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ORIGINAL PAPER

Microbial abundance in lacustrine sediments: a case study from Lake Van, Turkey

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Abstract The ICDP "PaleoVan" drilling campaign at Lake Van, Turkey, provided a long (>100 m) record of lacustrine subsurface sedimentary microbial cell abundance. After the ICDP campaign at Potrok Aike, Argentina, this is only the second time deep lacustrine cell counts have been documented. Two sites were cored and revealed a strikingly similar cell distribution despite differences in organic matter content and microbial activity. Although shifted towards higher values, cell counts from Lake Potrok Aike, Argentina, reveal very similar distribution patterns with depth. The lacustrine cell count data are significantly different from published marine records; the most probable cause is differences in sedimentary organic matter composition with marine sediments containing a higher fraction of labile organic matter. Previous studies showed that microbial activity and abundance increase centimetres to metres around geologic interfaces. The finely laminated Lake Van sediment allowed studying this phenomenon on the microscale. We sampled at the scale of individual laminae, and in some depth intervals, we found large differences in microbial abundance between the different laminae. This small-scale heterogeneity is normally overlooked due to much larger sampling intervals that integrate over several centimetres. However, not all laminated intervals exhibit such large differences in microbial abundance, and some non-laminated horizons show large variability on the millimetre scale as well. The reasons for such contrasting observations remain elusive, but indicate that heterogeneity of microbial abundance in subsurface sediments has not been taken into account sufficiently. These findings have implications not just for microbiological studies but for geochemistry as well, as the large differences in microbial abundance clearly show that there are distinct microhabitats that deviate considerably from the surrounding layers.

Keywords Subsurface biosphere · Deep biosphere · Lake Van · Cell counts · Lacustrine sediment

Introduction

For over two decades, microbial abundance has been quantified in deep marine sediments retrieved by the Integrated Ocean Discovery Program (IODP) and its predecessors (Parkes et al. 1994, 2000). Until the early 2000s, almost all ODP/IODP cell counts were carried out by the group of John Parkes at Bristol and later Cardiff, using the same highly standardized protocol (Cragg et al. 1990), thereby creating a very consistent data set. Subsequent analyses from other groups have used slightly different protocols, but the results of the different techniques usually agree very well. Over the years, a substantial number of sites from all major oceans were sampled, reaching sediment depths of over 2 km (Inagaki et al. 2012). Unless in situ temperatures of the drill cores exceeded the biological range, e.g. at Nankai Trough (Moore et al. 2001), microbial cells could be detected in all samples and the limits of life remain elusive (D'Hondt et al. 2015). Data can be accessed through the IODP database.

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The ODP data were used by several studies to calculate the abundance of micro-organisms in subseafloor sediments (Kallmeyer et al. 2012; Lipp et al. 2008; Whitman et al. 1998), coming to considerably different results. Despite the discussion about the total number of micro-organisms in subseafloor environments and their contribution to the global living biomass, it is commonly accepted that when plotted on a log–log plot, the decrease in cell abundance with depth follows a linear trend.

However, there are cases where the distribution of cells with depth deviates considerably from the aforementioned trend (Kallmeyer et al. 2012). The reasons can be diverse, e.g. rapid changes in sedimentary organic matter content due to changes in palaeoproductivity and/or preservation like in sapropel layers of the Mediterranean (Coolen et al. 2002), due to exceptionally high heat flow in the Nankai Trough (Moore et al. 2001), where cell concentration increases again at greater depth due to in situ thermogenic generation of microbial substrates (Horsfield et al. 2006) or at the base of continental margins, where mass-wasting events alter sediment accumulation rates (Cragg et al. 1995).

Samples for cell counts are usually taken with a cut-off syringe on a freshly cut core end, perpendicular to the sediment layers. Thus, the sample integrates over a certain depth interval and therefore time interval. Assuming typical sedimentation rates for high-productivity sediment (0.1 m ky^{-1}) or ultra-oligotrophic South Pacific Gyre (0.1 m My^{-1}), a 3-cm-long subcore will integrate over a few hundred to a few hundred thousand years, respectively (D'Hondt et al. 2009; Pälike et al. 2009). Depending on the location and its sedimentation rate, small or even large (i.e. glacial–interglacial cycles) temporal changes cannot be resolved.

Changes in environmental conditions may lead to changes in sediment composition and therefore interfaces of contrasting lithologies. It was shown that microbial abundance and activity increase a few tens of centimetres to metres around these geologic interfaces in both marine (Parkes et al. 2005) and terrestrial (Fry et al. 2009) subsurface environments. A study of lignites that were interbedded with sand and silt layers (Fry et al. 2009) revealed that the concentration of intact phospholipids, a marker for living micro-organisms, was lowest in the lignite, but increased in the silt layer immediately below the lignite before decreasing again with increasing distance from the lignite. The concentrations of water-extractable organic acids were highest in the lignite and decreased across the lignite/silt interface, indicating active consumption of these compounds in the more porous silt layer. Apparently, the organic substrates necessary for heterotrophic microbial metabolism are derived from the organic-rich lignite layer but cannot be consumed in situ. The organic substrates diffuse out of the lignites into the surrounding coarser sands and silts, where a more abundant microbial community consumes them. Diffusion of electron acceptors into the lignites is not a limiting factor as diffusion coefficients for low molecular weight organic acids are similar to those for electron acceptors like sulphate or nitrate (Jørgensen 2000).

Horsfield et al. (2002) introduced the term "host" and "feeder" lithology, referring to certain organic-poor but more porous lithologies that offer sufficient porosity and permeability for a microbial community (the host) and organic-rich layers with low porosity (the feeder), in which organic substrates like low molecular weight organic acids are generated and diffuse out into the host lithology.

