



RESEARCH ARTICLE

Co-applying mild alternate wetting and drying with biochar synergistically improves rice yield and quality

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Highlights

- Co-application of mild alternate wetting and drying (Mild AWD) and biochar significantly increased rice grain yield.
- Co-application of Mild AWD and biochar comprehensively improved rice grain quality by enhancing source–sink coordination and starch biosynthesis.
- A minimum soil water potential of -10 to -15 kPa at a depth of 15–20 cm was identified as the optimal threshold for Mild AWD in rice production.

Abstract

To address the dual challenges of water scarcity and rising demand for premium rice, this study investigated the synergistic effects of mild alternate wetting and drying (Mild AWD) irrigation combined with wheat straw biochar application on rice yield and grain quality. A two-year field experiment (2023–2024) was conducted with the hybrid rice cultivar Yongyou 2640, with two irrigation regimes: continuous flooding (CF) and Mild AWD (re-irrigation at a soil water potential of -10 to -15 kPa at 15–20 cm depth), with or without a one-time biochar application (10 t ha^{-1}). The results showed that co-application of Mild AWD and biochar significantly increased grain yield by 18.7% in 2023 and 13.4% in 2024 compared to CF alone. It also comprehensively improved grain quality: milling quality (head rice rate increased by 23.1–24.6%), appearance quality (chalkiness reduced by 36.4–38.2%), cooking and eating quality (higher peak viscosity and lower gelatinization temperature and enthalpy), and nutritional quality (increased glutelin and decreased prolamin content and starch digestion). These improvements were attributed to enhanced root activity alongside leaf photosynthetic rate, which promotes the accumulation of photoassimilates in vegetative organs and their translocation to grains. Moreover, elevated activities of key starch synthases further enhanced starch biosynthesis and accumulation, which underpinned the improved yield and superior quality. We also identified that a minimum soil water potential of -10 to -15 kPa at a depth of 15–20 cm represents the optimal threshold for Mild AWD in rice production. This research provides a cultivation approach for synergistically producing high-yield, high-quality rice, which shows promising potential for scalable implementation.

Keywords: water-saving irrigation, biochar, rice, yield, quality

1. Introduction

Rice (*Oryza sativa* L.), a key staple crop, underpins the food demand of close to 50% of the world population (Bin Rahman

and Zhang *et al.* 2023). The world population is projected to surpass 10 billion by 2050, while food demand per person will have doubled by that time. Meanwhile, growing incomes

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and improved living standards are fueling a shift in consumer preferences toward high-quality rice (Aznan *et al.* 2023). This trend is clearly evidenced in China's Yangtze River Delta region, where the focus of rice consumption shifted from standard to mid- and high-end varieties between 2015 and 2018, with high-end rice achieving an annual growth rate approaching 30% (Chen *et al.* 2023b). Regrettably, the escalating global warming and water scarcity are posing severe threats to rice productivity and grain quality, with further deterioration anticipated in the coming decades (Tuong and Bouman 2003; Tollefson 2021; FAO 2024; Gu *et al.* 2024; Itoh *et al.* 2024; Liu *et al.* 2024; Yu *et al.* 2024; Li *et al.* 2025). Research shows that water stress alone can lead to a 10–30% reduction in rice yield, with the specific extent depending on the growth stage at which the stress occurs and its severity (Zhang *et al.* 2018). Additionally, it impairs key grain quality traits such as reducing the head rice rate and increasing chalkiness (Han *et al.* 2025). This situation has led to an increasingly severe supply-demand imbalance: market demand for rice (including premium rice) continues to rise, while the potential for further improving yield and quality is increasingly limited. Thus, there is a pressing need to explore agronomic technologies that harmonize water-saving with quality improvement and yield increase.

The alternate wetting and drying (AWD) regime, characterized by re-irrigation at a soil water potential (SWP) of -15 to -25 kPa measured at 15–20 cm depth, is a water-saving irrigation method that reduces consumption through controlled wet–dry cycling and is widely used in global rice-growing regions (Ishfaq *et al.* 2020, 2021; Bo *et al.* 2024; Gao *et al.* 2024). Although the AWD regime reduces irrigation water consumption and improves water use efficiency, its impact on rice productivity remains controversial (Carrijo *et al.* 2017; Gao *et al.* 2024; Zhao *et al.* 2024). On one hand, several studies have demonstrated that the AWD regime can enhance grain filling by increasing root oxidation activity (ROA) and facilitating the translocation of photosynthates to grains, thereby improving rice yield (Chu *et al.* 2014; Li *et al.* 2018; Zhang *et al.* 2021; Zang *et al.* 2024; Xiao *et al.* 2025). On the other hand, other research has indicated that adopting the AWD regime does not increase rice yield and may even lead to a yield reduction (Carrijo *et al.* 2017; Gao *et al.* 2024). Regarding grain quality, research has shown that the AWD regime improves rice milling and appearance quality while increasing grain protein content (Ishfaq *et al.* 2021). Conversely, others suggest it exerts no significant impact on rice quality (Chen *et al.* 2021). These discrepancies may mainly stem from differences in SWP ranges, rice variety sensitivity, and interactions with agronomic practices (e.g., nitrogen management). Recent research has indicated that how the AWD regime influences rice yield formation hinges on whether the SWP falls beneath the critical threshold of -15 kPa in the soil drying stage (Carrijo *et al.* 2018; Bo *et al.* 2024). Notably, the mild alternate wetting and drying (Mild AWD) regime (SWP of -10 to -15 kPa) (Yang *et al.* 2017; Carrijo *et al.* 2018), a refined version of the AWD regime, could potentially provide a synergistic benefit through water conservation and grain yield enhancement. Despite extensive research on the impacts of the AWD regime on rice

cultivation, studies on the impacts of the Mild AWD regime remain limited, particularly regarding its comprehensive influences on rice yield and quality formation (e.g., milling and appearance quality, cooking and eating quality, and nutritional quality) and the underlying physiological mechanisms. Furthermore, the effectiveness of combining the Mild AWD regime with other agronomic practices in stabilizing yield and enhancing grain quality remains insufficiently explored.

Biochar, a solid product derived from the biomass pyrolysis (e.g., agricultural and forestry waste, organic waste) under oxygen-limited conditions, has proven to be a prospective agronomic amendment with specific relevance to water management in agricultural systems (Tomczyk *et al.* 2020; Kumar *et al.* 2023; He H *et al.* 2024; Jin *et al.* 2024). Biochar application enhances the soil pore system and water retention capacity, while also improving aggregate structure and optimizing root-zone moisture availability (Wang *et al.* 2019; Yang and Lu 2021; Chi *et al.* 2024). These traits may buffer transient water deficits under the Mild AWD regime and potentially synergize with its moisture regulation. Notably, biochar-induced improvements in root zone moisture availability may enhance Mild AWD's promotion of ROA and leaf photosynthetic rate (LPR), thereby further boosting the translocation of photosynthates to grains. Studies indicate that the application of biochar improves root morphology and physiology, as well as leaf photosynthesis, thereby promoting dry matter accumulation and nitrogen absorption, and ultimately enhancing rice productivity (Zhang *et al.* 2013; Ali *et al.* 2022; Chi *et al.* 2024). Furthermore, some research suggests that biochar enhances rice milling and appearance quality while reducing amylose content and altering starch pasting properties (Gong *et al.* 2020; Chen *et al.* 2023a). Conversely, others argue that these impacts are slight or barely noticeable (e.g., milling and appearance quality) (Niu *et al.* 2018). These discrepancies may primarily stem from variations in biochar characteristics (e.g., feedstock sources, pyrolysis temperature, application strategies) and soil hydrological conditions (e.g., SWP, drying cycle frequency), which are also main factors driving the inconsistent effects of the Mild AWD regime.

While the individual effects of biochar or Mild AWD have been extensively studied, the interactive effects of these two practices under field conditions remain unclear, particularly how biochar regulates soil–plant water relationships under Mild AWD and how this regulation further influences the physiological processes of rice yield and quality formation. Notably, biochar's ability to enhance soil water retention could theoretically buffer the mild water stress induced by AWD and stabilize the root-zone environment, addressing Mild AWD's dependence on stable moisture (Wang *et al.* 2019; Yang and Lu 2021; Chi *et al.* 2024). This study hypothesizes that their co-application could lead to superior benefits for both yield and quality formation. However, regarding the combined effects of biochar and Mild AWD irrigation, it remains to be verified whether they regulate crop physiological and population status, thereby influencing key starch synthases and protein fractions and ultimately improving rice quality. Accordingly, systematically investigating the combined effects and underlying mechanisms of biochar and the Mild AWD

regime is crucial for addressing the pressing issue of supply–demand imbalance in premium rice amid population growth and for enhancing the resilience of rice systems to climate change and water scarcity.

