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Study on the Influence of Elevated Carbon Dioxide Concentration on the Ecological Safety of Yiluo River in China¹

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Abstract: Ongoing elevations in atmospheric CO₂ concentrations are reshaping the biogeochemical functioning of aquatic ecosystems. However, the coupled responses of phosphorus fraction dynamics, nutrient stoichiometry and heavy metal geochemistry in inland fluvial systems remain severely underconstrained, leaving a knowledge gap that limits the predictive capacity for riverine carbon and nutrient cycles under future climate scenarios. This study selected the Yiluo River, a typical regulated tributary in the Yellow River Basin, as the research object, collected intact overlying water samples retaining in-situ suspended sediment matrices, and conducted a series of laboratory-controlled gradient CO₂ enrichment experiments to systematically quantify the cascading multi-element biogeochemical effects at the water-sediment interface. The results indicate that, under the moderate CO₂ conditions tested, significantly elevated dissolved Fe, Al and Zn concentrations in the water column promote the formation of stable Fe/Al-P and Zn-P phases on suspended particles, weaken the competitive suppression effect of Ca²⁺ on phosphorus sequestration, convert labile dissolved phosphorus into geochemically immobile phases, and reduce the potential endogenous eutrophication risk. CO₂-driven pH regulation may further act as a niche-filtering factor shaping phytoplankton community assembly, while the statistically significant negative correlation between particulate C/N and C/H ratios may enhance the chemical recalcitrance of particulate organic matter and the potential for fluvial carbon sequestration. This study identifies a mechanistic linkage between fluvial CO₂ dynamics, multi-element competitive interactions and phosphorus immobilization, providing localized empirical evidence that improves the understanding of inland river biogeochemical cycles under global change.

Keywords Carbon dioxide; Ilo River; Planktonic organisms; Water environment; Metal

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27 **1 Introduction**

28 The Yellow River, a cradle of Chinese civilization, functions as a vital hydrological corridor and a key component
29 of the East Asian continental ecological network, playing an irreplaceable role in sustaining regional agricultural
30 productivity and maintaining terrestrial-aquatic ecological connectivity^[1,2]. Its middle reaches traverse the highly
31 erodible Loess Plateau, transporting massive sediment loads downstream to form the agricultural plains of northern
32 China. The Yiluo River, a major first-order tributary in this region, originates from the confluence of the Yihe and Luohe
33 Rivers. Spanning multiple ecological zones and hosting unique aquatic biodiversity, the Yiluo River's main stem has
34 been designated a national-level aquatic germplasm reserve for the endemic Yellow River carp, underscoring its critical
35 ecological and conservation value^[3,4]. Despite this conservation status, the Yiluo River faces escalating pressures from
36 synergistic anthropogenic and climatic stressors. Intensive human activities, including industrial discharge, agricultural
37 runoff, and hydrological regulation, have degraded water quality and ecosystem integrity^[5,6,7]. Concurrently, global
38 climate change, particularly the rise in atmospheric CO₂, is altering fundamental aquatic processes such as carbonate
39 chemistry, nutrient availability, and trace metal speciation^[8,9,10]. While the individual impacts of nutrient pollution and
40 heavy metal contamination in the basin have been documented, the coupled ecosystem responses to elevated CO₂ —
41 spanning phytoplankton community dynamics, nutrient cycling (e.g., phosphorus mobilization and C-N coupling), and
42 heavy metal geochemistry — remain insufficiently understood.

43 This knowledge gap is significant. Existing studies on CO₂ effects in freshwater systems are largely fragmented,
44 focusing on isolated components such as single-species algal physiology^[11,12], rather than the integrated, multi-trophic
45 responses that determine ecosystem-level outcomes. The lack of holistic, mechanistic understanding hinders the ability
46 to predict how sensitive, ecologically valuable systems like the Yiluo River will respond to future climate scenarios,
47 thereby limiting the development of effective, science-based conservation and management strategies. To address these
48 knowledge gaps, this study was conducted in the confluence zone of the Yihe and Luohe Rivers — a representative area
49 integrating the core hydrological and ecological characteristics of the Yiluo River watershed. We implemented a series
50 of laboratory-controlled, gradient CO₂ enrichment experiments using in-situ water samples to simulate future scenarios.
51 The central objective was to systematically quantify the cascading effects of rising CO₂ concentrations on the coupled
52 biogeochemical processes at the water-sediment interface, with a focus on: (1) the directional redistribution of
53 phosphorus fractions and associated nutrient stoichiometry; (2) the consequent shifts in phytoplankton community
54 assembly and succession; and (3) the interplay with heavy metal geochemical behavior. By investigating these linkages,
55 this work aims to provide an experimental basis for understanding how inland fluvial ecosystems respond to global
56 change, offering insights relevant to the ecological management of the Yellow River Basin and other climate-vulnerable
57 freshwater systems.

