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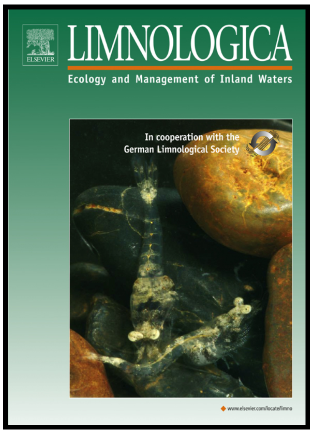


# Community-level physiological profiles in biofilms from streams differently affected by a volcanic eruption

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**Community-level physiological profiles in biofilms from streams differently affected by a volcanic eruption**

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**Abstract**

Volcanic eruptions are catastrophic events that allow testing different impacts on the ecosystems. Inputs of volcanic sediments into stream water may lead to drastic changes in the biota; however, the effects on biofilm functional traits remain poorly understood. This field study aimed to evaluate differences in structural and functional traits in the biofilm of streams differentially affected by the eruption of the Puyehue-Cordón Caulle Volcanic Complex (PCCVC, Chile, 2011). We examined differences in the structure (chlorophyll concentration, bacterial abundance, and biofilm organic matter and phosphorus content) and metabolic capacities (by use of Biolog EcoPlate™) in rock and sand biofilms from six streams with different distances from the volcanic source. Biofilms from streams near the volcano had higher phosphorus content and bacterial abundance. The Biolog EcoPlate™ indicated that there were differences in the use of some particular organic molecules among streams. On one hand, the use of substrate by biofilms from rocks and sand differed only in the non-affected streams. On the other hand, the biofilms of streams nearest the volcano were characterized by the use of galacturonic acid and this higher consumption can be related to the dominance of cyanobacteria. Our results suggest that volcanic sediment inputs, still occurring three years after the eruption, change biofilm communities, leading to physiological differences among streams.

**Keywords**: rock biofilm, sand biofilm, Biologs, organic matter, Patagonia

## Introduction

After a volcanic eruption, sediment inputs in aquatic environments produce numerous physical and chemical changes that affect the whole ecosystem (Ayris and Delmelle, 2012; Carrillo and Díaz-Villanueva, 2021; Modenutti et al., 2013). The common physical effects are the increase in turbidity and changes in the flow of water and sediments in the catchments (Canisius et al., 2009; Hayes et al., 2002; Janda et al., 1984). Sediments that fall into a water body may decant or be transported through streams and rivers (Hayes et al., 2002), depending on flow velocity and particle size. While particles <100 μm might be transported indefinitely, flow velocities under ~ 0.05 m $s^{-1}$ cause those particles to decant (Collinson and Thompson, 1989). In streams, volcanic ash can occupy the interstices between the rocks or completely cover the streambeds (Hayes et al., 2002) with severe effects on these habitats.

There are also important chemical and biological effects of volcanic sediments on aquatic environments. In this sense, studies after Mount St. Helens (MSH, USA, 1980) eruption registered an increase in bacterial growth rates and heterotrophic activity (Baross et al., 1982; Dahm et al., 1983; Wissmar et al., 1982). Similarly, after the eruption of the Puyehue-Cordón Caulle Volcanic Complex (PCCVC, Chile, 2011), the bacteria community of lakes was affected both structurally and physiologically (Elser et al., 2015; Modenutti et al., 2016). Floating pumice (volcanic sediments of 2–64 mm diameter) was soon colonized by bacteria communities containing taxa associated with the lake water and also taxa typical of sediment surfaces (Elser et al., 2015). Physiological changes were mainly related to the increase in phosphorus (P) which decreased the alkaline phosphatase activity and increased the bacteria respiration (Modenutti et al., 2016). The effects of volcanic sediment inputs on stream biofilms after MSH eruption showed a decrease in primary production (Cushing and Smith, 1982; Frey et al., 1983; Hawkins and Sedell, 1990; Steinman and Lamberti, 1988) and changes in the primary producer assembly (Rushforth et al., 1986; Steinman and Lamberti, 1988). Thus, the inputs of sediment may reduce light and dissolved gasses penetration, changing nutrient processing (Hanrahan et al., 2018a), primary production, and respiration (Marcarelli et al., 2015). In particular, the study on the effects of the PCCVC in streams indicated a decrease in the abundance of macroinvertebrates and fish immediately after the eruption (Lallement et al., 2016). Three years after the event, Carrillo et al. (2018) compared biofilms from affected and non-affected streams in a gradient of sediment deposition. These authors found both, substantial changes in the composition of primary producers (from diatoms in the non-afected streams to cyanobacteria as dominant in the affected streams) and a decrease in the enzyme alkaline phosphatase in the affected streams due to the increase in P.

Biofilms are responsible for most of the metabolism of streams (Bernhardt and Likens, 2002; Ylla et al., 2010). This consortium covers every solid substrate submerged in water and is constituted by algae, bacteria, ciliates, and microfauna, embedded in a polysaccharide matrix. However, there are structural and metabolic differences among biofilms according to the type of substrate (Hanrahan et al., 2018b; Marcarelli et al., 2015; Romaní et al., 2004; Romaní and Sabater, 2001). Fine sediments are more prone to movement, and more unstable than gravel or cobble (Knighton, 1998); thus, rocks carry higher biofilm biomass (Romaní and Sabater, 2001) and reach higher primary production (Marcarelli et al., 2015) than sand. On the other hand, biofilms on sand seemed to have a higher capacity to degrade polysaccharides and organic phosphorus compounds (Romaní et al., 2004).

