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PERSPECTIVE

Rethinking Photosynthesis: Intracellular Water Dynamics, Bicarbonate Photolysis, and Electrophysiological Coupling in Inorganic Carbon Assimilation

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Abstract

Photosynthesis is the fundamental biochemical process driving Earth's biogeochemical cycles. The traditional theory holds that water is the sole source of photosynthetic oxygen and atmospheric CO_2 the only inorganic carbon substrate. However, electrophysiological, isotopic, and physiological evidence from our systematic research prompts a re-examination of this paradigm, revealing an unrecognized coupling between plant inorganic carbon assimilation and intracellular water utilization. Our key findings are: (i) Intracellular water utilization rate is decoupled from atmospheric CO_2 assimilation, suggesting that terrestrial plants assimilate inorganic carbon from atmospheric CO_2, soil inorganic carbon, and internally recycled inorganic carbon derived from metabolic carbon turnover. (ii) Electrophysiologically quantified intracellular water utilization, reflecting photosynthetic water photolysis through electron transfer dynamics, may serve as a broader indicator of total photosynthetic capacity than leaf CO_2 flux. (iii) Bicarbonate photolysis—the first step of inorganic carbon assimilation—may contribute equally to photosynthetic oxygen evolution as water photolysis, forming a coupled chain reaction that minimizes diffusion and concentration losses to enhance efficiency. (iv) Plant growth and adaptation are jointly driven by chemical energy (ATP) and internal electrical energy stored in cellular electrical components, with energy allocation prioritizing stress responses under abiotic stressors. We propose a revised photosynthesis framework integrating bicarbonate photolysis, intracellular water–electrophysiology coupling, and multi-source inorganic carbon assimilation. This perspective highlights the theoretical significance of updating classical photosynthesis theory, its applied value for improving crop photosynthetic efficiency and stress tolerance, and outlines future directions to dissect the underlying molecular-electrophysiological mechanisms and their biotechnological translation.

Keywords: photosynthesis, bicarbonate photolysis, intracellular water dynamics, plant electrophysiology,

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inorganic carbon assimilation, energy allocation.

1 Introduction

Photosynthesis represents the only biological process capable of converting light energy into chemical energy, sustaining nearly all terrestrial and aquatic ecosystems and governing global cycles of carbon, oxygen, and other essential elements [1, 2]. For over eight decades, the classical photosynthesis model has dominated the field: photosynthetic oxygen evolution is exclusively derived from water photolysis, and the oxygen in photosynthetically fixed organic compounds originates solely from atmospheric CO_2, which is assumed to be the sole inorganic carbon source for carbon assimilation [3]. This model has served as the cornerstone of research in plant physiology, biochemistry, and ecology, and is universally presented in textbooks and scientific literature. Nevertheless, this traditional paradigm is increasingly confronted with unresolved contradictions and emerging experimental evidence. Initially, classical isotope-labeling experiments [3, 4], which corroborated the conclusion that "water serving as the exclusive oxygen source", failed to account for the rapid oxygen-isotope exchange between bicarbonate and water in chloroplasts, catalyzed by carbonic anhydrase (CA) (or PS II-like CA) [5, 6]. This oversight may have led to misinterpretation of the experimental outcomes. Secondly, measurements of leaf CO_2 flux, regarded as the gold-standard metric for evaluating photosynthetic rate, often fail to align with intracellular water-use rate in plants exposed to abiotic stresses such as drought, salinity, and bicarbonate stress (See Supplementary File). Although this inconsistency does not, by itself, establish the existence of multiple inorganic carbon sources, it suggests that conventional leaf CO_2 flux measurements may not fully reflect the total inorganic carbon assimilation processes considered in our framework. In this Perspective, the term intracellular water use rate refers to the rate of biologically active intracellular water utilization associated with photosynthesis-related processes, particularly those linked to electron-transfer-driven water photolysis, as inferred from plant electrophysiological measurements. It does not denote transpiration rate, bulk leaf water loss, or total tissue water content. Rather, it is intended to describe an intracellular functional component of water utilization that, in our framework, is associated with photosynthetic activity and inorganic carbon assimilation. Accordingly, it should be distinguished from conventional gas-exchange parameters such as leaf CO_2 flux, which primarily quantify atmospheric CO_2 assimilation rather than the broader intracellular water-linked processes considered here. This phenomenon is not readily accommodated within the classical water-only interpretation of photosynthetic oxygen evolution. Several independent observations are consistent with this view. In addition to our own studies, several independent lines of work have shown that bicarbonate has functionally important roles in photosynthetic systems, particularly in relation to Photosystem II (PSII). Classical experiments demonstrated bicarbonate-dependent stimulation of PSII-linked electron transport and oxygen evolution, indicating that bicarbonate can influence early photochemical events in PSII [7]. Subsequent biochemical and mechanistic studies further supported bicarbonate-related function on the acceptor side of PSII and also provided evidence consistent with a role near the water-oxidizing complex. In *Chlamydomonas reinhardtii*, studies on the PSII-associated carbonic anhydrase CAH3 further showed that carbonic-anhydrase-dependent bicarbonate handling can affect oxygen-evolution efficiency and facilitate proton removal on the donor side of PSII. At the same time, these studies do not by themselves establish the dual-substrate framework proposed here; rather, they provide a broader mechanistic context within which our interpretation may be considered [8]. First, the well-known bicarbonate effect, manifested as a marked stimulation of Hill reaction oxygen evolution following bicarbonate addition, indicates that bicarbonate plays a functionally important role in the oxygen-evolving system. Second, the lower thermodynamic free-energy requirement reported for bicarbonate photolysis relative to water photolysis suggests that bicarbonate-based oxygen release may be energetically feasible under appropriate physicochemical conditions [8]. Third, the Dole effect, in which atmospheric O_2 is enriched in ^{18}O relative to seawater by approximately 24‰, with the currently observed value being 23.56‰, has also been considered relevant to the interpretation of the isotopic origin and evolution of photosynthetic oxygen [10, 11]. Finally, electrophysiological studies indicating that plant cells function as bioelectrical systems, with measurable electrical properties such as resistance, capacitance, and inductive behavior, raise the possibility that internally organized electrical processes may contribute to reaction pathways not fully captured by the conventional framework (See

