

Volcanic ash stimulates growth of marine autotrophic and heterotrophic microorganisms

Rui Zhang^{1,2*}, Tao Jiang^{1,2}, Yuan Tian^{1,2}, Shucheng Xie³, Lian Zhou³, Qian Li^{1,2}, and Nianzhi Jiao^{1,2*}

¹State Key Laboratory of Marine Environmental Sciences, Xiamen University, Xiamen, Fujian, 361102, China

²Institute of Marine Microbes and Ecospheres, Xiamen University, Xiamen 361102, Fujian, China

³State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan 430074, China

ABSTRACT

Volcanic eruptions are considered to be some of the most important events affecting local and global ecosystems and climate. An increasing body of research has been concerned with discerning the influence of volcanic ash on marine ecosystems. However, studies on the responses of autotrophic and heterotrophic microorganisms to volcanic ash are rare. In this study we conducted two microcosm experiments in the low-nutrient and low-chlorophyll western Pacific Ocean and found that volcanic ash first stimulated the abundance of heterotrophic bacterioplankton, followed by phytoplankton bloom that included both picoeukaryotes and larger eukaryotes such as diatoms. Using terminal restriction fragment length polymorphism and clone library analyses, we observed an altered bacterial diversity and community structure with volcanic ash addition. Our study showed that volcanic ash affects the community composition of both heterotrophic bacterioplankton and phytoplankton in the surface ocean. These results elucidate the overlooked impacts of natural volcanic eruption events on microbial communities, which play important ecological and biogeochemical roles in the marine ecosystem.

INTRODUCTION

Volcanic eruptions are thought to be some of the most important events affecting local and global ecosystems and climate now and in Earth history (Robock, 2000). Several studies have highlighted the link between global cooling and massive volcanic eruptions (e.g., Bains et al., 2000). When the ash reaches the surface ocean, both macronutrients and micronutrients are injected into the water, possibly causing significant phytoplankton bloom, especially in nutrient-limited areas (Duggen et al., 2010). Consequently, atmospheric CO₂ can be reduced by the massive stimulation of marine primary productivity (Morel and Price, 2003). In recent years, the potential of volcanic ash as fertilizer resources of nutrients such as P, Si, N, and Fe for phytoplankton has been studied in several ocean habitats (Duggen et al., 2010).

Bacterioplankton play an important role in biogeochemical cycling in the ocean because of their high abundance and high genetic and metabolic diversity. They are also known to be closely associated with eukaryotic phytoplankton such as diatoms, providing vital nutrients that cannot be synthesized directly by phytoplankton (Amin et al., 2012). In Jiao et al. (2010) it was suggested that bacterioplankton can transform labile dissolved organic carbon (DOC) to refractory DOC through the microbial carbon pump (MCP) mechanism. This may be an important mechanism for the formation of a large dissolved organic matter (DOM) pool from Neoproterozoic Cryogenian to Ediacaran oceans, in which the biological pump can be ignored due to the absence of multicellular organisms (Ridgwell, 2011). Although bacterioplankton play a major role in the modern and historical ocean, their response to volcanic eruptions remains poorly

understood. In this study two microcosm experiments were conducted to determine how volcanic ash addition affects both heterotrophic bacterioplankton and phytoplankton in the oligotrophic western Pacific Ocean. To our knowledge, this is the first attempt to elucidate the response of the ecologically and biogeochemically important marine heterotrophic bacterioplankton to volcanic ash.

METHODS

Volcanic ash was sampled near the Eyjafjallajökull volcano in Iceland. Two volcanic ash addition experiments were conducted with surface seawater (5 m) collected using Niskin bottles attached to a Sea-Bird CTD (conductivity, temperature, depth) profiler in the western Pacific Ocean. Previous studies have shown that the chemical composition of volcanic ash from Iceland (including Eyjafjallajökull) and the Pacific rim (such as Sakura-jima, Japan) are similar (Jones and Gislason, 2008; Gislason et al., 2011). The volcanic ash leachate was prepared by adding 50 g volcanic ash into 20 L of seawater from which the macroorganisms and microorganisms, including viruses, had been removed by filtering through a 30 KD tangential filtration membrane package (Millipore USA). After 12 h of leaching, the suspension was filtered through 3 μm cellulose acetate membranes to remove large volcanic ash particles. The seawater for microcosm incubation was first filtered with a 20 μm prefilter and then dispensed into four 20 L polycarbonate bottles. The ash addition treatments were composed of 10 L of *in situ* seawater and 10 L of ash leachate and the control bottles contained 10 L of *in situ* seawater, and 10 L of 30 KD-filtered *in situ* seawater. All microcosms were incubated in on-deck flowing seawater incubators under ambient light conditions for 5 days. The trace metal concentration was measured by the inductively coupled plasma–mass spectrometry. Nutrients were measured using classic colorimetric methods with a Technicon AA3 AutoAnalyzer. The abundance of planktonic microorganisms (eukaryotes, cyanobacteria, heterotrophic bacteria, and viruses) was determined by flow cytometry. Microbial community structure was revealed by terminal restriction fragment length polymorphism and clone library analyses. The sequences retrieved in our study have been deposited in the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>) under accession numbers KF271011–KF271265 (for additional details, see the GSA Data Repository¹).

