Periphytic biofilms accumulate manganese, intercepting its emigration from paddy soil

Pengfei Sun, Mengning Gao, Rui Sun, Yonghong Wu, Jan Dolfing

PII: S0304-3894(21)00135-7

DOI: https://doi.org/10.1016/j.jhazmat.2021.125172

Reference: HAZMAT125172

To appear in: *Journal of Hazardous Materials*

Received date: 4 November 2020
Revised date: 29 December 2020
Accepted date: 15 January 2021

Please cite this article as: Pengfei Sun, Mengning Gao, Rui Sun, Yonghong Wu and Jan Dolfing, Periphytic biofilms accumulate manganese, intercepting its emigration from paddy soil, *Journal of Hazardous Materials,* (2020) doi:https://doi.org/10.1016/j.jhazmat.2021.125172

Periphytic biofilms accumulate manganese, intercepting its emigration from paddy soil

Pengfei Sun[a], Mengning Gao[a], Rui Sun[a], Yonghong Wu[a,b*], Jan Dolfing[c]

[a] *Zigui Ecological Station for Three Gorges Dam Project, State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, 71 East Beijing Road, Nanjing 210008, China*

[b] *College of Hydraulic & Environmental Engineering, China Three Gorges University, Hubei Yichang 443002, PR China*

[c] *Faculty Energy and Environment, Northumbria University, Newcastle upon Tyne, NE1 8QH, UK*

***Corresponding author**:

Dr. Yonghong Wu

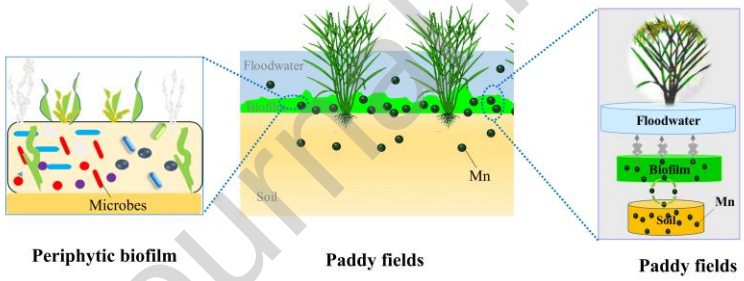
E-mail: yhwu@issas.ac.cn

Abstract

Manganese (Mn) in acidic paddy soil has large potential in emigrating from the soil and pollute adjacent ecosystems. Single microorganisms modulate the biogeochemistry process of Mn via redox reactions, while the roles of microbial aggregates (e.g. periphytic biofilm) in modulating its biogeochemical cycle is poorly constrained. Here we collected a series of periphytic biofilms from acidic paddy fields in China to explore how periphytic biofilm regulates Mn behavior in paddy fields. We found that periphytic biofilms have large Mn accumulation potential: Mn contents in periphytic biofilm ranged from 176 ± 38 to 797 ± 271 mg/kg, which

were 1.2-4.5 folds higher than that in the corresponding soils. Field experiments verified the Mn accumulation potential, underlining the biofilms function as natural barriers to intercept Mn emigrating from soil. Extracellular polymeric substances, especially the protein component, mediated adsorption was the main mechanism behind Mn accumulation by periphytic biofilm. Microorganisms in periphytic biofilms in general appeared to have inhibitory effects on Mn accumulation. Climatic conditions and nutrients in floodwater and soil affect the microorganisms, thus indirectly affecting Mn accumulation in periphytic biofilms. This study provides quantitative information on the extent to which microbial aggregates modulate the biogeochemistry of Mn in paddy fields.

Graphical abstract



Keywords: Periphytic biofilm; Mn bio-accumulation; Mn interception; Driving factors; Accumulation mechanisms.

1. Introduction

Manganese (Mn) is one of the most abundant elements on the surface of the Earth (Yu & Leadbetter, 2020). While it should be wary of that the Mn^{2+} contents widely fluctuate in soil, because Mn^{2+} in soil with high content and fluctuation would negatively affect the health of animals and even human via transformation

and migration (Jalali & Hemati, 2013). Microorganisms can alter the chemical forms of Mn via redox reactions and thus shift its biogeochemical cycle. Reduction of Mn by microorganisms using energy harnessed from MnO_2 as electron acceptor has been well documented (Henkel et al., 2019; Myers & Nealson, 1988), while the growth of microorganisms being able to use Mn(II) as electron donor and O_2 as electron donor, though long hypothesized, was documented only recently (Yu & Leadbetter, 2020). In nature, microorganisms generally grow in the form of microbial aggregates (Guilhen et al., 2017; Kim & Lee, 2016), and the role of microbial aggregates in the Mn cycle in the environment still needs to be established. It remains unknown whether microbial aggregates exhibit unique collective functions that are different from the way redox reactions of single microorganisms affect the Mn cycle in nature.

Periphytic biofilms growing in paddy fields are typical microbial aggregates. They grow ubiquitously like a lid on the interface between soil and floodwater (Larned, 2010; Wu, 2016), and have been shown to play important roles in regulating local N and P cycling, mainly via accumulation (Wu et al., 2016; Wu et al., 2018). Analogously, this leads to the hypothesis that periphytic biofilms could accumulate Mn, thereby potentially affecting efflux of Mn from paddy fields.

A periphytic biofilm is a mixture of prokaryotes and eukaryotes, with both the prokaryotes and the eukaryotes embedded in a matrix of extracellular polymeric substances (EPS) (Liu et al., 2019; Wu, 2016). Environmental factors (e.g. light and temperature) (Trabelsi et al., 2009) and soil nutrients (Flemming & Wingender,

2010; Freitas et al., 2017) shape its microbial community composition and diversity, which in turn affect its functional roles (Liu et al., 2019). Thus, analysis of the microbial composition of periphytic biofilms as shaped by different environmental factors would contribute to elucidating the underlying microbiological mechanisms by which periphytic biofilms fulfill their functional roles. Additionally, as the main abiotic component of periphytic biofilm (Bellinger et al., 2010; Sun et al., 2018a), EPS performs many functional roles such as adsorbing a diverse range of cationic elements from the environment (Flemming, 2016). This is due to EPS being rich in various negatively charged functional groups, such as carboxyl, carbonyl, etc. (Sun et al., 2018a). Based on the electric charge characteristics of EPS and Mn, this leads to a second hypothesis that EPS dominated adsorption processes may be partly accounting for Mn accumulation by periphytic biofilm.

