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A metagenomic snapshot of taxonomic and functional diversity in an Alpine glacier cryoconite ecosystem – Supplementary Information

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Supplementary Methods

Measurement of ecosystem productivity

In-situ 24 hour, triplicate light and dark incubations of 1 mL cryoconite debris from the holes sampled for metagenomics were used to estimate net ecosystem primary productivity by incubation 10 µL ^{14}C (DHI, Denmark, 1µCi) as Na bicarbonate. Incubations have been processed in sterile Whirlpaks (Lactan, Austria) which have been tested beforehand in a Hitachi spectrophotometer for light penetration as it would occur under natural conditions. Dark sets have been wrapped in tin foil to mimic total absence of light. Samples have been exposed in the respective cryoconite hole to ensure in situ conditions. Heterotrophic production was assessed by *in situ* incubations of ^{3}H-leucine by the modified microcentrifuge method (Kirchman, 2001) and by filtration method of Bell (1993). ^{3}H-leucine was added to a final concentration of 100 nM. Triplicate 1.5 ml samples and two control samples, collected with a syringe with a tube attached to its end in order to collect ca. 1.5 mL sediment material + water, were added into 2 ml microcentrifuge tubes. Samples were incubated for 4 hours. After incubation, 90 µl of 100% TCA were added to the samples. The tubes were then centrifuged at 16 000 g for 10 min, following washing, centrifugation and aspiration of the supernatant with 5% TCA and 80% ethanol. The final supernatant was aspirated and the remaining sediment weighted for the calculation of bacterial production on a weight basis. Finally, 1 ml of scintillation cocktail (Beckman, Ready Safe) was added and the samples counted by liquid scintillation (Beckman LSC 6000 IC).

For net ecosystem productivity estimation by changes in headspace dissolved inorganic carbon (DIC), incubations were conducted using debris from three sites on the glacier: two large cryoconite pools (Cryoconites R11, R12). Debris from the pool was representative of the cryoconite dispersed over much of the glacier. Debris from the thrust was noticeably lighter in colour and clay-like in consistency. It was rapidly melting out of an englacial thrust and was therefore thought to be derived from the glacier bed. These incubations were conducted using sediment layers of identical thickness to those found at the sampling site, which was approximately 0.2 – 0.5 cm. Triplicate light and dark incubations were conducted for one day using whirlpak bags. The dark incubations were foil – wrapped and left alongside the light incubations in the cryoconite pool. Changes in DIC in the light (unwrapped) incubations were normalised for dry sediment mass and used to represent net ecosystem production (NEP). Changes in the DIC content of dark incubations were used to deduce respiration rates (R), again normalised for dry sediment mass. Primary production was estimated from the difference of average NEP and R (Hodson et al, 2010).

In all cases, DIC change was established using the headspace method described in Hodson et al (2010) and employing a PP Systems EGM 4 infra-red gas analyser. No corrections for carbonate dissolution were required during the assays (due to there being no significant increase in dissolved Ca2+). Waters from the pool were used as a medium for all incubations and great care was taken to emplace the sediment layer without disruption, and to minimise shading by adjacent flasks.

DNA extraction

Within one month of storage at -80°C at Aberystwyth, DNA was extracted from ca. 250 mg (wet weight) aliquots of cryoconite debris using the PowerSoil DNA extraction kit (MoBio, Inc., Solana, California) as per the manufacturer's directions. Quality of extracted DNA was verified by

