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Microbial Community Composition and Activity Controls Phosphorus

Transformation in Rhizosphere Soils of the Yeyahu Wetland in Beijing,

China

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Zedong Teng[a], Yunyun Zhu[a], Min Li[a]*, Michael J. Whelan[b]

[a]College of Environmental Science and Engineering, Beijing Forestry University, Beijing 100083, China

[b]School of Geography, Geology and the Environment, University of Leicester, Leicester, UK

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*Corresponding Author

Ph.D., Professor

College of Environmental Science and Engineering

Beijing Forestry University, 60#

35# Qinghua East Rd., Haidian District,

Beijing 100083, P.R.China

Tel: 86 10 62336615

Email: *liminbjfu@126.com*

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Abstract:

Microorganisms in the rhizosphere of wetland plants can have a significant impact on phosphorus (P) interception. We investigated the seasonal pattern of microbial community structure and its relationship with to different P forms in the rhizosphere of three plants *Scirpus planiculmis*, *Zizania latifolia*, and *Phragmites australis* from the Yeyahu Wetland, China. Chloroform fumigation-extraction was used to determine the soil microbial biomass P (SMBP) and phospholipid fatty acids (PLFA) were used to characterize microbial community composition. P fractions in rhizosphere soil samples were also observed using sequential chemical fractionation. Results showed that the average total PLFA (TPLFA) contents of rhizosphere soils ranged from 34.9 to 40.7 nmol·g^{-1} and were highest in summer. Bacteria were predominant in the rhizospheres of all three plants, accounting for more than 63 % of TPLFA. Aerobic bacteria, represented by 16:0 PLFA, were most abundant. Both organic P (OP) and inorganic P (IP) accumulated in the rhizosphere during the winter die-back phase. Furthermore, both TPLFA and bacterial PLFA decreased with increases in highly resistant OP (HR-OP), occluded P (Oc-P) and Calcium-bound P (Ca-P). This suggests that bacteria play an important role in P transformation and can make use of various P forms. We also found that SMBP was significantly negatively correlated with labile OP (L-OP), moderately labile OP (ML-OP) and HR-OP, reflecting a high degree of cross correlation between SMBP and the PLFA indices.

1. Introduction

Phosphorus (P) is a key limiting nutrient in both aquatic and terrestrial ecosystems. In soils, most P is found in pools with low plant availability: bound to calcium, aluminum or iron minerals, or in organic compounds with low lability (Porder et al., 2007). Replenishment of soil P reserves through fertilization is common in agriculture, but the long-term sustainability of this practice is questionable, because the main source of fertilizer P is rock phosphate which is mined from non-renewable reserves. Only 50% of economically recoverable P reserves are forecast to remain by the middle of the 21st century (Ding et al., 2015). Furthermore, P can be lost from soils via the erosion of particles and via leaching of soil pore water (Haygarth et al.,1998; Heckrath et al., 1995), leading to eutrophication of freshwater (Correll et al., 1998) and marine ecosystems (Philippart et al., 2007) and elevated P concentrations in groundwater (Holman et al., 2008). In lakes, P can accumulate in sediment and be periodically released into overlying water under suitable environmental conditions, making remediation difficult (Ribeiro et al., 2008).

Wetlands represent important ecosystems which provide a number of essential ecosystem services including the provision of food and fiber resources, moderating hydrological variability (e.g. storing water at high flow and releasing it under dry conditions), regulating local climate and acting as an important habitat for wildlife. They can also play an important role in nutrient dynamics by encouraging nitrogen losses from water (via denitrification and plant uptake) and by retaining P via the trapping of sediment, plant uptake and a range of biological and chemical processes which reduce P mobility (Howard-Williams, 2010). Phosphorus transformation and transport in wetland systems is complex and involves numerous interactions between plants and microbes (Ahn et al., 2007) which are illustrated in Fig. 1. Many of the processes that regulate P availability are microbially-driven, such as

66   the solubilization of exchangeable mineral P via the secretion of organic and inorganic acids (Zhu et al.,

67   2018), the decomposition of soil organic matter (and associated P mineralization or the immobilization

68   of excess mineral P in the microbial biomass, which can be subsequently released) and the release of

69   mineral P by hydrolysis catalyzed by extracellular enzymes (Oehl et al., 2003; Chen et al., 2006; Qiu et

70   al., 2010).

71   Microbial communities are, therefore, essential for regulating plant-available P and overall

72   ecosystem function (Xu et al., 2017). In particular, microbes associated with the rhizosphere (the soil

73   region in close proximity to plant roots) are thought to play an especially important role in the

74   regulation of plant nutrient supply (Selvaraj et al., 2008). The rhizosphere is often characterized by

75   high microbial activity including transformation of organic substrates and the release of plant-available

76   nutrients (Wang et al., 2008). In return, plants supply carbon-rich compounds, such as carbohydrates,

77   via root exudates which can be metabolized by the microbial community, promoting growth and

78   further nutrient mobilization.

79   The characteristics and activity of microbial communities in the rhizosphere tend to be closely

80   aligned with vegetative change. For example, during early primary succession, levels of available P

81   increase due to microbial activity and changes in soil physicochemical characteristics (Bokhorst et al.,

82   2017). However, despite considerable advances in our understanding of plant-microbe interactions in

83   recent years, the exact nature of microbial - P species interactions in the rhizosphere of wetland plants

84   remains poorly understood. This knowledge gap potentially undermines our attempts to manage P

85   retention in wetland systems. Previous studies have shown that rhizodeposition can induce changes in

86   the composition of soil microbial communities by altering the quality and distribution of available

87   organic matter, which may affect P fractions over time (Marschner et al., 2001; Moreira et al., 2013).

88   This is of significance because the soil microbial biomass plays a central role in P cycling in soils

89   (Richardson et al., 2011). Previous studies have suggested that seasonal (phenological) changes to

90   vegetation can affect microbial community composition, with potential consequences for

91   decomposition dynamics and nutrient availability. However, seasonal relationships between P species

92   transformation and microbial community composition in the rhizosphere of different plants is currently

93   not well understood. The aim of this paper is, therefore, to elucidate the interactions between microbial

94   community composition and P transformation in wetland vegetation, with a particular focus on the

95   rhizosphere.

96   Specifically, we investigated seasonal variations in soil microbial biomass, changes in microbial

97   community composition, and fluctuation in the concentrations of different P fractions for three plant

98   species (*Scirpus planiculmis*, *Zizania latifolia*, and *Phragmites australis*) in the Yeyahu Wetland, China.
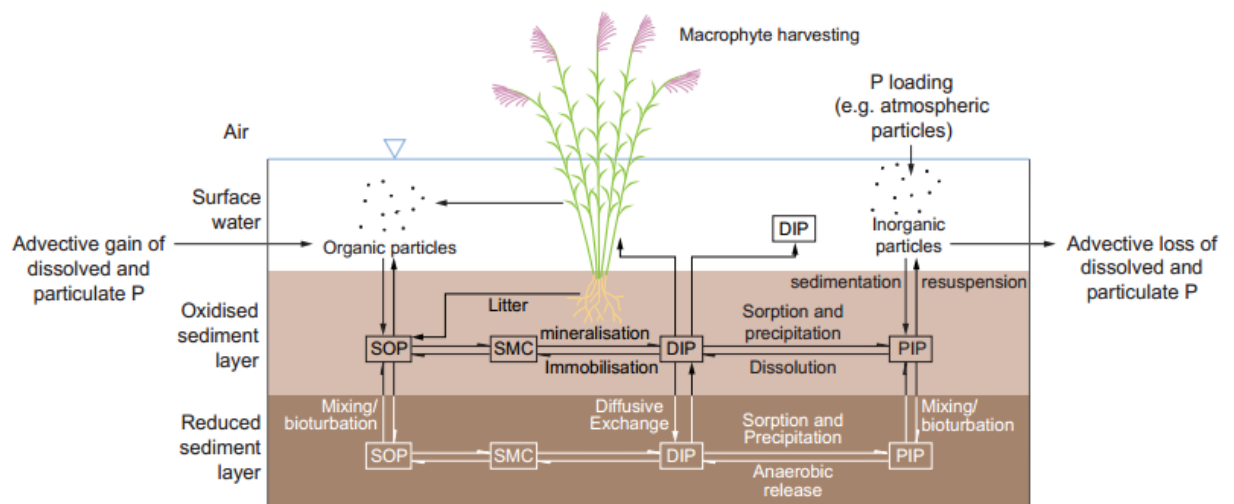

