Geomicrobiology of deep, low organic carbon sediments in the Woodlark Basin, Pacific Ocean

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Abstract

The distribution of bacterial populations and activity were determined at two Ocean Drilling Program sites (1109 and 1115) in the Woodlark extensional Basin, near Papua New Guinea, Pacific Ocean. These relatively deep water (1 150–2 211 m), low organic carbon (\( <0.4\%\)), low organic matter sedimentation sites, with average thermal gradients (\( <30\,^\circ\mathrm{C}/\mathrm{km}\)) are representative of large areas of ocean sediments. At a third site, 1118, with a higher thermal gradient (63\,^\circ\mathrm{C}/\mathrm{km}), only bacterial distributions and pore water acetate (bioavailable) concentrations were determined. Active bacterial populations were present to all depths at Sites 1109 and 1115, maximum depth 801 mbsf (metres below seafloor), and this represents the deepest and oldest marine sediments (\( <15\,\mathrm{Ma}\)) in which the presence of bacteria has been demonstrated using a combination of different approaches (direct microscopic and viable counts, radiotracer turnover and geochemical analysis). In addition, direct counts and geochemical data at Site 1118 provide strong evidence for significant bacterial populations to at least 842 mbsf (3.2\,\times\,10^5/cm³). Similar to previously studied subseafloor sediments, maximum bacterial populations and activity occurred in the upper \( <20\) m, with much lower rates (up to 10,000 times) in deeper layers. However, a depth integration of data demonstrated that the majority of bacterial cells and activity (sulfate reduction, methanogenesis, thymidine incorporation into DNA and acetate oxidation) occurred in the subsurface, below 20 m. For sulfate reduction this was dependent on the depth of sulfate penetration. Acetate was an important substrate for methanogenesis. Despite being degraded, acetate concentrations in subsurface layers remained relatively constant (\( \sim\,10\,\mu\mathrm{M}\)) and therefore, there must also have been deep acetate formation, which is consistent with the presence of acetogenic bacteria. These results extend the significance of deep acetate formation for the maintenance of subsurface bacterial populations to sediments representative of large areas of the ocean. © 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Deep marine sediment; Bacterium; Acetate; Sulfate reduction; Methanogenesis; Acetogen; Low organic carbon; Deep biosphere; Ocean Drilling Program

1. Introduction

Deep marine sediments have been estimated to contain a bacterial biomass equivalent to \( \sim 10\%\) of living carbon in the surface biosphere [1] and possibly much more [2,3]. Only within the last decade has this ‘deep biosphere’ been investigated [4], and comparatively little is known concerning the ecology of such environments. Generally, bacterial abundances decrease with increasing sediment depth in a broadly consistent manner which is described by the model of Parkes et al. (log cells = 7.98−0.57 log depth [1]). However, bacterial populations and their activity can actually increase where geochemical conditions change in deeper layers, such as the presence of brine incursions [5], and thermogenic gas [6]. An extreme example of elevated subsurface bacterial populations occurs in gas hydrate sediments, where bacterial abundances are so stimulated around the base of the deep gas hydrate zone [7–9] that some rates of bacterial activity are greater than those near the sediment surface. In the gas hydrate sediments at Blake Ridge [Ocean Drilling Program (ODP) Leg 164], the principal energy source for deep bacterial methane production is the low molecular mass organic acid, acetate [9]. Acetate formation in deep sediments is normally associated with the thermogenic alteration of organic matter at temperatures \( >80^\circ\mathrm{C}\) as part of the processes of fossil fuel formation.
At Blake Ridge, however, temperatures did not exceed 30°C, yet there is experimental evidence for the generation of acetate by bacteria, even at such a low temperature [11]. Gas hydrate-containing sediments, although of global significance [12], may reflect very different conditions compared to the majority of marine sediments. It is, therefore, important to establish the significance of deep acetate metabolism in non-hydrate sediments, but this has yet to be done.

Deep-sea sediment samples from ODP Leg 180, in the Western Woodlark Basin [13], offered an opportunity to investigate the microbial ecology of low organic carbon deep marine sediments in the absence of extremes of temperature, fluid incursions or gas hydrate deposits. In particular, the importance of deep acetate metabolism and its significance as a source for deep methane formation was investigated and contrasted with previous results for deep gas hydrate sites.

2. Materials and methods

2.1. Site description

Samples were obtained at three sites in June–August 1998 during a 2-month ODP cruise (Leg 180) in the Western Woodlark Basin, near Papua New Guinea. Samples for determination of bacterial populations and activity were taken from two sites (1109 and 1115) on the northern margin of the spreading tip in the Woodlark Basin extension system [13]. Bacterial populations (but not activity rates) were also determined in samples from a further site, 1118, nearer to the zone of ocean floor spreading.

Site 1109 was located on the Woodlark Rise, to the north of the Moreshy Seamount (Fig. 1), in a water depth of 2211 m. Sedimentological data from Site 1109 indicated that the site experienced progressive subsidence from sub-aerial to lagoonal, then shallow marine to deep water [13]. Unexpectedly, a massive dolerite was present at the base of the section (Fig. 2), from 773 to 802 metres below sea-floor (mbsf). This igneous layer was 66 million years old, predating the deepest sediments (~8.5 million years old) by over 50 million years [14]. The current thermal gradient was estimated at 31°C km⁻¹ [13]. Thus, Site 1109 provides an ideal ‘control’ site for deep sediment microbiological study. The thermal gradient is close to the oceanic sediment average (33°C/km [15]), and the full sediment column was sampled, unlike previous investigations [4]. The basement dolerite layer provided a bottom seal preventing vertical diffusion from deeper sediment, which has been a feature of all previous subsea-floor microbiological investigations [4]. In addition, no gas hydrates, brine incursions or high temperature thermogenic influences were present [13].

Site 1115 was located ~35 km to the north of Site 1109 (Fig. 1), in a water depth of 1150 m. The thick dolerite at the base of Site 1109 was not present, and far older sediments were sampled, to ~15.3 million years. The thermal gradient at Site 1115 was estimated to be 28°C km⁻¹, almost identical to that at 1109. No evidence of deep fluid flow was found at Site 1115 [13]. Site 1118 was situated ~9 km due south of Site 1109 (Fig. 1), in a water depth of 2303 m. Due to its proximity to the ocean spreading centre, the site has a higher thermal gradient (~63°C km⁻¹). The sediments are ~3.5 Ma, much younger than those at Sites 1109 and 1115. In addition, temperature measurements in the open hole during a logging run suggest migration of warm fluids at 700–800 mbfs [13].

