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Impacts of low pH and low salinity induced by acid rain on the photosynthetic activity of green tidal alga *Ulva prolifera*

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Abstract

Acid rain is a serious environmental problem and has obvious impacts on the growth, reproduction, and photosynthesis of terrestrial plants. *Ulva prolifera*, a main blooming species of green tides, was studied on its physiological response to acid rain. The photosynthetic parameters were determined under different conditions (salinity: 1, 10, 30‰; pH: 3.0, 3.5, 4.5; duration: 0.5, 1.0, 2.0 h) followed by 24-h recovering under natural conditions. Results showed 1-h treatment with pH 3.5 caused 50–70% reduction in the maximal quantum yield of PSII photochemistry (F_v/F_m) and effective quantum yield of PSII photochemistry (Φ_{PSII}) at normal salinity but when the low pH was combined with a salinity of 10‰ or lower, PSII activity was almost completely inhibited. Moreover, the low salinity (1‰ and 10‰) reduced the degree of photoprotection under low pH (3.5) conditions. Finally, we speculated if the pH of acid rain ≤ 3.5, with 1‰ salinity and 2-h rainfall time, the amount of *U. prolifera* and the scale of green tides would decrease.

Keywords: acid rain; chlorophyll fluorescence; nonphotochemical quenching; photosynthetic activity; *Ulva prolifera.*

Introduction

Acid rain, with pH values lower than 5.6, is one of the serious examples of regional air pollution (Charlson and Rodhe 1982). It is caused mainly by SO_2 dissolution and nitrogen oxides in the atmosphere (Shukla *et al.* 2013). Next to Western Europe and North America, China has become the third largest acid rain region in the world, especially in the southern and southwestern parts of China (Larssen *et al.* 2006). In China, Zhejiang Province was

Highlights

- The activity of *Ulva prolifera* exposed to different pH and salinity changed differently
- The low salinity could reduce NPQ of *U. prolifera* under low pH condition
- The study provided a reference for predicting the scale of green tides by acid rain

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Abbreviations: Chl – chlorophyll; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximal quantum yield of PSII photochemistry; NPQ – nonphotochemical quenching; q_E – energy-dependent quenching; RGR – relative growth rate; ROS – reactive oxygen species; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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an acid rain-affected region, in which Jinhua and Ningbo cities received large quantities of acid rain, and the pH was below 4.12 and 4.74, respectively (Zhang *et al.* 2007a, Ding *et al.* 2012). Additionally, acid rain was in the pH range of 3.24–6.56 in Wenzhou (Gao *et al.* 2016). In Shandong Province, Qingdao was a city with serious acid rain pollution, where the frequency of acid rain was high from 1993 to 2012, reaching 55% (Xing *et al.* 2017). Particularly, in 2008, the average pH of precipitation in Qingdao was 4.4 (Xing *et al.* 2017). In China, the main causes of acid rain are the burning of a large amount of coal with high sulfur contents and vehicle emissions (Kita *et al.* 2004). Emissions from motor vehicles generated concentrated nitrogen oxides and SO_2, which led to a gradual increase in acid rain (Singh and Agrawal 2008). Therefore, in the early 21st century, the Chinese government adopted a series of measures for energy conservation and emission reduction. However, acid rain pollution remained severe in the southern and southwestern regions, which directly or indirectly affected the physiological and ecological processes in plants, and plants were facing more and more adverse stress (Liu *et al.* 2019).

In recent years, related studies have shown that acid rain could directly affect higher plants by destroying the leaf structure, reducing chlorophyll (Chl) fraction, and changing Chl fluorescence characteristics and enzyme activity (Singh and Agrawal 2008, Zhou *et al.* 2020, Hu *et al.* 2021). A study has found that acid rain could destroy the structure and integrity of chloroplasts and weaken photosynthesis in plants (Mubarakshina *et al.* 2006). This is because acid rain destroys the coupling between the thylakoid electron transport chain and photophosphorylation, which makes chloroplasts unable to properly complete the absorption, transfer, and conversion of light energy (Sun *et al.* 2011). Most studies on acid rain involved the causes, temporal and spatial distribution, control measures, mechanisms of the damage to crops and conifers, and impacts on water or land ecosystems (Debnath *et al.* 2018, Ju *et al.* 2020, Zhang *et al.* 2020). However, studies on the impacts of acid rain on macroalgae in the intertidal zone were scarce (Polishchuk *et al.* 2016).

Nearly 6,500 macroalgae species have been recorded in the world and macroalgae have a variety of ecological functions, including material absorption and release, food supply, and space shelter for aquatic organisms (Zhang *et al.* 2007b). Macroalgae mainly distribute in the subtidal zone and intertidal zone. Under the tidal action, they are periodically exposed to amphibious environments. As a result, they often experience diurnal and seasonal fluctuations in physical environmental factors, such as temperature, light, osmotic pressure, and drought (Levin *et al.* 2015). During low tide, they are often exposed directly to acid rain that may last for several hours. Especially, at the lowest tide, acid rain falls to the intertidal zone, the algal ambient salinity may drop to 1‰ (Xu *et al.* 2018), and the pH drops to a lower value, thus the growth and photosynthesis of intertidal macroalgae could be affected by acid rain. Li *et al.* (2017) found that after cultivating *U. prolifera* in simulated acid rain (pH 4.4) for 3 d, the content of Chl *a*, the maximal quantum yield of PSII photochemistry (F_v/F_m), and effective quantum yield of PSII photochemistry (Φ_{PSII}) all declined, leading to a lower relative growth rate (RGR). Nonphotochemical quenching (NPQ) is one of the most important mechanisms of protection against rapid and high light, by which the excess light energy can be dissipated in heat by a light-harvesting complex (LHC), thus preventing the overexcitation of photochemical reactions (Baker 2008). Liu *et al.* (2020) exposed *U. prolifera* to acid (pH 4.0) and high light [1,200 μmol(photon) m^{-2} s^{-1}] for 1 and 3 h and found NPQ values were higher than that of *U. prolifera* cultured under normal conditions. The result showed that acid treatment and the combined treatment of acid and high light could induce energy dissipation. In addition, a study showed that the macroalgae diversity in the intertidal zone along the coast of Wenzhou, Zhejiang, China, has been seriously damaged over the past few years due to acid rain (Zheng *et al.* 2011). Most macroalgae populations were reduced and even disappeared in the Nanji Islands, Wenzhou, while several other macroalgae reproduced quickly, including *Corallina pilulifera*, *Lithophyllum okamurae*, and *Amphiroa anceps* (Sun *et al.* 2010, Tang *et al.* 2014).