To test the above hypotheses, we selected paddy fields located in the red soil region of south China as the research areas to collect periphytic biofilms, soil and floodwater samples. Soils in these areas are acidic (Dong et al., 2012), and the acidic soil conditions (e.g. low pH and Eh) could induce the dissolution of insoluble manganese oxides (MnO_2) into soluble Mn(II) (Huang et al., 2015; Shao et al., 2017), which increases the emigrate potential of Mn from the paddy soil to the adjacent receiving rivulets. In the face of high fluctuation of Mn in paddy fields, periphytic biofilm growing in such habitats is easier to show its response to Mn fluctuation.

Here we collected a total of 330 periphytic biofilm, corresponding paddy soil and floodwater samples. We also conducted field experiments to assess the impacts of periphytic biofilm on internal Mn behavior in paddy fields. The study answers the following two questions: (1) Can periphytic biofilm affect Mn transport in paddy fields via Mn accumulation? (2) What are the potential impact factors and mechanisms accounting for Mn accumulation by periphytic biofilm?

2. Methods and materials

2.1 Research areas and sample collection

The present study focusses on paddy soils in south China, the "red soil" region, where soils are acid, which enhances the availability and transferability of Mn. A total of 11 rice planting areas were selected for sample collection (Fig. S1A).

Per rice planting area ten sampling sites were randomly chosen within a radius of 1 km, for a total of 110 sampling sites. At each sampling site one periphytic biofilm, one corresponding paddy soil, and one floodwater sample were separately collected. All the samples were collected within 7-15 d after rice seedlings had been transplanted in 2018. Regarding sample collection, periphytic biofilm (about 50 g wet weight) was softly scraped from the surface of the soil using a sterilized stainless-steel knife, and visible clods were first removed and then washed several times with running floodwater to remove adhering soil from the biofilm samples until the effluent was no longer turbid. The washed biofilms were sealed in plastic sampling bags (Fig. S1B). Simultaneously, 100 mL of floodwater was bottled and 50 g of corresponding paddy soil from a depth of 0-20 cm (without periphytic biofilm) was

collected into sampling bags. All periphytic biofilm, soil, and floodwater samples were transported to the laboratory on ice and then stored at -20 ^{o}C before analysis. Additionally, the meteorological parameters (such as annual sunshine duration (h), annual effective accumulated temperature (^{o}C), and annual radiation intensity (MJ/m^2), etc.) of the sampling areas were collected from the website: http://data.cma.cn/site/index.html.

2.2 Field study

To verify the potential of periphytic biofilm to shift the biogeochemistry of Mn in paddy fields, field experiments were carried out in Changshu, Jiangsu, China (31.54°N, 120.63°E) in 2019. The biomass of periphytic biofilms in the experimental and control fields was manipulated, and then the Mn contents of these paddy soils and periphytic biofilms were quantified and compared. Specifically, two treatments were set: 1) in the experimental field (600 m^2), 2 kg of artificial carriers was employed after rice transplanting to induce the growth of periphytic biofilm. The carriers consisted of sodium alginate pellets containing WC medium (Sun et al., 2018a). During the preparation of sodium alginate pellets, distilled water was replaced by same-volume WC medium to dissolve sodium alginate, and then the pellets was prepared according to a previously described method (Sun et al., 2016a; Sun et al., 2015a); 2) in the control field (567 m^2), no carrier was applied and the periphytic biofilm in the field grew naturally. For both fields the transplanting date was 23 June 2019. Subsequently, paddy soils of both the experimental and control fields were collected on 24 June, 4 July, 14 July, 24 July, 2 August, and 23 August; additionally, periphytic biofilm

samples in both fields were collected on 4 July, 14 July, 24 July, 2 August, and 23 August. Mn contents in both the paddy soils and periphytic biofilm samples were quantified.

2.3 Sample analysis

Mn and Ca contents in periphytic biofilms and soils (0.5g, dry weight) were quantified using atomic absorption spectrometry with aqua regia digestion (Thermo Scientific™ iCE™ 3000, MA, USA). Additionally, 0.5 g dry weight of each periphytic biofilm/soil and 5 mL floodwater were digested by HNO_3-H_2O_2 in a digestion oven (JKXZ06-8B, China) before being used to measure total N (TN), total P (TP) contents in periphytic biofilm/soil; both TP and TN contents in biofilm, soil, and floodwater were quantified using a flow analyzer (FS3700, OI Analytical, USA). Total organic carbon (TOC) in periphytic biofilm, floodwater and soil were determined by the potassium dichromate method (Bao, 2005). EPS in periphytic biofilm were extracted using a modified alkaline extraction method, and protein and polysaccharide components were analyzed using the methods of a Bradford assay with bovine serum albumin as standard (Bio-Rad) and phenol-sulfuric acid, respectively (Sun et al., 2018a). The pH value of each floodwater sample was measured using a pH meter (Mettler Toledo FE28, Switzerland). For each sampling area, three samples were picked to analyze their microbial components, and the average values of the relative abundance of both prokaryotes and eukaryotes were calculated to summarize the microbial information of each sampling area.

2.4 16S and 18S rDNA gene amplicon sequencing and data analysis

To determine the microbial composition of periphytic biofilms, DNA was extracted from periphytic biofilm (2 g, wet weight) using DNA extraction kits (MOBIO 12888-100, Carlsbad, CA, USA). The contents and purities of the extracted DNA were measured using the NanoDrop One (Thermo Fisher Scientific, MA, USA). Then primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') and 528F (5'-GCGGTAATTCCAGCTCCAA-3') and 706R (5'-AATCCRAGAATTTCACCTCT-3') were respectively used for detecting the prokaryotes and eukaryotes diversities in periphytic biofilms using the HiSeq 2500 platform. Quality filtering on the raw reads were performed under specific filtering conditions to obtain the high-quality clean reads according to the Cutadapt (V1.9.1, http://cutadapt.readthedocs.io/en/stable/) quality control process. To detect and remove chimera sequences and finally obtained clean reads, the reads were compared with the reference database using UCHIME algorithm. Sequences analysis were performed by UPARSE software, of which sequences with ≥97% similarity were assigned to the same OTUs. Microbial sequences were then deposited into the NCBI databases with the accession number PRJNA543163.

