

Response of biogeochemical processes of the water-soil-plant system to experimental flooding-drying conditions in a eutrophic wetland: the role of *Phragmites australis*

M. C. Tercero · J. Álvarez-Rogel · H. M. Conesa ·
M. A. Ferrer · A. A. Calderón · A. López-Orenes ·
M. N. González-Alcaraz

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Abstract

Aims Assess the role of *Phragmites australis* in the temporal variability of physico-chemical and microbiological soil properties related to biogeochemical processes in eutrophic wetlands.

Methods A mesocosms experiment was performed with alternating flooding-drying conditions with eutrophic water at two nutrient levels, and soil Eh, pH, temperature, CO₂ emissions, dissolved organic carbon, carbon from microbial biomass, and *Phragmites* physiological activity were measured during 44 weeks.

Results In surface, Eh decreased with flooding and increased with drying regardless plant presence and nutrients content. In depth, *Phragmites* maintained oxic conditions. During warmer months, O₂ diffusion promoted by *Phragmites* hindered the drop of pH. Soil microbial respiration was stimulated in warmer months (soil temperature ~20–30 °C), as shown by larger CO₂ production, and higher aromaticity and phenolic compounds content in pore water. The latter occurred regardless the plant presence and nutrients content, although the combination of both contributed to a higher microbial population (shown by higher concentrations of carbon from microbial biomass).

Conclusions The presence of *Phragmites* and the nutrient concentration in the flooding water had a different role in the temporal evolution of the physico-chemical and microbiological soil properties in eutrophic wetlands, and this role was strongly influenced by soil depth and temperature.

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M. C. Tercero · J. Álvarez-Rogel · H. M. Conesa ·
M. A. Ferrer · A. A. Calderón · A. López-Orenes ·
M. N. González-Alcaraz
Departamento de Ciencia y Tecnología Agraria, E.T.S. de
Ingeniería Agronómica, Universidad Politécnica de
Cartagena, Paseo Alfonso XIII, 48, Cartagena 30203 Murcia,
Spain

M. N. González-Alcaraz (✉)
Department of Ecological Science, Faculty of Earth
and Life Sciences, VU University, De Boelelaan 1085,
1081 HV Amsterdam, The Netherlands
e-mail: m.n.gonzalezalcaraz@vu.nl

M. N. González-Alcaraz
e-mail: nazaret.gonzalez@upct.es

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Eutrophication · Temporal variability · Wetland

Introduction

Eutrophication is one of the main environmental concerns worldwide. The necessity to increase production according to socio-economic causes leads sometimes to large water and fertilizer applications, which might create a surplus of water enriched with nutrients (Goulding 2000). Lowland areas, such as coastal

wetlands, are prone to receive this eutrophic water that in turn might modify the balance of nutrients and the soil biogeochemical conditions decreasing the environmental quality (Smith et al. 1999). Particularly, in arid and semi-arid areas, fluctuations in the hydrological conditions are often linked to variations in the effluents from agricultural activities throughout the year and hence to changes in nutrient inputs (Sánchez-Carrillo and Álvarez-Cobelas 2001; Álvarez-Rogel et al. 2006; González-Alcaraz et al. 2012). In this scenario, natural wetlands are being increasingly valued due to their role as green filters (Mitsch and Gosselink 2007). For instance, the capacity of wetlands to remove nitrogen through volatilization, nitrification-denitrification, plant uptake and sedimentation processes has been demonstrated extensively (Reddy and DeLaune 2008). In many cases, the capacity for water amelioration is being augmented by artificially constructed wetlands (e.g., Vymazal 2007; Saeed and Sun 2012). The common management practices include the handling of vegetation (e.g., plant harvesting) and of agricultural drainage systems (e.g., interception of nutrient leachates by wetland systems), and the development of integrated wetland systems composed of natural and constructed wetlands (Mitsch et al. 2001; Thullen et al. 2002; Zhang et al. 2011). A better understanding of the biogeochemical processes occurring in the water-soil-plant system of wetlands is crucial to improve the knowledge about their functioning and management. In this sense, redox potential (Eh) and pH are master parameters mediating these key processes due to that the combination Eh-pH strongly influences the biogeochemistry of the system (Reddy and DeLaune 2008).

The Eh reflects the activity of soil microorganisms (Fiedler et al. 2007). Well-aerated soils, where microorganisms use free oxygen for their metabolism, have Eh values above $\sim +350$ mV, the threshold of oxic conditions at $\text{pH} \sim 7$ according to Vepraskas and Faulkner (2001) and Otero and Macías (2003). However, when a soil is flooded and oxygen concentration falls below 4 % (at $\text{pH} \sim 7$), soil microorganisms use other electron acceptors for the mineralization of organic matter and Eh values might decrease until suboxic ($\sim +350 > \text{Eh} > \sim +100$ mV) and anoxic ($\text{Eh} < \sim +100$) conditions (Vepraskas and Faulkner 2001; Otero and Macías 2003). Furthermore, the intensity of Eh changes depends on a range of factors: organic matter and nutrient availability, temperature, presence of plants, etc. (Fiedler et al. 2007; Reddy and DeLaune 2008). Hence, the high concentrations

of dissolved nitrogen, phosphorus and organic matter, such as those occurring in eutrophic ecosystems (Smith et al. 1999), play a major role in the behavior of the Eh-pH system. For example, increasing concentrations of dissolved organic carbon and nutrients provokes a peak of microbial activity, leading to the depletion of oxygen and then to a drop of the Eh values that might imply an increase of pH (Ponnamperuma et al. 1966; Stumm and Sulzberger 1992).

Phragmites australis (common reed) is one of the most common emergent plants growing in natural wetlands as well as a species usually planted in constructed wetlands (Ruiz and Velasco 2010) due to its contribution to enhance nutrient removal. The presence of *Phragmites* and other macrophytes creates suitable conditions for the sedimentation of suspended solids and prevents erosion by reducing water flow velocity (Lee and Scholz 2007). However, in many cases, this plant has been considered an invasive species (Findlay et al. 2003) since it forms dense monospecific stands that might decrease wetland biodiversity. *Phragmites* strongly influences soil biogeochemistry since it has some mechanisms for the adaptation to anaerobic conditions such as the presence of aerenchyma tissue (Jackson and Armstrong 1999). Aerenchyma provides a low-resistance, internal pathway for the exchange of gases between the emerged and submerged tissues (Visser et al. 2000). The gases supplied through aerenchyma diffuse into the rhizosphere and modify the Eh and pH conditions around the living roots, favoring the occurrence of a variety of microorganisms including aerobes and anaerobes (Nikolausza et al. 2008). Moreover, the growth of roots within soil aggregates helps to decompose organic matter and prevents clogging by creating channels for water to pass through the soil profile. In fact, there is an intimate connection between the biology, physics and chemistry of the rhizosphere that exhibits great spatial and temporal heterogeneity (Hinsinger et al. 2009).

Unlike the spatial changes of Eh and pH, the temporal variability of these two parameters has been poorly documented, especially over long-term periods (more than few hours or days). Investigating such long-time scales is difficult given the dynamic nature of the factors implied in the soil biogeochemistry, and requires detailed in situ monitoring. Furthermore, soil profiles usually show a heterogeneous layering with different physical and/or chemical characteristics at different depths, particularly in lowland areas such as wetlands, due to

different sedimentation events (Reddy and DeLaune 2008). The relations between the changes occurring in the rhizosphere environment and the behavior of the corresponding plants at different depths are key aspects to understand the biogeochemical processes in the water-soil-plant system, but also to increase the options to manipulate it to our advantage. The interest of this knowledge should be raised in the current scenario of global change (Philippot et al. 2009), even more under unfavorable conditions such as those in eutrophic systems. Therefore, the aim of the present study was to assess the role of *Phragmites australis* in the temporal variability of the physico-chemical and microbiological soil properties in eutrophic wetlands. To achieve this goal, a mesocosms experiment was performed with alternating flooding-drying conditions with eutrophic water and where several soil parameters were monitored and plant physiological activity measured during 44 weeks. We hypothesized that: 1) soil properties influencing by the biogeochemical processes are temporarily dynamics due to that they are affected by changes in factors such as soil moisture and temperature; 2) this dynamic changes at different soil layers, and might be altered both by the presence of the rhizosphere of *Phragmites* and the eutrophic water; 3) the physiological state of *Phragmites* might change over time due to variations in temperature and soil moisture conditions; 4) the influence of *Phragmites* on the rhizospheric soil might change over the experimental period in response to changes in factors such as temperature and soil moisture.

