant difference was calculated. Mean with the same letter are not significantly different at $P < 0.05$. The values on the bars represent mean of all treatments for each genotype, and the LSD ($P < 0.05$) value is presented for comparison between different genotypes. Abbreviations: aC_CP ambient CO_2 with control P, aC_LP ambient CO_2 with low P, eC_CP elevated CO_2 with control P, and eC_ elevated CO_2 with low P.

($P < 0.05$) lower in IR64 and Kasalath compared to IR64-Pup1, indicating inherent varietal variation.

The harvest index (HI), N harvest index (NHI), and P harvest index (PHI) exhibited significant changes ($P < 0.05$) under the different treatments (Table S1). The HI, which is a measure of reproductive efficiency and represents the ratio of grain to total above-ground dry matter, showed a significant increase in IR64 compared to the other two rice genotypes (Fig. S4A). This indicates that IR64 had a higher proportion of dry matter partitioning towards grain production. Under the influence of eCO_2, the HI was significantly reduced by 12.6% compared to aCO_2 suggesting that eCO_2 affected the reproductive efficiency, leading to a decrease in HI. Likewise, both the NHI and PHI showed maximum values in IR64 compared to the other two genotypes suggesting that IR64 has a higher proportion of N and P allocated to the grain. However, a significant reduction was observed in both NHI and PHI under eCO_2 (Figs. S4B–C) signifying that eCO_2 had a negative impact on the N and P partitioning to the grain. The effect of low P on all three indices (HI, NHI, and PHI) was non-significant compared to the control P treatment.

3.2.4. *Influence of CO_2 and P nutrition on association between nutrient content and grain yield*

The relationship between grain yield and the content of C, N, and P in different organs at both the growth stages (anthesis and maturity) was studied by linear regression (Fig. 4A–I). The results indicated that eCO_2 had a clear impact on these associations, and the variation was greater at maturity stages compared to anthesis. Under aCO_2 conditions, grain yield showed a significant ($P < 0.05$) positive association with C and P content in the leaves at both anthesis and maturity stages. However, under eCO_2, this association was non-significant for both nutrients. The N content in leaves showed a non-significant association with grain yield in most combinations, except aCO_2 at the maturity stage (Fig. 4A–D, G). Under aCO_2 conditions, the C, N, and P content in stem and sheath

