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Responses of testate amoebae assemblages (Amoebozoa: Arcellinida) to recent volcanic eruptions, inferred from the sediment record in Laguna Verde, southern Patagonia, Argentina

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Abstract There is little knowledge about testate amoebae in lakes and their responses to volcanic eruptions. To address this knowledge gap, we studied the paleoecology of these protists in a sediment record from Laguna Verde, a lake located east of the southern Patagonian Ice Field, in Santa Cruz Province, Argentina. The lake is under the influence of volcanic eruptions from Lautaro Volcano, Chile, $\sim$ 42 km WNW of Laguna Verde. We evaluated the response of 11 testate amoeba morphospecies in the lake to the last four Lautaro eruptions, using a 61-cm sediment core. Calcium (Ca), a major element of volcanic ash, was the most important variable explaining testate amoeba variability in the three zones determined by detrended correspondence analysis. We identified four declines in testate amoeba abundance and diversity associated with tephra deposition. Poisson regression analysis revealed that *Difflugia immanata*, *D. bidens*, and *D. glans* strain "glans" decrease after deposition of tephra layers. In contrast, *Centropyxis constricta* strain "constricta," *C. aculeata* strain "aculeata" and *Zivkovicia compressa* respond positively to ash deposition. Our findings suggest a high resilience of testate

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amoebae to stochastic events such as volcanism because the same assemblage (*D. immanata*, *D. bidens*, and *D. glans* strain "glans") inhabited the lake before and after the ashfalls. Nevertheless, several volcanic eruptions during the last ~ 300 years may have weakened this resilience and had a long-term effect on community diversity. Multivariate analysis showed that potassium (K) plays a significant role in shaping assemblage composition. Because of the low K content of the Lautaro tephra, we infer that higher potassium concentrations in sediments are not related with volcanic events, but rather, are associated with terrestrial input. Future studies, however, will be needed to identify the source of K in the sediment record and its relationship with testate amoebae assemblages. Our study demonstrates the potential for using lacustrine testate amoebae as environmental proxies, and illustrates the direct effects of volcanic ash deposition on their assemblage composition, diversity and distribution in southern Patagonia.

Keywords Protists · Bioindicators · Geochemistry · Patagonian lakes · Paleoecology · Volcanic impact · Tephra · Tephropaleoecology · Northern austral volcanic zone

Introduction

Tephra layers in lake sediments not only document past volcanic eruptions, but also provide information about the nature and strength of eruptions via their composition and spatial extent, respectively (Kilian et al. 2003; Stern 2007). Additionally, tephra layers can be dated, thereby providing chronological markers for lacustrine sediment sequences (Bogaard and Schmincke 1985). In aquatic ecosystems, ash deposition impacts biological communities in different ways. Payne and Blackford (2008) assigned the term "tephropalaeoecology" to studies that focus on identifying the impacts of tephra layers on different aquatic communities.

Volcanic events can be significant triggers of ecological change in lakes. Depending on tephra composition and limnological characteristics, dissolution of tephra can alter water chemistry, modify the lake ecosystem, and change the sediment composition (Hutchinson et al. 2019; Massaferro and Corley 1998;

Telford et al. 2004). In many cases, ashfall increases turbidity in lakes, reduces light penetration and modifies biotic communities (Barker et al. 2000; Hutchinson et al. 2019; Lotter and Birks 1993; Mayr et al. 2019).

Most published studies related to tephropaleoecology have focused on the impact of volcanic ash on freshwater diatoms and chironomid assemblages, with little information about the responses of other aquatic communities such as ostracods, cladocerans and testate amoebae. Hickman and Reasoner (1994) and Telford et al. (2004) found that the presence of tephra layers produced changes in pH and nutrients, causing pronounced changes in diatom assemblages. Other studies, however, argued that the response of diatom assemblages to volcanic ash also depends on other factors such as lake morphology (e.g. lake surface area, water volume), water chemistry, sedimentology, and thickness of the tephra (Hutchinson et al. 2019; Telford et al. 2004). Serra et al. (2016) found that even though diatoms and chironomids decrease in abundance associated with deposition of tephra layers, chironomids do not seem to decline as much as diatoms, which almost disappear after a tephra layer is deposited. For instance, Michelutti et al. (2016) explained that diatoms have a more dramatic response to ash deposition than chironomids because of the former's shorter life cycle. Conversely, other studies demonstrated that volcanic events are important factors that affect the chironomid community (Massaferro et al. 2005; Williams et al. 2016).

In Patagonia, the southernmost part of South America, several studies have shown different responses of chironomid assemblages to tephra layers. Massaferro and Corley (1998) studied a sediment sequence from Lake Mascardi that was deposited during the last 15 ka and documented complete absence of chironomids during volcanic events. Massaferro et al. (2005) analyzed a short core from Lake Morenito that spanned the last 100 years, and observed a distinct change in chironomid abundances during and after ash deposition. Similar results were found by Williams et al. (2016) in a record from Lake Moreno that spanned the last 700 years. The authors mentioned, however, that diversity and abundance of chironomid assemblages respond differently to different volcanic events, mainly depending on grain size. In a later publication about the environmental history of Laguna Toncek, Williams et al. (2019) claimed that

the different responses of chironomids to volcanic ashes were associated with their ecology and feeding habitats. Massaferro et al. (2018) indicated that volcanism seems to have been one of the main drivers of differential resilience and recovery of the chironomid fauna in two high-altitude lakes of northern Patagonia.

Testate amoeba is a term used to describe amoeboid protists that possess an extracellular shell (test), typically with a single, main opening. Some species have xenogenic tests, formed by agglutinated organic or mineral particles captured from the surrounding environment or from other organisms, including dwarf testate amoebae. Other taxa have autogenous tests that can be proteinaceous, siliceous or rarely, calcareous (Sigala and Escobar 2017; Todorov and Bankov 2019). Interest in testate amoebae as proxies in paleoenvironmental research is increasing. In the last few decades, studies have shown the potential of these protists as global paleoenvironmental indicators, especially in temperate lakes of Canada, where they have been used to infer past trophic status, pH and conductivity (Burbidge and Schröder-Adams 1998; Patterson et al. 1985; van Hengstum et al. 2010), or to evaluate human pollution (Kumar and Patterson 2000; Patterson et al. 2012). Additional testate-amoeba-based paleoenvironmental inferences have been made using sediment cores from Finland (Kihlman and Kauppila 2012), France (Wall et al. 2010), Italy (Asioli et al. 1996), England (Ellison 1995), China (Ndayishimiye et al. 2020) and Mexico (Krywy-Janzen et al. 2019; van Hengstum et al. 2010). In the Southern Hemisphere, until now, the only paleoecological study that used testate amoebae was conducted using sediments from Lago Fagnano (54°S), Tierra del Fuego, Argentina (Caffau et al. 2015). In that study, the authors reported 12 taxa from four gravity cores (each $\sim$ 90 cm long) and related greater abundances of some taxa (*D. oblonga* strain "oblonga" and *D. protaeiformis* strain "amphoralis") to higher total organic carbon (TOC) values, whereas *C. constricta* strain "constricta" and *D. urceolata* strain "lageniformis" tended to decrease under similar TOC conditions (Caffau et al. 2015).

