

Relative influences of submersed macrophytes and bioturbating fauna on biogeochemical processes and microbial activities in freshwater sediments

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SUMMARY

1. Invertebrates and aquatic plants often play a key role in biogeochemical processes occurring at the water–sediment interface of aquatic ecosystems. However, few studies have investigated the respective influences of plants and bioturbating animals on ecological processes (nutrient fluxes, benthic oxygen uptake, microbial activities) occurring in freshwater sediments.
2. We developed a laboratory experiment in aquaria to quantify the effects of (i) one invertebrate acting as a bioturbator (*Tubifex tubifex*); (ii) one submersed plant with a high sediment-oxygenating potential (*Myriophyllum spicatum*) and (iii) one submersed plant with a low sediment-oxygenating potential (*Elodea canadensis*).
3. The tubificid worms significantly increased the fluxes of nitrogen at the water–sediment interface (influx of nitrate, efflux of ammonium), whereas the two plant species did not have significant influences on these nitrogen fluxes. The differences in nitrogen fluxes between tubificid worms and plants were probably due to the bioirrigation process caused by *T. tubifex*, which increased water exchanges at the water–sediment interface. *Tubifex tubifex* and *M. spicatum* produced comparable reductions of nutrient concentrations in pore water and comparable stimulations of benthic oxygen uptake and microbial communities (percentages of active eubacteria and hydrolytic activity) whereas *E. canadensis* had a very weak influence on these variables. These differences between the two plants were due to their contrasting abilities to increase oxygen in sediments by radial oxygen losses (release of oxygen from roots).
4. Our study suggests that the bioirrigation process and radial oxygen loss are major functional traits affecting biogeochemical functioning at the water–sediment interface of wetlands.

Keywords: biogeochemical processes, bioturbation, microbial activity, radial oxygen loss, wetlands

Introduction

The role of biodiversity in the functioning of most ecosystems is highly influenced by the occurrence of a

single species or group of species (functional group) (Chapin, Schulze & Mooney, 1992; Hooper & Vitousek, 1997; Blondel, 2003). This functional predominance of a reduced number of species is linked to the particular functions the species play in the ecosystem (Hooper *et al.*, 2005). For instance, it has been demonstrated that the bioturbation activities of nereid species play a key role in a biogeochemical process (fluxes of nutrients) at the water–sediment interface of marine sediments

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(Emmerson *et al.*, 2001; Mermillod-Blondin, François-Carcaillet & Rosenberg, 2005a). Due to its strong bioturbation activity in sediments, this group of species masks the effects of other invertebrate species in the community. In freshwater sediments, it has also been shown that tubificid worms are a key group of bioturbators, strongly affecting organic matter processing in sediments (McCall & Fisher, 1980, Mermillod-Blondin *et al.*, 2001). Tubificid worms can stimulate microbial activities (Pelegri & Blackburn, 1995; Mermillod-Blondin *et al.*, 2005b), increase sediment permeability (Nogaro *et al.*, 2006) and modify the flux of pollutants at the water–sediment interface (Ciutat, Gerino & Boudou, 2007). Thus, regulation of ecosystem functions may be strongly influenced by specific functional groups (Giller *et al.*, 2004).

Lentic freshwater ecosystems such as cut-off channels are often characterized by a wide diversity of benthic invertebrates and macrophytes (Castella *et al.*, 1984; Godreau *et al.*, 1999; Bornette *et al.*, 2001). However, comparison of the respective influences of the main functional groups of species (plants and animals) on functioning of the water–sediment interface remains understudied. On one hand, several studies (see Krantzberg, 1985 for a review) have highlighted the significant role of macro-fauna bioturbation on biogeochemical processes occurring at the water–sediment interface (nutrient fluxes, respiration, organic matter processing). On the other hand, it has been shown that macrophytes can increase oxygenation and carbon content in sediments through radial oxygen loss (release of oxygen from roots) and root exudation, affecting microbial communities, redox reactions such as formation of iron oxyhydroxide precipitates (iron plaque) and the concentrations of several solutes (orthophosphates, ammonium, heavy metals) in pore water (Sand-Jensen, Prahl & Stokholm, 1982; Taylor, Crowder & Rodden, 1984; Risgaard-Petersen & Jensen, 1997; Mann & Wetzel, 2000; Karjalainen *et al.*, 2001). The aim of the present study was to compare the effects of species characterized by different functional traits (motility mode, capability of transferring O₂ into sediments) on biogeochemical processes to determine the main functional traits influencing nutrient dynamics at the water–sediment interface of lentic freshwater ecosystems.

Under experimental conditions, we compared the influences on water–sediment fluxes, pore water

solute and sedimentary microbial communities of three functional groups dominating cut-off channels of the Rhône river: (i) an invertebrate acting as bioturbator (*Tubifex tubifex*, McCall & Fisher, 1980); (ii) a submersed plant having a high sediment-oxygenating potential (*Myriophyllum spicatum*, L.; D. Lemoine, unpubl. data) and (iii) a submersed plant having a low sediment-oxygenating potential (*Elodea canadensis* Michx.; D. Lemoine, unpubl. data). According to the literature, we expected that the water–sediment fluxes and sedimentary processes at the water–sediment interface would be significantly influenced by two main biological traits: the mobility (bioturbation) of the organisms (animals versus plants) and the potential re-oxygenation capacity of submersed plants (*M. spicatum* versus *E. canadensis*). The mobility of organisms in sediments would determine the ability of organisms to produce fluxes of water and dissolved compounds (oxygen, nutrients) between sediment and overlying water. The re-oxygenation capacity of submersed plants would influence oxygen flux from overlying water to sediments and microbial activities in sediments.

Methods

Collection of sediment, plants and animals

The sediment and the two plant species were collected in a braided channel of the Rhône River (Lône de la Violette, 45°98'87"N and 5°28'28"E) on March 2006. The sediment was a muddy sand with a mean phi of 2.62. Contents of particulate organic carbon, particulate nitrogen and total phosphorus were 20.16 ± 1.72 , 1.019 ± 0.088 and 0.532 ± 0.050 g kg⁻¹ of dry sediment respectively. After collection, the plants were transported and kept in an aquarium at the laboratory until use. *Tubifex tubifex* were collected from an organically polluted section of the Semène River (45°20'27"N and 4°25'14"E) which is characterized by a monospecific community of tubificid worms (100% *T. tubifex*). At the laboratory, tubificid worms were kept for more than 30 days before use to acclimatize to experimental conditions (granulometry and food).

