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Effects of Volcanic Pumice Inputs on Microbial Community Composition and Dissolved C/P Ratios in Lake Waters: an Experimental Approach

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Abstract Volcanic eruptions discharge massive amounts of ash and pumice that decrease light penetration in lakes and lead to concomitant increases in phosphorus (P) concentrations and shifts in soluble C/P ratios. The consequences of these sudden changes for bacteria community composition, metabolism, and enzymatic activity remain unclear, especially for the dynamic period immediately after pumice deposition. Thus, the main aim of our study was to determine how ambient bacterial communities respond to pumice inputs in lakes that differ in dissolved organic carbon (DOC) and P concentrations and to what extent these responses are moderated by substrate C/P stoichiometry. We performed an outdoor experiment with natural lake water from two lakes that differed in dissolved organic carbon (DOC) concentration. We measured nutrient concentrations, alkaline phosphatase activity (APA), and DOC consumption rates and assessed different components of bacterial community structure using next-generation sequencing of the 16S rRNA gene. Pumice inputs caused a decrease in the C/P ratio of dissolved resources, a decrease in APA, and an increase in DOC consumption, indicating reduced P limitation. These changes in bacteria metabolism were coupled with modifications in the assemblage composition and an increase in diversity, with increases in bacterial taxa associated with biofilm and sediments, in predatory bacteria, and in bacteria with gliding motility. Our results confirm that volcanic eruptions have the potential to alter nutrient partitioning and light penetration in receiving waterways which can have dramatic impacts on microbial community dynamics.

Keywords Eruption · Bacteria diversity · Dissolved resources

Introduction

Explosive volcanic eruptions eject pyroclastic materials and ash that contain mostly silicates including feldspar, quartz, and volcanic glass [41]. These eruptions greatly alter the surrounding landscape and can have major effects on the adjacent water catchments [13, 14, 32]. Pumice is a light-colored, glassy, pyroclastic volcanic rock that is mainly composed of silicate glass with no crystal structure [4]. Ash is of similar chemical composition to pumice, but of smaller grain size [5]. Because of its low density, volcanic material is capable of floating in water for long periods of time. During an eruption, heat and lightning from volcanoes can make mineral forms of phosphorus more biologically available [40, 50]. Therefore, the impacts of pumice and volcanic ash on aquatic environments include the effect of floating pumice and suspended ash on light and nutrient availability in the water column [17, 32] and the fertilization effect by elements such as phosphorus (P) and iron [23, 29]. The fertilization effect of phosphorus and iron that occur after volcanic eruptions [18, 23, 29] is often related to an increase in the concentrations of chlorophyll *a* [23, 29]. Furthermore, paleolimnological evidence also shows that there was an increase in chlorophyll-derived pigments in sediments, indicating an increase in phytoplankton biomass following volcanic ash deposition [15].

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Since volcanic eruptions would increase P availability and the intracellular phosphate pool of planktonic microbes [19], a decrease in the alkaline phosphatase activity (APA) is expected. Conversely, based on the light–nutrient hypothesis [42], phytoplankton C/P ratio is expected to decrease sharply because the ash would simultaneously increase lake P concentrations (increasing P acquisition) while decreasing light availability (affecting C fixation). Since bacterial production is controlled by the availability of nutrients and organic carbon [9] and bacterial resource demands generally have a low C/P ratio [7], a pumice-induced decrease in C/P ratio of available substrates would be expected to cause an increase in bacterial growth efficiency and perhaps C processing [28]. However, predictions about changes in microbial assemblages are not straightforward as it has been recently shown that bacteria assemblages have a wide range in C/P ratios [20].

The ecological response of lakes to volcanism is highly variable. In the Mount St. Helens (USA) blast zone, a change in the lakes' trophic structure was directly related to enhanced concentrations of dissolved organic carbon (DOC) coming from destroyed forest and volcanic debris [49]. In contrast, during the 2011 Puyehue–Cordón Caulle eruption (Chile–Argentina), massive amounts of ash and pumice were discharged directly to the lakes and caused an increase in P concentrations in the ultraligotrophic lakes of the surrounding landscape [17, 32]. The massive amounts of ejected pumice created novel floating substrata for microbial life [17]. However, the impact of the pumice inputs on pelagic microbial communities remains unclear, especially for the dynamic period immediately after pumice deposition. The flexibility in C/P stoichiometry was observed to be greater in bacteria than in any species or assemblage [21], suggesting that bacteria respond very quickly to sudden environmental changes like volcanic eruptions. Up to now, we know of no studies considering sudden changes in C/P ratio and their consequences for bacteria community composition, metabolism and enzymatic activity over a short time scale following pumice input. Thus, the main aim of our study was to determine how ambient bacterial communities respond to pumice inputs in lakes that differ in DOC and P concentrations and to what extent these responses are moderated by substrate C/P stoichiometry. To test this, we performed an outdoor mesocosm experiment using lake water with two different DOC concentrations involving manipulations of light and pumice input and compared responses of bacterial assemblage composition and abundance, phytoplankton abundance and biomass, bacterial respiration, and enzymatic activities (APA).

Material and Methods

Experimental Design

We compared the impact of floating pumice on microbial communities from two contrasting lakes: Lake Escondido, a small shallow lake, and Lake Gutiérrez, a large deep lake (Table 1). Both lakes are located at 764 m above sea level in the Northern Patagonia Andes (Argentina; about 41° S and 71°W; for specific location, see Table 1). Lake Gutiérrez has a warm monomictic thermal regime and remains stratified during summer (December–April), while Lake Escondido exhibits a continuous thermal mixing regime. Lake Escondido generally has higher DOC and P concentrations than Lake Gutiérrez [33]. Because of their southern location and the predominance of strong western winds in the region, both lakes were relatively unaffected by the volcanic activity during the eruption of June 2011 [32].