Although it was shown that there is quite considerable heterogeneity in sediments (Fry et al. 2009; Soltwedel and Vopel 2001; Sørensen et al. 2007), the extent and the effects of heterogeneity in deep subsurface sediments have not yet been studied. Such knowledge may have great implications for models of global subsurface biosphere distribution. All current calculations of microbial abundance in subsurface sediments are based on the assumption that heterogeneity does not play a major role; therefore, cell distribution with depth can be described similar to diffusion-controlled concentration gradients of dissolved pore water constituents.

Despite the fact that geologic interfaces apparently stimulate microbial activity, this phenomenon has not been studied in much detail, especially not with regard to the question on which spatial scale these interfaces control microbial communities. The previous studies looked at changes around interfaces on the 10's of centimetre to metre scale, and smaller scales have not yet been studied, although diffusion becomes an increasingly important transport process with decreasing distance. However, any research on small-scale changes in microbial abundance, activity and community composition would also require changing the way samples for cell counting or other microbiological analyses are taken.

Despite being much easier to access than the open ocean, deep lacustrine sediments are much less studied; one of the main reasons is the lack of a geomicrobiological component in most lake drilling campaigns of the International Continental Drilling Program (ICDP). The only published long cell count record from a lacustrine environment with normal sedimentation is from Lake Potrok Aike, Argentina (Vuillemin et al. 2013, 2014). Other microbiological studies from ICDP projects that recovered deep sediments either did not perform cell counts (e.g. El'Gygytgyn Crater, (Brigham-Grette et al. 2013; Bischoff et al. 2014) or the sediments were deposited under very special conditions, e.g. as post-impact sediments in the Chesapeake Bay (Gohn et al. 2008; Cockell et al 2012), and therefore exhibit rather peculiar cell count profiles. Until now the PaleoVan project

is the only deep lake drilling campaign with a microbiological component that worked on multiple sites, all previous campaigns retrieved cores just from a single site.

The ICDP PaleoVan project at Lake Van, Turkey, was mainly driven by palaeoclimate research but also included a small geomicrobiology component. In summer 2010, long sediment cores were retrieved from two sites, recording palaeoenvironmental changes of the last 600 ky in this climate-sensitive area (Litt et al. 2009; Stockhecke et al. 2014).

A key feature of Lake Van sediment is the long intervals of annually laminated sediments, so-called varves (Landmann et al. 1996). Counting varves and tying them to dated events, e.g. volcanic eruptions, allow establishing a stratigraphy with annual resolution. Each varve consists of a lighter and a darker layer, and the timing of formation of each layer depends on the sedimentary regime (Zolitschka 2007). In the case of Lake Van, the lighter layer is formed in spring and summer by mineral precipitations and plankton blooms, and the darker layer is formed by organic and mineralogic detritus during fall and winter.

From a geomicrobiological perspective, Lake Van offered several unique opportunities:

1. Obtain a high-resolution record of microbial abundance in long (>100 m) lacustrine sediment cores.
2. Sampling two sites with different sedimentation rates and organic matter composition offers the chance to see whether and how these factors control microbial abundance and activity.
3. By quantifying cell numbers in single varves or even the different lighter and darker layers, it might be possible to assess microbial heterogeneity on the single laminae scale.

Materials and methods

Geologic setting

Lake Van is located on a high plateau in eastern Anatolia, Turkey, and is one of the largest terminal lakes in the world, having a surface area of 3570 km^2, a volume of 607 km^3 and a maximum depth of 460 m. It is the largest soda lake in the world, and the alkaline waters (alkalinity 155 m eq L^{-1}, pH 9.81, salinity 21.4 ‰) are the result of evaporation processes, hydrothermal activities and chemical weathering of volcanic rocks (Degens et al. 1984; Kempe 1977).

The bathymetry of Lake Van is characterized by basement highs and ridges, dividing the lake into two major basins and several smaller sub-basins (Utkucu 2006). The Tatvan Basin is located in the centre of the lake and represents the deepest and largest basin of Lake Van. Ahlat Ridge is a small ridge bordering the small Ahlat sub-basin, which is located between the shallow Northern Basin and the Tatvan Basin (Litt et al. 2009).

Site description

For the ICDP drilling operation in 2010, two drill sites were chosen. The first site was located in the centre of the Northern Basin (NB) at 260 metres below lake level (mbll), and the second site was located on top of the Ahlat Ridge (AR) at 375 mbll. The Northern Ridge separates the two drill sites. The distance between the two sites is ~10 km (Fig. 1).

The two drill sites were selected based on seismic data. Ahlat Ridge (AR) was drilled to recover a complete sedimentary section, reaching back about 600 ky. The Northern Basin (NB) site is located close to the northern shore of Lake Van in close proximity to the historically active volcanoes Nemrut and Süphan, which supplied numerous tephra layers to the lake sediments. These layers were of particular interest for the reconstruction of the temporal and compositional evolution of volcanic activity and associated volcanic hazards in the area (Litt et al. 2011).

Core sampling

Samples were obtained during the ICDP *PaleoVan* drilling campaign in summer 2010, using the Deep Lake Drilling System (DLDS) of DOSECC Inc. The sediment cores reached a maximum depth of approximately 143 m below lake floor (mblf) (Northern Basin) and 220 mblf (Ahlat Ridge). A hydraulic piston corer (HPC) was used for the upper approximately 100 m of sediment, and for deeper sections, a non-rotating (XN) and a rotating core bit (A) were used. Samples for regular cell counts were only taken from the core catchers because the cores themselves remained unopened at the drill site and were opened later at the IODP core repository in Bremen, Germany. No additional samples were taken from the opened cores as they had already experienced significant temperature fluctuations due to warming to room temperature for multi-sensor core logging, followed by storage at +4 °C for several months.

