Short communication

Effects of increasing organic matter loads on pore water features of vegetated (*Vallisneria spiralis* L.) and plant-free sediments

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ABSTRACT

The effects of organic enrichment on pore water chemistry of bare and *Vallisneria spiralis* L. colonized sediments were investigated. Substrates of three organic levels were created by adding different amounts of powdered fish feed (0, 5 and 10 g/l of sediment, respectively) to homogenized sediment and incubated with and without plants. Redox potential (Eh), reduced compounds (CH_4, Fe^{2+}, Mn^{2+}) and nutrients (PO_4^{3-}, NH_4^+) were analyzed at time zero and after 6, 10, 13 and 17 days. In control microcosms *V. spiralis* sediments displayed significantly higher Eh and lower CH_4, Fe^{2+}, Mn^{2+}, PO_4^{3-} and NH_4^+ concentrations than bare ones. In organic enriched microcosms methanogenesis became the main degradation pathway when other electron acceptor pools were depleted. However, lower levels of interstitial Fe^{2+}, Mn^{2+} and PO_4^{3-} were found in vegetated sediments compared to bare ones and this difference was maintained during the whole experimental time. Root oxygen release in the rizosphere seemed to be the main responsible of this outcome, as also suggested by the nitrification potential assay, indicating the maintenance of oxic microniches. *V. spiralis* can act as an *engineer species* in urban, organic impacted sediments due to its high tolerance against reduced conditions, which makes this macrophyte an interesting option in aquatic ecosystems restoration programs.

1. Introduction

Freshwater ecosystems in human-impacted watersheds are subjected to increasing rates of nutrient and organic matter (OM) supply (Nixon, 2009). Organic enrichment from enhanced primary production or terrestrial inputs (fertilizers, domestic sewage and aquaculture waste) accelerates oxygen consumption and build-up of potentially toxic compounds in pore water which becomes a hostile environment for roots. Radial oxygen loss (ROL) acts as a buffer against reduced conditions (Colmer, 2003; Lai et al., 2012), although above certain OM levels, it may not be sufficient to maintain an oxidized rhizosphere and root tissues exposure to phytotoxins can occur (van Wijck et al., 1992; Wu et al., 2009). Species-specific differences in macrophytes' response along chemically reduced pore water gradients are evident and some plants seem to be more tolerant to organic substrates (Wang and Yu, 2007; Pulido et al., 2010; Yu et al., 2010). The introduction of tolerant submersed vegetation in eutrophic OM-impacted aquatic bodies can improve the environmental state by the re-establishment of key ecosystemic functions (Chen et al., 2009; Zhang et al., 2010). The

comparison of pore water chemical environment in vegetated and unvegetated sediment, along steep OM gradients, can give multiple useful indications with this respect. In fact, chemical pore water composition provides evidences about the main microbial processes and the degree of coupling between reductions and oxidations. A significant difference in the presence or absence of plants suggests an active role of primary producers in the detoxification.

The ability of *Vallisneria* spp. (Hydrocharitaceae), a perennial freshwater vascular plant, to tolerate a wide range of environmental conditions makes it a suitable pioneer species for inclusion in restoration projects of eutrophic perturbed sites. A number of studies suggested that this genus performs photosynthesis in low light conditions (Harley and Findlay, 1994), grows in nutrient-rich waters (Xie et al., 2005), tolerates organic substrates (Xiao et al., 2006; Wang and Yu, 2007) and modifies sedimentary features, with positive feedbacks for aquatic ecosystem restoration (i.e. regeneration of ferric iron buffer and phosphorus retention in sediment, stimulation of coupled nitrification–denitrification with net nitrogen loss) (Wigand et al., 2000; Li et al., 2009; Pinardi et al., 2009). *Vallisneria spiralis* L. can colonize gravel or sandy bottom in lotic ecosystems (OM < 1%) as well as organic fluffy sediments in eutrophic ponds (OM > 10%) and such plasticity is likely due to its capacity to detoxify pore water. This is suggested by the markedly different color of sediment adjacent to roots compared to the

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Table 1
Results of the three-way ANOVA performed to test the effects of OM level, presence of plant and time on pore water chemistry.

