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

Haotian Chen[1, 2], Yunyi Gu[1, 2], Shengkai Yang[1, 2], Xiaohan Zhong[1, 2], Meijie Jia[1, 2], Wei Cai[1, 2], Kuanyu Zhu[1, 2], Junfei Gu[1, 2], Kaifeng Huang[3], Hao Zhang[1, 2], Zhiqin Wang[1, 2], Zujian Zhang[1, 2], Lijun Liu[1, 2], Jianhua Zhang[4, 5], Weiyang Zhang[1, 2#]

[1] *Jiangsu Key Laboratory of Crop Genetics and Physiology/Jiangsu Key Laboratory of Crop Cultivation and Physiology/ Agricultural College*, *Yangzhou University*, *Yangzhou 225009*, *China*

[2] *Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops*, *Yangzhou University*, *Yangzhou 225009*, *China*

[3] *School of Life Science*, *Guizhou Normal University*, *Guiyang 550001*, *China*

[4] *Department of Biology*, *Hong Kong Baptist University*, *Hong Kong 999077*, *China*

[5] *The State Key Laboratory of Agrobiotechnology*, *The Chinese University of Hong Kong, Hong Kong 999077*, *China*

Highlights[1]

Co-application of 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, 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

#Correspondence Weiyang Zhang, E-mail: wyz@yzu.edu.cn

Advanced Publication

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 and enhanced 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; 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; Xiao *et al*. 2025). On the other hand, other research has indicated that the adoption of the AWD regime does not increase rice yield and may even lead to a yield reduction (Carrijo *et al*. 2017; Gao *et al*. 2024). With respect to grain quality, research findings have indicated that the AWD regime exerts a positive effect on rice milling and appearance quality, while also 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), as 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 the moisture regulation of the regime. Notably, biochar-induced improvements in root zone

Advanced Publication

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 alternate wetting and drying 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 alternate wetting and drying 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 dependency 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, whether they regulate crop physiological and population status by enhancing the rewetting effect and compensatory effect, thereby further influencing key starch synthases and protein fractions, and ultimately improving rice quality, remains further verification. Therefore, 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 of premium rice under population growth and enhancing the resilience of rice systems to climate change and water scarcity.

Therefore, through a two-year field experiment, this study aims to systematically validate the synergistic effects of co-applying biochar and Mild AWD. 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) test whether biochar reinforces the rewetting and compensatory effects of Mild AWD by stabilizing root-zone moisture; (2) determine 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.

Advanced Publication

2. Material and methods

2.1. Plant materials and cultivation

From 2023 to 2024, researchers performed a two-year field trial at the Jiangwang Experimental Farm 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 days 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 hours prior to transplanting. Topdressing with urea (48 kg N ha^{-1} each) was administered at 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 via buffer strips with a width of 0.8 m and plastic barriers 0.5 m in depth 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 days 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 every irrigation method, experimental groups were configured to include biochar or not include it. For the groups receiving biochar, a one-time addition of 10 t ha^{-1} biochar was administered to each group prior to rice transplanting, specifically during the land preparation phase, and this application was limited to 2023 alone. The biochar used resulted from wheat straw pyrolysis carried out at a temperature of 500℃, 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 at 10.4, a carbon-to-nitrogen ratio of 52.7, and a surface area of 56.1 m^2 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

Advanced Publication

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) in accordance with the method reported 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 number. 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 ℃, then dried at 75 ℃ until they reached 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 via the anthrone-sulfuric acid colorimetric method, with reference to the protocol that was outlined by Cock *et al*. (1976). To minimize the impact of edge effects, four rice plants showing consistent growth were selected from the third row within each test unit for the determination of the above-mentioned indices.

Enzyme extraction and activity assays At the 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 Chen *et al*. (2025b). Every chemicals and enzymes essential to these assays were purchased from Sigma Chemical Company (St Louis, USA).

Final harvesting At the MA, assessments of grain yield and its constituent components were carried out following the methodology put forward by Yoshida *et al*. (1976). Grain yield was determined from a harvested section of 6.0 m^2 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 m^2 section within each experimental unit.

Milling and appearance quality At the 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℃ 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 ℃, passed over a 150 μm 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 underwent digestion with 10 mL concentrated sulfuric acid (98%) at 420 ℃ over a 90-min period, 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

Advanced Publication

sequentially extracted with distilled water, 5% NaCl, 70% ethanol, and 0.1% NaOH. Each fraction content was determined following a modified Coomassie brilliant blue assay based on Bradford (1976).

Thermal properties The thermal characteristics of starch were examined using a Netzsch DSC 200-F3 differential scanning calorimeter in accordance with 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).

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} through a standard thermal profile: heating from 50 to 95 ℃ at 12 ℃ min^{-1} and subsequent cooling to 50 ℃. Data analysis was conducted *via* Thermal Cycle for Windows software.

***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 ℃ with shaking. Aliquots were collected at intervals over 300 minutes, 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 allows for the conversion of glucose mass into 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 the significance threshold set at $P<0.05$. 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 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 a 18.7% gain in 2023 and an 13.4% gain 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 E). 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

Advanced Publication

3–6 days for the SWP to drop to –15 kPa under the Mild AWD regime. Notably, under identical irrigation conditions and measured on the identical 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 B). However, during the soil drying stages (D1, D2, D3, and D4), regardless of biochar application, LWP was markedly lower under the Mild AWD regime than under the CF regime. After rewatering (W1, W2, W3, and W4), LWP levels became comparable between the two irrigation regimes (Appendix B).

