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Phosphorus forms in the sediment of seagrass meadows affected mainly by fungi rather than bacteria: a preliminary study based on ^{31}P-NMR and high-throughput sequencing

by

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Abstract

Microorganisms play an important role in the circulation of phosphorus (P) in the sediment of coastal wetland ecosystems. In this study, solution ^{31}P nuclear magnetic resonance (NMR) was used to determine different forms of P in the sediments of four different seagrass meadows and a bare tidal flat, while high-throughput 16S and ITS rRNA gene sequencing was used to determine the microbial community composition. The solution ^{31}P-NMR spectra revealed six forms of the P compounds detected by the NaOH-EDTA extraction of sediments, where Ortho-P was the most dominant P compound, followed by Mono-P. The Po compounds were more varied in the seagrass meadow sediments and more abundant compared to the bare tidal flat. Bacterial communities in the sediments collected from *E. acoroides* and fungal communities in the bare tidal flat were relatively different from those at the other sites. The relative abundance of P-cycling-related fungi belonging to the phylum Ascomycota was 26.20% and was much higher than that of bacteria (only 0.29%) belonging to the class Bacilli. Mono-P was the major factor determining the distribution of P-cycling-related fungi and negatively correlated with the relative abundance of *Aspergillus* and *Trichoderma*. We believe that fungi can affect P forms in the sediment of seagrass meadows more than bacteria.

Key words: phosphorus forms, microbial community, solution ^{31}P-NMR, high-throughput sequencing, tropical seagrass sediments

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Introduction

Seagrass meadows provide a wide range of ecosystem services, including coastline stabilization, carbon sequestration, productive fisheries, nutrient cycling and reduction of bacterial pathogens (Brodersen et al. 2017; Ugarelli et al. 2017; Fraser et al. 2018). Phosphorus (P) in seagrass meadows has a pivotal role in several plant processes such as energy transfer, photosynthesis, respiration, enzyme regulation, as well as the synthesis of nucleic acids and membranes. It is estimated that tropical seagrasses require about 60–175 μmol P m^{-2} d^{-1} (Brodersen et al. 2017). P is mainly absorbed from the sediments through rhizomes and roots of seagrasses (McRoy et al. 1972). In tropical sedimentary environments, however, strong fixation of P in predominantly carbonate-rich sediments (Jensen et al. 1998; Nielsen et al. 2007) and adsorption of P to insoluble iron oxyhydroxides lead to strong nutrient limitation (Pagès et al. 2012). Various bound forms of P in the sediments have different bioavailability, which indicates the need for research on different proportions of P present in the sediment and factors affecting them. In addition, seagrasses as higher plants are adapted to the marine environment and their thick root systems can induce transformation and release of inorganic and organic P from the sediment (Yuan et al. 2015). Over the past few decades, P forms and their distribution in marine sediments have been extensively studied (Prasad & Ramanathan 2010; Bramha et al. 2014), but there is little research on P forms in seagrass sediments, which significantly reduces the understanding of the P cycle and P supplement in seagrass meadows, and consequently further reduces the conservation and restoration of declining seagrass beds from year to year.

Nuclear magnetic resonance (NMR) spectroscopy is a non-destructive and non-invasive technique for characterization and quantification of environmental samples without chromatographic separation or other pretreatments (Cade-Menun 2005). Currently, as the most popular method for analyzing P compounds (Cade-Menun 2005; Shinohara et al. 2012; Baldwin 2013; Cade-Menun & Liu 2014), NMR has been widely applied to study P forms in marine sediments (Liu et al. 2009; Shinohara et al. 2012; W. Li et al. 2015; Zhao et al. 2019). However, similar research on seagrass sediments is indeed scarce.

Microorganisms are the key drivers for the biogeochemical cycle of P in the sediments (Tapia-Torres et al. 2016). In natural environments, numerous microorganisms in the soil, sediment and plant rhizosphere effectively release P from the total pool of P through solubilization and mineralization (Bhattacharyya & Jha 2012). Many species of fungi and bacteria are able to solubilize P in vitro and some of them can mobilize P in plants (Zhu et al. 2011). These microorganisms solubilize insoluble inorganic (mineral) P and mineralize insoluble organic P, increasing the bioavailability of insoluble P in the substrate for plants (Sharma et al. 2013). Furthermore, some fungi have been reported to be able to traverse long distances within the soil more easily than bacteria, therefore they can have a greater effect on the solubilization of inorganic phosphate in soil as they typically produce and secrete more acids (Sharma et al. 2013). On the other hand, seagrasses are the only marine angiosperms that have true root systems, which oxygenate the surrounding rhizosphere sediments and create conditions that support higher levels of bacterial diversity compared with the adjacent unvegetated sediments (Garcia-Martinez et al. 2009). Unfortunately, we still lack a clear understanding of how seagrasses affect the microbial community composition in the sediments and how these microbial changes are reflected in the P forms and bioavailability.

In this study, ^{31}P-NMR was used to determine different forms of P in seagrass sediments. High-throughput 16S and ITS rRNA gene sequencing was used to analyze the microbial community composition, especially the P-cycling-related microbial community. There were two primary objectives of this study: (1) to investigate shifts in sediment P forms and microbial community composition via species of seagrasses; (2) to establish whether variables correlate with each other in order to discover how bacteria and fungi drive P cycles in seagrass sediments.

Materials and methods

Study sites and sampling

Xincun Bay is located in the southeast of Hainan Island, with only one narrow channel connected to the South China Sea. The main driving force behind the water flow is the irregular diurnal tide. The area of the bay is 22.5 km^2, with an average water depth of 4.2 m and a tidal range of 0.7 m; more than 3.2 km^2 of the total bay area is allocated for aquaculture. In 2002, seagrasses covered 2.0 km^2 of sand-mud beaches in Xincun Bay (Huang et al. 2006), but since then the meadows have been declining due to high anthropogenic pressure (Yang & Yang 2009). Sediment samples were collected from monospecific meadows of *Enhalus acoroides*, *Thalassia hemprichii*, *Cymodocea rotundata*, *Halophila ovalis* and the bare tidal flat in the intertidal zone of Xincun Bay in March 2018

(Fig. 1). Three plots were selected in each treatment. Five replicates of surface sediment samples were randomly collected in each species plot with a small acrylic plastic corer that penetrated to a depth of 5 cm. After the collection, sediment samples were placed on dry ice and immediately transported to the laboratory within 3 h. Some of them were stored at –20°C for 16S and ITS rRNA gene sequencing and the rest was dried by lyophilization for ^{31}P-NMR analysis.