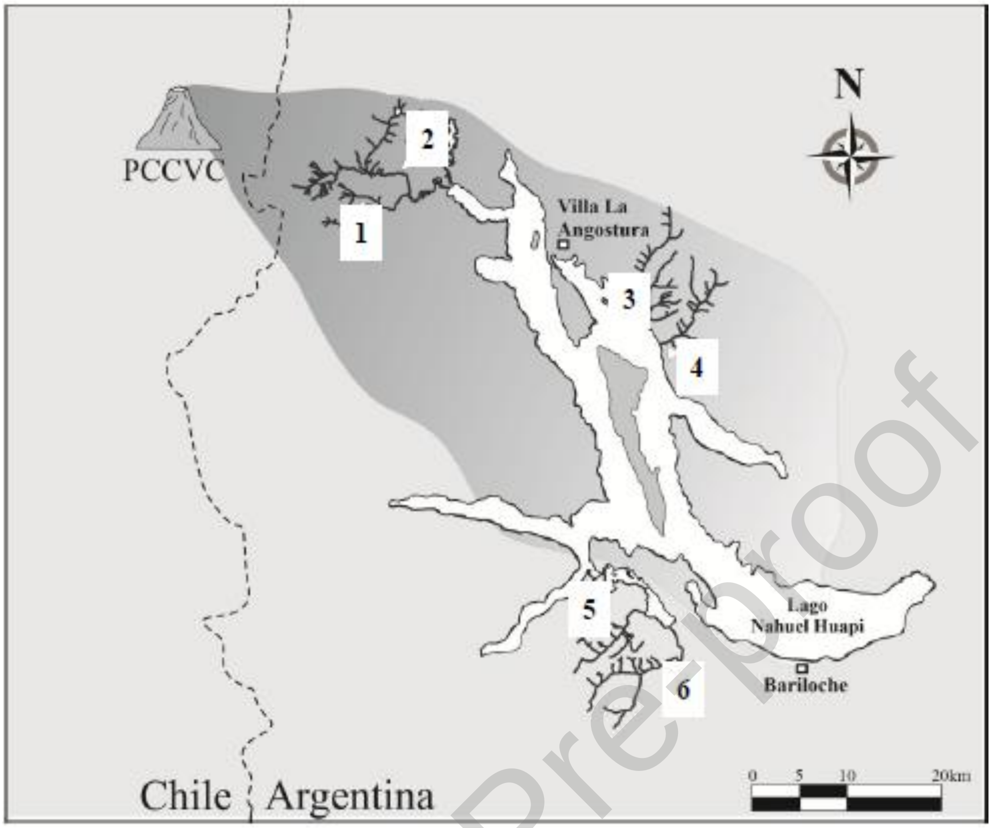
Bacterial communities differ in their abilities to consume organic substrates according to the pool of organic matter in which the communities develop (Comte and del Giorgio, 2009; Fasching et al., 2020; Ruiz-González et al., 2015). Differences in metabolic capacities were found in marine waters of different trophic states (Sala et al., 2006; Sala et al., 2020), in streams and lakes with different DOM concentrations (Bastidas Navarro et al., 2014; Díaz Villanueva, 2019; Díaz Villanueva et al., 2018) and in a river gradient from upstream to downstream sites (Freixa and Romaní, 2014). However, to our knowledge, there are no studies considering metabolic capacities concerning volcanic sediment inputs. Here, we presented a field survey analysing biofilm on rocks and sand from streams at increasing distances from the PCCVC. Volcanic eruptions might be seen as natural experiments, as they create a gradient of impacts in the environment that cannot be experimentally manipulated (Modenutti et al., 2013). We aimed to evaluate the response of stream biofilms to increased sediment loads, testing the hypothesis that changes in sediment loads and nutrient availability are reflected in the biofilm structure and community-level physiological profiles (CLPP). We tested this hypothesis by analysing the sediment and nutrient loads in six streams and the rock and sand biofilm structure and physiological response based on the Biolog technique (Biolog EcoPlate™ Hayward, CA, USA) (Zak et al., 1994). Although different methodological limitations have been indicated for Biolog EcoPlates (Kirk et al., 2004), the study of functional diversity in bacterial communities based on this technique can detect considerable variation in the ability of microbial communities to metabolize different organic compounds and can be used as a tool to evaluate changes in the metabolic capabilities of biofilms (Comte and Del Giorgio, 2010; Perujo et al., 2020; Ruiz-González et al., 2015; Sala et al., 2020).

## Material and methods

*Study site*

The study was carried out in six streams of the Nahuel Huapi basin, in the Nahuel Huapi National Park (41º00'S, 71º30'W, North Patagonia, Argentina) (Fig. 1). The area was glaciated extensively and repeatedly during the late Pleistocene. Thus, the landscape is dominated by glacial processes (moraines and glacial-fluvial plains) and also by volcanic events (Pereyra and Bouza, 2019). Climate is cold temperate, with the prevalence of west winds (westerlies) coming from the Pacific Ocean (Masiokas et al., 2008; Paruelo et al., 1998). Rainfall in this area is 2000 mm $year^{-1}$. The hydrological regime presents peak discharges in mid-autumn (rain) and spring (snowmelt) and the lowest values in late summer to early autumn. Headwaters (above 1000 m a.s.l.) are surrounded by a monospecific deciduous forest of *Nothofagus pumilio* (Poepp. & Endl.) Krasser, and below 1000 m a.s.l., the forest is mainly composed of the perennial forest of *N. dombeyi* (Mirb.) Oerst. and *Austrocedrus chilensis* (D.Don) Pic. Serm. & Bizzarri.

The area was differentially affected by the eruption of the Puyehue-Cordón Caulle Volcanic Complex (PCCVC, 40°30'S, 72°12'W, 2236 m a.s.l., Chile), on 4th June 2011. Given the predominance of westerly winds, a great part of the material fell in Argentinean Patagonia, leaving a gradient of sediment layer thickness (Elser et al., 2015; Gaitán et al., 2011). This layer varied not only in thickness but also in the particle size in the deposits (Pistolesi et al., 2015).




(Figure 1 was added at the end of the document for technical issues)

Figure 1: Map of the study area showing the location of the Puyehue-Cordón Cauye volcanic complex (PCCVC) and the extension of the ash plume (grey area). Sampling sites 1: Pereyra, 2: Pireco, 3: La Estacada, 4: Ragintuco, 5: Goye and 6: Casa de Piedra.



*Sample collection*

Sampling was carried out in six streams at different distances from the source (PCCVC, Fig. 1a, Table 1). At each sampling site, tephra layer thickness was measured (N = 5) in the adjacent riparian zone, where the soil profile was exposed. The tephra layers were easily recognized by their abrupt change of lithological and granulometric features (Carrillo et al., 2018).