Experimental set up

Twelve aquaria (50 cm long × 5 cm wide × 40 cm deep) were used for this experiment. All aquaria

were filled with 20 cm depth of wet sediment and 20 cm of overlying water. All aquaria were immersed in water baths (80 cm long × 80 cm wide × 80 cm deep) in which a constant renewal of water was maintained (mean water renewal: 4 h). Overlying water of each aquarium was aerated with an air pump to ensure its replacement with water supplied to the bath and to keep high dissolved oxygen concentrations (8.5–9.5 mg L⁻¹) in overlying water of all aquaria. The experiment was performed in a greenhouse (Plateforme de l'IFR 41, University Lyon 1) for three months (March–May 2006) under a controlled photoperiod (16-h light/8-h dark photoperiod) and at constant water temperature (18 ± 1 °C). Aquaria were maintained for 3 weeks to stabilize before introduction of plants. Plants were installed in six aquaria (three aquaria per plant species) by introducing three non-rooted shoots of each species per aquarium. Afterwards, plants were kept 1 week to develop their root system in the sediment. This procedure allowed us to avoid disturbing the water–sediment interface during plant introduction. After this week of plant installation, we introduced tubificid worms in three other aquaria, the three remaining aquaria acting as controls. Then, a total of four treatments (three aquaria per treatment) were tested: (i) control without macroorganisms; (ii) *E. canadensis* (Hydrocharitaceae); (iii) *M. spicatum* (Holoragaceae) and (iv) *T. tubifex* (Tubificidae). For plant treatments, we used three individuals of *E. canadensis* (individual dry biomass: 1.61 ± 0.29 g) and three individuals of *M. spicatum* (individual dry biomass: 2.03 ± 0.42 g) per aquarium to provide natural densities commonly tested in experimental studies (e.g. Barrat-Segretain, 2005). In the animal treatment, 300 *T. tubifex* were introduced per aquarium. The worm density used in this experiment (12 000 individuals m²) was typical for braided channels of the Rhône River (Martinet, 1993).

During the course of the experiment (45 days after worm introduction), oxygen consumption and water–sediment fluxes of nitrate, ammonium and phosphate were measured in all aquaria. At the end of the experiment, plants and tubificid worms were collected. Biomasses and ecophysiological variables of plants [radial oxygen loss, aerobic respiration, alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH) activities] were measured to characterize growth, health and activity in the aquaria. Tubificid

worms were collected and counted at the end of the experiment to assess mortality. At the end of the experiment, wet sediment was also collected at three depths (0–1, 4–6 and 8–10 cm) to analyse microbial variables (abundance of bacteria, percentage of active eubacteria, hydrolytic activity and denitrification potentials) and determine the concentrations of nitrate, ammonium and phosphate in interstitial water.

Plant biomass and physiological indicators

Radial oxygen loss measurement. Plants were carefully uprooted, washed and transported to the laboratory. For radial oxygen loss measurements, we used a titrimetric method using Ti³⁺-citrate added to solutions bathing intact root systems (Kludze, DeLaune & Patrick, 1994; Sorrell & Armstrong, 1994). Stock solutions of the reduced titanium (III) citrate buffer were prepared in a nitrogen-purged glove bag, using commercially available titanium (III) chloride in HCl solution (Sigma-Aldrich Chemical Co., Seelze, Germany), as described by Zehnder & Wuhrmann (1976) and Kludze *et al.* (1994). TiCl₃ was added to de-oxygenated 200 mM sodium citrate, and the pH of the titanium citrate adjusted to 5 with saturated sodium carbonate (Sorrell 1999). Root systems of intact plants were placed into Ti³⁺-citrate solution (2.5 mM) for 24 h in a growth chamber (photoperiod 16 h, light intensity = 250 μmol m⁻² s⁻¹ photons at 25 °C) before measuring oxidation of Ti³⁺ at 525 nm. Root systems were scanned to evaluate their surface areas and radial oxygen losses were calculated as nmol of O₂ cm⁻² min⁻¹.

Plant growth. Fresh plants were weighed before introduction to the aquaria. At the end of the experiment, plants were washed to eliminate clay and sand and weighed to obtain final fresh masses and estimate growth during the experiment. Using wet and dry masses of 10 specimens of each plant species, we determined the relationship between wet and dry weight (DW) of the two species. This relationship was used to express plant growth as mg of dry mass of plant per day.

Root respiration. Root oxygen uptake was measured using a Clark-type oxygen electrode (Hansatech, Kings Lynn, U.K.) at 25 °C. Measurements were made

on fresh roots with citrate buffer (pH 6.5) in respirometers with 15 mL cuvettes (Hansatech, Kings Lynn, U.K.) for 45 min. Previous tests have shown no effect of radial oxygen loss measurements on subsequent photosynthesis and aerobic respiration measurements. Root aerobic respiration was determined from changes over time in the concentration of O₂ in respirometers. Aerobic respiration was expressed as nmoles of O₂ h⁻¹ g⁻¹ of fresh biomass.

Alcohol dehydrogenase and lactate dehydrogenase activities. Root samples (c. 0.5 g fresh weight) were ground with liquid nitrogen and extracted in 5 mL of extraction buffer. The homogenates were centrifuged for 20 min at 30 000 g at 4 °C. The supernatants were immediately analysed for enzyme activities. The extraction buffer for ADH (EC 1.1.1.1) and LDH (EC 1.1.1.27) contained 50 mM Tris (pH 6.8), 5 mM MgCl₂, 5 mM mercaptoethanol, 1 mM EDTA, 1 mM EGTA, 0.5 mM thiamine pyrophosphate, 15% (v/v) glycerine and 0.1 mM proteinase inhibitor [4-(2)-aminoethyl-benzensulfonyl fluoride hydrochloride] (Biemelt *et al.*, 1999). ADH and LDH activities (nkat g⁻¹ of fresh biomass) were determined at 340 nm by the oxidation of NADH at 25 °C (Kato-Noguchi, 2000; Chen & Qualls, 2003).