RESULTS

The surface water of the two study stations is characterized by low nutrients and low chlorophyll, where the primary production is primarily limited by nitrogen (Li et al., 2015). Volcanic ash leachate addition significantly increased the concentration of nutrients such as nitrate, ammonium, phosphate, and silicate in the ash treatments (Fig. 1; Fig. DR1 in the Data

¹GSA Data Repository item 2017222, methods and experimental setup details, Figures DR1 and DR2, and Tables DR1 and DR2, is available online at <http://www.geosociety.org/datarepository/2017/>, or on request from editing@geosociety.org.

*E-mails: ruizhang@xmu.edu.cn; jiao@xmu.edu.cn

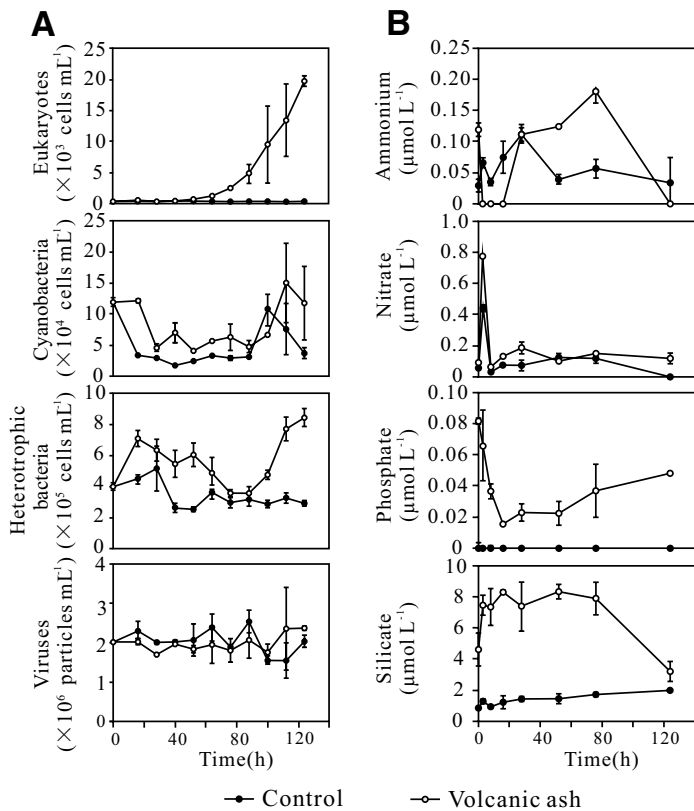


Figure 1. A: Dynamics of microbial planktonic abundance during incubation with volcanic ash manipulation at oligotrophic western Pacific Ocean (station N8–12). B: Dynamics of nutrient concentration.

Repository). During the experiment of station N8–12, ammonium, nitrate, and phosphate introduced by ash addition was consumed rapidly, and the concentration of ammonium and phosphate increased gradually after the initial decrease. Significant consumption of silicate was observed at the end of experiment. The concentration of nutrients showed generally similar and slightly different dynamics between two stations, suggesting that nutrient utilization was affected by differing microbial communities in them. In addition, volcanic ash leachate introduced certain amounts of Fe, Zn, and Cu, and low amounts of Ni, Co, Pb, and Cd (Table DR1). Generally, similar microbial population dynamics and successions were observed at the two stations along the incubation time course (Fig. 1; Fig. DR1). The abundance of heterotrophic bacterioplankton with volcanic ash manipulation increased by approximately twofold within ~20 h in both stations, followed by a steady decrease, while in the controls they remained relatively stable during incubation. Cyanobacteria (*Synechococcus* and *Prochlorococcus*) declined in the first 40 h and the decrease in the controls was more obvious than in the ash treatments (repeated measures multivariate analysis of variance, RM-MANOVA, treatment effect $p < 0.05$). After an ~40–60 h latent period, eukaryotic phytoplankton showed an exponential growth in volcanic ash treatments, whereas they remained stable in the controls (RM-MANOVA treatment effect $p < 0.05$). At the end of the incubation, the abundance of eukaryotes in the ash treatments reached 5.60×10^3 cells/mL and 1.96×10^4 cells/mL, which was 13-fold and 53-fold compared to the control at stations P1 and N8–12, respectively. The response of viral abundance to ash addition was not significant (RM-MANOVA treatment effect $p > 0.05$).