Factor	df	Eh F	CH_4 F	Fe^{2+} F	Mn^{2+} F	PO_4^{3-} F	NH_4^+ F
OM level	2	1336.18[***]	108.29[***]	1915.22[***]	2079.29[***]	479.63[***]	4932.80[***]
Presence of plant	1	174.16[***]	103.12[***]	100.82[***]	11.22[**]	285.71[***]	17.04[***]
Time	3	27.44[***]	0.89	36.38[***]	38.16[***]	12.16[***]	211.66[***]
OM level × presence of plant	2	102.74[***]	43.08[***]	0.35	4.53[*]	2.76	62.41[***]
OM level × time	6	22.90[***]	7.77[***]	25.38[***]	28.10[***]	1.30	60.18[***]
Presence of plant × time	3	9.43[***]	1.20	1.28	6.12[**]	2.09	0.33
OM level × presence of plant × time	6	33.10[***]	1.55	1.61	3.30[**]	1.28	1.22
Error	48						

[*] $p < 0.05$.
[**] $p < 0.01$.
[***] $p < 0.001$.

surrounding sediment and the absence of methane in interstitial water (Pinardi et al., 2009; Racchetti et al., 2010; Ribaudo et al., 2011).

The object of our research was to evaluate how the organic enrichment affects the redox related processes in bare and *V. spiralis* colonized sediments. We hypothesize significant differences in pore water chemistry as a result of less reduced conditions in vegetated sediments compared to the bare ones and we predict that such differences are a function of sedimentary OM content. To verify this assumption, we monitored pore water chemistry in plant-covered and plant-free sediment microcosms containing increasing OM amounts, simulating a strong eutrophication gradient.

2. Materials and methods

2.1. Experimental setup

The experiment was carried out during the exponential growth phase of *V. spiralis* (March, 2011) and lasted 17 days. Plants, sediment and water were collected from a shallow water eutrophic site of the Mincio River (Massimbona location, Northern Italy). Details of the study area are reported by Pinardi et al. (2009). About 100 healthy shoots were sampled randomly minimizing root damage and over 10 l of unvegetated sediment were collected from the top horizon (0–10 cm). Within 2 h from the collection, the sediment was sieved through a 2 mm mesh to remove coarse plant debris, macrofauna and stones, and homogenized. Average organic matter, C, N and P content were 9.90, 1.72, 0.56 and 0.06%, respectively (Pinardi et al., 2009; Racchetti et al., 2010). In the laboratory the sieved sediment was divided into three equally sized portions: one was not manipulated and served as control and the others were enriched with two different amounts of labile organic matter. Pellets of commercially available fish feed (49% organic C, 8% organic N and 1% organic P) were dried at 70 °C and ground to powder: 5 and 10 g of fish feed per liter of sediment were added to the two aliquots of sieved sediment, respectively, then carefully homogenized. Sediments of the three organic levels (C, +5 and +10) were transferred into cylindrical Plexiglas tubes (i.d. 3 cm, height 10 cm) and 24 microcosms were set up for each level, for a total of 72 microcosms. Aliquots of each type of sediment were collected for initial pore water characterization (see Section 2.2). Shoots were carefully washed several times with river water to remove the original sediment from roots and epiphytes from leaves. In 12 tubes of each OM level, one shoot randomly selected was transplanted. Microcosms were then maintained in tanks with river water at the temperature of 18–20 °C and subjected to a 12/12 h light/dark cycle at an irradiance of about 250 µmol photons m^{-2} s^{-1} (Photosynthetically Active Radiation, PAR) by means of fluorescent tubes. Water

temperature was measured with a YSI Multiple Probe (mod 556) and PAR intensity with a LI-192 Underwater Quantum Sensor and a LI-250A Light Meter (Li-Cor, Lincoln, NE, USA). Irradiance intensity and light/dark cycle length reflected the in situ average values for the sampling period. Water in the incubation tanks was stirred continuously with aquarium pumps and replaced weekly with fresh river water to prevent algal growth. Microcosms were exposed to the same water to eliminate possible effects of nutrient availability resulting from the release from enriched sediments.

During the 17-day incubation, 3 replicates for each experimental condition (C_V, control vegetated sediment; C_B, control bare sediment; +5$_V$, 5 g enriched vegetated sediment; +5$_B$, 5 g enriched bare sediment; +10$_V$, 10 g enriched vegetated sediment; +10$_B$, 10 g enriched bare sediment) were sacrificed after 6, 10, 13 and 17 days with the purpose of monitoring pore water chemistry changes. The first microcosm sacrifice was performed after 6 days, in order to allow the re-establishment of solute gradients in interstitial water and to give plants enough time to recover from the transplant stress. Sediment extrusion was performed in a glove-bag under N_2 atmosphere. Pore water was extracted by mixing and transferring sediment to 50-ml tubes followed by centrifugation for 10 min at about 3000 rpm. The supernatant was analyzed for Eh, CH_4, Fe^{2+}, Mn^{2+}, PO_4^{3-} and NH_4^+.