Benthic oxygen uptake

Measurements of benthic oxygen uptake were made in the dark (during the night) in weeks 1, 3 and 5. Each aquarium was individually sealed during oxygen measurement by stopping water renewal in the overlying water and blocking air supply. An oxygen sensor (Unisense, Aarhus, Denmark) coupled with a peristaltic pump was adapted to the aquaria to measure the O₂ decrease in the overlying water of each aquarium for 8 h (O₂ concentrations were recorded every minute). The use of a peristaltic pump enabled a continuous mixing of the water column, thereby preventing the occurrence of a vertical gradient of O₂. The water flow of the peristaltic pump was kept low (125 mL min⁻¹) to avoid inducing sediment resuspension. During measurements, dissolved oxygen concentration in aquaria never dropped below 80% of the initial concentration. Hourly-based oxygen uptakes were determined from changes over time in the concentration of O₂ in the water column (Kristensen & Hansen, 1999). Benthic oxygen uptake was

expressed as μmoles of O₂ h⁻¹ m⁻² of water–sediment interface.

Water–sediment fluxes of nutrients

Measurements of nutrient fluxes were made in the dark in weeks 2, 4 and 6. Each aquarium was isolated by stopping water renewal in the overlying water. Water samples (20 mL) were collected in the overlying water of each aquarium 0, 2, 4 and 8 h after isolation. Water samples for NH₄⁺, NO₃⁻ and PO₄³⁻ were taken using acid-washed 100 mL syringes, filtered through Whatman GF/F filters (Whatman, Maidstone, U.K.) and analysed within 24 h. Analyses of NH₄⁺, NO₃⁻ (NO₃⁻ + NO₂⁻) and PO₄³⁻ in water were performed using an automatic analyser Easychem Plus (Systea, Anagni, Italia) based on standard colorimetric methods (Grashoff, Ehrhardt & Kremling, 1983). The release rates of NH₄⁺, NO₃⁻ and PO₄³⁻ across the sediment–water interface were calculated from changes over time in the concentration of each species in the water column. The release rates were expressed as μmoles h⁻¹ m⁻² of water–sediment interface.

Vertical distribution of solutes (interstitial water) and sediment organic content

During aquaria dismantling (day 45), 40–45 mL of fresh sediment samples (with interstitial water) were collected at three depths (0–2, 4–6 and 8–10 cm) from each aquarium and centrifuged at 9168 g to collect interstitial water (supernatant). Interstitial water was filtered through Whatman GF/F filters and analysed for nitrate, ammonium and phosphate concentrations using an automatic analyser Easychem Plus (Systea). At the three sampled layers (0–2, 4–6 and 8–10 cm), per cent organic matter of sediment samples was determined by loss on ignition at 550 °C for 3 h following drying at 60 °C for 24 h.

Microbial analyses

One molecular probe was used on sediment samples to detect the Domain Bacteria (probe EUB 338, eubacteria). The use of this labelled rRNA-targeted nucleic acid probe allows an *in situ* identification of active microbial cells in their natural habitats (Amann, Glöckner & Neef, 1997). During aquaria dismantling (day 45), 2 g of wet sediment were immediately

collected from three layers (0–2, 4–6 and 8–10 cm). Sediment samples were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 h. Fixed samples were subsequently washed twice in PBS and were stored in ethanol and PBS (50 : 50) at -20°C . After storage (1 month), 0.5 g of fixed samples was homogenized in 4 mL of 0.1% pyrophosphate in PBS using a sonicator with a 2-mm-diameter probe (Sonicator XL 2020, Misonix, Inc., Farmingdale, NY, U.S.A.) at 100 W for two periods of 60 s. All homogenized samples were finally supplemented with the detergent NP-40 (Flucka, Buchs, Switzerland) to a final concentration of 0.01%. Aliquots (10 μL) of homogenized samples were spotted onto gelatine-coated slides and were hybridized with Cy3-labelled oligonucleotide probe (EUB 338) and concomitantly stained with the DNA intercalating dye DAPI (200 $\text{ng } \mu\text{L}^{-1}$, Sigma, Buchs, Switzerland) according to Schönholzer *et al.* (2002). Hybridizations were performed in 15 μL of hybridization buffer (0.9 M NaCl, 20 mM Tris/HCl, 5 mM EDTA, 0.01% SDS; pH 7.2) in the presence of 30% formamide, 1 μL of DAPI and 1 μL of the probe (25 $\text{ng } \mu\text{L}^{-1}$) at 37°C for 2 h. After hybridization, the slides were washed in buffer at 48°C for 15 min, rinsed with distilled water and air dried. Slides were mounted with Citifluor solution (Citifluor Ltd, London, U.K.) and the preparations were examined at $1000\times$ magnification with a BH2-RFCA Olympus microscope (Olympus, Tokyo, Japan) fitted for epifluorescence with a high-pressure mercury bulb (50 W) and filter sets BP 405 (for DAPI) and BP 545 (for Cy3). Bacteria from the samples were analysed in 20 fields per sample with up to 30 cells per field. Numbers of DAPI- and Cy3-bacteria were counted separately from the same field to calculate the percentages of active eubacteria (number of Cy3-bacteria hybridized with EUB338/number of bacteria counted with DAPI) from each analysed field.

Hydrolytic activity was measured using fluorescein diacetate (FDA) as substrate for hydrolases (Battin, 1997). Fresh sediment (0.4–0.6 g of wet sediment sample) was incubated in the dark with 3 mL of phosphate buffer (pH = 7.6) and 0.1 mL of FDA solution (2 mg mL^{-1}) at 15°C until the green colour of fluorescein became visible (0.5–1.5 h). Then, the reaction of FDA hydrolysis was stopped by freezing the sediment after addition of 3 mL of mercuric chloride solution (200 mg L^{-1}). Blank samples were prepared by using the same protocol on sediment

samples previously treated with acetone (50% final concentration). The absorbance of sample solutions (after incubation and fixation) was measured at 490 nm after filtration through a HAWP filter (mean pore size: 0.45 μm ; Millipore, Billerica, MA, U.S.A.). Results were expressed as micromoles of hydrolysed FDA $\text{h}^{-1} \text{g}^{-1}$ sediment DW. The average absorbance of blank samples was subtracted from that of the samples to obtain values corresponding to the hydrolytic activity mediated by microbes.