As revealed by terminal restriction fragment length polymorphism (T-RFLP) analysis (Fig. 2; Fig. DR2), the microbial diversity (Shannon index) at the beginning of incubation was 3.36 ± 0.16 , and then decreased significantly to 3.07 ± 0.10 and 2.69 ± 0.29 (two-paired *t*-test, $p < 0.05$) after incubation in the control and volcanic ash treatments, respectively.

The diversity index (Shannon, Chao, ACE, and Simpson indices calculated based on clone library analysis) is consistent with the T-RFLP results. Cluster analysis of T-RFLP fingerprints showed that the microbial community structure of samples from the ash treatments was distinct from that of the control samples. Phylogenetically, all clone libraries were dominated by Proteobacteria, Bacteroidetes, Cyanobacteria, and Bacillariophyta (diatoms). In the Proteobacteria, the percentage of Alphaproteobacteria increased from 46.7% to 58.4% and 59.7% after incubation. Flavobacteriia (Bacteroidetes) were observed only in samples after incubation, while Bacillariophyta were found only in volcanic ash-treated day 5 samples with a high proportion (24.7%). The *in silico* digestion of sequences obtained in the clone library was used to identify the possible phylogenetic affiliation of peaks observed in T-RFLP analysis. There were eight T-RFs that showed significant change after incubation in terms of the percentage of peak area (Fig. 2; Table DR2). Three T-RFs, 56.7 ± 0.5 bp (base pair), 88.5 ± 0.5 bp, and 309.6 ± 0.5 bp, which appeared in ash treatments but were absent in controls, were identified as *Nitzschia* (diatom), *Haslea* (diatom), and *Cellulophaga*, respectively. The Alteromonadaceae and/or Rhodobacteraceae (represented by a T-RF of 129.3 ± 0.5 bp) increased significantly in ash treatments.

DISCUSSION

Volcanic eruptions have long been considered to have significant impacts on marine ecosystems. In this study, flow cytometry, clone library, and DNA fingerprinting analyses showed the rapid succession and interaction of autotrophic and heterotrophic microbial populations with volcanic ash manipulation (Figs. 1 and 2; Figs. DR1 and DR2). Generally enhanced photosynthetic and growth rates of phytoplankton were observed in previous studies following ash enrichment or nutrient addition, but the responses vary among different species (e.g., Hoffmann et al., 2012). We observed a diatom bloom upon volcanic ash enrichment in the highly oligotrophic western Pacific Ocean, dominated with species of *Nitzschia* and *Haslea* belonging to the Bacillariophyta family. Studies from both laboratory cultivation and field bioassay have suggested that diatoms are likely to have considerably higher growth potentials than other algae when nutrient starvation is alleviated (e.g., Duggen et al., 2010). Addition of volcanic ash very likely relieved the nutrient depletion in surface western Pacific waters (Li et al., 2015), indicated in the higher nutrient concentrations (e.g., ammonium, nitrate, silica) than in the controls. Iron is probably not a limiting factor due to its high input rate from continental dust deposition and shelf water (Duce and Tindale, 1991). Increased diatom abundance and phytoplankton blooms are frequently observed in the volcanic ash layer on millennial scales, and the increased marine primary productivity is used to explain many global cooling events in geological history (Cather et al., 2009).

The response of heterotrophic bacterioplankton to volcanic ash has been neglected. In our study, the rapid growth of heterotrophic bacterioplankton and the simultaneous rapid decline in the ammonia concentration (Fig. 1; Fig. DR1) suggested that the inorganic nutrients leached from volcanic ash were first rapidly utilized by heterotrophic bacterioplankton. Bacterial cells usually have larger surface area to volume ratio and higher growth potential compared to phytoplankton (Havskum et al., 2003). Thus, bacterioplankton can compete with phytoplankton once nutrients are introduced by volcanic ash. Subsequently, the eukaryotes begin to grow. This suggests that the mortality of bacterioplankton releases nutrients and promotes phytoplankton growth. The bloom of eukaryotic phytoplankton was accompanied with the consumption of Si. In addition, the commonly found tight interaction between diatom and heterotrophic bacteria (e.g., *Alteromonas*) (Amin et al., 2012) might explain why they bloomed concurrently at the final stage in station N8–12 (Fig. 1).

In addition to their abundance and population succession and interaction, the response of microbial diversity and community structure to volcanic ash was also significant. Addition of volcanic ash reduced the diversity and altered the microbial community structure compared to the

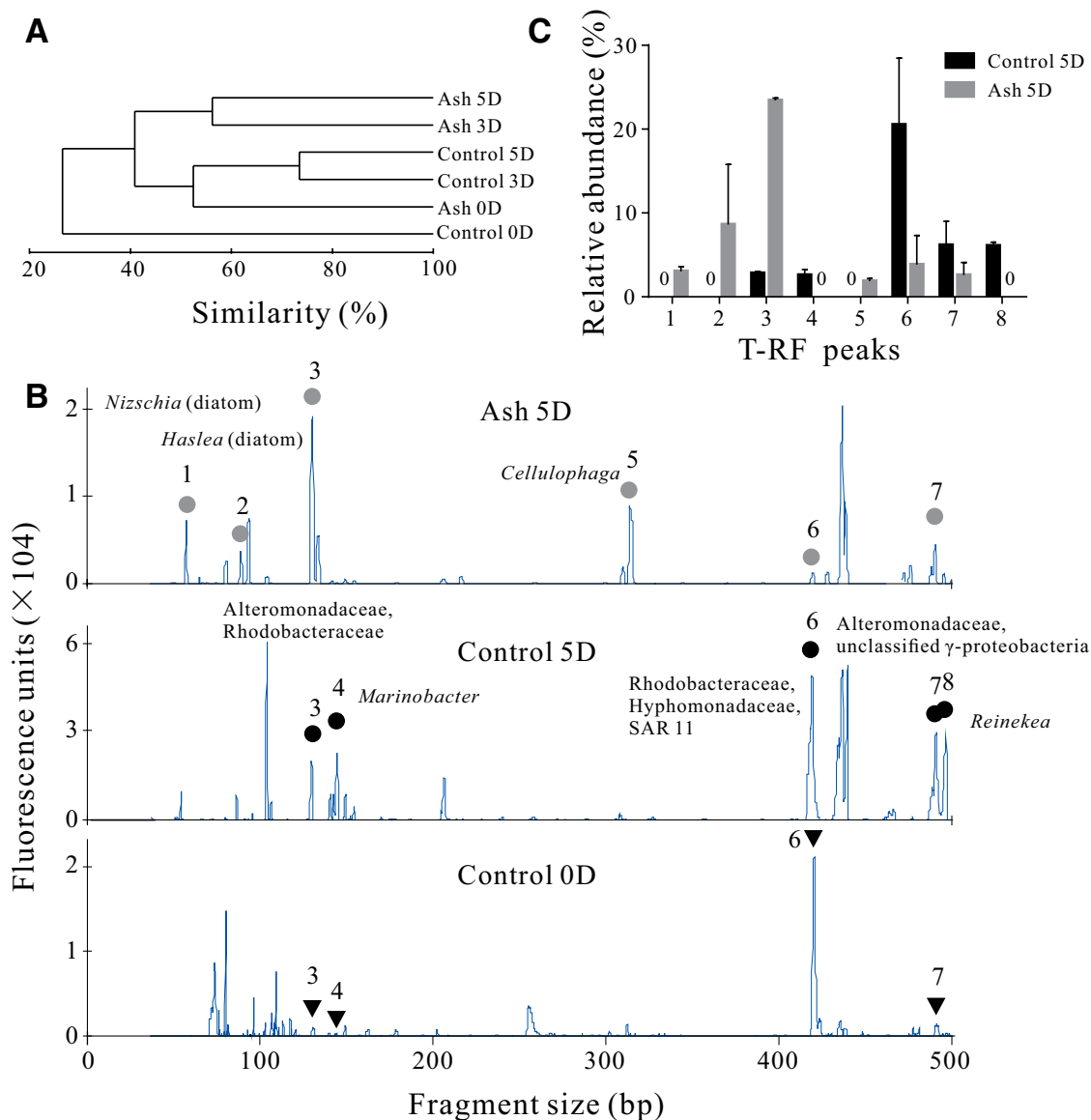


Figure 2. Microbial community structure altered by volcanic ash manipulation. A: The clustering dendrograms based on DNA fingerprinting analysis (terminal restriction fragment length polymorphism, T-RFLP) showing similarity of microbial community structure during 5-day incubation of volcanic ash treatments and control. B: Typical microbial community structure as revealed by T-RFLP analysis of polymerase chain reaction (PCR)-amplified 16S rRNA genes (bp—base pair). Eight peaks affected by volcanic ash manipulation are highlighted on T-RFLP profiling. Microbial groups that may generate these peaks are shown. C: Relative abundance of 8 T-RFLP peaks after 5-day incubation with volcanic ash manipulation.

controls (Fig. 2; Fig. DR2). Certain ecologically and biogeochemically important bacterial groups were possibly stimulated or inhibited by volcanic ash addition (Fig. 2; Table DR2). For example, Rhodobacteraceae and Alteromonadaceae dominated in the ash microcosms, and both are known as opportunistic companions of phytoplankton in using the dissolved organic matter produced by phytoplankton (Pinhassi et al., 2005). However, SAR11, the most abundant bacterial lineage in the modern ocean, is suppressed by volcanic ash addition. The shift in microbial community structure and the different responses of the major microbial lineages, as shown in our study, will lead to changes in the ecological functions of microorganisms. The consequence of this change has been shown in their influence on biogeochemical cycling in the modern ocean and on global climate change.