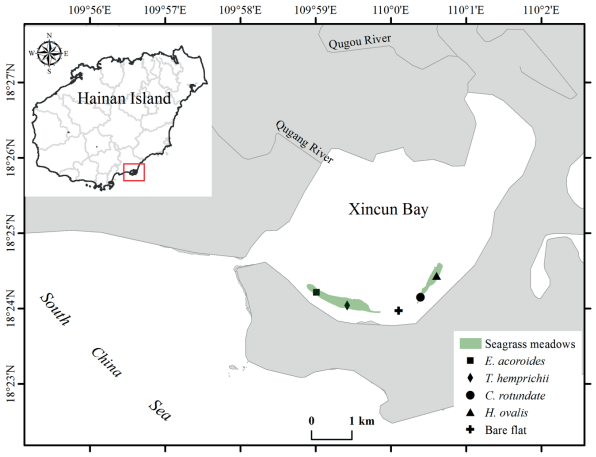

Figure 1

Location of five sampling sites with four monospecific meadows and the bare tidal flat at the intertidal zone of Xincun Bay

^{31}P-NMR analysis with NaOH-EDTA extracts

A 3.0 g sample of lyophilized sediment was extracted in 30 ml mixture of 0.25 mM NaOH and 0.05 mM Na_2EDTA for 16 h while shaking and then centrifugated (10 min, ~ 10 000 × *g*). The supernatants were frozen and freeze-dried. The freeze-dried material in the amount of 0.4 g for each sample was re-dissolved in 0.6 ml D_2O and 0.1 ml of 10 mM NaOH, centrifuged (25 min, 12 000 × *g*) and transferred to a 5 mm NMR tube. The ^{31}P-NMR spectra were obtained using the AVANCE IIITM 500 MHz spectrometer (Bruker) at 202.47 MHz. A pulse of 45°, an acquisition time of 0.68 s, a pulse delay of 4.32, a temperature of 20°C, and ~10 000 scans were used. Solution ^{31}P-NMR spectra were processed with 5 Hz line-broadening on MestReNova Software (version 12.0.0-20080). To obtain peak areas, different P compounds were identified by their chemical shifts by standardizing the orthophosphate peak to 6.0 ppm. Peaks in the raw spectrum with a signal-to-noise ratio exceeding 7 were fitted using the deconvolution method in MestReNova software. The percentage of individual P compounds was calculated as peak areas relative to the sum of all P peak areas based on previous reports (Shinohara et al. 2012; Baldwin 2013; Bramha et al. 2014).

DNA extraction, sequencing and analysis

The steps described below were conducted by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Microbial DNA was extracted from sediment samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's protocols. The final DNA concentration and purification were determined by a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), while DNA quality was checked using 1% agarose gel electrophoresis.

The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by a thermocycler PCR system (GeneAmp 9700, ABI, USA). PCR reactions for each sample were performed in triplicate in 20 µl mixtures containing 4 µl of 5 × FastPfu Buffer, 2 µl of 2.5 mM dNTPs, 0.8 µl of each primer (5 µM), 0.4 µl of FastPfu Polymerase and 10 ng of template DNA. The hypervariable regions of the fungal ITS gene were amplified with primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCG ATGC-3') by the same thermocycler PCR system as above. PCR reactions for each sample were performed in triplicate in 20 µl mixtures containing 2 µl of 10 × Buffer, 2 µl of 2.5 mM dNTPs, 0.8 µl of each primer (5 µM), 0.2 µl of rTaq Polymerase and 10 ng of template DNA. PCR reactions were conducted using the following program: 3 min of denaturation at 95°C, 27 cycles of 30 sat 95°C, 30 s for annealing at 55°C, 45 s for elongation at 72°C, and a final extension at 72°C for 10 min. The resulting PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the standard manual. Purified amplicons were pooled in equimolar ratios and paired-end sequenced (2 × 300) on the Illumina MiSeq platform (Illumina, San Diego, USA).

Processing of the sequencing data: the steps described below were also conducted/operated by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw fastq files were demultiplexed, quality filtered by Trimmomatic and merged by FLASH based on the following criteria: (i) reads were truncated at any site that received an average quality score < 20 over a 50 bp sliding window; (ii) primers were exactly

matched, allowing for a 2-nucleotide mismatch, and reads containing ambiguous bases were removed; (iii) sequences with overlaps longer than 10 bp were merged according to their overlapping sequence. Operational taxonomic units (OTUs) were clustered with a 97% similarity threshold using UPARSE (version7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S and ITSrRNA gene sequence was analyzed by the RDP classifier algorithm (http://rdp.cme.msu.edu/) against the 16S rRNA database (Silva 132/16S bacteria) and the ITS rRNA database (UNITE 8.0/its fungi) using a confidence level of 70% (Liu et al. 2019).

Statistical analysis

Statistical analyses of all parameters were performed using the IBM SPSS statistical software package, version 19 (IBM Corporation, New York, USA). Data from each treatment were analyzed using one-way analysis of variance (ANOVA), and Duncan's multiple range tests ($p < 0.05$) were performed for multiple comparisons. Non-metric multidimensional scaling (NMDS) plots were prepared in R with the vegan package (version 3.0.2). Redundancy analysis (RDA) was performed using CANOCO (version 5.0).

Results

P forms in the sediments of different seagrass meadows

The solution ^{31}P-NMR spectra revealed six forms of the P compound in the NaOH-EDTA extraction of sediments from various seagrass meadows, where orthophosphate (Ortho-P), pyrophosphate (Pyro-P) and polyphosphate (Poly-P) belong to inorganic P (Pi), orthophosphate monoesters (Mono-P), orthophosphate diesters (Di-P) and phosphonates (Phon-P) belong to organic P (Po) (Fig. 2), based on previously reported chemical shifts (Paytan et al. 2003; Cade-Menun 2015). Pi accounted for 61.36% to 93.40% of the TP extracted, much more than that of Po – from 6.60% to 38.64%. In general, Ortho-P accounted for the highest proportion and was followed by Mono-P, while the remaining forms contributed less in our study.

The variety of P compounds varied in the sediments of different seagrass meadows (Fig. 3). In *E. acoroides*, all six P forms were present, whereas in *T. hemprichii, C. rotundata* and *H. ovalis* – two Pi and two Po compounds were found, in the bare tidal flat – all three Pi forms were found, but only one Po

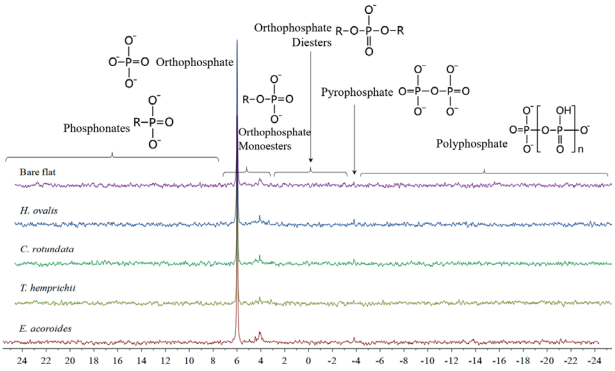

Figure 2

Solution ^{31}P-NMR spectra for NaOH-EDTA extracts of the sediments from different seagrass meadows

compound. The proportion of P forms in NaOH-EDTA extracts of sediments from different seagrass meadows was also very varied (Fig. 3). The *E. acoroides* meadows were characterized by the lowest proportion of Pi, while the content of Po was the highest of all samples – up to 38.64%, with Phon-P accounting for 19.05%. The P form and proportion were similar in *T. hemprichii* and *C. rotundata*. There was no Di-P in *H. ovalis*, but a small amount of Phon-P was found. In the sediment of the bare tidal flat, the proportion of Pi was the highest compared to the sediments with seagrasses, up to 93.40%, especially of Poly-P, which accounted for 10.78%. On the other hand, Mono-P accounted for only 6.6% and was the only Po form detected by ^{31}P-NMR spectroscopy in the sediment of the bare tidal flat. In general, seagrass sediments contained more forms and a higher proportion of Po compared to the bare tidal flat sediment, especially *E. acoroides* sediments.

