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Spatiotemporal variations and relationships of phosphorus, phosphomonoesterases, and bacterial communities in sediments from two Chilean rivers

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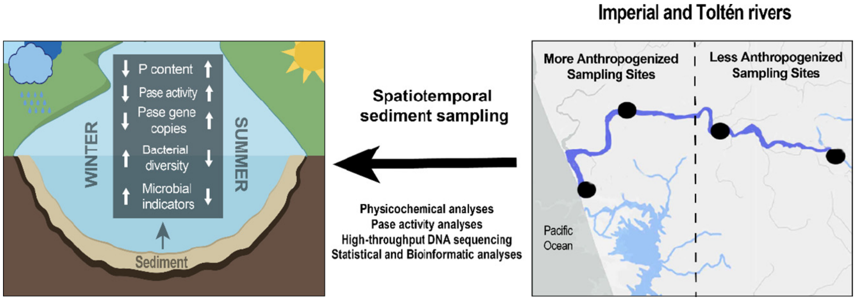
HIGHLIGHTS

- P contents, Pase activities, and bacterial community structures were studied in Chilean river sediments.
- Higher P contents and Pase activities were observed in summer and anthropized sites.
- Higher diversities, abundances, and predicted functionalities of bacteria was observed in winter.
- Relations between P content, Pase activity, and bacterial community structure were established.
- The Coxiellaceae family was suggested as a microbial indicator for anthropogenic impact in sediments.

GRAPHICAL ABSTRACT



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ABSTRACT

Rivers are important ecosystem services providers in southern Chile; however, the microbiology of most rivers are understudied. This is particularly true for phosphorus (P) recycling by bacterial activity. Here, we studied the physicochemical properties, P contents, phosphomonoesterase (Pase) activities, and bacterial Pase genes (*phoD*, *phoC*, and *phoX*) in Chilean river sediments (Imperial and Toltén rivers, Chile) collected in sites with less and more degrees of anthropogenic influence during the summer and winter seasons. The richness, diversity, composition, and predicted function of the bacterial communities were addressed, as well as the spatiotemporal relationships among P-associated parameters and bacterial communities. Our results showed spatiotemporal variations, highlighting higher values of the temperature, conductivity, nutrients (C, N, P), Pase activity, and Pase genes in sediments collected in sites more anthropogenized during summer. In contrast, higher richness, diversity, and abundance of total bacteria were found in sediments collected in winter. The phylum Proteobacteria and heterotrophy were the most relatively abundant bacterial taxa and predicted function, respectively. The

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Phosphomonoesterases
River sediment

differentiation among winter and summer sediments was also revealed and the relationships between bacterial community and P-associated parameters were confirmed. Our analysis also highlighted the positive relationship between Pase genes with Gemmataceae, Xanthomonadaceae, and Chitinophagaceae families, and between Pase activity and P_o with Chromatiaceae and Desulfobacteraceae families. This study represents an initial investigation aimed to unravel the compositions and relationships of bacterial communities with P recycling in these sediments, which are threatened by anthropogenic activity (*e.g.*, agriculture, industry, cities, *etc.*). This information may be used in the design of countermeasures to prevent deterioration of Chilean rivers, which are relevant as ecosystem service providers.

1. Introduction

Rivers are natural freshwater ecosystems where biogeochemical processes are directly involved in the recycling of nutrients such as nitrogen (N) and phosphorus (P) (Paerl, 2009). In these ecosystems, eutrophication can occur as a result of large P inputs from anthropogenic sources (*e.g.*, industries, agriculture, and cities), and where the slow-growing microorganisms may be overtaken by blooms of fast-growing microorganisms (*e.g.*, phytoplankton) (Camacho, 2013; Sinha et al., 2017). Eutrophic zones in rivers can persist for long periods due to multiple factors dominating the availability of inorganic P (P_i) fractions, mostly as dissolved P_i, which could be released from the hydrolysis of sedimentary organic P (P_o) by the action of microorganisms. Organic P ranges between 25 and 75% of the total P (TP) in river sediments (Lü et al., 2016; Ni et al., 2019a; Ni et al., 2019b) and can includes a large amount of insoluble forms such as inositol phosphates, phosphodiesters (PDEs), and phosphomonoesters (PMEs) (Baldwin, 2013). Some of the P_o forms in river sediments can be stabilized by complexation or precipitation with organic matter (OM) and metal ions (*e.g.*, iron, aluminum, and calcium). On the other hand, spatiotemporal variations in physicochemical and biological properties of river sediments can promote variable extent of hydrolysis of P_o by bacterial enzymatic activity releasing dissolved P_i, and feed to phytoplankton blooms (Worsfold et al., 2008; Kang et al., 2018; Yuan et al., 2019; Ni et al., 2019a, 2019b). Therefore, the eutrophication potential of different transects of a river could vary significantly both in space and time.

In recent years, the bulk of evidence has pointed to the direct role of benthic bacterial communities in recycling P and fueling eutrophication processes in freshwater ecosystems through the release of alkaline (ALP; EC 3.1.3.1) and acidic (ACP; EC 3.1.3.2) phosphomonoesterases (Pases) targeting PDEs and PMEs (Worsfold et al., 2008; Zhao et al., 2012; Yang et al., 2017). Both pH-dependent isoforms are ubiquitous in the environment and are sensitive to the P_i depletion stress (Yang et al., 2017; Pandey and Yadav, 2017; Ni et al., 2019b). Furthermore, the abundance of two homologs of ALP coding genes, *phoD* and *phoX*, as well as *phoC* class A gene for ACP, have been positively correlated with total Pase activities in the environment (Acuña et al., 2016; Sakurai et al., 2008; Sebastian and Ammerman, 2009; Fraser et al., 2017). Although this relationship is often a good indicator of bacterial activities concerning P_o status, the specific ecological roles of Pase-harboring bacterial populations in freshwater ecosystems are poorly understood (Zheng et al., 2019). In this sense, studies focused on unraveling the diversity, function, and structures of bacterial communities from estuarine, stream, and river sediments by using high-throughput DNA sequencing (HTS) analysis have been reported (Staley et al., 2013; Li et al., 2016; Lu et al., 2016; Kaestli et al., 2017; Roberto et al., 2018; Zhang et al., 2019a; Hu et al., 2020). Understanding the role of P in modulating the microbial community assembly by specific bacterial taxa as putative microbial indicators of P pollution and eutrophication is a crucial component in the P_o recycling of rivers. Thus, studies on the impacts of P content and Pase activity in river sediments by bacterial activity are required to understand the processes as well as to manage to alleviate the eutrophication in rivers.

Imperial and Toltén rivers are two important water bodies in southern Chile that harbor highly diverse wildlife, and facilitate the rise of the human settlements in the region through their effective ecosystem services (Peña-Cortés et al., 2011; Rojas et al., 2019). Their catchments have been historically threatened by intensive anthropogenic pressures (*e.g.*, agriculture, forestry, livestock, and aquaculture) that increase P inputs into their waters (Clapp, 1998; Pizarro et al., 2010; Fierro et al., 2015; Fierro et al., 2017). However, the effect of P enrichment on river flora and fauna of both rivers and their relations with eutrophic episodes have not been sufficiently investigated thus far (Pizarro et al., 2010; Montecino et al., 2016), particularly those microorganisms involved in P cycling in their sediments. With this research gap in mind, in this study we hypothesized that P content in sediments modulates the spatiotemporal variation and relationships among Pase activity, abundance of bacterial Pase genes, and composition of the bacterial communities in two Chilean rivers (Imperial and Toltén) during winter and summer seasons.

2. Material and methods

2.1. Study site description

The Imperial and Toltén river catchments are featured by a temperate rainy Mediterranean climate, with annual precipitations approximating ~1300 mm. The wet season appears in late autumn and winter, whilst the dryer season is in late spring and summer (Pizarro et al., 2010). A predominance of erosion reliefs characterizes their sections, consisting mostly of ridges and platforms in metamorphic rock. Morphogenetically, their units are associated with differential water erosion processes enhanced by the low vegetation cover resulting from deforestation and inappropriate land use. Consequently, their alluvial plains are associated with the accumulation of nutrient-rich eroded material, which is transported and deposited downstream by watercourses (Peña-Cortés et al., 2014, 2021). Both catchments are dominated by clay loam and volcanic ash-derived soils known for their high Po accumulating capacities (Borie and Rubio, 2003). Their percentages of land use are mainly represented by agriculture (1%), forestry (4–12%), and livestock (7–30%). Important point and nonpoint sources of water pollution have been identified in both catchments, although the trophic status of both water bodies has not been established yet (DGA, 2004; Pizarro et al., 2010).

2.2. Sampling and physicochemical properties of river sediments

Sediment samples from rivers were collected to a depth 10 cm (with overlying 5 m water column) at four sites in Imperial (Nueva Imperial [NI], Carahue [CA], Tranapuente [TR], and Nehuentue [NE]) and Toltén (Villarrica [VI], Pitrufquen [PI], Nueva Toltén [NT] and La Barra [LB]) rivers during the winter (W) of 2018 and the summer (S) of 2019 (Fig. 1). Among these sites, NI, CA, VI, and PI were categorized as “less” anthropogenically influenced whilst the samples TR, NE, NT, and LB were categorized as “more” anthropogenically influenced from the natural source (Andes mountains) to the sea coasts (downstream effluents) in both river catchments as recommended by Bol et al. (2018) based on the origin of dragged nutrients. The sampling was conducted following the US Environmental Protection Agency (EPA) recommendations

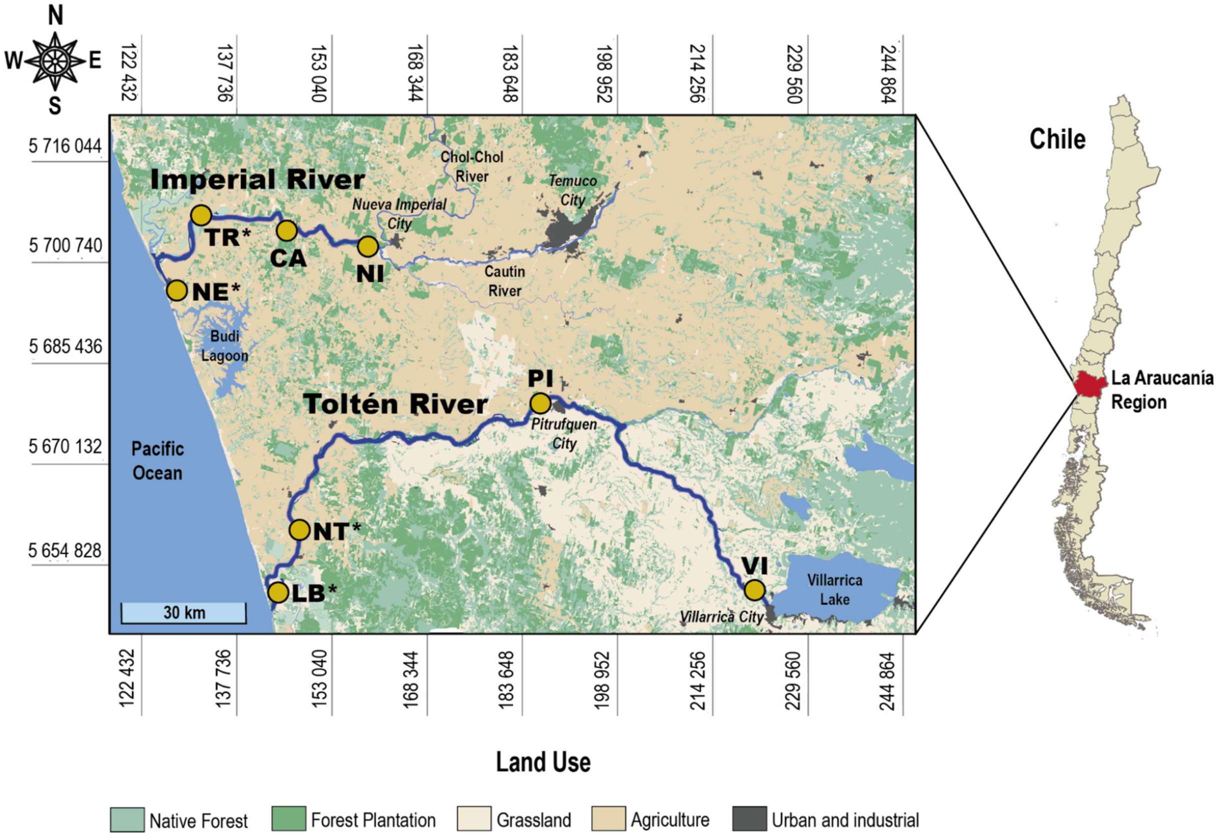

Fig. 1. Schematic distribution of sediment sampling sites from Imperial and Toltén rivers, La Araucanía Region, Chile. Sampling sites for Imperial River were coded as Nueva Imperial (NI), Carahue (CA), Tranapuente (TR), and Nehuentue (NE). Sites for Toltén River were coded as Villarrica (VI), Pitrufquen (PI), Nueva Toltén (NT), and La Barra (LB). In relation to the city downstream effluents and land use, the samples NI, CA, VI, and PI were categorized as 'less' anthropogenic influenced whilst the samples TR, NE, NT, and LB were categorized as 'more' anthropogenic influenced. Asterisks denote the more anthropogenically influenced sites in both rivers. The georeferencing is based on the universal transversal Mercator (UTM) system.

