Bacterial necromass as a source of organic carbon at the critical zone?

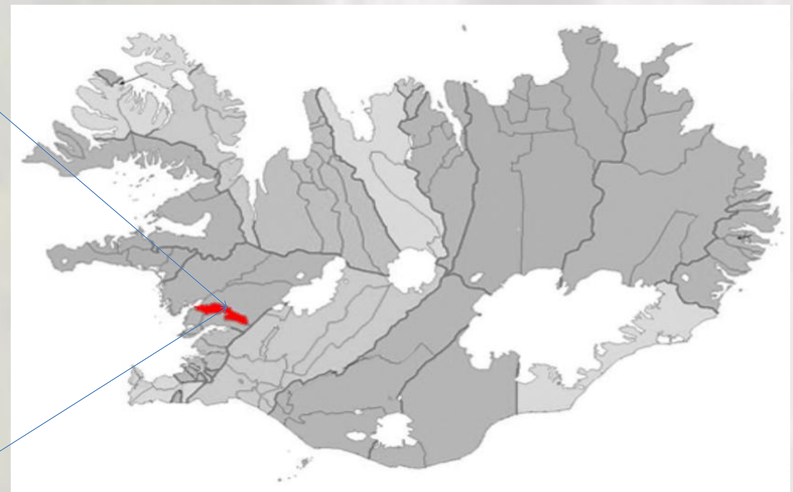
Stephen Summers[1,2], Charles S. Cockell[3] , Bruce Thomson[2] and Andrew S. Whiteley[2,4].
[1] Geomicrobiology Research Group, DPS, The Open University, Walton Hall, MK7 6AA, UK.
[2] Molecular Microbial Ecology Laboratory, Centre for Ecology and Hydrology, Benson Lane Crowmarsh Gifford, OX10 8BB, UK.
[3] School of Physics and Astronomy, James Clerk Maxwell Building, University of Edinburgh. EH9 3JZ, United Kingdom.
[4] School of Earth and Environment, University of Western Australia, Crawley, Australia.

Introduction

The critical zone, where soil comes into contact with bedrock, is an important region of weathering as this is where soil neogenesis occurs. Bacteria have been increasingly shown to contribute to mineral weathering [1, 2]; many of which are observed to be heterotrophic [3]. However the environment at the critical zone is often oligotrophic, potentially limiting the growth of biota. For areas surrounded by plants, there is a steady influx of carbon through leaf litter decomposition and photoautotrophic processes, however for areas with little evidence of plant matter, the question of where carbon originates can be posed.

One potential source of carbon was from the bacterial community itself. Bacteria deposited through melting snow packs or aerial deposition may provide one potential carbon source to the critical zone.

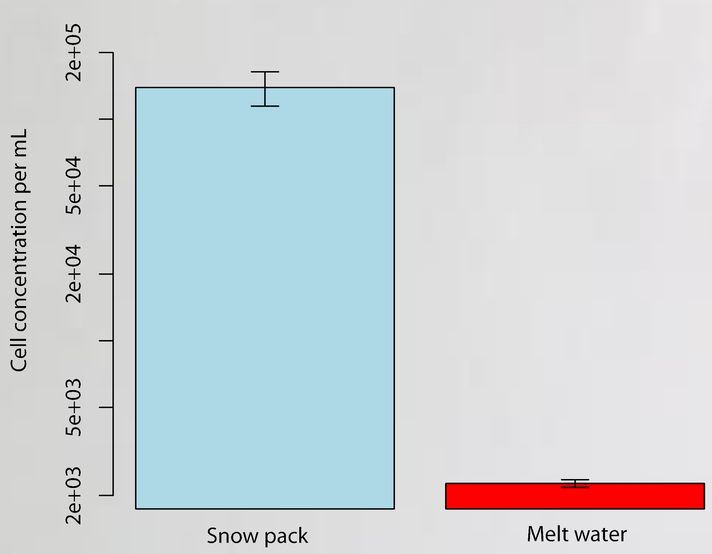
We investigated the potential for bacterial necromass to be recycled in the community by employing the use of stable isotope probing to track carbon utilization.



Methods

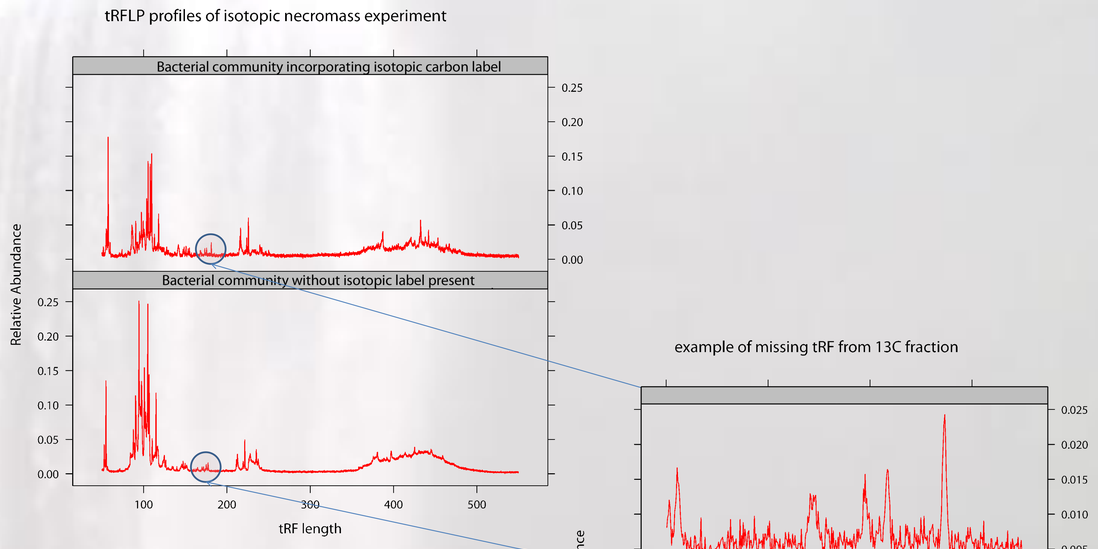
- Unvegetated soils around Skorradalur lake, Iceland were chosen for this study; previously this location was studied for differences in plant influences on basalt weathering rates [4], so lent itself to further analysis of the bacterial community.
- Bacterial immigration at sample site was observed by performing prokaryotic cell counts of snow packs above sample sites and melt water run off below sample sites, using flow cytometry. This gave an estimate of net bacterial influx to sample area.
- Bacterial isolates were cultivated using an ^{13}C isotopically labelled media. Following substantial growth, the bacterial cells were destroyed by autoclaving and the necrotic matter collected.
- Saprolite samples from critical zone were infused with ^{13}C isotopically labelled necromass and incubated at 5°C for up to one month.
- At regular intervals, samples were harvested and nucleic acids extracted from saprolite and analysed to detect evidence of ^{13}C incorporation into 16S rRNA genes. This was conducted through isopycnic centrifugation [5].

