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Diuron effects on photosynthesis and vertical migration of microphytobenthos: Potential rapid bioassessment of herbicide toxicity in coastal sediments

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Abstract :

The effects of herbicide diuron on photosynthesis and vertical migration of intertidal microphytobenthos (MPB) assemblages were investigated using chlorophyll fluorometry. The results shown diuron ≤ 60 μg L−1 had no obvious effect on MPB vertical migration during 24 h indicated by consistent rhythm. Low concentration of 10 μg L−1 diuron had no significant influence on MPB photosynthesis throughout, however, high concentrations of 40, 50, and 60 μg L−1 had significant impacts exhibited by decreased parameters of maximum relative electron transport rate (rETRmax), maximal PS II quantum yield (Fv/Fm) and non-photochemical quenching (NPQ). For middle concentrations of 20 and 30 μg L−1, above decreased 3 parameters recovered sooner or later after 2 h or 16.5 h. Comparatively, rETRmax, Fv/Fm and NPQ are concentration dependent and more sensitive than other parameters in assessing diuron toxicity. This study revealed the potential of using MPB assemblages and chlorophyll fluorometry for rapid assessing diuron toxicity in coastal sediments.

Highlights

► The photophysiology of MPB in sediments is very sensitive to diuron. ► The vertical migration of MPB in sediments is not impacted by diuron. ► Adverse diuron effects increase together with increasing herbicide concentrations. ► Photosynthetic parameters F_v/F_m, $rETR_{max}$, and NPQ are the most sensitive to diuron. ► Well-plates combined with imaging fluorimetry provide a rapid toxicity monitoring.

Keywords : Microphytobenthos, Photosynthesis, Vertical migration, Diuron, Herbicide toxicity, Bioassessment

1. Introduction

Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea, DCMU), as a urea-type herbicide has been effectively used to control a wide variety of grasses and weeds on cotton, coffee, sugar cane, and citrus farms (Coelho-Moreira et al., 2018). Apart from this, the organic biocide diuron has gained popularity as an antifouling agent in paints, especially to prevent fouling of algae on ship hulls and boats (Chesworth et al., 2004; Mukherjee et al., 2009). Incidentally, this kind of herbicide has been released to the freshwater and marine environments inevitably for more than 20 years. Due to its low solubility (42 mg mL^{-1} at 25 °C), long persistence in soil (4–8 months) and chemical stability in water, diuron tends to accumulate in the environment (Tixier et al., 2001). It has been detected not only in ground and freshwater systems (Nitschke and Schüssler,1998; Bruggen and Vandecasteele, 2003), but also in coastal water and sediments (Jones et al., 2003; Ali et al., 2014; Holmes, 2014).

As a universal PSII inhibitory herbicide, diuron can directly inhibits non-target photosynthetic organisms (Haynes et al., 2000; Jones, 2005). Besides, diuron's harms to human and animals have already been reported in numbers of literatures, causing diseases such as mutagenic, metabolic, abnormalities of the liver and spleen, and problems in the transport and release of natural hormones (US Government, 2005; Wong et al., 2013; Simões et al., 2017). All these make diuron a threat to the environment and human being, therefore, its ecological risk has been keenly concerned by countries over the world. It is declared that in 2011 Australia government has banned using it, and a series of assessment reports (US Government, 2005; Australian Government, 2011) and related researches have been carried out over the past two decades (OECD, 2006; Magnusson et al., 2008; Kumar et al., 2010; Negri et al., 2015).

In the published literatures, there are some standards dataset for accessing the toxicity of diuron on terrestrial plants (Australian Government, 2011; Holmes, 2014). In coastal ecosystems, some publications showed the toxicity of diuron for benthic photosynthetic organisms exposed through the water phase, which has been implicated in negative impacts on seagrass (Haynes et al., 2000), mangroves (Duke et al., 2005; Duke, 2008), coral (Jones et al., 2003; Negri et al., 2005, 2011), cyanobacteria (Stingaciu et al., 2019), and benthic microalgae (Magnusson et al., 2008, 2012). Standard toxicity tests have been conducted by exposing photosynthetic organisms to diuron in water (USEPA, 1996; OECD, 2006). In terms of risk characterization, the dataset had been consequently

applied in risk quotients of these organisms (Australian Government, 2011). Following these criteria, within the catchments and waters of the Great Barrier Reef, diuron is frequently detected in aquatic and marine water quality monitoring programs (Shaw et al., 2010; Kennedy et al., 2012; Lewis et al., 2012; Smith et al., 2012). However, those datasets have not yet established for benthic flora or fauna when exposed to diuron through the sediment due to short of investigations.

Microphytobenthos (MPB) is a group of photosynthetic organisms living in the upper several millimetres of sediments where light reaches, and frequently dominated by diatoms in coastal flats (Admiraal et al., 1984; MacIntyre et al., 1996; Underwood and Kromkamp, 1999). With short life cycle and relevant stable position in the sediment, benthic diatoms are very suitable indicators for monitoring the status of ecological environment. In freshwater system, there have been already numerous publications deliberated it (Kelly and Whitton, 1995; Potapova and Charles, 2007; Stevenson et al., 2010; Bere and Tundisi, 2012; Chen et al., 2016). It is widely used benthic diatoms as Biological Diatom Index (BDI; Coste et al, 2009) in the Water Framework Directive (EU Directive 2000/60/EC) for assessing freshwater system. Theoretically, MPB is very potential bioindicator to monitor or detect toxicity of pollutants in coastal ecosystems. Desrosiers et al. (2013) has proposed that using benthic diatoms for assessing water quality of coastal environments.