3.3. ROA and LPR

In comparison to the treatment without biochar, applying biochar generally brought about a notable rise in rice ROA under both irrigation regimes, with a more pronounced effect observed 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 C). During the soil drying period under the CF regime or the Mild AWD regime, 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, D2, D3, and D4). After rewatering (W1, W2, W3, and W4), the Mild AWD regime markedly increased the plant LPR, with the most pronounced effect observed when biochar was applied under the Mild AWD regime.

3.4. Tillering dynamics and LAI

The Mild AWD regime led to a decrease in the number of stems and tillers, along with the total leaf area of rice, during key growth stages compared to the CF regime independent of biochar application. However, it markedly enhanced the percentage of productive tillers and, at the HD, enhanced 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 D).

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, the use of biochar brought about a slight increase in NSC accumulation in stems (including leaf sheaths) at HD, and NSC translocation from stems (including leaf sheaths) to grains during the 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

Advanced Publication

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 starch accumulation amount 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 rewatering period). Independent of biochar application, the Mild AWD regime notably boosted the activities of AGPase, GBSS, SSS, and SBE when compared to the CF regime, with particularly pronounced increments observed when combined with biochar application (especially during the rewatering period). Additionally, after rewatering, AGPase and GBSS activities showed greater increases, followed by SSS, whereas SBE was relatively less affected by rewatering (Fig. 4).

3.8. Milling and appearance quality

Under the CF regime, applying biochar only slightly enhanced the brown rice rate, milled rice rate, and head rice rate of the grains, 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). Whether biochar was applied or not, relative to the CF regime, the Mild AWD regime markedly enhanced the milling and appearance quality of the grains, with a more pronounced effect when it was co-applied with biochar (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. Thermal properties of rice starch

Under the CF regime, applying biochar generally slightly 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 decreasing effect on the T_o, T_p, T_c, and ΔH_{gel} of rice starch (Table 1).

3.11. 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 brought about a significant rise in these viscosity parameters (PV, HV, BDV, FV) and decreased SBV of rice flour. Notably, these positive effects were further enhanced when it was co-applied with biochar (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 minutes 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. In comparison to 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 with the Mild AWD regime (Fig. 7).

4. Discussion

4.1. Effects of biochar co-applied with 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 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 dcreased 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 dcreased 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

Advanced Publication

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 dcreased 36.4–38.2%) (Fig. 5). These results demonstrate that co-applying the Mild AWD regime with biochar optimized starch synthesis and accumulation, enhanced grain structural stability, and thereby significantly improved milling and appearance quality.

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

Thermal and pasting 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 have 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*. 2025). Under normal irrigation, biochar application can also enhance rice taste by increasing PV, HV, and BDV, as well as 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 rice takes less time for cooking (Zhang *et al*. 2016).

The findings of our study demonstrated that, in comparison to the CF regime, the Mild AWD regime notably increased the PV, FV, HV, and BDV of rice flour, while decreasing the SBV, as well as 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 act as key indices for assessing the 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, whis 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

Advanced Publication

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 actually 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).

Starchy foods digestibility 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. In comparison to 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 of rice, thereby potentially lowering the risk of complications linked to 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

The mechanisms by which Mild AWD enhances rice grain yield are yet to be clarified. There are two potential reasons explaining the rise in rice grain yield 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 (Fig. 3-C and D; Appendices B and D). Consequently, the percentage of productive tillers was notably elevated under the Mild AWD regime. Minimising 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 plant during rewatering—effects not observed under the conventional CF regime or in soil-drying periods (Appendix C). 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 is indicative of a greater capacity for importing photoassimilates into the grain (Yang and Zhang 2010). Collectively, these two effects (i.e., the compensatory and rewatering effects) act synergistically to promote the accumulation of

Advanced Publication

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 the HD and its translocation rate, as well as with the final starch accumulation (Appendix F). Notably, applying biochar further amplified the compensatory effect and rewatering effect under the Mild AWD regime, laying an important material basis for the improvement of 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 differential 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 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.

Advanced Publication

4.6. Optimal thresholds of soil water level for rice production under 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 B and C). 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 reduces 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.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (32372214), the National Key Research and Development Program (2022YFD2300304), the Jiangsu Agriculture Science and Technology Innovation Fund, China (CX(23)1035), the Priority Academic Program Development of Jiangsu Higher Education Institutions, China (PAPD-2020-01), and the Top Talent Supporting Program of Yangzhou University, China.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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

During the preparation of this manuscript, the authors used generative Al 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.

References

Ali I, Zhao Q, Wu K, Ullah S, Iqbal A, Liang H, Zhang J, Muhammad I, Amanullah, Khan A, Khan A A, Jiang L. 2022. Biochar in combination with nitrogen fertilizer is a technique: To enhance physiological and morphological traits of Rice (*Oryza sativa* L.) by improving soil physio-biochemical properties. *Journal of Plant Growth Regulation*, **41**, 2406–2420.

Aznan A, Viejo C G, Pang A, Fuentes S. 2023. Review of technology advances to assess rice quality traits and consumer perception. *Food Research International*, **172**, 113105.

Bo Y, Wang X, Groenigen K J V, Linquist B A, Müller C, Li T, Yang J, Jägermeyr J, Qin Y, Zhou F. 2024. Improved alternate wetting and drying irrigation increases global water productivity. *Nature Food*, **5**, 1005–1013.

