Journal of Hazardous Materials 502 (2026) 140973

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Integrating alternate wetting and drying irrigation with duckweed for potential microplastic mitigation in rice ecosystems

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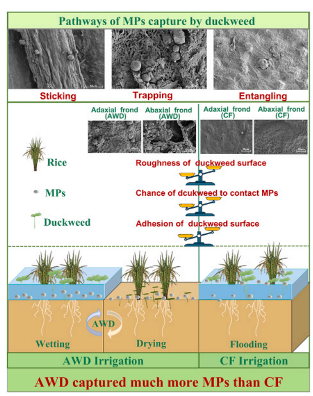
HIGHLIGHTS

- Alternate wetting and drying irrigation (AWD) resulted in duckweed capturing many more MPs, compared to CF.
- The increase in MPs captured under AWD was due to the higher capturing capacity of duckweed for MPs.
- The greatly increased MPs capturing capacity under AWD was primarily due to improved adhesion and physical interception.
- AWD showed greater retention of MPs on abaxial fronds than CF as indicated by well-modelled detachment curves of MPs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Adhesion
Caulobacterales
Capture
Detachment curve
Physical interception

ABSTRACT

Water–saving irrigation in rice fields can reduce water input and enhance water productivity, while widespread microplastics (MPs) contamination introduces an environmental risk. Duckweed is a hydrophyte that commonly floats in rice fields and can capture MPs. Regular harvesting of these contaminated plants prior to decomposition could prevent secondary pollution and ensures the permanent removal of accumulated MPs from the paddy ecosystem. However, it remains unknown whether water–saving irrigation, such as alternate wetting and drying irrigation (AWD), can promote MPs capturing through duckweed physio–anatomical modifications. In this study, MPs-contaminated rice-duckweed system was exposed to two irrigation regimes, i.e. conventional continuous flooding irrigation (CF) and AWD. The results showed that AWD led to duckweed capturing up to

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https://doi.org/10.1016/j.jhazmat.2025.140973
Received 20 June 2025; Received in revised form 18 December 2025; Accepted 27 December 2025
Available online 28 December 2025

16.0 –fold more MPs than under CF. We discovered that this increase was due to the considerably higher number of MPs particles per unit surface area (or per unit length) captured by duckweed, despite the reduction in duckweed coverage rate (42.4 %–48.8 %) and root length (23.2 %–67.7 %) during dry periods of AWD. We further revealed that the greatly increased MPs numbers per unit area on the frond of duckweed under AWD was primarily due to improved adhesion and physical interception, achieved through processes such as sticking, trapping, and entangling, facilitated by the rougher surface of duckweed. Notably, experimentally validated detachment curves of MPs, well fitted with linear with lower plateau models ($P < 0.01$), revealed that AWD resulted in greater capturing of MPs on abaxial fronds than CF. Our work uncovers a potential microplastic mitigation method in rice ecosystems by using duckweed combined with AWD in future.

1. Introduction

Rice is an essential staple food for over 50 % of the global population [1,2]. It is widely grown worldwide, with a total growing area of over 165 million ha in 2022 [3]. Continuously flooding irrigation (CF) is a typical irrigation method for rice fields widely adopted by farmers, which requires huge freshwater input (typically 1300 –1500 mm). Approximately 24 %–30 % of freshwater worldwide is applied in paddy rice fields [4]. However, the increasing scarcity of irrigation water in agriculture threatens the sustainability of rice production. Therefore, various water–saving techniques, such as alternate wetting and drying irrigation (AWD), have been proposed to reduce water and maintain reasonable yields. AWD can reduce water use by 25.7 %, increasing water productivity by 24.2 %, without significantly compromising rice yield as only a slight reduction of 5.4 % may occur [5]. Moreover, it can reduce the global warming potential induced by both methane and nitrous oxide emissions by 45 %–90 % and attenuate accumulation of heavy metals (e.g. mercury and arsenic) in grain [6]. However, other factors should also be considered when implement of AWD, such as soil properties (e.g., pH and soil organic carbon values), as well as the lower irrigation threshold and frequency of AWD [5]. For instance, an inappropriate lower AWD threshold setting may lead to greater yield reductions and decreased water use efficiency [5]. Nevertheless, with appropriate management practices, most of these negative impacts can be effectively mitigated in AWD. Therefore, AWD is the most promising water–saving irrigation technology for rice, which has been promoted globally [4,5,7].

Due to the use of plastic mulching, fertilizers, and the demand for wastewater reuse, microplastics (MPs) levels in agricultural fields, especially in paddy fields, are increased [8]. In China, the average abundance of MPs in farmland has reached 4537 items per kg of dry soil [9], while MPs in rice fields have approached a relatively high contamination level (10^3–10^4 items per kg of dry soil) [10]. Moreover, due to continuous weathering, fragmentation, and the enormous stock of microplastic residues in farmlands, studies estimate that environmental concentrations of MPs could be 10^{14}–fold higher than currently detected MP levels [11]. MPs have been shown to impair rice growth, reducing plant biomass and yield by disturbing root activity, photosynthesis, nutrient uptake, and oxidative balance [12–14]. Importantly, hydroponic experiments demonstrate that nano– and micro–sized polystyrene particles can be absorbed by rice roots and translocated into stems and leaves, raising concern for grain contamination and food–chain transfer [15]. These findings highlight that MPs are emerging contaminants that not only impact agricultural ecosystems, diminished crop productivity, and threaten biodiversity, but also pose health risks when crops adsorb these MPs that are subsequently consumed by humans, raising serious concerns about food chain contamination and food safety [16–18]. Therefore, reducing MPs in agricultural fields, especially rice fields, is urgently necessary.

Aquatic plants represent effective means for capturing MPs. Regular harvesting of these plants with adhered MPs, before decomposition occurs, prevents secondary pollution and facilitates the permanent removal of accumulated MPs from the paddy ecosystem. Duckweed (*Lemna minor* L.) is a common aquatic plant in paddy fields, known for its extensive growth in the floodwater of subtropical paddy fields [19,20]. The introduction of duckweed into paddy fields to establish a rice–duckweed co–culture system has been shown to offer several benefits, including the mitigation of nitrogen loss, absorption of heavy metals, suppression of weed growth, degradation of agrochemicals, and reduction of greenhouse gas emissions [21,22]. However, there is a gap in the literature regarding duckweed's impact on the capturing of MPs in the rice–duckweed system, especially under AWD.

On one hand, as mentioned above, AWD irrigation causes the paddy soil to undergo cycles of drying and wetting. The fluctuation of water depth under AWD may result in the migration of duckweed, which increases the chance to contact MPs suspended in the water as well as those settled on the soil surface. On the other hand, AWD can modify the physio–anatomical traits of duckweed [23], potentially influencing its capacity to capture MPs by altering surface roughness [24] and the stickiness of *Lemna minor* L. roots and fronds [25]. Thus, it is a plausible to hypothesize that (1) the growth, biochemical as well as physiological parameters of duckweed could be influenced by different irrigation regimes under microplastic–contaminated rice–duckweed system; (2) compared to CF, AWD could promote MPs capturing through duckweed physio–anatomical modifications. Therefore, the objectivity of this study was to investigate the potential mechanism of duckweed capturing MPs in rice–duckweed systems under different irrigation regimes, which can provide a feasible solution to reduce MPs contamination in paddy fields by using duckweed combined with reasonable irrigation regimes in future.

