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CORRESPONDENCE

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Geographic imprint and ecological functions of the abiotic component of periphytic biofilms

INTRODUCTION

In nature, most microorganisms grow as aggregates, flocs, or biofilms [1, 2], held together by a self-produced matrix of extracellular polymeric substances (EPSs) [3]. Periphytic biofilms are a typical example. Consisting of autotrophs and heterotrophs, these aggregates grow in both natural aquatic ecosystems (e.g., streams, wetlands, rivers, etc.) and constructed wetlands (e.g., paddy fields), where they play multiple, crucial roles in modulating element cycling [4]. Due to concerns about excessive fertilizer input in paddy fields, nitrogen (N) and phosphorus (P) management based on periphytic biofilm is receiving more and more attention [5]. For instance, the accumulation of P by periphytic biofilm is beneficial for minimizing its emigration from paddy fields to adjacent ecosystems [6].

The microbial component of periphytic biofilms has been relatively well studied [4, 6], whereas the abiotic component has received less attention. Little is known about EPS content and composition of the periphytic biofilm, and about the potential ecological functions of this matrix. It is well established that EPS is an important component of biofilms grown in natural aquatic ecosystems, and that the EPS composition of biofilms is sensitive to the factors that define their local habitats, such as light, temperature, and nutrient availability [7, 8]. This leads to the hypothesis that both the contents and the composition of EPS will vary greatly among biofilms growing in paddy fields and those in more natural aquatic ecosystems. Generally, proteins and polysaccharides, which account for over 70%–80% of the total mass of EPS, are the two main components of EPS, plus small amounts of other compounds, such as eDNA [9]. However, little is currently known about the main components of protein, polysaccharides, and eDNA of EPS in periphytic biofilms grown in paddy fields.

Geodistribution patterning is one of the central themes in macroecology and biogeography. As habitats of the periphytic biofilms, paddy fields in China are distributed over six geographical regions [10]. The differences in the physical geography of the habitats have resulted in regional differences in the microbial composition of periphytic biofilms [6]. Given that microorganisms are the primary factors affecting the components and contents of EPS in periphytic biofilms [9], this leads to a second hypothesis, namely, that the EPS characteristics of periphytic biofilms growing in different geographical regions' paddy fields will show distinct geodistribution patterns. However, so far, little is known about the geographical distribution patterns of EPS components in the periphytic biofilms. Furthermore, whether and how EPS composition is affected by physical geography characteristics, such as temperature, light, and precipitation, remains to be investigated.

The importance of EPS is determined by its ecological functions. EPS is known to function as the skeleton and protective barrier for microorganisms living in the biofilms [11, 12]. In recent years, we have conducted a series of studies into the roles of periphytic biofilm in paddy fields and found that periphytic biofilms shift the behavior of elements in paddy fields and that different biofilms have different potentials in regulating element cycling [6, 13]. Assuming that there are indeed dissimilarities in EPS contents and components between periphytic biofilms grown in different geographic regions, a third hypothesis guiding our work is then that such variations in EPS are associated with differences in nutrient accumulation in different periphytic biofilms.

To evaluate the above-mentioned hypotheses, we collected a total of 600 periphytic biofilm, soil, and floodwater samples from paddy fields on a nationwide scale in China to address the following questions: (1) What are the contents and main composition, and potential functions of EPS in periphytic biofilms growing in paddy fields? (2) Are there geographical differences between the EPS in periphytic biofilms along with the habitats, and if any, what are the main factors driving the geographical distribution of EPS?

RESULTS

Amounts, main compositions, and geodistribution of EPS in periphytic biofilms in paddy fields across China

Quantifying the EPS component in 200 periphytic biofilms (Figure 1A), it was found that EPS contents (dry weight) in the periphytic biofilms grown in the paddy fields in China varied from 11.3 to 64.9 g/kg (Figure 1A), with an average of 19.1 g/kg. As expected, proteins and polysaccharides were the two main components of the EPS. Protein levels were significantly higher (9.9–48.9 g/kg, Figure 1B) than polysaccharide levels (1.2–15.9 g/kg, $p < 0.001$, Figure 1B); the ratios of protein to polysaccharide varied from 1.8 to 20 (with an average value of 5.4, Figure 1A). The contents of eDNA in the EPS were relatively small, varying from 0.1 to 0.7 g/kg (Figure 1B). These results indicate that the protein component dominates the EPS.