Supplementary File). In this Perspective, we refer to this component as internal electrical energy, defined as an electro-physiologically inferred bioelectrical energy state associated with these measurable cellular electrical properties. This term is not intended to denote membrane potential alone, but rather a broader electrical state of the cell that may interact with biochemical energy metabolism, including ATP-dependent processes, in the regulation of photosynthesis, growth, and stress adaptation. This internal energy may participate in, or modulate, processes associated with bicarbonate and water electrolysis, leading to the release of oxygen and the subsequent assimilation of inorganic carbon [2]. On the other hand, it can be integrated with the chemical energy originating from ATP to support plant growth and stress adaptation.

Nevertheless, the functional correlation between this energy (electrical + chemical) pool and photosynthetic carbon-intracellular water metabolism is still inadequately characterized. Finally, our recent research directly investigated the interaction between bicarbonate and water in photosynthetic oxygen evolution and its implications for carbon neutrality. It offers useful insights into how a possible dual-substrate framework could be related to broader carbon cycling [1, 6]. At present, however, such broader ecological implications should be understood as conceptual extrapolations from organismal- and process-level observations, rather than as directly demonstrated ecosystem-scale conclusions. Over the past decade, our research group has addressed these critical knowledge gaps through a series of integrated studies that combine plant electrophysiology, physiological ecology, isotopic tracing, and molecular biology. Our work has systematically characterized the dynamics of plant intracellular water under abiotic stress, revealed the multisource nature of plant inorganic carbon assimilation, provided isotopic evidence for bicarbonate photolysis, and established the coupling relationships among plant electrical energy, chemical energy, and photosynthetic growth. In this Perspective, we synthesize the core findings of our systematic research, propose an integrated framework for photosynthesis, and outline future research directions aimed not only at extending this framework but also at testing several of its unresolved mechanistic aspects, including bicarbonate participation in oxygen evolution, the role of internal electrical energy, and the broader ecological significance of the proposed karst-photosynthesis coupling framework.

2 Summary of Our Previous Work

Our research has focused on resolving the discrepancies between classical photosynthesis theory and experimental observations. The core of this research lies in intracellular water dynamics, the sources of inorganic carbon assimilation, the role of bicarbonate in photosynthetic oxygen evolution, and the allocation of plant internal electrical energy, as inferred from electrophysiological parameters. Subsequently, we synthesize the key findings and conclusions as follows.

2.1 Decoupling between intracellular water use rate and atmospheric CO_2 assimilation is consistent with a multi-source inorganic carbon assimilation framework in terrestrial plants

We systematically measured leaf CO_2 assimilation (photosynthetic mode) and intracellular water-use rate, an electro-physiologically derived indicator of photosynthesis-related intracellular water utilization, across diverse plant species (including maize, rice, mangroves, tea plants, and medicinal herbs) under drought, salinity, bicarbonate, and heavy-metal stress (Table 1). While a small subset of cases exhibited matching patterns between the two parameters, the majority showed significant decoupling (See Supplementary File): for instance, Aegiceras corniculatum under salt stress displayed a photosynthetic mode of T1 (medium)–T2 (high)–T3 (low) but an intracellular water use rate of T1 (high)-T2 (medium)-T3 (low) [10]; Bletilla striata under bicarbonate stress exhibited a photosynthetic mode of CK (high)-T1 (medium)-T2 (low)-T3 (lowest) but an intracellular water use rate of CK (high)-T1 (high)-T2 (medium)-T3 (low).

This recurrent decoupling suggests that atmospheric CO_2 flux alone may not fully capture the total inorganic carbon assimilated by terrestrial plants. In our interpretation, this physiological inconsistency is consistent with the possibility that additional inorganic carbon sources contribute to photosynthesis under some conditions. This interpretation is further supported by our previous studies, which indicate that terrestrial plants may also assimilate inorganic carbon from soil bicarbonate and from internally recycled inorganic carbon derived from previously fixed organic carbon (See Supplementary File). Here, “internally recycled inorganic carbon” refers to inorganic carbon regenerated through

Table 1. Plant atmospheric CO_2 assimilation versus intracellular water use rate in plants.

