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Effects of the introduction of pre-treated wastewater in a shallow lake reed stand

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Abstract: Reed stands may be employed in the amelioration of water quality or even in the treatment of wastewater. In this study, the nutrient concentrations of (i) the above- and below-ground Common Reed (*Phragmites australis*) biomass, and (ii) surface and interstitial water were analyzed in a natural stand used in wastewater treatment. The reed stand was located in Hungarian part of Lake Fertő/Neusiedler See, by the shore near Fertőrákos Bay. The nitrate, phosphate and dissolved organic nitrogen concentrations of surface water were found to be higher on the inlet side of the reed stand compared to the outlet. The N and P concentrations in the above-ground biomass and P concentrations in the below-ground biomass increased after the introduction of pre-treated wastewater. The interannual differences in the characteristics of sediment interstitial water and in the nutrient content of reed tissues were assessed using statistical methods. The samples taken before and after the introduction of the pre-treated wastewater in the parcel formed different clusters. The results of the study provide further evidence that the nutrient retention capacity of natural stands of *P. australis* may be employed in the treatment of wastewater while protecting and preserving the valuable natural assets of the lake.

Keywords: nutrients; *P. australis*; pre-treated wastewater; water quality; statistical analysis

1 Introduction

Phragmites australis (Cav.) Trin ex Steudel is one of the most prolific and widespread emergent macrophytes in the world. While it is considered an invasive species in some places [1], it has also been shown to provide numerous cultural benefits, as well as being an important species in many wetland habitats [2–13].

It has functionally adapted to anaerobic conditions in sediments, on account of its ability to translocate oxygen into the rhizosphere via its well-developed aerenchima and to oxygenize and increase the redox potential of the otherwise anaerobic surrounding sediment [14, 15]. In this way, it can create favorable conditions for the aerobic and facultative anaerobic microorganisms in its root zone [16].

P. australis fulfills an important role in water purification because of its high filtering capacity: *i.e.* by bioremediation bacterial action on the surface of the roots, and by the uptake and incorporation of nutrients into its own biomass (which can then be partially removed by harvesting). Thanks to these characteristics, *P. australis* can be successfully used in phytoremediation water treatment [17–21]. However, high water-borne nutrient levels represent stressful conditions for reed stands and may affect the metabolism and the growth of the reed, leading in the end to the decline of reed-stands [22, 23].

The efficiency of *P. australis* in water purification and nutrient removal, along with its usefulness in the treatment of domestic and agricultural wastewater, has been thoroughly described, especially regarding the use of the Common Reed in constructed wetlands [11, 19, 24–26].

The aim of the study therefore was to determine the potential capacity of a natural stand of *P. australis* to assimilate nutrients from pre-treated wastewater in the reed stands on the western shore of Lake Fertő/Neusiedler See. The approach involved the measurement of (i) the changes in nutrient concentrations in surface and interstitial water at locations within the stand of Common Reeds and at the inlet and outlet of the pre-treated wastewater (ii) the nutrient content of the above- and below-ground biomass before and after the introduction of the wastewater.

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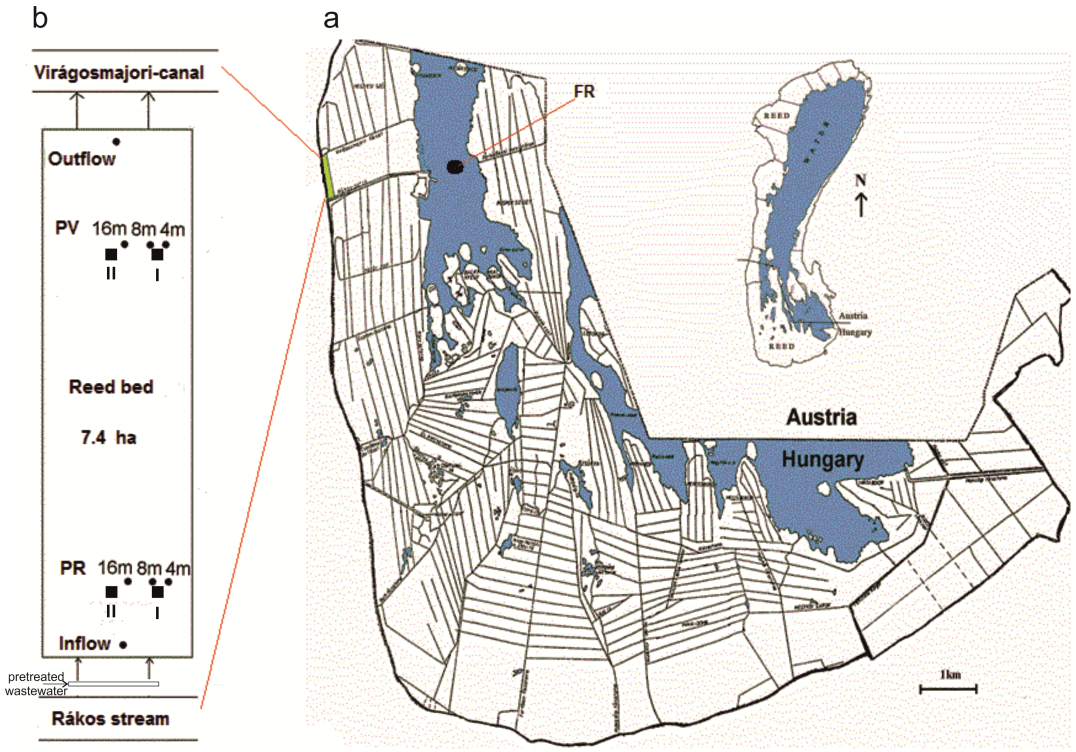

Figure 1: a) The Hungarian part of Lake Fertő/Neusiedler See. (area in blue: open water and inner ponds in the reed stands; area in white: reed stands with thin lines representing the canal system). b) Sketch of the filter bed delineated in the natural reed stand and used for the treatment of pre-treated wastewater, with sampling sites (PR: sites closed to the inlet, PV: sites close to the outlet, FR: reference site; black points: sampling sites for surface and interstitial water; black squares: reed sampling sites).