58
59
60

61 **2 Materials and methods**

62 **2.1 Experimental materials**

63 The water samples employed in this study were collected from the cross-sectional waters at the confluence of the
64 Yi River and Luo River (112° 52'7" E, 34° 42'30" N). This region is characterized by abundant water flow and diverse
65 habitats, serving as a critical hydrological junction in the upper reaches of the Yellow River Basin and holding high
66 research value. On May 21st, 2026, water samples were collected at a depth of 20 cm using a 2.5-L organic glass
67 sampler and promptly transported to the laboratory. A small portion of the samples was used for water quality indicator
68 determination, while the remaining samples were reserved for simulation experiments.

69 **2.2 Experimental methods**

70 A custom dynamic gas-blending system was employed to prepare precise CO₂-air mixtures. Ambient air was
71 purified through a two-stage filtration system containing soda lime and activated carbon, then mixed with high-purity
72 CO₂ (99.99%, v/v) in a stirred buffer tank. The resulting gas was continuously monitored and calibrated using an
73 infrared CO₂ analyzer (XLZ-1090GXH), with concentration maintained within $\pm 0.2\%$ of target values. Calibrated gas
74 was stored in nitrogen-purged PTFE cylinders. Experiments were conducted in CO₂-regulated growth chambers
75 (COHS-150) following 72-hour stability validation. Three distinct CO₂ concentration levels were established: 0.065%
76 (set a), 0.055% (set b), and 0.045% (set c). A mass flow controller maintained a constant gas exchange rate of 0.8
77 L·min⁻¹ within each chamber, ensuring homogeneous CO₂ distribution throughout the cultivation space. All treatments
78 maintained uniform environmental conditions: temperature at $20 \pm 0.2^\circ\text{C}$, photosynthetic photon flux density at 30 μmol
79 photons·m⁻²·s⁻¹, 12-hour photoperiod, and relative humidity at $65 \pm 3\%$. Each treatment included three independent
80 replicates. Chamber CO₂ concentrations and light intensities were verified daily, with samples collected at
81 predetermined intervals for physiological, growth, and biochemical analyses.

82 **2.3 Testing methods**

83 **2.3.1 Physical and chemical index testing**

84 Dissolved oxygen (DO) and pH value were measured using a dissolved oxygen analyzer (YSI 550A, USA) and a
85 pH meter (YSI pH60, USA), respectively. Total nitrogen (TN), ammonia nitrogen (NH₄-N), nitrate nitrogen (NO₃-N),
86 and organic carbon (OC) were determined in accordance with the alkaline potassium persulfate digestion method,
87 Nessler's reagent method, UV spectrophotometry, and microwave digestion method. Calcium (Ca) and magnesium (Mg)
88 were quantified using a flame atomic spectrophotometer (PE AA800). Water samples (1 L each) were filtered through
89 0.45 μm Whatman GF/C membranes (Kent, UK), and the chlorophyll a (Chl a) content was determined via the
90 acetone-ethanol mixture method^[13].

91 At the end of the experimental period, the overlying water was carefully decanted, and the underlying sediment
92 was collected for subsequent elemental composition analysis. Sediment samples were subjected to vacuum
93 freeze-drying and subsequently homogenized by grinding through a 100-mesh sieve. The quantification of major
94 organic elements (C, H, O, N, S) in sediment samples was performed using an elemental analyzer (EA3100, Eurovector).
95 Approximately 10 mg of each sample was subjected to high-temperature combustion, with elemental mass fractions
96 determined through the K-factor calibration method. Trace element analysis was conducted via inductively coupled

97 plasma optical emission spectrometry (ICP-OES, Plasma Quant 9100), following established analytical protocols for
98 sediment matrix analysis.

99 The six phosphorus fractions were separated via sequential extraction: Fe/Al-P with 0.5 mol NH₄F (pH 8.2) and
100 0.1 mol NaOH-0.5 mol Na₂CO₃; Cu-, Zn-, and Cr-P with 0.05 mol EDTA (pH 7.5, 6 h); Ca-P with 1 mol HCl (16 h); OP
101 after ashing (550 °C) and extraction with 1 mol HCl. Extracts were filtered (0.45 µm) and analyzed by the
102 molybdenum-antimony method, with Cu/Zn/Cr-P differentiated by ICP-OES. Results are expressed on a dry-weight
103 basis.