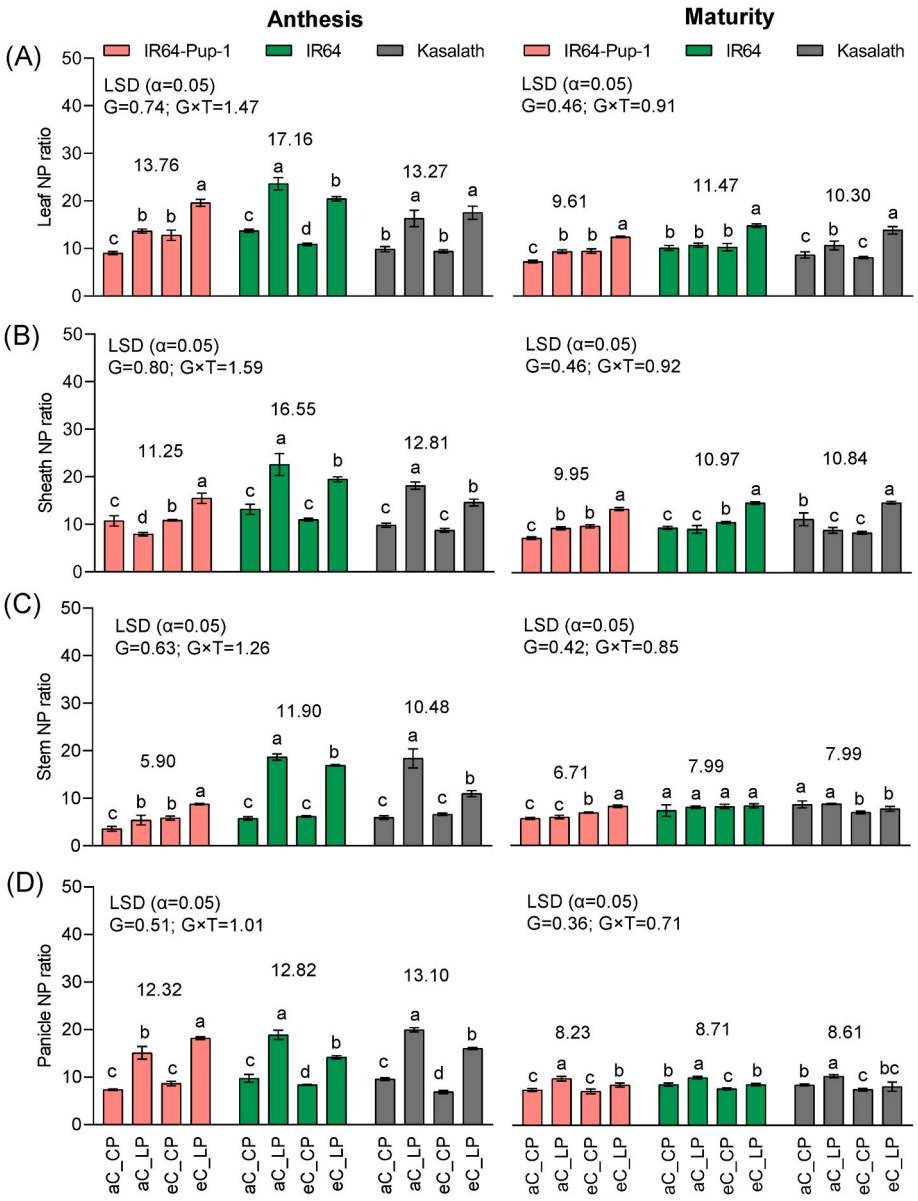

Fig. 3. Effect of CO_2 and P nutrition on the N:P ratio of (A) leaf, (B) sheath, (C) stem, and (D) panicle or grain of rice plants grown in soil with sufficient and low P in the OTC and natural ambient conditions. The data correspond to a mean ± SEm (n = 12). Data analysis was carried out using one-way ANOVA separately for different treatments for each genotype at both growth stages, and the least significant difference was calculated. Mean with the same letter are not significantly different at $P < 0.05$. The values on the bars represent mean of all treatments for each genotype, and the LSD ($P < 0.05$) value is presented for comparison between different genotypes. Abbreviations: aC_CP ambient CO_2 with control P, aC_LP ambient CO_2 with low P, eC_CP elevated CO_2 with control P, and eC_ elevated CO_2 with low P.

exhibited a significant positive association with grain yield at both growth stages (Fig. 4B, C, E, F, H, I). However, under eCO_2, this association was significant only at the anthesis stage, while no correlation was observed between grain yield and nutrient (C, N, and P) content in the stem and sheath at maturity.

4. Discussion

The elevated CO_2 has a positive impact on plant growth and yield, but the limited availability of P significantly reduced the impact of CO_2 fertilisation. Plant growth and productivity under sub-optimal nutrient conditions are generally improved by eCO_2 but this improvement cannot be sustained in the longer term unless sufficient nutrients are provided (Pandey et al., 2015a). The plant biomass accumulation is enhanced under eCO_2 by improving the CO_2 capture, primarily by C_3 plants through a "carbon fertilisation effect" (Long et al., 2015; Gojon et al., 2022). The ratio of net photosynthesis to respiration, which determines the amount of biomass produced by rice, was dramatically enhanced by eCO_2 (Sakai et al., 2001; Peng et al., 2004). Similar to earlier reports, we also observed a significantly enhanced biomass accumulation in all organs under eCO_2, irrespective of P levels, in all rice genotypes (Fig. S2) (Wang et al., 2013, 2019; Sakurai et al., 2014; Pandey et al., 2018). The interactive effects of CO_2 and low P also enhanced the shoot biomass in wheat and rye plants (Pandey et al., 2015a). Grain yield is a function of final biomass accumulation, and several reports found a significant increase in grain yield under eCO_2 (Long et al., 2006; Wang et al., 2013; Ainsworth and Long, 2005). The grain yield of rice grown under free-air CO_2 enrichment (FACE) system showed an increase by more than 12% compared to control CO_2 in China and Japan (Kim et al., 2003; Yang et al., 2006). Seneweera and Conroy (1997) reported up to 26% and 58%

Table 4
Effect of elevated CO_2 and P nutrition on N and P remobilization efficiencies from different organs of rice. Data corresponds to mean ± SEm (n = 12). *Abbreviation*: aC_CP ambient CO_2 with control P, aC_LP ambient CO_2 with low P, eC_CP elevated CO_2 with control P, eC_LP elevated CO_2 with low P, LSD least significant difference, df degree of freedom.

