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Seismic events as potential drivers of the microbial community structure and evolution in a paleo-ocean analog

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Anthropogenic perturbations profoundly affect aquatic ecosystem microbiomes and associated ecological functions. Comparatively, the effects of geological stresses on microbiome composition and stability remain poorly explored. Here, we monitored the archaeal, bacterial and microeukaryotes community structure over an 8-years period in Lake Dziani Dzaha (Comoros archipelago), that experienced a major earthquake swarm mid-survey, providing a rare opportunity to investigate the aftermaths of seismo-volcanic events on microbiome. Our results revealed the sensitivity of the aquatic microbial community towards seismicity and associated environmental changes, that triggered a major shift in microbiome composition and abundance with persisting consequences on structure and richness of the microbial ecosystem. Our findings suggest that seismological perturbations could be major drivers of the microbial community structure in aquatic environments through cascading effects on environmental conditions. Over geological time scales, such events may have been significant yet underestimated forces driving diversification and evolution of microbial communities.

Aquatic microbiomes are sentinels of environmental changes[1–3]. Large scale studies and time series have revealed that microbial community structure reacts rapidly following environmental perturbations such as pollutions[4,5], agriculture practices[6,7], and climate changes[8,9]. While the impact of a perturbation on the microbiome structure and associated functions depends on its intensity, duration and direction[10], the levels of resistance (the degree to which microbial composition remains unchanged after being disturbed) and resilience (the rate at which microbial composition returns to its original state after being disturbed) of microbial assemblages have been found to be positively correlated with the microbial richness[11], indicating that a biodiverse microbial ecosystem is more prone to recover from perturbations and maintain ecological functions[12,13]. While the effects of anthropogenic disturbances on microbiome have been extensively investigated, microbial community response toward geological hazards and associated consequences remains poorly explored. About 20,000 earthquakes of magnitude higher than Mw4 are reported around the globe each year (National Earthquake Information Center, https://www.usgs.gov/programs/earthquake-hazards). However, previous investigations on the aftermaths of earthquakes and seismic events on microbial community mostly focused on aquifer ecosystems and the consequences of exogenous water/microorganisms introduction for drinking water quality[14,15], leaving the effects of such large natural perturbation on surface aquatic microbiomes largely unexplored.

Lake Dziani Dzaha, located on the Petite Terre Island (Mayotte) in the Mayotte archipelago, is a maar; a low-relief volcanic crater; that formed around 7000 years ago following a phreatomagmatic eruption that trapped oceanic water within its caldera[16]. Isolated from the ocean, the system has evolved over millennia to form a meromictic hypersaline, alkaline lake[17]. This thalassohaline ecosystem displays original geochemical signatures such as the presence of growing stromatolites[18], strongly depleted carbon and nitrogen isotopic signatures, steep oxygen and sulfur gradients and warm temperature[16] that together define the lake as a possible modern analog of

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Proterozoic lakes and interior seas[19]. While no metazoans were previously detected, a large panoply of microorganisms has been previously identified in Lake Dziani Dzaha[20], with similarities to hypersaline environments such as soda lakes[21,22]. This includes two major primary producers, the pico-eukaryote *Picocystis salinarum* and the cyanobacterium *Limnospira platensis*, formerly *Arthrospira fusiformis*[23,24] and their potential respective phycosphere[25], various archaea such as *Woesearchaeota* and methanogenic lineages, and diverse heterotrophic bacteria[20,26]. Previous studies that characterized the geochemical environment and associated microbial community structure and network in 2014 and 2015, revealed a strong vertical stratification of the microbial assemblages depending on salinity, oxygen and sulfur concentrations[26,27]. While the main primary producers were identified throughout the 17.25 m of the water column, guilds of potential algicidal bacteria and organic matter recyclers differed between oxic and anoxic waters[20,27]. This vertical distribution of microbial assemblages also depended on precipitation and evaporation levels associated with the dry (April-August) and wet (October-February) seasons of the region, that modify the salinity gradient of the water column and the position of the halocline due to the dilution of the upper water layer by fresh rainwater[17] (Supplementary Fig. 1).

On May 2018, the Comoros archipelago has experienced an unexpected earthquake swarm, including a major volcano-seismic event of magnitude up to Mw5.9. After this intense volcano-seismic activity that was measured until late 2018, a continuous seismicity perdured, with signals associated with underground magmatic or hydrothermal fluid migration (https://www.ipgp.fr/observation/infrastructures-nationales-hebergees/revosima/). This volcano-seismic activity was related to the formation of a novel underwater volcano, Fani Maoré[28,29], that induced a subsidence of around 10–20 cm of the whole Mayotte archipelago. With the epicentres located about 5–50 km East offshore of Petite Terre Island, Lake Dziani Dzaha has been particularly impacted by the earthquake swarm. This offered an outstanding opportunity to investigate the ecology and stability of natural microbial ecosystems facing geological perturbation, providing a window on microbiome evolution and on the processes by which resistance, resilience or stabilization into an alternative state occur in a natural microbial system.

In this study, we analyzed the long-term dynamics of bacterial, archaeal and eukaryotic community composition of Lake Dziani Dzaha over 8 years (2014–2022) surrounding the 2018 seismic crisis to address the following questions: i) how could the natural microbiome be affected by direct and indirect consequences of a geologic crisis? and ii) are aquatic microbial ecosystems resistant, resilient or sensitive to geological perturbations and cascading aftereffects? Overall, this study provides first insights into the potential of seismicity as driver of community composition and evolution, and the possible consequences over geological time scales.

