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1 **Soil organic carbon enrichment triggers *in-situ* nitrogen interception by**
2 **phototrophic biofilms at soil-water interface: from regional to micro scales**

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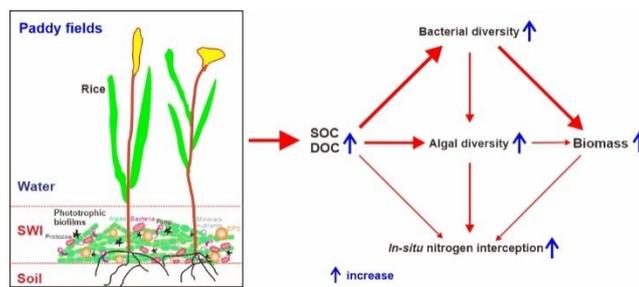
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18 **Graphical abstract**



19

20 **Abstract**

21 Phototrophic biofilms are easy to grow at sediment/soil-water interfaces (SWI) in
22 shallow aquatic ecosystems and greatly impact nutrient biogeochemical cycles.
23 However, the pathways by which they contribute to nitrogen interception and interact
24 with sediment/soil remains largely unknown. Here, we conducted a field investigation
25 in paddy fields in various regions of China and found that nitrogen immobilized in
26 biofilm biomass significantly positively correlated with soil organic carbon (SOC)
27 content. A microcosm experiment showed that this was due to increased bacterial and
28 algal diversity, biomass accumulation and inorganic nitrogen assimilation at high SOC
29 especially high dissolved organic carbon (DOC) levels. The metatranscriptomics results
30 further verified that many KO groups of PSII, PSI, AP and PC in antenna proteins and
31 Glutamate synthesis were distinctly expressed at elevated SOC and DOC levels. Our
32 results elucidated the effects and possible pathways of how SOC enrichment triggers
33 photosynthesis and nitrogen immobilization by phototrophic biofilms. The results will
34 provide meaningful information for *in-situ* nitrogen interception by using phototrophic
35 biofilms at SWI in human-made wetlands to change internal nitrogen cycling.

36 **Keywords:** DOC, Diversity, Nitrogen interception, Metatranscriptomics, Wetland

37 **Synopsis:** A national field investigation and a microcosm experiment together
38 elucidated that elevated soil organic carbon levels enhanced *in-situ* nitrogen
39 interception by phototrophic biofilms at soil-water interface.

40 Introduction

41 Nitrogen discharge from agro-ecosystems has been a main contributor to surface
42 water pollution, especially under the increasing food demand and overfertilization
43 conditions in developing countries.¹⁻³ Interception of excessive nitrogen in cropland has
44 long been challenging.^{4, 5} In paddy fields, the soil-water interfaces (SWI) form the
45 boundary between soil and water, and are the sites where the various nutrient cycles are
46 coupled.^{6,7} As a seasonal human-made wetland, the periodic alternation of wetting and
47 drying and fertilization in paddy fields initiates and drives formation and withering of
48 phototrophic biofilms at SWI.⁸ Mainly composed of algae, bacteria and abiotic
49 substances, phototrophic biofilms (also called periphyton⁹) form at SWI and flourish
50 during the tilling and shooting stages (about 1.5-3 months) of rice cultivation.^{1, 4, 8} By
51 assimilating inorganic nitrogen, phototrophic biofilms immobilize nitrogen in biomass
52 and effectively intercept *in-situ* nitrogen.¹

53 Studies have reported that phototrophic biofilms can accumulate up to 70 mg N/g
54 biomass,¹⁰ and that this nitrogen can be released into the sediment/soil in inorganic
55 forms when biomass gets mineralized.⁸ Reynaud and Roger¹¹ reported that in one
56 growth season, algal biomass accumulated in one ha of paddy fields ranged between
57 several hundred kg to more than one ton. Thus, in one ha paddy fields, phototrophic
58 biofilms may immobilize dozens of kilograms of nitrogen. In many places farmers build
59 ecological ditches or buffer strips to trap and filter nutrients from runoff.¹² Hence, even
60 though the paddy phototrophic biofilms may get washed away during heavy rains,
61 nitrogen immobilized in biofilm biomass is easier to intercept by ecological ditches than
62 nitrogen dissolved in water. Moreover, drainage and drying in paddy fields can prompt
63 the withering and decay of phototrophic biofilms¹, with consequent nitrogen release
64 into soil to supply to rice plants. However, the contribution of phototrophic biofilms to
65 *in-situ* nitrogen interception in paddy fields has been largely overlooked in the past.¹

66 Function and functionality of a community depend largely on its composition, and
67 due to complementarity effects, especially on its diversity.¹³⁻¹⁵ Assimilation is the main
68 pathway of inorganic nitrogen immobilization by phototrophic biofilms, however, both
69 algae and bacteria have phylogenetic differences in nitrogen uptake capacity for
70 different forms of nitrogen.^{16, 17} In soil, nitrogen exists in various forms and species-
71 rich communities have advantages in immobilizing different forms of nitrogen.^{15, 18}

72 Given the continuously changing physiochemical conditions at SWI, the community
73 structure and metabolic activities of phototrophic biofilms may vary greatly, resulting
74 in changes in biofilm growth and nitrogen immobilization capacity.¹⁹ However, current
75 studies on phototrophic biofilms mainly focus on their applications in nutrient removal
76 from wastewater, where they grow on artificial substrates and carbon and nutrient
77 exchanges with soil are absent.²⁰⁻²² Hence, the effects of soil physiochemical conditions
78 on the community structure and photosynthesis of phototrophic biofilm, and its
79 contribution to nitrogen interception in paddy fields deserve further studies.

