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Vallisneria spiralis L. adaptive capacity improves pore water chemistry and increases potential nitrification in organic polluted sediments

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Abstract

Background Macrophytes may modify benthic biodiversity and biogeochemistry via radial oxygen loss from roots. This condition contrasts sediments anoxia, allows roots respiration, and facilitates aerobic microbial communities and processes in the rhizosphere. Simultaneously, the rhizosphere can stimulate anaerobic microorganisms and processes via exudates or by favoring the build-up of electron acceptors as nitrate. As eutrophication often results in organic enrichment in sediments and large internal nutrients recycling, an interesting research question is to investigate whether plants maintain the capacity to stimulate aerobic or anaerobic microbial communities and processes also under elevated organic pollution.

Methods A manipulative experiment was carried out under laboratory-controlled conditions. Microcosms containing bare sediments and sediments transplanted with the macrophyte *Vallisneria spiralis* L. were created. The effect of the plant was investigated on sediments with moderate (8%) and elevated (21%) organic matter content, after an acclimatization period of 30 days. Chemical and physical parameters, microbial community composition and the potential rates of nitrification, denitrification and nitrate ammonification were measured at two different depths (0–1 and 1–5 cm) after the acclimatization period to evaluate the role of roots.

Results *Vallisneria spiralis* grew and assimilated pore water nutrients at the two organic matter levels and vegetated sediments had always nutrient-depleted porewaters as compared to bare sediments. Nitrifying microbes had a lower relative abundance and diversity compared to denitrifying bacteria. However, regardless of the organic content, in vegetated sediments nitrifiers were detected in deeper horizons as compared to bare sediments, where nitrification was confined near the surface. In contrast, potential denitrification rates were not affected by the presence of roots, but probably regulated by the presence of nitrate and by root-dependent nitrification. Potential nitrate ammonification rates were always much lower (< 3%) than potential denitrification rates.

Conclusions *Vallisneria spiralis* affects N-related microbial diversity and biogeochemistry at moderate and elevated organic matter content, smoothing bottom water–pore water chemical gradients and stimulating nitrification and nitrogen loss via denitrification. These results suggest the possibility to deploy *V. spiralis* as a nature-based

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solution to counteract eutrophication in freshwater systems impacted by high loads of organic matter, for example, downstream of wastewater treatment plants.

Keywords Macrophyte, Rhizosphere, Ammonium oxidation, Nitrate reduction, Microbial functional group, Nature-based solution

Background

Submerged phanerogams play a key role in freshwater systems providing a wide range of ecosystem services. Macrophytes actively take up nutrients like nitrogen (N) and phosphorous (P) from the porewater (Wigand et al. 2000) preventing their release to the water column. The canopy of macrophytes favors the local reduction of water flow, enhances sedimentation rates, and retains suspended matter (Sand-Jensen 1998; Kleeberg et al. 2010). Macrophytes develop aerenchyma tissues to ensure oxygen (O_2) supply to the roots that usually explore anoxic sediments. The release of O_2 from the roots can actively oxidize the rhizosphere, thus influencing the associated microbial community and its contribution to biogeochemical processes (Sand-Jensen et al. 1982; Jaynes and Carpenter 1986; Ribaudo et al. 2011; Sun et al. 2019).

Vallisneria spiralis L. is a submerged macrophyte with demonstrated capacity to provide different ecosystem services (Hauxwell et al. 2007; Li et al. 2010). It is a stoloniferous species capable of rapid clonal extension, that often forms dense meadows. It can colonize a wide range of substrates from gravel bottoms to muddy sediments in highly eutrophicated and turbid waters (Harley and Findlay 1994; Soana et al. 2012; Soana and Bartoli 2014). *V. spiralis* proliferates also in oligotrophic waters and may outcompete other macrophytes and become dominant at elevated water temperatures, suggesting a possible increase of its dispersion under the actual climatic preview (Hussner et al. 2014). *V. spiralis* has already been detected as an alien and/or invasive species in different aquatic ecosystems worldwide, with the exception of South America (Hussner et al. 2014; Wasekura et al. 2016; Gorham et al. 2021). In Europe, the plant is considered native only in some areas of the Mediterranean basin as the Po River watershed, where the present study was carried out (Hussner 2012).

Due to its high rates of radial oxygen loss (ROL) from the roots to the rhizosphere (Lemoine et al. 2012; Han et al. 2016; Marzocchi et al. 2019), *V. spiralis* can expand the oxic and suboxic sediment volume, enhancing the bacterial ability to degrade organic pollutants (Yan et al. 2011; Liu et al. 2014). The oxic conditions originating in the rhizosphere allow root respiration, influence oxygen- and redox-sensitive biogeochemical pathways, and maintain active coupled oxidative and reductive processes, avoiding the accumulation of metabolic end-products as Fe^{2+}, Mn^{2+}, H_2S or CH_4 in the pore waters (Begg et al. 1994; Risgaard-Petersen and Jensen 1997; Faußer et al. 2012; Vila-Costa et al. 2016). Indirect effects include the scavenging of soluble reactive phosphorus (SRP) from pore water due to coprecipitation with insoluble iron oxyhydroxides as plaques on the roots (Han et al. 2018; Marzocchi et al. 2019; Li et al. 2022; Magri et al. 2023). This precipitation is suggested to be partially mediated by microbial processes (Weiss et al. 2007) and it helps the plant to counteract stress condition induced by anoxia (Møller and Sand-Jensen 2008).

Vallisneria spiralis is demonstrated to significantly affect benthic nitrogen (N) cycling via combination of both direct (i.e., uptake) and indirect (i.e. O_2 release, exudate production or removal of sulphide toxicity) effects (Joye and Hollibaugh 1995; Racchetti et al. 2010, 2017; Soana et al. 2014, 2015). Inorganic N uptake from pore water decreases the chemical gradients with bottom water and internal recycling whereas O_2 release, the production of labile exudates and the oxidation of sulphides may promote coupled nitrification–denitrification in the rhizosphere. Moreover, the capacity of *V. spiralis* to colonize organic sediments can prevent processes as nitrate ammonification (DNRA) to becoming dominant over denitrification within dissimilative nitrate reduction pathways due to reduced sulphide toxicity to denitrifiers, and to different composition of detritus in vegetated sediments (Jiang et al. 2020, 2022; Zhao et al. 2020). *Vallisneria spiralis* therefore is expected to enhance both N retention and translocation within the benthic compartment and its permanent loss. With such environmental plasticity and prominent role on sediment biogeochemistry, *V. spiralis* is considered as an engineer species, and represents a potential nature-based solution to improve the chemical and biological quality of impacted aquatic ecosystems (Rai and Tripathi 2009; Liu et al. 2014). However, little is known about the biogeochemical response of *V. spiralis* vegetated sediments to an increase in the organic matter content, which is expected to occur because of nutrient enrichment and eutrophication. The quantification of such response is important to test the plasticity of *V. spiralis* meadows and to forecast the effects of *V. spiralis* transplant in organic-polluted sediments. In this framework, it is relevant to analyze whether the macrophyte affects biogeochemical

processes due to uptake or to the release of O_2 or exudates or due to better performances, higher growth and competitive advantage of specific microbial assemblages supported by the macrophyte, that ultimately affects the microbial community composition. The latter can be demonstrated via high-throughput sequencing techniques, that allow the characterization of the structure and diversity of microbial communities in various matrices, such as freshwater environments (Pang et al. 2016; Salmaso et al. 2018; Li et al. 2020).

In this work, individuals of *V. spiralis* were transplanted in microcosms containing sediments with two different levels of organic matter: moderate (8%) and elevated (21%) and conditioned for 1 month. The overall aim of the study was to analyze comparatively sedimentary features (pore water chemistry), microbial activity (potential rates of nitrification, denitrification and DNRA) and the relative abundance of bacteria and archaea in bare and vegetated sediments and along an organic gradient.

Fine-scale microbial community investigations have rarely been coupled with measurements of potential rates for specific key biogeochemical processes, particularly those related to the nitrogen cycle (Racchetti et al. 2010; Soana et al. 2012; Soana and Bartoli 2014; Bier et al. 2015; Lin et al. 2018). It was hypothesized that the proximity of oxic and anoxic microniches within the rhizosphere would increase microbial diversity and the relative abundance of aerobic and microaerophilic taxa in vegetated versus bare sediments (Vila-Costa et al. 2016; Martin et al. 2019; Moazzem et al. 2023). It was also hypothesized that the plasticity of *V. spiralis* results in higher ROL in more organic sediments, buffering the effects of organic matter enrichment on N-related microbial processes (e.g., inhibition of nitrification and dominance of DNRA over denitrification).