MPB shows high photosynthetic capacity and efficient protection against photoinhibition through both physiological and behavioural mechanisms (Serôdio et al., 2006; Cartaxana et al., 2011; Lavaud and Goss, 2014; Barnett et al., 2015; Laviale et al., 2015). Physiological photoprotection mainly depends on distinct xanthophyll pigments, diadinoxanthin and diatoxanthin, which support the dissipation of excessive light energy (the so-called non-photochemical quenching process) and protect PSII reaction centres (Barnett et al., 2015; Cartaxana et al., 2016; Frankenbach et al., 2018; Pniewski and Piasecka-Jędrzejak, 2020). Behavioural photoprotection is realized through the vertical migration of MPB within the uppermost layers of sediment, by which motile diatoms position themselves at suitable depth for optimal light conditions (Consalvey et al. 2004; Barnett et al., 2020). Disturbed or inhibited motility can impair MPB photosynthetic capacity, especially under high irradiance and/or prolonged illumination (Laviale et al., 2015; Cartaxana et al., 2016; Du et al., 2018; Frankenbach et al., 2018).

This study aimed through applying gradients of diuron on natural MPB assemblages to detect

their effects on MPB photosynthetic activities and behaviour characteristics of vertical migration, and monitor the duration of these effects during a whole day. The imaging chlorophyll fluorescence techniques were used to ensure prompt and non-destructive measurement, and assist further assessment of diuron toxicity in sediment.

2. Materials and methods

Sediment samples were collected from the intertidal mudflat of Aiguillon Bay (47°00' N, 1°05' W) La Rochelle, France, on 18th February, 2015. The sampling site is composed of fine muddy sediments (more than 90% of grain size < 63μm) where microphytobenthic biofilm is largely dominated by epipelic diatoms, especially species of genera *Navicula* and *Gyrosigma* (Du et al. 2017). The surface layers of sediment (top ~0.5 cm) were collected using a spatula.

In the laboratory, the sediment was sieved through a 500 μm mesh to remove the meio- and macrofauna, such as nematodes and sand hoppers, and was thoroughly mixed and spread in 4 cm deep plastic trays. The stock solution of diuron (0.05 g L^{-1}) was made up in 95% ethanol. In individual glass beakers, corresponding amounts of the solution were diluted with dH_2O and mixed well with sediment samples to reach final concentrations of 0, 10, 20, 30, 40, 50 and 60 μg L^{-1}. Beside the blank control of 0 μg L^{-1} diuron, to check the influence of solvent, the same amount of 95% ethanol that equal to that of stock solution used for 60 μg L^{-1} diuron was applied in sediment samples to be as the ethanol control. Each 3 mL well mixed sediments were transferred into a well of 24-well plates using pipette. Each concentration had 3 replicates, and two sets of well-plates were prepared. A minimum of 30 min was allowed for the well-plate system with diuron and MPB assemblages to stabilize before measurements started.

Chlorophyll fluorescence was measured using an Imaging-PAM fluorometer (Max/L, Walz, Germany) for 24h during 18th to 19th February. Before measuring fluorescence, areas of interest (AOIs) were defined under Live Video Mode, with circular samples of the same size as internal diameter of well in 24 well-plates. The same AOIs were used consistently over the course of the experiment. Fluorescence was induced by royal blue (450 nm) 3W Luxeon LEDs, with a standard intensity of 0.5 μmol photons m^{-2} s^{-1} and a modulation frequency between 1 and 8 Hz. The current fluorescence yield, F_t, for monitoring surface MPB abundance were measured at 900 s intervals

using one set of well-plate samples for 24 h. The other set of well-plate samples was incubated under "ambient filtered true light" conditions (i.e., light passing by the window of the laboratory, 24-h natural photoperiod, no direct sunlight). The room conditions in laboratory were stable around 15°C temperature and 60% humidity. The rapid light curves (RLCs) were measured to assess the photosynthetic activity, by exposing to 7 incremental steps of actinic light (30 s per step) ranging from 0 to 610 μmol photons m^{-2} s^{-1} (PAR, photosynthetically active radiation). The measurement of RLCs were taken after 15 min dark-adaptation at 6 different times during the whole experiment. To check the short-time effect of diuron, RLC measures were carried at 1 h intervals in the daytime of the first day. To monitor the persisting of diuron effect or MPB recovery from it, in the next day, RLC measures were taken at the beginning and the end of the day respectively. For each irradiance level (E), the relative electron transport rates (rETR) was calculated from the product of E and the PSII effective quantum yield ($Y(II) = (F_m' - F)/F_m'$), $rETR = E \times (F_m' - F)/F_m'$ (Genty et al., 1989), where the F is fluorescence yield, F_m' is maximum fluorescence under the highest actinic light of RLC. The photosynthetic parameters, the maximum relative electron transport rate ($rETR_{max}$), maximum light utilization coefficient (α) and the minimum saturation irradiance (E_k) were derived from the RLCs, referring the model of Platt et al. (1980), $ETR = ETR_{max} (1\text{-}\exp(-\alpha \times E/ETR_{max}))$, $E_k = ETR_{max}/\alpha$. The maximal PS II quantum yield (F_v/F_m) was calculated as $(F_m\text{-}F_0)/F_m$, where F_0 is the dark fluorescence yield and F_m is maximum fluorescence yield after dark adaptation. The non-photochemical quenching (NPQ) was calculated as $(F_m - F_m')/F_m'$ (Maxwell and Johnson, 2000).