2.5 Statistical analyses

All the statistical procedures were conducted with SPSS 16.0 software (SPSS Inc., Chicago, USA). The impact factors and mechanism for Mn accumulation by periphytic biofilm from the aspects of EPS components, environmental factors

(sunshine duration, effective accumulated temperature, and radiation intensity), nutrients in soil (TOC, TN, and TP) and floodwater (TOC, TN, TP, and pH) were analyzed using regression analysis. To explore the relationship of microbial compositions of periphytic biofilms with their Mn contents, a correlation matrix, running in R using the 'psych' package, was generated, and then the interaction network between prokaryotes, eukaryotes and Mn content in periphytic biofilm was analyzed and visualized using the software Gephi 0.9.2 (France). Partial Least Squares Path Modeling (PLS-PM) (Wang et al., 2016) was employed to illustrate the direct and indirect effects of prokaryotes, eukaryotes, and EPS on Mn accumulation in periphytic biofilm. PLS-PM was also employed to analyze how climate conditions, nutrients in soil and floodwater indirectly affect the microbial composition, EPS component, and Mn content in periphytic biofilm. PLS-PM were plotted using R Studio software. Figures were prepared using Origin 8.5 software (Origin Lab Inc., Massachusetts, USA). The prokaryotic and eukaryotic components of periphytic biofilms were presented as Heatmaps, and the composition of Mn oxidizing bacteria in periphytic biofilm was visualized via Circos software (http://circos.ca/).

3. Results

3.1 Mn accumulated in periphytic biofilm and its effect on Mn behavior in paddy soil

The Mn contents in periphytic biofilms collected in the tested rice planting areas varied significantly, from 107 to 1831 mg/kg, with average values ranging from 76 ± 38 to 797 ± 271 mg/kg (Fig. 1). The Mn contents in the corresponding paddy soils varied from 77 to 673 mg/kg, with average values ranging from 90 ± 9 to 376 ± 32

mg/kg. In all cases Mn content in periphytic biofilm was several folds (1.1-4.5) higher than that in the corresponding paddy soil ($p<0.05$, Fig. 1). The results verified the first hypothesis, that periphytic biofilms have excellent potential in accumulating Mn, which shows their potential to shift Mn biogeochemistry in paddy fields.

Following up on these field observations, we then performed a field experiment to verify and confirm the Mn accumulation potential of periphytic biofilms. Mn contents in periphytic biofilms collected from the control field were in the range of 328 to 560 mg/kg, with an average of 337±72 mg/kg. Mn contents in the soils of the control fields were in the range of 266 to 330 mg/kg, with an average of 291 ± 18 mg/kg (Fig. 2). Mn contents in periphytic biofilms of the control field was 1.2 folds higher than in the corresponding soils ($p<0.05$). The results verified and confirmed the excellent Mn accumulation capacity of periphytic biofilms.

The Mn contents of the periphytic biofilms in the experimental field, were in the range of 337 to 649 mg/kg, with an average of 465 ± 112 mg/kg (Fig. 2), while Mn contents in the corresponding soils were in the range of 278 to 339 mg/kg, with an average of 312 ±18 mg/kg (Fig. 2). Mn content in periphytic biofilms of the experimental field was 1.5 folds higher than in the corresponding soil ($p<0.05$). These results once again illustrate the high Mn accumulation capacity of periphytic biofilm.

Finally, special attention was paid to the Mn contents in soils of control and experimental fields to explore the effect of Mn accumulation on Mn behavior in paddy soil. The average Mn content of the experimental field (312 ±18 mg/kg) was higher than that of the control field (291 ± 18 mg/kg) ($p<0.05$, Fig. 2). The pattern

suggests that increasing the biomass of periphytic biofilm in paddy field enhanced the Mn content in the paddy soil, thus proving that the effect of periphytic biofilm accumulating Mn is intercepting Mn emigration from paddy soils. These results indicated that periphytic biofilm intercepts Mn emigration from paddy soils via accumulating Mn, shifting the biogeochemical cycle of Mn in such paddy fields.

3.2 Core community and Mn oxidizing bacteria in the periphytic biofilms

There were huge differences in microbial composition, at genus level, between periphytic biofilms collected from the selected rice planting areas (Fig. 3A). The core prokaryotic community (the most dominant genera) in each periphytic biofilm from the 11 sampling areas of TS, RH, QZ, NP, NB, HZ, CZ, YY, JZ, YC, and JJ were *Pirellula* (36.37%), *Planktothrix_NIVA-CYA_15* (20.20%), *Acinetobacter* (19.00%), *Acinetobacter* (21.37%), *Dinghuibacter* (7.66%), *Massilia* (5.75%), *Flavobacterium* (12.59%), *Proteiniclasticum* (13.33%), *Flavobacterium* (1.86%), *Flavobacterium* (15.44%), and *Bacteroides* (25.65%), respectively. Some of the organisms that were highly abundant in some biofilms were many folds lower in other biofilms and vice versa (Fig. 3A).

The core eukaryotic community in each periphytic biofilm consisted of *Characiopodium* (67.13%) in TS, *Aporcelaimellus* (24.30%) in RH, *Desmodesmus* (19.28%) in QZ, *Chlorotetraedron* (4.00%) in NP, *Paratripyla* (15.30%) in NB, *Chlorotetraedron* (16.44%) in HZ, *Heteromita* (13.85%) in CZ, *Aporcelaimellus* (12.45%) in YY, *Pythium* (6.12%) in JZ, *Nassarius* (10.63%) in YC, and *Peltodytes* (11.70%) in JJ (Fig. 3B).

Blasting the known Mn oxidizing bacteria against the bacteria in the periphytic biofilms, showed that there is a wide variety of known Mn oxidizing bacteria in periphytic biofilms (Fig. 3C). Given the abundance of Mn oxidizing bacteria in these periphytic biofilms, it can be concluded that periphytic biofilm is a habitat for Mn(II) oxidizers, and this suggests that Mn oxidizing bacteria in periphytic biofilm may have contributed to Mn accumulation via the formation of insoluble manganese oxides that precipitated in the biofilm.