Denitrification potentials were measured at the end of the experiment following the slurry technique (Furutani, Rudd & Kelly, 1984). About 10 g of wet sediment of each sediment layer were placed in 150 mL flasks supplemented with 5 mL of a feeding solution (2.2 g L^{-1} KNO_3 , 7.5 g L^{-1} glucose and 7.3 g L^{-1} glutamic acid) to optimize microbial activity. Flasks were purged thrice with He to perform incubations under anaerobic conditions. Then, pressure inside the flask was adjusted to atmospheric. After removal of 15 mL of He from the incubation flasks, 15 mL of C_2H_2 (10% v/v final volume) was added to inhibit N_2O reductase. All incubations were carried out at 20°C , in the dark while gently shaken. At $t = 3$ and 6 h, $\text{N}-\text{N}_2\text{O}$ was measured by gas chromatography on a MTI 200 microcatharometer (Agilent, Santa Clara, CA, U.S.A.) and DW of sediment were determined after drying at 60°C to express the results as μg of N g^{-1} sediment DW h^{-1} .

Data analyses

Biomass growth and ecophysiological variables measured on plants at the end of the experiment were compared between *E. canadensis* and *M. spicatum* using Student's *t*-tests. For fluxes (O_2 , $\text{N}-\text{NO}_3^-$, $\text{N}-\text{NH}_4^+$ and PO_4^{3-}), one-way repeated ANOVAs were run using organism treatment (control, tubificid worms, *E. canadensis* and *M. spicatum*) as main factor and time (three measurements during the course of the experiment were performed for each flux) as repeated factor. If the treatment effect was significant, Tukey *post hoc* tests were performed to determine which treatments differed. For vertical profiles of solutes in pore water and bacterial measurements performed at the end of the experiment, influences of the treatments and depths (three sediment layers were sampled) were tested using a two-way ANOVA and Tukey *post hoc* tests.

When necessary, data were log-transformed before statistical analysis to meet the assumptions of homoscedasticity and normality. The microbial variable, active eubacteria, being expressed as a percentage was arcsine transformed. Statistical analyses were performed using STATISTICA 5™ (Statsoft, Tulsa, OK, U.S.A.).

Results

Visual observations and physiology of plants

At the end of the experiment, 80–90% of *T. tubifex* were recovered from the aquaria, indicating low mortality. In aquaria containing tubificid worms, a dense network of galleries was observed in the top 5 cm of the sediment. *Myriophyllum spicatum* had a strong and deep fasciculate root system (15 cm deep) in the sediment and caused re-oxidation of the sediment through radial oxygen loss (Fig. 1a). In comparison, roots of *E. canadensis* were restricted to the top 5 cm of the sediment and this plant exhibited a lower radial oxygen loss than *M. spicatum* (Student's *t*-test, $t_{(0.05,16)} = 6.4$, $P < 0.001$). Biomass growth and aerobic respiration were significantly higher in *M. spicatum* than in *E. canadensis* (Fig. 1b,c, Student's *t*-test, $t_{(0.05,16)} = 7.1$ for biomass growth and $t_{(0.05,16)} = 18.3$ for respiration, $P < 0.001$). In contrast, *E. canadensis* exhibited significantly higher fermentative activities (ADH and LDH) in roots than *M. spicatum* (Fig. 1d,e, Student's *t*-tests, $t_{(0.05,16)} = 6.0$ for ADH and $t_{(0.05,16)} = 11.31$ for LDH, $P < 0.001$).

Fluxes

Benthic oxygen uptake measurements showed that macroorganisms tended to increase the global respiration of the system (Fig. 2a). Significant differences in oxygen uptake were measured among the treatments (one-way repeated ANOVA, $F_{(3,8)} = 18.4$, $P < 0.001$, treatment effect): *M. spicatum* and *T. tubifex* produced a higher benthic oxygen uptake than the control treatment (Tukey *post hoc* tests, $P < 0.005$ for comparison between *M. spicatum* and control treatments, $P < 0.005$ for comparison between *E. canadensis* and control treatments) whereas *E. canadensis* did not significantly influence oxygen uptake (Tukey *post hoc* test, $P > 0.13$). Moreover, benthic oxygen uptake increased during the experiment in all treatments

except in the treatment with *E. canadensis*, which did not show a significant evolution of oxygen uptake during the experiment (Fig. 2a). This difference in oxygen uptake evolution among treatments led to a significant statistical interaction between time and treatment (one-way repeated ANOVA, $F_{(3,8)} = 51.6$, $P < 0.001$, interaction 'time × treatment').

Nitrate flux measurements showed an influx of nitrate from overlying water to the sediment in all aquaria. Nitrate uptake was increased by the two plants and *T. tubifex* at week 2 (Fig. 2b). This effect of plants was not observed in the two other weeks of measurement, leading to overall non-significant influences of the two plants on nitrate fluxes during the experiment (Tukey *post hoc* tests, $P > 0.8$ for comparison between *E. canadensis* and control treatments, $P > 0.6$ for comparison between *M. spicatum* and control treatments). In contrast, tubificid worms stimulated nitrate uptake during the experiment (Tukey *post hoc* test, $P < 0.05$ for comparison between *T. tubifex* and control treatments).

Effluxes of ammonium from the sediment to the overlying water were measured for all treatments (Fig. 2b). Throughout the experiment, *T. tubifex* increased ammonium effluxes from the sediment compared to the other treatments (one-way repeated ANOVA, $F_{(3,8)} = 19.2$, $P < 0.001$, treatment effect). The net nitrogen flux at the water–sediment interface indicated an influx of nitrogen from overlying water to the sediment, N–NO₃ influxes being two- to threefold higher than N–NH₄ release rates from the sediment. Fluxes of phosphate between the sediment and the overlying water were very low during the experiment (0.1–0.8 μmoles h⁻¹ m⁻²) and were not significantly affected by the macroorganisms (one-way repeated ANOVA, $F_{(3,8)} = 3.0$, $P > 0.09$, treatment effect).