Sampling was conducted on 16th April 2014 coinciding with the lowest water discharge since summer and early autumn are the driest seasons in this area (Díaz Villanueva et al., 2016). All sampling sites were located around 750-800 m a.s.l. and were surrounded by the *Nothofagus dombeyi-Austrocedrus chilensis* forest. Stream morphology presents smooth step-pool habitats with a pool-riffle structure. Streambed roughness was determined by the dominance of cobble-boulder substrates, while depositional areas were covered by sand and silt. At each sampling site, we measured water temperature and conductivity with a multiprobe instrument (YSI 85, Yellow Spring, Ohio) and water velocity with a water flow meter (Global Water, California, USA). Water samples were taken in 2-L plastic bottles (Nalgene) to measure concentrations of total suspended solids (TSS), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), phosphorus (TDP), soluble reactive phosphorus (SRP), and iron (Fe). Samples were immediately transported to the laboratory in dark and thermally insulated containers.

For rock biofilm sampling, three cobbles (mean surfaces of 116 ± 7 $cm^2$) of each site were collected from three riffle areas of the main channel, at 10 m distance from each other, and put in individual hermetic bags. Also, three sand samples were collected (at 10 m distance from each other) by inserting a 1-cm diameter polyethylene tube into the sand, then transferring the top 0.5-cm of material in the core, with the help of a pipette, into a 50-mL sterile centrifuge tube for storage (Gómez et al., 2009). All samples were carried to the laboratory in dark and thermally insulated containers.

*Laboratory procedures*

Stream water (400 mL) was filtered through pre-combusted and pre-weight GF/F filters to quantify TSS concentration. Filters were dried for 48 h at 80°C and re-weight (± 0.01 mg). Filtered water was used to measure DOC, TDN, TDP, and SRP. DOC and TDN concentrations were determined with a high-temperature combustion analyser (Shimadzu TOC V-CSH) and a Total Nitrogen Unit (TNM-1), using potassium hydrogen phthalate as the standard. For TDP determinations, samples were digested with persulfate at 1.5 atm for 1 h and measured as SRP with the ascorbate-reduced molybdenum blue method (APHA, 2005). Iron was measured as $Fe^{+2}$ using 1,10-phenanthroline (Duarte et al., 2009).

The biofilm of rocks was obtained by scraping individual rocks with a brush and carefully rinsing them with Milli Q water to a known volume. The biofilm of sand was detached sonicating the sand into the tube in stream water for 2 min in a sonication bath (J.P. Selecta S.A., Abrera, Spain) operating at 40 W and 40 kHz (Romaní and Sabater, 2001). The obtained suspensions (both from rocks and sand) were used for the estimation of biofilm organic matter and phosphorus content, chlorophyll *a* concentration (Chl-*a*), bacterial counts, and community-level physiological profile. The scraped rocks were measured (three main axes) to estimate the surface (Graham et al., 1988). Only two-thirds of the surface was considered available for algal growth (Biggs and Close, 1989). Sand surface estimations were conducted as described by Romani et al. (2001). Sand samples were dried and grain sizes were estimated by granulometry. Grain size distribution was determined by sieving dry sediment through different sieves (250, 500, 1000, and 2000 µm). The conversion factors between the dry mass of sand and total grain surface area varied from 0.515 ± 0.087 to 3.120 ± 0.381 $cm^2$ per gram of sand.

Biofilm organic matter was estimated as ash-free dry mass (AFDM). A subsample of 5-10 ml was filtered on pre-combusted and pre-weighed Whatman GF/F filters and dried at 80 °C for 1.5 h. The filters were weighed and combusted at 550 °C for 1.5 h, then re-weighed, and AFDM was computed as the difference in mass before and after incineration (APHA, 2005). The biofilm P content was determined by filtering 5 mL of the suspension through acid-washed (10% HCl) GF/F filters and drying at 60 °C for 48 h. After combustion at 550 °C for 1.5 h, P content of ash was analysed using the ascorbate-reduced molybdenum method (APHA, 2005). Extractions of Chl-*a* were performed with 90% hot ethanol (Nusch, 1980); spectrophotometric readings were carried out at 665 nm and 750 nm. Corrections for pheophytin were performed by acidification with HCl. Slides for bacteria counting were prepared by filtering 1 ml of the suspension after being stained for 1 min with 2 % (V/V) DAPI (4,6-diamidino-2-phenylindole), through black polycarbonate filters 0.22 µm pore size (Poretics) (Porter and Feig, 1980). Counts were carried out at 1250X magnification with an Olympus BX50 epifluorescence microscope. Ten images for each sample were processed with an image analysis system (Image ProPlus; Media Cybernetics, Silver Spring, MD, USA).

*Community-level physiological profiles (CLPP)*

Biolog EcoPlate™ microplates (Biolog Inc., Hayward, California, USA) were used to determine differences in the CLPP of the biofilms developed in each stream. Each microplate contains three replicate wells of 31 carbon sources plus a tetrazolium salt, which is reduced to a coloured compound by active bacteria (Garland and Mills, 1991) that can be measured by colourimetric methods, and a blank with no substrate. The substrates were classified according to their chemical guilds as carbohydrates, carboxylic acids, polymers, amino acids, and amines (Am). Biofilms were obtained from the same homogenate from stones and sand used for biofilm Chl *a*. Microplates were inoculated under sterile conditions with 130 µl of the biofilm extract into each well and incubated at 20°C in dark conditions. The colour development in the plate was

measured after 48, 72, and 109 h of incubation (until maximum colour development was reached), spectrophotometrically at 590 nm (Chromate 4300, Awareness Technology). Detailed information on the EcoPlate is available at
http://www.biolog.com/pdf/milit/00A_012_EcoPlate_Sell_Sheet.pdf.

The overall colour development of each plate was expressed as the average well colour development (AWCD) defined as [∑(R - C)]/93, where R is the absorbance of each response well, C is the average of the absorbance of the control wells. Negative values were considered zero.