Materials and methods

Field sampling and initial soil characterization

Soil and plant material were collected from the Marina del Carmoli salt marsh, located on the coast of the Mar Menor lagoon (SE Spain, N 37° 41' 42"; W 0° 51' 31", Fig S1), which has been extensively studied (e.g., Álvarez-Rogel et al. 2007; González-Alcaraz et al. 2012). Three types of samples were taken: 1) soil (top 25 cm) and the corresponding plants of *Phragmites* growing above it were collected from a stand of this species; 2) soil (top 25 cm) from a bare area next to the *Phragmites* stand; 3) sand from a dune system of the marsh located next to the shoreline.

Five aliquots of the soil and sand samples were air-dried and sieved through a 2-mm mesh. Electrical conductivity (EC) and pH were determined in a 1:5 soil:water suspension after shaking for 2 h. Particle size distribution was determined by Robinson's pipette method, following organic matter oxidation with H₂O₂ and dispersion with Na₆P₆O₁₈.

Soil and sand subsamples (one for each aliquot) were grounded in an Agatha mortar for the determination of total CaCO₃, total organic matter and total nitrogen. Total CaCO₃ was determined by the Bernard calcimeter method, with 4 M HCl (Hulseman 1996; Muller and Gatsner 1971). Total organic matter was determined as loss on ignition (LOI) at 500 °C. Total nitrogen (TN) was determined in an automatic Leco CHN analyzer.

The characteristics of the collected materials appear in Table 1. The soil from the *Phragmites* stand was slightly less saline and had higher LOI and TN content than the other soil. Total CaCO₃, pH and texture were very similar between both soils. The sand was much less saline, with higher pH and CaCO₃ content than the aforementioned soils.

Experimental set-up and soil parameters measured

The experiment was performed in 12 methacrylate mesocosms (0.5×0.5×1 m³, Fig. 1) located inside a greenhouse located ~10 km inland from the Marina del Carmoli salt marsh. The mesocosms were filled with ~15 cm of the collected sand at the bottom (subsurface layer) and with ~25 cm of the collected soil (surface layer) above the sand. This simulates a typical soil profile from many zones of the Marina del Carmoli salt marsh, with a deeper sandy C horizon and a surface fine textured A horizon (Álvarez-Rogel et al. 2001, 2007). Six mesocosms were filled with the material (soil+plants) from the *Phragmites* stand, and six with the soil from the bare area. Each mesocosms was equipped, at each depth (surface and subsurface layers), with six 10-cm Rhizon® type samplers (pore diameter 0.1 µm) and three pH and Eh electrodes (Crison 50–50 and 50–55, respectively). The Rhizon® samplers were connected to 50-mL plastic syringes by means of polyethylene extension tubes to extract soil pore water. A drain cock was installed at the bottom of each mesocosm (Fig. 1). To allow the equilibration of the soil and the establishment of the plants, all the mesocosms were irrigated under similar conditions during 4 months with no eutrophic

Table 1 Characteristics of the soils and sand used in the experiment

	EC dS m ⁻¹	pH	CaCO ₃ g kg ⁻¹	LOI %	TN %	Texture
<i>Phragmites</i> soil	1.48±0.22	7.95±0.09	28±1	6.77±0.28	0.19±0.01	Loam
Bare soil	3.24±0.05	8.09±0.04	31±2	5.87±0.16	0.14±0.01	Silty Loam
Sand	0.23±0.01	8.70±0.04	59±1	3.46±0.04	0.08±0.01	Sand

Values are mean±SE ($n=5$). EC: electrical conductivity in the extract 1:5; LOI: loss on ignition; TN: total nitrogen

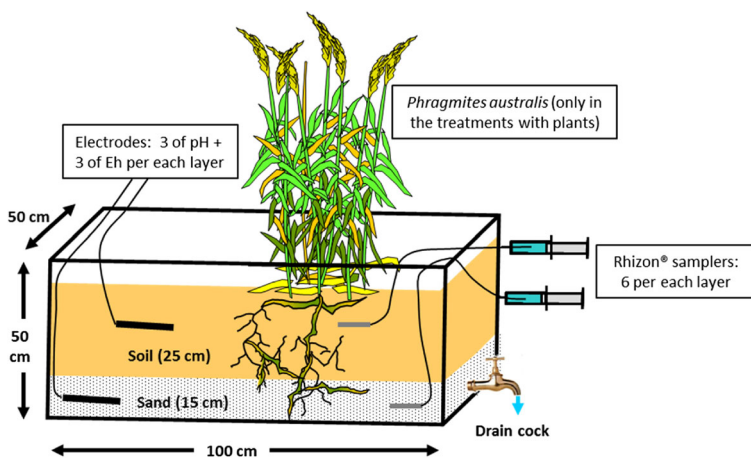
water. After that, the plants were cut at ground level and the experiment started ($t=0$).

The water regime consisted on flooding the mesocosms for 3–4 weeks (flooding phases) and on draining the mesocosms during the following 3–4 weeks (drying phases) (see Table S1 for details). The flooding-drying cycle was repeated six times during 44 weeks from April 2012 to April 2013. During the flooding phases, the water level was maintained ~5 cm above the soil surface by adding small quantities of water when necessary. The notation used throughout the paper is: F refers to flooding phases; D refers to drying phases; a number after F or D indicates the week of the experiment (e.g., F22 refers to week 22, which was a flooding week; D27 refers to week 27, which was a drying week).

To flood the mesocosms we used saline water with a similar composition to the water flowing throughout the salt marsh (2.5 g L⁻¹ Cl⁻, 1 g L⁻¹ SO₄²⁻, 0.3 g L⁻¹ Ca²⁺, 0.2 g L⁻¹ Mg²⁺, 0.1 g L⁻¹ K⁺, 1.5 g L⁻¹ Na⁺) with two levels of eutrophication. *Low Nutrients* level (LN) (EC 11.5 dS m⁻¹; pH 7.7): 20 mg L⁻¹ NO₃⁻, 0.5 mg L⁻¹ NH₄⁺, 0.6 mg L⁻¹ PO₄³⁻, 10 mg L⁻¹ dissolved organic carbon (DOC); and *High Nutrients* level (HN)

(EC 12.0 dS m⁻¹; pH 7.2): ten-fold more concentration than in the LN level. DOC consisted on a mixture of C₆H₁₂O₆ (glucose) and meat extract Sigma-Aldrich (46 % organic carbon) in a 2:1 ratio. The eutrophication levels were based on results obtained in previous field studies in the watercourses flowing into the salt marsh (Álvarez-Rogel et al. 2006; González-Alcaraz et al. 2012). Six mesocosms (three with *Phragmites* and three without plants) were flooded with the LN water and six (three with *Phragmites* and three without plants) with the HN water. Hence, the treatments assayed were: LN+no plant; HN+no plant; LN+*Phragmites*; HN+*Phragmites*.

Throughout the experiment, air temperature and moisture inside the greenhouse were recorded with a data logger LOG32. The frequency of monitoring and sampling was different for different parameters, depending on the sensibility of temporal changes (Table S1). The pH and Eh were regularly monitored (Table S1); the three measures from each layer were averaged to obtain a single value per depth and mesocosm). The Eh measurements were adjusted according to Vepraskas and Faulkner (2001), by adding +200 mV to the soil voltage (the value of the Ag/AgCl reference electrode at 20 °C). The temperature in the surface soil layers was measured

Fig. 1 Scheme of the experimental set-up

by inserting two thermometers until ~5 cm during pH and Eh measurements (Table S1).

Soil pore water samples were regularly extracted from each mesocosm (Table S1). The six sub-samples obtained from each layer were poured together to obtain a composite sample per depth and mesocosms, and frozen until subsequent analysis. DOC concentration was measured with an automatic analyzer TOC-VCSH Shimadzu. To ascertain the origin of the dissolved organic matter in pore water, several parameters were measured in selected representative samples. These samples were mainly chosen to represent inflexion points in the evolution of the physico-chemical soil conditions (pH and Eh) and the physiological activity of the plants. The content of total soluble phenolic compounds was assessed spectrophotometrically using the Folin-Ciocalteu method as described by Pérez-Tortosa et al. (2012); results are expressed in μmol gallic acid equivalents per liter. Aromaticity was estimated from calculations of carbon specific UV absorbance at 254 nm (SUVA_{254}) and expressed as $\text{L mg}^{-1} \text{cm}^{-1}$ according to Weishaar et al. (2003). UV absorbance readings were corrected taking into account the levels of nitrate determined previously in these samples. The fluorescence index (FI) was determined using a NanoDrop 3300 fluorospectrometer (Thermo Scientific). FI is defined as the ratio of the emission intensity at 450 to 500 nm under excitation at 370 nm (McKnight et al. 2001). To ensure a comparability of all water samples, DOC concentration was adjusted to 10 mg L^{-1} prior to carry out the UV and fluorescence measurements.