2.2. Pore water analyses

Redox potential was measured with a potentiometric electrode (Radiometer, DK) connected to a high impedance mV-meter (Crison micro pH 2002, ES). CH_4 was analyzed via gas chromatography (Fisons 9000 series) with flame ionization detector. Fe^{2+} and Mn^{2+} were determined by flame atomic absorption spectroscopy (Varian model AA240) after pore water filtration (Whatman GF/F) and acidification. PO_4^{3-} and NH_4^+ were analyzed with standard spectrophotometric techniques after pore water filtration (A.P.H.A., 1981).

2.3. Sediment analyses

Potential nitrification rates were measured on sediments from each experimental condition at the end of the incubation, according to Caffrey et al. (2007). A known volume of sediment (~1 ml) was added to 20 ml of filtered water from the site, enriched with NH_4Cl to give a final concentration of 100 µM and incubated in dark aerobic condition. After 20 h, an aliquot of the slurry was centrifuged at 3000 rpm for 10 min, filtered and analyzed for NO_3^- plus NO_2^- via standard spectrophotometric techniques (A.P.H.A., 1981). Potential nitrification rates were calculated from accumulation of $NO_2^- + NO_3^-$ over time and expressed in terms of sediment dry weight (DW).
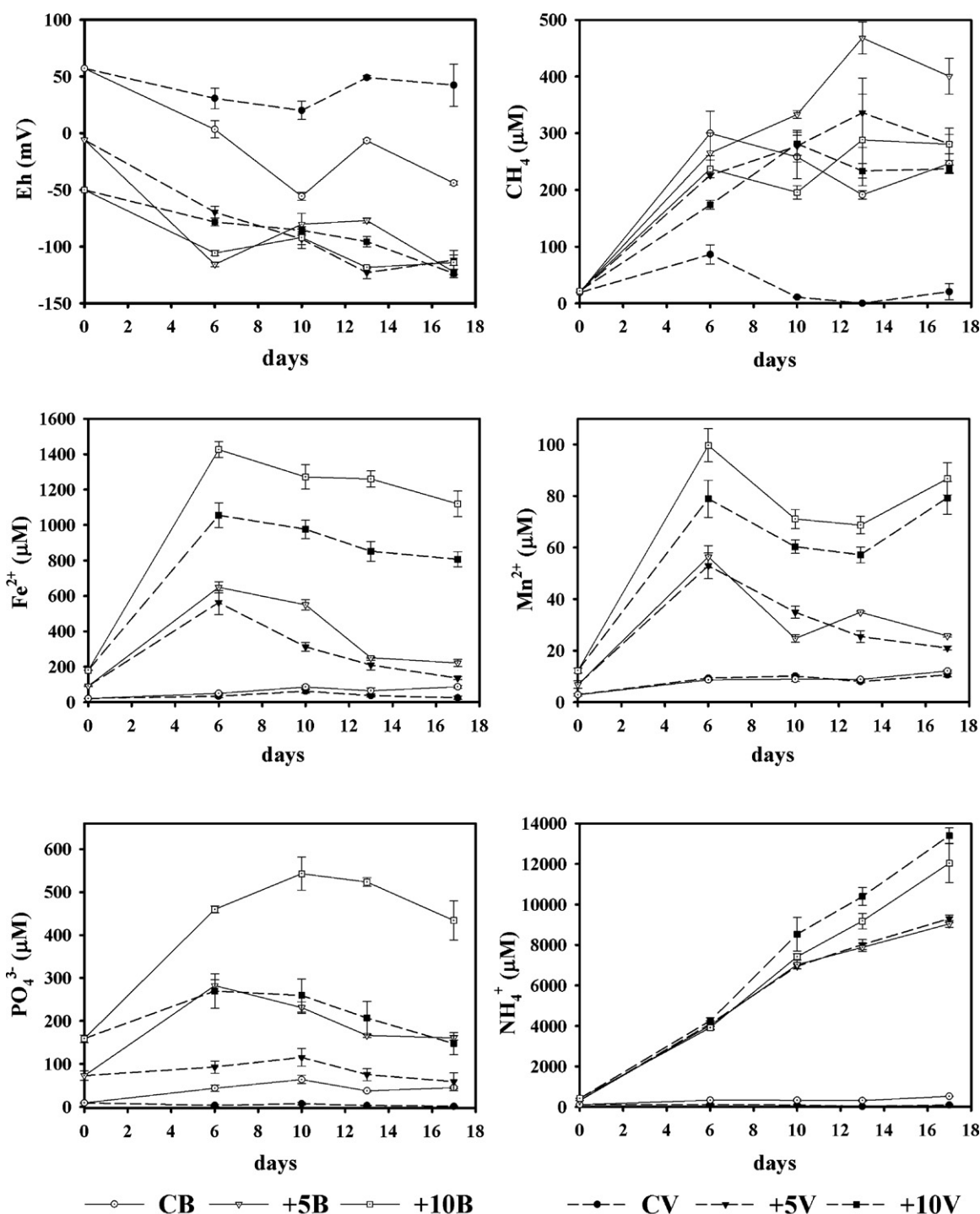
Fig. 1. Pore water features. Trends of pore water Eh, CH_4, Fe^{2+}, Mn^{2+}, PO_4^{3-} and NH_4^+ measured in each experimental condition during the 17-day incubation period (C_V, control vegetated sediment; C_B, control bare sediment; $+5_V$, 5 g enriched vegetated sediment; $+5_B$, 5 g enriched bare sediment; $+10_V$, 10 g enriched vegetated sediment; $+10_B$, 10 g enriched bare sediment; average ± standard error, $n = 3$).