In addition to pH, salinity could be dramatically reduced in the intertidal zone with the increase of precipitation in time and amount. Salinity affects the osmotic pressure, floatability, nutrient absorption, and photosynthetic oxygen evolution of algae cells to a certain point. Low salinity could inhibit the photosynthetic activity of *Ulva pertusa* and reduce the rate of oxygen evolution (Yamochi 2013). These results suggested the possibility of controlling a green tide of *U. pertusa* by a combination of exposure to air with low salinity. The impacts of low salinity on marine macroalgae vary from species to species, and the tolerance to low salinity depends mainly on their growth conditions and adaptive capacity (Karsten 2007). Low salinity affected the photosynthetic activity of macroalgae by altering osmotic pressure like in *Ulva fasciata* (Chen and Zou 2015) and *Griffithsia monilis* (Bisson and Kirst 1979). Allakhverdiev *et al.* (2002) found that salt stress inhibited the repair of photodamaged PSII in *Synechocystis*. However, few studies are available on the combined impacts of pH and salinity on the photosynthetic activity of macroalgae in the intertidal zone.

U. prolifera distributes in the intertidal zone and is known for its tolerance to a wide range of temperature and salinity (Gao *et al.* 2010). More importantly, it can trigger green tides, causing marine environmental pollution and economic losses (Yabe *et al.* 2009). Acid rain is one of the ten major environmental problems in the world (Larssen *et al.* 2006) and it is fundamentally unknown how acid rain mediates the impact of salinity changes on *U. prolifera*. Therefore, the combined impacts of pH and salinity on the F_v/F_m and Φ_{PSII} of *U. prolifera* in different durations were studied; 27 gradients of pH, salinity, and treatment duration were set up and NPQ values were measured to understand the ability of *U. prolifera* in self-regulation under acid rain.

Materials and methods

Sample collection and culture conditions: Samples of *U. prolifera* were collected from the intertidal zone at The

First Bathing Beach (36.06°N, 120.35°E), Qingdao, China on 20 July 2020 (Fig. 1), and the thalli were carried to laboratory in an icebox within 24 h. In the laboratory, the thalli were rinsed gently in filtered nature seawater at least three times to remove epiphytes, sediments, grazers, and other attached materials, and then cultured with filtered (0.22 µm) and autoclaved natural seawater (salinity of 30‰), in which $NaNO_3$ (100 µmol L^{-1}) and $KH_2PO_4 \cdot 12\,H_2O$ (10 µmol L^{-1}) were added. The thalli were cultured in an illuminated incubator (*GXZ-380C*, Ningbo, China) at 20 ± 1°C under irradiance of 100 µmol(photon) m^{-2} s^{-1} (12/12 h light/dark period) with ambient airing. After 2 d, tender and healthy thalli (3 cm in length) were chosen for experiments.

Experimental design: Well-grown *U. prolifera* (CK) was cultured in natural seawater (pH 8.2, salinity of 30‰). The pH and salinity were measured with a *P611* portable pH-meter (*YOKE*, Shanghai, China) and a portable salinometer (*DAIKELI*, Anhui, China). Salinities were set at 1‰ and 10‰ by diluting filterable natural seawater with distilled water, and pH of different salinity (1, 10, and 30‰) of seawater was set at 3.0, 3.5, and 4.5, using a mixed solution of H_2SO_4 (mol L^{-1}):HNO_3 (1 mol L^{-1}) = 2:1 (Li *et al.* 2017). Nine different conditions were simulated.

Treatments	pH 3.0	pH 3.5	pH 4.5
Salinity 1‰ (S 1)	S 1 – pH 3.0	S 1 – pH 3.5	S 1 – pH 4.5
Salinity 10‰ (S 10)	S 10 – pH 3.0	S 10 – pH 3.5	S 10 – pH 4.5
Salinity 30‰ (S 30)	S 30 – pH 3.0	S 30 – pH 3.5	S 30 – pH 4.5

The healthy thalli were submersed into these nine different conditions for 0.5, 1, and 2 h before Chl fluorescence determination. Each treatment was performed in triplicates ($n = 3$). Chl fluorescence was determined again after thalli were taken back into normal natural conditions for 24 h, and the culture conditions were consistent with those mentioned above.