Materials	Treatments	Atmospheric CO_2 assimilation	Intracellular water use rate
Zea mays L. var. ceratina	CK(Control, 75% field capacity); T1(moderate drought, 55%); T2 (severe drought, 35%) over 9 or 18 d.	CK (high) - T1 (moderate) - T2 (low)	CK (high)- T1 (moderate) - T2 (low)
Rice seedlings (Yixiangyou 876)	CK (Se^{4+},0 μM: Cd^{2+},0 μM: Si^{4+},0 μM); T1(Se^{4+},8 μM: Cd^{2+},6 μM: Si^{4+},0 μM); T2(Se^{4+},8 μM: Cd^{2+},6 μM: Si^{4+},10 μM)	CK (low) - T1 (moderate) -T2 (high)	CK (low) - T1 (moderate) - T2 (high)
Aegiceras corniculatum	T1 (0.1 M NaCl + 2 h); T2 (0.2 M NaCl + 4 h);T3 (0.4 M NaCl + 6 h)	T1 (moderate) - T2 (high) - T3 (low)	T1 (high) - T2 (moderate) - T3 (low)
Bletilla striata	CK (0 mM $NaHCO_3$); T1 (5mM $NaHCO_3$); T2 (10mM $NaHCO_3$); T3 (15mM $NaHCO_3$)	CK (high) - T1 (moderate) - T2 (low) - T3 (lowest)	CK (high) -T1 (high) -T2 (moderate) - T3 (low)
Zea mays L. var. Jingke 968	CK (0 mM $NaHCO_3$); T1(5mM $NaHCO_3$); T2 (10mM $NaHCO_3$)	CK (high) - T1(high) - T2 (low)	CK(moderate) - T1 (moderate) -T2 (moderate)*
Zea mays L. var. Tiannuo 182	CK (0 mM $NaHCO_3$); T1 (5mM $NaHCO_3$); T2 (10mM $NaHCO_3$)	CK (low) - T1 (low) - T2 (low)	CK (moderate) - T1 (high) - T2 (low)*
Cardamine violifolia	CK (0 mM HCO_3^-, 0 mM Se^{6+}, 0.27 mM Cd^{2+}); T1 (1 mM HCO_3^-, 0.46 mM Se^{6+}, 0.27 mM Cd^{2+}); T2 (15 mM HCO_3^-, 0.46 mM Se^{6+}, 0.27 mM Cd^{2+})	CK (low) - T1 (high) - T2 (low)	CK (low) - T1 (high) - T2 (low)
Rhizophora stylosa	T1 (0.1 M NaCl+4 h); T2 (0.2 M NaCl+4 h); T3 (0.4 M NaCl+4h)	T1 (low) - T2 (low) - T3 (low)	T1(low) - T2(low) - T3 (moderate)
Kandelia candel	T1 (0.1 M NaCl+4 h); T2 (0.2 M NaCl+4 h); T3 (0.4 M NaCl+4h)	T1 (moderate) - T2 (high) - T3 (low)	T1 (moderate) - T2 (high) - T3 (high)
Aegiceras corniculatum	T1 (0.1 M NaCl+4 h); T2 (0.2 M NaCl+4 h); T3 (0.4 M NaCl+4h)	T1 (moderate) - T2 (moderate) - T3 (low)	T1(low)-T2(low)-T3(low)
Brassica napus	T1 (125μM $H_2PO_4^-$); T2 (34μM $H_2PO_4^-$)	T1 (high) - T2 (low)	T1 (high) - T2 (low)
Tea plant seedlings Var. Zhonghuang No.2	D0 (soil moisture content 40%~45%); D5 (D0 without watering for 5 d); D10(D0 without watering for 10 d)	D0 (moderate) - D5 (moderate) -D10 (moderate)	D0 (high) - D5 (low) - D10 (low)
Tea plant seedlings Var. Wuniuzao	D0 (soil moisture content 40%~45%); D5 (D0 without watering for 5 d); D10(D0 without watering for 10 d)	D0(moderate) - D5(low) - D10(moderate)	D0(moderate) - D5(moderate) - D10(moderate)
Tea plant seedlings Var. Longjing 43	D0 (soil moisture content 40%~45%); D5 (D0 without watering for 5 d); D10(D0 without watering for 10 d)	D0 (low) -D5 (low) - D10 (low)	D0 (high) - D5 (moderate) - D10 (low)
Coix lacryma-jobi Var. Yizhu 1	CK (0 mM $NaHCO_3$); T1 (2mM $NaHCO_3$); T2 (7mM $NaHCO_3$); T3 (12 mM $NaHCO_3$)	CK (moderate) - T1(high) - T2 (Medium-low) - T3 (low)	CK (low) -T1 (high) - T2 (low) - T3 (low)
Morus alba	T1 (Soil moisture content: 21.54%); T2 (Soil moisture content:7.90%)	T1 (high) - T2 (low)	T1 (high) - T2 (high)
Broussonetia papyrifera	T1 (Soil moisture content: 21.54%); T2 (Soil moisture content:7.90%)	T1 (high)- T2 (low)	T1 (high) - T2(low)

Note: Data were obtained from our group's previous experiments and reanalyzed for the present study. Raw data are available upon request.