104 2.3.2 Plankton species composition analysis

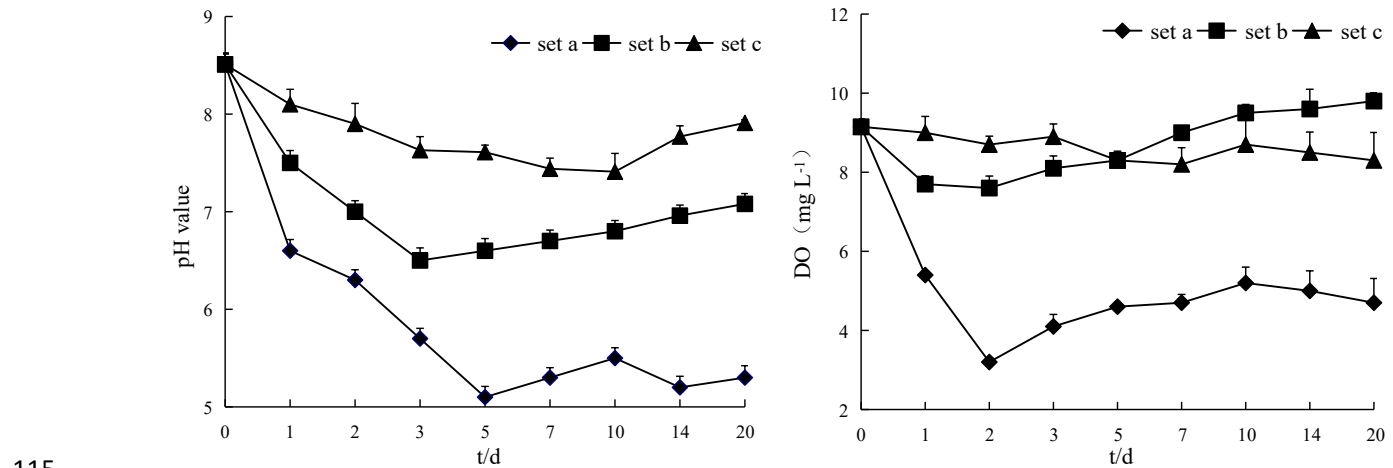
105 To provide biomass information on phytoplankton, 1 L of fresh samples were fixed with Lugol's iodine solution^[14],
106 and then identified and counted under a microscope at 400× magnification after a 24-hour settling period. For each
107 subsample, the abundance of algal cells was examined three times, and the average value was calculated.

108 2.4 Data statistical analysis

109 Origin 2024 and SPSS 18 were used for plotting and analysis of variance (ANOVA), respectively. The
110 experimental groups were compared using LSD method. The correlation between the comprehensive variables was
111 analyzed through Pearson Correlation, and the significant difference level was $p < 0.05$. All experimental results are
112 reported as the mean values of three independent replicates ($n = 3$).

113 3 Results

114 3.1 Temporal Dynamics of pH and DO Concentrations in Overlying Water



115
116 Fig.1 Changes of pH value and DO concentration in overlying water

117 As illustrated in Figure 1, there was a statistically significant difference in pH value between set a and set b
118 treatments ($p < 0.05$). On Day 20, the measured pH values across groups ranked as: set c > set b > set a. The DO in set a
119 exhibited a sharp significant decline within the initial 48 h, followed by a gradual increase. The initial DO level in set b
120 was marginally lower than that in set c, and this relative order was fully reversed after 5 days of treatment. Throughout
121 the entire experimental duration, both pH and DO concentrations in set c remained largely stable with negligible
122 temporal variation.

123 3.2 Temporal Dynamics of Ca²⁺ and Mg²⁺ Concentrations in Overlying Water

As shown in Figure 2, Ca^{2+} concentrations in set b and set c exhibited a statistically significant decrease within the initial days, followed by a subsequent stable plateau. Ca^{2+} levels in set c showed a slight declining trend and attained a steady state on Day 7. On Day 20, the final Ca^{2+} content across treatments ranked as: set a > set c > set b. For Mg^{2+} , all three groups showed a significant sharp decline on Day 1, and Mg^{2+} concentrations in all treatments stabilized after 2 days.