2 Materials and Methods

2.1 Site description

The study site is located in Lake Fertő/Neusiedler See, a large, shallow, reed-dominated lake, situated on the Hungarian-Austrian border with a surface area of 309 km^2 and with a regulated outflow. Common Reed covers 54% of the lake, and 85% of the Hungarian part (75 km^2). *P. australis*, is the dominant species in the littoral zone and it has been shown to play an important role in the biogeochemical nutrient cycle of the lake through the uptake, storage and decomposition [27–31]. The lake has been declared a biosphere reserve by UNESCO, as well as a Ramsar wetland, National Park, and World Heritage site. A detailed description of the lake is given in Löffler [32].

The landward edge of a wide inhomogeneous reed stand–the area of the present study-is situated near Fertőrákos Bay on the lake. A 7.4 ha area of the natural reed stand was set aside for the subsequent cleaning of the pre-treated wastewater (bottom left: 47.715993, 16.667188; bottom right: 47.716195, 16.668347; top left: 47.721653, 16.665602; top right: 47.722043, 16.666504). The parcel was provided with hydro-mechanical devices regulating the introduction of the water (Fig. 1).

From May to October a dosing rate of 300 m^3 day^{-1}, while from November to April 250 m^3 day^{-1} of pre-treated effluent was discharged to the reed bed. As a consequence of this dosing, the water depth over the wetland increased to 5 and 25 cm in summer and winter, respectively. Water flow across the reed bed was not uniform, because the soil surface of the bed was uneven. The difference in height across the parcel in the direction of the outlet was about 3 cm in every hundred meters [33].

The sampling sites (PR and PV; Fig. 1) were located 20 m from the inlet and outlet at each site. We established sampling plots at 4, 8, and 16 m from the edge of the reed bed to the interior in order to sample the interstitial water. The surface water samples were taken from the inlet, outlet, and at 4, 16 m at PR and PV sites (Fig. 1). At the same time, a reference site was selected at Fertőrákos Bay (FR) for the comparison of surface water characteristics. The reed samples were taken from the PR and PV sites close to the surface and interstitial sampling sites at 4 and 16 m, respectively (Fig. 1).

2.2 Surface water and interstitial water

Surface and interstitial water samples were collected on four dates in 2004, both before (April 4), and after the introduction of the pre-treated wastewater (June 13, August 1, October 17). Interstitial water was collected from depths of 0–20 and 20–40 cm using triplicate PVC tubes (5 cm diameter, previously purged) the lower 20 cm of which was perforated. Reducing interaction time of the samples with air to a minimum was a priority in the sampling process. Subsamples were filtered in the field for certain laboratory analyses and $ZnCl_2$ was added to the sub-samples taken for S^{2-} determination. The temperature, pH, Eh and oxygen concentration of the surface and interstitial water samples was determined *in situ* with a Hydrolog 2100 field instrument (Grabner, Vienna).

The samples were transported to the laboratory in a cooler box and stored in a refrigerator until the chemical analyses, which began the following day. The NH_4^+, NO_2^-, NO_3^-, and SO_4^{2-} concentrations were determined using a Dionex DX-120 ionchromatograph, after filtering the samples at 0.45 µm in the field and then 0.2 µm in the laboratory (using Chromafil filters). Dissolved organic (DOC), inorganic (DIC), total (DTC) carbon, and dissolved total nitrogen (DN) concentrations were measured using a LiquiTOC analyser, from previously filtered samples (using 0.45 µm Chromafil filters). The PO_4^{3-} was determined spectrophotometrically, using the molybdenum blue method. The S^{2-} concentrations of surface and interstitial water were measured employing iodometric titration [34].

2.3 Reeds

The reeds were sampled on two dates (in August 2003 and August 2004) to determine shoot length, density, inflorescence, basal diameter, living leaf (number, surface), leaf area index (LAI), shoot dry mass, and biomass using the procedures briefly described in the present study. More information can be found in [3] and [35].

The Reeds were harvested from randomly selected 0.25 m^2 quadrats at each site in 4 replicates by cutting shoots at the sediment surface. The current year aboveground biomass and the standing litter (old shoots) were determined separately. Eight samples of rhizomes were collected using 1 m long sampling tubes with a diameter of 19.5 cm [36, 37] at the PR and PV sites separately. The rhizomes were separated into living, senescing, and dead categories based on the colour, branching and consistency of the rhizome system.

The C, N, S concentrations of the above-ground and below-ground biomass were determined using a Fisons NA1500 NCS-analyser, and the P concentrations with the molybdenum blue method after the digestion with sulphuric acid.

2.4 Data analysis

Besides the calculation of the descriptive statistics of the datasets, uni-and multivariate statistical methods were applied to investigate the differences between the sampling sites in space and time. The significant differences between the group's means (i) in time for all the sampling events at one site, and (ii) in space for one sampling time and all the sites were investigated using One-way ANOVA. The homogeneity of variances was assessed using F-tests. In the case of both tests a $p = 0.05$ significance level was applied [38, 39].

To answer the question of which sampling sites and times are similar to each other, hierarchical cluster analysis was used with Ward's method [40] and squared Euclidean distance. This is a widely known multivariate classification method in which each case starts in a separate cluster and joins up to the other clusters as the linkage distance grows, until only one cluster remains [41]. This method has been successfully applied in hydrology [42–45], hydrogeology [46], geology [47–51], chemistry [52] and anthropology [53] to find similar and homogeneous groups of observations.

The validity of the groupings was verified using linear discriminant analysis (LDA), which separates the observations with linear planes resulting in a percentage of correctly classified cases [54, 55]. During the analysis, a predictive model was built for group membership. The model consists of discriminant functions based on linear combinations of the predictor variables [44].