Treatments	Genotypes	NRE leaf	NRE sheath	NRE stem	Total NRE	PRE leaf	PRE sheath	PRE stem	Total PRE	NUtE	PUtE
aC_CP	IR64-Pup-1	0.69	0.68	0.50	0.76	0.61	0.53	0.69	0.74	43.78	319.44
	IR64	0.74	0.77	0.77	0.82	0.65	0.68	0.82	0.80	43.84	379.79
	Kasalath	0.61	0.79	0.64	0.75	0.56	0.81	0.75	0.76	40.14	345.47
aC_LP	IR64-Pup-1	0.70	0.45	0.59	0.77	0.56	0.54	0.63	0.69	44.96	430.09
	IR64	0.67	0.72	0.83	0.80	0.27	0.30	0.61	0.59	44.79	448.57
	Kasalath	0.58	0.81	0.68	0.75	0.35	0.62	0.33	0.54	41.71	425.51
eC_CP	IR64-Pup-1	0.69	0.56	0.31	0.71	0.57	0.51	0.41	0.67	40.15	305.95
	IR64	0.62	0.71	0.72	0.75	0.59	0.70	0.79	0.77	42.32	347.86
	Kasalath	0.61	0.76	0.67	0.75	0.55	0.75	0.68	0.75	50.74	384.51
eC_LP	IR64-Pup-1	0.62	0.44	0.40	0.69	0.40	0.34	0.36	0.55	40.91	380.59
	IR64	0.72	0.72	0.78	0.80	0.61	0.64	0.55	0.73	49.68	457.63
	Kasalath	0.56	0.70	0.62	0.73	0.44	0.70	0.45	0.66	49.82	447.51
LSD (5%)	Genotypes (G), *df-2*	0.04	0.05	0.07	0.02	0.07	0.09	0.08	0.03	1.15	16.68
	CO_2 (C), *df-1*	0.03	0.04	0.05	0.02	0.06	0.07	0.06	0.02	0.94	13.61
	Phosphorus (P), *df-1*	0.03	0.04	0.05	0.02	0.06	0.07	0.06	0.02	0.94	13.61
	G × C, *df-2*	0.06	0.06	0.09	0.03	0.10	0.12	0.11	0.04	1.64	23.58
	C × P, *df-1*	0.05	0.05	0.08	0.02	0.09	0.10	0.09	0.03	1.34	19.25
	G × P, *df-2*	0.06	0.06	0.09	0.03	0.10	0.12	0.11	0.04	1.64	23.58
	G × C × P, *df-2*	0.08	0.09	0.13	0.04	0.15	0.17	0.15	0.06	2.31	33.35