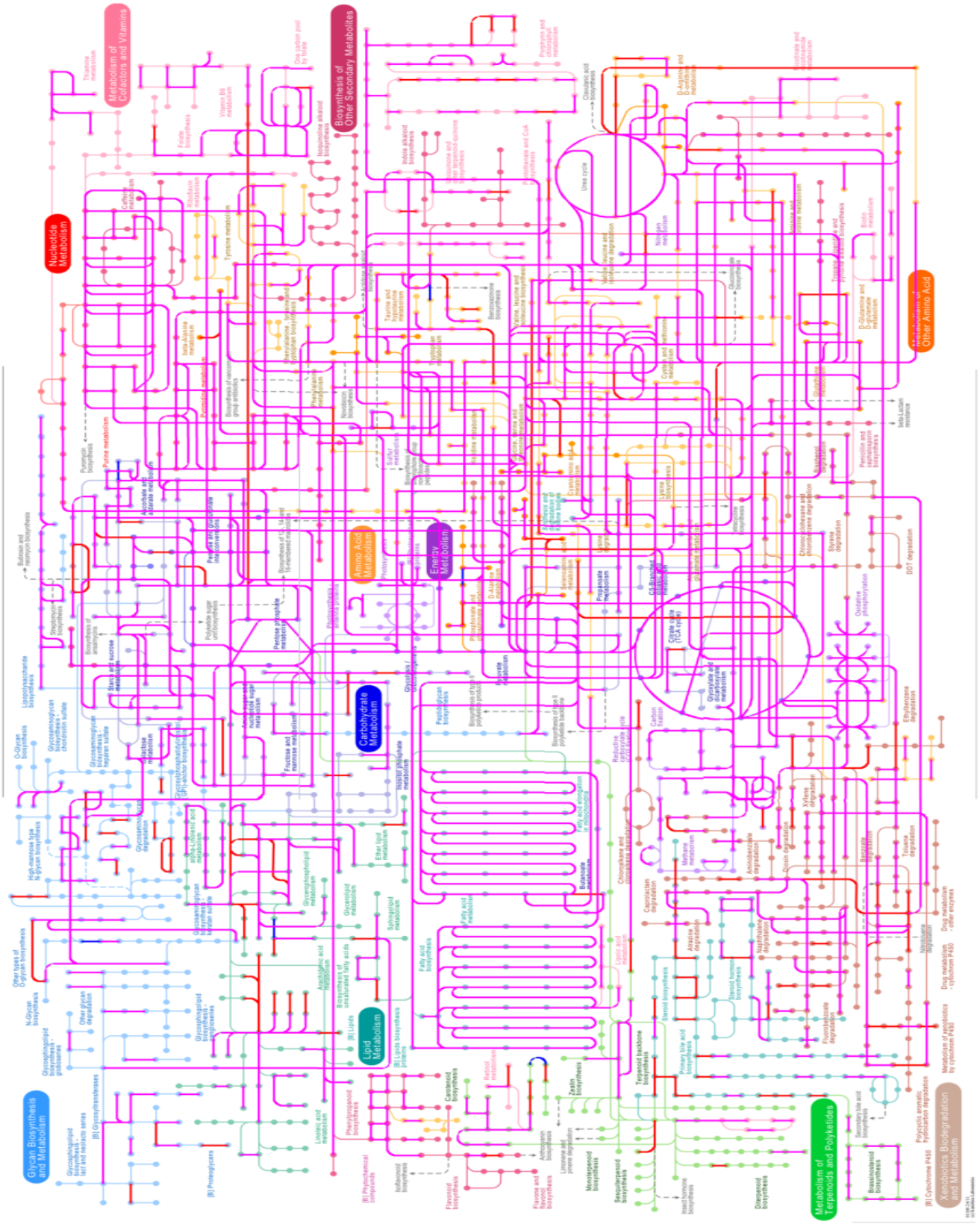
spectrophotometry (Nano-Drop 1000; as A_{260} and $A_{260/280}$ ratio) and bacterial 16S rRNA gene PCR with 27F-1389R primers exactly as detailed previously (Edwards et al., 2011; Edwards et al., 2013); products of expected size (~1.3kbp) were observed following 30 cycles of amplification including one minute annealing steps at 53 °C and elongation for 1minute; 16S rRNA gene T-RFLP and amplicon pyrosequencing will be reported elsewhere. All procedures were conducted aseptically using aerosol-resistant tips and certified DNA-free plasticware in bleach disinfected laminar flow hoods.