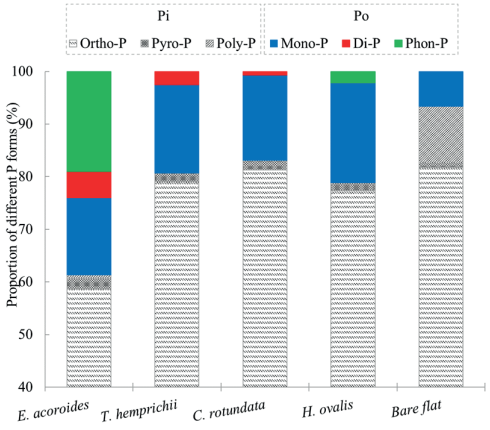

Figure 3

Proportion of P forms in solution ^{31}P-NMR spectra for NAOH-EDTA

Microbial community composition

The analysis of 16S and ITS rRNA gene sequences in 15 samples provided a total of 463 575 and 936 900 valid sequences clustered into 2874 OTUs and 1283 OTUs at 97% sequence similarity, respectively. They were assigned to 53 phyla and 514 genera in the case of bacteria, and seven phyla and 356 genera in the case of fungi. In the case of bacteria, there were 1622 core OTUs in five groups; 51, 5, 20, 6 and 4 unique OTUs were identified in the sediments from *E. acoroides*, *T. hemprichii*, *C. rotundata*, *H. ovalis,* and the bare tidal flat, respectively. In the case of fungi, there were 116 core OTUs in five groups; 197, 120, 151, 107 and 159 unique OTUs were identified in sediments from *E. acoroides*, *T. hemprichii*, *C. rotundata*, *H. ovalis* and the bare tidal flat, respectively. The ratio of the core OTUs of bacteria was 56.44%, which was much higher than that of fungi (9.04%).

Differences in the diversity of bacterial and fungal communities among all samples are presented in Table 1. The richness indices, Ace and Chao, showed significantly the lowest value for the bacterial community in *E. acoroides* sediments (2147, 2142), followed by the bare tidal flat and *H. ovalis* (2392, 2408), while *C. rotundata* (2424, 2414) and *T. hemprichii* (2426, 2428) showed the highest richness values. There was no significant difference in the bacterial Shannon and Simpson indices among the samples. With regard to the fungal community diversity in the sediment, the highest values of the Ace and Chao indices were recorded for *E. acoroides* (303, 303), followed by *H. ovalis* (256, 259) and the bare tidal flat (246, 246). A significant difference was found in the Shannon index and the Simpson index for fungi. The lowest diversity of the fungal community was recorded for *T. hemprichii* (2.82, 0.188), followed by *H. ovalis* (3.87, 0.071) and *E. acoroides* (3.91, 0.064), while *C. rotundata* (4.12, 0.040) and the bare tidal flat (4.37, 0.029) showed higher diversity. NMDS plots based on the confidence intervals ($p < 0.05$) showed no overlap among different sites, which proves that bacterial (stress = 0.105) and fungal communities (stress = 0.087) in the sediment at five sites were different, especially between *E. acoroides* and other sites of bacterial communities, between the bare tidal flat and other sites of fungal communities (Fig. 4). In other words, *E. acoroides* can clearly affect sediment bacterial communities, just as seagrasses can affect sediment fungal communities.

The relative abundance of bacterial (> 1% of the total reads) and fungal phyla under different treatments are shown in Figure 5. The dominant bacterial phyla were Proteobacteria (from 29.11% to 38.62%), Bacteroidetes (15.72% to 22.16%), Firmicutes (8.91% to 13.53%), Chloroflexi (8.21% to 11.92%), Actinobacteria (4.65% to 8.96%), Acidobacteria (3.02% to 3.33%), Latescibacteria (1.86% to 2.44%), Cyanobacteria (0.65% to 2.75%), Verrucomicrobia (1.03% to 2.34%) and Planctomycetes (1.08% to 1.92%; Fig. 5A). According to the Duncan test, the relative abundance of Bacteroidetes and Firmicutes was significantly higher in the bare tidal flat than in the

Table 1

The number of operational taxonomic units (OTUs) and Alpha diversity indices of bacterial and fungal communities. The values are means followed by a standard error. Different letters indicate statistically significant differences ($p < 0.05$) according to the Duncan test.

	Samples	OTUs	Shannon Index	Simpson Index	Ace Index	Chao Index
Bacteria	*E. acoroides*	1832 (124)^{a}	5.72 (0.24)^{a}	0.012 (0.004)^{a}	2147 (104)^{a}	2142 (99)^{a}
	T. hemprichii	2091 (6)^{b}	5.88 (0.22)^{a}	0.011 (0.005)^{a}	2426 (53)^{b}	2428 (59)^{b}
	C. rotundata	2081 (54)^{b}	5.72 (0.39)^{a}	0.019 (0.015)^{a}	2424 (15)^{b}	2414 (48)^{b}
	H. ovalis	2105 (103)^{b}	6.08 (0.11)^{a}	0.007 (0.001)^{a}	2392 (83)^{b}	2408 (95)^{b}
	bare tidal flat	1937 (15)^{a}	5.93 (0.07)^{a}	0.009 (0.001)^{a}	2313 (38)^{b}	2316 (63)^{b}
	F test	7.427	1.233	1.058	9.295	7.555
	p value	0.005	0.357	0.426	0.002	0.005
Fungi	*E. acoroides*	299 (63)^{b}	3.91 (0.45)^{b}	0.064 (0.033)^{a}	303 (65)^{b}	303 (65)^{b}
	T. hemprichii	219 (40)^{ab}	2.82(0.48)^{a}	0.188 (0.093)^{b}	226 (41)^{ab}	231 (43)^{ab}
	C. rotundata	207 (58)^{a}	4.12 (0.41)^{b}	0.040 (0.023)^{a}	211 (58)^{a}	210 (57)^{a}
	H. ovalis	251 (22)^{ab}	3.87 (0.19)^{b}	0.071 (0.007)^{a}	256 (21)^{ab}	259 (26)^{ab}
	bare tidal flat	241 (27)^{ab}	4.37 (0.03)^{b}	0.029 (0.002)^{a}	246 (28)^{ab}	246 (29)^{ab}
	F test	1.907	8.451	5.796	1.810	1.694
	p value	0.186	0.003	0.011	0.203	0.227

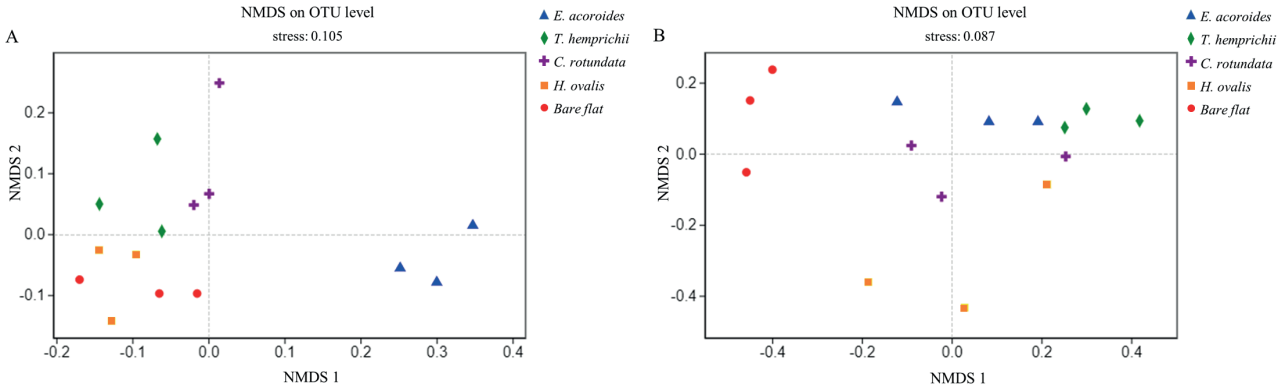

Figure 4

NMDS plots of the sediment bacterial (A) and fungal OTUs (B) based on the unweighted UniFrac metric for all samples