Materials and methods

Sediment and macrophyte sampling

Sediments with moderate (M_{OM}) and elevated (E_{OM}) OM content were collected in July 2022 from two shallow sites (0.5 m deep) within the Mincio River, northern Italy. Sediment sampling was carried out by hand, via transparent plexiglass liners (inner diameter 8 cm, height 30 cm). At each site the upper 0–10 cm sediment horizon was extruded from the liners and transferred into a 30 L bucket. Nearly 20 L of sediment was collected from each site. Organic-enriched sediments were collected from the Vallazza riverine wetland, located downstream the wastewater treatment plant of the city of Mantova (45° 08′ 01.6″ N, 10° 48′ 34.6″ E). The Vallazza wetland lies within a contaminated Site of National Interest (SIN). SINs are defined by the Italian law as sites with elevated levels of pollution in the soil and/or sediment where remediation actions are in progress or must be undertaken (ISPRA 2021). The polluted area comprises nearly 1000 ha of lacustrine, riverine and wetland ecosystems receiving effluents generated by a 100,000 AE (Equivalent inhabitants) wastewater treatment plant and impacted by different industrial activities that have released large amounts of heavy metals and hydrocarbons (Provincia di Mantova 2021). Sediments with lower organic matter content were collected from a station 20 km upstream the Vallazza site (45° 16′ 42.3″ N, 10° 42′ 29.9″ E), outside the SIN, from a natural, densely vegetated riverine marginal area. At this site, nearly 50 individuals of the submersed macrophyte *Vallisneria spiralis* were also carefully collected by hand and rinsed in situ to remove sediments from roots and epiphytic organisms from leaves. Similar plants in terms of leaf number and height and with visually healthy rhizosphere (whitish, well developed root hairs) were chosen in order to limit the variability of macrophyte-related measurements.

Experimental setup

In the laboratory, sediments from both sites were separately sieved (1 mm mesh) to remove large debris and macrofauna and homogenized. After three days, the water overlying sediments was syphoned off and sediments were transferred into bottom-capped plexiglass microcosms (height = 10 cm, inner diameter = 7 cm, $n = 24$) laterally covered with aluminum foil. Four different treatments, each with 6 replicates, were compared. Half of the microcosms were filled with M_{OM} sediments and half with E_{OM} sediments. Within each sediment type, in 6 microcosms two individuals of *V. spiralis* were transplanted, simulating a density of 520 individuals m^{-2}, whereas 6 microcosms contained bare, unvegetated sediment. Afterwards, all microcosms were submerged and pre-incubated in a 100 L aquarium filled with unfiltered, continuously aerated and gently stirred water from the Mincio river, collected at the upstream station and maintained at 24 °C and under a 12:12 h light and dark cycle. Halogen lamps produced an irradiance of 250 μE m^{-2} s^{-1}, which represents the average daily irradiance during summer at the study sites. The microcosms were maintained in the aquarium for 30 days, replacing nearly 30% of the water every 4 days (Additional file 1: Figs. S1 and S2). The preincubation period allowed the establishment of steady conditions and stable chemical gradients in bare and vegetated sediments and the microbial communities to develop (Stocum and Plante 2006).

Measurements of sediment features and of microbial activity and diversity

After the preincubation period, four microcosms from each treatment were randomly collected and sacrificed.

Two layers (0–1 and 1–5 cm) were considered for sediment characterization, for the measurement of microbial activity and for nucleic acid extraction.

Physical and chemical analyses

Sediments from the microcosms were extruded and sliced and each slice was homogenized. In the presence of plants, the sediment from the 1–5 cm layer within the roots was carefully collected with a sterilized spatula. From each sediment homogenate, a volume of 5 ml was collected with a cut-off syringe for porosity and water content determination. To this purpose, sediments were weighed wet and after having been dried at 60 °C for 48 h. Organic matter content (OM) was measured as percentage of weight loss by ignition (450 °C, 2 h) from the dried, powdered sediment. Chlorophyll a (Chl-a) was extracted from 1 ml of fresh sediment (only 0–1 cm layer) with 5 ml of 90% acetone for 24 h at 4 °C in the dark. The slurry was then centrifuged, and the supernatant filtered for spectrophotometric determination of pigments according to Lorenzen (1967). Porewater was extracted from the wet sediment by centrifugation under N_2 atmosphere at 4200 rpm for 10 min. Afterwards, the supernatant was filtered (Whatman GF/F) and analyzed for NH_4^+ and soluble reactive phosphorous (SRP) by spectrophotometry (A.P.H.A. 1981; Valderrama 1977).

Slurries incubation

Subsamples of the sediment homogenate from the two layers were incubated under aerobic and anaerobic conditions to measure potential rates of nitrification (PN), denitrification (PD), and dissimilatory nitrate reduction to ammonium (PDNRA). Potential nitrification rates were estimated via oxic incubation in 100 ml Erlenmeyer flasks ($n=32$: 4 replicates per 2 sediment layers per 4 treatments) of slurries containing 40 ml of 0.22 µm-filtered in situ water enriched with NH_4^+ to a final concentration of 200 µM and 2 ml of sediment and maintained on a shaker at 24 °C. Water samples (5 ml) were collected at the beginning and end of the dark incubation, that lasted ~ 20 h. Collected samples were centrifuged at 4200 rpm for 3 min, and the GF/F filtered supernatant was later analyzed for combined nitrite and nitrate concentrations ($NO_x^- = NO_2^- + NO_3^-$) via spectrophotometry (A.P.H.A. 1981; Rodier 1978).

Potential NO_3^- dissimilatory reduction processes (denitrification and DNRA) were measured in anoxic incubations. To this purpose, 2 ml of sediments were transferred into 12 ml exetainers containing filtered and anoxic in situ water ($n=256$: 4 replicates per time series ($n=4$ times) per 2 sediment layers per 4 treatments per 2 potential activities) and continuously suspended on a rotating shaker. An overnight preincubation was necessary to consume all dissolved oxygen traces and $^{14}NO_3^-$ by microbial nitrate reduction. Thereafter, all exetainers were added with $^{15}NO_3^-$ to a final concentration of 200 µM. With respect to potential denitrification measurements, immediately after the $^{15}NO_3^-$ addition microbial activity was terminated in the first exetainer of every replicated time series of all treatments by adding 200 µl of 7 M $ZnCl_2$. Afterwards, every 3 h this same procedure was applied to the second, third and fourth exetainer. This made it possible to analyze the linearity of the production of labelled ^{15}N-N_2 along with the course of the experiment.

The experimental procedure to measure potential DNRA rates was similar but exetainers were not poisoned with $ZnCl_2$. They were centrifuged at 2700 rpm for 3 min and the supernatant was filtered (GF/F) and frozen for later $^{15}NH_4^+$ analyses (see details below). This also allowed to check the linearity of the $^{15}NH_4^+$ production along the course of the experiment.

The produced ^{15}N-N_2 was analyzed by membrane inlet mass spectrometry (MIMS, Bay instruments, USA). The produced $^{15}NH_4^+$ was also analyzed by MIMS after the oxidation of NH_4^+ to N_2 by the addition of alkaline hypobromite (Warembourg 1993). Efficiency of the alkaline hypobromite oxidation was quantified after its addition to solution of defined NH_4^+ concentration spanning the range of NH_4^+ in the samples. Before adding the oxidant, all samples were bubbled with air to remove ^{15}N-N_2 traces. Slopes of the linear regression of concentration against incubation time were used to calculate production rates of labelled N_2 ($p^{29}N_2$ and $p^{30}N_2$) and NH_4^+ ($p^{15}NH_4^+$), respectively. Time points deviating from linearity were excluded from the calculations because they suggest depletion of the $^{15}NO_3^-$ added. All rates were expressed as N amount produced or consumed per gram of fresh sediment per day.

DNA extraction and sequencing

Sediments for molecular analyses were selected from all microcosms, by collecting at least 2 ml of surface (0–1 cm) and subsurface (1–5 cm) sediment with a sterile spatula. Thirty-two samples in total were collected and stored at − 80 °C until extraction. Microbial DNA was extracted from 450 to 500 mg of homogenized sediment using the NucleoSpin Soil kit (Macherey–Nagel, Düren, Germany). The DNA was extracted using lysis buffer SL1 with Enhancer solution SX according to the manufacturer's instructions. The DNA was eluted into 50 µl elution buffer SE. Successful DNA extraction was confirmed by electrophoresis on a 1% agarose gel and DNA concentration was assayed using the dsDNA HS Assay Kit 0.2–100 ng μl^{-1} with the Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, California, US). The DNA

was then sent to Novogene Europe (Cambridge, UK, Novogene) for PCR amplification, library preparations, and sequencing. For bacterial community composition, the hypervariable region V3-V4 of the 16S rRNA gene was amplified using universal degenerate primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′). For archaeal community composition, the 16S rRNA gene in the hypervariable region V4-V5 was amplified with primers Arch519F (5′-CAGCCGCCGCGGTAA-3′) and Arch915R (5′-GTGCTCCCCCGCCAATTCCT-3′). Multiplexing with unique index handles were constructed by Novogene Europe using in-house protocols. The final pooled libraries were sequenced using a paired-end setup (2 × 250 bp) on the Illumina NovaSeq 6000 platform by Novogene Europe.