2. Materials and methods

2.1. Experimental design

The experiment was conducted in four rectangle chambers (Caiduoduo, Haier, Qingdao, China) from July 21 to September 11, 2023, in the campus of Hohai University, Nanjing, China (31°86" N, 118°60" E). Each chamber (length × width × depth of 50 × 27 × 34 cm) consisted of four uniform tanks. The conditions in the chamber were adjusted as 25 °C day/night air temperature and 12 h photoperiod (7:00–19:00) using a full–spectrum lighting system.

The rice seedlings were well–watered (water levels of 1 –3 cm) during the first 10 days after transplanting (DAT) in all tanks, with an initial duckweed density of 52.1 g m^{-2} released just after transplanting. The duckweed species (genus: Lemna; family: Lemnaceae) used in this study was obtained from the Shuangying Agricultural Supplies Store (Kunshan, China). After10 DAT, the seedlings were exposed to two irrigation regimes, i.e. CF and AWD. The water level in CF was maintained at 0 –3 cm. For AWD, when the soil water content reached 60 %–70 % of saturation (lower limit), the rice field was re–flooded to 3 cm above the soil surface, and this procedure was repeated (Fig. 1). Each treatment was replicated four times, resulting in a total of 8 tanks (plots), and the experimental design was complete randomized.

To observe MPs under varying treatments, dry hydrophobic powder of polyethylene MPs particles ($(C_2H_4)_n$) (Science & Technology Polymer, Foshan, China) with 0.909 g per kg of dry soil was uniformly supplied to each tank with irrigation water just after transplanting. These sphere

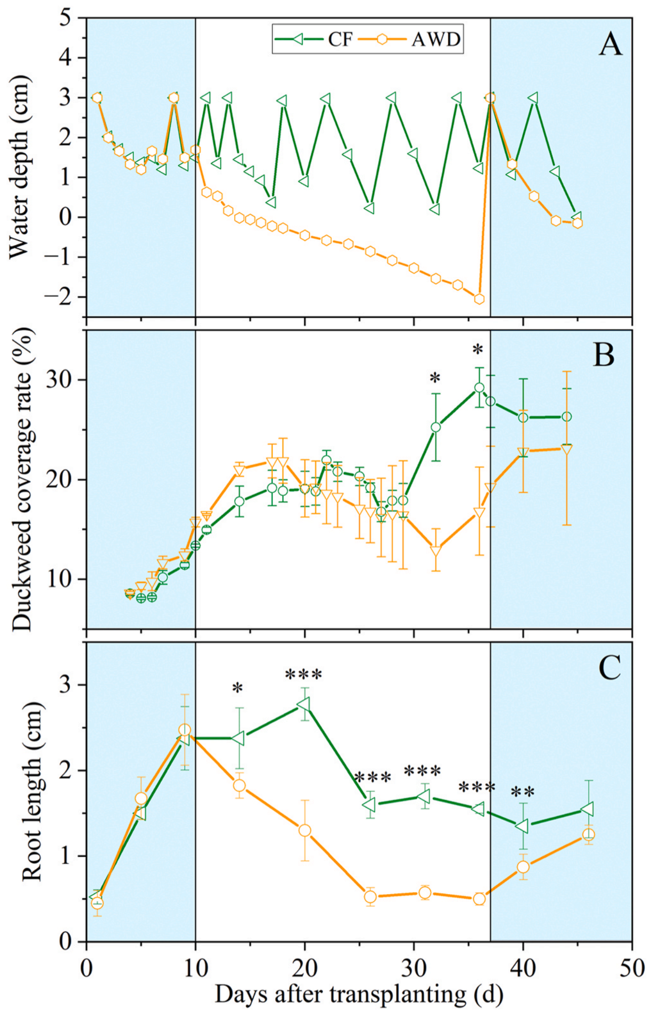

Fig. 1. The dynamics of water depth (A), coverage rate (B) and root length (C) of duckweed under alternate wetting and drying (AWD) and continue flooding (CF) regimes. *, **, and *** indicate significant differences between CF and AWD by Student's t − test at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. Values are the means ± SE (n = 4). The left and right shaded areas represent the phase before AWD and re−watering phase of AWD, respectively.

MPs had a diameter of 1 −100 µm (mean = 24.5 µm), with a peak fluorescence at 605 nm (Fig. S1) and a density of 0.98 cm^{-3}. The concentration of MPs was 1.66 × 10^7 items g^{-1} [24]. In order to characterize the infrared spectra of MPs, Fourier transform infrared spectroscopy (FTIR, Nicolet IS10, Thermo Fisher, Waltham, MA, USA) was used at 6 DAT (Fig. S1B).

The experimental soil was collected from a nearby paddy field, which had not been subjected to plastic film mulching and sewage irrigation. All the soil samples in the 0–20 cm layer of the paddy field were sieved through ≤ 2 mm mesh, and subsequently were thoroughly homogenized. The detailed physical and chemical properties of soil are shown in Table 1. Before the experiment, fertilizers (0.5 g pure urea, 0.05 g K_2SO_4, and 0.2 g KH_2PO_4 for each tank) were thoroughly mixed with the dry soil during soil filling, and no additional fertilization was supplied later. A total of 1.1 kg of mixed dry soil was filled into each tank.

2.2. Measurements and methods

2.2.1. Coverage rate and root length of duckweed

To observe the growth dynamics of duckweed, the coverage rate of duckweed (ratio of duckweed coverage area to water surface area in the tank) was determined by using a camera (IMX890, Sony, Tokyo, Japan) at 15, 17, 18, 20 −23, 25 −29, 32, 36, 37, 40, and 44 DAT. Images were vertically taken from 10 cm above the top of the tank and subsequently analyzed using Photoshop software (Adobe Systems Inc., San Jose, USA) to calculate duckweed coverage rates.

Four duckweed colonies were randomly collected at 1, 5, 9, 14, 20, 26, 31, 36, 38, 40, and 46 DAT, respectively, and individually photographed. After that, the root lengths were calculated using ImageJ software (version 1.8.0, NIH, Bethesda, USA).

2.2.2. Biochemical indicators of duckweed

To examine the biochemical status of duckweed, superoxide dismutase (SOD, U g^{-1} FW), malondialdehyde (MDA, nmol g^{-1} FW), catalase (CAT, U g^{-1} FW) and peroxidase (POD, U g^{-1} FW) were evaluated at 35 (1 day before irrigation of AWD) and 49 DAT (13 days after irrigation of AWD). 0.2 g of duckweed was randomly removed from the four tanks of each treatment. After sampling, the duckweed was preserved in liquid nitrogen and measured using assay kits (Nanjing Jiancheng Bioengineering Institute, China). The detailed measurement methods of SOD, MDA, CAT, and POD were described elsewhere [26, 27].