(Taft and Jones, 2001). For each site, nine sediment samples were randomly collected using a Petersen-like grab sampler over 10 m radius. From each sampling site, three samples were randomly chosen and homogeneously mixed *in situ* to fill sterile flasks with 500 mL of composite sample, yielding three composite samples per sampling site. Thus, a total of 48 sediments samples (2 rivers × 4 sites × 2 seasons × 3 composite samples) were collected. Each composite sample was then aseptically placed into hermetic plastic flasks, and then temperature (T°), conductivity (Cond), dissolved oxygen (DO), and pH were measured *in situ* with a HI 9829 multiparameter sensor (Hanna Instruments, Inc., Rhode Island, USA).

After sampling, all sediment samples were immediately kept on ice (4 °C), and transported to the Applied Microbial Ecology Laboratory (EMALAB) at the Universidad de La Frontera, Chile. Then, each composite sample was subdivided into three subsamples for different analyses. One split was immediately subjected to the measurement of enzymatic activity (ALP and ACP), whilst the second set was frozen at −80 °C for further genomic DNA (gDNA) extraction, and the third was freeze-dried in a LyoQuest HT40 freeze-drier (Telstar®). The freeze-dried sediment was sieved at 150 μm size fraction and stored at room temperature for the measurement of carbon (C), N, and P contents as follows. Total carbon (TC) and total nitrogen (TN) in 1.5–2.5 mg in freeze-dried sediment samples measured using an automated elemental analyzer EA 3000 (Eurovector, Milano, IT) according to the method described by Yang et al. (2010). The elemental composition was calculated by interpolation into a suitable calibration curve ($r^2 = 0.98$) using EDTA as standard (99.4% purity; LECO®, USA) and expressed as mg of C or N per kg of dried weight (dw sediment mg kg^{-1} dw).

All data on physicochemical analyses were spatiotemporally contrasted by one-way ANOVA with Tukey's honestly significant difference (HSD) test.

2.3. Phosphorus content in river sediments

The contents of P_i, P_o, and TP in freeze-dried sediment samples were measured after sequential extraction using the method described by Ivanoff et al. (1998), which fractionates P into readily labile P_i and P_o, microbial P_o, moderately labile P_i, moderately labile P_o (fulvic acid-bonded fraction), and non-labile P_o (humic acid-bonded fraction, and residual P_o) P pools using the following order of extractants: i) 0.5 M of $NaCO_3$ at pH 8.5 and incubated at room temperature for 16 h, ii) 1 M of HCl and incubated at room temperature for 3 h, iii) 0.5 M of NaOH and incubated at room temperature for 16 h, and iv) incinerated with 1.0 M of H_2SO_4 at 550 °C for 1 h. The extracted P_i in each fraction was determined by the molybdenum blue method (Murphy and Riley, 1962), and concentrations obtained by absorbance interpolation into a suitable standard curve ($r^2 = 0.99$) and expressed as mg kg^{-1} dw. The P_o in each extracted fraction was firstly hydrolyzed with appropriate extractant (Rowald and Haygarth, 1997) and then the P_i released was measured by the molybdenum blue method. The P_o content was calculated as the difference between the hydrolyzed and dissolved P_i values. Similarly, the total P_i and P_o content in each sediment was the sum of P_i and P_o in each pool, whilst the total P (TP) was the sum of both P forms. The data obtained from each P pool were spatiotemporally analyzed by one-way ANOVA with Tukey's honestly significant difference (HSD) test. Detailed results on sequential extraction are shown in Supplementary Table ST1.

2.4. Phosphomonoesterase activity in river sediments

The activity of ALP and ACP were analyzed according to the Tabatabai and Bremner (1969) method using pure para-nitrophenyl phosphate (*p*-NPP) (Sigma-Aldrich, St. Louis, USA). Briefly, 0.2 g of fresh sediment samples were incubated with 1 mL of modified universal buffer (MUB) containing *p*-NPP (0.05 M) at pH 11.0 for ALP and 6.5 for ACP. The reactions were incubated for 1 h at 37 °C and then stopped by adding 1 mL of CaCl (0.5 M) and 4 mL of NaOH (0.5 M), respectively. To avoid potential errors caused by *p*-NPP adsorption in the sediment, blanks were prepared receiving the *p*-NPP solution promptly after incubation. Precipitates in samples and blanks were then removed by filtration and concentration of *p*-nitrophenol (*p*-NP) formed in the supernatant was determined by absorption at 420 nm using a microplate spectrophotometer (Multiskan™ GO, Thermo Fisher Scientific, Inc., MA, USA). The values of absorbances were interpolated into a suitable calibration curve ($r^2 = 0.97$) and expressed as milligrams of *p*-NP released by 1 g sediment per hour dw (mg *p*-NP g^{-1} h^{-1} dw).

The data obtained from Pase activity were spatiotemporally contrasted by one-way ANOVA with Tukey's honestly significant difference (HSD) test.

2.5. Bacterial phosphomonoesterase genes in river sediments

The extraction of gDNA from the sediments (~150 mg dw) was carried out using DNeasy® PowerBiofilm Kit (QIAGEN, Carlsbad, CA, USA) according to the manufacturer protocol. The gDNA concentrations were determined with a broad range Quant-iT™ dsDNA Assay Kit (Thermo Fisher Scientific) and measured in a Qubit4™ Fluorometer (Thermo Fisher Scientific). The extracts of gDNA were stored at −20 °C until analysis.

The abundance of bacterial Pase genes (*phoC*, *phoD*, and *phoX*) and total bacterial community based on 16S rRNA genes were estimated by quantitative polymerase chain reaction (qPCR) with a StepOne Real-Time PCR System (ThermoFisher Scientific, Inc., Waltham, MA, USA) using PowerUp™ SYBR™ Green Master Mix (Applied Biosystems™, Foster City, CA, USA) and ~ 25 ng μL^{-1} of gDNA. The primer sets and conditions used for quantification of *phoC* (Fraser et al., 2017), *phoD* (Sakurai et al., 2008), *phoX* (Sebastian and Ammerman, 2009), and 16S rRNA (Shade et al., 2013) genes by qPCR are shown in Supplementary Table ST2. The copy numbers of targeted genes were calculated using standards of each gene, built with dsDNA gBlock® Gene Fragments (Integrated DNA Technologies, Inc. Iowa, USA), and the equation ([concentration of the dsDNA gBlock® Gene Fragment in ng μl^{-1}] × [molecular weight in fmol ng^{-1}] × [Avogadro's number] = copy number) following the method described by Whelan et al. (2003). Absolute quantification (AQ) of bacterial genes was expressed as copy number per gram of dw (gene copy g^{-1} sediment dw) and later used to determine the relative quantification (RQ) of Pase genes relative to 16S rRNA genes as described by Acuña et al. (2016).

The data obtained from Pase gene quantification were spatiotemporally contrasted by one-way ANOVA with Tukey's honestly significant difference (HSD) test.

2.6. Relationship of P-associated parameters in river sediments

Principal component analysis (PCA) built in R *stats* v3.6.2 package was used to determine the structure and relationship between physicochemical properties, P content, Pase activity, and bacterial Pase genes present in sediments. The PCA was visualized with the *ggbiplot* function in R. As physicochemical properties were not determinant in PCA, this result was complemented by analysis of Pearson's correlation coefficients of P content, Pase activity, and bacterial Pase genes to determine the significance (*P*-value) of correlations using the *BSDA* function in R. The results of both analyses were considered to select gDNA samples for the composition, spatiotemporal variation and microbial indicators of bacterial community in river sediments.

2.7. Composition, spatiotemporal variation and microbial indicators of bacterial community in river sediments

Based on PCA and Pearson's correlation results, gDNA extracts obtained in Section 2.4 were selected, contrasting samples collected from more and less anthropogenically influenced sampling sites. The selected samples were from: NI and TR from Imperial River and PI and LB from Toltén River collected during summer and winter.

The gDNA extracts were used for the preparation of 16S rRNA gene libraries following the instructions described in the Illumina™ Metagenomic Sequencing Library Preparation (Illumina, 2013) with slight modifications. Briefly, ~1 ng μL^{-1} of gDNA samples were subject to amplification of the V3 ~ V4 region of 16S rRNA by endpoint PCR using 1 μM of the universal Bakt_341f and Bakt_805r primer set and Takara MightyAMP™ Hotstart DNA Polymerase (Takara Bio, Inc., Shiga, Japan). The PCR products were purified with Pronex® Size Selective Purification System, reamplified, and indexed using index adapters from the Nextera v2 kit (Illumina, Inc., San Diego, CA, USA) and KAPA HiFi HotStart ReadyMix (F. Hoffmann-La Roche Ltd., Basel, Switzerland). Indexed PCR products were verified with a D1000 Screentape kit in an Agilent Tapestation 4150 fragment analyzer (Agilent, Inc., Santa Clara, CA, USA). Then, DNA libraries were adjusted, pooled, mixed with 20% PhiX Sequencing Control v3, loaded into MiSeq Kit V3 (600-cycles), and sequenced in an Illumina MiSeq (Illumina, Inc.) available in the Scientific and Technological Bioresource Nucleus (BIOREN) from Universidad de La Frontera. The resulting raw sequence reads were trimmed and processed as described by Zhang et al. (2020b). The obtained sequences were clustered into species-level operational taxonomic units (OTUs) and taxonomy was determined at 97% of similarity cutoff based on GreenGenes version 13.8 (https://greengenes.secondgenome.com) database (McDonald et al., 2012). Communities datasets were rarefied at a sampling depth of 9000 reads for the diversity analysis.

Differences among communities were firstly compared by Wilcoxon signed-rank test for alpha diversity indexes, including richness (Chao1) and diversity (Shannon's entropy, Pielou's evenness, and Faith's phylogenetic indexes), whereas a principal coordinate analysis (PCoA) based on Bray Curtis dissimilarity matrix was used for beta diversity determination. The taxonomic composition of the bacterial community, estimated as relative abundance, was then assessed at the phyla level for each sediment sample. Additionally, the FAPROTAX database and software were used to predict the functions in the bacterial community based on the current literature of cultured strains as described by Louca et al. (2016). Then, phyla and families with relative abundances (> 1.0%), were explored for biological relevant features by linear discriminant analysis (LDA) effect size (LEfSe) with a discriminant analysis score of 2.0, whereas *IndicSpecies* test was carried out to determine the strength and statistical significance of the relationship between occurrence and abundance and group of sites (De Cáceres et al., 2010). Each of these statistical analyses and visualizations were performed by using QIIME2 and R package vegan (version 2.5–6) platforms. In the same sense, co-occurrence networks were generated using the SparCC algorithm at the family level according to the described by Friedman and Alm (2012). Those edges with a significance of $P < 0.01$ and correlation >0.8 were retained for the network constructions using Gephi (http://gephi.github.io/). The node sizes were plotted according to their relative abundances and those with a higher degree (> 5), lower betweenness centrality (< 1,000), and relative abundance (≥ 1%) were recognized as keystone taxa (Berry and Widder, 2014).

2.8. Relationship between P-associated parameters and bacterial community composition in river sediments

A constrained partial redundancy analysis (RDA) was performed to identify the relationship among bacterial families with physicochemical properties and P-associated parameters (P content, Pase activity, and bacterial Pase genes). For the data set standardization, bacterial family information was transformed using the Hellinger method implemented in the *decostand* function from the vegan package; meanwhile, the explanatory variables were centered and scaled. To compare the degree of variation in predictor variables, a variation partitioning step based on redundancy analysis was used. The significance of the models was tested by an ANOVA-like test using 999 permutations. All analyses were used as implemented in the *rda*, *varpart*, and *anova.cca* functions in Vegan package version 2.5–6 (https://cran.r-project.org/web/packages/vegan/index.html; Oksanen et al., 2017). Additional information about RDA results, inertia, r^2, and the significance of its construction are detailed in Supplementary Tables ST3 and ST4.

2.9. Data availability

The raw data from HTS are deposited in the Sequence Read Archive (SRA) of NCBI under the BioProject accession number PRJNA665803.

3. Results

3.1. Physicochemical properties of river sediments

The analysis of physicochemical properties in river sediments showed spatiotemporal variations in some parameters analyzed in both rivers (Table 1). Spatially, Cond showed significantly ($P < 0.01$) higher values in more anthropogenically influenced sampling sites (from 42.3 to 2024.7 μS cm^{-1}) compared with those values in less anthropogenically influenced sampling sites (from 36.7 to 224.8 μS cm^{-1}). Temporally, significantly ($P < 0.01$) higher values of Cond (from 56.7 to 2024.7 μS cm^{-1}) and T° (from 18 to 20.9 °C) were registered during summer compared with those values of Cond (from 36.7 to 47.3 μS cm^{-1}) and T° (from 8.5 to 10.2 °C) observed in winter. In contrast, DO showed significantly ($P < 0.01$) lower values for all sites (from 5.2 to 10.3 mg L^{-1}) during summer compared with winter (from 8.8 to 14.4 mg L^{-1}).