3.3 Mechanisms underlying Mn accumulation in periphytic biofilm

Partial Least Squares Path Modeling (PLS-PM) was conducted to illustrate the direct and indirect effects of microorganisms, EPS, nutrients in soil and floodwater, and climatic conditions on Mn accumulation in periphytic biofilms (Fig. 4). The overall effects (including direct and indirect effects) of prokaryotes and eukaryotes on Mn accumulation in periphytic biofilm are -0.25 and -0.60 (Fig. 4), respectively. The results suggest that microorganisms in periphytic biofilms may overall decrease Mn accumulation in periphytic biofilm. The EPS component in periphytic biofilm on the other hand showed a large and positive direct effect on Mn content (path coefficient=0.78), suggesting that EPS-dominated adsorption may be the main mechanism behind Mn accumulation in periphytic biofilm.

Besides the direct effects, factors such as climatic factors and nutrients in paddy soil and floodwater indirectly affect the Mn accumulation by affecting the microbial composition in periphytic biofilm (Fig. 4). Combining their direct and indirect effects, the overall effects of climatic factors and nutrients in paddy soil and floodwater on

Mn accumulation in periphytic biofilm are respectively -0.22, -0.22, and 0.14.

3.4 Impact factors driving Mn accumulation in periphytic biofilm

We further analyzed the separate effect of each potential impact factor on Mn accumulation to verify the results of PLS-PM. As the co-occurrence patterns between prokaryotes, eukaryotes and the Mn contents in periphytic biofilms indicated, among the nine genera of prokaryotes that are significantly co-correlated with Mn contents in periphytic biofilms, eight of them showed negative correlations ($r<0$, $p<0.05$, Fig. 5), and only one genus, *Pseudomonas* spp. ($r=0.687$, $p=0.019$, Fig. 5), showed a positive correlation with Mn accumulation. For eukaryotes, all three genera that significantly correlated with Mn contents in periphytic biofilms showed negative correlations ($r<0$, $p<0.05$, Fig. 5). Notably, there were more negative than positive correlations in the network for both prokaryotes and eukaryotes with the Mn content. Integrating the effect of prokaryotes and eukaryotes, the total effect of microorganisms on Mn accumulation appears be negative (Fig. 5), which echoes the results of PLS-PM.

Once the roles of biotic components in periphytic biofilm in facilitating Mn accumulation had been excluded, we then focused on the effect of the main abiotic component, EPS, on Mn accumulation. EPS content was positively correlated with the Mn content of the periphytic biofilm ($r=0.265$, $p=0.006$, Fig. 6A), suggesting that EPS contributes to Mn accumulation in periphytic biofilm. EPS contributing to Mn accumulation may be related to its protein components, which was found to be positively correlated with Mn accumulation ($r=0.320$, $p=0.001$, Fig. 6A). These results verified the second hypothesis that EPS dominated adsorption may be the

underlying mechanism accounting for Mn accumulation by periphytic biofilm.

pH in floodwater had a significantly negative correlation with Mn content in periphytic biofilm ($r=-0.349$, $p<0.001$, Fig. 6B), while the Ca and phosphorus (Fig. 6B) content of the periphytic biofilm were positively correlated with its Mn content, indicating a possibility that the more Mn accumulated in periphytic biofilms, the more Ca and TP contents in periphytic biofilms.

4. Discussion

The acidic and flooded conditions of the paddy fields in the red soil region imply that soluble Mn in these environments can easily reach high levels and has high risk of emigration (Shao et al., 2017). Fortunately, and similar to their roles in accumulating nutrients and metals (Wu et al., 2016; Wu et al., 2018; Yang et al., 2016), the ubiquitous periphytic biofilms (Wu, 2016) in paddy fields have the capability to accumulate Mn from paddy soil. As a result, Mn accumulation by periphytic biofilm intercepts the emigration of Mn from soil into adjacent ecosystems (c.f. Fig. 1C) (Reddy et al., 2013), thereby shifting the biogeochemical cycle of Mn in paddy fields.

Previous studies mainly focused on the effects of laboratory-cultured single bacteria, such as *Pseudomonas putida* GB-1 (Parikh & Chorover, 2005), *Chara braunii* (Amirnia et al., 2019), Acremonium-like hyphomycete fungus KR21-2 (Tani et al., 2004a), etc., on the chemical forms of Mn. In the present study, the role of microbial aggregates in the Mn cycle in paddy fields was well established. Single bacterium modulates Mn forms by promoting the formation of Mn oxides, while

periphytic biofilms affect the biogeochemical cycling of Mn in nature via a collective function of Mn accumulation. However, it still needs further exploration whether microorganisms in periphytic biofilms regulate Mn cycling as laboratory-cultured single ones.

Periphytic biofilm is a microbial aggregate (Sun et al., 2018b; Wu et al., 2016), and microorganisms could directly modulate the biogeochemistry of Mn (Henkel et al., 2019; Myers & Nealson, 1988). However, in the periphytic biofilms, only one genus of *Pseudomonas* spp. showed significant positive correlation with Mn accumulation, this may be due to that some bacteria (e.g. *Pseudomonas putida* GB-1) belonging to *Pseudomonas* spp. are manganese oxidizing bacteria (Andy et al., 2013). Manganese oxidizing bacteria affect the forms of Mn mainly by accelerating the process of Mn(II) oxidation and promoting the formation of Mn oxides (Parikh & Chorover, 2005; Tani et al., 2004a). The resulting Mn oxides will precipitate in the biofilm, which leads to the enrichment of Mn in biofilm. The formation of Mn oxides in biofilm has a deeper influence, that is, the Mn oxides would affect the environmental fate of other elements through co-precipitation and adsorption reactions (Nelson et al., 2002; Tani et al., 2004b). In contrast, most of non-Mn oxidizing bacteria in periphytic biofilm act negatively to Mn accumulation. Thus, the overall effect of microorganisms is against Mn accumulation in periphytic biofilm.