The CO_2 emissions from soil surface were measured in situ with a SRC-1 Respiration System CIRAS-2 during flooding and drying phases (Table S1). The results are expressed as $\text{g m}^{-2} \text{h}^{-1}$.

After finishing each phase of flooding and drying conditions, three soil aliquots were collected from the upper soil layer of each mesocosms and poured together to obtain a composite sample. These samples were frozen until subsequent analysis of microbial biomass carbon (MBC). MBC was determined by the fumigation-extraction method (Vance et al. 1987). Ten grams of fresh sample were fumigated with chloroform, whereas, other 10 g were not fumigated. The C was extracted with 0.5 M K_2SO_4 from fumigated and non-fumigated samples and measured with a TOC-VCSH Shimadzu equipment. MBC was calculated by the expression: $\text{MBC} = \text{C extracted} \times 2.66$ (Vance et al. 1987),

where C extracted is the difference between the C extracted from the fumigated samples and the C extracted from the non-fumigated samples.

Photosynthesis and fluorescence measurements on *Phragmites* plants

Photosynthetic gas exchange and chlorophyll fluorescence determinations were performed regularly (Table S1) at midday (at least 5 replicates per mesocosm) using the first fully expanded leaf from the apex of the plant. A portable infrared gas analyzer (CIRAS-2, PP system) was used to estimate the net photosynthesis rate at a photosynthetic photon flux density level of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ and at a CO_2 concentration of $350 \mu\text{mol mol}^{-1}$. The measurements of chlorophyll fluorescence were made on the upper (adaxial) surface of leaves using a portable modulated fluorimeter (FMS-2, Hansatech Instruments Ltd.). Leaves were dark adapted for 30 min using leaf clips (Hansatech Instruments Ltd.) to determine F_0 (minimum fluorescence yield) and F_m (maximum fluorescence yield) in this stage and, then exposed for 15 min to actinic light ($500 \mu\text{mol m}^{-2} \text{s}^{-1}$) to obtain F_s (steady-state fluorescence yield), F_m' (light-adapted maximum fluorescence) and the minimal fluorescence level in the light-adapted state (F_0'). Calculations for photochemical quenching [$qP = (F_m' - F_s) / (F_m' - F_0')$] and other fluorescence-related parameters were carried out following the recommendations described by Maxwell and Johnson (2000).

Statistical analyses

For each treatment and soil layer, the relations among variables were analyzed by Spearman's rank correlations. Repeated measures ANOVA (RM-ANOVA) was carried out to compare the evolution of different variables over time and among treatments for each soil layer. The factors included in the analysis were: (1) an inter-subjects factor, treatment, with four levels (*LN+ no plant*; *HN+ no plant*; *LN+ Phragmites*; *HN+ Phragmites*); and (2) an intra-subject factor, time -the repeated factor, with many levels as sampling days (e.g., for Eh and pH $n=49$; for DOC $n=39$; for fluorescence index $n=21$; Table S1). The dependent variables were the parameters analyzed in pore water, soil and plants. When data did not fulfill the sphericity requirement for Mauchly's sphericity test, univariate F statistics using a

corrector index of epsilon were applied, based on either Greenhouse–Geisser, Huynh–Feldt or Lower-bound corrections (SPSS Inc., 2006). A significant effect of time indicates that changes of a dependent variable (ie. the dynamic) were significant along the experiment, regardless the treatment. A significant effect of time x treatment interaction indicates that the evolution of a dependent variable over time differed among treatments. A significant effect of treatment indicates that the average values of a dependent variable were different among treatments. When the treatment was a significant factor, Bonferroni post-hoc tests were performed to identify differences. Statistical analyses were performed with SPSS 17.0 (SPSS 2008). Differences were considered significant at $P < 0.05$.

Results

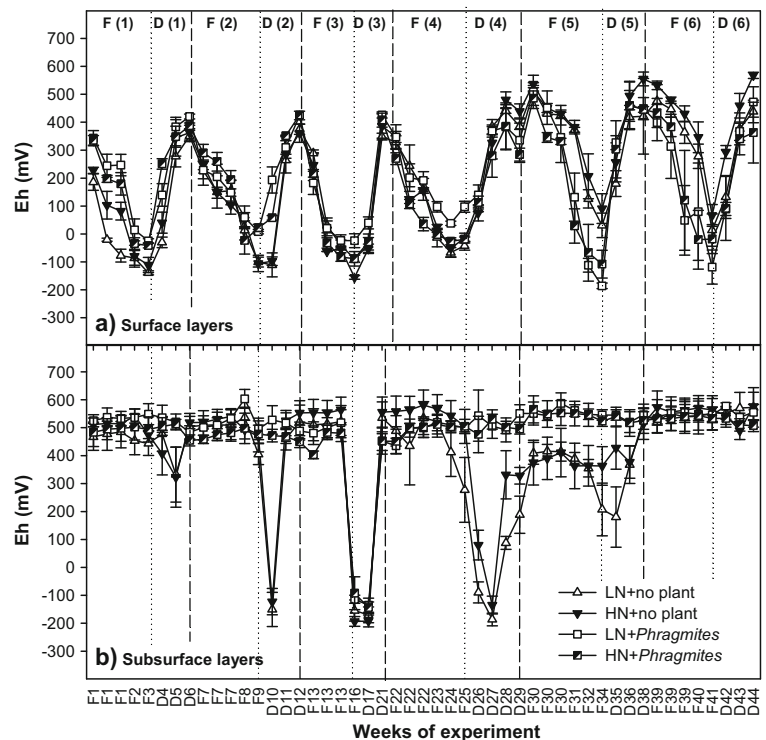
Changes in Eh, pH and temperature

The behavior of the Eh was different for surface and subsurface layers (Fig. 2). In the surface layers (Fig. 2a), the RM-ANOVA indicated that the Eh evolved significantly along the experiment (time effect, $P < 0.001$;

Table S2) regardless the treatment. The Eh values decreased during flooding phases (minimum ~ -200 mV in F34) and increased during drying phases (until $> +400$ mV), regardless the presence of plants and the type of water (non-significant effect of the treatment and time x treatment interaction). In the sandy subsurface layer (Fig. 2b), the evolution of the Eh along the experiment was also significant (time effect, $P < 0.001$; Table S2). However, in this case, it was affected by the treatment, with a significant effect of the interaction time x treatment ($P = 0.028$; Table S2) which indicated that not all the treatments changed in the same way over time. Besides, there were significant differences among the average Eh values (treatment effect, $P = 0.007$; Table S2): the treatments with *Phragmites* showed higher values. In the treatments without plants, the Eh values dropped at the end of the flooding phases and the beginning of the drying phases (minimum ~ -200 mV in weeks F16–D17) except between weeks F41–D42. However, in the treatments with *Phragmites*, the Eh hardly varied (average $\sim +500$ mV) except in weeks F16–D17 when it decreased to similar values than in the treatments without plants.

The temporal changes of pH (Fig. 3) along the experiment were significant for both surface and subsurface

Fig. 2 Evolution of redox potential (Eh) during the experiment in surface (a) and subsurface (b) layers. Values are mean \pm SE ($n=3$). F refers to flooding and D to drying phases. In the upper part of the graph, numbers in parentheses after F or D indicate the flooding or drying phase. In the x-axes, the numbers indicate the week of the experiment (e.g., F22 was the 22th week of the experiment, which belonged to the fourth flooding period -F(4)-; D35 was the 35th week of the experiment, which belonged to the fifth drying period -D(5)-). During the first week of each flooding period data were taken during three alternating days (Table S1)



