

Physical geography shapes the distribution characteristics of extracellular polymeric substances in periphytic biofilms

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Abstract

Background: Extracellular polymeric substances (EPS) are the glue that holds periphytic biofilms together. However, little is known about the chemical composition of the EPS and whether and if so how these components vary over different geographic regions. Therefore, we collected a large series of periphytic biofilms from paddy fields in different geographic regions to study the content, geographical distribution, and potential ecological function of EPS in periphytic biofilms, as well as the function of microbial components on EPS in periphytic biofilm.

Results: EPS accounted for 1.3-2.9% of the total biomass of periphytic biofilms, while the protein content in periphytic biofilm was 1.8 to 20 times higher than that of the polysaccharides. The geographical distribution of EPS in periphytic biofilms follows the typical latitudinal diversity gradient theory: EPS contents decreased with increasing latitudes, but the ratio of protein to polysaccharide in periphytic biofilms showed the opposite trend. Physical geography characteristics, including temperature and light, are the principal forces driving the geographical distribution of EPS in periphytic biofilms, affecting their microbial composition. Prokaryotes in periphytic biofilm negatively affect the EPS content in periphytic biofilm, suggesting that prokaryotes may function as EPS consumers, while eukaryotes may be EPS producers, because they positively affect the EPS content in periphytic biofilm. Functionally, EPS components contribute to N and P accumulation in periphytic biofilms, as well as enhance the total organic carbon content in periphytic biofilm.

Conclusion: These analyses provide invaluable information on the biogeochemistry of EPS in periphytic biofilms and their potential role in modulating nutrient cycles in paddy fields.

Background

Microorganisms in biofilms comprise of a complete food chain including producers, consumers and decomposers [1], and live in their own micro-environment supported by a self-produced matrix of extracellular polymeric substances (EPS) [2, 3]. EPS functions as the skeleton of the biofilm, nutrients source, and protective barrier for the microorganisms in biofilms[4]. EPS originating from single organisms has been well studied [4, 5], while in contrast, our understanding of the EPS in biofilms of complex communities, such as those growing in paddy fields, is far from complete [5].

Analogous to natural biofilms growing in natural aquatic ecosystems (e.g. stream, wetlands, and rivers, etc.) [6], periphytic biofilms growing in paddy fields are important due to their potential significance for both agronomy and environmental protection [7]. However, the study of periphytic biofilms is still in its infancy due to periphytic biofilms have been chronically ignored by both agronomists and ecologists. Most of the recent studies on periphytic biofilms have focused on the relationship between their microbial composition and their ecological functions [7, 8], while the all-important abiotic composition of the periphytic biofilm, specifically the EPS component, has received only limited attention.

Empirically, we can infer that EPS are most likely a main abiotic components in periphytic biofilms. This inference is based on knowledge of biofilms (periphyton) growing in aquatic ecosystems [2], however, the entirely different survival environment, and the availability of nutrients imply that the microbial composition, content and components of EPS may vary greatly between biofilms grown in man-made paddy fields and those grown in natural aquatic ecosystems [2]. Thus a study on the content, components, and driving forces for the geographical distribution of EPS in periphytic biofilms, as well as their potential function (environmental and agronomic effects) in periphytic biofilms has been long overdue.

Therefore, we collected a total of 200 periphytic biofilm samples at nationwide scale (from south to northeast China with geographical regions varying from 22°25' N to 47°16' N) from China's paddy fields in 2018 to address the following questions: 1) What are the content and composition of EPS in periphytic biofilms? 2) Are there geographical differences between the EPS in periphytic biofilm, and if any, what are the driving forces for the geographical distribution? 3) How do the biotic components affect the abiotic components in biofilm? And 4) What are the potential ecological roles of EPS in periphytic biofilm?

Results

The contents, components and geographical distribution of EPS in biofilms

EPS contents (dry weight) in periphytic biofilm varied from 1.28–2.88% of the total biofilm mass (Fig. 1a). On average, EPS accounted for 1.9% of the biomass of periphytic biofilm, which provides a direct evidence that that EPS is an important abiotic component in periphytic biofilms. In periphytic biofilms, the contents of protein are much larger than the contents of polysaccharides (Fig. 1b), and the ratio of protein to polysaccharide varied from 1.8 to 20 (Fig. 1a), with an average value of 5.4.

Here, we employed the latitudinal diversity gradient (LDG) theory to evaluate the geographical distribution pattern of EPS content in periphytic biofilm. We found that EPS content in periphytic biofilm decreased significantly with increasing latitude ($r = 0.3294$, $p < 0.0001$, red line in Fig. 2). In addition, we found that the ratio of protein to polysaccharide in periphytic biofilm increased significantly with the increasing latitudes ($r = 0.2742$, $p = 0.0005$, blue line in Fig. 2). The results showed that both the EPS content and the ratio of protein to polysaccharide in periphytic biofilm have significant geographical distribution characteristics, namely, the higher the latitude at which a periphytic biofilm grows, the lower its EPS content, and the higher its ratio of protein to polysaccharide.

Driving forces for the geographical distribution of EPS in biofilm

Partial Least Squares Path Modeling (PLS-PM) was employed to synthesize the data and analyze the forces driving the geographical distribution of EPS in periphytic biofilm focusing on climate, paddy soil composition, and floodwater characteristics. Microbes are the primary executors changing the component and content of EPS in periphytic biofilm [13], all the other factors such as climate, paddy soil, and floodwater are secondary: they affect the microbial components first, and (thus) only indirectly affect the geographical distribution pattern of EPS in periphytic biofilm (Fig. 3). The total effect of climate on eukaryotes and prokaryotes is 0.55 and 0.69, respectively. While the total effects of paddy soil on eukaryotes and prokaryotes are respectively 0.28 and 0.19, and the total effects of floodwater on eukaryotes and prokaryotes are 0.45 and 0.40, respectively (Fig. 3). Combined their direct and indirect effect on microbial composition together, the total effect of climate, soil and floodwater on the geographical distribution of EPS are -0.41, -0.11, and -0.23, respectively. By contrast, the total contribution of these three factors on the microbial components which in turn shift the geographical distribution of EPS is in the following order: Climate > floodwater > paddy soil. Thus we can conclude that climate factors are the principle forces driving the geographical distribution of EPS in periphytic biofilm. Among the analyzed climate factors, both sunshine duration (path coefficient = 0.91) and radiation intensity (path coefficient = 0.63) showed positive effect (Fig. 3). Additionally, the factor of effective accumulated temperature (EAT) showed significantly negative effect (path coefficient = -0.92, Fig. 3) on the geographical distribution of EPS in periphytic biofilm. These patterns suggest a role for physical geography in determining the geographical distribution of EPS in periphytic biofilm.