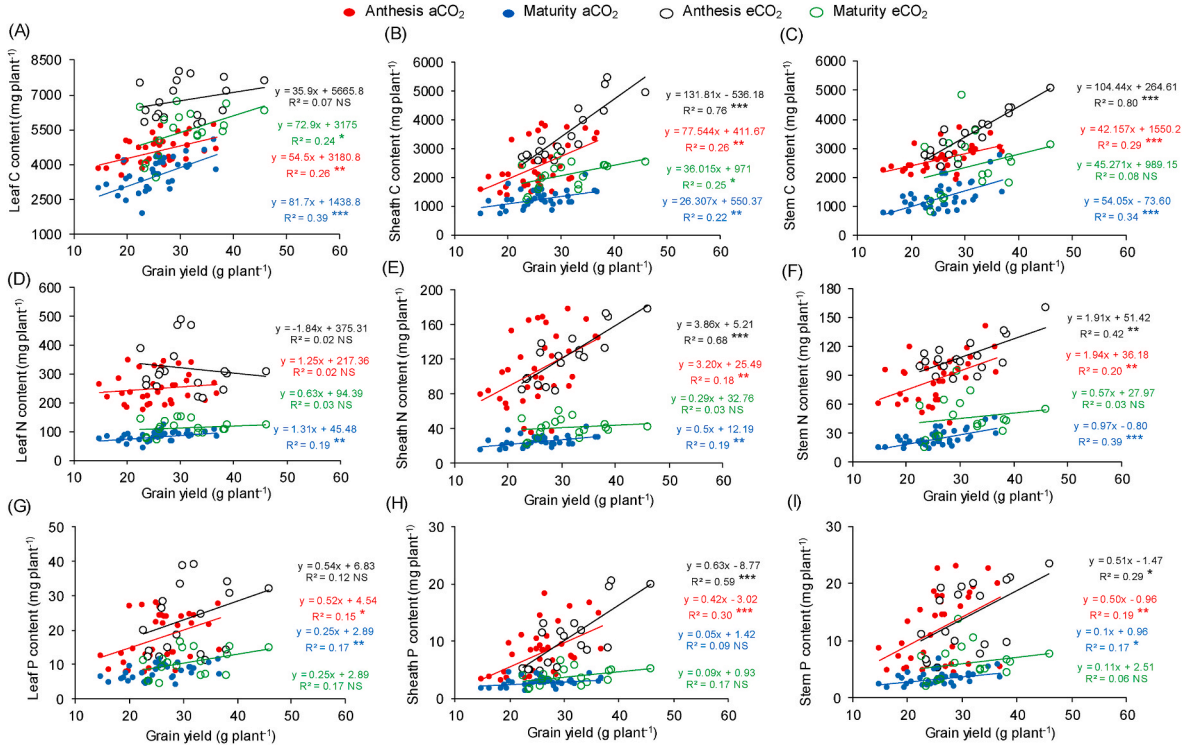

Fig. 4. Linear regression between grain yield and (A) leaf C content, (B) sheath C content, (C) stem C content, (D) leaf N content, (E) sheath N content, (F) stem N content, (G) leaf P content, (H) sheath P content, and (I) stem P content at anthesis and maturity of rice plants grown in soil with sufficient and low P in the under different CO_2 levels.

increases in total above-ground biomass and grain productivity, respectively in rice under eCO_2 compared to aCO_2 with a wide range of P levels. Our results are in accordance with these studies resulting in an increased total biomass and grain yield under eCO_2 as compared to aCO_2 irrespective of P levels (Fig. S3A) suggesting that eCO_2 helps mitigate the adverse effect of low P stress.

The C, N, and P are essential nutrients for plant growth and development, and their stoichiometric ratios reflect the elemental interrelationships of plants. Our results agree with earlier reports presenting a similar increase in C concentration in all organs under eCO_2 (Baslam et al., 2012; McKenzie et al., 2016; Du et al., 2019). Under eCO_2, photosynthesis increased due to the increase in ribulse-1,5 bisphosphate carboxylase/oxygenase (RuBisCo) activity which stimulates carbohydrate production in plants (Thompson et al., 2017). However, plant C accumulation was significantly reduced under low P conditions exhibiting explicit interrelationships between these nutrients (Table 1). Unlike C, the N concentrations was significantly reduced in most of the organs except grain under eCO_2 due to the dilution effect caused by increased biomass accumulation under eCO_2 (Bloom et al., 2010; Wang et al., 2019). Earlier studies have shown that under eCO_2, the overall concentrations of N in plants decreases due to the accumulation of non-structural carbohydrates (Gifford et al., 2000; Lieffering

et al., 2004; Taub and Wang, 2008; Yuan and Chen, 2015). Another reason for reduction in plant N concentration is the acclimations of plants to eCO_2 at leaf-level. A meta-analysis of 125 studies on rice plants found that under CO_2, the concentration of RuBisCo, the key enzyme involved in photosynthesis and a major component of leaf N content, was significantly reduced by more than 20% as compared to aCO_2 (Wang et al., 2015). Additionally, the N remobilized from different organs such as leaves, sheath, and stems, also plays a significant role in lowering N concentration in vegetative tissues under low P and eCO_2 conditions (Table S2). Furthermore, the concentration of N in grains was found to be significantly reduced under low P and eCO_2 conditions. Previous reports have also found a decrease in grain N concentration under eCO_2 (Dier et al., 2019; Jin et al., 2019), but the specific effect of low P and eCO_2 interaction on grain N concentration has not been extensively studied.