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100   **Fig. 1** Schematic illustration of phosphorus transformations and migration in wetland systems under varying conditions

101   of hydrology, phosphorus loading and vegetation. DIP is dissolved inorganic P; SOP is sediment/soil organic P; PIP is

102   particulate inorganic P and SMC is the soil microbial community (modified from Ahn et al., 2007).

2. Methods

2.1 Study area

The study was conducted in the Yeyahu Wetland in Yanqing country, China, to the northwest of Beijing (40°25'N~40°30'N; 115°47'E~115°54'E). This is the largest wetland in the Beijing area and is characterized by mudflats, open water and vegetated marshes. These components are linked together by the Guishui River, which floods seasonally. The wetland receives contaminated water from domestic sewage, limited industrial pollution and diffuse-source agricultural pollution (including sediment from soil erosion). However, detailed information on the P budget of the wetland is currently unavailable. The soils of the wetland are mainly fluvial in origin. The climate is continental monsoon, with four distinct seasons. The mean annual temperature is 8.9 °C and the mean annual precipitation is 463 mm (Gong et al., 2007). Emergent vegetation in the area is dominated by *P. australis*, *Z. latifolia* and *S. planiculmis*. Soil sampling was conducted at a site close to the northeastern catchment boundary (Fig. 2). All three dominant emergent plants are present in this area along a gradient from open water to dry land. *S. planiculmis* is mainly present along the edge of open water, *Z. latifolia* is dominant in seasonally flooding mudflats and *P. australis* is present in shallow open water.

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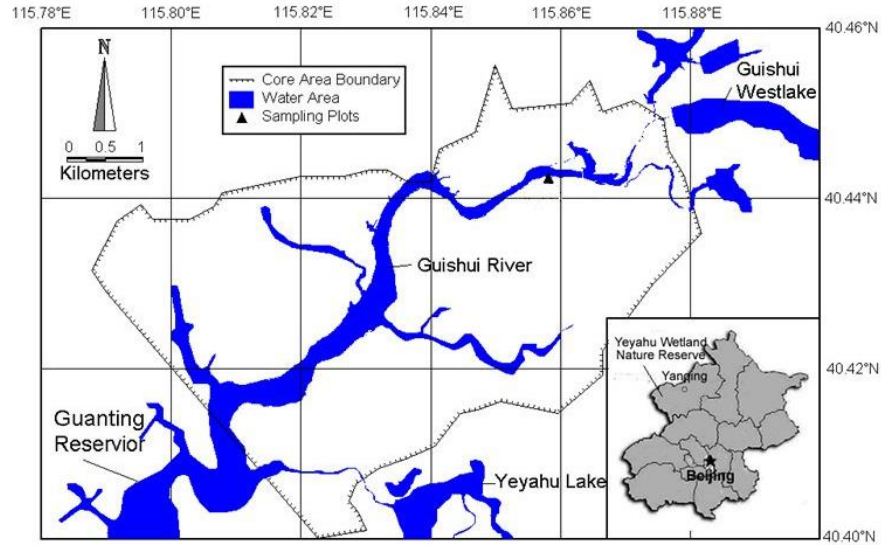
Fig. 2 Location map of the Yeyahu Wetland and the sampling site (black triangle).

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2.2 Experimental Design and Soil Collection

Samples of rhizosphere soils were collected from each of the three dominant wetland plant species (i.e. five healthy plants were selected for each species) at three stations located in a core area of the wetland between March and October, 2015. Soils from each sampling site were mixed to form a composite soil sample.　Roots were carefully excavated and loose soil shaken off and discarded. The remaining soil still attached to the roots (hence forth referred to as the rhizosphere soil) was swept off with a brush and collected. Care was taken to avoid cross contamination between different samples.

The collected samples were immediately placed in a refrigerated sealed container. One subsample of sieved fresh soil was stored at 4 °C and analyzed within ten days of sampling for phospholipid fatty acids (PLFAs) and microbial biomass P. Another subsample was air-dried at room temperature, ground, homogenized and passed through a standard 100-mesh stainless steel sieve. Several measurements such as soil physicochemical properties and P fractions were determined on the sieved fraction.

135   pH was measured in a 1: 2.5 mix of dried soil and deionized water. Moisture content (MC) was

136   determined gravimetrically from mass loss following oven-drying at 105 °C. Organic matter (OM)

137   content was measured using a colorimetric method after digestion with $K_2Cr_2O_7/H_2SO_4$ at 165 °C in an

138   oil bath (Bowman et al., 1978). Alkali-hydrolysable nitrogen (AHN) is an index of the potential

139   capacity of the soil to supply N. It was determined by an alkali solution diffusion method (Roberts et

140   al., 2009). Triplicate samples were used for all determinations and results were presented as replicate

141   means ± standard errors.

142   **2.3 Soil Microbial Community**

143   Lipid extraction and PLFA analysis were carried out using a modified protocol described by

144   Frostegard et al. (2011) and Strickland et al. (2010). Briefly, 6 g of soil was incubated in a solution of

145   methanol, chloroform and citrate buffer in a ratio of 2: 1: 0.8 by volume, shaken for 2 h and

146   centrifuged. The chloroform phase was then collected and stored. Phospholipids were separated from

147   glycolipids and neutral lipids by sequential elution with chloroform (6 mL), acetone (6 mL) and

148   methanol (3 mL) on 3 mL silica solid phase extraction columns, saponified and methylated to

149   fatty-acid methyl esters (FAMEs) (Ding et al., 2015). The phospholipid fraction was then methylated

150   with a methanol: toluol (1:1) solution (1 mL) and 0.2 $mol·L^{-1}$ methanolic KOH (1 mL) and heated at

151   37 °C for 15 min. After incubation, 0.3 mL of 1 $mol·L^{-1}$ acetic acid and 1 mL chloroform were added

152   and the bottom phase was removed and dried. Finally, the samples were re-dissolved in 75 μL of

153   hexane and identified using gas chromatography (GC) (6890N, Agilent, USA) equipped with a mass

154   selective detector (5975C, Agilent, USA).

155   GC conditions were as follows: The oven temperature was raised from 50 °C to 180 °C at

156   12 $°C·min^{-1}$ and then to 220 °C at 6 $°C·min^{-1}$, to 240 °C at 15 $°C·min^{-1}$, and finally to 260 °C at

157 15 °C·min^{-1}, where it was held for 2 min. The detector temperature was 280 °C and the ionisation

158 energy was 70 eV. The abundance of individual FAMEs was expressed as nmol·g^{-1} of dry soil and

159 classified according to standard nomenclature (Tunlid et al., 1989). Concentrations of each PLFA were

160 estimated using fatty acid 19: 0 as an internal standard. The sum of PLFAs indicated below were

161 considered to be representative of the total PLFAs of the soil microbial community. In addition, PLFAs

162 were assigned to different microbial taxonomic groups based on previously published PLFA biomarker

163 data (shown in Table 1).