Microbes in periphytic biofilm had direct effect on the EPS composition. PLS-PM analysis showed that prokaryotes in periphytic biofilms exerted a negative effect (path coefficient = -0.63, Fig. 3) on EPS production by periphytic biofilm, suggesting that prokaryotes in periphytic biofilm may function as the main EPS consumer. While eukaryotes on the other hand exerted a positive effect (path coefficient = 0.46, Fig. 3), implying that eukaryotes may be producers of EPS in periphytic biofilms.

In our periphytic biofilm samples, a total of 130 genera of prokaryotes belonging to 16 phyla and 145 genera of eukaryotes belonging to 23 phyla were found. This indicates that a wide diversity of both prokaryotes and eukaryotes are present in periphytic biofilms, and that eukaryotes are slightly more varied than prokaryotes. For eukaryotes, *Aporcelaimellus*, *Paratripyla*, *Characiopodium*, *Heteromita*, *Desmodesmus*, *Chlorotetraedron*, *Rhabdolaimus*, *Halteria*, *Pythium*, and *Chaetomium* are the genera most frequently present in the individual top 10 of most abundant genera in a specific periphytic biofilm (Fig. 4a); while for prokaryotes the corresponding top 10 genera are *Flavobacterium*, *Acinetobacter*, *Pirellula*, *Dinghuibacter*, *Massilia*, *UTCXF1*, *Bacteroides*, *Luteolibacter*, *Clostridium_sensu_stricto_13*, and *Proteiniclasticum* (Fig. 4b).

Both prokaryotes and eukaryotes are significantly related to EPS content in periphytic biofilm (Fig. 5). Most of the prokaryotes in biofilm, except for a few bacteria (e.g. *Azospira*, *Dechloromonas*, *Paludibacterium*, *Phreatobacter*, *Prevotella*), had negative correlation with the EPS content. On the contrary, most of eukaryotes, except for *Rhogostoma*, in periphytic biofilm showed significantly positive correlation with the EPS in periphytic biofilm. In summary, the correlation analysis results (c.f. Figure 5)

echo the results of eukaryotes showing a positive effect on EPS in periphytic biofilm while prokaryotes show the opposite roles (c.f. Figure 3). The correlation analysis results further support our speculation that eukaryotes are possibly EPS producers in periphytic biofilm, while prokaryotes may act as EPS consumers.

Furthermore, we found that EPS in periphytic biofilm was significantly affected by the availability of nutrients such as phosphorus in paddy soil ($r=-0.155$, $p = 0.03$, Fig. 6a) and $\text{NH}_4^+\text{-N}$ in floodwater ($r = 0.175$, $p = 0.017$, Fig. 6b).

Potential environmental and agronomic effects of EPS in periphytic biofilms

As periphytic biofilm grows in paddy fields, we thus pay close attention to their possible roles in regulating nutrients (C, N, and P) cycling in paddy fields. Herein, we found that periphytic biofilm showed great potential in nutrients accumulating (Fig. 7a), and the concentration of TN ($r = 0.372$, $p < 0.001$, green line in Fig. 7b) and TP ($r = 0.272$, $p < 0.001$, red line in Fig. 7b) in periphytic biofilm were significantly related to the EPS composition, which may partly explain why periphytic biofilm could accumulate plenty of N and P. In other words, EPS contribute to assisting periphytic biofilm accumulating N and P, thus shifting the biogeochemical cycling of N and P in paddy fields. For the environmental effect, periphytic biofilm accumulating N and P increases the retention time of N and P in paddy fields and then prevents N and P loss from paddy fields in runoff [14].

Additionally, we found that EPS in periphytic biofilm also showed significantly positive correlation with TOC in periphytic biofilm ($r = 0.302$, $p < 0.001$, Fig. 7b). The results suggest that EPS from periphytic biofilms may be a source of TOC in paddy soils, and then improve the fertility of paddy soils, which is the agronomic effect of EPS in periphytic biofilm. In summary, we revealed two novel ecological functions of EPS in periphytic biofilms: 1) EPS in periphytic biofilms contributes to accumulating N and P and then decreasing the loss of nutrients from paddy fields; 2) EPS in periphytic biofilms helps to improve the TOC levels and hence the fertility of paddy soils.

Discussion

EPS are considered as the skeleton of biofilms, exerting important ecological functions [15]. However, we know very little about the EPS component in periphytic biofilms. Herein, we quantitated, for the first time, the EPS contents (dry weight) in periphytic biofilm, providing a direct evidence for EPS being an important component in periphytic biofilm. For the component of EPS, in most EPS, polysaccharides are the most abundant component [16, 17], while for EPS in periphytic biofilms the opposite is the case (Fig. 1b). The growth environment of periphytic biofilm would affect its EPS composition, and in paddy fields, the low ratio of carbon to nitrogen would result in the high ratio of protein to polysaccharide in EPS of periphytic biofilm [18, 19], which was verified by our results of C/N in paddy soil showing negative effect on the ratio

of protein to polysaccharide in periphytic biofilm (Supplementary Fig. S1). The LDG theory is usually applied to evaluate microbial geographical distribution patterns [11, 20], here we found that the LDG theory can also be employed to evaluate the EPS geographical distribution patterns in periphytic biofilm.