Bradford M M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248–254.

Advanced Publication

Cao S, Liu B, Wang D, Rasheed A, Xie L, Xia X, He Z. 2024. Orchestrating seed storage protein and starch accumulation toward overcoming yield-quality trade-off in cereal crops. *Journal of Integrative Plant Biology*, **66**, 468–483.

Carrijo D R, Akbar N, Reis A F B, Li C, Gaudin A C M, Parikh S J, Green P G, Linquist B A. 2018. Impacts of variable soil drying in alternate wetting and drying rice systems on yields, grain arsenic concentration and soil moisture dynamics. *Field Crops Research*, **222**, 101–110.

Carrijo D R, Lundy M E, Linquist B A. 2017. Rice yields and water use under alternate wetting and drying irrigation: A meta-analysis. *Field Crops Research*, **203**, 173–180.

Chen H, Jia M, Luo S, Liu Y, Zhang Y, Zhu K, Wang W, Xu Y, Gu J, Zhang H, Wang Z, Liu L, Zhang J, Yang J, Zhang W. 2025a. Controlled soil drying mitigates the effects of high-temperature stress on rice quality by enhancing starch accumulation and stabilizing starch structure and functional properties. *Carbohydrate Polymers*, **362**, 123688.

Chen H, Luo S, Ma Y, Jia M, Cai W, Liu Y, Zhu K, Wang W, Xu Y, Gu J, Zhang H, Wang Z, Liu L, Zhang J, Yang J, Zhang W. 2025b. Brassinosteroids regulate amylose more sensitively than amylopectin: Essential for high-amylose premium rice. *Food Chemistry*, **493**, 146021.

Chen L, Guo L, Deng X, Pan X, Liao P, Xiong Q, Gao H, Wei H, Dai Q, Zeng Y, Zhang H. 2023. Effects of biochar on rice yield, grain quality and starch viscosity attributes. *Journal of the Science of Food and Agriculture*, **103**, 5747–5753.

Chen M, McClung A M, Rohila J S, Barnaby J Y. 2021. Effects of alternate wetting and drying irrigation management and air temperature during grainfill on rice grain physicochemical and functionality traits of US inbred varieties. *Cereal Chemistry*, **98**, 980–993.

Chen P, Liu J, Zhao X. 2023. Demand elasticity estimation and market potential of middle and high-end rice in China: A case study of the Yangtze River delta market. *Chinese Journal oF Rice Science*, **37**, 102-112. (in Chinese)

Chi W, Nan Q, Liu Y, Dong D, Qin Y, Li S, Wu W. 2024. Stress resistance enhancing with biochar application and promotion on crop growth. *Biochar*, **6**, 43.

Chu G, Chen T, Wang Z, Yang J, Zhang J. 2014. Reprint of "Morphological and physiological traits of roots and their relationships with water productivity in water-saving and drought-resistant rice". *Field Crops Research*, **165**, 36–48.

FAO (Food and Agriculture Organization of the United Nations). 2024. Land and water statistics. [2024-8-9]. http://www.fao.org/faostat/en/#data/QC

Gao R, Zhuo L, Duan Y, Yan C, Yue Z, Zhao Z, Wu P. 2024. Effects of alternate wetting and drying irrigation on yield, water-saving, and emission reduction in rice fields: A global meta-analysis. *Agricultural and Forest Meteorology*, **353**, 110075.

Gebre B A, Zhang C, Li Z, Sui Z, Corke H. 2024. Impact of starch chain length distributions on physicochemical properties and digestibility of starches. *Food Chemistry*, **435**, 137641.

Gong D, Xu X, Wu L A, Dai G, Zheng W, Xu Z. 2020. Effect of biochar on rice starch properties and starch-related gene expression and enzyme activities. *Scientific Reports*, **10**, 16917.

Han S, Liu X, Makowski D, Ciais P. 2025. Meta-analysis of water stress impact on rice quality in China. *Agricultural Water Management*, **307**, 109230.

Advanced Publication

He C, Deng F, Yuan Y, Huang X, He Y, Li Q, Li B, Wang L, Cheng H, Wang T, Tao Y, Zhou W, Lei X, Chen Y, Ren W. 2024. Appearance, components, pasting, and thermal characteristics of chalky grains of rice varieties with varying protein content. *Food Chemistry*, **440**, 138256.

He H, Long L, Fu Z. 2024. Improving rice quality through biochar application in China: A meta-analysis. *Journal of Environmental Management*, **371**, 122701.

He W, Wang L, Lin Q, Yu F. 2021. Rice seed storage proteins: Biosynthetic pathways and the effects of environmental factors. *Journal of Integrative Plant Biology*, **63**, 1999–2019.

Hu N, Qi W, Zhu J, Zhao F, Zheng M, Zhao C, Yan J, Liu J. 2025. Effect of endogenous protein on starch before and after post-harvest ripening of corn: Structure, pasting, rheological and digestive properties. *Food Chemistry*, **473**, 143039.

Huang M, Li L, Cheng X, Qiu R, Wang Y, Zhou X, Zong X. 2025. Study on the influence mechanism of endogenous protein components on the characteristics of distiller's grains starch. *Food Chemistry*, **486**, 144689.

Ishfaq M, Akbar N, Zulfiqar U, Ali N, Ahmad M, Anjum S A, Farooq M. 2021. Influence of water management techniques on milling recovery, grain quality and mercury uptake in different rice production systems. *Agricultural Water Management*, **243**, 106500.