**Unpublished data.*

metabolic carbon turnover within the plant system and potentially available for reassimilation, rather than to an external environmental carbon source. Thus, the lack of coordination between atmospheric CO_2 assimilation and intracellular water use rate is considered here as supportive evidence within a broader body of observations, rather than as a standalone demonstration. Intracellular water

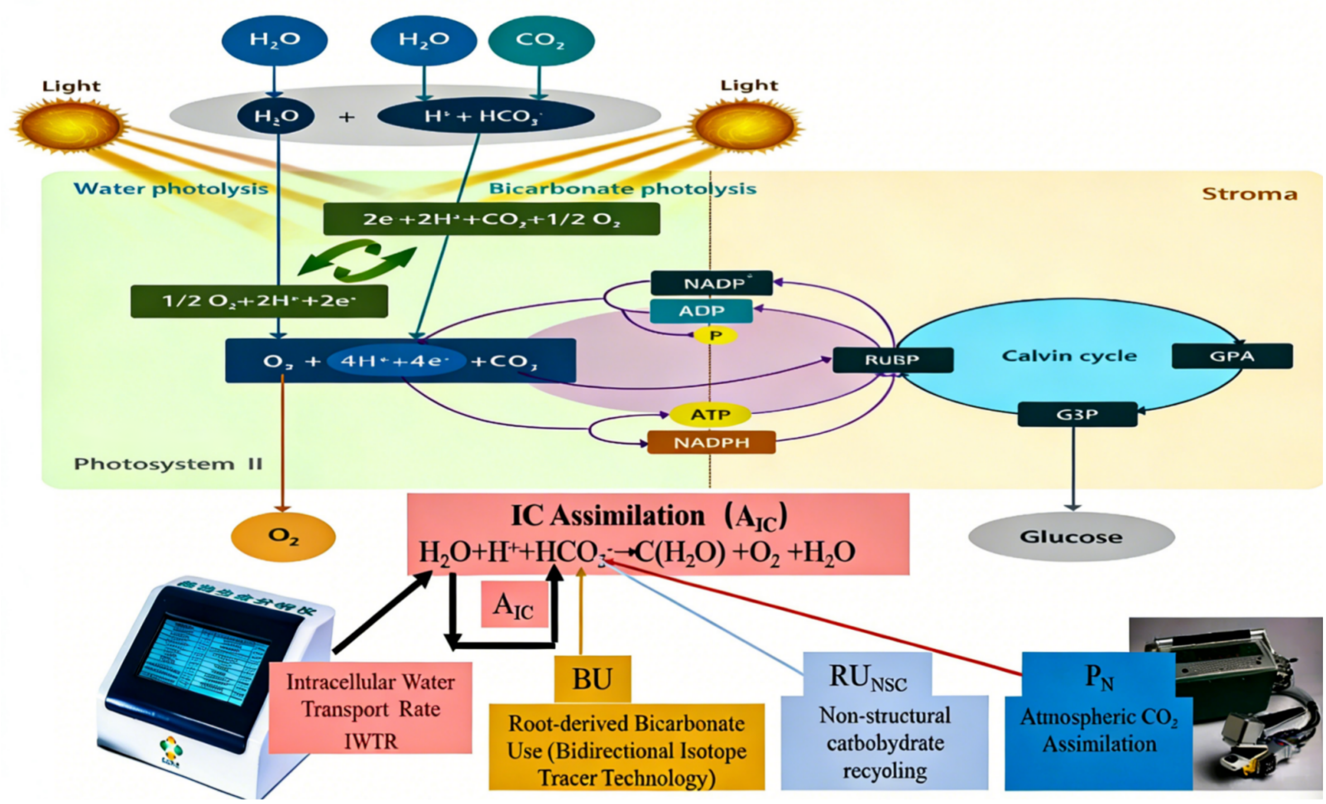

Figure 1. Coupling relationship between intracellular water transport and photosynthetic inorganic carbon assimilation in leaves (Note: Bicarbonate photolysis is the first step in inorganic carbon assimilation. The stoichiometric relationship between water photolysis and bicarbonate photolysis in photosynthetic oxygen evolution is 1:1. The assimilation of photosynthetic inorganic carbon includes atmospheric carbon dioxide assimilation (P_N), root-derived bicarbonate (inorganic carbon) use (BU), and recycling of non-structural carbohydrates (RU_{NSC}))[14–16].

photolysis, which is reflected by the intracellular water use rate, supports total inorganic carbon assimilation (atmospheric + soil + recycled), while leaf CO_2 flux only quantifies atmospheric CO_2 assimilation.

2.2 Electrophysiological measurement of intracellular water utilization is a comprehensive indicator of total photosynthetic capacity

Our electrophysiological observations are consistent with the view that, beyond its role as a solvent, intracellular water is closely linked to photosynthesis-related processes, particularly those, in our framework, associated with electron-transfer-driven water photolysis. We therefore interpret the intracellular water use rate derived from plant electrophysiological measurements as an indicator of photosynthesis-related intracellular water utilization, especially the electron-transfer-linked component associated with water photolysis. In this Perspective, this interpretation is used as a working framework rather than as a universally established conclusion. Within this framework, the intracellular water use rate may provide complementary information to leaf CO_2 flux and serve as a broader indicator of total photosynthetic activity. By contrast, the conventional measurement of leaf CO_2 flux primarily reflects atmospheric CO_2 assimilation and therefore captures only one component of the total inorganic carbon assimilation considered in our model (Figure 1).