*Data analysis*

The correlation of environmental variables (TSS, DOC, SRP, TDP, TDN, molar N:P, and Fe) and biofilm parameters (TP, OM, Chl *a* concentration, and bacterial abundance) with the distance from the volcano were tested with linear regressions (Pearson correlations). Principal Component Analysis (PCA) on environmental variables and biofilm parameters was performed after normalizing variables to identify those most responsible for the spatial variation among streams, using PRIMER v.6.1.6 (Primer-E Ltd 2006., Plymouth, UK). Variables that were strongly correlated with others in the initial analysis were removed (TDP correlated with SRP, TSS correlated with Fe, and biofilm P with biofilm Chl *a*, with $p < 0.001$ and $r > 0.9$) and a further analysis was carried out with the remaining variables (temperature, conductivity, flow velocity, DOC, TDN, molar dissolved N:P, SRP, Fe, biofilm OM, Chl a concentration, and bacterial abundance).

To analyze differences in organic carbon utilization by stream biofilms from the two types of substrates (rocks and sand communities), plate colour development (normalized absorbance) was carried out with two-way repeated measures ANOVA (two way RM-ANOVA) with the substrate (rock and sand) and stream (N = 6) as factors and temporal series of absorbance (from t = 48 to t = 109 h) as the repeated measure (Perujo et al., 2020). The comparison of the use of the different chemical guilds was performed by dividing the sum of the absorbance of molecules of one type by the number of substrates of that guild (CH=10, CA=8, Po=4, AA=6, Am=2).

We performed a similarity analysis to compare the use of organic compounds by the different communities (N = 12). The standardized matrix of absorbance from the Biolog EcoPlate™ was used to generate the similarity matrices using Bray-Curtis index. A non-metric multidimensional scaling (NMDS) analysis was applied to visualize the ordination of sites according to these matrices. Vectors were plotted according to their correlation (Pearson correlation) with NMDS axis 1 and 2.

**Results**

Environmental variables in stream water differed among streams (Table 1). We found also differences in the granulometry of benthic sediments (Fig. 2) since the particles >2 mm diameter were more frequent in Goye and Casa de Piedra (the two farthest streams) and the smallest size classes (<0.5 cm) were more abundant in La Estacada and Ragintuco.

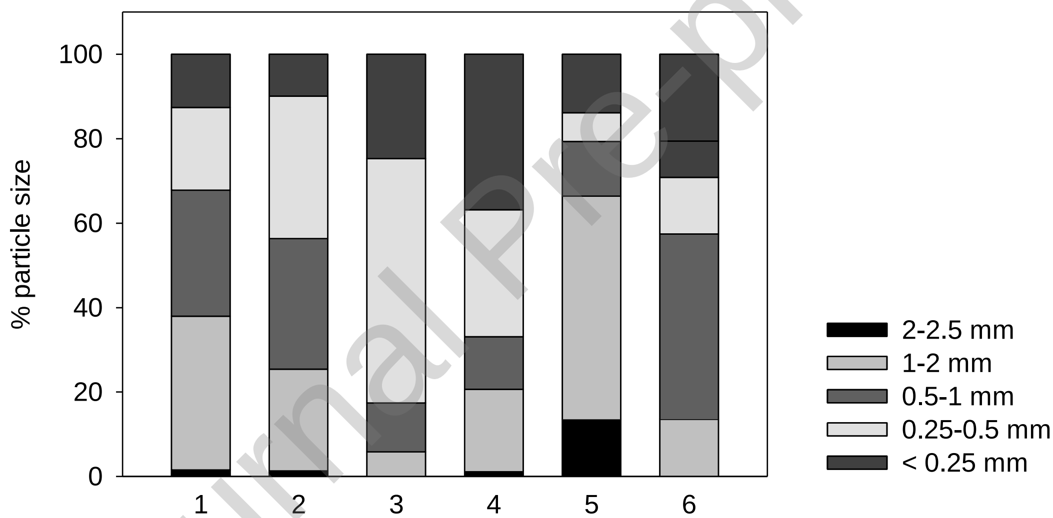



Figure 2: Granulometry of sand sediments in the six studied streams, 1: Pereyra, 2: Pireco, 3: La Estacada, 4: Ragintuco, 5: Goye, 6: Casa de Piedra.

We also observed differences in some features of the biofilms from rocks and sand. Organic matter per surface unit was one to two orders of magnitude higher in the sand samples than in the rocks. On the contrary, biofilm P content, Chl *a*, and bacterial abundance per biomass were one or two orders of magnitude higher in the rocks than in the sand (Table 2). Biofilm on rocks differed significantly in bacterial abundance, being higher in the two streams closest to the volcano, while biofilm on sand differed in the four parameters measured (Table 2).

Table 1: Environmental variables (mean ± standard error, where it corresponds) in the six studied streams and results of the comparison among them (one way ANOVA)

| Stream | 1.Pereyra | 2.Pireco | 3.La Estacada | 4.Ragintuco | 5.Goye | 6.Casa de Piedra | F | P |
|---|---|---|---|---|---|---|---|---|
| Distance to PCCVC (km) | 32 | 35 | 63 | 65 | 83 | 89 | | |
| Altitude (m asl) | 997 | 817 | 793 | 786 | 838 | 853 | | |
| Catchment area (km$^{2}$) | 10.2 | 73.7 | 53.0 | 46.3 | 32.9 | 62.7 | | |
| Q (L s$^{-1}$) | 55 | 680 | 329 | 219 | 417 | 512 | | |
| Tephra layer depth (cm) | 20 | 43 | 12 | 10 | 0 | 0 | | |
| TSS (mg L$^{-1}$) | 3.68 ± 0.36$^{a}$ | 12.57 ± 0.75$^{b}$ | 10.40 ± 2.63$^{ab}$ | 9.15 ± 1.71$^{ab}$ | 0.56 ± 0.56$^{a}$ | 0.37 ± 0.37$^{a}$ | 9.70 | <0.001 |
| DOC (mg L$^{-1}$) | 1.49± 0.01$^{a}$ | 1.75 ± 0.01$^{b}$ | 1.63 ± 0.01$^{c}$ | 1.15 ± 0.01$^{d}$ | 0.40 ± 0.00$^{e}$ | 0.80 ± 0.00$^{f}$ | 15171 | <0.001 |
| SRP (µg L$^{-1}$) | 6.5 ± 0.0$^{ab}$ | 10.7 ± 0.9$^{ab}$ | 25.2 ± 0.9$^{a}$ | 12.2 ± 0.1$^{ab}$ | 0.8 ± 0.2$^{b}$ | 1.1 ± 0.6$^{b}$ | H=16.22 | 0.006 |
| TDP (µg L$^{-1}$) | 7.2 ± 0.3$^{a}$ | 10.8 ± 0.4$^{b}$ | 26.0 ± 1.2$^{c}$ | 13.0 ± 0.6$^{d}$ | 1.9 ± 0.1$^{e}$ | 1.5 ± 0.1$^{e}$ | 277.73 | <0.001 |
| TDN (µg L$^{-1}$) | 88.39 ± 1.77$^{a}$ | 89.43 ± 1.79$^{a}$ | 57.83 ± 1.01$^{b}$ | 36.73 ± 0.73$^{c}$ | 37.77 ± 0.76$^{c}$ | 30.03 ± 0.60$^{d}$ | 481.928 | <0.001 |
| N:P (molar) | 5.63 ± 1.59$^{a}$ | 17.34 ± 1.04$^{b}$ | 25.56 ± 0.31$^{c}$ | 16.78 ± 0.43$^{c}$ | 39.16 ± 2.5$^{d}$ | 32.38 ± 3.02$^{d}$ | 91.27 | <0.001 |
| Fe (µg L$^{-1}$) | 10.77 ± 0.23$^{ab}$ | 29.88 ± 0.46$^{a}$ | 15.60 ± 0.01$^{ab}$ | 14.91 ±0.23$^{ab}$ | 6.16 ± 0.12$^{ab}$ | 5.70 ± 0.11$^{b}$ | H=16.56 | 0.005 |