Ishfaq M, Farooq M, Zulfiqar U, Hussain S, Akbar N, Nawaz A, Anjum S A. 2020. Alternate wetting and drying: A water-saving and ecofriendly rice production system. *Agricultural Water Management*, **241**, 106363.

Itoh H, Yamashita H, Wada K C, Yonemaru J. 2024. Real-time emulation of future global warming reveals realistic impacts on the phenological response and quality deterioration in rice. *Proceedings of the National Academy of Sciences of the United States of America*, **121**, e2316497121.

Jenkins D J A, Wolever T M S, Jenkins A L. 1988. Starchy foods and glycemic index. *Diabetes Care*, **11**, 149–159.

Jin P, Chen Z, Wang H, Lv R, Hu T, Zhou R, Zhang J, Lin X, Liu Q, Xie Z. 2024. 12-year continuous biochar application: Mitigating reactive nitrogen loss in paddy fields but without rice yield enhancement. *Agriculture, Ecosystems & Environment*, **375**, 109223.

Kumar A, Bhattacharya T, Shaikh W A, Roy A, Chakraborty S, Vithanage M, Biswas J K. 2023. Multifaceted applications of biochar in environmental management: A bibliometric profile. *Biochar*, **5**, 11.

Li W, Yang K, Hu C, Abbas W, Zhang J, Xu P, Cheng B, Zhang J, Yin W, Shalmani A, Qu L, Lv Q, Li B, He Y, Lai X, Xiong L, Zhang Q, Li Y. 2025. A natural gene on-off system confers field thermotolerance for grain quality and yield in rice. *Cell*, **188**, 3661-3678.e21.

Li Z, Li Z, Letuma P, Zhao H, Zhang Z, Lin W, Chen H, Lin W. 2018. A positive response of rice rhizosphere to alternate moderate wetting and drying irrigation at grain filling stage. *Agricultural Water Management*, **207**, 26–36.

Lin L, Zhang L, Cai X, Liu Q, Zhang C, Wei C. 2018. The relationship between enzyme hydrolysis and the components of rice starches with the same genetic background and amylopectin structure but different amylose contents. *Food Hydrocoll*, **84**, 406–413.

Liu X, Ciais P, Makowski D, Liang J. 2024. Warming leads to lower rice quality in East Asia. *Geophysical Research Letters*, **51**, e2024GL110557.

Advanced Publication

Lu X, Ma R, Zhan J, Wang F, Tian Y. 2022. The role of protein and its hydrolysates in regulating the digestive properties of starch: A review. *Trends in Food Science & Technology*, **125**, 54–65.

Ma Z, Zhu Y, Wang Z, Chen X, Cao J, Liu G, Li G, Wei H, Zhang H. 2024. Effect of starch and protein on eating quality of *japonica* rice in Yangtze River Delta. *International Journal of Biological Macromolecules*, **261**, 129918.

MacNeill G J, Mehrpouyan S, Minow M A A, Patterson J A, Tetlow I J, Emes M J. 2017. Starch as a source, starch as a sink: The bifunctional role of starch in carbon allocation. *Journal of Experimental Botany*, **68**, 4433–4453.

Niu T, Zheng G, Jiang Y, Zhao T, Zhang L, Zhong W, Li M, Chen L, Zhou J, Xie B. 2018. Effects of biochar on yield and quality of kengeng 5. *China Rice*, **24**, 76–79. (in Chinese)

Peng B, Kong H, Li Y, Wang L, Zhong M, Sun L, Gao G, Zhang Q, Luo L, Wang G, Xie W, Chen J, Yao W, Peng Y, Lei L, Lian X, Xiao J, Xu C, Li X, He Y. 2014. *OsAAP6* functions as an important regulator of grain protein content and nutritional quality in rice. *Nature Communications*, **5**, 4847.

Peng B, Liu Y, Sun X, Zhao Q, Qiu J, Tian X, Peng J, Zhang Z, Wang Y, Huang Y, Pang R, Zhou W, Qi Y, Sun Y, Wang Q, He Y. 2024. The *OsGAPC3* mutation significantly affects grain quality traits and improves the nutritional quality of rice. *Frontiers in Plant Science*, **15**, 1470316.

Prathap V, Ali K, Singh A, Vishwakarma C, Krishnan V, Tyagi A. 2019. Starch accumulation in rice grains subjected to drought during grain filling stage. *Plant Physiology and Biochemistry*, **142**, 440–451.

Purakayastha T J, Bera T, Bhaduri D, Sarkar B, Mandal S, Wade P, Kumari S, Biswas S, Menon M, Pathak H, Tsang D C W. 2019. A review on biochar modulated soil condition improvements and nutrient dynamics concerning crop yields: Pathways to climate change mitigation and global food security. *Chemosphere*, **227**, 345–365.

Bin Rahman A N M R, Zhang J H. 2023. Trends in rice research: 2030 and beyond. *Food and Energy Security*, **12**, e390.

Ramasamy S, ten Berge H F, Purushothaman S. 1997. Yield formation in rice in response to drainage and nitrogen application. *Field Crops Research*, **51**, 65–82.

Shi L, Zou Z, Zhu C, Wang H, Lin L, Wang J, Wei C. 2024. Structures, gelatinization properties and enzyme hydrolyses of starches from transparent and floury grains of rices subjected to field natural extreme high temperature. *Food Chemistry*, **459**, 140392.