Therefore, through a two-year field experiment, this study aims to systematically investigate the synergistic effects of co-applying biochar and Mild AWD on rice production. By integrating measurements of plant physiological status (e.g., ROA, LPR, non-structural carbohydrate translocation, starch synthase activity), yield components, and comprehensive grain quality, we seek to: (1) determine whether co-applying biochar and Mild AWD synergistically regulates rice yield and grain quality formation; (2) elucidate how this combined practice further regulates the biosynthesis of storage substances such as starch and proteins to optimize grain quality; and (3) identify the optimal soil water potential threshold for Mild AWD to synergistically enhance both yield and quality. This work will provide a mechanistic scientific foundation and practical guidance for ensuring food security and promoting the sustainable production of premium rice.

2. Materials and methods

2.1. Plant materials and cultivation

From 2023 to 2024, researchers performed a two-year field trial at the Jiangwang Experimental Farm of Yangzhou University in Jiangsu Province, China (32°36'N, 119°33'E; 21 m above sea level), covering the typical rice-growing seasons (13 June–14 October 2023 and 15 June–22 October 2024). The soil at the experimental location was a sandy loam. Key soil properties included an organic matter level at 16.2 g kg⁻¹, alkali-hydrolyzable nitrogen at 91.1 mg kg⁻¹, Olsen phosphorus at 30.4 mg kg⁻¹, and exchangeable potassium at 128 mg kg⁻¹. The three-line *indica-japonica* hybrid rice cultivar Yongyou 2640, widely grown in the region, was selected for its robust yield capacity and adaptability. Pregerminated seedlings were nursery-raised for 25 d before being transplanted into the field with two seedlings per hill at a spacing of 0.16 m×0.25 m. Fertilization followed a split-application protocol: a basal dose of 96 kg N ha⁻¹ (urea), 30 kg P ha⁻¹ (calcium superphosphate), and 40 kg K ha⁻¹ (potassium chloride) was blended into the soil 24 h prior to transplanting. Topdressing with urea (48 kg N ha⁻¹ each) was administered at the mid-tillering stage (MT), jointing stage (JT), and spikelet differentiation stage. Standard agronomic practices for weed, pest, and disease control were maintained throughout the growing season, aligning with local recommendations to safeguard yield potential. A meteorological station adjacent to the experimental field recorded meteorological data throughout the rice growing season (Appendix A).

2.2. Irrigation and biochar treatments

This study employed a 2×2 factorial design, evaluating two irrigation methods: continuous flooding (CF) and Mild AWD; and two rates of biochar application: 0 and 10 t ha⁻¹. Four treatment combinations were established using a randomized complete block design, with each combination replicated four times. Test plot units (each 6.0 m×4.0 m, length×width) were

partitioned using 0.8 m-wide buffer strips and 0.5 m-deep plastic barriers to avoid cross-plot contamination. Initiating 7 days post-transplanting and extending to the rice ripening stage, the two irrigation regimes were executed. Under the CF regime, the field was kept with a 2–3 cm water depth throughout the entire rice growing season, without mid-season drainage, and irrigation was discontinued 7 d prior to harvest in accordance with local agricultural conventions. For the Mild AWD regime, irrigation was initiated when the average SWP at 15–20 cm depth dropped to –10 to –15 kPa, and then plots were rewatered to a standing water depth of 2–3 cm. SWP was monitored using five tensiometers (Institute of Soil Science, Nanjing, China) per experimental unit. To ensure spatially representative measurements, the tensiometers were evenly distributed within each plot: one at the plot center and four at positions 1 m from each corner, avoiding proximity to border rows or irrigation channels. All plots were individually equipped with irrigation and drainage facilities to ensure precise water management. For each irrigation method, experimental groups were configured to include or not include biochar. For the groups receiving biochar, a one-time addition of 10 t ha⁻¹ biochar was applied to each group prior to rice transplanting, specifically during the land preparation phase, and this application was limited to 2023. The biochar used was obtained from wheat straw pyrolysis at 500°C, sourced from Biochar Future Eco-environmental Technology Co., Ltd. (Guangdong, China). Its key properties included 50% carbon content, 30% moisture content, 37% ash content, 18% volatile phenol content, a pH of 10.4, a carbon-to-nitrogen ratio of 52.7, and a surface area of 56.1 m² g⁻¹. As biochar was applied only in 2023, the ‘-C’ treatments in the 2024 season specifically represent the residual (or legacy) effects of the initial biochar application.

2.3. Sampling and measurements

Measurement of leaf water potential (LWP), LPR, and ROA Under the Mild AWD regime, SWP at plot centers reached approximately –10 to –15 kPa on days 15 (2023) and 25 (2024) (D1), days 22 (2023) and 39 (2024) (D2), days 43 (2023) and 52 (2024) (D3), and days 98 (2023) and 101 (2024) (D4); corresponding rewatering occurred on days 17 (2023) and 27 (2024) (W1), days 24 (2023) and 41 (2024) (W2), days 45 (2023) and 57 (2024) (W3), and days 100 (2023) and 103 (2024) (W4). These periods (D1–W4), which correspond to mid-tillering, panicle initiation to booting, heading to flowering, and mid-grain filling stages, represent critical stages for rice water demand during growth and development (Zhuang *et al.* 2014; Zhang *et al.* 2023).

LWP was measured using the pressure chamber method with a Model 3000 instrument (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Readings were recorded daily between 9:00 and 11:00 a.m. on the corresponding dates (D1–W4), with 10 leaves selected per plot as replicate samples. Concurrently, the LPR of the topmost fully expanded leaves was determined using a Li-6800 portable photosynthesis system (Li-Cor, Lincoln, NE, USA) on the same dates (D1–W4). The same sampling protocol was applied for photosynthetic rate measurements, with 10 leaves selected

per plot as replicates. On the same dates (D1–W4), ROA was measured by evaluating the oxidation of α -naphthylamine (α -NA) as described by Ramasamy *et al.* (1997).

Tillering dynamics and leaf area index (LAI) At the JT, heading stage (HD), and maturity stage (MA) of rice, 20 representative rice hills showing consistent growth were chosen in each test plot to determine the stem and tiller numbers. After leaf clipping, the LAI was assessed with a leaf area meter (Li-Cor 3050, LI-COR, USA).

Determination of aboveground and underground biomass and accumulation of non-structural carbohydrates (NSC) At the mid-tillering stage (MT), JT, HD, and MA of rice, from each test plot, four representative rice hills with steady growth were sampled. The plants were separated into 3 to 4 portions, namely roots, stems, leaves (blades and sheaths), and panicles (from the HD onwards). These samples were first deactivated by heating for 30 min at 105°C, then dried at 75°C until a constant weight, and the biomass of each organ was determined. The dry matter samples assayed at HD and MA were first dried to constant weight, ground into powder, and thoroughly homogenized. Thereafter, the NSC content was determined and computed using the anthrone-sulfuric acid colorimetric method, as outlined by Cock *et al.* (1976). To minimize edge effects, four rice plants showing consistent growth were selected from the third row in each test unit for the determination of the above-mentioned indices.

Enzyme extraction and activity assays At HD, approximately 80–100 panicles with synchronous heading dates were chosen from each test plot and labeled. During the mid-filling stage (corresponding to D4 and W4), 10–15 tagged panicles were randomly selected, and 30–50 grains showing uniform growth were sampled for enzyme activity determination. The activities of ADP-glucose pyrophosphorylase (APGase), granule-bound starch synthase (GBSS), soluble starch synthase (SSS), and starch branching enzyme (SBE) were quantified following modified protocols from Zhang *et al.* (2025). All chemicals and enzymes essential to these assays were purchased from Sigma Chemical Company (St Louis, USA).

Final harvesting At MA, assessments of grain yield and its constituent components were conducted following the methodology described by Yoshida *et al.* (1976). Grain yield was determined from a harvested section of 6.0 m² and standardized to a moisture content of 14%. For yield components (panicles per square meter, spikelets per panicle, filled grain percentage, and 1,000-grain weight), we conducted assessments on a randomly chosen 2 m² section within each experimental unit.