Prokaryote cell concentrations in snow melt waters



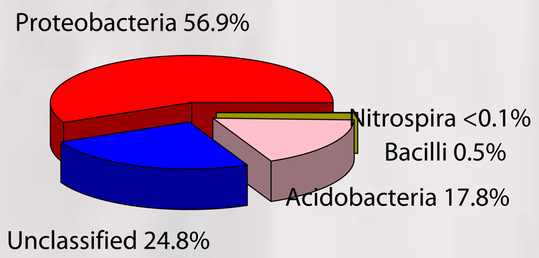
Flow cytometry cell counts indicating the concentration of prokaryote cells present in snow prior to spring melt (1.39 x 10^5 cells mL^{-1}) and prokaryote cell concentrations of melt water below sample site (2.43 x 10^3 cells mL^{-1})

tRFLP profiles of isotopic necromass experiment

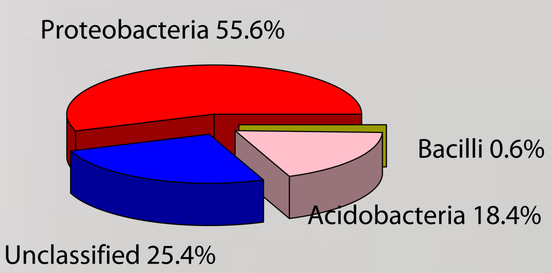


tRFLP electropherograms indicating the bacteria utilizing both forms of isotopic carbon molecules. Each peak is representative of a taxonomic group. The presence of a peak in lower plot that is not evident in upper plot signifies that no isotopic label was incorporated by that taxa.

Bacterial community utilising Carbon 12



Bacterial community utilising Carbon 13



Results and Discussion

The number of prokaryote cells entering the soil matrix from snow melt is substantial with cell numbers as high as 4.4 x 10^5 cells mL^{-1}present in the frozen ice packs. This influx of cells offers one potential source of carbon only during the snow melt in spring. Measures of bacteria depositing from air would be required to estimate annual bacterial immigration to soil.

Incorporation of ^{13}C within the nucleic acids of bacteria occurred following incubations of 24 hours. The bacterial community were able to metabolise the labelled necromass; to the degree that no statistically significant differences were detected between the communities metabolizing ^{12}C from soil matrix and those incorporating necromass labelled with ^{13}C (ANOSIM, $P \leq 0.05$).

Apparent from relative abundance plots is that communities are highly similar, yet some tRF peaks are present in ^{12}C fractions and not the ^{13}C fractions, hence not observed as incorporating isotopic label from bacterial necromass into nucleic acids:

tRF length (^{12}C)	Taxon	Trophic ecology
115	*Rhodoplanes*	Potentially phototrophic bacteria
125	*Methylocystaceae*	Methylotrophic
127	*Beijerinckiaceae*	Chemoheterotrophic (N_2 fixing bacteria)
130	Unclassified	
134	*Nitrospira*	Chemolithoautotrophic
177	*Acidobacteria*	Not Known
226	*Acidobacteria*	Not Known
232	Unclassified	
242	*Acidobacteria*	Not Known

Most bacteria at the critical zone exhibit a heterotrophic ecology, potentially utilizing many different carbon sources. One carbon source shown here, is the necrotic matter of bacterial species; as ~90% of tRFs measured showed the presence of isotopically labelled carbon from bacterial necromass. It can be suggested that necromass is providing a proportion of the organic carbon required for these communities to maintain growth in oligotrophic conditions.

References:
1.Cockell, C.S., et al., *Bacteria in Weathered Basaltic Glass, Iceland.* Geomicrobiology Journal, 2009. **26**(7): p. 491-507.
2.Cockell, C.S., et al., *Alteration textures in terrestrial volcanic glass and the associated bacterial community.* Geobiology, 2009. **7**(1): p. 50-65.
3.Uroz, S., et al., *Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil.* Applied and Environmental Microbiology, 2007. **73**(9): p. 3019.
4.Moulton, K.L. and R.A. Berner, *Quantification of the effect of plants on weathering: Studies in Iceland.* Geology, 1998. **26**(10): p. 895.
5.Neufeld, J.D., et al., *DNA stable-isotope probing.* Nature Protocols, 2007. **2**(4): p. 860-866.

Acknowledgments:
We wish to thank Daniel Read (CEH, UK) for his help in operations of the flow cytometer and Jason Stephenson (University of Warwick, UK) for helping to carry out the centrifugation. This work was co-funded by The Open University, UK and The Centre For Ecology and Hydrology, UK.