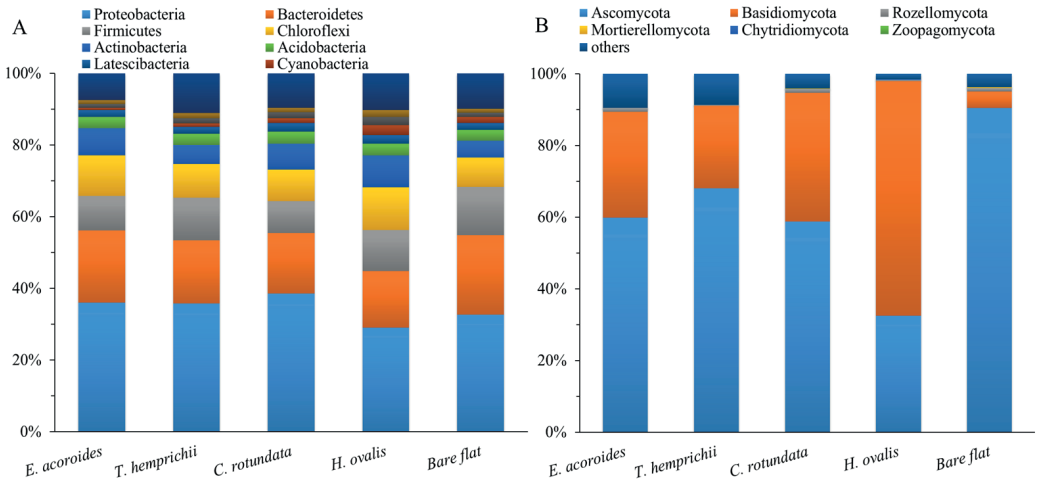

Figure 5

Relative abundance of bacterial (A) and fungal (B) phyla

seagrasses, and there was no significant difference between different seagrasses. The dominant fungal phyla were Ascomycota (32.56% to 90.51%), Basidiomycota (4.57% to 65.40%) and Rozellomycota (0.14% to 1.07%; Fig. 5B). Interestingly, the relative abundance of Ascomycota was significantly the highest, whereas that of Basidiomycota was significantly the lowest in the bare tidal flat. On the other hand, the relative abundance of Ascomycota was significantly the lowest, while that of Basidiomycota was significantly the highest in the sediment of *H. ovalis*.

Previous studies explored and screened P-cycling-related bacteria and fungi (Khan et al. 2007; Sharma et al. 2013; Li et al. 2019). At the genus level, only five bacteria and 15 fungi were screened, and the average percentage was respectively 0.29% of the total bacteria and 26.20% of the total fungi. Figure 6 shows the relative abundance of the top five genus-level P-cycling-related bacteria and fungi, which on average accounted for 100% and 97.97% of the total relative abundance of the P-cycling-related bacteria and fungi, respectively. The relative abundance of P-cycling-related bacteria, ranging from 0.12% to 0.60%, was very low and there was no significant difference between the treatments (Fig. 6A). The top five genus-level P-cycling-related bacteria were *Bacillus, Streptomyces, Staphylococcus, Pseudomonas,* and *Paenibacillus*, which belong mainly to the Bacilli class and only *Streptomyces* belongs to Actinobacteria. Compared with bacteria, the relative abundance of P-cycling-related fungi (15.98% to 39.31%) was higher, with *H. ovalis* and the bare tidal flat having the highest abundance, followed by *E. acoroides* and *C. rotundata*, *T. hemprichii* with the lowest abundance (Fig. 6B). The top five genus-level P-cycling-related fungi were *Cladosporium*, *Penicillium*, *Aspergillus*, *Trichoderma* and *Candida*, belonging to different classes but the

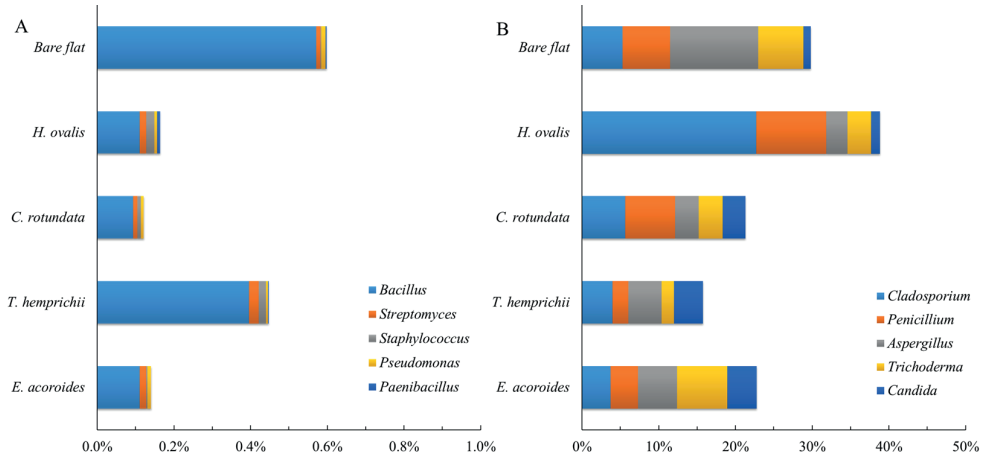

Figure 6

Relative abundance of the top five genus-level P-cycling-related bacteria (A) and fungi (B) detected by ^{31}P-NMR. Blue arrows represent different genera of microbes, while red arrows represent P forms. Correlation between P forms and RDA axes is shown by both the length and angle of the arrows.

same phylum Ascomycota. The dominant fungi were *Cladosporium* and, according to the Duncan test, the relative abundance of *H. ovalis* was significantly higher compared to other treatments. The lowest relative abundance of *Penicillium* was observed in *T. hemprichii*, while the highest in *H. ovalis*, and the difference between them was statistically significant. The highest relative abundance of *Aspergillus* was observed in the bare tidal flat and was significantly higher compared to other treatments. The highest relative abundance of *Trichoderma* was recorded in *E. acoroides*, while the lowest in *T. hemprichii*, and the difference between them was significant. In addition, the highest relative abundance of *Candida* was recorded in *E. acoroides*, and the lowest in the bare tidal flat, and the difference between them was significant.

Relationship between P-cycling-related microbial community composition and P forms

Redundancy analysis and a Monte Carlo permutation test were performed to determine the relationships between the top five genus-level P-cycling-related microbes and the sediment P forms. Visualized through RDA ordination (Fig. 7), all of the P form variables explained statistically 38.31% of the

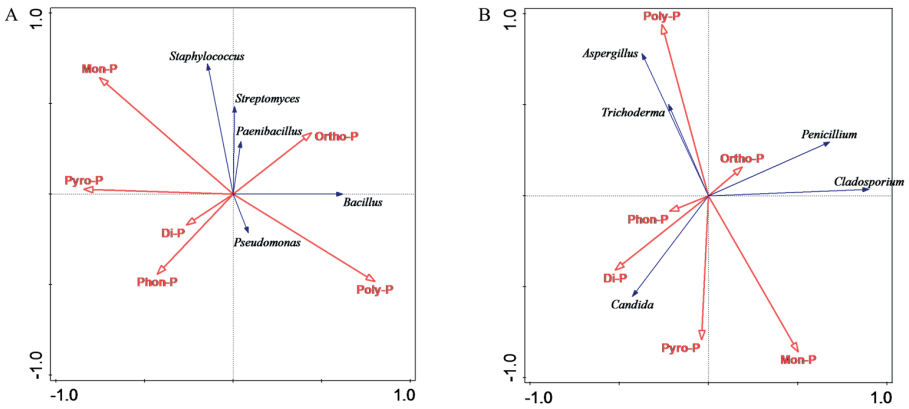

Figure 7

Redundancy analysis (RDA) for the top five genus-level P-cycling-related microbes (A for bacteria and B for fungi) and sediment P forms detected by ^{31}P-NMR. The blue arrows represent different genera of microbes, whereas variables for P forms are shown with red arrows. The correlation between P forms and RDA axes are shown by both the length and angle of the arrows.