EPS in periphytic biofilms grown in Chinese paddy fields showed a significant geographical distribution pattern: the EPS contents in periphytic biofilms significantly decreased with the increasing latitude of the habitats ($r = 0.3294$, $p < 0.0001$, red line in Figure 1C). Additionally, the ratio of the two main components of protein and polysaccharide in EPS also showed a significant but contrary geographical distribution pattern: the ratio of proteins to polysaccharide in periphytic biofilms increased significantly with the increasing latitude of the habitats ($r = 0.2742$, $p = 0.0005$, blue line in Figure 1C). The results showed that the higher the latitude at which a periphytic biofilm grows, the lower is its EPS content, and the higher is its ratio of protein to the polysaccharide. Thereby, the hypothesis that the EPS in periphytic biofilm growing in different geographical

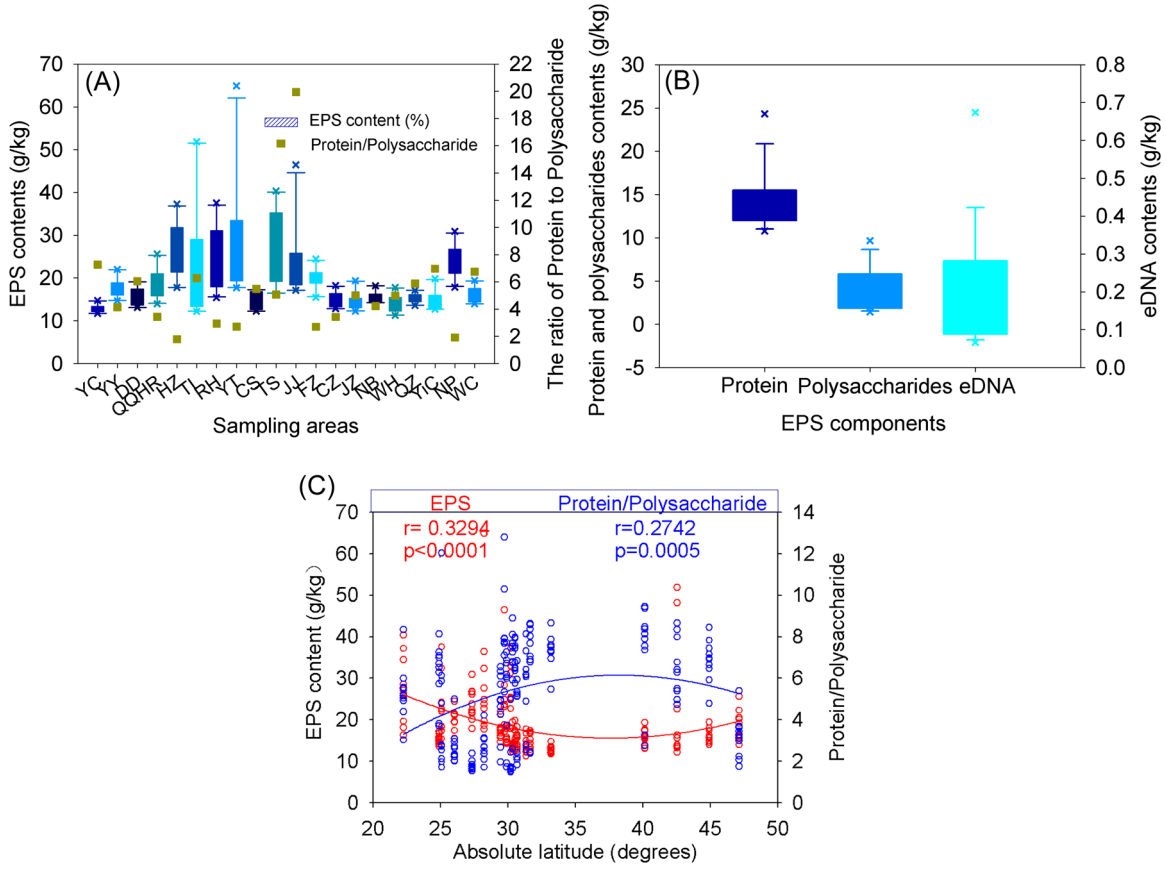

FIGURE 1 EPS contents (g/kg) in periphytic biofilms (boxes in A) and the ratios of protein to polysaccharide in the EPS (squares in A); contents of these three main components of protein, polysaccharides, and eDNA contents (g/kg) in the EPS (B); EPS contents in periphytic biofilms exhibit a contrasting pattern across the latitudinal gradient, while the ratio of protein to polysaccharide (protein/polysaccharide) in periphytic biofilms exhibit the same patterns across the latitudinal gradient (C). Second-order polynomial fits are shown in blue (protein/polysaccharide) and red (EPS content). Periphytic biofilms were collected from the 20 sampling areas of CS, Changshu; CZ, Chizhou; DD, Dandong; FZ, Fuzhou; HZ, Hangzhou; JJ, Jiujiang; JZ, Jingzhou; NB, Ningbo; NP, Nanping; QQHR, Qiqihar; QZ, Quanzhou; RH, Renhua; TL, Tieling; TS, Taishan; WC, Wuchang; WH, Wuhu; YC, Yancheng; YiC, Yichang; YT, Yingtan; YY, Yueyang. EPS, extracellular polymeric substances.

regions' paddy fields might show geographical distribution patterns was verified.