The experiment was performed in the growing season following the volcanic explosive event (summer, 11–26 February 2012). Epilimnetic lake water was collected on 11 February 2012 and gently filtered through 200-μm-mesh plankton net to remove macrozooplankton and then incubated for 15 days in 20-L experimental units in a water bath with a thermostat held at 18±1 °C (similar to summer surface lake temperatures). The incubation system was located outdoors to experience natural solar radiation and setup within 1 h after sampling. We tested three different treatments for each lake community using four replicates per treatment: Control, treatment with natural lake water covered with a neutral screen to prevent photoinhibition; Pumice, treatment with natural lake water with floating pumice (~1 g cm^{-2}); and Dark, treatment with natural lake water completely covered with aluminum foil in order to test if the effect of pumice cover was equivalent to dark conditions. Fresh pumice was collected the week after the event in June 2011, sterilized (450 °C), and kept in sterile plastic bags in the laboratory. Pumice rocks used in the experiment were 1.05±0.16 cm (mean±s.e.) in diameter. Due to their porous structure, rocks remained floating at surface during the entire experimental period. Light was measured at

Table 1 Features and initial conditions of the two lakes: Gutiérrez and Escondido

	Lake Escondido	Gutiérrez
Location	41° 03′ S, 71° 34′ W	41°10′ S, 71° 23′ W
Area (km^2)	0.06	16.4
Maximum depth (m)	111	8
Initial conditions		
DOC (mg L^{-1})	4.27	0.68
TDP (μg L^{-1})	5.70	1.67
NH_4 (μg L^{-1})	11.31	7.24
NO_3 (μg L^{-1})	9	7
N.P (dissolved atomic)	136	197
C/P (dissolved atomic)	2265	1053

DOC dissolved organic carbon, *TDP* total dissolved phosphorus, *DIN* dissolved inorganic nitrogen, *NH_4* ammonium, *NO_3* nitrate, *N/P* ratio of DIN/TDP, *C/P* ratio of DOC/TDP

subsurface in the different treatments using a PUV500B submersible radiometer (Biospherical Instruments) and yielded the following results: Control, 11 % of surface photosynthetically active radiation (PAR=400–700 nm), and Pumice, 0.1 % of surface PAR. Based on these measurements, we estimated the equivalent depth for each lake that the experimental conditions were mimicking (Lakes Escondido and Gutiérrez; Control treatment, 3.5- and 20-m depth, and Pumice treatment, 10.5- and 60-m depth, respectively). Each unit was gently shaken manually twice a day.

Sampling and Laboratory Procedures

Nutrient and Chlorophyll a Concentration

At the start (T_0), and after 5, 10, and 15 days of incubation (T_5, T_{10}, and T_{15}, respectively), we sampled water in each treatment using a sterile tube and syringe in order to estimate phosphorus (P; total dissolved phosphorus, TDP), dissolved organic carbon (DOC), chlorophyll *a* (Chl *a*) concentration, and bacterial abundance. Samples for phytoplankton abundance and alkaline phosphatase activity (APA) were obtained at T_0, T_{10}, and T_{15}. Finally, nitrogen (as nitrate and ammonium) and bacterial community composition were determined at T_0 and T_{15}. Dissolved oxygen concentration and pH were monitored during each sampling event; these parameters did not differ among treatments nor changed during the experiment. pH remained near neutral (7.2) and dissolved oxygen was always in 100 % saturation.

Phosphorus concentrations were determined according to APHA [1]. TDP was determined on Whatman GF/F filtered lake water and analyzed by the molybdate reaction after persulfate digestion. Dissolved organic carbon (DOC) was determined on filtered lake water (pre-combusted GF/F Whatman filters) using a Shimadzu™ TOC-VCSH Organic Carbon Analyzer. Ammonium was measured with the indophenol blue method, and nitrate analysis was carried out using Lachat QC8000 flow injection systems (detection limit 0.85 μg N L^{-1}).

Chlorophyll *a* was estimated on 0.2-μm Nucleopore filters extracted in hot ethanol following Nusch [35] and determined by fluorometric analysis (Turner Designs model 10 AU fluorometer).

Phytoplankton and Bacterial Abundance

Phytoplankton were enumerated to genus and/or species level in 50-mL chambers with an inverted microscope following the Utermöhl technique [45]. Because of the dominance of nanoflagellate cells, enumerations were also performed on samples stained with DAPI (final concentration 2 %*v*/*v*) and filtered onto 1-μm polycarbonate black membrane filters. Cells were counted by epifluorescence microscopy at ×1250 magnifications, using both UV and blue (U-MWB filter) light, to distinguish heterotrophic nanoflagellates (HNF) from autotrophic (ANF) and mixotrophic nanoflagellates (MxNF).

Total bacterial abundance determination was performed by staining cells collected on polycarbonate black membrane filters (0.2-μm pore size) with fluorochrome 4,6-diamidino-2-phenylindole (DAPI, final concentration 2 %*v*/*v*) [37]. Bacterial cells were counted at ×1250 magnification with an Olympus BX50 epifluorescence microscope using UV light (U-MWU filter). Cells were counted using an image analysis system (Image ProPlus; Media Cybernetics, Silver Spring, MD, USA).

Bacterial Community Structure

DNA Extraction and Sequencing

DNA was extracted from water and pumice samples collected at the end of the experiment on day 15. Water samples (50 mL) from each treatment (four independent replicates for each treatment except Lake Escondido Control and Lake Gutiérrez Dark treatments, for which one replicate from each treatment was lost) were filtered through a 20-mm-mesh net prefilter and followed by a 0.2-μm Nucleopore filter (25-mm diameter). The Nucleopore filters were aseptically transferred into MoBio PowerWater DNA Isolation kit Bead Tubes. Additionally, from the Pumice treatment, pumice rocks were collected, rinsed with 0.2-μm filtered lake water, and kept in sterile 50-mL tubes for genetic analysis. Filter and pumice samples were stored at −80 °C until extraction.

DNA from water samples was extracted using MoBio PowerWater DNA Isolation kit according to the manufacturer's protocol. Pumice samples were first sonicated with a bead mixture using a Branson 450 cup horn sonifier at duty cycle 50 % and output 4. The sample was sonicated for four cycles of 2.5 min while resting on ice between cycles. DNA was then extracted according to the manufacturer's protocol for MoBio PowerMax Soil Isolation kit. Purity of DNA was determined by PCR amplification with universal bacterial primers (8 F/1492R) and quantified using Nanodrop (Nanodrop, DE, USA).