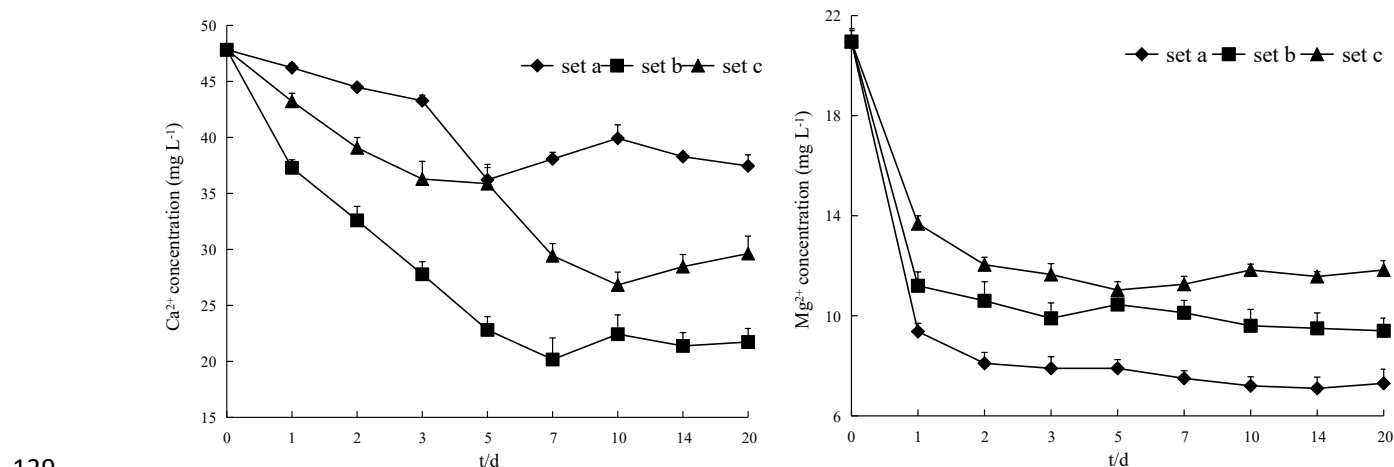


Fig.2 Changes of calcium and magnesium ions contents in overlying water

3.3 Temporal Dynamics of Ammonia Nitrogen and Nitrate Nitrogen Concentrations in Overlying Water

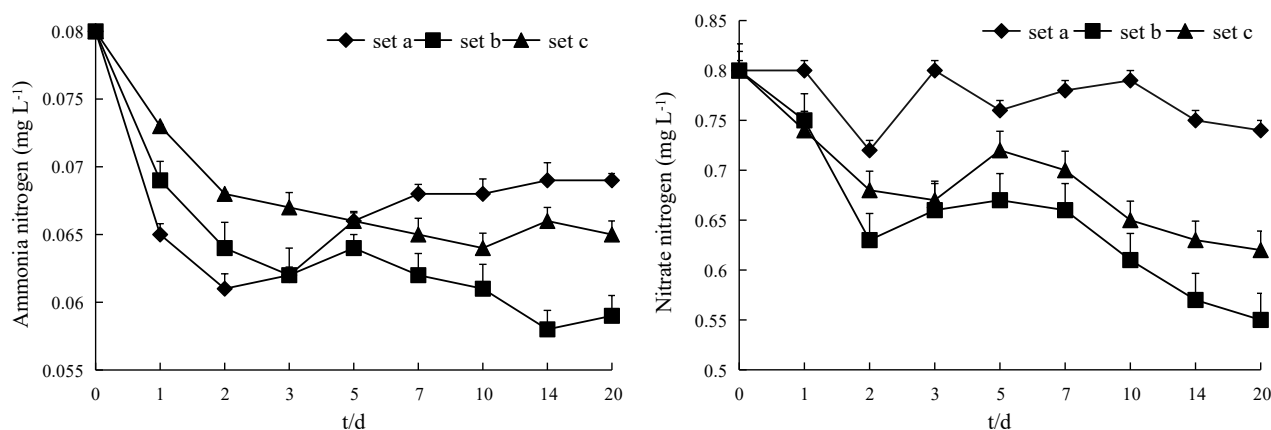


Fig.3 Changes of ammonia nitrogen and nitrate nitrogen concentrations in overlying water

The dominant dissolved inorganic nitrogen species in the water samples was nitrate nitrogen, while ammonia nitrogen remained at a relatively lower level (Figure 3). The ammonia nitrogen concentration in set a exhibited an initial declining phase followed by a subsequent increasing trend. After Day 7, the ammonia nitrogen content in set b was significantly lower than that in both set a and set c ($p < 0.05$). The temporal variation patterns of nitrate nitrogen across all three groups were highly comparable, and the final nitrate concentration at the end of the experiment followed the order: set a>set c>set b.

3.4 Comparison of Phytoplankton Biomass

The total phytoplankton density in set b was the highest, with Chlorophyta as the dominant group, followed by Diatoms (Figure 4). Diatoms dominated in both set a and set c, with their abundance far exceeding that of Chlorophytes

144 ($p < 0.01$). Except for Cryptophytes, the phytoplankton densities in set a were significantly lower than those in set b and
 145 set c. The content of Chl a was the highest in set b, showing no significant difference from set c, while both were
 146 significantly higher than that in set a ($p < 0.01$).