The groupings of the sampling sites were also assessed based on the measured variables' stochastic connections using a powerful pattern recognition technique, principal component analysis (PCA) [56]. The principal components are uncorrelated and are obtained as a linear combination of the original variables [57]:

$$z_{ij} = a_{i1}x_{1j} + a_{i2}x_{2j} + \ldots + a_{im}x_{mj}$$

where z is the component loading, a is the component score, x is the measured value of the variable, i is the component number, j is the sample number, and m is the total number of variables [58]. The pattern of the observations' grouping can be visualized by plotting the scores

Table 1: Chemical characteristics of surface and interstitial water in the investigated reed parcel (PR: sites close to the inlet, PV: sites close to the outlet, FR: reference site; sites for sampling of the surface and interstitial water: PR 4, 8, 16 m, PV 4, 8, 16 m from the edge of the parcel).

Sampling sites			surface water							interstitial water					
			Inflow	PR		PV		Outflow	FR	PR			PV		
Parameter	Unit			4	16	4	16			4	8	16	4	8	16
T	°C	min	10.0	8.8	8.5	8.1	7.6	5.9	7.8	8.4	8.2	9.0	9.1	8.9	8.9
		max	23.2	16.8	17.6	18.0	18.1	22.5	22.1	17.2	17.2	17.6	18.1	18.5	18.5
Conductivity	µS cm^{-1}	min	1464	1103	1547	922	905	790	2227	1219	1336	1676	1316.5	1319	1086.5
		max	1965	1507	1744	1093	1215	1184	3030	1645	1716.5	2410	2045	1883	2174
pH	-	min	7.7	7.0	7.2	7.2	7.0	7.9	8.8	6.5	6.6	6.4	6.5	6.5	6.5
		max	8.3	7.8	7.4	7.8	8.0	8.2	9.3	7.4	7.2	7.1	7.4	7.4	7.4
DO	mg l^{-1}	min	0.25	0.64	0.80	0.56	1.06	2.25	6.90	0.51	0.75	0.61	0.61	0.79	0.77
		max	5.40	2.30	2.14	6.33	3.41	5.20	10.39	1.45	3.10	2.15	1.70	1.90	2.39
Oxygen saturation	%	min	2.3	6.6	8.0	5.9	11.5	20.8	77.0	5.0	7.5	6.1	6.5	8.6	7.7
		max	64.0	24.0	18.7	57.5	32.8	60.0	108.0	34.5	32.5	22.5	18.0	20.5	22.3
S^{2-}	mg l^{-1}	min	<LOD	<LOD	4.07	1.65	1.53	<LOD	<LOD	0.50	4.71	5.26	0.63	1.53	1.65
		max	<LOD	6.79	6.92	6.01	12.67	<LOD	<LOD	22.86	24.36	25.99	15.02	13.78	14.89
PO_4^{3-}	mg l^{-1}	min	6.00	0.34	4.48	0.14	0.05	0.17	0.04	0.30	2.62	0.39	0.42	0.67	0.54
		max	15.25	6.53	15.28	0.64	0.50	1.17	0.12	6.00	8.50	5.68	4.50	4.04	3.78
Cl^-	mg l^{-1}	min	n.a.	70.43	159.12	50.32	47.85	n.a.	261.20	78.09	88.59	87.33	57.36	60.14	57.70
		max	n.a.	159.29	213.63	105.27	81.67	n.a.	376.44	269.16	249.54	301.91	126.63	114.06	176.91
NO_3^-	mg l^{-1}	min	0.404	0.040	0.269	0.040	0.087	0.196	0.121	0.163	0.118	0.107	0.108	0.086	0.129
		max	16.080	19.265	11.462	1.302	0.381	1.771	0.484	2.295	0.605	1.916	2.401	0.426	0.490
SO_4^{2-}	mg l^{-1}	min	196.20	105.54	179.64	6.47	6.08	10.02	365.50	18.99	45.57	17.71	2.81	3.25	3.84
		max	306.67	206.35	302.27	193.16	172.78	41.72	572.12	181.08	152.23	265.52	137.78	119.48	145.08
Na^+	mg l^{-1}	min	245.06	122.23	229.40	59.52	55.41	73.65	104.82	119.76	154.48	135.70	125.16	105.94	79.87
		max	358.47	487.15	776.71	147.46	156.36	122.83	492.09	580.19	625.67	900.82	390.91	274.82	327.31
K^+	mg l^{-1}	min	26.85	12.01	25.14	12.31	11.27	11.44	14.31	6.50	9.61	4.70	8.36	10.62	11.48
		max	51.45	18.33	48.97	22.83	27.62	25.45	49.00	19.12	27.30	52.60	27.83	30.04	29.22
Mg^{2+}	mg l^{-1}	min	26.31	54.56	36.91	61.45	55.79	62.36	90.60	57.65	48.08	60.58	113.65	116.27	109.26
		max	75.36	166.67	130.73	184.36	157.41	81.23	193.88	171.00	196.66	268.32	246.84	214.65	212.19
Ca^{2+}	mg l^{-1}	min	78.94	120.86	89.52	87.58	69.58	76.72	18.77	96.71	114.44	128.79	134.39	138.07	117.44
		max	89.14	297.71	219.67	267.53	223.68	90.17	136.54	255.02	310.59	359.71	314.22	295.36	248.18
DIC	mg l^{-1}	min	88.34	90.95	100.33	94.37	101.72	99.41	120.71	111.68	135.79	144.23	172.50	173.69	145.33
		max	117.67	145.12	128.08	140.89	119.69	125.55	177.38	177.56	186.90	291.01	252.22	231.80	221.07
DOC	mg l^{-1}	min	17.51	19.29	17.89	19.66	21.78	29.39	19.02	21.52	21.82	27.62	31.14	34.82	36.04
		max	33.60	26.67	26.97	32.21	32.11	39.84	43.04	41.09	49.05	63.06	50.96	79.01	88.62
DN	mg l^{-1}	min	3.19	1.99	2.44	2.06	1.90	2.30	1.50	2.28	2.59	2.74	2.46	2.43	2.53
		max	6.77	8.23	8.72	4.71	4.21	2.74	2.62	5.69	4.91	5.97	4.99	5.65	6.30

of the first and second principal components on a scatterplot [59].