Table 2: Rock and sand biofilm parameters (mean ± standard error) in the six studied streams and results of the comparison among them (one way ANOVA)

| Stream | 1.Pereyra | 2.Pireco | 3.La Estacada | 4.Ragintuco | 5.Goye | 6.Casa de Piedra | F | P |
|---|---|---|---|---|---|---|---|---|
| **Biofilm on rocks** | | | | | | | | |
| OM (mg cm$^{-}$ | 0.07 ± 0.01 | 0.05 ± 0.01 | 0.34 ± 0.14 | 0.18 ± 0.06 | 0.23 ± 0.02 | 0.22 ± 0.08 | 2.738 | 0.071 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2) | | | | | | | | |
| P (µg mg$^{-1}$) | 1.73 ± 0.57 | 0.84 ± 0.15 | 0.74 ± 0.26 | 1.27 ± 0.23 | 0.93 ± 0.41 | 1.29 ± 0.10 | 1.976 | 0.155 |
| Chl *a* (µg mg$^{-1}$) | 5.83 ± 0.78 | 1.98 ± 0.79 | 1.93 ± 0.68 | 1.29 ± 0.45 | 2.05 ± 1.54 | 3.67 ± 1.42 | 2.929 | 0.059 |
| Bacteria ($10^{6}$ cells mg$^{-1}$) | 60.18 ± $3.97^{a}$ | 72.58 ± $3.00^{a}$ | 14.77 ± $2.87^{b}$ | 15.82 ± $0.61^{b}$ | 16.10 ± $1.06^{b}$ | 16.15 ± $4.20^{b}$ | 87.341 | <0.001 |
| **Biofilm on sand** | | | | | | | | |
| OM (mg cm$^{-2}$) | 0.82 ± $0.11^{a}$ | 2.14 ± $0.32^{a}$ | 14.77 ± $1.07^{b}$ | 4.58 ± $0.71^{a}$ | 9.83 ± $0.67^{c}$ | 3.70 ± $0.34^{a}$ | 71.775 | <0.001 |
| P (µg mg$^{-1}$) | 0.05 ± $0.02^{a}$ | 0.44 ± $0.05^{b}$ | 0.13 ± $0.02^{a}$ | 0.13 ± $0.01^{a}$ | 0.10 ± $0.07^{a}$ | 0.09 ± $0.04^{a}$ | 11.462 | <0.001 |
| Chl *a* (µg mg$^{-1}$) | 0.019 ± 0.006 | 0.004 ± 0.002 | 0.0010 ± 0.0001 | 0.008 ±0.004 | 0.009 ± 0.001 | 0.027 ± 0.005 | H = 12.11 | 0.033 |
| Bacteria ($10^{6}$ cells mg$^{-1}$) | 11.21 ± $0.76^{a}$ | 1.23 ± $0.18^{b}$ | 4.89 ± $1.50^{ab}$ | 3.55 ± $0.79^{ab}$ | 1.26 ± $0.33^{b}$ | 5.00 ± $0.99^{ab}$ | H = 14.38 | 0.013 |

Tephra layer thickness in the riparian zone of sample sites was negatively related to the distance to the volcano (Pearson coefficient = 0.87, P = 0.025). The concentration of DOC and TDN were negatively related to the distance to the volcano. In addition, bacterial abundance on rocks, among the biofilm parameter, were also negatively related to distance (Table 3).

Table 3: Relations (Pearson Correlation coefficient and P value between brackets) of environmental variables and biofilm parameters with distance from PCCVC

| | | | |
|---|---|---|---|
| TSS | | -0.576 (0.231) | |
| **DOC** | | **-0.820 (0.046)** | |
| SRP | | -0.308 (0.553) | |
| TDP | | -0.291 (0.576) | |
| **TDN** | | **-0.955 (0.003)** | |
| N:P | | 0.459 (0.360) | |
| Fe | | -0.685 (0.134) | |
| | Rocks | | Sand |
| Organic matter | 0.703 (0.119) | | 0.399 (0.511) |
| Total P | -0.233 (0.656) | | -0.468 (0.350) |

| | | |
|---|---|---|
| Chl *a* | -0.333 (0.518) | 0.435 (0.388) |
| Bacteria | **-0.883 (0.020)** | -0.395 (0.440) |

The ordination of samples in the PCA explained 66.5% of the total variance in the two first components (PC1 and PC2). We observed segregation of the samples of non-affected streams, Goye and Casa de Piedra (both, biofilm from rock and sand) to the positive values of PC 1 (41.0 % explained variation) and the affected streams to the negative values (Fig. 3). This axis was positively correlated with dissolved molar N:P and negatively correlated with SRP, Fe, and DOC. On the other hand, PC 2 (25.5% explained variation) segregated the rock samples Pereyra, Pireco, Goye, and Casa de Piedra in the positive values and most sand samples to the negative values. This axis was positively correlated with Chl *a*, and bacteria and negatively correlated with OM.