Vertical distribution of solutes (interstitial water)

Nitrate concentrations were not significantly different among depths (Fig. 3a, two-way ANOVA, $F_{(2,24)} = 0.24$, $P > 0.7$, depth effect) and were not influenced by macroorganisms (two-way ANOVA, $F_{(3,24)} = 0.19$, $P > 0.9$, treatment effect). Concentrations of ammonium and phosphate significantly increased with sediment depth (Fig. 3b,c, two-way ANOVA, $F_{(2,24)} = 122.7$ for ammonium and $F_{(2,24)} = 138.4$ for phosphate, $P < 0.001$, depth effect). There was a

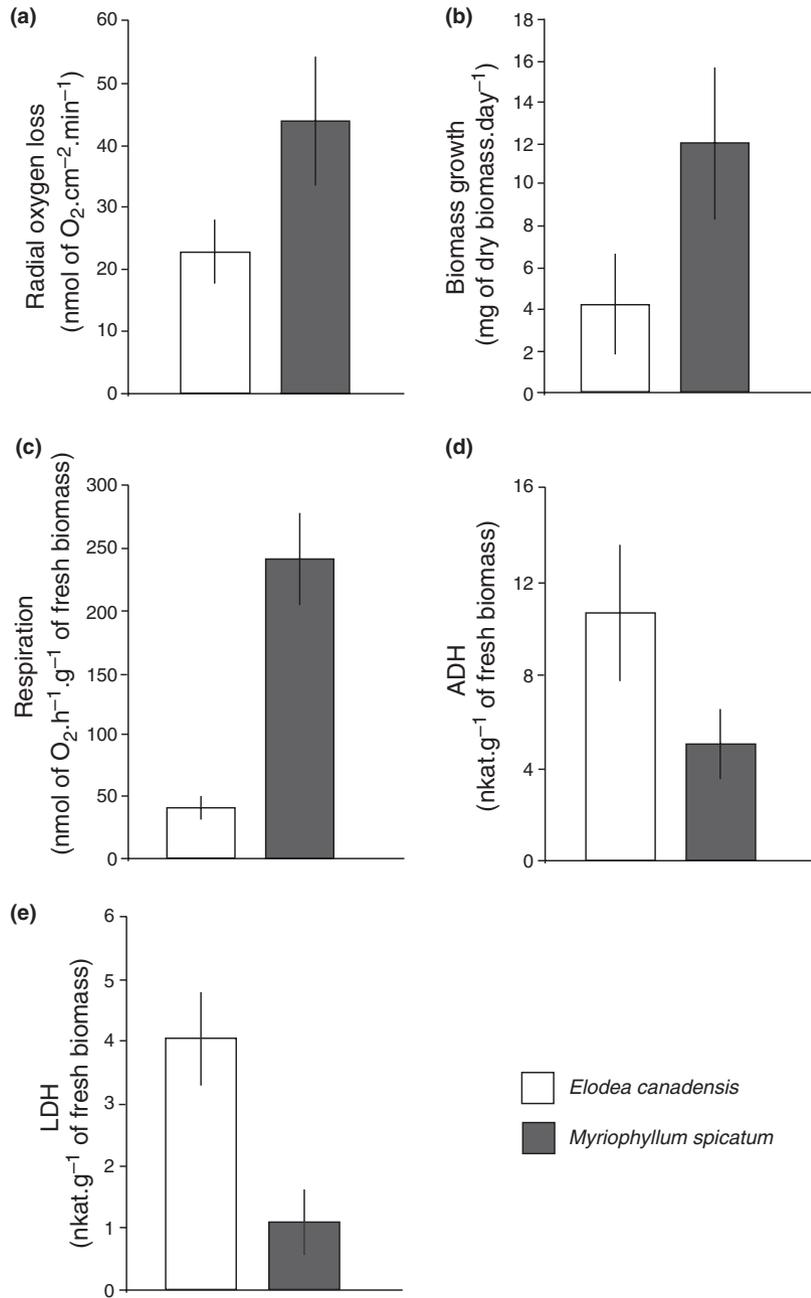


Fig. 1 (a) Radial oxygen loss, (b) biomass growth, (c) respiration rate, (d) ADH activity and (e) LDH activity in *Elodea canadensis* and *Myriophyllum spicatum* during the experiment (mean \pm SD, $n = 9$).

significant difference in ammonium concentrations in pore water among treatments (two-way ANOVA, $F_{(3,24)} = 13.0$, $P < 0.001$, treatment effect). *Tubifex tubifex* and *Myriophyllum spicatum* significantly reduced ammonium concentrations compared to the control treatment (Tukey *post hoc* tests, $P < 0.001$ for comparison between *T. tubifex* and control treatments, $P < 0.001$ for comparison between *M. spicatum* and

control treatments) whereas *E. canadensis* did not have a significant influence on ammonium in pore water (Tukey *post hoc* tests, $P > 0.1$). Figure 3b also shows that all macroorganisms tended to reduce ammonium concentrations in pore water collected in the top layer of sediments whereas such a trend was only observed with *M. spicatum* and *T. tubifex* in the deeper layers of the sediment. Phosphate concentrations in pore water

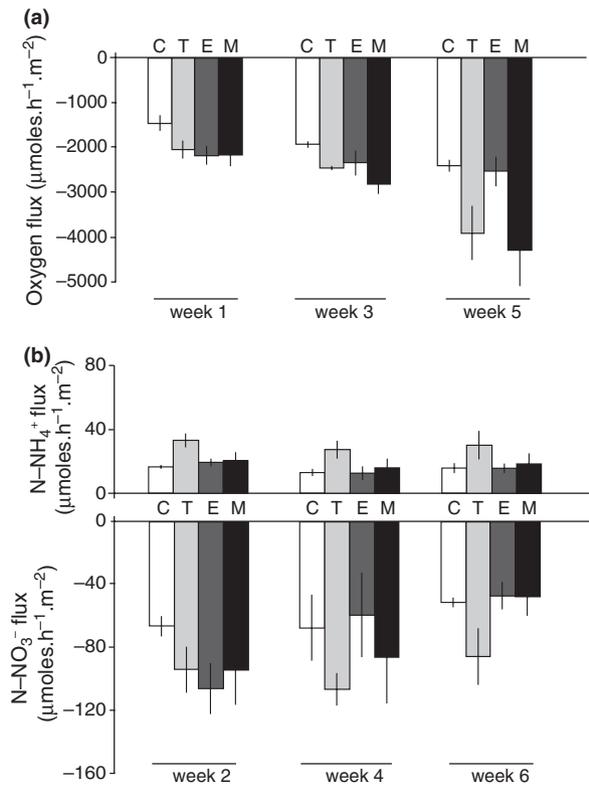


Fig. 2 Fluxes of (a) dissolved O₂ and (b) N (N-NH₄⁺ and N-NO₃⁻) measured in aquaria for the four treatments on three dates during the experiment (means ± SD, *n* = 3 aquaria per treatment). C, control; T, *Tubifex tubifex*; E, *Elodea canadensis*; M, *Myriophyllum spicatum*. Positive values indicate an efflux from the sediment to the overlying water and negative values indicate an influx from the overlying water to the sediment.

were significantly influenced by treatments (Fig. 3c, two-way ANOVA, $F_{(3,24)} = 3.2$, $P < 0.05$, treatment effect). This result was due to *M. spicatum*, which significantly reduced phosphate concentrations in the deepest sediment layer (Tukey *post hoc* test, $P < 0.02$, effect of *M. spicatum*) whereas the two other species did not have significant effects on phosphate concentrations in pore water (Tukey *post hoc* tests, $P > 0.4$ for comparison between *E. canadensis* and control treatments, $P > 0.8$ for comparison between *T. tubifex* and control treatments).