Interaction network between microbial communities and EPS in periphytic biofilms

In the present periphytic biofilm samples, a total of 130 genera of prokaryotes and 145 genera of eukaryotes were identified. The eukaryotes in periphytic biofilms grown in paddy fields mainly consist of microeukaryotes, such as green algae and meiofauna, such as nematodes (Figure 2A). Specifically, Heteromita, Desmodesmus, Aporcelaimellus, Paratripyla, Characiopodium, Chlorotetraedron, Tubificoides, Chaetomium, Rhabdolaimus, and Pythium are frequently present in the individual top 10 of most abundant genera in a specific periphytic biofilm, thus are the core eukaryotic communities in periphytic biofilms (Figure 2A); the core prokaryotic communities consist of Flavobacterium, Acinetobacter, Cyanobium_PCC-6307, Dinghuibacter, Massilia, UTCFX1, Bacteroides, Luteolibacter, Clostridium_sensu_stricto_13, and Proteiniclasticum (Figure 2B). Thus, it can be concluded that the microbial community of periphytic biofilms growing in paddy fields mainly consists of prokaryotes, microeukaryotes, and meiofauna.

On the basis of the results of the co-occurrence patterns between prokaryotes, eukaryotes, and the EPS contents in periphytic biofilms, it was found that a total of 15 genera of prokaryotes and eight genera of eukaryotes were significantly related to the EPS accumulation in periphytic biofilms (Figure 2C). Four genera (Prevotella_9, Dechloromonas, Paludibacterium, and Azospira) of prokaryotes showed significantly positive correlations with the EPS accumulation, while eleven genera (Sphingomonas, Flavobacterium, RB41, Nocardioides, Adhaeribacter, JGI_0001001.H03, Phreatobacter, Arcticibacter, Flavisolibacter, UTCFX1, and Nitrospira) had significantly negative correlations (Figure 2C). Notably, there were more negative than positive correlations in the network for prokaryotes. This suggests that the negative effects of prokaryotes on the EPS contents in periphytic biofilms may outweigh the positive effects, then potentially makes prokaryotes negatively affect the EPS content in periphytic biofilms. Conversely, the correlations were more positive in the network for eukaryotes. Six genera (Pinnularia, Heterolepidoderma, Lepidochaetus, Sminthurides, Halichaetonotus, and Chaetonotus) of eukaryotes showed significant positive correlations with the EPS accumulation in periphytic biofilms, while only two genera (Rhogostoma and Pythium) showed significant negative correlations (Figure 2C). This suggests that the total effect of eukaryotes on EPS accumulation in periphytic biofilms may be positive.

Impact factors driving the geographical distribution of EPS in periphytic biofilms

Both the prokaryote and eukaryote showed a direct effect on the geographical distributions of EPS in periphytic biofilms (Figure 2D). As analyzed by PLS-PM, prokaryotes in periphytic biofilms showed a negative effect (path coefficient = −0.63, Figure 2D) on the geographical distributions of EPS, while eukaryotes exerted a positive effect (path coefficient = 0.46, Figure 2D). Specifically, the effect of eukaryotes included a weak direct effect (path coefficient = 0.09, Supporting Information Table S1) plus a strong indirect effect (path coefficient = 0.37, Supporting Information Table S1) on the prokaryotes. These results suggest that prokaryotes negatively affected the geographical distributions of EPS, while eukaryotes showed a positive effect mainly by indirectly affecting the prokaryotes.

Climatic factors, soil, and floodwater characteristics are the indirect factors, affecting the microbial components, driving the geographical distribution of EPS in periphytic biofilms (Figure 2D). As analyzed by PLS-PM, the total effects of climatic factors on eukaryotes and prokaryotes were 0.55 and 0.69, respectively; the total effects of paddy soil on eukaryotes and prokaryotes were, respectively, 0.28 and 0.20, and the total effects of floodwater on eukaryotes and prokaryotes were 0.45 and 0.40, respectively (Figure 2D). Combining the effect of each factor on microbial composition and the effect of microbial composition on EPS content, the total effect of climatic factors, soil, and floodwater on the geographical distribution of EPS are −0.41, −0.11, and −0.23, respectively (Supporting Information Table S1).

