Using *Ipomoea aquatic* as an environmental-friendly alternative to *Elodea nuttallii* for the aquaculture of Chinese mitten crab

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Elodea nuttallii (EN) is widely used in Chinese mitten crab (CMC) rearing practice, but it is not a native aquatic plant and cannot endure high temperature. Thus, large EN mortality and water deterioration events could occur during high temperature seasons. The aim of this study was to identify the use of local macrophytes in CMC rearing practice, including *Ipomoea aquatic* (IA) and *Oryza sativa* (OS). A completely randomized field experiment was conducted to investigate the crab yield, water quality, bacterioplankton community and functions in the three different systems (EN, IA, and OS). Average crab yields in the different macrophyte systems did not differ significantly. The IA and OS systems

significantly decreased the TN and NO_3^--N quantities in the outflow waters during the rearing period compared to the EN system, and the IA and OS plants assimilated more nitrogen than the EN plant. Moreover, the significant changes of bacterioplankton abundances and biodiversity in the three systems implied that cleanliness of rearing waters was concomitantly attributed to the differential microbial community and functions. In addition, principle component analysis successfully differentiated the bacterioplankton communities of the three macrophytes systems. Environmental factor fitting and the co-occurrence network analyses indicated that pH was the driver of bacterioplankton community structure. Functional predictions using PICRUSt (v.1.1.3) software based on evolutionary modeling indicated a higher potential for microbial denitrification in the IA and OS systems. Notably, the OS plants stopped growing in the middle of the rearing period. Thus, the IA system rather than the OS system could be a feasible and environmental-friendly alternative to the EN system in CMC rearing practice.

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2 **aquaculture of Chinese mitten crab**

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13 **Abstract**

14 *Elodea nuttallii* (EN) is widely used in Chinese mitten crab (CMC) rearing practice, but it is not

15 a native aquatic plant and cannot endure high temperature. Thus, large EN mortality and water

16 deterioration events could occur during high temperature seasons. The aim of this study was to

17 identify the use of local macrophytes in CMC rearing practice, including *Ipomoea aquatic* (IA)

18   and *Oryza sativa* (OS). A completely randomized field experiment was conducted to investigate

19   the crab yield, water quality, bacterioplankton community and functions in the three different

20   systems (EN, IA, and OS). Average crab yields in the different macrophyte systems did not

21   differ significantly. The IA and OS systems significantly decreased the TN and NO_3^--N

22   quantities in the outflow waters during the rearing period compared to the EN system, and the IA

23   and OS plants assimilated more nitrogen than the EN plant. Moreover, the significant changes of

24   bacterioplankton abundances and biodiversity in the three systems implied that cleanliness of

25   rearing waters was concomitantly attributed to the differential microbial community and

26   functions. In addition, principle component analysis successfully differentiated the

27   bacterioplankton communities of the three macrophytes systems. Environmental factor fitting

28   and the co-occurrence network analyses indicated that pH was the driver of bacterioplankton

29   community structure. Functional predictions using PICRUSt (v.1.1.3) software based on

30   evolutionary modeling indicated a higher potential for microbial denitrification in the IA and OS

31   systems. Notably, the OS plants stopped growing in the middle of the rearing period. Thus, the

32   IA system rather than the OS system could be a feasible and environmental-friendly alternative

33   to the EN system in CMC rearing practice.

34   **Introduction**

35   The Chinese mitten crab (CMC), *Eriocheir sinensis,* is considered an invasive species in

36   Europe and North America (Brodin & Drotz 2014; Hanson & Sytsma 2008), but it is an

37   expensive delicacy in Asia (Chen & Zhang 2007). In 2014, 796,621 tons of farmed Chinese

38 mitten crabs were produced (Zeng et al. 2013); crabs were primarily bred in ponds and lakes

39 (Zeng et al. 2013). The mitten crabs produced in Yangcheng Lake, Suzhou, China, are of high

40 quality and have high economic value (Gu et al. 2013). Most of the crabs produced in

41 Yangcheng Lake are exported to Shanghai, Hong Kong, and high-profit foreign markets.

42 Aquatic plants are required for mitten crab farming. The plants provide shelter for the crabs

43 during exuviation, which is an important part of crab growth (Meng et al. 2013). In addition,

44 aquatic plants assimilate excess nutrients, improve water cleanliness, and absorb solar radiation

45 to maintain cool water temperatures. These properties increase crab growth, yield, and quality

46 (Zhan & Yang 2015).

47 *Elodea nuttallii* (EN), a perennial aquatic plant native to North America, provides these

48 benefits, and is thus widely used in CMC aquaculture (Wang et al. 2016). However, EN cannot

49 withstand high temperatures (Zhan & Yang 2015). In Yangcheng Lake, summer air and water

50 temperatures typically reach 35–40°C and 26–34°C, respectively, which frequently leads to

51 massive EN die off, resulting in serious water quality deterioration (Wu et al. 2016). Under such

52 conditions, crab growth is negatively affected due to loss of shelter for exuviation hiding places

53 and poor water quality (Zhan & Yang 2015). Consequently, alternative aquatic plants are

54 required to facilitate mitten crab aquaculture in areas such as Yangcheng Lake.

55 Local plants that have adapted to local conditions are the best candidates for EN replacement.

56 For example, *Ipomoea aquatica* (IA) is a semiaquatic, tropical/subtropical plant that might be

57 applicable to mitten crab aquaculture (Zhang et al. 2014). IA grows well in shallow waters,

58   withstands high temperatures, and efficiently removes nutrients (e.g., nitrogen and phosphorous)

59   from water bodies (Liu et al. 2007; Wei et al. 2017). Furthermore, the tender shoots and leaves of

60   IA are consumable, and providing additional economic value. Alternatively, *Oryza sativa* (OS) is

61   a submerged grain crop that is common in Asia, and its assimilation ability of nitrogen and

62   phosphorus is similar to IA (Nawaz & Farooq 2017). OS is thus another candidate for EN

63   replacement.

64   Therefore, in this study, we aimed to answer three questions. Which of the two locally adapted

65   plants would most adequately replace EN, similarly improving crab yield and quality? How do

66   the candidate plant systems affect water quality and the associated environmental characteristics?

67   Considering microorganisms play important roles in nutrient conversion in wastewater (Daims et

68   al. 2006), are the two plant systems associated with different bacterioplankton communities that

69   differentially affect water quality? Answers to these questions are of critical importance to crab

70   farmers, and those who are concerned with water quality. Despite the importance of these

71   questions, they remain unanswered. We thus aimed to address the above questions by evaluating

72   candidate aquatic plant systems, and exploring the associated bacterioplankton communities.