Chl fluorescence parameters: The photosynthetic parameters were measured at room temperature (20 ± 1°C) using a *Closed FluorCam FC 800-C* (*PSI*, Czech Republic). The thalli were kept in darkness for 15 min and then the minimal fluorescence yield of the dark-adapted state (F_0) was measured. The intensity of measuring flashes is 0.02 µmol(photon) m^{-2} s^{-1} in 10-µs pulse to remain nonactinic. The maximal fluorescence yield of the dark-adapted state (F_m) was measured when a saturation pulse was applied. Saturation pulse was cold white light, and its irradiance and pulse length were 3,370 µmol(photon) m^{-2} s^{-1} and 800 ms, respectively. The actinic light treatment was applied for 60 s. Five saturation pulses were given during actinic light and the intervals were 10, 10, 20, and 20 s. The F_v/F_m was calculated as $F_v/F_m = (F_m - F_0)/F_m$ and the Φ_{PSII} was calculated as $\Phi_{PSII} = (F_m' - F_s)/F_m'$, where F_m' is the maximal fluorescence level from algae induced by a saturation pulse under active light of 104 µmol(photon) m^{-2} s^{-1} (close to the cultured irradiance) and F_s is steady-state fluorescence yield. NPQ reflects the downregulation of PSII as a protective mechanism against excess light intensity, which was calculated as $NPQ = (F_m - F_m')/F_m'$.

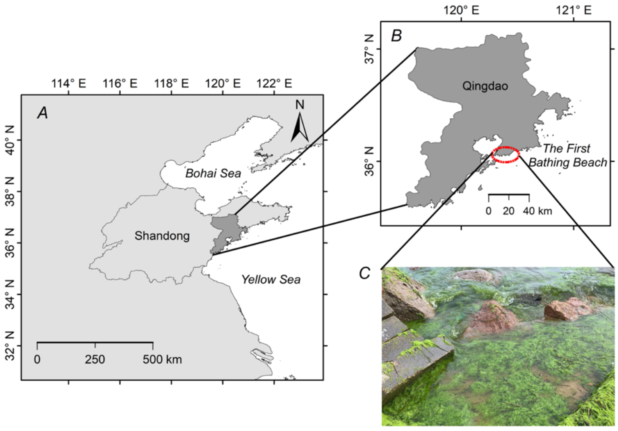

Fig. 1. The sampling place for *Ulva prolifera* in The First Bathing Beach, Qingdao, Shangdong, China. (*A*,*B*) The geographical position; (*C*) the sampling scene of the intertidal zone in the place.

Statistical analysis: All data were analyzed using *Origin 8.5* (*OriginLab*, USA) and *SPSS 25.0* (*IBM*, Chicago, USA). A map was made by *ArcGIS 10.2* (*ESRI*, USA). Statistical differences under the different treatment conditions were analyzed with the repeated-measures analyses of variance (*RM-ANOVA*), followed by *Tukey*'s honestly significant difference posthoc test. Before all statistical analyses, the homogeneity of variances was verified by *Levene*'s test. Partial eta squared (η^2) was used to measure the effect size. $P<0.05$ was considered significant and a confidence interval of 95% was set for all tests. All data were expressed as mean ± standard deviation (SD, $n = 3$).

Results

Impacts of pH and treatment duration on F_v/F_m and Φ_{PSII}: The impacts of pH and treatment duration on the F_v/F_m and Φ_{PSII} of *U. prolifera* are shown in Fig. 2. The F_v/F_m and Φ_{PSII} declined significantly with the decrease of pH (Fig. 2). At pH 3 after 0.5 h, the F_v/F_m and Φ_{PSII} declined significantly and only 70.8 and 64.1% of the CK could be recovered, respectively (Fig. 2). The F_v/F_m and Φ_{PSII} were severely lowered and could not recover with the treatment duration increase. The F_v/F_m and Φ_{PSII} of 1-h treatment were only 22.9 and 21.4% of those in 0.5-h treatment, respectively, and those in 2-h treatment declined to 0. The F_v/F_m and Φ_{PSII} could recover and approach the CK level when *U. prolifera* was under pH 3.5 for 0.5 h-duration treatment. However, with treatment duration increase, the F_v/F_m and Φ_{PSII} could recover partially only (1 h) or failed to recover (2 h).

RM-ANOVA analysis showed that pH and treatment duration had an interactive effect on the F_v/F_m and Φ_{PSII}

of *U. prolifera* (Table 1S, *supplement*). Moreover, pH was demonstrated as the most important contributor to the F_v/F_m and Φ_{PSII} change as the magnitude of η^2 indicated (F_v/F_m: 1.622 > 0.237; Φ_{PSII}: 1.426 > 0.094).

F_v/F_m and Φ_{PSII} under low pH conditions: We studied how salinity affected photosynthetic activity under low pH (3.5) conditions (Fig. 3). The F_v/F_m remained at a normal level after treatment for 0.5 h (Fig. 3*A*). But under long-time treatment (1 and 2 h), high salinity (S 30) was more conducive to maintain the F_v/F_m and Φ_{PSII} than low salinity (S 1 and S 10) (Fig. 3) did. The F_v/F_m and Φ_{PSII} treated in salinity of 30‰ for 1 h were 87.3 and 81.5% of CK, while those treated in salinity of 10‰ could only reach 29.2 and 23.7% of CK, respectively.

In addition, *RM-ANOVA* analysis showed that salinity and treatment duration had an interactive effect on the F_v/F_m and Φ_{PSII} of *U. prolifera* under low pH (3.5) conditions (Table 2S, *supplement*). The order of contribution to the variability of F_v/F_m and Φ_{PSII} as shown in η^2 was salinity < treatment duration (F_v/F_m: 0.577 < 1.379; Φ_{PSII}: 0.119 < 0.775).