Upon retrieval of the core on the drilling rig, the core catcher was briefly inspected for its integrity and, if it looked undisturbed, transferred into a short piece of core liner, capped and labelled like a regular core. In many cases, the core catchers obtained with the HPC tool were up to 10-cm-long intact core sections that were as well preserved as the regular cores, whereas core catchers obtained with the XN or A tool were usually just small-cm-sized pieces or crumbles.

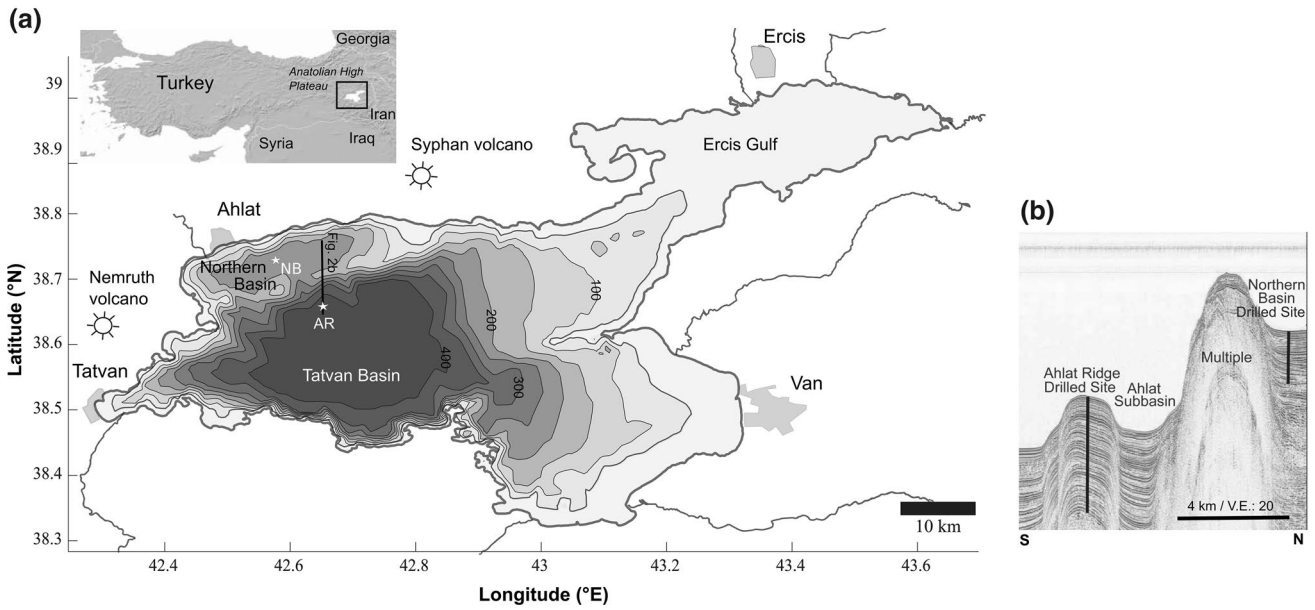

Fig. 1 **a** Bathymetric map of Lake Van, showing the major basins, volcanoes and cities. The two drill sites are marked with stars (*AR* Ahlat Ridge; *NB* Northern Basin). **b** Seismic profile along a N–S transect, showing the basins and ridges. The figure was originally published in Glombitza et al. (2013). Sulphate reduction controlled by organic matter availability in deep sediment cores from the saline, alkaline Lake Van (Eastern Anatolia, Turkey). Frontiers in Microbiology, 4. doi:10.3389/fmicb.2013.00209

The reason for the poor quality of the core catcher samples obtained with the XN or A is that the core bit exerts too much pressure and/or torque on the sediment, causing it to break or shear. Even in cases where a semi-intact sample was recovered with the XN or A tool, due to the mechanical fracturing of the semi-consolidated sediment, chances were high that drilling fluid had completely penetrated the sample along the cracks and therefore rendered it unsuitable for microbiological or biogeochemical investigations. Therefore, core catchers were only sampled if the sediment appeared to be undisturbed, which was the case for the majority of HPC core catchers and we, therefore, only used those and limited the depth range of our study to 100 mbsf and 120 mbsf at AR and NB, respectively. In order to retrieve the undisturbed sediment–water interface, short gravity cores of 65–75 cm length were retrieved at both drill sites.

For the microlayer study, whole-round cores (WRCs) from the Ahlat Ridge were used. These WRCs were originally taken for noble gas analyses (Tomonaga et al. 2011b). Sampling for noble gases requires transferring the sediment into gas-tight copper tubes without any atmospheric contact (Tomonaga et al. 2011a). This was achieved by hydraulically squeezing a WRC that was fitted with a gas-tight sleeve connected to the copper tube. The sediment exits through a hole in the core liner in the middle of the WRC. As only the central part of the WRC was squeezed into the copper tube, the remainder was supposed to be usable for investigating microbial abundance on the microlayer level. However, upon subsampling the ends of the squeezed sections in the home laboratory, we found that the vast majority of the originally straight layers were deformed and folded, most probably due to the squeezing of the WRC. Only small sections of relatively undisturbed material could be identified and sampled from a few WRC. Due to the deformation, it was impossible to exactly measure the thickness of the layers, as they varied depending on their position.

Subsampling and sample processing

For the regular cell count samples, a 2-cm^3 sample was retrieved from the centre of a visibly undisturbed core catcher, using a sterile 3-ml cut-off syringe. Before inserting the syringe into the sediment, the surface was scraped off with a sterile scalpel. Cell count samples from the short gravity cores were taken in a similar fashion, the core was sectioned by pushing it out of its liner, and a sterile 3-ml cut-off syringe was inserted into the centre of each section.