Despite the proven utility of testate amoebae as climate and human-impact indicators, there are almost no studies about the impact of past volcanic events on these aquatic communities, except for a few studies from Alaska. There, volcanic impacts were related to testate amoebae communities from peatlands, showing that more studies are needed (Payne and Blackford 2008; Payne 2012; Payne and Egan 2019). Additionally, Delaine et al. (2016) identified the potential of testate amoebae to detect microtephra using X-ray spectrometry on sediments from Puyehue National Park, Chile, as some species collect tephra particles to build their shells.

To address this knowledge gap, we studied the paleoecology of lacustrine testate amoebae in a 61-cm-long sediment core from Laguna Verde, southern Patagonia, a lake located in a region that has been impacted by frequent volcanic activity since the Late Glacial (Fontijn et al. 2014; Mayr et al. 2019; Stern et al. 2007). This information enabled us to identify the response of lacustrine testate amoebae assemblages to four volcanic events during the past $\sim$ 300 years. Additionally, we provide new paleoecological information about testate amoebae from lakes of southern South America.

Study site

Laguna Verde (49.2090°S 72.9728°W, 560 m asl) is a lake located close to Estancia Los Huemules. The site is 17 km north of the village El Chalten, in southern Patagonia, Argentina (Fig. 1a). The regional geology includes metamorphic Paleozoic rocks, volcanic rocks from the Jurassic, and Cretaceous rocks (Giacosa et al. 2012). Laguna Verde has a surface area of 0.029 km², a maximum diameter of 305 m and a maximum water depth of 5.8 m. Since 2015, standard limnological measurements were recorded sporadically during the summer season, and included electrical conductivity (20–30 μS cm^{-1}), pH (6.8–7.4) and temperature (5.2–10.2 °C) (Mayr et al. 2019). The lake is immersed in a dense wet forest of southern beech (*Nothofagus pumilio*), is surrounded by a reed belt of bulrush (*Schoenoplectus* sp.), and also hosts submersed aquatic macrophytes (*Potamogeton* sp.). The catchment area is mountainous and has slope angles of up to 40° on the western side of the lake. Mean annual precipitation at the meteorological station of Estancia Los Huemules, for the period 2007–2016, was 930 mm. Laguna Verde is strongly influenced by ashfall from Volcán Lautaro, located 42 km WNW of the lake (Fig. 1b) (Mayr et al. 2019).
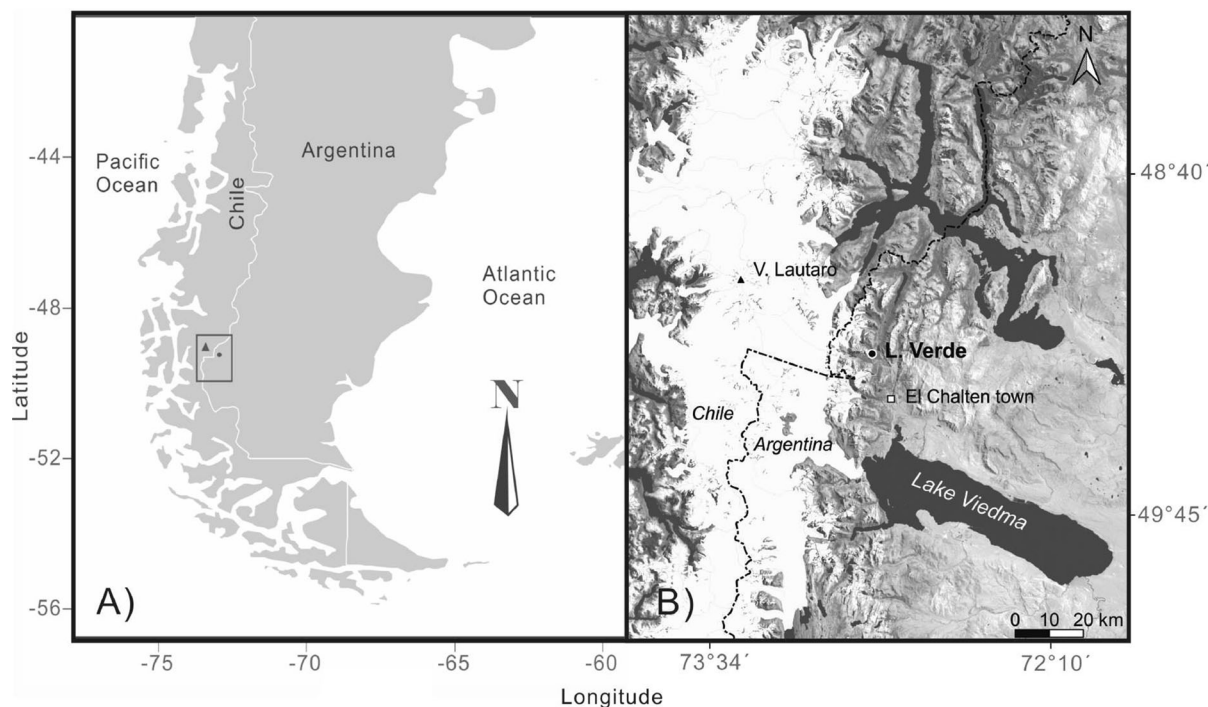
Fig. 1 a Study site in South America. **b** Close-up map showing the location of Laguna Verde. In both maps the lake is indicated by a circle and Lautaro Volcano by a triangle

Materials and methods

Sampling

In April 2018, a 61-cm-long sediment core (VER18-1 = 49°12′33″S, 72°58′21″W) was recovered with a 6-cm-diameter hammer-corer (Aquatic Research Instruments, USA) deployed from a rubber boat in 4.7 m of water. The surface of the recovered sediment core was fixed with floral foam and the core tube was capped and transported to the lab where it was cut lengthwise, photographed and subsampled at 1-cm intervals for geochemical and subfossil testate amoebae analyses.