We observed significant influence of P nutrition and eCO_2 on C:N:P stoichiometry in rice plants. The assimilation of C through photosynthesis is directly influenced by N concentrations because N is required for the synthesis of RuBisCo. Therefore, increased N demand under eCO_2 led to significantly higher C:N ratios in the plants (Lieffering et al., 2004; Roy et al., 2012). The low P stress also caused an increase in C:N ratio in most of the organs exhibiting a synergistic relationship between N and P. Low P stress, independent of CO_2 level, caused a significant reduction in N and P concentrations in most of the organs; however, the reduction was greater under eCO_2 (Tables 1 and 2). Unlike C:N ratio, the C:P ratio exhibited a different trend in response to eCO_2. At both the growth stages, the leaves and grain C:P ratio increased under eCO_2, while the stem and grain C:P ratio were unaffected. The total P uptake by plants increased under eCO_2 due to significantly higher biomass production but the P concentration was reduced because of the dilution effect (Jin et al., 2012, 2013). Earlier studies have demonstrated that the magnitude and direction of growth response of plants to eCO_2 depends on P availability (BassiriRad et al., 2001; Fernando et al., 2014; Jin et al., 2013, 2015, 2019). Under eCO_2, the external phosphatic fertilizer application rates must be re-assessed as the plant's internal P requirement increases (Yang et al., 2006; Jin et al., 2015).

The remobilization efficiency of N from leaves (NRE_L) and sheath (NRE_{SH}) was significantly influenced by eCO_2, while P remobilization efficiency (PRE_L and PRE_{SH}) was unaffected. On the other hand, remobilization of N (NRT_{ST}) and P (PRE_{ST}) from stem exhibited similar reduction pattern under eCO_2. Yuan and Chen (2015) also observed that the C:N and C:P ratios in deciduous and woody angiosperms were unaltered by eCO_2, indicating that plant species responds differently to eCO_2 in terms of C:N and C:P ratios. Likewise, the N:P ratio was also influenced by eCO_2 at both P levels and in all plant organs. A significant reduction in N:P ratio was observed as compared to control conditions. The increase in C:N and C:P ratio exhibited a reduction in N and P concentrations under eCO_2, but the increase in the N:P ratio indicated that the reduction in P concentration was lesser than the reduction in N concentration (Loladze, 2014; Deng et al., 2015; Du et al., 2019).

The linear regression showed that eCO_2 influences the C, N, and P concentrations depending on organ type, plant growth stage, and the plant nutritional status (Fig. 5). A positive ($P < 0.01$) association was observed between leaf C and P concentrations at both growth stages under eCO_2 but it was non-significant under aCO_2. This indicates that the rate of photosynthesis increases under eCO_2 to assimilate more C requiring more ATPs, thereby leading to higher leaf P demand (Khlyntseva et al., 2009; Liang et al., 2015). A similar trend was also found between the association of C and P concentrations in other components (sheath, stem, and grain) at maturity. Our results are consistent with the finding of Wang et al. (2019), who reported a positive correlation between C assimilation and N and P concentrations in rice and wheat under eCO_2. However, the effect of eCO_2 on the relationship between C and N concentrations was lesser than C and P concentrations.