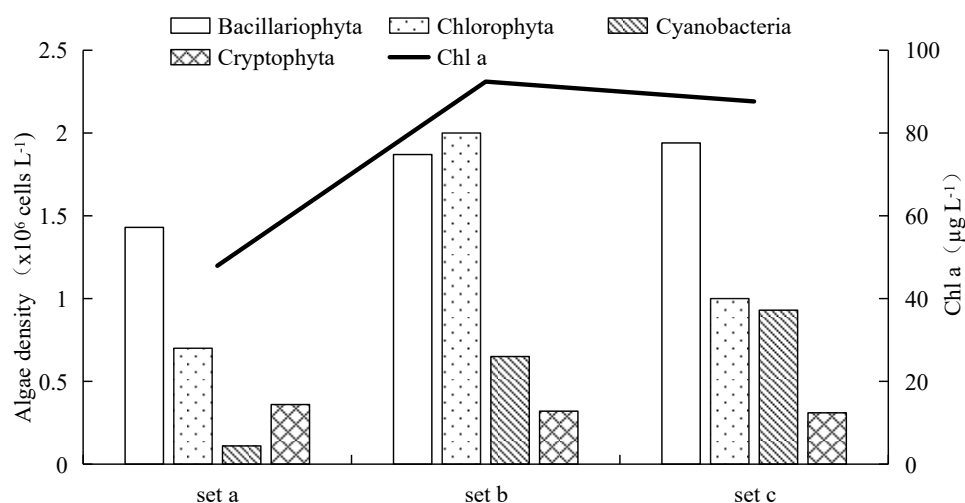


Fig.4 Phytoplankton biomass in overlying water

3.5 Variations in multi-element contents of sediments across different treatments

Table 1 Major organic element compositions of sediment samples across different treatments

Elemental content	C/%	N/%	S/%	H/%	O/%	C/N	C/S	C/H	C/O
set a	60.73	6.63	1.12	17.50	14.02	9.16	54.22	3.47	4.33
set b	61.57	6.58	0.77	11.54	19.54	9.36	79.96	5.34	3.15
set c	49.81	6.26	0.64	7.09	37.12	7.96	77.83	7.03	1.34

As shown in Table 1, the mass ratios of the five major organic elements in the sediment exhibited statistically significant differences across treatments. The carbon mass fraction ranked as set b > set a > set c. The atomic C/H and C/S ratios in set a were significantly lower than those in set b and set c ($p < 0.01$).

Table 2 Multi-element contents of sediment samples across different treatments

Elemental content	P	Ca	Mg	Cd	Sn	Cr	Mn	Al	Fe	Cu	Zn	As
set a	0.53	1.04	0.88	0.07	0.05	0.11	0.02	0.26	0.35	0.26	0.25	0.01
set b	0.55	1.36	1.24	0.12	0.09	0.17	0.02	0.34	0.38	0.17	0.26	0.01
set c	0.41	1.17	0.95	0.10	0.07	0.23	0.01	0.23	0.31	0.13	0.22	0.02

Significant variations were observed in sediment between phosphorus and trace metal element contents (Table 2). The mass fractions of Ca, Mg, Cd and Sn followed the consistent order: set b > set c > set a, while P, Al, Fe and Zn exhibited the ranking: set b > set a > set c. The Cu content decreased in the order of set a > set b > set c, whereas Cr content showed the opposite trend, ranking as set c > set b > set a.

3.6 Contents of different phosphorus fractions in sediments across treatments

Significant differences in phosphorus fractions were observed (Figure 5). The OP, Fe/Al-P, and Ca-P collectively constituted the dominant phosphorus forms across all treatments, followed by Cu-P, while Cr-P consistently showed the lowest concentrations. Specifically, the OP was the predominant phosphorus speciation in set a. The Fe/Al-P and Zn-P contents in set b were significantly higher than those in the set a and set c groups ($p < 0.05$). The Ca-P content in set c was the highest, while its Cu-P and Zn-P concentrations were significantly lower than those in set a and set b ($p < 0.05$).