2.3 Isotopic and thermodynamic observations consistent with a possible contribution of bicarbonate photolysis to photosynthetic oxygen evolution

We re-evaluated classical ^{18}O isotope-labeling experiments and found that the rapid CA (PS II-like CA)-catalyzed oxygen-isotope exchange between bicarbonate and water in chloroplasts [1, 6] masked the actual contribution of bicarbonate to oxygen evolution. Specifically, labeled bicarbonate ^{18}O was rapidly exchanged with water ^{16}O, leading to the misinterpretation that oxygen evolution was derived solely from water. More broadly, independent studies across other photosynthetic systems have shown that bicarbonate-related processes can affect PSII function, chloroplast carbon handling, and inorganic-carbon acquisition, supporting a broader mechanistic discussion, even though they do not directly validate the full interpretation advanced in this Perspective [12, 13]. Our novel dual-element (C/O) bidirectional isotope-tracing culture technology directly suggested that bicarbonate carbon is incorporated into photosynthetic products in microalgae and that bicarbonate-derived oxygen contributes to photosynthetic oxygen evolution. Thermodynamically, bicarbonate photolysis ($H^+ + HCO_3^- \rightarrow 1/2O_2 + 2e^- + 2H^+ + CO_2$) requires a standard free energy of 24.8 kcal/mol, which is significantly lower than the 37.3 kcal/mol required for water photolysis [9], rendering it a thermodynamically favorable reaction. Based on these findings, we further proposed a dual-substrate (bicarbonate/water) model of photosynthetic oxygen evolution, which predicts a 1:1 molar stoichiometry between bicarbonate and water photolysis, consistent with geological evidence (the Dole effect) and the global carbon-water balance [1, 2, 6]. Unlike the classical stepwise parallel reactions of water photolysis and CO_2 assimilation, bicarbonate photolysis generates CO_2 in situ, which can immediately enter the Calvin cycle, thereby forming a coupled chain reaction without diffusion, delay, or concentration loss and resulting in substantially higher quantum efficiency, carbon-assimilation efficiency, and energy-use efficiency [1, 2, 6]. This dual-substrate mechanism not only enhances photosynthetic performance but also plays a crucial role in global carbon neutrality by promoting efficient inorganic carbon sequestration [1, 2, 6]. This coupled chain reaction inherently establishes a strong coordinative relationship between the intracellular water transport (use) rate in leaves and the photosynthetic inorganic carbon assimilation rate, as shown in Figure 1. Simultaneously, the photolysis of bicarbonate, as the initial stage of inorganic carbon assimilation, might also be propelled by internal electrical energy [2].

2.4 Plant growth and adaptation are fueled by a dual energy system of chemical energy (ATP) and internal electrical energy

Electrophysiological investigations across diverse plant species exposed to drought, salinity, and bicarbonate stress (See Supplementary File) suggest that plant cells maintain an internal electrical energy state, reflected in measurable cellular electrical properties, including resistance, capacitance, and inductive behavior, which can be quantitatively characterized using electrophysiological parameters. In our framework, this internal electrical energy is considered alongside ATP-derived chemical energy as a potential contributor to plant growth and environmental adaptation. Previous studies have demonstrated consistent growth patterns linked to these two energy forms. Maize (*Zea mays* var. *ceratina*) displayed vigorous growth under conditions characterized by high atmospheric inorganic carbon assimilation and high internal electrical energy, but slow growth under low atmospheric inorganic carbon assimilation and low internal electrical energy; a similar trend was observed in paper mulberry (*Broussonetia papyrifera*). In the mangrove species *Aegiceras corniculatum*, rapid growth occurred under high internal electrical energy coupled with moderate atmospheric inorganic carbon assimilation. In contrast, moderate growth was supported by high atmospheric inorganic carbon assimilation and low internal electrical energy, whereas slow growth corresponded to low values of both parameters. In *Bletilla striata* subjected to T1 treatment, despite only moderate atmospheric inorganic carbon assimilation, rapid growth was still achieved, consistent with its high internal electrical energy. A separate study on two maize cultivars showed that growth in 'Jingke 968' was driven by the combined effects of atmospheric inorganic carbon assimilation and internal electrical energy. In contrast, growth in ' Tiannuo 182 ' was governed by adaptive traits that were also modulated by these two energy components.

Furthermore, under abiotic stresses such as high salinity and waterlogging, plants undergo a pronounced shift in energy allocation: energy invested in stress responses increases significantly, whereas that allocated to growth decreases, reflecting a trade-off strategy that sacrifices growth potential to enhance stress tolerance (See Supplementary File). We have further established that electro-physiologically based water-salt transport dynamics are closely coupled with the expression of key salt-transport genes, including *SOS1*, *NHX1*, and *VHAc1*, supporting the use of electrophysiological parameters as real-time, non-destructive indicators of plant stress adaptation (See Supplementary File). For instance, in *Aegiceras corniculatum*, high salinity downregulates *SOS1* (reducing Na^+ efflux) and upregulates *VHAc1* (enhancing vacuolar H^+ pumping), thereby compromising vacuolar Na^+ sequestration. These molecular alterations are precisely mirrored by changes in electrophysiological parameters, including reduced active salt transport rate and elevated passive salt transport rate (See Supplementary File).