73   **Material and methods**

74   **Pond construction, seedling preparation, and floating system construction**

75   All of the experiments were performed in Lianzigang, Suzhou, China (31°27'40.18"N,

76   120°43.5'5.32"E). This region has a subtropical monsoon climate, with an annual average

77 rainfall of 1076.2 mm (http://www.pmsc.cn/). Annual average temperature in Lianzigang is 15–

78 17°C, with high temperatures of 35–40°C in July and August (http://www.pmsc.cn/). All of the

79 experiments were conducted between May 1 and November 30, 2017.

80 The crab-rearing system used here consisted of a water inlet, a water outlet, a micro-porous

81 aeration system, a pond, floating macrophytes, and vertical posts to anchor the macrophytes (Fig.

82 1). The pond, which was mechanically excavated, had a total area of 1,000 m^2 and was 1.5-m

83 deep. The pond was separated into nine sub-ponds with cement walls that were 0.4 m wide and

84 1.5 m high. Each sub-pond had an area of ~100 m^2 and an independent staff gauge. Sub-pond

85 bottoms were left in a natural state to allow crabs to burrow, and to facilitate EN root

86 establishment and growth. IA, OS, and EN were grown in separate sub-ponds with three

87 replicates per plant using a completely randomized design.

88 Sub-ponds were disinfected using sodium hypochlorite. After disinfection, water was pumped

89 into each sub-pond to a depth of ~10 cm. Basal fertilizer (45 kg ha^{-1} of compound-fertilizer;

90 N:P$_2$O$_5$:K$_2$O = 15:15:15) was applied 2–3 days before seedling transplantation.

91 OS and IA seedlings were prepared by sowing seeds on a patented nutritional matrix (0.16 m^2)

92 containing sufficient nutrients for seedling growth. The matrix had sufficient buoyancy to allow

93 seedlings to float on the surface of the water. Seeds were germinated on the matrix, grown for 30

94 days in a greenhouse at 25°C, and then transferred to the bottom of the appropriate sub-ponds.

95 Seedling matrices were arranged side by side until seedling coverage reached 60% of the total

96 water surface area. Additional water was then pumped into each sub-pond to increase the water

97   depth to 20 cm, and seedlings were allowed to re-establish growth for 5–7 days. Upon growth re-

98   establishment, water was again gradually and gently pumped into the sub-ponds to increase

99   water depth to 1.0 m. At this depth, all of the seedlings were floating. Seedlings were fixed in

100 place using ropes attached to buoys, which were fastened to posts in the sides of the cement

101 dividers. EN seedlings were directly transplanted from other ponds. EN cluster spacing was 0.5

102 m × 0.5 m, with forty seedlings per cluster. The transplanted EN plants covered ~60% of the

103 total water surface area. Upon growth re-establishment, water was pumped into the EN sub-

104 ponds to a depth of 1.0 m.

105 **Crab pond management, nutrient measurements, and crab yield determination**

106 Crab ponds were managed using standard Chinese mitten crab rearing methods (Zhan & Yang

107 2015). Juvenile crabs, with an average weight of 15 g, were purchased from a local company

108 (Su'an Fishery Co., Ltd., Nantong, China) and added to the experimental sub-ponds at a density

109 of about 12,000 individuals per hectare. Crab feed (bait) was purchased from the Tongwei Group

110 (http://www.tongwei.com/). The nutrient composition of the feed was varied to meet the

111 different needs at each of the growth stages. Crabs were fed twice per day dependent on growth

112 stage, as recommended by the bait manufacturer. Crabs were not dosed with antibiotics or

113 chemical fishery drugs. A micro-porous aeration system was used nightly to ensure sufficient

114 aeration. Approximately 5 cm of water was pumped out of all ponds each week, and replaced

115 with an equal volume of fresh water from Yangcheng Lake (depending on local precipitation).

116 Outflow water samples were taken every 7–10 days during the rearing period, and

117 immediately frozen at -20°C. At the end of the experiment, nutrients in the outflow samples were

118 measured using an auto analyzer (SKALAR SAN++, Breda, the Netherlands); the nutrients

119 measured included total nitrogen (TN), total phosphorous (TP), ammonium-N (NH_4^+-N), nitrate-

120 N (NO_3^--N), and nitrite-N (NO_2^--N). Absolute cumulative nutrient quantity in discharged water

121 was measured by nutrient concentration in discharged water and outflow water quantity

122 estimated by staff gauge. Outflow pH was measured using a WTW portable pH meter (ProfLine

123 3310, Weilheim, Germany). Inlet water samples were taken and measured every month during

124 the rearing period, and average nutrient concentrations were finally calculated. Dissolved oxygen

125 (DO) concentrations were not measured, because preliminary studies identified large spatial and

126 temporal variations in DO concentrations (Fig. S1) due to uncontrollable factors (i.e., air

127 temperature, air pressure, water disturbances, aeration, activities of aquatic organisms, and

128 photosynthesis of plants and algae) (Dai et al. 2013).

129 The biomass yields (TBM) of EN, IA, and OS were estimated by dry matter productivity in

130 unit area (DM) and aquatic plant areas (APA) in each pond. The fresh plant tissues in 1 m^2 area

131 were collected and oven-dried with 3 replicates in each pond at the end of the experiment, and

132 average dry weight in 1 m^2 area was considered as DM. APA was equal to the 60% of total area

133 of each pond. Thus, the TBM could be calculated by the equation: TBM = DM×APA. Trimmed

134 plant tissues were included in the biomass production estimates. As IA and EN has a sprawling

135 growth pattern, it was periodically cut back outside of the rope-restricted area to maintain ~60%

136 coverage to keep constant aquatic plant coverage (Trimming details see Fig. S1-S2). Nutrients

137 assimilated by the plants were calculated based on plant biomass and nutrient concentrations.

138 The tissue-mixed plant samples that were collected in different growth stages were used for

139 nutrient concentration measurement. Table S1 showed the average nutrient concentrations of

140 plants.

141   To assess crab production, mature crabs were harvested using crab traps, and remaining crabs

142 were captured by hand at night, after the pond water was completely drained. Males and females

143 were manually separated and weighed.

144 **Characterization of bacterioplankton communities**

145   As crab rearing is sensitive to high air temperatures (Yuan et al. 2017), the bacterioplankton

146 communities during periods of high temperatures were more interesting. Thus, the

147 bacterioplankton community was assessed on July 2, 2017, when the maximum air temperature

148 reached 37°C. To obtain sufficient bacterioplankton biomass for community profiling via DNA

149 sequencing, we collected 10 L of water at 20 cm depth from each replicate pond. Water samples

150 were filtered through a 0.22-μm polycarbonate membrane (Millipore, Billerica, MA, USA).