The interaction of CO_2 and low P was more pronounced in Kasalath and IR64-Pup1 compared to IR64. A significant increase in total biomass and yield attributes (number of productive tillers, test weight, and grain yield) were recorded in IR64-Pup1 as compared to IR64 (Figs. 1 and 2). Additionally, in response to eCO_2 with low P conditions, IR64-Pup1 showed lower N concentration but higher P concentration than IR64 indicating that *Pup1* QTL had no significant effect on plant N uptake but improved P uptake. As reported, *Pup1* QTL not only maintains P homeostasis by regulating the expression of P starvation-responsive genes but also the expression of root system architecture-related genes by modulating DNA methylation in different locations resulting in enhanced root growth (Chin et al., 2011). Recently, Kumar et al. (2022) compared the methylation patterns of CG, CHG, and CHH (where H = A, T, and G) contexts (DNA methylation contexts in plant genes) in two contrasting rice genotypes (Pusa-44 and its near-isogenic line introgressed with the *Pup1* QTL called NIL-23) under P stress. They found a significant reduction in the methylations of CG, CHG, and CHH in both root and shoot of Pusa-44 compared to NIL-23 under P stress indicating the role of *Pup1* QTL in epigenetic modification in modulating gene expression under P stress.

In conclusion, this study demonstrated that the growth is stimulated under increased atmospheric CO_2 conditions due to the "CO_2 fertilisation

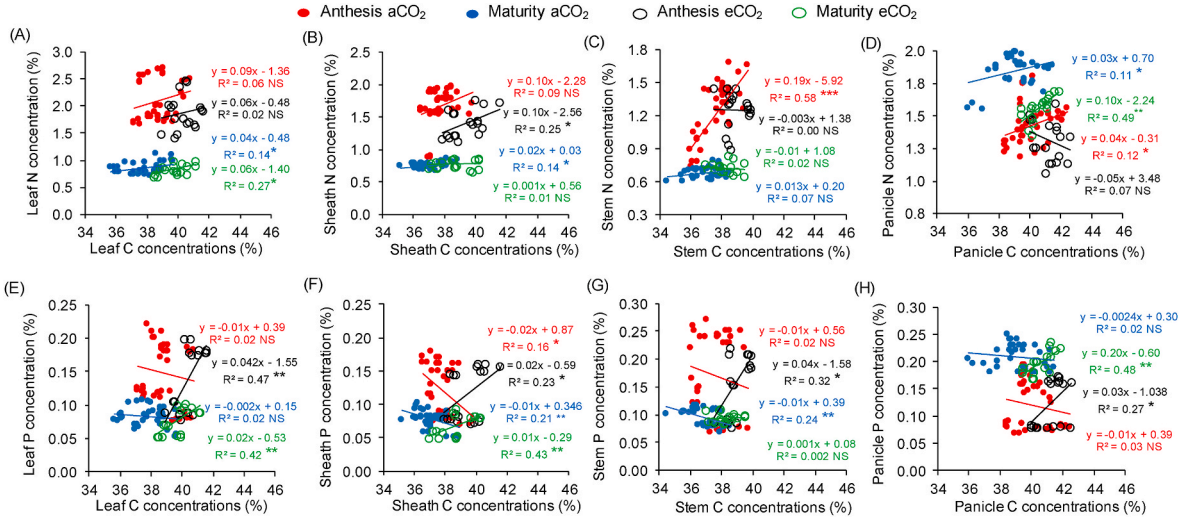

Fig. 5. Linear regression between nutrient concentration in different organs at anthesis and maturity of rice plants grown in soil with sufficient and low P under different CO_2 levels.

effect", but under limited P availability, this improvement cannot be sustained in the longer term unless sufficient nutrients are provided. Due to the combined effect of eCO_2 and low P stress, the N and P concentrations decreased in all plant components, which significantly altered the plant stoichiometric ratios compared to control conditions in all three genotypes. The C:N and C:P ratios significantly increased in all plant components under eCO_2 with low P, whereas the N:P ratio decreased under eCO_2 but increased under low P. As compared to IR64, higher P uptake was recorded in IR64-Pup1 under eCO_2 conditions; however, lower N concentrations were found in the tissues of IR64-Pup1 under eCO_2 indicating that the *Pup1* QTL had no significant effect on plant N uptake. The positive effect of eCO_2 on biomass accumulation and grain yields was recorded under both sufficient and low P conditions in all genotypes, but the performance of *Pup1* QTL-containing lines, Kasalath and IR64-Pup1, was superior than IR-64.

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CRediT authorship contribution statement

Sandeep Sharma: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **D.H. Raviteja:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Tarun Kumar:** Writing – review & editing, Methodology. **Prem S. Bindraban:** Writing – review & editing. **Renu Pandey:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2024.108657.

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