Tollefson J. 2021. IPCC climate report: Earth is warmer than it's been in 125,000 years. *Nature*, **596**, 171–172.

Tomczyk A, Sokołowska Z, Boguta P. 2020. Biochar physicochemical properties: Pyrolysis temperature and feedstock kind effects. *Reviews in Environmental Science and Bio/Technology*, **19**, 191–215.

Tuong T P, Bouman B A. 2003. Rice production in water-scarce environments. [2003-8-6]. https://doi.org/10.1079/9780851996691.0053

Wang D, Li C, Parikh S J, Scow K M. 2019. Impact of biochar on water retention of two agricultural soils-A multi-scale analysis. *Geoderma*, **340**, 185–191.

Xiao Z, Zhang N, Zhang Y, Zhu K, Wang W, Zhang W, Gu J, Zhang G, Liu L, Zhang J, Yan X, Zhang H. 2025. Synergistic mechanism of moderate wetting drying irrigation improving rice productivity and environmental sustainability. *Field Crops Research*, **332**, 110035.

Advanced Publication

Xu Y, Gu D, Li K, Zhang W, Zhang H, Wang Z, Yang J. 2019. Response of grain quality to alternate wetting and moderate soil drying irrigation in rice. *Crop Scienc*e, **59**, 1261–1272.

Xu Y, Huang Y, Weng X, Traye I D, Tang S, Jiang X, Zhu K, Zhang W, Zhang H, Wang Z, Yang J. 2025. Cooking and eating quality of rice as affected by nitrogen fertilizers and alternate wetting and moderate drying irrigation at panicle stage. *Agricultural Water Management*, **318**, 109715.

Yang C, Lu S. 2021. Effects of five different biochars on aggregation, water retention and mechanical properties of paddy soil: A field experiment of three-season crops. *Soil and Tillage Research*, **205**, 104798.

Yang J, Zhang J. 2010. Grain-filling problem in 'super'rice. *Journal of Experimental Botany*, **61**, 1–5.

Yang J, Zhou Q, Zhang J. 2017. Moderate wetting and drying increases rice yield and reduces water use, grain arsenic level, and methane emission. *The Crop Journal*, **5**, 151–158.

Yang T, Xiong R, Tan X, Huang S, Pan X, Guo L. 2022. The impacts of post-anthesis warming on grain yield and quality of double-cropping high-quality *indica* rice in Jiangxi Province, China. *European Journal of Agronomy*, **139**, 126551.

Yoshida S, Forno D A, Cock J H, Gomez K. 1976. *Laboratory Manual for Physiological Studies of Rice*. International Rice Research Institute, The Philippines.

Yu Q, Dai Y, Wei J, Wang J, Liao B, Cui Y. 2024. Rice yield and water productivity in response to water-saving irrigation practices in China: A meta-analysis. *Agricultural Water Management*, **2**, 109006.

Zhang C, Zhou L, Zhu Z, Lu H, Zhou X, Qian Y, Li Q, Lu Y, Gu M, Liu Q. 2016. Characterization of grain quality and starch fine structure of two *japonica* rice (*Oryza sativa*) cultivars with good sensory properties at different temperatures during the filling stage. *Journal of Agricultural and Food Chemistry*, **64**, 4048–4057.

Zhang H, Zhang Y D, Huang Y, Wang B. 2023. Study on water consumption and growth characteristics of rice under different irrigation modes. *Water Saving Irrigation*, **4**, 25-31. (in Chinese)

Zhang S, Wang R, Jiang H, Zhang L, Zhang J. 2018. Meta-analysis of influence of water stress on China's paddy yield. *Jiangsu Agricultural Sciences*, **46**, 51-54. (in Chinese)

Zhang W, Meng J, Wang J, Fan S, Chen W. 2013. Effect of biochar on root morphological and physiological characteristics and yield in rice. *Acta Agronomica Sinica*, **39**, 1445–1451. (in Chinese)

Zhang W, Yu J, Xu Y, Wang Z, Liu L, Zhang H, Gu J, Zhang J, Yang J. 2021. Alternate wetting and drying irrigation combined with the proportion of polymer-coated urea and conventional urea rates increases grain yield, water and nitrogen use efficiencies in rice. *Field Crops Research*, **268**, 108165.

Zhao C, Qiu R, Zhang T, Luo Y, Agathokleous E. 2024. Effects of alternate wetting and drying irrigation on methane and nitrous oxide emissions from rice fields: A meta-analysis. *Global Change Biology*, **30**, e17581.

Zhao D, Zhang C, Li Q, Liu Q. 2022. Genetic control of grain appearance quality in rice. *Biotechnology Advances*, **60**, 108014.

Zhuang D, Si Z, Li F. 2014. Experimental research on water consumption law of paddy under different irrigation mode. *Water Saving Irrigation*, **8**, 1-3, 11. (in Chinese)

Advanced Publication

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

Year/Treatment[2)]	T_o (℃)	T_p (℃)	T_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	2893 d	1925 c	968 c	2376 c	–517 a
Mild AWD	64.4 a	69.1 b	75.2 b	10.4 b	3027 b	1985 b	1041 b	2467 b	–560 b
CF-C	65.5 a	70.2 ab	76.8 a	11.7 a	2943 c	1945 bc	998 c	2411 c	–532 ab
Mild AWD-C	62.1 b	67.6 c	73.0 c	9.16 c	3441 a	2230 a	1210 a	2834 a	–606 c
2024									
CF	65.3 a	70.2 a	76.5 a	11.7 a	2875 c	1918 c	957 c	2371 c	–504 a
Mild AWD	63.7 b	69.1 a	74.9 b	10.2 b	2999 b	1976 b	1022 b	2456 b	–542 b
CF-C	65.0 a	69.2 a	75.5 ab	11.3 ab	2908 c	1934 c	974 c	2388 c	–520 a
Mild AWD-C	62.3 c	66.0 b	72.3 c	9.09 c	3386 a	2202 a	1184 a	2767 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 $P=0.05$ ($n=4$).