Supplementary Table 1: Comparison metagenomes (publically available)

Label	Metagenome detail	Reference	MG-RAST ID
Polar desert	Polar desert soil, Garwood Valley, Antarctica	Fierer *et al.* (2012)	4477900.3
Polar desert	Polar desert soil, Lake Bonney Valley, Antarctica	Fierer *et al.* (2012)	4477901.3
Polar desert	Polar desert soil, Lake Fryxell Valley, Antarctica	Fierer *et al.* (2012)	4477902.3
Polar desert	Polar desert soil, Lake Hoare Valley, Antarctica	Fierer *et al.* (2012)	4477903.3
Polar desert	Polar desert soil, Wright Valley, Antarctica	Fierer *et al.* (2012)	4477904.3
Polar desert	Polar desert soil, Lake Bonney Valley, Antarctica	Fierer *et al.* (2012)	4477803.3
Hot desert	Hot desert soil, Mojave Desert, California, USA	Fierer *et al.* (2012)	4477805.3
Hot desert	Hot desert soil, Chihuahuan Desert, Galisteo, New Mexico, USA	Fierer *et al.* (2012)	4477872.3
Hot desert	Hot desert soil, Chihuahuan Desert, Sevilleta LTER, New Mexico, USA	Fierer *et al.* (2012)	4477873.3
Soil	Tropical forest soil, Misiones, Argentina	Fierer *et al.* (2012)	4477875.3
Soil	Boreal forest soil, Bonanza Creek LTER, Alaska, USA	Fierer *et al.* (2012)	4477876.3
Soil	Temperate deciduous forest soil, Calhoun Experimental Forest, South Carolina, USA	Fierer *et al.* (2012)	4477877.3
Soil	Temperate coniferous forest soil, Duke Forest, North Carolina, USA	Fierer *et al.* (2012)	4477899.3
Soil	Temperate grassland soil, Konza Prairie LTER, Kansas, USA	Fierer *et al.* (2012)	4477804.3
Soil	Tropical forest soil, Manu National Park, Peru	Fierer *et al.* (2012)	4477807.3
Soil	Arctic tundra soil, Toolik Lake LTER, Alaska, USA	Fierer *et al.* (2012)	4477874.3
Sludge	Phosphorus Removing (EBPR) Sludge Community soil, Thornside, Australia	Martin *et al.* (2006)	4441092.3
Sludge	Phosphorus Removing (EBPR) Sludge Community soil	Martin *et al.* (2006)	4441093.3
Freshwater	GS020 Shotgun - Fresh Water - Panama Canal - Lake Gatun - Panama	Rusch *et al.* (2007)	4441590.3
Freshwater	GS020 Shotgun - Fresh Water - Panama Canal - Lake Gatun - Panama	Rusch *et al.* (2007)	4443679.3
Antarctic lake	AntarcticaAquatic_2 - ACE LAKE, ANTARCTICA	NCBI project ID 33179	4443680.3
Antarctic lake	AntarcticaAquatic_4 - MARINE DERIVED LAKE	NCBI project ID 33179	4443681.3
Antarctic lake	AntarcticaAquatic_5 - MARINE DERIVED LAKE	NCBI project ID 33179	4443682.3
Antarctic lake	AntarcticaAquatic_1 - MARINE DERIVED LAKE	NCBI project ID 33179	4443683.3
Antarctic lake	AntarcticaAquatic_6 - ACE LAKE, ANTARCTICA	NCBI project ID 33179	4443684.3
Microbial mat	mis_wdu polar microbial mat	Varin *et al.* (2010)	4445126.3
Microbial mat	whi_wdu polar microbial mats	Varin *et al.* (2010)	4445129.3
Microbial mat	mis_wdu polar microbial mat	Varin *et al.* (2010)	4449590.3
Stromatolite	Stromatolite T1S2 HBC	Khodadad & Forster (2012)	4449591.3
Ice	Schneefernerice	Simon *et al.* (2009)	4492048.3
Ice	Schneefernerice	Simon *et al.* (2009)	4492064.3
Ice	Schneefernerice	Simon *et al.* (2009)	4492068.3

Supplementary Table 2: Breakdown of cryoconite metagenome functionally enriched categories at e <1x10^{-2}