Purified DNA samples were sent to Michigan State University Research Technology Support Facility for paired-end sequencing with the Illumina MiSeq platform using the standard V2 reagent kit (Illumina, CA, USA). Amplicon libraries were prepared according to [6] using 12-bp barcodes for multiplex sequencing. Briefly, DNA extracts from each treatment were used as templates in PCR reactions using primers 515 F/806R targeting the V4 region of the bacterial 16S rRNA gene. The reverse primers also contained 12-bp barcodes for sample identification and Illumina linker sequences. The sequences were then demultiplexed using Illumina Bcl2Fastq software (Version 1.8.4).

Sequence Analysis

A total of 17,106,106 reads were obtained from the sequencing. The sequences were screened for quality control using the bioinformatics software mothur (v.1.33.0) [39]. The initial screening involved minimum length (100 bp), no ambiguous bases, and maximum homopolymer of 5 bases. The sequences were then aligned based on the SILVA database followed by chimera removal using the UCHIME function [12] in mothur. After screening, a total of 9,687,004 sequences with an average of 322,900 reads/library were used for further analysis. The sequence libraries were deposited to NCBI Sequence Read Archives with the Biosample accession numbers SAMN03245106–SAMN03246115 under Bioproject ID PRJNA267509. The sequences were clustered by the average neighbor-joining method into operational taxonomic units (OTUs) defined as sequences with at least 97 % similarity. The taxonomic identity of the OTUs was determined from a representative sequence using the Ribosomal Database Project Release 11.2 Classifier tool [46]. Diversity indices for the sequence libraries were calculated using mothur without normalization because the library sizes were of less than 15 % difference. Significant differences (p value <0.05) in diversity indices were determined by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test in R statistical software v3.1.0 [43]. OTUs that appeared only once in the entire library were omitted from further analysis. The bacterial community composition for each lake was described using Bray–Curtis similarity index based on the relative abundance of the OTUs. The relationships were visualized using non-metric multidimensional scaling (NMDS) using the vegan package v2.0-10 in R. Significant differences were identified using one-way analysis of similarities (ANOSIM) calculated in mothur. OTUs representative of particular treatments were identified using the Dufrene–Legendre indicator species analysis [11] in R with the lab dsv package.

APA and DOC Bioassays

APA was determined at T_0, T_{10}, and T_{15} according to Hoppe [25] using methylumbelliferyl phosphate, MUP (36 μM), in Tris buffer, pH 8.3. Water from the different treatments (4 mL) was analyzed as unfiltered water (total) and as filtrate from 2-μm Nucleopore filters (<2 μm). Temperature was stabilized in a water bath of 20 °C for 10 min and then 0.5 mL MUP solution was added to all samples. Fluorescence was measured with a Perkin Elmer LS45 fluorometer immediately and after 45 min (this interval was previously chosen based on the activity in the sample). Values are given in nM L^{-1} h^{-1}.

Short-term bacterial respiration and DOC consumption experiments (DOC bioassays) were performed at T_0 (lake water) and after 10 days of experimentation (T_{10}) for the three treatments. We selected the T_{10} because at this time, we observed a maximum availability of phosphorus in the Pumice treatments (see "Results" section). Bioassays consisted of incubating water filtered through sterile polycarbonate (2-μm Nucleopore) filters to exclude nanoflagellates. The incubations were performed in triplicate using 500-mL ground-stoppered Erlenmeyer flasks in an incubation chamber in the dark at 20 °C [22]. The concentration of dissolved oxygen was measured every 6 h with an optical-oximeter (ODO) with non-invasive oxygen fluorescent sensors (PreSens) located inside the flasks. The O_2 consumption rates were estimated as the slope of the O_2 change over time fitted to a least squares regression. Oxygen consumption rates were converted to CO_2 production to provide short-term estimates of organic carbon consumption using a respiratory quotient of 1 [30]. After 2 days of incubation, the sample was transferred to 500-mL bottles, previously washed with HCl, and sterilized. DOC decay analysis was performed every 2–4 days up to 28 days by sampling 40 mL in pre-combusted vials (450 °C) to which 40 μL of 5 M sulfuric acid had been added.

Data Analyses

Since the samples were taken at different times (T_0, T_5, T_{10}, and T_{15}), the results were analyzed with two-way repeated measures ANOVA (RmANOVA) separately for each of the two lakes (i.e., Lake Escondido and Lake Gutiérrez). For each analysis, the between-subjects factor was experimental treatment (Pumice, Dark, and Control) and the within-subjects factor was time in days (T_0, T_5, T_{10}, and T_{15}). The variables analyzed were TDP, DOC, and Chl *a* concentrations. Normality (Kolmogorov–Smirnov test), homoscedasticity (Levene's test), and sphericity (Mauchly test) assumptions were previously verified. Greenhouse–Geisser's statistics were used to compute the adjusted p level of the F-test when sphericity was not fulfilled. For a posteriori multiple comparisons, the Tukey test was applied to determine significant differences between individual treatments and times. Statistical analyses were carried out with SPSS 18. When samples were taken only at the final time (T_{15}), differences between treatments were analyzed with one-way ANOVA.

Respiration experiments were analyzed by calculating a k value that represents the DOC decay. A first-order decay model based on the multiG model [47] with only one reactive member was applied to this single decay curve. In all experiments, there was a relatively large residual portion of the total DOC that was present at the end of the incubation. Thus, the equation was as follows:

$$G_T(t) = G_{Lab} \cdot e^{-kt} + G_{Re}$$

where G_T is the total DOC concentration at time t, G_{Lab} and G_{Res} are the labile and residual pools, respectively, k is the first-order decay constant, and t is the time of decomposition.

The k value represents the shape of the overall DOC decline, thereby indicating the evolution of the initial consumption over time. High values of k represent a strong inflexion in the DOC versus time curve, suggesting the rapid exhaustion of a highly reactive pool and a rapid transition to refractory carbon. Conversely, low values of k imply relatively constant DOC consumption over time, suggesting a more homogenous composition of the labile pool [22].