Results

Drift of the physicochemical environment of the lake

The physico-chemical environment of the lake was monitored since 2007[16,17]. At the beginning of the microbial community survey (2014–2015), Lake Dziani Dzaha waters were strongly stratified by a salinity gradient, with a moving halocline, depending on the seasonal precipitation and evaporation levels (Supplementary Fig. 1). High sulfide (up to 6 mM), methane (up to 2 mM) and ammonium (up to 4.5 mM) concentrations were measured in the anoxic monimolimnion, while oxygen was detected only in the first meter of the water column even when the halocline was located deeper (Supplementary Fig. 1). Unfortunately, due to logistical and financial constraints, the number of sampling campaigns and measured physicochemical parameters decreased after 2017, precluding for redundancy analyses. Nonetheless, available data documented a drift of the environmental conditions since 2017 with a progressive decline of the salinity in the monimolimnion, an increase of the oxygen penetration and a strong depletion of the sulfide and methane content (Supplementary Fig. 1).

8-years survey of the lake microbial abundance

Microbial (non-photosynthetic cells) and pico-eukaryote abundances in Lake Dziani Dzaha were estimated from 2014 to 2022 using flow cytometry (Fig. 1). To avoid clogging of the cytometer, cell counts were carried out on prefiltered samples (20 µm mesh filters) and therefore excluded most of the filamentous *Limnospira* cells and large particles. Counting of *Limnospira* cells on raw water samples was also performed to complete the microbial abundance survey (Fig. 1). For each sampling campaign, the number of cells per mL of lake water was relatively stable throughout the water column but increased by an average of 3.7 times at the water-sediment interface (17 m) for prokaryotic cells while decreasing by 3.8 and 4.1 times below 14 m for pico-eukaryotic and *Limnospira* cells respectively. From Apr. 2014 to Nov. 2015, prokaryotic (excluding *Limnospira*), pico-eukaryotic and *Limnospira* abundances throughout the water column averaged at $1.78 \pm 0.2 \times 10^8$, $1.16 \pm 0.7 \times 10^6$ and $1.4 \pm 0.9 \times 10^6$ cells per mL of lake water, respectively. In 2017, prokaryotic abundance was an order of magnitude lower (1.59 ± 0. 57

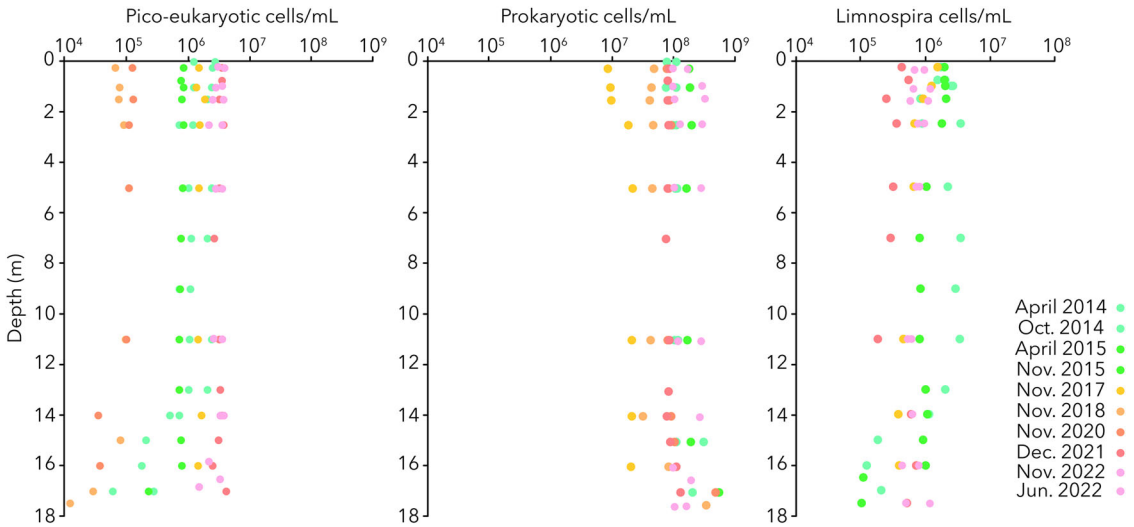

Fig. 1 | Interannual abundance of microbial cells throughout the water column of Lake Dziani Dzaha. Number of pico-eukaryotic and prokaryotic cells per mL of lake water was determined by flow cytometry on prefiltered samples excluding *Limnospira* cells. Number of *Limnospira* cells was estimated on raw water subsamples by microscopy following Utermöhl method for phytoplankton counting. Abundance scale is logarithmic. Samples collected before the seismic events are colored in green, whereas samples corresponding to the seismic event are in yellow and post event samples in red/purple.

$\times 10^7$ cells per mL, one-way ANOVA, $p < 0.01$), then progressively increased from 2018 to 2021 until reaching the initial level of abundance, around 1 $\times 10^8$ cells per mL. The number of *Limnospira* cells above 13 m also decreased in 2017 by a factor two (7.21×10^5 cells per mL, one-way analysis of variance - ANOVA, $p < 0.04$) and remained low in 2021 with $3.4 \pm 1.2 \times 10^5$ cells per mL before a slight increase in 2022 without reaching 2014–2015 levels. By contrast, a significant drop of pico-eukaryote abundance was measured in 2018 and 2020, with an average of $8.6 \pm 2.8 \times 10^4$ cells per mL of water (one-way ANOVA, $p < 0.001$), before a complete return to the initial abundance by 2021.

Microbial community structure shifts over time

Archaeal, bacterial and microeukaryotic communities of the lake were characterized by high-throughput sequencing of ribosomal RNA genes. Our sampling strategy, that extended from 2014 to 2022, investigated a minimum of 7 different depths (0.25 m, 1 m, 2.5 m, 5 m, 11 m, 15 m and 17 m – max. depth of the lake: 17.25 m) per sampling campaign and distinguished the "large" (>3 µm) and "small" (3–0.2 µm) size fractions, leading to a total of 163 samples (Fig. 2).