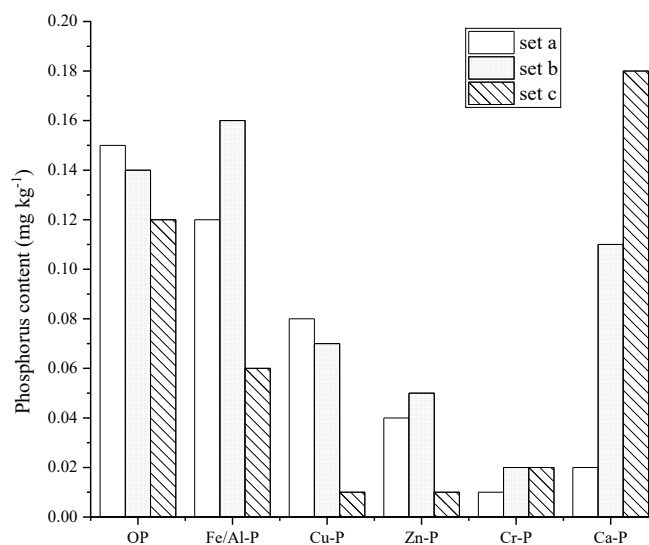


Fig.5 Comparison of different phosphorus component contents

3.7 Pearson correlation analysis of ecological factors

Table 3 Pearson correlation analysis of ecological factors

	pH	NH ₃ -N	NO ₃ -N	TOC	Chl.a	C/N	C/S	C/H	C/O	P	Ca	Mg	Cd	Sn	Cr	Mn	Al	Fe	Cu	Zn	As
DO	.51	-.41	.17	.74*	-.45	.31	-.24	-.16	.07	-.21	-.26	-.32	.17	-.06	-.37	-.20	-.36	-.37	-.25	-.25	-.38
pH		-.27	.76*	-.91**	-.92**	.08	-.11	.42	-.20	-.69*	-.88**	-.64*	.92**	-.87**	-.60*	.46	-.13	-.26	.15	.33	-.46
NH ₃ -N			.74*	.51	-.84**	-.35	-.61*	.47	.46	-.60*	-.60*	-.42	.64*	-.61*	-.40	.34	-.06	-.15	.25	.25	-.30
NO ₃ -N				.63*	.03	-.61*	-.37	.52	-.19	-.73*	.88**	.74*	-.55	-.14	.32	-.31	-.24	.06	-.29	-.12	-.23
TOC					.91**	.53	.40	-.06	.27	.64*	.63*	.41	-.68*	.68*	.38	-.43	.01	.09	-.34	-.34	.26
Chl.a						.47	.33	-.42	.15	.73*	-.30	.42	-.19	-.50	.35	.83**	-.09	.03	.86**	-.50	.23
C/N							.61*	-.76**	.20	.66*	.61*	.51	-.28	-.33	.17	.50	.46	-.09	.35	.07	-.38
C/S								-.54	-.31	-.29	.55	.17	.32	.07	-.65*	-.19	.28	.47	-.03	.26	-.14
C/H									-.49	-.64*	-.22	.03	-.21	-.19	.27	-.21	-.05	-.41	-.62*	.17	-.12
C/O										.43	.32	.14	.36	-.16	-.32	-.20	.58	.43	.52	.26	.18
P											.51	.26	.57	.75*	.23	-.09	.69*	-.60*	-.52	-.52	.09
Ca												.87**	-.96**	.81**	.85**	-.23	.43	.55	-.07	-.07	.73*
Mg													-.69*	.42	.99**	.28	.81**	.89**	.42	.42	.97**
Cd														-.95**	-.66*	.50	-.14	-.28	.36	.36	-.50
Sn															.37	-.77**	-.19	-.05	-.65*	-.65*	.19
Cr																.33	.84**	.91**	.47	.47	.98**
Mn																	.79**	.69*	.99**	.98**	.50
Al																		.99**	.87**	.82**	.93**
Fe																			.80**	.79**	.97**
Cu																				1.00**	.63*
Zn																					.66*

** . Correlation is significant at the 0.01 level (2-tailed), * . Correlation is significant at the 0.05 level (2-tailed).

As presented in Table 3, the pH value showed significant correlations with elements including P, Ca, Mn, Cd and Sn. The TOC content was positively correlated with DO, and exhibited a significant negative correlation with pH value. The Chl a content showed significant positive correlations with P, Cu and Mn contents in the sediment. The phosphorus content in the sediment was significantly positively correlated with Al and Fe concentrations, while significant positive correlations were also detected among Al, Fe, Cu and Zn. The sediment C/H ratio exhibited a significant negative correlation with the C/N ratio.