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165 **Table 1**. PLFA biomarkers used for identifying microbial types.

Species of microbial	PLFA biomarkers	Reference
Bacteria	14:0, 15:0, a15:0, i15:0, 16:0, i16:0, 16:1w7t, 16:1w9, 17:0, a17:0, i17:0, cy17:0, 18:1w7, cy19:0	Frostegard and Baath (1996); Smolander (1999)
Aerobic bacteria	15:0, a15:0, i15:0, i16:0, 16:1w7t, 16:1w9t, 17:0, a17:0, i17:0, 18:1w7t	Zhang, Q. F.,et al., (2009)
Anaerobic bacteria	Cy17:0, 18:1w7c, cy19:0	Zhang, Q. F.,et al., (2009)
Fungi	18:1w9, 18:2w6, 18:3w3, 18:3w6	Beese (1992); White (1996)
Protozoa	10Me16:0, 10Me17:0, 10Me18:0	Zhu, Y.Y., (2016)
Actinomycetes	10Me16:0, 19Me17:0, 10Me18:0	Wu, Y. P., (2009)
Gram positive bacteria (GP)	16:1w6c, 18:0 2OH, 17:0 3OH, 18:1w7c	Frostegard et al., (2011)
Gram negative bacteria (GN)	i15:0, a15:0, i16:0, a16:0, i17:0, a17:0, i18:0	He et al., (2009)

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167 **2.4 Soil microbial biomass phosphorus (SMBP)**

168 SMBP was measured using the chloroform fumigation-extraction technique (Brookes et al., 1982)

169 on fresh soil samples (stored at 4 °C). Three sets of soil samples, non-fumigated, fumigated, and

170 P-spiked, were extracted with NaHCO$_3$, and the P content in all extracts was determined by

171   spectrophotometry at 700 nm on a U-1000 spectrophotometer (T6, China) using the ascorbic

172   acid-molybdate reaction. The SMBP concentration (B_P: mg·g^{-1}) was calculated from

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$$B_P = \frac{E_{pi}}{K_p \cdot R_{pi}}$$
Eq. 1

174   where R_{pi} is the proportion of the P-spike recovered in each non-fumigated soil sample; E_{pi} is the

175   difference (P$_{i\ \text{fumigated}}$ - P$_{i\ \text{non-fumigated}}$), in which P$_{i\ \text{fumigated}}$ is the inorganic P concentration (ug·g^{-1}) in

176   NaHCO$_3$ extracts of fumigated soil and P$_{i\ \text{non-fumigated}}$ is the inorganic P concentration (ug·g^{-1}) in

177   NaHCO$_3$ extracts of non-fumigated soil. K$_P$ is a constant which was set to 0.4 to account for the

178   efficiency of P extraction from the lysis of microbial cells (Shi et al., 2012).

179   **2.5 Soil P fractions via sequential chemical extraction**

180   A sequential chemical extraction procedure, proposed by Zhang et al. (2011), was used to measure

181   different inorganic P (IP) forms in rhizosphere soil. Organic P (OP) fractionation was based on the

182   scheme described by Li et al. (2013), which was modified from the method of Ivanoff et al. (1998) to

183   improve the OP extraction efficiency and to more-clearly distinguish the inorganic and organic P

184   fractions in each extract.

185   **2.6 Statistical analysis**

186   SPSS 18.0 was employed for all statistical analyses. Differences in individual soil PLFAs and

187   soil physicochemical characteristics between the three plants and months were tested with one-way

188   analysis of variance (ANOVA) and two factorial variance analysis. The normality of input data was

189   tested using the Anderson-Darling method.   Pearson product moment correlations were used to assess

190   the strength of any relationships between soil microbial community structure, soil environmental

191   characteristics and different P fractions. Correlations and differences between means were deemed

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192   statistically significant at $p<0.05$.

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3. Results

3.1 Soil physicochemical characteristics

The physical and biochemical characteristics of soil samples from each plant species over time are shown in Fig. 3. The study soil was mildly alkaline. The results of ANOVAs suggested that there were no significant differences in the rhizosphere pH between plant species ($p>0.05$) and no significant change over time was observed ($p>0.05$) (March to October) (Fig. 3a). In contrast, there were significant differences between plant species ($p<0.01$) and season ($p<0.01$) for rhizosphere soil AHN content (Fig. 3b). Soil moisture content values were influenced by degree of inundation and were lowest in October for all three species (Fig. 3c). Soil OM contents exhibited a similar temporal pattern to AHN (low in the summer and higher in spring and autumn) and are shown in Fig. 3d. Significant differences were observed in the interaction between plant species and season for AHN ($p<0.01$) and OM ($p<0.01$) according to ANOVAs.

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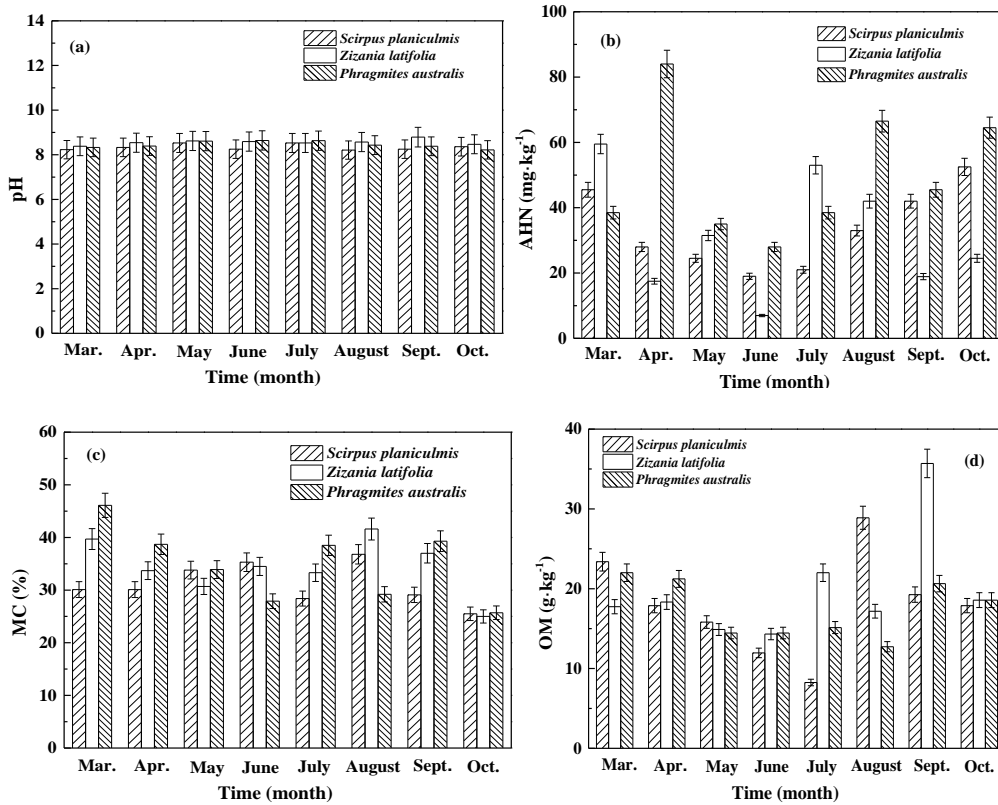
Fig. 3 Physicochemical characteristics of rhizosphere soils of *S. planiculmis*, *Z. latifolia*, and *P. australis* in the Yeyahu

Wetland. AHN is alkali-hydrolysable nitrogen; MC is moisture content; OM is organic matter.