Spatial and temporal differentiation was not present in sediment pH, where the range from slightly acidic (6.0) to neutral (7.1). Similarly, the TN and TC contents did not show a clear spatial distribution. However, sediment collected during the summer season showed significantly ($P < 0.01$) higher values of TC (from 19.7 to 59.8 mg kg^{-1} dw) and TN (from 1.8 to 5.8 mg kg^{-1} dw) than those values of TC (from 4.8 to 34.7 mg kg^{-1} dw) and TN (from 0.7 to 2.7 mg kg^{-1} dw) observed in winter.

3.2. Phosphorus content in river sediments

The analysis of P contents in sediments showed spatiotemporal variations for P fractions in both rivers. Spatially, the contents of P_i (from 152.7 to 286.5 mg kg^{-1} dw), Po (from 410.2 to 1313.1 mg kg^{-1} dw) and TP (from 608.8 to 1599.6 mg kg-^{1} dw) were significantly ($P < 0.01$) higher in more anthropogenically influenced sites compared with the contents of P_i (from 119.3 to 194.8 mg kg^{-1} dw), P_o (from 272.5 to 946 mg kg^{-1} dw), and TP (from 391.8 to 1117.5 mg kg-^{1} dw) in less anthropogenically influenced sites (Table 1). Temporally, the contents of P_i (from 140.2 to 286.5 mg kg^{-1} dw), P_o (from 572 to 1313.1 mg kg^{-1} dw), and TP (from 717.3 to 1599.6 mg kg^{-1} dw) in sediment collected in summer were significantly ($P < 0.01$) higher than those values of P_i (from 119.3 to 209.7 mg kg^{-1} dw), P_o (from 272.5 to 753.6 mg kg^{-1} dw) and TP (391.8 to 869.8 mg kg^{-1} dw) in the winter.

3.3. Phosphomonoesterase activity in river sediments

Analogous to the physicochemical properties and P content, spatiotemporal variations of Pase activities were also found in both river sediments (Table 2). Spatially, the activities of ACP (from 2.8 to 23.7 μg PNP

Table 1
Physicochemical properties and phosphorus contents in sediments from Imperial and Toltén rivers collected during winter and summer seasons.

River/Season	Physicochemical properties							P content		
	Sample	T (°C)	Cond (μS cm^{-1})	DO (mg L^{-1})	pH_{H20}	TC (mg kg^{-1} dw)	TN (mg kg^{-1} dw)	TP (mg kg^{-1} dw)	P_i (mg kg^{-1} dw)	P_o (mg kg^{-1} dw)
Imperial										
Winter	NI_W	9.8 ± 0.04[a] Bcd[b]	39.0 ± 1.7 Bc	10.6 ± 0.2 ABab	6.8 ± 0.2 ABab	34.7 ± 3.3 Ac	2.7 ± 0.6 Ac	754.1 ± 10.7 Ad	148.3 ± 3.5 Abc	605.8 ± 9.6 Ad
	CA_W	9.0 ± 0.01 Dd	36.7 ± 0.6 Bc	11.9 ± 0.8 Aa	6.0 ± 0.1 Cd	13.6 ± 1.1 Bd	1.7 ± 0.03 ABcd	391.8 ± 36.0 Be	119.3 ± 11.8 Bc	272.5 ± 34.2 Be
	TR_W*	9.4 ± 0.08 Ccd	47.3 ± 1.5 Ac	8.8 ± 1.0 Bbc	6.3 ± 0.2 BCcd	30.3 ± 1.1 Ac	2.5 ± 0.6 Ac	877.8 ± 27.7 Abcd	160.3 ± 1.3 Abc	753.6 ± 65.6 Abcd
	NE_W*	10.2 ± 0.06 Ac	44.7 ± 0.6 Ac	11.7 ± 0.6 Aa	7.1 ± 0.1 Aa	16.8 ± 2.9 Bd	0.7 + 0.1 Bd	869.8 ± 63.8 Acd	170.0 ± 3.5 Ab	710.2 ± 49.2 Acd
Summer	NI_S	19.7 ± 0.02 Ab	138. ±34.2 Bb	5.2 ± 0.4 Bd	6.4 ± 0.1 ABbcd	59.8 ± 1.1 Aa	5.8 ± 0.4 Aa	1117.5 ± 73.1 Bb	171.4 ± 3.1 Bb	946.0 ± 70.5 Bb
	CA_S	19.9 ± 0.03 Aab	224.8 ± 2.2 Aa	7.3 ± 0.2 Ac	6.4 ± 0.1 Bbcd	15.5 ± 1.1 Cd	1.8 ± 0.1 Ccd	717.3 ± 77.1 Cd	140.2 ± 26.4 Bbc	577.1 ± 53.0 Cd
	TR_S*	20.9 ± 0.03 Aa	225.93 ± 2.3 Aa	8.6 ± 0.5 Abc	6.6 ± 0.1 ABabc	43.4 ± 3.9 Bb	4.2 ± 0.4 Bb	992.1 ± 45.5 BCbc	177.2 ± 5.0 Bb	814.9 ± 44.6 BCbc
	NE_S*	19.5 ± 0.9 Ab	235.8 ± 0.8 Aa	8.3 ± 0.1 Ac	6.8 ± 0.1 Aab	46.5 ± 1.5 Bb	5.5 ± 0.3 Aa	1599.6 ± 102.9 Aa	286.5 ± 11.0 Aa	1313.1 ± 92.2 Aa
Toltén										
Winter	VI_W	10.0 ± 0.1 Ad	45.7 ± 0.6 Ac	11.1 ± 0.6 Bbc	6.9 ± 0.1 Aa	4.8 ± 0.2 Ce	0.8 ± 0.02 Bc	508.0 ± 8.2 Ccd	194.8 ± 10.3 Aab	313.1 ± 7.5 Cd
	PI_W	9.0 ± 0.01 Be	43.7 ± 0.6 ABc	14.4 ± 0.5 Aa	6.2 ± 0.1 Bcde	17.1 ± 0.1 Bcd	1.0 ± 0.1 ABc	418.8 ± 6.6 Dd	135.9 ± 10.4 Bd	282.9 ± 3.8 Cd
	NT_W*	8.5 ± 0.06 *Cf*	42.3 ± 0.6 Bc	12.5 ± 0.6 ABb	6.0 ± 0.1 Be	25.3 ± 3.0 Abcd	1.1 ± 0.1 Ac	608.8 ± 15.0 Bc	198.6 ± 17.5 Aab	410.2 ± 8.0 Bc
	LB_W*	9.0 ± 0.04 Be	44.5 ± 0.7 ABc	12.4 ± 0.4 Bb	6.2 ± 0.1 Bde	17.0 ± 1.8 Bd	1.1 ± 0.1 Ac	729.8 ± 37.4 Ab	209.7 ± 16.3 Aa	520.1 ± 21.1 Ab
Summer	VI_S	18.0 ± 0.01 Bb	56.7 ± 1.5 Bbc	8.8 ± 0.2 Ad	6.9 ± 0.04 Aa	19.7 ± 1.3 Bbcd	2.6 ± 0.3 Bb	752.0 ± 26.9 Bb	180.0 ± 6.2 Aabc	572.0 ± 28.4 Bb
	PI_S	17.6 ± 0.05 Cc	65.7 ± 5.2 Bb	6.9 ± 0.7 Be	6.4 ± 0.04 Bbcd	28.5 ± 3.1 Bb	4.2 ± 0.4 ABa	890.0 ± 48.4 Aa	157.4 ± 5.8 Abcd	732.6 ± 44.0 Aa
	NT_S*	18.2 ± 0.05 Bb	67.0 ± 1.7 Bb	10.3 ± 0.03 Acd	6.5 ± 0.02 Bb	44.3 ± 4.6 Aa	4.5 ± 0.6 Aa	864.2 ± 19.5 ABa	172.6 ± 8.0 Aabcd	699.3 ± 7.8 Aa
	LB_S*	18.6 ± 0.03 Aa	2024.7 ± 11.2 Aa	9.7 ± 0.5 Acd	6.5 ± 0.1 Bbc	26.5 ± 1.0 Bbc	2.8 ± 0.4 Bb	912.3 ± 44.1 Aa	152.7 ± 11.0 Acd	759.6 ± 38.7 Aa

T: temperature; Cond: conductivity; DO: dissolved oxygen; TC: total carbon; TN: total nitrogen; TP: total phosphorus; P_i; total inorganic phosphorus; P_o: total organic phosphorus.

[a] Values represent the mean ($n = 3$) ± standard deviation.

[b] Capital letters in the same column represent spatial significant differences ($P < 0.01$) among samples inside an individual season of each river, whilst small letters in the same column represent temporal significant differences ($P < 0.01$) among samples from both seasons of each river.

* Asterisks denote the more anthropogenically influenced sampling sites in both rivers.

Table 2

Phosphomonoesterase activity and quantification of bacterial phosphomonoesterase genes in sediments from Imperial and Toltén rivers collected during winter and summer seasons.

River/Season	Sample	Phosphomonoesterase activity (mg PNP g^{-1} h^{-1} dw[a])		Absolute Quantification[b] (gene copy g^{-1} sediment dw)				Relative Quantification		
		ACP	ALP	16S rRNA (×10^{10})	*phoC* (×10^{7})	*phoD* (×10^{6})	*phoX* (×10^{5})	*phoC* (×10^{-4})	*phoD* (×10^{-5})	*phoX* (×10^{-6})
Imperial										
Winter	NI_W	3.04 ± 0.8[c] Bde[d]	1.0 ± 0.1 Bb	0.054 ± 0.012 Aa	1.86 ± 30.0 ABde	7.32 ± 1.5 Aa	0.13 ± 2.5 Ab	367 ± 170 Bc	13.8 ± 352 Bd	24.4 ± 168 Bb
	CA_W	2.0 ± 0.6 Bde	0.3 ± 0.04 Cb	0.6 ± 0.36 Bbc	59.5 ± 22.9 Be	62.0 ± 15.7 Bc	56.7 ± 45.5 Be	18 ± 305 Abc	12.8 ± 771 Bd	0.13 ± 0.01 Bc
	TR_W*	12.4 ± 1.3 Abc	3.8 ± 0.2 Aab	0.048 ± 0.47 Aa	6.76 ± 2.3 Acd	5.47 ± 1.0 Aa	8.56 ± 1.1 Abc	13.8 ± 356 Abc	11.3 ± 151 Bd	0.18 ± 0.005 Bc
	NE_W*	9.4 ± 0.9 Abcd	1.2 ± 0.3 Bb	0.79 ± 49 Bb	9.57 ± 6.7 Abc	6.90 ± 1.4 Aa	0.29 ± 0.19 Aab	46 ± 22 Abc	93.8 ± 21 Ac	4.6 ± 2.4 Ab
Summer	NI_S	7.4 ± 0.6 BCcde	3.1 ± 0.4 ABab	0.15 ± 1.4 Ad	0.3 ± 7.7 ABab	4.20 ± 26.0 Aab	5.84 ± 1.7 Bbc	0.2 ± 6.2 Aa	2.8 ± 36.7 Aa	3.9 ± 1.3 Bb
	CA_S	1.0 ± 0.05 Ce	0.8 ± 0.1 Bb	3.6 ± 1.8 Be	1.48 ± 22.8 Ce	45.2 ± 13.2 Bc	97.2 ± 19.6 Dde	5.0 ± 2.5 Bb	1.4 ± 27.9 BCbc	3.0 ± 0.88 Bb
	TR_S*	16.2 ± 2.2 ABb	6.8 ± 2.5 ABa	0.11 ± 2.7 Ad	0.16 ± 3.3 Babc	2.23 ± 38.0 Ab	2.47 ± 45.7 Ccd	0.15 ± 52 ABa	2.1 ± 44.1 ABab	2.3 ± 0.27 Bb
	NE_S*	23.7 ± 4.4 Aa	8.3 ± 1.8 Aa	0.22 ± 4.7 Acd	0.47 ± 0.12 Aa	2.26 ± 55.0 Ab	0.69 ± 0.12 Aa	0.21 ± 1.4 Aa	1 ± 742 Cc	31 ± 1.5 Aa
Toltén										
Winter	VI_W	1.0 ± 0.1 Bc	0.4 ± 0.1 Cd	47 ± 24 Ccd	39.1 ± 12.0 *Cf*	45.3 ± 15.4 Cd	88.6 ± 35.1 Bc	9.3 ± 3.6 ABcd	0.1 ± 1.8 Bb	20 ± 4.1 Ac
	PI_W	0.8 ± 0.5 Bc	0.9 ± 0.3 BCcd	2.3 ± 42 ABcd	53.1 ± 35.6 BCf	2.11 ± 1.0 Bc	4.37 ± 2.7 ABab	2.2 ± 1.1 Bd	8.7 ± 2.8 Bb	0.17 ± 7.5 Ac
	NT_W*	4.1 ± 1.0 Ab	2.1 ± 0.3 Abc	3.2±34 Aa	9.45 ± 2.2 Abc	8.26 ± 1.2 Aab	6.26 ± 1.5 Aab	0.3 ± 0.1 Ac	0.26 ± 5.8 Ab	0.20 ± 7.1 Ac
	LB_W*	2.8 ± 0.4 ABbc	1.8 ± 0.3 ABbc	1 ± 23 BCbc	2.49 ± 1.2 ABde	3.24 ± 1.2 ABbc	2.32 ± 92.0 ABbc	0.36 ± 0.14 Ac	0.32 ± 5.4 Ab	0.23 ± 3.9 Ac
Summer	VI_S	1.9 ± 0.2 Cbc	1.1 ± 0.1 Ccd	1.5 ± 34 Bab	80.9 ± 12.7 Cef	2.27 ± 21.5 Bc	4.83 ± 85.0 Bab	5.8 ± 1.8 Bd	0.16 ± 2 Bb	0.35 ± 0.12 Cc
	PI_S	10.1 ± 1.2 Aa	3.6 ± 0.5 Aa	3.2 ± 52 Aa	0.47 ± 0.12 Aa	3.42 ± 10.3 Bbc	5.02 ± 1.5 Bab	0.017 ± 0.15 Ab	0.11 ± 1.9 Bb	0.15 ± 2.2 Cc
	NT_S*	7.5 ± 1.4 ABa	2.9 ± 0.2 ABab	1.5 ± 23 Bab	0.35 ± 7.0 Aab	0.13 ± 5.4 Aa	0.13 ± 87.0 Aa	0.023 ± 0.37 Aab	0.01 ± 0.49 Aa	0.87 ± 0.14 Bb
	LB_S*	4.1 ± 0.5 BCb	2.0 ± 0.5 BCbc	28 ± 570 Cd	7.63 ± 2.2 Bcd	5.31 ± 61.1 ABabc	4.44 ± 90.7 Bab	0.027 ± 3 Aa	0.02 ± 0.28 Aa	0.016 ± 0.12 Aa