Statistical analysis was carried out using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). To test the effect of diuron on migration, separate one-way ANOVAs were run on F_t for each treatment, followed by the Tukey's HSD- post-hoc tests to compare paired difference. To compare the effects of different diuron concentrations on photosynthesis, T-test were run for each treatment on the variables of rETR, $rETR_{max}$, α, E_k, F_v/F_m and NPQ at individual measuring time.

3. Results

3.1 Chlorophyll fluorescence yield (F_t) variations

Indicated by F_t, distinct variations of surface MPB abundance along with experiment time were illustrated in Figure 1. Under weak measuring light of F_t (0.5 μmol photons m^{-2} s^{-1}), the natural

MPB assemblage which had been artificially mixed homogenously with sediments still exhibited their endogenous migration up to the surface. Besides the initial rapid increase at the beginning, there was steeply ascending F_t from AM 3:30 to 5:00 during the early morning of the next day before sunrise, and followed by relatively smoothly increase till the midday around 12:00. Relatively decreased F_t happened around PM 20:30 and AM 2:00 (the next day) during night. In the next day, after midday peak, the F_t slowly declined until the end of experiments. Averagely, the Ft of the next day was twice higher than that of the first day. Overall, there was no significant difference between the different treatments for all measured F_t (Tukey HSD test, $P > 0.05$), especially for treatments of diuron concentration less than 40 μg L^{-1}, there was no concentration dependent difference along with the experiment time. However, it illustrated two trends which are worthy of further study. One is the blank control (0 μg L^{-1}) presented the largest variation (SD, ±0.717) throughout the whole experiment with relatively lower F_t during night and higher F_t during day than other treatments. The another is the high concentration of 50 or 60 μg L^{-1} generally induced the lowest F_t. Especially for 60 μg L^{-1} in the next day, the difference became significant with 20 or 40 μg L^{-1} since 10:49 (after 18.5h exposure, paired t-test, $P < 0.05$), but still not significant with the remained treatments including controls (paired t-test, $P > 0.05$). Overall, the results indicated that no significant diuron effects on vertical migration of MPB within 60 μg L^{-1} concentration during 24h experiment period.

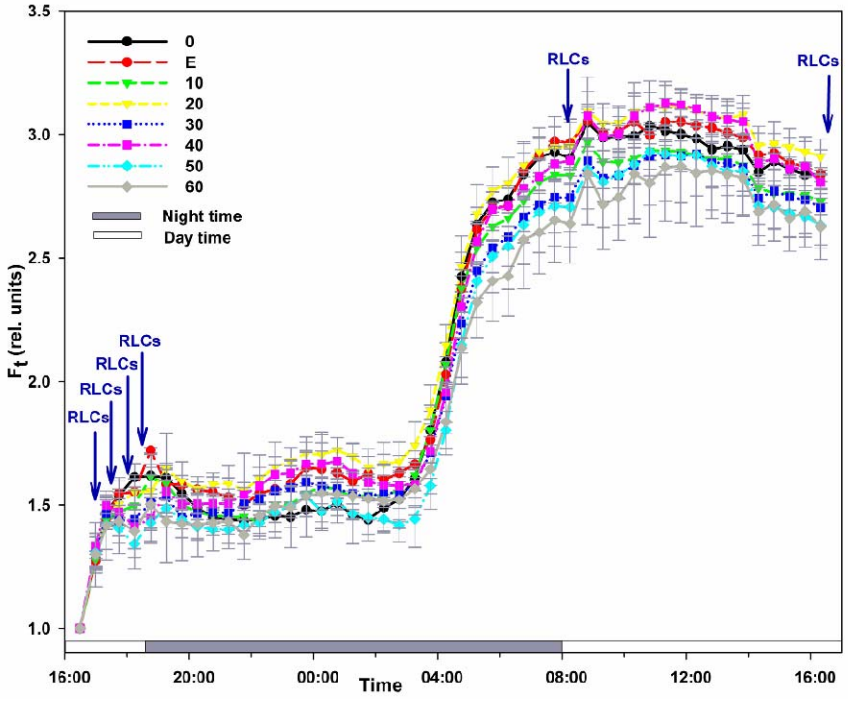


Fig. 1. F_t variations along with time under different treatments (shown as 30 min interval and normalized to initial values). Concentrations of 0, 10, 20, 30, 40, 50, 60 $\mu g \cdot L^{-1}$ diuron are represented by numbers, and ethanol control is labelled as E; dark grey bar, night time; blank bar, day time of natural photoperiod. RLCs (rapid light curves) arrows point the timing at which RLCs were performed.