151 DNA was extracted from the filtered biomass using a FastDNA Spin Kit for soil (MP bio, Solon,

152 OH, USA), following the manufacturer's protocols. Extracted DNA concentration was

153 determined with a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington,

154 NC, USA); DNA quality was assessed with gel electrophoresis on a 1% agarose gel. The V4

155 hypervariable region of the bacterial 16S rRNA gene was amplified using PCR, with the primers

156 563F (5'-AYTGGGYDTAAAGVG-3') and 802R (5'-TACNVGGGTATCTAATCC-3')

157   (Cardenas et al. 2010), following previously described protocols (Wang et al. 2017). The

158   amplified PCR products were purified using an AxyPrep DNA Gel Extraction Kit (Axygen

159   Biosciences, Union City, CA, USA), and quantified using a QuantiFluor-ST kit (Promega,

160   Madison, WI, USA), following the manufacturer's instructions. Purified amplicons were pooled

161   in equimolar concentrations and sequenced on an Illumina MiSeq platform (Illumina, San Diego,

162   CA, USA) at Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China), following standard

163   protocols.

164   Raw sequence reads were demutiplexed using QIIME (v.1.9.1) (Caporaso et al. 2010a).

165   Barcoding adapters and PCR primers were cleaved using cutadapt (v.1.16) (Martin 2011). Low-

166   quality reads were removed from the dataset with USEARCH10 (Edgar & Flyvbjerg 2015),

167   using the "fastq_filter" command with the parameters maxee=1 and truncqual=15. The

168   remaining paired-end reads were merged using the "fastq_mergepairs" command in

169   USEARCH10 (Edgar & Flyvbjerg 2015). Merged read abundances were normalized across

170   samples by randomly subsampling 28,000 sequences from each sample. A zero-radius OTU

171   (zOTU) table was produced from the sequence reads using the Unoise3 algorithm (Edgar 2018).

172   The taxonomic classification of each zOTU was assigned using the UCLUST algorithm against

173   the Silva (SSU123) 16S rRNA database with default parameters (Caporaso et al. 2010b; Edgar

174   2010).

175   An additional OTU table was generated to predict bacterioplankton functions. The abundance-

176   normalized sequences were clustered at the 97% nucleotide similarity cutoff level into OTUs

177   using the "pick_closed_reference_otus.py" function in QIIME (v.1.9.1) (Caporaso et al. 2010b).

178   OTUs were taxonomically classified using the UCLUST algorithm (Edgar 2010) against the

179   Greengenes (gg_13_5) reference database (DeSantis et al. 2006) with default parameters. The

180   resulting OTU table was analyzed using PICRUSt (v.1.1.3) (scripts "normalize_by_funtion.py",

181   "predict_metagenomes.py", "categorize_by_function.py", and "metagenome_contributions.py")

182   (Langille et al. 2013). The PICRUSt algorithm produced a table of functional Kyoto

183   Encyclopedia of Genes and Genomes (KEGG) orthologs (KOs). To obtain OTU-specific counts

184   of genes (da Fonseca et al. 2019; Fan et al. 2018) associated with nitrification and denitrification,

185   the PICRUSt script "metagenome_contributions.py" with -l option was applied to selected KOs

186   (K00370, K00371, K00374, K02567, K02568, K00368, K15864, K04561, K02305, K00376,

187   K10535, K10944, K10945, and K10946) (Script S1).

188   **Statistical analysis**

189   Statistical analyses were primarily performed in R (v.3.3.2) (Team 2013). To identify

190   significant differences among treatments, the Levene and Kolmogorov-Smirnov tests were used

191   to check the homogeneity of variances and data normality, respectively. One-way ANOVA was

192   used to determine the significance among the treatments, and Tukey's HSD test was then applied

193   for multiple comparisons. If the measurement variable did not meet the normality assumption, a

194   Kruskal-Wallis test was performed instead of one-way ANOVA. We considered $P < 0.05$ to be

195   statistically significant, unless otherwise noted.

196   The bacterioplankton communities were analyzed using the vegan package (Dixon 2009) in R.

197 The matrix of zOTU abundances was transformed prior to distance-based analyses using the

198 "decostand" function with the Hellinger method (Legendre & Gallagher 2001). Principal

199 component analyses (PCAs) were performed using the "rda" function in vegan to visualize

200 differences among the bacterioplankton communities from the different macrophyte systems.

201 Environmental variables were then fitted and projected onto an ordination using "envfit"

202 function in vegan based on 1,000 permutations.

203 To investigate the correlation of microbial taxa and environmental variables, we constructed a

204 co-occurrence network using CoNet (Faust & Raes 2016), based on the zOTU table and the

205 environmental variables. In the co-occurrence analysis, the read count matrix was first filtered,

206 and only those zOTUs with at least seven minimum occurrence values across the nine samples

207 were retained. Pair-wise associations among zOTUs and environmental factors were calculated

208 using the Pearson, Spearman, Kendall, Bray-Curtis, and Kullback-Leibler correlation methods.

209 The initial top and bottom edge numbers were set at 1,000. For each edge and each measure of

210 association, 1,000 permutation scores and 1,000 bootstrap scores were computed. The resultant

211 networks were visualized using Cytoscape (v.3.2.1) (Shannon et al. 2003).

212 **Results**

213 **System performance, crab yield, and water quality**

214 The macrophyte systems floated steadily throughout the whole experiment despite two

215 moderate windstorms. Thus, the floating systems described here were suitable for plant growth

216   on water surfaces. All of the plants grew well initially (between May and August). However, OS

217   began to go to seed (indicating the start of the reproductive stage) in early August, about 25 days

218   earlier than the normal (September, if OS is grown in a field). After early August, OS plants

219   yellowed and the roots darkened. In contrast, IA and EN grew well until the end of the

220   experiment.

221   Crab yields among the three macrophyte systems did not differ significantly. The average

222   yields of male and female crabs were ~740 kg ha^{-1} and ~267 kg ha^{-1}, respectively (Fig. 2).

223   Individual weight is one of the most important factors determining the value of commercial crabs.

224   Here, the individual weight distributions did not differ significantly among plant systems ($P >$

225   0.05). The median weights of male and female crabs were 168 g and 109 g, respectively (Fig. 2).

226   The nitrogen assimilated by IA was estimated to be 118 kg ha^{-1}, based on macrophyte biomass

227   weight and nutrient concentrations. This level of assimilation was 3.5 times that of EN, and 1.5

228   times that of OS (Fig. 3). In contrast, the phosphorous assimilated by IA, EN, and OS did not

229   differ significantly.