2.4. Statistical analyses

The effect of OM enrichment, presence of *V. spiralis* and time on pore water features (Eh, CH_4, Fe^{2+}, Mn^{2+}, PO_4^{3-}, NH_4^+) was tested by means of a three-way ANOVA. Potential nitrification rates was tested via two-way ANOVA, with OM enrichment and presence of *V. spiralis* as factors. Correlative relationships among pore water variables were identified using the Pearson's Correlation Coefficient (r). Differences were considered not significant if $p > 0.05$.

Normality and homoscedasticity of data were examined and Box–Cox transformation was used when necessary. Statistical analyses were performed with R statistical package (R Development Core Team, 2011).

3. Results

OM level, presence of plant and time all had a highly significant effect on interstitial water chemistry evolution (Table 1 and Fig. 1).

The presence of roots, in particular in the early stage of the incubation, significantly affected Eh values, which were comparatively less reduced than in bare sediments. During the course of the experiment, redox potential of C_V microcosms remained always above 0 mV, while a decline from +57 to about −56 mV was observed in C_B microcosms. In enriched sediments, Eh values underwent similar trends with a steady decrease (down to −133 mV), but a significant distinction between vegetated and bare microcosms was observed only up to day 6. At the end of the experiment the presence of *V. spiralis* did not appreciably affect redox status in enriched sediments.

Plant presence exerted a significant influence on the whole set of pore water features investigated (Table 1). In C_v microcosms CH_4 concentrations decreased significantly from day 10 down to less than 40 μM during the rest of the incubation. On the contrary, in C_B sediments values were always higher than 180 μM. CH_4 concentrations in enriched sediments were constantly greater (up to 470 μM) than in C ones, but the patterns were more erratic and the effect of plant presence not always clear, due to large gas bubble formation and CH_4 loss with sediment resuspension. CH_4 concentrations were negatively correlated with redox potential ($r = -0.709$, $p < 0.01$; pooled data, $n = 81$). Dissolved iron and manganese ranged from 6 to 1370 μM and from 4 to 107 μM, respectively. Differences between vegetated and bare sediments were not so evident in control microcosms, where Fe^{2+} and Mn^{2+} concentrations were always less than 115 and 12 μM, respectively. Reduced metals accumulated quickly in enriched sediment pore water from day 1 to day 6, both in vegetated and bare microcosms. Afterwards, in +10 sediments Fe^{2+} concentrations remained essentially unchanged from day 6 till the end of the experiment, while in +5 sediments they decreased significantly. Mn^{2+} concentrations were more erratic with no clear trend with time. Fe^{2+} and Mn^{2+} concentrations were negatively correlated with redox potential (for Fe^{2+}, $r = -0.629$, for Mn^{2+} $r = -0.679$, $p < 0.01$; pooled data, $n = 81$). PO_4^{3-} concentrations were undetectable or up to 12 μM in C_v microcosms while always greater than 30 μM in C_B microcosms. In enriched sediments values increased up to 300 and 620 μM in +5 and +10 sediments, respectively. Phosphorus and metal concentrations were highly correlated (for Fe^{2+}, $r = 0.920$; for Mn^{2+} $r = 0.822$; $p < 0.01$, pooled data, $n = 81$). NH_4^+ concentrations exhibited distinct trends in control and enriched sediments. Values were always lower than 160 and 540 μM in C_v and C_B microcosms, respectively. In enriched sediments NH_4^+ concentrations increased quickly and constantly, reaching about 9000 and 12,000 μM in +5 and +10 sediments, respectively (pooled data). From day 6, in +10 treatment, NH_4^+ concentrations were generally higher in vegetated microcosms than in bare ones.