The sediment was immediately transferred into a centrifuge tube filled with 8 ml of 0.2-μm-filtered Lake Van water, amended with 2 vol% formalin. The sample was thoroughly shaken to form a homogeneous slurry. Samples were stored at +4° C until analysis in the home laboratory within the next few months.

For the microlayer study, four core sections were examined. Three of the core sections (2D9H1, 26.3 mbsf; 2E13H5, 38.6 mbsf; and 2D20H3, 58.9 mbsf) were

laminated with alternating light and dark ochre layers, and one section (2D9H1) additionally contained a thin ash layer as well as a very thin red layer of unknown composition. One core section (2D17H3, 50.7 mbsf) was not laminated, light ochre in colour, and used as a control. From this core, layers of about 1 mm thickness were scraped off and treated like a microlayer. Collection of sample material from single layers turned out to be difficult, because even those sections that were considered to be unaffected by the squeezing turned out to be compromised upon closer inspection. The layers were compressed and exhibited a slight waviness or microfolding, making it very difficult to precisely extract single layers without removing parts of a deeper layer. Because of the limited amount of material available, each layer was scraped off with a sterile scalpel and put it into a vial filled with 2 ml of 0.2-µm filter-sterilized 1× phosphate-buffered saline (PBS), amended with 2 vol% formalin instead of filtered Lake Van water. The suitability of 1× PBS was tested to avoid any artefacts caused by the different salinities of the fixative solution. The colour of each single layer was visually determined, as well as any additional information about mineral composition or other notable features. Unfortunately, the amount of material that could be retrieved was very limited and completely used up for cell counting, so there was no chance for further sedimentological or geochemical investigations.

The vials were weighed with and without sample to obtain the exact wet weight of the sediment in the vial. Using the sediment density data from the multi-sensor core logger, we were able to calculate the volume and hence the dilution factor of each sample, which varied from 1:2 to 1:30, but was mostly in the range of 1:5 to 1:12.

Although cell numbers were generally high enough to be measured directly without cell separation, background fluorescence caused by a high amount of particulate organic matter and fine clay particles covering the cells made microscopic analysis difficult. It was therefore decided to subject all samples to a cell separation treatment according to the protocol of Kallmeyer et al. (2008), with slight modifications due to the variable concentration of the slurries. The amount of chemicals used for each extraction varied depending on the respective dilution. For example, 500 µl carbonate dissolution mix (CDM), 50 µl methanol (MeOH), 50 µl detergent mix (DM) and 350 µl NaCl solution (2.5 % with 0.1 vol% NaN_3) were added to 50 µl slurry with a dilution factor of 1: 5. For different dilution factors, the volumes of the reagents were changed accordingly.

Prior to cell extraction, the sample was first checked for its carbonate content. As described in detail by Kallmeyer et al. (2008), calcium interferes with the cell extraction by binding to the complexing agents in the detergent mix and thereby drastically lowering its efficiency. As the main calcium-containing mineral is calcium carbonate, it had to be removed by dissolution through addition of a carbonate dissolution mix (CDM), a sodium acetate–acetic acid buffer solution containing 20 mL L^{-1} (0.43 M) glacial acetic acid and 35 g L^{-1} (0.43 M) sodium acetate. CDM has a high acidity but a moderate pH (4.6), in order to avoid any damage to the cells but to rapidly dissolve any carbonate minerals. After all carbonates were dissolved, the sample was centrifuged to settle out all particles. The supernatant was carefully removed and kept for later analysis. To remove the last traces of calcium, the pellet was resuspended in saline solution of the corresponding salinity and centrifuged again. The resulting carbonate-free sample was treated as a regular sample.

To the carbonate-free slurry, 10 vol% each of a detergent mix (37.2 g L^{-1} (100 mM) disodium EDTA dihydrate, 44.6 g L^{-1} (100 mM) sodium pyrophosphate decahydrate, 10 mL L^{-1} (1 % vol/vol) Tween 80) and methanol are added and the sample is placed on a vortex shaker for 30 min. The detergent mix consists of complexing agents (EDTA, pyrophosphate) that remove calcium from the molecular structure of the extracellular polymeric substances (EPS), which are used to bind the cells to the sediment grains, a surfactant (Tween 80) to lower the surface tension and act as an detergent and an alcohol (methanol) to dissolve lipids, which are also part of the EPS.

After vortexing, the slurry was underlain by a layer of 50 % (w/v) Nycodenz density liquid (density 1.25 g cm^{-3}), followed by centrifugation in a swing-out rotor (3000×*g*, 20 min). A sediment pellet formed at the bottom, whereas the separated cells float in the supernatant. The supernatant was carefully siphoned off, the pellet was resuspended in 2.5 % NaCl solution, and the entire process was repeated, but this time with an additional sonication step (10 min at 20 °C, Bandelin, Germany; Model: Sonorex Digitec 103H bath, set to max. power 640 W) prior to vortexing to dislodge remaining cells.

The two different types of supernatants from the carbonate dissolution and the cell separation were not pooled to avoid renewed carbonate precipitation. Before filtering the supernatants onto a 0.2-µm-pore-size black polycarbonate membrane (Whatman Cyclopore), they were treated with hydrofluoric acid (final concentration 0.1 %) for 20 min in order to dissolve all remaining sediment particles (Morono et al. 2009). First, the supernatant from the carbonate dissolution is passed through the filters and then rinsed with PBS or a cell-free solution of the same salinity, followed by the second supernatant and another rinsing. Each filtration step ran until the filter was completely dry.