ACP: acid phosphomonoesterase; ALP: alkaline phosphomonoesterase.

[a] Milligrams of para-nitrophenyl phosphate released by 1 g sediment per hour dry weight.

[b] Quantification by qPCR with an efficiency from 86% to 103%.

[c] Values represent the mean ($n = 3$) ± standard deviation.

[d] Capital letters in the same column represent spatial significant differences ($P < 0.01$) among samples inside an individual season of each river, whilst small letters in the same column represent temporal significant differences ($P < 0.01$) among samples from both seasons of each river.

* Asterisks denote the more anthropogenically influenced sampling sites in both rivers.

g^{-1} h^{-1} dw) and ALP (from 1.2 to 8.3 μg PNP g^{-1} h^{-1} dw) were significantly ($P < 0.01$) higher in the more anthropogenically influenced sites compared with those values of ACP (from 0.8 to 10.1 μg PNP g^{-1} h^{-1} dw) and ALP (0.3 to 3.6 μg PNP g^{-1} h^{-1} dw) in less anthropogenically influenced sites. Temporally, sediments collected in summer generally showed significantly ($P < 0.01$) higher values of ACP (from 1.0 to 23.7 μg PNP g^{-1} h^{-1} dw) and ALP (from 1.2 to 8.3 μg PNP g^{-1} h^{-1} dw) compared with those values of ACP (from 0.8 to 12.4 μg PNP g^{-1} h^{-1} dw) and ALP (from 0.3 to 3.8 μg PNP g^{-1} h^{-1} dw) in the winter.

It is noteworthy, however, to mention that the ACP activity, in general, was significantly higher than ALP in sediments analyzed.

3.4. Bacterial phosphomonoesterase genes in river sediments

The P content and Pase activity, and the abundances of 16S rRNA and bacterial Pase genes showed spatiotemporal variations in sediments from both rivers.

The sizes of the total bacterial communities based on the enumeration of the copies of 16S rRNA genes ranged from 2.8×10^9 to 5.4×10^{12} 16S rRNA gene copies g^{-1} sediment dw (Table 2). Despite some significant ($P < 0.01$) differences among sediments that varied spatially detected, separation among more anthropogenically influenced sites (from 2.8×10^9 to 4.8×10^{12} 16S rRNA gene copy g^{-1} sediment dw) and the less anthropogenically influenced sites (from 4.7×10^9 to 5.4×10^{12} 16S rRNA gene copy g^{-1} sediment dw) was not significant. However, significant ($P < 0.01$) higher abundances of total bacteria in sediments collected in winter (from 4.7×10^9 to 5.4×10^{12} 16S rRNA gene copy g^{-1} sediment dw) were found than that in summer (from 2.8×10^9 to 2.2×10^{11} 16S rRNA gene copy g^{-1} sediment dw).

In relation to the abundance of bacterial Pase genes (Table 2), significantly ($P < 0.01$) higher abundances of *phoC* (from 2.49×10^7 to 4.7×10^8 copies g^{-1} sediment dw), *phoD* (from 2.23×10^6 to 1.3×10^7 copies g^{-1} sediment dw), and *phoX* (from 2.32×10^5 to 2.9×10^6 copies g^{-1} sediment dw) were found spatially in more anthropogenically influenced sites compared with those values of *phoC* (from 3.91×10^6 to 4.7×10^8 gene copy g^{-1} sediment dw), *phoD* (from 4.52×10^5 to 7.32×10^6 copies g^{-1} sediment dw) and *phoX* (from 5.67×10^4 to 1.3×10^6 copies g^{-1} sediment dw) observed in less anthropogenically influenced sites. Temporally, significant ($P < 0.01$) higher abundances of *phoC* (from 8.09×10^6 to 4.7×10^8 copies g^{-1} sediment dw), *phoD* (from 4.52×10^5 to 1.3×10^7 copies g^{-1} sediment dw), and *pho*X (from 9.72×10^4 to 1.3×10^6 gene copy g^{-1} sediment dw) were observed in sediment samples collected in summer compared with those values of *phoC* (from 2.49×10^7 to 4.7×10^8 copies g^{-1} sediment dw), *phoD* (4.53×10^5 to 2.9×10^7 copies g^{-1} sediment dw) and *phoX* (from 5.67×10^4 to 2.9×10^6 copies g^{-1} sediment dw) observed in winter.

Comparing the relative abundance of bacterial Pase genes in relation to total bacterial abundance measured as 16S rRNA genes (Table 2), the relative abundances of *phoC* (from 1.38×10^{-5} to 2.7×10^{-2}), *phoD* (from 1.13×10^{-6} to 2.0×10^{-3}) and *phoX* (from 3.1×10^{-7} to 1.6×10^{-4}) genes were significantly ($P < 0.01$) higher in more anthropogenically influenced sites than those observed for *phoC* (from 3.67×10^{-6} to 1.7×10^{-2}), *phoD* (from 1.28×10^{-6} to 2.0×10^{-4}) and *phoX* (from 2.04×10^{-7} to 3.5×10^{-5}) in less anthropogenically influenced sites. Temporally, the relative abundances of *phoC* (from 5.0×10^{-4} to 1.7×10^{-2}), *phoD* (from 1.0×10^{-5} to 2.0×10^{-3}) and *phoX* (from 3.1×10^{-7} to 1.6×10^{-4}) genes were significantly ($P < 0.01$) higher in sediments collected in summer compared with the values of *phoC* (from 3.67×10^{-6} to 3.6×10^{-3}), *phoD* (from 1.28×10^{-6} to 3.2×10^{-4}) and *pho*X (from 2.04×10^{-7} to 2.3×10^{-5}) collected in winter.

Coincident with Pase activity, *phoC* (ACP) was the most abundant Pase gene (from 8.09×10^6 to 4.7×10^8 copies g^{-1} sediment dw) in sediment samples, followed by *phoD* (from 4.52×10^5 to 1.3×10^7 copies g^{-1} sediment dw) and *phoX* (from 5.67×10^4 to 2.9×10^6 copies g^{-1} sediment dw), dependent on sites and seasons.

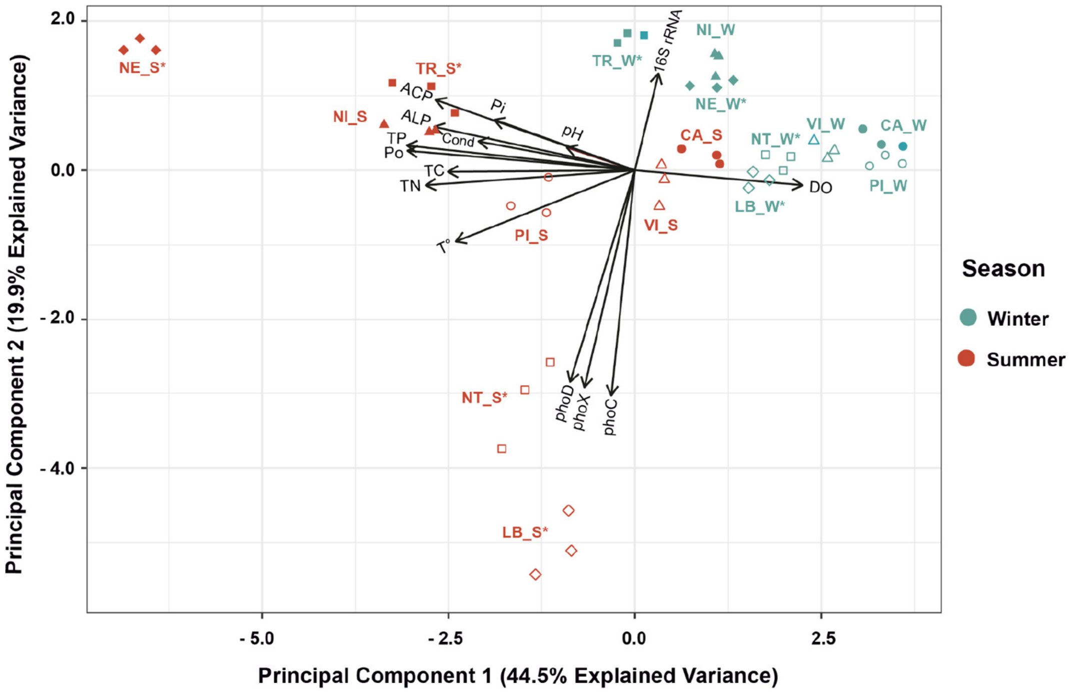

Fig. 2. Principal component analysis (PCA) of physicochemical properties, phosphomonoesterase activity, and bacterial phosphomonoesterase genes (*phoC*, *phoD*, *phoX*) in sediments from Imperial and Toltén rivers collected during winter (W) and summer (S) seasons. Sites for Imperial River were coded as Nueva Imperial (NI), Carahue (CA), Tranapuente (TR), and Nehuentue (NE). Sites for Toltén River were coded as Villarrica (VI), Pitrufquen (PI), Nueva Toltén (NT), and La Barra (LB). T°: temperature; DO: dissolved oxygen; Cond: electric conductivity; TC: total carbon; TN: total nitrogen; P_i: total inorganic phosphorus; P_o: total organic phosphorus; TP: total phosphorus; ACP: acid phosphomonoesterase; ALP: alkaline phosphomonoesterase. Asterisks denote the more anthropogenically influenced sites in both rivers.

Table 3
Pearson's correlation between P content (P_i, P_o, and TP), phosphomonoesterase activity (ACP and ALP), and abundance of bacterial phosphomonoesterase genes (*phoC*, *phoD* and *phoX*) in sediment samples from Imperial and Toltén rivers during winter and summer seasons.

		ACP	ALP	*phoC*	*phoD*	*phoX*	P_i	P_o	TP
Imperial	ACP	1							
	ALP	0.861[**]	1						
	phoC	0.580[**]	0.658[**]	1					
	phoD	0.056	0.134	0.564[**]	1				
	phoX	0.673[**]	0.595[**]	0.666[*]	0.239	1			
	P_i	0.858[**]	0.766[**]	0.648[**]	0.122	0.903[**]	1		
	P_o	0.827[**]	0.752[**]	0.743[**]	0.156	0.819[**]	0.816[**]	1	
	TP	0.834[**]	0.760[**]	0.694[**]	0.106	0.992[**]	0.911[**]	0.988[**]	1
Toltén	ACP	1							
	ALP	0.927[**]	1						
	phoC	0.734[**]	0.754[**]	1					
	phoD	0.211	0.317	0.809[**]	1				
	phoX	0.184	0.255	0.374	0.935[**]	1			
	P_i	−0.199	−0.174	0.773[**]	−0.147	−0.22	1		
	P_o	0.725[**]	0.712[**]	0.743[**]	0.626[**]	0.657[**]	−0.206	1	
	TP	0.710[**]	0.679[*]	0.732[**]	0.617[**]	0.637[**]	−0.060	0.989[**]	1

ACP: acid phosphomonoesterase; ALP: alkaline phosphomonoesterase; P_i: total inorganic phosphorus; P_o: total organic phosphorus; TP: total phosphorus.