Bioinformatics

The raw amplicon data was processed in DADA2 (Callahan et al. 2016), with the following quality trimming parameters: truncLen = c (227,227), maxEE = 2, truncQ = 2, maxN = 0, rm.phix = TRUE. Primer sequences had already been removed by the sequencing facility. To ensure the filtering was successful, both the raw and filtered data were visualized as FastQC 0.11.9 reports (Andrews 2010) and combined using MultiQC 1.12 (Ewels et al. 2016). The error model was run with parameters: nbases = 1e8, MAX_CONSIST = 30; the merging step using minOverlap = 10, and the chimera removal step with the parameter: method = "consensus. The SILVA nr99 v138.1 database (Quast et al. 2012) was then used to annotate the ASVs, and singletons, chloroplasts, and mitochondria sequences were removed from the final ASV table. The final amplicon sequence variant (ASV) data was normalized as relative abundances and further visualized and analyzed using the software Explicet 2.10.5 (Robertson et al. 2013). Bacterial and archaea Shannon's *H* alpha diversity index was calculated after subsampling to the lowest sample size (bacteria: 13590 counts, archaea 1120 counts) using the software Explicet 2.10.15. The raw sequencing data has been uploaded to NCBI GenBank and are available under BioProject PRJNA996757.

Statistics

The effects of sites (E_{OM}, M_{OM}), sediment layer (0–1; 1–5 cm), and the presence/absence of *V. spiralis* were assessed for their impact on N-cycling processes (PN, PD, PDNRA), porewater nutrient concentrations (SRP, NH_4^+), physical property (density, water content, porosity, OM) and bacterial and archaea Shannon's *H* alpha diversity using a non-parametric multivariate analysis of variance (PERMANOVA) (*adonis2* function in R) based on the Bray–Curtis dissimilarity index. The *p*-values for each variable were determined after 999 permutations. Pairwise PERMANOVA (*pairwise.adonis* function in R, package *vegan*) based on the Bray–Curtis dissimilarity index was subsequently performed for each variable investigated to test significance between single treatments. PERMANOVA was used instead of a parametric counterpart like MANOVA because most of the variables did not meet the required conditions of equality of variance between groups and normal distribution. The two requirements have been previously assessed with Levene's and Shapiro–Wilk tests, respectively. The relationships among N-cycling pathways, porewater, and other sedimentary properties were analyzed using Pearson's *r* correlation index. All the previous statistical analyses were performed using RStudio 06.1 (RStudio Team 2020) using the packages *vegan*, *dplyr*, and *GGally*. The 16S rRNA gene ASV data (normalized as relative abundance %) were used to construct non-metric multidimensional scaling (NMDS) plots based on the Bray–Curtis dissimilarity index and PERMANOVA (9999) tests using the software Past 4.07b (Hammer et al. 2001). Similarity of percentages (SIMPER) analysis from the *vegan* package was used to discriminate species responsible for dissimilarities between the first and second layers at each site and treatment. Statistical significance was set at $p \leq 0.05$.

Results

Sediment properties

During the 30 days conditioning period, in all vegetated microcosms and regardless the OM content, *V. spiralis* produced new leaves, stoloniferous growth and appeared healthy. This confirms the plasticity of this species and its capacity to grow under organic-enriched conditions. The microcosms without plants developed a surficial 3 to 6 mm thick light brown oxidized sediment layer overlaying homogeneously dark brown sediments (Additional file 1: Fig. S2). *Vallisneria spiralis* roots growing in the proximity of the transparent microcosm walls were surrounded by 1–3 mm thick light-brown halos suggesting oxidized conditions in the sediment adjacent to roots, generated by ROL (Additional file 1: Fig. S2). Such subsurface injection of oxygen likely contributed to decreasing redox gradients and to expanding the oxidized layer at the water–sediment interface, that resulted 6–10 mm thick (Additional file 1: Fig. S2).

Sediments from the two sampling sites had different organic matter content (PERMANOVA, $p < 0.001$) but similar mean porosity and density (0.88 ± 0.045 and 1.14 ± 0.09 g cm^{-3}, respectively, pooled data from the two layers, Table 1). The Vallazza site had an average OM content of 21.22 ± 1.71% (pooled data from the two layers), whereas at the upstream site the OM content

Table 1 General features of superficial (0–1 cm) and deep (1–5 cm) sediments and pore water from different treatments

Treatment	Site	Layer	Density	Porosity	OM	SRP	NH_4^+	Chl-a
Bare sediment	M_{OM}	0–1	1.18 ± 0.02	0.90 ± 0.01	8.22 ± 0.14	1.84 ± 1.02	79.47 ± 35.30	7.33 ± 0.82
Bare sediment	M_{OM}	1–5	1.24 ± 0.05	0.80 ± 0.04	8.04 ± 0.03	5.65 ± 3.86	703.40 ± 77.75	–
Bare sediment	E_{OM}	0–1	1.04 ± 0.02	0.92 ± 0.02	20.41 ± 0.57	13.96 ± 1.64	68.76 ± 16.55	2.43 ± 0.53
Bare sediment	E_{OM}	1–5	1.08 ± 0.03	0.92 ± 0.03	20.84 ± 0.41	243.45 ± 23.78	812.69 ± 100.80	–
Planted	M_{OM}	0–1	1.17 ± 0.01	0.88 ± 0.01	8.28 ± 0.21	1.40 ± 0.64	21.66 ± 13.60	7.44 ± 1.57
Planted	M_{OM}	1–5	1.25 ± 0.04	0.82 ± 0.02	8.01 ± 0.16	0.73 ± 0.22	39.93 ± 20.39	–
Planted	E_{OM}	0–1	1.04 ± 0.01	0.91 ± 0.02	21.62 ± 0.33	7.64 ± 1.81	13.46 ± 6.88	2.68 ± 0.20
Planted	E_{OM}	1–5	1.08 ± 0.01	0.91 ± 0.01	22.03 ± 0.39	44.35 ± 45.69	72.35 ± 68.74	–

Reported numbers [density (g/cm^3); porosity and OM (%); SRP (µmol $P\text{-}PO_4^{3-}$/L); NH_4^+ (µmol $N\text{-}NH_4^+$/L); Chl-a (mg/m^2)] are averages ± SE ($n = 4$)

averaged 8.14 ± 0.53%. During the incubation period, *V. spiralis* slightly but significantly increased the OM content of vegetated *versus* bare sediments, likely due to root growth and detachment, release of exopolysaccharides and sedimented leave fragments (PERMANOVA, $p < 0.05$, Table 1).

The NH_4^+ concentration in the pore water was similar in M_{OM} and E_{OM} microcosms (PERMANOVA, $p > 0.60$, Table 1 and Fig. 1A). However, regardless the OM content, vegetated sediments had always much lower NH_4^+ concentrations as compared to bare sediment, in particular in the 1–5 cm horizon where concentrations differed by one order of magnitude (PERMANOVA, $p < 0.001$). The NH_4^+ concentration always increased with depth and differences between layers were highly significant, in particular in bare sediments where concentration

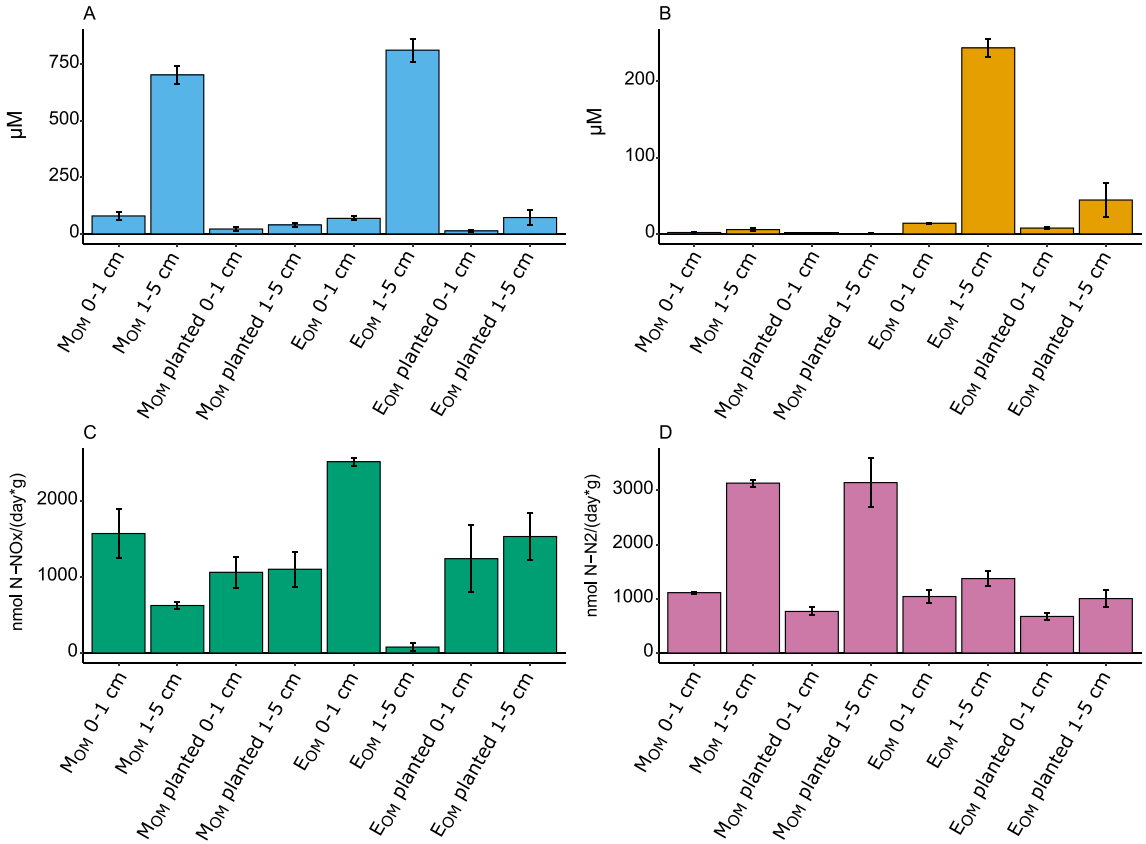

Fig. 1 Pore water concentrations of dissolved ammonium (NH_4^+, **A**) and soluble reactive phosphorus (SRP, **B**), and potential rates of denitrification (PD, **C**) and nitrification (PN, **D**). Concentrations are expressed in µM whereas rates are expressed in nmol of N produced per day (d) per gram (g) of wet sediment. Bars represent standard error (se). M_{OM} = moderate organic matter, E_{OM}: elevated organic matter