Microbial analyses

The abundance of bacteria ranged from 5×10^8 to 7×10^8 bacteria g⁻¹ of dry sediment. Abundance did not significantly vary among depths (Fig. 4a, two-way ANOVA, $F_{(2,24)} = 0.9$, $P > 0.4$, depth effect) or among treatments (two-way ANOVA, $F_{(3,24)} = 1.1$, $P > 0.3$, treatment effect). The percentage of active eubacteria and hydrolytic activity significantly decreased with depth (Fig. 4b,c, two-way ANOVAS, $F_{(2,24)} = 137$ for active eubacteria and $F_{(2,24)} = 29.7$ for hydrolytic activity, $P < 0.001$, depth effect) and were influenced by the occurrence of macro-organisms (two-way ANOVAS, $F_{(3,24)} = 11.7$ for active eubacteria and $F_{(3,24)} = 12.3$ for hydrolytic activity, $P < 0.001$, treatment effect). The three macro-organisms did not have the same influence on

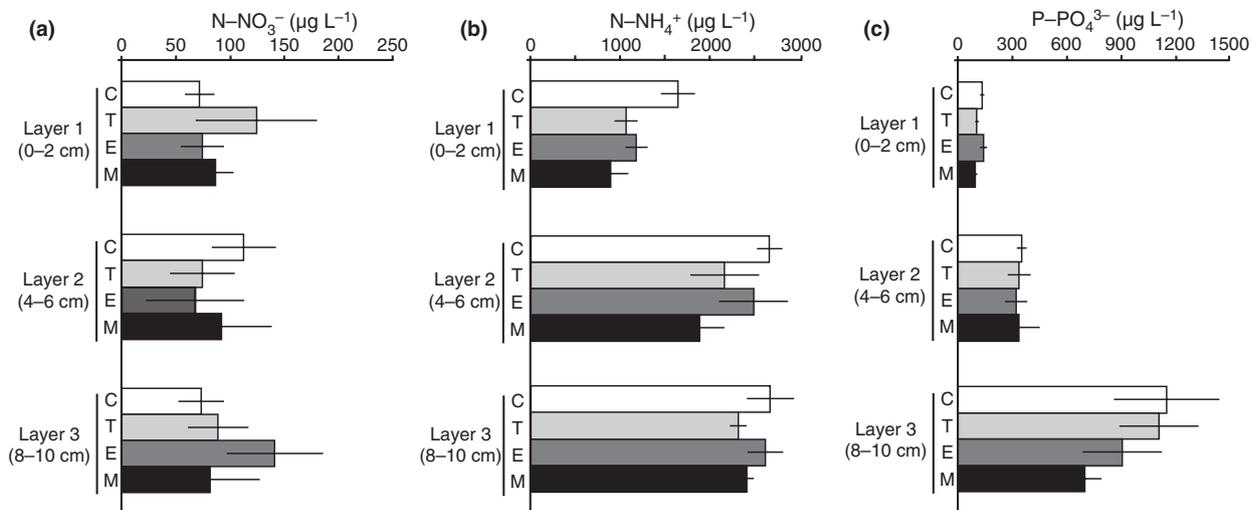


Fig. 3 Concentrations of (a) N-NO₃⁻, (b) N-NH₄⁺ and (c) P-PO₄³⁻ measured in pore water in three sediment layers of the aquaria for the four treatments (mean ± SD, *n* = 3 aquaria per treatment). C, control; T, *Tubifex tubifex*; E, *Elodea canadensis*; M, *Myriophyllum spicatum*.

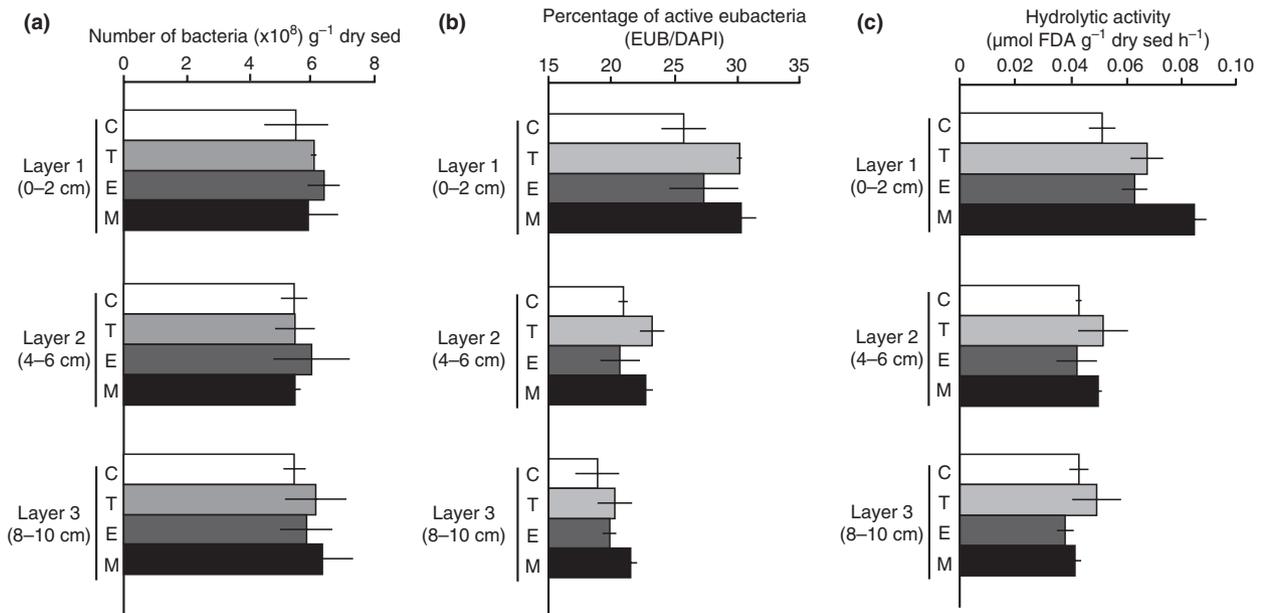


Fig. 4 (a) Number of bacteria, (b) percentage (EUB/DAPI) of active eubacteria and (c) hydrolytic activity measured in three sediment layers of the aquaria for the four treatments (mean \pm SD, $n = 3$ aquaria per treatment). C, control; T, *Tubifex tubifex*; E, *Elodea canadensis*; M, *Myriophyllum spicatum*.