2.5 Intracellular water dynamics are tightly coupled with inorganic carbon assimilation and plant growth patterns

By comparing plant growth modes, intracellular water use rates, and internal electrical energy patterns inferred from electrophysiological parameters (Table 1), we found that plant growth modes were largely congruent with intracellular water use rate modes rather than with atmospheric CO_2 assimilation modes alone. For example, *Broussonetia papyrifera* under water stress showed reduced leaf growth accompanied by a synchronous decrease in intracellular water use rate, whereas *Morus alba* adopted a "protect the core" strategy, reducing leaf number while maintaining intracellular water use rate and normal growth (See Supplementary File). This coupling is consistent with the interpretation that intracellular water dynamics may be closely linked to plant growth through their relationship with total inorganic carbon assimilation. In the framework proposed here, intracellular water use is therefore treated as a candidate integrative process rather than as a definitively established sole driver of growth. On this basis, we proposed a coupling model of leaf intracellular water use and photosynthetic inorganic carbon assimilation (Figure 1), in which intracellular water photolysis is functionally associated with multi-source inorganic carbon assimilation and, consequently, with plant growth. This model is intended as an interpretative framework for integrating the observed relationships among intracellular water dynamics, inorganic carbon assimilation, and growth patterns, and it remains to be further tested mechanistically.

3 Rethinking & elevation

Our systematic investigation has addressed several apparent inconsistencies within the classical photosynthesis framework and suggests reconsidering aspects of photosynthetic oxygen evolution, inorganic carbon assimilation, and plant energy metabolism. Our work directly challenges two core tenets of classical photosynthesis theory. Subsequently, we elevate our findings by considering their theoretical significance, application value, and conceptual refinement to develop a novel, comprehensive understanding of photosynthesis.

3.1 Theoretical Significance: Updating the Classical Photosynthesis Model and Redefining Core Processes

Our work raises questions regarding two central assumptions in classical photosynthesis theory: (1) water is the sole source of photosynthetic oxygen; we provide observations that we interpret as being consistent with the hypothesis that bicarbonate photolysis may contribute to oxygen evolution alongside water photolysis within the framework proposed here; (2) atmospheric CO_2 is the only inorganic carbon source for terrestrial plants, whereas our previous findings suggest that terrestrial plants may assimilate inorganic carbon from multiple sources, including atmospheric CO_2, soil inorganic carbon, and recycled inorganic carbon. We thus propose a revised photosynthesis framework [1, 6] (Figure 1) that integrates:

- **Dual-substrate oxygen evolution:** We propose that bicarbonate- and water-associated photolytic processes may operate in a coupled manner, potentially approaching a 1:1 relationship within the framework of our model, with bicarbonate photolysis potentially contributing to a more tightly coupled carbon-assimilation sequence.
- **Multi-source inorganic carbon assimilation:** Total photosynthetic carbon assimilation = atmospheric CO_2 assimilation + soil inorganic carbon assimilation + recycled inorganic carbon assimilation.
- **Electrophysiological coupling:** Intracellular water dynamics (as reflected in electrophysiological parameters) serve as the core link between water/bicarbonate photolysis and multi-source inorganic carbon assimilation, with electrophysiological measurements providing a comprehensive indicator of total photosynthetic capacity.
- **Dual energy system:** Photosynthesis produces both ATP (chemical energy) and the electron transport system, which generates internal energy (stored in cellular electrical components), both of which jointly regulate plant growth and stress adaptation.

This revised framework is intended as an interpretative model that may help explain several long-standing inconsistencies in the field, including the decoupling of CO_2 assimilation and intracellular water-use rate, and the broader discussion surrounding isotopic observations such as the Dole effect. Rather than being presented as a definitive replacement of the classical model, it is offered here as a broader conceptual framework for re-examining the relationships among photosynthetic oxygen evolution, inorganic carbon assimilation, and plant bioelectrical function. In addition, our work suggests that bicarbonate may play a broader role in photosynthesis than simply serving as a CO_2 source via dissociation ($H^+ + HCO_3^- \rightarrow CO_2 + H_2O$). Within the framework proposed here, bicarbonate is considered a possible participant in both oxygen-evolution-associated and carbon-assimilation-related processes, supporting a potentially important conceptual shift in how bicarbonate function is considered in photosynthesis research.

3.2 Application Value: New Strategies for Improving Crop Photosynthetic Efficiency and Stress Tolerance

Our findings have profound practical implications for agricultural biotechnology, crop breeding, and ecological restoration, providing novel targets and strategies for improving plant photosynthetic efficiency and stress tolerance:

- **Improving photosynthetic efficiency:** The coupled chain reaction of bicarbonate photolysis and carbon assimilation exhibits a significantly higher energy-use efficiency than the classical stepwise reaction. Engineering crops to enhance bicarbonate uptake, transport, and photolysis capacity (e.g., overexpressing bicarbonate transporters, chloroplast CA, or photosystem II (PSII) bicarbonate-binding proteins) can directly improve photosynthetic quantum efficiency and carbon assimilation efficiency; this is a new direction for high-yield crop breeding, especially for crops grown in alkaline/karst soils (rich in bicarbonate).
- **Enhancing abiotic stress tolerance:** Our discovery of the plant dual energy system (chemical + electrical) and stress-induced energy reallocation provides a new target for improving stress tolerance. Modulating plant electrophysiological parameters (e.g., enhancing cellular internal electrical energy storage) or engineering energy allocation pathways to maintain growth under stress can improve crop tolerance to drought, salinity, and bicarbonate stress. This is particularly valuable for agricultural production on marginal lands (e.g., saline-alkali soils).
- **Non-destructive and rapid monitoring of plant photosynthesis and stress status:** Plant electrophysiological parameters that characterize intracellular water dynamics and inorganic carbon assimilation can serve as real-time, non-destructive biomarkers of total photosynthetic capacity and stress adaptation. Developing more portable electrophysiological sensors based on these parameters can enable precision agriculture (e.g., real-time monitoring of crop photosynthetic status and targeted irrigation/fertilization) and ecological monitoring (e.g., assessing mangrove salt-stress adaptation).
- **Optimizing microalgal biofuel production:** Microalgae efficiently utilize bicarbonate for photosynthesis, with a photosynthetic efficiency far higher than that of terrestrial plants. Our dual-element isotope tracing technology and insights into bicarbonate utilization mechanisms can guide the optimization of microalgal culture conditions (e.g., adjusting bicarbonate concentration) to improve biofuel production efficiency.