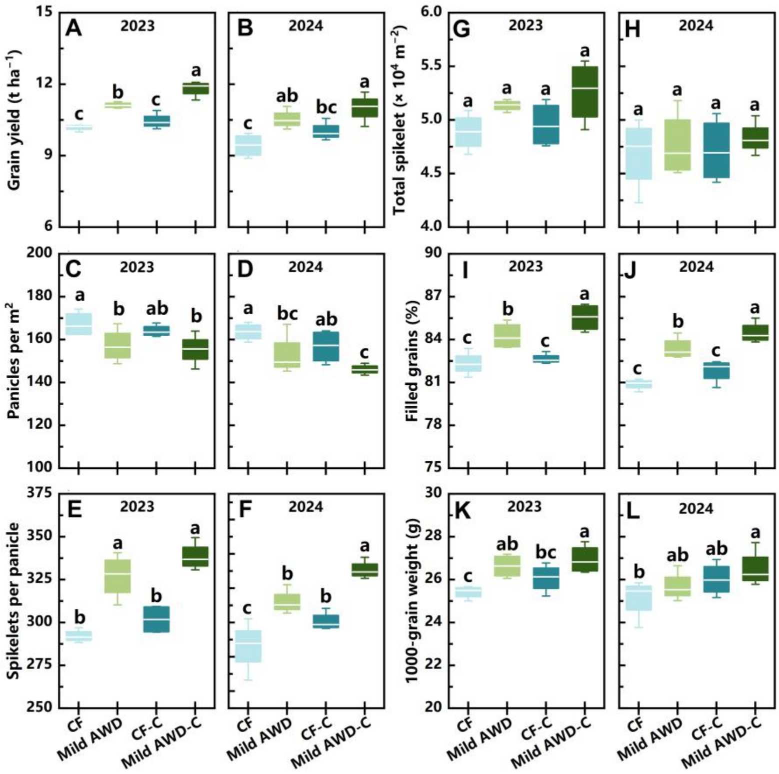

Fig. 1 Grain yield and its components of rice as affected by irrigation and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively, respectively. C indicates biochar application. Bars mean SD (*n*=4). Different letters above the SD bars indicate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

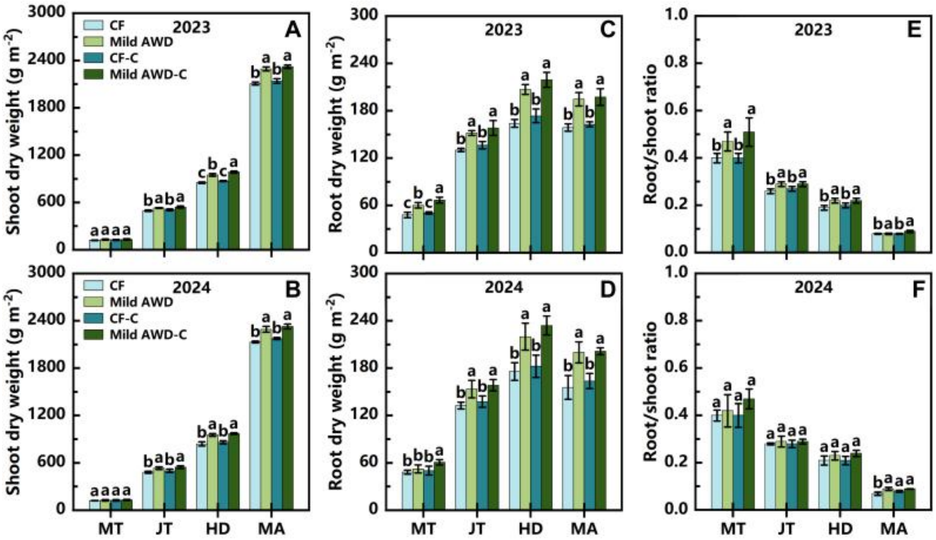

Fig. 2 Dry matter accumulation dynamics in rice under different irrigation regimes and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C means the application of biochar. MT, JT, HD and MA represent mid-tillering, jointing stage, heading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