As expected based on previous reports[17,23], microeukaryotic community composition was strongly dominated by a single operational taxonomic unit (OTU) related to the phototrophic green algae *Picocystis salinarum*, that represented the large majority of the 18S rRNA sequences in both large (24–99% of the 18S rRNA gene sequences) and small (74–99% of the 18S rRNA gene sequences) fractions, at each depth and throughout the 8 years of survey (Fig. 2). Other protists, detected in minor proportions, included various zooplankton species affiliated to marine ciliates (e.g., *Hypotrichia*, *Lacrymaria*, *Cyclidium*) and bacterivorous zooflagellates (*Jakoba*) and biflagellates (*Planomonas*).

Regarding bacterial community composition, while most of the bacterial sequences recovered from the large size fraction were related to a single OTU affiliated to the filamentous cyanobacterium *Limnospira platensis*, the small size fraction harbored a large panoply of bacteria that differed depending on depth, season and years (Fig. 2). In 2014 and 2015, bacterial community assemblages depended on stratification of the water column, that is strongly influenced by the balance between precipitation and evaporation of the dry and wet seasons. This vertical stratification of the bacterial community was particularly visible in 2014 and 2015 and to a lesser extent in 2020 on both large and small fractions and clustered monimolimnion samples together on NMDS and compositional analyses (Fig. 3 and Supplementary Figs. 2 and 3). SIMPER analysis, based on the rarefied OTU table, indicated that dissimilarity between monimolimnion samples and upper oxygenated samples of 2014 and 2015 sampling periods (average Bray–Curtis dissimilarity: 66.16%, PERMANOVA, $p = 0{,}0001$) resulted from differential proportions of *Rhodobacteraceae* (15.63% contribution to the dissimilarity) and *Nitriliruptoraceae* (7.2%), that are detected in larger proportion in the upper water layer and *Dethiobacteraceae* (23.5%) and *Bacteroidetes* clades of ML635J (19.6%), *Balneolaceae* (4.9%) and *Sparospiraceae* (4.3%), that are found in the anoxic and sulfidic monimolimnion. For the first time, no stratification of bacterial community was detected in Nov. 2017 and the community was significantly different from previous years (PERMANOVA, $p < 0.0001$). The bacterial community was stable throughout the depth with predominant *Limnospira platensis* in the large size fraction and high proportions of typical surface lineages *Nitriliruptoraceae* (57.6% of the bacterial community) and *Rhodobacteraceae* (14.9%) in the small size fraction.

However, analysis at the OTU level, revealed that the bacterial community experienced a large turnover (dissimilarity rate: −0.2 per year) with a change of more than half of the OTUs (110 OTUs) between 2015 and 2017, including the loss of 70 OTUs (Fig. 4). For comparison, the number of changes in OTU composition between 2015 and 2017 was approximatively twice as the number of seasonal changes observed in 2014 and 2015. This changes of the bacterial community composition in 2017 was associated by a strong decrease of Simpson and Shannon alpha-diversity indexes at the OTU level (pairwise Mann–Whitney tests, $p < 0{,}002$, Supplementary Fig. 4), indicating a major perturbation of the community richness. This remodeling of the community, also detectable on NMDS and compositional analyses (Fig. 3 and Supplementary Figs. 2 and 3), continued in 2018 with 54 novel OTUs emerging from below the detection threshold. The bacterial community was composed of a mix of lineages previously detected in the monimolimnion and surface waters, with equivalent proportions of *Dethiobacteraceae* and *Bacteroidetes versus Nitriliruptoraceae* and *Rhodobacteraceae*. After 2018, while the vertical stratification of the bacterial community was slightly detectable at the water/sediment interface, the bacterial community composition in the water column diverged with time and increasing oxygen penetration (Fig. 3) and was composed of equivalent proportions of *Microbacteriaceae*, *Nitriliruptoraceae*, *Rhodobacteraceae*, *Paracaedibacteraceae*, *Thiotrichaceae*, *Saprospiraceae*, *Balneolaceae*, *Lentimicrobiaceae* and increasing proportions of *Anaerolineae* and *Patescibacteria* (Fig. 2).

Archaeal community was mainly composed of *Woesearchaeota*, *Thermoplasmata* and various methanogenic archaea such as members of *Methanomicrobiales*, *Methanocalculus* and *Methanofastidiosales* lineages (Fig. 2). As observed for bacteria, the archaeal community was seasonally stratified in 2014 and 2015, in both large and small size fractions, with members of the *Methanofastidiosales* and *Woesearchaeota* in upper water layers (together explaining 46.7% of the dissimilarity between surface and monimolimnion samples, PERMANOVA, $p = 0{,}0012$) and increasing proportion of *Thermoplasmata* and *Methanomicrobiales* in the monimolimnion (Figs. 2 and 3). Stratification was lost in 2017 and while the community was still dominated by members of *Woesearchaeota*, *Thermoplasmata* and *Halobacteriales* more than 80% of the archaeal OTUs changed (Fig. 4). In 2018 and 2020, the archaeal community was enriched in *Methanofastidiosales* throughout the water column, representing up to 55% of the archaeal 16S rRNA genes in the large size fraction of Nov. 2018, while *Thermoplasmata* proportion strongly decreased. In 2021 and 2022, number of changes in the archaeal OTU composition decreased and the proportion of *Methanofastidiosales* declined to the benefit of *Methanomicrobiales* and *Woesearchaeota* lineages.