Results

Nutrient and Chlorophyll *a* Concentrations

The two lakes differed in dissolved C/P ratio (DOC/TDP) at T_0, with the C/P atomic ratio in Lake Escondido (2265 C/P) higher than in Lake Gutiérrez (1053 C/P; Table 1). Experimental manipulations produced highly significant differences in P concentration between treatments for both lakes (TDP, RmANOVA $p<0.001$). In particular, the Pumice treatment resulted in a significant increase in P concentration relative to initial values in both lakes (post hoc Tukey test, $p<0.001$; Fig. 1a, b). DOC concentration did not differ among treatments (RmANOVA, $p>0.05$) in the higher-DOC lake, Lake Escondido (DOC >4 mg L^{-1}; Fig. 1c). However, for low-DOC Lake Gutiérrez (DOC <1 mg L^{-1}), pumice addition resulted in a significant increase in DOC concentration (RmANOVA, $p<0.001$; post hoc Tukey test, $p<0.001$; Fig. 1d). Regardless of the lake, at T_{10}, pumice input (Pumice treatment) strongly decreased dissolved C/P ratio (e.g., to 140 in Lake Gutiérrez and to 400 in Lake Escondido; Fig. 2, note change in the graph scales).

Chl *a* differed between treatments similarly in both lakes (RmANOVA, $p<0.001$; Fig. 1e, f). While Chl *a* declined during the incubation in the Dark treatment, an initial decline was followed by an increase in the Pumice treatment in both lakes. This resulted not only in differences between treatments but also in a significant treatment × time interaction ($p<0.001$ in both lakes). Nitrogen was analyzed at T_0 and T_{15} in both lakes, but no differences in dissolved inorganic nitrogen (DIN= NH_4+NO_3) was observed between treatments (one-way ANOVA, $p>0.05$; Supplementary Fig. S2).

Phytoplankton and Bacterial Abundance

In both lakes, phytoplankton was dominated by mixotrophic nanoflagellates, in particular by *Plagioselmis lacustris* and *Chrysochromulina parva*. The abundance of these two species was positively correlated with Chl *a* concentration ($r^2=0.32$, $p<0.001$; Supplementary Fig. S3a, b). The HNF assemblage had a somewhat different response: All treatments showed a similar increase in HNF during the first 10 day followed by a moderate decrease (Supplementary Fig. S3c, d).

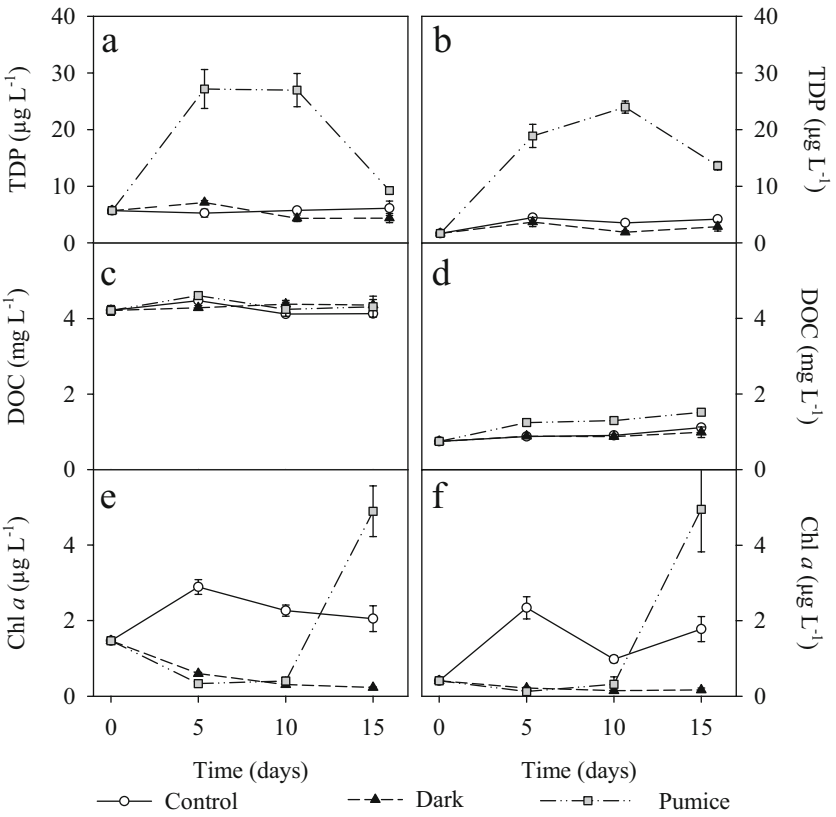

Fig. 1 Total dissolved phosphorus (TDP), dissolved organic carbon (DOC), and chlorophyll *a* (Chl *a*) concentrations in the different treatments during the experiment. **a**, **c**, **e** Lake Escondido; **b**, **d**, **f** Lake Gutiérrez

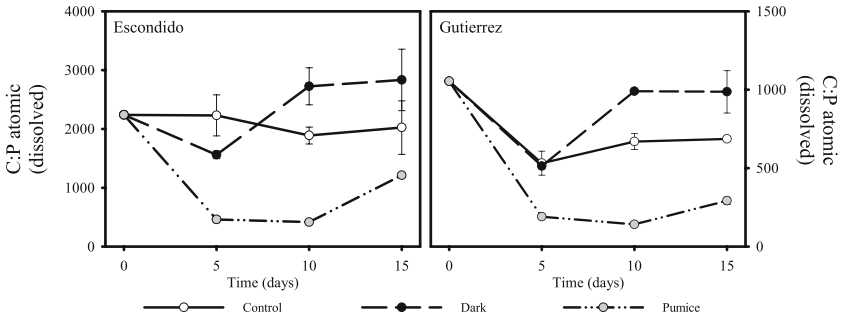

Fig. 2 Dynamics of dissolved C/P atomic ratio (calculated based on dissolved organic carbon, DOC, and total dissolved phosphorus, TDP) in the different treatments during the experiment. Please note the change in scale

At the start of the experiment, bacterial abundances differed between lakes (4×10^6 cell mL^{-1} in Lake Escondido vs 2×10^6 cell mL^{-1} in Lake Gutiérrez). During the experiment, bacterial abundance decreased in the Pumice treatment (to 2×10^6 cell mL^{-1} in the Lake Escondido experiment and to 1×10^6 cell mL^{-1} in the Lake Gutiérrez experiment) but remained relatively constant in the Control and Dark treatments (Supplementary Fig. S4).