2.2. Shipboard sample handling

2.2.1. Whole round cores

All samples used for measurement of bacterial activity and estimation of viable bacterial numbers were taken from 25 cm whole round cores (WRC), obtained from Site 1109 (14×25 cm samples, 0.05–660.79 mbsf) and 1115 (12×25 cm samples, 1.20–797.89 mbsf). The samples were taken using a specially constructed cutting rig [16], and sealed with sterile core end caps under a flow of sterile oxygen-free nitrogen (OFN) to maintain anaerobic conditions. Capped WRC were stored in gas-tight anaerobic bags [17] in the ship’s cold room at 4°C for up to 6 weeks during the cruise, and transported back to the laboratory in insulated trunks containing wet ice and ice-packs. The samples remained cold throughout transportation.

2.2.2. Direct bacterial enumeration

Total bacterial numbers were determined by epifluorescence microscopy using acridine orange, as previously described [18].

2.2.3. Pore waters

Pore waters were collected using a titanium squeezer from 15-cm core sections, from which the outer few millimetres of potentially contaminated sediment had been removed [13]. Pore water was extruded through Whatman No. 1 filters fitted on a titanium screen by applying pressures up to ~4150 psi with a hydraulic press. Pore water was then filter-sterilised (0.1 μm) and stored, frozen, in 2.5-ml capped vials before transportation back to the laboratory for determination of acetate concentrations by HPLC.

2.3. Laboratory sample handling

2.3.1. Whole round cores

On arrival at the laboratory the WRC samples were stored in a constant-temperature room at 4°C prior to further handling. Apart from cores taken by rotary coring, samples were processed within 3 weeks of arrival. Cores obtained using rotary drilling required the development of
special handling techniques for semi-lithified sediments [19]. Thus, rotary core samples were stored for up to 3 months prior to subsequent handling.

2.3.1.1. Non-rotary cores. All sample handling was performed under aseptic, anaerobic conditions [16]. The WRC were cut into 5-cm sections, from each of which ten 5-ml syringe subcores were removed for radiotracer activity measurements: (i) methanogenesis from bicarbonate, (ii) turnover of acetate to methane and CO₂, (iii) sulfate reduction and (iv) thymidine incorporation. In addition, one 5-ml syringe subcore was removed for most probable number (MPN) viable counts [20]. Syringe subcores were taken from the centre of the WRC, avoiding sediment near the core liner, to prevent the possibility of contamination [21] and sealed with sterile Suba Seals.

2.3.1.2. Rotary cores. Leg 180 was the first ODP cruise on which detailed microbiological studies have been conducted on highly consolidated sediments from

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Fig. 1. Map showing location of Sites 1109, 1115 and 1118 in the Western Woodlark Basin, Papua New Guinea. The sites were sampled during ODP Leg 180 in June–August 1998. 1 and 2 denote 1000 and 2000 m depth contours.
rotary cores. The surface of rotary core samples are subject to more contamination than other core samples, although the interiors are still uncontaminated [21]. In addition, as they often have a smaller diameter than the core liner, and are highly compacted, they cannot be handled by vertical syringe subcoring. A special hydraulic cutting rig, therefore, had to be developed to obtain an uncontaminated subcore from the centre of the RCB core under sterile, anoxic conditions [19], and without the core collapsing and introducing surface contamination to the rest of the sample. The subcored material was crushed to a fine grain size under sterile, anoxic conditions. This powder was slurried (25% v/v) with sterile mineral salts (MS) solution, and then 10-ml aliquots transferred to 20-ml serum vials, and sealed under OFN with a butyl rubber septum. The composition of the MS solution was as follows: 0.2 g l⁻¹ KH₂PO₄; 0.25 g l⁻¹ NH₄Cl; 30.0 g l⁻¹ NaCl; 3.0 g l⁻¹ MgCl₂6H₂O; 0.5 g l⁻¹ KCl; 0.15 g l⁻¹ CaCl₂2H₂O. The pH was adjusted to 7.5, then the solution was autoclaved and cooled under 80:20 (v/v) N₂:CO₂.

2.3.2. Potential activity measurements

During sample collection and subsequent handling, it is presently impossible to maintain in situ conditions. Incubation of each sample at its in situ temperature would make direct comparison of rates difficult, thus incubations were conducted at the mean down core temperature (12°C). In addition, it is not possible to obtain and subsequently handle samples at in situ pressures. Thus, bacterial activity rates are described as ‘potential’ rates, as they may differ from those in situ. Furthermore, slurrying of rotary core samples may have an additional effect on rate measurements [22]. However, at these sites, there was no discernable differences between rate measurements made in intact cores and slurries.

2.3.2.1. Injection schedule. Intact 5-ml ‘syringe’ subcores and/or slurried samples were allowed to equilibrate overnight at 12°C before incubation with radiotracers. Isotopes were injected into the syringe subcores using a specially designed rig [16] which allowed an even distribution of isotope along the centre line of the subcore; slurry samples were injected through the butyl rubber septum and then thoroughly shaken. Each group of ten samples was divided into one time-zero control and triplicate samples for each of three incubation periods. Time-zero subcores were prechilled at 4°C, injected and immediately frozen and stored in anaerobic bags at −20°C. The other injected subcores were sealed in anaerobic bags and incubated at 12°C for varying periods (Table 1). Incubations were terminated by freezing at −20°C and subcores were stored frozen prior to analysis. In all cases, time-zero control results were subtracted from experimental data before calculation of potential activity rates. Slurry samples were similarly treated but were not incubated in anaerobic bags.