2.2.3. The physiological parameters of duckweed

Rapid light curves were measured on 19, 22, 37, and 51 DAT at the photosynthetically active radiation (PAR) levels of 1500, 1150, 850, 650, 450, 300, 200, 150, 100, 70, 50, and 0 µmol m^{-2} s^{-1} using a MINI−PAM−II portable pulsed fluorimeter (Walz, Nürnberg, Germany). Randomly selected duckweed colonies for each tank were placed on a soaked filter paper to keep them alive, then subjected to a dark acclimation period of 15 min at a temperature of 23 ± 2 °C before the measurements. The duckweeds need to ensure coverage of the probe. The maximum photosynthetic electron transfer rate (ETR_{max}, µmol m^{-2} s^{-1}) and half−saturation intensity (I_k, µmol m^{-2} s^{-1}) can be determined as [28]

$$ETR = \frac{PAR}{(aPAR^2 + bPAR + c)} \quad (1)$$

$$ETR_{max} = 1/(b + 2\sqrt{ac}) \quad (2)$$

$$I_k = c/(b + 2\sqrt{ac}) \quad (3)$$

where a, b, and c are empirical coefficients.

2.2.4. Three−dimensional excitation−emission matrices (3D EEMs) fluorescence spectroscopy for dissolved organic matter in surface water

50 ml water samples with 2 randomly selected duckweed colonies were first collected from each tank at 6 h after rewatering, then centrifuged at 4000 rpm for 15 min, and subsequently filtered through 0.22 µm pore−size membranes to obtain water samples with dissolved organic matter. The filtered samples were then scanned for emission wavelengths (E_m) ranging from 280 to 500 nm at 2 nm intervals and for excitation wavelengths (E_x) ranging from 220 to 400 nm at 10 nm intervals by using a spectrophotometer (model F−4700 FL, Hitachi Ltd,

Table 1
Soil physical and chemical properties.

Mass (g)	Clay content (%)	Silt content (%)	Sand content (%)	Saturated soil water content (%)	Soil bulk density (g cm^{-3})	Soil organic carbon (g kg^{-1})	Soil organic matter (g kg^{-1})	Ammonium nitrogen (g kg^{-1})
1100	22.23	48.63	28.15	45.95	1.23	9. 83	16.95	1.81

Tokyo, Japan). Contours of E_x/E_m at different locations can identify the composition of dissolved organic matter, such as humic acid−like, protein−like, and fulic acid−like [29].

2.2.5. DNA extraction, amplicon sequencing, and data preprocessing in microorganisms

To assess microbial abundance, 50 ml water samples with 2 randomly selected duckweed colonies were first collected from each tank at 6 h after re−watering, and subsequently filtered through a 0.22 −micron pore size filter paper for sampling. The V4 −V5 regions of the 16S rRNA gene for the bacteria were amplified by two−step PCR using the primer pair 515 F−Y and 926 R [30]. The amplicons were extracted from 2 % agarose gel electrophoresis, purified with the AxyPrep DNA gel extraction kit (Axygen Biosciences, CA, USA), and Qubit ® 3.0 (Life Invitrogen) was utilized for quantification. Amplicons were subjected to paired−end sequencing on the Illumina MiSeq sequencing platform using PE300 chemical (Majorbio Bio−Pharm Technology Ltd., Shanghai, China).

2.2.6. Number of MPs

Four colonies of randomly selected duckweed from each tank were used to determine the quantity of MPs per unit area (1 mm^2 unit for both sides of fronds and 1 mm unit for the root system). The number of MPs per unit area was then quantified using a research-grade microscope (BX41, Olympus Corporation, Tokyo, Japan) together with a portable microscope (DinoCapture2.0, AnMo, Taiwan, China). The total number of MPs captured by each part (both sides of the front and roots) is the area of fronds (or total root length) multiplied by the quantity of MPs per unit area for each part.

2.2.7. Microscopic structure on the surface of duckweed and MPs

Four colonies of randomly selected duckweed from each tank were used to observe the MPs structure by the scanning electron microscope (SEM) method (Apireo, Thermo Fisher, Waltham, MA, USA). All collected samples were first coated with a thin layer of Au/Pd (approximately 10 nm). Meanwhile, the elemental composition was analyzed using energy−dispersive X − ray spectroscopy (EDS) (UltimMax 65, Oxford Instruments, Oxford, UK).

2.2.8. Detachment curves of MPs on duckweed

Four randomly picked duckweed colonies per tank were placed into 20 ml centrifuge tubes and completely covered with distilled water. Subsequently, tubes were centrifuged at 500, 1500, 2000, 3000, and 4000 rpm for 5 min, respectively, using a centrifuge (800D XINIU, Shanghai, China). The initial and final items of MPs retained on the duckweed was counted and recorded for each centrifugation speed. A linear with lower plateau model [31], experimentally validated and applied here as the MP detachment curve in this study, was used to fit the data as follows:

$$y = \begin{cases} y_o + k(x - x_o) & x \le x_o \\ y_o & x > x_o \end{cases} \quad (4)$$

where y is the relative retention of MPs, y_o is the retained plateau level, x_o is the breakpoint suction, k is the detachment rates, x is the suction (converted from centrifugation speed).

2.3. Statistical analysis

A non−linear regression procedure was applied to fit the rapid light curves of duckweed frond and detachment curves for adaxial and abaxial fronds as well as roots of duckweed under AWD and CF, by using the SPSS software for Windows (SPSS Statistics, USA). Student's t − test (SPSS Statistics, USA) was used to examine whether there were differences between all duckweed−related traits subjected to AWD and CF. Pearson correlation analysis was conducted to test the relationships between the relative abundance of bacterial community compositions in the inoculum and y_o, x_o, and k of the detachment curves on abaxial fronds based on pooled data of the two irrigation regimes by using Origin software (Origin lab, USA).

3. Results

3.1. Physical interception of MPs by duckweed

The effectiveness of physical interception of MPs depends on contact areas with MPs (i.e. the coverage rate of fronds and root length) and surface roughness of duckweed. Here, we found that the coverage rate of duckweeds was significantly lower compared to CF, reducing by 48.8 % and 42.4 % (Fig. 1 B), respectively, at 18 (32 DAT) and 22 (36 DAT) days after surface water disappeared (Fig. 1 A). After rewatering (37 DAT), the coverage rate of duckweed was non−significantly different between AWD and CF (Fig. 1 B). The root length of duckweed under AWD was also significantly reduced (23.2 %−67.7 % during the drying period), relative to CF, since surface water was absent (Fig. 1 C).

The surface roughness can be reflected by the biochemical and physiological indices resulting from destroyed cell membrane structures. In this study, more folds (Fig. 2 C, I) and porous structures (Fig. 2 F) were formed under AWD compared to CK (Fig. 2 B, D, H). Hence, the capability of physical interception of MPs was increased, as indicated by more items captured per frond and root area (Fig. 3). Moreover, AWD induced significantly greater MDA (53.3 %), SOD (25.6 %), POD (51.7 %), and CAT (120.3 %) contents before rewatering, compared to CF, but did not significantly affect MDA, CAT, and POD after rewatering (Fig. 4). This indicates that the cell membrane structures are disrupted and plants increase their ability to scavenge oxygen free radicals during the dry period of AWD. The destroyed cell membrane structures during the dry period of AWD further supported by the significantly smaller ETR_{max} and I_k during the dry period of AWD than CF (Table 2).