Bacterial Community Structure

Community analysis using the 16S rRNA gene V4 region identified a total of 9182 and 8422 OTUs for Lake Escondido and Lake Gutiérrez, respectively. The initial bacterial community of each lake consisted mainly of *Gammaproteobacteria*, *Alfaproteobacteria*, *Flavobacteria*, *Sphingobacteria*, and *Firmicutes* (the latter only in Lake Escondido; Supplementary Fig. S5). These phyla made up 96.8 and 98.6 % of the Lake Escondido and Lake Gutiérrez bacterial community, respectively. However, both lakes differed in OTU composition, sharing only 36.5–49.4 % of their total richness. The high DOC Lake Escondido had higher abundance of *Actinobacteria* (*Proteobacteria*). On the other hand, in the low-DOC Lake Gutiérrez, *Brevundimonas* and *Caulobacter* (*Alphaproteobacteria*) and *Chryseobacterium* (*Bacteroidetes*) were the dominant OTUs.

At the final sampling time, bacterial communities in the control treatment for each lake consisted again mainly of *Gammaproteobacteria*, *Alfaproteobacteria*, and *Sphingobacteria* (Fig. 3, pie chart Control). Marked differences in the community structure can be observed in the NMDS ordination plot (Fig. 3, ordination plot). Bacterial communities from each quadruplicate in a given treatment yielded nearly identical fingerprint patterns, indicating the reproducibility of the experimental procedures (Fig. 3). ANOSIM showed significant differences in community structure between treatments: Control, Dark, and Pumice (PW= water from the treatment and P=floating pumice rocks, Fig. 3). In the Dark treatment, an increased abundance of *Actinobacteria* was observed for both Escondido and Gutiérrez lake water. In both lakes, the Pumice treatment resulted in a distinct assemblage composition in the water and on the pumice, with a decrease in *Gammaproteobacteria* and *Actinobacteria*, especially on the pumice rocks. In the water of the Pumice treatment, there was an increase in *Sphingobacteria*. On the pumice rocks, *Deltaproteobacteria*, *Flavobacteria*, and unclassified *Bacteroidetes* also became more abundant. OTUs from these taxa were rare members (<0.1 %) in the Control and Dark treatments. Consistent with the increased evenness measured for Pumice rock bacterial community (Table 2), 11 bacterial classes were found to have abundance of more than 1 % while only 5 classes in the other treatments had abundance of more than 1 %.

The Pumice treatment significantly increased richness in Lake Escondido water, resulting in increased bacterial diversity (observed richness, $F_{3,11}=11.71$, p value <0.001; Chao richness, $F_{3,11}=4.39$, p value=0.03; diversity, $F_{3,11}=14.94$, p value <0.001). In both lakes, pumice rocks in the Pumice treatment had bacterial communities that were significantly greater even in richness (Escondido, $F_{3,11}=5.91$, p value= 0.012; Gutiérrez, $F_{3,11}=6.92$, p value=0.001), which led to a significant increase in community diversity in Lake Gutiérrez ($F_{3,11}=6.87$, p value=0.007; Supplementary Fig. S6).

The Dufrene–Legendre indicator species analysis identified a total of 1295 and 1125 OTUs for the different treatments for Lake Escondido and Lake Gutiérrez water. Phylogenetic relationships of the indicator OTUs with relative abundance of more than 1 % are presented in Fig. 4. The increased bacterial diversity in the Pumice treatment of both lakes is evidenced in the more evenly distributed bubble sizes for rocks from the Pumice treatment (Fig. 4, bubble size represents $\log_2$ of relative abundance). The Control treatment was characterized by only 3–4 OTUs because it has a very similar community structure as the Dark treatment, as is also observed in the ordination plot (Fig. 3). Indicator species for the Control and Dark treatments included oligotrophic bacteria such as *Caulobacter* spp. and *Sphingomonas* spp., and newly characterized species such as *Planktophila limnetica* and *Aquirestis calciphila*. The Control treatment of Lake Escondido was also characterized by high dominance of *Acinetobacter calcoaceticus*. On the other hand, *Chryseobacterium gregarium* thrived in the Dark treatment of Lake Gutiérrez while the Verrucomicrobium *Luteolibacter algae* was enhanced in the Dark treatment of Lake Escondido. The Pumice treatment showed more diverse