Microbes are the primary executors changing the component and content of EPS in periphytic biofilm [13]. Prokaryotes in periphytic biofilms exerted a negative effect on EPS production by periphytic biofilm. This may be due to, although some bacteria such as prokaryotic bacteria and algae can produce EPS [13, 26, 27], most prokaryotes in periphytic biofilm consuming EPS as their carbon or energy sources [21, 22], and the effect of prokaryotes consuming EPS may be larger than that of prokaryotes producing EPS, the overall effect of prokaryotes on EPS in periphytic biofilm thus is negative. Based on the positive effect of eukaryotes on EPS production by periphytic biofilm, which suggests that eukaryotes may be producers of EPS in periphytic biofilms [23, 24]. The reasoning that because prokaryotes negatively affect the EPS content in periphytic biofilm, someone else needs to produce the EPS to maintain the micro-environment they growing; thus it must be the eukaryotes.

Climatic factors, including temperature and light, principally drive the geographical distribution of both the EPS content and the ratio of protein to polysaccharide in periphytic biofilm. Theoretically, the lower the latitude, the longer the sunshine duration and the higher the radiation intensity. The higher lighting intensity and duration, the more production of EPS [25]. Additionally, being well corresponded to the previous findings [26, 27], the EAT showed significantly negative effect on the geographical distribution of EPS in periphytic biofilm. This is because EPS play important roles in protecting microbes in biofilm against adverse environment [4], microbes in biofilm would secret more EPS to help them resist stress posed by low temperatures [28]. The scarcity of resources (e.g. nutrients) affects the biomass of microbes such as fungi and bacteria [29], and this in turn, nutrients in paddy fields would affect the EPS content in periphytic biofilm. Both N and P are essential for the growth of periphytic biofilm [30], while the high concentration of P in paddy soil is expected to negatively affect the production of EPS [31]. The positive correlation of $\text{NH}_4^+\text{-N}$ in floodwater with EPS content in periphytic biofilm suggests that high $\text{NH}_4^+\text{-N}$ in floodwater stimulates EPS production by periphytic biofilm [32].

Ecologically, the high protein content in EPS could contribute to protecting microbes in periphytic biofilm against adverse growth conditions such as water stress, heavy metals, pesticides, and insecticides [4, 33, 34]. In the present study, we found two new ecological function of EPS in periphytic biofilm, for the first one of EPS assisting N and P accumulation by periphytic biofilm, this is because EPS has abundant functional groups such as carboxyl, carbonyl, etc. [9], providing various binding sites for N and P, thus facilitating the accumulation of N and P by periphytic biofilm. For the second one, as proteins and polysaccharides being the main components of EPS [4], EPS in periphytic biofilms may be a source of TOC in paddy soils, and then expects to improve the fertility of paddy soils [5]. In summary, the environmental and agronomic effects of EPS in paddy fields show: 1) the potential in decreasing nutrients loss, via facilitating the accumulation of N and P by periphytic biofilm, from paddy fields, and 2) the periphytic biofilm with high content of EPS expects to improve the TOC levels and hence the fertility of paddy soils.

Conclusion

EPS is an important abiotic component in periphytic biofilm, accounting for 1.3–2.9% of the total biomass of periphytic biofilms. Its protein content is 1.8 to 20 times higher than that of the polysaccharides. Prokaryotes appear to function as the main EPS consumers, while eukaryotes are suggested to be EPS producers. Functionally, in addition to enhancing the total organic carbon content in periphytic biofilms EPS components also contribute to N and P accumulation in periphytic biofilms. Our results provide invaluable information on the biogeochemistry of EPS in periphytic biofilms and their potential role in modulating nutrient cycles in paddy fields.

Methods And Materials

Sampling areas description and samples collection

Periphytic biofilm, paddy soil and floodwater samples were collected individually from a series of paddy fields in different geographical regions of China, ranging from 22°25' N to 47°16' N (Fig. 8A). The paddy fields sampled covered the main rice planting areas in northeast, central, and south China. All samples were collected 7–15 days after the transplanting in 2018. In total, twenty sampling areas were selected (Fig. 8A); per sampling area ten sampling sites were randomly chosen within a radius of 10 km; one periphytic biofilm, one corresponding paddy soil, and one floodwater sample were separately collected per sampling site. Thus, 200 periphytic biofilm, 200 paddy soil, and 200 floodwater samples were collected from the twenty sampling areas. Climate data (SD (h), RI (MJ/m²), and EAT (°C)) of each sampling areas were retrieved from the website: <http://data.cma.cn/site/index.html>.

Regarding sample collection, about 50 g wet periphytic biofilm was collected per sampling site by scraping biofilm from the surface of paddy fields, and then the biofilm samples were washed in floodwater for 5 times to remove soil from the biofilm before being putting into sampling bags (Fig. 8B). Meanwhile, 100 mL of 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 for further study.

Samples Analyses

The extraction and analysis of EPS (including polysaccharide and protein) in periphytic biofilm followed our previous method [9]. 0.5 g dry weight of each periphytic biofilm/soil and 5 mL floodwater were first digested by HNO₃-H₂O₂ in a digestion oven (JKXZ06-8B, China) before being used to measure TN and TP; both TN and TP were quantified using a flow analyzer (FS3700, OI Analytical, USA). Determination of TOC in soils was by potassium dichromate method [10]. The pH value of each floodwater sample was measured using a pH meter (Mettler Toledo FE28, Switzerland). The concentrations of NH₄⁺-N, NO₃⁻-N, and PO₄³⁻ in floodwater and soil were measured using a flow analyzer (FS3700, OI Analytical, USA). DNA

was extracted using DNA extraction kits (MOBIO 12888-100, Carlsbad, CA, USA) from the corresponding samples. The concentration and purity of extracted DNA were measured using a NanoDrop One (Thermo Fisher Scientific, MA, USA). The V4V5 region of 16S rDNA was amplified using primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') to identify the diversity of prokaryotes (bacteria and archaea); the V4 region of 18S rDNA was amplified using primers 528F (5'-GCGGTAATTCCAGCTCCAA-3') and 706R (5'-AATCCRAGAATTTACCTCT-3') to identify the diversity of eukaryotes in periphytic biofilm. 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 controlled process. To detect and remove chimera sequences, the reads were compared with the reference database using UCHIME algorithm, and then these clean reads finally obtained. Sequences analysis were performed by UPARSE software, of which sequences with $\geq 97\%$ similarity were assigned to the same OTUs. Microbial sequences were then deposited into the NCBI databases with the accession number (PRJNA543163).