Subsystems	Mean rank of Metagenomes(n=32)	Cryoconite Metagenome rank	Contigs assigned at Subsys Level 2	
Regulation & cell signalling	12	7	-	21210
			Programmed Cell Death and Toxin-antitoxin Systems	1807
			Proteolytic pathway	26
			Quorum sensing and biofilm formation	1504
			Regulation of virulence	5745
Membrane transport	13	9	-	17191
			ABC transporters	18436
			Protein and nucleoprotein secretion system, Type IV	4892
			Protein secretion system, Type I	70
			Protein secretion system, Type II	5741
			Protein secretion system, Type III	187
			Protein secretion system, Type V	195
			Protein secretion system, Type VI	5756
			Protein secretion system, Type VII (Chaperone/Usher pathway, CU)	83
			Protein secretion system, Type VIII (Extracellular nucleation/precipitation pathway, ENP)	359
			Protein translocation across cytoplasmic membrane	9511
			Sugar Phosphotransferase Systems, PTS	999
			Uni- Sym- and Antiporters	1184
Fatty acids, lipids and isoprenoids	15	12	-	12903
			Fatty acids	30765
			Isoprenoids	16038
			Phospholipids	12239
			Triacylglycerols	313
Stress response	17	14	-	4942
			Acid stress	1485
			Cold shock	357
			Dessication stress	11
			Detoxification	2639
			Heat shock	12584
			Osmotic stress	7588
			Oxidative stress	25451
			Periplasmic Stress	2047
Aromatic compound metabolism	18	15	-	8114
			Anaerobic degradation of aromatic compounds	5985
			Metabolism of central aromatic intermediates	12292
			Peripheral pathways for catabolism of aromatic compounds	19942
Sulphur metabolism	21	20	-	8602
			Inorganic sulfur assimilation	7951
			Organic sulfur assimilation	5545
Motility & chemotaxis	22	21	-	7003
			Flagellar motility in Prokaryota	14436
			Social motility and nonflagellar swimming in bacteria	13



Supplementary Figure 1: KEGG-mapped metabolic pathways of the Rotmoosferner cryoconite metagenome. Represented pathways are highlighted in pink. A full resolution version of this figure is available on MG-RAST (4491732.3)

References

Bell, R.T. (1993). Estimating production of heterotrophic bacterioplankton via incorporation of tritiated thymidine. In: Kemp PF. Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, p. 495-503.

Edwards, A; Anesio, A.M.; Rassner, S.M.; Sattler, B.; Hubbard, B.P.; Perkins, W.T.; Young, M. and Griffith, G.W. (2011) Possible interactions between bacterial diversity, microbial activity and supraglacial hydrology of cryoconite holes in Svalbard. ISME Journal. 5:150-160.

Edwards, A.; Rassner, S.M.; Anesio, A.M.; Worgan, H.J.; Irvine-Fynn, T.; Williams, H.; Sattler, B.; & G.W. Griffith. (2013) Contrasts between the cryoconite and ice-marginal bacterial communities of Svalbard glaciers. Polar Research. 32:19648.

Fierer N, Leff JW, Adams BJ, Nielsen UN, Bates ST, Lauber CL et al (2012). Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proceedings of the National Academy of Sciences.

Hodson, A., Cameron, K., Bøggild, C., Irvine-Fynn, T., Langford, H., Pearce, D., and Banwart, S. (2010) The structure, biogeochemistry and formation of cryoconite aggregates upon an Arctic valley glacier; Longyearbreen, Svalbard. Journal of Glaciology 56: 349-362.

Kirchman, D. (2001) Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. Methods in Microbiology 30: 227-237.

Khodadad CLM, Foster JS (2012). Metagenomic and Metabolic Profiling of Nonlithifying and Lithifying Stromatolitic Mats of Highborne Cay, The Bahamas. PloS One 7.

Martin HG, Ivanova N, Kunin V, Warnecke F, Barry KW, McHardy AC et al (2006). Metagenomic analysis of two enhanced biological phosphorus removal (EBPR) sludge communities. Nature Biotechology 24: 1263-1269.

Rusch DB, Halpern AL, Sutton G, Heidelberg KB, Williamson S, Yooseph S et al (2007). The Sorcerer II Global Ocean Sampling expedition: Northwest Atlantic through Eastern Tropical Pacific. PloS Biology 5: 398-431.

Simon C, Wiezer A, Strittmatter AW, Daniel R (2009). Phylogenetic Diversity and Metabolic Potential Revealed in a Glacier Ice Metagenome. Applied and Environmental Microbiology 75: 7519-7526.

Varin T, Lovejoy C, Jungblut AD, Vincent WF, Corbeil J (2010). Metagenomic profiling of Arctic microbial mat communities as nutrient scavenging and recycling systems. Limnology and Oceanography 55: 1901-1911.