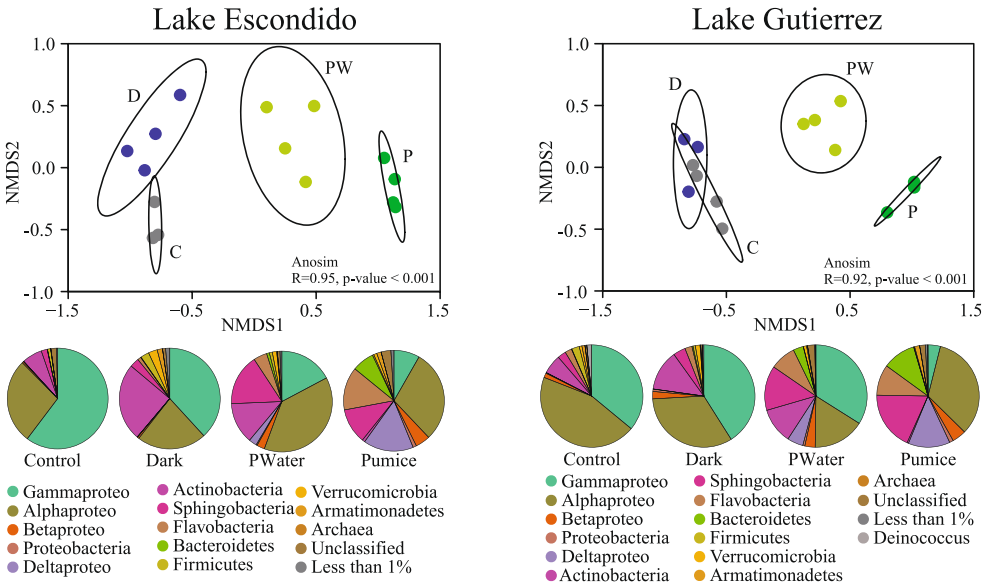

Fig. 3 Non-metric dimensional scaling (NMDS) plot based on Bray–Curtis similarity index of bacteria community structure in the different samples of Lake Escondido and Lake Gutiérrez. References: *C*=Control treatment, *D*=dark treatment, *P* and *PW*: Pumice treatments (PW=Pumice water, P=pumice rocks). The *ellipse* around each treatment represents the 95 % confidence interval of the centroid. The difference in community structure is significant based on analysis of similarity (ANOSIM)

bacteria taxa, including *Caulobacter* spp. that were rare in the Control and Dark treatments. Noticeably, in the Pumice treatment, there was a clear increase of predatory bacteria such as *Bdellovibrio* spp., *Peredibacter* spp., and *Bacteriovorax* spp., and gliding bacteria such as *Flavobacterium* spp. and *Sediminibacterium* spp. (Fig. 4). While these genera were found in both water and rock samples of the Pumice treatment, they were more abundant on the pumice rock.

APA and DOC Bioassays

APA (total and <2 μm) decreased only in the Pumice treatment. The decrease was higher in the water from Lake Gutiérrez than that from Lake Escondido (50 vs 30 %). In particular, there was a strong positive relationship between APA (total and <2 μm) and dissolved C/P ratio (r^2=0.68, p<0.001, for total; and r^2=0.56, p<0.001, for <2 μm; Fig. 5).

Experimental treatments caused a significant shift in DOC decomposition rates in both lakes. Initially, we observed that the DOC decay rate (k) was low and very similar in both lakes (~0.005 day^{-1}). However, after 10 days of experimentation (which coincided with higher availability of P in the Pumice treatment, Fig. 1a, b), we observed that the k value differed between treatments in both lakes (one-way ANOVA; Lake Escondido $F_{2,6}$=30.4, p<0.00; Lake Gutiérrez, $F_{2,6}$=384, p<0.001). In both lake experiments, k was significantly higher in the Pumice treatment than in the Dark or Control treatments (post hoc Tukey test, p<0.03 in Lake Escondido and p<0.003 in Lake Gutiérrez; Fig. 6). The k values increased twofold in Lake Escondido and fourfold in Lake Gutiérrez under the

Table 2 Diversity indices for bacterial communities from the different experimental treatments

Diversity Index	Lake	Control	Dark	Pumice	Pumice Rocks
Observed richness	Escondido	1478±264 a	1999±521 a	4457±1232 b	2645±357 a
	Gutierrez	2262±379	2053±590	3329±438	2337±1189
Chao estimated richness	Escondido	3578±1062 a	4948±1434 ab	9842±4409 b	5981±237 ab
	Gutiérrez	5168±377	4980±1591	6664±714	5997±3184
Simpson evenness	Escondido	0.002±0.00 a	0.007±0.005 ab	0.004±0.002 a	0.011±0.003 b
	Gutiérrez	0.004±0.003 a	0.004±0 a	0.005±0.001 a	0.012±0.004 b
Simpson diversity	Escondido	3.25±0.85 a	11.49±4.92 ab	16.91±6.74 b	29.45±5.99 c
	Gutiérrez	9.64±6.97 a	9.07±3.37 a	17.15±5.33 ab	23.54±3.13 b

The values are presented as averages±1 standard deviation of four replicates (except Lake Escondido Control and Lake Gutiérrez Dark that only have three replicates). Different letters indicate significant differences between treatments for each lake

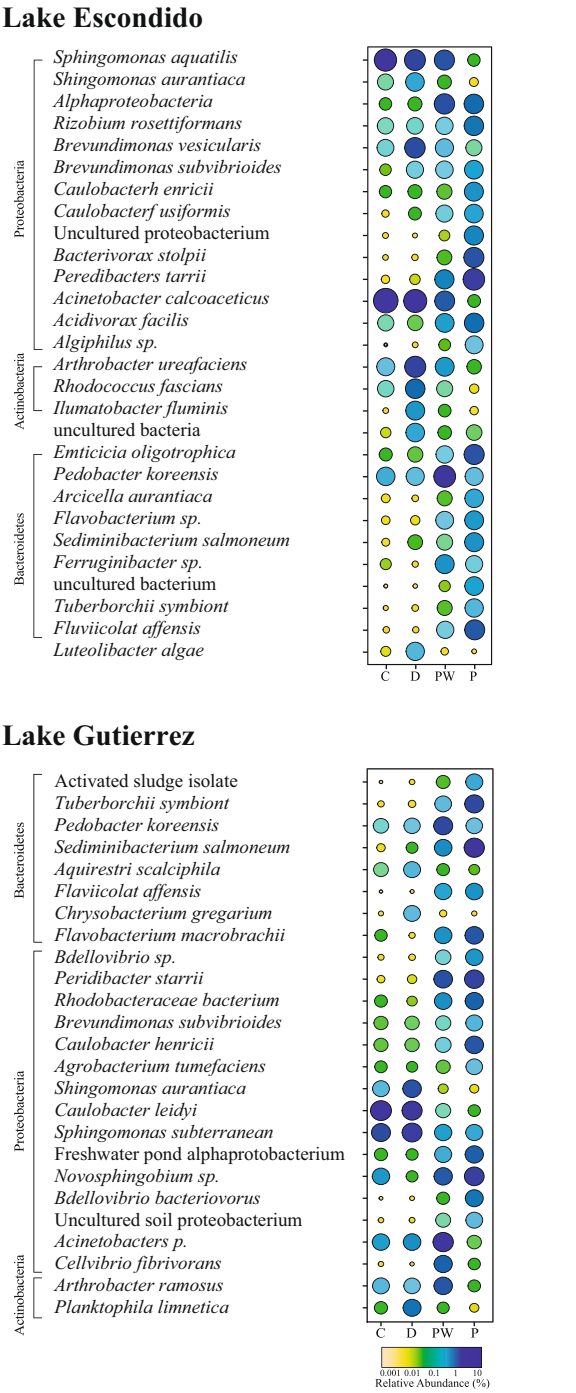

Fig. 4 Representative OTUs (Legendre species indicator analysis) in the different samples of Lake Escondido and Lake Gutiérrez. Only indicator OTUs that have a relative abundance of 1 % or more are included. The relative abundance of each OTU is presented as both the bubble size (in $\log_2$ scale) and fill color (in $\log_{10}$ scale). If there is no isolate that is at least 90 % similar, then an uncultured relative was selected

Pumice treatment, while in the Control and Dark treatments, k remained very similar to that of T_0 (Fig. 6, graph bars).