230   The average TN, TP, NH_4^+-N and NO_3^--N concentrations of inlet waters in the rearing period

231   were 1.25 ± 0.44 (Mean ± SD) mg L^{-1}, 0.03 ± 0.02 mg L^{-1}, 0.25 ± 0.43 mg L^{-1} and 0.57 ± 0.47

232   mg L^{-1}, respectively (Fig. 4). The TN content in 31.3% of the samples from the EN system

233   exceeded the Environmental Quality Standards for Surface Water (GB3838-2002) limit for type

234   III water. In contrast, the TN content in 9.4% and 15.6% samples from the OS and IA systems,

235   respectively, exceeded the type III water quality limit (GB3838-2002) (Fig. 4). The number of

236   samples that exceeded the TP and NH_4^+-N type III water quality limits did not differ

237   substantially among treatments. No samples exceeded the water quality limit for NO_3^--N (10 mg

238   L^{-1}). Cumulative curve analysis indicated that amount of TP and NH_4^+-N accumulated over the

239   course of the experiment (up to November 27) did not differ significantly among different plant

240   systems. However, the levels of accumulated TN and NO_3^--N were significantly higher in the EN

241   system than in the IA or OS systems (Fig. 5). Average pH was 7.48 in the IA system, 8.16 in the

242   EN system, and 7.9 in the OS system (Fig. 6).

243   **Bacterioplankton   communities   and   predicted   functions   associated   with**

244   **nitrification/denitrification**

245   The abundances of nearly all of the bacterioplankton phyla differed significantly among

246   macrophyte systems (Fig. 7 A). The abundances of *Acidobacteria*, *Chloroflexi*, *Firmicutes*, *β-*

247   *Proteobacteria,* and *δ-Proteobacteria* were significantly higher in the OS and IA systems as

248   compared to the EN system, while the abundances of *Actinobacteria*, *Armatimonadetes,* and *γ-*

249   *Proteobacteria* were significantly lower. OTU-based diversity (α-diversity) was higher in the OS

250   and IA systems than in the EN system (Fig. 7 B), suggesting that bacterioplankton communities

251   were more complex in the OS and IA systems than in the EN system.

252   PCA indicated that the bacterioplankton communities were discrete among the different plants,

253   with the first two axes explaining 83.67% of the total variation (Fig. 8 A). After fitting

254   environmental variables (Table S2) and bacterioplankton community ordination, we found that

255   pH was significantly associated with community differences (Bonferroni-adjusted $P < 0.05$) (Fig.

256   8 A). The co-occurrence network analysis also identified pH as the only environmental factor

257   that co-varied with certain taxa. These pH-correlated taxa included zOTUs belonging to the

258   phyla *Acidobacteria*, *Chloroflexi*, *Firmicutes*, *Proteobacteria,* and *Planctomycetes* (Fig. 8 B).

259   The total average abundance of co-occurring zOTUs was 7.7%, suggesting that pH influenced

260   the relative abundance of the most abundant members of each community.

261   Potential functions of the bacterioplankton communities were predicted using PICRUSt. We

262   focused specifically on functional genes associated with nitrification and denitrification, as these

263   processes are highly related to nitrogen cycling and are likely to affect nitrogen concentration in

264   pond water. Genes associated with all of the steps of denitrification (*napA*, *napB*, *nirK*, *norB*,

265   *norC,* and *nosZ*; (Kanehisa et al. 2017)) were generally more abundant in the IA and/or OS

266   systems than in the EN system. However, the abundances of *narI*, *narG,* and *narH,* which are

267   only involved in the reduction of nitrate to nitrite (Kanehisa et al. 2017), were lower in the IA

268   and/or OS systems (Fig. 9). The abundances of the nitrifying genes *pmoA-amoA*, *pmoB-amoB,*

269   and *pmoC-amoC,* which are involved in the oxidation of ammonia to hydroxylamine (Kanehisa

270   et al. 2017), were significantly higher in the OS system than in the IA and EN systems. The

271   abundance of *hao* was not significantly different among the three macrophyte systems, although

272   the abundances of *narG* and *narH* were lower in the IA and OS systems than in the EN system

273   (Fig. 9).

274   **Discussion**

275   The macrophyte system described here floated steadily throughout the whole experiment. In

276 addition, mitten crab yield and quality did not differ significantly among plant systems (Fig. 2).

277 Thus, our results demonstrated that local plants were a feasible alternative to EN. Indeed, our

278 data indicated that IA was be the best local replacement for EN, as this plant grew well in ponds

279 and provided sufficient shade. In contrast, OS is not a suitable alternative to EN, as it stopped

280 growing in the middle of the experimental period. The mechanisms underlying the different

281 performances of OS and IA were related to the different growth behaviors and nutrient

282 requirements of the two species. OS has several distinctive growth stages, including tillering,

283 heading, ripening and et al. (Zhang et al. 2018), which typically require different levels of

284 nutrients (Fairhurst et al. 2007). For example, during the tillering stage, OS biomass increases

285 much faster than during other stages of OS growth, and consequently requires a more intensive

286 nutrient supply (Nawaz & Farooq 2017). If the nutrient supply needs are not met (i.e., due to low

287 nutrient concentrations in the water), many tillers become non-productive, and the few remaining

288 productive tillers enter the reproductive stage earlier than normal (Nawaz & Farooq 2017). In

289 submerged paddy fields, the intensity of nutrient supply is manipulated by top-dressing fertilizers

290 (Fairhurst et al. 2007). However, this technique cannot be used in crab-rearing ponds because it

291 would pollute the water.

292   Unlike OS, IA does not exhibit obvious physiological differences among growth stages. IA

293 has sprawling growth, with biomass increasing over rearing time (from May to November)

294 (Shaltout et al. 2010). Thus, low concentrations of nutrients in the water are sufficient. We

295 consequently concluded that IA is an attractive macrophytic alternative to EN in crab-rearing

296   ponds.