3.3 Conceptual sublimation: proposing the "karst-photosynthesis coupling" mechanism and redefining photosynthesis as a driver of biogeochemical cycles

Our work extends beyond plant physiology and suggests a possible karst-photosynthesis coupling framework through which organismal observations may be connected to broader ecological and geochemical implications. At present, however, this linkage should be understood as a conceptual extension of plant-level physiological and isotopic findings, rather than as a directly demonstrated ecosystem-scale mechanism. Accordingly, the ecosystem-scale significance of this proposed framework is treated below as a future research priority requiring field validation and integrated biogeochemical modeling. Bicarbonate (the core substrate of karst processes) acts as a direct substrate for photosynthetic oxygen evolution and carbon assimilation; bicarbonate photolysis produces CO_2 that is immediately fixed by the Calvin cycle, while karst processes generate bicarbonate from carbonate rock dissolution ($CaCO_3+CO_2+H_2O \rightarrow Ca_2^+ + 2HCO_3^-$). This forms a closed loop: karst processes supply bicarbonate to photosynthesis, and photosynthesis consumes bicarbonate to drive carbon sequestration and oxygen evolution, coupling the terrestrial carbon cycle (karst processes) with the biological carbon cycle (photosynthesis). This proposed coupling may provide a useful conceptual link among the lithosphere, hydrosphere, atmosphere, and biosphere, and may contribute to carbon-water interactions across these spheres. It may have broader implications for carbon-water interactions and biogeochemical cycling, although these larger-scale consequences remain to be validated beyond the plant and laboratory scales considered here [1]. Furthermore, it is posited that bicarbonate photolysis represents an ancestral photosynthetic pathway. Considering its relatively lower thermodynamic free - energy requirement in comparison to water photolysis, bicarbonate photolysis might have evolved earlier than water photolysis in primitive photosynthetic organisms (such as cyanobacteria). Subsequently, as atmospheric concentrations of CO_2 and bicarbonate decreased, water photolysis became evident. This hypothesis offers a novel perspective for investigating the evolutionary origin of oxygenic photosynthesis, a crucial event in Earth's evolution. Finally, our work supports the view that photosynthesis may also be usefully considered from a bio-electrochemical perspective (rather than a purely biochemical process): plant cells function as bioelectrical units, and photosynthetic energy conversion involves both chemical energy (ATP) and internal electrical energy, with electrophysiological dynamics mediating the coupling of water/bicarbonate photolysis and inorganic carbon assimilation. This bio-electrochemical perspective expands the scope of photosynthesis research, bridging plant electrophysiology and photosynthesis biology, a new interdisciplinary research direction.

4 Future directions & perspectives

Although the framework advanced here integrates several observations from electrophysiological, isotopic, and physiological studies, several important uncertainties remain. In particular, the precise molecular basis of bicarbonate participation in photosynthetic oxygen evolution, the extent to which electrophysiological parameters reflect causal rather than correlative aspects of intracellular water–carbon coupling, the phylogenetic breadth of the proposed pathway, and the ecosystem-scale relevance of the karst–photosynthesis coupling concept all require further validation. These open questions are accompanied by substantial experimental challenges, including rapid bicarbonate–water oxygen isotope exchange, the difficulty of resolving bicarbonate-related processes within PSII under physiological conditions, and the need to bridge controlled laboratory observations with field-scale biogeochemical behavior. Accordingly, the following directions are presented as testable priorities intended to refine, challenge, and, where necessary, delimit the framework proposed in this Perspective. Based on our systematic work and the revised photosynthesis framework, we outline five future research priorities intended to test key assumptions, address unresolved mechanisms, and evaluate the broader applicability of the framework proposed here. These directions focus on mechanistic depth, technical expansion, and applied translation, and are expected to drive major advances in photosynthesis research and agricultural biotechnology.

4.1 Elucidate the molecular-electrophysiological mechanism of bicarbonate photolysis in PSII

A key unresolved question is the molecular mechanism of bicarbonate binding and photolysis in the PSII oxygen-evolving complex (OEC). Our dual-substrate model predicts that bicarbonate binds to the PSII OEC, but the specific binding site, interaction with the Mn_4CaO_5 cluster, and photolysis reaction pathway remain unclear. Future work will integrate cryo-electron microscopy (cryo-EM) and FTIR spectroscopy to: (1) identify the specific bicarbonate-binding site in the PSII OEC under physiological pH (7.5–8.2); (2) characterize the dynamic interaction between bicarbonate and the Mn4CaO5 cluster during the Kok-Joliot cycle; (3) clarify the electrophysiological signals generated by bicarbonate photolysis and their coupling with inorganic carbon assimilation; (4) identify PSII proteins that modulate bicarbonate binding and photolysis (e.g., CA-like proteins, bicarbonate transporters). If successful, such work could clarify whether bicarbonate plays a direct mechanistic role in PSII and provide a stronger basis for evaluating, refining, or rejecting aspects of the dual-substrate model. Also, it will lay the groundwork for engineering PSII to enhance bicarbonate utilization.