Having ruled out the roles of microorganisms in facilitating Mn accumulation, we then focused on the effect of EPS, which is an abundant abiotic component in periphytic biofilm (Sun et al., 2018a), on Mn accumulation to seek the underlying

Mn accumulation mechanism. Both the results of regression analysis and PLS-PM suggest that EPS-mediated, especially protein-mediated, adsorption may be the main mechanism for Mn accumulation in periphytic biofilm. This is because EPS is rich in negatively charged groups such as carboxyl and carbonyl, and these functional groups are expected to contribute to the complexation reaction between the negative charged EPS (Sun et al., 2016b; Sun et al., 2015b) and positive charged Mn ions. By contrast, climatic factors showed indirect effects on the Mn accumulation by periphytic biofilms (c.f. Fig. 4). This is because EPS-mediated adsorption accounts for Mn accumulation by periphytic biofilms, and the contents and components of EPS in microbial aggregates are sensitive to the climatic factors defining the local habitat such as light and temperature (Trabelsi et al., 2009; Wang et al., 2009). Additionally, being similar to the effect of pH on Mn content in soil (Barber, 1995), pH in floodwater also affects the forms and transferability of Mn (Brown et al., 2019), it is then affect Mn accumulation in periphytic biofilm. Furthermore, periphytic biofilm could capture Ca and P (Li et al., 2017; Liu et al., 2019), and the accumulation of Mn enhances the capture of Ca and P by periphytic biofilms (Peng et al., 2019). This is due to Mn accumulation by microbes is a process of the formation of Mn oxides (Amirnia et al., 2019; Peng et al., 2019), and the biogenic Mn oxides could offer extra binding sites for a variety of elements such as uranium, cadmium, hydrogen**,** arsenic, et al., (Parikh & Chorover, 2005; Ren et al., 2020; Yu et al., 2013).

In conclusion, the present study suggests the biogeochemical implications of periphytic biofilm accumulating Mn: periphytic biofilm based *in situ* Mn interception

techniques in paddy fields can be developed to prevent Mn emigration from paddy soil. Based on our results of driving forces behind Mn accumulation by periphytic biofilm, we propose the *in-situ* Mn interception technique based on the following two approaches:

1) Bio-manipulation. We have confirmed that EPS, especially the protein components, are the main factors enhancing the Mn content in periphytic biofilm, thus techniques could be developed to stimulate the secretion of protein-rich EPS by periphytic biofilm; in addition, it found that some microorganisms belonging to *Pseudomonas* spp. showed a positive effect on Mn accumulation, thus the technique of bio-manipulation could be employed to enhance the abundance of such microorganisms which contribute to Mn accumulation;

2) Bio-design, that is artificial cultivation of periphytic biofilm. Based on the results, manganese oxidizing bacteria and EPS-producers can be directly added or stimulated to enhance the EPS content of the biofilm. For instance, certain microorganisms belonging to *Pseudomonas* spp., which are not only manganese oxidizing bacteria (Andy et al., 2013) but also EPS-producers (Boyle et al., 2013; Cristina et al., 2002), could be added during the process of artificial culture of the biofilm, and thereafter, the cultured biofilm can be applied into paddy fields to intercept the movement of Mn from soil to floodwater, and our unpublished data has shown that using artificially cultured biofilm is feasible and efficient in intercepting P loss from paddy fields. In short, it is expected that the findings of periphytic biofilm accumulating Mn will provide a new perspective of the role of periphytic biofilm in

modulating the biogeochemical cycle of Mn in paddy fields, and a basis/framework for developing a technique of periphytic biofilm based *in-situ* Mn interception in paddy fields.

5. Conclusion

Periphytic biofilms growing in paddy fields in the red soil region of south China showed excellent Mn accumulation potential, and via this way, periphytic biofilms shift the behavior of Mn in paddy fields. Field experiments were conducted to verify the Mn accumulation potential, and found that the more biofilm in paddy fields, the higher Mn in the paddy soils, proving the potential of periphytic biofilm in intercepting Mn movement. Extracellular polymeric substances-mediated adsorption was the main mechanism behind Mn accumulation by periphytic biofilm. Microorganisms in general appeared to show negative effects on Mn accumulation in periphytic biofilms. Climatic conditions, and nutrients in floodwater and soil impact the microbial composition, thus indirectly affect Mn accumulation in periphytic biofilms. This study provides valuable information on how and why could microbial aggregates modulate the biogeochemistry of Mn in paddy fields.

Credit authorship contribution statement

Pengfei Sun: Methodology, Writing - original draft. Mengning Gao: Methodology. Rui Sun: Methodology. Yonghong Wu: Supervision, Conceptualization, Writing review & editing. Jan Dolfing: Writing review & editing.

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.

Acknowledgements

This work was supported by National key research and development program (2017YFD0800105 and 2018YFC0213300), the National Natural Science Foundation of China (41701301, 41825021, 31772396, and 813070000), and the Natural Science Foundation of Jiangsu Province (BK20171104). This work was also supported by Chinese Academy of Sciences Interdisciplinary Innovation Team.

Appendix A. Supplementary material

Supporting Information to this article can be found online at***

References

Amirnia, S., Asaeda, T., Takeuchi, C., Kaneko, Y. 2019. Manganese-mediated immobilization of arsenic by calcifying macro-algae, *Chara braunii*. Sci. Total Environ., **646**, 661-669.

Andy, B., Valarie, C., Julia, D., Allison, N., Sophia, H., Vu, H., Peter, J., Fernanda, E., Johnson, H.A. 2013. Manganese (Mn) oxidation increases intracellular Mn in *Pseudomonas putida* GB-1. PLos One, **8**(10), e77835.

Bao, S.D. 2005. Agricultural and Chemistry Analysis of Soil. Beijing, China.

Barber, S.A. 1995. Soil nutrient bioavailability, a mechanistic approach. Q. Rev. Biol., **161**(2), 140-141.

Bellinger, B.J., Gretz, M.R., Domozych, D.S., Kiemle, S.N., Hagerthey, S.E. 2010. Composition of extracellular polymeric substances from periphyton assemblages in the Florida Everglades. J. Phycol., **46**(3), 484-496.

Boyle, K.E., Heilmann, S., van Ditmarsch, D., Xavier, J.B. 2013. Exploiting social evolution in biofilms. Curr. Opin. Microbiol., **16**(2), 207-212.

Brown, C.J., Barlow, J.R.B., Cravotta, C.A., Lindsey, B.D. 2019. Factors affecting the occurrence of lead and manganese in untreated drinking water from Atlantic

and Gulf Coastal Plain aquifers, eastern United States—Dissolved oxygen and pH framework for evaluating risk of elevated concentrations. Appl. Geochem., **101**, 88-102.

Cristina, G.S., Jos, P., Borden, A.J., Van Der, Jost, W., Hans-Curt, F., Busscher, H.J., Mei, H.C., Van Der. 2002. Influence of extracellular polymeric substances on deposition and redeposition of *Pseudomonas aeruginosa* to surfaces. Microbiology, **148**(4), 1161-1169.

Dong, W.Y., Zhang, X.Y., Wang, H.M., Dai, X.Q., Sun, X.M., Qiu, W.W., Yang, F.T. 2012. Effect of different fertilizer application on the soil fertility of paddy soils in red soil region of Southern China. PLos One, **7**(9), 9.