Statistical analysis

All the statistical procedures were conducted by SPSS 16.0 software (SPSS Inc., Chicago, USA). Statistical significance for the effects of driving forces on the geographical distribution of EPS content in periphytic biofilm was analyzed with R using the 'vegan' package. Regression analysis was used to examine whether the theory of LDG is applicable to EPS content patterns and the ratio of protein to polysaccharide in periphytic biofilm [11]. Correlation analysis was used to analyze the potential function of EPS in regulating nutrient (C, N, and P) cycling. PLS-PM was employed, using R, to comprehensively understand how characteristics of soil (TOC, TN, and TP) and floodwater (pH, TN, and TP), climate factors (SD, RI, and EAT) affect microbes (prokaryotes and eukaryotes) in periphytic biofilm in conjunction with the effect of the latter on the EPS content (geographical distribution) of the biofilm [12]. All the figures were generated with SigmaPlot 10.0 software (Systat Software Inc., California, USA), except for the abundances of eukaryotes and prokaryotes, which were visualized with Circos software (<http://circos.ca/>).

Declarations

Data availability

Observed data such as temperature, light, and precipitation, etc. were acquired from National Meteorological Information Center of China (<http://data.cma.cn/site/index.html>.) The data that support the findings of this study are available from the corresponding author upon request.

Author Contributions

Yonghong Wu and Pengfei Sun designed and conceived the study. Pengfei Sun, Ying Xu, Rui Sun, and Mengning Gao conducted the study. Pengfei Sun analyzed the results and wrote the first draft. Jan Doling revised the manuscript. All authors commented on the draft and discussion of results.

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Availability of data and materials

Supplementary information is available in the online version of the paper. Correspondence and requests for materials should be addressed to Yonghong Wu (yhwu@issas.ac.cn).

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Competing Interests statement

The authors declare no competing financial interests.

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Figures

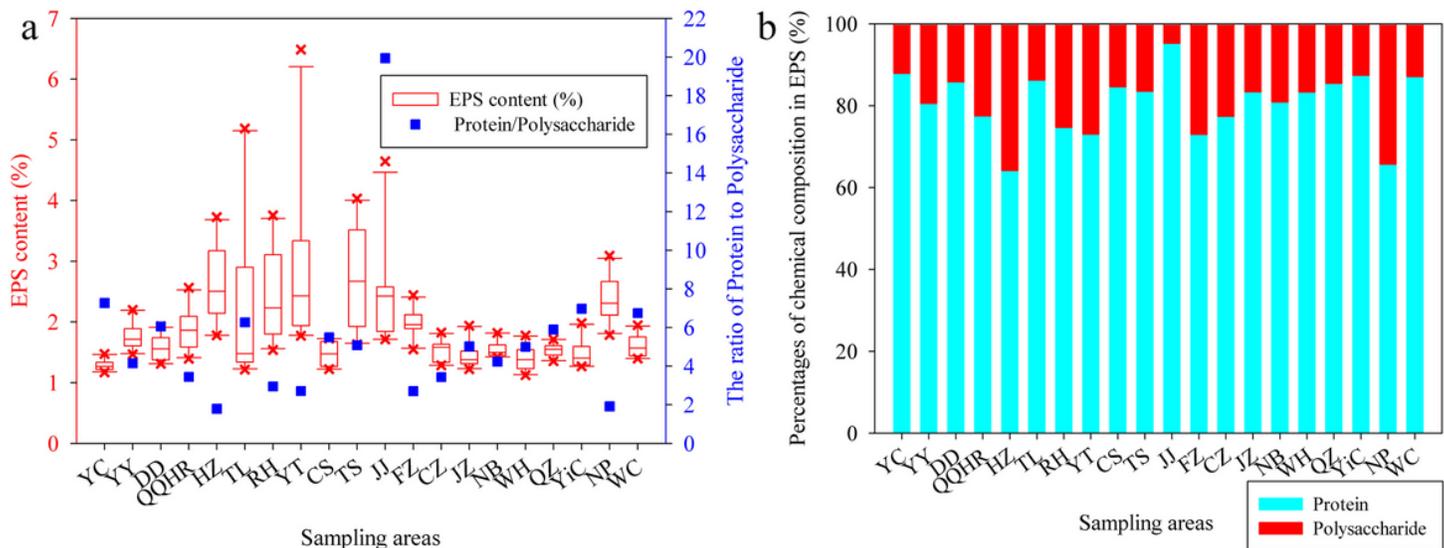


Figure 1

The EPS content in periphytic biofilms from different geolocations (red boxes in a) and the ratios of protein to polysaccharide in EPS from different periphytic biofilms (blue points in a), and the chemical composition of EPS in periphytic biofilms collected from the 20 sampling areas (b). TS: Taishan, RH: Renhua, QZ: Quanzhou, FZ: Fuzhou, NP: Nanping, CS: Changshu, YC: Yancheng, NB: Ningbo, HZ: Hangzhou, WH: Wuhu, CZ: Chizhou, YY: Yueyang, JZ: Jingzhou, YiC: Yichang, JJ: Jiujiang, YT: Yingtan, TL: Tieling, DD: Dandong, WC: Wuchang, QQHR: Qiqihar.