3.2 Rapid light curves (RLCs) and photosynthetic parameters

RLCs generated by PAM fluorometers presented MPB photosynthetic activity of different treatments. It showed a similar relation pattern of different treatments at 6 measuring time (Fig. 2). The RLCs of blank and ethanol control always accompanied closely and presented increasing rETR along with incremental light intensity more rapidly than other treatments. On the contrary, the high diuron concentrations of 50 and 60 μg L^{-1} constantly induced the most slowly increasing rETR along with light intensity. After 1.5 h exposure, the RLCs of 10 μg L^{-1} diuron almost overlapped with those of blank or ethanol control. The RLCs of 40 μg L^{-1} diuron always closed to those of 50 μg L^{-1} diuron. The RLCs of 20 and 30 μg L^{-1} diuron were always in the middle of all curves at each measuring time.

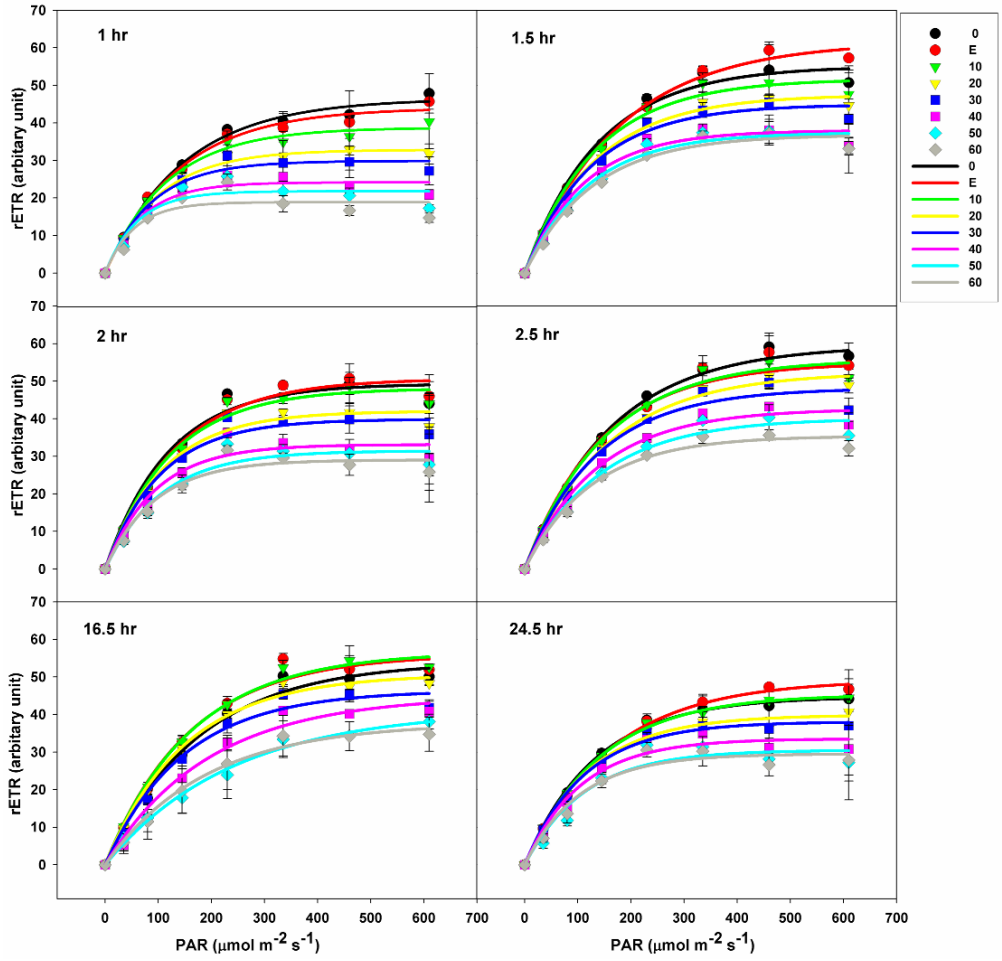


Fig. 2. Rapid light curves (RLCs) with corresponding fitting curves of the different treatments at 6 measuring time, which are marked by blue arrows in Fig.1 with starting timing 0 hr of the experiment beginning. Treatments symbols of 0, 10, 20, 30, 40, 50, 60 μg·L^{-1} diuron are represented by numbers and ethanol control by E. The monochrome lines represent corresponding fitting curves (Platt et al., 1980).

Five key photosynthetic parameters derived from fluorescence measurement were illustrated their variation under different treatments at 6 measuring time (Fig.3).

Revealed by $rETR_{max}$, the diuron toxicity distinctly affected MPB photosynthetic ability under different treatments (Fig. 3a). It showed that the $rETR_{max}$ under high concentrations of 40, 50 and 60 μg L^{-1} were significantly lower than other treatments ($P < 0.01$ or 0.05), especially at the early period ($P < 0.01$). There was no difference of 10 μg L^{-1} diuron with controls on $rETR_{max}$ throughout the whole experiment ($P > 0.05$). Comparing to blank control, the inhibition of 20- 60 μg L^{-1} diuron on $rETR_{max}$ was more distinct under 1 h exposure, but for lower concentrations of 20 and 30 μg L^{-1} diuron, the inhibition recovered from 2 h. However, for high concentrations of 40 to 60 μg L^{-1}