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3.2 The seasonal distribution of microbial community structure

Seasonal variations in the PLFA content of the sampled rhizosphere soils are shown in Fig. 4.

Total PLFAs (TPLFAs), bacterial PLFAs (BPLFAs) and fungal PLFAs (FPLFAs) (which can be viewed

as indicators of total microbial biomass, bacterial biomass and fungal biomass, respectively) all

changed significantly with season in the rhizosphere soils of each plant. The ranges of TPLFA contents

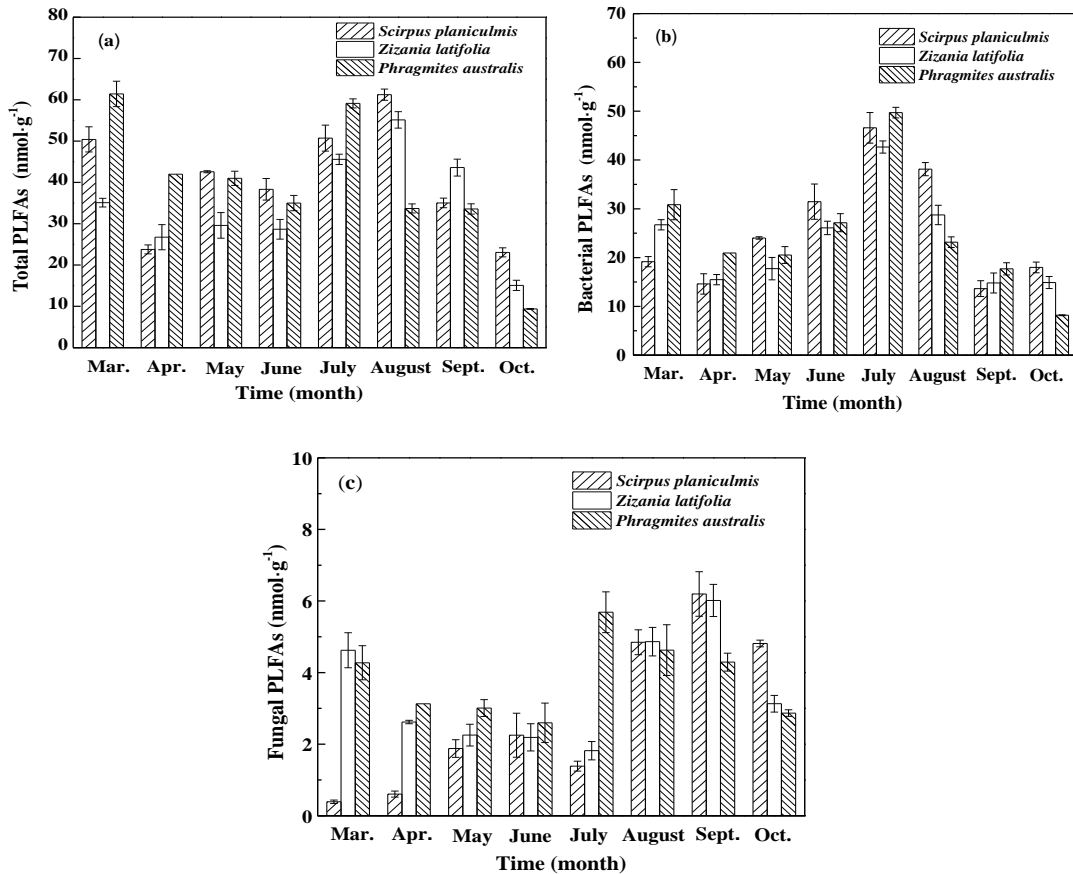for *S. planiculmis*, *Z. latifolia* and *P. australis*, respectively were 23.1-61.2 nmol·g^{-1}, 15.1-55.1

nmol·g^{-1} and 9.4-59.1 nmol·g^{-1} (Fig. 4a). The TPLFAs decreased between March and April but then

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221 increased to a maximum in July or August, before decreasing again in the autumn. The maximum

222 TPLFA content (61.2 nmol·g^{-1}) was observed in the rhizosphere of *S. planiculmis* in August, with the

223 minimum value (23.1 nmol·g^{-1}) observed in October. The TPLFA trends for *Z. latifolia* and *P. australis*

224 were similar to those for *S. planiculmis*, although the maximum TPLFA content for *P. australis* was

225 observed in July rather than in August. These patterns suggest that the wetland microbial biomass

226 varies seasonally (degrees of freedom [df] =7, F=5.134, *p*=0.003), presumably in response to plant

227 phenology, temperature and level of inundation but does not vary with plant species (df=2, F=0.365,

228 *p*=0.704).

229   There were also clear annual cycles in the concentrations of BPLFAs and FPLFAs in the samples

230 collected from each plant. There was a prominent peak in BPLFA concentration in July for all three

231 plants (Fig. 4b) and a peak in FPLFA concentration in September (Fig. 4c) for *S. planiculmis* and *Z.*

232 *latifolia* (with peak fungal concentration in *P. australis* occurring in July). The ratio of BPLFA to

233 TPLFA in the soils from all three plants was always greater than 63%, implying that bacteria are the

234 dominant microbe in the rhizospheres of these wetland plants. The ranges of BPLFA contents in the

235 rhizosphere soils of *S. planiculmis*, *Z. latifolia*, and *P. australis* were 13.6-46.6 nmol·g^{-1}, 14.80-42.7

236 nmol·g^{-1} and 8.2-49.7 nmol·g^{-1}, respectively (Fig. 4b). Significantly lower FPLFA contents were

237 typically observed in the early growth stages of *S. planicumis* (Fig. 4c). This could be due to the

238 relatively short roots of this species and lower associated fungal activity. In contrast, the FPLFA

239 contents were higher in the other two plants in March and April. This may reflect a maintenance of

240 fungal growth via litterfall under these species.

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Fig. 4 Seasonal distributions of (a) total PLFAs, (b) bacterial PLFAs and (c) fungal PLFAs contents in rhizosphere soils

of *S. planiculmis*, *Z. latifolia*, and *P. australis* in the Yeyahu Wetland.

The seasonal distributions of ratios of bacteria: fungi (B: F) and Gram negative bacteria: Gram

positive bacteria (GN: GP) under the three plants are illustrated in Fig. 5. Over most of the sampling

period, the B: F ratios were clearly higher in the rhizosphere soils of *S. planiculmis* than under the
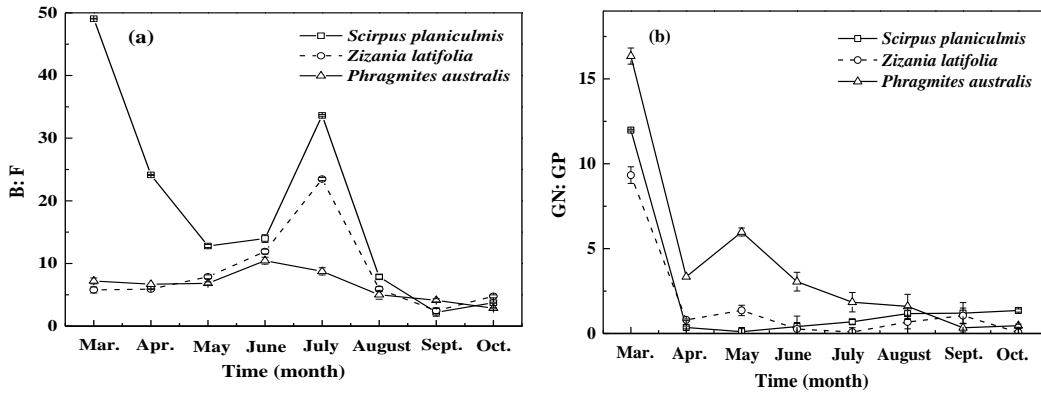
other two plants (Fig. 5a). Peak B: F ratios were typically observed in July or June, except in the case

of *S. planiculmis*, for which B: F peaked in March. The GN: GP ratios of all three plants were highest

in March with an apparent secondary peak in May for *P. australis*. Late summer GN: GP ratios were all

relatively low (Fig. 5b).