bacterial variance, with axis 1 explaining 38.23% of the variance and axis 2 explaining 0.08% (Fig. 7A). One of the issues emerging from these findings is that different forms of P have different effects on the abundance of P-cycling-related bacteria. Of the P form variables considered in this study, the Pyro-P negatively correlated with *Bacillus* and significantly explained the variations of P-cycling-related bacteria ($p < 0.05$), indicating that this was the principal factor affecting the P-cycling-related bacterial communities. All of the P fraction variables explained 73.63% of the fungal variance, with axis 1 explaining 61.90% of the variance and axis 2 explaining 11.73% (Fig. 7B). Mono-P and Di-P were suggested to significantly explain the variability in P-cycling-related fungi composition ($p < 0.05$). Mono-P, the main factor determining the distribution of P-cycling-related fungi, was negatively correlated with *Aspergillus* and *Trichoderma*. Di-P, another important factor, was positively correlated with *Candida* and negatively correlated with *Penicillium* and *Cladosporium*. By comparison, the other factors had no obvious effect on P-cycling-related fungal communities. The main finding of this research is presented in Figure 8.

Discussion

Species-specific differences in P forms in seagrass meadows

To our knowledge, little data has been published so far on P forms using ^{31}P-NMR in seagrass meadows. The results from ^{31}P-NMR spectroscopy showed that Ortho-P was the most dominant P compound in all our samples. The proportion of Ortho-P, accounting for 58.61% to 81.70% in Xincun Bay sediments, was similar to other marine and estuary sediments (Watson et al. 2018; Prüter et al. 2019). The Poly-P signal was not observed by the solution-state NMR spectra in the seagrass meadows, except for *E. acoroides*. Poly-P was considered a labile form of P with a half-life of 0.8 to 2.0 years and could be transformed to Pyro-P or tri-metal-phosphate catalyzed by various metal ions, which consequently was rarely detectable by the solution-state ^{31}P-NMR in sediments (Liu et al. 2009; Ding et al. 2010; Xie et al. 2019). Pyro-P was observed in all samples and its highest percentage was found in the surface sediments of *E. acoroides*, which could indicate high activity of soil fungi in the transformation of Mono-P and Di-P into Pyro-P (Koukol et al. 2008).

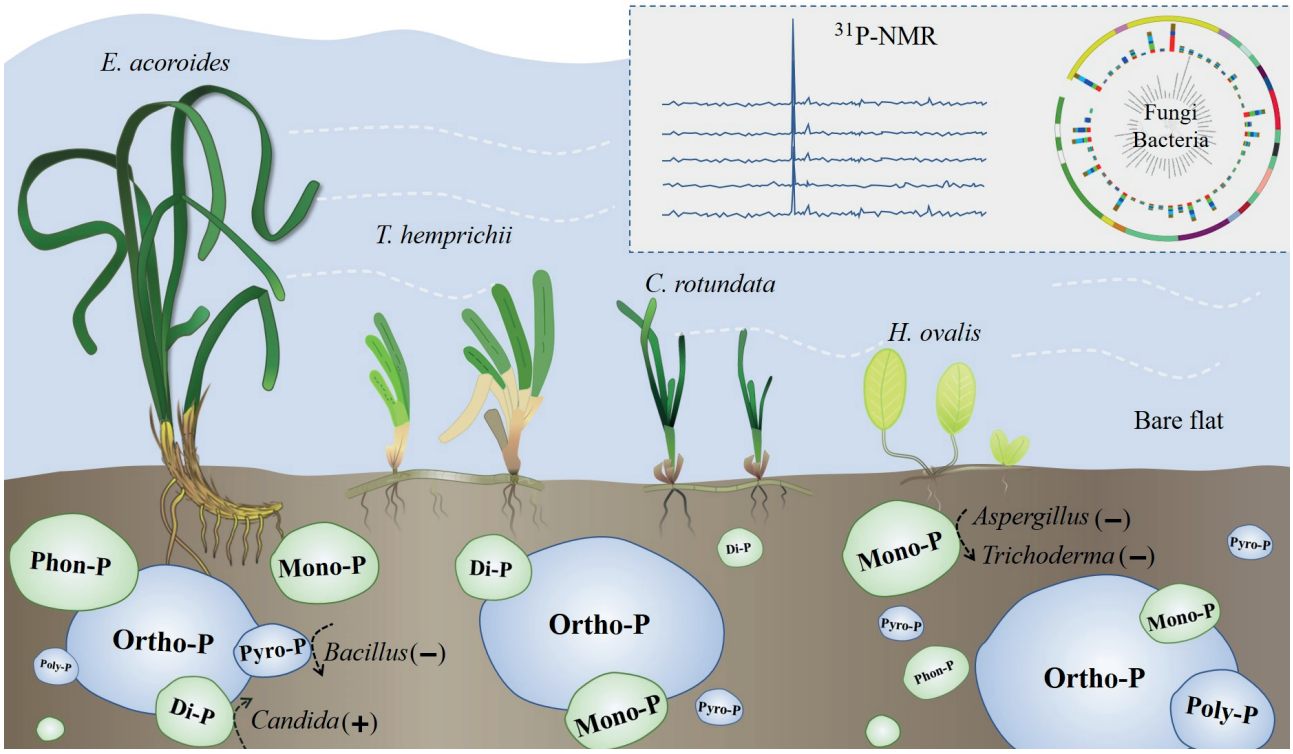

Figure 8

Main findings of this research

Mono-P was the dominant Po form in these sediments; it is derived from plants and was detected in all samples. Degradation and mineralization of Mono-P is an important source of net primary productivity (Yuan et al. 2015). The usual Mono-P species to be determined are *myo*-inositol hexakisphosphate and its stereoisomers, such as D-*chiro*-inositol hexakisphosphate neoinositol hexakisphosphate and *scyllo*-inositol hexakisphosphate (Turner et al. 2003). Some of the Mono-P species may be unidentified Di-P degradation products (Schneider et al. 2016). A small portion of Di-P was detected in *E. acoroides, T. hemprichii* and *C. rotundata*. Being a type of unstable Po compound, Di-P is usually degraded within a few days and mineralized faster than Mono-P, while playing a key role in the P cycle (Dell'Anno & Danovaro 2005; Reitzel et al. 2006). In addition, Di-P can be degraded to Mono-P during sample preparation and extraction (Turner et al. 2003), so caution should be taken in the interpretation as these concentrations do not necessarily reflect the actual content in the sediments. The observed absence or low proportion of diesters, ranging from 0% to 4.98%, in the sediments could be attributed to easy degradation in nature and the extraction time of 16 h in this study. Phon-P in the present study appeared to be greatly variable, ranging from 0% to 19.05% in different samples, which is consistent with many other reports (Benitez-Nelson et al. 2004; Sannigrahi & Ingall 2005). The highest Phon-P proportion of 19.05% in *E. acoroides* sediments is probably explained by the interception of organophosphorus herbicides from farmland and insecticides from the nearby aquaculture. It is not possible for the secretion and degradation of seagrasses to produce such a high Phon-P concentration naturally.

The present study has shown species-specific differences in P forms in seagrass meadows, especially in *E. acoroides*. Yuan et al. (2015) reported that the proportion of organic P in total P in the sediments varied in different plants. On the one hand, according to the existing studies (Duarte & Chiscano 1999) and our research, the biomass and production of *E. acoroides* are much higher compared to other seagrasses, so litter production of *E. acoroides* is also higher. On the other hand, *E. acoroides*, the largest (over 1 m high) seagrass species in Xincun Bay, can capture more different floating organic matter from water into sediments by slowing down the water flow (Komatsu et al. 2004). The vertical carbon flux can be estimated at 557.6 g C m^{-2} yr^{-1} in *E. acoroides* meadows (Wahyudi et al. 2016). The higher concentration and greater variety of organic matter in *E. acoroides* meadows result in higher organic P content and more abundant P forms compared to other seagrass meadows.