percentages of active bacteria and hydrolytic activity in the sediment: *M. spicatum* and *T. tubifex* significantly increased the percentage of active bacteria (Tukey *post hoc* tests, $P < 0.001$ for comparison between *M. spicatum* and control treatments, $P < 0.005$ for comparison between *T. tubifex* and control treatments) and hydrolytic activity (Tukey *post hoc* tests, $P < 0.001$ for comparison between *M. spicatum* and control, $P < 0.001$ for comparison between *T. tubifex* and control treatments) whereas *E. canadensis* did not significantly influence these microbial variables in comparison with the control (Tukey *post hoc* tests, $P > 0.5$ for active eubacteria and $P > 0.9$ for hydrolytic activity). Statistical results also showed that the influences of *M. spicatum* and *T. tubifex* were the highest in the top sediment layer, no significant effect of these two macroorganisms being measured at the deepest sediment layer (Tukey *post hoc* tests, $P > 0.3$ for active eubacteria and $P > 0.9$ for hydrolytic activity, comparison between *M. spicatum* and control treatments; $P > 0.9$ for active eubacteria and $P > 0.8$ for hydrolytic activity, comparison between *T. tubifex* and control treatments). Moreover, *M. spicatum* produced a higher hydrolytic activity in the top sediment layer of the sediment than the treatment with *T. tubifex* (Tukey *post hoc* test, $P < 0.05$, comparison between *M. spicatum* and *T. tubifex* treatments).

Denitrification potentials and organic matter in the sediment were not significantly affected by the occurrence of macroorganisms (Fig. 5a,b, two-way ANOVAs, $F_{(3,24)} = 1.3$ and $P > 0.25$ for denitrification potentials, $F_{(3,24)} = 2.2$ and $P > 0.1$ for organic matter, treatment effect). Denitrification potentials sharply decreased with depth from the top sediment layer to the medium sediment layer (Fig. 5a, two-way ANOVA, $F_{(2,24)} = 34.7$, $P < 0.001$, depth effect). Such vertical distribution was clearly linked to the vertical distribution of organic matter in the sediment which had the same pattern (Fig. 5b, two-way ANOVA, $F_{(2,24)} = 69.0$, $P < 0.001$, depth effect).

Discussion

Realism of the experiment

It is important to be cautious when considering the applicability of our results to the natural environment. The influences of plants and invertebrates at the water–sediment interface of benthic systems greatly depend on hydrodynamic conditions (Palmer *et al.*, 1997). For instance, the effects of invertebrate bioturbation (sediment reworking, biogenic structure building, bioirrigation) on microbial processes differ in diffusion-dominated (fine sediments, low intersti-

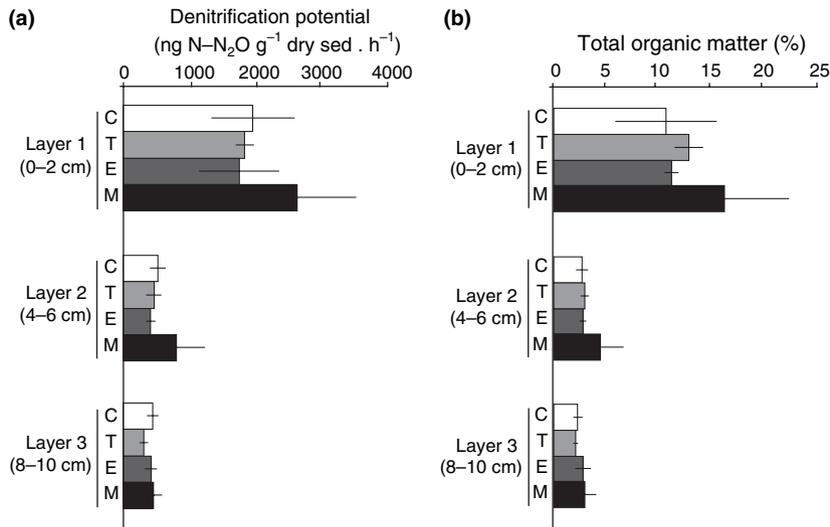


Fig. 5 (a) Denitrification potential and (b) percentage of total organic matter measured in three sediment layers of the aquaria for the four treatments (mean \pm SD, $n = 3$ aquaria per treatment). C, control; T, *Tubifex tubifex*; E, *Elodea canadensis*; M, *Myriophyllum spicatum*.

tial flow rates) and advection-dominated (coarse sediments, advection of water into sediments) benthic environments (Mermillod-Blondin & Rosenberg, 2006). In the same way, the effect of macrophytes at the water–sediment interface also depends on hydrodynamic context. For instance, macrophytes can reduce hydrodynamic energy from currents and waves, inducing the deposit of fine sediment and the anoxification of sediments (Neira *et al.*, 2006; Bos *et al.*, 2007). This effect is therefore strongly dependent on hydrodynamic conditions at the water–sediment interface. Our experiment was designed to reproduce a diffusion-dominated benthic system where water dynamics were low at the water–sediment interface. Because of the context-specific effects of the organisms, our results should be applicable to water–sediment interfaces of cut-off channels of rivers, lakes and ponds.