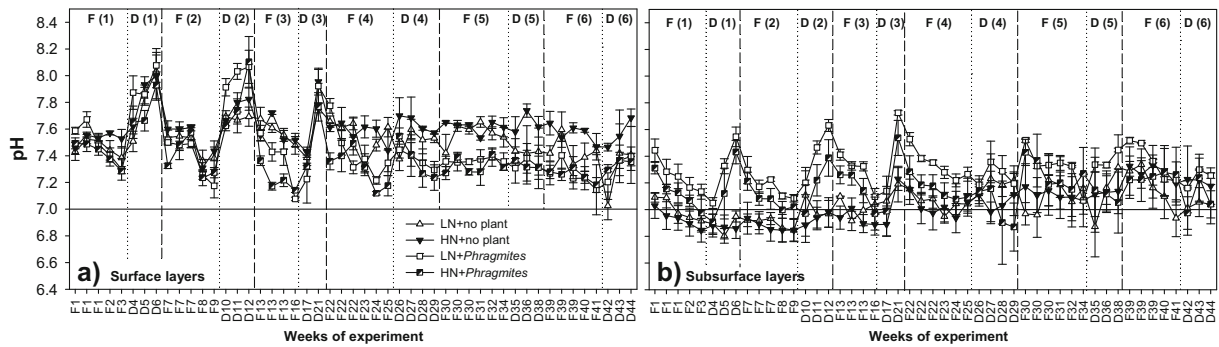


Fig. 3 Evolution of pH during the experiment in surface (a) and subsurface (b) layers. Values are mean \pm SE ($n=3$). F refers to flooding and D to drying phases. In the upper part of the graph, numbers in parentheses after F or D indicate the flooding or drying phase. In the x-axes, the numbers indicate the week of the

experiment (e.g., F22 was the 22th week of the experiment, which belonged to the fourth flooding period -F(4)-; D35 was the 35th week of the experiment, which belonged to the fifth drying period -D(5)-). During the first week of each flooding period data were taken three alternating days. (Table S1)

layers (time effect, $P \leq 0.025$; Table S2). In both cases, the interaction time \times treatment was significant ($P \leq 0.015$) indicating that the changes of pH over time were different in the different treatments assayed. In the absence of plants, the pH values in the surface layers (Fig. 3a) were clearly higher than in the subsurface layers (Fig. 3b), but this difference was not so evident for the treatments with *Phragmites*. Moreover, it was clear that there were two periods in the evolution of pH: a first period (between weeks F1 and D21) in which the pH increased during drying and decreased progressively during flooding phases in both layers; and a second period (between weeks F22 and D44) in which the pH oscillations were less pronounced. When the RM-ANOVA was applied separately for the data of these two periods, the results were different for the surface and subsurface layers (Table S2). In the first 21 weeks of the experiment (weeks F1 to D21), the effect of time was significant at both depths ($P < 0.001$; Table S2). The time \times treatment interaction was significant only in the subsurface layer ($P = 0.010$; Table S2), where the treatment HN+no plant had a significant lower pH than the treatment LN+*Phragmites* (treatment effect, $P = 0.017$; Table S2). In the second period (weeks F22 to D44), the pH also changed significantly at both depths (time effect, $P \leq 0.008$), but there was non-significant time \times treatment interaction, neither in the surface layer nor in the subsurface layer. In this second period, the treatment HN+no plant showed a significant higher pH than the treatment HN+*Phragmites* in the surface layer (treatment effect, $P = 0.015$; Table S2). However, there were no differences among treatments in the subsurface layer.

The highest air temperature values occurred during the third flooding-drying period (days 90–145, weeks F13–D21), decreasing from day 150 (week D22) onwards in accordance with an increase of air moisture (Fig. 4a). The soil temperature was very similar among the different treatments (range of variation < 0.1 °C) and hence the data of the different mesocosms were pooled together (Fig. 4b). The soil temperature progressively increased from ~ 17 °C in week F1 to ~ 30 °C in week F16 and decreased until reaching ~ 10 °C in week F30. Between weeks F39 and D44 there was a tendency to increase up to ~ 17 – 20 °C in the last two weeks of the experiment.

Changes in DOC, CO₂ and MBC

The concentration of DOC (Fig. 5) significantly evolved during the experiment at both layers (time effect, $P < 0.001$; Table S2). In the surface layer (Fig. 5a), the temporal changes were not affected by the treatments (non-significant effect of treatment and time \times treatment interaction). However, in the subsurface layer (Fig. 5b), the treatments showed different evolution (significant time \times treatment interaction, $P = 0.012$; Table S2), and, besides, the treatment HN+*Phragmites* showed significant higher DOC concentration than the treatment LN+no plant (treatment effect, $P = 0.029$; Table S2). The strongest DOC oscillations were found between weeks F1 and D21. In these first 21 weeks of the experiment the results of the RM-ANOVA were similar than those obtained when we analyzed together the data for the 44 weeks of the experiment. However, between weeks

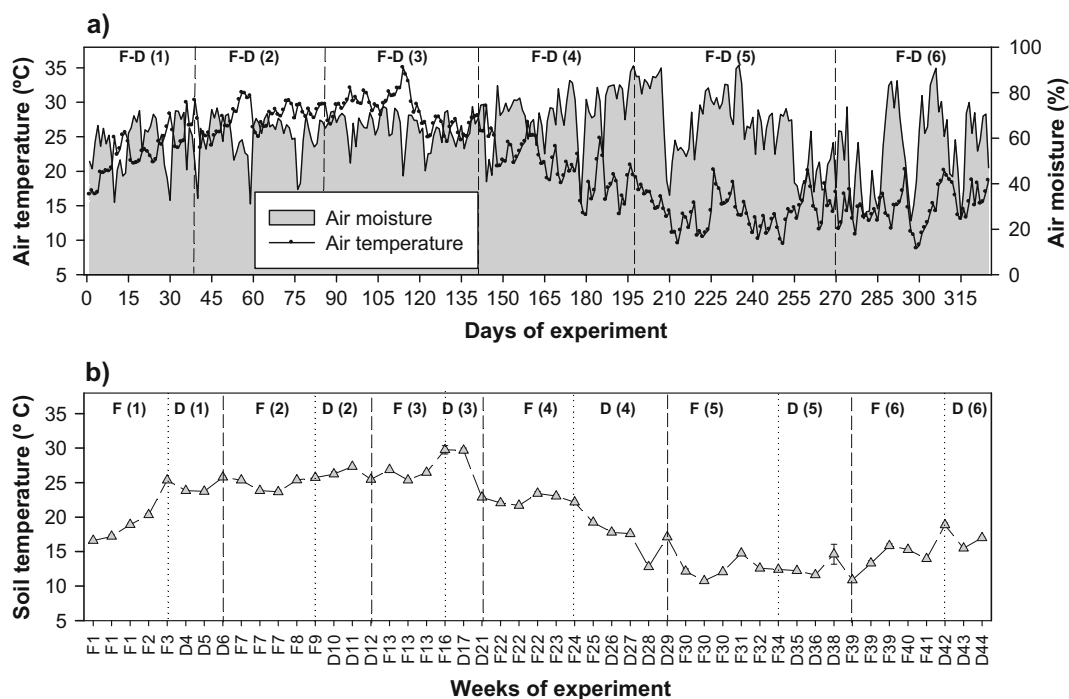


Fig. 4 **a** Evolution of daily average air temperature and moisture inside the greenhouse during the experiment. **b** Evolution of soil temperature (top 5 cm) during the experiment (values are mean \pm SE, $n=12$). F refers to flooding and D to drying phases. In the upper part of the graph, numbers in parentheses after F or D indicate the flooding or drying phase. In the x-axes, the numbers

indicate the week of the experiment (e.g., F22 was the 22th week of the experiment, which belonged to the fourth flooding period -F(4)-; D35 was the 35th week of the experiment, which belonged to the fifth drying period -D(5)-). During the first week of each flooding period data were taken three

alternating days (Table S1) (e.g., F22 and F25, a progressive increase of DOC concentrations was observed, followed by a drop until week F30 and a tendency to stabilization between this week and the end of the experiment. When RM-ANOVA was applied to the data of this second period, the temporal evolution was also significant for both layers (time effect, $P \leq 0.006$; Table S2) but without differences among the treatments.

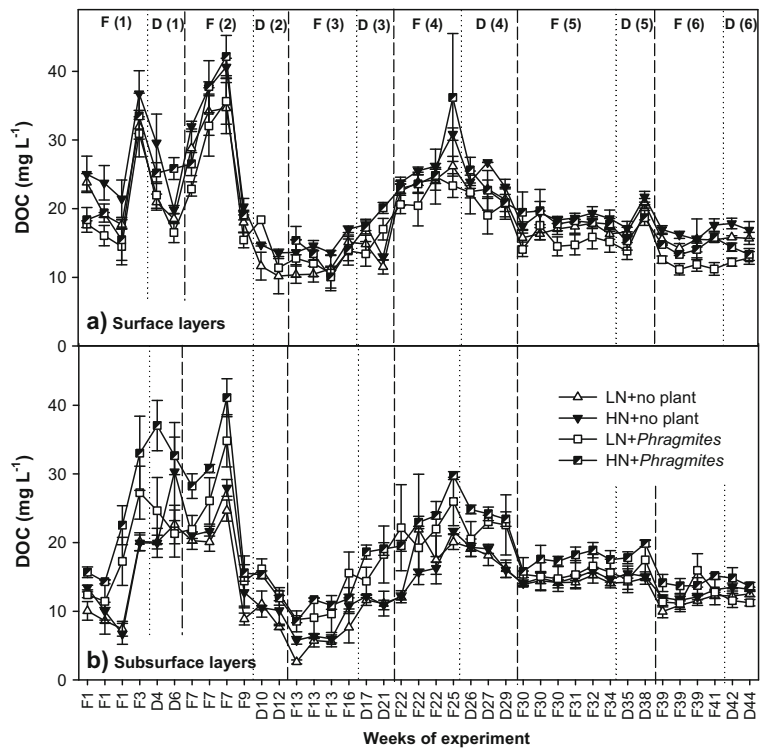
Regarding the nature of DOC (Fig. 6), the evolution of $SUVA_{254nm}$, phenolic content and fluorescence index along the experiment showed two periods with different behavior: the strongest temporal oscillations occurred in the first three flooding-drying phases (weeks F3 to D21) and the lowest in the last two cycles (weeks F30 to D44), with a stabilization of the measurements. The three parameters significantly varied over time (time effect, $P < 0.001$; Table S2) when considering the 44 weeks together and also independently for each of the two periods described above, but without differences among treatments.