NPQ variations: The impacts of pH and treatment duration on the NPQ of *U. prolifera* are described in Fig. 4*A*. The NPQ of CK was 0.1 ± 0.005, but that of the three pH (3, 3.5, 4.5) treatments increased to 0.21 ± 0.11, 0.58 ± 0.08, and 0.25 ± 0.03 in 0.5 h, respectively. In addition, we studied how salinity affected NPQ under low pH (3.5) conditions (Fig. 4*B*). The results showed NPQ under salinity of 30‰ was much higher than that under salinity of 1‰ and 10‰ (Figs. 4*B*, 5). After treatment for 1 h, NPQ under salinity of 30‰ was 2.68 times and 3.26 times that under salinity of 1‰ and 10‰, respectively;

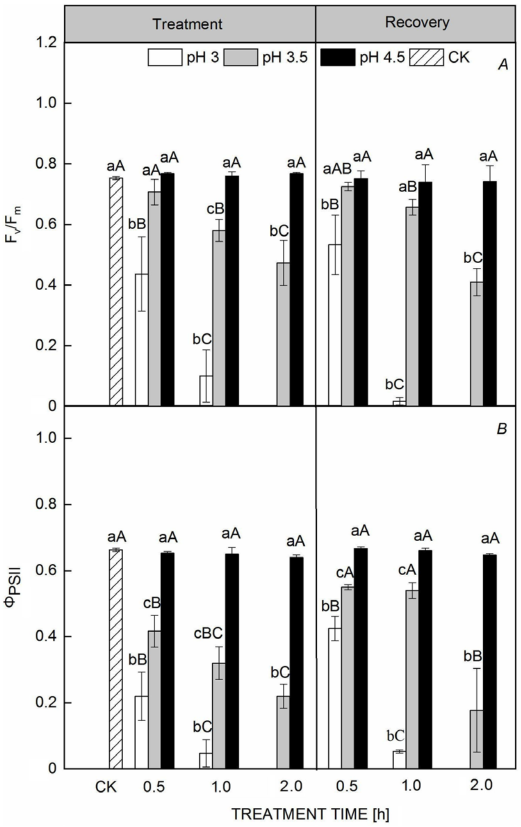

Fig. 2. The maximal quantum yield of PSII photochemistry (F_v/F_m) (*A*) and effective quantum yield of PSII photochemistry (Φ_{PSII}) (*B*) of *Ulva prolifera* after treatment in a different pH at salinity 30‰ for a different duration (0.5, 1, and 2 h), and those after recovery under natural growth condition for 24 h. CK is the well-grown *U. prolifera* under normal growth conditions. *The different lowercase letters* over the bars indicate significant differences (compared to CK) between pH treatments at the same treatment duration or recovery duration, while *the different uppercase letters* over the bars indicate significant differences (compared to CK) between treatment duration or recovery duration at the same pH. Data are represented as means ± SD and the values are the means of three replications. P<0.05 was considered significant.

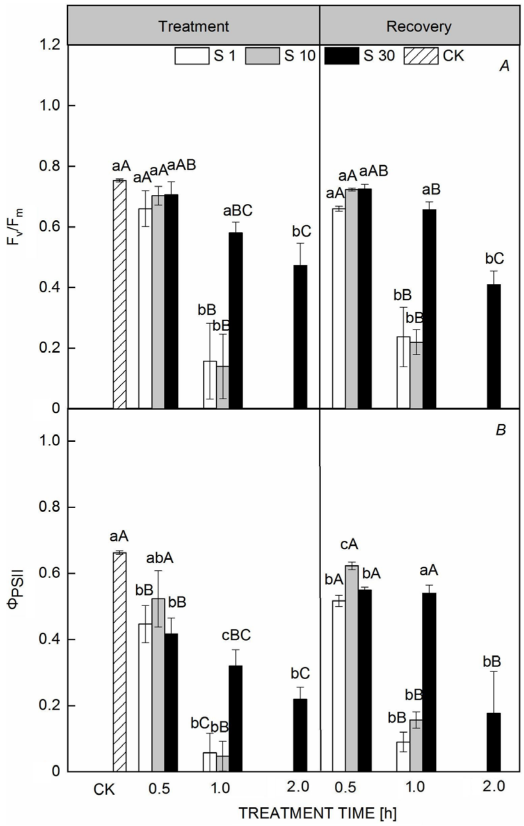

Fig. 3. The maximal quantum yield of PSII photochemistry (F_v/F_m) (*A*) and effective quantum yield of PSII photochemistry (Φ_{PSII}) (*B*) of *Ulva prolifera* after treatment in a different salinity at pH 3.5 for a different duration (0.5, 1, and 2 h), and those after recovery under natural growth conditions for 24 h. CK is the well-grown *U. prolifera* under normal growth conditions. *The different lowercase letters* over the bars indicate significant differences (compared to CK) between salinity treatments at the same treatment duration or recovery duration, while *the different uppercase letters* over the bars indicate significant differences (compared to CK) between treatment duration or recovery duration at the same salinity. Data are represented as means ± SD and the values are the means of three replications. P<0.05 was considered significant.