Discussion

In this study, we build on previous work that characterized the seasonal and vertical fluctuations of the microbial community of the lake in 2014 and 2015[20,27] and extended our analyses until 2022. This interannual monitoring of Lake Dziani Dzaha, covered the volcano-seismic crisis that occurred in the region in 2018, providing a unique opportunity to investigate the effect of geological events and their cascading aftereffects on environmental conditions on natural microbiome structure. Geochemical, abundance and microbial (bacterial, archaeal, and eukaryotic) community composition data allowed us to propose a putative chrono-sequence of changes occurring with the increasing seismicity of the region. Before 2017, the microbial community followed a stable seasonal pattern with a strong vertical stratification of the microbial processes[27]. A major shift was detected in the chemistry, OTU composition and evenness and microbial abundance and community structure in Nov. 2017. This particular date witnessed the lowest prokaryote abundances, suggesting an important microbial mortality, as well as a maximum in composition dissimilarity for bacterial communities (smaller fraction) compared to 2015. Other changes include an increase in the O_2 penetration depth, a strong depletion of sulfides in the water column and a decrease of temperature and salinity throughout the water column (Supplementary Fig. 1), leading to the absence of stratification of the microbial community and a significant lost of microbial richness (Supplementary Fig. 4) with a bloom of *Nitriliruptoraceae* lineages in the small size fraction (3–0.2 µm). Genomic data available for *Nitriliruptoraceae* recovered from soda lake sediments reported a sulfide oxidation-based metabolism for theses lineages[21], potentially explaining the observed sulfide depletion. While no seismic events were registered in the region before May 2018, our observations suggest that major changes were already occurring in the lake as early as Nov. 2017. Based on the disappearance of the deep chemocline (e.g. deep water layer of high H_2S, CH_4 and NH_4 concentrations

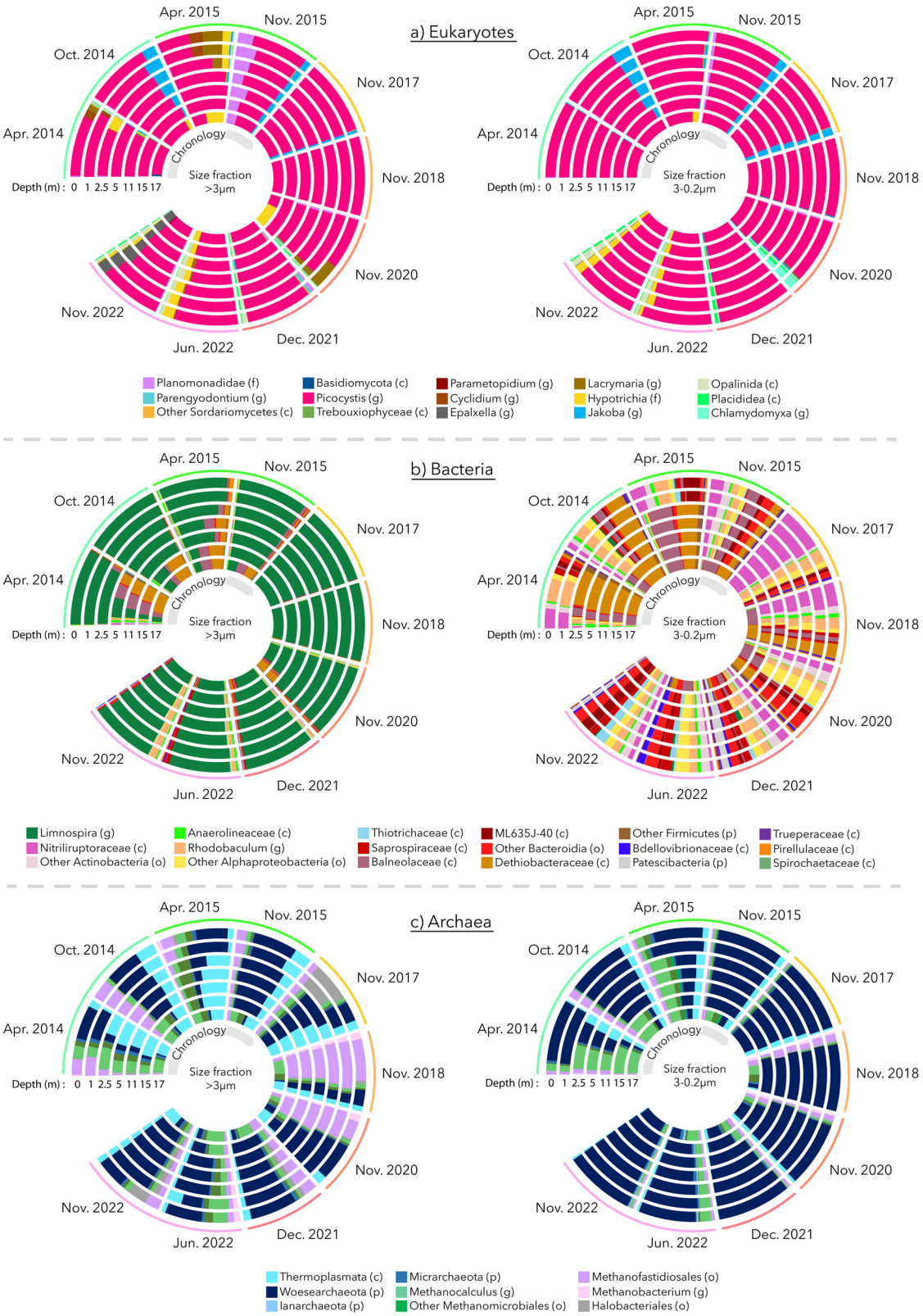

Fig. 2 | Interannual microbial community composition. a Microeukaryotic, **b** bacterial and **c** archaeal community structure in Lake Dziani Dzaha. Left graphs present the communities identified in >3 µm size fraction while right graphs show the communities detected in the 3–0.2 µm size fraction. Results are based on the relative proportions of 18S and 16S rRNA gene sequences. Apr. April, Oct. October, Nov. November, Dec. December, Jun. June. For Archaea, blue, green and purple colors indicate members of the DPANN superphylum, *Halobacterota* and *Euryarchaeota* phyla respectively. Letters in brackets indicate the taxonomic rank: p: phylum, o: order, c: class and g: genus.