2.3.2.2. Methanogenesis from bicarbonate. Sample subcores and slurries were injected with 7.2 μl (4.8 μCi) of sodium ¹⁴C-bicarbonate solution (Amersham UK, diluted with filter-sterilised (0.2 μm), degassed distilled water) prior to incubation. Rates of methanogenesis from bicarbon-
ate were determined from the amount of 14CH4 produced, and the concentration of pore water carbon dioxide calculated from alkalinity data [6]. Frozen subcores were ejected into 5 ml of 1 M NaOH in a 20-ml serum vial, which was crimp sealed with a butyl rubber septum and allowed to thaw. 14CH4 was stripped from the vial by flushing with OFN (100 ml min⁻¹ for 30 min), oxidised to 14CO2 in a CuO furnace operated at 850°C [6], and trapped in β-phenylethylamine (10% v/v in ‘OptiScint 3’ (Wallac, UK) scintillation cocktail for liquid scintillation counting (LSC). The extraction system was modified to include a moisture trap (100 ml, packed with silica gel and anhydrous sodium sulfate) rather than a cold trap. Slurried samples were processed similarly, with incubations terminated by the injection of 0.5 ml of 10 M NaOH, prior to freezing.

### 2.3.2.3. Acetate turnover
Replicate syringe subcores and slurries were injected with 7.4 µl (1.5 µCi) of undiluted [1-(2)14C]acetate (Amersham UK) prior to incubation. Incubations were terminated, and processed for analysis of labelled 14CH4 as described above. 14CO2 was determined in the same sample after acidification with 2.0 ml of 2 M H2SO4, and a further 30 min flushing period. Methane and carbon dioxide production rates were calculated based on the proportion of labelled gas produced, the pore water acetate concentration (bioavailable acetate determined using the enzymatic method of King [23,24]) and porosity.

### 2.3.2.4. Sulfate reduction
Subcores and slurries were injected with 7.2 µl (3.6 µCi) of 35S sodium sulfate solution (Amersham UK, diluted with filter-sterilised (0.2 µm), degassed distilled water) prior to incubation. Rates of sulfate reduction were determined from the proportion of 35S-labelled sulfide produced [25] using a single stage distillation for total reduced inorganic sulfide [26], pore water sulfate concentrations and porosity.

Frozen syringe subcores were ejected into 10 ml of 20% (w/v) zinc acetate solution in a conical flask, and allowed to thaw with occasional mixing. A magnetic stirrer and 10 ml of degassed 35% NaCl were added. The flask was attached to a distillation rig, and the headspace flushed with OFN for 15 min at 80 ml min⁻¹ before addition of 5 ml 95% (v/v) ethanol, 25 ml CrCl2 and 5 ml conc. HCl. The flasks were heated to 80°C and distilled for 40 min, trapping 35S-labelled sulfide in 10 ml of 10% (w/v) zinc acetate. Aliquots (9 ml) were removed for determination of 35S activity by LSC.

### 2.3.2.5. Thymidine incorporation into DNA
Samples were injected with 25 µl (18 µCi) of undiluted [27] thymidine (83 Ci mmol⁻¹, Amersham UK) prior to incubation. After incubation frozen samples were transferred to a 13-ml centrifuge tube containing 5 ml of 20% (w/v) aqueous trichloroacetic acid (TCA) solution, and allowed to thaw, mixing thoroughly at intervals. Labelled DNA was extracted and purified from the sediment by an acid-base hydrolysis method [27,28]. Sediment was rinsed (three times with 5 ml of 5% (w/v) TCA, two times with 5 ml of 95% (v/v) ethanol, all at 4°C) and dried overnight before hydrolysis with 1M NaOH at 37°C for 1 h. After centrifugation (2500×g for 10 min at 4°C), the supernatant was removed, cooled to 4°C, and DNA precipitated by addition of 1.5 ml of 20% (w/v) TCA in 3.6 M HCl, 50 µl of a saturated solution of unlabelled ‘carrier’ DNA and Kieselguhr. Following centrifugation (2500×g for 10 min at 4°C) and rinsing (once each with 5% (w/v) TCA, 95% (v/v) ethanol, all at 4°C), labelled DNA was extracted from the pellet in 5% TCA at 100°C for 30 min, and activity determined by LSC.

### 2.4. Enumeration of viable bacteria
An MPN technique was used to estimate numbers of viable anaerobic fermentative heterotrophs [29], heterotrophic acetogens and autotrophic acetogens (modified from [30]). This procedure involved six to ten dilution levels from the original sediment, descending serially in triplicate one in five dilutions. Incubation was conducted at 12°C until no further positive vials developed (up to 26 months). Positive growth was scored by microscopic analysis, and for the acetogen cultures, confirmed by measurement of acetate production (≥0.5 mM) by Dionex ICE chromatography [31]. Numbers of viable bacteria were calculated according to Hurley and Roscoe [32].

All MPN enrichments were performed in 7-ml serum
Fermentative heterotroph medium: MS plus 1.0 mg l\(^{-1}\) Resazurin; 0.5 g l\(^{-1}\) casamino acids; 0.1 g l\(^{-1}\) yeast extract. The pH was adjusted to 7.5 with NaOH and the medium autoclaved. Once autoclaved, the following were added from sterile, stock solutions: 3.0 ml l\(^{-1}\) of ‘Combined Vitamin Solution’ [40.0 mg l\(^{-1}\) 4-aminobenzoic acid; 10.0 mg l\(^{-1}\) p-(-)-biotin; 100.0 mg l\(^{-1}\) thiamine-HCl; 20.0 mg l\(^{-1}\) folic acid; 100.0 mg l\(^{-1}\) pyridoxine-HCl; 50.0 mg l\(^{-1}\) riboflavin; 50.0 mg l\(^{-1}\) nicotinic acid; 50.0 mg l\(^{-1}\) dL-calcium pantothenate; 50.0 mg l\(^{-1}\) lipoid acid; 50.0 mg l\(^{-1}\) cyanocobalamin]; 3.0 ml l\(^{-1}\) of ‘Trace Elements 1’ [190.0 mg l\(^{-1}\) CoCl\(_2\)·6H\(_2\)O; 100.0 mg l\(^{-1}\) MnCl\(_2\)·4H\(_2\)O; 70.0 mg l\(^{-1}\) ZnCl\(_2\); 62.0 mg l\(^{-1}\) H\(_2\)BO\(_3\); 36.0 mg l\(^{-1}\) Na\(_2\)MoO\(_4\)·2H\(_2\)O; 24.0 mg l\(^{-1}\) NiCl\(_2\)·6H\(_2\)O; 17.0 mg l\(^{-1}\) CuCl\(_2\)·2H\(_2\)O; 1.5 g l\(^{-1}\) FeCl\(_3\)·4H\(_2\)O (dissolved first in 10 ml 25% (v/v) HCl)]; 3.0 ml l\(^{-1}\) of selenite/tungstate [3.0 mg l\(^{-1}\) Na\(_2\)SeO\(_3\)·5H\(_2\)O; 4.0 mg l\(^{-1}\) Na\(_2\)WO\(_4\)·2H\(_2\)O; 30.0 ml l\(^{-1}\) of saturated NaHCO\(_3\) solution (84.0 g l\(^{-1}\)); 3.0 ml l\(^{-1}\) of Na\(_2\)S solution (12.0 g l\(^{-1}\)); 10.0 ml l\(^{-1}\) of a mixed solution of 0.5 g glucose, 0.5 g glycerol in 10 ml water]. The pH of the medium was readjusted to 7.5 and dispersed under a N\(_2\)·CO\(_2\) head space into sterile vials containing 2.5 mg chitin, 2.5 mg cellulose.