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Fig. 5 Seasonal distributions of B: F (a) and GN: GP (b) ratios in rhizosphere soils of *S. planiculmis*, *Z. latifolia*, and *P.*

australis in the Yeyahu Wetland. B: F is the ratio of bacterial to fungal biomass; GN: GP is the ratio of Gram-negative

to Gram-positive bacterial biomass.

The results of cluster analysis on the PLFA data from the soil sampled from the three plants are

presented in Table 2. PLFA biomarkers in July and August were chosen for this analysis based on the

seasonal pattern of PLFAs. There were no significant differences between plant species in the

predominant bacteria present (characterized by PLFA 16:0 as a marker for aerobic bacteria) during the

period of most vigorous plant growth. The observed abundance of PLFA markers for fungi and

anaerobic bacteria were lower than those for aerobic bacteria, suggesting that the root systems in July

and August provide an oxygen-rich habitat for aerobic bacteria (Kirk and Kronzucker, 2005).

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269 **Table 2**. Results of cluster analysis of microbial community attributes in rhizosphere soils of *S. planiculmis*, *Z. latifolia*,

270 and *P. australis* in the Yeyahu Wetland.

Plants	Type	Content (nmol·g⁻¹)	Characteristics	PLFA biomarkers	Indicator species
S. planiculmis	I	16.06	HC, HF	16:0	AB
	II	8.35-13.98	MC, HF	i16:0, 16:1w7c	AB
	III	0.19-2.38	LC, LF	14:0, 15:0, a15:0, i17:0, a17:0, 18:1w7t, 18:2w6,9, 18:1w9c, 20:4w6,9,12,15, 10Me18:0	F, AB, Pr, Ac
Z. latifolia	I	27.01	HC, HF	i16:0	AB
	II	14.10	MC, HF	16:0	AB
	III	0.07-3.50	LC, LF	14:0, 15:0, a15:0, i17:0, a17:0, 17:0, 16:1w7c, 18:1w7, 18:2w6,9, 18:1w9	F, AB, AN
P. australis	I	19.20	HC, HF	16:0	AB
	II	1.58-3.19	MC, MF	i16:0, 16:1w7c, 18:2w6,9, 18:1w9t	AB, F
	III	0.04-0.60	LC, LF	14:0, 15:0, a15:0, i17:0, a17:0, 18:1w7, 18:1w9, 10Me17:0	F, AB, Pr, AN, Ac

271 **Type**: The results are divided into three categories according to the PLFA characteristics

272 **HC:** High content, **MC:** Medium content, **LC:** Low content, **HF:** High frequency **MF:** Medium frequency, **LF:** Low frequency.

273 **AB**: Aaerobic bacteria, **F**: Fungi, **Pr**: Protozoa, **Ac**: Actinomycetes, **AN**: Anaerobic bacteria.

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275 **3.3 Soil phosphorus fractions and microbial biomass phosphorus**

276   Temporal patterns of different P fractions extracted from rhizosphere soils for the three plant

277 species are shown in Fig. 6. Total IP (TIP) increased, under all three plant species, during the early

278 growth stage (March to May) and then dipped in the summer months, before increasing again in the

279 autumn. The IP in all cases was dominated by Calcium-bound P (Ca-P), which accounted for

280 77.9-92.5 % of TIP (reflecting the consistently alkaline pH of this system). The rank order of IP

281 fractions was: Ca-P > occluded P (Oc-P) > Iron-bound P (Fe-P) > exchangeable P

282   (Ex-P) >Aluminium-bound P (Al-P).

283   There was also a pronounced seasonal cycle in the concentrations of Total OP (TOP) observed

284   under all three plant species (Fig. 6b, d and f). Peak TOP concentrations were observed in September

285   and October and lowest concentrations were observed in July under all three plants. The dominant OP

286   fraction in all cases was highly resistant OP (HR-OP), followed by moderately resistant OP (MR-OP),

287   moderately labile OP (ML-OP) and labile OP (L-OP). The concentrations of HR-OP varied from 52.9

288   mg·kg^{-1} to 132 mg·kg^{-1}, accounting for between 31.7 % and 84.5 % of TOP. There were no significant

289   differences in the HR-OP: TOP ratios in the rhizosphere soils associated with the three different plants,

290   indicating that the composition of OP in this system was relatively stable.

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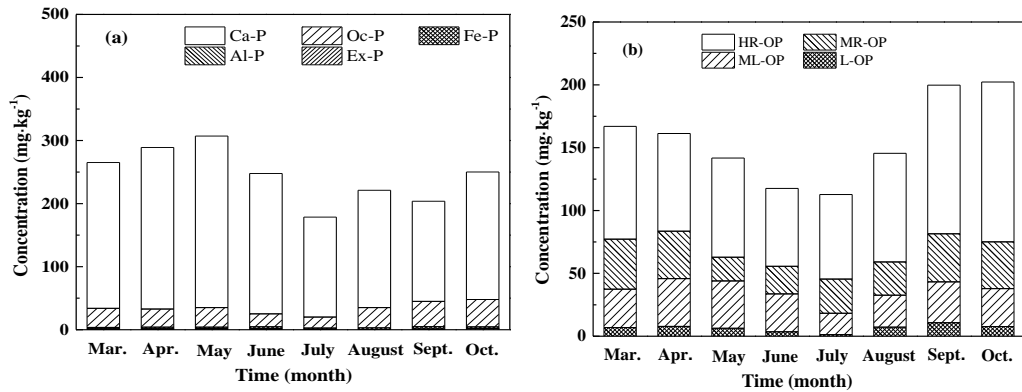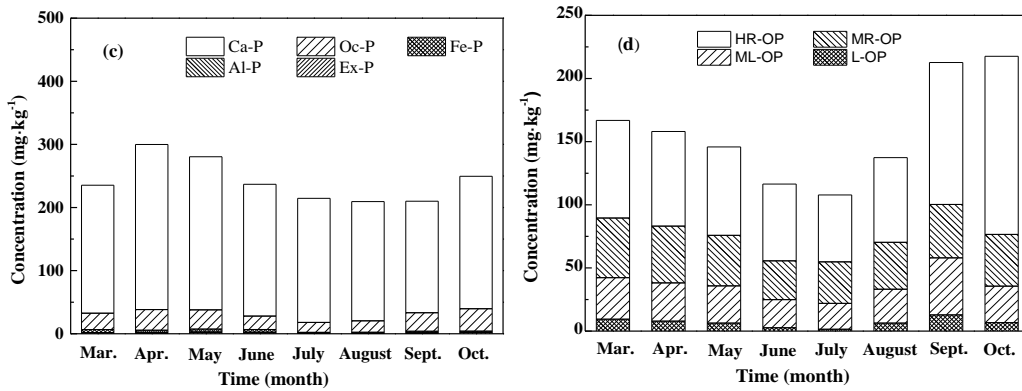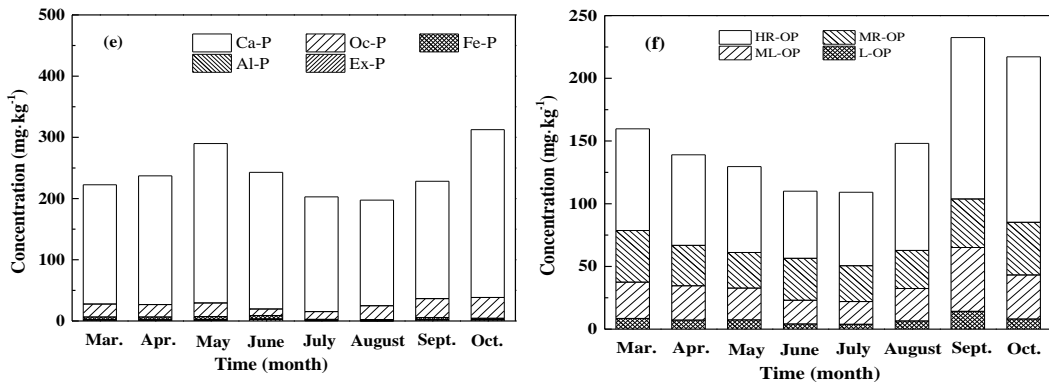
Fig. 6 Seasonal concentration patterns of different inorganic phosphorus (IP) and organic phosphorus (OP) fractions in