The cells were stained with SYBR Green I (Noble and Fuhrman 1998) according to the protocol of (Morono et al. 2009) and counted using a fluorescence microscope (Leica DM2500, light source Leica EL 6000, excitation filter: BP

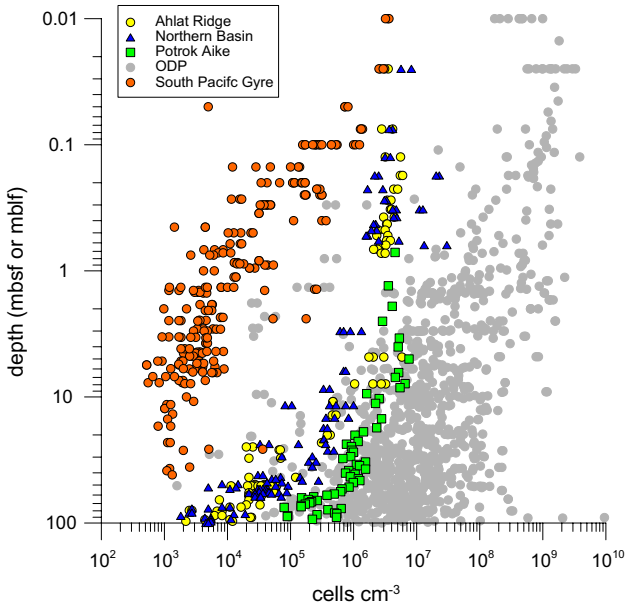

Fig. 2 Subsurface microbial cell counts from the two ICDP drilling projects at Lake Van (Ahlat Ridge; *yellow circles*; Northern Basin: *blue triangles*) and Potrok Aike (*green squares*). For comparison, records of marine subseafloor microbial cell counts from the South Pacific Gyre (*orange circles*), the most oligotrophic oceanic province and counts from more productive coastal and upwelling regions are plotted as well (*grey circles*). The *red circles* indicate cell counts from individual microlayers, taken from the Ahlat Ridge drill core

480/40, dichromatic mirror: 505, suppression filter 527/30, 100× objective).

For organic carbon and nitrogen analysis, the samples were freeze-dried and homogenized. Samples were analysed for total carbon (TC) and total nitrogen (TN) using an elemental analyser (HEKAtech Euro EA). Total inorganic carbon (TIC) content was determined using a titration coulometer (Coulometric Inc. 5011 CO2-Coulometer). Total organic carbon (TOC) was calculated as TOC = TC − TIC and the C/N ratio as C/N = TOC/TN. TOC and C/N data were previously published (Glombitza et al. 2013). All data from Potrok Aike were taken from the literature (Vuillemin et al. 2013).

Results

Cell counts from samples taken with cut-off syringes from both drill sites (Ahlat Ridge, Northern Basin) are almost indistinguishable from each other (Figs. 2, 3). They start in the 10^6 cells cm^{-3} range at the sediment–water interface and remain almost constant over the upper metre. Between 1 and 100 mblf (metres below lake floor), they drop steadily by about two to three orders of magnitude. When plotting them on a double log plot, they fall along a curve, whereas marine cell counts fall more or less along

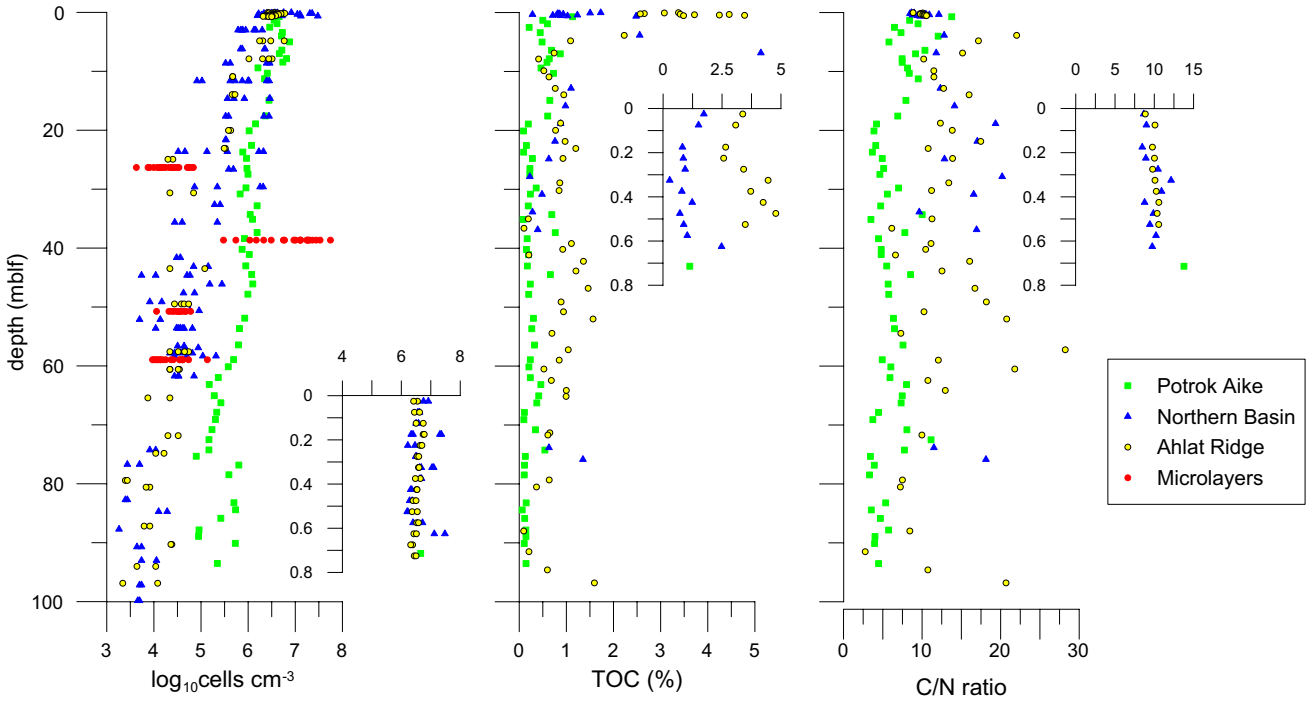

Fig. 3 Compilation of cell counts, TOC concentration and C/N ratios of the two Lake Van sites and Potrok Aike. *Symbols* are identical to Fig. 2, please note the linear depth scale