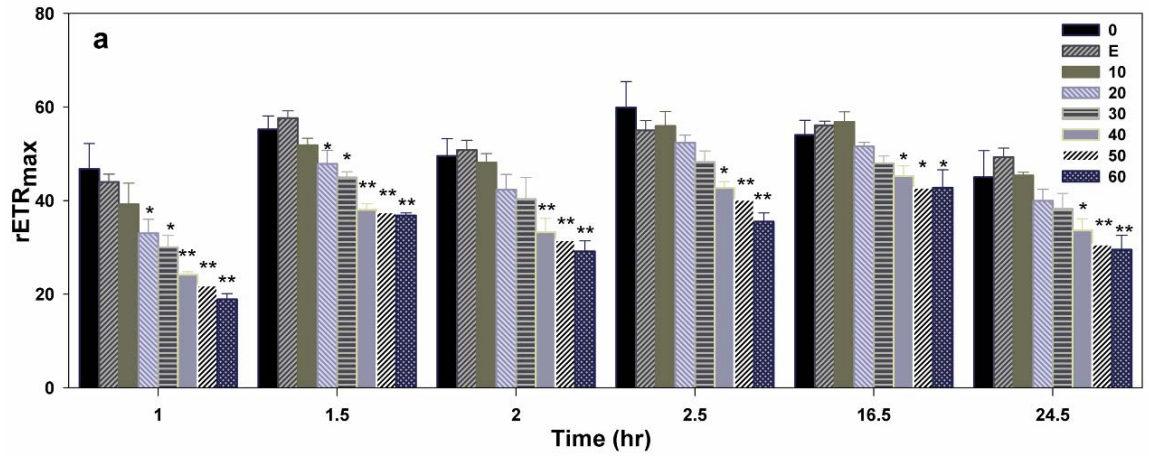
diuron, no recovery happened until the end of the experiment (24.5 h).

The diuron effect on the maximum light utilization coefficient (α) exhibited less difference than on $rETR_{max}$ (Fig. 3b). There was no difference of α among all treatments under 1 h exposure. Comparing to blank control, 10-30 μg L^{-1} diuron had no significant effect on α throughout the experiment ($P > 0.05$), 40 μg L^{-1} diuron induced significantly different α from 1.5 h to 16.5 h exposure ($P < 0.01$ or 0.05), while 50 and 60 μg L^{-1} diuron resulted in significant difference from 1.5 h till to the end of the experiment (24.5 h) ($P < 0.01$ or 0.05).

For the minimum saturation irradiance (E_k), only 40-60 μg L^{-1} diuron caused significantly different E_k with controls under 1 h exposure ($P < 0.05$, Fig.3c). After 1 h, no significant difference existed in E_k of each treatment from control.

The maximal PS II quantum yield (F_v/F_m) which indicated the photosynthetic capacity presented the similar trend as the $rETR_{max}$ (Fig. 3d). Besides no difference on F_v/F_m between 10 μg L^{-1} diuron and controls ($P > 0.05$), the difference among treatments was still distinct throughout the experiment. For 20 and 30 μg L^{-1} diuron, the significant impacts on F_v/F_m recovered for a longer time as 16.5 h and 24.5 h respectively, however, for high concentrations of 40 to 60 μg L^{-1} diuron, the effects were significant throughout the whole experiment ($P < 0.01$ or 0.05).