Sediment nitrification activity significantly differed among treatments ($p < 0.001$) (Fig. 2). Nitrification rates in control sediments were generally higher than 150 nmol NO_x^- g_{DW}^{-1} h^{-1} and not statistically different between vegetated and bare microcosms ($p > 0.05$). Rates in enriched vegetated substrate were 5 and 25-fold lower for +5 and +10 treatments, respectively, than those measured in control ones and were not detectable in bare sediments.

4. Discussion and conclusions

4.1. OM enrichment, shift to anaerobic metabolism and accumulation of potentially toxic end-products in pore water

Aquatic environments impacted by human activities as urban canals and ponds are generally enriched with high amounts of labile organic matter that turns sediment reduced and harmful to roots (Cotano and Villate, 2006; Poté et al., 2008; Pulido et al., 2010). To mimic the chemical conditions that are established in such situations we added an easily degradable source
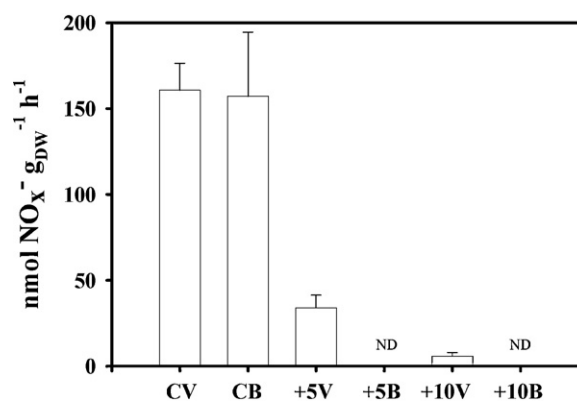

Fig. 2. Nitrification activity in sediments. Potential nitrification rates measured in sediments of each experimental condition on day 17 (C_V, control vegetated sediment; C_B, control bare sediment; $+5_V$, 5 g enriched vegetated sediment; $+5_B$, 5 g enriched bare sediment; $+10_V$, 10 g enriched vegetated sediment; $+10_B$, 10 g enriched bare sediment; average ± standard error, $n = 3$). Rates in $+5_B$ and $+10_B$ sediments were not calculated as the accumulation of $NO_2^- + NO_3^-$ during the assay was not detectable.

of OM with a low C/N ratio (∼7) and a high protein content to a sediment with an elevated OM background. The chosen levels of organic enrichment simulated those of highly impacted sediments as those below fish farms, which represent an extreme of eutrophication gradient (Holmer et al., 2007). Fish feed inputs led to immediate stimulation of mineralization processes and to accumulation of anaerobic metabolism end-products. Enriched microcosms showed dark brown sediments and no oxidized halos were evident in the rhizosphere. Release of gas bubbles suggested that methanogenesis and methanotrophy were decoupled and that electron acceptors as nitrate or oxidised iron or manganese species were depleted within sediments. Although not measured in this study, free S^{2-} may have been present: sulfide smell and very dark sediments could be in fact detected during microcosm handling. The decrease of pore water Fe^{2+} and Mn^{2+} after day 6 in both enriched treatments suggested the precipitation of iron monosulfides or pyrite and manganese sulfide (Webb et al., 1998).