3.2. Microbial abundance and 3D EEMs fluorescence spectroscopy for dissolved organic matter in surface water

Adhesion is another crucial factor affecting MPs capture. Under stress conditions, microorganisms secrete extracellular polymeric substances (composed of proteins, carbohydrates, amino acids, among others, involved in dissolved organic matter), enhancing adhesion [32]. Here, we observed a significantly ($P < 0.05$) greater abundance of the orders Propionibacteriales (156.1 %), Chitinophagales (233.1 %), and *Caulobacterales* (146.1 %) under AWD, compared to CF (Fig. 5). Meanwhile, *Caulobacterales* and Propionibacteriales showed a significant ($P < 0.05$) positive correlation with the retained plateau level (y_o) and detachment rates (k) of the detachment curves of MPs on duckweed (Fig. 6). Moreover, 3D EEMs fluorescence spectroscopy showed that AWD induced humic acid−like, fulvic acid−like, and especially protein−like substances, as indicated by peak intensity (Fig. 7). The higher protein−like substances further demonstrated that AWD enhanced the adhesion capability compared to CF.

3.3. Number of captured MPs

We further assessed which irrigation regime could capture more MPs through duckweed. We found that at the initial stage, for both irrigation regimes, the duckweeds, especially adaxial fronds (Fig. 3A), could rapidly capture MPs as duckweed and MPs were both floating at the water surface (Fig. 3). As floating MPs (insoluble in water) sink, there are fewer contact opportunities between duckweeds and MPs under CF, leading to less and almost stable capturing MPs later on (Fig. 3 A−D). However, the MPs numbers per unit aera captured by duckweed significantly ($P < 0.05$) increased after surface water was absent under AWD, especially for abaxial fronds. From 10–50 DAT, the number of MPs per unit area on the adaxial and abaxial fronds, as well as the root

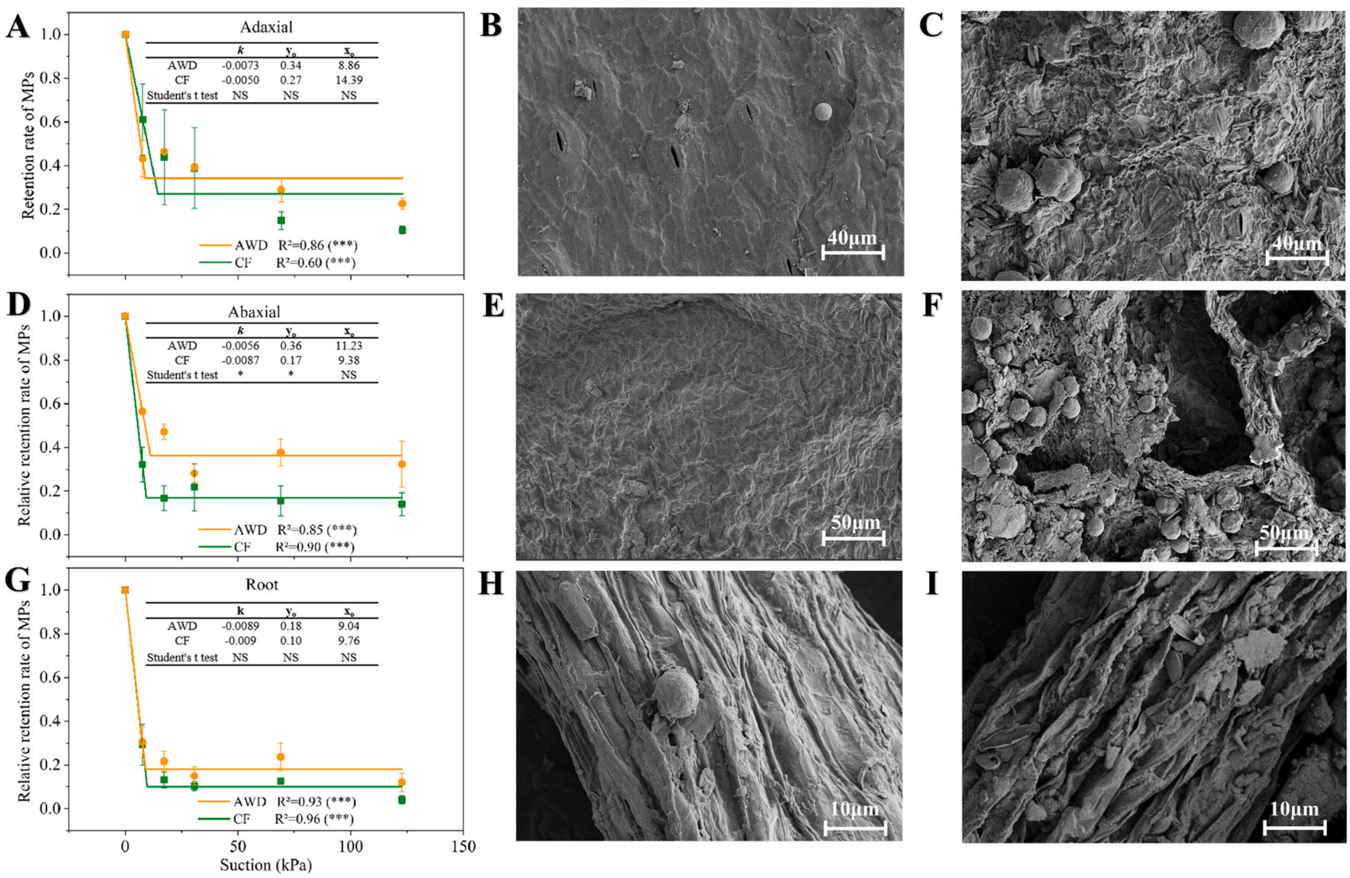

Fig. 2. Detachment curves on adaxial (A) and abaxial (D) fronds and roots (G) of duckweed under alternate wetting and drying (AWD) and continuous flooding (CF) irrigation regimes. Each curve was fitted using a linear with lower plateau model based on pooled data of three treatment replications (n = 18). y_o is the retained plateau level, x_o is the breakpoint suction, and k is the detachment rate. Figure also shows the scanning electron microscope images of the adaxial (B, C) and abaxial (E, F) fronds and roots (H, I) of duckweed under AWD (C, F, I) and CF (B, E, H), respectively. *, ** and *** indicate significant differences between CF and AWD at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. NS shows non−significance. The values shown are the means ± SE (n = 4).

system of duckweed, increased by 1.4 −2.6, 5.7 −30.9, and 1.6 −8.9 −fold, respectively. This resulted in an overall increase of 0.7 −16.0 −fold in the total number of MPs captured by duckweeds during the dry period of AWD relative to CF. This capture of MPs by duckweeds (277, 827, 112 items per mm^2 (or per mm), respectively, for adaxial and abaxial fronds and root system) generally further increased right after the rewatering under AWD (Fig. 3). This improved capture under AWD could be achieved through the enhanced availability of adhesion and physical interception via stuck (Fig. 8 A, B), trapped (Fig. 8 C, D), and entangled (Fig. 8 E, F) processes benefited from the rougher duckweed surface.