297   Based on the Chinese national water quality standards (GB3838-2002) (SEPA), 31.3% of the

298   outflow samples from the EN system contained TN levels in excess of limits for type III waters

299   (Fig. 4). Thus, the outflow from ponds using the EN system is likely to pollute the downstream

300   environment. Fewer outflow samples from the IA and OS systems, as compared to the EN

301   system, had TN levels above the type III water quality limits. These data suggest IA or OS

302   systems would generate less environmental pollution than EN systems. This suggestion was

303   reinforced by the absolute cumulative nutrient quantities in outflow samples (Fig. 5), which

304   showed that the TN and NO_3^--N concentrations were significantly higher in the EN system, as

305   compared to the IA and OS systems. Indeed, IA and OS assimilated more nitrogen from the

306   water than did EN (Fig. 3). Thus, it was possible that IA and OS more effectively improved

307   water quality than EN, which is a desirable property for macrophytes grown in crab-rearing

308   ponds. An alternative explanation was that the differences in water quality among plant systems

309   were a result of the activities of macrophyte-specific bacterioplankton communities.

310   Bacterioplankton communities differed significantly among the three macrophyte systems (Fig.

311   6), possibly because of the different root deposits produced by the three plants. Terrestrial and

312   macrophytic plants release unique root exudates that drastically alter bacterioplankton

313   community structure (Baudoin et al. 2003; Casamatta & Wickstrom 2000; Nelson et al. 2013;

314   Tanaka et al. 2012; Zhao et al. 2013). Bacterioplankton community composition might also be

315   regulated by pH, as pH was the only environmental factor that was significantly associated with

316 the relative abundances of specific bacterioplankton taxa (Fig. 8). These results were consistent

317 with previous reports, which indicated that pH affects the community structures of both

318 terrestrial bacteria (Fierer & Jackson 2006; Lauber et al. 2009; Rousk et al. 2010) and

319 bacterioplankton (Ren et al. 2015). Moreover, the significant increases in bacterioplankton

320 diversity with lower water nutrients in the IA and OS treatments (Figs. 5, 7 B) indicated that

321 biodiversity helped improve water quality, as observed in other aquatic systems (Cardinale 2011;

322 Gregoracci et al. 2012).

323   The predicted functions of the bacterioplankton community also differed among the

324 macrophyte systems, particularly those related to nitrification and denitrification. Most

325 denitrifying genes, especially those associated with the reduction of nitrite, nitric oxide, and

326 nitrous oxide, were more abundant in the IA and OS bacterioplankton communities, as compared

327 to the EN bacterioplankton community (Fig. 9). Moreover, abiotic environmental factors usually

328 control the denitrification process, i.e., pH, temperature and organic carbons (OC). The IA and

329 OS systems that could provide labile OC derived from root exudates (Wang et al. 2018; Xu et al.

330 2008; Zhu & Cheng 2011) favored denitrification. Overall, the biotic and abiotic influences on

331 denitrification in the IA and OS systems were thought to be co-occurring, consistent with the

332 conclusion in riparian wetlands (Xiong et al. 2017). In addition, concentrations of NO_3^--N (and

333 TN) were lower in the IA and OS systems than in the EN system. Thus, the IA and OS systems

334 might have a higher denitrification potential than the EN system. Meanwhile, there were no

335 obvious differences in the abundance patterns of nitrification-associated genes among

336   macrophyte systems. The genes responsible for the oxidation of ammonium to hydroxylamine

337   (*pmoA-amoA*, *pmoB-amoB,* and *pmoC-amoC*) were significantly more abundant in the OS

338   system (but not the IA system) as compared to the EN system, but the abundances of these genes

339   were relatively low across all of the plant systems, with only ~2–20 copies per sample. In

340   addition, the gene abundances in nitrification process usually could not accurately predict

341   nitrification potential, which can be affected by other abiotic factors (Francis et al. 2005; Rocca

342   et al. 2015; Yao et al. 2018). Thus, nitrification potential may not vary significantly among these

343   three macrophytes, and further studies are required to validate these coupled nitrification-

344   denitrification processes in future.

345   **Conclusions**

346   EN is routinely cultivated in ponds used for mitten crab aquaculture. However, EN

347   temperature sensitivity often leads to plant deterioration and decreased water quality at high

348   ambient temperatures, negatively affecting crab production. Here, we successfully designed a

349   floating system to support the growth of IA and OS on the surfaces of crab-rearing ponds. We

350   then compared the crab yield, outflow water quality, and bacterioplankton communities among

351   ponds with EN, IA, and OS macrophyte systems. Our results indicated that IA growth behavior

352   was preferable to that of OS. Crab yields did not differ significantly among systems. Moreover,

353   outflow water quality, as indicated by TN and NO_3^--N concentrations, was better in the IA and

354   OS systems than in the EN system, due to the greater nitrogen assimilation of IA and OS as

355   compared to EN. In addition, the microbial communities associated with IA and OS had a greater

356 denitrification potential than the microbial community associated with EN. Thus, our results

357 indicated mitten crabs could be successfully reared using native aquatic plant. Specifically, IA

358 was a suitable and environmental-friendly replacement for EN, but OS was not.

359 **Data Availability**

360 Demultiplexed sequences and metadata are available from the NCBI Sequence Read Archives

361 (SRA) under accession number SRP136316.