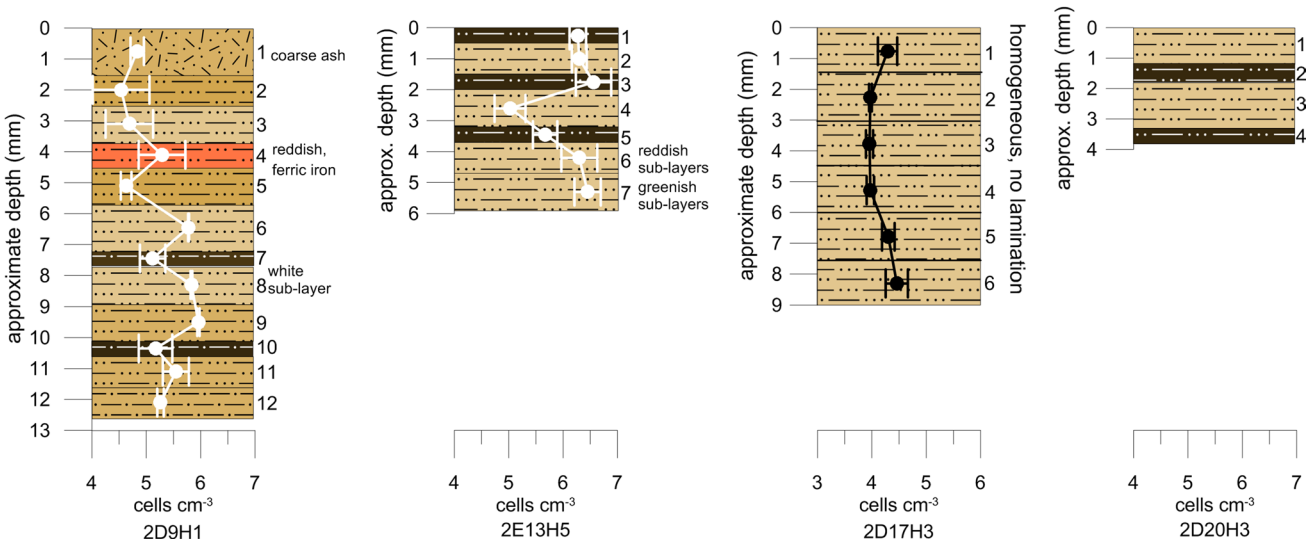

Fig. 4 Cell abundance in the different microlayers. The *y*-axis on the *left* of each panel gives an approximate scale, and the *numbers on the right* indicate the number of each layer. See main text for description of the different lithologies and further information

a straight line (Fig. 2). The almost vertical South Pacific Gyre cell count profile between 10 and 100 mbsf with values around 10^3 cells cm^{-3} is an artefact caused by a cut-off of lower cell counts due to the minimum detection limit of the counting method, which is around 10^3 cells cm^{-3}.

The Lake Van cell counts show relatively little scatter, and reproducibility between replicate measurements is usually better than 30 %, which is quite good, considering the drop in cell abundance over several orders of magnitude (Figs. 2, 3).

Unfortunately, the uppermost sample at Potrok Aike was taken at 0.75 mblf, so only a single sample overlaps with the depth range of the short gravity cores from Lake Van. Cell counts from both lakes start in the same range around 10^6 cells cm^{-3}, but the Potrok Aike counts show almost no decrease over the upper 10 mblf before following the same trend as the Lake Van counts, albeit shifted to higher values by about one order of magnitude.

TOC values of Potrok Aike and both Lake Van sites are all very similar and mainly stay below 1 %, with some single samples reaching up to 2 %. In the upper 0.8 mblf, some higher values of up to 2 and 5 % can be found for Northern Basin and Ahlat Ridge, respectively (Fig. 3).

The C/N ratios of Potrok Aike fall into a relatively narrow range between 3 and 8, with some scattered single samples reaching values of up to 12. The uppermost sample reaches 13.8, which is the highest value of the entire drill core. The data from Lake Van show a rather different picture. Generally, the values are higher, but the short gravity cores from both sides have almost identical values around 10 with very little scatter and only a very slight increase with depth, and the values from greater depths scatter between 5 and 25 with no apparent depth trend (Fig. 3).

The results from the microlayer study clearly show that there is considerable scatter with regard to cell abundances between the different layers and even in non-laminated sediment (Fig. 4). However, when plotting cell abundance of all layers together with the standard cell counts of the respective side, the microlayer cell counts scatter around the neighbouring normal cell counts (Fig. 3).

Core 2D9H1 has a diverse lithology, composed of several dark and light layers as well an ash layer (layers 1) and a reddish, probably ferric iron-rich layer (layer 4). While layers 1–3 exhibited very similar cell counts around 6×10^5 cells cm^{-3}, the following layers showed a zig-zag pattern with odd-numbered layers remaining in the same range as the previous layers, whereas the even-numbered layers reveal increasing cell numbers with values of up to 10^7 cells cm^{-3}. There is no obvious correlation between cell counts and sediment colour. The two very dark layers have lower cell counts than the neighbouring lighter layers. Elevated cell counts can be found in the fine reddish layer and three light-coloured layers, but other light-coloured layers (e.g. layers 3 and 12) have rather low cell counts.