3.4. Detachment curves of MPs on duckweed

Finally, we employed a linear with lower plateau model to estimate the relationships between the relative retention of MPs and suction, which could be well ($P < 0.001$) captured by the model (Fig. 2 A, D, G). Student T − test showed that there were no significant ($P > 0.05$) differences of y_o, k, and breakpoint suction (x_o) between AWD and CF for adaxial front and roots of duckweed (Fig. 2 A, G). However, for abaxial frond, slowly declined k and higher value of y_o indicate greater retention capability of MPs under AWD (Fig. 2 D).

4. Discussion and conclusions

Larger−sized MPs (1 −100 µm) are less readily absorbed by water hyacinths. Hence, the removal of larger−sized MPs by aquatic plants is primarily through two mechanisms, *i.e.* physical interception [24,33] and adhesion *via* hydrogen bonding or electrostatic interactions [17]. Physical interception involves entangled, stuck, and trapped methods [17], and its effectiveness is closely linked to the morphology of duckweed, such as contact areas with MPs (the number or coverage rate of fronds and root length) and surface roughness. More folds [17] and porous structures [34,35] caused by cell membrane disruption under abiotic stress could induce a rougher surface, thereby greatly enhancing MPs' capture. These cell membrane damages in duckweed can be indicated by biochemical indicators (e.g. MDA, SOD, POD, and CAT) and physiological indices (e.g. ETR_{max} and I_k). When plants experience abiotic stress, their cell membrane structures are prone to disruption, resulting in increased levels of MDA [36,37] and reduced ETR_{max} and I_k. Additionally, plants tend to mitigate oxidative stress by enhancing the activity of antioxidant enzymes, such as SOD, POD, and CAT [36,38]. Accordingly, we observed that AWD resulted in significantly greater values of MDA, SOD, POD, and CAT (Fig. 4) and lower values of ETR_{max} and I_k, compared to CF, during the dry period (Table 2). These differences diminished after re−watering of AWD, except for SOD (Fig. 4). These results indicated that while AWD resulted in lower coverage rate and root length of duckweed (Fig. 1), its much rougher surface (Fig. 2 C, F, I) as indicated by increased folds (Fig. 2 C, I), more porous structure (Fig. 2 F), and significantly higher developed interfacial area ratio values (Fig. S2, $P < 0.05$), due to cell membrane damage, led to a more efficient capture of MPs through physical interception compared to CF. This was achieved via stuck (Fig. 8 A, B), trapped (Fig. 8 C, D), and entangled (Fig. 8 E, F) processes. Consequently, the number of MPs per unit area on the adaxial frond, abaxial frond, and root system of duckweed increased by 1.7 −2.6, 5.7 −30.9, and 1.6 −8.9 −fold during the

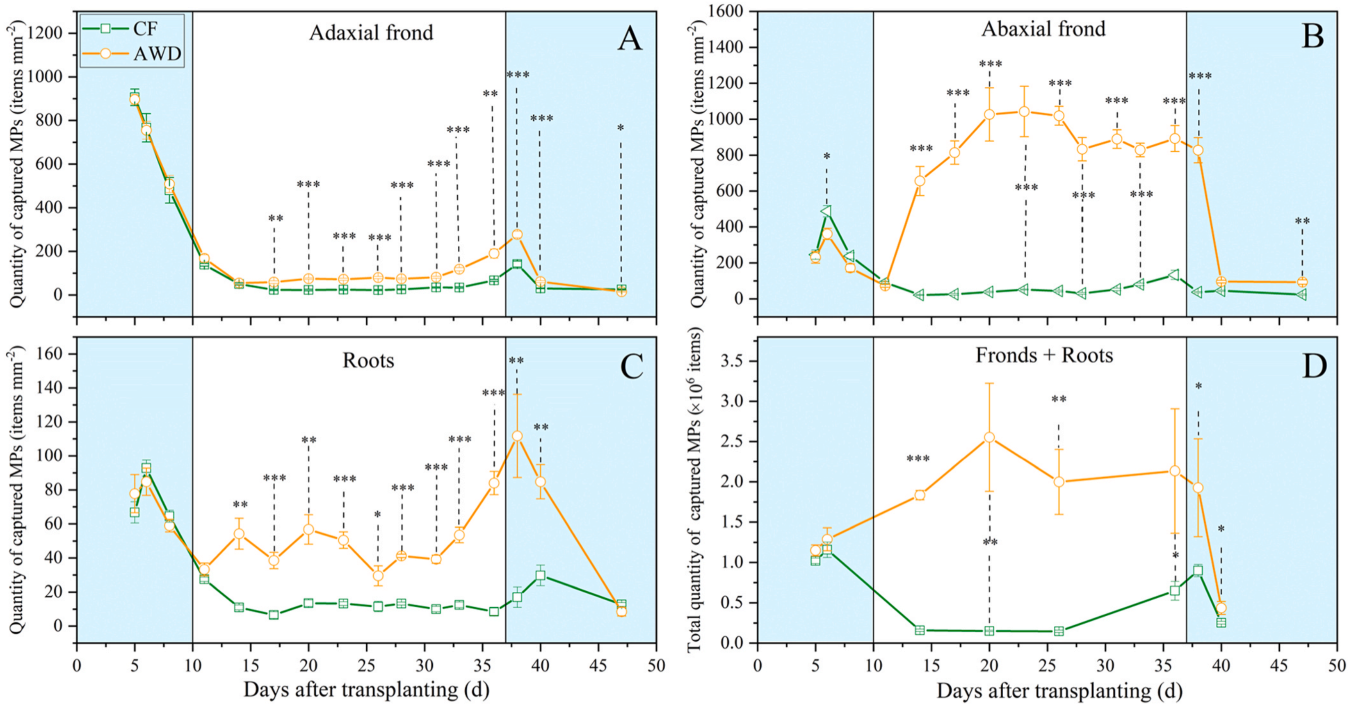

Fig. 3. Quantity of captured microplastics (MPs) per unit area on the adaxial (A) and abaxial (B) frond of duckweed, as well as on the root system (C), and overall capturing quantity (D) under alternate wetting and drying (AWD) and continues flooding (CF) irrigation regimes. *, **, and *** indicate significant differences between CF and AWD by Student's t − test at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. Values are the means ± SE (n = 4). The left and right shaded areas represent the phase before AWD and re−watering phase of AWD, respectively.