gradients were very steep (PERMANOVA, $p<0.001$). Similar results were found for SRP concentrations, that were positively correlated with those of NH_4^+ (Pearson's $r=0.69$, Additional file 1: Fig. S4). SRP concentrations increased with depth and were lower in vegetated versus bare sediments (PERMANOVA, $p<0.005$).

Potential rates of nitrification, denitrification and DNRA

The presence of *V. spiralis* significantly enhanced the potential rates of nitrification in both sediment types whereas it did not produce clear effects on potential denitrification and nitrate ammonification.

Potential nitrification rates (PN) were significantly different among treatments (PERMANOVA, $p<0.05$). In non-vegetated sediment higher PN was measured in the 0–1 cm compared to the 1–5 cm layer (E_{OM} and M_{OM}: $p<0.05$). The presence of *V. spiralis* resulted in a significant increase of PN in subsurface sediments and difference between layers were not significant in vegetated microcosms (E_{OM}: $p=0.52$; M_{OM}: $p=0.90$) (Additional file 1: Fig. S3B). The highest (2.51 ± 0.12 µmol N-NOx d^{-1} g^{-1}) and the lowest (0.08 ± 0.11 µmol N-NOx d^{-1} g^{-1}) PN differed by a factor of nearly 30 and were measured in the 0–1 and 1–5 cm layer of the non-vegetated E_{OM}, respectively (Fig. 1C). The layer where lower PN was detected was simultaneously the layer with the highest concentration of SRP and NH_4^+ in the pore water as evidenced also by negative and significant correlation coefficients (Pearson's r; SRP $=-0.46$ NH_4^+ $=-0.58$) (Additional file 1: Fig. S4).

Average, depth weighed PN measured in the 0–5 cm layer were 0.7, 1.1, 0.5 and 1.4 µmol N-NOx d^{-1} g^{-1} in bare and vegetated M_{OM} and in bare and vegetated E_{OM} sediments, respectively. In both M_{OM} and E_{OM} sediments the presence of plants determined a significant growth of the community of nitrifying microbes in the rhizosphere, below O_2 penetration depth (PERMANOVA, $p<0.05$). *Vallisneria spiralis* produced the largest effect in the E_{OM} sediments, where depth weighed PN increased by a factor 2.8, whereas at M_{OM} it increased by a factor of 1.6.

Potential denitrification rates (PD) were significatively different between layers, sites and treatments (PERMANOVA, $p<0.001$) (Fig. 1D). PD were always higher in the 1–5 cm layer. In the M_{OM} the subsurface layer had rates ~ 3.3 times higher than in the 0–1 cm layer (PERMANOVA, $p<0.01$). In the E_{OM} sediments differences in PD between layers were lower, with PD being ~ 40% higher in the 1–5 cm layer. In the presence of *V. spiralis* PD tended to slightly decrease in both M_{OM} and E_{OM} sediments (Fig. 1D). The highest PD was detected in the M_{OM} 1–5 cm layer (3.14 ± 0.60 µmol N-N_2 d^{-1} g^{-1}, pooled data of bare and vegetated sediments) (Fig. 1D). Potential rates of nitrate ammonification were generally low and always < 3% of PD, suggesting that DNRA was a less relevant nitrate reduction pathway as compared to denitrification. PDNRA rates were significantly higher in the 1–5 cm layer except for the vegetated M_{OM} (PERMANOVA, $p<0.01$). DNRA results were significantly different between vegetated and non-vegetated sediments only in the M_{OM} (PERMANOVA, $p<0.05$). PDNRA rates were positively correlated with NH_4^+ concentration as evidenced by the Pearson's coefficient of 0.68 (Additional file 1: Fig. S4).

Microbial diversity

The 16S rRNA gene amplicon sequencing with subsequent DADA2 analysis yielded 13,590 amplicon sequence variants (ASVs) for bacteria and 1120 for archaea data. Shannon's *H* alpha diversity in the 16S rRNA gene data was 10.1 ± 0.05 (mean ± standard error, $n=4$) for bacteria and 6.6 ± 0.14 (mean ± standard error, $n=4$) for the archaea data. The difference between 16S rRNA gene data Shannon's *H* alpha diversity was significantly higher in the bacterial group compared to the archaeal groups (One-way ANOVA, $F_{(1,62)}=560$, $p<0.001$). Contrasting the two sediment types, archaeal Shannon's *H* was statistically different between the M_{OM} and E_{OM} sites (One-way ANOVA, $F_{(7,31)}=21.05$, $p=0.001$) while there was no difference between the bacterial diversity. At the M_{OM} non-vegetated sediment, in the layer 0–1 cm, bacterial diversity tended to be lower (9.65 ± 0.265, Shannon index) (Fig. 2), as compared to all the other layer and treatments (10.2 ± 0.3, Shannon index, pooled data) (Fig. 3). The archaeal diversity was always lower than the

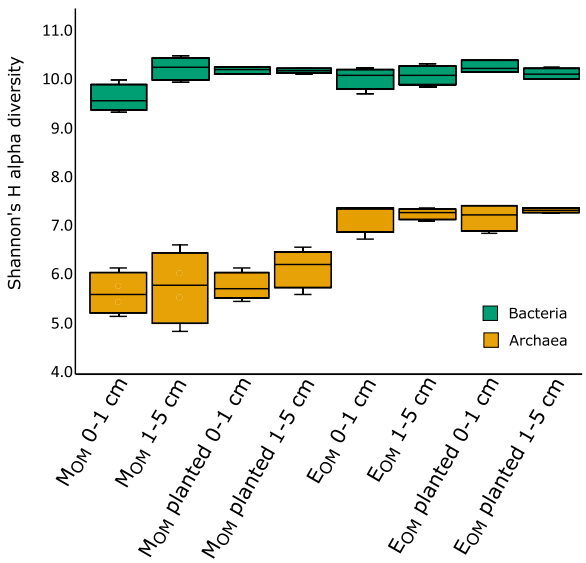

Fig. 2 Shannon's *H* alpha diversity index of both bacterial and archaeal datasets calculated with the software Explicet 2.10.15