The statistical analyses were performed using R Software. Besides number of basic functions of base and stats packages, lda from MASS and PCA from FactoMineR package were used during the analysis.

3 Results

3.1 Surface and interstitial water

3.1.1 Overview

The chemical characteristics of surface and interstitial water were found to be quite diverse (Table 1). The pH of the surface water fell between 7.0 and 8.3, with the highest values measured at the inlet (8.3) and outlet (8.2). The pH values at the reed bed sites were lower than the pH values measured in surface water at the reference site (FR: 8.8–9.3). The pH of the interstitial water was lower than that of the surface water in each case.

The electrical conductivity of surface water decreased proceeding from the inlet (1464–1965 µS cm^{-1}) towards the outlet (790–1184 µS cm^{-1}). Most of the time the electrical conductivity of interstitial water increased with sediment depth, and EC values were always higher in surface water at the reference site (Table 1).

The values for dissolved oxygen (DO) concentration at the inlet and outlet did not differ remarkably from each other, and in most cases were higher than in the reed stand. The oxygen concentration also decreased from the edge (4 m) towards to interior (16 m) of the parcel. The DO concentration in the interstitial water was lower than in the surface water in most cases. At reference site, the DO concentration of the surface water was 4-5 times higher than in the reed parcel.

Higher PO_4^{3-} concentrations were measured at the inlet (6.0–15.25 mg l^{-1}) than at the outlet (0.17–1.17 mg l^{-1}). In the reed stand the PO_4^{3-} concentrations of the surface and interstitial water were higher at the PR sites in most cases. The phosphate concentration in surface water at the reference site was almost always lower (avg. 0.12 mg l^{-1}) than the values measured in the reed stand.

The SO_4^{2-} concentration in the surface water was higher at the inlet (range = 196.2–306.7 mg l^{-1}) than at the outlet (range = 10.0–41.7 mg l^{-1}; Table 1). The SO_4^{2-} concentrations of surface and interstitial water were both higher at the PR sites. The SO_4^{2-} concentrations of surface water were almost always higher than in the interstitial water, and these concentrations decreased with depth. The SO_4^{2-} concentrations were highest at the reference site (Table 1).

The concentration of S^{2-} was always beneath the limit of detectability in the surface water at the inlet, outlet, or even at the reference site. However, in surface water in the reed stand it varied between 1.5 and 12.7 mg l^{-1}. It was almost always lower than in the interstitial water (Table 1).

Ammonium and NO_2^- were not detectable in either surface or interstitial water. Nitrate concentrations were higher at the inlet compared to the outlet (Table 1) and decreased from the inlet to the outlet, but there was no consistent pattern across the distances and sites when the four sampling dates were compared. At the reference site, the NO_3^- concentrations were always lower than the values measured at the outlet (Table 1).

The DN (total dissolved nitrogen) of the surface water at the inlet varied between 3.19 and 6.77 mg l^{-1} and it was higher than at the outlet (2.3–2.7 mg l^{-1}). DN concentrations in the surface water within the reed bed were highest at the PR sites and decreased from the inlet towards the outlet. The DN in the surface water at the outlet was almost the same as that at the reference site (FR range = 1.50–2.62 mg l^{-1}).

Higher DOC concentrations were measured in the surface water at the outlet compared to the inlet. The DOC concentration of the interstitial water increased with the depth and it was higher than that in surface water.

3.1.2 Multivariate results

The cluster results of the surface water samples indicated that the samples taken before and after the introduction of the pre-treated wastewater in the parcel (PR and PV) formed two different groups, regardless of which site they came from, but in the meanwhile this process did not affect the reference site in the open water of Fertőrákos Bay (Fig. 2a). Here, the samples remained in one group before and after the introduction of pre-treated wastewater. With the grouping of the interstitial water samples, it became clear that before the introduction of the pre-treated wastewater all the samples from all the sites formed one group, while after it, these separated according to which site they were measured at, near the outlet and the inlet, PV and PR, respectively (Fig. 2b). These groupings were justified by the results of the linear discriminant analysis.

Using PCA the principal component (PC) loadings and scores were obtained for the interstitial water samples. The first two PCs explained 62–74% of the total variance. The scores of these two PCs were plotted against each other on scatterplots in the case of all sampling events. These plots