80 In paddy fields, soil organic carbon (SOC), affects soil texture, water content and
81 pH, and provides essential substrate and nutrients for phototrophic biofilms.²³⁻²⁵
82 Formation of phototrophic biofilms starts with colonization by heterotrophic bacteria
83 and the production of adhesive extracellular polymeric substances (EPS) as a matrix on
84 solid surfaces, subsequently followed by the immigration and adhesion of algae.^{22, 26, 27}
85 During this process, sufficient SOC supply facilitates the colonization of various
86 bacterial species and secretion of polysaccharides, which help different algal cells to
87 adhere to the biofilm matrix and help stabilize the biofilm community with high
88 diversity.^{27, 28} Via this pathway nitrogen immobilization capacity of phototrophic
89 biofilms gets influenced, but the driving effects and underlying mechanisms of SOC
90 still remain largely unknown.^{10, 22} In the complex mixture of components that SOC is,
91 dissolved organic carbon (DOC) such as humic acids, proteins, vitamins and amino
92 acids is the most labile and active fraction with potent effects on the growth of algae
93 and bacteria.^{29, 30} To make full use of phototrophic biofilms for *in-situ* nitrogen retention
94 in paddy soils, the effects of SOC and DOC deserve intensive study, especially in China,
95 where nitrogen is excessively used in paddy fields and retention is paramount.³¹ High-
96 throughput sequencing technologies such as the full-length 16S and 18S rRNA gene
97 amplicon sequencing and metatranscriptomics should enable to precisely identify the
98 functioning species and reveal the potential activities of complex microbial
99 communities and the underlying mechanisms that regulate them in phototrophic
100 biofilms grown under varying SOC conditions in paddy fields.^{32, 33}

101 In this study, we conducted a large-scale field investigation and microcosm
102 experiments to elucidate the effects of SOC on the community characteristics and
103 nitrogen immobilization functions of phototrophic biofilms and the underlying

104 mechanisms. Our research hypothesis was that SOC affects both the community
105 structure and the nitrogen immobilization functions of paddy phototrophic biofilms. We
106 addressed the following three questions: (1) How do the community structure and
107 nitrogen immobilization capacity of phototrophic biofilms vary along SOC gradients?
108 (2) What are the effects of community structure of paddy phototrophic biofilms on
109 nitrogen immobilization? (3) What is the underlying mechanism of the influence of
110 SOC on nitrogen interception by phototrophic biofilms at SWI?

111 **Materials and methods**

112 *Collection and analysis of paddy phototrophic biofilms*

113 To obtain a general relationship between SOC and phototrophic biofilm functions
114 in paddy fields, 20 sites in three of the six main rice planting regions in China³⁴ were
115 selected (Fig. S1). They were South China (region I), the middle and lower reaches of
116 Yangtze River (region II), and Northeast China (region III). Paddy phototrophic
117 biofilms were collected during May to July in 2016 and 2017, depending on the rice
118 transplanting time in the different regions. Samples were collected about 20 days after
119 rice transplanting, at which time the plants were in tillering stage and phototrophic
120 biofilms were thick. Specifically, about 200 g biofilm biomass (wet weight) was peeled
121 off from the submerged soil surface with tweezers and razor knives. Meanwhile, after
122 having first scraped off and removed the attaching phototrophic biofilms from soil,
123 individual samples of about 500 g surface soil (top 0-5 cm) were collected with a shovel.
124 At each site, ten samples were collected from ten rice planting parcels within an area of
125 10 km². After being transported to the laboratory preserved on ice, paddy phototrophic
126 biofilms samples were stored at -20°C and then freeze-dried to measure their nitrogen
127 contents. Soil samples were air-dried under indoor conditions and then crushed and
128 passed through 0.15 mm sieves to measure their contents of soil total organic carbon
129 (SOC) and soil total nitrogen (STN).

130 *Microcosm experiment under varying SOC conditions*

131 To elucidate the mechanism as to how SOC influences the growth and nitrogen
132 accumulation of phototrophic biofilms, a microcosm experiment was conducted.
133 Specifically, surface soil (0-30 cm) collected from a paddy field in Jurong, Jiangsu

134 Province, China was air-dried, crushed and passed through 1 mm sieve for a pot
135 experiment. The characteristics of the paddy soil were as follows: pH 5.8 ± 0.1 , SOC
136 $2.32\pm 0.2\%$, total nitrogen (TN) 1.72 ± 0.12 g/kg, total phosphorus (TP) 0.42 ± 0.04 g/kg,
137 and DOC 272.2 ± 3.4 mg/kg. To obtain a gradient of SOC content, 80 g paddy soil was
138 mixed with 0.4 g, 0.8 g, 1.2 g, 1.6 g, 2.0 g and 2.4 g fermented and crushed peanut shell
139 (organic carbon content of 41%, piece size of 1-2 mm) respectively, designated as
140 treatments A, B, C, D, E and F. A paddy soil without adding peanut shell was set as
141 control (CK); all the treatments were set up in triplicates. The mixture of soil and peanut
142 shell was placed in crystallizing dishes (diameter 90 mm). To provide sufficient nutrient
143 and mimic fertilizer application in paddy fields, NaNO_3 and KH_2PO_4 were diluted in
144 distilled water at concentrations of 2.8 NO_3^- -N mg/L and 0.15 PO_4^{3-} -P mg/L
145 respectively, and in each dish 140 ml of the abovementioned medium was added,
146 resulting in a water layer of 1 cm. To prevent atmospheric deposition, the crystallizing
147 dishes were covered with sealing film with holes for aeration. The microcosm
148 experiment was conducted in a greenhouse in Nanjing, China, and lasted for 30 days
149 during May to July 2020 with temperatures of 18-30°C. To compensate for evaporation,
150 every two days distilled water was added to dishes to keep a water layer of 1 cm.

151 On day 2, soil samples were collected from each dish to measure SOC and DOC.
152 On day 30, dense phototrophic biofilms had formed at SWI and were collected for
153 analysis. The wet biomass of phototrophic biofilms was first weighed and then part of
154 it was quick-frozen with liquid nitrogen and preserved at -80°C for full-length 16S and
155 18S rRNA sequencing and metatranscriptomic analysis. Part of the wet biomass was
156 used to measure EPS contents. The rest of the biomass was freeze-dried to measure the
157 water content and total nitrogen content.

158 *Chemical analyses*

159 SOC and STN contents of soil samples were analyzed using a C/N analyzer
160 (FLASH 2000 NC Analyzer, Thermo Scientific) after removing carbonate in soil by
161 adding 0.1 M HCl. DOC was measured using a TOC analyzer (LCPH, Shimadzu, Japan)
162 after an extraction of 2.5 g dry soil in 15 ml ultra-pure water. Dry weight (biomass) of
163 phototrophic biofilms from the microcosm experiment was obtained by multiplying the
164 wet weight by the moisture content. Nitrogen content of phototrophic biofilm biomass
165 (biomass N) collected from both paddy field and the microcosm experiment was

166 measured using an ICP-OES (ICPOES730, Agilent, U.S.A) by using 20 mg freeze-
167 dried biomass per sample. EPS of phototrophic biofilms was extracted from 200 mg
168 wet biomass using NaOH and polysaccharide and protein contents were determined by
169 anthrone-H₂SO₄ colorimetry and the bicinchoninic acid (BCA) method, respectively³⁵
170 and expressed as mg/g dry biomass by multiplying with the moisture content of each
171 phototrophic biofilm sample.