Milling and appearance quality At MA, 30–50 tagged panicles were harvested. After threshing, the grains were air-dried until their moisture content stabilized at 15%. The determination of milling quality (brown rice rate, milled rice rate, and head rice rate) and appearance (chalkiness, chalky grain rate, and chalky area) was performed with reference to Chen *et al.* (2025b). The leftover milled head rice was pulverized to powder, sieved using a 200-mesh screen, and preserved at 4°C for later analytical procedures.

Starch isolation, starch accumulation and apparent amylose content (AAC) Starch was isolated from dehulled

grains using a slightly modified version of the protocol developed by Shi *et al.* (2024). To elaborate, 15 g rice powder was blended with distilled water at a 1:15 weight-to-volume ratio. The slurry was then filtered and centrifuged to obtain crude starch, which was subsequently purified with 2% SDS, then rinsed with distilled water and ethanol. The purified starch was oven-dried at 40°C, passed over a 150 μ m sieve, and further dried until constant weight was achieved. Total starch and AAC were determined using colorimetric methods adapted from Prathap *et al.* (2019) and Lin *et al.* (2018). Additionally, the total starch content per grain multiplied by the grain weight equals the starch accumulation per grain.

Total protein content and protein fraction content The nitrogen content in rice powder was determined *via* the Kjeldahl method (He C *et al.* 2024). A 0.50 g sample was digested with 10 mL concentrated sulfuric acid (98%) at 420°C for 90 min, with nitrogen quantified automatically using a Kjeltec 8400 analyzer (FOSS, Denmark). Crude protein content was calculated through the multiplication of nitrogen values using a conversion factor of 5.95. Protein fractions (albumins, globulins, alcohol-soluble proteins, and glutelins) were sequentially extracted with distilled water, 5% NaCl, 70% ethanol, and 0.1% NaOH. Each fraction content was determined using a modified Coomassie brilliant blue assay according to Bradford (1976).

Pasting properties analysis The pasting characteristics of rice flour were evaluated using a rapid visco analyzer (RVA-3D, Newport Scientific, Australia) according to the method of Shi *et al.* (2024). A suspension of 2.5 g flour in 25 mL distilled water was tested at 160 r min⁻¹ using a standard thermal profile: heating from 50 to 95°C at 12°C min⁻¹, followed by cooling to 50°C. Data analysis was conducted *via* Thermal Cycle for Windows software.

Thermal properties The thermal characteristics of starch were examined using a Netzsch DSC 200-F3 differential scanning calorimeter according to the protocol outlined by Shi *et al.* (2024). The thermal parameters determined included the onset temperature (T_o), peak gelatinization temperature (T_p), conclusion temperature (T_c), and gelatinization enthalpy (ΔH_{gel}), which were then analyzed using Proteus Thermal Analysis software (version 5.1.0).

In vitro digestion rate The *in vitro* starch digestibility was determined according to modified methods from Chen *et al.* (2025b). A mixture containing 100 mg of dry starch (M_0) in sodium acetate buffer was enzymatically digested using porcine pancreatin and amyloglucosidase at 37°C with shaking. Aliquots were collected at intervals over 300 min, and the reaction was stopped with anhydrous ethanol. Glucose content (M_1) was measured using a GOPOD Assay Kit (Megazyme, Ireland). The starch digestion rate (%) = $M_1 \times 0.9 / M_0 \times 100$. The use of a 0.9 coefficient enables the conversion of glucose mass to starch mass.

2.4. Statistical analysis

Statistical analyses were conducted using SAS/STAT version 9.2, which included analysis of variance (ANOVA). Mean comparisons were performed using the least significant difference (LSD) test, with a significance threshold of $P < 0.05$.

Figures were prepared with Origin 2021. A random forest analysis was performed to identify the vital predictors of yield, using the randomForest package in R 4.0.2.

3. Results

3.1. Grain yield and yield components of rice

Under the CF regime, applying biochar (relative to CF alone) led to only marginal improvements in rice yield, with increments of 4.44% (2023) and 5.20% (2024). However, under Mild AWD, applying biochar substantially boosted yield, showing rises of 5.24 and 6.07% in the same years. In the absence of biochar, the Mild AWD regime alone resulted in significantly higher yields compared to the CF regime alone, increasing by 12.8% in 2023 and 6.89% in 2024. The combination of biochar and Mild AWD additionally elevated yield compared to CF alone, with grains of 18.7% in 2023 and 13.4% in 2024. The highest yields were consistently achieved with the combined application (Fig. 1). Random forest analysis quantified the contributions of key yield components, revealing that although Mild AWD slightly reduced panicle number per plot compared to CF, this reduction was outweighed by a significant increase in spikelets per panicle. The resulting synergistic improvements in spikelets per panicle, filled grain rate, and 1,000-grain weight were the primary drivers of the overall yield increase (Fig. 1; Appendix B). Despite not being reapplied in 2024, biochar still demonstrated a yield-enhancing effect. This indicates that biochar exerts a persistent and stable agronomic effect in the farmland ecosystem.

3.2. SWP and LWP

The variation in paddy SWP differs significantly with irrigation

regimes. Under sunny weather conditions, it takes 3–6 d for the SWP to drop to -15 kPa under the Mild AWD regime. Notably, under identical irrigation conditions and measured on the same date, SWP showed no statistically significant variation regardless of biochar application. Meanwhile, whether under the traditional CF regime or the Mild AWD regime, biochar application (or not) has no significant effect on rice LWP (Appendix C). However, during the soil drying stages (D1–D4), regardless of biochar application, LWP was markedly lower under the Mild AWD regime than under the CF regime. After rewatering (W1–W4), LWP levels became comparable between the two irrigation regimes (Appendix C).

3.3. ROA and LPR

Compared with the treatment without biochar, applying biochar generally led to a notable rise in rice ROA under both irrigation regimes, with a more pronounced effect under the Mild AWD regime (especially during the rewatering period). With or without biochar application, ROA was notably greater under the Mild AWD regime than under the CF regime, and the enhancing effect of Mild AWD on ROA was more prominent when biochar was applied (particularly during the rewatering period) (Appendix D). During the soil drying period under the CF or Mild AWD regimes, biochar application exerted no notable impact on LPR. However, during the rewatering period under the Mild AWD regime, applying biochar markedly elevated the LPR of plants. Irrespective of whether biochar was applied, LPR under the Mild AWD regime did not show a significant decrease compared with that under the CF regime over the soil drying period (D1–D4). After rewatering (W1–W4), the Mild AWD regime markedly increased plant LPR, with the greatest effect observed when biochar was applied under this regime (Appendix D).

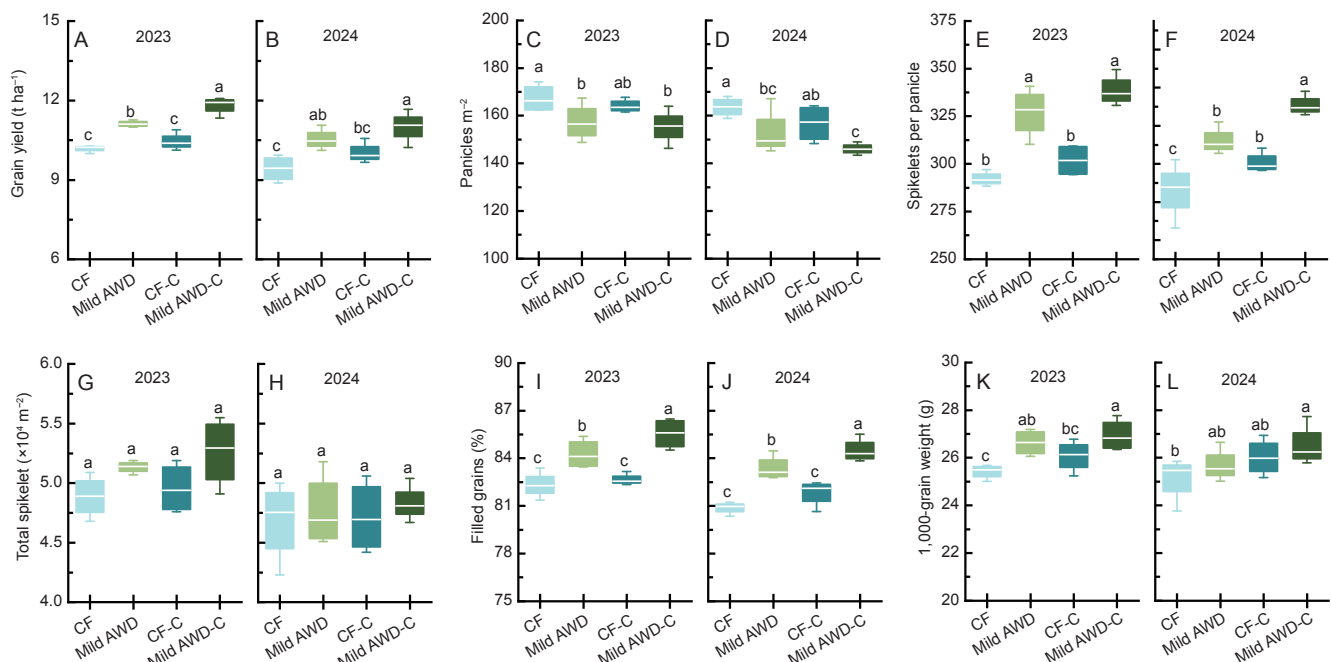


Fig. 1 Grain yield and its components of rice as affected by irrigation and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C indicates biochar application. Bars mean SD ($n=4$). Different letters above the SD bars indicate the least significant difference at the $P=0.05$ level.