Heterotrophic acetogen medium: MS plus 1.0 mg l\(^{-1}\) Resazurin; 0.1 g l\(^{-1}\) yeast extract. Adjust pH to 7.2±0.2 with NaOH, autoclave, cool under N\(_2\)·CO\(_2\). Once cool, the following were added from sterile, stock solutions (composition as above except otherwise stated): 3.0 ml l\(^{-1}\) of ‘Combined Vitamin Solution’; 3.0 ml l\(^{-1}\) of ‘Trace Elements 1’; 2.0 ml l\(^{-1}\) of selenite/tungstate; 30.0 ml l\(^{-1}\) saturated NaHCO\(_3\) solution, 3.0 ml l\(^{-1}\) Na\(_2\)S solution; 5.0 ml l\(^{-1}\) of Al-K(SO\(_4\))·12H\(_2\)O solution (10 mg l\(^{-1}\)); 20 mg of syringic acid (dissolved in 5 ml of 0.1 M NaOH and filter-sterilised). The pH of the medium was readjusted to 7.2±0.2 and dispersed under a N\(_2\)·CO\(_2\) head space into sterile vials.

Autotrophic acetogen medium: As for heterotrophic acetogen medium (above) minus syringate, and cooled and dispersed under H\(_2\)·CO\(_2\).

3. Results

3.1. Site 1109

3.1.1. Bacterial abundance and growth

Bacteria were most abundant near the sediment surface at Site 1109 (3.3×10\(^6\) cells ml\(^{-1}\) at 0.005 mbsf), and decreased rapidly with depth, reaching 1.2×10\(^6\) cells ml\(^{-1}\) by 746 mbsf (Fig. 2a), a >99% decrease. Despite this large decrease, bacteria were observed in all samples analysed. Dividing cells were also present in every sample, and followed a similar depth trend to the total population. The distribution of total bacterial populations, divided and dividing cells reflect three zones. (1) A narrow near-surface zone (top ~20 mbsf) in which bacterial numbers decreases rapidly with increasing depth from the surface. (2) A middle zone, from ~20 to ~580 mbsf, where total bacterial numbers fit the model of Parkes et al. [1]) for typical bacterial distributions in deep marine sediments. (3) A deep zone, below 580 mbsf, where numbers of total bacteria and dividing cells were lower than the Parkes model (Fig. 2a).

Culturable bacteria were detected in every sample (Fig. 2b). In the near-surface zone, numbers of fermentative heterotrophs and autotrophic acetogens decreased with increasing depth from a surface maximum (5050 and 94400 cells ml\(^{-1}\) respectively). In the middle zone numbers of fermenters and autotrophic acetogens increase to a subsurface maximum of 1.33×10\(^6\) and 344 cells ml\(^{-1}\) respectively at 366 and 164 mbsf. In the deep zone, no autotrophic acetogens were detected, and fermentative heterotrophs only occurred in the bottom sample (661 mbsf). Numbers of heterotrophic acetogens decreased with increasing depth from the near-surface (maximum 150 cells ml\(^{-1}\)), but were relatively constant, and although low throughout the remainder of the depth profile (Fig. 2b), they are the highest viable count in the deep zone. Potential bacterial growth rates decreased rapidly from the sediment surface to 11.8 mbsf (Fig. 2f), then decreased at a slower rate below with positive growth, albeit at very low rates, down to 366 mbsf (0.5 pmol ml\(^{-1}\) day\(^{-1}\) thymidine incorporation rate). At 438 mbsf, no thymidine incorporation was measured, and below this, rates of thymidine incorporation were positive, but very low (<0.05 pmol ml\(^{-1}\) day\(^{-1}\)).

3.1.2. Geochemistry and bacterial activity

Pore water sulfate concentrations decreased with increasing depth, from 28.1 mM at the sediment surface to 0 at 106.9 mbsf (Fig. 2c). Bacterial sulfate reduction rates were highest in the uppermost ~5 m of sediment, coincident with rapid sulfate removal. Maximum sulfate reduction rates occurred at 1.15 mbsf at 4.75 nmol ml\(^{-1}\) day\(^{-1}\). Below ~5 m, rates of sulfate reduction declined to <0.06 nmol ml\(^{-1}\) day\(^{-1}\). Near the sulfate-methane interface there was a small increase in rates to 0.16 nmol ml\(^{-1}\) day\(^{-1}\) at 89.1 mbsf. Below this depth, where sulfate concentrations were very low, potential sulfate reduction was not significantly different from blank values, except at 164.3 and 366.4 mbsf, at 0.02 nmol ml\(^{-1}\) day\(^{-1}\).