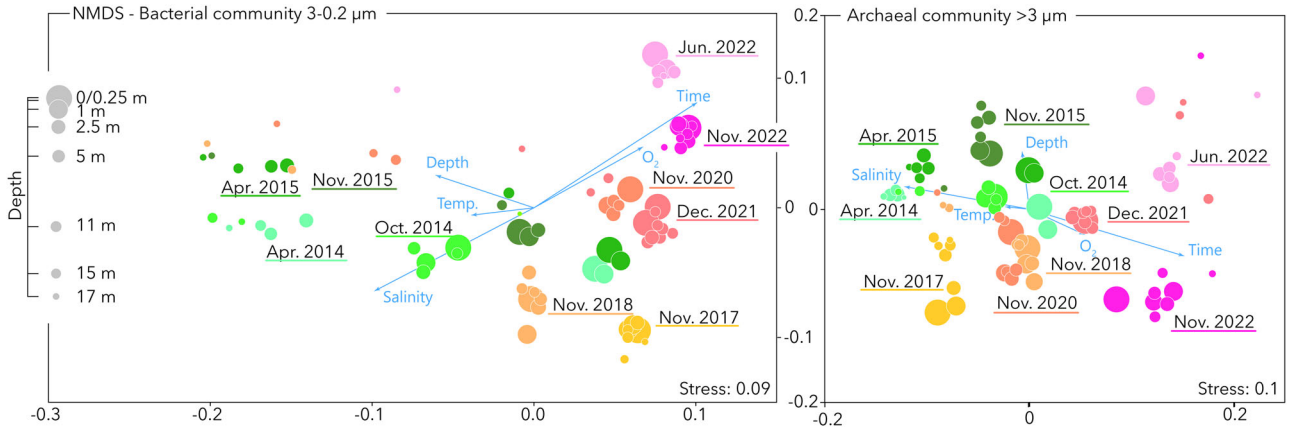

Fig. 3 | Non-Metric Multidimensional Scaling (NMDS) of the bacterial and archaeal community. NMDS were calculated based on normalized OTU tables. Samples were colored by sampling campaign as in Fig. 2 and size of the dots is related to the sampling depth. Correlation coefficients between each environmental variable and the NMDS scores are presented as blue vectors from the origin. Lengths of the vectors are scaled to the correlation coefficients. Time represents the number of months between each sampling time and the day of the major earthquake (May 18, 2018). NMDS of the bacterial large fraction (>3 µm) and archaeal small fraction (3–0.2 µm) were uninformative (Stress >0.15) due to the overrepresentation of *Limnospira fusiformis* and *Woesearchaeota* in the respective datasets and are therefore not presented but are available in Supplementary Fig. 2. Compositional data analyses (PCA plots based on centered logratio (CLR) OTU tables) are also presented in Supplementary Fig. 3.

