SM2. Sediment dark carbon fixation (DCF) and Heterotrophic Microbial Production (HMP) - Analytical procedures

The rates of the dark carbon fixation (DCF) and heterotrophic microbial production (HMP) were determined by Passos et al. (in preparation). Rates of DCF in the water column and sediments were estimated using radiolabeled-inorganic carbon incorporation (14C-bicarbonate), proposed by Steemann-Nielsen (1952) and Teixeira (1973), with slight modifications (Reinthaler et al., 2010). Three samples, one blank and two sample replicates, of 50 ml (water) and 5 g (sediment) were incubated with 10 µCi 14C-bicarbonate (specific activity 56 mCi mmol−1, Perkin Elmer, USA) in the dark at in situ temperature for 12 h. Incubations were terminated by the addition of formaldehyde (2% final concentration), and the water samples were filtered into 0.22 µm-membranes using a vacuum pump and manifold. To remove the remaining 14CO 2, the membranes and sediment samples were then exposed to concentrated HCl fumes. Afterwards, the filters and sediment were placed into scintillation vials with 5 mL of scintillation cocktail (Optiphase Hisafe 3, Perkin Elmer) at the "Multiusuário de Radioisótopos" Laboratory of IOUSP. After being left in the dark for at least 24 h, the samples were counted for 30-60 minutes per sample, respectively, with the use of a liquid scintillation counter (Perkin Elmer Tricarb 2810 TR). The resulting disintegrations per minute (DPM) were converted into production rates of carbon per volume and time (µgC.m-3.h-1) (Steemann-Nielsen, 1952; Teixeira, 1973).

The HMP in the water column and sediments were estimated using the 3H-leucine incorporation method from Kirchman et al. (1985), and modified by Smith and Azam (1992), Svensson et al. (2001) and Santoro et al. (2013). Five samples, two blanks and three sample replicates, of 1 ml (water) and 1 g (sediment) were placed into Eppendorfs with 10 nM 3H-leucine (specific activity 125.6 Ci mmol−1, Perkin Elmer, USA), and incubated in the dark at in situ temperature for 6 h. Before incubating, the sediment samples were combined with filtered water (0.22 µm membranes) and then homogenized to form a slurry. Incubations were terminated by the addition of formaldehyde (2% final concentration). At the "Multiusuário de Radioisótopos" laboratory, the samples were treated with 5% trichloroacetic acid, Milli-Q water and ethanol through successive steps of centrifugation for protein extraction. Subsequently, 1.5 ml of scintillation cocktail (Optiphase Hisafe 3, Perkin Elmer) was added to the Eppendorf, and after being left in the dark for at least 24 h, the water and sediment samples were counted for 30-60 minutes per sample, respectively, with the use of a liquid scintillation counter (Perkin Elmer Tricarb 2810 TR). The resulting disintegrations per minute (DPM) were converted into production rates of carbon per volume and time (µgC.m-3.h - 1), considering the theoretical protein/carbon conversion factor of 0.86 (Simon and Azam, 1989).