Below ~100 mbsf, methane concentrations increased rapidly from ~5 to ~6000 ppmv. Concentrations then stabilised between 1000 and 10000 ppmv down to 600 mbsf. Below ~600 mbsf, methane decreased, reaching 5
ppmv by 720 mbsf. Throughout the depth profile, \( \text{C}_1/\text{C}_2 \) ratios (methane:ethane ratios) did not drop below 1000 (high ratios indicate biogenic, whilst low ratios indicate thermogenic methane [33]). This, combined with the low temperature (\( \approx 25^\circ C \)), indicates that the methane was biogenic in origin, which is consistent with active methanogenesis being measured throughout the core. Methanogenesis from both \( \text{H}_2:\text{CO}_2 \) and acetate were highest in near-surface sediment, and occurred at broadly similar rates. \( \text{H}_2:\text{CO}_2 \) methanogenesis was maximal at 8.4 pmol ml\(^{-1}\) day\(^{-1}\) at 19.6 mbsf (Fig. \( \text{d} \)), below the peak in sulfate reduction (Fig. \( \text{c} \)). Acetoclastic methanogenesis occurred at slightly faster rates in near-surface sediment, at 24 pmol ml\(^{-1}\) day\(^{-1}\) at 0.05 mbsf (Fig. \( \text{e} \)). In deeper sediments, rates of \( \text{H}_2:\text{CO}_2 \) methanogenesis were very low (range 0.2–2.1 pmol ml\(^{-1}\) day\(^{-1}\) below 89.1 mbsf), but significantly different from blank values down to 661 mbsf, except for one zero measurement at 164 mbsf. Rates of deep acetate methanogenesis were generally \( \leq 1 \) pmol ml\(^{-1}\) day\(^{-1}\), although there were subsurface peaks at 238 mbsf (3.6 pmol ml\(^{-1}\) day\(^{-1}\)) and 517.5 mbsf (7.5 pmol ml\(^{-1}\) day\(^{-1}\)). Therefore, there was active methanogenesis within the broad zone of subsurface \( \text{CH}_4 \) gas (Fig. \( \text{d} \)), including an important contribution from acetate metabolism. Rates of acetate oxidation to \( \text{CO}_2 \) were very similar to those for acetate methanogenesis (Fig. \( \text{e} \)), although near-surface rates were slightly lower (4.4 pmol ml\(^{-1}\) day\(^{-1}\) at 1.15 mbsf). Additional evidence for bacterial activity in the deepest zone was provided by increases in ammonia and alkalinity between 430 and 550 mbsf [13].

Pore water acetate concentrations were very low, in the range \( \leq 10 \) \( \mu \text{M} \) (Fig. \( \text{e} \)). Concentrations down to \( \sim 550 \) mbsf were in the range of 4–10 \( \mu \text{M} \). Below \( \sim 550 \) mbsf, acetate concentrations tended to decrease even further, into the range of 1–2 \( \mu \text{M} \), apart from a single peak at 701 mbsf (9 \( \mu \text{M} \)). These low acetate concentrations are consistent with the generally low organic carbon concentrations, \(<1\%\), throughout the core, average 0.42\% [13].

### 3.2. Site 1115

#### 3.2.1. Bacterial abundance

Bacteria were highest near the sediment surface \( (2.8 \times 10^9 \text{ cells} \text{ ml}^{-1} \text{ at } 0.01 \text{ mbsf}) \), and decreased rapidly with depth, reaching \( 3.5 \times 10^5 \text{ cells} \text{ ml}^{-1} \) by 801 mbsf (Fig. \( \text{a} \)). Dividing cells were observed in every sample analysed, with the exception of the deepest sample (801 mbsf), and followed a similar trend to total populations. Similar to Site 1109, distributions of total bacterial populations, divided and dividing cells at Site 1115 can be interpreted in three zones. (1) A narrow near-surface zone (top \( \sim 20 \) mbsf) in which bacterial numbers decreases rapidly with increasing depth from the surface. (2) A middle zone, from \( \sim 20 \) to \( \sim 430 \) mbsf, where bacterial population distributions fit the model of Parkes et al. [1] for typical subsea-floor sediments. (3) The deepest zone (below 430 mbsf), where the gradient of decreasing bacterial numbers was 100 times more rapid than the Parkes model (Fig. \( \text{a} \)).

Culturable bacteria occurred in every sample (Fig. \( \text{b} \)). However, apart from a clear decrease from the surface in numbers of fermentative heterotrophs, MPN counts of all three types were relatively constant, and low, throughout
the depth profile. Growth rates, as reflected by thymidine incorporation, were also very low throughout the depth profile (Fig. 3f), in the range of 0.3 pmol ml\(^{-1}\) day\(^{-1}\). At two points, no thymidine incorporation was measured (555 and 736 mbsf) but in all other samples the thymidine incorporation rates were significantly different from blank values.

### 3.2.2. Geochemistry and bacterial activity

Pore water sulfate decreased with increasing depth, from 28.4 mM near the sediment surface to zero at 198.7 mbsf (Fig. 3c). This was coincident with high near-surface rates of bacterial sulfate reduction, 1.26 nmol ml\(^{-1}\) day\(^{-1}\) at 1.2 mbsf (Fig. 3b). Rates subsequently decreased with depth reaching 0.43 nmol ml\(^{-1}\) day\(^{-1}\) at 87.4 mbsf. Below 87 mbsf, sulfate reduction was ≲ 0.01 nmol ml\(^{-1}\) day\(^{-1}\), but always significantly higher than blank values.