4 Discussion

The continuous accumulation of CO₂, primarily attributed to anthropogenic emissions, is a well-established driver of global environmental change^[15-17]. While CO₂ serves as the fundamental substrate for aquatic primary production, its rising concentration exerts multifaceted and often indirect regulatory effects on ecosystem structure and function, which have become a central focus in contemporary limnology and oceanography. Conventional theory posits that elevated CO₂ levels can enhance phytoplankton growth by reducing the metabolic energy expenditure associated with active inorganic carbon uptake via carbon-concentrating mechanisms (CCMs)^[18-22]. This “CO₂ fertilization” effect predicts a positive correlation between CO₂ availability and algal biomass. Contrary to this expectation, our experimental results indicated a more complex response pattern. Specifically, set a, despite sustaining the highest dissolved CO₂ concentration, exhibited significantly lower total algal biomass and Chl-a content compared to set b and set c. This apparent paradox underscores that the direct physiological benefit of increased CO₂ may be overridden by secondary environmental alterations it induces. The key to resolving this paradox lies in the concomitant shift in water chemistry. The most pronounced difference among treatments was the divergent pH trajectory: while the pH value in set b recovered to near-neutral levels, it in set a maintained a persistently acidic environment. This pH differential emerged as a key mediator governing microbial community responses. The growth of many phytoplankton taxa, particularly Cyanobacteria, is optimized within a neutral to alkaline pH range (7.0–9.5)^[23,24]. Consequently, the suppressed Cyanobacterial biomass in set a and its relative dominance in set c are consistent with this fundamental physiological constraint. Additionally, a striking exception was observed within the Cryptophyte community. Cryptophyte biomass was significantly higher in set a than the other treatments. This indicates a distinct ecological strategy, where Cryptophytes may outcompete other taxa under low-pH stress, potentially through superior acid tolerance or a release from competition with pH-sensitive groups like Cyanobacteria. This finding may carry ecological implications: CO₂-induced acidification may not simply suppress overall productivity but can selectively favor specific algal lineages, thereby altering competitive hierarchies and successional pathways. Such a shift could elevate the risk of blooms dominated by acid-tolerant taxa, such as certain Cryptophytes, under future high-CO₂ scenarios. Furthermore, the structural integrity of Chl-a is known to be compromised under acidic conditions^[25, 26]. The observed gradient in Chl-a content mirrored the overall biomass trend and provides independent corroborating evidence that neutral conditions are more favorable for maintaining photosynthetic apparatus stability. This consistency between biomass and Chl a content supports the interpretation that pH, rather than CO₂ concentration alone, was a principal factor contributing to the observed community dynamics in this experimental system.

Moreover, ammonia nitrogen and nitrate nitrogen in overlying water of set a and set c were significantly higher than that in set b. This pattern provides empirical evidence consistent with the competitive advantage of Diatoms under high dissolved inorganic nitrogen regimes, in line with the well-documented niche partitioning characteristics reported for phytoplankton^[27]. Notably, this study observed a highly consistent co-variation between water column Mg^{2+} concentration and Diatom biomass, following the gradient of set c>set b>set a,. This positive correlation differs from the field investigation in the Lijiang River^[28], which documented a significant negative relationship between Mg^{2+} availability and Diatom biomass. In-depth mechanistic attribution suggests that this discrepancy arises from two non-mutually exclusive drivers. First, inherent differences in background water quality properties between the two study systems may fundamentally alter the physiological pathways through which Mg^{2+} mediates Diatom growth and metabolic activity. Second, the inter-group CO_2 concentration gradient established in the present experiment represents a potentially important modulating variable that was not explicitly controlled in the Lijiang River field survey. This contrast suggests a potentially important regulatory role of CO_2 availability in modulating the interaction dynamics between aquatic autotrophs and their geochemical environment. From the perspective of nutrient stoichiometry in the sediment, the significantly lower C/N ratio in set c relative to set a and set b indicates a state of relative nitrogen surplus, which may elevate the potential risk of nitrogen release and subsequent water column eutrophication. Furthermore, Pearson Correlation Analysis showed a statistically significant negative correlation between sediment C/H and C/N ratios ($P<0.05$). This co-variation pattern suggests that, along the gradient of increasing CO_2 concentration, organic compounds undergo directional molecular modification in the sediment: enhanced hydrogenation reactions increase the saturation of organic molecular backbones, while the concurrent reduction of nitrogen functional group abundance further elevates the chemical recalcitrance of sedimentary organic matter. This coupled structural stabilization process may contribute to the long-term stabilization of sedimentary organic carbon and to carbon sequestration in this system.