rhizosphere soils associated with *S. planiculmis* (a. IP; b. OP), *Z. latifolia* (c. IP; d. OP) and *P. australis* (e. IP; f. OP).

Ca-P is Calcium-bound P; Oc-P is occluded P；Fe-P is Iron-bound P; Al-P is Aluminum-bound P; Ex-P is exchangeable

P; HR-OP is highly resistant OP; MR-OP is moderately resistant OP; ML-OP is moderately labile OP；L-OP is labile

OP.

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302   The seasonal pattern of SMBP is shown in Fig. 7. There was a pronounced increase in SMBP

303   between May and July which may have been driven by increases in temperature, soil moisture content

304   (July is warmer and wetter than spring and autumn in this system) or may have been stimulated by

305   plant-microbe interactions (e.g. "priming" by root exudates: Spohn et al., 2013). There is often ice

306   cover in the Yeyahu wetland in winter which may influence the size and activity of the microbial

307   biomass. Waters began to thaw in early April 2015 and the plants started growing at the same time. For

308   most of the year the SMBP concentrations in samples from all three plants were quite similar, except

309   for April and July when SMBP levels were higher under *P. australis* and in October when SMBP levels

310   were particularly low under *Z. latifolia*.

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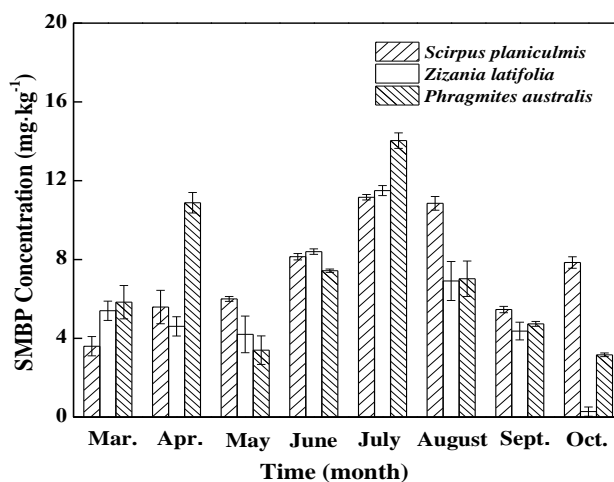
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313   **Fig. 7** Seasonal variations of soil microbial biomass phosphorus (SMBP) in rhizosphere soils of *S. planiculmis*, *Z.*

314   *latifolia*, and *P. australis* in the Yeyahu Wetland.

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317   **3.4 The relationships between PLFA, soil environmental characteristics and different P forms**

318   The correlation analysis suggested that significant relationships exist between microbial

319   community composition and soil properties (Table 3). Positive correlations were observed between

320   TPLFA and (i) MC ($r = 0.665^{**}$, $p<0.01$) and (ii) OM ($r = 0.604^{**}$, $p<0.01$). This implies that the

321   microbial biomass increases in the presence of elevated resources and (unsurprisingly) that microbes in

322   this system are well adapted to high moisture content. A positive correlation was also observed

323   between FPLFA and AHN ($r = 0.506^{*}$, $p<0.05$). This suggests that enhanced development of fungi may

324   be partly responsible for increasing nitrogen availability. Soil OM and N content are important factors

325   for soil microbial growth and activity. Soils with high nutrient availability tend to be conducive to

326   microbial accumulation and retention and, therefore, tend to support higher levels of microbial activity.

327

328   **Table 3**. Pearson correlation coefficients (*r*-values) between PLFA and soil environmental characteristics in rhizosphere

329   soils from the Yeyahu Wetland.

	TPLFAs	BPLFAs	FPLFAs	GP	GN	MC	pH	AHN	OM
TPLFAs	1.00	0.715^{**}	0.208	0.226	0.590^{**}	0.665^{**}	0.095	-0.030	0.604^{**}
BPLFAs		1.00	0.017	0.371	0.093	0.348	0.172	-0.255	0.654^{**}
FPLFAs			1.00	-0.077	0.211	0.338	0.077	0.506^{*}	0.152
GP				1.00	-0.289	0.022	0.192	-0.218	0.117
GN					1.00	0.406^{*}	-0.156	0.366	0.302
MC						1.00	0.111	0.014	0.238
pH							1.00	-0.379	-0.102
AHN								1.00	-0.077
OM									1.00

330   ****Significant at *P*<0.01；*Significant at *P*<0.05**

331

332   Correlation coefficients between total, bacterial and fungal PLFAs and the concentrations of

333   different forms of OP in sampled rhizosphere soils are shown in Table 4. There were strong

334   correlations between TPLFAs and BPLFAs, reflecting the dominance of bacterial markers in the PLFA

335   mix. TPLFAs and BPLFAs were also strongly correlated with SMBP ($p<0.01$) which underpins the

336   utility of the PLFA method as an indicator of microbial biomass. TPLFAs were negatively correlated

337   with HR-OP (r = -0.534**, $p<0.01$). Correlations between BPLFAs and L-OP, ML-OP and HR-OP

338   were also highly significant ($p<0.01$) and negative (r values -0.696**, -0.706** and -0.615**,

339   respectively). Unsurprisingly, SMBP was also significantly negatively correlated with L-OP (r =

340   -0.608**, $p<0.01$), ML-OP (r = -0.593**, $p<0.01$) and HR-OP (r = -0.552**, $p<0.01$), reflecting a high

341   degree of cross correlations between SMBP and the PLFA indices.

342

343   **Table 4**. Pearson correlation coefficients (r-values) between PLFAs, different OP forms and SMBP in rhizosphere soils

344   from the Yeyahu Wetland.

	TPLFAs	BPLFAs	FPLFAs	GP	GN	L-OP	ML-OP	MR-OP	HR-OP	SMBP
TPLFAs	1.00	0.715**	0.208	0.226	0.590**	-0.169	-0.394	-0.128	-0.534**	0.556**
BPLFAs		1.00	0.017	0.371	0.093	-0.696**	-0.706**	-0.392	-0.651**	0.819**
FPLFAs			1.00	-0.077	0.211	0.397	0.179	0.013	0.431*	0.101
GP				1.00	-0.289	-0.294	-0.229	-0.180	-0.063	0.349
GN					1.00	0.257	-0.027	0.258	-0.062	-0.019
L-OP						1.00	0.846**	0.574**	0.667**	-0.608**
ML-OP							1.00	0.363	0.613**	-0.593**
MR-OP								1.00	0.439*	-0.358
HR-OP									1.00	-0.552**
SMBP										1.00

345   **Significant at $P<0.01$；*Significant at $P<0.05$

346

Analogous correlation coefficients for IP are shown in Table 5. There were highly significant negative correlations between TPLFAs and Ex-P ($p<0.01$), Oc-P ($p<0.05$) and Ca-P ($p<0.05$). Correlations between SMBP and various IP fractions were also negative (and, in the case of Oc-P, highly significant). The only significant correlation between FPLFAs and IP fractions was with Ca-P (which was highly significant and negative, $p<0.01$). Unsurprisingly, most P fractions were positively correlated with one another, although these relationships were not always significant.