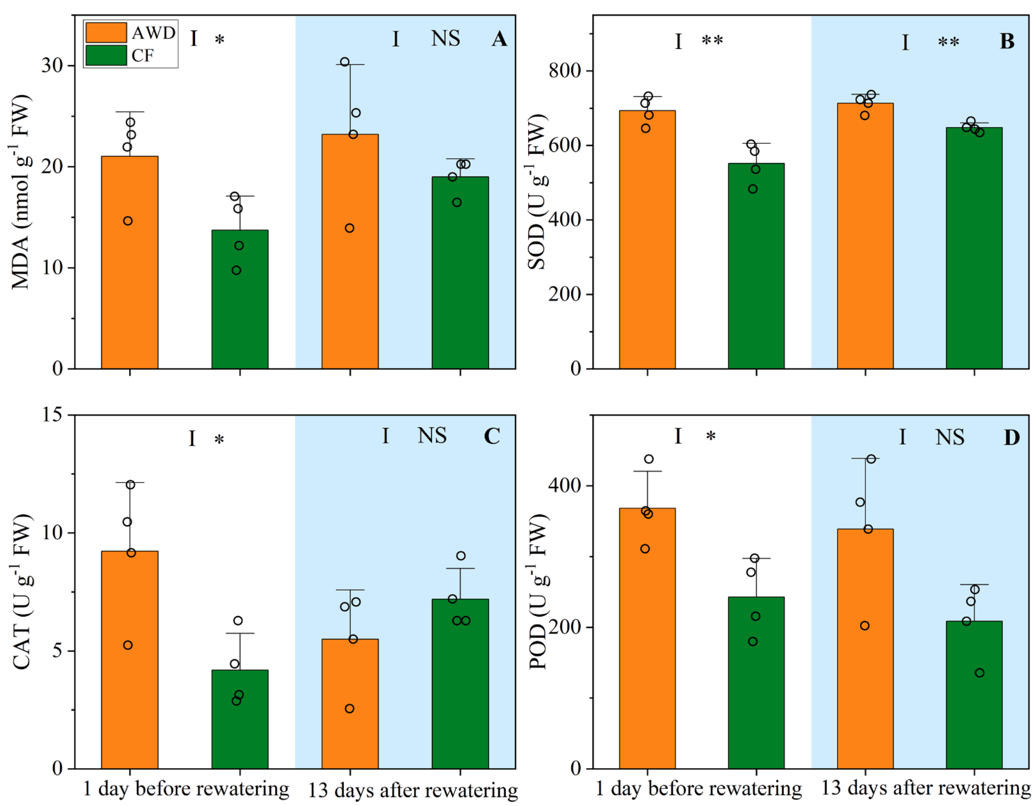

Fig. 4. Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) in duckweed subjected to alternate wetting and drying (AWD) and continuous flooding (CF) irrigation regimes on 1 day before irrigation and 13 days after irrigation under AWD. * and ** indicate significant differences between CF and AWD at $P < 0.05$ and $P < 0.01$, respectively. NS shows non−significance. Values are the means ± SE (n = 4).

Table 2
Maximum photosynthetic electron transfer rate (ETR_{max}) and half−saturation intensity (I_k) subjected to alternate wetting and drying (AWD) and continues flooding (CF) irrigation regimes at 19, 22, 37 and 51 days after transplanting (DAT). Values are the means ± SE (n = 4). Different letters within a column indicate significant differences at $P < 0.05$.

	19 DAT		22 DAT		37 DAT		51 DAT	
	ETR_{max}	I_k	ETR_{max}	I_k	ETR_{max}	I_k	ETR_{max}	I_k
CF	65.5 ± 3.2a	246.5 ± 4.6a	62.1 ± 0.6a	211.5 ± 3.4a	55.7 ± 3.4a	184.6 ± 13.9a	60.6 ± 0.6a	222.3 ± 2.5a
AWD	55.5 ± 0.8b	192.1 ± 4.0b	57.0 ± 0.8b	189.6 ± 1.4b	39.9 ± 2.1b	125.6 ± 11.4b	63.2 ± 1.8a	214.2 ± 4.2a

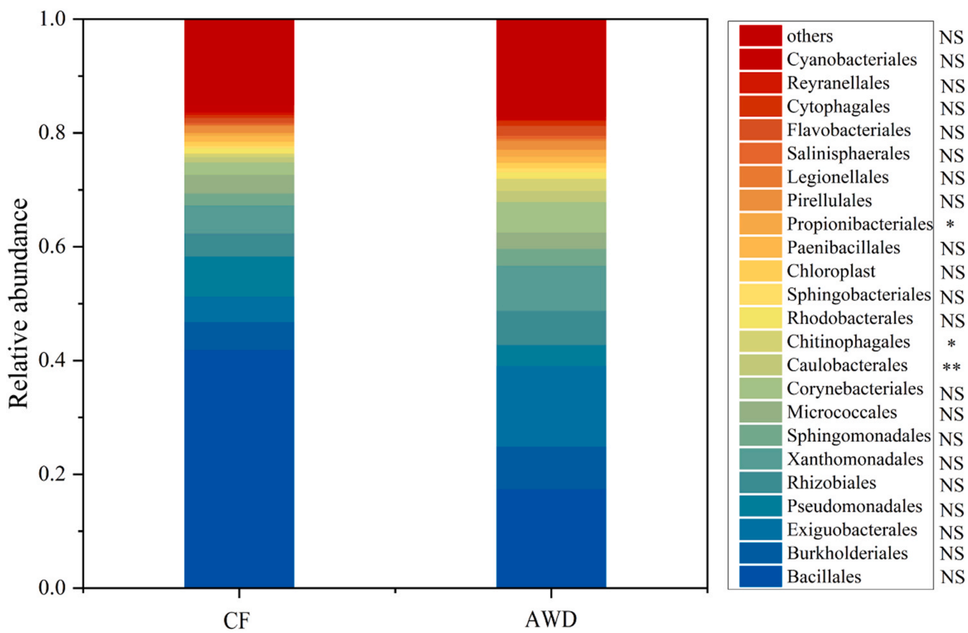

Fig. 5. Relative abundance of bacterial community compositions in the inoculum under alternate wetting and drying (AWD) and continuous flooding (CF) irrigation regimes. * and ** indicate significant differences between CF and AWD at $P < 0.05$ and $P < 0.01$, respectively. NS shows non−significance. 'Other' means taxa with less than 1 % relative abundance in all samples and unassigned taxa.

drying period of AWD, compared to CF (Fig. 3). Moreover, we also observed that the total number of MPs captured by duckweeds increased by 0.7 −16.0 times during the dry period of AWD, relative to CF (Fig. 3 D).