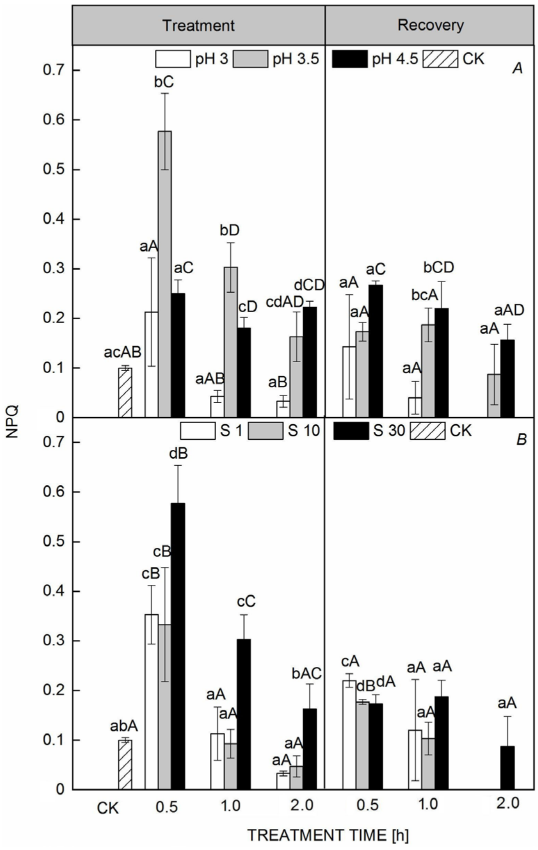

Fig. 4. (*A*) The nonphotochemical quenching (NPQ) of *Ulva prolifera* after treatment in a different pH at salinity 30‰ for a different duration (0.5, 1, and 2 h), and those after recovery under natural growth conditions for 24 h. *The different lowercase letters* over the bars indicate significant differences (compared to CK) between pH treatments at the same treatment duration or recovery duration, while *the different uppercase letters* over the bars indicate significant differences (compared to CK) between treatment duration or recovery duration at the same pH. (*B*) The NPQ of *U. prolifera* after treatment in a different salinity at pH 3.5 for a different duration, and those after recovery under natural growth conditions for 24 h. *The different lowercase letters* over the bars indicate significant differences (compared to CK) between salinity treatments at the same treatment duration or recovery duration, while *the different uppercase letters* over the bars indicate significant differences (compared to CK) between treatment duration or recovery duration at the same salinity. CK is the well-grown *U. prolifera* under normal growth conditions. Data are represented as means ± SD and the values are the means of three replications. $P<0.05$ was considered significant.

and for 2h-duration treatment, NPQ under salinity of 30‰ was 4.94 times and 3.47 times that under salinity of 1‰ and 10‰, respectively.

Impacts of pH, salinity, and treatment duration on F_v/F_m and Φ_{PSII}: An acid rain often lasts for several hours, resulting in low pH and salinity. Therefore, we selected 0.5, 1, and 2 h to evaluate the impacts of pH and salinity on the F_v/F_m and Φ_{PSII} of *U. prolifera* (Fig. 6). When salinity and treatment duration did not change, the decrease of pH inhibited the F_v/F_m and Φ_{PSII} of *U. prolifera* (Figs. 6, 7). Low salinity (S 1 and S 10) inhibited the F_v/F_m and Φ_{PSII} of *U. prolifera* under low pH (3, 3.5) conditions (Figs. 6, 7). Importantly, the F_v/F_m and Φ_{PSII} were not significantly affected at pH 4.5 (Figs. 6, 7). Under pH 3.5 and salinity of 30‰, the F_v/F_m and Φ_{PSII} of *U. prolifera* treated for 0.5 h could restore to 96.3 and 83.0% of CK level after 24-h recovering. However, the F_v/F_m and Φ_{PSII} continued declining and could not recover with treatment duration increased and salinity decreased. When pH dropped to 3, the F_v/F_m and Φ_{PSII} declined significantly and could not recover (Fig. 6).

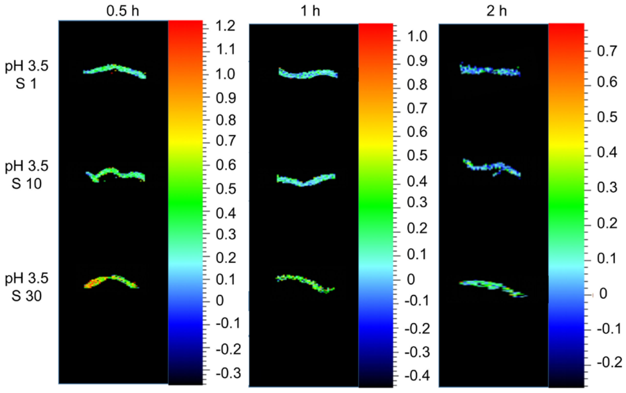

Fig. 5. Fluorescence imaging of nonphotochemical quenching (NPQ) after treatment in a different salinity at pH 3.5 for a different duration (0.5, 1, and 2 h). *The different colors* represent different NPQ values. S 1, S 10, and S 30 represent salinity of 1‰, 10‰, and 30‰, respectively.

Results of *RM-ANOVA* showed that pH, salinity, and treatment duration had an interactive effect on the F_v/F_m and Φ_{PSII} of *U. prolifera* (Table 1). The effects that contributed to the variability of F_v/F_m and Φ_{PSII} in the order of η^2 were pH > salinity > pH × salinity (F_v/F_m: 6.545 > 0.506 > 0.264; Φ_{PSII}: 5.257 > 0.116 > 0.052).