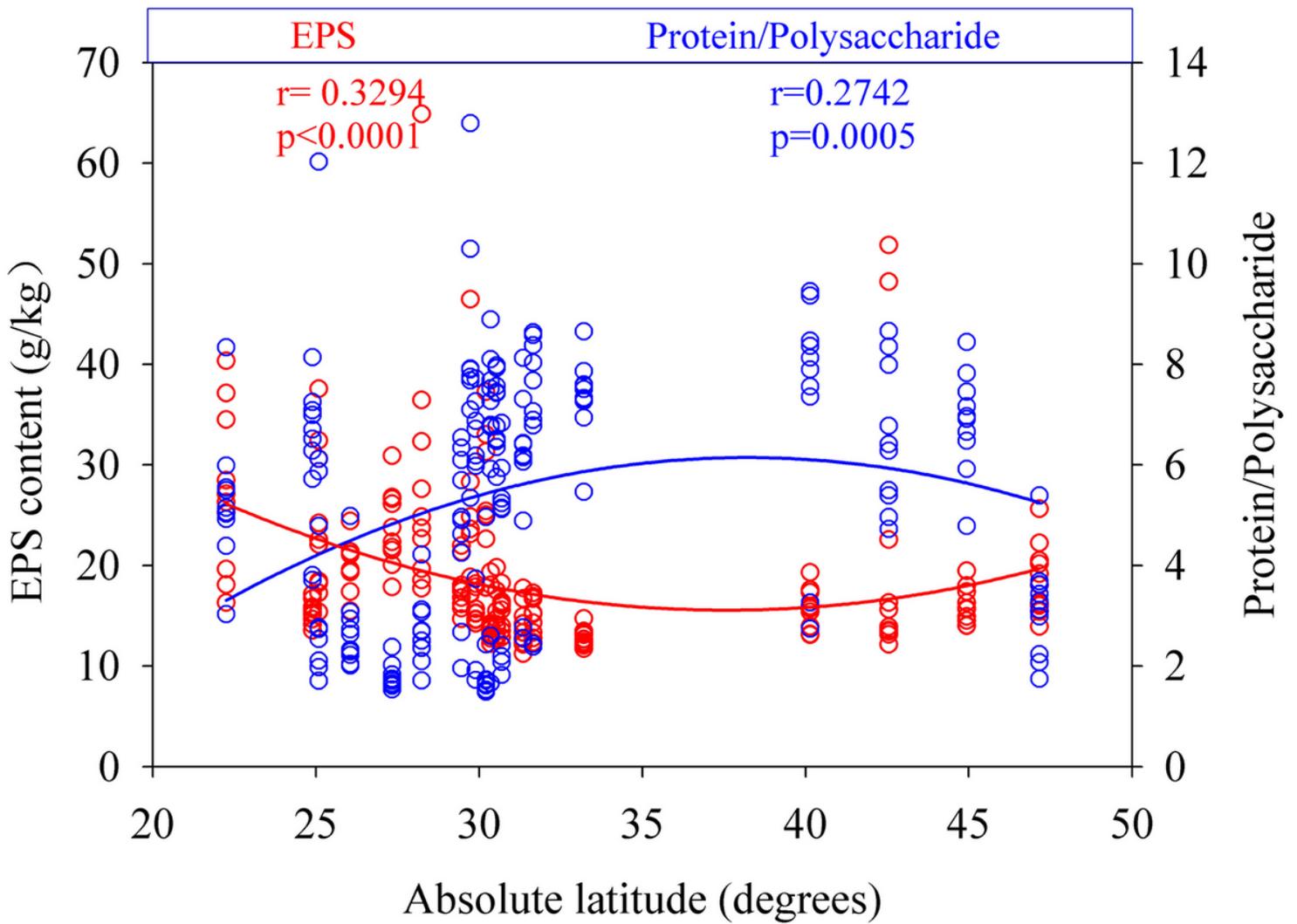


Figure 2

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 same patterns across the latitudinal gradient. Second order polynomial fits are shown in blue (protein/polysaccharide) and red (EPS content), respectively. The regression parameters of EPS contents and protein/polysaccharide are given at the top of the figure.

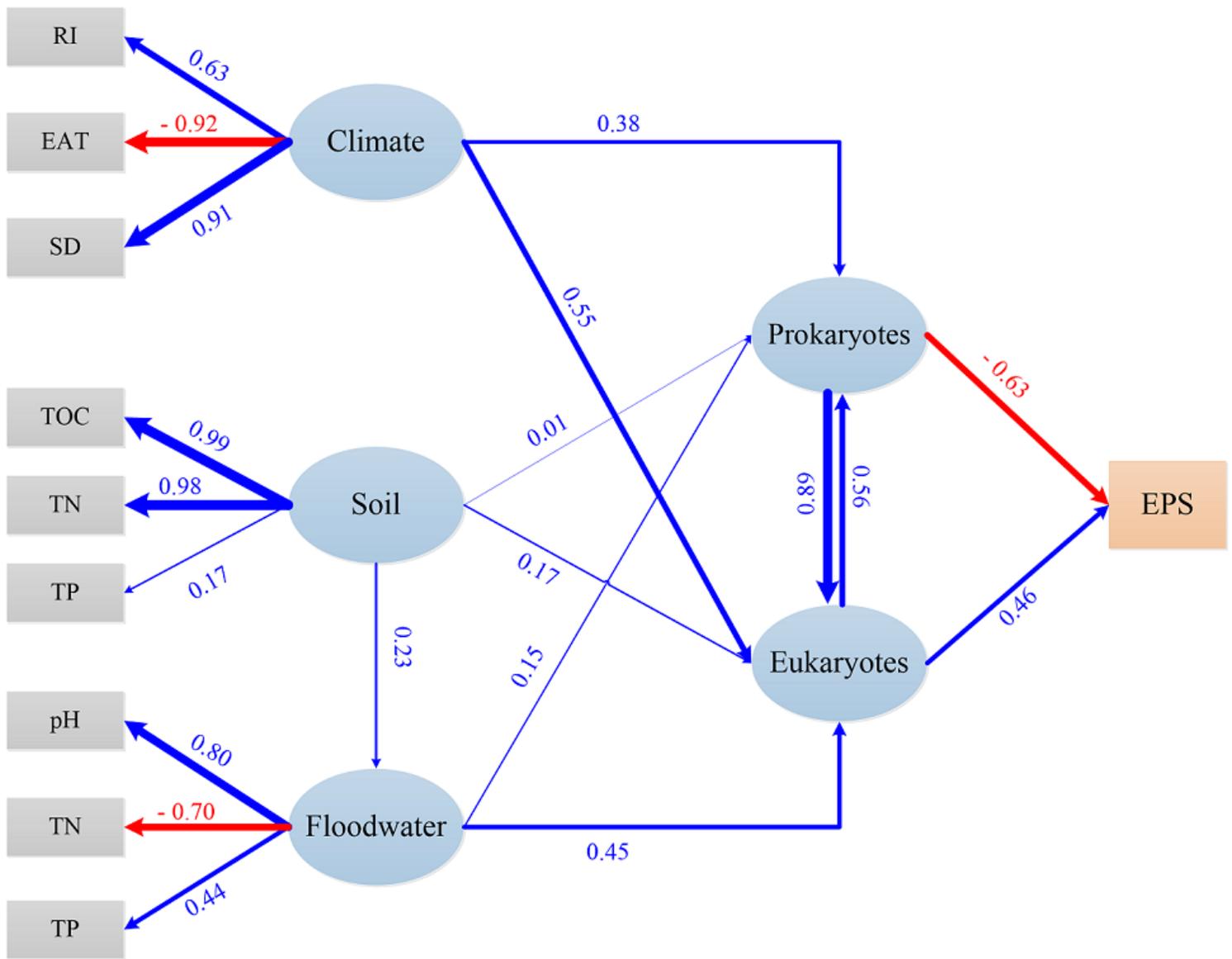


Figure 3

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 microbes in periphytic biofilm, and their effect on EPS in periphytic biofilms, as analyzed by PLS-PM. Blue arrows in the model present positive effect, while red arrows in the model show 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.