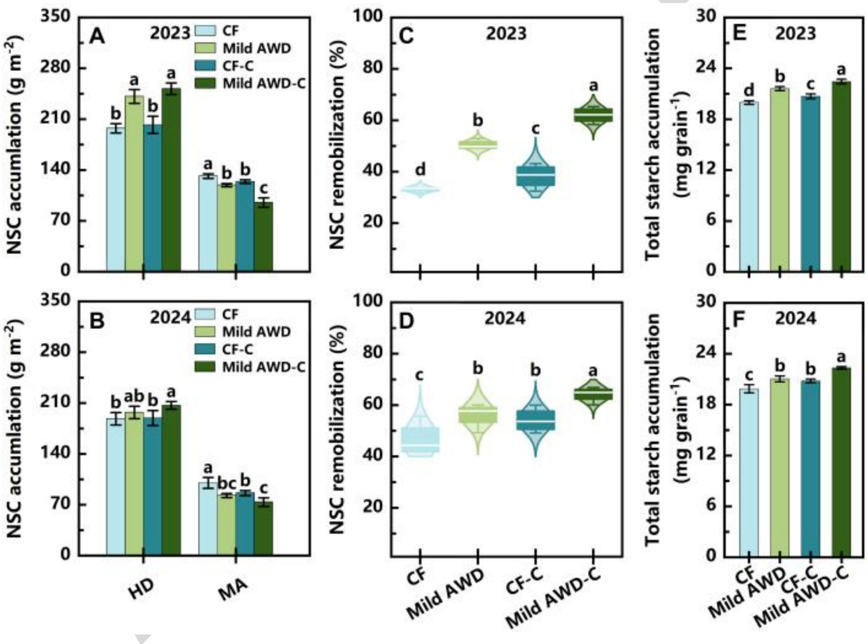

Fig. 3 Dynamics of non-structural carbohydrate accumulation and translocation in rice stems and starch accumulation in grains under contrasting irrigation regimes and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C means the application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

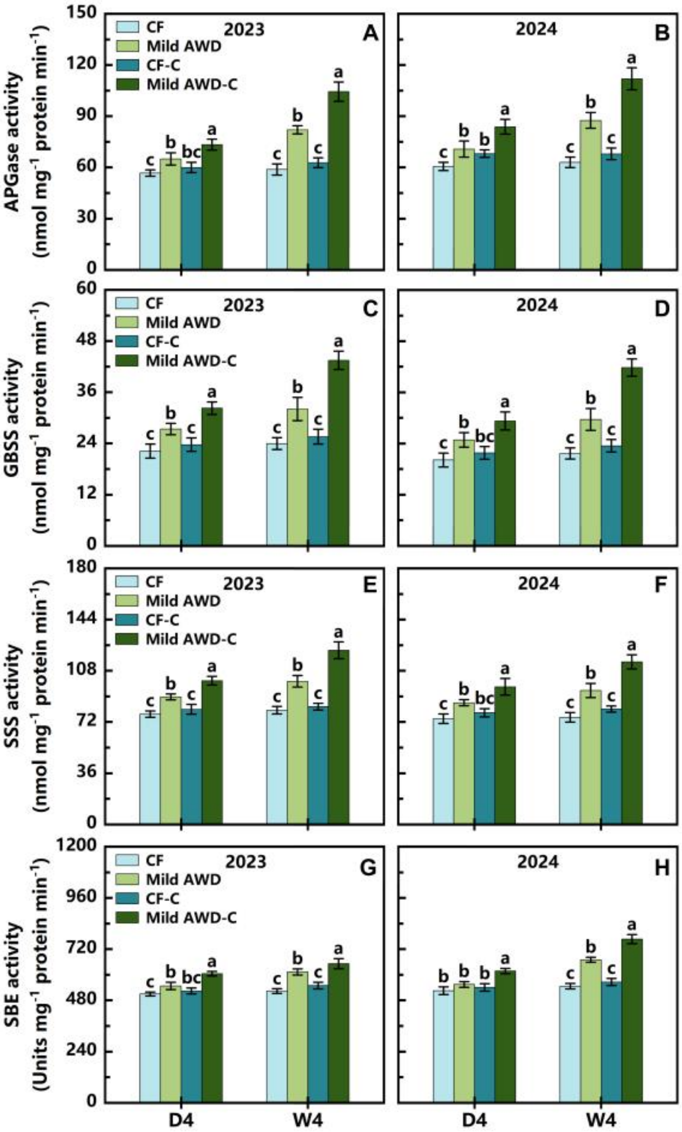

Fig. 4 Activities of key starch biosynthetic enzymes in rice grains at mid-grain filling stage under contrasting irrigation and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C means the application of biochar. AGPase, ADP-glucose pyrophosphorylase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

Advance Publication

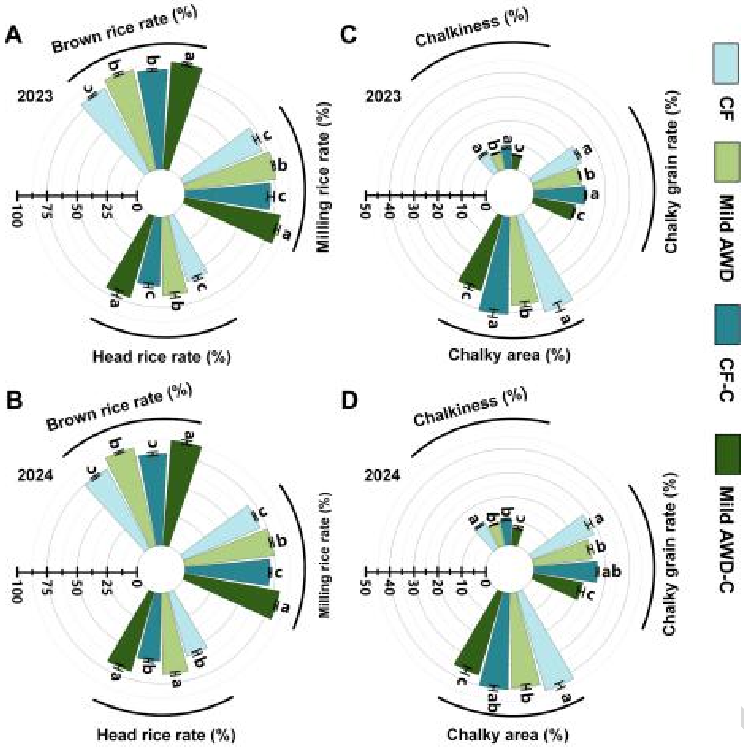

Fig. 5 Milling and appearance quality of rice grains under contrasting irrigation regimes and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C means the application of biochar. Bars mean SD (n=4). Different letters above the SD bars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