[*] Asterisk denotes a $P < 0.05$ significant statistical in the correlations among parameters.

[**] Asterisks denote a $P < 0.01$ significant statistical in the correlations among parameters.

3.5. Relationship of P-associated parameters in river sediments

The PCA showed that it explained 64.4% of the variability of physicochemical parameters, Pase activity, and bacterial Pase genes (Fig. 2). The PCA confirmed the spatiotemporal variations of samples observed in the previous analyses, particularly the differentiation between samples collected in summer with those collected in winter in both rivers. PCA also showed that Pase activities (ACP and ALP), P fractions (P_i, P_o, and TP), C and N contents (TC and TN), Cond, and pH were positively correlated with most samples collected from Imperial river during summer (TR, NI, and NE). Interestingly, bacterial Pase genes (*phoC*, *phoD,* and *phoX*) were positively correlated with the samples NT and LB collected from Toltén river during summer, whilst T° was positively correlated with the sample PI collected from Toltén river during summer. In contrast, the abundance of 16S rRNA genes was positively correlated with samples collected during winter from Imperial river (NE, TR, and NI) whilst the OD was positively correlated with the samples collected from Toltén river (LB, NT, PI, and VI) during winter.

Regarding the Pearson test (Table 3), in general terms, the results showed significant ($P < 0.01$) positive correlations between P fractions, Pase activities, and Pase genes for both rivers, with some exceptions, mainly between *phoD* and Pase activities (ACP and ALP) and P fractions (P_i, P_o, and TP) in Imperial river, and between both Pase activities (ACP and ALP) and Pase gene (*phoD* and *phoX* genes) and P_i fraction in Toltén river.

3.6. Composition of the bacterial community in river sediments

Concerning the bacterial community of the selected contrasting spatiotemporal samples from Imperial (NI and TR) and Toltén (PI and LB) rivers in both seasons, the alpha-diversity analysis showed higher richness and diversity in the sediments of both rivers during winter than summer (Table 4). The values of Chao1 index ranged from 1525.5 to 2252.3 in winter whilst the values in summer ranged from 374.4 to 650. The values of Shannon entropy, Pielou's evenness, and Faith's phylogenetic index ranged from 9.5 to 10.7, from 0.95 to 0.97, and from 74.8 to 130.4 in winter, respectively, whilst the values in summer ranged from 7.6 to 8.7, from 0.94 to 0.95, and from 32.1 to 61.8, respectively. The beta diversity analyses confirmed these observations, where a remarkable difference in the seasonality between winter and summer sediments was also revealed by PCoA (Fig. 3a).

The analysis of the taxonomic composition of bacterial communities in the sediments (Fig. 3b) showed the Proteobacteria phylum as the most abundant taxa in the selected sediments, showing percentages of relative abundances from 47.3 to 51.4 % independently of temporality and seasonality. Proteobacteria was followed by the phylum Actinobacteria with higher relative abundances in winter (from 13.4 to 17.6%) than summer (from 5.0 to 9.9 %). In contrast, the phylum Bacteroidetes showed higher relative abundances in summer (from 6.2 to 12.5 %) than winter (from 5.7 to 7.1 %). Other dominant phyla found in the sediments were Acidobacteria (from 6.0 to 10.5%), Chloroflexi (from 4.4 to 8.8%), Planctomycetes (from 1.9 to 8.0%), Firmicutes (from 0.6 to 8.4%) and Verrucomicrobia (from 1.2 to 5.5%). Based on the FAPROTAX analysis (Louca et al., 2016), 29.5% and 30.5% of registered taxa were assigned for the Imperial and Toltén rivers, respectively. From these, higher percentages of gene sequences were associated with heterotrophy function in both rivers, with relative abundances ranging from 37.4 to 38.4% (Fig. 3c). Other functions predicted in bacterial communities from sediments in both rivers were assigned to the respiration of sulfur compounds, methylotrophy,

Table 4
Alpha diversity (mean ± standard deviation) among bacterial communities in sediment samples from Imperial and Toltén rivers during winter and summer seasons.

River	Season	Sample	Chao1	Shannon entropy	Pielou's evenness	Faith's phylogenetic
Imperial	Winter	NI_W	1525.5 ± 134.5[†] B[‡]	9.5 ± 0.2 AB	0.97 ± 0.01 A	74.8 ± 11.3 A
		TR_W[*]	2178.6 ± 182.5 A	9.7 ± 0.1 A	0.97 ± 0.01 A	77.2 ± 2.8 A
	Summer	NI_S	394.4 ± 270.2C	7.6 ± 1.3 B	0.94 ± 0.01 B	32.1 ± 13.8 B
		TR_S[*]	374.4 ± 69.0C	7.9 ± 0.3 AB	0.94 ± 0.01 B	37.9 ± 5.9 B
Toltén	Winter	PI_W	1598.2 ± 430.4 AB	9.8 ± 0.3 AB	0.95 ± 0.01 AB	111.7 ± 18.5 AB
		LB_W[*]	2252.3 ± 712.9 A	10.7 ± 0.3 A	0.97 ± 0.01 A	130.4 ± 27.1 A
	Summer	PI_S	650.0 ± 341.4 B	8.6 ± 0.9 B	0.95 ± 0.01 AB	61.8 ± 22.6 B
		LB_S[*]	627.0 ± 219.6 B	8.7 ± 0.6 B	0.94 ± 0.01 B	58.3 ± 12.2 B

[†] Values represent the mean (n = 3) ± standard deviation.

[‡] Capital letters in the same column represent temporal significant differences ($P < 0.01$) among samples from both seasons of each river.

[*] Asterisks denote the more anthropogenically influenced sampling sites in both rivers.

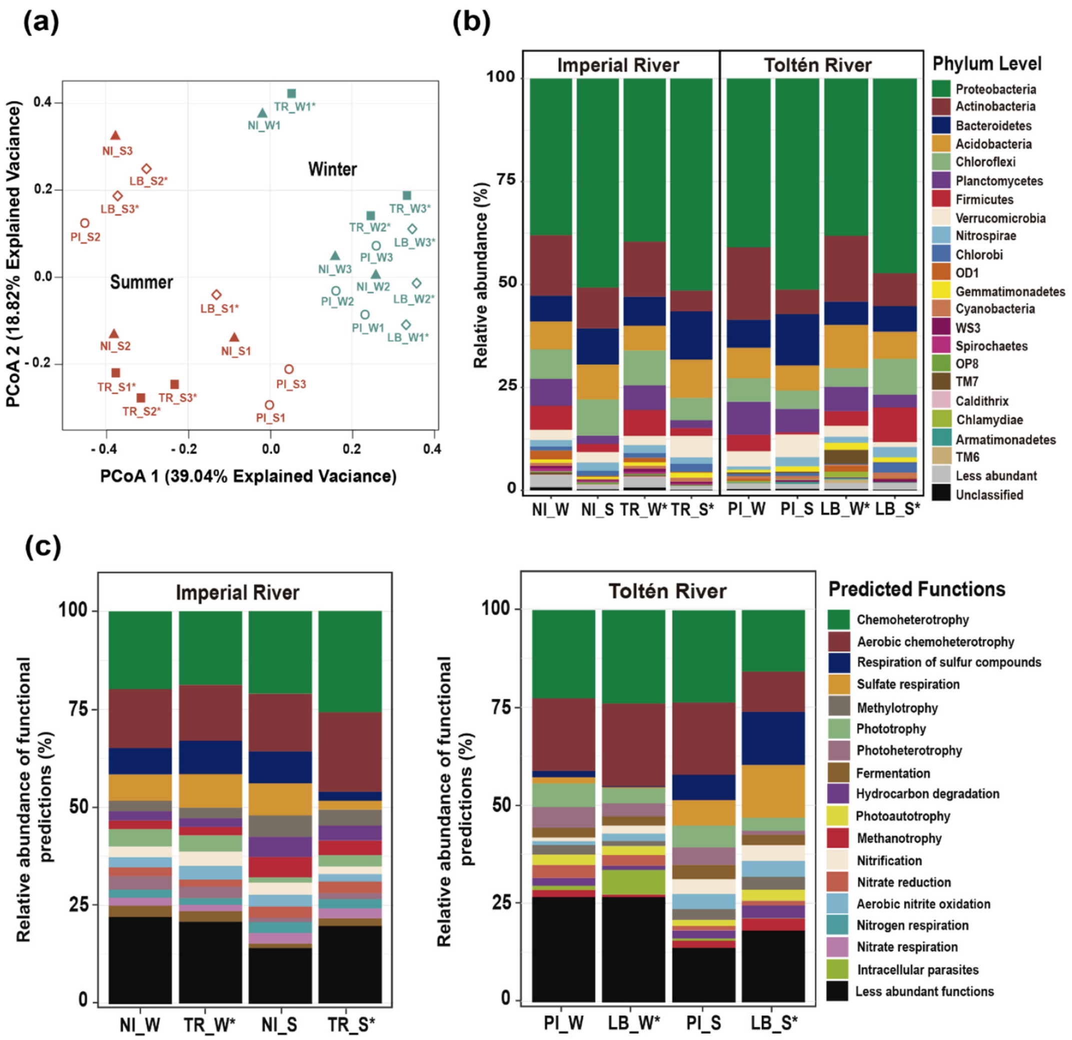

Fig. 3. (a) Principal coordinate analysis (PCoA) of bacterial communities in selected sediment samples from Imperial and Toltén rivers collected during winter (W) and summer (S) seasons. (b) Average of relative abundance at phylum taxonomic level in the total bacterial communities in selected sediment samples from Imperial and Toltén rivers collected during W and S seasons. (c) Average of relative abundance of functions predicted in the total bacterial communities in selected sediment samples from Imperial and Toltén rivers collected during W and S seasons. Selected sediment samples for Imperial River were Nueva Imperial (NI) and Tranapuente (TR), whilst the selected sediment samples for Toltén River were Pitrufquen (PI) and La Barra (LB). Asterisks denote the more anthropogenically influenced sites in both rivers.

phototrophy, photoheterotrophy, fermentation, and hydrocarbon degradation, among others. Interestingly, higher relative abundances of sequences were assigned to respiration of sulfur compounds (from 6.5 to 13.5%) and sulfate respiration (from 6.5 to 13.6%) in sediments from Toltén river during summer compared with those observed in winter (from 0.3 to 1.6%), and those from Imperial river (from 2.3 to 8.5%).

The LEfSe analysis (De Cáceres et al., 2010) suggested mainly four phyla (14 families) as predicted microbial indicator for sediments in both rivers (Fig. 4a). In detail, the Proteobacteria (Bradyrhizobiaceae, Sphingomonadaceae, Coxiellaceae, Hyphomicrobiaceae), Chlamydiae, Actinobacteria (Gaiellaceae, Nocardioidaceae, EB1017, and Mycobacteriaceae), Firmicutes (Clostridiaceae), Planctomycetes (Gemmataceae) were suggested for winter whilst Proteobacteria (Crenotri-chaceae, OM60, Alcaligenaceae, and Chromatiaceae) was the only phyla suggested for summer. The results of *IndicSpecies* analysis proposed 11 families as microbial indicators for winter in sediments, without any exclusivity for summer (Table 5). The suggested families included: Sphingomonadaceae, Mycobacteriaceae, EB1017, Nocardioidaceae, Paenibacillaceae, Caulobacteraceae, Koribacteraceae, Isosphaeraceae, Alicyclobacillaceae, Coxiellaceae, and Ktedonobacteraceae.

In co-occurrence network analysis, the Spearman correlation analysis resulted in 136 nodes and 397 edges for the winter network, whilst 57 nodes and 111 edges were found for the summer (Fig. 4b). The networks also showed different degrees of modularity (MD; 0.569–0.383) and figure density (FD; 0.04–0.07). Interestingly, positive (blue lines) and negative (red lines) interactions among nodes were distributed close equally in both season networks; however, the existence of modules almost entirely featured by positive interplays was more prominent for the winter network. This analysis also suggested the presence of keystone taxa within the microbial community, which have been defined as those taxa that produce a severe impact in the ecosystem assembly when they are removed (Berry and Widder (2014). Based on nodes with a high degree (> 5), low betweenness centrality (< 1000), and relative abundance ≥1%, 17 putative keystone taxa were suggested in Fig. 4b and Supplementary Table ST5 for both seasons and rivers. In this sense, keystone taxa from 13 families were exclusively associated with winter (0319_6A21, Clostridiaceae, Ellin6075, Intrasporangiaceae, Methylocystaceae, Alcaligenaceae, Coxiellaceae, Thermodesulfovibrionaceae. Caulobacteraceae. Gaillaceae, Cytophagaceae, Planctomycetaceae, and OM60), one family for summer (Xanthomonadaceae), and three families for both seasons (Chromatiaceae, Desulfobacteraceae, Desulfobulbaceae).