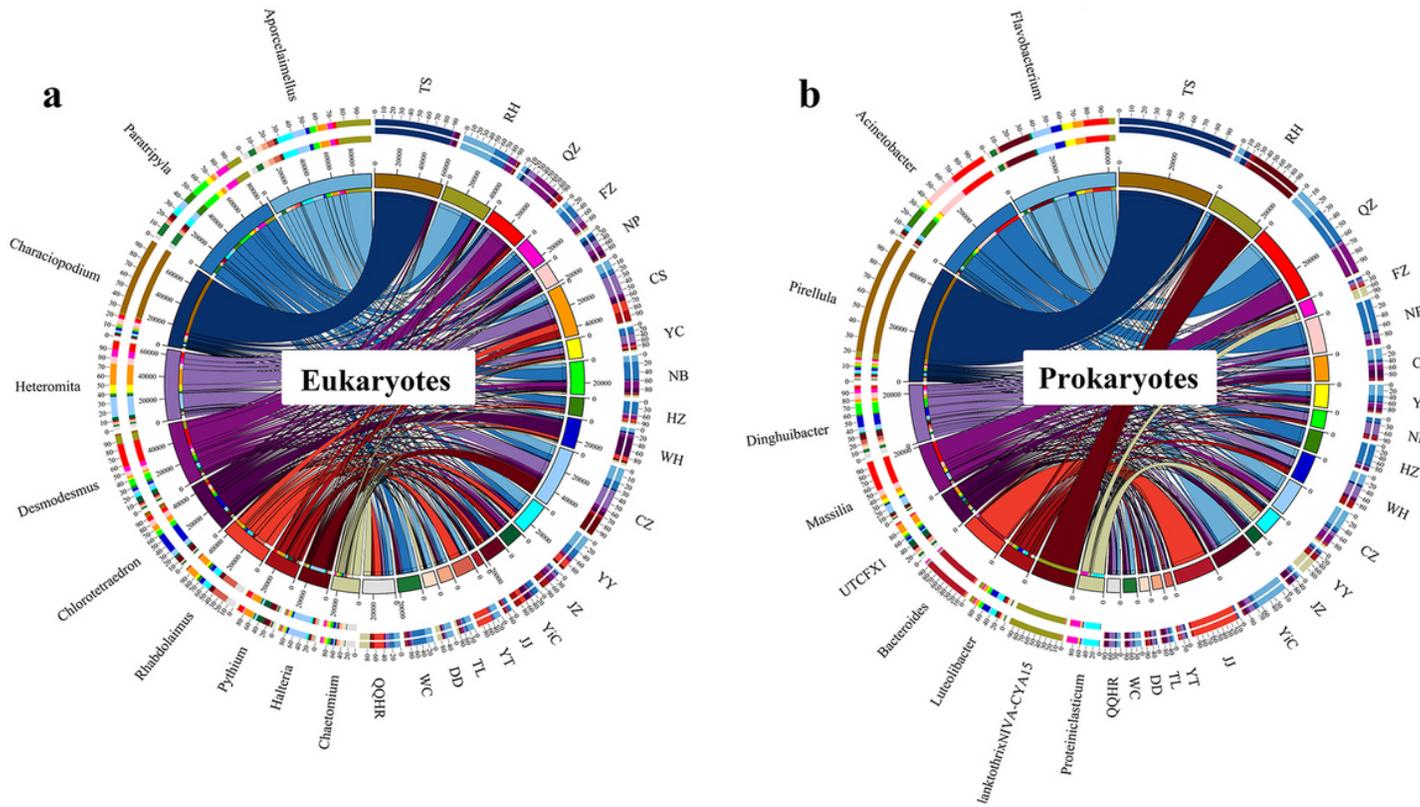


Figure 4

Distribution of eukaryotes (a) and prokaryotes (b) with the highest abundance at genus level in the top 10 in periphytic biofilm. The length of the bars of each sample on the outer-ring represents the percent of microbes in each sample.