By contrast, the total contribution of these three external factors to the EPS is in the following order: climatic factors > floodwater > paddy soil. Thus, we can conclude that climatic factors may be the principal forces driving the geographical distribution of EPS in periphytic biofilm grown in large-scale paddy fields. Among the analyzed climatic factors, both sunshine duration (path coefficient = 0.91) and radiation intensity (path coefficient = 0.63) showed positive effects on the geographical distribution of EPS in periphytic biofilms (Figure 2D). Additionally, the effective accumulated temperature showed a significant negative effect (path coefficient = −0.92, Figure 2D). These patterns suggest a role for climatic factors, especially temperature and light, in determining the geographical distribution of EPS in periphytic biofilms.

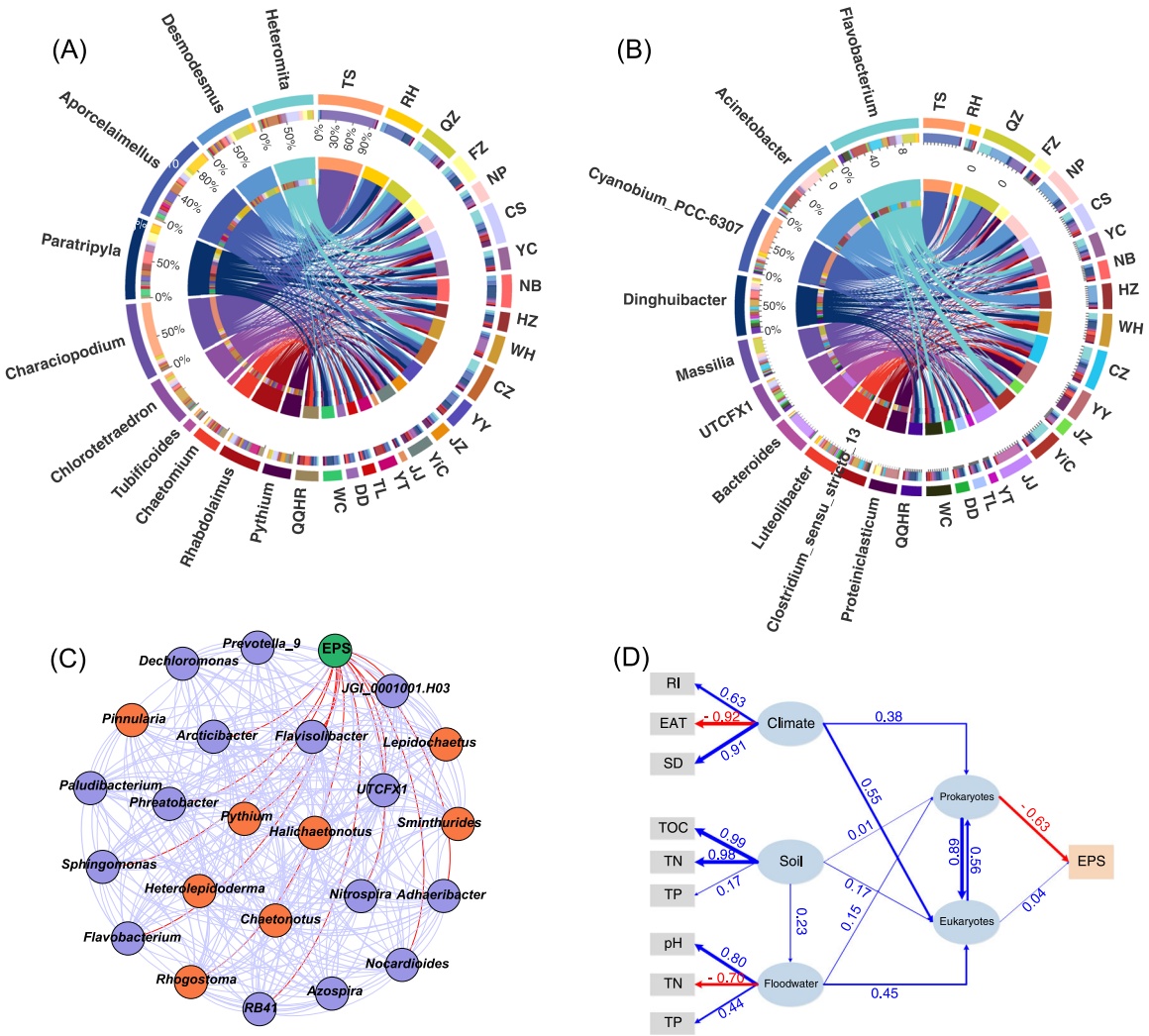

FIGURE 2 Distribution of the core community of eukaryotes (A) and prokaryotes (B) which with the highest abundance at genus level in the top 10 in periphytic biofilm; interaction networks between prokaryotes, eukaryotes, and extracellular polymeric substances (EPS) accumulation in periphytic biofilms (C); and synthesis of the effects of paddy soil (total organic carbon [TOC], total nitrogen [TN], and total phosphorus [TP]), floodwater (pH, TN, and TP), and climate (sunshine duration [SD], radiation intensity [RI], and effective accumulated temperature [EAT]) on microorganisms in periphytic biofilm, and their effect on the geographical distribution patterns of EPS in periphytic biofilms, as analyzed by Partial Least Squares Path Modeling (D). In panels (A) and (B), the length of the bars of each sample on the outer ring represents the percent of microorganisms in each sample. In C, the co-occurring