Coincidently, the families Gaiellaceae, Nocardioidaceae, EB1017, Mycobacteriaceae, Clostridiaceae, OM60, Alcaligenaceae, Chromatiaceae,

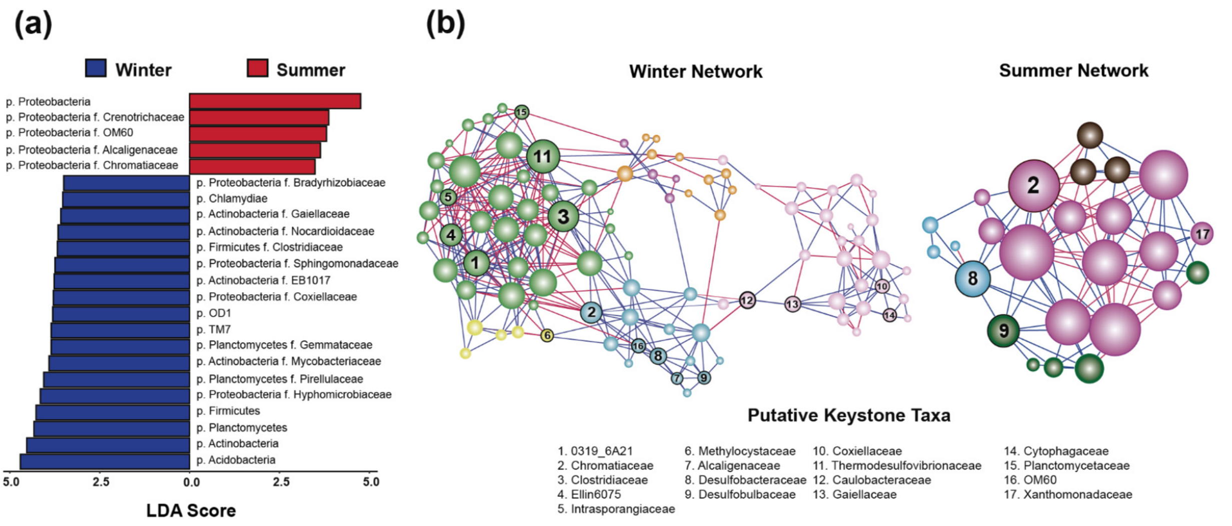

Fig. 4. Linear discriminant analysis (LDA) effect size (LEfSe) (a) and co-occurrence network analysis (b) of bacterial communities in selected sediment samples from Imperial and Toltén rivers collected during winter and summer seasons. For LEfSe, phyla (p.) and families (f.) with relative abundances >1.0% are shown with their respective LDA score. For network analysis, nodes (bacterial families) were colored according to their modulatory scores and fitted in the function of their relative abundances (%). Lines represent positive (blue) and negative (red) interactions among nodes. Putative keystone taxa for winter (1, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, and 16), summer (17), and both (2, 8, and 9) seasons were highlighted and enlisted. See also Supplementary Table ST3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Caulobacteraceae were highlighted as microbial indicators or key taxa in at least two of the analysis carried out, mainly in winter, whilst the Coxiellaceae family was highlighted as a microbial indicator or key taxon in the three analyses.

3.7. Relationship between P-associated parameters and bacterial community composition in river sediments

The results from the partial RDA demonstrated that ACP, ALP, P_i, P_o, *phoC*, and pho*D* were the best predictor of the data variance, resulting in 38.67% of the species variability explanation (Fig. 5). In this analysis, RDA1 and RDA2 axis explained 29.34% of the variability of the bacterial communities and 73.31% of the relationship between bacterial communities and P-associated parameters in the samples ($P < 0.05$). In this context, the relationships between bacterial community and P-associated parameters were best explained by P_i (23.53%), followed by ALP (21.95%), P_o (16.01%), *phoC* (12.45%), ACP (7.13%), and *phoD* (6.15%). The RDA analysis did not reveal a spatiotemporal separation for the samples; however, positive relationships between some families with P-associated parameters were also revealed. As shown in the plot, independently of temporality and seasonality, the samples PI, NI, and TR were positively related to *phoD* and *phoC* genes, and the families Coxiellaceae, Gemmataceae, Xanthomonadaceae, Chitinophagaceae, and Comamonadaceae. Similarly, ALP was positively related to NI, TR, and PI samples, independently of temporality and seasonality. In contrast, the samples LB were seasonally contrasted, where LB in winter showed a positive relationship with P_i, whilst LB in summer showed a higher relationship with ACP and P_o, and the families Chromatiaceae and Desulfobacteraceae.

Table 5
List of the best-associated bacterial families in sediment samples from both Imperial and Toltén rivers during winter or summer seasons as revealed by the *IndicSpecies* analysis.

Bacterial Family	Relative Abundances (%)		*IndicSpecies* metrics	
	Winter	Summer	A[a]	B[b]
Sphingomonadaceae	1.8	0.6	0.91	1
Mycobacteriaceae	1.26	0.08	0.98	1
EB1017	1.15	0.23	0.95	1
Nocardioidaceae	1.06	0.07	0.98	1
Paenibacillaceae	0.57	0.01	0.99	1
Caulobacteraceae	0.47	0.1	0.95	1
Koribacteraceae	0.34	0.01	0.91	1
Isosphaeraceae	0.26	0.04	0.97	1
Alicyclobacillaceae	0.22	0.03	0.96	1
Coxiellaceae	0.11	0.04	0.92	1
Ktedonobacteraceae	0.07	0	0.99	1

[a] Means family is only present in one season. Values closer to 1 represent higher exclusivity.

[b] Means family is present inside each sample for the same season. Values closer to 1 represent a more homogeneous distribution.

4. Discussion

4.1. Physicochemical properties and phosphorus contents of river sediments

The analysis of physicochemical properties showed spatiotemporal variations in many of the analyzed parameters in both river sediments (Table 1). Spatial and/or temporal variations in physicochemical parameters in river sediments have previously been reported in diverse studies (Yang et al., 2010; Li et al., 2016; Kaestli et al., 2017; Roberto et al., 2018; Zhang et al., 2019b). Spatially, higher values of Cond in sediments were found in more anthropogenically influenced sites. This result could be attributed as the result of seawater intrusion and meager tidal mix since these sites are located at 2–10 km closer to each estuarine, and is consistent with past reports from the Ten Mile Creek from USA (Yang et al., 2010), Jiaolai River from China (Li et al., 2016), and Darwin Harbour estuary from Australia (Kaestli et al., 2017). The hypereutrophication and disturbance of the anthropogenically influenced points of rivers have also indicated to increase the Cond in Chilean rivers (Fierro et al., 2017; Fierro et al., 2018) and creeks such as Tinkers Creek from USA (Roberto et al., 2018). Temporally, higher values of Cond and T° were registered in sediments during summer, which can be attributed to the reduction of rainfalls during summer in Chile, with the concomitant lower tributary river inputs and electrolyte, nutrient, and heavy metal pollutant concentrations, as observed in other freshwater ecosystems (estuaries and creeks) (Li et al., 2016; Roberto et al., 2018). In contrast, the lower values of DO observed in sediments during summer may be the result of higher consumption of oxygen by microbial communities and higher T° in sediments and water column

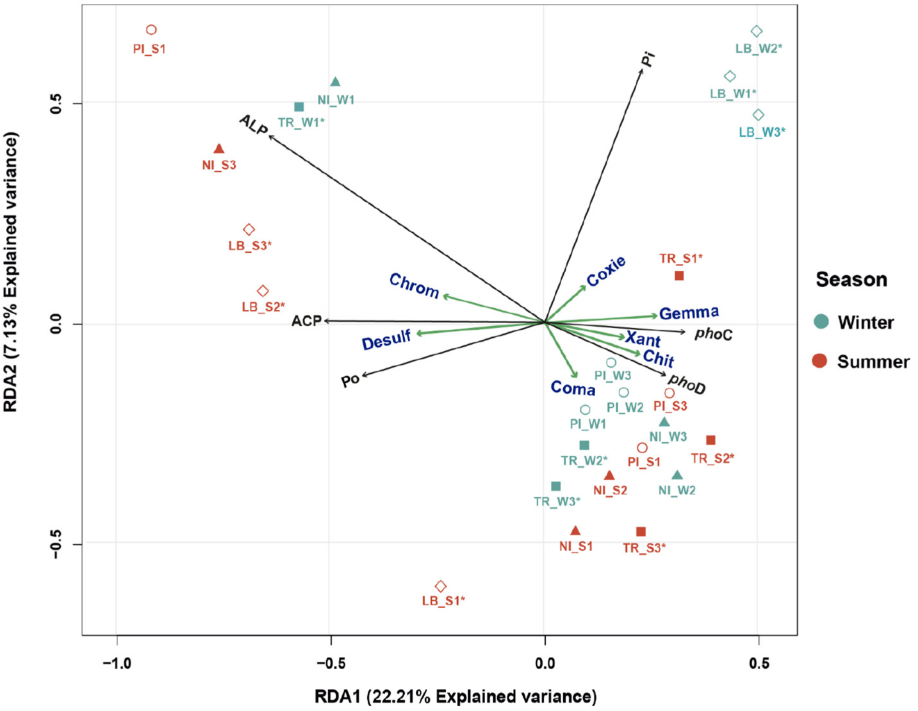

Fig. 5. Redundancy analysis (RDA) of phosphorus content, phosphomonoesterase activity, bacterial phosphomonoesterase genes (*phoC* and *phoD*), and the total bacterial community at the family taxonomic level in sediments from Imperial (filled dots; NI and TR samples) and Toltén (empty dots; PI and LB) rivers collected during winter (W) and summer (S) seasons. Green arrows represent featured bacterial families, being Desulfobacteraceae (Desulf), Chromatiaceae (Chrom), Coxiellaceae (Coxie), Gemmataceae (Gemma), Xanthomonadaceae (Xant), Chitinophagaceae (Chit), and Comamonadaceae (Com). Asterisks denote the more anthropogenically influenced sites in both rivers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

during the summer season as observed in sediments from the highly anthropized Ganga River in India (Jaiswal and Pandey, 2019), from Chinese drinking water reservoirs (Zhang et al., 2014) or waters from some human-impacted Chilean lakes (Pizarro et al., 2016).

Sediment samples collected during summer also showed higher contents of nutrients (TC and TN), possibly as a result of dilution factor by the common water level decrease that accompanies this season. Studies conducted by Pandey and Yadav (2017) have similarly reported an increase of levels of C and N during dry seasons in the Ganga River. Although the Imperial and Toltén rivers differ on many characteristics from the Ganga River, studies conducted by Jaiswal and Pandey (2019) on this latter explain that increasing levels of C and N during dry seasons are mainly regulated by anthropogenic wastewater inputs, an aspect intimately related to increased oxygen consumption (Jaiswal and Pandey, 2019). However, this seems to be no to strictly limited to summer due to the existence of evidence of higher levels of C and N for similar lotic systems during the colder seasons (Roberto et al., 2018; Zhang et al., 2019a), or even no significant changes of nutrient contents in Chinese rivers between warmer and colder seasons (Li et al., 2016; Zhang et al., 2019b). In this context, our results are reliable since the nutrients from anthropogenic runoff such as nitrate (NO_3^-) in the waters of the Imperial and Toltén rivers have demonstratedly increased during the last 20 years of monitoring, but with erratic annual fluctuation (Pizarro et al., 2010). The spatial fluctuations observed in both rivers appear to have greater forcefulness based on observations made by other studies in rivers and creeks from China, USA, and Brazil (Li et al., 2016; Köchling et al., 2017; Roberto et al., 2018; Yang et al., 2010; Zhang et al., 2020a), which conclude that the most distal downstream locations (coincidently with the more anthropogenically influenced points) are capable to harbor higher levels of C and N in sediments.

Following with the content of nutrients in sediments, the analysis of P content also showed spatiotemporal variations for P fractions in both rivers (Table 1; ST1). Spatial and/or temporal variations of P fractions in sediments of freshwater ecosystems have also been reported (Yang et al., 2010; Pandey and Yadav, 2017; Hu et al., 2020; Zhang et al., 2020a). Higher contents of P_i, $P_{o,}$ and TP were found in sediments collected from more anthropogenically influenced sites and sediments collected during summer. Spatially, it has been described that the P accumulation in distant downstream locations is broad depending on the sediment texture, being fine reactive particles (such as silt and clay) over the non-reactive coarser particles (such as sand) as important P-carriers responsible for its mobility along the river course (Yang et al., 2010; Gireeshkumar et al., 2013; Yuan et al., 2019). In this context, the predominance of fine sediment texture (> 80% of fine sand, silt, and clay) and higher P contents, especially P_o, were coincident in the more anthropogenically influenced sites, whilst coarsest textures (> 50% of medium and coarse sand) and lower P contents were presented in the less anthropogenically influenced sites (data not shown). In our study, the higher P contents were coincident with sites with higher TC contents. Furthermore, the affinity of P and N species to other elements and OM contents also affect the trend of distribution along the river transect (Bai et al., 2009).