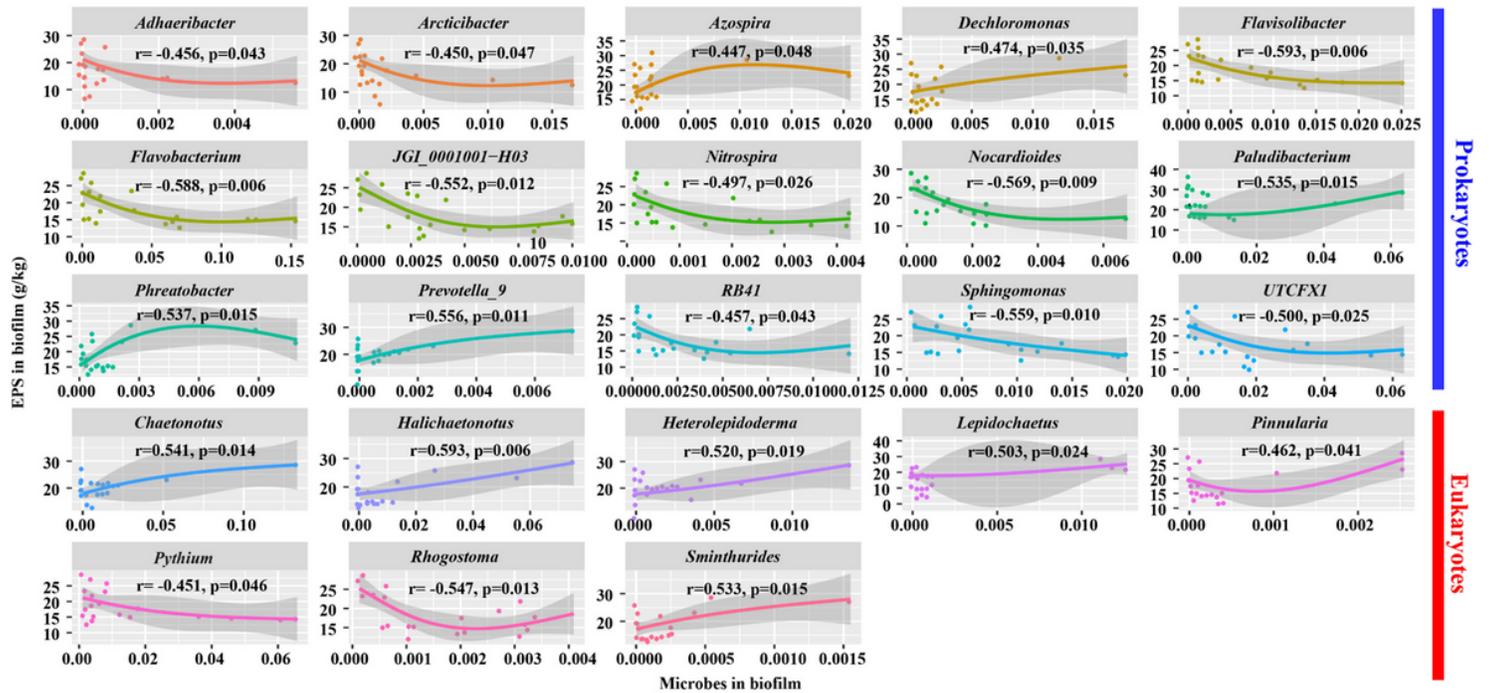


Figure 5

Prokaryotes and eukaryotes exhibit contrasting effect on EPS content in periphytic biofilm. Horizontal coordinates represent the abundance of each prokaryote and eukaryote, while vertical coordinates are the content of EPS in biofilm (g/kg).

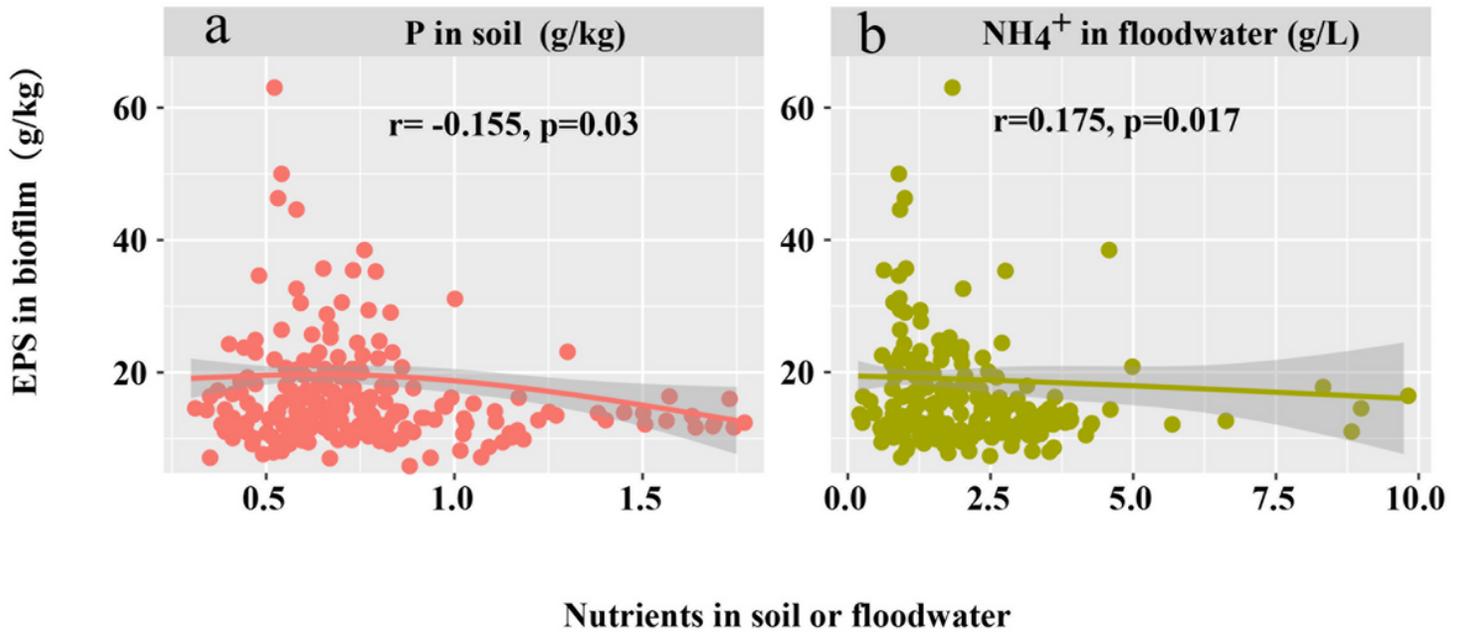


Figure 6

Nutrients in paddy soil (a) and floodwater (b) show significant effect on EPS content in periphytic biofilm.