Discussion

The low pH induced by acid rain can alter community structure and species richness (Raut *et al.* 2012, Gao *et al.* 2016) *via* damaging their cell membrane, destroying photosystem, or disordering signal transduction (Liu *et al.* 2014). In this study, we found that F_v/F_m and Φ_{PSII} of *U. prolifera* kept normal levels under pH 4.5. Li *et al.* (2017) also found similar results and no significant differences in RGR of *U. prolifera* were observed between normal seawater (pH 8.2) and pH 4.4 treatment. Differently, when *U. prolifera* was under pH 3.5 for 0.5 h, F_v/F_m and Φ_{PSII} declined sharply but could recover to CK level after 24-h recovering, while the F_v/F_m and Φ_{PSII} could not recover after longer treatments (1 and 2 h). On the other hand, when the pH dropped to 3, F_v/F_m and Φ_{PSII} could not recover at all treatment durations. The possible reason is that *U. prolifera* is composed of monolayer cells, which is favorable for the entry of H^+ (Liu *et al.* 2020). Gómez *et al.* (2004) found that H^+ could cause photoinhibition and further affect the

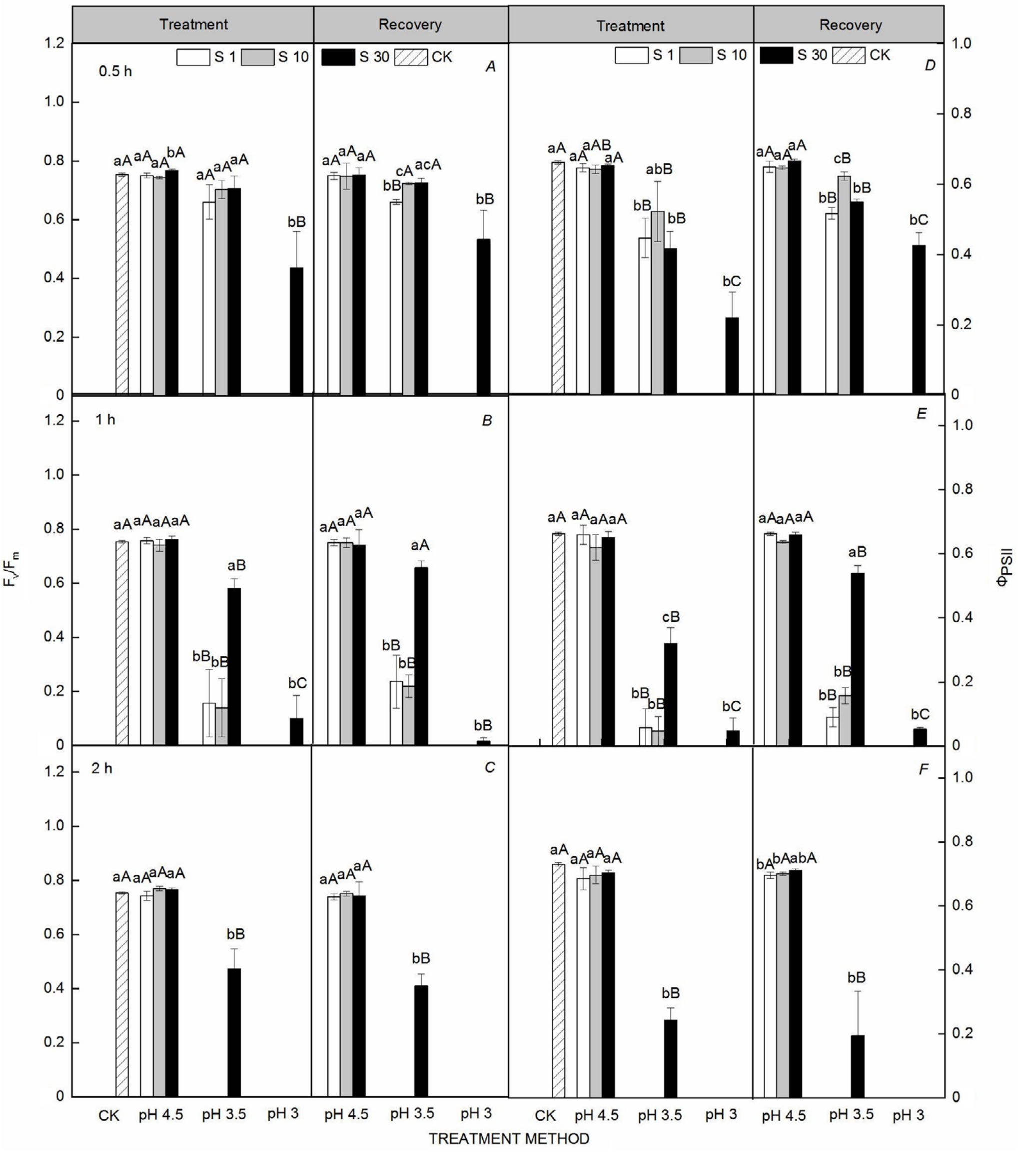

Fig. 6. The maximal quantum yield of PSII photochemistry (F_v/F_m) (*A−C*) and effective quantum yield of PSII photochemistry (Φ_{PSII}) (*D−F*) of *Ulva prolifera* after treatment under different pH and salinity values for a different duration (0.5, 1, and 2 h), and those after recovery under natural growth conditions for 24 h. CK is the well-grown *U. prolifera* under normal growth conditions. *The different lowercase letters* over the bars indicate significant differences (compared to CK) between salinity treatments at the same treatment duration or recovery duration and the same pH, while *the different uppercase letters* over the bars indicate significant differences (compared to CK) between pH at the same salinity and treatment duration or recovery duration. Data are represented as means ± SD and the values are the means of three replications. $P<0.05$ was considered significant.

protein synthesis and degradation in PSII. Ma *et al.* (2020) found that H^+ led to the degradation of PSII reaction center proteins D1, and reduced the ability to utilize light energy in red alga *Pyropia yezoensis*. In addition, some studies found that H^+ could destroy Rieske FeS protein and cytochrome (Cyt) *f* protein in Cyt b_6f complex, further damage PSI, and reduce the photosynthetic activity of *U. prolifera* (Yamori *et al.* 2011, Liu *et al.* 2020). In severe cases, H^+ could cause DNA damage and irreversibly destroy the photosynthetic system (Gómez *et al.* 2004, Milligan *et al.* 2009). As shown in Table 1S, the impact of pH on the F_v/F_m and Φ_{PSII} of *U. prolifera* was dominant in the order of η^2. Therefore, we thought that severe acid rain pollution (low pH or longer duration) would destroy the photosynthetic activity of *U. prolifera*.