Flemming, H.-C. 2016. EPS—Then and Now. Microorganisms, **4**(4), 41.

Flemming, H.-C., Wingender, J. 2010. The biofilm matrix. Nat. Rev. Microbiol., **8**, 623.

Freitas, F., Torres, C.A.V., Reis, M.A.M. 2017. Engineering aspects of microbial exopolysaccharide production. Bioresource Technol., **245**, 1674-1683.

Guilhen, C., Forestier, C., Balestrino, D. 2017. Biofilm dispersal: multiple elaborate strategies for dissemination of bacteria with unique properties. Mol. Microbiol., **105**(2), 188-210.

Henkel, J., Dellwig, O., Pollehne, F., Herlemann, D., Leipe, T., Schulz-Vogt, H. 2019. A bacterial isolate from the Black Sea oxidizes sulfide with manganese(IV) oxide. P. Natl. Acad. Sci. USA., **116**, 12153-12155.

Huang, X., Shabala, S., Shabala, L., Rengel, Z., Wu, X., Zhang, G., Zhou, M. 2015. Linking waterlogging tolerance with Mn^{2+} toxicity: a case study for barley. Plant Biol., **17**(1), 26-33.

Jalali, M., Hemati, N. 2013. Chemical fractionation of seven heavy metals (Cd, Cu, Fe, Mn, Ni, Pb, and Zn) in selected paddy soils of Iran. Paddy Water Environ, **11**(1), 299-309.

Kim, S.K., Lee, J.H. 2016. Biofilm dispersion in *Pseudomonas aeruginosa*. J. Microbiol., **54**(2), 71-85.

Larned, S.T. 2010. A prospectus for periphyton: recent and future ecological research. J. N. Am. Benthol. Soc., **29**(1), 182-206.

Li, J.-y., Deng, K.-y., Hesterberg, D., Xia, Y.-q., Wu, C.-x., Xu, R.-k. 2017. Mechanisms of enhanced inorganic phosphorus accumulation by periphyton in paddy fields as affected by calcium and ferrous ions. Sci. Total Environ., **609**, 466-475.

Liu, J., Sun, P., Sun, R., Wang, S., Gao, B., Tang, J., Wu, Y., Dolfing, J. 2019. Carbon-nutrient stoichiometry drives phosphorus immobilization in phototrophic biofilms at the soil-water interface in paddy fields. Water Res., **167**, 115129.

Myers, C.R., Nealson, K.H. 1988. Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. Science, **240**(4857), 1319-21.

Nelson, Y.M., Lion, L.W., Shuler, M.L., Ghiorse, W.C. 2002. Effect of oxide formation mechanisms on lead adsorption by biogenic manganese (hydr)oxides, iron (hydr)oxides, and their mixtures. Environ. Sci. Technol., **36**(3), 421-5.

Parikh, S.J., Chorover, J. 2005. FTIR Spectroscopic Study of biogenic Mn-oxide formation by *Pseudomonas putida* GB-1. Geomicrobiol. J., **22**(5), 207-218.

Peng, L., Deng, X., Song, H., Tan, X., Gu, J.D., Luo, S., Lei, M. 2019. Manganese enhances the immobilization of trace cadmium from irrigation water in biological soil crust. Ecotox. Environ. Safe., **168**, 369-377.

Reddy, M.V., Satpathy, D., Dhiviya, K.S. 2013. Assessment of heavy metals (Cd and Pb) and micronutrients (Cu, Mn, and Zn) of paddy (*Oryza sativa* L.) field surface soil and water in a predominantly paddy-cultivated area at Puducherry (Pondicherry, India), and effects of the agricultural runoff on the elemental concentrations of a receiving rivulet. Environ. Monit. Assess., **185**(8), 6693-6704.

Ren, Y., Bao, H., Wu, Q., Wang, H., Gai, T., Shao, L., Wang, S., Tang, H., Li, Y., Wang, X. 2020. The physical chemistry of uranium (VI) immobilization on

manganese oxides. J. Hazard. Mater., **391**, 122207.

Shao, J.F., Yamaji, N., Shen, R.F., Ma, J.F. 2017. The key to Mn homeostasis in plants: regulation of Mn transporters. Trends Plant Sci., **22**(3), 215-224.

Sun, P., Hui, C., Wang, S., Khan, R.A., Zhang, Q., Zhao, Y.H. 2016a. Enhancement of algicidal properties of immobilized *Bacillus methylotrophicus* ZJU by coating with magnetic Fe_3O_4 nanoparticles and wheat bran. J. Hazard. Mater., **301**(6), 65-73.

Sun, P., Hui, C., Wang, S., Wan, L., Zhang, X., Zhao, Y. 2016b. *Bacillus amyloliquefaciens* biofilm as a novel biosorbent for the removal of crystal violet from solution. Colloids Surf. B., **139**(12), 164-170.

Sun, P., Lin, H., Wang, G., Zhang, X., Zhang, Q., Zhao, Y. 2015a. Wheat bran enhances the cytotoxicity of immobilized *Alcaligenes aquatilis* F8 against *Microcystis aeruginosa*. PLos One, **10**(8), e0136429.

Sun, P., Zhang, J., Esquivel-Elizondo, S., Ma, L., Wu, Y. 2018a. Uncovering the flocculating potential of extracellular polymeric substances produced by periphytic biofilms. Bioresource Technol., **248**, 56-60.

Sun, P.F., Lin, H., Wang, G., Lu, L.L., Zhao, Y.H. 2015b. Preparation of a new-style composite containing a key bioflocculant produced by *Pseudomonas aeruginosa* ZJU1 and its flocculating effect on harmful algal blooms. J. Hazard. Mater., **284**, 215-221.

Sun, R., Sun, P., Zhang, J., Esquivel-Elizondo, S., Wu, Y. 2018b. Microorganisms-based methods for harmful algal blooms control: A review. Bioresource Technol., **248**, 12-20.

Tani, Y., Miyata, N., Ohashi, M., Ohnuki, T., Seyama, H., Iwahori, K., Soma, M. 2004a. Interaction of inorganic arsenic with biogenic manganese xxide produced by a Mn-oxidizing fungus, strain KR21-2. Environ. Sci. Technol., **38**(24), 6618-6624.