Once sulfate had been depleted, methane concentrations increased rapidly (Fig. 3d) to levels above 1000 ppm from 250 to 450 mbsf, and above 20 000 ppm from 572 to 802 mbsf (Fig. 3d). The methane minimum at ≈ 550 mbsf coincided with a change in sedimentation resulting from the emergence of the forearc sequence [13]. The C\(_1\)/C\(_2\) ratios generally exceeded 1500^3000. These high ratios are consistent with a biogenic origin for the methane [13], and this is also supported by the low temperatures (max ≈ 25°C) and the presence of active methanogenesis. Bacterial methanogenesis from both H\(_2\):CO\(_2\) and acetate was highest in near-surface sediment. H\(_2\):CO\(_2\) methanogenesis decreased from a maximum of 20.2 pmol ml\(^{-1}\) day\(^{-1}\) at 1.2 mbsf to ≲ 1 pmol ml\(^{-1}\) day\(^{-1}\) below 87.4 mbsf (Fig. 3d), with positive rates consistently measured (with the exception of two zeros at 236.6 and 797.9 mbsf). Acetate methanogenesis decreased from 2.8 pmol ml\(^{-1}\) day\(^{-1}\) at 1.2 mbsf (Fig. 3e). In deeper sediments, rates of acetate methanogenesis were generally ≲ 1 pmol ml\(^{-1}\) day\(^{-1}\), although there was a subsurface peak at 310 mbsf (8.86 pmol ml\(^{-1}\) day\(^{-1}\)). There was active acetoclastic methanogenesis throughout the core to ≈ 800 mbsf, except for one zero measurement (736 mbsf), which was due to a corresponding zero acetate concentration. This deep methanogenesis from both acetate and H\(_2\):CO\(_2\) corresponds to the broad subsurface zone of methane (Fig. 3d), however, there was no change in the already low rates in the methane minimum zone. Rates of acetate oxidation to CO\(_2\) were similar to those for acetoclastic methanogenesis (Fig. 3e), and generally low throughout (≲ 1 pmol ml\(^{-1}\) day\(^{-1}\)). The exception was a subsurface peak around 400 mbsf which coincided with a broad zone of slightly elevated acetate concentrations in which acetate methanogenesis also was stimulated, but at a shallower depth (310 mbsf). Further evidence for deep bacterial activity was provided by a broad maximum in ammonia between 200 and 450 mbsf and again at the base of the hole [13].

Organic carbon was again low, averaging 0.34%. Pore water acetate concentrations were also low, although higher than at Site 1109, up to ≈ 20 μM (Fig. 3e). In the uppermost ~ 430 mbsf, acetate concentrations were generally higher than those below ~ 430 mbsf (5–13 μM above 430 mbsf; 2–4 μM below). Above 430 mbsf, acetate concentrations were also more variable, including the broad elevated subsurface zone previously described (around 400 mbsf).

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Fig. 4. Depth profiles of bacterial populations and activities in sediments at Site 1118: a: Total bacterial populations (●) and dividing and divided cells (○). The solid line shows Parkes’ general model for bacterial distributions in marine sediments [4], and dotted lines represent 95% prediction limits. b: Pore water sulfate (●). c: In situ methane (○). d: Pore water acetate (+).
3.3. Site 1118

3.3.1. Bacterial abundance

The uppermost \(\sim 200\) mbsf were not cored at Site 1118, and only samples for direct counts were obtained from the remainder of the hole. Bacteria were observed in all samples from 505 (\(1.6 \times 10^6\) cells \(\text{cm}^{-3}\)) to 842 mbsf (Fig. 4a). Bacterial populations to 750 mbsf were within the prediction limits of Parkes’ general model [1]. Bacterial numbers, however, appear to decrease more rapidly than predicted in the indurated material below \(\sim 750\) mbsf, although porosity remains fairly high (40–50\% [13]). Despite this population decrease, significant numbers of bacteria were detected in the deepest sample, \(3.2 \times 10^5\) cells \(\text{cm}^{-3}\), at 842 mbsf. Dividing and divided cells follow a similar trend to total bacterial numbers, and were observed in all samples.

3.3.2. Geochemistry and bacterial activity

Although bacterial activities were not determined, geochemical measurements were made [13], and these reflect active microbial processes. Depletion of pore water sulfate by \(\sim 240\) mbsf (Fig. 4b) demonstrates active sulfate reduction, and the subsequent rapid increase in biogenic methane concentrations (\(C_1/C_2\) ratios > 1000 [13]) confirms deeper methanogenic activity. Below \(\sim 700\) mbsf, there was an increase in pore water sulfate concentration, associated with fluid flow. This sulfate increase is accompanied by a rapid decrease in methane (Fig. 4c), indicating potential anaerobic oxidation of methane via sulfate reduction [34,35]. Acetate concentrations were generally low, and \(\leq 10\) \(\mu\text{M}\), however, within the potential deep methane oxidation zone, there were two depths where acetate concentrations increased markedly to \(\sim 20\) \(\mu\text{M}\). Organic carbon concentrations at Site 1118 were low, averaging 0.28 wt\%, [13]. Downhole ammonia profiles also confirm that bacterial degradation of organic matter is continuing deep within the sediments [13].

4. Discussion

The populations and activity of bacteria are greatest near the sediment surface (Figs. 2 and 3), presumably reflecting the input of fresh organic carbon. With increasing depth, populations and activities decrease, although there are some limited subsurface peaks. All the bacterial activity measurements are maximal in the top \(\sim 20\) m of sediment (Figs. 2 and 3), and then decrease rapidly. Despite this, pore water sulfate continues to be removed, and reaches \(\sim\) zero, in the uppermost \(\sim 100–200\) m. This low sulfate concentration is reflected in very low rates of sulfate reduction below 200 mbsf. Immediately below the sulfate depletion, biogenic methane accumulates. This methane accumulation indicates that active methanogenesis must be occurring in situ, and this is confirmed by direct measurement of both acetoclastic and \(\text{H}_2:\text{CO}_2\) methanogenesis. Although measured rates of methanogenesis are very low, methanogenesis is consistently present even in the deepest layers (maximum \(\sim 800\) mbsf).

Rates of \(\text{H}_2:\text{CO}_2\) and acetate methanogenesis were similar, which was surprising as based on \(\delta^{13}\text{C}\) values, \(\text{H}_2:\text{CO}_2\) is thought to be the major substrate for methanogenesis in marine sediments [36]. However, there have been few direct determinations of acetate methanogenesis in deep marine sediments and those where it has been measured have found significant acetate metabolism [11,9]. This is consistent with the significant role for acetate metabolism recognised in other deep environments [30,37].