Core 2E13H5 shows higher scatter between the replicate measurements of each layer, leading to a higher standard deviation. Cell numbers in layers 1–3 are uniformly in the low 10^6 cells cm^{-3} range, before dropping by over an order of magnitude in layer 4 and steadily increasing again to 3×10^6 cells cm^{-3} in layers 5–7. There is no apparent correlation with sediment colour.

The non-laminated core 2D17H3 shows a distinct pattern with cell counts decreasing by about a factor of five

towards the centre of the section before going back up again. Cell abundances in layers 2–4 are lower than the cell counts in layers 1, 5 and 6.

The deepest sample for the single laminae study was core 2D20H3. Only four individual layers could be extracted: two light ones (layers 1 and 3) and two dark ones (layers 2 and 4). From all four core sections used for this study, these four samples show the best reproducibility between the replicates and the smallest differences between the different layers. Despite the small (<1 std. dev.) differences, this section still shows a very distinct pattern with higher cell counts associated with darker layers.

Discussion

Cell abundance is a parameter that provides information about the net habitability of an environment. The total size of a microbial community depends on many different factors, e.g. supply of electron donors and acceptors, sufficient porosity and availability of water.

The normal cell counts from both Lake Van drill sites are basically indistinguishable from each other, and the overall shape of the cell count profile of Potrok Aike is surprisingly similar to the two Lake Van sites. However, compared to Lake Van, the cell counts from Potrok Aike are shifted to higher values by on order of magnitude. There is only a single cell count in the upper 1 mblf from Potrok Aike, preventing any comparisons with Lake Van in the uppermost depth interval.

In comparison with marine cell count records, the three long lacustrine cell count records show a different depth trend. When plotted on a log–log plot (Fig. 2), marine cell counts decrease more or less linearly, whereas the lacustrine data fall along a curve, showing less decrease in cell abundance over the upper 10 mbsf and then turning towards an increasingly steep gradient. This gradient becomes much steeper than cell counts from the South Pacific Gyre (IODP Exp. 329 Scientists 2011), which already show a more rapid decrease with depth than other deep subseafloor cell count records from the ODP.

The differences between marine and lacustrine subsurface cell distribution raise the question about the parameters that control microbial abundance in deep subsurface environments. Sediment properties like porosity or permeability are highly variable at both Lake Van sites due to rapid changes from laminated sediments to chaotic, mass-wasting sediments of variable composition and tephra layers of variable thickness. All lithologies can be found at both sites (Stockhecke et al. 2014), albeit in variable thickness and stratigraphic position. Still, at any given depth, cell abundance at both sites is very similar, independent of lithology in the respective sample. Despite some differences in the uppermost 10 mblf, cell counts from Potrok Aike also show a very similar depth distribution despite a heterogeneous and diverse sedimentology (Vuillemin et al. 2014). Apparently, sedimentological features do not seem to have a large impact on microbial subsurface cell distribution.

Differences in organic matter composition might provide some explanation of the different shapes of cell distributions with depth and the general differences between lacustrine and marine records. Organic matter in lacustrine sediments is mainly composed of allochthonous terrestrial plant material plus some fraction of eroded fossil organic matter; both are rather recalcitrant and resist microbial degradation. There is also a variable but normally small fraction of autochthonous algal biomass, which is more bioavailable (Meyers and Ishiwatari 1993). Marine organic matter contains a much higher fraction of autochthonous algal biomass and is therefore more reactive. This compositional difference is reflected in the C/N ratios, and while fresh marine sedimentary organic matter usually has C/N ratios between 4 and 10, lacustrine organic matter can reach values of 20 or higher and often shows more scatter (Meyers 1994).

When looking at the C/N ratios of Lake Van and Potrok Aike, it becomes clear that this parameter does provide some explanation, but it cannot explain all observed features. The C/N ratios of the two Lake Van sites are typical for a lacustrine environment, but the Potrok Aike record resembles much more a marine record by having values mainly below 10 and very little scatter. The lower C/N ratios of Potrok Aike indicate fresher material, which is more readily available for biodegradation and therefore might explain the higher cell counts. However, over the upper 20 mblf at Potrok Aike, C/N ratios and TOC concentrations are shifted towards slightly higher values, indicating an increased input of more recalcitrant organic matter. The change in organic matter composition coincides with the onset of the LGM (Vuillemin et al. 2013).

Over this depth interval, C/N ratios of Potrok Aike and the two Lake Van sites are more similar than at greater depths, the cell counts are also somewhat closer to each other, especially in the upper 10 mblf. Below 10 mblf, the Lake Van cell counts start to decrease, whereas the Potrok Aike counts remain almost constant down to about 20 mblf before starting to decrease at a similar rate as the Lake Van counts, leading to the aforementioned difference of 1 order of magnitude. Except for the upper 20 mblf, where TOC concentrations at Potrok Aike are almost identical to those from Lake Van, they are usually lower, despite higher cell counts. This indicates that the C/N ratio has a stronger impact on cell abundance than TOC concentration.