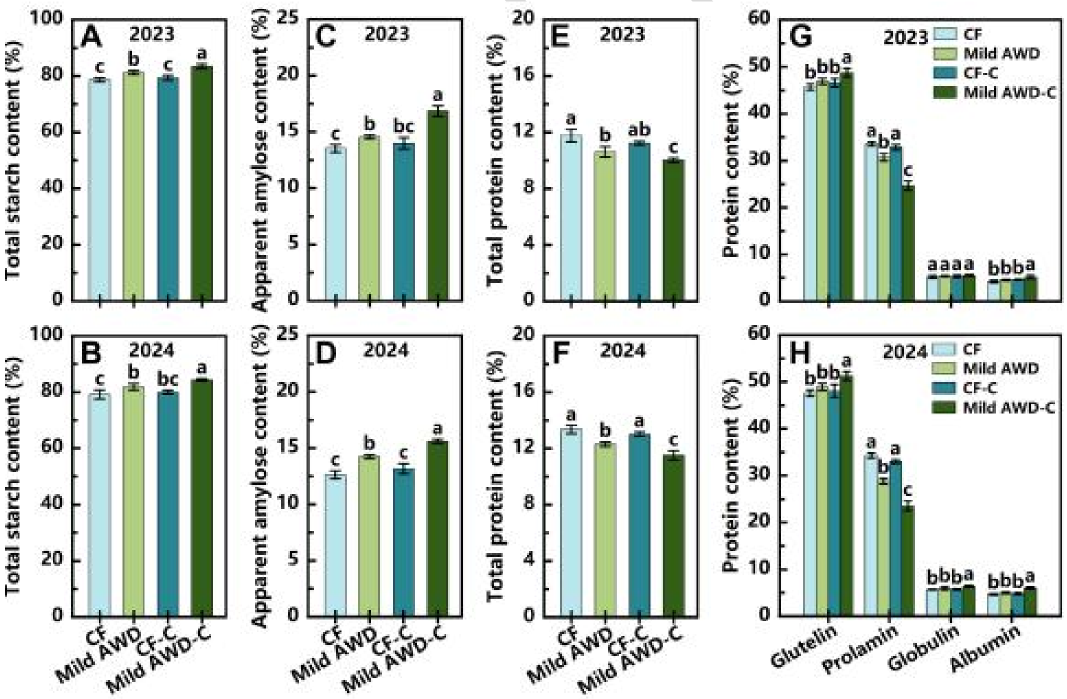

Fig. 6 Composition of major components in rice grains under contrasting irrigation regimes and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C means the application of biochar. Bars mean SD (n=4). Different letters above the SD bars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

Publication

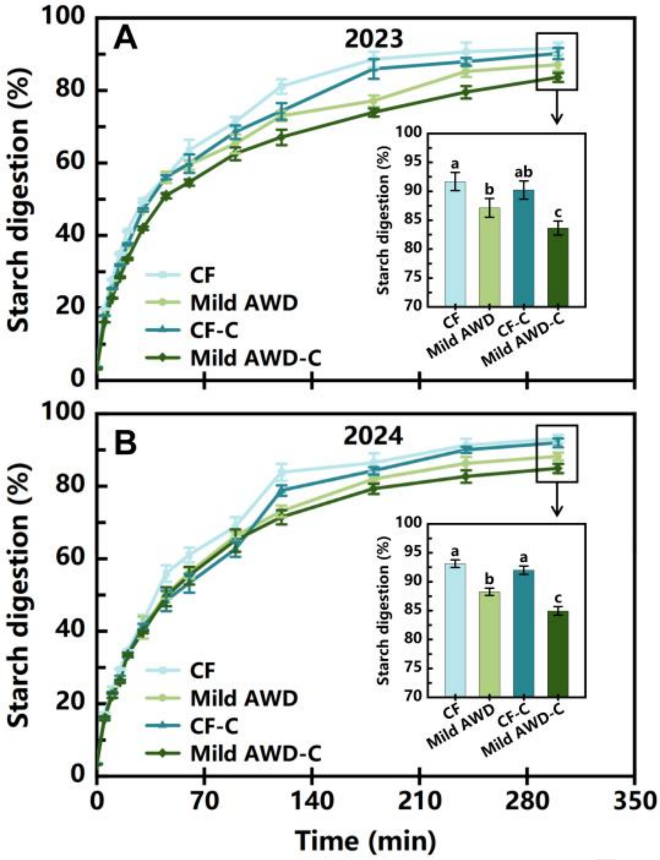

Fig. 7　Starch digestion characteristics of rice grains under contrasting irrigation and biochar treatments. CF and Mild AWD denote continuous flooding and mild alternate wetting and drying, respectively. C means the application of biochar. Bars mean SD (n=4).　Different letters above the SD bars indicate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