Table 5. Pearson correlation coefficients (*r*-values) between PLFA, different IP forms and SMBP in rhizosphere soils from the Yeyahu Wetland.

	TPLFAs	BPLFAs	FPLFAs	GP	GN	Ex-P	Al-P	Fe-P	Oc-P	Ca-P	SMBP
TPLFAs	1.00	0.715**	0.208	0.226	0.590**	-0.576**	-0.197	-0.095	-0.500*	-0.443*	0.556**
BPLFAs		1.00	0.017	0.371	0.093	-0.456*	-0170	-0.268	-0.686**	-0.442*	0.819**
FPLFAs			1.00	-0.077	0.211	-0.216	-0.252	0.005	0.158	-0.582**	0.101
GP				1.00	-0.289	-0.336	-0.272	-0.567**	-0.091	-0.222	0.349
GN					1.00	-0.140	-0.097	0.277	-0.028	-0.226	-0.019
Ex-P						1.00	0.537**	0.537**	0.188	0.419*	-0.461*
Al-P							1.00	0.415*	-0.254	0.535**	-0.257
Fe-P								1.00	-0.064	0.203	-0.189
Oc-P									1.00	0.171	-0.557**
Ca-P										1.00	-0.432*
SMBP											1.00

**Significant at *P*<0.01；*Significant at *P*<0.05

Soil microbes, including both bacteria and fungi, play an important role in soil P immobilization by transforming P from active (inorganic) forms into soil microbial biomass P (SMBP). This is reflected in the negative correlation between SMBP and L-OP, as shown in Fig. 8.

363
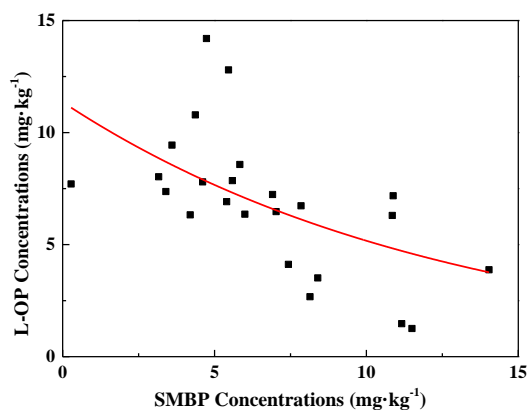
Fig. 8 The relationship between soil microbial biomass P (SMBP) and soil active P (L-OP) in rhizosphere soils from the

Yeyahu Wetland. The red line shows the best fit exponential relationship ($Y=13.357e^{-0.119X}$, $r^2=0.4168$).

366

4. Discussion

Marked seasonal patterns were revealed in the size and composition of the microbial communities

associated with the rhizosphere soil sampled from three commonly-occurring emergent wetland plants.

Seasonality was also observed in the sizes of a number of organic and inorganic P pools in these

rhizosphere soils, which were variously correlated with soil microbial characteristics. Here, we discuss

possible explanations for the observed phenomena and explore the implications of our findings for

understanding and managing wetland processes.

4.1 Seasonal dynamics of microbial community in different rhizosphere soils

PLFAs are widely used in microbial ecology as indicators of both the size and composition of the

microbial community (Zelles, 1999; Frostegard et al., 2011). We observed significant and consistent

seasonal changes in the concentrations of TPLFAs, BPLFAs and FPLFAs in soil samples from the

rhizosphere of the different plants (Fig. 4). Previous studies have shown that microbial community

379 composition is influenced by a combination of soil properties (Huang et al., 2016; Liu et al., 2012) and

380 other environmental factors (Steenwerth et al., 2008), including the effect of toxic stressors (Frostegard

381 et al., 1993; Butler et al., 2012). Pearson product moment correlations suggest that the soil organic

382 matter content and soil nitrogen content appear to be important factors affecting the microbial

383 community in the rhizospheres of all three plants. Total PLFAs were significantly correlated with a

384 more conventional measure of the size of the microbial biomass P (SMBP), except for the March

385 sampling (when SMBP was low but TPLFA concentration was high). The seasonal pattern in microbial

386 biomass which peaked in July or August can be explained in a number of ways, including the seasonal

387 development of the wetland vegetation which is widely believed to have a symbiotic relationship with

388 the microbial community in the rhizosphere (e.g. via the exchange of labile carbon in root exudates for

389 enhanced mineralization of N and P: e.g. Wheatley et al., 1990; Nobili et al., 2001; Spohn et al., 2013).

390 Other factors which may have been influential include temperature (Schostag et al., 2015), soil water

391 content (Brockett et al., 2012) and the wider availability of soil organic matter (with associated

392 mineralisable C, N and P: Jirout et al., 2011). We observed that bacteria account for the largest fraction

393 of PLFAs extracted from the rhizosphere soil under all three plants, suggesting that bacteria may be

394 more abundant than fungi in the soils sampled. The temporal trend for BPLFAs was similar to that for

395 TPLFAs, confirming the importance of bacterial PLFA as a contributor to PLFAs. Bacteria typically

396 belong to the main decomposers in soil and are known to mediate many biogeochemical processes and

397 associated ecosystem functions (Strickland & Rousk, 2010). FPLFA concentrations varied with plant

398 type and plant growth stage. Maximum concentrations were observed late in the growing season,

399 inferring that litter inputs may be more influential for the fungal community than factors like

400 temperature (Santonja et al., 2017).

401　　　　The ratio of bacterial to fungal (B: F) PLFAs varied with plant type. Different plants produce

402　　different quantities and qualities of root exudates and have different growth habits. This means that

403　　they support the flora of the rhizosphere to different degrees and at different times (Niu et al., 2012).

404　　Our data show that the B: F ratio in the rhizosphere varied significantly with different plant species

405　　(distinctly higher for *S. planiculmis* during March and April than with the other two plants). This

406　　discrepancy may have been the result of differences in moisture content and nutrient availability. *S.*

407　　*planiculmis* mainly inhabit the littoral zone, where flood and ebb can cause alternate wetting and

408　　drying which can change redox status and nutrient supply. Rewetting of dry soil can stimulate C and N

409　　mineralization (Haynes and Swift, 1989; Gordon et al., 2008) and can, hence, lead to an increase in

410　　soluble P concentrations in the soil solution (Dinh et al., 2016). This could influence the relative

411　　abundance of bacteria and fungi, which have different life history strategies and sensitivities. For

412　　example, some studies have shown that bacterial communities are more sensitive to soil moisture

413　　variations than fungal communities (Paul and Clark, 1989; Kaisermann et al., 2015) in part because

414　　fungi, by virtue of their hyphal systems, are better able access parts of the soil at moisture contents

415　　which severely limit bacteria movement and solute diffusion (Wilson and Griffin, 1975). The increases

416　　in B: F ratios observed for all three plants in July (Fig. 5a) may have been the result of abundant

417　　rainfall and high soil water contents in this period which could have reduced oxygen concentrations.