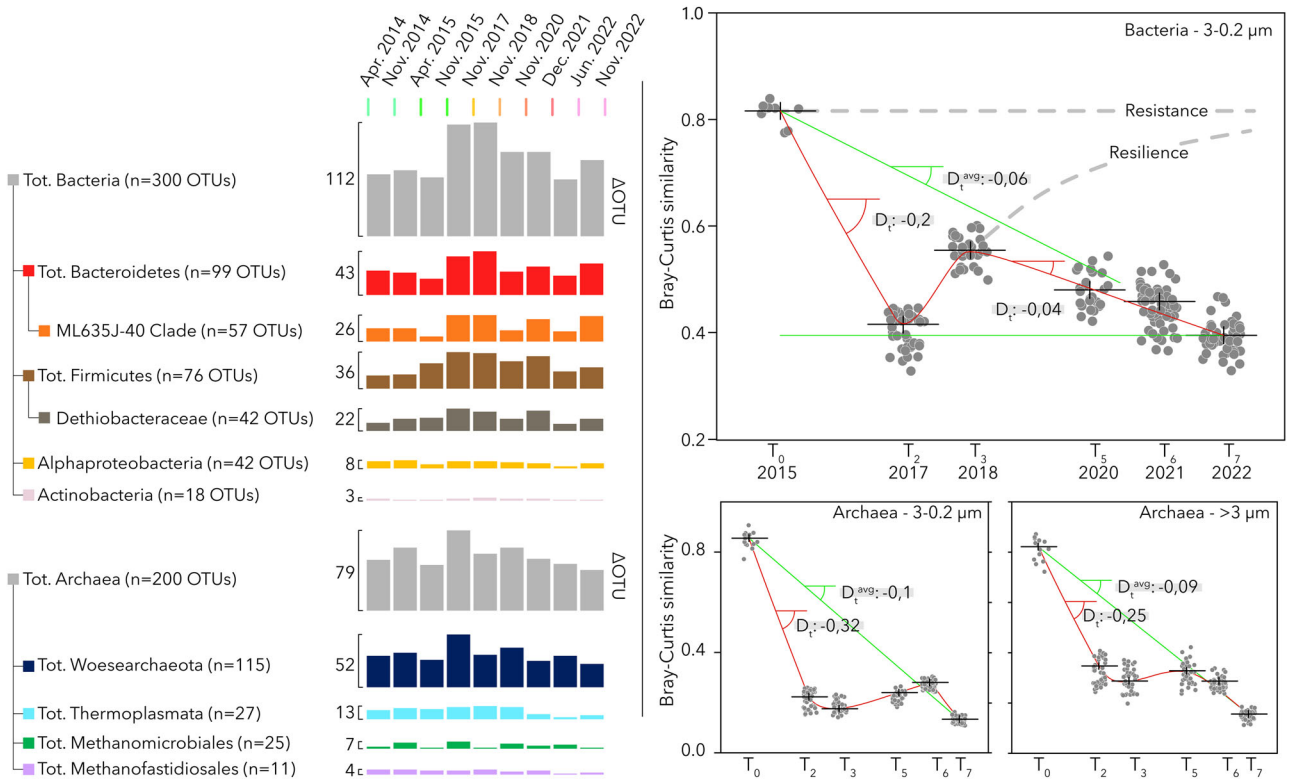

Fig. 4 | Overall change in microbial community composition per year. The left panel shows the delta OTU (disappearance or detection of a novel OTU from between two consecutive years) for dominant bacterial and archaeal groups in >3 and 3−0.2 µm pore-size filters combined. The right panel shows the Bray–Curtis dissimilarity rate of the bacterial and archaeal community over time. For dissimilarity rate analyses, only samples collected in the upper water layer and during the same season (October, November and December) were included. Each gray dots indicates Bray–Curtis similarity values calculated from pairwise comparisons between 2015 samples and subsequent years and average values are represented by black horizontal lines. Green line represents the average dissimilarity rate between community composition identified from 2015 to 2022 with the coefficient of the line D_t indicating the Bray–Curtis dissimilarity rate per year. The red line represents the changes of the dissimilarity rate between 2015 community composition and all subsequent years. Dashed gray lines represent theoretical resistance/stability and resilience patterns.

observed in Oct. 2014 and Nov. 2015) and on the changes in lake biochemistry and microbiome, this perturbation likely involved an unprecedent complete mixing and oxygenation of the water column. Although the contribution of meteorological forcing in mixing process at the bottom of the lake has not been quantified yet, the morphology of the crater that forms a steep pit at the sampling point likely limits its action. However, bubbling, that has been observed in the lake since 2012, has strongly intensified following the geologic crisis as a probable consequence of magmatic fluid

migration. Monitoring of O_2 concentration in the water column at bubbling points revealed that this effervescence, composed of CO_2 of magmatic origin generates a convective plume in the water column, convoying oxygen from the surface to deep waters[30]. Therefore, we hypothesize that the major perturbation of the lake microbiome and biochemistry is likely to be due to an undetected pre-2018 seismicity that increased gas release in the water column. Although a larger scale analysis is required, our results might suggest that aquatic microbiomes could be early sentinels of geologic disturbances, that a monitoring of the microbial abundance (e.g. number of cells per mL) would have detected. The identification in 2018 of a bacterial community composed of a mix of deep and surface dominant lineages, as well as the increase of the relative proportion of potential methanogens (*Methanofastidiosales*) in the large size fraction is consistent with rapid and frequent shifts between oxic and anoxic conditions as well as increased migration of anoxic sediments/particles in the water column, triggered by a higher bubbling associated with seismicity. Likewise, the depth distribution of primary producers is consistent with a well-mixed environment where green-algae are more competitive than filamentous bloom-forming cyanobacteria[31]. Previous experiment applying an artificial oxygenation pulse on stratified lake, demonstrated a rapid recovery of the microbial community[32]. By contrast, the further development of a (micro) aerobic complex and original community, concomitantly with the recurrent detection of oxygen down the water column, suggest that the seismic crisis generated novel environmental conditions leading after 2020 to a new persisting ecological state that overtook the seasonal pattern of the ecosystem as revealed by the June 2022 samples. The repetition of stresses of seismic origin (e.g. frequent episodes of mixing and oxygenation associated with bubbling) did not allow a recovery of the microbial community to its original state, suggesting that it applies a long-lasting "press" disturbance on the ecosystem, resulting in a continued response of the microbial community[33]. Our results suggest that, in addition to its intensity or direction, the frequency of the disturbance could also be a strong controlling factor shaping the ecological response of an ecosystem.

