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Two-dimensional distribution of living benthic foraminifera in anoxic sediment layers of an estuarine mudflat (Loire estuary, France)

A. Thibault de Chanvalon et al.

Correspondence to: A. Thibault de Chanvalon (athibaultdc@gmail.com, aubin.thibault-de-chanvalon@univ-nantes.fr)

1 Supplementary materials

S1 Solid geochemistry

The two cores were used to constrain geochemistry. They were stored at *in situ* temperature until processing, and were processed in a field laboratory. The first core was dedicated to solid phase geochemistry and microelectrode profiling (see section 2.2.3). The solid phase was characterized by total organic carbon, reactive iron, manganese and phosphorus. After profiling, the core was sub-sampled using a tube of 3 cm diameter and sliced every 2 mm until 2 cm and every 5 mm until 5 cm depth (Fig 2 A). After slicing, samples were immediately frozen with carbonic ice. Within a week, sampled were freeze-dried, the weight difference before and after freeze-drying served to calculate porosity. Next, samples were manually ground using an agate mortar and separated into two aliquots for chemical analyses.

The first aliquot of freeze-dried sediment (between 50 and 150 mg) was incubated in 10 mL of a solution of ascorbic acid (buffered at pH 8) during 24 hours to extract the reactive solid phase. This technique is commonly used (Anschutz et al., 1998; Hyacinthe et al., 2001; Hyacinthe and Van Cappellen, 2004; Kostka and Luther III, 1995) and allows to extract both amorphous Fe(III) oxyhydroxides (Kostka and Luther III, 1994) supposedly close to those reduced by microorganisms (Hyacinthe et al., 2006) as well as Mn(III) and Mn(IV) oxides (Anschutz et al., 2005). After extraction, samples were centrifuged (15 mn at 3000 rpm) and the supernatant was diluted in Ultrapure© HCl (1% weight). Next, samples were analyzed on ICP-AES (Thermo Scientific iCAP 6300 Radial), incertitude is 1, 8 and 4% for respectively iron, phosphorus and manganese (twice the relative standard deviation of ICP-AES triplicates). The second aliquot, between 1.5 and 3 mg, was used for organic carbon analysis. It was performed on EA1110 CHN/S/O (Thermo Fisher) after 1h-extraction in a HCl saturated atmosphere. Each chromatograph was inspected visually. Accuracy was verified with standards (MS-61 and B2150) and incertitude, calculated from standard deviation for ten replicates from standard MS-61, was 4.5%.

S2 1D Pore water analysis

Oxygen, dissolved iron, manganese and phosphorus were analyzed. The core dedicated to oxygen profiling and solid phase remained emerged in the *in situ* temperature tank. The

sediment water interface was roughly visually estimated during profiling. During data treatment, the interface was repositioned according to the break visible in the O_2 profile after the start of the concentration decrease. 18 oxygen profiles (each time two profiles were measured simultaneously) were realized using Clark's type electrodes (50µm tip diameter) mounted on an automated micromanipulator (Unisense©, Denmark) within the first 5 mm at a 100 µm vertical resolution. Profiling was done within 1 hour after sampling.

Diffusive Equilibrium in Thin film in one dimension probes (DET 1D, adapted from Davison and Zhang, 1994; Krom et al., 1994) were used for dissolved iron, manganese and phosphorus. Two probes were prepared from DGT-Research© supports, less than one week before deployment. Each support corresponds to 75 cells of 22 µL and has a vertical resolution of 2 mm. They were cleaned during 1 week using 10 % Suprapur Merck nitric acid and rinsed three time with milli-Q water (Millipore©). A solution (1.5% w/w) of agarose in Milli-Q water was poured into the probe, the excess gel was removed with a Teflon-coated razor blade and then covered with a PVDF hydrophilic membrane (0.2 µm size pore, Millipore©) (Metzger et al., 2007, 2014). Each probe was conserved in a wet clean plastic bag and finally bubbled with N_2 during 6h before deployment in the third core. After one night incubation in the core at *in situ* temperature, probes were retrieved and DET gel pieces were sampled using a small plastic tip and eluted in 5mL of a 0.01 mol L^{-1} suprapur© Merck nitric acid solution (dilution factor of the pore water of about 200). Iron, manganese and phosphorus were then analyzed by ICP-AES (Thermo Scientific iCAP 6300 Radial). Sodium was supposed constant through the sediment column, and used as internal standard. Incertitude is less than 10% for dissolved iron and manganese and 30% for phosphorus.

S3 2D pore water analysis

The DET 2D probe was analyzed in order to obtain the concentrations of dissolved iron, dissolved reactive phosphate (DRP) and the qualitative distribution of H_2S (Cesbron et al., 2014). The 2D DET probe was unfrozen during 10 minutes at ambient temperature; next, the plastic-coated aluminum plate was taken out and the polyacrylamide thin-film was taken off. The PVC adhesive film was scanned with a common commercial flatbed scanner (Canon Canoscan LiDE 600F) and analyzed in blue intensity (from RGB decomposition) with ImageJ© software. The unfrozen gel is laid on a white board and recovered by a reactive gel.

The reactive gel was a 0.46mm thick polyacrylamide gel incubated during 1 hour in a reactive solution containing ascorbic acid 3 10^{-2}M, sulfuric acid 5.58 10^{-1}M, potassium antimony(III) tartrate hydrate 3.2 10^{-4}M, ammonium molybdate tetrahydrate 1.86 10^{-2}M and ferrozine 1.22 10^{-2}M, final concentrations. This is an improvement compared to Cesbron et al. (2014) as only one reactive gel is made, instead of two, reducing handling time considerably.

Twenty five minutes after contact, a picture (reflectance analysis) of superposed gels was taken with a hyperspectral camera (HySpex VNIR 1600) and analyzed with the software ENVI (Environment for Visualizing Image, RSI) to obtain DRP and dissolved iron concentrations. The resolution (length of pixels) was 211*216µm^2. The HySpex VNIR 1600 camera is sensitive to 160 channels (spectral resolution of 4.5 nm), which is much more precise than the three channels of 100 nm resolution from standard RGB (Red, Green, Blue) images. Standards, made following (Cesbron et al., 2014) gave one end-member spectrum for each measured species (mean of 2470±5 pixels) and a third end-member spectrum for the background (Fig 4). Next, after logarithmic transformation of reflectance, linear combination between these three end-members applied on each pixel (of both standard and probe gels), gave the proportion of each one expressed on that pixel. For the two chemical species, this proportion was multiplied by the respective known concentration of end-members (here 18.58 µM for DRP and 253.56 µM for dissolved iron). Next, a calibration with the standard is made (six points for each species: from 3.52 to 59.31 µM for DRP and from 16.46 to 253.56 µM for iron). The exactness of the method is verified by 1) comparison between measured+calculated and real concentrations of standards (mean difference of 4,4% for iron and 7,3% for DRP), 2) the expression of background end-members from linear combination (here 0.95±0.06 compared to the theoretical value of 1.00) and 3) the error from linear combination, here of 3.4±0.5%. The estimated complete incertitude is then 9,8% for iron and 11,2% for DRP.

To compare the geochemical species distribution (at submillimeter resolution) with foraminiferal density (at centimeter resolution), a handmade R code was written allowing the decrease of chemical resolution from 0.2 mm down to 1 cm. As 1 centimeter is equal to 46.3*47.4 pixels, the code takes for each centimeter the average concentration of 46*47=2162 pixels. Thus 0.3*0.4 pixels are lost for each centimeter square which correspond to 1.27% of the surface *i.e.* 2.3 cm^2 for the entire gel. This loss is attributed to each side, and then neglected.