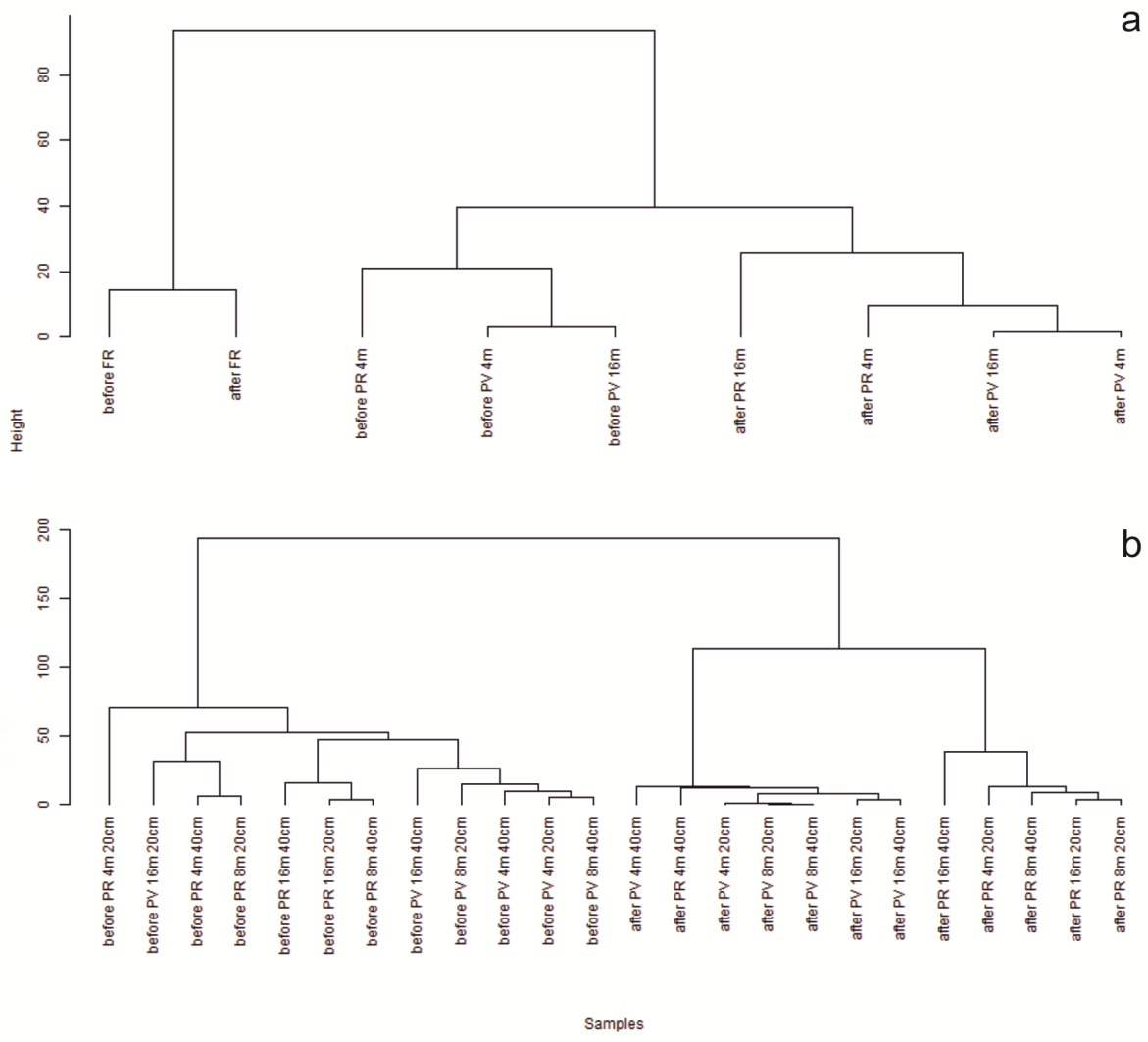

Figure 2: Grouping of the sampling sites based on the results of the cluster analysis conducted on the surface (a) and interstitial water samples (b).

confirmed the results of the cluster analysis; the samples originating from the sites near the inlet (PR) and the outlet (PV) separated from each other (A1) in spite of the fact that not the total variance of the dataset but only a part of it was considered during the analysis.

3.2 Characteristics of the Common Reed

3.2.1 Above ground

Regarding the point when the above-ground biomass reached its maximum, shoot height varied between sites and from 2003 to 2004 (Table 2). The average shoot height was smaller in most cases at the PV than at the PR site. The differences in shoot basal diameter between the PR and PV sites were notable in both years (Table 2). The number of living leaves varied between 7 and 10 per shoot at both sites; the only year when they differed significantly was 2004. The morphometry of the shoots (shorter and thinner shoots) indicated a change in 2004, leading to a considerable decrease in dry mass. This also affected biomass as well (Table 2). As a combined result of shoot density increase and the previously discussed decrease in shoot morphometry, a decrease in biomass was recorded at the PV sites (Table 2). Considerable differences in the assimilating surface were recorded between the sites and from

Table 2: Biometrical parameters and biomass of the *Phragmites australis* (PR: sites close to the inlet, PV: sites close to the outlet)
(* represents the significant differences (p = 0.05) between the investigated years at the same sampling sites; the letters indicate the significant differences between the sampling sites in the given year; > marks the significant differences between the investigated years; values in **bold** indicate that the sampling site differed significantly from another site(s) in the investigated year or from the values from among the same sites in time).

Parameters/Sampling sites	Unit	2003					2004			
		PR I	PR II	PV I	PV II		PR I	PR II	PV I	PV II
Above-ground										
shootlength	**cm**	**252.5^{b}**	**213.3^{a}**	**244.5^{b*}**	**249.8^{b*}**	>	**227.1^{b}**	215.5^{ab}	**201.4^{ab*}**	**198.8^{a*}**
density	**No m^{-2}**	84	72	54.7	66.7		92	118	92	64
inflorescence	**No m^{-2}**	9.3	16	28	18.7		10	16	10	25
rate of inflorescence	**%**	**10.7^{a}**	19.7^{ab}	**50.0^{b*}**	**27.3^{b}**		**11.5^{a}**	**13.8^{a}**	**12.5^{a*}**	**41.3^{b}**
basal diameter	**mm**	**10.1^{b*}**	**7.6^{a*}**	**6.5^{a}**	**7.2^{a*}**	>	**7.1^{c*}**	**5.9^{ab*}**	**6.1^{b}**	**5.4^{a*}**
living leaf	**shoot^{-1}**	9.3	9.3	10.1	10.1		**7.4^{a}**	**8.0^{ab}**	**9.7^{bc}**	**10.6^{c}**
LAI (leaf area index)	**m^{2}m^{-2}**	2.4	1.5	1	1.3		1.8	2.1	1.7	1.2
shoot dry mass	**g shoot^{-1}**	**38.1^{b*}**	**25.1^{a}**	**18.3^{a}**	**22.3^{a}**	>	**26.3^{b*}**	19.6^{ab}	**17.5^{a}**	**18.0^{a}**
biomass	**kg m^{-2}**	2.1	1.7	1.7	2		**2.2^{b}**	2.0^{ab}	**1.3^{a}**	1.6^{ab}
nutrient standing stock										
P	**g m^{-2}**	**1.9^{c}**	**0.8^{b}**	**0.5^{a}**	0.7^{ab}		1.9	1.7	1.3	0.7
N	**g m^{-2}**	**36.7^{c}**	**15.6^{b}**	**9.6^{a}**	16.1^{ab}		28.8	22.4	20.9	13.2
C	**g m^{-2}**	**1070.0^{b}**	**696.7^{b}**	**382.8^{a}**	648.1^{ab}		1058.6	1017.0	700.1	470.6
S	**g m^{-2}**	**5.2^{c*}**	**2.1^{b}**	**1.2^{a}**	3.5^{abc}	>	**1.2***	2.9	3.1	2.2
Below-ground										
living rhizome	**kg m^{-2}**	3.4	2.5	1.1			**4.3^{b}**	2.7^{ab}	**1.8^{a}**	**1.8^{a}**
living root	**kg m^{-2}**	0.3^{ab}	**0.3^{b}**	**0.2^{a}**			0.5	0.4	0.3	0.2
senescence rhizome	**kg m^{-2}**	**0.7***	1.4	**1.0***			**1.4^{b*}**	**1.4^{b}**	**0.3^{a*}**	**0.2^{a}**
senescence root	**kg m^{-2}**	0.2	0.4	0.4			**0.4^{bc}**	**0.6^{c}**	**0.2^{a}**	**0.2^{ab}**
bud biomass	**kg m^{-2}**	0.3	0.3	0.1			0.1	0.1	0.1	0.1