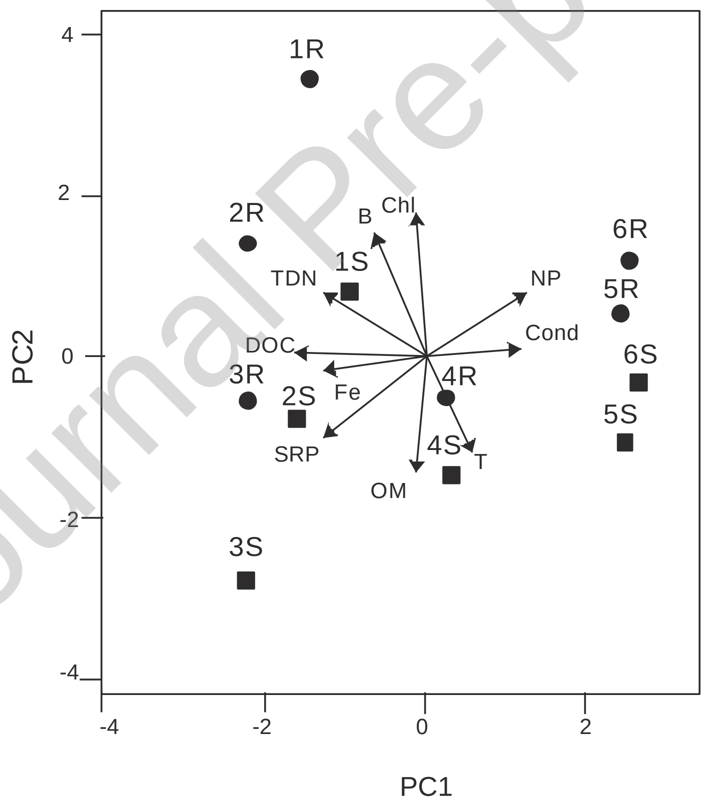



Figure 3: Sample distribution of biofilms from rock (R) and sand (S) in the six streams (1 to 6 according to Fig. 1) in the PCA based on environmental variables (Table 1) and biofilm parameters (Table 2).

The analysis of the use of organic substrates in biofilms showed that the development of colour in Biolog EcoPlate™ (AWDC) from t = 48 h to t = 109 h differed among substrates depending on the stream (two-way RM-ANOVA, interaction term F = 4.59, P = 0.010). Differences in colour development between sand and rocks were only significant in the farthest streams, Goye and Casa de Piedra (*a posteriori*, $P < 0.05$).

The biofilm of all streams was able to use 20-24 substrates (Table 4), while seven substrates were not consumed (D,L- α -Glycerol Phosphate, i-Erythritol, γ-Hydroxybutyric Acid, α -Ketobutyric Acid, L-Phenylalanine, L-Threonine, and Glycyl-L-Glutamic Acid). When the substrates were analyzed according to the chemical guilds, amino acids were the most used and polymers were the least used substrate (Table 4).

Table 4: Absorbance of each organic substrate of the Biolog Ecoplate at 109 hs incubationin the rock and sand biofilms from the six studied streams. Numbers on the left indicate the number in the NMDS on Figure 4. Colors according to chemical guilds: Polymers in green, Carbohydrates in orange, Carboxylic acids in yellow, Amino acids in blue, and Amines in violet. Greyscale indicated absorbance level:

0-0.1 0.1-0.5 0.5-1 1-2 >2

| | | Rocks | | | | | | Sand | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1.Pereyra | 2.Pireco | 3.La Estacada | 4. Ragintuco | 5.Goye | 6.Casa de Piedra | 1.Pereyra | 2.Pireco | 3.La Estacada | 4. Ragintuco | 5.Goye | 6.Casa de Piedra |
| 2 | Tween 40 | 0.62 | 1.08 | 0.87 | 0.36 | 0.29 | 0.42 | 1.18 | 0.97 | 1.05 | 0.43 | 0.73 | 0.72 |
| 3 | Tween 80 | 0.44 | 1.35 | 0.87 | 0.26 | 0.30 | 0.42 | 1.07 | 0.92 | 1.31 | 0.51 | 0.71 | 0.66 |
| 4 | α-Cyclodextrin | 0.16 | 0.12 | 0.30 | 0.07 | 1.24 | 1.44 | | | 0.06 | | 0.15 | 0.06 |
| 5 | Glycogen | 1.65 | 1.40 | 1.23 | 0.04 | 1.12 | 1.37 | 0.82 | 0.51 | 1.04 | 1.57 | 1.66 | 1.52 |
| 6 | D-Cellobiose | 1.50 | 0.14 | 0.96 | 1.41 | 1.10 | 1.86 | 0.31 | 0.18 | 0.90 | 1.87 | 2.13 | 2.05 |
| 7 | α -D-Lactose | 2.04 | 1.02 | 2.05 | 2.23 | 1.10 | 1.89 | 2.24 | 2.30 | 2.79 | 2.23 | 1.88 | 1.81 |
| 8 | β-Methyl-D-Glucoside | 1.57 | 1.95 | 1.72 | 2.23 | 1.39 | 1.57 | 2.48 | 1.52 | 2.06 | 1.90 | 1.53 | 1.70 |
| 9 | D-xyloside | 0.97 | 0.73 | 0.39 | 0.51 | 1.47 | 0.26 | | 0.16 | | 0.89 | 0.73 | 0.46 |
| 10 | D-Mannitol | 2.17 | 2.62 | 2.65 | 2.13 | 1.52 | 1.86 | 2.31 | 2.20 | 1.53 | 2.00 | 2.01 | 2.15 |
| 11 | N-Acetyl-D-Glucosamine | 1.96 | 2.17 | 1.69 | 2.06 | 1.26 | 1.89 | 3.01 | 2.44 | 2.47 | 1.85 | 1.82 | 1.87 |
| 13 | Glucose-1-Phosphate | 0.64 | 0.87 | 0.64 | 0.45 | 0.93 | | 0.50 | 0.63 | 0.75 | 1.21 | 0.87 | 0.44 |
| 14 | D-Galactonic Acid γ -Lactone | 1.20 | 1.10 | 1.41 | 1.70 | 1.29 | 1.43 | 1.56 | 1.57 | 1.39 | 1.37 | 1.44 | 1.21 |
| 1 | Pyruvic Acid Methyl Ester | 1.44 | 1.47 | 1.70 | 1.59 | 1.26 | 1.34 | 1.38 | 1.73 | 1.69 | 1.38 | 1.32 | 1.20 |
| 12 | D-Glucosaminic Acid | 1.68 | 1.55 | 1.48 | 1.22 | 1.67 | 0.79 | 1.19 | 1.69 | 1.16 | 1.54 | 1.42 | 1.35 |
| 15 | D-galacturonic Acid | 1.86 | 2.23 | 2.07 | 1.76 | 0.92 | 0.51 | 2.64 | 2.36 | 1.99 | 1.69 | 1.44 | 1.24 |
| 16 | 2-Hydroxy Benzoic Acid | | | | | 0.73 | 0.67 | | | | | 0.40 | |
| 1 | 4-Hydroxy | 1.47 | 1.62 | 1.20 | 1.27 | 1.35 | 1.66 | 1.51 | 1.51 | 1.20 | 1.27 | 1.07 | 1.03 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | Benzoic Acid | | | | | | | | | | | | |
| 18 | Itaconic Acid | 1.13 | 1.26 | 1.23 | 1.18 | 1.18 | 0.56 | 1.41 | 1.42 | 0.53 | 1.40 | 1.14 | 1.27 |
| 19 | D-Malic Acid | 0.85 | 0.82 | 0.65 | 2.02 | 1.72 | 1.55 | 0.09 | 1.84 | 2.08 | 0.82 | 1.63 | 1.50 |
| 20 | L-Arginine | 2.15 | 1.95 | 2.40 | 2.04 | 2.17 | 2.30 | 0.90 | 1.55 | 1.32 | 1.91 | 1.79 | 2.13 |
| 21 | L-Aparagine | 2.00 | 2.59 | 2.48 | 2.29 | 2.09 | 2.17 | 2.98 | 2.91 | 2.53 | 2.10 | 2.10 | 2.24 |
| 22 | L-Serine | 1.88 | 1.51 | 1.36 | 1.82 | 1.70 | 1.56 | 2.30 | 1.75 | 1.59 | 1.15 | 1.41 | 1.12 |
| 23 | Phenylethylamine | 1.10 | 0.40 | 0.89 | 1.40 | 1.71 | 1.18 | 0.39 | 0.16 | 0.41 | 1.13 | 0.41 | 1.56 |
| 24 | Putrescine | 0.63 | 0.97 | 0.75 | 0.86 | 0.93 | 1.32 | 1.02 | 0.84 | 1.08 | 0.77 | 0.94 | 1.19 |

The use of the individual substrates differed among streams (Fig. 4). The distribution of sites in the NMDS showed segregation of the samples of rocks from the farthest streams (Goye and Casa de Piedra) to the right and samples of sand from the nearest streams (Pereyra and Pireco) to the left.

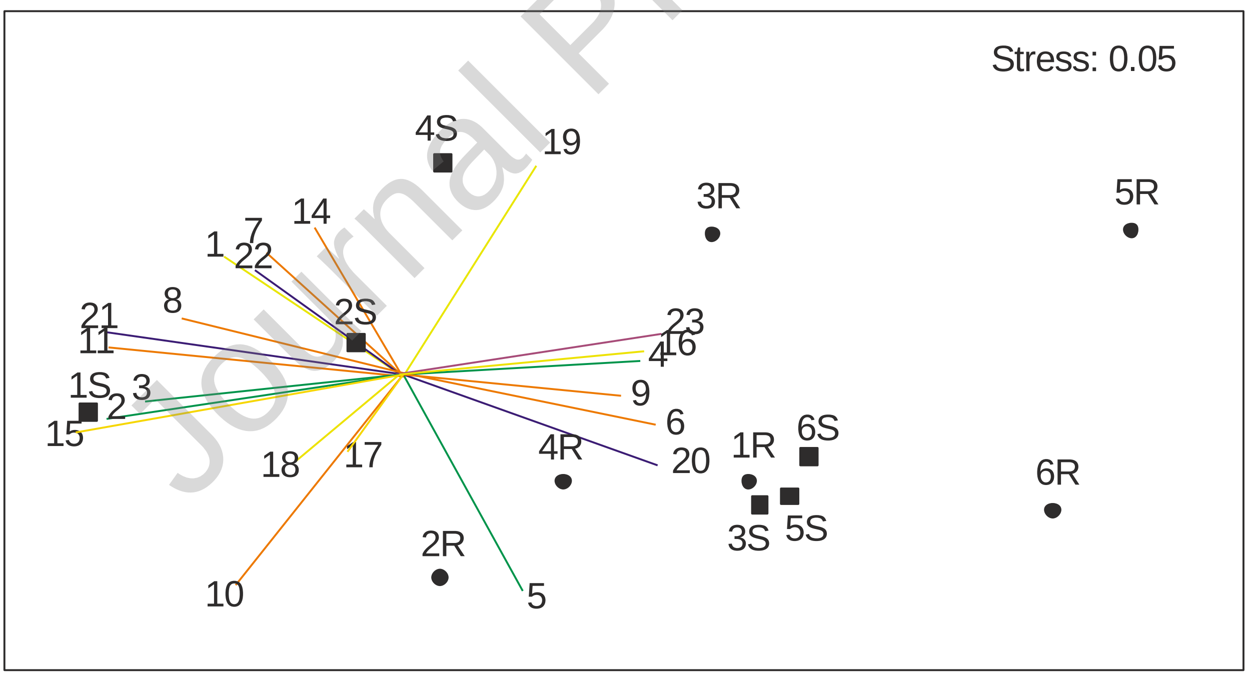



Figure 4: Sample distribution in MDS plot, according to similarity index based on the use of organic carbon substrates in Biologs Ecoplates by biofilm communities from rocks (circles, capital letters) and sand (squares, lowercase letters), of streams with different impact of the

volcanic eruption. The numbers of each vector refer to the substrates as indicated in Table 4, the colour of vectors accords to chemical guilds: carbohydrates in orange, carboxylic acids in yellow, polymers in green, amino acids in blue, and amines in violet.