Woodlark Basin sites lie beneath a relatively deep water column (2211 and 1150 m at 1109 and 1115 respectively). This depth reduces the input of photosynthetically derived material [38], and hence, the rate of organic matter sedimentation and organic carbon content is low. There were, however, periods of relatively high sedimentation but the bulk of this material was inorganic and thus the flux of organic carbon remained low [13]. This sedimentation regime also means that the age of the accumulated sediments is considerable, with the deepest sediments at Site 1115 reaching 15.3 million years old, thus any organic matter remaining in these sediments must be highly recalcitrant. The thermal gradient of the sites is average (\(\leq \sim 30^\circ\text{C} \text{km}^{-1}\)), and there is no evidence for any previous heating of the sediments [13]. Thus, there are limited heating effects to activate buried organic carbon, as has been demonstrated in the laboratory and for other sediments [11]. The Woodlark Basin setting – low organic matter flux and low organic carbon concentrations, cool, deep water environments – is typical of the majority of deep-sea sediments (sediments in >1000 m of water account for 87\% of all ocean sediments [38]), and is in marked contrast to most of the previous sites studied in detail for deep sediment microbiology. Previous research has investigated areas such as the high productivity Peru Margin [5], with elevated organic carbon concentrations; and sediments containing gas hydrates such as the Cascadia Margin [7] and Blake Ridge [9]. Hence, it is not surprising that bacterial populations and activity rates are generally lower in the Woodlark Basin than at these upwelling and hydrate sites (Table 2). In addition, there is no deep stimulation of bacterial activity at Sites 1109 and 1115, as occurred in the Japan Sea in response to the influx of thermogenic methane [6]. However, similar to previously studied sites, maximal rates of bacterial activity occur in near-surface sediments (top \(\sim 20\) m), with much lower rates in deeper layers, up to 10 000 times lower (Table 2). This could indicate that deep bacteria may be largely dormant or even dead, and thus have little impact on deep geochemical processes, and that their significance for global bacterial biomass may have been overestimated [3].

Despite these very low rates of bacterial activity in the deeper zones of Woodlark Basin sediments, deep layers still contain significant bacterial populations (\(1.2 \times 10^6\)}
cells ml\(^{-1}\) at 746 mbsf, 3.5 \times 10^5 cells ml\(^{-1}\) at 801 mbsf, 3.2 \times 10^5 cells cm\(^{-3}\) at 842 mbsf at 1109, 1115 and 1118 respectively). In addition, the deepest zones at 1109 and 1115 account for a considerable proportion of the total (depth-integrated) bacterial population, 11.2% and 14.4% respectively (Table 3). The presence of both culturable and dividing cells and measurable DNA replication (thymidine incorporation) and other activities reinforces the continuous presence of active and viable bacterial populations at depth at these sites. In fact, the bulk of the depth-integrated bacterial cells occur in the subsurface (below 20 m, Table 3) and this is consistent with the majority of depth-integrated bacterial activity also occurring in this zone. Considerable deep bacterial activity is consistent with deep geochemical changes, such as continued sulfate removal, biogenic methane and ammonium production and alkalinity increases (CO\(_2\) formation). Subsurface bacterial activity is so significant because the low/very low rates continue over such large depth intervals. The deepest samples measured, Site 1115 at 801 mbsf and \(~15\) Ma, represent the deepest and oldest marine sediments in which the presence of bacteria has been confirmed by a combination of different microbiological approaches, and extends previous reports by 110 m and \(~5\) Ma [4]. In addition, the results from Site 1118, although there is only direct count and geochemical data (Fig. 4a), provides strong evidence for the presence of significant bacterial populations to at least 842 mbsf.

The significance of deep sulfate reduction seems to depend on the depth of sulfate penetration and hence, bacterial sulfate-reducing activity (Figs. 2c and 3c). At Site 1109, where rates are highest sulfate is removed by \(~107\) mbsf, the upper 20 m of sediment accounts for the majority (65%) of all sulfate reduction (Table 3). In contrast, at Site 1115, where rates are lower sulfate penetration is almost double that at 1109 (\(~199\) mbsf), sediments below 20 m are responsible for the majority of sulfate reduction (72%). Total methanogenesis, which is inhibited in the near-surface due to the presence of sulfate and hence competition for substrates by sulfate-reducing bacteria [39], is always dominated by deeper zones. This consistent with methane accumulation in these zones, despite the overall low rates of methanogenic activity (Figs. 2d and 3d).

A key issue is what energy sources are still available in sediments deposited over 10 Ma ago, even to fuel these

### Table 2

Comparison of maximum rates of potential bacterial activity in subsea-floor sediments from the Woodlark Basin and four previous investigations, Peru Margin [29], Japan Sea [6], Cascadia Margin [7] and Blake Ridge hydrate (recalculated from [9]).

<table>
<thead>
<tr>
<th>Activity</th>
<th>Depth (m)</th>
<th>Peru Margin</th>
<th>Japan Sea</th>
<th>Cascadia Margin</th>
<th>Blake Ridge hydrate</th>
<th>Woodlark Basin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfate reduction (nmol ml(^{-1}) day(^{-1}))</td>
<td>top 20</td>
<td>4.13</td>
<td>24.77</td>
<td>6.85</td>
<td>400.3</td>
<td>4.75</td>
</tr>
<tr>
<td></td>
<td>20–500</td>
<td>0.0016</td>
<td>0.0042</td>
<td>0.0014</td>
<td>1.91</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>below 500</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>5.89</td>
<td>0.005</td>
</tr>
<tr>
<td>Methanogenesis (from HCO(_3^-)) (nmol ml(^{-1}) day(^{-1}))</td>
<td>top 20</td>
<td>0.183</td>
<td>0.64</td>
<td>0.0145</td>
<td>0.16</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>20–500</td>
<td>0.046</td>
<td>0.09</td>
<td>0.272</td>
<td>2.73</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>below 500</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Methanogenesis (from acetate) (nmol ml(^{-1}) day(^{-1}))</td>
<td>top 20</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.18</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>20–500</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.34</td>
<td>0.009</td>
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<tr>
<td></td>
<td>below 500</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>1.24</td>
<td>0.008</td>
</tr>
<tr>
<td>Methane oxidation (nmol ml(^{-1}) day(^{-1}))</td>
<td>top 20</td>
<td>nd</td>
<td>nd</td>
<td>18.58</td>
<td>0.085</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>20–500</td>
<td>nd</td>
<td>nd</td>
<td>134.53</td>
<td>173.62</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>below 500</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>56.67</td>
<td>nd</td>
</tr>
<tr>
<td>Thymidine incorporation (fmol ml(^{-1}) day(^{-1}))</td>
<td>top 20</td>
<td>nd</td>
<td>476.5</td>
<td>nd</td>
<td>4070.2</td>
<td>2320</td>
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<tr>
<td></td>
<td>20–500</td>
<td>nd</td>
<td>0.4</td>
<td>nd</td>
<td>1341.3</td>
<td>920</td>
</tr>
<tr>
<td></td>
<td>below 500</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>204.6</td>
<td>50</td>
</tr>
</tbody>
</table>

nd = no data.