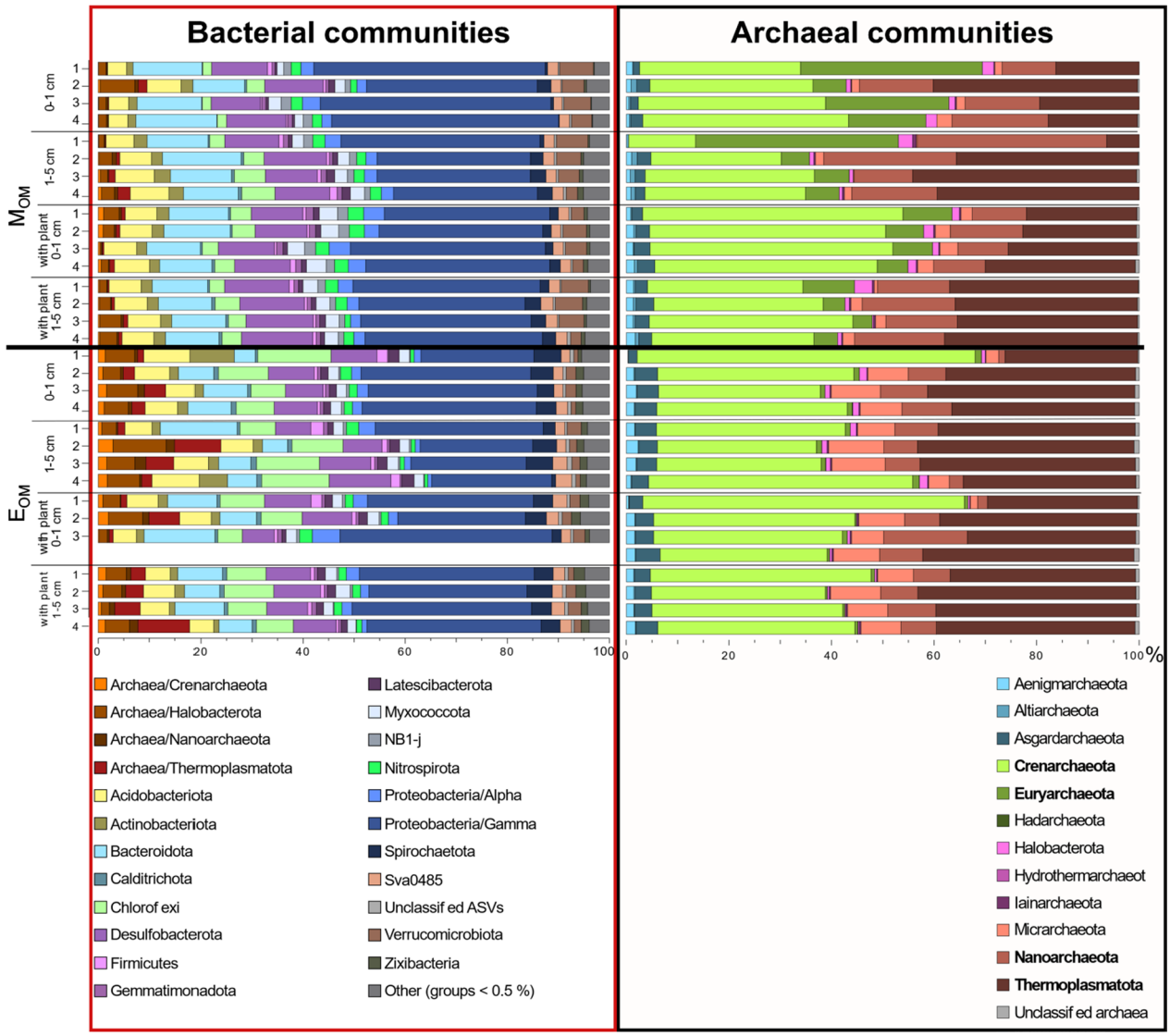

Fig. 3 Bar plots of relative abundance of 16S rRNA gene of bacterial and archaeal communities described at phylum levels. Bar plots of significantly differential abundant taxa, over 0.2% for bacteria and 0.5% for archaeal communities' relative abundance based on ASVs (the cutoff was based on an ASV having > 0.2% in at least one sample for bacteria and > 0.5% for archaea) are reported. Phyla < 0.5% and non-significant phyla > 0.5% are summarized for archaea and phyla < 0.2% and non-significant phyla > 0.2% are summarized for bacteria. Bar plots of the four different replicates (except for the bacteria missing data E_{OM} with plant) for each treatment of the elevated organic matter (E_{OM}) and moderate organic matter (M_{OM}) are reported

bacterial diversity in both M_{OM} and E_{OM} (PERMANOVA, $p < 0.001$) (Fig. 3): the lowest value was found in the non-vegetated M_{OM}, layer 0–1 cm as was found for bacteria (5.68 ± 0.43, SI). E_{OM} sediment in the two treatments and layers had an archaea Shannon diversity index always higher than 7.2 compared to the M_{OM} that was always lower than 6.2 (Fig. 2).

The relative abundance analysis showed that the dominating archaea phyla were Crenarchaeota (37.9 ± 10.0%) and Thermoplasmatota (33.0 ± 9.0%) followed by Nanoarchaeota (11.7 ± 6.9%) and Micrarchaeota (4.6 ± 3.4%) (Fig. 2). The most abundant groups among the phyla identified (ASVs > 0.5%) were Bathyarchaeia class and the *Nitrosarchaeum* genus from the phylum of Crenarcheaota, and the benthic group D and DHVEG a class of Thermoplasmata phylum found mainly in M_{OM} (Additional file 1: Fig. S5).

The 16S rRNA gene amplicon sequencing showed Gammaproteobacteria (33 ± 7.05%) as the major bacterial phylum followed by Bacteroidota (10 ± 2.94%) and

Desulfobacterota (10 ± 2.25%). The other phyla are Chloroflexi (6 ± 3.45%) and Acidobacteriota (6 ± 1.62%) (Fig. 2). The most abundant groups among the phyla identified (ASVs > 0.2%) were Nitrosomanadaceae and Rodocyclaceae families and *Ignavibacterium* genus from Bacteroidota phylum found mainly in M_{OM} and *Denitratisoma*, *Candidatus* Competibacter and *Thiobacillus* genera from Gammaproteobacteria phylum, found mainly in E_{OM} (Additional file 1: Fig. S6).

Only three bacterial clades (Nitrospira, and four strains from Nitrosomonadaceae family) and one archaea (Nitrosarcheum), with a mean relative abundance over the total higher than 0.5%, occurred to be able to perform one or more steps of nitrification (Additional file 1: Figs. S5, S6). Among the more abundant taxa identified involved in nitrification there were individuals of *Nitrospira*, a mesophilic genus of ammonia oxidizing Crenarchaeota (Li et al. 2020) that is able to carry both steps of nitrification from NH_4^+ to NO_3^- alone (Daims et al. 2015), showing a significant increase in abundance in vegetated M_{OM} (0–1 cm) respect to the other treatments (Fig. 4A). The Nitrosomonadaceae, a family that is able to oxidize NH_4^+ to NO_2^-, has a relative abundance positively correlated with the PN rates. Higher relative abundance was always detected in the 0–1 cm layer and the differences between the two layers decreased when *V. spiralis* individuals were present (Fig. 4D).

A higher diversity of microorganisms performing denitrification was detected as compared to the diversity of microorganisms performing nitrification. Among them, *Denitratisoma* a betaproteobacterium (Fig. 4B) showed higher abundance in both layers of the vegetated E_{OM} (0–1 cm and 1–5 cm); the Competibacteriaceae family (Fig. 4C) from the Gammaproteobacteria phylum had higher abundance in E_{OM} compared to M_{OM}. In vegetated

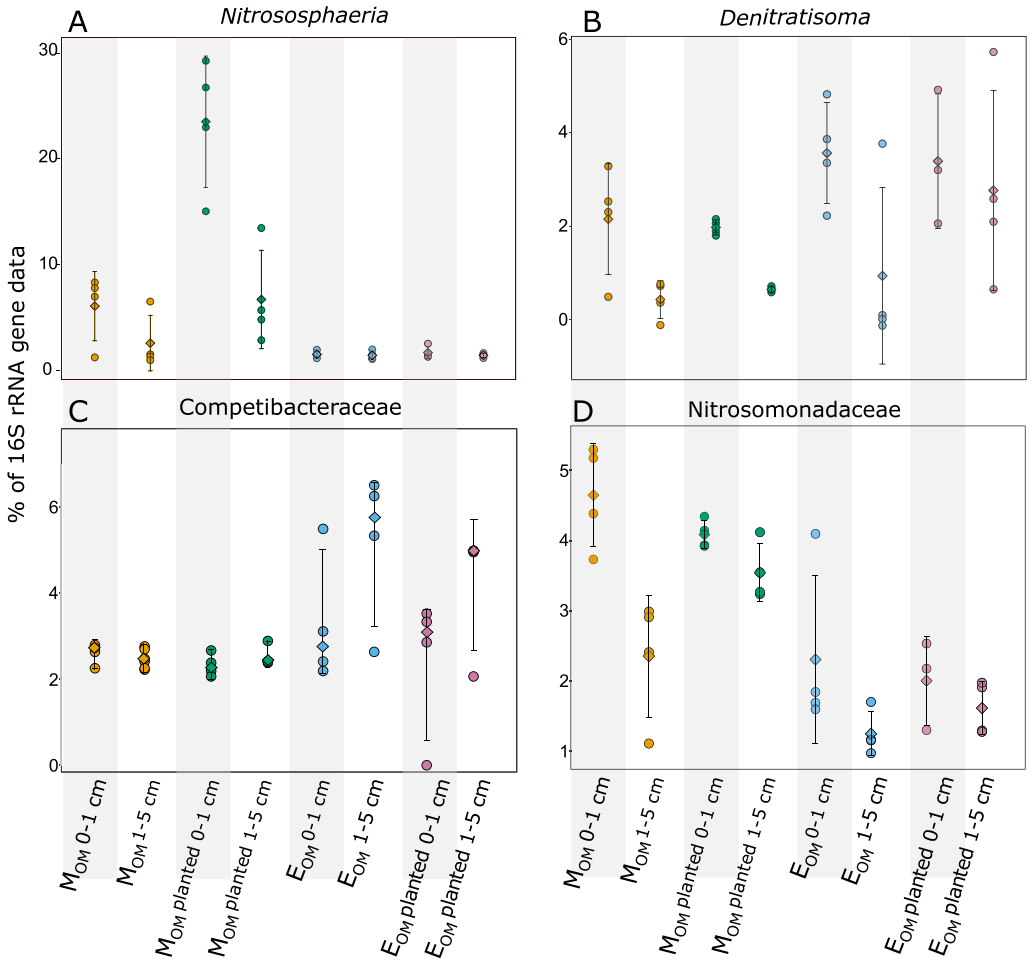

Fig. 4 Dot plots of the % of the abundance of the main genera and families of archaea and bacteria for each treatment associated with N-cycle. The genus *Nitrososphaera* and the family Nitrosomonadaceae are generally associated to nitrification while the genus *Denitratisoma* and the family of Competibacteraceae are generally associated to denitrification process

sediments, the relative abundance of Competibacteraceae was higher than in non-vegetated ones (Fig. 4C, Additional file 1: Fig. S5). The major representative of the Competibacteraceae was *Candidatus* Competibacter which is proved to have the ability to host genes for denitrification and it was detected to perform it in pure cultures (McIlroy et al. 2014; Rubio-Rincón et al. 2017).