The capacity of using substrates that contains P (Glucose-1-Phosphate and D,L- α -Glycerol Phosphate) was low (D,L-α-Glycerol Phosphate was not used) and Glucose-1-Phosphate was similarly used in the six streams (Table 4). On the other hand, N-Acetyl-D-Glucosamine was more used by the biofilms of Pereyra and Pireco (two-way RM ANOVA, F=0.012).

**Discussion**

The ordination analysis (PCA) showed a clear segregation of stream sampling sites, with the two farthest sites (Goye and Casa de Piedra) clearly different from the four nearest streams (Fig. 3). In accordance, we observed a gradient in tephra deposit thickness in the riparian zone of streams (Table 1). Although we did not find differences in TSS concentration in the streams according to the distance from the volcano, sediment granulometry differed between streams. The lack of a clear gradient in the four nearest streams might be due to (not exclusive) 1) the low number o streams studied, 2) differences in the slopes and the size of their catchments, and 3) to differences in the grain size of the sediments. According to Pistolesi et al. (2015), deposits at a distance of 28-37 km from the volcano (PCCVC) were characterized by a great proportion of lapilli (particles of 2 to 64 mm in diameter) and presented 45-32 cm thick. Similar thickness layers were measured by this study in Pireco stream (at 35 km from the volcano) almost three years after the eruption (Table 1). Deposits at 48 km (near La Estacada and Ragintuco) were 12 cm thick, similar to our measurements in La Estacada, and the proportion of lapilli and ash was almost equal (Pistolesi et al., 2015). Finally, deposits at 100 km (near Bariloche) were only 2 cm thick and exclusively composed of lapilli (without the ash layer) and were completely lost at the time of our study. These differences in the proportion of ash deposits, higher at intermediate distance, might have lead to the lack of a clear gradient in some variables, as SRP.

Our hypothesis on the effect of sediment loads and nutrient availability on the biofilm structure and community-level physiological profiles (CLPP) was confirmed. Sediment loads affected biofilm features and the metabolic capacities to use specific organic molecules of stream biofilms that colonize rocks and sand. Biofilms from rocks had a higher proportion of P, Chl *a*, and bacteria per unit of OM mass, but biofilms from sand had higher OM per surface area, suggesting a higher accumulation of non-living OM in the sediments. Similarly, Marcarelli et al. (2015) found 4.2-fold higher chlorophyll concentrations in rock biofilms than in sand, but 8-fold higher ash-free dry mass on sand substrates. Substrate heterogeneity and substrate-specific biofilms influence biogeochemical processes in streams (Hanrahan et al., 2018b). In particular, sand aggregations are sites of accumulation and degradation of OM (Marcarelli et al., 2015; Romaní et al., 2004), and the differences observed in the use of some substrates of the Biolog EcoPlate™ suggest that bacterial communities from sand and rock biofilms potentially had different capacities in substrate consumption.

The use of Biologs technique to analyze the CLPPs response of bacterial communities to different impacts has been extensively discussed (Kirk et al., 2004). However, the method is relatively easy to use, reproducible and produces a large amount of data reflecting metabolic characteristics. Bacterial communities are usually very similar in the use of organic substrates due to a high functional redundancy of the assemblages and most communities can potentially use the same organic substrate (Sala et al., 2020). However, the analysis of CLPPs were very useful to reveal the functional response and capacities of community trait structure underlying bacterial processing of dissolved organic matter (Ruiz-González et al., 2015). Therefore, small differences in the use of substrates (as observed in the present study) may be indicative of different responses of the bacterial community to environmental resource availability.

Stream biofilms are dynamic matrices that change the composition of photosynthetic and heterotrophic microbes, and extracellular polymers in response to environmental conditions (Battin et al., 2016). Here, we observed differences in the consumption of organic substrates by the biofilm of streams affected by the PCCVC eruption. Biofilms from rocks and sand of all streams consumed more amino acids than the other chemical guilds (in particular, L-Aparagine, L-Arginine, and L-Serine). Amino acids are a high-quality resource produced by primary producers, especially by diatoms (Bruckner et al., 2011; Kalscheur et al., 2012), which dominated in the biofilms of non-affected streams (Carrillo et al., 2018). The higher use of amino acids in rocks than in sand in the non-affected streams (Table 4) might be related to the observed higher biomass (higher Chl *a* concentration) of primary producers (diatoms) in the biofilm. This higher use of amino acids has been previously observed in biofilms from Andean-

Patagonian streams (Díaz Villanueva, 2019), differing from other studies carried out with plankton from lakes in the same area in which carbohydrates and polymers were the most consumed substrates (Díaz Villanueva et al., 2018).

We also found differences in the use of some individual molecules by the biofilms from the streams nearest the PCCVC. In particular, these communities were characterized by the use of galacturonic acid and this higher consumption can be related to changes in the primary producer biomass. While diatoms dominated the biofilm of non-affected streams, cyanobacteria increased their abundances (99.9% of total algal biovolume) in the affected streams (Carrillo et al., 2018). There is a close relationship between autotrophs and heterotrophic bacteria in biofilms, where the excretion product of the autotrophs provides energy and nutrient to heterotrophs (Bengtsson et al., 2018; Wagner et al., 2017). Here, our findings on the differential use of specific molecules reveal that sediment input affected biofilm communities and their metabolic capacities. In particular, the communities of rocks and sand from the streams with higher sediment loads had similar CLPP responses. On the contrary, we found differences in biofilms communities from rock and sand in Goye and Casa de Piedra. We further suggest that changes in primary producers' composition toward Cyanobacteria dominance in the affected environments (Carrillo et al., 2018) would generate changes in bacterial communities' potential metabolic capacities towards the use of certain molecules.



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**Declaration of Competing Interest**

The authors report no declarations of interest

**Author contributions**

VDV: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - original draft. UC: Data curation; Formal analysis; Investigation. BM: Funding acquisition; Project administration; Resources; Supervision; Writing - review & editing.

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***Availability of data and material*** (data transparency): Data supporting this publication are available from the corresponding author upon reasonable request.

**Declaration of interests**

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: