Zhang et al., 2017, Volcanic ash stimulates growth of marine autotrophic and heterotrophic microorganisms: Geology, doi:10.1130/G38833.1.

METHODS SUMMARY：FURTHER DETAILS

Sample collection and experimental setup

The volcanic ash used in the microcosm experiment was sampled near the Eyjafjallajökull volcano in Iceland on 17 April 2010, three days after the eruption. The uppermost layer of ash was removed and only the middle layer of ash was collected, in order to avoid possible contamination.

Two volcanic ash addition experiments were conducted with surface seawater (5 m) collected using Niskin bottles attached to a Sea-Bird CTD profiler in the western Pacific Ocean from November to December, 2011: Stations P1 (129°35' E, 17°35' N) and N8-12 (133°41' E, 6°22' N). At each station, the volcanic ash-leachate was prepared by adding 50 g volcanic ash into 20 L of seawater from which the macro- and micro-organisms, including viruses, had been removed by filtering through a 30 KD tangential filtration membrane package (Millipore, USA). After 12 hours of leaching, the suspension was filtered with 3 μm cellulose acetate membranes to remove large volcanic ash particles. The seawater for microcosm incubation was first passed through a 20 μm prefilter, and then dispensed into four 20 L polycarbonate bottles (Nalgen). Each of the two control bottles contained 10 L of *in situ* seawater, and 10 L of 30 KD-filtered *in situ* seawater. The volcanic ash addition treatments were composed of 10 L of *in situ* seawater and 10 L of ash-leachate. Using this experimental setup, the abundance of bacteria in control and treatment could be comparable. The controls and treatments were incubated in on-deck flowing seawater

incubators under ambient light conditions for 5 days.

Nutrient analysis

Seawater for nutrient analysis was collected in 200 mL polypropylene bottles and stored at -20°C. The NH_4^+ was analyzed on deck, using the indophenol blue spectrophotometric method (Pai et al., 2001). NO_2^-, NO_3^-, PO_3^- and SiO_3^{2-} were measured using classic colorimetric methods with a Technicon AA3 Auto-Analyzer (Bran-Lube) in the laboratory. The trace metal concentration (Fe, Zn, Cu etc.) of the seawater after contact with the volcanic ash was measured by the inductively coupled plasma-mass spectrometry (ICP-MS).

Flow cytometric analysis

For determination of the abundance of planktonic microorganisms (eukaryotes, cyanobacteria, heterotrophic bacteria and viruses), 2 mL of subsamples were fixed with glutaraldehyde (0.5% final concentration), quickly frozen in liquid nitrogen, and then stored at -80°C until flow cytometry analysis. The samples were analyzed on an Epics Altra II Flow Cytometer (Beckman Coulter, USA), equipped with an external quantitative sample injector (Harvard Apparatus PHD 2000). Procedures were as described by Jiao et al. (2002). Flow cytometry data were analyzed with FCS Express V3 software. Repeated measures of the multivariate analysis of variance (RM-MANOVA) were used to test if prokaryote abundance of the control and treatment had significant difference across the incubation times for the same suite of response variables. Replicate microcosms were grouped based on volcanic ash addition or not (O'Brien and Kaiser, 1985).

DNA extraction and T-RFLP analysis

The total DNA was extracted using the phenol-chloroform extraction method which was a modification of the Wilson Method (Wilson, 1987). To obtain T-RFLP fragments, the 16S rRNA gene was amplified using the forward primer 27F, labeled at the 5' end with 6-carboxyfluorescein phosphoramidite, and the reverse primer 907R (Lane, 1991). Amplification of the 50 μL PCR reaction was performed following the process mentioned by Zhang et al. (2008). PCR products were purified using the QIA quick PCR Purification Kit (Qiagen Inc., Valencia, CA). Fluorescently labeled PCR products were digested by two restriction enzymes (Msp I and Rsa I) and analyzed using the MegaBACE platform (Amersham). We used the peak area percentage (% of the total area) to standardize the T-RFLP data and the percentage peak area of less than 0.5% was removed (Zhang et al., 2008). The matrix data of peak area percentages were analyzed with clustering analysis using PRIMER 5 software (Clarke and Gorley, 2001) based on log(x+1) transformation of percentage values and Euclidean distance.

Clone library construction and phylogenetic analysis

For the construction of clone libraries, bacterial 16S rRNA gene sequences were amplified using the same protocol. PCR products from the experiment conducted at Station N8-12 were cloned and 100 clones were picked out randomly from each sample for sequencing. The sequences obtained have been checked on Bellerophon (http://comp-bio.anu.edu.au/bellerophon/bellerophon.pl) to remove the

chimeric sequences. The qualified sequences were clustered as operational taxonomic units (OTUs) at an overlap identity cut-off of 97% sequence similarity using mothur (http://www.mothur.org). The diversity estimators (Shannon–Wiener, Chao, ACE, Chao 1, and Simpson) for each clone library were also calculated using mothur.

Phylogenetic assignment of T-RFs

In order to phylogeneticly assign major peaks (T-RFs) with possible microbial groups, all the sequences from clone libraries were analyzed with *in silico* digestion and were compared to the corresponding T-RFs. Briefly, microbial 16S rRNA gene sequences and recognition sites of restriction enzymes (e.g., Msp I and Rsa I), as same as those used in T-RFLP analysis, were submit to NEBcutter V2.0 (http://tools.neb.com/NEBcutter2/). These will generated certain DNA sequence fragments digested by restriction enzymes, depending on the number and position of recognition sites. Theoretically, the 5' terminal fragment is the same one shown in T-RFLP analysis. Therefore, the microbial groups generating specific T-RFs can be identified with phylogenetic analysis of the 16S rRNA gene sequences (see above). Detailed background information of this analysis can be found in previous studies (e.g., Fitzjohn and Dickie, 2007; Kent et al., 2003; Kitts, 2001; Shyu et al., 2007; Szubert et al., 2007).

References cites in the supplementary online materials

Clarke, K. R., and Gorley, R. N., 2001, PRIMER v5: user manual/tutorial, Primer-E

Limited.

Fitzjohn, R. G., and Dickie, I. A., 2007, TRAMPR: an R package for analysis and matching of terminal-restriction fragment length polymorphism (TRFLP) profiles: Molecular Ecology Notes, v. 7, no. 4, p. 583-587.

Jiao, N., Yang, Y., Koshikawa, H., Harada, S., and Watanabe, M., 2002, Responses of picoplankton to nutrient perturbation in the South China Sea, with special reference to the coast-wards distribution of Prochlorococcus: Acta Botanica Sinica, v. 44, no. 6, p. 731-739.

Kent, A. D., Smith, D. J., Benson, B. J., and Triplett, E. W., 2003, Web-based phylogenetic assignment tool for analysis of terminal restriction fragment length polymorphism profiles of microbial communities: Applied and Environmental Microbiology, v. 69, no. 11, p. 6768-6776.

Kitts, C. L., 2001, Terminal restriction fragment patterns: a tool for comparing microbial communities and assessing community dynamics: Current issues in intestinal microbiology, v. 2, no. 1, p. 17-25.

Lane, D., 1991, 16S/23S rRNA sequencing: Nucleic acid techniques in bacterial systematics, p. 125-175.

O'Brien, R. G., and Kaiser, M. K., 1985, MANOVA method for analyzing repeated measures designs: an extensive primer: Psychological Bulletin, v. 97, no. 2, p. 316.

Pai, S., Tsau, Y., and Yang, T., 2001, pH and buffering capacity problems involved in the determination of ammonia in saline water using the indophenol blue

spectrophotometric method: Analytica Chimica Acta, v. 434, no. 2, p. 209-216.

Shyu, C., Soule, T., Bent, S. J., Foster, J. A., and Forney, L. J., 2007, MiCA: A web-based tool for the analysis of microbial communities based on terminal-restriction fragment length polymorphisms of 16S and 18S rRNA genes: Microbial Ecology, v. 53, no. 4, p. 562-570.

Szubert, J., Reiff, C., Thorburn, A., and Singh, B. K., 2007, REMA: A computer-based mapping tool for analysis of restriction sites in multiple DNA sequences: Journal of Microbiological Methods, v. 69, no. 2, p. 411-413.

Wilson, K., 1987, Preparation of genomic DNA from bacteria: Current protocols in molecular biology, p. 241-245.

Zhang, R., Thiyagarajan, V., and Qian, P. Y., 2008, Evaluation of terminal-restriction fragment length polymorphism analysis in contrasting marine environments: FEMS Microbiol Ecology, v. 65, no. 1, p. 169-178.

SUPPLEMENTARY FIGURE AND TABLE

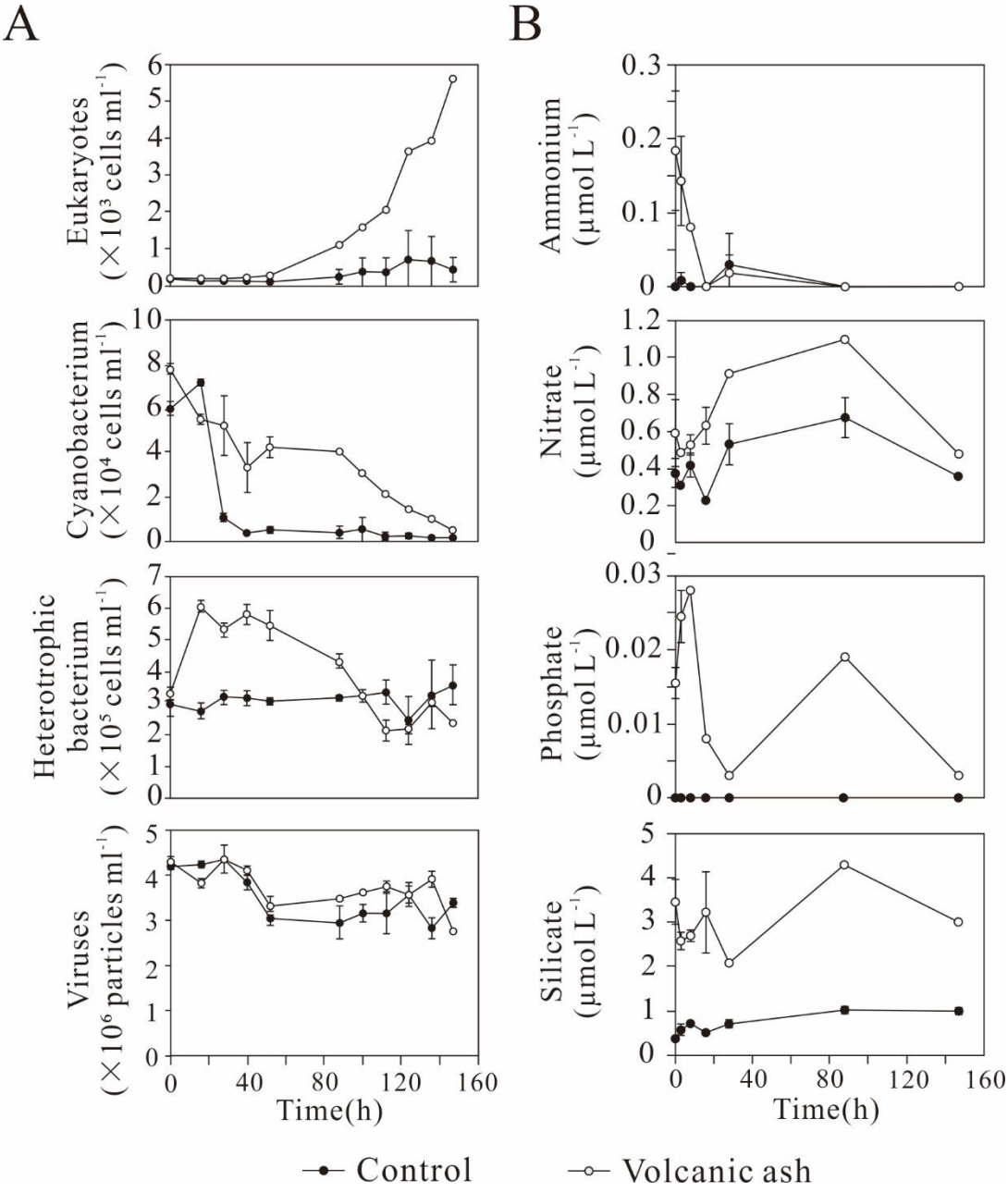

Fig. DR1. Dynamics of microbial planktonic abundance (A) and nutrient concentration (B) during incubation with volcanic ash manipulation at oligotrophic western Pacific Ocean (Station P1).

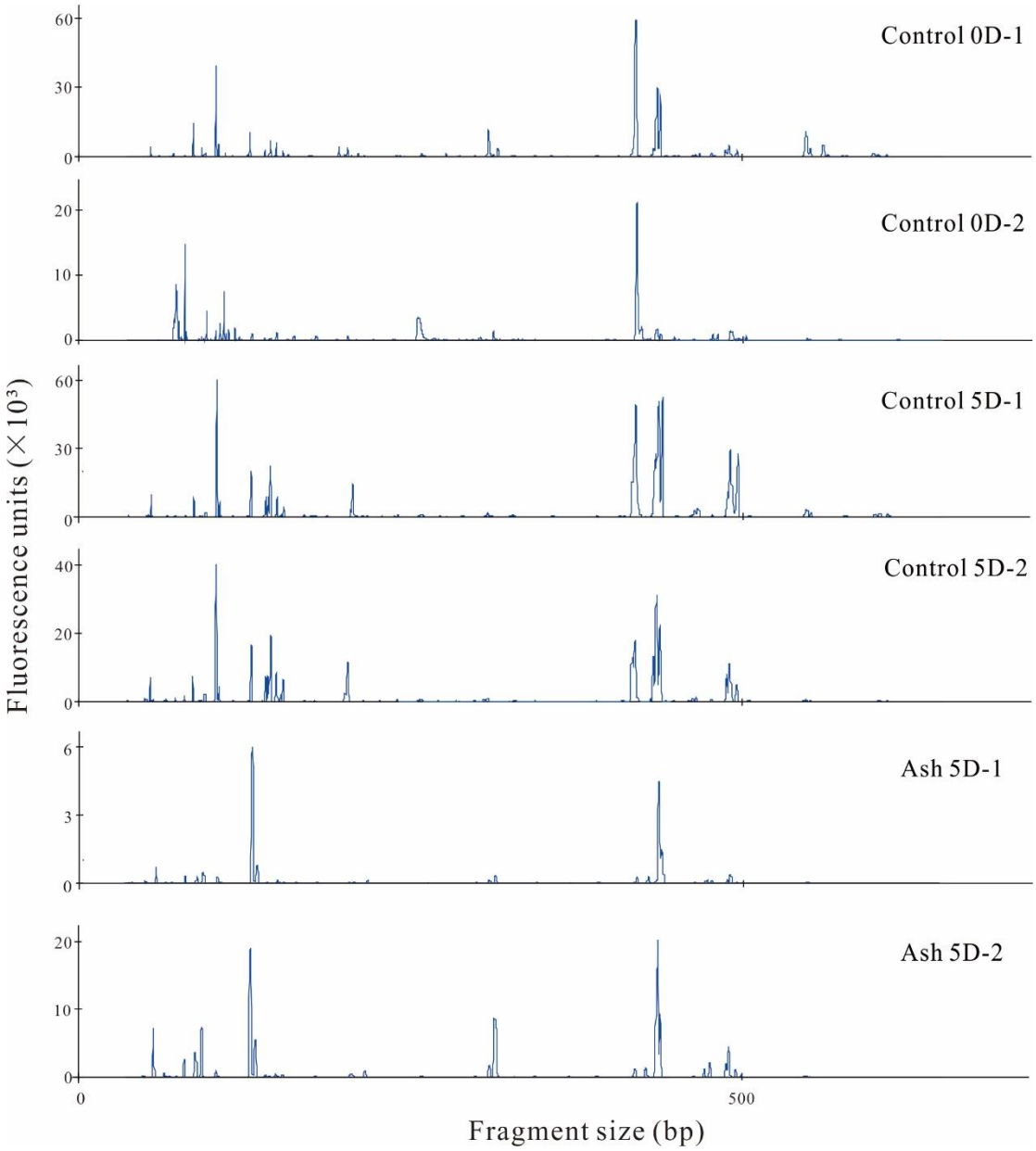

Fig. DR2. Microbial community structure as revealed by T-RFLP analysis of PCR-amplified 16S rRNA genes in controls and ash treatments.

Table S1: Trace metal-release from the Eyjafjallajökull volcanic ash. The results are expressed as trace metal release in micromoles per L of ash leachates after 12-hours contact time when mixing 2.5 g of ash with 1 L of seawater.

Trace metal	^{57}Fe	^{59}Co	^{60}Ni	^{63}Cu	^{66}Zn	^{111}Cd	^{206}Pb	^{207}Pb	^{208}Pb
	μmol/L								
Concentration	31.234	0.009	0.033	1.457	0.147	0.000	0.000	0.000	0.000

Table S2: The effects of volcanic ash manipulation on microbial community composition as revealed by percentage of peak area (% of the total area±standard deviation) in T-RFLP analysis.

Possible effects	T-RFs	Taxonomic groups	Control	Ash
Promote	56.7±0.5	*Nitzschia* (diatom)	0	3.04±0.56
	88.5±0.5	*Haslea* (diatom)	0	8.60±7.18
	129.3±0.5	Alteromonadaceae, Rhodobacteraceae	2.81±0.25	23.42±0.28
	309.6±0.5	*Cellulophaga*	0	1.86±0.32
Inhibit	144.0±0.5	*Marinobacter*	2.59±0.68	0
	495.7±0.5	*Reinekea*	6.09±0.43	0
	419.6±0.5	Rhodobacteraceae, Hyphomonadaceae, SAR11	20.05±7.95	3.85±3.46
	490.7±0.5	Alteromonadaceae, unclassified Gammaproteobacteria	6.15±2.87	2.59±1.51