3.4. Tillering dynamics and LAI

Compared to the CF region, the Mild AWD regime, regardless of biochar application, reduced the number of stems and tillers and the total leaf area of rice during key growth stages. However, it markedly increased the percentage of productive tillers and, at the HD, increased the effective leaf area and effective leaf area ratio. Under either the CF or Mild AWD regime, applying biochar further increased the proportion of productive tillers and the effective leaf area of rice compared to without biochar application, and co-applying the biochar with the Mild AWD regime showed a better promoting effect (Appendix E).

3.5. Aboveground and belowground biomass

In the early growth periods (MT and JT), no notable variations were observed in the dry weight of aboveground parts, dry weight of roots, or the root-to-shoot biomass ratio of rice across the treatments. In the middle and late growth stages (HD and MA), relative to their respective control groups, both applying biochar and the Mild AWD regime notably elevated the dry weight of shoots, the dry weight of roots, and the root-to-shoot ratio, with the most pronounced effects observed when they were applied in combination (Fig. 2).

3.6. Non-structural carbohydrate (NSC) accumulation, translocation, and starch accumulation

Under the CF regime, using biochar slightly increased

the NSC accumulation in stems (including leaf sheaths) at HD and promoted the NSC translocation from stems (including leaf sheaths) to grains during MA; however, these effects did not reach statistical significance. Under the Mild AWD regime, applying biochar markedly enhanced NSC accumulation in stems (including leaf sheaths) at HD and NSC translocation from stems (including leaf sheaths) to grains at MA. Irrespective of biochar application, the Mild AWD regime induced higher NSC accumulation in stems (including leaf sheaths) when compared to the CF regime at HD to some extent, and markedly promoted NSC translocation to grains, with the most pronounced regulatory effect observed when co-applying the Mild AWD regime and biochar. The amount of starch accumulation is consistent with the changes in NSC translocation (Fig. 3).

3.7. Enzyme activities

Under the CF regime, biochar application generally increased the activities of AGPase, GBSS, SSS, and SBE in rice grains throughout the filling stage, while the enhancing effect of biochar on these enzyme activities was more significant under the Mild AWD regime (especially during the rewetting period). Independent of biochar application, the Mild AWD regime notably boosted the activities of AGPase, GBSS, SSS, and SBE compared with the CF regime, with particularly pronounced increments observed when combined with biochar application (especially during the rewetting period).

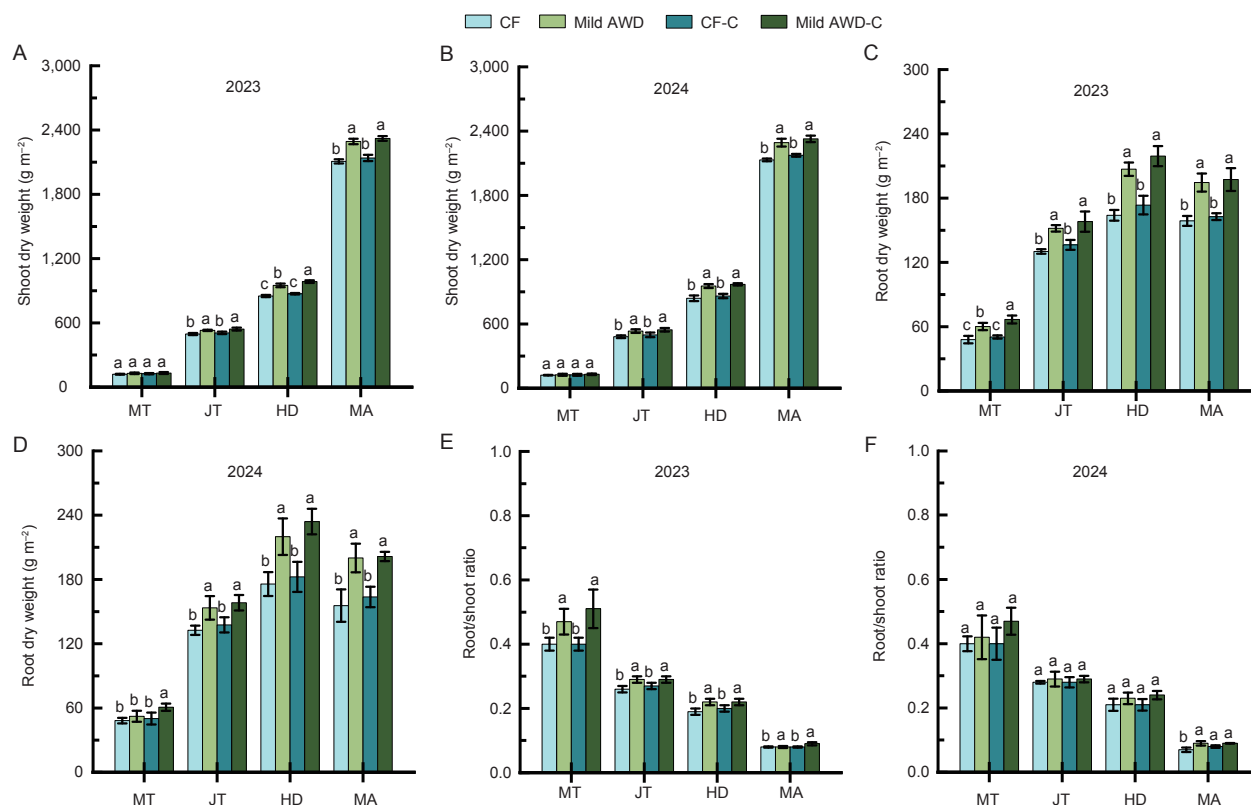


Fig. 2 Dry matter accumulation dynamics in rice under different irrigation regimes and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C means the application of biochar. MT, JT, HD, and MA represent the mid-tillering, jointing, heading, and maturity stages, respectively. Bars mean SD ($n=4$). Different letters above the SD bars indicate the least significant difference at the $P=0.05$ level.