year to year (Table 2). The leaf area index (LAI) was higher at the PR than at the PV sites.

3.2.2 Below-ground

The majority of the living rhizomes and roots is situated in the upper sediment layer, above the first horizontal rhizome (20–30 cm). Differences between the investigated years were found in the course of the analysis of variance of the different parameters (Table 2). Living rhizome biomass varied between 2.5–4.3 and 1.1–1.8 kg m^{-2} at sites PR and PV respectively, while the total living biomass (rhizome, root, buds) varied between 3.1–4.9 kg m^{-2} at the PR sites, and between 1.4 and 2.1 kg m^{-2} at the PV sites.

In the rhizome system, the senescence dry mass varied between 0.5 and 1.9 kg m^{-2} at the investigated sites. The senescence rhizomes made up less than half of the total below-ground biomass.

3.2.3 Nutrient concentrations and standing stock

The highest C concentrations were measured in the living roots and senescence rhizome and the lowest ones in living leaves (Table 3), while higher P and N concentrations were measured in the inflorescence, leaves, the living rhizome, and roots than in the stem and senescence rhizome. The S concentration was relatively variable in the examined reed organs. The living and senescence roots and living rhizomes contain the highest S concentration in contrast to above-ground organs.

In 2004 the stem contained more P and C, the living leaves and senescence roots more N, P and C, the living roots more N and C, the senescence rhizomes more P and N and the living rhizomes more C than in 2003. In the above-ground organs there were no remarkable differences in the S concentrations, nevertheless in the living leaf and the underground organs the S concentration was higher in 2003.

The C standing stock of living shoots varied between 383 and 1070 g m^{-2} in 2003 and between 471 and 1059 g m^{-2} in 2004. Similar to the case of the above-ground biomass, the C standing stock was also lower at PV sites than at the PR sites (Tables 2 and 4). As for the below-ground measurements, differences were found between the C standing stock of living (440–2.210 g m^{-2}) and senescence biomass (220–912 g m^{-2}).

The N standing stock of the annual above-ground phytomass varied between 9.6 and 36.7 g m^{-2} in 2003 and between 13.2 and 28.8 g m^{-2} in 2004. It was higher in both years at the PR sites. Considerable differences were recorded at the individual sites between the N standing stock of the below-ground living and decaying biomass (4–63 g m^{-2} and 3.9–15.4 g m^{-2}, respectively).

As for the P standing stock of the new shoots, it varied between the PR and PV sites (Table 2). It was similar in 2004 (0.7–1.9 g m^{-2}) and in 2003 (0.5–1.9 g m^{-2}). There was 0.8–3.0 and 0.3–1.2 g m^{-2} P standing stock in the below-ground organs (living and decaying biomass, separately).

As for the above- and the below-ground organs, their samples were separated by cluster analysis into two groups, before and after the introduction of pre-treated wastewater (Figs. 3a and 3b respectively). These groupings were confirmed by the results of the linear discriminant analysis as in the case of the analysis of the water samples.

4 Discussion

Nutrients in water and in sediment interstitial water

Simultaneous physical, chemical, and biological processes affected the pre-treated wastewater as it flowed through the reed parcel, towards the Virágosmajor-Canal. Nutrient removal by *P. australis* is achieved via two major processes: the absorption by the plant itself and microbial activity in the rhizosphere [16, 60, 61]. As a result, the concentration of the nutrients noticeably decreased between the inlet and the outlet. It was shown that the nutrient removal efficiency of the reed beds depends on (i) the loading rates, (ii) the distribution/spread of the water at the inlet, (iii) the nutrient species occurring (organic or inorganic N and P forms), and (iv) the abiotic environmental conditions [62].

At the sites in the vicinity of the inlet (PR), the PO_4^{3-} concentration of the water was significantly higher than at the sites close to the outlet (PV). This happened presumably via the phosphate uptake of the reeds, bacteria, and algae and through phosphate absorption, complexation, and precipitation with metals and clay particles [60, 63].

The decrease in nitrate concentration in the water from the inlet to the outlet suggested that N removal was taking place in the system. The major N removal mechanisms in the reed bed systems used for wastewater treatment are the combined nitrification and denitrification processes [16, 60]. The internal oxygen transport of *P. australis* to its rhizosphere makes the simultaneous existence of oxidised, anoxic, and reduced zones possible, as also the co-occurrence of these processes [16, 64, 65].