Tani, Y., Ohashi, M., Miyata, N., Seyama, H., Iwahori, K., Soma, M. 2004b. Sorption of Co(II), Ni(II), and Zn(II) on biogenic manganese oxides produced by a

Mn-oxidizing fungus, strain KR21-2. J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng., **39**(10), 2641-60.

Trabelsi, L., Ben Ouada, H., Bacha, H., Ghoul, M. 2009. Combined effect of temperature and light intensity on growth and extracellular polymeric substance production by the cyanobacterium *Arthrospira platensis*. J. Appl. Phycol., **21**(4), 405-412.

Wang, J., Pan, F., Soininen, J., Heino, J., Shen, J. 2016. Nutrient enrichment modifies temperature-biodiversity relationships in large-scale field experiments. Nat. Commun., **7**, 13960.

Wu, Y. 2016. Periphyton: Functions and Application in Environmental Remediation. Elsevier, New York.

Wu, Y., Liu, J., Lu, H., Wu, C., Kerr, P. 2016. Periphyton: an important regulator in optimizing soil phosphorus bioavailability in paddy fields. Environ. Sci. Pollut. Res., **23**(21), 21377-21384.

Wu, Y., Liu, J., Rene, E.R. 2018. Periphytic biofilms: A promising nutrient utilization regulator in wetlands. Bioresource Technol., **248**, 44-48.

Yang, J., Tang, C., Wang, F., Wu, Y. 2016. Co-contamination of Cu and Cd in paddy fields: Using periphyton to entrap heavy metals. J. Hazard. Mater., **304**, 150-158.

Yu, H., Leadbetter, J.R. 2020. Bacterial chemolithoautotrophy via manganese oxidation. Nature, **583**(7816), 453-458.

Yu, Q., Sasaki, K., Tanaka, K., Ohnuki, T., Hirajima, T. 2013. Zinc sorption during bio-oxidation and precipitation of manganese modifies the layer stacking of biogenic birnessite. Geomicrobiol. J., **30**(9), 829-839.

Figure captions

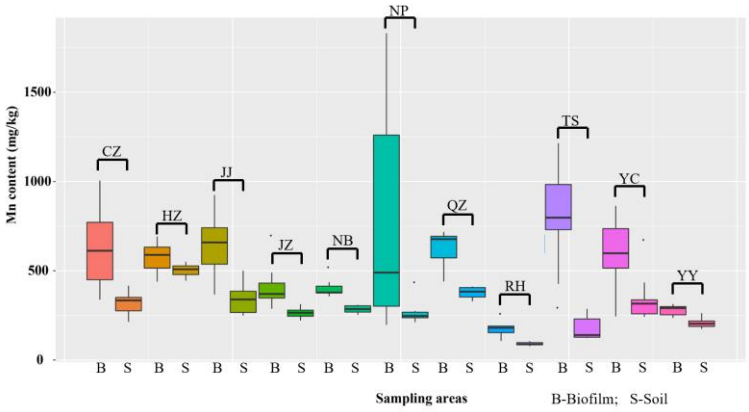


Figure 1 The Mn contents in the paddy biofilms and the corresponding soils in the 11 sampling areas. The sampling areas are: TS: Taishan, RH: Renhua, QZ: Quanzhou, NP: Nanping, NB: Ningbo, HZ: Hangzhou, CZ: Chizhou, YY: Yueyang, JZ: Jingzhou, YC: Yichang, JJ: Jiujiang. The letter 'B' on horizontal axis represents Mn contents in periphytic biofilms, while 'S' on horizontal axis represents Mn contents in paddy soils.

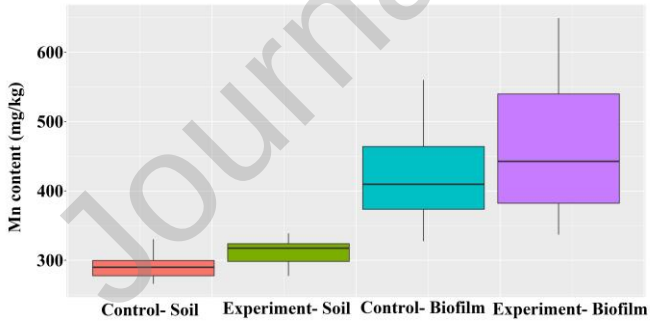


Figure 2 The Mn contents in periphytic biofilms and the corresponding soils collected from the experimental and control fields during the field experiment. Control-Soil, Control-Biofilm, Experiment-Soil, and Experiment-Biofilm represent Mn contents in the soil and biofilm samples collected from control field, and soil and biofilm samples collected from experimental field, respectively.