172 *16S and 18S rRNA amplicon analysis*

173 To determine the community composition of phototrophic biofilms of the
174 microcosm experiment, DNA was extracted from wet biomass using the CTAB/SDS
175 method.³⁶ The primers 27F (AGRGTTYGATYMTGGCTCAG) & 1492R
176 (RGYTACCTTGTTACGACTT) and Euk-A (AACCTGGTTGATCCTGCCAGT) &
177 Euk-B (GATCCTTCTGCAGGTTACCTAC) were used for PCR amplification of
178 full-length 16S rRNA and 18S rRNA genes respectively.³⁷ The PCR reactions were
179 carried out with TransStart® FastPfu DNA Polymerase (TransGen Biotech, Beijing,
180 China). Electrophoresis on 2% agarose gel was performed to detect and remove
181 undesired PCR products. The gels were then extracted with QIAquick Gel Extraction
182 Kit (Qiagen, Venlo, NL) and purified with AMPure PB beads (PacBio, CA,
183 USA). Sequencing libraries were generated using the SMRTbell™ Template Prep Kit
184 (PacBio). Sequencing was performed on the PacBio Sequel platform at Novogene
185 (Tianjin, China). After sequencing, raw sequences were first processed through the
186 PacBio SMRT portal and aligned to each other to generate circular consensus sequences.
187 Then the files were used for amplicon size trimming and sequences smaller than 1340
188 bp or larger than 1640 bp were removed. The reads were compared with the reference
189 database using UCHIME Algorithm to detect and remove chimera sequences.
190 Operational Taxonomic Unit (OTU) classification was performed with a 97% identity
191 cut off using Uparse v7.0.1001. The SSU rRNA Database of Silva Database was used
192 to annotate taxonomic information. In this study, the taxonomic structure compositions
193 of algae and bacteria in phototrophic biofilms were selected to calculate the relative
194 abundance of different groups at each classification level.

195 *Metatranscriptomics analysis*

196 The total RNA was extracted using PowerSoil™ Total RNA Isolation Kit (MoBio)

197 with RNA quantity and quality assessed with the Bioanalyzer 2100 (Agilent). RNA
198 libraries were prepared as rRNA depletion and stranded method. The ribosomal RNA
199 was firstly depleted from total RNA using the rRNA Removal Kit and then fragmented
200 into 250-300 bp fragments and reverse-transcribed into cDNA. The cDNA fragments
201 were amplified using PCR. Then, the library concentration was measured by the Qubit®
202 fluorometer and adjusted to 1 ng/μL. After building the library and pooling of different
203 samples, samples were sequenced on Illumina (PE150) at Novogene Co., LTD.
204 Thereafter, raw reads were processed through in-house perl scripts to get clean reads
205 by removing the poor quality-based and adapter sequences. After that, de novo
206 assembly was performed on the clean reads with Trinity and sequences of all samples
207 were integrated and repeat was deleted using CH-HIT-EST to obtain unigene dataset.

208 In order to obtain comprehensive gene function information, the database KEGG
209 was used for gene function annotations. To quantify the differential expression of genes,
210 the clean reads of each sample were mapped to reference sequence which was spliced
211 by Trinity. Quantification of the transcripts and genes was performed with the
212 Fragments Per Kilobase of transcript sequence per Millions base pairs (FPKM)-
213 normalization method using RSEM. DESeq2 was used for differential expression
214 analysis and the resulting p values were adjusted using Hochberg's approach and genes
215 with $|\log_2(\text{Fold Change})| > 0$ & $p_{\text{adj}} < 0.05$ were assigned as differentially expressed.
216 Moreover, the differentially expressed genes were aligned to the KEGG Pathway
217 Database to identify the enriched pathways, using the hypergeometric distribution
218 model. Then, the proportion of differentially expressed genes mapping to each KO
219 group relative to all KEGG annotated genes at that KO group was calculated, referring
220 to as rich factor.

221 *Statistical analysis*

222 Box plots were employed to show the SOC and biomass nitrogen contents in
223 phototrophic biofilms collected from the 20 sampling sites in China. One-way ANOVA
224 was used to test the differences in SOC, DOC, biomass, biomass N and EPS contents
225 among different treatments by applying a significance level of 0.05 with SPSS 22.0.
226 Using the OTUs, the community diversity of algae and bacteria were separately
227 evaluated by Shannon-Wiener index using the package 'vegan' in R.³⁸ To evaluate the

228 correlations between SOC, DOC, and phototrophic biofilm biomass, nitrogen
229 immobilization capacity, diversity and community composition, Spearman correlation
230 analyses for pairwise complete data were performed using package ‘psych’ in R, and a
231 correlation heatmap was used to visualize the results using package ‘pheatmap’ in R.³⁹
232 To elucidate the effects of SOC and DOC on biomass accumulation and nitrogen
233 immobilization in phototrophic biofilms, a structural equation model (SEM) was set up.
234 Models with different structures were evaluated using χ^2 , p value, and RMSEA. SEM
235 was conducted using the package ‘lavaan’ in R.⁴⁰

236 *Data deposition*

237 Data sets of the phototrophic biofilm amplicon and metatranscriptomics sequences
238 of the microcosm experiment are available through NCBI BioProject accessions
239 PRJNA693353 and PRJNA693642.

240 **Results**

241 *SOC in paddy fields and in situ nitrogen content of phototrophic biofilms*

242 Before zooming in on the effects of SOC on the community structure and nitrogen
243 immobilization functions of paddy phototrophic biofilms we first established the SOC
244 in paddy fields under study, measured the nitrogen contents of phototrophic biofilms in
245 those fields, and tested the working hypothesis that nitrogen content would increase
246 with increasing SOC. SOC levels in the topsoil varied greatly, from 7.6 to 39.1 g/kg
247 (Fig. 1A), while STN contents were 0.3-6.1 g/kg (Fig. S2). The *in-situ* nitrogen contents
248 of paddy phototrophic biofilms varied from 3.0 (± 1.1 S.D.) to 16.0 (± 5.1 S.D.) mg TN/g
249 dry biomass (Fig. 1B). From south to northeast (regions I&III, Fig. S1) in China, the
250 biomass nitrogen contents of paddy phototrophic biofilms showed an increasing trend,
251 while from west to east along the Yangtze River (region II), there was a decreasing
252 trend. Despite the regional difference, the biomass nitrogen content had a significant
253 positive correlation with SOC (Fig. 1C, $r = 0.345$, $p < 0.001$), indicating potential
254 effects of SOC on nitrogen immobilization by phototrophic biofilms at SWI in paddy
255 fields.

256 *Characterizing phototrophic biofilm community and N immobilization at varying SOC*

257 Zooming in on the effects of SOC and the labile fractions DOC on the community
258 structure and the nitrogen interception functions of paddy phototrophic biofilms we
259 then set up a controlled microcosm experiment. In this experiment SOC conditions were
260 shown to have distinct effects on the community compositions of phototrophic biofilms.
261 Generally, diversity of both bacteria and algae increased at elevated SOC levels. The
262 cyanobacterial families *Nostocaceae* and *Leptolyngbyaceae* were abundant in all the
263 treatments (Fig. S4A), yet *Nostocaceae* had higher relative abundance in the six
264 treatments than in the control (CK), while the relative abundance of *Leptolyngbyaceae*
265 decreased with increasing SOC and DOC contents. The bacterial family
266 *Alphaproteobacteria*, had significantly positive correlations with DOC ($r = 0.453$, $p <$
267 0.05) and SOC ($r = 0.557$, $p < 0.01$), while others such as *Chitinophagaceae*,
268 *Planctomycetales*, *Sphingomonadaceae* and *Spirosomaceae* had significant difference
269 between treatments (all $p < 0.05$), but without significant correlations with SOC and
270 DOC (all $p > 0.05$). For algae (Fig. S4B), *Carteria*, *Characiochloris*, *Chlamydomonas*
271 and *Desmodesmus* were abundant genera. DOC and SOC had negative but insignificant
272 correlations with some algae genera including *Desmodesmus*, *Nautococcs*,
273 *Spirosomaceae* and *Chlamydomonas*.

274 With regards to biofilm biomass and nitrogen contents of that biomass (biomass
275 N), both showed an increasing trend with increasing SOC levels (Fig. S3), with the
276 highest biomass and biomass N observed at the highest initial SOC and DOC contents
277 (i.e. treatment F). Moreover, the biomass N had significantly positive correlations with
278 algal diversity (Fig. 2, $r = 0.673$, $p < 0.01$), bacterial diversity ($r = 0.549$, $p < 0.01$),
279 biomass ($r = 0.813$, $p < 0.01$), DOC content ($r = 0.782$, $p < 0.01$), SOC content ($r =$
280 0.617 , $p < 0.01$), polysaccharide contents ($r = 0.564$, $p < 0.01$), protein contents ($r =$
281 0.548 , $p = 0.011$), and the relative abundance of alga *Uronema* ($r = 0.589$, $p < 0.01$).
282 However, the bacteria family *Leptolyngbyaceae* had significantly negative correlation
283 with biomass N ($r = -0.581$, $p < 0.01$). Similar to biomass N, the biomass (represented
284 by dry weight) had significantly positive correlations with DOC ($r = 0.942$, $p < 0.01$),
285 SOC ($r = 0.856$, $p < 0.01$) and protein content ($r = 0.478$, $p = 0.028$). These positive
286 correlations indicated that SOC was important in driving the growth of phototrophic
287 biofilms. Among the various components in SOC, As the bioactive part, DOC most
288 probably had driven the growth of phototrophic biofilms. Besides, the significantly
289 positive correlation between biomass and algal diversity ($r = 0.695$, $p < 0.01$) suggested

290 potential impacts of algal diversity on biomass accumulation.

291 *Ecological effects of SOC and DOC on N immobilization by phototrophic biofilms*

292 Considering the high activity and availability of DOC to phototrophic biofilms,
293 two structural equation models (SEM) were set up to assess the effects of both SOC
294 and DOC on the phototrophic community structure, EPS content, biomass
295 accumulation and nitrogen immobilization (Fig. 3). The DOC model (Fig. 3B) had
296 better performance (e.g. lower χ^2 value and higher p value) and explained more of the
297 variance (60.4%) in nitrogen immobilization than the SOC model (56.8%, Fig. 3A).
298 Specifically, the models indicated that both SOC and DOC had strong and positive
299 effects on biomass (path coefficients: 0.827 and 0.78, both $p < 0.001$), bacterial
300 diversity (path coefficients: 0.269 and 0.35, $p = 0.032$ and 0.029 respectively), with the
301 strongest effects on biomass. DOC also had strong effects on polysaccharide contents
302 (path coefficient: 0.249, $p = 0.043$), while SOC had weak effects. Bacteria are pioneers
303 in the formation of phototrophic biofilms with strong and positive effects of bacterial
304 diversity on polysaccharide and algal diversity in the two models. Algae are the main
305 contributors to biomass accumulation in phototrophic biofilms, and here algal diversity
306 had strong and positive effects on biomass (path coefficient: 0.433, $p < 0.001$) and
307 biomass N (path coefficient: 0.197, $p = 0.042$) in the DOC model. However, in the SOC
308 model algal diversity had weak effects on biomass (path coefficient: 0.136, $p > 0.05$).
309 Similar to the significantly positive correlations between biomass and biomass N (Fig.
310 2), the SEM results verified the strong and positive effects of biomass on nitrogen
311 immobilization in biomass (path coefficients: 0.68 and 0.664, both $p < 0.001$).
312 Polysaccharide, which plays key roles in the initial colonization of phototrophic
313 biofilms, had weak and insignificant effects on algal diversity (path coefficients: 0.054,
314 $p > 0.05$) and biomass nitrogen contents (path coefficients: 0.04, $p > 0.05$) of the mature
315 phototrophic biofilms in the two models. However, polysaccharide had stronger effects
316 on biomass in the SOC model (path coefficient: 0.25, $p < 0.05$) than the DOC model
317 (path coefficient: 0.061, $p > 0.05$). Overall, SOC especially DOC had impacts on
318 biomass accumulation and nitrogen immobilization of phototrophic biofilms via
319 influencing the community.

320 *Metatranscriptomics of phototrophic biofilms along SOC gradients*

321 Differentially expressed genes of phototrophic biofilms from the six treatments
322 compared to the control were annotated with KEGG orthology (KO) allowing the
323 relative expression of KO gene families within a KEGG module to be examined.
324 Proportion of the differentially expressed gene numbers of KEGG modules to the total
325 KEGG annotated gene numbers yielded the rich factor, which highlighted the
326 differences between phototrophic biofilms cultivated under varying SOC and DOC
327 conditions. Overall, the rich factor at low SOC and DOC levels was lower than that at
328 high SOC or DOC levels (Fig. 4). Photosynthesis antenna proteins and photosynthesis
329 were the two KEGG modules for which the different expression increased most under
330 conditions of increased SOC and DOC, and expression of these two pathways peaked
331 at treatment F, representing about 10% and 6% of mapped KEGG genes respectively.
332 In addition, other pathways including ribosome, protein export, carbon fixation in
333 photosynthetic organisms and nitrogen metabolism were also differentially expressed.
334 As indicated by the color in Fig. 4, the rich factors of photosynthesis antenna proteins,
335 photosynthesis and carbon fixation increased with increasing SOC and DOC contents.
336 For instance, the rich factor of photosynthesis antenna proteins increased from 0.061 in
337 treatment A to 0.108 in F. For photosynthesis, the rich factor was 0.043 in treatment A,
338 while it was 0.056 in treatment F. For carbon fixation in photosynthetic organisms, the
339 rich factor increased from 0 in treatment A to 0.024 in F.

340 Plasticity in the KEGG module expression patterns, especially the expression of
341 photosynthesis and carbon fixation, suggested that SOC or DOC enrichment had
342 elevated growth rate of phototrophic biofilms. This was in accordance with the biofilm
343 biomass observed under varying SOC and DOC conditions (Fig. S3E). Another
344 significantly expressed KEGG module related to growth rate was ribosome, with a rich
345 factor of 2.5-3.4% in A-F, further verifying the increase in biomass accumulation under
346 high SOC and DOC conditions. Besides the growth-related modules, nitrogen
347 metabolism KEGG module was differentially expressed (1.1-2.1%), indicating
348 potential effects of SOC or DOC on nitrogen metabolism of phototrophic biofilms.

349 To elucidate the effects of SOC and DOC on growth and nitrogen immobilization
350 in more detail, KO gene families related to photosynthesis and nitrogen metabolism
351 were further examined (Figs. 5 and 6). The relative expression of these individual KO
352 gene families varied greatly, but the enzymes related to a pathway exhibited a similar,

353 apparently coordinated response. For example, the different expressions of enzymes for
354 key processes in PSII (e.g. *PsbA*, *PsbD*, *PsbC* and *PsbB*), PSI (e.g. *PsaA* and *PsaB*),
355 and photosynthesis antenna proteins (e.g. *ApcA*, *ApcE*, *CpcB*, *CpcC* and *CpcG*) all
356 tended to increase as SOC and DOC levels increased (Fig. 5). For nitrogen
357 immobilization, the distinctly expressed KO gene families were mainly *GLT1* and *GLU*
358 in glutamate synthase (Fig. 6), with larger rich factors at high SOC and DOC levels.
359 This affirmed the potent effects of SOC and DOC on inorganic nitrogen immobilization
360 especially the transformation of ammonia to L-Glutamate.

361 Discussion

362 Nitrogen immobilization by phototrophic biofilms is a complex process being
363 affected by a mixture of factors including the structure and the physiological status of
364 the community, and the nutrient supply.^{22, 41, 42} Here, we answered three questions
365 aimed at elucidating the effects of SOC on nitrogen immobilization by phototrophic
366 biofilms on paddy soils and identifying the pathways behind this immobilization, and
367 thereafter proposing regulating measures for increasing the *in-situ* nitrogen interception
368 by phototrophic biofilms. Based on analyses of phototrophic biofilms collected in
369 paddy fields with different levels of SOC and on microcosm experiments, we present
370 the first empirical evidence that *in-situ* SOC level is central to influencing the
371 community structure, photosynthesis and ultimately inorganic nitrogen immobilization
372 by phototrophic biofilms in paddy fields. Our results show that at high SOC, and
373 especially at high DOC levels, biofilm biomass and nitrogen immobilization were
374 linked with high community diversity and distinct expression of photosynthesis and
375 nitrogen assimilation related gene groups (Figs. 4-6).

376 Species diversity is key in influencing the functioning of a community or
377 ecosystem, and a common pattern is that biomass production increases with diversity.⁴³⁻
378 ⁴⁵ Due to the resource partitioning of nutrients by different species, the species-rich
379 community has higher capacity of completely using different forms of nutrients, with
380 biotic feedbacks on community functions.^{14, 15, 46} In this study, our microcosm
381 experiment verified that both biomass production and nitrogen immobilization
382 increased with algal diversity. Algae, the main contributor to biomass yield and nitrogen
383 immobilization in phototrophic biofilms, mainly take up inorganic nitrogen including
384 nitrate and ammonia.^{20, 47} However, in soil/sediment more than 90% nitrogen exists in

385 organic forms, such as amino acid nitrogen, amino sugar nitrogen, hydrolysable
386 unidentified nitrogen and non-hydrolysable nitrogen, which can't be directly
387 assimilated by algae.⁴⁸ Mineralization of organic matter by bacteria is the main way
388 through which organic nitrogen is transformed into inorganic nitrogen bioavailable to
389 algae.⁴⁹ In other words, the complementarity of species-rich bacteria community must
390 have facilitated the mineralization of organic nitrogen in soil.⁵⁰ That should be part of
391 the reason that bacterial diversity had positive effects on algal diversity.

392 For algae, nitrogen immobilization is an active transport process with a high
393 demand on ATP production, which is related to photosynthesis.⁴⁷ In return, the
394 increased uptake of nitrogen by algae prompts algal growth and photosynthesis. [Ross
395 et al.](#)¹⁷ reported that high nitrogen uptake by filamentous algae *Cladophora parriaudii*
396 and *Cladophora coelothrix* was followed by high daily growth rate and yielded biomass
397 rich in carbohydrates. Here, we found distinctly different expressions of many KO
398 groups in photosynthesis at high SOC levels (Fig. 5), indicating potent effects of SOC
399 enrichment on triggering photosynthesis. Besides, the KO group of glutamate synthesis
400 was distinctly expressed at high SOC levels (Fig. 6), indicating the effects of SOC on
401 inorganic nitrogen assimilation of phototrophic biofilms. In summary, photosynthesis,
402 biomass accumulation and nitrogen immobilization of phototrophic biofilms are
403 complementary to each other, closely related to SOC levels.

404 In soil, organic matter is the substrate and energy source for growth of
405 heterotrophic bacteria and nitrogen mineralization.^{31, 50} Here bacterial diversity had
406 significant and positive correlations with the SOC and DOC contents (Fig. 2), implying
407 that sufficient SOC supply especially DOC facilitated the formation of species-rich
408 community. In return, species-rich bacterial community releases more inorganic
409 nitrogen into soil via mineralization, with consequent effects on enhancing inorganic
410 nitrogen assimilation by algae. This was consistent with the study of [Berthrong et al.](#)⁵¹
411 that green manure treated soil (SOC enrichment) had higher soil microbial diversity,
412 accumulated more nitrogen in microbial biomass than the control (12.8 vs. 10.5 $\mu\text{g N/g}$
413 dry soil), and also mineralized twice as much nitrogen as the control (44 vs. 23 $\mu\text{g N/g}$
414 dry soil). By elucidating the cause-effect relationships among SOC, community
415 structure, photosynthesis, and nitrogen metabolism in the controlled microcosm
416 experiments, we verified the positive correlations between SOC and nitrogen contents

417 in phototrophic biofilms from paddy fields. Overall, the underlying pathways of SOC
418 enrichment triggering nitrogen interception by phototrophic biofilms at SWI is
419 postulated that: (1) SOC especially DOC enrichment facilitates the formation of
420 species-rich community and prompted photosynthesis; (2) the high diversity induced
421 higher biomass accumulation and nitrogen immobilization; (3) the elevated
422 photosynthesis prompted the synthesis of L-Glutamate and thereby the immobilization
423 of inorganic nitrogen.

424 **Supporting information**

425 Sampling sites location and STN contents in Chinese paddy fields, content
426 changes of DOC, SOC, biomass, biomass N, and EPS, community composition of
427 phototrophic biofilms, and the number of reads mapped to reference of the microcosm
428 experiment.

429 **Declaration of Competing Interest**

430 The authors report no declarations of interest.

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580 **Figure legend**

581 **Fig. 1** The box plots of total organic carbon content in soil (SOC, %, A) and total
582 nitrogen contents in paddy phototrophic biofilms biomass (biomass N, % of dry weight,
583 B), C: Correlationship between SOC and nitrogen immobilization capacity of paddy
584 phototrophic biofilms (i.e. biomass N contents) from the 20 sampling sites over China
585 (n = 200, I: South China, II: the middle and lower reaches of Yangtze River, III:
586 Northeast China).

587 **Fig. 2** Correlation heatmap of SOC, DOC and phototrophic biofilm community
588 characteristics including abundant genera and families, diversity, biomass, EPS
589 components and nitrogen contents in biomass in the microcosm experiment. Different
590 color intensities represent Spearman correlation coefficients. Significant correlations
591 are indicated with asterisks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

592 **Fig. 3** The effects of (A) soil organic carbon (SOC) and (B) dissolved organic carbon
593 (DOC) on nitrogen immobilization in phototrophic biofilms using the structural
594 equation model (SEM). The red line represents the significantly effect, and grey line
595 represents insignificant effect. The width of arrows indicates the strength of the causal
596 effects. R^2 represents the proportion of variance explained for each variable. For SOC,
597 $\chi^2 = 5.820$, $p = 0.221$, $df = 3$, $GFI = 0.988$, $RMSEA = 0.112$. For DOC, $\chi^2 = 0.894$, $p =$
598 0.925 , $df = 4$, $GFI = 0.998$, $RMSEA = 0.000$. Numbers above arrows indicates the
599 standard path coefficient. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Bacteria: bacterial
600 diversity; Algae: algal diversity.

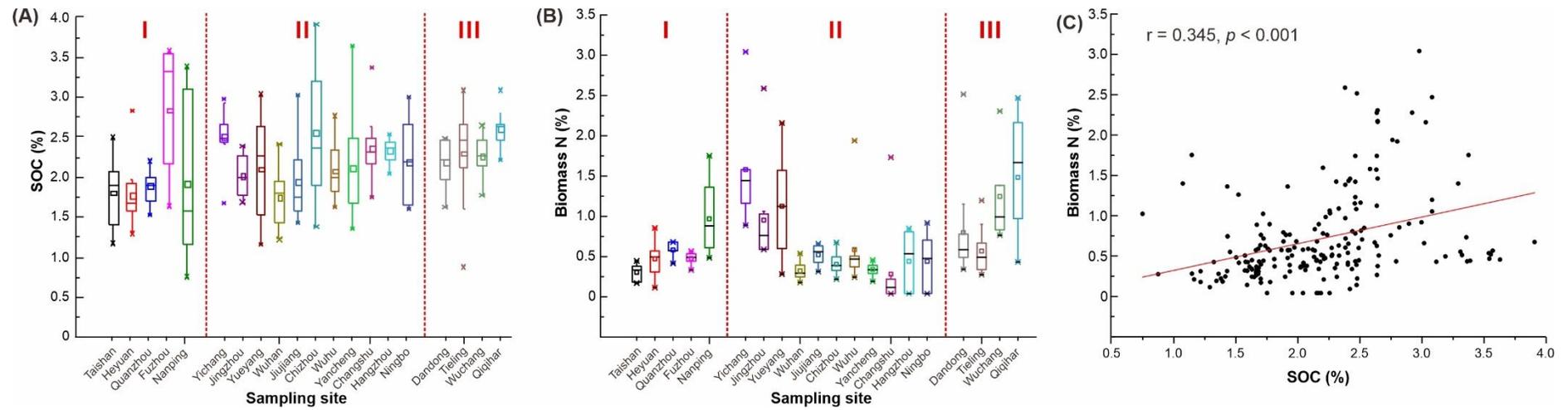
601 **Fig. 4** The enriched KO groups for phototrophic biofilms under varying SOC conditions
602 (Treatments A-F vs. CK). Color indicates the proportion of differentially expressed
603 gene numbers of each KEGG module to the total annotated gene numbers at that KEGG
604 module.

605 **Fig. 5** Schematic model depicting the different expression of KO gene families
606 associated with photosynthesis pathways for phototrophic biofilms under varying SOC
607 conditions (Treatments A-F vs. CK). Color indicates the proportion of differentially
608 expressed gene numbers of each KEGG module relative to the total annotated gene
609 numbers at that KEGG module.

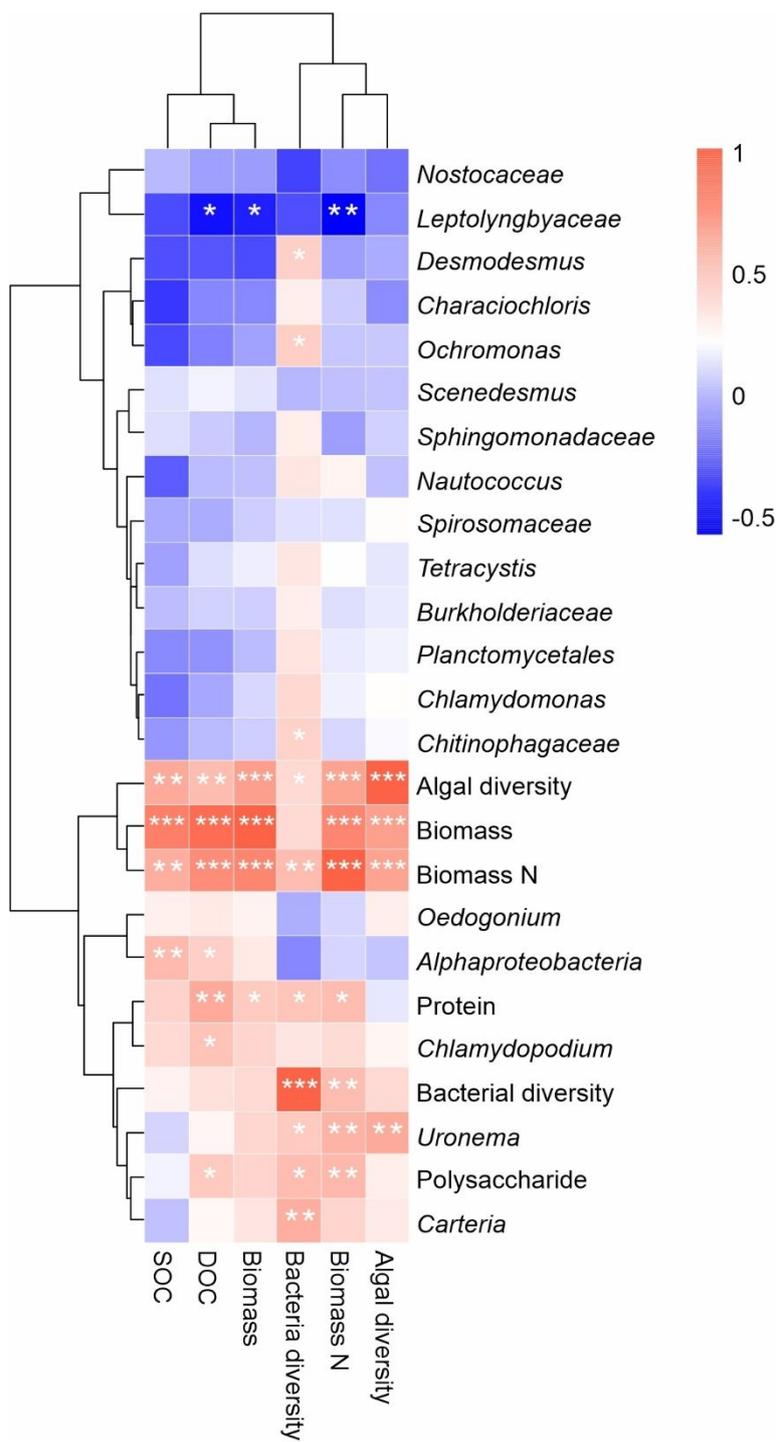
610 **Fig. 6** Schematic model depicting the different expression of KO gene families
611 associated with nitrogen metabolism pathways including nitrate reduction,
612 denitrification, nitrogen fixation, nitrification, and Glutamate synthesis for phototrophic
613 biofilms under varying SOC conditions (Treatments A-F vs. CK). Color indicates the
614 proportion of differentially expressed gene numbers of each KEGG module relative to
615 the total annotated gene numbers at that KEGG module.

616 **Figures**617 **Fig. 1**

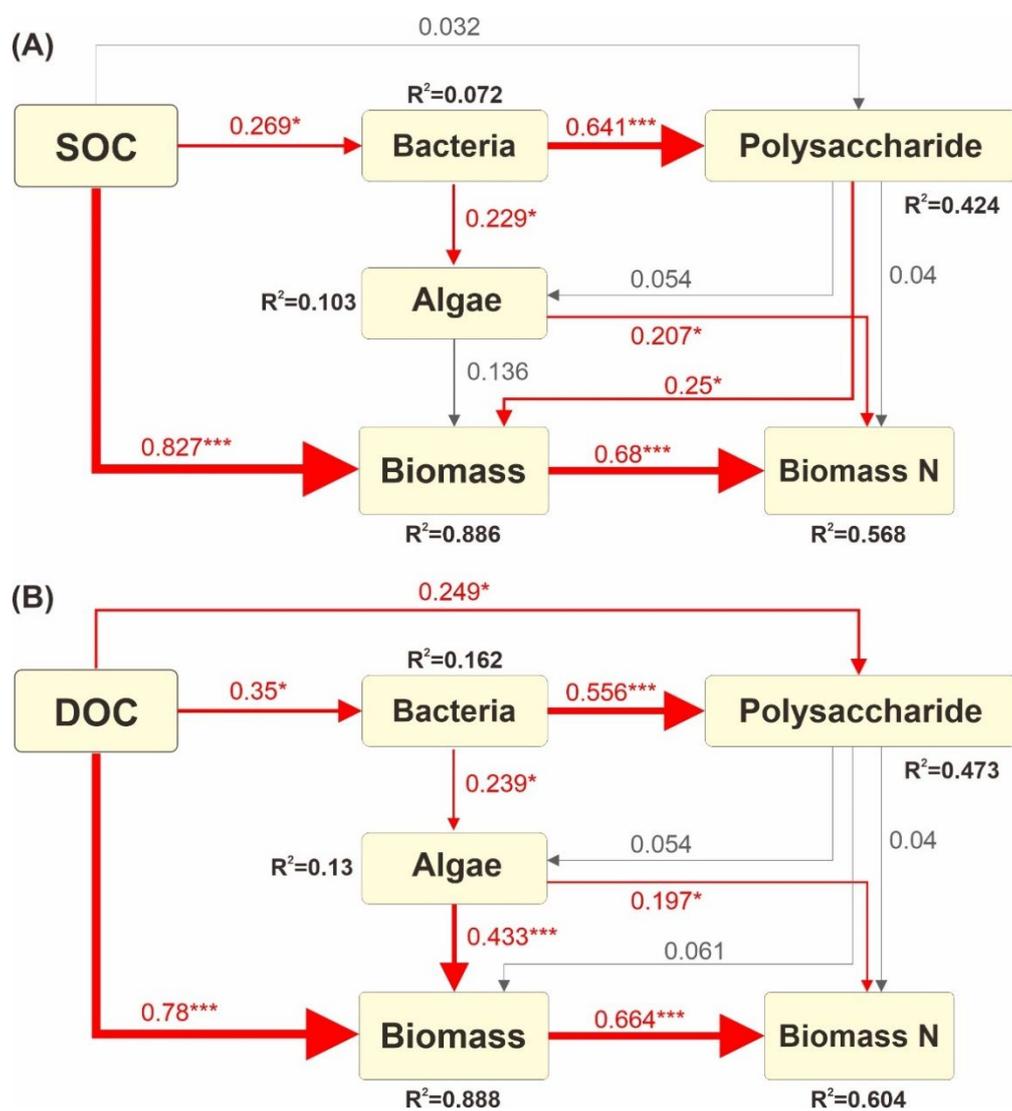
618



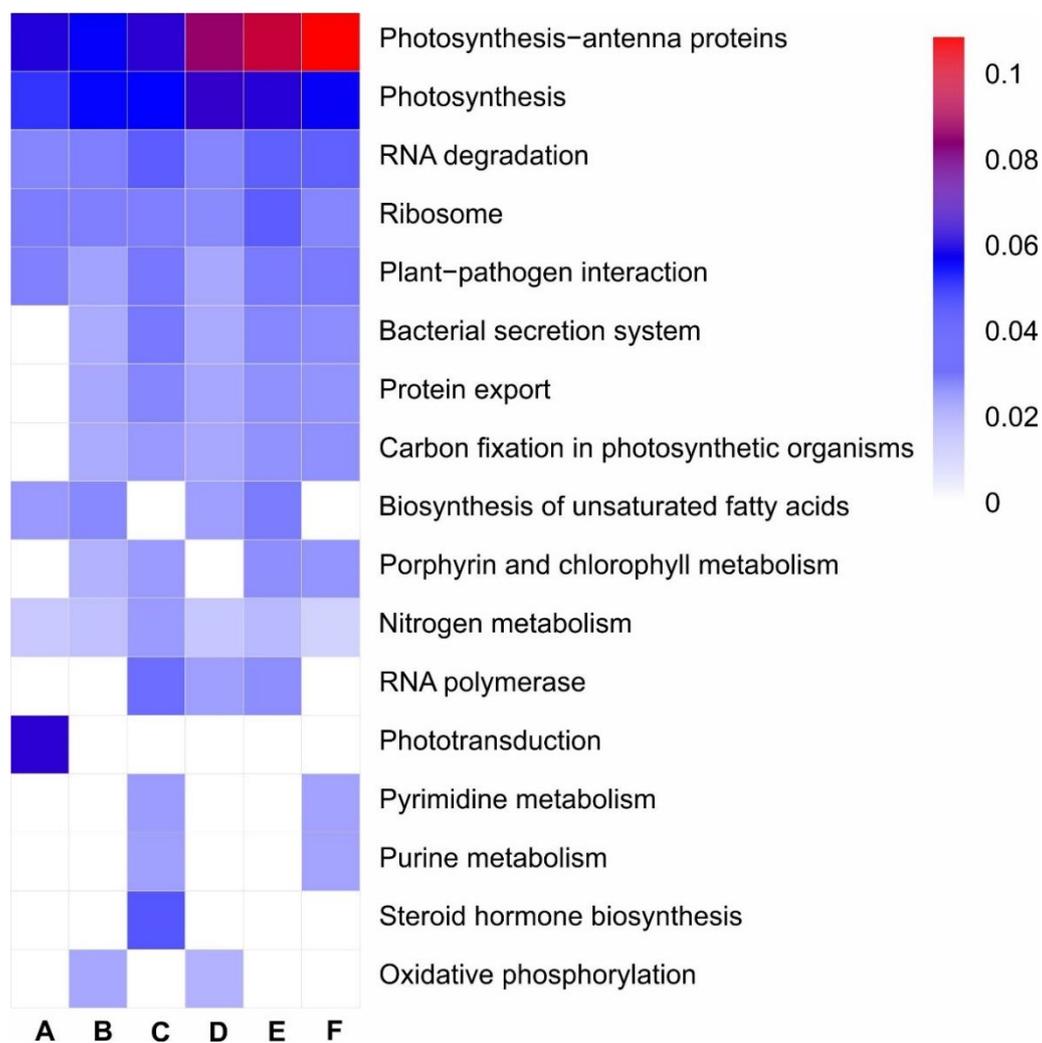
619 **Fig. 2**



620

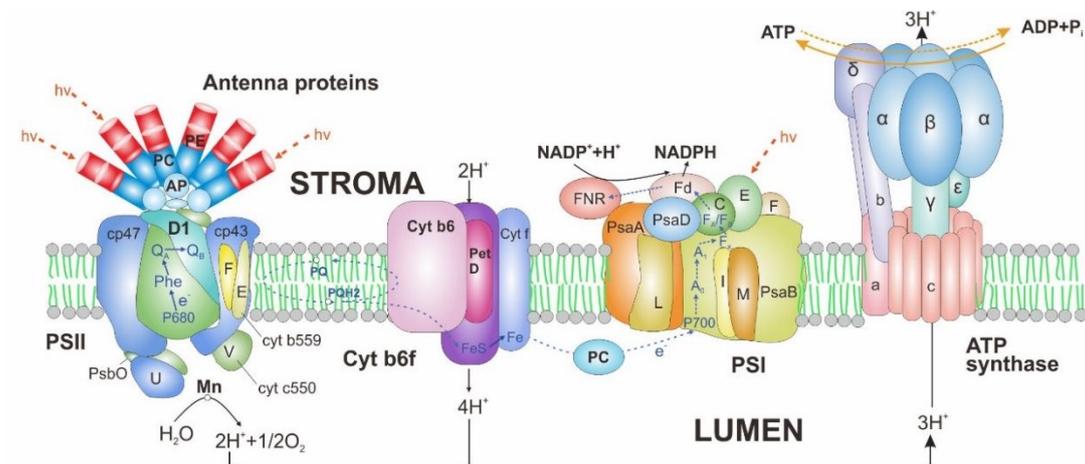
621 **Fig. 3**

622

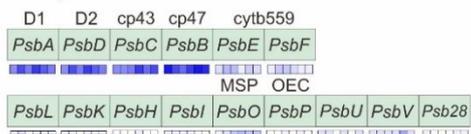
623 **Fig. 4**

624

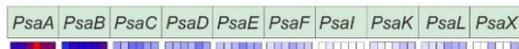
625 **Fig. 5**



Photosystem II



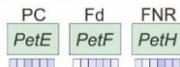
Photosystem I



Cytochrome b6/f complex



Photosynthetic electron transport



F-type ATPase



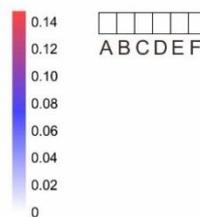
Allophycocyanin (AP)



Phycocyanin (PC)/Phycocerythrin (PEC)



Phycocerythrin (PE)



626

627 **Fig. 6**