Studies that compared microbial abundance and activity in deep marine sediments (e.g. (D'Hondt et al. 2004) usually found that both parameters correlate positively

with each other, and high metabolic activity is reflected in elevated cell counts. However, Lake Van deviates somewhat from this general rule. While both Lake Van drill sites reveal almost identical cell count records, there are significant differences in sulphate reduction rates (SRR), which is the quantitatively most important electron acceptor process in these sediments (Glombitza et al. 2013). This difference cannot be explained by differences in TOC content or C/N ratio. For SRR, Glombitza et al. (2013) concluded that differences in the composition and therefore availability of the sedimentary organic matter caused the different rates. Although TOC and C/N ratios were similar at both sites, there were distinct compositional differences in the dissolved organic carbon (DOM) pool, with higher concentrations of more bioavailable compounds like organic acids in sediments from Northern Basin, which also exhibited higher SRR. At Potrok Aike, no direct turnover rate measurements were taken. The only parameter that provides some information about microbial activity is ATP concentration. As metabolic activity requires ATP as an energy supply, it can be used as a measurement of total microbial activity. There was generally good agreement between total cell counts and ATP concentration, and hot spots of microbial activity could be detected (Vuillemin et al. 2013).

This study of lamina-scale microbial activity reveals that there is a much larger heterogeneity in these sediments than what can be inferred from the normal 2 cm^3 syringe samples, as the latter integrates the record over a depth interval of about 2 cm. However, the four core sections that were used for the microlayer study did not provide a clear correlation between visible sedimentary layers and cell abundance. While two laminated core section (2D9H1, 2E13H5) showed significant differences between different microlayers, no clear trend became visible, e.g. lighter-coloured layers being associated with higher cell counts or vice versa. In fact, in section 2D9H1, the two dark layers had some of the lowest cell abundances, whereas section 2D20H3 showed the opposite with dark layers having the highest cell counts, although the differences between individual layers were not significant (<1 std. dev.) in this section.

In several cases, neighbouring microlayers showed almost identical cell counts, independent of colour or sedimentology. One core section (2D20H3) did not reveal any significant difference between its layers, although the microlayers clearly differed in colouration. Interestingly, even the one core section that did not have any lamination (2D17H3) exhibited changes in cell abundance.

Due to the suboptimal preservation of the core sections and the extremely small amount of material available for analysis, it was not possible to determine other parameters like TOC or perform other more detailed organic geochemical or molecular biological analyses. Although the sample material was mechanically deformed, we consider this to have little to no effect on cell distribution, as most sedimentary microbes are not free living in the pore space, but attached to sediment particles (Fenchel 2008; Fry 1988; Kallmeyer 2011), their motility is much lower than that of planktonic organisms (Fenchel 2008; Kallmeyer 2011).

While Horsfield et al. (2002) based their concept of host and feeder lithologies on a substantial geochemical data set, we can only draw indirect conclusions about the controls on microbial abundance on the microscale. Cell abundances scatter by up to 1.5 orders of magnitude between the different microlayers. The differences that Fry et al. (2009) reported for cell abundances around interbedded lignite and sand layers in a drill core from New Zealand are somewhat lower, not exceeding one order of magnitude. Also, Coolen et al. (2002) only reported comparatively small changes in cell abundance between inside and outside of Mediterranean sapropels. They interpreted these findings, together with detailed geochemical and molecular biological analyses, as an indication for ongoing modification of several hundred thousand year old sapropels. While it has been shown in several studies that micro-organisms are able to subsist entirely on very old organic matter (Petsch et al. 2001; Røy et al. 2012), the question that is arising from the results from this study focuses on a somewhat different subject: Why does marked heterogeneity occur on such a microscale when diffusion should even out all concentration gradients? On the millimetre or submillimetre scale, the time that is necessary to transport a small molecule like acetate over a distance of a millimetre is in the range of seconds to minutes (Jørgensen 2000). Using the lowest published maintenance energy requirement values, Coolen et al. (2002) calculated that the sapropels would have been completely degraded after about 10,000 years. As the sapropels are still not fully degraded, they conclude that the in situ activity must be orders of magnitude lower. Assuming a similarly slow metabolic activity in deep Lake Van sediments, it becomes even more puzzling why there is such a heterogeneity in microbial abundance. At such low metabolic rates, diffusion will surely suffice to supply all micro-organisms in the immediate surrounding with organic substrates. The diffusivity of electron acceptors is in the same range as small organic molecules, so this is also not a limiting factor. Soltwedel and Vopel (2001) showed that bacterial abundance and biomass change in response to organism-generated habitat heterogeneity in deep-sea sediments. However, their study focused on the cm-scale, not down to such small scales as our study. Still, their findings indicate that organisms themselves can alter their environment to such an extent that it influences microbial abundance.

The fact that some microlayers exhibit massive differences in cell abundance, while others do not remains an unsolved issue. More detailed research might provide

important information about the finer controls on microbial activity and abundance in subsurface sediments.

The average of all microlayer cell counts from each core section falls very closely to the neighbouring normal cell counts. This means that the cell counts made from the 2-cm^3 plugs are integrating very well over the different heterogeneous microlayers.

Conclusions

Cell counts from the two PaleoVan ICDP drill sites offer a unique insight into the distribution of microbial cells in lacustrine sediments. As the Lake Van cell counts are in good agreement with data from Lake Potrok Aike, it appears that the deviation in depth distribution from marine cell counts represents a general feature and is not associated with site-specific conditions.

There is a considerable scatter in cell abundance between different microlayers, although no immediate correlation with certain lithologies or sediment colours could be seen. Interestingly, even non-laminated sediment sections revealed considerable scatter, while some laminated sections had relatively uniform cell counts. With the currently available data, it is difficult to identify the controls on microbial abundance in lacustrine sediment. Given the large heterogeneity, future research should include research on the microscale to obtain more detailed information about the finer mechanisms that control microbial life in deep subsurface sediments, independent of whether they are from marine or lacustrine environments.

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