The rate of CO_2 emissions (Fig. 7) significantly changed along the experiment (time effect, $P < 0.001$;

Table S2), but with similar dynamic in the four treatments assayed (non-significant effect time \times treatment interaction). The higher CO_2 emissions were observed between weeks F1 and D21, with peaks during the drying phases. The treatment LN+no plant produced significantly (treatment effect, $P=0.032$; Table S2) lower CO_2 emissions (average $0.15 \pm 0.02 \text{ g m}^{-2} \text{ h}^{-1}$) than the treatment HN+*Phragmites* (average $0.28 \pm 0.02 \text{ g m}^{-2} \text{ h}^{-1}$).

In relation to the MBC (Fig. 8), when the RM-ANOVA was applied to the whole experiment (weeks F3 to D44), non-significant effects of time, treatment and time \times treatment interaction were found (Table S2). However, between weeks F1 and D21, the changes in MBC were significant over time (time effect, $P=0.019$; Table S2). During this first period, the treatments showed significant different dynamics (effect time \times treatment interaction, $P=0.030$; Table S2), with significant higher MBC values in the treatment HN+*Phragmites* than in those without plants (treatment effect, $P=0.013$; Table S2). From weeks F22 to D44 there was neither temporal evolution nor effects of the treatments.

Fig. 5 Evolution of dissolved organic carbon (DOC) in pore water during the experiment in surface (a) and subsurface (b) layers. Values are mean \pm SE ($n=3$). F refers to flooding and D to drying phases. In the upper part of the graph, numbers in parentheses after F or D indicate the flooding or drying phase. In the x-axes, the numbers indicate the week of the experiment (e.g., F22 was the 22th week of the experiment, which belonged to the fourth flooding period - F(4); D35 was the 35th week of the experiment, which belonged to the fifth drying period -D(5)-). During the first week of each flooding period data were taken during three alternating days (Table S1)



Physiological conditions of *Phragmites*

The evolution of the photosynthesis-related parameters in *Phragmites* is shown in Fig. 9. Plants were inactive from week D27 (November) onwards; hence data could be collected only until this period. The changes of net photosynthesis (Fig. 9a) over time were significant (time effect, $P=0.001$; Table S2) but not affected by the nutrients level in the water (non-significant effect of the treatment). However, the photosynthetic capacity of *Phragmites* was slightly higher in the LN treatment. During the first two flooding-drying phases (weeks F1 to D12), net photosynthesis tended to increase during flooding (maximum $\sim 12.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ in week F3) and to decrease during drying (minimum $\sim 2.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ in week D6). This behavior was different between weeks F13 and D27: there was a general increase of net photosynthesis from week F13 to D19 and a general decline from week F22 onwards, coincidental with an extensive drying of the leaves (personal authors' observations). The photochemical quenching (qP, Fig. 9b) was almost constant from week F1 to D19 and decreased markedly from week F22 onwards (significant effect of time, $P=0.001$; Table S2), but without significant time \times treatment interaction nor differences between treatments.

Discussion

In relation with our initial hypothesis, the results showed that: 1) there was a strong temporal component in the biogeochemical behavior of the soils due to changes in factors such as temperature and soil moisture content; 2) the behavior described in the previous point was modulated depending on the depth and the presence/absence of *Phragmites* and its physiological state in different periods of the year; 3) the concentration of nutrients in the flooding water seemed to have a minor role in the whole response of the system, but it was somewhat relevant for the microbial biomass.

Temporal changes of the biogeochemical conditions in the surface layers

The oscillations of the Eh values in the surface layers followed a typical pattern, decreasing during the flooding phases (reaching anoxic conditions, $\text{Eh} < \sim +100 \text{ mV}$ according to Otero and Macías 2003) and increasing during drying phases (reaching oxic conditions, $\text{Eh} > \sim +350 \text{ mV}$ according to Otero and Macías 2003). This pattern was observed regardless the presence of *Phragmites* and the nutrient level in the flooding

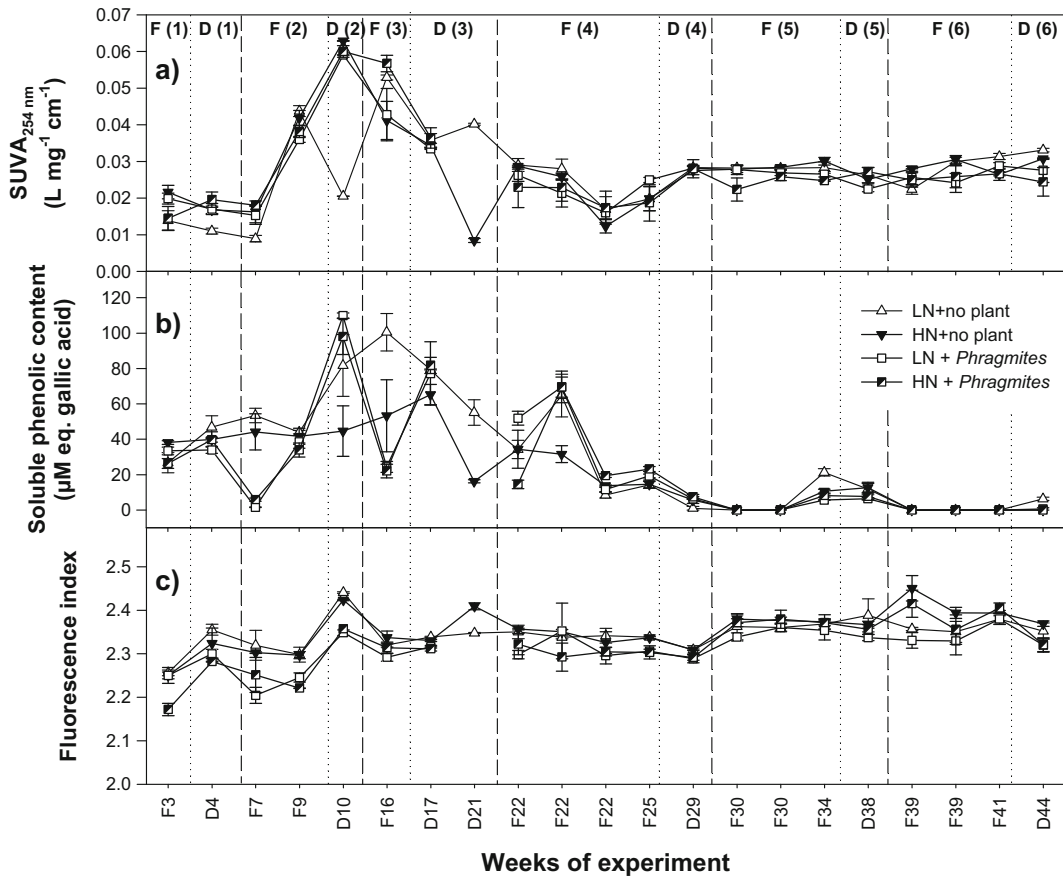


Fig. 6 Evolution of specific UV absorbance at 254 nm ($SUVA_{254nm}$) (a), soluble phenolic content (b), and fluorescence index (c) in pore water during the experiment. Values are mean \pm SE ($n=3$). F refers to flooding and D to drying phases. In the upper part of the graph, numbers in parentheses after F or D indicate the

flooding or drying phase. In the x-axes, the numbers indicate the week of the experiment (e.g., F22 was the 22th week of the experiment, which belonged to the fourth flooding period -F(4)-; D35 was the 35th week of the experiment, which belonged to the fifth drying period -D(5)-)