Non-biological analyses

Color and lithologic variations were described according to standard procedures, before cores were subsampled. Sediment color was determined with a Munsell soil color chart (Munsell 2000) on the fresh (unoxidized) sediment surface, after core opening. We used Loss-On-Ignition (LOI) to estimate organic matter (OM) content. We weighed 1 g of dried

sediment into a nickel capsule and then heated it in a muffle furnace to 550 °C (LOI-550) for 6 h. The OM content was determined as the difference between the dry weight and ash weight (Heiri et al. 2001).

The smoothed surface of one core half was photographed and scanned with an X-ray fluorescence (XRF) core scanner (ITRAX, Cox Analytics) equipped with a chromium tube for qualitative elemental composition. Sixteen elements were measured, of which three (Ca, K, Ti) were used for interpretation. Elemental composition was recorded in counts (cts) that were normalized by dividing by the coherent radiation (coh) (Hahn et al. 2014). Tephra layers were identified macroscopically and confirmed by Ca/coh peaks. The succession of tephras in core VER18-1 was correlated with that in adjacent core VER17-1, which was dated using ^{210}Pb and ^{137}Cs and related to known eruptions of Lautaro Volcano (Mayr et al. 2019) (Electronic Supplementary Material [ESM] Fig. S1).

Testate amoebae analysis

A volume of 1 cm^3 of wet sediment from each subsample was sieved through a 53-μm mesh to retain amoebae tests of a size easily identifiable under a stereomicroscope. All tests were extracted with a fine paintbrush, counted, and kept in semi-permanent preparations on cover slides with glycerin. Taxonomic identification was carried out with an optical microscope and a ZEISS EVO MA10 scanning electron microscope (SEM), using specialized literature for this protist group (Kumar and Dalby 1998; Sigala et al. 2015). We validated the taxonomic identifications with literature that reported regional testate amoebae (Caffau et al. 2015; Fernandez et al. 2015). Lacustrine testate amoebae species can display broad eco-phenotypically controlled morphological variability. Therefore, it is common to assign informal, infra-subspecific 'strain' names to eco-phenotypes to avoid inadvertent and unwarranted descriptions of new species. Although the International Code of Zoological Nomenclature stipulates that infra-subspecific-level designations have no status (ICZN 1999), in testate amoebae studies they are useful for distinguishing environmentally distinct populations in lacustrine environments (Patterson et al. 2012).

Statistical analyses

Total abundance of tests (individuals/cm^3) was used to compare assemblages along the core and relative abundance of taxa (%) to identify dominance for each assemblage. Diversity was calculated for each sample, using the Shannon Diversity Index (SDI), to evaluate the stress level of testate amoebae. According to Patterson and Kumar (2002), SDI values should be between 0.1 and 1.5 if an assemblage is dominated by one or two taxa. Such values can be related to stressful conditions for testate amoebae, whereas higher values (1.5–2.5) are considered to indicate less stressful conditions (Sigala et al. 2018; Cockburn et al. 2020).

For the multivariate analysis, we standardized the testate amoeba relative abundance with square root transformation and non-biological information was transformed by standardization to zero mean and unit variance (Borcard et al. 2011). Detrended Correspondence Analysis (DCA) was performed with all species present in each sample. DCA was used to explore taxa assemblage patterns and the underlying structure of the dataset. In addition, DCA helped to interpret the zonation according to the distance between contiguous samples, which in turn represents the ecological turnover in standard deviation units (SD). A value of 4 SD represents a complete turnover of species and higher values represent an important ecological change between samples (Correa-Metrio et al. 2014).

A multivariate redundancy analysis (RDA) was carried out to evaluate the relationship between sediment geochemistry and amoebae assemblages in the core. Environmental variables were pre-selected using variance inflation factors (VIF). The VIF measures how much variance is influenced between independent variables. A high VIF (> 20) between environmental variables indicates strong collinearity (Borcard et al. 2011), and those were removed. We then used a forward selection to find the explanatory variables in the RDA (Blanchet et al. 2008). Analysis of variance (ANOVA) was used to recognize the significance of the remaining variables. To determine the effect of a tephra layer on each testate amoeba taxon from Laguna Verde, a generalized linear model (GLM) was applied to evaluate the abundance of each taxon as a function of Ca concentration, using Poisson regression (Correa-Metrio et al. 2014).

To calculate Diversity (Shannon Index) and run multivariate analyses (DCA, RDA), the Vegan package (Oksanen et al. 2019) was used. Abundance, diversity, multivariate analysis (DCA) and non-biological variables were plotted stratigraphically with the Rioja package (Juggins 2020). All variables were standardized to avoid effects of unit heterogeneity and all statistical analyses were carried out in the R environment version 3.5.3 (R Core Team 2020).

Results

Sediment description and geochemical analyses

Throughout core VER18-1, sediments consist of laminated, clayey olive-grey mud (Munsell colors: 5Y4/2, 5Y5/2, 5Y6/2) intercalated with four greyish to violet-greyish layers (5Y6/1, 7.5YR6/2). The sequence is interrupted by four macroscopically visible tephra layers (VT4: 46–42 cm, VT3: 24–17 cm, VT2: 13–11 cm, VT1: 6–8), confirmed by peaks of Ca (Fig. 2). Titanium (Ti) also increases within the tephra layers, contrary to potassium (K) and
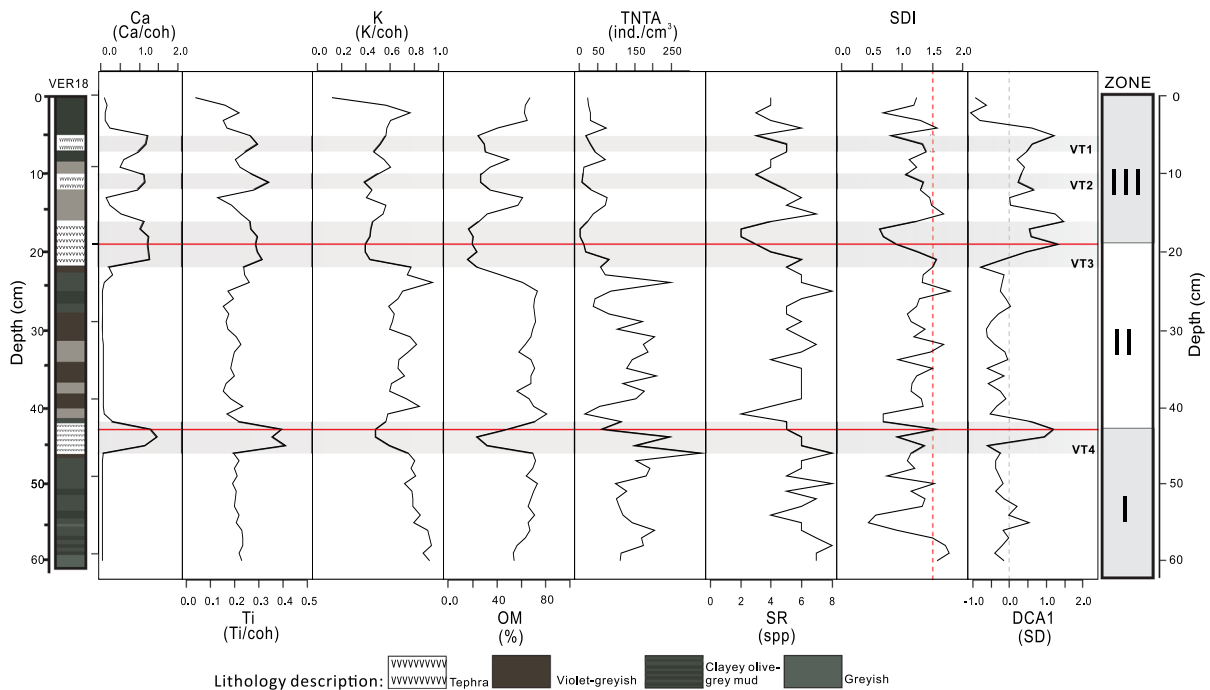
Fig. 2 Lithological description of core VER18, geochemical and statistical profiles showing calcium (Ca/coh), titanium (Ti/coh), potassium (K/coh), organic matter (OM), the total number of testate amoebae per cm (TNTA), Species Richness (SR), Shannon Diversity Index (SDI) with dashed red line indicating stressful values (SDI ≤ 1.5), Detrended Correspondence Analysis of Axis 1 sample scores (DCA1) in standard deviation units (SD) and the zonation based on testate amoebae. Red lines indicate zones identified by DCA > 1 SD. Grey horizontal bars highlight tephra layers