Comparing to above 4 parameters, the non-photochemical quenching (NPQ) exhibited more distinct difference among treatments at each measuring time, except at the dawn of the second day (16.5h, Fig.3e). The 10 μg L^{-1} diuron still showed no significant effect on NPQ indicated by no difference with the controls ($P > 0.05$). The 20 and 30 μg L^{-1} diuron had identical significant effects on lowering NPQ from 1 h to 2.5 h exposure ($P < 0.01$ or 0.05). At the dawn of the second day (16.5h), it showed the effects of 20 and 30 μg L^{-1} diuron were not significant due to the whole dropping of NPQ values, however, for 30 μg L^{-1} diuron the effect became significant again at 24.5 h along with increased NPQ of all treatments ($P < 0.05$). The high diuron concentrations of 40, 50 and 60 μg L^{-1} brought significant impacts on NPQ over whole experiment ($P < 0.01$ or 0.05) and exhibited significant difference among themselves in the first day from 1.5 h to 2.5 h exposure ($P < 0.01$ or 0.05).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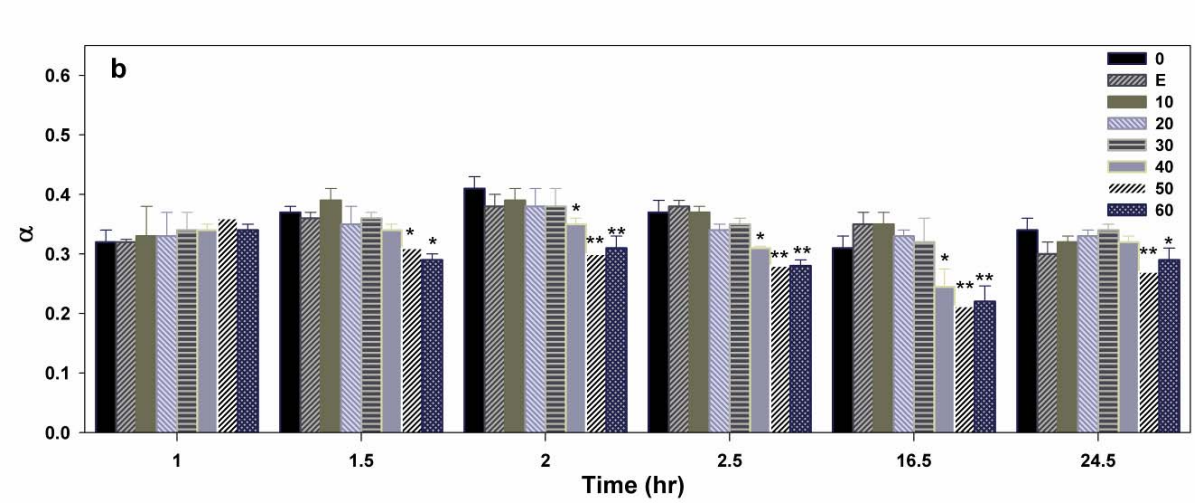
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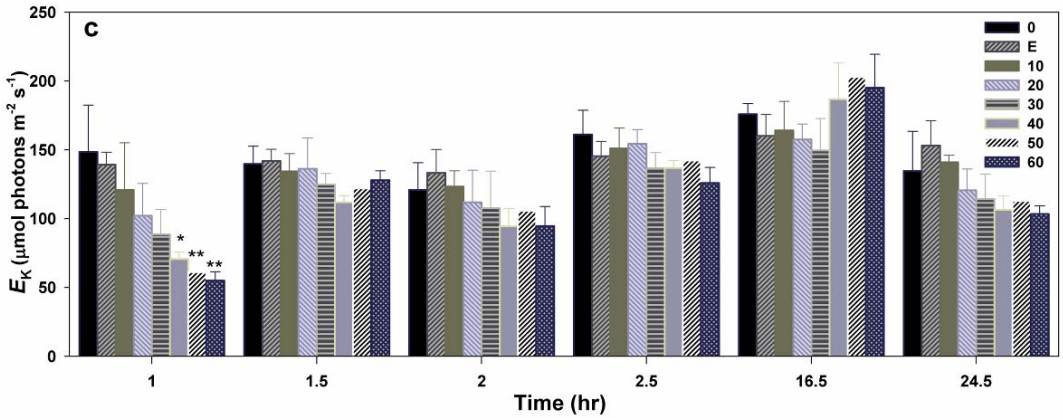
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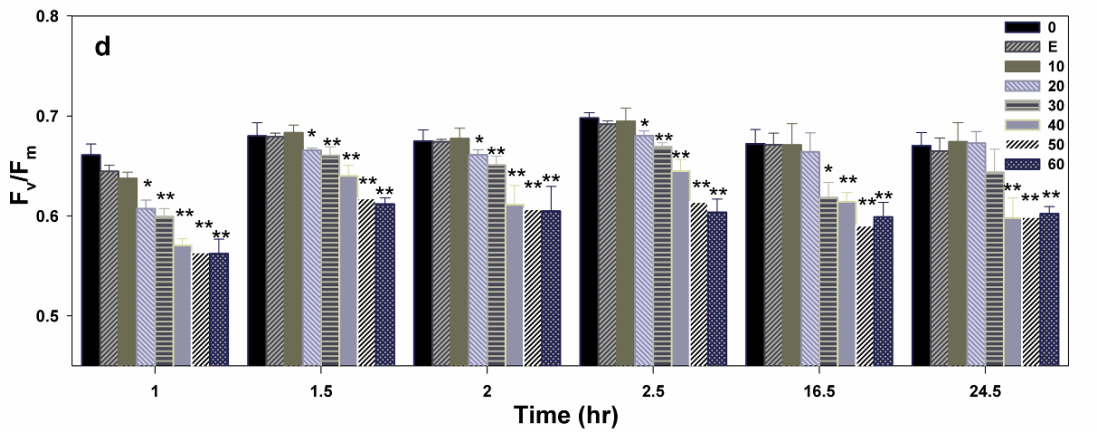

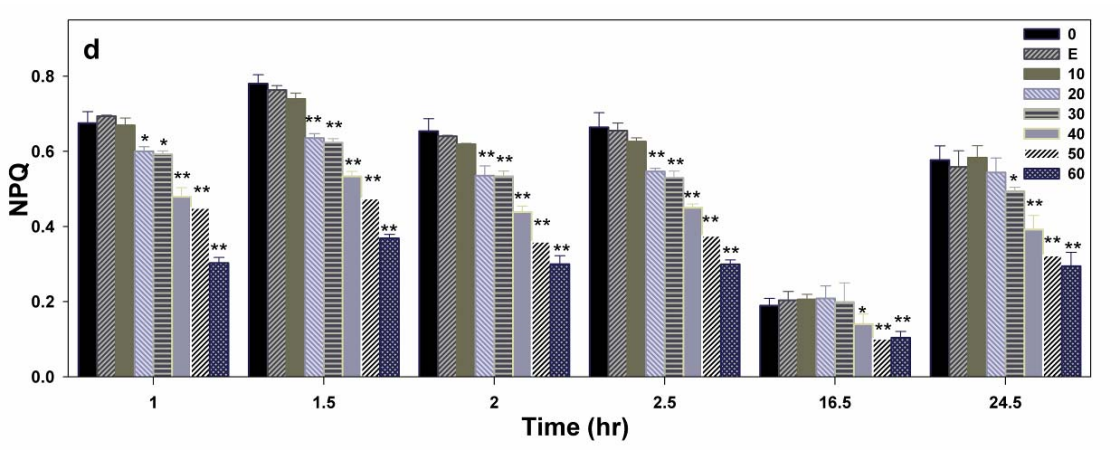

Fig 3. Variations of photosynthetic parameters under different treatments as a function of experiment time (hr). a, maximum relative electron transport rate ($rETR_{max}$); b, light utilization coefficient (α); c, minimum saturation irradiance (E_k); d, maximal PS II quantum yield (F_v/F_m); e, non-photochemical quenching (NPQ). Concentrations of 0, 10, 20, 30, 40, 50, 60 $\mu g \cdot L^{-1}$ diuron are represented by numbers, and ethanol control is labelled as E. Symbols * and ** denote the statistical difference of each treatment from the blank control (0) significantly by $P < 0.05$ and $P < 0.01$, respectively.