Discussion

Our experiment showed that pumice inputs caused substantial changes in the microbial communities (composition, enzymatic activity, and DOC consumption) and that these changes could be due to shifts in both light and nutrient (P) availability. While light was strongly reduced in the Pumice treatment, because of its porosity and glassy structure [4], pumice cannot be considered as an impervious cover of the lake surface. In the Lake Gutiérrez experiment, the Pumice treatment imposed a light level close to the depth at which the natural deep chlorophyll maximum (DCM) develops [31] while, in the Lake Escondido experiment, the light level was close to that exhibited at the bottom of the lake where active phytoplanktonic cells have been observed [3]. Therefore, in our experiment, there was still sufficient light penetrating the pumice layer in both lake waters for photosynthetic growth. In deep Andean lakes, mixotrophic cells (*Plagioselmis lacustris* and *Chrysochromulina parva*) are dominant phytoplankters particularly at the DCM level [31], while in shallow Andean lakes, nanoflagellates tend to inhabit the entire, illuminated water column [3]. Indeed, we observed a similar pattern of increase in mixotrophic nanoflagellates both in the Pumice treatment (this experiment in both lake waters) and in nature (shallow and deep lakes) following the eruption in the more strongly affected lakes in the area [32].

The different treatments resulted in different nutrient concentrations as P concentrations increased up to 20-fold with pumice addition. This increase probably overestimates the effect in nature because, although the pumice per surface area in our experiment was comparable to the floating pumice in small lakes (~1 g cm^{-2}), the amount of pumice per volume (20 L) was considerably higher. Nevertheless, we observed a maximum increase of P up to tenfold in shallow Lake Piré, a lake that was completely covered by pumice following the 2011 Puyehue–Cordón Caulle eruption (data unpublished), indicating that our experiment was comparable to actual concentrations after volcanic eruptions. The P increase in the water in our experiment was observed with a concurrent decrease in APA activity (both in total and <2 μm) and an increase in Chl *a* concentration, suggesting that with pumice additions, P was no longer limiting of microbial growth or at least that the severity of P limitation had decreased. A previous study showed that there is a P concentration threshold for the stimulation of phosphatase enzyme production [27], and our data suggest that, due to the volcanic eruption, this threshold can be surpassed rapidly.

Bacterioplankton are significant consumers of the dissolved organic matter pool in freshwater ecosystems [2] and P has been observed to be important in regulating the rate of organic carbon consumption by aquatic bacteria [10]. In addition, osmotrophic algae and heterotrophic protozoa are known to be very effective in dissolved C assimilation [8, 44].

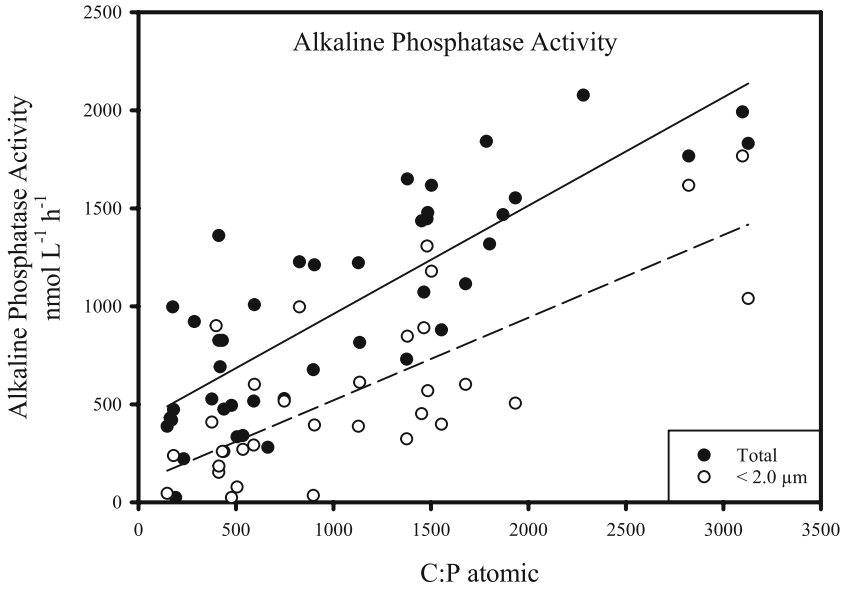

Fig. 5 Relationship of alkaline phosphatase activity (APA; total and <2 μm) with C/P dissolved atomic ratio

However, our bacterial respiration and DOC consumption experiments were carried out with filtered lake water; therefore, we assumed that osmotrophic protists were precluded. We found an increase in the carbon consumption rate by bacteria in the Pumice treatment (high P concentration) in both lakes. However, the C consumption curve reached a minimum plateau (~4 mg L^{-1} in Lake Escondido and ~0.8 mg L^{-1} in Lake Gutiérrez) after an initial elevated C consumption rate. This plateau was almost at the same level as in the other treatments and the initial control. This change in the C consumption function (from a steeper slope in the first 48 h, but reaching the same asymptotic C level thereafter) may indicate that the recalcitrant C pool did not change with addition of pumice. Consequently, pumice inputs to these systems may be driving microbial growth to organic C limitation since the labile, but not the recalcitrant form, of C is consumed rapidly. Therefore, the impact of increased P availability due to volcanic eruption would imply not only changes in autotrophic abundances (increases in biomass) but also shifts in the regulation of microbial metabolism, as was observed in our DOC bioassays.