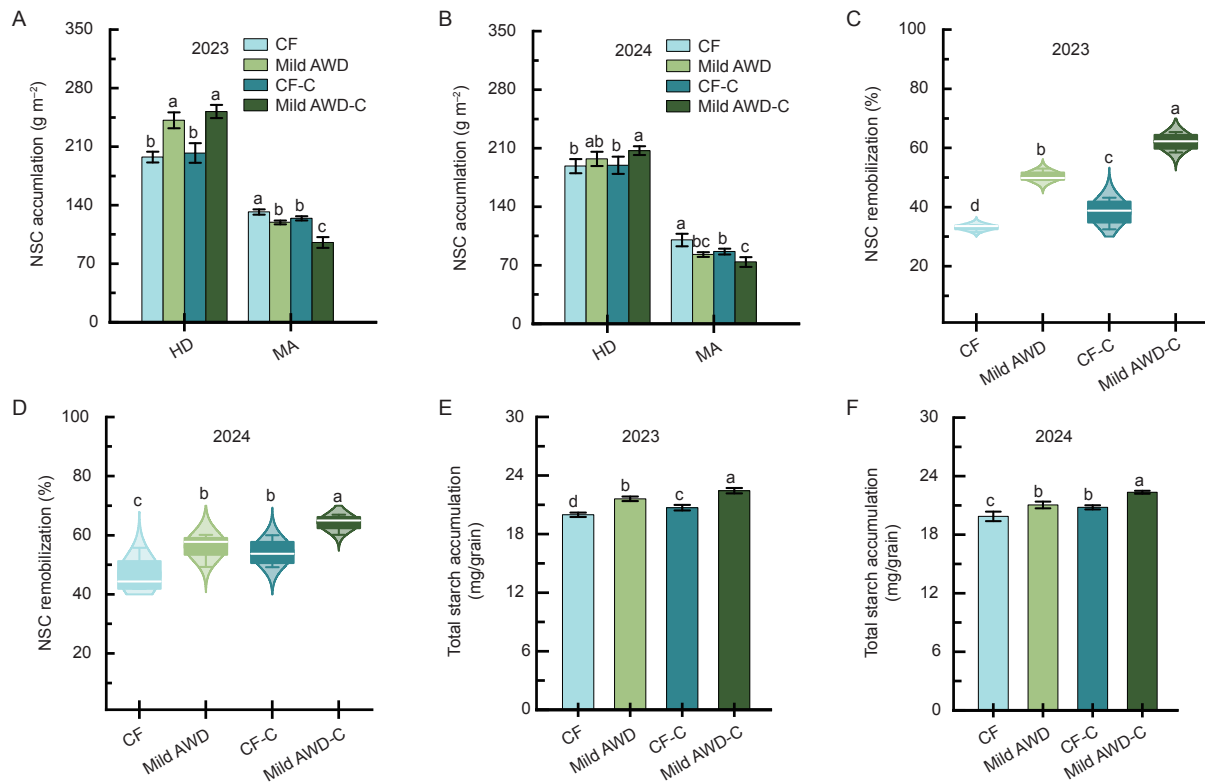


Fig. 3 Dynamics of non-structural carbohydrate (NSC) accumulation and translocation in rice stems and starch accumulation in grains under contrasting irrigation regimes and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C means the application of biochar. HD and MA represent the heading and maturity stages, respectively. Bars mean SD ($n=4$). Different letters above the SD bars indicate the least significant difference at the $P=0.05$ level.

Additionally, after rewetting, AGPase and GBSS activities showed greater increases, followed by SSS, whereas SBE was relatively less affected (Fig. 4).

3.8. Milling and appearance quality

Under the CF regime, applying biochar only slightly increased the brown rice rate, milled rice rate, and head rice rate, while reducing the chalkiness, chalky grain rate, and chalky area. Under the Mild AWD regime, applying biochar notably enhanced the milling and appearance quality of grains (e.g., higher head rice rate and lower chalkiness). Compared with the CF regime, the Mild AWD regime, whether biochar was applied or not, markedly enhanced gain milling and appearance quality; the effect was more pronounced when biochar was co-applied (Fig. 5).

3.9. Composition of major components in rice

Under the CF regime, applying biochar boosted the total starch content and AC of grains while decreasing the total protein content, though these changes were not significant. On the other hand, under the Mild AWD regime, applying biochar led to significant alterations in the aforementioned indices. With or without biochar application, compared to the CF regime, the Mild AWD regime markedly boosted both total starch and AC of grains and significantly decreased the total protein content, with a more pronounced effect when combined with biochar application (Fig. 6-A–F). In terms of

protein fractions, under the CF regime, biochar application had negligible effects on the contents of prolamin, glutelin, globulin, and albumin. In the absence of biochar, grain prolamin content was significantly reduced under the Mild AWD regime relative to the CF regime. In contrast, the concentrations of glutelin, globulin, and albumin were comparable under both irrigation regimes. When the Mild AWD regime was co-applied with biochar, the contents of albumin, glutelin, and globulin increased significantly, while the prolamin content decreased further, with the most pronounced changes observed (Fig. 6-G and H).

3.10. Pasting properties of rice flour

In comparison to their corresponding control treatments (CF or CF-C), the application of biochar and the Mild AWD regime brought about a rise in the peak viscosity (PV), hot paste viscosity (HV), breakdown viscosity (BDV), and final viscosity (FV), while decreasing the setback viscosity (SBV) of rice flour, with the effect of the Mild AWD regime being more significant. Overall, irrespective of biochar application, the Mild AWD treatment led to a significant rise in these viscosity parameters (PV, HV, BDV, and FV) and a decrease in SBV of rice flour. Notably, these positive effects were further enhanced when biochar was co-applied (Table 1).

3.11. Thermal properties of rice starch

Under the CF regime, applying biochar generally slightly

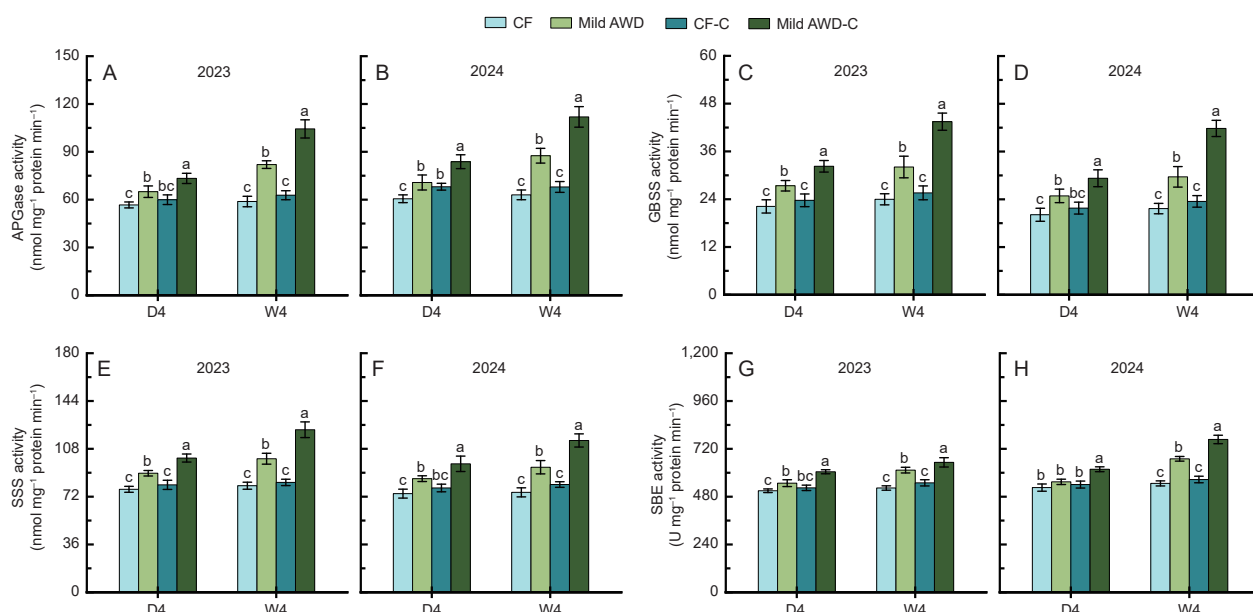


Fig. 4 Activities of key starch biosynthetic enzymes in rice grains at the mid-grain filling stage under contrasting irrigation and biochar treatments. A and B, ADP-glucose pyrophosphorylase (AGPase). C and D, granule-bound starch synthase (GBSS). E and F, soluble starch synthase (SSS). G and H, starch branching enzyme (SBE). CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C means the application of biochar. D4 represents measurements taken on day 98 (2023) and day 101 (2024) after transplanting, when soil water potential (SWP) reached -10 to -15 kPa under the Mild AWD regime; W4 corresponds to measurements collected on day 100 (2023) and day 103 (2024) after rewetting. Bars mean SD ($n=4$). Different letters above the SD bars indicate the least significant difference at the $P=0.05$ level.