Similarly, Gao *et al.* (2016) reported that the photosynthetic properties of *Ulva conglobata* decreased irreversibly under a low pH of 3, indicating that it could not survive after encountering low pH induced by acid rain. Conversely, the photosynthetic performance of *Corallina* sp. could recover quickly in normal seawater after low pH treatment; the reason was that they could

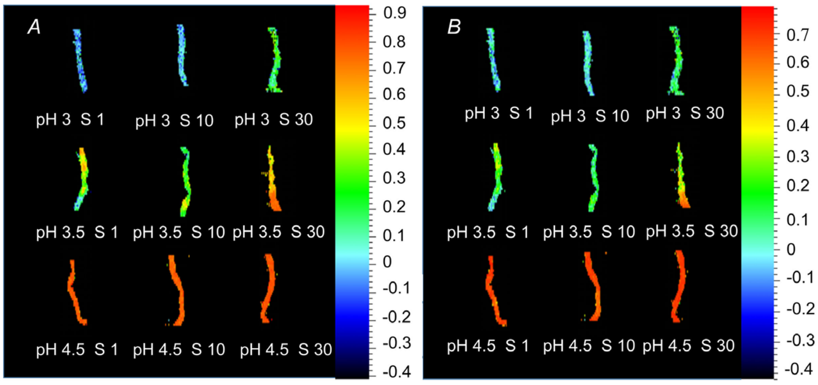

Fig. 7. Fluorescence imaging of maximal quantum yield of PSII photochemistry (F_v/F_m) (*A*) and effective quantum yield of PSII photochemistry (Φ_{PSII}) (*B*) after treatment under different pH and salinity values for 1-h duration. *The different colors* represent different F_v/F_m (*A*) and Φ_{PSII} (*B*) values. S 1, S 10, and S 30 represent salinity of 1, 10, and 30‰, respectively.

Table 1. Repeated-measure *ANOVA* (*RM-ANOVA*) statistics for impacts of salinity, pH, and treatment duration on the maximal quantum yield of PSII photochemistry (F_v/F_m) and effective quantum yield of PSII photochemistry (Φ_{PSII}) of *Ulva prolifera*. η^2 – Partial eta squared; df – degree of freedom; *F* – value of *F* statistic; *P* – *P* value.

Variable	η^2	df	Mean square	*F*	*P*	η^2	df	Mean square	*F*	*P*
	F_v/F_m in treatment stage					F_v/F_m in recovery stage				
	Within-subjects									
Time (T)	0.737	2	0.369	88.849	< 0.01	0.893	2	0.447	240.593	< 0.01
T × S	0.003	4	0.001	0.186	> 0.05	0.012	4	0.003	1.563	> 0.05
T × pH	0.746	4	0.187	44.981	< 0.01	0.748	4	0.187	100.802	< 0.01
T × S × pH	0.456	8	0.057	13.746	< 0.01	0.563	8	0.070	37.885	< 0.01
Error in T	0.149	36	0.004			0.067	36	0.002		
	Between-subjects									
Salinity (S)	0.506	2	0.253	109.119	< 0.01	0.409	2	0.205	107.377	< 0.01
pH	6.545	2	3.272	1,412.168	< 0.01	5.262	2	2.631	1,381.018	< 0.01
S × pH	0.264	4	0.066	28.525	< 0.01	0.303	4	0.076	39.770	< 0.01
Error in S	0.042	18	0.002			0.034	18	0.002		
	Φ_{PSII} in treatment stage					Φ_{PSII} in recovery stage				
	Within-subjects									
T	0.384	2	0.192	89.334	< 0.01	0.675	2	0.337	184.924	< 0.01
T × S	0.014	4	0.003	1.596	> 0.05	0.034	4	0.009	4.720	< 0.01
T × pH	0.419	4	0.105	48.721	< 0.01	0.593	4	0.148	81.264	< 0.01
T × S × pH	0.181	8	0.023	10.516	< 0.01	0.381	8	0.048	26.086	< 0.01
Error in T	0.077	36	0.002			0.066	36	0.002		
	Between-subjects									
S	0.116	2	0.058	40.320	< 0.01	0.268	2	0.134	87.650	< 0.01
pH	5.257	2	2.628	1,828.952	< 0.01	4.848	2	2.424	1,583.134	< 0.01
S × pH	0.052	4	0.013	8.985	< 0.01	0.120	4	0.030	19.545	< 0.01
Error in S	0.026	18	0.001			0.028	18	0.002		

regulate the pH of ambient seawater through dissolving their $CaCO_3$. In addition, *Sargassum vulgare* could adapt to a low pH by upregulating the activities of oxidative metabolic enzymes (Kumar *et al.* 2017). The different responses to low pH are species-specific (Gao *et al.* 2016).