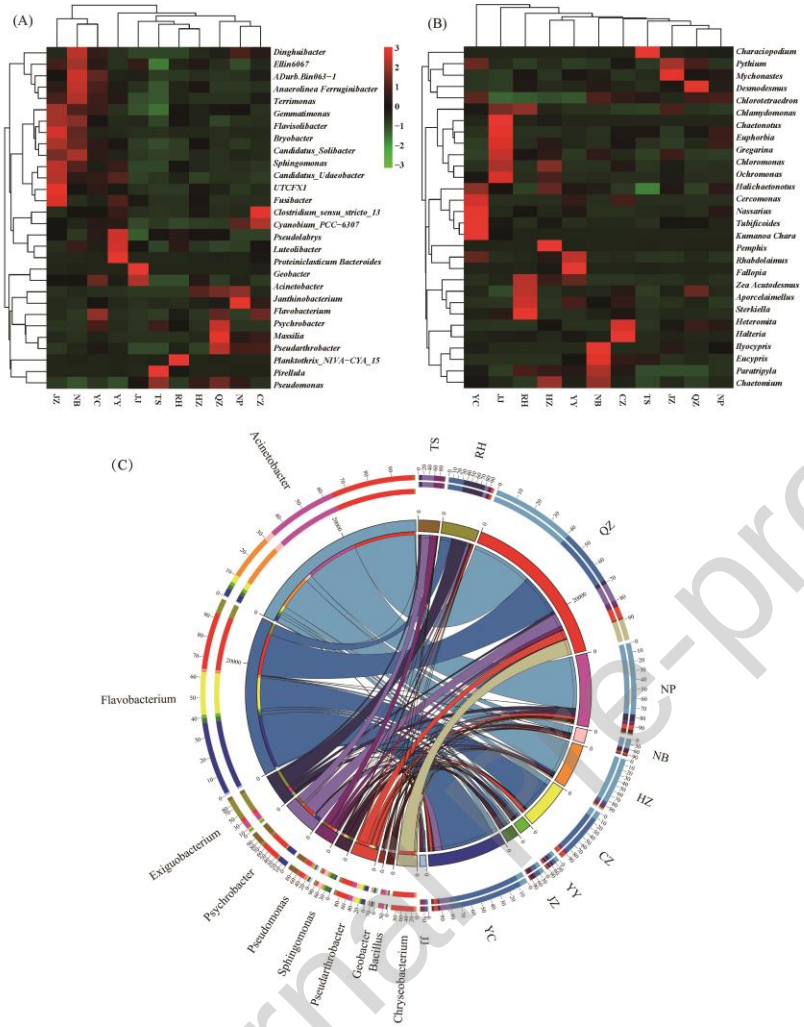


Figure 3 Distribution of core prokaryotes (A) and eukaryotes (B) in periphytic biofilm, and the distribution of the top 10 most abundant Mn oxidizing bacteria in individual periphytic biofilms (C). Each column in (A) and (B) is labeled with the sample name, and each row depicts the results from a single microorganism with the highest abundance at genus level in the top 30. The scale of -3 to 3 is Z score. The length of the bars on the outer-ring in (C) represents their percentages in each sample. TS: Taishan, RH: Renhua, QZ: Quanzhou, NP: Nanping, NB: Ningbo, HZ: Hangzhou, CZ: Chizhou, YY: Yueyang, JZ: Jingzhou, YC: Yichang, JJ: Jiujiang.

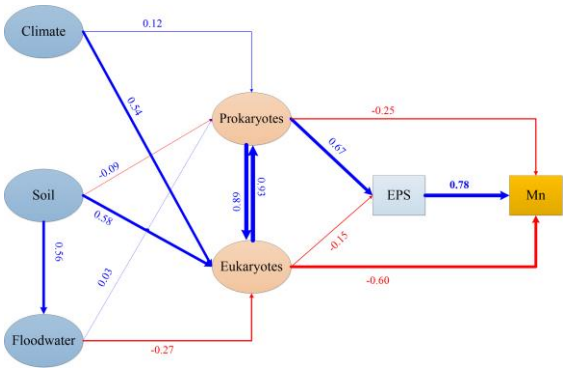


Figure 4 The effects of nutrients in soil and floodwater, climatic conditions, and the relative abundances of prokaryotes and eukaryotes on Mn contents in periphytic biofilms analyzed using Partial Least Squares Path Modeling (PLS-PM). Blue arrows indicate positive effects, while red arrows indicate negative effects. The width of the line indicates the strength of the effect: the thicker the line, the stronger the effect.

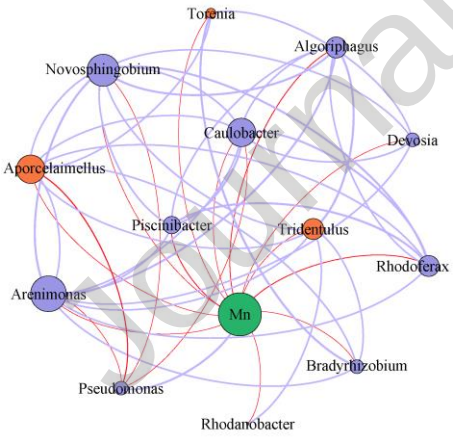


Figure 5 Interaction network between prokaryotes, eukaryotes and Mn contents in periphytic biofilms. The co-occurring networks are colored by genera. The size of each node is proportional to the number of connections (that is, degree), and the thickness of each connection between two nodes (that is, edge) is proportional to the value of Spearman's correlation coefficients. A blue edge indicates a positive

interaction between two individual nodes, while a red edge indicates a negative interaction. The blue nodes are prokaryotes, while the red nodes are eukaryotes and the green node is Mn content.

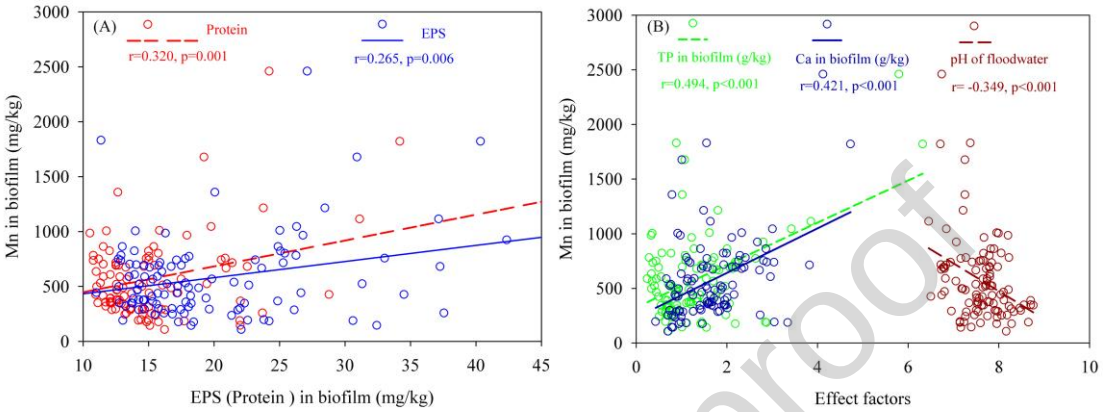


Figure 6 Regression analysis of EPS and protein in periphytic biofilm (g/kg) versus Mn content (mg/kg) in periphytic biofilm (A), and pH in floodwater, Ca content in periphytic biofilm, and the content of total phosphorus in periphytic biofilm (g/kg) versus Mn content (mg/kg) in periphytic biofilm (B).

Credit authorship contribution statement

Pengfei Sun: Methodology, Writing - original draft. Mengning Gao: Methodology. Rui Sun: Methodology. Yonghong Wu: Supervision, Conceptualization, Writing review & editing. Jan Dolfing: Writing review & editing.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Periphytic biofilm has excellent Mn accumulation and interception potentials.
- EPS dominated adsorption is the mechanism behind Mn accumulation in periphytic biofilm.
- Ca and P in periphytic biofilm enhance the accumulation of Mn.
- Microbes in general show negative effects on Mn accumulation in periphytic biofilms
- Environmental factors affect Mn accumulation by affecting microbes in periphytic biofilms.