Recently, it has been shown that high P availability can select for stoichiometrically homeostatic bacterial strains [20]. Thus, different bacteria assemblages are very likely to have occurred in our Pumice treatment due to the changes in

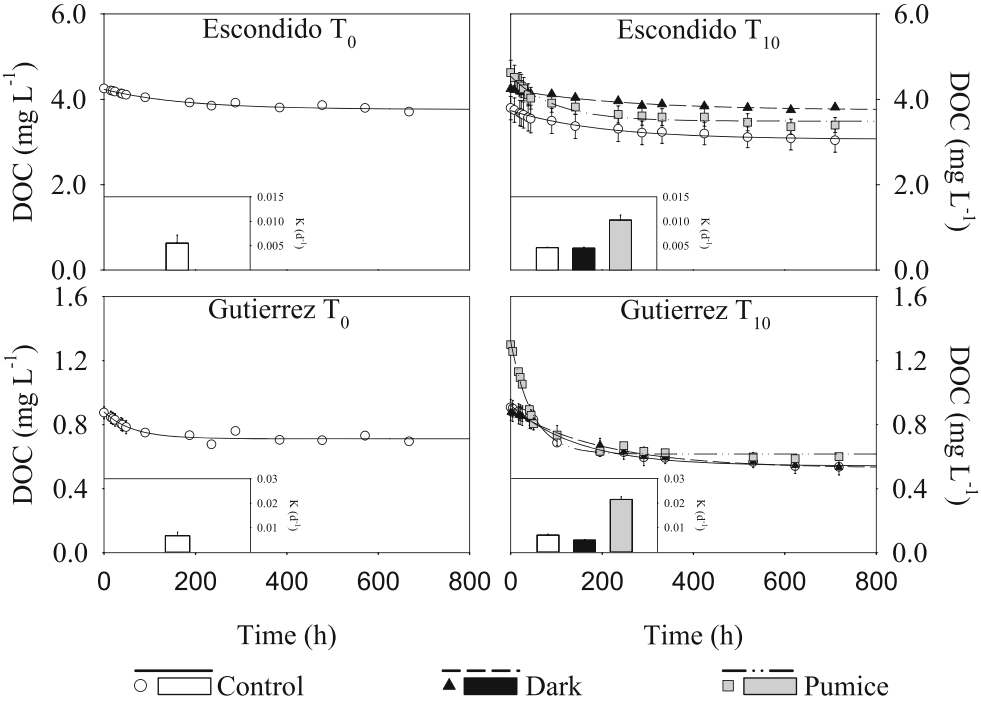

Fig. 6 Results of the DOC consumption experiments at that of the experiment (T_0) in Lake Escondido and Lake Gutiérrez waters and after 10 days of experimentation (T_{10}) in the three treatments: Control, Dark, and Pumice. Graph bar inside indicates the constant decay rate (k) of a first-order decay model. The difference in scale between lakes is due to differences in DOC concentration

the dissolved C/P ratio. In fact, in addition to the observed changes in APA and DOC consumption, we observed a noticeable increase in bacterial diversity in the treatment with added pumice. In particular, we observed an increase of bacteria associated with biofilm and sediments on the pumice rocks such as *Rhodobacteraceae*, *Proteobacteria* (*Cellvibrio*), and *Bacteroidetes* (*Sediminibacterium*) [16, 38], present in less than 0.1 % in the other treatments. Species such as *Flavobacterium* sp. and close relatives of the recently isolated *Fluviicola taffensis* members of the class *Flavobacteria* have the capability to move across surfaces by gliding motility [24], which probably allows them to be more competitive on pumice rocks than in the water. We also observed a significant increase in *Caulobacter* spp. on the pumice rocks (3.7 % in both lakes). Caulobacters are oligotrophic bacteria that contribute to C cycling in low nutrient environments but are generally found in low abundances (<0.1 % in all water samples). stalked caulobacters were previously shown to have a preference for association with diatoms as opposed to other algae. One of the explanations for this association is that Caulobacters might have a preference for siliceous surfaces [36]. The increased abundance of *Caulobacter* 16S rRNA gene sequences found on pumice rock supports this hypothesis. The porous surface of pumice rocks in the Pumice treatment provides increased microniches for the high diversity of bacteria community. The spatial heterogeneity can lead to promote niche differentiation for the diverse biofilm-forming species such as *Caulobacter*, *Sphingomonas*, and *Flavobacteriaceae*, supporting the high diversity of bacteria in the Pumice treatment. In addition, predatory bacteria also increased on the pumice rocks. These predator populations may have potential function in controlling and shaping bacterial communities through selective predation [48] as was observed in our experiments in which bacterial diversity and equitability increased in the Pumice treatment.

Interestingly, in the Pumice treatment, bacterial abundances decreased in the water concomitant with an increase in DOC consumption. This outcome may result from a top–down effect, i.e., increased predation by predaceous bacteria. Nevertheless, the increase in DOC consumption in the Pumice treatment (bioassays without protists) suggested that bacteria in water, although less abundant, were more efficient in consuming DOC because of changes in P supply that affected bacterial metabolism [10]. Alternatively, the decrease of major taxa such as *Acinetobacter calcoaceticus* (OTU001, from 28 to 55 % in Control and Dark to 7.3 % in Pumice water) may have reduced nutrient competition for fast-growing opportunistic species such as members of the *Flavobacteriales*, especially in the high DOC Lake Escondido [34]. At high dissolved C/P ratios, as was observed in the two lakes, bacterial production is P-limited [26].

In conclusion, our experiment demonstrated that pumice inputs from volcanic eruptions substantially decrease the C/P ratio of dissolved resources in lakes and cause changes in the bacteria assemblage composition. We showed that, over short incubation time, a concurrent decrease in APA activity and an increase in C consumption occur as a consequence of the decrease in P limitation due to the floating pumice. At the same time, the bacteria assemblages shift toward higher bacterial diversity due to increased abundance of rare taxa.

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