Meaningful difference in the microbial community, especially in the fungal community

Tropical seagrasses, generally P-limited owing to the strong P fixation capacity of carbonate-rich sediments, can surprisingly form densely vegetated meadows in such low-nutrient environments (Brodersen et al. 2017). The high P mobilization in tropical seagrass sediments could potentially be further supported by microorganisms (Vazquez et al. 2000). Microorganisms have been increasingly recognized as pivotal players in seagrass ecology (Ugarelli et al. 2017; Brodersen et al. 2018). Previous studies focused mainly on the effect of microorganisms on carbon, nitrogen and sulfur cycles, but research on the effects on the P cycle is limited. Jiang et al. (2015) reported that *T. hemprichii* hosted the highest abundance of bacteria, followed by *E. acoroides* and *C. rotundata*, but in this study *H. ovalis* was characterized by the highest abundance of bacteria. Similarly to the findings of Hurtado-McCormick et al. (2019) regarding the *Zostera muelleri* seagrass microbiome in the sediment where Flavobacteria, Chronaxies, and Desulfobacteraceae dominated the bacteria communities at the order level, we found that Flavobacteriaceae, Clostridiales and Desulfobacteraceae were the main orders and they were present in similar numbers. The finding presented in this study that Ascomycota and Basidiomycota dominated in the *E. acoroides* sediment thus far supports the idea of Wainwright et al. (2019) at the fungal phyla level. Contrary to the previous studies, it is somewhat surprising that high abundance of Tremellomycetes and Cystobasidiomycetes was detected at the class level, 45.97% and 16.38% in the *T. hemprichii* sediment respectively, and very low abundance, ranging from 0% to 1.24%, in the bare tidal flat sediment. Physicochemical parameters of the sediments are significantly modified by seagrasses. The bacterial abundance and biomass in the rhizosphere is about twice as high as in the nearby non-vegetated sediments (Jiang et al. 2015; Ugarelli et al. 2017). The seagrass rhizosphere microbiome shows statistically significant differences in the composition compared to adjacent vegetated sediment communities (Cucio et al. 2016; Ettinger et al. 2017). This case study confirms the meaningful difference in the microbial community between the bare tidal flat and seagrass meadows, especially in the case of the fungal community between treatments.

Phosphorus forms affected mainly by fungi rather than bacteria

In the natural environment, numerous bacteria and fungi in the soil and sediment, playing an important role in the P cycle, are effective in releasing P from total P through acidification, secretion of organic acids or protons, chelation exchange reaction, mineralization of Po by acid phosphatases, phytases, phosphonates and C-P lyases (Sharma et al. 2013; Dipta et al. 2019; Y. Li et al. 2019). In seagrass sediments, the dominant genus of P-cycling-related bacteria was *Bacillus*, followed by *Streptomyces* and *Staphylococcus* (Fig. 6). These results are similar to those obtained in the study by Li et al. (2019) on the sediments from Lake Sancha, who also found that the most phosphate-solubilizing bacteria strains belonged to the genus *Bacillus*, followed by *Paenibacillus*. Bacteria with a high potential for solubilizing phosphate from the rhizosphere of marine plants *Avicennia* belong mainly to *Bacillus*, *Pseudomonas* and *Acinetobacter* (Teymouri et al. 2016). Of all microorganisms, it was found that fungi have a greater capacity to solubilize insoluble phosphate than bacteria (Sharma et al. 2013; Dipta et al. 2019). In this study, the proportion of P-cycling-related fungi is much higher than that of P-cycling-related bacteria, ranging from 26.20% to 0.29%. Some soil fungi species are well known for their strong potential for P-solubilization or mobilization of Po in soils and sediments, such as *Penicillium* and *Aspergillus* (Mercl et al. 2020). *Penicillium* species are considered a key group of fungi in the P cycling due to their ability to solubilize inorganic P through the release of organic anions (Richardson et al. 2011). Consistent with the aforementioned literature, this study also found that *Cladosporium*, *Penicillium* and *Aspergillus* were the main fungi at the genus level in the seagrass meadows.

Po is an important component of the P pool, but it cannot be used directly by plants and microorganisms. The degradability of Po compounds depends mainly on physicochemical and biochemical properties of their molecules, nucleic acids and phospholipids. Sugar phosphates decompose easily, whereas phytic acid, polyphosphates and phosphonates decompose relatively slowly (Dipta et al. 2019). Previous studies on P-cycling-related microorganisms focused mainly on Pi-cycling-related bacteria and only a few studies investigated Po-cycling-related bacteria and fungi that can excrete phosphatase to degrade organic phosphorus, such as phytates, phosphomonoesters and phosphotriesterases, thereby enhancing the available soil phosphorus level (Khan et al. 2007; Sun et al. 2017). Different microorganisms secrete different enzymes. According to the summary by Dipta et al. (2019), *Bacillus*, *Aspergillus* and *Trichoderma* discussed in this study can secrete alkaline phosphatase, acid phosphatase and phytase; *Pseudomonas* and *Penicillium* can secrete alkaline phosphatase and acid phosphatase; *Streptomyces* can secrete phytase, while *Aspergillus* and *Trichoderma* can secrete phytase. Phytate (*myo*-inositol hexakisphosphate) was quantitatively the most important Mono-P. Therefore, Mono-P was negatively correlated with *Aspergillus* and *Trichoderma* in this study.

Conclusions

Ortho-P was the most dominant P compound in all sediment samples, accounting for 58.61% to 81.70%, followed by Mono-P. In general, seagrass sediments provided a greater variety and contribution of Po compounds compared to the bare tidal flat, especially in *E. acoroides*. The diversity of the microbial community in sediment samples from different seagrass meadows varied. The P-cycling-related bacteria and fungi belong mainly to the class Bacilli and the phylum Ascomycota, respectively. The relative abundance of P-cycling-related fungi was 26.20%, which was much higher than that of bacteria – 0.29%. Pyro-P was the main factor determining the distribution of P-cycling-related bacteria and was negatively correlated with *Bacillus*. Mono-P was the main factor determining the distribution of P-cycling-related fungi and was negatively correlated with *Aspergillus* and *Trichoderma*. In this study, sediment samples were collected only from the lagoon with sandy sediments in one spring season. The effects of other types of coasts, other sediment environments and other seasons on P forms and the microbial community in seagrass meadows need to be further discussed. Due to the P forms affected by microorganisms, especially fungi, more research will be needed to focus on the contribution rate, the related biochemical and molecular biological mechanisms of bacteria and fungi in the P cycling process of seagrass meadows.

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References

Baldwin, D.S. (2013). Organic phosphorus in the aquatic environment. *Environ. Chem.* 10(6): 439–454. DOI: 10.1071/EN13151.

Benitez-Nelson, C. R., O'Neill, L., Kolowith, L.C., Pellechia, P. & Thunell, R. (2004). Phosphonates and particulate organic phosphorus cycling in an anoxic marine basin. *Limnol. Oceanogr.* 49(5): 1593–1604. DOI: 10.4319/lo.2004.49.5.1593.

Bhattacharyya, P.N. & Jha, D.K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J. Microbiol. Biotechnol.* 28(4): 1327–1350. DOI: 10.1007/s11274-011-0979-9.