Influence of mobility on water–sediment fluxes

Our results corroborated our hypothesis that mobility has a major influence on exchanges of water and solutes between the sediment and the overlying water. *Tubifex tubifex* produced higher fluxes of nitrogen at the water–sediment interface (influx of nitrate, efflux of ammonium) than either plant species. Such results suggest that animals have a higher potential for increasing water–sediment fluxes at the interface than macrophytes. In both marine and lake sediments, the influence of invertebrates on water–sediment fluxes has often been related to their

bioirrigation potential (Sandnes *et al.*, 2000; Svensson, Enrich-Prast & Leonardson, 2001; Vopel, Thistle & Rosenberg, 2003). For instance, Mermillod-Blondin *et al.* (2004) determined that the fluxes of ammonium at the water–sediment interface were positively correlated to the water fluxes induced by animals (measured using bromide as a water tracer). In our experiment, both the movements of *T. tubifex* in their galleries and the occurrence of a dense gallery network, which increased the exchange area between sediment and overlying water, stimulated nitrogen fluxes at the water–sediment interface. In contrast, macrophytes did not increase the flux of water between the benthic and the pelagic habitat: they did not bioirrigate the sediment and their mode of root colonization did not increase the water–sediment exchange area, the structures produced by plants being filled with roots.

Influence of mobility and radial oxygen loss on benthic oxygen uptake and concentration of nutrients in pore water

Despite differences in nitrogen fluxes induced by *M. spicatum* and *T. tubifex*, these two taxa produced a comparable stimulation of benthic oxygen uptake. The physiological respiration of macroorganisms could only explain <8% (*T. tubifex*) and 2% (*M. spicatum*) of oxygen uptake stimulations measured at the end of the experiment (week 6). Thus, benthic oxygen uptake stimulations were mainly due to a stimulation of microbial processes as revealed by the

positive effects of tubificid worms and *M. spicatum* on the percentages of active bacteria and FDA hydrolysis. The similar benthic oxygen fluxes measured with these two organisms resulted from two different mechanisms: the stimulation of benthic oxygen fluxes by *T. tubifex* was due to an increase of water-sediment exchanges (Krantzberg, 1985) whereas the benthic oxygen uptake stimulation due to *M. spicatum* was linked to a transfer of dissolved oxygen from the leaves to the roots (expressed as radial oxygen loss).

The transfer of dissolved oxygen from the overlying water to the sediment clearly explained the stimulation of microbial processes in the uppermost layer but also the lower pore water concentrations of ammonium and orthophosphate measured with *M. spicatum* and the lower pore water concentrations of ammonium measured with *T. tubifex* in comparison with values reported in the control. As demonstrated by several authors (Wigand, Stevenson & Cornwell, 1997; Gilbert, Aller & Hulth, 2003; Lewandowski, Laskov & Hupfer, 2007), the presence of dissolved oxygen in organic sediments produced an oxidation of ammonium to nitrate (by nitrification) and a binding of orthophosphate with sediments. In the sediment, the reduction of ammonium concentration due to macroorganisms was not linked to production of nitrate. This result was probably due to high denitrification rates occurring in the sediment. If nitrification was stimulated by animals and plants, the nitrate so produced was apparently denitrified or used by organisms (microorganisms or plants) subsequently to nitrification. Similarly, Karjalainen *et al.* (2001) observed that *Lobelia dortmanna* L., a plant that is very efficient at releasing oxygen into sediment, had no influence on nitrate concentration in interstitial water. The occurrence of a strong coupling between nitrification and denitrification in our experiment was supported by measurements showing that sediments acted as a nitrogen sink rather than a source of nitrogen for overlying water. Arango *et al.* (2007) showed that the quantity of benthic organic matter was a key driver of denitrification in streams characterized by high nitrate concentrations. In our system, the large amount of organic matter present in the uppermost sediment layer also had a strong positive effect on denitrification potentials. As a consequence, the possible accumulation of nitrate in this uppermost sediment layer, recognized as the main nitrification zone in lentic

systems (Aller, 1988; Kristensen, 2000), was reduced due to strong denitrification activity. Such occurrence of aerobic and anaerobic processes in the same sediment layer was linked to the heterogeneity of sediments, which induced the coexistence, at a millimetre scale, of both aerobic and anaerobic micro-zones.

Influence of radial oxygen loss capacity on biogeochemical processes

Our results supported our second hypothesis: radial oxygen loss due to plants was a major process for sedimentary microbial activity and concentrations of solutes in pore water. Whereas *M. spicatum* stimulated microbial activities and benthic oxygen uptake, *E. canadensis* which exhibited a twofold lower radial oxygen loss had a very small effect on the system. The high radial oxygen loss and the strong and deep fasciculate root system (15 cm deep) exhibited by *M. spicatum* also produced a reduction of ammonium and orthophosphate concentrations in pore water compared to the other plant species. In our experiment, the radial oxygen losses of the two species were positively linked to their abilities to develop and grow in the organic sediment used. The positive effect of radial oxygen loss and transfer of dissolved oxygen to sediments by plants was probably due to a reduction of anaerobic conditions by root system, limiting the production of toxic products such as ammonia (Smolders *et al.*, 1996) and the occurrence of fermentative processes that are poorly efficient for plant growth (John & Greenway, 1976). In our experiment, the differences in radial oxygen loss between the two plant species could explain why fermentative activities (ADH and LDH) were higher in roots of *E. canadensis* than *M. spicatum*. As a consequence, the growth of *E. canadensis* was reduced and this species exhibited lower photosynthetic and respiration activities than *M. spicatum*.

Importance of functional traits to ecosystem functioning

Our comparative study on the effects of plants and animals in organic sediments clearly demonstrated that the effects of organisms on ecosystem processes are linked to biological traits such as bioturbation mode or sediment-oxygenating potential. For instance, mobility which increases water exchange at the interface (bioirrigation process) had a major

influence on nutrient exchanges between water and sediments (e.g. flushes of nutrients from sediments). Radial oxygen losses by plants determined the degree of re-oxidation of sediments and, consequently, the effects of plants on sedimentary microbial processes and concentrations of solutes in pore water. Moreover, oxygen releases by roots that reduced anaerobic conditions in sediments had a very significant influence on plant growth. It is clear that quantification of the role of macroorganisms in wetlands needs to consider the functional traits having the strongest influence on biogeochemical processes (C and N cycles). As determined in other aquatic ecosystems (Pearson, 2001; Gerino *et al.*, 2003; Norling *et al.*, 2007), we need detailed information on ecophysiology, feeding mode, mobility mode, degree of mobility and mode of feeding habit of organisms to link benthic communities to ecosystem functioning.

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