networks are colored by genera. The size of each node is proportional to the number of connections (i.e., degree), and the thickness of each connection between two nodes (i.e., 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 one is EPS. In (D), blue arrows in the model present a positive effect, while red arrows in the model show a negative effect. The thickness of the line in the model represents the strength of the effect, and the thicker the line, the stronger the effect. CS, Changshu; CZ, Chizhou; DD, Dandong; FZ, Fuzhou; HZ, Hangzhou; JJ, Jiujiang; JZ, Jingzhou; NB, Ningbo; NP, Nanping; QQHR, Qiqihar; QZ, Quanzhou; RH, Renhua; TL, Tieling; TS, Taishan; WC, Wuchang; WH, Wuhu; YC, Yancheng; YiC, Yichang; YT, Yingtan; YY, Yueyang.

EPS shapes the function of periphytic biofilm in nutrient accumulation

Nutrient accumulation is one of the ways in which periphytic biofilms modulate nutrient cycles in paddy fields. The concentrations of TN and TP in periphytic biofilm varied from 0.8 to 12.5 g/kg and 0.3 to 6.3 g/kg, respectively (Figure 3A). The concentrations of TN ($r = 0.372$, $p < 0.001$, Figure 3B) and TP ($r = 0.272$, $p < 0.001$, Figure 3C) in periphytic biofilm were significantly related to the EPS contents, which may partly explain why periphytic biofilm could accumulate considerable amounts of N and P.

Additionally, there is abundant TOC in periphytic biofilm; the amount of TOC in periphytic biofilm varied

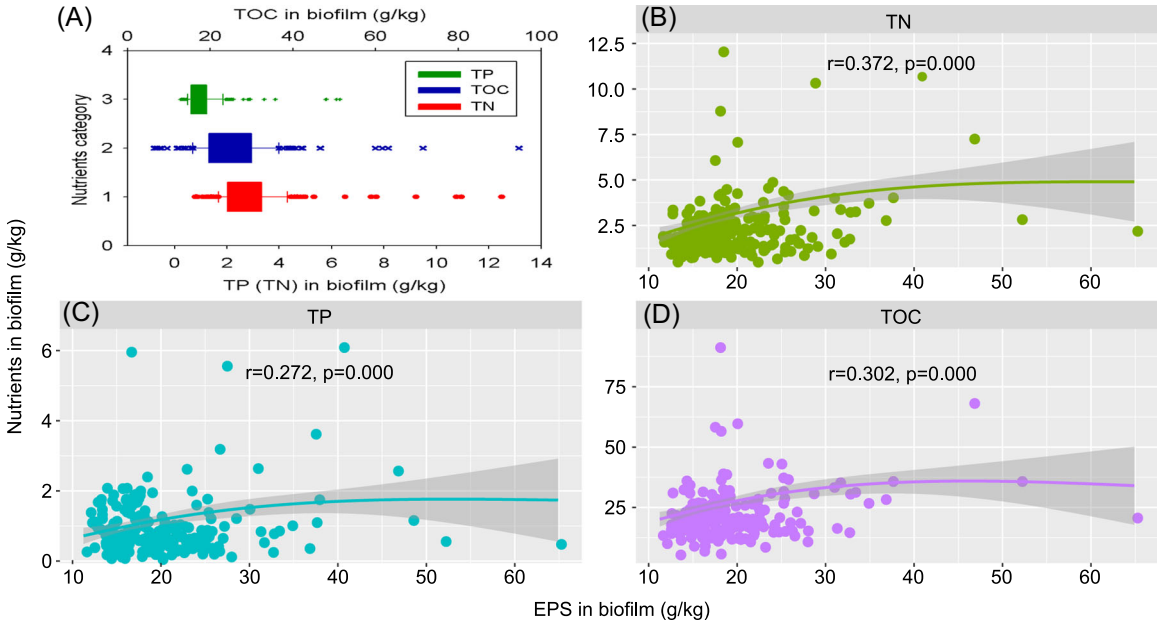

FIGURE 3 Quantities of TN, TP, and TOC in periphytic biofilms (A), and the relationship between the EPS in periphytic biofilms with the TN (B) and TP (C) contents, and TOC (D) contents in periphytic biofilms. EPS, extracellular polymeric substances; TN, total nitrogen; TOC, total organic carbon; TP, total phosphorus.