Stability of an ecosystem is its capacity to return at pre-disturbance state after or during a perturbation and combines resistance and resilience processes[13]. Due to logistical constraints, experimental studies typically focus on short-term return to pre-perturbation state while theoretical works (mathematical modeling) study long-term recovery[34]. With these contrasted time scales, experimental validations of theoretical conclusions of ecosystems' responses to perturbation remain rare and the integration of theoretical and empirical approaches has been identified as one of the main challenges for ecological stability studies[35]. The seismic crisis experienced in the surrounding environment of Lake Dziani Dzaha offers a rare opportunity to explore ecosystem stability and resilience during and after a geological perturbation. While the frequency, amplitude and direction of the perturbation did not allow a return to original state, we observed several patterns predicted by theoretical ecology[35]. First, immediately and after one year following the seismic crisis, that we putatively positioned in time when the degassing and bubbling intensified in the water column during 2017, major changes were associated with the dominant species (*Nitriliruptoraceae*, *Rhodobacteraceae*, *Dethiobacter*, *Bacteroidetes*), that led to a drastic shift in OTU evenness (Supplementary Fig. 4), supporting that short-term response to perturbation is dependant to abundant species[35]. This alteration of the dominant species had strong repercussion on the microbial abundance of the system, suggesting increased cell mortality due to disturbed conditions. Secondly, after three years following the crisis (2020), the non-phototrophic bacterial community has assembled from members of *Microbacteriaceae*, *Paracaedibacteraceae*, *Thiotrichaceae*, *Saprospiraceae*, *Bdelovibrionaceae*, *Lentimicrobiaceae*, *Anaerolineae* and *Patescibacteria* that were very little or not detected before the crisis (Fig. 2), with a strong remodeling of bacterial and archaeal OTUs (Fig. 4) and a complete recovery of the evenness of the community (Supplementary Fig. 4). This supports that long term response of microbial community can be attributed to minority, rare or dormant lineages, that have been awaken and may benefit from long-term changes in ecosystem characteristics. This result, theoretically explained by the independency of long-term recovery process with the initial perturbation, could for example be related to the recurrent oxygenation of the water column in Lake Dziani Dzaha that selects aerobic lineages with sulfur-oxidation potential (*Thiotrichaceae*)[36], algicidal (*Saprospiraceae*, *Microbacteriaceae*)[37,38] and bacterivorous metabolism (*Bdelovibrionaceae*)[39] or oxygen resistant fermenters (*Lentimicrobiaceae*, *Patescibacteria*)[40]. The volcano-seismic sequence that occurred in Mayotte provided a unique natural experiment, and our findings support the hypothesis that distinct sets of taxa determine the short-term and long-term dynamic of ecosystem after perturbation[35] and extend these projections to major perturbations that move ecosystem community structure away from their initial state into alternative stable states. The new ecological state of Lake Dziani Dzaha is characterized by similar evenness than before the perturbation (Supplementary Fig. 4), suggesting that although the community composition shifted, its complexity returned to the initial state. Interestingly, while the evenness recovered, the overall bacterial diversity (iChaO) of the lake decreased, from an average of 169.6 ± 36 before 2017 to 118.9 ± 23 since 2020 (Pairwise Mann–Whitney tests, $p < 0{,}04$). This suggest that the reduction of environmental constraints (e.g., high sulfide concentration, high salinity, anoxia) allows a larger equitability in the community composition with increasing proportions of interactive metabolisms as illustrated by the detection of potential host-dependant lineages of *Paracaedibacteraceae*[41] and *Patescibacteria*[42,43]. The decrease of OTU diversity could be associated with the homogenisation of the lake environmental conditions (e.g. one environment with both aerobic and anaerobic niches before perturbation vs one micro-aerobic environment after the disturbance). Large diversity and evenness are typically associated with more robust and resilient ecosystems[11,13], therefore while perturbations can be negatively seen, they could also provide a long term positive priming effect by enhancing resistance and resilience of ecosystems through the development of a richer community from the microbial seed bank, as observed after other perturbations[44,45].

Interestingly, we observed in NMDS and compositional analyses that in 2018 and to a lesser extend in 2020 and 2022, the bacterial community composition at the bottom layer of the lake partially recovered, with predominant *Balneolaceae* and *Firmicutes* as detected in 2014 and 2015. These results suggest that the microbial community of the bottom waters, that are likely to return more rapidly under anoxia after mixing events (Supplementary Fig. 1), might be more resilient to the disturbance. This partial recovery might be favored by incomplete mixing events due to a lower bubbling activity and therefore supports that both frequency and intensity of the disturbance shape the microbial community response.

Although our results confirm that microbial ecosystems are overall sensitive to perturbations, the same OTUs of the photosynthetic eukaryote *Picocystis salinarum* and cyanobacterium *Limnospira platensis* remained strongly dominant throughout the survey, suggesting that the main primary producers of Lake Dziani Dzaha were resistant to the perturbation, and highlighting different sensitivities depending on the species. Nonetheless, cells counting revealed a decrease of *Limnospira* abundance since 2017, suggesting that although the original single *Limnospira* species remain predominant, its population size has been affected by the perturbation. This decline could be explained by the sensitivity of filamentous bloom-forming cyanobacteria to strong turbulency[31]. Likewise, cytometry revealed that *Picocystis* abundance was significantly reduced in 2018 and 2020, highlighting a stress on their population size. This observation has been detected one year after the prokaryotic abundance crash, highlighting a better resistance to the disturbance. This lag phase could be explained by a secondary effect of the bacterial community remodeling such as by modifications of their phycosphere composition[25] or the predator community. Finally, the better recovery of *Picocystis* population size observed at the end of our survey compared to *Limnospira* might be explained by the better fitness of green algae over cyanobacteria in well-mixed systems[31].

By its isotopic signatures, and original geochemistry, Lake Dziani Dzaha was considered as a modern analog of some Proterozoic ecosystems[19], allowing the investigation of the ecology and evolution of

potentially primitive microbiomes. While keystone species (i.e., primary producers) were remarkably resistant and/or resilient, our results demonstrated that the volcano-seismic crisis that occurred in the Comoros archipelago, 50 km away from the lake, drove the microbial community of this system to an alternative ecological state with increased species evenness and shifts in non-phototrophic taxa composition as a result of seismic-induced alteration of the lake environmental conditions. Based on these results we hypothesise that at geological time scales, earthquake and seismic crises could have been major drivers of the microbial community structure in early aquatic environments, possibly leading to their overall diversification of microbial communities.

Methods

Study site

Lake Dziani Dzaha is a volcanic crater lake located on the Petite Terre Island of Mayotte in the Comoros archipelago (latitude 12°46.237'S, longitude 45°17.315'E). The lake formed after a phreatomagmatic eruption that occurred between 7000-4000 years ago, imprisoning oceanic waters which evolved with time into a warm hypersaline and hyperalkaline environment. The lake has a surface area of 0.236 km^2 and a maximum depth of 18 m in the central area of the crater[16], where samples were collected. Geochemical environment of the lake since 2007 has been previously characterized[16,17,29] and is summarized in Supplementary Fig. 1. Briefly, temperature, salinity and O_2 concentration were measured in situ using a YSI 6600 probe that has been lowered in the water. For SO_4^{2-}, H_2S, CH_4 and NH_4, water from Niskin bottles has been subsampled and conditioned/poisoned for off-site measurements as described[16,17,29].