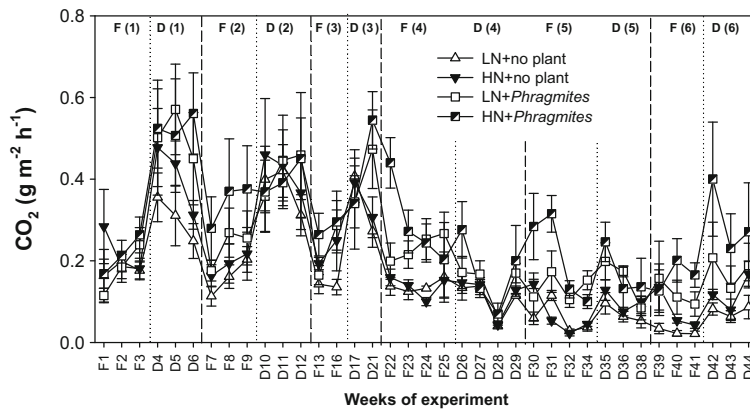
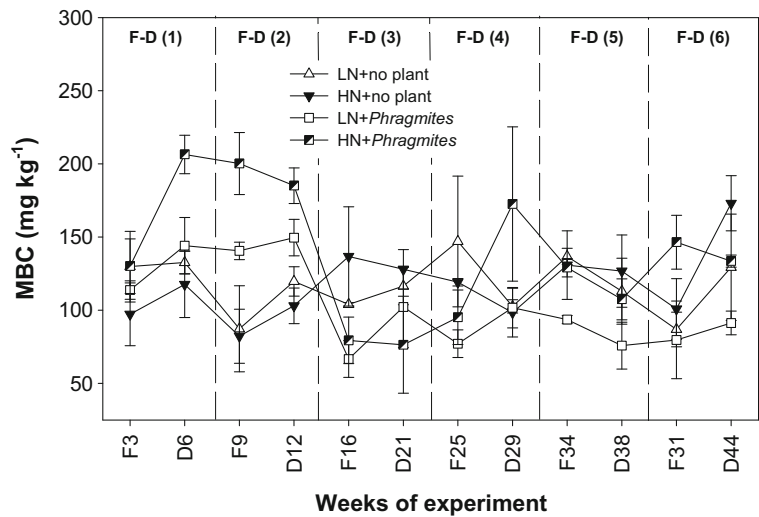


Fig. 7 Evolution of CO_2 emissions from soil surface during the experiment. Values are mean \pm SE ($n=3$). F refers to flooding and D to drying phases. In the upper part of the graph, numbers in parentheses after F or D indicate the flooding or drying phase. In

the x-axes, the numbers indicate the week of the experiment (e.g., F22 was the 22th week of the experiment, which belonged to the fourth flooding period -F(4)-; D35 was the 35th week of the experiment, which belonged to the fifth drying period -D(5)-)

Fig. 8 Evolution of carbon from microbial biomass (MBC) in the upper soil layers during the experiment. Values are mean±SE ($n=3$). F refers to flooding and D to drying phases. In the upper part of the graph, numbers in parentheses after F or D indicate the flooding or drying phase. In the x-axis, the numbers indicate the week of the experiment (e.g., F9 was the 9th week of the experiment, the end of the second flooding period; D29 was the 29th week of the experiment, the end of the third drying period)



water, which indicated that: the soil acted as a suitable environment for the activity of microorganisms; the oxygen diffused into the soil during the drying phases; and there were alternative electron-acceptors than oxygen for anaerobic activity during flooding phases (Reddy and Delaune 2008). The behavior described was favored by the composition of the flooding water

that included a substantial amount of soluble organic matter (substrate for microorganisms) and nitrate (a suitable electron acceptor for anaerobic metabolism).

The changes observed in soil pH could be attributable to several factors acting together. In flooded soils, a drop in Eh values implies H⁺ consumption that might lead to an increase of pH (Ponnamperuma et al. 1966; Stumm

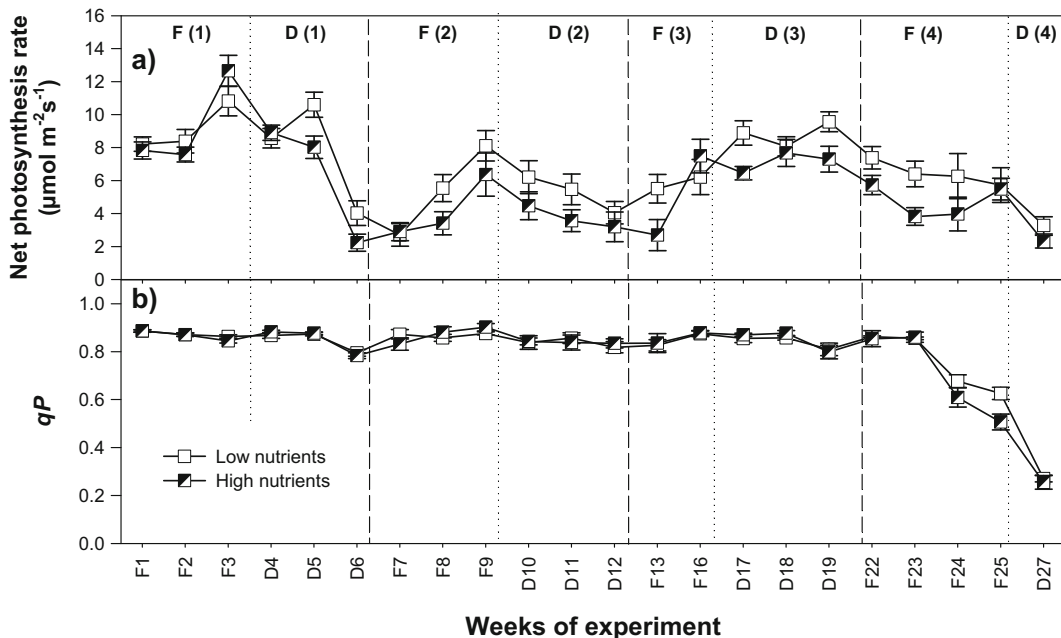


Fig. 9 Evolution of net photosynthesis rate (a) and photochemical quenching (qP) (b) in the first fully-expanded leaves of *Phragmites australis* along the experiment. Values are mean±SE ($n=3$). F refers to flooding and D to drying phases. In the upper part of the graph, numbers in parentheses after F or D indicate the flooding or drying phase. In the x-axes, the numbers indicate the week of the

experiment (e.g., F9 was the 9th week of the experiment, which belonged to the second flooding period; D19 was the 19th week of the experiment, which belonged to the third drying period). Plants were inactive from week D27 onwards; hence data could not be collected between weeks D28 and D44

and Sulzberger 1992). Hence, the higher pH values reached in the surface layers between weeks F1 and D21 could be related with their lower Eh values (Fig. 10a and c). However, the whole picture was more complex at both depths, and the changes over time were affected by the flooding-drying conditions, the microbial activity and the presence/absence of plants. Indeed, in the surface layers both Eh and pH values decreased during flooding and increased during drying phases (significant positive correlation for all the treatments, $r \geq 0.669$, $P \leq 0.05$). Our results were in accordance with the dynamic of the CO_2 - H_2CO_3 system (Reddy and DeLaune 2008). The CO_2 dissolved in pore water might form H_2CO_3 , a weak acid that leads to a decrease in pH. This process is favored when the soil is flooded, due to that the excess of water impedes gas exchange and leads to an elevated pCO_2 and low pO_2 (Greenway et al. 2006; Hinsinger et al. 2009). The strong drop of pH values observed in the surface layers during the first four flooding phases (Fig. 3) could be related to an increase of pCO_2 and a decrease of pO_2 . An intense organic carbon consumption by microorganisms as response to the pulses of eutrophic water (as shown by the drop of Eh during flooding phases, Fig. 2) implied the production of CO_2 , which remained trapped underwater. Then during the drying phases, when oxygen penetrated into the soil, the pO_2 increased and the pCO_2 decreased (as shown by larger CO_2 emissions, Fig. 7), leading to an increase of pH that reached values up to ~ 8.0 . From week F22 onwards the changes of pH did not match with the variations of Eh probably due to a slowdown of microbial activity leading to a lower CO_2 production, as explained in the following paragraphs.