Table 3: The element concentration (mg g^{-1}) of the above-ground and below-ground reed organs (Legend see Table 2).

P. australis	Nutrient	2003					2004			
		PR I	PR II	PV I	PV II		PR I	PR II	PV I	PV II
inflorescence	P	1.7	1.7	1.6	1.5		1.7	2.2	2.3	1.4
	N	21.0	18.3	18.8	17.5		23.3	23.2	23.9	21.5
	C	412.3	434.6	411.9	413.3		440.6	461.0	470.6	464
	S	2.5	1.8	1.7	2.4		1.0	0.8	2.45	2.5
living leaf	P	1.0	**0.8***	**1.1***	1.0	<	1.3^{ab}	**1.3^{ab*}**	**1.4^{b*}**	**1.2^{a}**
	N	25.3	**22.0***	**24.6***	**25.1***	<	30.0^{ab}	**29.5^{a*}**	**32.1^{b*}**	**32.4^{ab*}**
	C	**388.2^{ab*}**	**412.7^{b*}**	**382.6^{a*}**	415.0^{ab}	<	**435.6***	**443.6***	**450.9***	435.2
	S	**2.8***	2.1	2.4	3.8	>	**1.5^{a*}**	3.3^{abc}	**3.0^{b}**	**3.4^{c}**
stem	P	**0.6^{b}**	**0.4^{a*}**	**0.3^{a*}**	**0.3^{a*}**	<	**0.6^{b}**	**0.6^{b*}**	**0.6^{b*}**	**0.4^{a*}**
	N	**8.8^{b}**	**5.8^{a}**	**5.3^{a}**	**6.1^{a}**		**7.1^{b}**	**4.6^{a}**	6.3^{ab}	**4.5^{a}**
	C	**380.3^{a*}**	**415.5^{c*}**	**394.2^{b*}**	427.1^{abc}	<	**441.9***	**450.5***	**440.9***	444.7
	S	**1.7***	1.1	**0.9***	1.6		**0.2^{a*}**	1.0^{ab}	**1.6^{b*}**	**1.6^{b}**
living rhizome	P	**0.9***	0.6	**0.7***			**0.6^{a*}**	**0.6^{a}**	**1.0^{b*}**	**0.7^{a}**
	N	**10.6^{b}**	**4.3^{a}**	**3.4^{a}**			**12.6^{b}**	**5.0^{a}**	5.7^{ab}	11.3^{ab}
	C	**448.2^{a}**	**454.0^{b}**	**411.4^{ab*}**		<	438.5^{ab}	**439.2^{a}**	**489.4^{b*}**	**550.1^{b}**
	S	**5.0***	**8.3***	**2.8***		>	**0.8***	**0.5***	**0.5***	0.5
senescence rhizome	P	0.3	**0.2***	0.2		<	0.3	**0.3***	0.5	0.3
	N	**6.2^{b}**	**3.8^{a*}**	**5.3^{ab*}**		<	**7.2^{b}**	**5.2^{a*}**	**7.4^{b*}**	4.6^{ab}
	C	**468.6^{a}**	**468.9^{a*}**	**450.0^{b}**		>	527.1^{ab}	**446.6^{a*}**	**476.0^{b}**	**483.7^{b}**
	S	6.7	**5.5***	3.8		>	2.6^{ab}	**1.7^{b*}**	**1.5^{b}**	**0.2^{a}**
living root	P	**0.8^{b}**	**0.6^{a}**	**0.6^{a}**			0.9	0.7	0.9	0.8
	N	**11.2^{b}**	10.5^{ab}	**6.9^{a*}**		<	11.6	10.3	**11.0***	10.1
	C	**435.9^{a}**	**470.0^{b}**	**443.0^{ab*}**		<	538.0^{ab}	**444.7^{a}**	**483.8^{b*}**	483.5^{ab}
	S	**8.4***	**7.5***	**6.5***		>	**2.1^{b*}**	**2.0^{b*}**	**1.1^{a*}**	2.0^{ab}
senescence root	P	**0.8^{b}**	**0.6^{a*}**	**0.7^{b}**		<	0.8	**1.1***	0.9	0.8
	N	**12.8^{b}**	**10.3^{a*}**	**9.9^{a*}**		<	11.3	**13.1***	**13.0***	12
	C	437.6	487.5	**433.0***		<	**368.1^{a}**	**439.8^{b}**	**489.4^{c*}**	**475.4^{bc}**
	S	**7.5***	**6.2***	**5.8***		>	**2.0^{ab*}**	**2.5^{b*}**	**1.5^{a*}**	**1.2^{a}**

Table 4: The element standing stock (g m^{-2}) of the below-ground reed organs (Legend see Table 2).

P. australis	Nutrient	2003				2004			
		PR I	PR II	PV I		PR I	PR II	PV I	PV II
living rhizome	P	2.8	1.7	0.7		2.6	1.8	1.9	1.2
	N	34.4	11.1	3.4		57.7	13.9	10	19.3
	C	1526.3	1132.9	371.2		1942.8	1185.6	894	986.3
	S	**14.9***	20.5	**3.1***	>	**3.6^{b*}**	**1.5^{a}**	**0.8^{a*}**	**0.6^{a}**
senescence rhizome	P	0.2	**0.2***	0.2	<	**0.4^{bc}**	**0.5^{c*}**	**0.2^{ab}**	**0.1^{a}**
	N	**4.5***	5.2	**5.1***		**10.3^{b*}**	**7.4^{b}**	**2.7^{a*}**	**1.0^{a}**
	C	333.2	664.2	**432.0***	<	**759.4^{b}**	**644.0^{b}**	**167.6^{a*}**	**114.2^{a}**
	S	4.8	**7.8***	**3.7***	>	3.6^{abc}	**2.4^{c*}**	**0.5^{b*}**	**0.1^{a}**
living root	P	0.2	0.2	0.1		**0.4^{c}**	**0.3^{bc}**	**0.2^{ab}**	**0.2^{a}**
	N	3.4^{ab}	**3.4^{b}**	**1.0^{a}**		5.7	4.1	3.1	2.4
	C	132.4	160.2	68.4		267.3	176	139.5	122.5
	S	2.6	**2.6***	1.1	>	**1.0^{b}**	**0.8^{b*}**	**0.3^{a}**	0.5^{ab}
senescence root	P	0.2	0.2	0.3		**0.3^{bc}**	**0.7^{c}**	**0.1^{a}**	**0.2^{ab}**
	N	3.1	4.3	3.6		**3.9^{bc}**	**8.0^{c}**	**2.1^{a}**	**2.9^{ab}**
	C	105.5	204.2	154.3		130.2^{ab}	**268.1^{b}**	**79.1^{a}**	**106.2^{a}**
	S	**1.8***	2.5	**2.0***	>	**0.7^{b*}**	**1.5^{c}**	**0.3^{a*}**	**0.3^{ab}**