Bramha, S.N., Mohanty, A.K., Padhi, R.K., Panigrahi, S.N. & Satpathy, K.K. (2014). Phosphorus speciation in the marine sediment of Kalpakkam coast, southeast coast of India. *Environ. Monit. Assess.* 186(10): 6003–6015. DOI: 10.1007/s10661-014-3836-0.

Brodersen, K.E., Koren, K., Mosshammer, M., Ralph, P.J., Kuhl, M. et al. (2017). Seagrass-mediated phosphorus and iron solubilization in tropical sediments. *Environ. Sci. Technol.* 51(24): 14155–14163. DOI: 10.1021/acs.est.7b03878.

Brodersen, K.E., Siboni, N., Nielsen, D.A., Pernice, M., Ralph, P.J. et al. (2018). Seagrass rhizosphere microenvironment alters plant-associated microbial community composition. *Environ. Microbiol.* 20(8): 2854–2864. DOI: 10.1111/1462-2920.14245.

Cade-Menun, B.J. (2005). Characterizing phosphorus in environmental and agricultural samples by P-31 nuclear magnetic resonance spectroscopy. *Talanta.* 66(2): 359–371. DOI: 10.1016/j.talanta.2004.12.024.

Cade-Menun, B.J. (2015). Improved peak identification in ^{31}P-NMR spectra of environmental samples with a standardized method and peak library. *Geoderma.* 257–258: 102–114. DOI: 10.1016/j.geoderma.2014.12.016.

Cade-Menun, B.J. & Liu, C.W. (2014). Solution phosphorus-31 nuclear magnetic resonance spectroscopy of soils from 2005 to 2013: A review of sample preparation and experimental parameters. *Soil Sci. Soc. Am. J.* 78(1): 19–37. DOI: 10.2136/sssaj2013.05.0187dgs.

Cucio, C., Engelen, A.H., Costa, R. & Muyzer, G. (2016). Rhizosphere microbiomes of european seagrasses are selected by the plant, but are not species specific. *Front. Microbiol.* 7: 440. DOI: 10.3389/fmicb.2016.00440.

Dell'Anno, A. & Danovaro, R. (2005). Extracellular DNA plays a key role in deep-sea ecosystem functioning. *Science* 309(5744): 2179–2179. DOI: 10.1126/science.1117475.

Ding, S., Bai, X., Fan, C. & Zhang, L. (2010). Caution needed in pretreatment of sediments for refining phosphorus-31 nuclear magnetic resonance analysis: Results from a comprehensive assessment of pretreatment with ethylenediaminetetraacetic acid. *J. Environ. Qual.* 39(5): 1668–1678. DOI: 10.2134/jeq2009.0396.

Dipta, B., Bhardwaj, S., Kaushal, M., Kirti, S. & Sharma, R. (2019). Obliteration of phosphorus deficiency in plants by microbial interceded approach. *Symbiosi.* 78(2): 163–176. DOI: 10.1007/s13199-019-00600-y.

Duarte, C.M. & Chiscano, C.L. (1999). Seagrass biomass and production: A reassessment. *Aquat. Bot.* 65(1–4): 159–174. DOI: 10.1016/S0304-3770(99)00038-8.

Ettinger, C.L., Voerman, S.E., Lang, J.M., Stachowicz, J.J. & Eisen, J.A. (2017). Microbial communities in sediment from *Zostera marina* patches, but not the *Z. marina* leaf or root microbiomes, vary in relation to distance from patch edge. *Peerj.* 5: e3246. DOI: 10.7717/peerj.3246.

Fraser, M.W., Gleeson, D.B., Grierson, P.F., Laverock, B. & Kendrick, G.A. (2018). Metagenomic evidence of microbial community responsiveness to phosphorus and salinity gradients in seagrass sediments. *Front. Microbiol.* 9: 1703. DOI: 10.3389/fmicb.2018.01703.

Garcia-Martinez, M., Lopez-Lopez, A., Calleja, M.L., Marba, N. & Duarte, C.M. (2009). Bacterial community dynamics in a seagrass (*Posidonia oceanica*) meadow sediment. *Estuaries Coasts* 32(2): 276–286. DOI: 10.1007/s12237-008-9115-y.

Huang, X., Huang, L., Li, Y., Xu, Z., Fong, C.W. et al. (2006). Main seagrass beds and threats to their habitats in the coastal sea of South China. *Chin. Sci. Bull.* 51: 136–142. DOI: 10.1007/s11434-006-9136-5.

Hurtado-McCormick, V., Kahlke, T., Petrou, K., Jeffries, T., Ralph, P.J. et al. (2019). Regional and microenvironmental scale characterization of the *Zostera muelleri* seagrass microbiome. *Front. Microbiol.* 10: 1011. DOI: 10.3389/fmicb.2019.01011.

Jensen, H.S., McGlathery, K.J., Marino, R. & Howarth, R.W. (1998). Forms and availability of sediment phosphorus in carbonate sand of Bermuda seagrass beds. *Limnol. Oceanogr.* 43(5): 799–810. DOI: 10.4319/lo.1998.43.5.0799.

Jiang, Y.F., Ling, J., Wang, Y.S., Chen, B., Zhang, Y.Y. et al. (2015). Cultivation-dependent analysis of the microbial diversity associated with the seagrass meadows in Xincun Bay, South China Sea. *Ecotoxicology.* 24(7–8): 1540–1547. DOI: 10.1007/s10646-015-1519-4.

Khan, M.S., Zaidi, A. & Wani, P.A. (2007). Role of phosphate-solubilizing microorganisms in sustainable agriculture – A review. *Agron. Sustainable Dev.* 27(1): 29–43. DOI: 10.1051/agro:2006011.

Komatsu, T., Umezawa, Y., Nakakoka, M., Supanwanid, C. & Kanamoto, Z. (2004). Water flow and sediment in *Enhalus acoroides* and other seagrass beds in the Andaman Sea, off Khao Bae Na, Thailand. *Coast. Mar. Sci.* 29(1): 63–68. DOI: 10.15083/00040824.

Koukol, O., Novak, F. & Hrabal, R. (2008). Composition of the organic phosphorus fraction in basidiocarps of saprotrophic and mycorrhizal fungi. *Soil Biol. Biochem.* 40(9): 2464–2467. DOI: 10.1016/j.soilbio.2008.04.021.

Li, W., Joshi, S.R., Hou, G., Burdige, D.J., Sparks, D.L. et al. (2015). Characterizing phosphorus speciation of Chesapeake

Bay sediments using chemical extraction, ^{31}P-NMR, and X-ray absorption fine structure spectroscopy. *Environ. Sci. Technol.* 49(1): 203–211. DOI: 10.1021/es504648d.

Li, Y., Zhang, J., Zhang, J., Xu, W. & Mou, Z. (2019). Characteristics of inorganic phosphate-solubilizing bacteria from the sediments of a eutrophic lake. *Int. J. Environ. Res. Public Health*. 16(12): 2141. DOI: 10.3390/ijerph16122141.

Liu, J., Wang, H., Yang, H., Ma, Y. & Cai, O. (2009). Detection of phosphorus species in sediments of artificial landscape lakes in China by fractionation and phosphorus-31 nuclear magnetic resonance spectroscopy. *Environ. Pollut.* 157(1): 49–56. DOI: 10.1016/j.envpol.2008.07.031.

Liu H., Pan F., Han X., Song F., Zhang Z. et al. (2019). Response of soil fungal community structure to long-term continuous soybean cropping. *Front. Microbiol.* 9: 3316. DOI: 10.3389/fmicb.2018.03316.

McRoy, C.P., Nebert, M. & Barsdate, R.J. (1972). Phosphorus cycling in an eelgrass (*Zostera marina* L.) ecosystem. *Limnol. Oceanogr.* 17(1): 58–67. DOI: 10.4319/lo.1972.17.1.0058.