The relationship of P_o and P_i contents in river sediments is affected by increasing temperature (seasons), salinity, dissolved oxygen, and microbial activity (Zhang et al., 2014; Kang et al., 2018). However, substantial seasonal effect in P_i forms is not apparent in some river sediments (Yang et al., 2010; Zhang et al., 2019b), where the accumulation of P_o-rich particles in the downstream sediments of the Cochin (Gireeshkumar et al., 2013) and Min River (Hu et al., 2020) estuaries are contributed by runoff fluxes of tributary rivers during dryer seasons. The extent of differences between seasons and runoff fluxes often controls the dominance of source-driven or process-driven P signals. Besides, since primary production increase during the warmer seasons, processes such as deposition of particulate P_o from detritus or dead aquatic biomass can serve as sources of labile and non-labile P_o in the surface layer of river sediments (Bai et al., 2009; Jiang et al., 2018; Ni et al., 2019b; Yuan et al., 2020). Moreover, as water flow decreases during warmer and dryer seasons in Chile, the contribution of urban and agricultural areas with wastewaters becomes high and cause erosion of soil particles enriched with PMEs and PDEs, together facilitates the sequestration of Po in fine sediment particles (Bol et al., 2018). Similar trends have been observed in tributary streams in the Avon River, UK receiving higher wastewater input, where decreased P content was observed during stormy periods and increased P content during low water in summer (Bowes et al., 2005).

4.2. Phosphomonoesterase activity and bacterial phosphomonoesterase genes in river sediments

Spatiotemporal variations of Pase activities were also observed in both river sediment samples (Table 2) Spatial and/or temporal variations of Pase activities have previously been reported in river sediments (Mateo et al., 2010; Labry et al., 2016; Pandey and Yadav, 2017; Yuan et al., 2020). In general terms, higher activity of ACP and ALP were found in sediments from more anthropogenically influenced sites and sediments collected in summer, being coincident with the previous observations for P and TC distribution. These results are in concordance with reports describing increasing ACP levels in rivers and salt marsh sediments receiving OM-rich and long-term N and P fertilized soil particles in South Carolina, USA (Huang and Morris, 2005). Additionally, Mateo et al. (2010) demonstrated that measurements *in situ* of ALP activities in waters of the Spanish Muga river increase in order to the increasing temperature and PMEs and PDEs. Labry et al. (2016) also conclude that ALP activity is promoted during warmer seasons by an increased bacterial activity, where P_o hydrolysis into phosphate is maximum in the Aulne and Elorn macrotidal estuaries. Similarly, superficial sediments receiving higher inputs of P from human sources in the Jiulong Estuary Mangrove Reserve have shown higher ACP and ALP activities by an increased microbial activity with higher temperatures during spring (Jiang et al., 2018).

It is noteworthy that ACP activity was higher than ALP in studied sediment samples. Most of the studies on the Pase activity of rivers and freshwater ecosystems have historically been focused on determining ALP, hindering the estimation of ACP order its alkaline isoform (Mateo et al., 2010; Labry et al., 2016; Pandey and Yadav, 2017; Yuan et al., 2020). However, ACP activity at least 3-fold higher than ALP activity in sediments (pH ranging 5.3 to 6.2) has been reported in tidal freshwater marshes on the Cooper River from South Carolina (USA) (Huang and Morris, 2005). Similarly, Jiang et al. (2018) have found higher levels of ACP than ALP in sediments from Jiulong Estuary Mangrove Reserve (China).

Following with the Pase assessment, spatiotemporal variations of total bacteria and bacterial Pase genes in sediments collected from both rivers were also observed (Table 2). Studies describing spatial or temporal variation o abundances of bacterial Pase genes in freshwater ecosystems are very scarce. Morrison et al. (2016) found abundances of *pho*D gene higher (from 10^{11} to 10^{12} copies g^{-1} dw) compared with our study (from 10^6 to 10^7 gene copy g^{-1} dw) in the Florida Everglades sediments receiving high water inputs from closer agricultural areas. In contrast, Chen et al. (2019) found similar *phoD* gene copies to our study, ranging from 2.20×10^7 to 3.13×10^7 copies g^{-1} dw, in microcosms confected with sediments from Taihu Lake from China. Similarly, Wan et al. (2020) found *phoD* gene abundances from 10^6 to 10^8 copies mL^{-1} in waters of the eutrophic Lake Nanhu in China. In the case of *phoX*, our counts were significantly lower (from 10^5 to 10^6 gene copy g^{-1} dw) than those described by Morrison et al. (2016) with *phoX* abundances from 10^{11} to 10^{12} copies g^{-1} dw in the Florida Everglades sediments, whereas Dai et al. (2018) noticed similar values (from 10^5 to 10^6 copies mL^{-1}) in water samples from Taihu Lake (China) under active decomposition of *Microcystis* biomass. Otherwise, to our knowledge, no studies are describing the abundance of *phoC* in sediments or water from freshwater ecosystems thus far, being this gene mostly studied in terrestrial environments, such soils, and where its abundance ranges from 10^6 to 10^7 g^{-1} soil (Fraser et al., 2017; Zheng et al., 2019), which are lower than those found in our study (from 10^7 to 10^8 gene copy g^{-1} dw).

Coincidently with other studied parameters, significantly higher abundances of bacterial Pase genes were observed in more anthropogenically influenced sites and sediment samples collected in summer. Zhang et al. (2019a) observed significantly higher abundances of *phoD* genes during summer than autumn in suspended particles of the Taihu Lake, which at the same time presented peaks in downstream locations of tributary rivers, closer to their mouth zones. Dai et al. (2018) working with water mesocosms from the eutrophic Lake Taihu, showed the *phoX* genotype can increase from $10^{\times}$ to 10^6 copies mL^{-1} during cyanobacterial blooms and decomposition of death biomass in the warmer periods. Likewise, Fan et al., 2019 found that the abundances of *phoD* and *phoX* in sediments were influenced by temporal variations in the eutrophic Chaohu Lake, having *phoX* a key role in the P solubilization during summer.

It is noteworthy that our results also showed higher abundances of Pase genes coincidently in sediments with higher Pase activities. In this sense, studies conducted by Morrison et al. (2016) and Chen et al., 2019 with sediments showed a narrow relationship between the increase of Pase activities and higher abundances of *phoX* and *phoD*. A similar phenomenon has been observed in terrestrial ecosystems, such as soils, where microbial activity increased by C compounds inputs resulted in the growth of Pase harboring bacterial populations with the concomitant increasing abundance of bacterial Pase genes and total Pase activity (Acuña et al., 2016; Luo et al., 2017). Higher abundances ACP gene (*phoC*) compared with ALP genes (*phoD* and *phoX*) was also observed in our study. As mentioned above, studies on the relationship between Pase genes for aquatic ecosystems have mainly been focused on the diversity of ALP genes (*phoX* and *phoD*), and therefore, information on *phoC* is very scarce. Thus, our results provide useful information for the understanding of P recycling bacterial genes in sediments (not only for Chilean rivers), especially considering that *phoC* constituted the most abundant of the three studied P_o mineralizing genes. Nevertheless, results on abundances for each Pase gene, especially *phoD* and *phoC*, should be taken with caution because *phoC* and *phoD* primer sets, used in this study, were designed to target members of the Proteobacteria phylum from soil (Sakurai et al., 2008; Acuña et al., 2016; Fraser et al., 2017). In this sense, advances in the design and implementation of novel primers set based on the metagenome database from rivers and related aquatic ecosystems could provide better coverage on the diversity of Pase genes in further studies (Ragot et al., 2015; Acuña et al., 2016).

Finally, it is necessary to note that despite that significant differences in the abundance of total bacteria in sediments among less and more anthropogenically influenced sites was not observed, higher abundances of total bacteria in sediments were found in winter when rain occurs intensively in southern Chile. Although the Imperial and Toltén rivers catchments differ on climatological features from the tundra, higher abundances of total bacteria have been previously observed in autumn when precipitation is higher and land nutrients reach the waters of the Revelva River system in Norway (Kosek et al., 2019). Similarly, Luo et al. (2019) studying sediments from the Lancang River, a watercourse that feeds the Gongguoqiao hydroelectric dam in China, discovered that bacterial abundances were higher during wet than dry seasons since increasing water flows was able to remove superficial sediment implying nutrient recirculation, oxygenation, and bacterial dead biomass runoff.

4.3. Relationship of P-associated parameters in river sediments

Concerning the relationship of P-associated parameters in river sediments, PCA not only confirmed the spatiotemporal variations of sediment samples but also showed that Pase activities, P fractions, C and N contents, Cond and pH were positively correlated with the most of samples collected from Imperial river during summer (Fig. 2). As discussed above, the increase of temperature and nutrient contents in the sediment and water of rivers and other freshwater ecosystems during summer is generally accompanied by higher microbial activity, including Pase activity involve in P_o mineralizing and P_i releasing from biomass (Acuña et al., 2016; Dai et al., 2018; Luo et al., 2017; Pandey and Yadav, 2017; Zhang et al., 2019a). In this context, Ni et al. (2019b) reported positive linear correlations among deposition of PMEs and Pase activity in sediments of Erhai Lake, describing this relationship as a preceding event of a new algae bloom. Ma et al. (2019) reported positive correlations between the bioavailability of sedimentary dissolved P_o and ALP with a substrate transformation rate into dissolved P_i during summer in Chinese lake. Similar correlations have been reported by Yuan et al. (2020) in hypereutrophic lake sediments, which huge predominance of non-labile P_o lead to gradually transform into moderately labile P_o and dissolved P_i by ALP. However, although our results suggest a closer relationship between P_o content, Pase activity, and Pase genes, this triad is still controverting due to the strong spatiotemporal heterogeneity of Pase-harboring bacterial communities observed in freshwater ecosystems (Pandey and Yadav, 2017; Zhang et al., 2019a). Interestingly, PCA also showed positive correlations between the abundance of bacterial Pase genes and summer sediments in Toltén river, between the abundance of bacteria and winter sediments in Imperial river, and between OD and winter sediments in Toltén river. To our knowledge, differences in the correlations found in similar rivers located in close geographical areas have not been reported thus far. These differences in the correlation found may be influenced by the spatiotemporal heterogeneity of both rivers or by bias during sampling or selection of sites not considered in this study.

4.4. Composition of the bacterial community in river sediments

About the bacterial community of the selected contrasting spatiotemporal samples in both rivers and seasons, the alpha-diversity analysis showed higher richness and diversity in the sediments during winter in both rivers (Table 4). Zhang et al. (2019b) found 3-fold magnitude of differences in alpha diversity of bacteria among sediment samples obtained from wet and dry seasons in Nanfei River (China). Similarly, the Fengle-Hangbu, Nanfei, and Zhegao tributary rivers of the Chaohu Lake (China) presented higher richness and diversity in the wet seasons than dryer seasons (Zhang et al., 2019a). In contrast, seasonal differences in alpha diversity were not observed among warmer and colder seasons in freshwater ecosystems, such as the Jiaolai River (Li et al., 2016) and the Tinkers Creek (Roberto et al., 2018). In our study, the higher alpha diversities in sediments during winter (rainy season) may be attributed to the higher water flows, which moderately enriched the rivers with nutrients (N and P) and provide allochthonous particles containing attached bacteria from other sources (*e.g.*, eroded soils) which can precipitate in sediments increasing their bacterial diversity as reported by Zhang et al. (2019a). In the case of beta diversity, the PCoA confirmed the remarkable difference in the seasonality between winter and summer sediments (Fig. 3a). Zhang et al. (2019a) also observed seasonal differences in the beta diversity for the bacterial community of sediments from the Fengle-Hangbu, Nanfei, and Zhegao tributary rivers during wet and dry seasons. A similar observation was also reported in sediments of the Tinkers Creek (Roberto et al., 2018) and Nanfei River (Zhang et al., 2019b), corroborating the seasonal changes in the bacterial communities for sediments from rivers as observed in our study.

Proteobacteria phyla was the most abundant taxa in the selected sediments followed by the phyla Actinobacteria, Bacteroidetes, Acidobacteria, Chloroflexi, Planctomycetes, Firmicutes, and Verrucomicrobia (Fig. 3b). Several studies have reported Proteobacteria as the most abundant phylum in sediments from several Chinese rivers, such as Jiaolai (Li et al., 2016), Jaboatão (Köchling et al., 2017), Fengle-Hangbu, Nanfei, and Zhegao rivers (Zhang et al., 2019a; Zhang et al., 2019b). In these studies, Actinobacteria, Bacteroidetes, Acidobacteria, Chloroflexi, Planctomycetes, Firmicutes, and Verrucomicrobia were also considered as abundant phyla, but in lower relative abundance compared with Proteobacteria. Similarly, Proteobacteria, Bacteroidetes, and Actinobacteria have also been reported as dominant taxa in Mississippi River (USA) (Staley et al., 2013). With respect to the functionality of bacterial taxa in sediments, FAPROTAX analysis assigned higher percentages of gene sequences to heterotrophy function in both rivers (Fig. 3c). It is known that oxic heterotrophic bacteria are responsible of many ecological functions and energy flow in superficial sediments from lotic ecosystems (Findlay, 2010). In this context, the decomposition of OM pools and mobilization of several nutrients in river sediments are conducted by heterotrophic bacteria under aerobic conditions (Findlay, 2010), including some heterotrophs, such as members of Planctomycetes and Bacteroidetes phyla in surface sediments from the Changjiang Estuary (Ye et al., 2016). In waters from the River Frome (UK), high P concentrations was correlated with the increasing of heterotrophic and photosynthesis functions, leading to the deterioration of water quality (Bowes et al., 2011). Variations in the heterotrophic function have also been used by researchers as an indicator of nutrient status and ecological quality of sediments from Shedu River (Fu et al., 2020) and sediment disturbances of Kewaunee and Ahnapee rivers with agriculturally derived wastewaters enriched with manure (Beattie et al., 2020).