Many anaerobic microorganisms were detected in the absence of *V. spiralis*. Methanosaeta relative abundance was lower in the M_{OM}, compared to unvegetated E_{OM} in the 1–5 cm layer (Additional file 1: Fig. S6). The effect of the plant to oxidize and decrease anaerobic niches in the 1–5 cm layer sediment was easily detectable by the significantly lower abundance of *Methanosaeta* in the vegetated mesocosms. Other microorganisms detected, with anaerobic metabolisms, that could be impacted by the ROL were Amicenantales and some Gammaproteobacteria and the archaea *Methanoregula*, the latter one is a methanogen (Additional file 1: Fig. S6) (Bräuer et al. 2006). Instead, the relative abundance of other genera, like *Ignavibacterium* and *Desulfatiglans* that are obligatory anaerobic chemoorganoheterotroph and anaerobic sulfate reducers, respectively (Shin et al. 2019; Bei et al. 2021), were dependent on the site, with higher abundance in the M_{OM} (Additional file 1: Fig. S6).

Multivariate analyses comprising the total operational taxonomic units (OTUs) matrices showed significant effects of sediment types in shaping both bacterial and archaeal communities as shown by NMDS (Fig. 5). The microorganism community was strongly different between the two studied sediment types and this factor was the most important in shaping the microbial community composition, as outlined in Fig. 5. Both bacterial and archaeal communities differed significantly between M_{OM} and E_{OM} ($p < 0.0001$, PERMANOVA 9999) (Fig. 5). Within sediment types, the presence of *V. spiralis* did not result in pervasive effects on the microbial community, but the plant specifically affected a few groups of microorganisms.

Discussion

Effects of *V. spiralis* on pore water chemistry

Under eutrophic and organic-enriched conditions, benthic macrophytes are generally replaced by fast growing, opportunistic algal pelagic primary producers (Scheffer and Carpenter 2003) due to combination of higher nutrient availability and sediment toxicity (Sand-Jensen and Møller 2014). Indeed, the accumulation of labile organic matter in sediments results in higher microbial activity, depletion of electron acceptors and build-up of phytotoxic solutes in pore water (Møller and Sand-Jensen 2008). Such cascade of events can be resisted by tolerant macrophytes, as *V. spiralis*, that can alter the amount of O_2 released from the roots to detoxify the surrounding sediment (Lemoine et al. 2012; Soana and Bartoli 2014).

By comparing seasonal O_2 and CO_2 fluxes measured in light and dark incubations, Soana and Bartoli (2014) postulated that *V. spiralis* modulates ROL along the seasons, increasing O_2 transport to the roots during summer, when microbial activity increases. The same can happen over much shorter time scales, for example when *V. spiralis* meadows retain large amounts of suspended labile organic matter transported by rivers or, as in the present study, when the plant is transplanted into much more organic sediment. *Vallisneria spiralis* survived the transplant, grew into organic sediments and it was able to modify pore water chemistry, microbial activity and specific microbial groups. Other studies support the evidence that *V. spiralis* has a competitive advantage over other submerged macrophytes under eutrophic conditions, due to its adaptive behavior. Indeed, it displays fast rates of biomass production and clonal expansion, resilience to physical disturbances, rapidity to repair damaged tissues and it has the capacity to contrast anaerobic and sulphidic sedimentary conditions via ROL (Xiao et al. 2006; Li et al. 2010; Marzocchi et al. 2019).

In this study ROL was not measured, but previous investigations addressed, demonstrated, and quantified O_2 leaking by *V. spiralis* roots. Marzocchi et al. (2019) quantified *V. spiralis* ROL with planar optodes in moderately OM enriched sediments into 324 ± 107 μmol h^{-1} m^{-2} of root surface and this value is in the higher range of values reported for submerged macrophytes (Wang et al. 2018). In the present work, active ROL in E_{OM} sediments is evidenced by the visible, light brown halos surrounding roots along their whole length and not confined to their tips, over a much darker sediment background (Additional file 1: Fig. S2) (Flessa 1994). Similar halos surrounding roots were present also in plants transplanted into the M_{OM} treatment, suggesting that under less-enriched and less-stressful conditions the plant releases O_2 in excess to roots metabolic requirements and in excess to the real needs to detoxify sediments. The presence of roots and ROL resulted in significantly lower concentrations of both SRP and NH_4^+ in pore water, as also reported by Racchetti et al. (2010) and Wang et al. (2015). Such decrease likely depends upon direct (i.e., uptake) and indirect effects (i.e., coprecipitation with iron oxy-hydroxides as plaques on the roots for SRP and nitrification for NH_4^+) (Jaynes and Carpenter 1986; Risgaard-Petersen and Jensen 1997; Sand-Jensen 1998; Racchetti et al. 2017; Wang et al. 2018; Magri et al. 2023). The ROL-dependent oxidation of iron could be microbially mediated, as evidenced by the metagenomic analysis that detected clades involved

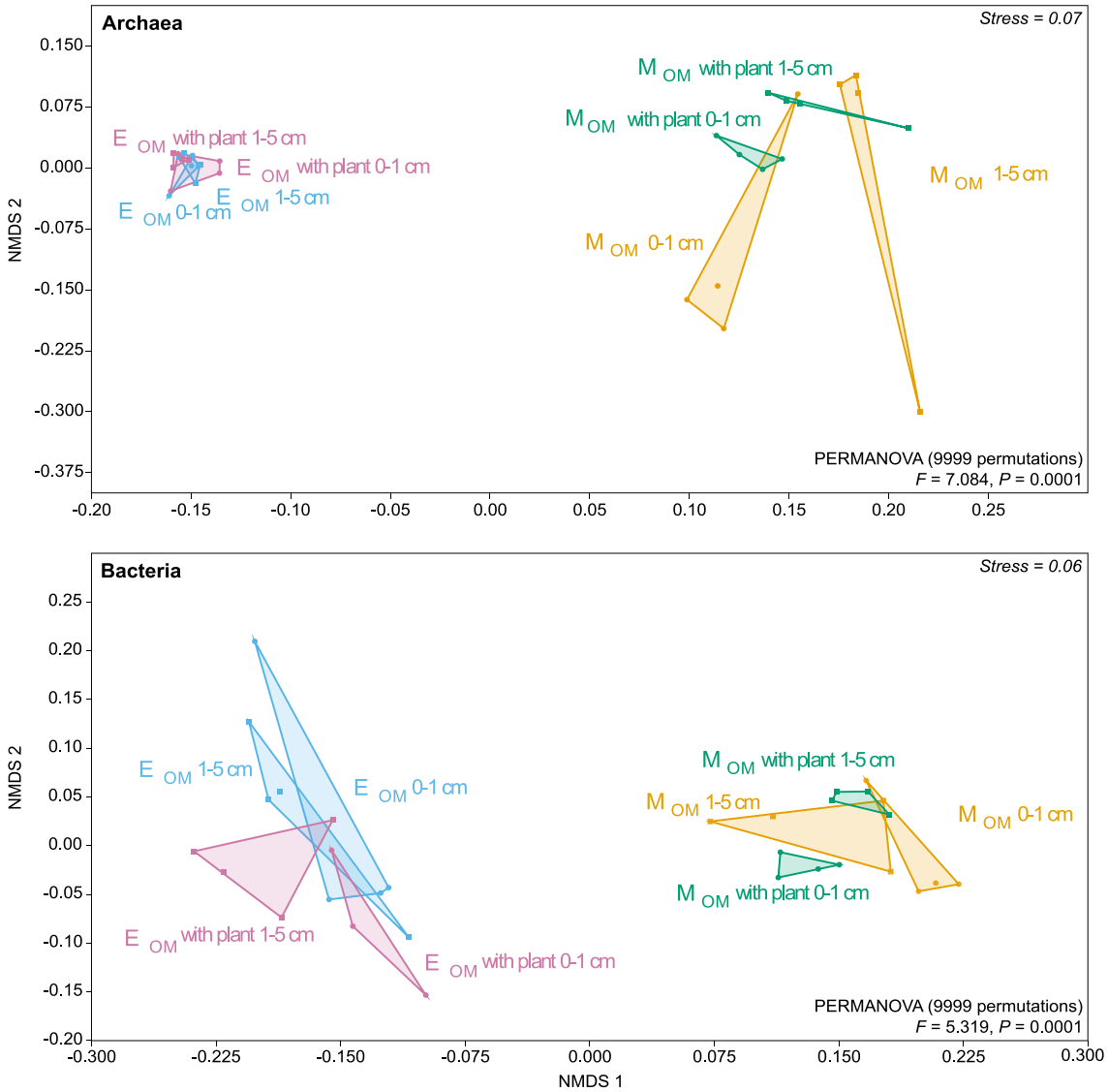

Fig. 5 Non-metric multidimensional scaling (NMDS) based on the microorganisms relative abundance (%) showing the beta diversity (Bray–Curtis) of bacterial and archaeal communities is reported. The PERMANOVA results are based on testing all treatments together and show the pseudo-*F* values

in the iron oxidation and reduction as *Syderoxydans* and *Geobacter,* respectively (Weiss et al. 2007; Kato et al. 2012). Besides reducing dissolved metals concentrations in pore water, ROL may produce another service to *V. spiralis* as a buffer modulating iron acquisition by the plants by facilitating its translocation inside the roots at controlled rates (Gentner 1977). The co-precipitation of Fe(III) and SRP is not limiting P uptake by the roots, as the iron plaques formation and dissolution may mobilize SRP upon plant needs (Wan et al. 2020).