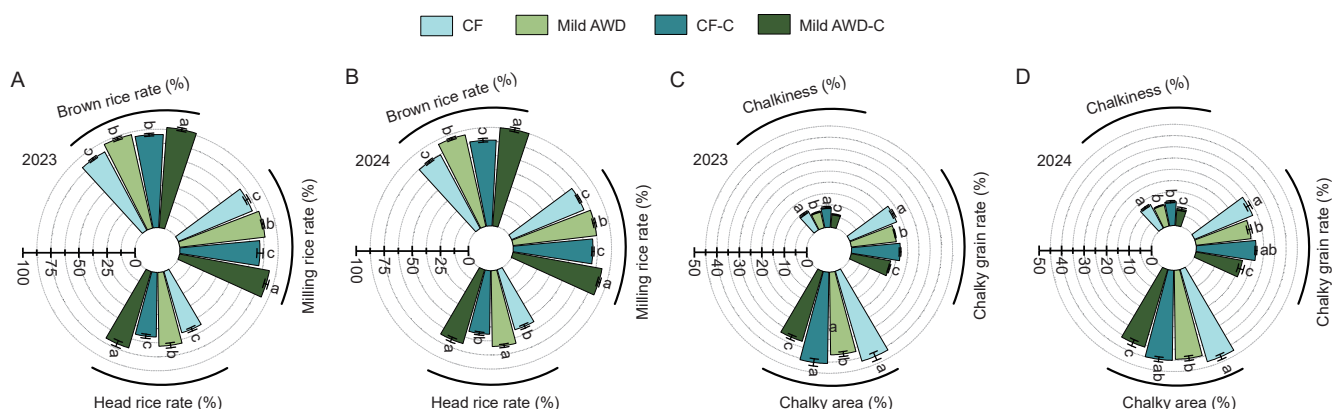


Fig. 5 Milling and appearance quality of rice grains under contrasting irrigation regimes and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C means the application of biochar. Bars mean SD ($n=4$). Different letters above the SD bars indicate the least significant difference at the $P=0.05$ level.

decreased the T_o , T_p , T_c , and ΔH_{gel} of rice starch, though these changes were not significant. Under the Mild AWD regime, however, the same parameters followed a similar trend with statistically significant differences. Irrespective of whether biochar was applied, the Mild AWD regime notably lowered the T_o , T_p , T_c , and ΔH_{gel} of rice starch when relative to the CF regime. Co-applying the Mild AWD regime and biochar exerted a more notable decrease in the T_o , T_p , T_c , and ΔH_{gel} of rice starch (Table 1).

3.12. In vitro digestion rate of rice starch

During the initial stage of enzymatic hydrolysis, the starch

digestion patterns were similar among all treatments. Divergence in the digestion curves became evident after 30 min of hydrolysis, revealing the following order of digestion rates: CF>CF-C>Mild AWD>Mild AWD-C. Specifically, regardless of the irrigation regime, biochar application reduced the digestion rate of rice starch, but this reduction reached a significant level only under the Mild AWD regime. Compared with the CF regime, the Mild AWD regime markedly retarded the digestion rate of rice starch, irrespective of biochar application, with the most pronounced effect observed when biochar was co-applied (Fig. 7).

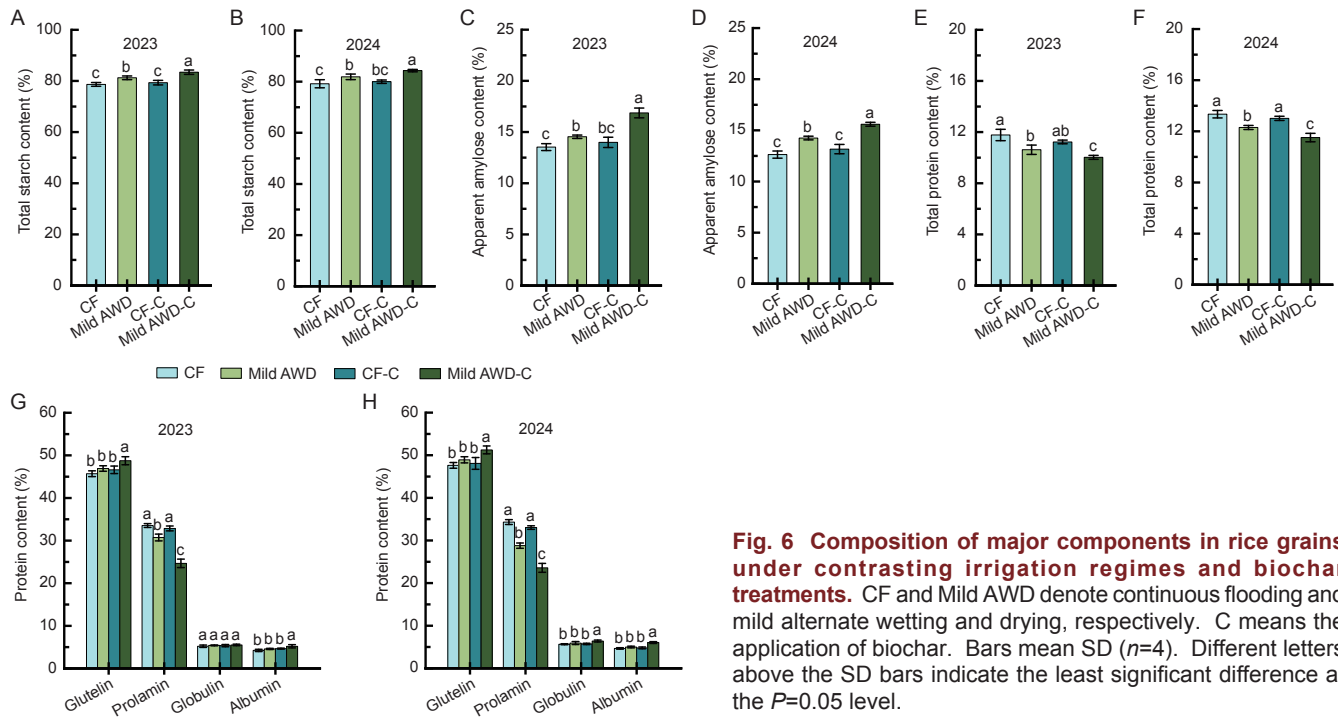


Fig. 6 Composition of major components in rice grains under contrasting irrigation regimes and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C means the application of biochar. Bars mean SD ($n=4$). Different letters above the SD bars indicate the least significant difference at the $P=0.05$ level.

4. Discussion

4.1. Effects of biochar co-applied with the Mild AWD regime on rice yield formation

In paddy agroecosystems, numerous investigations have explored the impacts of biochar or Mild AWD applied alone on rice yield, but existing conclusions remain highly controversial (Carrijo *et al.* 2017; Gao *et al.* 2024). Research has revealed that the regulatory impacts of biochar or Mild AWD used individually on rice yield are comprehensively shaped by a range of factors (e.g., regional climatic traits, soil physical and chemical properties, the intensity of soil drying), and biochar-related aspects (e.g., feedstock origins, pyrolysis techniques, and application approaches) (Carrijo *et al.* 2018; Purakayastha *et al.* 2019). Findings from this research suggest that Mild AWD regimes, especially when used in combination with biochar, markedly boosted rice yield (Fig. 1). This indicates that synergy for rice yield enhancement can be achieved through integrating a Mild AWD regime (i.e., maintaining SWP at -10 to -15 kPa at a depth of 15–20 cm) co-applied with biochar application.

4.2. Effects of biochar co-applied with the Mild AWD regime on rice milling and appearance quality

Chalkiness and head rice rate are key determinants of rice's market acceptability and economic worth. Notably, chalkiness not only impairs rice milling quality but also diminishes the eating quality of cooked rice (Zhao *et al.* 2022). It is well documented that the Mild AWD regime improves brown rice rate and head rice rate while reducing grain chalkiness relative to the CF regime (Xu *et al.* 2019; Ishfaq *et al.* 2021). In contrast, biochar application has been found to exert minimal or negligible effects on these parameters under normal irrigation regimes (Niu *et al.* 2018). Consistent with these prior findings, our results demonstrate that, relative to

CF, the Mild AWD regime markedly enhanced the milling and appearance quality of grains (e.g., head rice rate increased 15.5–21.3%, chalkiness decreased 18.7–21.2%), while biochar application under CF conditions showed no significant effects (e.g., head rice rate increased 1.17–1.50%, chalkiness decreased 6.42–11.0%) (Fig. 5). Chalkiness is typically formed due to insufficient starch accumulation during grain filling, which results in weak bonding between starch granules and increases susceptibility to breakage during milling, thereby reducing the head rice rate (Chen *et al.* 2025a). Our data further demonstrated a positive correlation among ROA, LPR, starch synthase activity, and starch accumulation, with the latter showing a positive correlation with head rice rate and a negative correlation with chalkiness (Appendix F). Most remarkably, when the Mild AWD regime and biochar were applied together, they synergistically enhanced these quality traits to a greater extent than either practice alone (e.g., head rice rate increased 23.1–24.6%, chalkiness decreased 36.4–38.2%) (Fig. 5). These results demonstrate that co-applying the Mild AWD regime with biochar optimized endosperm starch synthesis and accumulation, enhanced grain structural stability, and thereby significantly improved milling and appearance quality.