Other functions predicted in bacterial communities from sediments in both rivers were assigned to respiration of sulfur compounds, sulfate respiration, methylotrophy, phototrophy, photoheterotrophy, fermentation, hydrocarbon degradation, among others. Although these functions are more commonly reported for anoxic environments, superficial sediments present several oxic and anoxic microsites where ubiquitous anoxic bacterial populations can cohabit and alter functions of a wide range of anthropized freshwater ecosystems (Luo et al., 2020). In this context, Beattie et al. (2020) found higher occurrences of fermentation, nitrate reduction, nitrate respiration, and sulfate respiration in sediments from the Kewaunee and Ahnapee rivers (USA) receiving wastewater from agricultural lands. Similar findings were reported by Ma et al. (2020) in artificial sediments formulated with polluted groundwater from Chinese aquifers, where sulfur-related functions were attributed to blooms of Desulfobulbaceae and Desulfobacteraceae members. Functions associated with the recycling of C (*e.g.*, methylotrophy and hydrocarbon degradation), sulfur, and nitrogen have also been noticed in sediments from polluted water bodies such as Huangjinxia reservoir (Li et al., 2020) and Taihu Lake and their tributary rivers (Yan et al., 2020).

As predicted microbial indicators for sediments in both rivers, the LEfSe and *IndicSpecies* analysis highlighted several families (such as Bradyrhizobiaceae, Sphingomonadaceae, Coxiellaceae, Gaiellaceae, Clostridiaceae, and Alcaligenaceae) (Fig. 4a; Table 5), mainly in winter. All these families have been reported in several rivers and lakes. For example, Bradyrhizobiaceae and Sphingomonadacea have been documented as dominants in water samples taken from anoxic sediments of Chinese Yangtze and Han rivers (Ma et al., 2020). Sphingomonadacea has also been found at a high proportion in sediments from the Tinkers Creek (Roberto et al., 2018) and Taihu Lake (China) (Huang et al., 2017). Gaiellaceae and Clostridiaceae families were identified as decomposers in sediments of eutrophic Chaohu Lake (Fan et al., 2019). Similarly, fecal anaerobe Clostridiaceae members have also been reported in sediments of the heavy eutrophic Jiaolai River (Li et al., 2016) and Buffalo Creek (Kaestli et al., 2017). Members of Alcaligenaceae are fecal anaerobic denitrifying bacteria found in the Jianghan Plain formed by the Yangtze and Han rivers in China (Ma et al., 2020) and waters of the Russian Yenisei River (Gladyshev et al., 2015).

Complementarily, the co-occurrence network analysis showed as keystone taxa 13 families exclusively associated with winter (such as Clostridiaceae, Alcaligenaceae, 0319_6A21, Thermodesulfovibrionaceae, Ellin6075, Methylocystaceae, Cytophagaceae, and Planctomycetaceae), 1 family for summer (Xanthomonadaceae) and 3 families for both seasons (Chromatiaceae, Desulfobacteraceae, Desulfobulbaceae). All these families have also been reported in several rivers and lakes. For example, the families 0319_6A21 and Thermodesulfovibrionaceae are abundant in ammonia and nitrite enriched sediment samples from brackish water shrimp aquaculture ponds (Baskaran et al., 2020). Thermodesulfovibrionaceae has also been documented in sediments of the moderated eutrophic Taihu and Huangda lakes (Huang et al., 2017). Scarce information on Elli6075 and Methylocystaceae is available for freshwater ecosystems, but both families have been naturally found in oxygenated riverine microbial mats of Rio de la Saba (Mexico) (Pineda-Mora et al., 2020) and waters of the Russian Yenisei River (Gladyshev et al., 2015). Cytophagaceae is also a family naturally found in several aquatic ecosystems forming part of mats (Pineda-Mora et al., 2020). Planctomycetaceae family are ubiquitous in aquatic environments were contribute to the carbon and nitrogen cycle (Ward et al., 2006). In the case of the Xanthomonadaceae family highlighted in summer, members of this family are aerobic chemoheterotrophs and potential ammonia and nitrite oxidizers described as part of microbial mats (Pineda-Mora et al., 2020). Regarding Desulfobacteraceae and Desulfobulbaceae indicated in both seasons, both families are widely spread in freshwater and marine sediments, where are capable to survive and grown in anoxic sediments from the Changjiang Estuary (Ye et al., 2016), aquaculture fish cages on the coast of Japan (Kondo et al., 2012), sediments from the Buffalo Creek with higher amount of sulfur (Kaestli et al., 2017), anaerobic zones of the Jaboatão River (Köchling et al., 2017), the urban polluted Tinker Creek (Roberto et al., 2018) and cyanobacteria-dominated sediments of Taihu Lake (Fan et al., 2018).

Interestingly, the Coxiellaceae family was highlighted as a microbial indicator or key taxa in the three analyses carried out. This family harbor obligated intracellular bacteria, but its ecology and role in freshwater ecosystems is unknown. Nevertheless, some opportunistic human pathogen species have attracted the attention of microbiologists, such as *Coxiella burnetii* that causes the zoonotic Q fever and can be transmitted by waters contaminated with domestic ruminant feces (Duron et al., 2018). Currently, reports indicating the occurrence of Coxiellaceae in several freshwater environments, such as sediments of tundra Revelva River (Kosek et al., 2019), Sweden wetlands (Graells et al., 2018), mangroves of the Boguaçú River (Huergo et al., 2018) and Alberche River (Lezcano et al., 2017), are increasing; and therefore, urgent the protection of rivers from the impact of agricultural and urban discharges.

4.5. Relationship between P-associated parameters and bacterial community composition in river sediments

The RDA demonstrated the relationships between bacterial community and P-associated parameters were best explained by the variation in P_i, followed by ALP, P_o, *phoC* gene, ACP, and *phoD* gene. As discussed above, positive correlations among P_o mineralization, P_i release, bacterial ACP and ALP, and co-occurrence of higher abundances of Pase genes are expected and reported in the sediment of anthropized rivers (Huang and Morris, 2005; Bai et al., 2009; Morrison et al., 2016; Zhang et al., 2019a). Although the RDA analysis did not reveal a spatiotemporal variation among sites, a positive relationship between ALP genes (*phoD* and *phoC*) and the families Coxiellaceae, Gemmataceae, Xanthomonadaceae, Chitinophagaceae, and Comamonadaceae was found. Similarly, a positive relation between ACP and P_o, and the families Chromatiaceae and Desulfobacteraceae was also present in summer sediments. These families are known for Pase-harboring bacterial groups inside Proteobacteria (Sebastian and Ammerman, 2009; Fan et al., 2019), Bacteroidetes (Ye et al., 2016; Zhang et al., 2019b), Planctomycetes (Martinez et al., 2014; Valdespino-Castillo et al., 2014), and Acidobacteria (Zhang et al., 2019a) phyla. Therefore, our results suggest that specific members of these families could be a crucial component in the mobilization and cycling of P in the rivers from southern Chile during eutrophication processes in colder and warmer seasons. These results are consistent with past findings in which Coxiellaceae has been related to cyanobacterial blooms in rivers, being the protecting effect by this later phototroph a factor for the establishment of the former (Lezcano et al., 2017). Xanthomonadaceae are potential decomposers of P-rich detritus of phytoplankton deposited in superficial sediments across the full year-long (Wang et al., 2012; Lezcano et al., 2017). The isolation of Xanthomonadants, known for P_o-solubilizer, was made from sediments of the Lake Taihu in China, the abundance of which depends on the OM content in sediment, thereby supporting Po enzymatic hydrolysis (Zhou et al., 2011). Chitinophagaceae has been described as a potential degrader of a wide range of biopolymers in P polluted sediments of the Brazilian Mina stream (Costa et al., 2015), Maozhou and Pearl rivers (China) (Liao et al., 2020), and Yangtze Estuary (China) (Guo et al., 2019). Comamonadaceae is a dominant bacterial community in wastewater and is recognized by its ability to accumulate polyphosphate (poly-P) at higher rates (Willems, 2014; Ge et al., 2015). Comamonadaceae members have also been reported as a potential decomposer of cyanobacterial biomass in anoxic sediments of the Taihu Lake (Li et al., 2012; Fan et al., 2018) and Fenghe River (Lu et al., 2016).

Decomposition of dead biomass consumes oxygen and thereby promote anoxia in sediments, which facilitate the reduction of Fe^{3+} to Fe^{2+} by degraders such as Comamonadaceae, Gaiellaceae, Clostridiaceae, and Gammataceae. The reductive dissolution of Fe minerals release P, which increase the flux of dissolved P_i and P_o in the water column (Klump and Martens, 1981; Joshi et al., 2015; Fan et al., 2018; Jung et al., 2019). Similarly, members of Chromatiaceae and Desulfobacteraceae families can couple OM respiration with the sulfate or Fe^{3+} reduction and release of P from sediments (Sinkko et al., 2013; Lü et al., 2016; Reyes et al., 2017; Fan et al., 2018). For example, a positive correlation among Desulfobacteraceae individuals with TP, TN, and OM contents in sediments of the East Dongting Lake supports the notion that OM provides a sufficient electron source for the activity of iron and sulfate reducers and promote the recurrent transformation of nutrients in sediments (Reimers et al., 1996; Joshi et al., 2015; Huang et al., 2017). Sulfate reduction has been reported to alleviate the inhibiting effect of ALP activity caused by the Fe^{+2} produced and AlOOH colloids over, thereby increasing hydrolyzing efficiency in surface sediments (Marvin-Di Pasquale and Capone, 1998; Zhao et al., 2019). Otherwise, since the discussed bacterial groups harbor the potential to modulate the biochemistry of P, the results suggest direct participation of these bacterial groups in the P recycling.

5. Conclusions

This study revealed significant differences in the spatiotemporal variation physicochemical and microbial properties in sediments from the Imperial and Toltén rivers in Chile. Higher contents of TP, P_i, $P_{o,}$ and TC were observed in sediments collected from the more anthropogenically influenced sites during summer. The activity of ACP and ALP followed a similar trend to that of P_o demonstrating an enzyme expression controlled by the substrate in river sediments. Interestingly, ACP was significantly higher than ALP isoform, constituting a unique finding for this type of field environment. Bacterial Pase genes also showed a spatiotemporal variation that is correlated with P contents demonstrating to be suitable molecular indicators of Pase activity and P status of the analyzed sediments. Among Pase genes, the *phoC* gene was by far the most abundant compared with *phoD* and *phoX* genes. The spatiotemporal variations also showed positive correlations among the P-related parameters in summer. In relation to the composition of the bacterial community, higher richness and diversity were found in the winter season. The taxonomic assignment proposed temporal differences with higher abundances of specific bacterial groups (such as Proteobacteria and Bacteroidetes) in more anthropogenically impacted sites during summer. In these sites, bacterial functions such as heterotrophy, respiration of iron and sulfate compounds, and hydrocarbon degradation were found to be in higher proportions. In addition, a series of bacterial families (many of them are coincident through the bacterial community analyses) were suggested to be microbial indicators of the trophic status of the sediments. These indicators were found in a higher number and diversity during the winter than in summer. Additionally, Coxiellaceae, Xanthomonadaceae, Chitinophagaceae, Comamonadaceae, Gemmataceae, and Desulfobacteraceae families were associated with studied P-related parameters, suggesting their potential role for the P recycling in sediments. Thus, given the relationship between P-related parameters and the spatiotemporal shifts of the bacterial community structures, the present study presents valuable information regarding a better understanding of the role of sediment bacteria in P recycling within eutrophic rivers. At national level, data from our studies should be discussed and considered as part of the design of public policies to prevent the deterioration of rivers, which are relevant as ecosystem service providers in southern Chile. At international level, data from our studies should be useful for further studies assessing the role of bacterial communities in P cycling, surveying the abundance and expression of bacterial Pase genes by HTS and proteomics, in rivers placed in regions with similar climatic (Mediterranean), geological (*e.g.*, ash-derived volcanic soils) and land use (*e.g.*, agriculture, aquaculture and urbanization) characteristics.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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