Beyond its direct biological effects, elevated CO_2 was also associated with a significant redistribution of key metal elements at the water-sediment interface, which may in turn influence broader aspects of aquatic ecological security. Pearson Correlation Analysis revealed that Chl-a content was strongly and positively correlated with sedimentary Cu and Mn ($P<0.01$). As essential trace elements, Cu and Mn are vital for biological growth, yet their excessive accumulation in the water column can induce metal toxicity and compromise ecosystem stability^[29, 30]. In the present study, the total Cu content in sediments of set a and set b increased significantly compared to set c, with a concurrent and marked increase in the Cu-P fraction. This sequestration of Cu into the sediment phase likely attenuated its potential adverse effects on water safety, a finding consistent with prior observations^[26]. Conversely, the Cr content exhibited a decreasing trend with rising CO_2 , and set a showed the lowest Cr-P content, suggesting a potential risk of chromium remobilization under acidic conditions. Accordingly, this study observed a non-monotonic transformation phenomenon where Fe/Al-P surpassed Ca-P in abundance under moderate CO_2 partial pressure conditions, becoming the dominant phosphorus binding phase. This result differs from the conclusions of previous research, which focused on extreme leakage scenarios in nearshore marine carbon sequestration^[29]. Their system's CO_2 partial pressure far exceeded the natural fluctuation thresholds of aquatic environments, leading to irreversible dissolution of Fe/Al oxides under strongly acidic conditions, thus preventing structural reorganization of phosphorus forms. In contrast, the gradient settings of this study precisely align with the CO_2 concentration rise in the Yi Luo River Basin. The corresponding pH range falls

242 within the sensitive coupling zone for both Fe/Al-P and Ca-P. This operational difference may account for the divergent
243 phosphorus cycling response patterns observed between the two research settings. Furthermore, the findings of this
244 study suggest a possible extension of the classical early phosphorus cycle model^[30]. While the traditional framework
245 posits Ca-P as the overwhelmingly dominant form in weakly alkaline sediments, it does not account for the competitive
246 overtaking by Fe/Al-P under moderate, progressive acidification. The results indicate that, under moderate CO₂
247 elevation, the interfacial adsorption capacity of Fe/Al oxides may override the ionic competitive advantage of Ca²⁺
248 through coupled geochemical processes. This may extend the applicability of the classical model from marine systems
249 toward the alluvial sediment systems characteristic of the middle and lower reaches of the Yellow River. The observed
250 “nonlinear response of Fe-C-P dynamics to aquatic acidification” is broadly consistent with patterns reported in other
251 aquatic systems. It aligns with the theoretical framework for deep-sea sediments^[31] and resonates with the “CO₂
252 elevation regulates phosphorus bioavailability” mechanism identified in watershed-scale rice paddy ecosystems^[32].
253 Together, these parallels help refine the theoretical understanding of phosphorus cycling responses to increasing
254 atmospheric carbon at the water-sediment interface.

255 In summary, the results of this study indicate that, under the experimental conditions applied, moderate CO₂
256 enrichment, accompanied by aquatic acidification, induced multifaceted ecological responses in the studied riverine
257 system. Key outcomes include a restructuring of phytoplankton assemblages driven by altered N and Mg²⁺
258 stoichiometry, a transition in dominant sedimentary phosphorus speciation from Ca-bound to Fe/Al-bound phases, and
259 the chemical stabilization of sedimentary organic carbon via enhanced aliphaticization and N-depletion. These findings
260 collectively indicate that the response of this experimental system to rising CO₂ was governed by the interplay between
261 direct biotic effects and indirect, geochemically mediated feedback loops, rather than by a simple fertilization effect.

262 Nevertheless, this study has several limitations that should be noted when interpreting the results. First, the water
263 and sediment samples used in this experiment were collected only from the confluence section of Yiluo River, which
264 cannot fully represent the spatial heterogeneity of CO₂ response patterns across different tributaries, upper reaches and
265 downstream sections of the watershed. Future studies could expand the spatial coverage of sampling points to verify the
266 universality of the observed multi-element coupling mechanisms across the entire basin. Second, the 20-day
267 experimental duration captured the short-term biogeochemical responses of the water-sediment interface, but long-term
268 cumulative effects and ecosystem adaptive evolution over seasonal and annual time scales were not addressed.
269 Longer-term positioning experiments and field in-situ monitoring are needed to validate the persistence of the
270 phosphorus speciation transformation and phytoplankton community shifts observed in this study. Third, the present
271 conclusions are drawn from a controlled laboratory microcosm experiment with three replicates per treatment, in which
272 CO₂-enriched gas was supplied continuously under fixed temperature, light and hydrodynamic conditions. Such a
273 simplified system inevitably differs from the complex hydrodynamic, light and temperature regimes of the natural Yiluo
274 River, and the absolute magnitude of the observed responses should therefore be extrapolated to field conditions with
275 caution.

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350 Data Availability

351 All data generated or analysed during this study are included in this published article and its supplementary
352 information files.

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