organic matter (OM), which decrease in tephras to values below their means (Fig. 2).

The four tephra layers are correlated to a previously studied sediment record from Laguna Verde (VER17-1, Mayr et al. 2019), in which each tephra was geochemically analyzed and compared to historic eruptions of Lautaro Volcano. Three of the four tephra were dated: VT1 corresponds to the CE 1959–1960, VT2 to the CE 1933 and VT3 to the CE 1876–1878 eruptions. The oldest tephra (VT4) could not be identified and dated (Mayr et al. 2019).

Testate amoeba assemblages

A total of 11 taxa were identified (Fig. 3): *Difflugia immanata*, *D. bidens*, *D. glans* strain "glans", *D. elegans*, *D. oblonga* strain "oblonga", *D. oblonga* strain "tenuis", *Centropyxis constricta* strain "constricta", *C. aculeata* strain "aculeata", *Lagenodifflugia vas*, *Lesquereusia modesta,* and *Zivkovicia compressa*. No taxon, however, was present in all samples. To visualize the main ecological changes

through time, zonation of the stratigraphic profile is presented based on changes of DCA Axis 1 scores (Fig. 2). That analysis enabled identification of three distinct zones (I to III).

Zone I (61–45 cm)

In this zone, mean abundance of amoebae is 159.8 ind/cm^3 with a maximum of 330 ind/cm^3 at 48 cm (Fig. 2). Species richness (SR) values are between 4 and 8. Shannon Index values are between 0.44 and 0.92 (58–56, 51, 46 cm) and 1.79 (61 cm). Seven species are present in this zone. The dominant taxon is *D. glans* strain "glans" in almost all the samples (Fig. 3), with a relative abundance of > 50%, followed by *D. immanata* (> 20%). In the middle part of Zone I (58–54 cm), *D. glans* strain "glans" increases its abundance to almost 90%. *Centropyxis constricta* strain "constricta" and *D. oblonga* strains "oblonga" and "tenuis" also increase, while taxa like *D. immanata, L. vas, D. elegans* and *D. bidens* disappear, only recovering their abundance at 53 cm. Finally, at
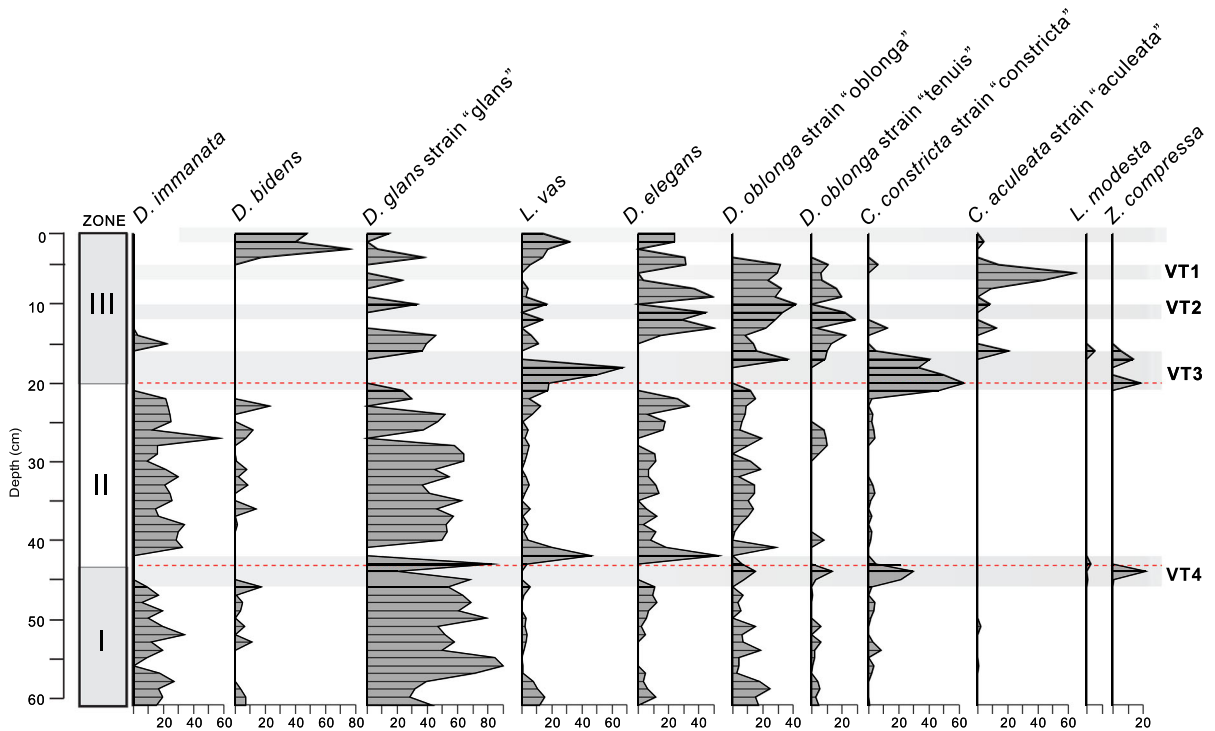
Fig. 3 Zonation based on testate amoebae and relative abundances of testate amoebae from Laguna Verde: *Difflugia immanata, Difflugia bidens, Difflugia glans* strain "glans", *Lagenodifflugia vas, Difflugia elegans, Difflugia oblonga* strain "oblonga", *Difflugia oblonga* strain "tenuis", *Centropyxis constricta* strain "constricta", *Centropyxis aculeata* strain "aculeata", *Lesquereusia modesta, Zivkovicia compressa*. Grey horizontal bars indicate tephra layers. Red lines indicate zones identified by DCA > 1 SD (Fig. 2)

