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Supplementary material

Microbially mediated reduction of Fe^{III} and As^{V} in Cambodian sediments amended with ^{13}C-labelled hexadecane and kerogen

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Quantification of extractable PLFAs and ^{13}C-incorporation

Methods

Phospholipid fatty acids (PLFAs) were generated from the phospholipid fraction, after the addition of an internal standard (540 ng of tetracosane-d_{50}; ISOTEC). Base hydrolysis was conducted by adding 0.5 M, 95 % methanolic NaOH and heating (70 °C, 60 min).[1,2] The fractions were derivatized using bromotriflouride (BF_3) in methanol and heated (70 °C; 60 min) to convert the PLFAs into their corresponding methyl esters.[3] To convert any alkanols into their corresponding trimethylsilyl ethers, bis(trimethylsilyl)trifluroacetamide (BSTFA) was added to these fractions and heated (70 °C; 60 min). The extracted hydrocarbon fraction and PLFAs were analysed using an Agilent 789A gas chromatograph interfaced to an Agilent 5975C MSD mass spectrometer using the same operational parameters as described previously.[4] In addition to GC-MS, incorporation of ^{13}C label into phospholipid fatty acids was qualitatively assessed with a ThermoQuest DeltaPlus XL GC–isotope ratio mass spectrometer, as described previously.[5]

Results

Extraction of PLFAs was performed on the original sediment before incubations and on the 'sediment + ^{13}C-hexadecane' and 'sediment + ^{13}C-kerogen' treatments at the end of the incubations (t = 8 weeks). Compared to the indigenous PLFA content of the sediment (0.19 μg g^{-1} sediment), the total concentration of the PLFAs remained relatively unchanged in both incubations (0.8 and 0.2 μg g^{-1} of sediment respectively). Due to the low amounts of extracted PLFAs, it was not possible to quantify

^{13}C-incorporation, as the difference in $\delta^{13}C$ values of PLFAs in the control samples and the labelled samples was less than analytical error.

16S rRNA gene amplicon pyrosequencing

Methods

PCR of the V1-V2 hypervariable region of the bacterial 16S rRNA gene was performed using tagged fusion universal bacterial primers 27F[6] and 338R,[7] synthesised by IDTdna (Integrated DNA Technologies, BVBA, Leuven, Belgium). The fusion forward primer (5′ CCATCTCATCCCTGCGTGTCTCCGACTCAGNNNNNNNNNNAGAGTTTGATGMTGGCTCA G 3′) contained the 454 Life Sciences 'Lib-L Primer A', a 4 base 'key' sequence (TCAG), a unique ten-base barcode 'MID' sequence for each sample, and bacterial primer 27F. Roche's MID-1 barcode (ACGAGTGCGT) was used for '*t* = 0, sediment', MID-2 (ACGCTCGACA) for '*t* = 8 weeks sediment', MID-3 (AGACGCACTC) for '*t* = 8 weeks, sediment+hexadecane'. The reverse fusion primer (5′ CCTATCCCCTGTGTGCCTTGGCAGTCTCAGTGCTGCCTCCCGTAGGAGT 3′) contained the 454 Life Sciences 'Lib-L Primer B', a 4 base 'key' sequence (TCAG), and bacterial primer 338R.

The PCR amplification was performed in 50-μL volume reactions using 0.5 μL (2.5 units) FastStart High Fidelity DNA polymerase (Roche Diagnostics GmbH, Mannheim, Germany), 1.8 mM MgCl2, 200 μM of each dNTP, 0.4 μM of each forward and reverse fusion primers. The PCR conditions included an initial denaturing step at 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and a final elongation step at 72 °C for 5 min. PCR products were loaded onto an agarose gel, and following gel electrophoresis, bands of the correct fragment size (~410 bp) were excised, purified using a QIAquick gel extraction kit (QIAGEN, GmBH, Hilden, Germany), and eluted in 30 μL of DNAse free H_2O. The purified PCR products were quantified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA), and pooled so that the mixture contained equal amounts of DNA from each sample. The emulsion PCR and the pyrosequencing run were performed at the University of Manchester sequencing facility, using a Roche 454 Life Sciences GS Junior system.

The 454 pyrosequencing reads were analysed using Qiime 1.6.0 release,[8] and de-noising and chimera removal was performed in Qiime during OTU picking (at 97 % sequence similarity) with usearch.[9] Taxonomic classification of all reads was performed in Qiime using the Ribosomal Database Project (RDP)[10] at 80 % confidence threshold.

Table S1. Number of pyrosequencing reads obtained in this study, reads remaining after denoising and chimera check, and observed OTUs (at 97 % similarity level)

Sample	Number of reads	Reads after denoising and chimera check	Observed OTUs
t = 0, sediment	2158	1888	125
t = 8 weeks, sediment	2369	2056	132
t = 8 weeks, sed+^{13}C-hexadecane	4440	3768	188
Total	8967	7712	226

Table S2. Taxonomic classification according to the RDP (at 80 % confidence threshold) of the pyrosequencing reads obtained in this study

Taxonomy	sediment *t* = 0	sediment *t* = 8 weeks	sed + ^{13}C-hex *t* = 8 weeks
Betaproteobacteria; f__Gallionellaceae; Other	30.1 %	42.2 %	45.9 %
Deltaproteobacteria; f__Geobacteraceae; g__Geobacter	3.6 %	6.9 %	7.6 %
Betaproteobacteria; f__Rhodocyclaceae; g__Azospira	14.5 %	5.7 %	4.7 %
Acidobacteria; f__Holophagaceae; g__Geothrix	3.5 %	5.3 %	4.2 %
Epsilonproteobacteria; g__Sulfurospirillum	1.3 %	3.7 %	4.2 %
Firmicutes; c__Clostridia; f__Coriobacteriaceae; g__	1.5 %	0.6 %	3.7 %
Spirochaetes; f__Spirochaetaceae; g__Treponema	2.1 %	2.9 %	2.6 %
Betaproteobacteria; g__Hydrogenophaga	9.7 %	4.7 %	2.0 %
Bacteroidetes; o__Bacteroidales; Other	0.8 %	1.3 %	1.7 %
Acidobacteria; f__Holophagaceae; Other	5.8 %	1.1 %	1.6 %
Deltaproteobacteria; f__Geobacteraceae; g__	0.4 %	1.4 %	1.6 %
Betaproteobacteria; f__Comamonadaceae; Other	4.2 %	3.9 %	1.5 %
Betaproteobacteria; f__Gallionellaceae; g__	1.7 %	1.8 %	1.5 %
Deltaproteobacteria; f__Pelobacteraceae; Other	3.3 %	0.2 %	0.9 %
Other	17.5 %	18.3 %	16.3 %

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