In relation to the DOC dynamic, during the first two flooding phases, the concentrations tended to increase (Fig. 5a), probably due to the accumulation of organic compounds resulting from an incomplete decomposition of organic matter under water-saturated conditions (Simões et al. 2011). However, during soil drying phases this pool could be rapidly mineralized due to the stimulation of aerobic microorganisms (Reddy and DeLaune 2008), as showed by the peaks in CO_2 emissions in weeks D4-D6 and D10-D12 (Fig. 7). However, between weeks F13 and F16 (third flooding phase -F(3)) DOC concentrations did not increase (Fig. 5a), which could be attributable to a higher organic matter consumption by microorganisms favored by higher soil temperatures (Fig. 4b) (Conant et al. 2008). This explanation is supported by the drastic drop of Eh values

between the first ($\sim +200$ mV, F13) and the third (~ 0 mV, F16) day after flooding (Fig. 2a). Moreover, in all the treatments, a significant negative correlation ($r \leq -0.330$, $P < 0.05$) was obtained between soil temperature and Eh and a positive correlation ($r \geq 0.660$, $P < 0.05$) between CO_2 and soil temperature, indicating that microbial activity and hence oxygen consumption that leads to a drop of Eh values under water saturation was stimulated in warmer months, as previously stated (Reddy and DeLaune 2008). The negative effect of the low temperature on microbial activity was more evident from week F30 to the end of the experiment (week D44) with soil temperatures ~ 10 – 15 °C (Fig. 4b), as showed by a tendency to a lower CO_2 production (Fig. 7). The latter was ameliorated in the treatment HN+*Phragmites*, in which the combination of a higher nutrient content and the presence of rhizosphere could contribute to maintain the microbial activity at slightly higher levels than in the other three treatments.

In the mesocosms there were two sources of organic carbon: native soil organic carbon and organic compounds added with the flooding water. The carbon from both sources was not identified, but it is to be expected that microorganisms consumed the latter more easily because of containing higher glucose concentrations. This agrees with De Nobili et al. (2001) who found that trace amounts of simple and easily degradable substances such as glucose could stimulate microbial activity by shifting the soil microorganisms from dormancy to activity. In fact, the similar DOC concentrations (between ~ 10 and ~ 40 mg L^{-1} , Fig. 5a) regardless the nutrient level suggest that most of the organic compounds added with the flooding water were consumed during the first flooding hours. In this way, the input of exogenous organic carbon into the mesocosms did not substantially modify the ratio of autochthonous to allochthonous DOC, as indicated by a FI above 2 (Fig. 6c), which points that the dissolved organic compounds (measured as DOC) had mainly an autochthonous origin (Wang et al. 2013). In fact, the turnover of labile organic carbon is very fast and often may approach a rate of 5–10 times per day (DeBusk and Reddy 1998; Reddy and DeLaune 2008). Hence, the results observed for DOC concentrations (Fig. 5a) should be mainly attributable to the sum of two elements: organic carbon from microbial origin and organic compounds originated from the microbial attack to both stable fractions of soluble organic matter (mainly composed by alkyl and aromatic compounds)

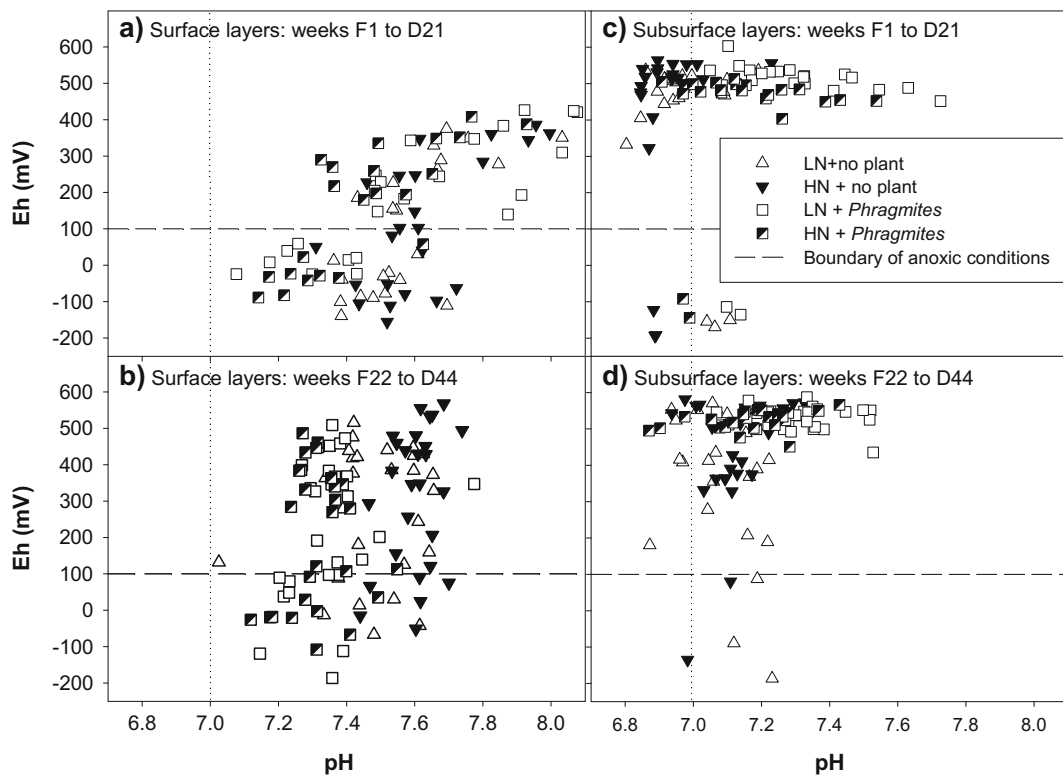


Fig. 10 Eh vs. pH in surface and subsurface layers during the experiment. F refers to flooding and D to drying phases. Numbers after F or D indicate the week of the experiment (e.g., F22 was the 22th week of the experiment; D21 was the 21th week of the

and particulate organic matter. The analysis of aromaticity shown by $SUVA_{254}$ (Fig. 6a) indicates an aromatic moieties enrichment in DOC during the first three flooding-drying phases (weeks F3 to D21, Fig. 5a), in accordance with the highest soil temperature in the mesocosms (Fig. 4b). This higher aromaticity, together with the higher content of phenolic compounds during these weeks (Fig. 6b), suggests more intense microbial activity and hence an increase of the degradation rates of organic recalcitrant compounds until soil temperature fell down in week F22 (Fig. 4b).

At the view of the results obtained from this study, the presence of *Phragmites* had a minor role, if any, in the content and dynamic of DOC during the experiment in the surface layer. These results contrast with those obtained by Lindén et al. (2014) who found a noticeable effect of *Pinus sylvestris* on soil organic matter degradation after incubation with glucose. According to these authors, fresh carbohydrates would activate soil microorganisms and increase their capacity to extract nutrients from organic matter in a phenomenon known as

experiment). The vertical dotted line indicates neutral conditions ($pH=7$) and the horizontal dashed line the boundary of anoxic conditions ($Eh < +100$ mV)

rhizosphere priming effect (Zhu and Cheng 2011). Due to that there are many uncertainties in the mechanisms underlying the priming effect (Lindén et al. 2014 and references cited therein), it is difficult to explain the apparent discrepancy between the literature revised and the results obtained in our experiment. Perhaps, the differences might be attributable to the different size and morphology in the root systems of the two plant species considered (*Phragmites* in the present study and *Pinus* in Lindén et al. 2014).

The MBC is an indicator of the microbial population in the soil (Jenkinson et al. 2004), which is expected to be more developed in soils with plants, since the rhizosphere provides a better structure and resources for microorganisms to adhere to and perform the processes necessary for elements transformation (Caffery and Kemp 1990). In the experiment, there were significant higher MBC concentrations in the treatments with plants during the first 12 weeks (Fig. 8), but the values dropped sharply in week F16 probably as a consequence of several factors that could act together. A drastic decrease

in DOC concentrations between weeks D10 and F13 (Fig. 5a) indicates a peak of consumption of soluble organic carbon by microorganisms favored by the high temperature (Fig. 4b). The oxygen consumption associated with the improved metabolic activity of microorganisms led to the strongest decrease of Eh values between weeks F13 and F16 in surface and even in subsurface layers (Fig. 2). Then, the decrease of DOC concentrations in combination with the strong anoxic conditions reached could have damaged the microorganisms (Mentzer et al. 2006; Somenahally et al. 2011), leading to a decrease of the MBC concentrations. Our results agree with the findings of McLatchey and Reddy (1998) who found a decrease of 20-fold in MBC when Eh decreased below ~ -100 mV.

Temporal changes of the biogeochemical conditions in the subsurface layers

As in the surface layers, the evolution of Eh in the subsurface sandy layers was not affected by the nutrient concentration in the flooding water. However, the presence of *Phragmites* strongly modulated the dynamic of Eh in the subsurface layers (Fig. 2b), with lower Eh values in those treatments without plants.