from 6.4 to 94.6 g/kg (blue bar in Figure 3A). The EPS component in periphytic biofilm showed a positive correlation with TOC in periphytic biofilm ($r = 0.302$, $p < 0.001$, Figure 3D). The results indicate that the higher EPS content, the more TOC in periphytic biofilm. Thus, we concluded that EPS has important roles in shaping the functions of paddy periphytic biofilms in nutrient accumulation.

DISCUSSION

Previously, even the most basic information on the EPS component of periphytic biofilms grown in paddy fields was not systematically available. Thus, we quantitatively analyzed both the content and composition of the EPS component in periphytic biofilms comprehensively collected from the main paddy fields in China. The results quantitatively substantiated that EPS is an important abiotic component in periphytic biofilms and that EPS in paddy field biofilms varies greatly between different rice-growing provinces and those grown in natural aquatic ecosystems. Protein and polysaccharides are generally the two main components of EPS [14, 15]. In some natural (oligotrophic) environments (such as oceans and the Everglades), EPSs are primarily composed of polysaccharides [9]; but in the EPS in periphytic biofilms grown in the eutrophic environments of paddy fields, protein is the most abundant component. The results suggest that the trophic status of the habitat is an important factor affecting the composition of EPS in biofilm, which extends a similar observation of Durmaz and Sanin [16] who reported that the carbon to nitrogen ratio of the wastewater affects the EPS composition of activated sludge flocs. In terms of function, proteins and polysaccharides in EPS have different functional groups [11, 14], and the function of the protein component largely affects the functions of EPS [15]. It is tempting to speculate that the high protein content of periphytic biofilms in rice paddies affects and possibly promotes their potential to sequester N and P. In contrast, eDNA in the periphytic biofilms mainly affects the process of biofilm formation [17]. Thus, research to test this hypothesis should not only be scientifically interesting but may also have practical applications.

Microorganisms are the main producers and consumers of the EPS component in periphytic biofilms; their activity will directly change the composition and content of EPS in periphytic biofilms [9]. Here we found that the microbial community of periphytic biofilms grown in paddy fields is different from those grown in natural aquatic ecosystems, which are largely dominated by various phototrophic algae [18]. Periphytic biofilms growing in paddy fields are mainly dominated by chemotrophic microorganisms, which may be a result of the abundant supply of nitrogen and phosphorus, and limited light caused by rice canopy shading.

Prokaryotes in periphytic biofilms may exert negative effects on the EPS content in periphytic biofilm which was indicated by the results of co-occurrence patterns (cf. Figure 2C). This is because although some bacteria can produce EPS [9], EPS-producing prokaryotes apparently accounted for only a small part of the whole prokaryotic microbial population in periphytic biofilms. Additionally, EPSs are a good substrate due to their abundance of monosaccharides, such as maltose, D-xylose, mannose, and D-fructose [15]. For the microorganisms in periphytic biofilms, autotrophic microorganisms (such as cyanobacteria) do not require EPS as carbon or energy sources, while heterotrophic prokaryotes, which dominate the periphytic biofilms (cf. Figure 2A,B), need to consume the EPS for their growth [19]. Therefore, on the basis of most prokaryotes consuming EPS versus only a few producing EPS, the overall effect of prokaryotes on the EPS content in periphytic biofilm may be negative.

Eukaryotes showed positive effects on EPS accumulation (path coefficient = 0.46, Figure 2D), which was supported by the co-occurrence pattern results (cf. Figure 2C). The total positive effect of eukaryotes on the EPS consists of two parts: a weak direct effect and a strong indirect effect through affecting the prokaryotes, with the indirect effect being the most important of the two (Supporting Information Table S1). The reason for the weak direct effect may be that only a few eukaryotic microorganisms can produce EPS to maintain their grown microenvironment [20]. A possible reason for the observed strong indirect effect is that the predatory behavior of some eukaryotes on prokaryotes, such as nematodes feeding on bacteria [21], might control the abundance of the prokaryotes, reducing the consumption of EPS by prokaryotic microorganisms and then indirectly increase the accumulation of EPS in periphytic biofilms.