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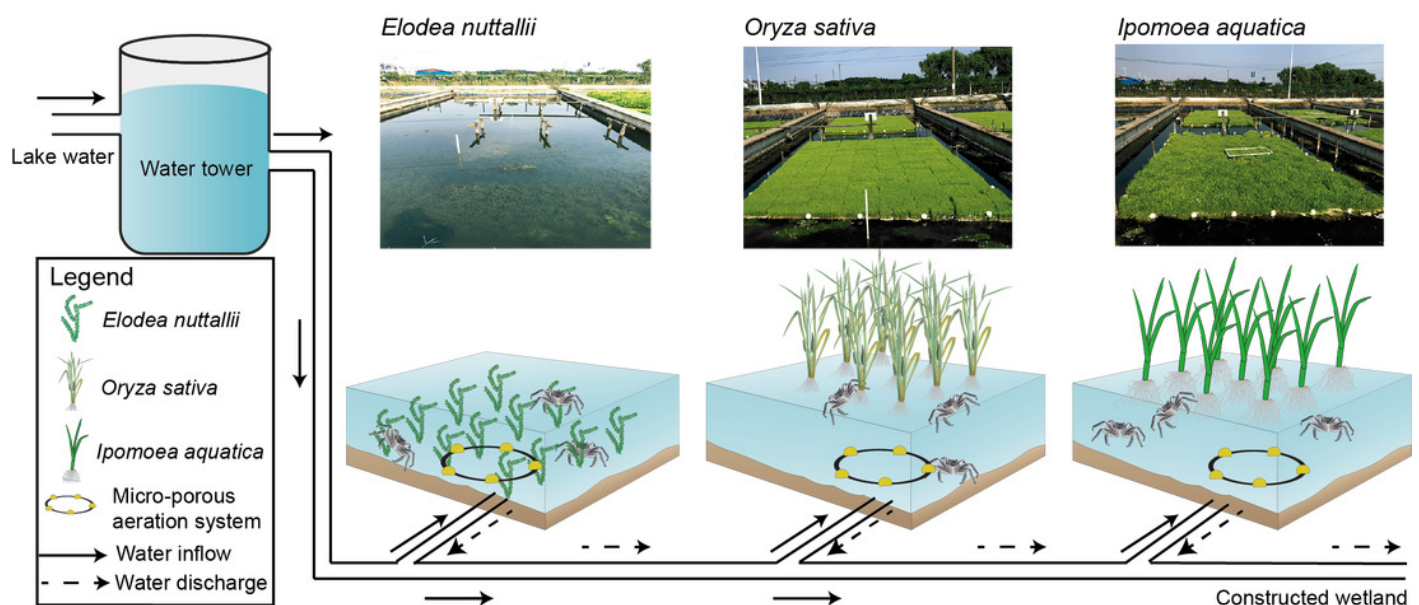
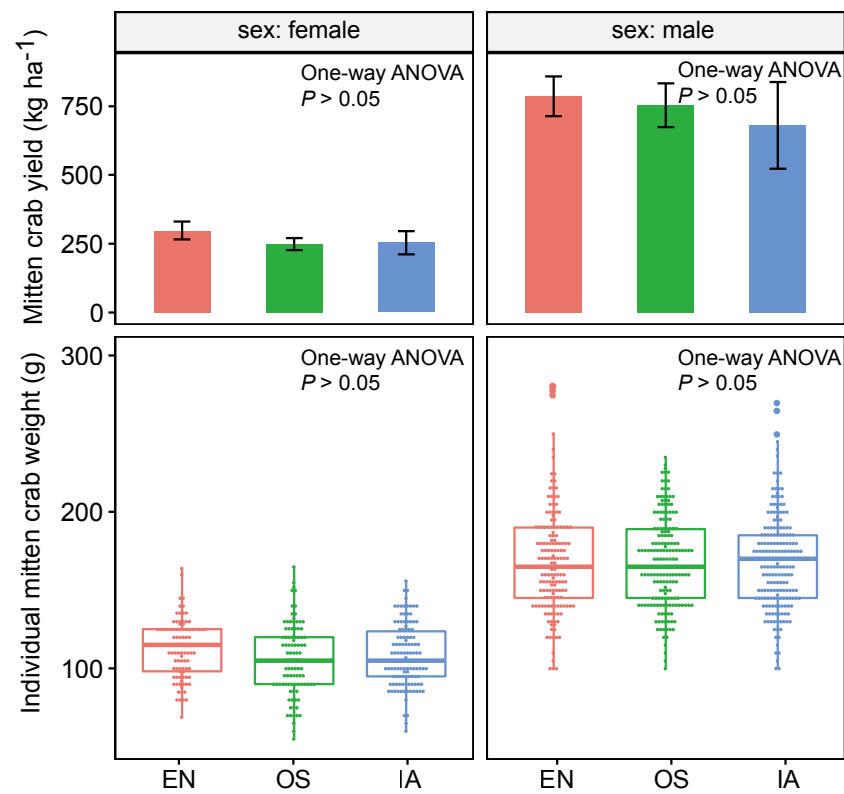
Figure 1

Experimental design and schematics for three macrophyte systems.

Elodea nuttallii (EN), *Oryza sativa* (OS), and *Ipomoea aquatica* (IA). Each macrophyte was planted in a separate sub-pond (three replicate ponds per species). The sub-ponds were connected by a series of PVC pipes. Pond water was replenished from the Yangcheng Lake water, and discharged water flowed into a constructed wetland. The growth of all three macrophytes was restricted to 60% of the pond areas. A micro-porous aeration system was used nightly to ensure sufficient aeration. Photo credit: Linlin Shi.

The yield and individual weight distributions of the Chinese mitten crabs did not differ significantly among macrophyte systems.

Error bars denote the standard deviations of the mean (n=3). EN, *Elodea nuttallii*; OS, *Oryza sativa;* IA, *Ipomoea aquatica*.

Assimilation of nitrogen and phosphorous by *Elodea nuttallii* (EN), *Ipomoea aquatica* (IA), and *Oryza sativa* (OS).

Different letters above bars represent significant differences among plant systems ($P < 0.05$; Tukey's HSD test). Error bars denote the standard deviations of the means (n=3).

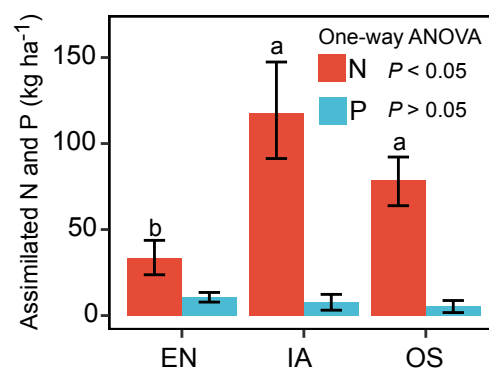



Figure 4 (A-D) separately shows the TN, TP, NH_4^+–N and NO_3^-–N concentrations in the rearing pond.

Error bars denote the standard deviations of the means (n=3). The brown dashed lines represent the average nutrient concentrations in the inlet waters during the rearing period. The grey dashed lines represent the type III water quality limits from the Chinese Environmental Quality Standard for Surface Water (GB3838-2002). NO_3^--N concentrations were all below the type III water quality limit (10 mg L^{-1}). EN, *Elodea nuttallii*; OS, *Oryza sativa*; IA, *Ipomoea aquatica*.