the top of Zone I (46–44 cm), *C. constricta* strain "constricta" becomes the dominant taxon and *L. modesta* and *Z. compressa* appear. The rest of the taxa are absent or present at low abundances (Fig. 3).

Zone II (44–21 cm)

In the first part of this zone (44–42 cm), the total number of individuals is < 55 ind/cm^3 and the Shannon Index values are low (< 1) (Fig. 2). *C. constricta* strain "constricta" (40%) is the dominant taxon. All the other species, except *L. vas, D. elegans* and *D. oblonga* strain "oblonga" (Fig. 3) are absent. Species richness increases above 40 cm to the end of the zone. At that time, *D. glans* strain "glans" recovers and becomes dominant, whereas the rest of the assemblage does not change substantially (Fig. 3). At the end of Zone II (22–20 cm), the abundance of testate amoebae decreases, as do Shannon Index values (Fig. 2). Some taxa disappear, such as *D. immanata, D. elegans and D. bidens,* whereas *C. constricta* strain "constricta"

(50%) and *L. vas* (20%) become the dominant taxa of the assemblage.

Zone III (20–0 cm)

This zone shows two important ecological changes, both coincident with VT3 (Fig. 2). Testate amoebae abundance is very low (mean 31 ind/cm^3), and only three samples in this zone have > 50 ind/cm^3 (9–5 cm). Species richness shows fluctuations along the zone, with only three samples (16, 14, 5 cm) with a high species richness and Shannon Index values (SR > 5 spp./cm; SDI > 1.5) (Fig. 2). The first ecological change is in the first part of Zone III (20–17 cm; Fig. 2), some taxa disappear, such as *D. immanata, D. bidens, D. glans* strain "glans", *D. elegans,* and *C. aculeata* strain "aculeata", whereas *D. oblonga* strains "tenuis" and "oblonga" are absent, only recovering to greater abundance at 16 cm, where the second ecological change is detected by DCA axis 1 (Fig. 2), with *L. vas* and *C. constricta* strain
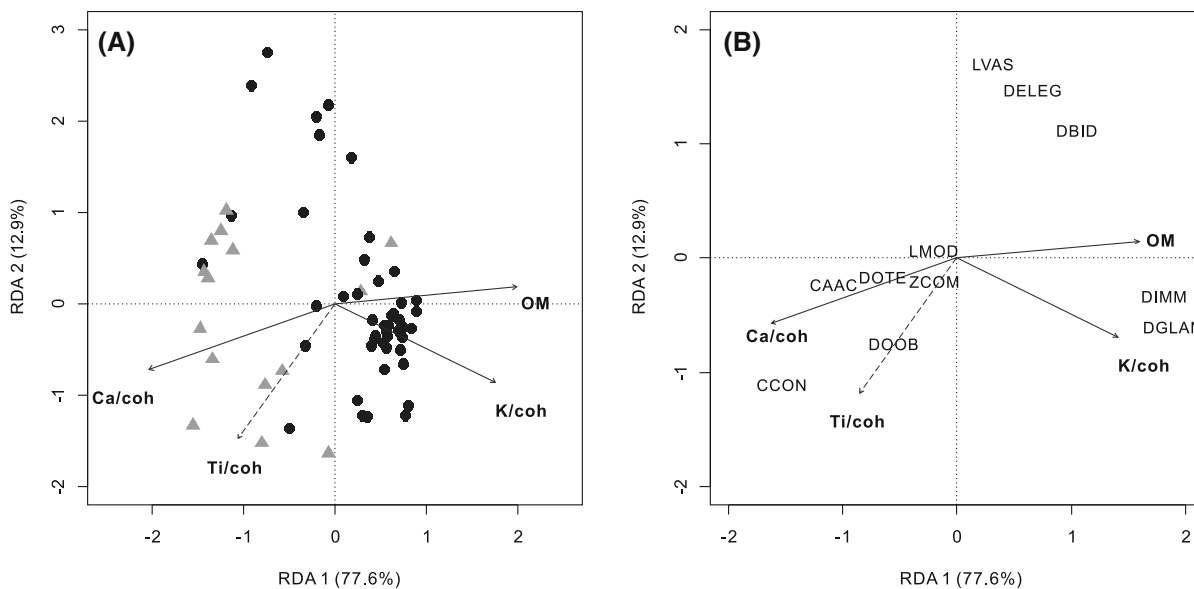
Fig. 4 a RDA as sample ordination biplot. Gray triangles represent the tephra layers. b RDA biplot by species ordination. *D. immanata* (DIMM), *D. bidens* (DBID), *D. glans* strain "glans" (DGLAN), *L. vas* (LVAS), *D. elegans* (DELEG), *D. oblonga* strain "oblonga" (DOOB), *D. oblonga* strain "tenuis" (DOTE), *C. constricta* strain "constricta" (CCON), *C. aculeata* strain "aculeata" (CAAC), *Lesquereusia modesta* (LMOD), *Z. compressa* (ZCOM). Significant vectors are drawn as solid arrows in **a** and **b** and are related to selected environmental variables ($p < 0.05$)

"constricta" as dominant taxa. Recovery of the full assemblages between 15 and 13 cm occurs, showing an increase in the total number of individuals to 70 ind/cm^3, as well as increases in species richness (5 spp.) and the Shannon Index (> 1) (Fig. 2). Some taxa such as *D. glans* strain "glans" and *D. elegans* reappear, while *L. vas* and *C. constricta* strain "constricta" decrease. Taxa such as *D. oblonga* strains "oblonga" and "tenuis" and *C. aculeata* strain "aculeata" increase their abundance in contrast to their trends in Zone II (Fig. 3). From 12 to 5 cm there is a general decrease in abundances, species richness and Shannon Index (Fig. 2). From 5 cm to the top of the core, some species such as *D. immanata, L. modesta, Z. compressa, C. constricta* strain "constricta" and *D. oblonga* strains "tenuis" and "oblonga" disappear, but taxa like *D. bidens, D. glans* strain "glans" *D. elegans* and *L. vas* reappear in the assemblage (Fig. 3).