In addition to physical interception, adhesion through hydrogen bonding or electrostatic interactions is another critical pathway for aquatic plants, such as duckweed, to capture MPs [32]. Under stress conditions, microorganisms and phytoplankton can be triggered to secrete extracellular polymeric substances or dissolved organic matter. These substances, composed of proteins, carbohydrates, amino acids, and other compounds, exhibit increased viscosity with a higher protein content or protein−to−carbohydrate ratio, consequently affecting the adhesion of MPs to aquatic plants [32]. In particular, extracellular proteins in extracellular polymeric substances are crucial in pollutant adsorption, providing binding sites with carboxyl, amine, and hydroxyl groups, involving electrostatic forces and hydrogen bonds [39]. Accordingly, we found that the abundance of bacteria such as *Caulobacterales* was significantly higher under AWD (Fig. 5). Previous study has shown that *Caulobacterales*, an order within the *Alphaproteobacteria*, employ a polar adhesin known as the holdfast for surface attachment and biofilm formation in freshwater environments [40]. The holdfast generates an exceptionally strong adhesive force (70 N mm^{-2}) when attached to a surface [41]. As the abundance of *Caulobacterales* increases, the production of extracellular secretions—including the holdfast—is also expected to rise, potentially enhancing the adhesion of MPs to biological surfaces [40]. Moreover, bacteria of this order secrete protein-like substances [41,42], and a higher protein content or protein-to-carbohydrate ratio is generally associated with increased viscosity [32], which may further promote MP adhesion. In this study, the elevated protein−like content (Fig. 7) was likely secreted by the higher abundance of *Caulobacterales* (Fig. 5) under AWD. Interestingly, a significant ($P < 0.05$) correlations was observed between the abundance of *Caulobacterales* and the retained plateau values on the abaxial fronds surface (Fig. 8D). The higher plateau values under AWD indicate a greater proportion of strongly adhered MPs relative to total adhered MPs, which is consistent with a potential contribution of increased *Caulobacterales* abundance and elevated secretion viscosity under abiotic stress [40] to enhanced MP adhesion. While the holdfast adhesin of *Caulobacterales* has been proposed as a potential contributor to MP adhesion, and the observed association with increased protein-like DOM provides supportive evidence, it should be noted that no direct evidence currently demonstrates that secretions from this bacterial group specifically bind to the polyethylene MPs used in this study. Consequently, the proposed linkage remains suggestive rather than definitive, and a mechanistic confirmation requires future quantitative analysis of EPS composition and direct measurements of adhesive force. Furthermore, It is noteworthy that the different detachment rates and retained plateau values of detachment curves under various irrigation regimes may relate not only to adhesion but also possibly to physical interception [33,43] and biogenic calcite precipitation on the MPs surface facilitated by sessile cyanobacteria [44]. The entrapment of MPs in porous structures formed by decayed gas cavities (Fig. 8 C, D; Fig. S3), the tight sealing of MPs by stomata (Fig. 8 G, H; Fig. S4), and biogenic calcite precipitation (Fig. 8 I, J; Fig. S5) in the MPs surface may contribute to the observed greater plateau values and detachment rates of detachment curves, although we were unable to quantitatively distinguish the relative

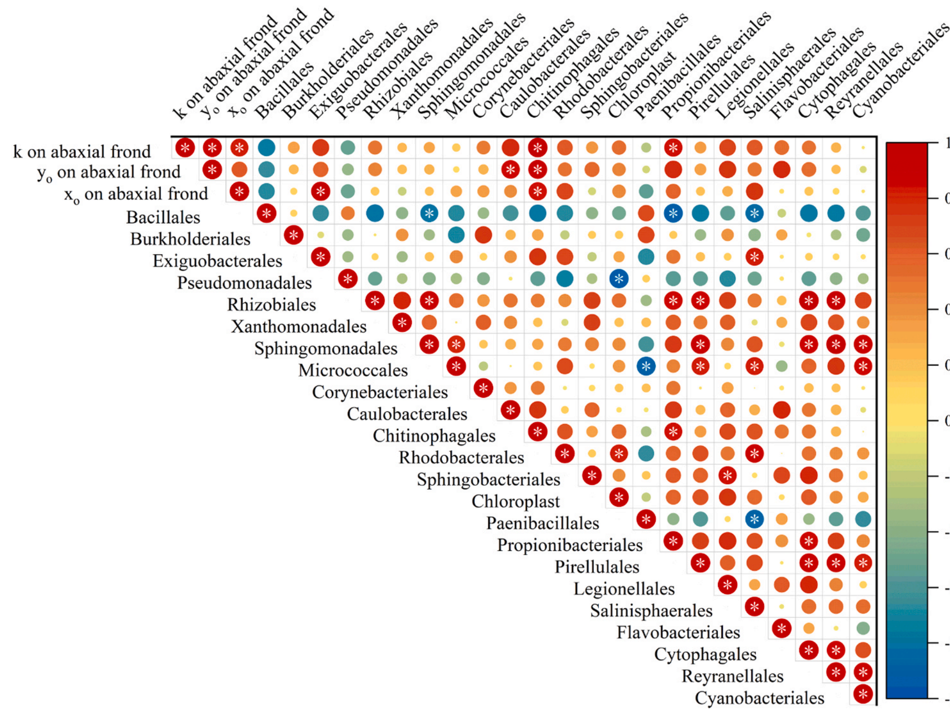

Fig. 6. Pearson correlations between bacterial communities (as shown in Fig. 5) and the retained plateau level (y_o), breakpoint suction (x_o), and detachment rate (k) of detachment curves on abaxial fronds (as shown in Fig. 2) based on the pooled data of three replications under two irrigation regimes (n = 6). * indicates the significance level at $P < 0.05$.

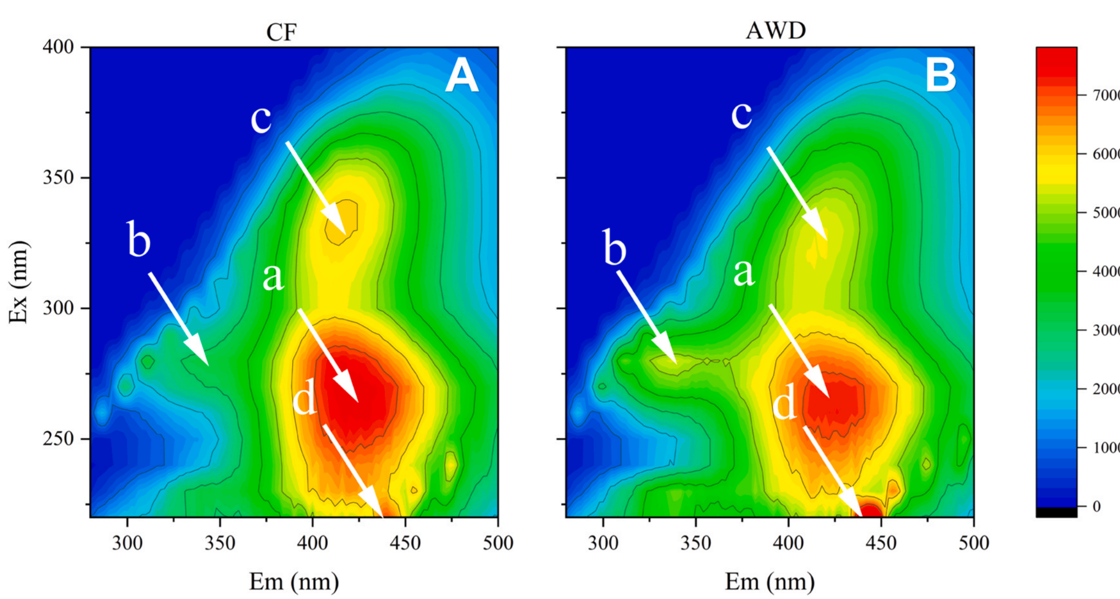

Fig. 7. Typical contours of excitation–emission matrices for dissolved organic matter under alternate wetting and drying (AWD) and continuous flooding (CF) irrigation regimes. E_m and E_x are emission and excitation wavelengths. Specifically, peaks *a* and *c*, located at E_x/E_m of 260/425 and 325/425, were identified as humic acid–like, peak b, located at Ex/Em of 285/330 nm, was identified as protein–like, and peak d located at 220/445 was identified as fulvic acid–like.

contributions of physical interception, microbial adhesion, and calcite precipitation to MP capture in this study.