Analogously, the decrease of pore water NH_4^+ concentrations is due to uptake and to nitrification, which is favored by the simultaneous availability of NH_4^+ and O_2 (Racchetti et al. 2010). Indeed, the subsurface vegetated sediment layer exhibited significantly higher potential nitrification rates than bare sediments. Different studies have demonstrated that the nitrate produced within sediments is denitrified upon diffusion into anaerobic layers (Racchetti et al. 2017). As plants need ROL to survive, an indirect cost of O_2 leakage is the unintentional N loss via coupled nitrification–denitrification (Risgaard-Petersen and Jensen 1997). However, if this can represent a constraint in nutrient-limited sandy sediments, in organic sediments with

large ammonification potential as those tested in the present study such N loss does not affect plant growth (Soana et al. 2015).

As diffusive fluxes are generated by concentration gradients, with the available data we can speculate that at the M_{OM} and E_{OM} site the presence of *V spiralis* decreased the NH_4^+ gradients calculated in bare sediments (250 and 297 μM cm^{-1}, respectively) by a factor of 35 and 12, respectively. *Vallisneria spiralis* also significantly reduced the SRP gradients calculated in bare sediments (2 and 92 μM cm^{-1} at M_{OM} and E_{OM}, respectively). At M_{OM} the SRP gradient was set to an undetectable level by the plant, whereas at E_{OM} it was reduced by a factor of 7. Lower chemical gradients in vegetated sediments of both sites suggest lower regeneration of nutrients as compared to bare sediments.

Effects of *V. spiralis* on potential nitrification and the diversity of nitrifying microbes

The biochemical capacity to oxidise NH_4^+ to NO_2^- and NO_2^- to NO_3^- is limited to a narrow group of slowly growing aerobic, chemoautotrophic microbes (Canfield et al. 2005; Flemming et al. 2016) as the genes for nitrification are specific of a small number of microbial groups in the tree of life (Nelson et al. 2016). The measurement of potential nitrification capacity is a good proxy of the number of microbes capable to perform these oxidations. Differences in potential nitrification within or between sediment types are likely due to different combinations of O_2 and NH_4^+ availability in sediments, favouring or depressing the growth of nitrifiers (Henriksen et al. 1981). In organic matter poor sediments, microbial respiration, ammonification rates and NH_4^+ availability are expected to be low whereas O_2 penetration depth is expected to be elevated. Under these circumstances, NH_4^+ more than O_2 regulates nitrification. Under moderate or elevated organic matter content, on the contrary, NH_4^+ production and availability in sediments are high whereas microbial respiration confines O_2 penetration in the upper few millimeters, limiting the horizon where nitrification occurs and regulating the process (Wang et al. 2015).

In vertical profiles, potential nitrification rates in sediments devoid of plants and burrowing macrofauna generally peak in surface sediments due to the co-occurrence of O_2 and NH_4^+ in pore water and decrease steeply due to anoxic conditions (Benelli et al. 2020). Potential nitrification activity is measured also in strictly anoxic sediments, suggesting that nitrifiers can remain viable also in the absence of O_2 where they will use other electron acceptors. In contrast, potential nitrification rates change in the presence of plants or burrowing animals as via ROL or bioirrigation, plants and macrofauna can expand the oxic sediment volume (Benelli et al. 2020). Indeed, high rates of potential nitrification are reported along the burrow walls of macrofauna and in sediments adjacent to roots (Mayer et al. 1995; Soana and Bartoli 2014; Racchetti et al. 2017; Carpintero Moraes et al. 2018). Such microniches represent elective spots for nitrifiers due to the simultaneous availability of O_2 and NH_4^+ (Rysgaard et al. 1993).

Results from this study align with this general evidence: in the microcosms containing bare sediments, regardless of the organic matter content, potential nitrification rates peaked at the 0–1 cm depth whereas the community of nitrifiers and its potential ammonium oxidation capacity were much lower (M_{OM}) or almost undetectable (E_{OM}) at the 1–5 cm subsurface sediment layer, despite large NH_4^+ availability. In vegetated sediments, PN was reduced in the upper layer likely due to decreased vertical NH_4^+ gradients and concentrations whereas they significantly increased, suggesting the active growth of nitrifiers, in the 1–5 cm layer, in particular in the E_{OM} treatment. The latter is probably due to ROL by *V. spiralis* in a NH_4^+ richer porewater environment. The O_2 leaked by *V. spiralis* in heavily organic enriched sediments was therefore in excess of the needs of root respiration. Similar results were previously detected for other macrophytes such as *Oryza sativa* and *Phragmites australis* with nitrification rates directly dependent on root development, ROL and root density (Li and Wang 2013; Li et al. 2021).

Marzocchi et al. (2019) demonstrated that ROL in *V. spiralis* occurs both during night and day. During the day ROL is stimulated by the macrophyte photosynthetic rates whereas during the night it occurs due to concentration gradients between bottom and pore water, driving O_2 transport across the plant aerenchyma. This suggests that the presence of plants always guarantees the presence of O_2 microniches within the rhizosphere, favoring the slow growth of the nitrifiers community. It also suggests that nitrification rates vary during day and night as a consequence of different O_2 availability and of higher competition between bacteria and plants during the day. Risgaard-Petersen and Jensen (1997) measured higher rates of coupled nitrification–denitrification in *Lobelia dortmanna* vegetated than in bare sandy sediments due to the high macrophyte ROL. Within rooted sediments, they measured higher rates in the dark than in the light due to competition for N between the plant and the bacteria. Racchetti et al. (2017) reported a different result for *V. spiralis* growing in muddy, organic sediment, where coupled nitrification–denitrification rates were always higher in the light due to higher O_2 availability under conditions of N excess and absent plant-microbes competition. In vegetated sediments, organic enrichment can

alleviate N limitation and ultimately result in higher N losses via nitrification and denitrification.

The relative abundance of nitrifying organisms was not significantly correlated with potential nitrification rates. Process rates and functional groups relative abundances frequently appear disjoint, without a clear quantitative correlation. For example, in the surface layer of non-vegetated E_{OM} treatment the highest PN was measured, but none of the clades identified had a proportionally higher abundance in this condition compared to the other treatments. This can be due to the fact that many taxa are capable of different biochemical pathways depending on environmental settings that are variable along time and space (Louca et al. 2018). Furthermore, the screening of 16S rDNA genes explores the vast majority of the environmental microbial diversity and large abundances of some common and generalist microorganisms could mask and hide changes in the relative abundance of specific functional groups of interest. Lastly, the measured PN depends on the species involved in the process, the specific environmental conditions, and the competitions with other microorganisms, together governing the kinetics and the rate of the pathway investigated (Nelson et al. 2016; Van Huynh et al. 2023). This explains why it was possible to identify the relative abundance of clades performing nitrification, but it was not possible to address and quantify their relative contribution to the absolute value of the measured PN. It is also important to remark that PN likely overestimates true nitrification rates in sediments as the laboratory method is based on a slurry where possible limiting factors (i.e., O_2, NH_4^+ and diffusion) are removed.

Effects of *V. spiralis* on potential denitrification and nitrate ammonification and the microbial diversity of denitrifying and nitrate ammonifying microbes

Potential denitrification rates measured in the present study had similar order of magnitude as PN; however, they showed different vertical profiles and were not stimulated by the presence of plants. On the contrary, in particular at the E_{OM} sites, PD tended to be lower in vegetated versus bare sediments. Such tendency can be due to ROL creating oxic and oxidized zones reducing the volume of sediments where strictly anaerobic denitrifiers could grow. Indeed, the latter frequently comprise a significative proportion of the overall functional group (Pishgar et al. 2019). *Vallisneria spiralis* is also able to control the growth of biofilms on leaves, on the benthic floor and in the overlying waters through release of allelopathic substances (Xian et al. 2006; Gette-Bouvarot et al. 2015). However, competition for N between plants and microbes is not expected at the E_{OM} site. At the M_{OM} site potential denitrification increased significantly from the surface to the subsurface sediment layer in both bare and vegetated sediments whereas at the E_{OM} site there was a slight tendency towards higher rates in subsurface sediments, but differences were not significant. Increasing rates of denitrification along the vertical profile have been previously reported in saturated soil of constructed wetlands with a variety of plant species (Pelissari et al. 2018).