Statistical analyses

Redundancy analysis

Redundancy Analysis (RDA) is a canonical ordination method that in this case provides a quantification of the proportion of the variance in testate amoebae data explained by non-biological proxies. The variance inflation factor (VIF), together with the forward selection approach of the RDA, enabled selection of Ca, K, and OM as the most important variables without collinearity ($p < 0.01$, Fig. 4). These three variables together explain 23.4% of the variance. The variable with the highest explanatory proportion is Ca (18.9%, F = 11.84, $p = 0.001$), followed by K (3.10%, F = 2.69, $p = 0.001$), whereas OM is low (1.40%, F = 2.13, $p = 0.06$; Table 1, Fig. 5).

RDA Axis 1 (77.6%) is strongly correlated with Ca concentration. The ordination indicates that samples corresponding to tephra layers are positioned in the negative part of Axis 1 and have a different assemblage compared to the rest of the core. Principal taxa associated with the Ca vector are *Z. compressa, L. modesta, D. oblonga* strain "tenuis", *D. oblonga* strain "oblonga" and both centropyxid species (*C. aculeata* strain "aculeata" and *C. constricta* strain "constricta"). In contrast, RDA Axis 2 (12.9%) shows a correlation with K and OM. Associated with the K vector are *D. immanata* and *D. glans* strain "glans", whereas *D. bidens, D. elegans*, and *L. vas* are associated with OM (Fig. 4).

Table 1 Variance partitioning results showing the percentages of testate amoebae distribution variation explained by geochemistry and Redundancy Analysis (RDA) for each variable

Variable	Variance	Variance explained (%)	F	Pr(> F)	RDA1	RDA2
Ca	6.51	18.90	11.84	0	−0.9	−0.31
K	1.48	3.10	2.69	0.01	0.78	−0.38
OM	1.17	1.40	2.13	0.06	0.88	0.08
Ti	0.81	0.80	1.47	0.19	−0.47	−0.65
Residuals	30.79	75.50				

Poisson regression

A General Linear Model was used to statistically assess the relation of Ca with each taxon. The estimated coefficient of logistic regression shows that 10 of 11 taxa are significantly associated with Ca concentration (Fig. 5). Positively associated are *Z. compressa*, *L. modesta*, *C. aculeata* "aculeata" and *C. constricta* strain "constricta". Contrary to *D. immanata*, *D. bidens*, *D. glans* strain "glans", *D. elegans*, *L. vas* and *D. oblonga* strain "oblonga" have a negative association with Ca. *D. oblonga* strain "tenuis" is the only taxon without a significant association with Ca.

Discussion

Effect of tephra deposition on testate amoebae assemblages

Our results show a strong response of testate amoebae to the volcanic events recorded in Laguna Verde sediments. This is clearly visible when comparing the scores of DCA Axis 1 with Ca, representing the tephra layers (Fig. 2). For instance, the DCA stratigraphic Axis 1 shows the most significant ecological turnover, in perfect agreement with Ca peaks (Fig. 2). In RDA analysis, Ca is the first explanatory variable, explaining 18.9% (F = 11.84, p = 0.001) of total amoebae variability (Fig. 4). Considering that Ca is a proxy for volcanic ashfalls, it is evident that tephra layers had a clear effect on testate amoebae assemblages throughout the core. Indeed, there is a general decrease in the total number of testate amoebae (< 100 ind/cm^3), species richness (< 3 spp.) and Shannon Index values (SDI < 1) during ash deposition (Fig. 2), which is evident at the end of Zone I at VT4 (46–42 cm) and throughout Zone III for each tephra (VT3: 24–17 cm, VT2: 13–11 cm, VT1: 8–6 cm). According to these results, we suggest that ashfalls produced, in general, a negative effect on amoebae abundance and diversity. Similar responses have also been seen with other aquatic groups such as diatoms (Lotter and Birks 1993) and in some cases, with chironomids (Massaferro et al. 2005; Williams et al. 2016) in that species richness and population size were reduced as a consequence of pulsed volcanic events (Massaferro and Corley 1998). Recently, Wang et al. (2020) mentioned that stochastic processes explained a significantly higher percentage of a modern community assemblage of testate amoebae than deterministic processes over seven years, although deterministic processes (temporality) were more influential in certain years. In our study of Laguna Verde, ashfalls were the pulse or the stochastic process that had a strong influence on assemblages of testate amoebae.

Some assemblages, however, characterized by the centropyxids *L. modesta* and *Z. compressa,* are typical for tephra samples (Fig. 3). These taxa have a positive relation with Ca (Figs. 4, 5), which means that their abundances increase during or immediately after the tephra layer. Payne and Egan (2019) also identified some species of testate amoebae that respond positively to volcanic events, increasing their abundances during the ashfall. In the case of the assemblages of Laguna Verde, the dominance of centropyxid taxa within the tephra layers (Fig. 3) could indicate that those taxa are pioneers that colonize immediately after volcanic events. *Centropyxis* was previously reported as the earliest colonizer and the taxon most tolerant of extreme environmental conditions, and its presence is related to lakes with hyper-saline conditions (van Hengstum et al. 2010), low oxygen concentrations (Charqueño Celis et al. 2019), and high concentrations
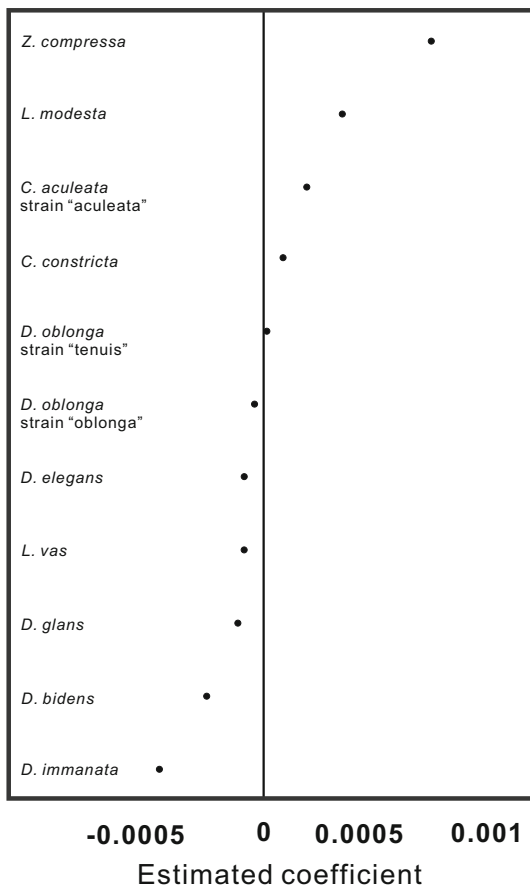
Fig. 5 Estimated Poisson regression coefficients. Coefficients of taxon abundance as a function of Ca are considered to be a proxy for tephra deposition. Points show the estimated value of the coefficient, whereas horizontal black lines show their 95% confidence intervals. Regression is significant only in the event that horizontal lines do not cross the zero-line marked by a vertical black line