Studying the geographical distribution pattern of biodiversity contributes to addressing one of the most basic scientific issues of macroecology and biogeography [22]. Here, we employed multiple regression analysis [23] to evaluate the geographical distribution patterns of EPS in periphytic biofilms growing in paddy fields. Then, Partial Least Squares Path Modeling [24] was employed to synthesize the data and analyze the direct and indirect impact factors driving the geographical distribution of EPS in periphytic biofilm. Climatic factors, including temperature and light, principally drive the geographical distribution of EPS content in periphytic biofilms. Theoretically, the lower the latitude, the longer the sunshine duration and the higher the radiation intensity is, and the higher the light intensity and duration are, the more production of EPS is to be expected, which agrees well with our results. Additionally, in line with the previous findings [25, 26], the effective accumulated temperature showed a significant negative effect on the geographical distribution of EPS in periphytic biofilm. This is because EPSs play important roles in protecting microorganisms in biofilm against adverse conditions [11, 27]. For example, microorganisms in biofilms secrete more EPS to help them resist stress posed by low temperatures [28, 29]. Therefore, maybe certain climatic factors would lead to the decreased synthesis of EPS by periphytic biofilms, resulting in the difference in the EPS content in periphytic biofilms grown in different rice planting areas. In addition, it is well known that climatic factors, such as lighting and temperature, can affect the growth of microbes thus shifting the microbial community structure in periphytic biofilms [4, 18]. As prokaryotes and eukaryotes potentially showed different roles in the EPS content in periphytic biofilms, thus the climate-induced difference in the structures of prokaryotes and eukaryotes in different periphytic biofilms (cf. Figure 2A,B) may be another important factor determining the geodistribution of EPS (biomass or content) across China.

In spite of the quantitative importance of EPS in periphytic biofilms and the above correlation analyses notwithstanding, conclusive evidence for the significance of the EPS component in paddy periphytic biofilms is still lacking. Accumulation is one efficient way of periphytic biofilm in modulating nutrients/elements cycling in paddy fields [4, 6]. In the present study, we quantified the accumulation potential of C, N, and P in periphytic biofilm, and found that the EPS component shapes the functions of periphytic biofilm in the accumulation of the three nutrients.

It is known that the high protein content of EPS contributes to protecting microbes in periphytic biofilm against adverse growth conditions, such as water stress, heavy metals, pesticides, and insecticides [11]. In the present study, we found two new roles of EPS in periphytic biofilm. First, EPS is instrumental in N and P accumulation by periphytic biofilms; this is because EPS has abundant functional groups, such as carboxyl, carbonyl, and so forth [30], providing various binding sites for N and P, thus facilitating the accumulation of N and P by periphytic biofilm. Second, with proteins and polysaccharides being the main components of EPS [11], EPS in periphytic biofilms may be a source of TOC in paddy soils and then expects to improve the fertility of paddy soils [31].

CONCLUSION

EPS is an important abiotic component of periphytic biofilm, with protein, polysaccharides, and eDNA being their main components. Prokaryotes and eukaryotes may have different effects on the abiotic component in periphytic biofilms; that

is, prokaryotes may show negative effects on EPS contents in periphytic biofilms, while eukaryotes may potentially exert positive effects. EPS in periphytic biofilms shows a significant geographic imprint: EPS contents of periphytic biofilms decrease with increasing latitude, while the ratio of protein to polysaccharide component of the EPS shows the opposite trend. Temperature and light are principal factors driving the geographical distribution of EPS in periphytic biofilms. Further, the data indicate that beyond the known function of the skeleton, EPS affects the propensity of periphytic biofilms to accumulate nutrients.

METHODS

Sampling area description

Sampling areas were paddy fields in different geographical regions of China, ranging from 22°25′ N to 47°16′ N (Supporting Information Figure S1A). The sampled paddy fields were located in northeast, central, and south China, covering over 93% of the typical rice planting areas in China. All samples were collected 7–15 days after the transplanting. In total, 20 sampling areas were selected; per sampling area, 10 sampling sites were randomly chosen within a radius of 1 km; one periphytic biofilm, one floodwater sample, and one corresponding paddy soil were separately collected per sampling site. Thus, 200 periphytic biofilm, 200 floodwater, and 200 paddy soil samples were collected from the 20 sampling areas. Climatic data (sunshine duration, radiation intensity, and effective accumulated temperature) of the individual sampling areas were retrieved from http://data.cma.cn/site/index.html.