Sample collection and nucleic acid extraction

Samples were collected during ten sampling campaigns from 2014 to 2022 (Apr. 2014, Nov. 2014, Apr. 2015, Nov. 2015, Nov. 2017, Nov. 2018, Nov. 2020, Dec. 2021, Jun. 2022 and Nov. 2022), spanning the seismic crisis of the region that mainly occurred mid 2018. 1.2 liter of water was collected at seven different depths (0.25 m, 1 m, 2.5 m, 5 m, 11 m, 15 m and 17 m) from the center of the crater using a horizontal Niskin bottle. Additional depths were also sampled depending on the sampling campaigns for a total of 81 water samples. For each sample, 30 to 50 mL of water were filtered through 3 µm pore-size polycarbonate filters (Merck Millipore, Burlington, MS, USA) to collect the large-size fraction of the microbial community then subsequently through 0.2 µm pore-size polycarbonate filters to recover the small-size fraction. Filters were then stored at −20 °C until nucleic acid extractions.

Nucleic acids were extracted from all filters (3 µm and 0.2 µm) using Power Water DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) or ZymoBIOMICS DNA Miniprep kit (Zymo Research, Freiburg, Germany) according to the manufacturer's recommendation. For both kits, lysis of the bacterial cells was based on similar procedure (bead beating) and affinity columns, allowing comparison of the microbial community composition.

PCR amplification of ribosomal RNA genes, sequencing and analysis

Eukaryotic, bacterial and archaeal community composition of the samples was determined by high throughput sequencing, using three distinct primer sets targeting the V4 region of the 18S rRNA-encoding gene (Euk515F-Euk915R) for microbial eukaryotes, the V3-V5 region of the 16S rRNA gene for Bacteria (S-D-Bact-0343-a-S-15/S-D-Bact-0907-a-A-19) and the V4 region of the 16S rRNA gene for Archaea (S-D-Arch-0519-a-S-15/S-D-Arch-0911-a-A-20)[46], with Illumina adapters fused to the 5' end. Amplifications were carried out in triplicates then PCR products were pooled and purified. PCR conditions, amplification product purification and library preparation were previously detailed[20]. Negative controls of DNA extraction and PCRs were included as samples in sequencing libraries. Amplicons were sequenced using an Illumina HiSeq 2500 rapid run (2*300 bp) or Illumina MiSeq runs (2*300 bp). Reads were assembled into single paired-end sequences using PEAR v.0.9.8[47], curated and clustered into operational taxonomic units (OTUs) using SWARM v.3[48] with a local clustering threshold of 3 bases. Chimeric sequences and singletons were removed using VSEARCH[49] and taxonomic classification was performed by BLASTn algorithm against Silva v.138 database[50]. This procedure was automated in the FROGS pipeline[51]. Contaminant sequences, identified in the control samples were removed from the dataset as well as sequences affiliated to untargeted kingdom (ex. Bacteria for 18S rRNA gene sequencing), chloroplast and mitochondria. For comparison, datasets were randomly rarefied to the lowest number of sequences per sample (8981, 1498 and 763 for micro-eukaryotes, bacteria and archaea respectively) before statistical analyses (Non-Metric Multidimensional Scaling NMDS, SIMilarity PERcentage SIMPER analyses, PERMANOVA, pairwise Mann–Whitney tests and alpha and beta diversity indexes). Bray–Curtis (dis)similarity indices were also calculated on normalized OTU tables. Explanatory power of environmental variables (temperature, salinity, depth and oxygen profiles as well as "time", defined as the number of months between sampling periods and May 2018) in structuring microbial communities, were added in NMDS as vectors with length scaled to the correlation coefficients between each environmental variable and the NMDS scores. In addition, compositional data analyses were performed on the OTU datasets. OTU tables were transformed by center logratio (CLR) then variance based PCA plots with 100 bootstraps were performed for the ordination of the samples. Aitchison indexes were also calculated to explore (dis)similarity between samples. Statistical analyses and ordinations were performed using Past v.4[52] software following recommendations from the GUide to STatistical Analysis in Microbial Ecology (GUSTA ME)[53].

Estimation of microbial abundance by flow cytometry and microscopy

For each sampling campaign, discrete water samples (1.6 mL) were collected at each depth for cell enumeration using flow cytometry. Samples were preserved using filtered formaldehyde (2% final concentration) then frozen in liquid nitrogen before storage at −80 °C until processing. To avoid clogging of the cytometer, water samples were prefiltered on 20 µm pore-size filters, discarding *Limospira* cells and large aggregates from cell counts. Prokaryotic cells were stained using SYBR-green I and quantified using a FACSAria flow cytometer (Becton Dickinson, San Jose, CA, USA) based on SYBR-green fluorescence and forward-scattered light. Pico-eukaryotic cells were distinguished using phycocyanin and chlorophyll fluorescence as previously detailed[23]. In addition, *Limnospira* cells were counted using an inverted microscope following standardized Utermöhl method for the enumeration of phytoplankton.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Raw sequence data are available on NCBI under bioprojects ID PRJNA1042917 and PRJNA1043039 for bacterial and archaeal 16S rRNA gene sequences respectively and PRJNA1043056 for eukaryotic 18S rRNA reads. Source data (OTU tables) are available in Figshare using https://doi.org/10.6084/m9.figshare.26097094.

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Author contributions

A.V. designed the study, analyzed the data and wrote the manuscript. L.A.C. contributed to samples collection and data production and revised the manuscript. H.A., C.B., C.L. collected fundings and contributed to samples collection and manuscript discussion and revision. D.J., A.G. and M.A. contributed to fundings, collected samples, produced data and discussed the manuscript. P.G., C.C. and C.R. produced data. Additional fundings were provided by M.T. and S.D. also collected and conditioned samples. P.M.O. discussed and revised the manuscript. M.H. acquired fundings, designed the study, collected samples, produced data and contributed to data analysis and manuscript discussion and writing.

Competing interests

The authors declare no competing interests.

Additional information

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