418　　The activity of fungi and actinomycetes is often inhibited by low oxygen tensions (Vinten and Smith,

419　　1993) which may have limited fungal growth (Yuste et al., 2011).

420　　　　　The GN: GP ratio also varied seasonally in all three plants and reached a peak in March. Soil

421　　nutrient levels and substrate contents were high in March due to the organic matter and nutrient

422　　accumulation from the previous autumn, reducing the need for GP and GN bacteria to compete for

423 nutrients (Bartelt-Ryser et al., 2005). Previous studies have shown that GN bacteria are active

424 heterotrophs in contrast with GP bacteria (Wang et al., 2017), which may have resulted in a higher GN:

425 GP ratio in March in the presence of available nutrient and energy resources. However, nutrients

426 availability decreased sharply thereafter along with the growth of plants. GP bacteria tend to be more

427 competitive under conditions with limited nutrient availability (Waldrop et al., 2004). Thus, a

428 systematic reduction in available nutrients may have caused a decrease in the GN: GP ratio.

429 The most abundant bacteria were aerobic, represented by PLFA markers 16:0, 16:1w7c, i16:0

430 under *S. planiculmis*; i16:0, 16:0 under *Z. latifolia* and 16:0, i16:0, 16:1w7c under *P. australis*,

431 respectively. Fatty acid 16:0 has been reported as being ubiquitous in many microbial communities

432 (Moeskops et al., 2010). The dominance of aerobic bacteria is unsurprising because the root systems of

433 many wetland plants are known to act as conduits for oxygen transport, particularly under conditions

434 of active plant growth (Kirk and Kronzucker, 2005).

435 **4.2 Seasonal variations in phosphorus fractions in different rhizosphere soils**

436 Total inorganic P increased slightly during the early stages of plant growth (Fig. 6), which could

437 be related to increased microbially-mediated mineralization with increased temperatures or as a

438 consequence of priming by root exudates (Nobili et al., 2001; Spohn et al., 2013; Karasawa et al., 2015)

439 coupled with relatively low plant nutrient requirements (Bernadine et al., 2015). The attached layer of

440 mucigel in plant roots can not only provide a nutrient source for rhizosphere microorganisms, but may

441 also help retain various enzymes released from plant roots (Wright et al., 2009). There may also have

442 been an effect of OP carry-over via the litter left behind by dead plant biomass in previous years (Wang

443 et al., 2017). The main form of IP was Ca-P, which is relatively stable and is considered a permanent P

444 store (Dotaniya et al., 2013). Previous studies have shown that Ca-P content is commonly driven by

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445 pH. High concentrations are normally found in high pH soils due to a reduced concentration of free

446 Fe and Al ions and a decreasing solubility of Ca-P minerals at increasing pH, resulting in the formation

447 of insoluble calcium salts (Haynes, 1982; Yang et al., 2011; Wang et al., 2017).

448 Total organic P decreased systematically in the soils of all three plants from March to July and

449 then increased to October (Fig. 6). The decrease over the main period of plant growth may reflect

450 enhanced mineralization with warming temperatures (and perhaps enhanced by priming in the

451 rhizosphere). This is reflected to some extent in an increase in TIP to May, after which plant uptake is

452 likely to have removed any available (dissolved) IP resulting from OP mineralization. The increase of

453 OP in autumn may reflect plant senescence and enhanced litter (and associated nutrient) inputs (Cao,

454 2012; White et al., 2012; Kopáček et al., 2017). Increased acid phosphatase activity and litter fall can

455 accelerate the release of OP into the soil solution and improve P availability (Zhu et al., 2017). The fact

456 that maximum HR-OP concentrations occurred in October under all three plants could be connected to

457 litter and plant residue inputs during senescence, which starts in late September in this system.

458 **4.3 Potential interactions between the microbial community and phosphorus fractions**

459 Both TPLFAs and BPLFAs were negatively correlated with different forms of soil P (in effect, the

460 higher the microbial biomass, the lower the extractable P). Microbial communities promote mineral

461 dissolution (e.g. via the secretion of organic acids: Zhu et al., 2018), organic matter mineralization

462 (Hoyle et al., 2018) and improve plant nutrition (Gadd et al., 2010), although they can also immobilize

463 P via uptake if P is in short supply relative to other resources (Sarker et al., 2018). By increasing CO_2

464 partial pressures via respiration they may also be able to reduce local pH (depending on how buffered

465 the system is: Kim et al., 2003). This could increase phosphate adsorption to charged surfaces (Haynes,

466 1982) and increase the concentrations of metal cations which fix P via the formation of insoluble

27

467   precipitates: Specifically Ca in alkaline soils and Fe and Al in acidic soils (Hinsinger et al., 2001).

468   Enhanced respiration will also deplete dissolved oxygen concentrations which could promote the

469   reduction of Fe^{3+} to Fe^{2+} in mineral complexes, which has been shown to release P into the soil

470   solution (Carlyle et al., 2001). The temporal variations in the size and composition of rhizosphere

471   microbial communities which we observed in this study are undoubtedly linked to the changes in the

472   abundance of different P fractions and with interactions with the plants (e.g. uptake of P and N and the

473   return of resources to the soil via plant litter and root exudates). Bacteria typically accounted for >

474   63 % of the rhizosphere flora by PLFA abundance and can make use of HR-OP, Oc-P and Ca-P.

475   Although there was no significant correlation between GN and GP and the concentrations of different

476   OP fractions, a significant negative relationship (p<0.01) was observed between GP and Fe-P. This

477   suggests that Gram-positive bacteria may be able to activate (and deplete) Fe-P in soils.

478   There are many different organic P compounds in soil including phosphomonoesters, phosphodiesters

479   (including phospholipids), nucleic acids, phytic acid and phosphotriesters (Behera et al., 2014).

480   Nucleic acids and phytic acid tend to be relatively more abundant and phospholipids much less so. In

481   all cases, OP must be mineralized into plant-available IP ($H_2PO_4^-$, HPO_4^{2-} and $PO4^{3-}$). The negative

482   relationship between SMBP and most forms of OP and IP suggests that microorganisms play an

483   important role in mobilizing P which can be subsequently taken up by the plant or immobilized by

484   microorganisms themselves (Turner et al., 2012). The microbial biomass P can itself be mineralized

485   once the organism dies (Richardson et al., 2011). The strongest relationships were observed between

486   SMBP and the OP fraction suggesting that SMBP acts as an important hub for OP transformation.

487   Finally, it has been shown that the accumulation of labile P can be quickly precipitated as Fe, Al and

488   Mn minerals (Costa et al., 2016), and that, with microorganisms present, Al-P and Ca-P can be

489 transformed into ML-OP, Oc-P and residual P (Yin et al., 2013).

490

5. Conclusions

492 Consistent and systematic seasonal patterns in different P fractions and in indicators of the size

493 and composition of the microbial biomass were observed in the rhizosphere soils associated with three

494 wetland plants. Significant correlations were observed which suggest that these patterns are linked.

495 Although it is difficulty to tease these relationships apart, they are undoubtedly influenced by the

496 seasonal cycle of plant growth and senescence (and the associated close interactions between plants

497 and the soil microbial community, particularly in the rhizosphere). The negative correlations observed

498 between soil P concentrations and indicators of microbial abundance (e.g. SMBP, TPLFA and BPLFA)

499 suggest that microbes can make use of HR-OP, Oc-P and Ca-P in plant rhizospheres. These results

500 demonstrate that microorganisms are the main driving force for the transformation of P and can have a

501 significant impact on P interception by wetland plants. However, the precise mechanisms involved

502 still need to be explored by further experiments which should target P transformation by phosphate

503 solubilizing microorganisms at the molecular and genetic levels.

504

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