Mercl, F., Garcia-Sanchez, M., Kulhanek, M., Kosnar, Z., Szakova, J. et al. (2020). Improved phosphorus fertilization efficiency of wood ash by fungal strains *Penicillium* sp. PK112 and *Trichoderma harzianum* OMG08 on acidic soil. *Appl. Soil Ecol.* 147: 103360. DOI: 10.1016/j.apsoil.2019.09.010.

Nielsen, O.I., Koch, M.S. & Madden, C.J. (2007). Inorganic phosphorus uptake in a carbonate-dominated seagrass ecosystem. *Estuaries Coasts.* 30(5): 827–839. DOI: 10.1007/bf02841337.

Pagès, A., Welsh, D.T., Robertson, D., Panther, J.G., Schäfer, J. et al. (2012). Diurnal shifts in co-distributions of sulfide and iron(II) and profiles of phosphate and ammonium in the rhizosphere of *Zostera capricorni*. *Estuar. Coast Shelf Sci.* 115: 282–290. DOI: 10.1016/j.ecss.2012.09.011.

Paytan, A., Cade-Menun, B.J., McLaughlin, K. & Faul, K.L. (2003). Selective phosphorus regeneration of sinking marine particles: Evidence from ^{31}P-NMR. *Mar. Chem.* 82(1–2): 55–70. DOI: 10.1016/s0304-4203(03)00052-5.

Prasad, M.B.K. & Ramanathan, A.L. (2010). Characterization of phosphorus fractions in the sediments of a tropical intertidal mangrove ecosystem. *Wetlands Ecol. Manage.* 18(2): 165–175. DOI: 10.1007/s11273-009-9157-3.

Prüter, J., Leipe, T., Michalik, D., Klysubun, W. & Leinweber, P. (2019). Phosphorus speciation in sediments from the Baltic Sea, evaluated by a multi-method approach. *J. Soils Sediments* 20(3): 1676–1691. DOI: 10.1007/s11368-019-02518-w.

Reitzel, K., Ahlgren, J., Gogoll, A., Jensen, H.S. & Rydin, E. (2006). Characterization of phosphorus in sequential extracts from lake sediments using ^{31}P-nuclear magnetic resonance spectroscopy. *Can. J. Fish. Aquat. Sci.* 63(8): 1686–1699. DOI: 10.1139/f06-070.

Richardson, A.E., Lynch, J.P., Ryan, P.R., Delhaize, E., Smith, F.A. et al. (2011). Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant Soil.* 349(1–2): 121–156. DOI: 10.1007/s11104-011-0950-4.

Sannigrahi, P. & Ingall, E. (2005). Polyphosphates as a source of enhanced P fluxes in marine sediments overlain by anoxic waters: Evidence from ^{31}P-NMR. *Geochem. Trans.* 6(3): 52–59. DOI: 10.1063/1.1946447.

Schneider, K.D., Cade-Menun, B.J., Lynch, D.H. & Voroney, R.P. (2016). Soil phosphorus forms from organic and conventional forage fields. *Soil Sci. Soc. Am. J.* 80(2): 328–340. DOI: 10.2136/sssaj2015.09.0340.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H. & Gobi, T.A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springerplus.* 2: 587. DOI: 10.1186/2193-1801-2-587.

Shinohara, R., Imai, A., Kawasaki, N., Komatsu, K., Kohzu, A. et al. (2012). Biogenic phosphorus compounds in sediment and suspended particles in a shallow eutrophic lake: A ^{31}P-nuclear magnetic resonance (^{31}P-NMR) study. *Environ. Sci. Technol.* 46(19): 10572–10578. DOI: 10.1021/es301887z.

Sun, W., Qian, X., Gu, J., Wang, X. J., Li, Y. et al. (2017). Effects of inoculation with organic-phosphorus-mineralizing bacteria on soybean (*Glycine max*) growth and indigenous bacterial community diversity. *Can. J. Microbiol.* 63(5): 392–401. DOI: 10.1139/cjm-2016-0758.

Tapia-Torres, Y., Rodríguez-Torres, M.D., Elser, J.J., Islas, A., Souza, V. et al. (2016). How to live with phosphorus scarcity in soil and sediment: Lessons from bacteria. *Appl. Environ. Microbiol.* 82(15): 4652–4662. DOI: 10.1128/AEM.00160-16.

Teymouri, M., Akhtari, J., Karkhane, M. & Marzban, A. (2016). Assessment of phosphate solubilization activity of rhizobacteria in mangrove forest. *Biocatal. Agric. Biotechnol.* 5: 168–172. DOI: 10.1016/j.bcab.2016.01.012.

Turner, B.L., Mahieu, N. & Condron, L.M. (2003). Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. *Soil Sci. Soc. Am. J.* 67(2): 497–510. DOI: 10.2136/sssaj2003.4970.

Ugarelli, K., Chakrabarti, S., Laas, P. & Stingl, U. (2017). The seagrass holobiont and its microbiome. *Microorganisms.* 5: 81. DOI: 10.3390/microorganisms5040081.

Vazquez, P., Holguin, G., Puente, M.E., Lopez-Cortes, A. & Bashan, Y. (2000). Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biol. Fertil. Soils.* 30(5–6): 460–468. DOI: 10.1007/s003740050024.

Wahyudi, A.A.J., Rahmawati, S., Prayudha, B., Iskandar, M.R. & Arfianti, T. (2016). Vertical carbon flux of marine snow in *Enhalus acoroides*-dominated seagrass meadows. *Reg. Stud. Mar. Sci.* 5: 27–34. DOI: 10.1016/j.rsma.2016.01.003.

Wainwright, B.J., Zahn, G.L., Zushi, J., Lee, N.L.Y., Ooi, J.L.S. et al. (2019). Seagrass-associated fungal communities show distance decay of similarity that has implications for seagrass management and restoration. *Ecol. Evol.* 9(19): 11288–11297. DOI: 10.1002/ece3.5631.

Watson, S.J., Cade-Menun, B.J., Needoba, J.A. & Peterson,

T.D. (2018). Phosphorus forms in sediments of a river-dominated estuary. *Front. Mar. Sci.* 5: 302. DOI: 10.3389/fmars.2018.00302.

Xie, F., Li, L., Song, K., Li, G., Wu, F. et al. (2019). Characterization of phosphorus forms in a Eutrophic Lake, China. *Sci. Total Environ.* 659: 1437–1447. DOI: 10.1016/j.scitotenv.2018.12.466.

Yang, D. & Yang, C. (2009). Detection of seagrass distribution changes from 1991 to 2006 in Xincun Bay, Hainan, with satellite remote sensing. *Sensors* 9(2): 830–844. DOI: 10.3390/s90200830.

Yuan, H.Z., Pan, W., Ren, L.J., Liu, E.F., Shen, J. et al. (2015). Species and biogeochemical cycles of organic phosphorus in sediments from a river with different aquatic plants located in Huaihe river watershed, China. *Int. J. Phytoremediation.* 17(1–6): 215–221. DOI: 10.1080/15226514.2013.876969.

Zhao, G., Sheng, Y., Jiang, M., Zhou, H. & Zhang, H. (2019). The biogeochemical characteristics of phosphorus in coastal sediments under high salinity and dredging conditions. *Chemosphere.* 215: 681–692. DOI: 10.1016/j.chemosphere.2018.10.015.

Zhu, F., Qu, L., Hong, X. & Sun, X. (2011). Isolation and characterization of a phosphate-solubilizing halophilic bacterium *Kushneria* sp. YCWA18 from Daqiao Saltern on the coast of Yellow Sea of China. *Evid. Based Compl. Altern. Med.* 2011: 615032. DOI: 10.1155/2011/615032.