Degradation of the added labile OM may have led to the formation and accumulation in pore water of reduced inorganic and organic species at levels toxic to freshwater macrophytes. Ammonium and iron stress could occur, since interstitial concentrations in enriched substrates were widely greater than those found in previous toxicity studies (van Wijck et al., 1992; Britto and Kronzucker, 2002). Despite this, all *V. spiralis* specimens were alive during the course of the 17-day experiment. However, plants in enriched substrates showed signs of stress, probably as a consequence of oxygen shortage and high levels of phytotoxins in pore water. Root decay (blackening and increasing flaccidity) was visible in all the specimens exposed to organic rich substrates. Plants were anchored with just the primary root and shedding of all the lateral fine roots was evident. This could be a strategy to minimize oxygen loss and maintain the sufficient supply to primary root tissue (Colmer, 2003). Moreover plants in enriched substrates produced plagiotropic stolons that spread horizontally above ground and formed new ramets; previous studies have shown that *V. spiralis* can escape hostile sediment patches by clonal growth (Xiao et al., 2006).

4.2. Effect of V. spiralis on pore water chemistry

N and P theoretical uptake during the first six days of the experiment was calculated assuming for *V. spiralis* preferential assimilation of NH_4^+ and PO_4^{3-} from the roots, a mean net spring

growth rate of $0.055\,d^{-1}$ and a conservative N and P content of $30.7\,mg\,N\,g_{DW}^{-1}$ and of $7.4\,mg\,P\,g_{DW}^{-1}$, respectively (Pinardi et al., 2009). Each plant assimilated on average $\sim\!260\,\mu mol$ of N, and such uptake was appreciable only in control sediments. Here, pore water NH_4^+ concentrations increased in bare sediments while they remained almost constant in the presence of *V. spiralis*, suggesting a tight coupling between ammonification and assimilation. In +5 and +10 treatments ammonification largely exceeded plant uptake. Higher NH_4^+ concentrations measured towards the end of the experiment in vegetated enriched microcosms could be explained by ROL and stimulation of OM degradation or by root exhudates, representing an additional source of easily degradable OM (Karjalainen et al., 2001).

A combination of indirect (co-precipitation with ferric iron) and direct effects (assimilation, estimated in $\sim\!29\,\mu mol$ per plant per 6 days period) resulted in significantly lower PO_4^{3-} concentrations in the presence of *V. spiralis* in all organic levels. In control microcosms, P regeneration was coupled to assimilation, while in +5 and +10 treatments P mineralization largely exceeded plant uptake, stressing the importance of other plant-mediated indirect processes as the precipitation of phosphorous in insoluble forms.

In enriched microcosms, lower concentrations of Fe^{2+} and Mn^{2+} were measured in *V. spiralis* rooted sediments from day 6 till the end of the experiment. We calculated that, in order to explain the different concentrations of the two metals in vegetated and bare sediment, 2.8 ± 1.8 and $8.4 \pm 3.0\,nmol$ of oxygen equivalents per g of fresh sediment per day were needed in +5 and +10 treatments, respectively. Considering the very limited penetration depth in enriched substrates, such oxygen amount was likely provided by ROL (Colmer, 2003). Li et al. (2011) reported for *V. natans* an oxygen loss of about $1000\,\mu mol\,O_2\,g_{DW}\,root^{-1}\,d^{-1}$. Supposing a similar rate of release for *V. spiralis*, the oxygen amount supplied by plants in the enriched microcosms (0.89 ± 0.69 and $7.03 \pm 2.02\,\mu mol\,O_2\,g_{DW}\,root^{-1}\,d^{-1}$ in +5 and +10 treatments, respectively) represented only <1% of ROL. This outcome suggests either lower rates of oxygen loss or the presence of other oxygen consuming processes.

The nitrification potential test provided another evidence that roots maintained a residual functionality even under highly stressful conditions, as some activity was detectable in vegetated but not in unvegetated enriched microcosms. The decline in nitrification activity along the OM gradient was probably a result of oxygen shortage due to high sedimentary demand not completely balanced by ROL. Moreover a variety of compounds, such as sulfide and ammonia, could have adversely affected the growth and activity of the nitrifying community (Sears et al., 2004).

In conclusion, we provide multiple, indirect evidences based on the analysis of pore water that *V. spiralis* is a tolerant macrophyte that can withstand large perturbations of sedimentary features. Since organic enrichment occurring in most impacted aquatic environments, including urban areas, is generally moderate and progressive compared to that reproduced in the present study, we are convinced that *V. spiralis* could be considered as a valid option in restoration programs aiming at the recovery of organic enriched sediments.

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