4.3. Effects of biochar co-applied with the Mild AWD regime on rice cooking and eating quality (ECQ) of rice

Pasting and thermal properties are key indicators of cooked rice texture and taste, and core criteria for evaluating rice ECQ (Lu *et al.* 2022; Ma *et al.* 2024). Prior research has demonstrated that the Mild AWD regime can improve rice ECQ by increasing PV and BDV, while decreasing HV, FV, SBV, and gelatinization temperature (GT) (Xu *et al.* 2025a, b; 2026). Under normal irrigation, biochar application can also

Table 1 Thermal behavior and pasting properties of rice starch as affected by irrigation and biochar treatments¹⁾

Year/Treatment ²⁾	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J g ⁻¹)	PV (cP)	HV (cP)	BDV (cP)	FV (cP)	SBV (cP)
2023									
CF	65.9 a	70.8 a	77.0 a	11.8 a	2,893 d	1,925 c	968 c	2,376 c	-517 a
Mild AWD	64.4 a	69.1 b	75.2 b	10.4 b	3,027 b	1,985 b	1,041 b	2,467 b	-560 b
CF-C	65.5 a	70.2 ab	76.8 a	11.7 a	2,943 c	1,945 bc	998 c	2,411 c	-532 ab
Mild AWD-C	62.1 b	67.6 c	73.0 c	9.16 c	3,441 a	2,230 a	1,210 a	2,834 a	-606 c
2024									
CF	65.3 a	70.2 a	76.5 a	11.7 a	2,875 c	1,918 c	957 c	2,371 c	-504 a
Mild AWD	63.7 b	69.1 a	74.9 b	10.2 b	2,999 b	1,976 b	1,022 b	2,456 b	-542 b
CF-C	65.0 a	69.2 a	75.5 ab	11.3 ab	2,908 c	1,934 c	974 c	2,388 c	-520 a
Mild AWD-C	62.3 c	66.0 b	72.3 c	9.09 c	3,386 a	2,202 a	1,184 a	2,767 a	-619 c
Analysis of variance									
Year (Y)	NS	*	*	NS	**	NS	**	*	NS
Irrigation (I)	**	**	**	**	**	**	**	**	**
Biochar (B)	**	**	**	NS	**	**	**	**	**
Y×I	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y×B	NS	NS	NS	NS	NS	NS	NS	NS	NS
I×B	*	*	**	NS	**	**	**	**	**
Y×I×B	NS	NS	NS	NS	NS	NS	NS	NS	NS

¹⁾ T_o , onset temperature; T_p , peak temperature; T_c , conclusion temperature; ΔH_{gel} , gelatinization enthalpy; PV, peak viscosity; HV, hot paste viscosity; BDV, breakdown viscosity; FV, final viscosity; SBV, setback viscosity.
²⁾ CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C indicates biochar application. *, $P<0.05$; **, $P<0.01$; NS, not significant at $P=0.05$. Different lowercase letters within the same column indicate significant differences among treatments in the same year at $P=0.05$ ($n=4$).

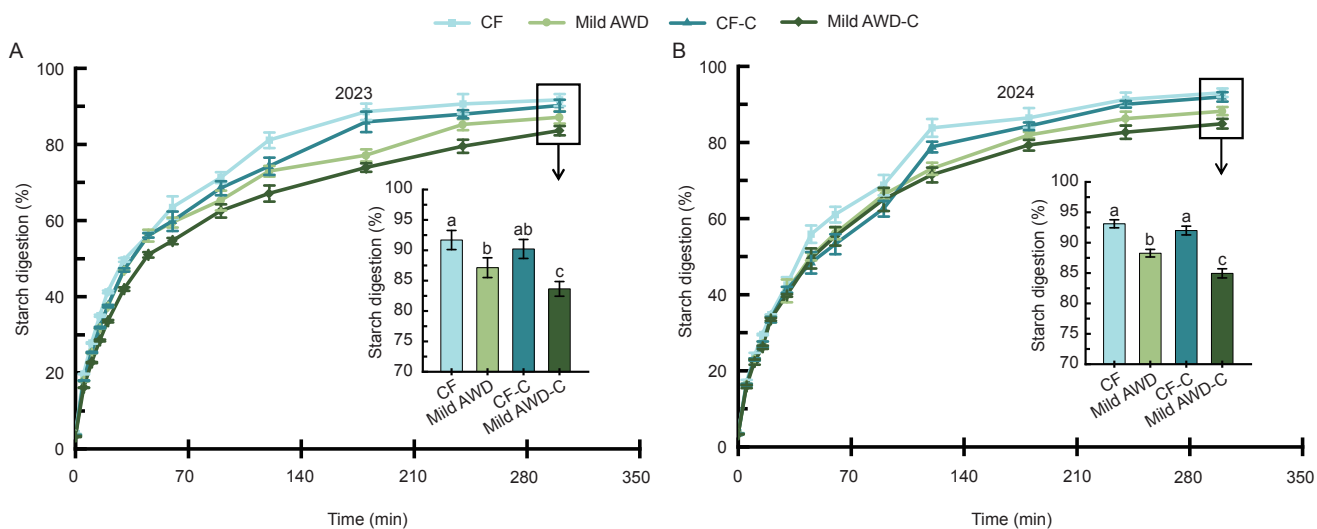


Fig. 7 Starch digestion characteristics of rice grains under contrasting irrigation and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C means the application of biochar. Bars mean SD ($n=4$). Different letters above the SD bars indicate the least significant difference at the $P=0.05$ level.

enhance rice taste by increasing PV, HV, and BDV, while reducing GT (Gong *et al.* 2020). It has been verified that greater PV and BDV, along with lower SBV, signal superior ECQ of rice (Yang *et al.* 2022). Additionally, better cooking quality is associated with lower GT and ΔH_{gel} , meaning that rice takes less time to cook (Zhang *et al.* 2016).

The findings of our study demonstrated that, compared with the CF regime, the Mild AWD regime notably increased the PV, FV, HV, and BDV of rice flour, while decreasing the SBV and the GT and ΔH_{gel} of starch. Under the CF regime, biochar application showed an analogous trend, but the impact lacked statistical significance (Table 1). These results

further confirm that both treatments (i.e., Mild AWD or CF-C) alone can improve rice ECQ to some extent. The combined application of the Mild AWD regime and biochar significantly enhanced the activity of GBSS (Fig. 4-C and D), leading to a marked increase in AAC (Fig. 6-C and D), which showed a negative correlation with GT and ΔH_{gel} (Appendix F). Consequently, the elevated AAC directly contributed to the observed reduction in GT and ΔH_{gel} . Moreover, the overall boost in starch synthesis and accumulation resulted in a dilution of grain protein content. Since a negative correlation exists between protein content and starch gelatinization efficiency (He *et al.* 2021; Lu *et al.* 2022), the reduced

protein proportion further facilitated starch pasting, leading to higher PV and BDV. Notably, the optimal rice ECQ was achieved with the combined use of Mild AWD and biochar, demonstrating a synergistic optimization of the AAC and protein balance. In conclusion, Mild AWD combined with biochar optimizes rice ECQ by both promoting amylose synthesis to modify starch thermal properties and reducing protein content to alleviate its inhibitory effect on starch gelatinization.

4.4. Effects of biochar co-applied with Mild AWD regime on rice nutritional quality

Proteins in cereals are a vital source of protein in the human diet, and their composition and content serve as key indices of nutritional quality (Peng *et al.* 2014, 2024). Earlier research has demonstrated that applying the Mild AWD regime reduces the protein content in milled rice and alters the proportion of protein components relative to the CF regime (Xu *et al.* 2019, 2025). Moreover, under the CF regime, biochar application markedly enhances the protein content in *indica* rice but exerts no significant effect on *japonica* rice (He C *et al.* 2024). Notably, during this study, under the CF regime, applying biochar exerted no significant effect on protein fractions, which may be attributed to factors such as rice variety, biochar feedstock type, or soil properties. Under the Mild AWD regime, the total protein content and prolamin content were notably reduced compared with those under the CF regime, whereas globulin and albumin contents showed no significant differences, and glutelin content increased significantly. Interestingly, when the Mild AWD regime and biochar were co-applied, all protein fractions except prolamin (whose content decreased further) increased significantly. Given that prolamin is poorly digestible and lysine-deficient, whereas glutelin (and albumin) are highly digestible and lysine-rich (Peng *et al.* 2014; He *et al.* 2021), the observed shifts indicate that the reduction in total protein under Mild AWD is primarily driven by the decline in low-nutritional-value prolamin. Consequently, the Mild AWD regime may not compromise the overall nutritional quality of cereal proteins; rather, it may improve the nutritional profile by increasing the relative proportion of high-quality proteins. This beneficial effect is synergistically enhanced when biochar is co-applied, leading to a more favorable protein composition for nutritional quality (Fig. 6-G and H). We propose that this optimization of protein composition is directly linked to the enhanced carbon sink strength under the combined treatment. The strong demand for starch synthesis preferentially directs nitrogen resources toward synthesizing high-quality components such as glutelin, while reducing the synthesis of prolamin. This reflects a coordinated carbon–nitrogen metabolism, where dominant carbon metabolism regulates the compositional allocation of nitrogen metabolism (MacNeill *et al.* 2017; Cao *et al.* 2024).