Building on these mechanistic insights, it is important to recognize certain limitations of the current study. While our results demonstrate that AWD enhances MP capture by duckweed through combined effects of surface roughness, microbial adhesion, and potential biogenic calcite precipitation, several aspects remain unquantified. For instance, we were unable to distinguish the relative contributions of physical interception, microbial adhesion, and calcite precipitation, which limits the ability to identify the dominant mechanism under different irrigation regimes. Additionally, although periodic harvesting of MP–laden duckweed has been proposed as a strategy to mitigate secondary pollution, practical considerations such as harvesting frequency, labor requirements, and post–harvest management were not explicitly evaluated. Future research should integrate techno–economic analysis and environmentally sound disposal or resource–recovery pathways, such as bioenergy conversion, to improve the real–world applicability of duckweed-based MP mitigation strategies.

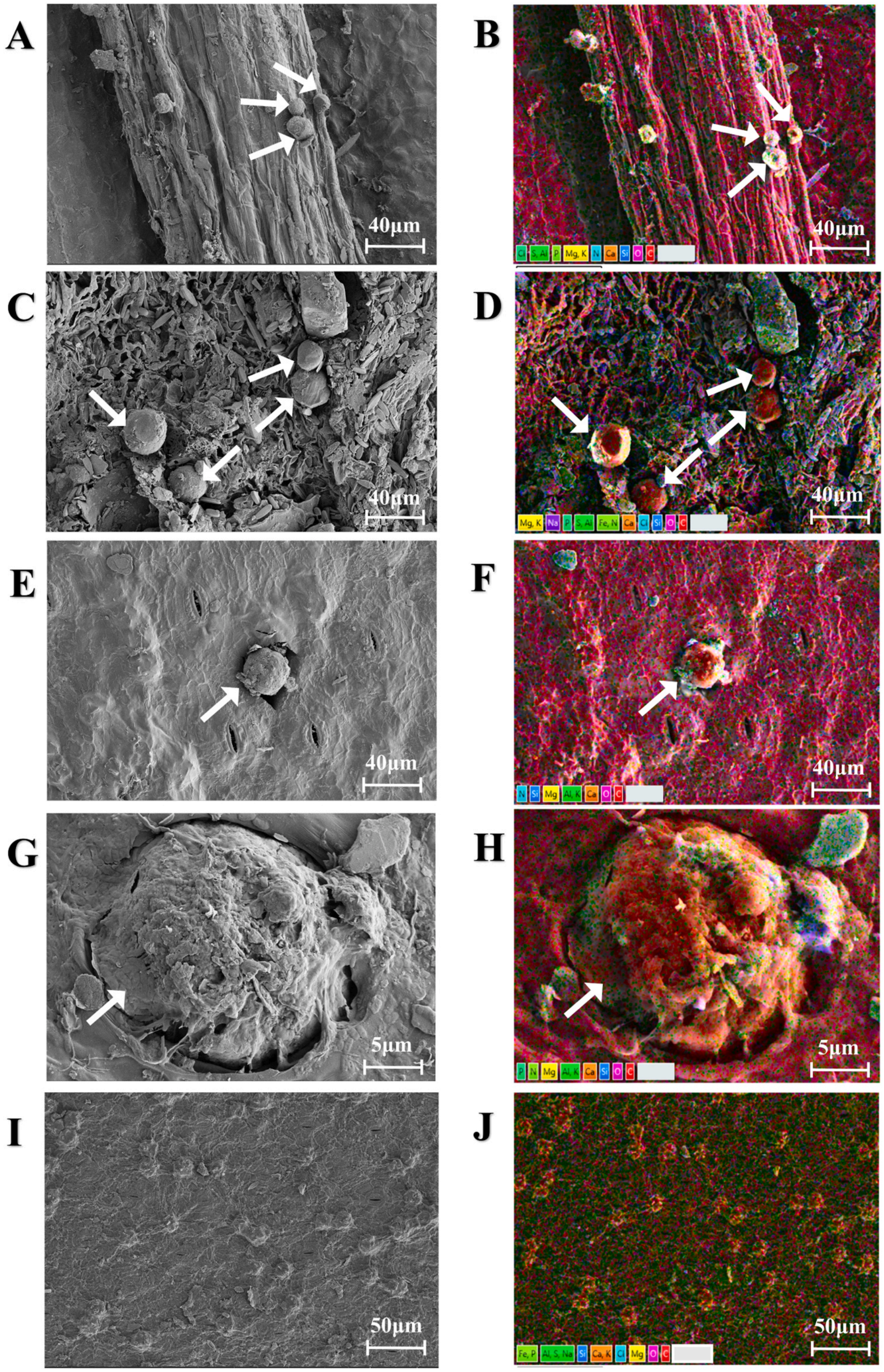

Fig. 8. Scanning Electron Microscope (A, C, E, G, I) coupled with Energy Dispersive X − ray spectroscopy analysis (B, D, F, H, J) of duckweed samples with the captured MPs. The microplastics (MPs; indicated by arrows) are 'entangled' (A, B) in roots, 'trapped' in back of leave surface (C, D) and stomata (G, H), 'stuck' (E, F) in front of leave surface, and enveloped by biogenic calcite precipitation in the front surface of duckweeds (I, J). The EDS images are used to characterize and identify MPs.

Despite these limitations, this study provides novel insights into mitigating the risk of MPs in rice fields *via* duckweed modification. In particular, it establishes a conceptual framework for understanding how AWD influences MP retention through plant physio-anatomical changes and microbial interactions. Future work should expand on this proof−of−concept by exploring a broader range of microplastic types, sizes, and shapes, different duckweed species, and extended field trials under realistic environmental conditions to generalize these findings and refine the mechanistic understanding of MP capture dynamics.

Environmental implication

This work shows that the total number of microplastics captured in duckweed increased by 0.7–16.0 times during the drying period of alternate wetting and drying irrigation (AWD) compared to conventional continuous flooding irrigation. This increase is attributed to the greater capturing capacity of microplastics by duckweed, resulting from its rougher structure and enhanced adhesion, despite the reduction in coverage rate and root length during the dry phase of AWD by harnessing duckweed capabilities. This study provides novel insights into mitigating the risk of MPs in rice fields through duckweed modification.

CRediT authorship contribution statement

Evgenios Agathokleous: Writing – review & editing, Validation. **Zhenchang Wang:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Cheng Hong:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Rangjian Qiu:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Yaosheng Wang:** Writing – review & editing, Validation. **Hiba Shaghaleh:** Writing – review & editing, Validation. **Hamoud Yousef:** Writing – review & editing, Validation.

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.

Acknowledgments

We acknowledge support from the National Natural Science Foundation of China (52579037, 52322904).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.140973.

Data availability

Data will be made available on request.

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