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Sustained effects of volcanic ash on biofilm stoichiometry, enzyme activity and community composition in North- Patagonia streams

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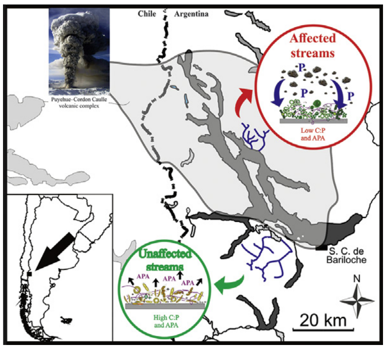
HIGHLIGHTS

- Volcanic deposits changed habitat and biofilm composition near the volcano.
- Affected streams had higher suspended solids and P concentrations than unaffected streams.
- Biofilm C:P ratio and alkaline phosphatase activity were lower in affected streams than in unaffected streams.
- Lower dissolved N:P ratio in affected streams led to higher Cyanobacteria biomass.

GRAPHICAL ABSTRACT



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ABSTRACT

Volcanic eruptions are extreme perturbations that affect ecosystems. These events can also produce persistent effects in the environment for several years after the eruption, with increased concentrations of suspended particles and the introduction of elements in the water column. On 4th June 2011, the Puyehue-Cordón Caulle Volcanic Complex (40.59°S-72.11°W, 2200 m.a.s.l.) erupted explosively in southern Chile. The area affected by the volcano was devastated; a thick layer of volcanic ash (up to 30 cm) was deposited in areas 50 km east of the volcano towards Argentina. The aim of the present study was to evaluate the effect of volcanic ash deposits on stream ecosystems four years after the eruption, comparing biofilm stoichiometry, alkaline phosphatase activity, and primary producer's assemblage in streams which were severely affected by the volcano with unaffected streams. We confirmed in the laboratory that ash deposited in the catchment of affected streams still leach phosphorus (P) into the water four years after eruption. Results indicate that affected streams still receive volcanic particles and that these particles release P, thus stream water exhibits high P concentration. Biofilm P content was higher and the C:P ratio lower in affected streams compared to unaffected streams. As a consequence of less P in unaffected streams, the alkaline phosphatase activity was higher compared to affected streams. Cyanobacteria increased their abundances (99.9% of total algal biovolume) in the affected streams suggesting that the increase in P may positively affect this group. On the contrary, unaffected streams contained a diatom dominant biofilm. In this way, local heterogeneity was created between sub-catchments located within 30 km of each other. These types of events should be seen as opportunities to gather valuable ecological information about how severe disturbances, like volcanic eruptions, shape landscapes and lotic systems for several years after the event.

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1. Introduction

Explosive volcanic eruptions are extreme perturbations that affect ecosystems due to the ejection and emission of gases, ash, pumice, and lava (Franklin et al., 2000; Lindenmayer et al., 2010; Sousa, 1984). This material arrives in the biosphere directly from the Earth's interior, introducing new elements into ecosystems (Jones and Gislason, 2008; Óskarsson, 1980; Stewart et al., 2006; Witham et al., 2005). Many studies have examined how fresh ash deposition introduces a range of potentially toxic elements into the ecosystem, such as arsenic, copper, zinc, fluoride, and molybdenum (Cronin et al., 2014; Duggen et al., 2007; Ruggieri et al., 2011; Stewart et al., 2006). Ash and pumice can also deliver different key nutrient into the waters, such as iron or phosphorus, that can stimulate marine or lacustrine primary producers (Boyd et al., 2000; Frogner et al., 2001; Hamme et al., 2010; Modenutti et al., 2013). In addition to this enrichment effect, the increased concentrations of suspended particles (volcanic ash) in the water column may also positively affect phytoplankton growth by reducing photoinhibition in very clear lakes (Modenutti et al., 2013).

Besides the immediate effects, volcanic ash can also produce persistent effects in the environment lasting several years after the eruption (Dale et al., 2005). Volcanic material that falls into water bodies immediately affects the aquatic communities, while much of the material that falls inland can reach the streams decades after the event due to rain and snowmelt (Bisson, 2005). In this way, accumulation of ash in stream channels increases total suspended solids and changes the riparian zone, causing habitat loss that affects macroinvertebrate communities for years after eruption (Lallement et al., 2016; Miserendino et al., 2012). Additionally, following the initial physical effects, mechanical pyroclast disaggregation resulting from natural post-eruptive processes (i.e. avalanches, lahars, and fluvial transport) could produce new fresh particle surfaces with the increase of fine-grained particles that can also leach elements (Genareau et al., 2016). In this sense, there are very few studies investigating the effect of element inputs after the occurrence of volcanic eruptions on aquatic environments.

One of the elements responsible for fertilization effects after addition of ash is phosphorus (Modenutti et al., 2013). Phosphorus (P) recycling in streams is intimately associated with microorganisms attached to the bottom (the biofilm) (Dodds, 2003; Mulholland et al., 1994). In P-limited environments, algae and bacteria can acquire P from organic molecules by releasing extracellular enzymes, such as alkaline phosphatase, into the biofilm matrix (Sand-Jensen, 1983). Therefore, estimation of alkaline phosphatase activity (APA) can be used to detect P deficiency in aquatic ecosystems (Münster and Chróst, 1990). Since volcanic ash releases P, a decrease in P limitation would affect the N:P ratio (Modenutti et al., 2013) and this new environment would, in turn, affect biofilm composition since cyanobacteria would be favoured, increasing N_2 fixation under an increased P supply (Marcarelli and Wurtsbaugh, 2006).

Northern Patagonia is an active volcanic region with historically high eruption frequency (Inbar et al., 1995). On 4th June 2011, the Puyehue-Cordón Caulle Volcanic Complex (40.59°S-72.11°W, 2200 m.a.s.l.) erupted explosively in southern Chile. The volcano produced 1.46 km^3 of rhyolitic volcanic material (or 0.2–0.4 km^3 dense rock equivalent pyroclastic deposits) (Silva Parejas et al., 2012), a similar amount to that erupted by Mount St. Helens in 1980. The total area (7.5 million hectares) affected by the volcano was covered by 9.5×10^{11} kg of pyroclast material, which fell differentially south-eastwards, depositing a thick layer (up to 30 cm) in areas 50 km east of the volcano towards Argentina (Gaitán et al., 2011). This event created an opportunity for the study of ecological disturbance following explosive eruptions. The aim of the present study was to evaluate the effect of volcanic ash deposits on stream ecosystems, analysing biofilm stoichiometry, APA and composition of primary producer communities four years after the eruption. Our hypothesis is that volcanic ash still releases P to the aquatic environment and this affects P content, APA and primary producer's composition in the biofilm. We tested this hypothesis by comparing streams which received different ash discharges in the Nahuel Huapi catchment. Our predictions are: 1) streams with high levels of suspended solids (TSS) will have higher P concentrations both in the water and in the biofilm; 2) biofilms with low P availability in the environment will present higher levels of APA; and 3) differences in P availability will be reflected in the composition of primary producer communities.

2. Material and methods

2.1. Study area

After the eruption of the Puyehue, the predominant westerly winds of Patagonia generated a gradient (NW-SE) in the thickness of the deposited material (Elser et al., 2015; Gaitán et al., 2011) (Fig. 1). In the affected area (50 km from the volcano), the pyroclastic layers are mostly characterized by bimodal grain-size distributions, alternating lapilli layers capped by multiple fine ash layers (Pistolesi et al., 2015). The mineralogy and chemical composition of these deposits were studied immediately after the eruption and they are mainly composed of silicate glass (Caneiro et al., 2011; Castro et al., 2013; Shkinev et al., 2016). It is common that in fresh volcanic particles P occurs primarily in acid soluble forms and its solubility decreases with chemical weathering (Nanzyo et al., 1993). Adsorption of volcanic salt aerosols is the main mechanism responsible for the presence of soluble compounds on volcanic particles (Smith et al., 1982; Smith and Kalff, 1983; Taylor and Stoiber, 1973).

Pristine (non-weathered) pumice produced by the Puyehue leached 90 μg P g^{-1} (Modenutti et al., 2013). The most affected lakes showed an increase of total suspended solids and in light attenuation, higher P concentrations and phytoplankton biomass relative to pre-eruption conditions (Modenutti et al., 2013). By 2012, lake transparency had greatly recovered (Balseiro et al., 2014); however, thick pyroclastic deposits persist in the catchments (Fig. 2). Sampling was carried out in four streams according to the presence/absence of ash deposits (Fig. 1). We chose the streams based on the NW-SE gradient of deposit thickness and previous exploratory sampling, in order to select comparable systems regarding stream order, size, slope, vegetation cover and bottom morphology. Two types of streams were selected: Affected streams (A-streams), La Estacada (A1) and Ragintuco (A2), located closer to the volcano (approximately 50 km), with a 10-cm layer of ash in the floodplain; and Unaffected streams (U-streams), Goye (U1) and Casa de Piedra (U2), located 80 km from the volcano, without the presence of volcanic ash in the floodplain. The A-streams discharge into Lake Nahuel Huapi and have a N-S exposition. The U-streams discharge into Lake Moreno, which is connected with Lake Nahuel Huapi, and have a SW-NE exposition (Fig. 1). The streams are fourth order streams and drain pristine areas, where the only human activity is hiking.

Streambeds were dominated by a mixture of crystalline igneous, volcanic, and plutonic rocks with approximately 50% of boulders, 40% of cobbles, and 10% of gravel and sand, with volcanic deposits in the interstices of rocks in A-streams. The streams are surrounded by perennial native forests of *Nothofagus dombeyi* and *Austrocedrus chilensis*. The area receives approximately 2000 mm $year^{-1}$ as rainfall and snow, following a bimodal hydrological regime with high discharges in late autumn and spring (rainfall and snowmelt, respectively) and the lowest discharge in summer–early autumn.

2.2. Leachate experiment

In order to investigate volcanic deposits as a source of P in the environment, we performed a laboratory experiment in which we quantified P released from lapilli four years after the eruption. In addition, we assessed whether the effect of milling increased P leachate. We assessed the milling effect using two grain-size particles: Large size

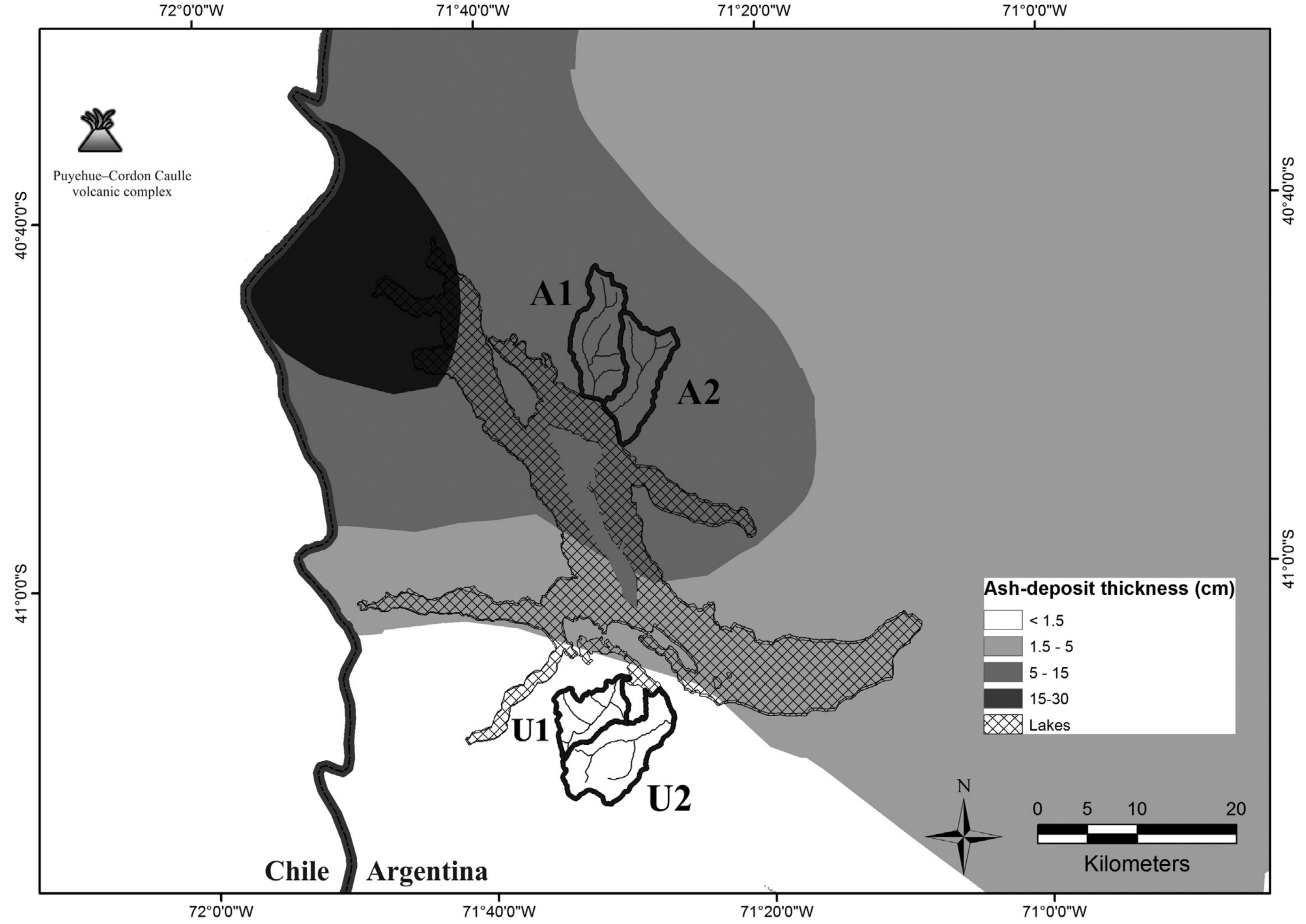

Fig. 1. Area affected by volcanic deposits along the West–East gradient layers depth in Nahuel Huapi catchment. Affected streams: A1 and A2. Unaffected streams: U1 and U2. PCCVC: Puyehue Cordón Caulle volcanic complex located in Chile (40.59°S-72.11°W, 2200 m.a.s.l.).

"lapilli" (2.0 ± 0.1 cm diameter) and Small size particles "ash" (<500 μm), obtained from the milling of lapilli. Volcanic material was obtained from the deposits that remained in the riparian zone of the A-streams (Fig. 2). The samples were collected with a sterile shovel excluding the upper 4 cm layer to avoid soil debris, and transported to the laboratory in sterile polyethylene bags. In the laboratory, sediments were immediately dried at 60 °C for 48 h. Then, four lapilli (2.0 ± 0.1 cm diameter) were mechanically milled in a manual mortar and sieved

Fig. 2. Photograph of the deposits of volcanic material at the riparian zone of an affected stream during March 2015 sampling.

through a 500-μm sieve to obtain ash particles <500 μm. Leaching was carried out by adding 1 g of volcanic particles (Large or Small size) to 250 mL Milli Q water in sterile Erlenmeyer flasks (4 replicates each). The flasks were gently and continuously agitated in a shaker at 140 rpm in an incubator at 15 °C. Samples for P measurement were taken after 24 h (following Cronin et al., 1997), and 48 h as suggested by Witham et al. (2005). The water was then filtered through 0.7-μm GF/F filters and P was determined using the ascorbate-reduced molybdenum method (APHA, 2005) (see *Laboratory procedures*). The results were expressed as μg P per g ash.

2.3. Field sampling

Streams were sampled on three occasions, one in high discharge conditions (October 2014), and two in low discharge conditions (March 2015 and April 2015). On each sampling occasion, temperature, oxygen concentration, and conductivity were measured with a multiprobe (YSI 85, Yellow Spring, Ohio) and pH was measured with a pHmeter (HANNA, HI8424). Turbidity was measured as NTU (Nephelometric Turbidity Units) with a portable turbidity meter (Lutron TU-2016, Taipei, Taiwan). Flow velocity was measured with a water flow meter (Global Water, California, USA). Water samples for nutrient concentration measurements were collected in acid-washed 2-L plastic containers and immediately transported to the laboratory.

For biofilm sampling, three rocks (cobbles, surface area 90 ± 4 cm^2 approximately), were collected at each sampling site and individually stored in a plastic bag according to Biggs and Close (1989), from riffle areas of the main channel of the stream, avoiding the shore line. Rocks were transported to the laboratory under dark conditions and in thermally insulated containers.

2.4. Laboratory procedures

Stream water was filtered through pre-combusted GF/F filters to determine dissolved organic carbon and dissolved nutrient concentrations. Dissolved organic carbon (DOC) concentration was determined with a high-temperature combustion analyser (Shimadzu TOC V-CSH), using potassium hydrogen phthalate as the standard. Total dissolved nitrogen (TDN) was analysed in filtered lake water using a TN-M1 unit on the Shimadzu TOC V-CSH. Total dissolved phosphorus (TDP) was analysed after digestion with potassium persulphate at 125 °C at 1.5 atm for 1 h. Phosphorus concentration was measured using the ascorbate-reduced molybdenum blue technique (APHA, 2005). Total suspended solids (TSS) were quantified by filtering 400 mL stream water through pre-combusted and pre-weighed GF/F filters. Water N:P ratios (atomic) were calculated as TDN to TDP atomic concentrations.

The biofilm was obtained by scraping individual rocks with a brush and carefully rinsing them with Milli Q water. The obtained suspension was used for the estimation of biofilm phosphorus (P), carbon (C) and nitrogen (N) content, total organic matter as ash-free dry mass (AFDM), alkaline phosphatase activity (APA), chlorophyll *a* (Chl-*a*) concentration, bacterial and algal abundance, and autotroph biovolume and composition (Romaní and Sabater, 2001). The biofilm P content was determined by filtering 5 mL of the suspension through acid-washed (10% HCl) GF/F filters and dried 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). Measurements of C and N in biofilm were conducted by filtering another 5 mL of the homogenized sample through pre-combusted GF/F filters, dried at 60 °C for 48 h and analysed with a Thermo Finnigan EA 1112 CN elemental analyser (Thermo Scientific, Milano, Italy). The results were expressed as C:N and C:P atomic ratios.

Subsamples for ash-free dry mass (AFDM) determination were 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).

Determinations of APA in the homogenized sample were carried out during 30 min of incubation, according to Hoppe (1983), using methylumbelliferyl phosphate (MUP; 36 μM) in Tris buffer (pH 8.3). Temperature was stabilized in a water bath at 25 °C (to optimize the temperature of the enzymatic activity) for 30 min; 0.5 mL MUP solution was then added to all samples, blanks and MUP standards. Fluorescence was measured with a Perkin Elmer LS45 fluorometer. Values were expressed as nM MU g $AFDM^{-1}$ h^{-1}.

A subsample of the suspension was used for Chl-*a* extraction, using 90% hot ethanol (Nusch, 1980); spectrophotometric readings were carried out at 665 nm and 750 nm. Corrections for phaeophytin were performed by acidification with HCl. Autotrophic index was calculated as the relationship between Chl-*a* and AFDM concentrations to estimate the relative importance of the photosynthetic fraction in the community (Havens et al., 1996).

Determination of bacterial abundance involved fixing a subsample in filtered formaldehyde at a final concentration of 2% *v*/v. Abundance of bacterial cells was determined by filtering 250 μL subsample, previously stained with 4′,6′-diamidino-2-phenylindole (DAPI) at a final concentration of 0.2% *w*/*v*, according to Porter and Feig (1980). Counting was performed on 0.2-μm black polycarbonate filters (Poretics) under an epifluorescence microscope (Olympus BX50) at ×1250 magnification using a UV light (U-MWU filter). Twenty fields per filter were counted.

For algal composition analysis, a subsample (10 mL) of the suspension was fixed with acid Lugol's solution. Identifications were performed under microscope (Olympus BX50) at 1000×, to genus level when possible, according to the website AlgaeBase (Guiry and Guiry, 2017). Besides, for Cyanobacteria we followed Komárek and Anagnostidis (1989, 1999, 2005) and Komárek et al. (2014). For diatoms, 1 mL of the samples was treated with peroxide in order to oxidise organic matter to facilitate the identification. Samples were mounted in Naphrax ® and examined under microscope at 1000×. Identifications were performed according to Krammer and Lange-Bertalot (1986, 1988, 1991). For algal abundance determinations, three aliquots were examined in a chamber of 20 μL volume under a direct microscope (Olympus BX50) at 400× magnification and at least 60 random images of each aliquot were obtained with a digital camera (Olympus DP 70). Cells were counted and measured using an image analysis system (Image ProPlus; Media Cybernetics, Warrendale, PA, USA). Biovolume was estimated with best fitting geometric models, using average dimensions of a minimum of 20 cells per species per sample (Hillebrand et al., 1999). The biovolume of macroscopic spherical colonies of *Nostoc* was calculated by measurement of individual colonies at 50× magnification.

2.5. Data analysis

Statistical analyses were performed using SPSS 18 software and SigmaStat version 12.5 (Systat Software Inc., San Jose, CA, USA.). Statistical differences between P concentrations in leachates of volcanic material were assessed using one-way repeated measures of Analysis of Variance (RM-ANOVA), with particle size (Small and Large) as the between-subject factor and leachate time (24 h and 48 h) as the within-subject factor.

Differences in stream water physical and chemical variables and biofilm parameters (biofilm P and C content, C:P and C:N ratio, APA, Chl-*a*, AFDM, Chl-*a*:AFDM, bacterial and algal abundance and algal biovolume) between stream types were also analysed using one-way RM-ANOVA. We used “Impact” as the between-subject factor (with two levels: A-streams and U-streams) and “Time” as the within-subject factor (with three levels: October, March and April). A posteriori Bonferroni's tests were used to compare main effects. These contrasts are based on the linearly independent paired comparisons between the estimated marginal means.

Table 1
Physiochemical parameters of stream water in affected (A) and unaffected (U) streams during the study period and results of the one-way RM-ANOVA. Values correspond to individual measurements at each of the two sites for each stream type.

Time	October 2014		March 2015		April 2015		Statistics		
Impact	A	U	A	U	A	U	Impact	Time	Impact × Time
TSS (mg L^{-1})	5.9	0.3	6.2	2.4	3.5	0.3	<0.001*	0.004*	0.031*
	6.1	0.2	7.2	2.2	2.5	0.4			
Turbidity (NTU)	4.1	1.6	4.1	2.1	3.2	0.6	0.001*	0.032*	0.627
	4.2	1.5	4.7	1.9	2.9	0.9			
TDP (µg L^{-1})	5.8	6.2	15.1	6.9	5.0	2.1	0.037*	0.023*	0.047*
	5.3	6.7	12.2	4.68	4.9	2.05			
DOC (mg L^{-1})	0.6	0.4	0.8	0.5	0.9	0.6	0.078	0.140	0.507
	0.6	0.4	0.7	0.4	0.6	0.5			
TDN (µg L^{-1})	53.8	28.5	55.1	57.8	57.8	37.8	0.227	0.101	0.059
	58.7	28.6	42.8	71.9	36.7	30.0			
N:P (atomic)	20.4	10.2	8.1	18.5	25.6	39.2	0.119	0.153	0.044*
	24.3	9.4	7.7	34.0	16.8	32.4			
Flow velocity (cm s^{-1})	55.6	51.0	17.4	27.1	35.5	22.0	0.748	0.008*	0.154
	63.7	59.6	14.0	29.7	25.7	29.7			
Temperature (°C)	5.7	4.7	11.4	10.6	7.9	5.9	0.161	0.001*	0.303
	7.0	6.2	13.8	11.5	8.6	5.2			
Oxygen (%)	94.0	92.0	63.0	91.4	38.7	91.7	0.016*	0.069	0.060
	91.0	86.4	85.0	100.0	37.8	93.2			
Conductivity (µS cm^{-1})	17.0	11.7	50.0	37.0	43.4	29.6	0.017*	0.018*	0.842
	25.2	9.8	46.3	42.4	38.7	33.6			
pH	7.5	7.5	7.4	7.4	7.3	7.5	0.937	0.091	0.091
	7.2	7.1	7.2	7.2	7.3	7.1			

* statistic significance.

Normality and sphericity assumptions were verified previously. The Greenhouse–Geisser correction was used to compute adjusted P-level of F-test when sphericity was not met. The main effects were compared using Bonferroni's tests. The magnitude of the difference between treatments was estimated by the Effect Size Analysis (h^2) in ANOVA as:

$$h^2 = SS_{effect}/SS_{total}$$

where SS_{effect} is the Sum of Square for the factors and SS_{total} is the total Sum of Squares for all effects, interactions and errors in the ANOVA.

3. Results

3.1. Ash P leachate and stream water characteristics

The leachate experiments showed that small particles (ash) released three-fold more P than large particles (lapilli), after both 24 h and 48 h (one-way RM-ANOVA, no interaction, $P_{size} = 0.018$ and $P_{time} < 0.001$; Fig. 3). This results show that after four years since the eruption particles still release P and the mechanical milling effectively increases the P release.

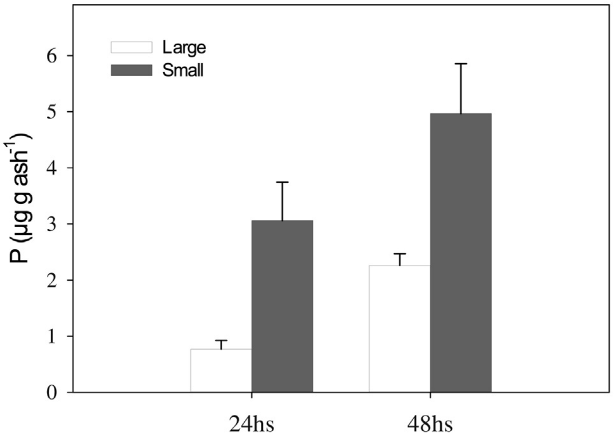

Fig. 3. Phosphorus released by the two different sizes of volcanic particles (Large and Small) after 24 h and 48 h in the leaching experiment. Error bars represent ± standard error.

The A-streams and U-streams differed in TSS concentration and TDP (Table 1). TSS was positively related to both water turbidity and P (linear regression $r^2 = 0.97$, d.f. = 11, $P < 001$; and $r^2 = 0.53$, d.f. = 11, $P < 0.01$ respectively). These results suggest that A-streams still receive volcanic particles (more TSS and turbidity) and that these particles release P (3-fold higher in A-streams than in U-streams). In contrast, we did not observe differences in DOC or TDN concentration (Table 1). The N:P ratio differed between stream type and the lowest value (below 16) was observed in A-streams in March (Table 1). Finally, there were no differences between the studied streams in terms of flow velocity, water temperature and pH (Table 1).

3.2. Biofilm

Biofilm P content was higher in A-streams (Figs. 4a and 3b; Table 2), and the C:P ratio was always lower (Fig. 4c; Table 2). As a consequence of the high P content in the biofilm of A-stream, APA was lower than in the biofilm of the U-stream (Fig. 4d; Table 2). The biofilm C:N ratio was lower in A-streams (C:N = 8 ± 0.1) than in U-streams (C:N = 10 ± 0.4; Table 2).

Although Chl-*a* concentration did not differ between stream types, total autotroph cell number, biovolume and biofilm AFDM were higher in A-streams (Fig. 5a, b and c; Tables 2 and 3). Thus, the autotrophic index (Chl-*a*:AFDM) was lower in A-streams (Fig. 5d, Table 2). However, bacterial abundances did not differ between stream types (Table 3).

Composition of the autotrophic community differed between stream types. While in U-streams the assemblage was dominated by Bacillariophyta (76.9% and 89.9% of biovolume in March and April, respectively), biofilms in A-streams were dominated by Cyanobacteria (99.9% of total algal biovolume) in particular in March and April (Fig. 6). Among the Cyanobacteria, the most abundant identified genera were *Nostoc* (90%), *Calothrix* (4%) and *Homeothrix* (3%). In particular, in March and April *Nostoc* reached almost 100% of cell abundance and biovolume, developing macroscopic colonies. This condition was not observed in U-streams in which the assemblage was a cohesive thin

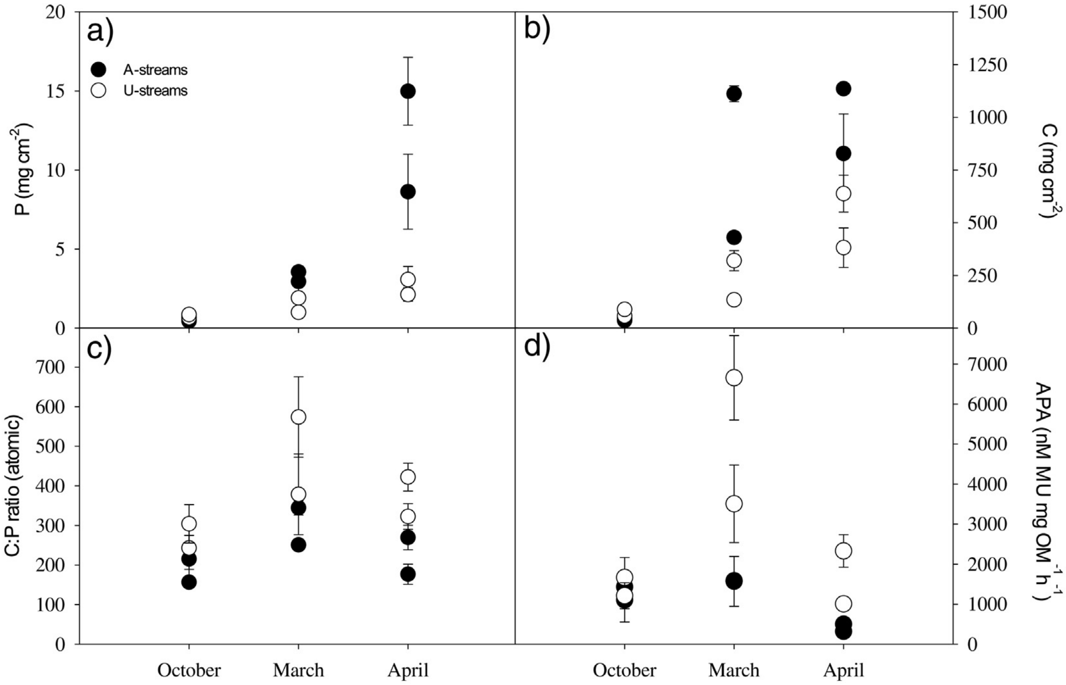

Fig. 4. Biofilm variables on the three sampling dates (October 2014, March 2015 and April 2015) in Affected streams (A1 and A2) and Unaffected streams (U1 and U2). a) Biofilm phosphorus content, b) Biofilm carbon content, c) Biofilm C:P atomic ratio, and d) Alkaline Phosphatase Activity (APA). Error bars represent ±standard error.

layer dominated by diatoms, with *Gomphoneis herculeana*, *Hannaea arcus* and *Syendra ulna* accounting for >80% of the biovolume. On the contrary, in A-streams the diatom species *Rhopalodia gibba* and *Epithemia adnata* were observed always in very low abundance (<1%). Finally, Chlorophyta were only found in U-streams.

4. Discussion

Our results show that four years after the volcanic eruption, headwater streams that were originally heavily affected contained more suspended solids and higher P concentrations in the water than other streams in the catchment. This condition can be related to the presence of deposits in their watershed that release P, particularly if the rocks are subjected to mechanical milling as performed in our laboratory experiments. Therefore, our results demonstrate that volcanic deposits in the catchments may represent an important source of solids and phosphorus introduced into aquatic environments several years after eruption. Precipitation not only is responsible for mobilizing volcanic deposits into the water (Bisson, 2005) but it also increases chemical weathering rates. Shkinev et al. (2016) found that 15% of the P of the Puyehue fresh ash was lost after the first rain. But volcanic material may continue to release P, as mechanical milling of pumice introduces nutrients into the water with important implications for chemical cycles (Genareau et al., 2016). Once volcanic ash enters a water body, it releases ions and elements according to their adsorption/desorption equilibrium constant, and these ions become available for primary producers (Frogner et al., 2001). Pristine (non-weathered) ash emitted by the Puyehue volcano released 90 μg P per gram of pyroclasts (Modenutti et al., 2013). We showed that after four years of weathering, this volcanic material continues to leach P, and this leaching increases with mechanical disaggregation, probably as a result of exposure of new, reactive particle surfaces. As a consequence of the difference in P concentration in the water (3-fold higher in A-streams than in U-streams), we observed differences in biofilm elemental composition, enzymatic activity and algal assemblage between the two types of streams.

Biofilm structure and function are sensitive to changes in nutrient content and other factors, such as temperature, flow velocity, and light, which determine not only the biofilm community composition, but also its thickness, relative abundance of autotrophs/heterotrophs,

Table 2
Results of the one-way RM-ANOVA for the biofilm parameters of stream water in affected (A) and unaffected (U) streams during the study period. Values correspond to individual measurements at each of the two sites for each stream type.

Source of variation	Impact			Time			Impact × Time		
Biofilm parameters	F	*P*	h^2	F	*P*	h^2	F	*P*	h^2
P (mg cm^{-2})	34.92	<0.001*	**0.777**	27.46	<0.001*	0.733	14.67	0.003*	0.595
C (mg cm^{-2})	15.48	0.003*	0.607	41.70	<0.001*	**0.807**	8.44	0.002*	0.458
C:P ratio (atomic)	13.89	0.004*	**0.581**	5.42	0.0130*	0.351	0.30	0.740	0.029
C:N ratio (atomic)	8.91	0.02*	**0.497**	1.31	0.29	0.127	4.87	0.02*	0.351
APA (nM MU mg OM^{-1} h^{-1})	15.23	0.003*	0.604	16.89	<0.001*	**0.628**	7.91	0.009*	0.442
Chl *a* (μg cm^{-2})	0.94	0.360	0.086	28.11	<0.001*	**0.738**	0.045	0.930	0.004
Biovolume (mm^3 cm^{-2})	113.01	<0.001*	**0.919**	5.69	0.011*	0.363	5.69	0.011*	0.362
AFDM (mg cm^{-2})	31.22	<0.001*	0.757	45.60	<0.001*	**0.820**	18.41	<0.001*	0.648
Chl *a*: AFDM	31.61	<0.001*	**0.760**	10.52	0.001*	0.513	2.22	0.134	0.182

* statistic significance. Bold indicate the highest effect size (h^2)

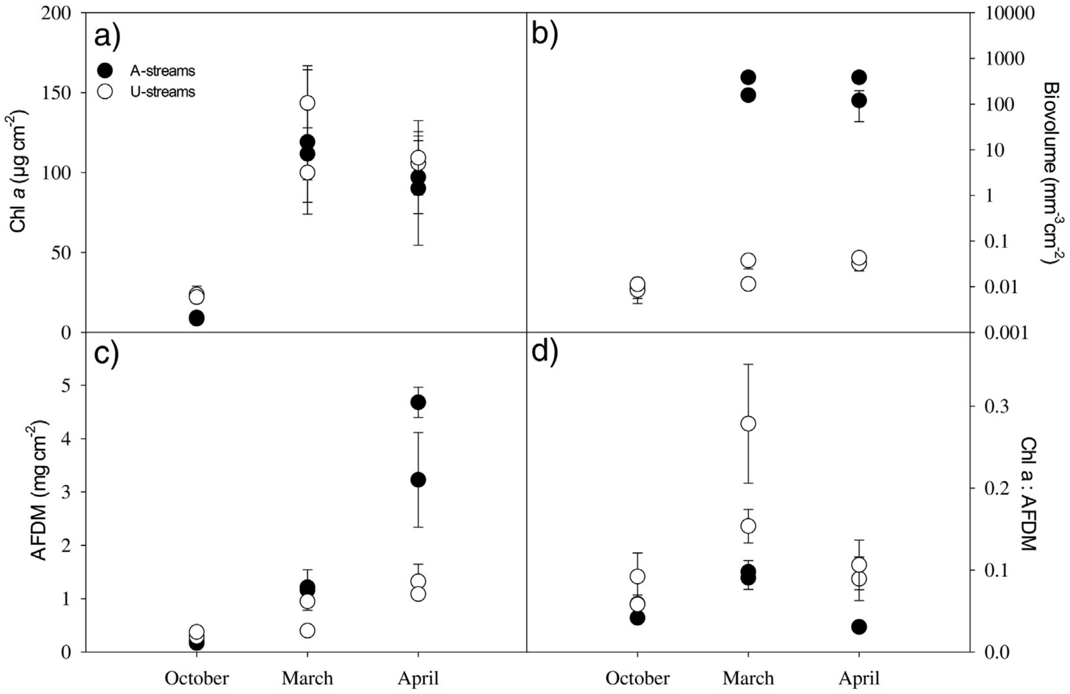

Fig. 5. Biofilm variables on the three sampling dates (October 2014, March 2015 and April 2015) in Affected streams (A1 and A2) and Unaffected streams (U1 and U2). a) Chlorophyll *a* concentration, b) Total algal biovolume, c) Ash free dry mass (AFDM), and d) Chlorophyll *a*/AFDM ratio. Error bars represent ±standard error.

and ultimately its function (Battin et al., 2016; Francoeur and Biggs, 2006; Romaní et al., 2013; Sekar et al., 2002). P uptake depends on different factors including water P concentration, biofilm P content, forms of P available, and the growth stage and thickness of the biofilm (Cotner and Wetzel, 1992; Sand-Jensen, 1983).The relationship between the concentration of elements in the water and in biofilms (i.e. the capacity of the biofilm to accumulate a specific element) might be subjected to biofilm internal processes and element limitation. In P-limited environments, additional P may be quickly taken up by microorganisms (Rier and Stevenson, 2001), leading to increases in P content in biofilm (Hill and Fanta, 2008; Horner et al., 1990; Kamjunke et al., 2015). Although algae and bacteria can also obtain P from organic matter, this mechanism requires the synthesis of alkaline phosphatase (Jansson et al., 1988). Thus, higher APA in biofilms of streams with low P content reflects the severe P-limitation in these streams. The lack of differences in P-content between stream types in October might be attributed to the low biomass of biofilm in the spring. Flow velocity is recognized as a major factor affecting biofilm variations in a given habitat (Battin et al., 2003; Stevenson et al., 1996), and in temperate mountain streams, snowmelt produces extremely high increases in discharge (~60 cm s^{-1} flow velocity in our studied streams), causing biofilm drag and slough.

Inputs of P in ultraoligotrophic environments, such as Patagonian Andean catchments, may lead to changes in the assemblage/composition of the primary producers. Modenutti et al. (2013) found that in deep lakes, the immediate effect of pyroclastic inputs was a change in the phytoplankton biomass and composition from a community dominated by the nanoflagellate *Crysochromulina parva* (Haptophyceae) to a dominance of Cryptophyceae (in particular *Plageoselmis lacustris*) and diatoms (particularly *Tabellaria flocculosa* and *Aulacoseira granulata*).

The algal composition of the biofilm in the study area (pre-eruption condition) was highly dominated by diatoms (Díaz-Villanueva and Albariño, 1999; Díaz-Villanueva and Modenutti, 2004; Gaglioti, 1992). Immediately after the eruption, streams were severely affected by ash accumulation, turbidity increase and changes in the riparian zone (Lallement et al., 2016). Four years after the eruption, differences in biofilm autotroph dominance between affected and unaffected streams were observed. Cyanobacteria increased their abundances in the A-streams suggesting that the increase in P may positively affect this group. On the contrary, U-streams retained a diatom dominant biofilm.

The ability of Cyanobacteria to fix atmospheric N_2 favours them when N is limiting (Marcarelli and Wurtsbaugh, 2006), in particular when N:P ratios are below 16 (Ahn et al., 2002; Biggs and Smith, 2002; Levine and Schindler, 1999; Smith and Kalff, 1983; Stancheva et al., 2013). When environmental conditions are favourable, cells can rapidly multiply, forming benthic blooms (Fristachi et al., 2008). This pattern was observed in A-streams, where macroscopic *Nostoc* colonies dominated the biofilm. Although no molecular identification was carried out for cyanobacteria in these streams, the presence of *Nostoc* was confirmed by Illumina MiSeq platform associated with pyroclastic

Table 3
Results of algal and bacterial abundance and one-way RM-ANOVA of biofilm of stream water in affected (A) and unaffected (U) streams during the study period.

Time	October 2014		March 2015		April 2015		Statistics					
Impact	A	U	A	U	A	U	Impact		Time		Impact × Time	
							F	*P*	F	*P*	F	*P*
Algal cell number (10^5cell cm^{-2})	4.3 ± 0.17	6.6 ± 0.02	2.5 ± 0.2 10^5	1.2 ± 0.5	1.1 ± 0.7 10^5	8.2 ± 2.2	28.8	<0.01*	153.4	<0.01*	34.7	<0.01*
	4.7 ± 0.03	3.1 ± 0.09	4.1 ± 2.2 10^5	2.6 ± 0.7	1.9 ± 1.2$10^5$	3.2 ± 0.5						
Bacterial cell number (10^6cell cm^{-2})	2.6 ± 0.14	3.3 ± 0.19	13.9 ± 1.0	20.0 ± 1.7	36.8 ± 4.3	27.3 ± 1.6	0.1	0.74	14.4	0.015*	1.2	0.38
	5.6 ± 0.44	4.4 ± 0.28	14.0 ± 1.1	17.6 ± 1.2	22.1 ± 1.6	16.1 ± 0.6						

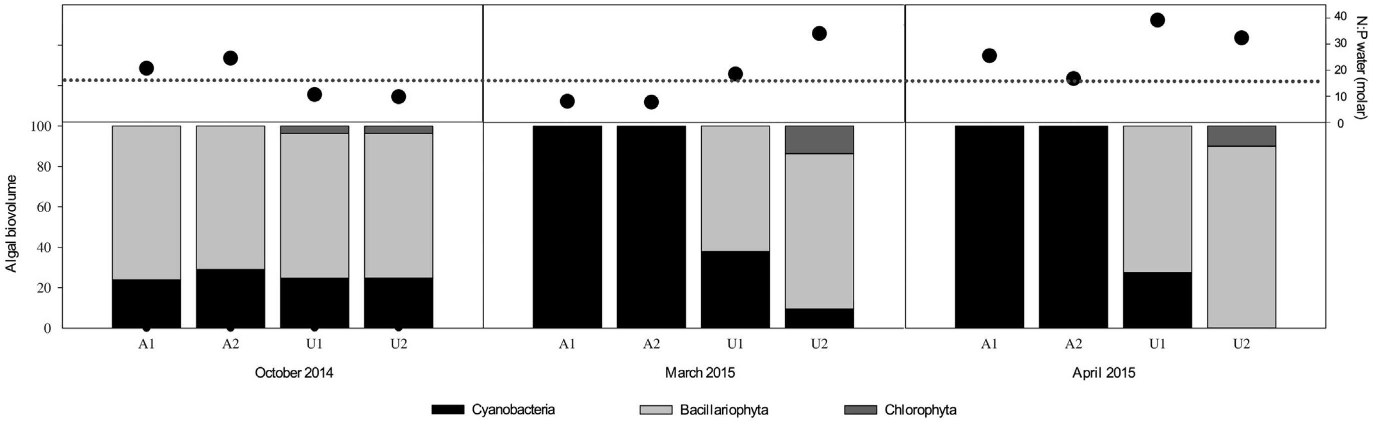

Fig. 6. Biovolume of Cyanobacteria (black bars), Bacillariophyta (light grey bars) and Chlorophyta (dark grey bars) in Affected streams (A1 and A2) and Unaffected streams (U1 and U2) and N:P atomic ratio for each stream on each sampling date (October 2014, March 2015 and April 2015).

inputs from the same volcanic event (Modenutti et al., 2016). In *Nostoc* colonies, extracellular polysaccharides were found to account for >60% of dry weight of the matrix (Hill et al., 1997). The capacity of *Nostoc* to generate a thick layer of polysaccharide matrix is a possible explanation for the high C content in biofilm from A-streams, resulting in lower autotrophic index (Chl-*a*:AFDM) than in U-streams. These modifications in community composition lead to changes in the stoichiometry of the assemblage. The lowest C:N ratio of biofilm in A-streams could be associated with the ability of Cyanobacteria to fix atmospheric N_2.

In environments with high TSS, particle deposition in the biofilm matrix can have negative effects on biofilm biomass (Boulêtreau et al., 2010). In these environments, the polymeric matrix results in an adaptive response that can protect biofilm from erosion (Flemming and Wingender, 2010). Filamentous Cyanobacteria, for example, have the capacity to build structures which enable them to colonize highly erodible substrates (Garcia-Pichel and Wojciechowski, 2009; Vignaga et al., 2013). The presence of *Nostoc* in streams affected by volcanic ash was previously observed by Cushing and Smith (1982) after the 1980 Mount St. Helens eruption. The high capacity of *Nostoc* to produce massive polysaccharide structures forming macroscopic colonies mentioned above may confer on this genus a great advantage in post-eruptive environments. Therefore, it is possible that both nutrient conditions and a harmful environment (high TSS) could have acted simultaneously, favouring the bloom of *Nostoc* in the A-streams. As benthic cyanobacteria have received less attention than their planktonic freshwater counterparts (Fetscher et al., 2015; Quiblier et al., 2013), little is known about the physicochemical variables promoting their proliferation in rivers (Wood et al., 2017). The absence of *Nostoc* colonies in U-streams, despite N:P ratios below 16, may be related to the low P concentrations in these streams, as nitrogenase activity needs P concentrations >8 μg P L^{-1} (Stewart et al., 1970).

5. Conclusions

We conclude that volcanic deposits that persist in the catchments for several years after the volcanic event represent a source of solids (ashes and pumice) and P for the streams. These inputs affect the biofilm communities: the autotroph assemblage composition, the elemental ratios and the enzymatic responses. Since biofilm communities in low order streams are important for nutrient and carbon cycling, a change in biofilm metabolism is expected to affect nutrient stream cycles which would be transported downstream. We also observed a change in the autotroph dominance with Cyanobacteria in the affected streams. These types of events should be seen as opportunities to gather valuable ecological information about how severe disturbances, like volcanic eruptions, shape landscapes and lotic systems for several years after the event.

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References

Ahn, C.Y., Chung, A.S., HM, Oh., 2002. Rainfall, phycocyanin, and N:P ratios related to cyanobacterial blooms in a Korean large reservoir. Hydrobiologia 474:117–124. https://doi.org/10.1023/A:1016573225220.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, AWWA, Washington, D.C.

Balseiro, E., Souza, M.S., Serra Olabuenaga, I., Wolinski, L., Bastidas Navarro, M., Laspoumaderes, C., et al., 2014. Effect of the Puyehue-cordon Caulle volcanic complex eruption on crustacean zooplankton of Andean lakes. Ecol. Austral 24, 75–82.

Battin, T.J., Kaplan, L.A., Newbold, J.D., Cheng, X., Hansen, C., 2003. Effects of current velocity on the nascent architecture of stream microbial biofilms. Appl. Environ. Microbiol. 69, 5443–5452.

Battin, T.J., Besemer, K., Bengtsson, M.M., Romani, A.M., Packmann, A.I., 2016. The ecology and biogeochemistry of stream biofilms. Nat. Rev. Microbiol. 14:251–263. https://www.nature.com/articles/nrmicro.2016.15.

Biggs, B.J.F., Close, M.E., 1989. Periphyton biomass dynamics in gravel bed rivers: the relative effects of flows and nutrients. Freshw. Biol. 22:209–231. https://doi.org/10.1111/j.1365-2427.1989.tb01096.x.

Biggs, B.J.F., Smith, R.A., 2002. Taxonomic richness of stream benthic algae: effects of flood disturbance and nutrients. Limnol. Oceanogr. 47, 1175–1186.

Bisson, P.A., 2005. Responses of Fish to the 1980 Eruption of Mount St. Helens. In: Dale, V.H., Swanson, F.J., Crisafulli, C.M. (Eds.), Ecological Responses to the 1980 Eruption of Mount St. Helens. Springer:pp. 163–181 https://doi.org/10.1007/0-387-28150-9_12.

Boulêtreau, S., Sellali, M., Elosegi, A., Nicaise, Y., Bercovitz, Y., Moulin, F., et al., 2010. Temporal dynamics of river biofilm in constant flows: a case study in a riverside laboratory flume. Int. Rev. Hydrobiol. 95:156–170. https://doi.org/10.1002/iroh.200911203.

Boyd, P.W., Watson, A.J., Law, C.S., Abraham, E.R., Trull, T., Murdoch, R., et al., 2000. A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. Nature 407, 695–702.

Caneiro, A., Mogni, L., Serquis, A., Cotaro, C., Wilberger, D., Ayala, C., et al., 2011. Análisis de cenizas volcánicas del Cordón Caulle (complejo volcánico Puyehue-Cordón Caulle). Comisión Nacional de Energía Atómica.

Castro, J.M., Schipper, C.I., Mueller, S.P., Militzer, A.S., Amigo, A., Parejas, C.S., et al., 2013. Storage and eruption of near-liquidus rhyolite magma at Cordón Caulle, Chile. Bull. Volcanol. 75:702. https://doi.org/10.1007/s00445-013-0702-9.

Cotner, J.B., Wetzel, R.G., 1992. Uptake of dissolved inorganic and organic bphosphorus compounds by phytoplankton and bacterioplankton. Limnol. Oceanogr. 37: 232–243. https://doi.org/10.4319/lo.1992.37.2.0232.

Cronin, S.J., Hedley, M.J., Smith, R.G., Neall, V.E., 1997. Impact of Ruapehu ash fall on soil and pasture nutrient status 1. October 1995 eruptions. N. Z. J. Agric. Res. 40: 383–395. https://doi.org/10.1080/00288233.1997.9513260.

Cronin, S.J., Stewart, C., Zernack, A.V., Brenna, M., Procter, J.N., Pardo, N., et al., 2014. Volcanic ash leachate compositions and assessment of health and agricultural hazards from 2012 hydrothermal eruptions, Tongariro, New Zealand. J. Volcanol. Geotherm. Res. 286:233–247. https://doi.org/10.1016/j.jvolgeores.2014.07.002.

Cushing, C.E., Smith, S.D., 1982. Effects of Mt. St. Helens Ashfall on Lotic Algae and Caddisflies. J. Freshw. Ecol. 1:527–538. https://doi.org/10.1080/02705060.1982.9664071.

Dale, V.H., Swanson, F.J., Crisafulli, C.M., 2005. Disturbance, survival, and succession: understanding ecological responses to the 1980 eruption of Mount St. Helens. In: Dale, V.H., Swanson, F.J., Crisafulli, C.M. (Eds.), Ecological Responses to the 1980 Eruption of Mount St. Helens. Springer, New York:pp. 3–11 https://doi.org/10.1007/0-387-28150-9_1.

Díaz-Villanueva, V., Modenutti, B.E., 2004. Experimental analysis of grazing by the mayfly Meridialaris chiloeensis on different successional stages of stream periphyton. Int. Rev. Hydrobiol. 89, 263–277.

Díaz-Villanueva, V., Albariño, R.J., 1999. Feeding habit of Notoperla archiplatae (Plecoptera) larvae in a North Patagonia Andean stream, Argentina. Hydrobiologia 412:43–52. https://doi.org/10.1023/a:1003896115219.

Dodds, W.K., 2003. The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. J. Phycol. 39, 840–849.

Duggen, S., Croot, P., Schacht, U., Hoffmann, L., 2007. Subduction zone volcanic ash can fertilize the surface ocean and stimulate phytoplankton growth: Evidence from biogeochemical experiments and satellite data. Geophys. Res. Lett. 34 n/a-n/a. https://doi.org/10.1029/2006GL027522.

Elser, J.J., Bastidas, M., Corman, J.R., Emick, H., Kellom, M., Laspoumaderes, C., et al., 2015. Community structure and biogeochemical impacts of microbial life on floating pumice. Appl. Environ. Microbiol. 81:1542–1549. https://doi.org/10.1128/AEM.03160-14.

Fetscher, A.E., Howard, M.D.A., Stancheva, R., Kudela, R.M., Stein, E.D., Sutula, M.A., et al., 2015. Wadeable streams as widespread sources of benthic cyanotoxins in California, USA. Harmful Algae 49:105–116. https://doi.org/10.1016/j.hal.2015.09.002.

Flemming, H.-C., Wingender, J., 2010. The biofilm matrix. Nat. Rev. Microbiol. 8, 623.

Francoeur, S.N., Biggs, B.J., 2006. Short-term Effects of Elevated Velocity and Sediment Abrasion on Benthic Algal Communities, Advances in Algal Biology: A Commemoration of the Work of Rex Lowe. Springer, pp. 59–69.

Franklin, A.B., Anderson, D.R., Gutierrez, R.J., Burnham, K.P., 2000. Climate, habitat quality, and fitness in Northern Spotted Owl populations in northwestern California. Ecol. Monogr. 70, 539–590.

Fristachi, A., Sinclair, J.L., Hall, S., Berkman, J.A.H., Boyer, G., Burkholder, J., et al., 2008. Occurrence of cyanobacterial harmful algal blooms workgroup report. In: Hudnell, H.K. (Ed.), Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Springer New York, New York, NY:pp. 45–103 https://doi.org/10.1007/978-0-387-75865-7_3.

Frogner, P., Gíslason, S.R., Óskarsson, N., 2001. Fertilizing potential of volcanic ash in ocean surface water. Geology 29:487–490. https://doi.org/10.1130/0091-7613(2001)029<0487:fpovai>2.0.co;2.

Gaglioti, P.V., 1992. Variación espacial y estacional en la estructura de las comunidades de diatomeas epilíticas de un arroyo andino. Su relación con factores abióticos. Ecol. Austral 2, 77–86.

Gaitán, J.J., Ayesa, J.A., Umaña, F., Raffo, F., Bran, D.B., 2011. Cartografía del área afectada por cenizas volcánicas en las provincias de Río Negro y Neuquén. Informe PROEVO. Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental S.C. de Bariloche, p. 8.

Garcia-Pichel, F., Wojciechowski, M.F., 2009. The evolution of a capacity to build supra-cellular ropes enabled filamentous cyanobacteria to colonize highly erodible substrates. PLoS One 4, e7801. https://doi.org/10.1371/journal.pone.0007801.

Genareau, K., Cronin, S., Stewart, C., Bhattacharyya, S., Donahoe, R., 2016. Posteruptive impacts of pyroclastic deposits from basaltic andesite stratovolcanoes on surface water composition. J. Geophys. Res. Biogeosci. 121, 1275–1287.

Guiry, M.D., Guiry, G.M., 2017. AlgaeBase. World-wide electronic publication. National University of Ireland, Galway http://www.algaebase.org.

Hamme, R.C., Webley, P.W., Crawford, W.R., Whitney, F.A., DeGrandpre, M.D., Emerson, S.R., et al., 2010. Volcanic ash fuels anomalous plankton bloom in subarctic northeast Pacific. Geophys. Res. Lett. 37, L19604. https://doi.org/10.1029/2010gl044629.

Havens, K.E., East, T.L., Meeker, R.H., Davis, W.P., Steinman, A.D., 1996. Phytoplankton and periphyton responses to in situ experimental nutrient enrichment in a shallow subtropical lake. J. Plankton Res. 18, 551–566.

Hill, W.R., Fanta, S.E., 2008. Phosphorus and light colimit periphyton growth at subsaturating irradiances. Freshw. Biol. 53, 215–225.

Hill, D.R., Keenan, T.W., Helm, R.F., Potts, M., Crowe, L.M., Crowe, J.H., 1997. Extracellular polysaccharide of Nostoc commune (Cyanobacteria) inhibits fusion of membrane vesicles during desiccation. J. Appl. Phycol. 9:237–248. https://doi.org/10.1023/a:1007965229567.

Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollingher, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. 35:403–424. https://doi.org/10.1046/j.1529-8817.1999.3520403.x.

Hoppe, H.G., 1983. Signiticance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl substrates. Mar. Ecol. Prog. Ser. 11:299–308. https://doi.org/10.3354/meps011299.

Horner, R.R., Welch, E.B., Seeley, M.R., Jacoby, J.M., 1990. Responses of periphyton to changes in current velocity, suspended sediment and phosphorus concentration. Freshw. Biol. 24:215–232. https://doi.org/10.1111/j.1365-2427.1990.tb00704.x.

Inbar, M., Ostera, H., Parica, C., Remesal, M., Salani, F., 1995. Environmental assessment of 1991 Hudson volcano eruption ashfall effects on southern Patagonia region, Argentina. Environ. Geol. 25, 119–125.

Jansson, M., Olsson, H., Pettersson, K., 1988. Phosphatases; origin, characteristics and function in lakes. In: Persson, G., Jansson, M. (Eds.), Phosphorus in Freshwater Ecosystems: Proceedings of a Symposium held in Uppsala, Sweden, 25–28 September 1985. Springer Netherlands, Dordrecht :pp. 157–175. https://link.springer.com/chapter/10.1007/978-94-009-3109-1_10.

Jones, M.T., Gislason, S.R., 2008. Rapid releases of metal salts and nutrients following the deposition of volcanic ash into aqueous environments. Geochim. Cosmochim. Acta 72:3661–3680. https://doi.org/10.1016/j.gca.2008.05.030.

Kamjunke, N., Mages, M., Büttner, O., Marcus, H., Weitere, M., 2015. Relationship between the elemental composition of stream biofilms and water chemistry—a catchment approach. Environ. Monit. Assess. 187 (432). https://doi.org/10.1007/s10661-015-4664-6.

Komárek, J., Anagnostidis, K., 1989. Modern approach to the classification system of cyanophytes 4 – Nostocales. Algol. Stud. 56, 247–345.

Komárek, J., Anagnostidis, K., 1999. Cyanoprokaryota. 1. Teil: Chroococcales, 19/1., Süβwasserflora von Mitteleuropa. Gustav Fischer, Stuttgart, p. 548.

Komárek, J., Anagnostidis, K., 2005. Cyanoprokaryota. 2. Teil: Oscillatoriales, Süßwasserflora von Mitteleuropa; Band 19/2. Elsevier, Műnchen, p. 759.

Komárek, J., Kaštovský, J., Mareš, J., Johansen, J.R., 2014. Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. Preslia 86, 295–335.

Krammer, K., Lange-Bertalot, H., 1986. Bacillariophyceae 1. Teil: naviculaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (Eds.), Süsswasserflora von Mitteleuropa. G. Fischer, Jena.

Krammer, K., Lange-Bertalot, H., 1988. Bacillariophyceae. 2. Teil: bacillariaceae, epithemiaceae, surirellaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (Eds.), Süsswasserflora von Mitteleuropa. G. Fischer, Jena.

Krammer, K., Lange-Bertalot, H., 1991. Bacillariophyceae. 3. Teil: centrales, fragilariaceae, eunotiaceae. In: Ettl, H., Gerloff, J., Heynig, H., Mollenhauer, D. (Eds.), Süsswasserflora von Mitteleuropa. G. Fischer, Jena.

Lallement, M., Macchi, P.J., Vigliano, P., Juarez, S., Rechencq, M., Baker, M., et al., 2016. Rising from the ashes: changes in salmonid fish assemblages after 30 months of the Puyehue–cordon Caulle volcanic eruption. Sci. Total Environ. 541:1041–1051. https://doi.org/10.1016/j.scitotenv.2015.09.156.

Levine, S.N., Schindler, D.W., 1999. Influence of nitrogen to phosphorus supply ratios and physicochemical conditions on cyanobacteria and phytoplankton species composition in the Experimental Lakes Area, Canada. Can. J. Fish. Aquat. Sci. 56, 451–466.

Lindenmayer, D.B., Likens, G.E., Franklin, J.F., 2010. Rapid responses to facilitate ecological discoveries from major disturbances. Front. Ecol. Environ. 8, 527–532.

Marcarelli, A.M., Wurtsbaugh, W.A., 2006. Temperature and nutrient supply interact to control nitrogen fixation in oligotrophic streams: an experimental examination. Limnol. Oceanogr. 51, 2278–2289.

Miserendino, M.L., Archangelsky, M., Brand, C., Epele, L.B., 2012. Environmental changes and macroinvertebrate responses in Patagonian streams (Argentina) to ashfall from the Chaiten Volcano (May 2008). Sci. Total Environ. 424:202–212. https://doi.org/10.1016/j.scitotenv.2012.02.054.

Modenutti, B.E., Balseiro, E.G., Elser, J.J., Bastidas Navarro, M., Cuassolo, F., Laspoumaderes, C., et al., 2013. Effect of volcanic eruption on nutrients, light, and phytoplankton in oligotrophic lakes. Limnol. Oceanogr. 58:1165–1175. https://doi.org/10.4319/lo.2013.58.4.0000.

Modenutti, B.E., Balseiro, E.G., Bastidas Navarro, M.A., Lee, Z.M., Souza, M.S., Corman, J.R., et al., 2016. Effects of volcanic pumice inputs on microbial community composition and dissolved C/P ratios in lake waters: an experimental approach. Microb. Ecol. 71:18–28. https://doi.org/10.1007/s00248-015-0707-3.

Mulholland, P.J., Steinman, A.D., Marzolf, E.R., Hart, D.R., DeAngelis, D.L., 1994. Effect of periphyton biomass on hydraulic characteristics and nutrient cycling in streams. Oecologia 98:40–47. https://doi.org/10.1007/bf00326088.

Münster, U., Chróst, R.J., 1990. Origin, composition, and microbial utilization of dissolved organic matter. In: Overbeck, J., Chrost, R.J. (Eds.), Aquatic Microbial Ecology. Springer, pp. 8–46.

Nanzyo, M., Dahlgren, R., Shoji, S., 1993. Chemical Characteristics of Volcanic Ash Soils. In: Shoji, S., Nanzyo, M., Dahlgren, R. (Eds.), Developments in Soil Science. Elsevier: pp. 145–187 https://doi.org/10.1016/S0166-2481(08)70267-8.

Nusch, E.A., 1980. Comparison of different methods for chlorophyll and phaeopigment determination. Arch. Hydrobiol. Beih. 14, 14–36.

Óskarsson, N., 1980. The interaction between volcanic gases and tephra: fluorine adhering to tephra of the 1970 Hekla eruption. J. Volcanol. Geotherm. Res. 8, 251–266.

Pistolesi, M., Cioni, R., Bonadonna, C., Elissondo, M., Baumann, V., Bertagnini, A., et al., 2015. Complex dynamics of small-moderate volcanic events: the example of the 2011 rhyolitic Cordón Caulle eruption, Chile. Bull. Volcanol. 77:3. https://doi.org/10.1007/s00445-014-0898-3.

Porter, K.G., Feig, Y.S., 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25, 943–948.

Quiblier, C., Wood, S., Echenique-Subiabre, I., Heath, M., Villeneuve, A., Humbert, J.F., 2013. A review of current knowledge on toxic benthic freshwater cyanobacteria – ecology, toxin production and risk management. Water Res. 47:5464–5479. https://doi.org/10.1016/j.watres.2013.06.042.

Rier, S.T., Stevenson, R.J., 2001. Relation of environmental factors to density of epilithic lotic bacteria in 2 ecoregions. American Benthological Society.]–>J. N. Am. Benthol. Soc. 20, 520–532.

Romaní, A.M., Sabater, S., 2001. Structure and activity of rock and sand biofilms in a mediterranean stream. Ecology 82:3232–3245. https://doi.org/10.1890/0012-9658(2001)082[3232:SAAORA]2.0.CO;2.

Romaní, A.M., Amalfitano, S., Artigas, J., Fazi, S., Sabater, S., Timoner, X., et al., 2013. Microbial biofilm structure and organic matter use in mediterranean streams. Hydrobiologia 719:43–58. https://doi.org/10.1007/s10750-012-1302-y.

Ruggieri, F., Fernández-Turiel, J.-L., Saavedra, J., Gimeno, D., Polanco, E., Naranjo, J.A., 2011. Environmental geochemistry of recent volcanic ashes from the Southern Andes. Environ. Chem. 8:236–247. https://doi.org/10.1071/EN10097.

Sand-Jensen, K., 1983. Physical and chemical parameters regulating growth of periphytic communities. In: Wetzel, R.G. (Ed.), Periphyton of Freshwater Ecosystems: Proceedings of the First International Workshop on Periphyton of Freshwater Ecosystems held in Växjö, Sweden, 14–17 September 1982. Springer Netherlands, Dordrecht: pp. 63–71 https://doi.org/10.1007/978-94-009-7293-3_12.

Sekar, R., Nair, K.V.K., Rao, V.N.R., Venugopalan, V.P., 2002. Nutrient dynamics and successional changes in a lentic freshwater biofilm. Freshw. Biol. 47, 1893–1907.

Shkinev, V.M., Ermolin, M.S., Fedotov, P.S., Borisov, A.P., Karandashev, V.K., Spivakov, B.Y., 2016. A set of analytical methods for the estimation of elemental and grain-size composition of volcanic ash. Geochem. Int. 54:1252–1260. https://doi.org/10.1134/s0016702916130176.

Silva Parejas, C., Lara, L.E., Bertin, D., Amigo, A., Orozco, G., 2012. The 2011–2012 eruption of Cordón Caulle volcano (Southern Andes): evolution, crisis management and current hazards. EGU General Assembly 2012, Vienna, Austria, p. 9382.

Smith, R.E., Kalff, J., 1983. Competition for phosphorus among cooccuring freshwater phytoplankton. Limnol. Oceanogr. 28, 448–454.

Smith, D.B., Zielinski, R.A., Rose, W.I., Huebert, B.J., 1982. Water-soluble material on aerosols collected within volcanic eruption clouds. J. Geophys. Res. Oceans 87:4963–4972. https://doi.org/10.1029/JC087iC07p04963.

Sousa, W.P., 1984. The role of disturbance in natural communities. Annu. Rev. Ecol. Syst. 15:353–391. https://doi.org/10.1146/annurev.es.15.110184.002033.

Stancheva, R., Sheath, R.G., Read, B.A., McArthur, K.D., Schroepfer, C., Kociolek, J.P., et al., 2013. Nitrogen-fixing cyanobacteria (free-living and diatom endosymbionts): their use in southern California stream bioassessment. Hydrobiologia 720:111–127. https://doi.org/10.1007/s10750-013-1630-6.

Stevenson, R.J., Bothwell, M.L., Lowe, R.L., Thorp, J.H., 1996. Algal Ecology: Freshwater Benthic Ecosystem. Academic press.

Stewart, W.D., Fitzgerald, G., Burris, R., 1970. Acetylene reduction assay for determination of phosphorus availability in Wisconsin lakes. Proc. Natl. Acad. Sci. 66, 1104–1111.

Stewart, C., Johnston, D.M., Leonard, G.S., Horwell, C.J., Thordarson, T., Cronin, S.J., 2006. Contamination of water supplies by volcanic ashfall: a literature review and simple impact modelling. J. Volcanol. Geotherm. Res. 158:296–306. https://doi.org/10.1016/j.jvolgeores.2006.07.002.

Taylor, P.S., Stoiber, R.E., 1973. Soluble material on ash from active central American volcanoes. GSA Bull. 84:1031–1042. https://doi.org/10.1130/0016-7606(1973)84<1031:SMOAFA>2.0.CO;2.

Vignaga, E., Sloan, D.M., Luo, X., Haynes, H., Phoenix, V.R., Sloan, W.T., 2013. Erosion of biofilm-bound fluvial sediments. Nat. Geosci. 6, 770.

Witham, C.S., Oppenheimer, C., Horwell, C.J., 2005. Volcanic ash-leachates: a review and recommendations for sampling methods. J. Volcanol. Geotherm. Res. 141:299–326. https://doi.org/10.1016/j.jvolgeores.2004.11.010.

Wood, S.A., Atalah, J., Wagenhoff, A., Brown, L., Doehring, K., Young, R.G., et al., 2017. Effect of river flow, temperature, and water chemistry on proliferations of the benthic anatoxin-producing cyanobacterium Phormidium. Freshw. Sci. 36:63–76. https://doi.org/10.1086/690114.