of heavy metals such as arsenic (Patterson and Kumar 2002). Indeed, this genus is a typical r-strategist, capable of surviving under conditions of low food availability and able to colonize stressed (anaerobic) environments, where it feeds on denitrifying bacteria (Baross et al. 1982). Smith (1985) observed the presence of *Centropyxis* as a pioneer, together with a microbial succession, after a volcanic eruption in Telephon Bay, Antarctica. Such changes could be also linked to shifts in predation of methanotrophic bacteria by centropyxids after tephra deposition (Baross et al. 1982; Payne 2010, 2012). The increase and dominance of *C. constricta* strain "constricta" within tephra layers of Laguna Verde may, however, be

related to pH changes caused by ash deposition in the lake, because this taxon has also been identified as one of the few amoebae tolerant of low-pH conditions (Patterson et al. 2013).

Autogenous taxa, common in lacustrine environments (Fernandez et al. 2015; Sigala et al. 2018), are almost absent from our record, and only represented by *L. modesta*. Their tests, however, are a mix of siliceous and small mineral grains, so they must be considered pseudo-autogenic (Fig. 6). This taxon shows increases in abundance in the last part of tephra layers VT3 and VT4, coincident with the decrease of *C. constricta* strain "constricta" (Fig. 3). This amoeba should be related with a pH increase after tephra deposition, given that this species has been reported in high-pH environments (8.3–9.0) (Quin et al. 2013: Velho 2003). It could mean that *L. modesta* has an advantage in colonization of the environment, even over other autogenous amoebae. Although it is possible that some xenogenic taxa benefited over proteic or siliceous amoebae because of the high availability of shell material, our finding could be related to taphonomic issues or to the size of the mesh we used for sample preparation, because there are other tolerant autogenous amoebae that are smaller than the taxa we found, e.g., *Arcella* sp. < 20 μm. Future studies should also analyze the fraction smaller than 53 μm to confirm the presence of other autogenous taxa preserved in tephra layers or other parts of the core.

Post-volcanic deposition contains assemblages composed mainly of *Difflugia oblonga* strains "oblonga" and "tenuis", *D. elegans* and *L. vas* (Fig. 3). These amoebae are typical of environments rich in organic matter and nutrients (Burbidge and Schröder-Adams 1998; Caffau et al. 2015). Despite the fact that both strains of *D. oblonga* are not fully correlated with OM on the RDA plot (Fig. 4b), abundance peaks of both taxa coincide with OM after tephra layers (Figs. 2, 3). Those results indicate an increase of biologic activity after a volcanic event. Mayr et al. (2019) claimed that tephra layers had an effect on the nitrogen cycle at Laguna Verde, reducing uptake of this element by primary producers. Other studies have shown an increase of lacustrine nutrients after tephra deposition, associated with allochthonous input that increased productivity (Urrutia et al. 2007).

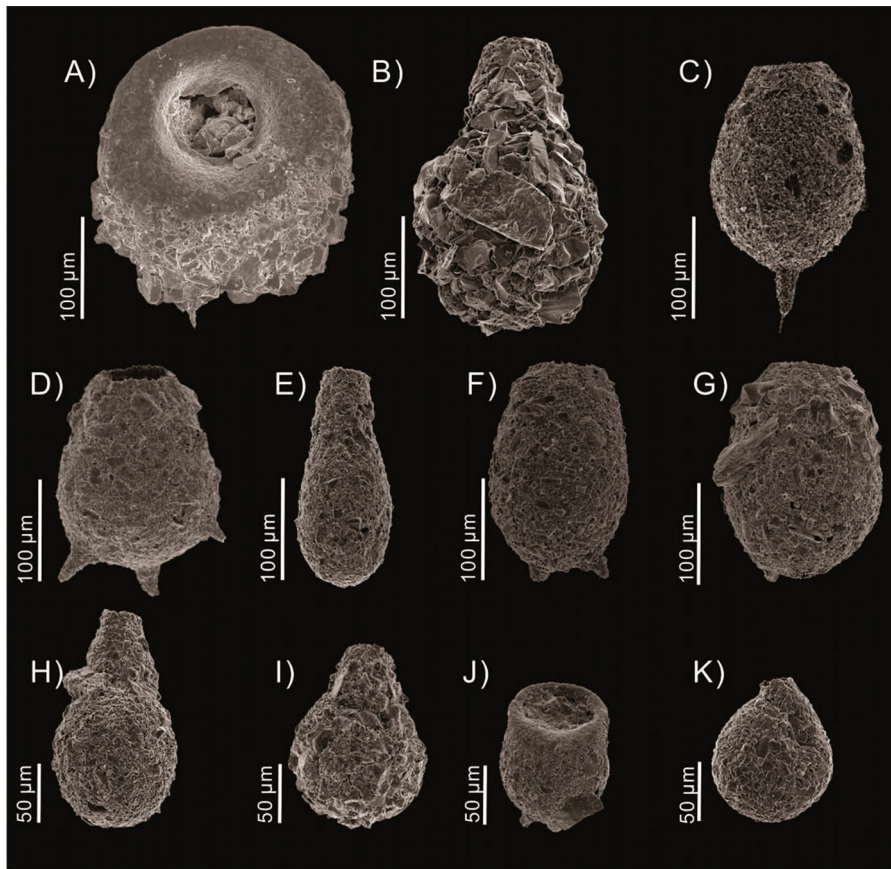Some difflugids are among the amoebae most vulnerable to tephra impacts. Poisson results show that

Fig. 6 Testate amoebae from Laguna Verde: **a** ventral view of *Centropyxis aculeata* strain "aculeata"; **b** *Lagenodifflugia vas;* **c** lateral view of *Difflugia elegans;* **d** lateral view of *Difflugia immanata*; **e** lateral view of *Difflugia oblonga* strain "tenuis"; **f** lateral view of *Difflugia bidens;* **g** lateral view of *Difflugia glans* strain "glans"*;* **h** lateral view of *Difflugia oblonga* strain "oblonga"; **i** lateral view of *Zivkovicia compressa;* **j** ventral view of *Centropyxis constricta* strain "constricta"*;* **k** lateral view of *Lesquereusia modesta*