4. Discussion

Diuron effects on microphytobenthos (MPB) photosynthesis

Diuron inhibition on photosynthesis has been observed in all kinds of aquatic plants and algae, with differences in both toxicity degree and minimum toxicity concentration both of which depend on exposure time. For brown macroalga *Saccharina japonica*, after 2 weeks exposure to diuron, it

presented a concentration dependent decrease on photosynthetic parameters of F_v/F_m and ETR_{max} along with incremental concentration from 0.1 to 0.4 mg L^{-1} (in water) and a complete inhibition of electron transport at 0.4 mg L^{-1} (Kumar et al., 2010). For seagrass *Zostera marina*, the EC20 of diuron revealed that the lowest concentration was 0.5 μg L^{-1} for significant effect on growth and photosynthetic efficiency in 10 days (Chesworth et al., 2004). Other two tropical seagrass *Halodule uninervis* and *Z. mueller* were significantly inhibited by 0.3 μg L^{-1} diuron on their photosynthetic efficiency (F_v/F_m and Y(II)) over a 11-week exposure period (Negri et al., 2015). In the first study of diuron toxic effects on tropical MPB (diatom *Navicula* sp. and chlorophyte *Nephroselmis pyriformis*), growth and photosynthetic efficiency were reduced by 10% when exposed to diuron concentrations as low as 0.5~2μg L^{-1} for 3 days (Magnusson et al., 2008).

These above studies were all carried out for a longer exposure time from 3 days to 11-week and tested the diuron toxicity in water. However, in the present study, natural MPB assemblages was used to test the diuron toxicity in sediments during shorter time within 24.5 h. It shown that the lowest diuron concentration of 10 μg L^{-1} had no obvious effect on MPB photosynthesis, while, from 20 μg L^{-1} to 60μg L^{-1}, it presented a concentration dependent decrease in the photosynthetic activity. Under this situation, the minimum toxicity concentration on MPB could be 40μg L^{-1} for 1 h till to 24.5 h. Because the impact of 20 or 30 μg L^{-1} was mostly evident within 2 h and could recovered sooner or later (2 h or 16.5 h) according to individual parameters, if using them as minimum toxicity concentration, the testing time and parameters should be considered together. Nevertheless, our study suggested a very short time as 1 h for assessing diuron toxicity. Cho et al. (2008) also proposed that short time as 2 h for suspended microalgae to predict EC50 values using a photosynthetic activity measurement system (substantially dissolved oxygen meter) for rapid toxicity assessment. It corroborated our study on supplying a potential rapid method instead of the conventional algal assay, in which 96 h is recommended for assessing toxicity based on algal growth rate (U.S. EPA, 1996 and OECD guidelines, 2002).

Previous studies almost tested diuron toxicity in water on aquatic photosynthetic organisms. Even for MPB Magnusson et al. (2008 and 2010) used suspended cells of tested species and developed an artificial epiphytic biofilm (2012) for assessing, it still tested water phase toxicity. Without directly applying diuron in sediments, it hardly reflects the natural response of benthic flora

in coastal sediments. Furthermore, diuron, and its toxic degradation products, could persist longer in sediments than in water, potentially imposing longer lasting toxicity (Tixier et al., 2001). Therefore, through mimicking the exposure of MPB in freshly collected mudflat sediments to diuron, our study is specifically practical for assessment diuron toxicity in coastal sediment.

It was also noteworthy that, in this study, the recovery of MPB photosynthetic ability under 20 and 30 μg L^{-1} diuron concentrations was different according to respective parameters, i.e. recovery was observed after 2 h exposure for $rETR_{max}$, while it needed the overnight period for NPQ and F_v/F_m. Such recovery was also observed in other aquatic photosynthetic organisms and very much species-dependent (Negri et al., 2005; Magnusson et al., 2012; Burns et al., 2015; Esteves et al., 2017). Among them, diatoms are often considered as more resistant to and more rapidly recoverable from diuron toxicity (Juneau et al., 2007; Magnusson et al., 2012; Stachowski-Haberkorn, et al., 2013), which possibly most thanks to the capability of diatom frustules (porous silica cell-wall) to sort and filter nutrients from harmful agents (De Tomassi et al., 2017).

Diuron toxicity assessment on MPB using chlorophyll fluorescence-derived photosynthetic parameters

To assess the environmental toxicity of a contaminant, a biological sensitive parameter with clear concentration-dependent ranging values needs to be defined as a solid toxicity indicator. The chlorophyll fluorescence-derived photosynthetic parameters firstly contribute to a rapid, sensitive and non-invasive assessment based on photosynthetic activity (Juneau et al. 2007; Baker 2008; Murchie et al. 2013). Furthermore, coupled with a well-plate system, the imaging fluorometry (Imaging-PAM) used in this study realized a monitoring on multi-samples at the same time.