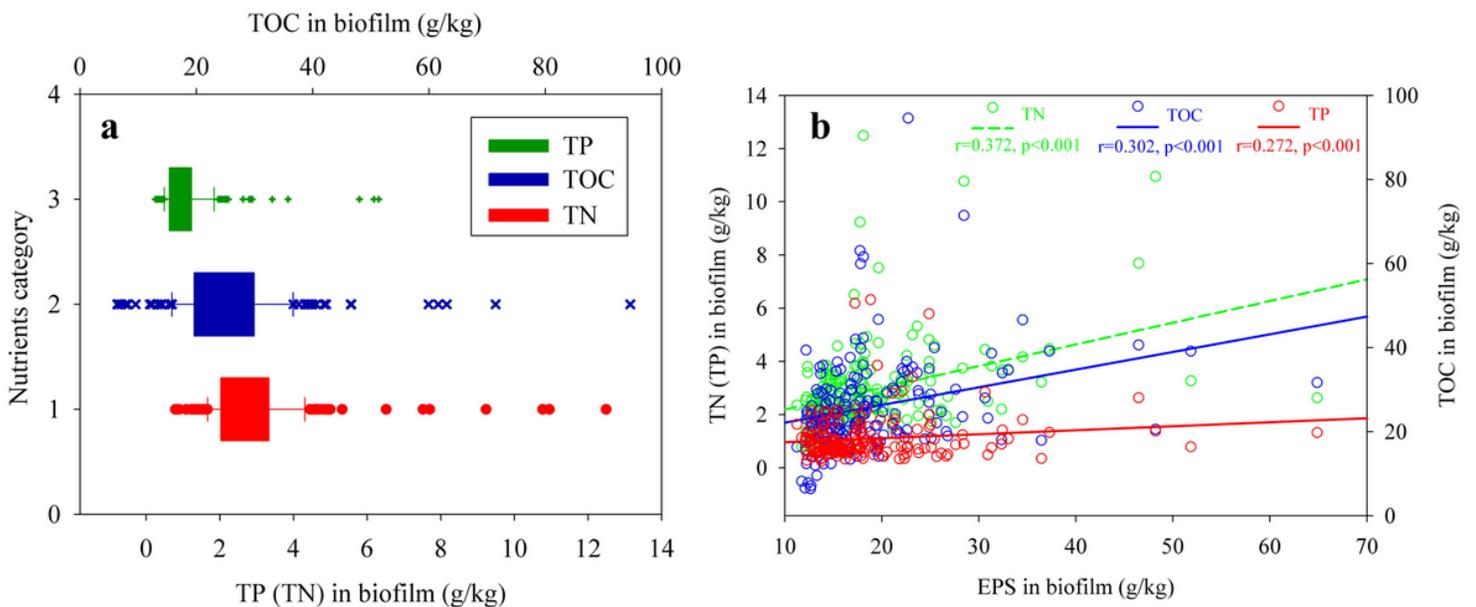


Figure 7

The quantities of TN, TP, and TOC in periphytic biofilms (a), and the increasing EPS in periphytic biofilms correlates with enhanced N and P contents and TOC content in periphytic biofilms (b).

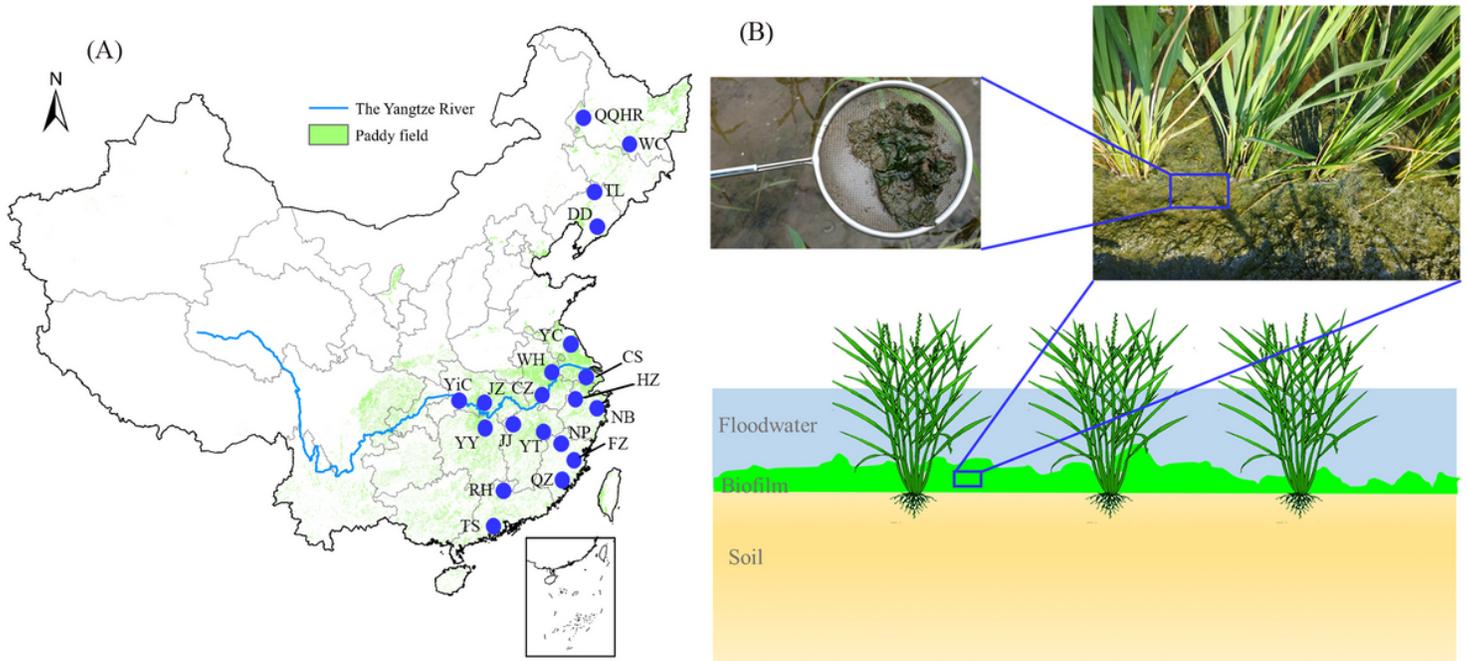


Figure 8

Map of the twenty sampling areas located in different rice growing areas across China (A) and the diagram of collecting periphytic biofilm (B). The twenty sampling areas are, TS: Taishan, RH: Renhua, QZ: Quanzhou, FZ: Fuzhou, NP: Nanping, CS: Changshu, YC: Yancheng, NB: Ningbo, HZ: Hangzhou, WH: Wuhu, CZ: Chizhou, YY: Yueyang, JZ: Jingzhou, YIC: Yichang, JJ: Jiujiang, YT: Yingtian, TL: Tieling, DD: Dandong, WC: Wuchang, QQHR: Qiqihar. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.