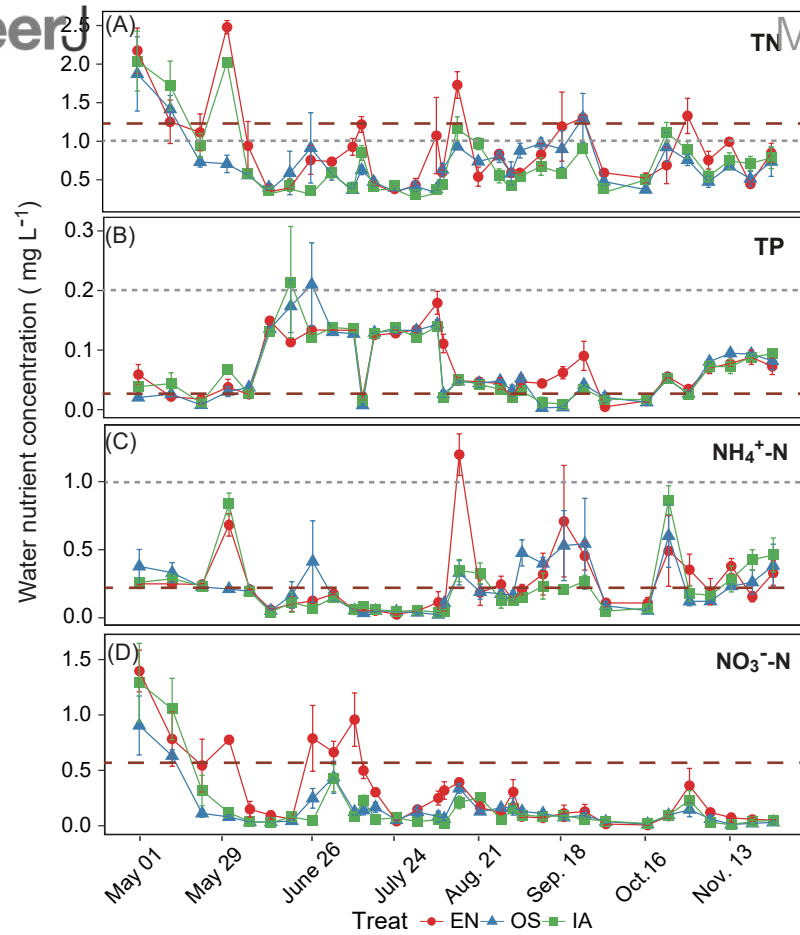
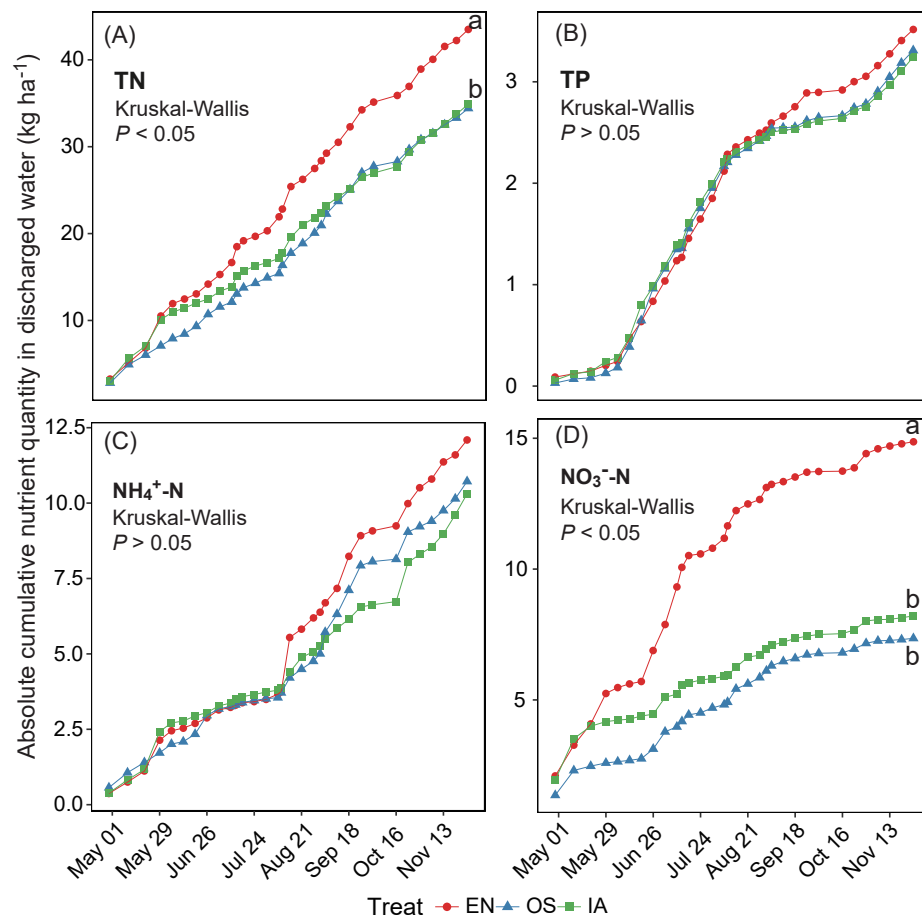
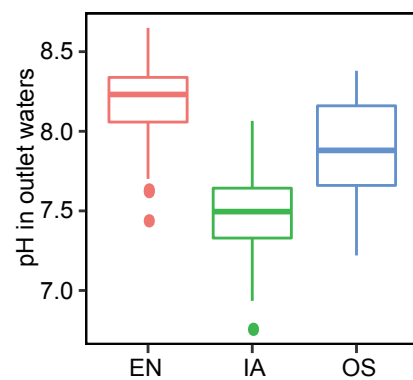
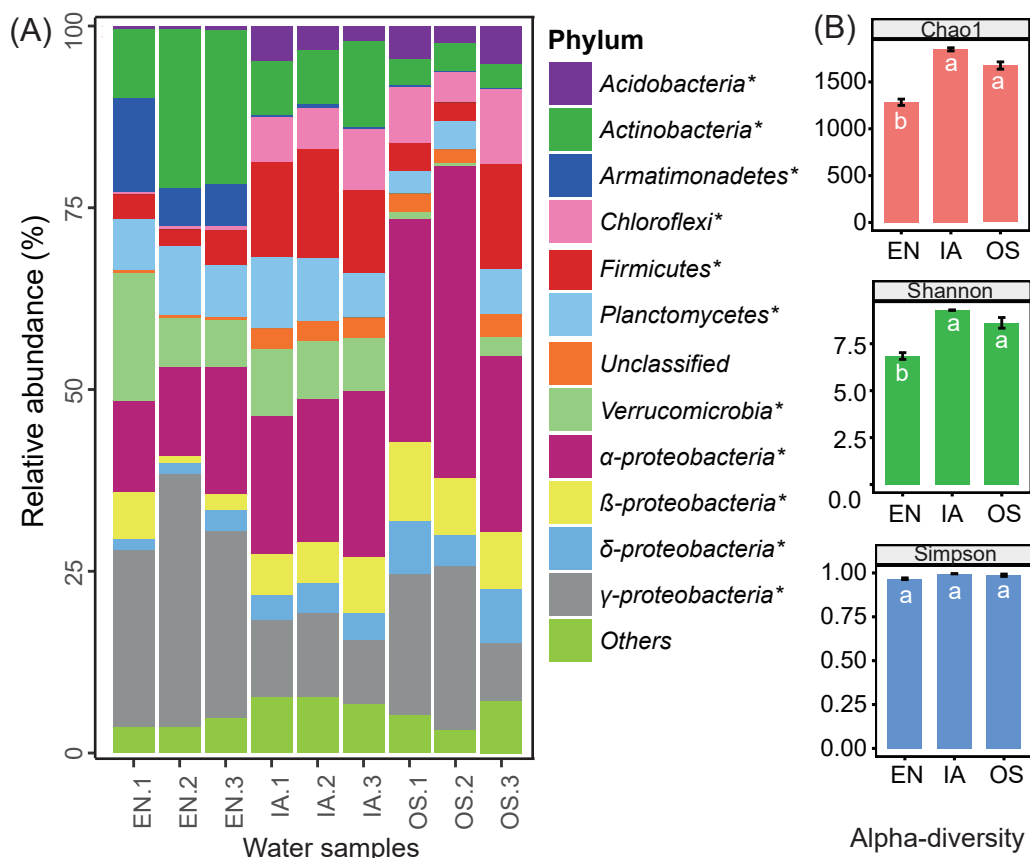
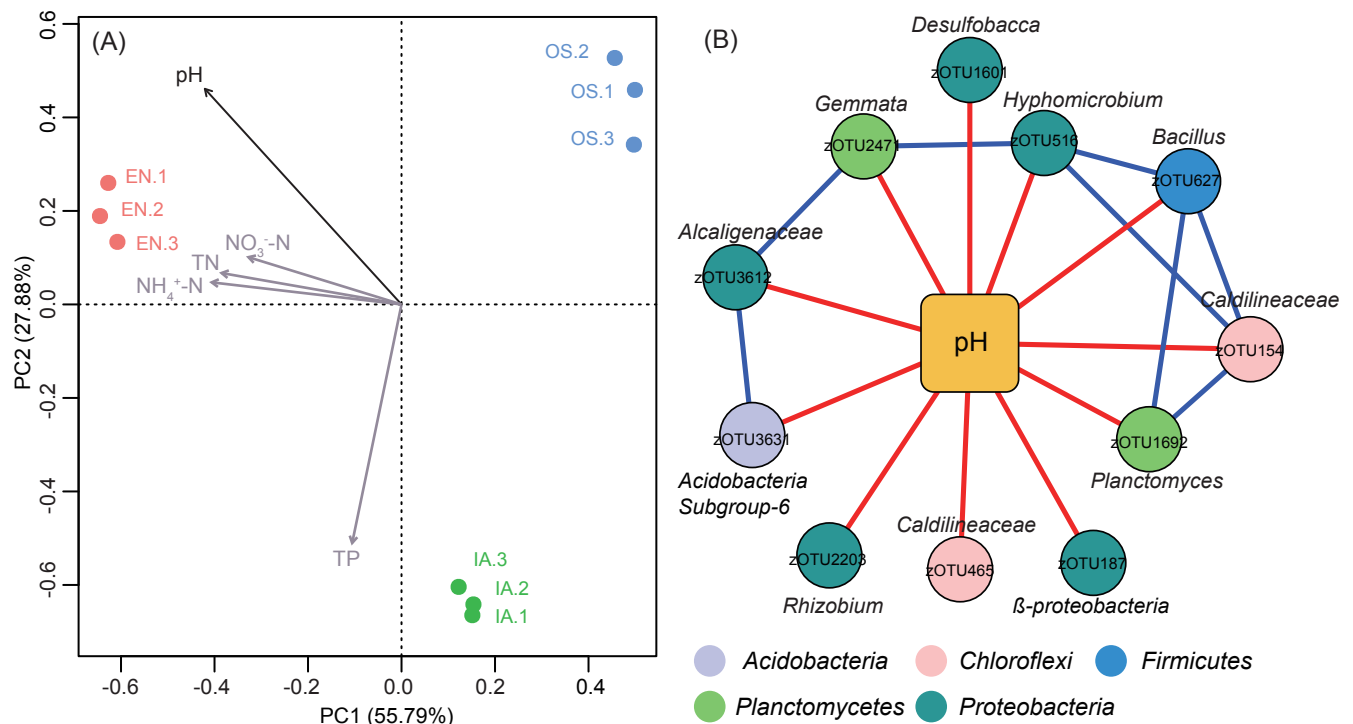



Figure 5 (A-D) separately shows the absolute cumulative TN, TP, NH_4^+–N and NO_3^-–N quantities in rearing period.

The statistical significance of differences in cumulative nutrient quantity among macrophyte systems was determined with a Kruskal-Wallis test (n=3) at the end of the rearing periods (November 27). Different lowercase letters represent significant differences ($P < 0.05$; Tukey's HSD test). EN, *Elodea nuttallii*; OS, *Oryza sativa*; IA, *Ipomoea aquatica*.

pH of the outflow water samples during the rearing period.

EN, *Elodea nuttallii*; OS, *Oryza sativa;* IA, *Ipomoea aquatica*.

Bacterioplankton abundances and diversity indices of the three macrophytes systems.

(A) The relative abundances of the dominant bacterial phyla in the bacterioplankton communities, and (B) the associated alpha diversity. Asterisks indicate significant differences in abundances among the macrophyte systems ($P < 0.05$ level; one-way ANOVA; n=3). Error bars denote the standard errors of the means. Different letters above bars represent significant differences among treatments ($P < 0.05$; Tukey's HSD test; one-way ANOVA; n=3). EN, *Elodea nuttallii*; OS, *Oryza sativa*; IA, *Ipomoea aquatica*.

Bacterioplankton community structure and environmental factors.

(A) Principal component analysis (PCA) of bacterioplankton community composition and environmental variables. The significant environmental variable ($P < 0.05$) is shown with a black arrow; other factors are shown in gray. EN, *Elodea nuttallii*; OS, *Oryza sativa;* IA, *Ipomoea aquatica*. (B) Subnetwork showing the correlation between bacterial taxa and pH. Squared nodes correspond to environmental parameters and circle nodes correspond to zOTUs. Circle nodes not assignable to genus are labeled with the names of higher taxonomic ranks, and node colors represent phyla. Red and blue colors of edge represent negative and positive correlations, respectively.

The abundances of the bacterioplankton genes associated with nitrification and denitrification among macrophyte systems, as predicted by PICRUSt.

(A) and (B) shows the denitrification and nitrification predicted gene abundances, respectively. Error bars denote the standard deviations of the means (n=3). Different lowercase letters above bars represent statistically significant differences ($P < 0.05$; one-way ANOVA followed by Tukey's HSD tests). EN, *Elodea nuttallii*; OS, *Oryza sativa;* IA, *Ipomoea aquatica*.