In most hydric soils, lower Eh values in deeper layers has been reported (Reddy and DeLaune 2008; Rostaminia et al. 2011) due to the decrease in oxygen concentration in depth (Reddy and DeLaune 2008). However, when environmental conditions are not favorable for microbial activity, oxygen is not consumed and the Eh does not decrease, even in saturated soils (Vepraskas and Faulkner 2001). The latter may explain the high Eh values in the subsurface layers (Fig. 2b) with scarce organic matter content and sandy texture (Table 1). In fact, the peaks of low Eh values in the treatments without plants occurred at the beginning of the drying phases (weeks D10, D17 and D27; Fig. 2b), and hence could be mainly related with the vertical flow of anoxic water that infiltrated from the upper to the deeper layers when the drain cocks were open, more than with the consumption of oxygen by microorganisms. Therefore, the flow of water was facilitated by the sandy texture of the deeper layer. In the treatments with *Phragmites*, the higher Eh values, even during drying phases, could be partially attributable to the architecture of *Phragmites* rhizome system that contributes to hollow the soil, facilitating air penetration (Hinsinger et al. 2009). However, the main mechanism to explain the

more oxidized environment in the presence of *Phragmites* rhizosphere probably was the diffusion of oxygen via aerenchyma which was enhanced by the sandy texture of the deeper layer. *Phragmites* has the ability to introduce oxygen in the soil through its rhizomes (Dickopp et al. 2011) via aerenchyma (Armstrong 1979; Dickopp et al. 2011) to guarantee the respiration of their root cells, which results in a local build-up of oxygen around the roots (Armstrong et al. 2000) that increases the oxygen partial pressure (Philippot et al. 2009). This phenomenon was more intense in subsurface than in the surface layers, as shown by the greater differences between treatments with and without plants in deeper layers. This agrees with the findings of Schöttelndreier and Falkengren (1999) that indicated that the process is rather confined to the apical region of the roots and rhizomes.

The only exception when the Eh dropped up to anoxic conditions in the subsurface layers of the treatments with *Phragmites* was seen in weeks F16–D17 (Fig. 2b), the warmest weeks during the experiment (average diurnal soil temperature ~ 30 °C, average diurnal air temperature ~ 35 °C; Fig. 4). During this period, most of the leaves and stems of *Phragmites* were dried, and it was expected that the capacity to aerate the soil decreased. In fact, the ability of *Phragmites* to aerate soils is based on two mechanisms: suction due to the venturi effect at the end of dead culms and pressurization due to gradients in water vapor concentration (Colmer 2003). For the former mechanism to be operative it is necessary the presence of dead, broken (both tall and short) culms whereas pressure-driven through gases flow requires living tissues to generate the water vapor concentration gradients between microporous chambers located in the plant and the surrounding bulk air. In our study, *Phragmites* plants were not disturbed by factors such as fauna or wind. Hence, there were absence of broken culms and that, together with the air stillness inside the greenhouse and the high environmental moisture (~ 30 – 35 %, Fig. 4), could help to understand the failure in subsurface layers aeration during the hottest period of the experiment.

From week D29 onwards, the pattern of Eh variation in the subsurface layers of the treatments without plants clearly changed. The Eh values were higher than in the previous weeks of the experiment, without falling below $\sim +300$ - $+200$ mV during the fifth drying phase and with no changes during the last flooding-drying phase (weeks F39–D44, Fig. 2b). The latter could be

related to the slowdown of the microbial activity during autumn and winter.

Regarding the pH, in the absence of plants, the sandy subsurface layers clearly showed lower pH values than the surface ones regardless the nutrients concentration of the flooding water (Fig. 3). This result is noteworthy since the initial characterization of the materials used in the experiment showed that the pH was higher in the sandy ones (Table 1). Probably, the scarce chemical reactivity of the sand particles hindered its capacity to prevent the drop of pH along the experiment. Our findings highlight that the processes occurring in pore water during flooding events can modify per se the soil environment in a different way depending on the soil depth and the nature of the soil materials. The latter points the need to consider soil layering as a factor in this kind of studies. The differences between both layers were less evident in the presence of *Phragmites* between weeks F1 and D21, and disappeared from week F22 onwards (Figs. 3 and 10b and d). Between weeks F22 and D44, the pH tended to increase in the absence of plants (Fig. 10d), probably due to the consumption of H^+ associated with the larger duration of the decrease of Eh (Fig. 10d). Hence, in our experiment, the plants could have hindered the decrease of pH while they were physiologically more active, by introducing O_2 into the soil (weeks F1 to D21) so decreasing the pCO_2 . From week F22 onwards a lower activity, probably due to the lower temperature, reduced the influence of the rhizosphere and pH values decreased.

The evolution of the physiological parameters of *Phragmites* supported the interpretation done in the previous paragraph. Net photosynthesis rate displayed oscillations during the entire experiment in relation to the hydric status of the soils, increasing during the flooding phases and decreasing during the drying ones (Fig. 9a). However, from the third drying period this pattern changed: between weeks D17 and D19 net photosynthesis was more or less constant, to decline from week D22 onwards. The latter could probably indicate a switch in the whole metabolism of *Phragmites* that would lead the plant to prioritize the filling of the rhizome system in response to environmental conditions announcing the end of the warm season. On the contrary, qP values did not change until the fourth flooding-drying phase (Fig. 9b), indicating that there was not apparent slow down to photochemical reactions until that time. The qP value gives an indication of the proportion of photosystem II (PSII) reaction centers that are

open. Then, low qP values indicate a limited chloroplast capacity to channeling electrons from water to electron acceptors involved in further redox reactions (Maxwell and Johnson 2000). A noticeable decrease in qP values was observed in week F24 (Fig. 9b), regardless the plant nutrient level, indicating the photoinhibition phenomenon that might eventually lead to chloroplast dismantling and subsequent cell death. In fact, a general decay of aboveground plant parts (authors' visual observation) was observed after week F23, in accordance with the drop of soil temperature (Fig. 4b), making impossible the determination of photosynthetic parameters beyond week D27.

Conclusions

The presence of *Phragmites australis* and the concentration of nutrients in the flooding water had a different role in the temporal evolution of the physico-chemical and microbiological soil properties related to the biogeochemical processes in eutrophic wetlands, and this role was strongly influenced by the soil depth, the nature of the soil materials, and the temperature.

In the upper fine textured soil layers, Eh dynamics was mainly influenced by the existence of alternating flooding-drying conditions, regardless the presence of plants, the water nutrient load and the soil temperature. However, the evolution of the Eh values in the subsurface sandy layers was affected by the presence of plants. *Phragmites* contributed to maintain high Eh values (oxic conditions), which were mainly attributable to the capacity of this species to conduct oxygen from the shoots to the roots via aerenchyma which results in a local build-up of oxygen. The latter was facilitated by the sandy materials of the subsurface layer and modulated by the physiological performance of the plant (measured as photosynthetic activity), which decreased during the warmest period (air temperature reaching $\sim 40^\circ C$), leading to a decrease of its capacity to aerate the soils that resulted in a drop of the Eh values in the subsurface layers.

The dynamic of pH was mainly attributable to the CO_2 - H_2CO_3 system. The role of *Phragmites* in the latter process was modulated by the soil depth and the physiological activity of the plant which, in turn, was influenced by temperature. During the warmer months, the diffusion of O_2 promoted by the rhizosphere of

Phragmites hindered the drop of pH (mainly in the subsurface sandy layers).

The activity of the soil microorganisms was stimulated in warmer months (soil temperature from ~20 to ~30 °C), as shown by larger CO₂ production and higher aromaticity in pore water. The latter occurred regardless the presence of rhizosphere and nutrient content, although the combination of these two factors contributed to a higher MBC. Due to that, our observations suggest that the temporal dynamic of DOC (quantity and quality) was mainly attributable to the variations of organic C from microbial attack to native soil organic matter, with *Phragmites* playing a minor role (if any).

Our findings point out the importance of incorporating the temporal and vertical scales when reed-beds wetlands are managing to be used to cope with eutrophic inputs. We have demonstrated that the existence of soil layers with different characteristics determines the biogeochemical behavior of eutrophic wetlands along the time. *Phragmites* modulates this behavior in function of two aspects: 1) the depth and the characteristics of the soil layers and, 2) the season of the year that determines the physiological state of the plants. Although management practices such as vegetation harvesting must be planned according to the specific objectives of each case (e.g., water retention, nutrient removal), the seasonal variability of the biogeochemical conditions and the physiological state of the plants should be also taken into account.

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