Sample collection

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; to minimize soil contamination of the biofilms, distinct, visible clods were collected and washed several times with running floodwater to remove adhering soil from the biofilm samples until the effluent was no longer turbid. Water in periphytic biofilms was drained and then the samples were sealed in plastic sampling bags (Supporting Information Figure S1B). Additionally, 100 ml of the corresponding floodwater was bottled, and 100 g of the paddy soil (0–20 cm without periphytic biofilm) was collected. All the samples were transported on ice to the laboratory and stored at −20°C until further analysis.

Sample analysis

EPSs in the periphytic biofilm were extracted and quantified using a modified alkaline extraction method, and protein and polysaccharide components were quantified using the Bradford assay with bovine serum albumin as standard (Bio-Rad) and the phenol–sulfuric acid assay, respectively [30], and the eDNA contents in EPS were quantified using a microspectrophotometer (Bei Jing Kai Ao K5600). Part of periphytic biofilm and soil samples was pretreated at 60°C to constant weight in an oven (GZX-9140ME) before further analysis. In all, 0.5 g (dry weight) of each periphytic biofilm or soil sample and 5 ml floodwater was digested with HNO_3–H_2O_2 in a digestion oven (JKXZ06-8B) and subsequently used to measure total nitrogen (TN) and total P (TP) contents; both TN and TP were quantified using a flow analyzer (FS3700, OI Analytical). Total organic carbon (TOC) in periphytic biofilm was determined with the potassium dichromate method. The pH value of each floodwater sample was measured using a pH meter (Mettler Toledo FE28).

16S and 18S rDNA amplicon sequencing

For the 10 collected periphytic biofilm samples from each sampling area, 2 g periphytic biofilm was, respectively, taken and mixed well, and then divided into three parts to analyze their microbial communities. The average values of the relative abundance of both prokaryotes and eukaryotes were calculated to summarize the microbial information of each sampling area. Prokaryotes and eukaryotes in each sample were analyzed via 16S and 18S rDNA high-throughput sequencing on the HiSeq. 2500 platforms, respectively. The methods of DNA extraction and purification, sequencing, quality control, sequence analysis, sequences deposition into the National Center for Biotechnology Information (NCBI) database, and so forth, were as described previously [4]. Microbial sequences were then deposited in the NCBI under accession number PRJNA854262, and the Genome Warehouse in National Genomics Data Center [32, 33], Beijing Institute of Genomics, Chinese Academy of Sciences/China National Center for Bioinformation, under accession number GWHBOSZ00000000.

Statistical analysis

A correlation matrix was generated in R using the "psych" package, and then the interaction network between prokaryotes, eukaryotes, and EPS content in periphytic

biofilm was analyzed and visualized using the software Gephi 0.9.2 (France). The effect of EPS on nutrients (C, N, and P) accumulation in periphytic biofilm was analyzed using regression analysis. The potential geographical distribution patterns of EPS contents in periphytic biofilms and the ratio of protein to polysaccharide in EPS were analyzed using regression analysis [34]. Statistical significance for factors driving the geographical distribution of EPS content in periphytic biofilm was analyzed in R using the "vegan" package. Partial Least Squares Path Modeling was employed to evaluate how characteristics of soil (TOC, TN, and TP) and floodwater (pH, TN, and TP), climatic factors (sunshine duration, radiation intensity, and effective accumulated temperature) affect microorganisms (including prokaryotes and eukaryotes) in periphytic biofilm in conjunction with the effect of the latter on the geographical distribution of EPS contents in periphytic biofilms [21]. All the statistical procedures were conducted with SPSS 16.0 (SPSS Inc.). The abundances of eukaryotes and prokaryotes were visualized with Circos software (http://circos.ca/), and other figures were generated with SigmaPlot 10.0 software (Systat Software Inc.).

AUTHOR CONTRIBUTIONS

Yonghong Wu and Pengfei Sun conceived the study. Pengfei Sun and Rui Sun collected samples and data. Pengfei Sun analyzed the data and wrote the draft. Yonghong Wu, Yingyao Liu, Rui Sun, and Jan Dolfing gave advice on experimental design and revised the manuscript. All authors reviewed the manuscript draft and discussion of results.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available (repository name, e.g., "figshare"). All the sequencing data have been deposited in the Genome Warehouse in National Genomics Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences/China National Center for Bioinformation, under accession number GWHBOSZ00000000 that is publicly accessible at https://ngdc.cncb.ac.cn/gwh, BioProject accession number PRJCA012051, as well as NCBI under submission number PRJNA854262. The data and scripts used are saved in GitHub https://github.com/SunPFei/PLS-PM-for-EPS.git or https://gitee.com/pengfei-sun0913/pls-pm-for-eps.git. Supplementary materials (figures, tables, scripts, graphical abstract, slides, videos, Chinese translated version, and update materials) may be found in the online DOI or iMeta Science http://www.imeta.science/.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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