### Table 3

Relative percentage of depth-integrated bacterial activities occurring in three depth zones of deep marine sediments in the Woodlark Basin.

<table>
<thead>
<tr>
<th>Site</th>
<th>Depth Zone</th>
<th>Activity</th>
<th>1109 Near-surface</th>
<th>1115 Near-surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>1109</td>
<td>upper 20 m</td>
<td>Sulfate reduction</td>
<td>65.63</td>
<td>27.58</td>
</tr>
<tr>
<td></td>
<td>upper 20 m</td>
<td>Methanogenesis from H(_2):CO(_2)</td>
<td>11.62</td>
<td>12.37</td>
</tr>
<tr>
<td></td>
<td>upper 20 m</td>
<td>Methanogenesis from acetate</td>
<td>4.89</td>
<td>3.12</td>
</tr>
<tr>
<td></td>
<td>upper 20 m</td>
<td>Acetate oxidation to CO(_2)</td>
<td>6.84</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>upper 20 m</td>
<td>Thymidine incorporation</td>
<td>10.6</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>upper 20 m</td>
<td>Bacterial populations</td>
<td>21.1</td>
<td>23.6</td>
</tr>
<tr>
<td>1115</td>
<td>upper 20 m</td>
<td>Sulfate reduction</td>
<td>65.63</td>
<td>27.58</td>
</tr>
<tr>
<td></td>
<td>upper 20 m</td>
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<td>11.62</td>
<td>12.37</td>
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<tr>
<td></td>
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<td>3.12</td>
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<tr>
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<td></td>
<td>upper 20 m</td>
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<tr>
<td></td>
<td>upper 20 m</td>
<td>Bacterial populations</td>
<td>21.1</td>
<td>23.6</td>
</tr>
</tbody>
</table>

See text for description of three zones chosen.
low rates of bacterial activity. Although there is a small amount of organic carbon at these sites (<0.4%), this presumably survived due to its recalcitrance. Low concentrations of bioavailable acetate are present (≤10 μM), and although 14C-acetate tracer measurements show that this is being degraded, surprisingly acetate concentrations remain relatively constant (Figs. 2 and 3). Therefore, acetate production must also be occurring. Acetate production has been documented in deep gas hydrate sites at Blake Ridge, Atlantic Ocean [9], although here acetate concentrations increased dramatically, reaching 14,922 μM at 691 mbsf, over 1000 times the concentrations in Woodlark Basin sediments. This emphasises not only the general importance of deep acetate formation, but also how exceptional the conditions are in deep sediments at Blake Ridge. Indeed, at Blake Ridge, deep acetate methanogenesis may contribute to the large volumes of CH4 that may be accumulated within and below deep gas hydrates [40]. Heating during burial was demonstrated as a mechanism for increasing the reactivity of organic matter and thus acetate production in Blake Ridge sediments [11,4] and this could also be occurring in Woodlark Basin sediments. However, the combination of low organic matter concentrations and only an average thermal gradient would limit this process. Thus, the consistently low deep acetate concentrations may reflect a steady state between production and consumption, with concentrations approaching the minimum values reached in bioassay measurements of marine pore water acetate (2 μM, [41,24]). Thus, continuing low turnover of acetate may maintain bacterial populations in very deep and ancient sediments. Deep acetate formation in Woodlark Basin sediments is consistent with the regular presence of culturable acetogens (Figs. 2b and 3b). Although the thermal gradient at Site 1118 was higher, the fluid flow introducing sulfate to the deepest sediments results in an influx of fresh electron acceptors which presumably prevents consistent acetate accumulation. However, it is interesting to note that the highest deep acetate concentrations of any site occur below 686 mbsf at Site 1118 (Fig. 4d). The introduction of sulfate at depth coincides with removal of methane, which indicates that active anaerobic methane oxidation is occurring at depth. Anaerobic methane oxidation has been directly demonstrated at other deep sediment sites (Cascadia Margin [8] and Blake Ridge [9]) and would provide additional energy for deep bacterial processes.

Even the low rates of deep acetate degradation in Woodlark Basin sediments, however, seem too high, as these would utilise the small organic carbon pool in an unrealistically short time (≈136 and ≈317 years at 1109 and 1115 respectively). With the much higher acetate turnovers measured in Blake Ridge sediments, Egeberg and Barth [42] suggested that additional acetate and other volatile fatty acids could be supplied by upward migration from even deeper sediments. However, at Site 1109, the dolerite basement would prevent such migration, and presumably migration is also not important at other sites as the pore water acetate concentrations and turnover are very similar at Sites 1109 and 1115 (Figs. 2 and 3). Therefore, it seems that deep acetate is being produced in situ, albeit at rates less than those measured by the ‘potential activity’ radiotracer approach. Deep acetate generation may seem surprising in 15 Ma sediments, but the same process has been observed in other deep subsurface environments [37,43], including Cretaceous Atlantic Plain sediments, at similar temperatures (20–35°C) and in the presence of aceticogenic bacteria [30].

5. Summary

The deep sediments of the Woodlark Basin are representative of large areas of oceanic sediment. The sediments lie beneath deep water, they have low organic matter flux and low organic carbon concentrations, and have a globally average thermal gradient. The consistent presence of significant bacterial populations in these sediments reinforces the suggestion that bacteria are ubiquitous in deep marine sediments [4], and supports the contention of Whitman et al. [3] that subseaﬂoor bacterial biomass represents a large proportion of the global total. Data from Sites 1109 and 1115 demonstrate that despite maximal rates of bacterial activity occurring in near-surface sediments, microbial processes continue to extreme depth (≈800 mbsf) in deep marine sediments, albeit at generally very low rates. However, depth integration of bacterial processes demonstrates that the majority of both bacterial populations and activity occur in the subsurface. Depth distributions of pore water acetate and turnover demonstrate that deep acetate generation may play a major role in maintaining these deep bacterial populations as has been suggested for other subsurface environments.

Acknowledgements

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