To the best of our knowledge, only a few studies report potential denitrification rates measured in freshwater sediments. Benelli et al. (2020) measured comparatively potential denitrification rates in the rhizosphere of different aquatic macrophytes and in adjacent bare sediments collected from oligotrophic French lakes. They report rates that are one order of magnitude lower than those reported in the present study likely due to the much lower organic matter content in sediments (range 0.1–0.3%). They report large heterogeneity of PD vertical profiles measured in bare sediments, sometimes decreasing along with depth, and sometimes increasing in subsurface sediments, likely due to small scale heterogeneity in the vertical distribution of organic matter and microbial communities, in turn depending on factors as sediment reworking by bioturbating macrofauna. They also report contrasting effect of macrophytes on PD, sometimes stimulating, sometimes depressing, and sometimes not significantly affecting the process as compared to reference values measured in adjacent bare sediments. Such contrasting results are likely due to the oligotrophic settings of the systems in which measurements were made. Under such conditions, plants releasing O_2 and labile carbon can stimulate the coupled growth of nitrifiers and of denitrifiers. The latter can be favored by the production of nitrate and by the dissolved organic carbon inputs from roots, which represent a significant increase of the total organic carbon in sediments. Simultaneously, under oligotrophic conditions some plants can release allelopathic molecules that inhibit nitrifiers and denitrifiers to avoid significant N losses in a N-limited sedimentary environment.

The sediment tested in the present study had organic matter content averaging 8 and 21% at M_{OM} and E_{OM}, respectively, and the labile OM released by roots as exopolysaccharides (EPS) and exudates likely represent an irrelevant fraction as compared to the large background. This can explain why *V. spiralis* did not stimulate potential denitrification, despite exudates that were demonstrated to be a suitable substrate for denitrifiers (Wu et al. 2017). Lower rates of potential denitrification rates at E_{OM} suggest some sort of toxicity of organic sediments to denitrifiers, for example via production and accumulation of free sulphides in pore water (Christensen et al. 2000; Lu et al. 2018). Such inhibition may

lead to the dominance of DNRA, and to the shift from net N loss to net N recycling (from the dominance of a N_2- to a NH_4^+-producing process). However, unexpectedly, potential DNRA did not prevail over PD in the deep sediment layer of E_{OM}. It is proved that the relative importance of DNRA increases in sulphidic and anoxic environment where the end products of anaerobic metabolism, for example, Fe(II), foster DNRA (Nizzoli et al. 2010; Robertson and Thamdrup 2017). Despite this, other unknown processes acted to maintain low PDNRA rates, that represented a minor fraction of total nitrate reduction rates. Measured PDNRA activity was in agreement with measurements carried out with the isotope pairing technique in intact cores collected from mesotrophic lowland lakes by Nizzoli et al. (2010), where rates of DNRA represented < 3% of the denitrification rates as in this study.

Members of Competibacteraceae were probably among the major contributors as denitrifiers (Fig. 4, Additional file 1: Fig. S6). They present a bi-phasic metabolism that enables them to survive under cyclic aerobic and anerobic conditions: during the anerobic periods they are able to survive using an array of different electron acceptors including NO_3^-. This was proven through culture experiment and supported by the detection of functional genes for denitrification (McIlroy et al. 2014; Rubio-Rincón et al. 2017). Other denitrifying microorganisms detected were Rhodocyclales and Burkholderiales, that are frequently found in paddy soils and wastewater treatment plants (Takayuki et al. 2008; Wang et al. 2020; Yang et al. 2020). The detected taxa responsible for denitrification were so numerous and diverse that it was not possible to find specific functional clades, responsible for the high PD rates in the subsurface layer of the M_{OM} sediment (Fig. 1D). This suggests how the coupling of metagenomic and potential rates measurement does not provide direct evidence linking single clades to specific biogeochemical pathways as denitrification. Indeed, the latter can be performed by different species of microbes, genetically capable of multiple biochemical pathway, that can be activated and regulated by environmental constraints (Louca et al. 2018).

Conclusions

Results from this study support the evidence that *V. spiralis* is a plastic macrophyte capable to grow in organic enriched sediments, hosting a microbial community significantly different from the moderately OM enriched site. Such plasticity is probably due to the adaptive capacity of the macrophyte to alter ROL and contrast the potential phytotoxicity of anoxic and chemically reduced sediment. The ROL performed to enable *V. spiralis* survival under stressful conditions provides numerous side effects shaping the composition of the rhizospheric microbial community and the environmental processes due to the modification of the redox potential and oxygen content of the sediment itself. *V. spiralis* decreased significantly the concentrations of NH_4^+ and SRP in subsurface sediments where most of the plant roots develop, due to combination of uptake and O_2 leakage stimulating nitrification and SRP precipitation, with the net results to decrease nutrient release from sediments and the risk of eutrophication. The potential nitrification rates increased significantly in the sediment layer hosting roots, suggesting the growth of nitrifiers sustained by the presence of microoxic niches in the proximity of roots. Alternatively, available data support the evidence that *V. spiralis* promotes specific conditions (oxic) under which some the potential biochemical pathways of microbes are expressed. This is to say that the macrophyte facilitates aerobic microorganisms. Potential denitrification rates were not affected by the presence of roots but in situ denitrification and N losses are likely higher in vegetated sediments. Indeed, vegetated sediments can produce nitrate below the oxygen penetration depth defined by diffusion. Surprisingly, potential rates of nitrate ammonification represented a minor fraction of nitrate reduction in both M_{OM} and E_{OM} sediments and were not affected by the presence of the macrophyte. The analysis of the microbial community composition confirmed the higher abundance and diversity of microorganisms capable to denitrify as compared to the microorganisms capable to perform nitrification, regardless the OM content. It also evidenced large differences between sediment type whereas differences between bare and vegetated sediments were limited to specific groups. The 16S rRNA gene abundance investigation revealed that, among the microorganisms involved in nitrification, *Nitrospira*, the family Nitrosomonadaceae and the archaea *Nitrosarcheum* were detected. Among the denitrifying microorganisms, the 16S rRNA gene abundance analysis detected *Candidatus* Competibacter, Rhodocyclales and Burkholderiales. Overall, results from this study suggest that *V. spiralis* can represent a nature-based solution to contrast eutrophication in organic-rich sediments and that its transplant can decrease internal N and P recycling, increase P retention in insoluble forms and N loss via nitrification and denitrification.

Abbreviations

ROL	Radial oxygen loss
PN	Potential nitrification
PD	Potential denitrification
PDNRA	Potential dissimilatory nitrate reduction to ammonium
OM	Organic matter
SRP	Soluble reactive phosphorous

ASV	Amplicon sequence variant
AE	Equivalent inhabitant
M_{OM}	Moderate organic matter
E_{OM}	Elevated organic matter

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13717-024-00506-8.

Additional file 1: Fig. S1 Graphical overview of the experimental setup: (A) representation of the different microcosms created for the experiment, with 4 treatments each with 6 replicates; (B) photo of the tank where microcosms were preincubated for 30 days; (C) biogeochemical and molecular measurements carried out in 2 sediment layers of the 4 treatments. **Fig. S2.** Photos of vegetated and bare sediment microcosms (a and b) after the 30 days preincubation, just before measurements; surface of bare sediments, colonized by microphytobenthos (c); washed and dried roots of *Vallisneria spiralis*, with reddish-brown iron oxy-hydroxide plaques (d); preincubation tank with microcosms (e). **Fig. S3.** Pairwise correlation matrixes of the *p*-value obtained from PERMANOVA with 999 permutations. The obtained numerical matrix is visually displayed through a heatmap where stronger colors indicate higher significance (lower *p*-value). (A) potential denitrification rates (PDR); (B) potential nitrification rates (PNR); (C) ammonium (NH_4^+); (D) soluble reactive phosphorus (SRP). **Fig. S4.** Scatterplot matrix of pore water concentration of NH_4^+ and SRP and of potential rates microbial N transformations (PNR, PDR, PDNRA). **Fig. S5.** Heat map of Archaea assemblage relative DNA abundances considering taxa > 0.5% at least in one sample detected in each of the four treatments of two sites. The color intensities range from dark purple (highest relative abundance) to light blue (lowest relative abundance). **Fig. S6.** Heat map of 16S rDNA gene communities' assemblage relative abundances considering taxa > 0.2% at least in one sample detected in each of the four treatments of two sites. The color intensities range from dark red (highest relative abundance) to light red (lowest relative abundance).

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Author contributions
Conceptualization and investigation by LM, CF, MB, MZ, GV. Formal analysis, validation, and data curation by LM, CF, MZ, EB, GV. Writing—original draft: LM, CF, GV. Writing—review and editing: LM, EB, MB. Project administration: MB.

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Availability of data and materials
The dataset supporting the conclusions of this article is included within the article (and its additional file).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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