The digestibility of starchy foods directly affects human health, as rapid digestion causes a sharp rise in blood glucose, increasing the risk of metabolic diseases like diabetes (Jenkins *et al.* 1988). Cereals of high quality that contain high amylose content exhibit diminished enzymatic ability to digest starch, a factor that endows them with slow

digestion and glucose-lowering properties (Gebre *et al.* 2024). Nevertheless, information regarding the impacts of either the Mild AWD regime or biochar application on starch digestibility of rice remains limited. In this research, applying biochar exerted nearly no impact on the starch digestion rate under the CF regime. Compared with the CF regime, applying the Mild AWD regime markedly slowed down the starch digestion rate of rice. However, when biochar was co-applied with the Mild AWD regime, the starch digestion rate decreased to its lowest level, showing an inverse correlation with the increased AAC (Fig. 7; Appendix F). This aligns with earlier findings that the starch digestion rate exhibits a negative correlation with amylose content (Gebre *et al.* 2024; Chen *et al.* 2025a). In addition to the elevated AAC, the protein matrix can also impose physical constraints on starch granules, thereby modifying their internal structure (Hu *et al.* 2025; Huang *et al.* 2025). Such structural modifications persist after starch isolation and purification and may thus contribute to a further reduction in digestion rate. The results suggest that, driven by the enhancement in AAC, co-applying the Mild AWD regime and biochar synergistically lowers the starch digestion rate in rice, thereby potentially lowering the risk of complications associated with fast changes in blood glucose levels.

4.5. Mechanisms underlying the co-application of biochar and Mild AWD for enhancing grain yield and quality in rice

In this study, two potential mechanisms may explain the rise in rice grain yield and quality observed under the Mild AWD regime. The first aspect is the ‘compensatory effect’. Relative to the CF regime, the Mild AWD regime decreased the maximum tiller number and total leaf area at the HD, while significantly increasing the productive tiller number, effective leaf area, shoot dry weight, root dry weight, and root/shoot ratio, as well as NSC remobilization (Figs. 2 and 3-C and D; Appendix E). Consequently, the percentage of productive tillers was notably elevated under the Mild AWD regime. Minimizing surplus vegetative growth improves canopy quality by limiting water consumption from nonproductive tillers and reducing transpiration from excessive leaf area. The second is the ‘rewatering effect’, where the Mild AWD regime significantly enhances ROA and LPR of plants during rewatering — effects not observed under the conventional CF regime or in soil-drying periods (Appendix D). Furthermore, the rewatering effect also significantly enhanced the activities of key enzymes involved in starch biosynthesis (e.g., AGPase, GBSS, SSS, and SBE) (Fig. 4). The elevated activity of sucrose-to-starch conversion enzymes indicates a greater capacity to import photoassimilates into the grain (Yang and Zhang 2010; Zhang *et al.* 2025). Collectively, these two effects (i.e., the compensatory and rewatering effects) act synergistically to promote the accumulation of photosynthetic products in rice and enhance the transport and reallocation of NSC from source organs (vegetative organs) to sink organs (grains). Meanwhile, under the Mild AWD regime, the capacity of sink organs (grains) to absorb photosynthates was further improved, a process mainly

driven by the activity of sucrose invertase, thereby enhancing sink activity (the capacity to accommodate photosynthates imported from source organs) and ultimately leading to a marked boost in starch synthesis and accumulation in rice grains. Correlation analysis indicated that ROA and LPR were significantly positively correlated with starch synthase activity, NSC accumulation at HD and its translocation rate, and final starch accumulation (Appendix F). Notably, applying biochar further amplified the compensatory and rewatering effects under the Mild AWD regime, laying a solid foundation for improving rice yield and quality.

Under the Mild AWD regime, GBSS (which catalyzes amylose biosynthesis) exhibited the most substantial upregulation, whereas SSS and SBE (involved in amylopectin synthesis) showed comparatively attenuated responses (Fig. 4). This difference in enzyme activities directly resulted in a significant elevation in AAC (Fig. 6-C and D). Concurrently, the marked increase in starch synthesis and accumulation likely diluted the total protein content in grains, thereby primarily driving the observed decrease in its percentage. This phenomenon aligns with the ‘dilution effect’ in cereal crops — a mechanism where prioritizing carbon allocation to the primary sink (i.e., starch in rice grains) reduces the relative proportion of other storage compounds (e.g., proteins) in the final grains (MacNeill *et al.* 2017; Cao *et al.* 2024). Collectively, the coordinated modulation of starch metabolic enzymes — with a particular emphasis on the marked upregulation of amylose biosynthesis — and the dilution effect on proteins drive a holistic enhancement of rice grain quality. These improvements include enhanced textural properties, optimized cooking characteristics, and balanced nutrition, underscoring the potential of the Mild AWD regime as a sustainable means to optimize cereal grain quality.

4.6. Optimal thresholds of soil water level for rice production under the mild AWD regime

The extent of soil drying is a critical factor governing the yield-promoting effect of the AWD regime (Carrijo *et al.* 2018; Bo *et al.* 2024). According to several studies, if the midday LWP remains above -1.2 MPa without significant inhibition of photosynthesis, such conditions may be considered a central sign that rice plants are under optimal soil drying. Additional evidence shows that under the Mild AWD regime, even during water-sensitive growth stages, maintaining a SWP of -15 kPa at 15–20 cm depth can satisfy rice water demands (Yang *et al.* 2017). In this research, under the Mild AWD regime, the SWP, LWP, and LPR all met these thresholds (Appendices C and D). Importantly, despite differences in soil moisture content among soil types, steady SWP values helped maintain consistent plant water status (Zhang *et al.* 2021). Hence, it is recommended to adopt a SWP of -15 kPa at 15–20 cm depth, or a midday LWP between -0.7 and -1.0 MPa, as a general guideline for mild water management. This strategy not only helps maximize rice yield but also enhances grain quality. Notably, a key agronomic finding is the significant residual effect of the one-time 2023 biochar application: its yield-improving and comprehensive grain quality-enhancing effects remained statistically significant in

the subsequent season. This persistent efficacy underscores biochar’s long-term agronomic value and substantially boosts the integrated practice’s economic viability and practicality, as the initial biochar investment can be effectively amortized over multiple growing seasons.

Since this was a two-year, single-site study and since biochar impacts are highly dependent on factors such as feedstock type, production method, and dosage, future research should employ multi-year, multi-location trials involving diverse rice cultivars, soil types, climates, and agronomic practices to robustly assess the reproducibility of these findings. Such an approach will establish a more robust foundation for ensuring the wide applicability and long-term sustainability of these outcomes in real-world farming systems.

5. Conclusion

Co-applying the Mild AWD regime and biochar synergistically enhances rice yield and improves overall grain quality. Biochar amplifies the ‘rewatering effect’ and ‘compensatory effect’ under the Mild AWD regime, thereby significantly increasing plant ROA and LPR to promote NSC accumulation and translocation from vegetative organs to grains. Concurrently, increased activities of key starch synthases (e.g., AGPase, GBSS, SSS, and SBE) enhance starch biosynthesis and accumulation, establishing a physiological foundation for improved yield and quality. It also optimizes protein composition (e.g., increasing glutelin and decreasing prolamin) to improve nutritional quality and reduce *in vitro* starch digestibility, potentially lowering postprandial glycemic response. These findings provide a novel water-saving agronomic strategy for synergistic improvements in rice productivity and quality. This strategy shows great potential for advancing sustainable rice production amid global water scarcity and rising demand for high-quality staples, and future studies should systematically validate its effectiveness across diverse soil types, biochar feedstocks, and climatic conditions.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used generative AI tools to assist in improving the clarity and readability of the language. All content generated was carefully reviewed and revised by the authors as necessary, and the

authors take full responsibility for the final content of the article.

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