Long-time (2 h) treatment of low pH (3.5) could destroy the photosynthetic activity of *U. prolifera* in this study (Fig. 2). The reason might be that the low pH impaired the membrane electrochemical potential and enzyme activity (Milligan *et al.* 2009) and further damaged the PSII of *U. prolifera*. Salinity may affect the osmotic balance and electrochemical gradient of plant cells by changing the osmotic pressure of seawater (Gacia *et al.* 2007). Besides, salinity also affects nutrient uptake, chloroplast structure, and photosynthetic oxygen evolution (Pancha *et al.* 2015), playing a decisive role in macroalgae growth (Gustavs *et al.* 2009), reproduction, and photosynthesis (Mantri *et al.* 2011). However, can salinity and pH affect interactively the photosynthetic activity of *U. prolifera*? According to *RM-ANOVA* statistics, pH, salinity, and treatment duration showed an interactive impact on F_v/F_m and Φ_{PSII} (Table 1). The order of the contribution to the variability of F_v/F_m and Φ_{PSII} (Table 1) as indicated by η^2 was pH > salinity, indicating that the impact of pH on the photosynthetic activity of *U. prolifera* was greater than that of salinity.

In this study, NPQ at pH 3.5 was higher than that of CK (Fig. 4*A*), which is consistent with the result of Liu *et al*. (2020) that NPQ of *U. prolifera* was markedly higher at pH 4 than that of control. The thermal dissipation of light energy, referred to as NPQ, is the fastest and most flexible response towards excess light among the known photoprotection mechanisms (Goss and Lepetit 2015). The NPQ mechanism has been widely found in various plants, such as algae, mosses, and vascular plants (Gerotto *et al.* 2011). Under a moderate light intensity, the production of NPQ was related to the proton gradient (ΔpH) *in vivo* and *in vitro* of the thylakoid, which could reduce the pressure of electron transport and avoid the damage caused by reactive oxygen species (ROS) to cells (Peers *et al*. 2009). The green algae could express PsbS and LhcSR proteins, by which NPQ could be induced (Peers *et al*. 2009). PsbS, a Chl-binding protein in PSII, can sense the intrathylakoid lumen pH and is crucial for NPQ (Li *et al*. 2000).

LhcSR is a light-induced transcript (Gagné and Guertin 1992), which accumulates under environmental conditions known to induce photooxidative stress, including deprivation of carbon dioxide (Miura *et al*. 2004), sulphur (Zhang *et al*. 2004), or iron (Naumann *et al*. 2007), as well as high light (Ledford *et al*. 2004). Peers *et al*. (2009) found that the accumulation of LhcSR proteins was induced by high light and correlated with energy-dependent quenching (q_E) capacity in *Chlamydomonas*. The q_E type quenching represents, under most light conditions, the main constituent of NPQ. It is the fastest in terms of induction and relaxation (Derks *et al*. 2015). In addition, NPQ can prevent photochemical overexcitation in a xanthophyll cycle (Baker 2008). When the lumen pH drops to about 5, the violaxanthin de-epoxidase (VDE) gene, a key gene in the xanthophyll cycle, can be activated to produce zeaxanthin to regulate the xanthophyll cycle and induce q_E (Mozzo *et al*. 2008). However, in Bryopsidales, a monophyletic branch of the Ulvophyceae (Leliaert *et al.* 2012), the short-term inducible NPQ is neither related to a pH-dependent mechanism, nor modulated by the activity of the xanthophyll cycle, even when the xanthophyll cycle is triggered under high light growth conditions, as seen in *Caulerpa* (Christa *et al*. 2017). Therefore, whether the increase of NPQ depends on pH and whether it is related to the xanthophyll cycle still needs further research.

In addition, we found that NPQ under low salinities (S 1 and S 10) was lower than that under high salinity (S 30) at a low pH (3.5) (Fig. 4*B*). Probably, the low salinity weakened the photoprotection induced by the low pH (3.5) and lost the ability to dissipate excess energy timely, resulting in the damage to the photosynthetic activity of *U. prolifera*. Why did the low salinity reduce the degree of photoprotection induced by low pH on *U. prolifera*? When *U. prolifera* was in a low-salinity environment, it absorbed water to balance the osmotic pressure, destroying cytomembrane and chloroplast membrane structures (Pancha *et al*. 2015), and further damaged the photoprotection mechanism. Meanwhile, to adapt to the low-salinity environment, the thalli might voluntarily lose some ions and soluble cellular components to reduce their osmotic pressure (Feng *et al*. 2016) at the expense of the destruction of their photosynthetic ability. In addition, the capacity of photoprotection declined with the increase of treatment duration (Fig. 5). Li *et al*. (2017) confirmed that long-time acid treatment harmed the photoprotection to *U. prolifera*, thus reducing the ability to self-regulate the xanthophyll cycle, which led to NPQ decline. Therefore, we speculated that if the pH of acid rain was ≤ 3.5, with the salinity decrease (salinity 1‰ and 10‰) and the rainfall time increase (1 and 2 h), the photosynthetic activity of *U. prolifera* would be destroyed and the scale of green tides would decrease.

Conclusion: The impact of pH on the photosynthetic activity of *U. prolifera* was greater than that of salinity; the photosynthetic activity of *U. prolifera* treated under 2-h treatment duration with the low pH (3.5) could not recover to normal levels after 24-h recovering in natural seawater. Moreover, low salinity (salinity 1‰ and 10‰) reduced the degree of photoprotection under low pH (3.5) conditions, thereby impairing the photosynthetic activity. Finally, we speculated that if the pH of acid rain ≤ 3.5, with the salinity decrease (salinity 1‰ and 10‰) and the rainfall time increase (1 h and 2 h), the amount of *U. prolifera* and the scale of green tides would decrease.

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