4.2 Explore the evolutionary origin of the bicarbonate photolysis pathway

We hypothesize that bicarbonate photolysis is an ancestral photosynthetic pathway, but its evolutionary origin remains uncharacterized. Future research endeavors will encompass the following aspects: (1) Employ isotope tracing and electrophysiology techniques to examine the bicarbonate photolysis capacity in primitive photosynthetic organisms, such as cyanobacteria, green algae, and early land plants; (2) Conduct phylogenetic analysis of bicarbonate transporters, chloroplast carbonic anhydrase (CA), and photosystem II (PSII) bicarbonate-binding proteins to reconstruct the evolutionary trajectory of the bicarbonate photolysis pathway; (3) Probe into the co-evolution of bicarbonate photolysis with atmospheric CO_2/bicarbonate concentrations across geological time scales. This research may help clarify whether bicarbonate-associated processes represent an evolutionarily ancient feature, or instead a more limited or derived phenomenon, and may refine their possible relevance to early oxygenation and carbon-cycle evolution.

4.3 Develop electrophysiological-based precision agriculture technologies and stress tolerance biomarkers

Our research has demonstrated that plant electrophysiological parameters can serve as comprehensive indicators of total photosynthetic capacity, intracellular water dynamics, nutrient transfer and transport, energy storage and allocation, metabolic activity, growth potential (growth rate), health status, and stress adaptation. However, these parameters have not yet been translated into practical agricultural technologies. Future work should therefore focus on: (1) identifying core electrophysiological biomarkers of total photosynthetic capacity, intracellular water dynamics, nutrient transfer and transport, energy storage and allocation, metabolic activity, growth potential (growth rate), health status, and stress tolerance (e.g., drought, salinity, and bicarbonate stress) in major crops such as maize, rice, and wheat through large-scale screening; (2) developing more rapid, self-adaptive, portable, and high-throughput plant electrophysiological sensors for real-time, non-destructive monitoring of crop growth potential (growth rate), health status, photosynthetic status, and stress levels in the field; (3) integrating these sensors with precision-agriculture systems, such as unmanned aerial vehicles and intelligent irrigation platforms, to enable targeted management strategies, including variable-rate fertilization and drought early warning; and (4) using electrophysiological biomarkers for high-throughput screening of crop germplasm with high photosynthetic efficiency and enhanced stress tolerance, thereby accelerating breeding progress. Collectively, this work could help assess whether plant electrophysiology can be translated into robust precision-agriculture tools, while also clarifying its present limitations.

4.4 Engineer crops to enhance bicarbonate use for improved photosynthetic efficiency and alkaline/karst soil adaptation

Alkaline and karst soils account for a substantial proportion of global marginal lands, and their high bicarbonate levels are often associated with constraints on crop growth. At the same time, our work suggests that bicarbonate may represent a potentially valuable inorganic carbon source for photosynthesis. However, a central challenge will be to determine whether the targeted traits are causally linked to enhanced photosynthetic performance under realistic growth conditions, rather than merely correlated with bicarbonate-rich environments. Future research should therefore investigate strategies to enhance bicarbonate uptake, transport, and photolysis capacity in crops, to improve adaptation to alkaline soils and potentially increase photosynthetic efficiency. Independent work in algae, C_3 plants, and aquatic macrophytes indicates that bicarbonate transport and utilization strategies are diverse across photosynthetic organisms, suggesting that bicarbonate-related physiology is broader than any single experimental platform and should be evaluated comparatively. Such efforts may include: (1) overexpressing plasma membrane and chloroplast bicarbonate transporters to enhance bicarbonate uptake and its transport into chloroplasts; (2) engineering chloroplast carbonic anhydrase (CA) to enhance CA-catalyzed bicarbonate dehydration and/or related photolysis-associated processes; (3) modifying the PSII oxygen-evolving complex (OEC) to improve bicarbonate binding and potential photolysis capacity; and (4) stacking these traits to develop “bicarbonate-efficient” crop varieties that may utilize soil bicarbonate more effectively for photosynthesis, thereby improving growth and yield in alkaline and karst soils. If validated experimentally, this line of research could help unlock the agricultural potential of marginal lands and contribute to global food security.

4.5 Characterize the Karst-Photosynthesis Coupling Mechanism at the Ecosystem Scale and Its Role in Global Carbon Sequestration

Our karst-photosynthesis coupling model is currently based on laboratory studies, and its ecosystem-scale function and contribution to global carbon sequestration remain unclear. Future work should integrate field monitoring, isotope tracing, and biogeochemical modelling to: (1) characterize the carbon sequestration rate associated with karst-photosynthesis coupling and its response to climate change, including elevated CO_2 and warming; (2) develop a biogeochemical model that integrates karst processes with photosynthetic carbon assimilation, including bicarbonate photolysis, to predict its role in the global carbon-water balance under future climate scenarios; and (3) explore the potential of karst ecosystem restoration to enhance karst-photosynthesis coupling and carbon sequestration. Collectively, this work could clarify the broader ecological significance of bicarbonate photolysis and offer a new perspective on climate change mitigation and carbon sequestration, with potential relevance to global carbon-neutrality goals [2].

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Conflicts of Interest

The authors declare no conflicts of interest.

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Ethical Approval and Consent to Participate

Not applicable.

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