The most severe mass extinction of marine faunas across the Permian-Triassic boundary ca. 252.2 Ma is associated with significant microbial change and volcanic activities (Xie et al., 2005, 2007; Cao et al., 2009). In particular, the main extinction horizon of faunas occurring at volcanic ash bed 25 at the Meishan section in China (the Global Stratotype Section and Point for the Permian-Triassic boundary) is immediately followed by the rapid expansion of cyanobacteria at bed 26, as shown by the biomarker records. The association between volcanic ash, cyanobacterial expansion, and mass extinction of the fauna is further observed at beds 28 and 29

at the Meishan section, where the second episode of faunal extinction at volcanic ash bed 28 is followed by a second phase of cyanobacterial expansion at bed 29 (Xie et al., 2005, 2007). Although cyanobacterial expansion may be in response to limited bioavailable nutrients in shallow water due to the development of anoxic conditions, resulting in enhanced fixation of atmospheric nitrogen (Cao et al., 2009; Jia et al., 2012), our study indicates an alternative interpretation: enhanced volcanic activities might also have contributed, partly if not wholly, to the microbial expansion by adding nutrients to the marine water.

Furthermore, acritarch and prasinophyte algae were also found to have cobloomed with the cyanobacteria immediately after the faunal mass extinction that occurred at the volcanic ash bed across the Permian-Triassic boundary (Jia et al., 2012). These eukaryotes could be the opportunistic species during the faunal crisis. Our volcanic ash experiments, showing the increased abundance of both cyanobacteria and eukaryotes, provide another possibility of interpreting the microbial changes during the faunal mass extinctions in critical periods of Earth's history that enabled changes of the oceanic chemistry resulting in the microbial expansion of opportunistic species. Consequently, intensive and extensive volcanism could have important roles not only in driving the faunal mass extinction documented previously (Xie et al., 2007), but also in triggering microbial changes, leading to their co-occurrence identified in ancient rocks.

CONCLUSIONS

Volcanic ash derived from volcanic eruptions is one of the most important factors affecting the global climate, as suggested by both geological and modern oceanography investigations. Investigation of the influence of volcanic ash on marine microbial populations is crucial for elucidating the process and mechanisms of the ecological and biogeochemical consequences of volcanic eruption. Our microcosm study revealed that volcanic ash leachate affected the development and succession of marine autotrophic and heterotrophic microbial populations in a typical oligotrophic ocean. Increased abundance, decreased diversity, and changed community structure were observed for both bacterioplankton and phytoplankton, likely due to the nutrients (such as nitrogen and silicate) provided by the volcanic ash leachate. The ecological characteristics in marine microbial populations altered by volcanic ash associated with large-scale volcanic eruption events in the history of the Earth may affect the biogeochemical cycling mediated by them, which should be further considered in paleogeological studies.

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