D. immanata, *D. glans* strain "glans" and *D. bidens* have the most negative response related to Ca (Fig. 5). Those taxa display low abundance or are absent from tephra layers, whereas they reach high abundance values and are dominant during the first part of Zone I and the middle part of Zone II (Fig. 4), depths where no tephra is present. It is possible that the composition of the volcanic ashes altered the lake sediments and thus also the habitats of the taxa. Indeed, many species of centropyxids are generalists and capable of using mineral grains from their environment for their tests, including tephra (Delaine et al. 2016), whereas other taxa, particularly difflugids, require specific grains or even prefer specific diatoms to build their tests (Patterson et al. 2015). An example is shown in the SEM photo of *D. immanata,* which possesses a test made mainly of valves from the diatom *Aulacoseira*

and mineral sediments (Fig. 6). Both species, i.e. the amoeba *D. immanata* and diatom *Aulacoseira*, show declines in abundance during tephra deposition (Fig. 3; Mayr et al. 2019).

Despite their sensitivity to environmental changes, our results show that most testate amoebae from Laguna Verde are resilient organisms and that assemblages recovered rapidly after volcanic events and returned to initial relative abundances. Previous paleoecological studies dealing with the effects of volcanic events on chironomids in Patagonia (Massaferro et al. 2005; 2018; Williams et al. 2016) also suggested high resilience of these communities, with reestablishment of previous conditions and community structure shortly after volcanic events. It is, however, necessary to compare biological responses among different types of lakes, because the

susceptibility to disturbance and ecosystem resilience are highly dependent on local factors such as lake and watershed morphology, hydrology and vegetation cover (Massaferro et al. 2018). Moreover, comparing the responses of different aquatic communities during and after events such as volcanic ashfalls, would enable a better understanding of lake ecological processes. For example, Michelutti et al. (2016) studied sediments from a lake in Ecuador and observed that after a volcanic event, chironomids only changed in overall abundance, whereas diatoms displayed a change in their specific composition (relative abundances), which may be related to the very short life span of diatoms. According to our results, testate amoebae, which also have short life cycles, respond much like diatoms to volcanic events.

Zone II shows a complete recovery of the amoeba assemblage after volcanic ash deposition, to a pre-tephra state of the taxa, with increases in abundance and diversity (Fig. 2). Zone III, however, records low species richness and Shannon Index values, even well after volcanic ash deposition (Fig. 2). This is interpreted as a response to periodic volcanic activity of Lautaro Volcano during the last $\sim$ 150 years (Fontijn et al. 2014; Mayr et al. 2019), which must have led to a gradual loss of amoebae diversity. Recurrent volcanic activity does not provide enough time for community reestablishment. The consequent loss of resilience makes the system more vulnerable in the sense that it could easily shift to an unfavorable state for amoebae communities This highlights the importance of *tephropaleoecology* studies to improve our understanding of how the frequency of volcanic ash deposition into lakes impacts the diversity of aquatic communities (Brown et al. 2019; Smith et al. 2019).

Non-volcanic events and testate amoebae at Laguna Verde

This study showed that volcanic events are the most important drivers of testate amoebae assemblage composition and diversity in Laguna Verde. Other factors, however, such as climate change, may also play a role in community shifts observed in the sediment record. In our case, K is the second most significant explanatory variable of the amoebae assemblage (Fig. 4), which could be correlated with allochthonous input to the lake (Davies et al. 2015). Lautaro tephra composition is mainly rhyolitic, with a low K content (Smith et al. 2019). Thus, stratigraphic variations in K in sediments of Laguna Verde are interpreted as being related to input of siliciclastic (not volcanic) material to the lake, which in turn suggests hydrological changes in the catchment (Davies et al. 2015). In the case of Ti, although it is also an index of input from the watershed, its presence in the Lautaro tephra precludes its use as an indicator of other environmental impacts (Smith et al. 2019). In our study, variations in K seem to be related to changes in abundance of less tolerant taxa such as *D. immanata, D. glans* strain "glans", *D. elegans* and *D. bidens* (Figs. 4, 5). In fact, *D. bidens* has been shown to be indicative of allochthonous input to lakes, reflecting both human impacts and climate change (Patterson and Kumar 2002; Patterson et al. 1985). These findings are promising, however more work needs to be done to better understand the relationships between changes in geochemistry and the testate amoebae assemblages.

There is a strong need to increase our knowledge of the biodiversity, distribution and ecology of testate amoebae in lakes, especially in the Southern Hemisphere, and to better correlate changes in testate amoebae assemblages to non-volcanic events such as climate and/or human activities, which also influence these protists. Only then will we be able to fully realize the potential of testate amoebae as environmental and climate proxies in Patagonian lakes.

Conclusions

Documented eruption events of Lautaro Volcano during the last $\sim$ 300 years had strong influences on benthic testate amoebae assemblages in Laguna Verde. Tephra layers found in the lake sediments create stressful conditions for these protists, causing abrupt decreases in their abundance and diversity, as well as complete ecological turnover throughout the record. All tephra layers are rich in Ca and are characterized mainly by the presence of opportunistic taxa such as centropyxids. Immediately after these tephra layers, the presence of *Difflugia oblonga* strains "oblonga" and "tenuis", *D. elegans* and *L. vas*, is related to an increase in organic matter content. In contrast, the most vulnerable difflugids to volcanic ash impact (*D. immanata*, *D. glans* strain "glans" and *D. bidens*), are more abundant or only present (*D.*

immanata, a taxon that is less resilient than others) in pre-tephra-deposition sediments and are negatively affected by frequent volcanic activity.

Paleoecological reconstructions provide an excellent opportunity to generate information about the influence of volcanic events on aquatic communities and to elucidate their capacity for resilience following stochastically recurrent events. In our study, testate amoebae from Laguna Verde document strong resilience and rapid recovery to pre-tephra conditions. Nevertheless, following persistent events over a short period of time, assemblages displayed reduced resilience and no sign of recovery, leading to irreversible loss of diversity.

Our results also indicate that testate amoebae in Laguna Verde may have responded to environmental changes other than volcanism, but responses to some, like changes in runoff, may be obscured by the strong volcanic signal. Future paleolimnological studies should compare past shifts in the amoeba community with recent climate changes and human activities around the lake, to better understand how testate amoeba species assemblages respond to stressors not associated with volcanic events.

This study highlights the potential of lacustrine testate amoebae as biological proxies that can be used to understand past climate and environmental changes in the Southern Hemisphere. A better understanding of their taxonomy and modern ecological requirements is a prerequisite for future studies.

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