As referenced above, several previous studies also used chlorophyll fluorometry to assess the toxicity of diuron on benthic-living photosynthetic organisms, such as F_v/F_m and ETR_{max} used for brown macroalga (Kumar et al., 2010), F_v/F_m and Y(II) for tropical seagrass (Negri et al., 2015), Y(II) for tropical benthic microalgae (Magnusson et al., 2008 and 2010). Juneau et al. (2007) reviewed chlorophyll fluorescence-derived parameters and considered the F_v/F_m may not be the best parameter for evaluating toxic effect of herbicides due to its relatively low sensitivity, and rather, proposed better use Y(II) and NPQ since they better integrate the physiological status of the plant.

In the present study, five derived parameters, $rETR_{max}$, α, E_k, F_v/F_m, as well as NPQ, were assessed in determining diuron effect. Although all photosynthetic parameters were lowered during the 24.5 h diuron treatment, the effective concentrations as well as the timings differed among parameters. The α showed a delay reaction over 1 h, and was the most insensitive of the parameters examined, exhibiting relatively stable values especially under 0-30 μg L^{-1} diuron range. The other derived parameter, E_k presented a more volatile and irregular trends. Comparatively, $rETR_{max}$, F_v/F_m and NPQ were more sensitive and reliable parameters with clear concentration-dependent ranging values. Although for most tested parameters, the minimum toxicity concentration could be 40μg L^{-1} diuron as its throughout significant influence, on the other hand, with respect to individual parameters such as $rETR_{max}$ and F_v/F_m, it could be the lowest as 20μg L^{-1} for only 1 h exposure time. Besides, compared to others, NPQ could better distinguish the difference among individual diuron concentrations. Juneau et al. (2007) proposed to use a combination of various fluorescence parameters to provide complementary information on the mode of herbicide action. Therefore, based on our study and previous researches, we suggested $rETR_{max}/F_v/F_m$ and NPQ as the combination indicators for diuron toxicity in sediments. Our study also validated the utility of imaging chlorophyll fluorometry as a rapid and reliable technique to measure sub-lethal toxicity threshold.

Indirect effect of diuron on MPB photosynthesis inhibition

Because photosynthesis is a central process that supports the whole cell metabolism, its inhibition could cause potential effects on other activities. Staats et al. (2000) observed that the secretion of exopolysaccharide (EPS) by motile benthic diatoms was decreased after diuron inhibition on photosynthesis. EPS keenly associates with diatoms motility, especially in building the trails for diatom motility on substrate surface (Edgar and Pickett-Heaps,1984; Hoagland et al. 1993, Molino and Wetherbee, 2008). Even Coquillé et al. (2015) suggested to use diatom motility features as endpoints of herbicide metolachlor toxicity. However, in our study, there was no evidence of diuron-driven differences in vertical migration of natural MPB assemblages during 24.5 hours. Instead, in our conditions, MPB abundance at the surface of sediment indicated by F_t went on varying at the function of photoperiod (and probably of the tidal cycle), with striking increase of F_t before day starts (Fig.1) as previously reported before (Consalvey et al. 2004; Barnett et al. 2020). It

was consistent with the observation of Staats et al. (2000) that *in situ* patterns of diuron-treated-MPB vertical migration in the dark did not differ from those exposed to light during 24 h field experiment on mudflat surface. Both studies indicated that although light steers MPB vertical migration, the decrease in photosynthetic activity itself does not seem to directly influence this directive motility of MPB in a short time as 24 h. This phenomenon, from other side, also endows MPB a special ability to actively avoid further exposing to diuron as benthic motile diatoms did in escaping from herbicide metolachlor (Coquillé et al. 2015) and directing to dissolved silicate (Bondoc et al. 2016). Further study needs to explore the possible combined results, because it is crucial in the framework of using chlorophyll fluorometry to bioassess diuron and photosynthesis-impacting contaminants in coastal sediments.

As natural assemblages in coastal flats, MPB community dynamics in species composition and biomass are close related to a complex set of environmental factors (MacIntyre et al. 1996; Underwood and Kromkamp 1999). The effect of diuron on MPB also depends on environmental factors such as light and temperatures (Camuel et al., 2017; Chaumet et al., 2020). Therefore, it should be considered these factors when assessing diuron toxicity *in situ* in different seasons and regions. Furthermore, the influence of diuron on MPB photosynthesis inevitably firstly impacts MPB functions as primary producer in coastal ecosystem. Secondly, according to the findings of class-specific responses of marine benthic microalgae (Magnusson et al.,2008, 2010 and 2012) and species-specific sensitivity of benthic diatoms (Larras et al., 2012; Wood et al., 2016) to herbicide, chronic exposures to diuron also could in turn affect the sensitivity and community structure of MPB (Magnusson et al., 2012).

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Declaration of competing interest

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

CRediT authorship contribution statement

Guo-ying Du: planning, conceptualization, methodology, performed the experiments and drafted the original manuscript, **Xue-feng Zhong** and **Shuai Che**: performed the data analysis, editing the manuscript; **Christine Dupuy**: project administration and supervision, funding acquisition, **Johann Lavaud**: planning, methodology, supervision, reviewing and editing the manuscript.

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