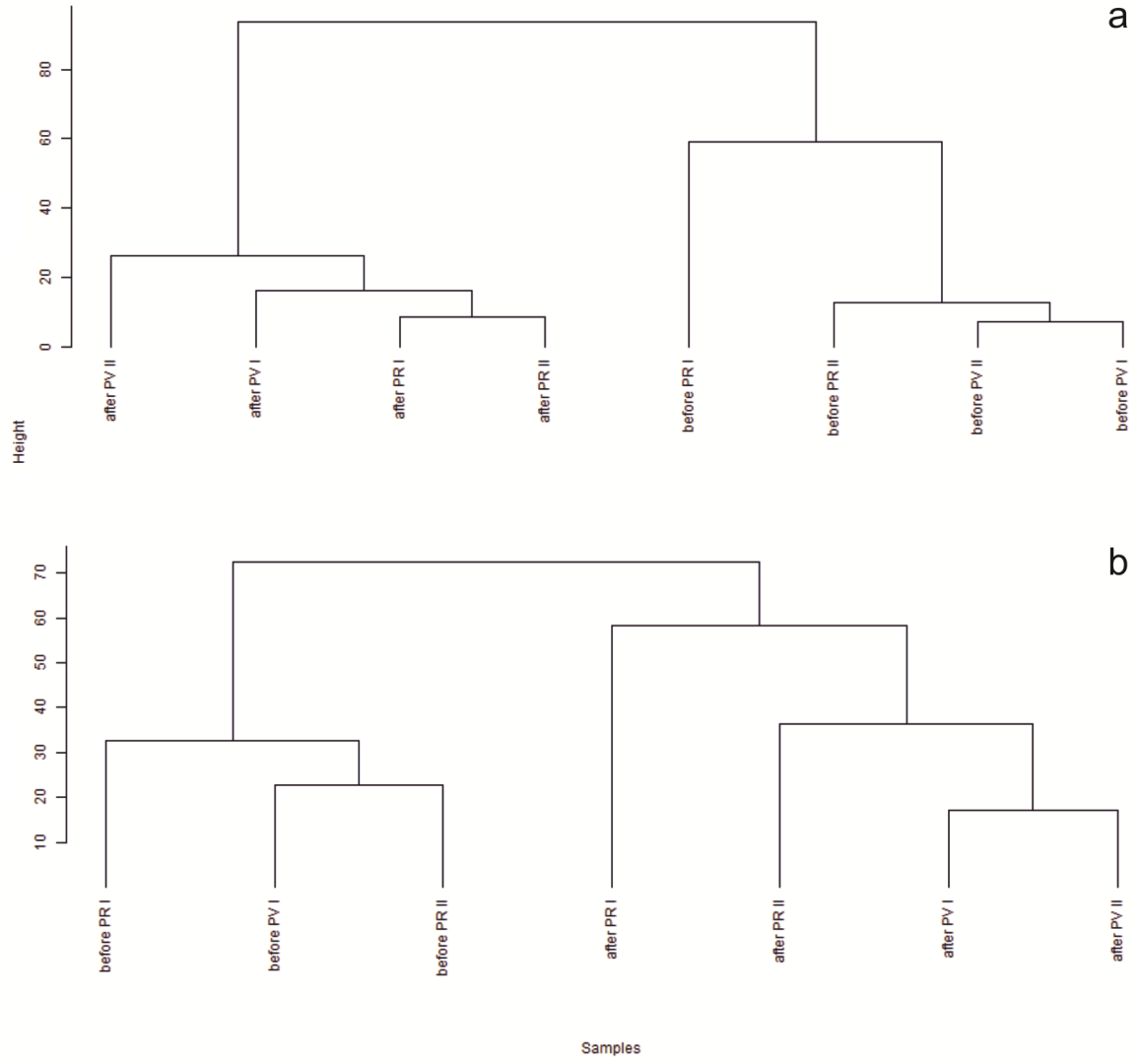

Figure 3: Grouping of the sampling sites based on the results of the cluster analysis conducted on the above-ground (a) and below-ground reed samples (b).

Aerobic bacteria are responsible for oxidizing ammonia, heavy metals and other toxic compounds. Nitrogen can also be taken up by the reeds and incorporated into its own biomass [60]. Ammonia was not detectable in the surface and interstitial water during the investigation period, which may be explained by the efficient ammonium-N uptake of reeds and algae and by its removal through the nitrification-denitrification processes.

The phosphate and nitrate concentrations measured in the surface and interstitial water in this study were similar to those of *e.g.* [66], which were obtained in different reed stands of Lake Fertő/Neusiedler See; however, both of these were higher than the results of [67]. The reason for this may lie in the great fall in the water level of the whole lake and the partial drying out of the parcel on the shoreline in 2003.

The concentration of DOC in the surface water was higher at the outlet than at the inlet. However, in the reed stand even these values were exceeded. This might have been due to the contribution of the DOC originating from the decaying plant material accumulating in the sediment, as reflected by the high organic matter content of the sediment. [68], studying the nutrient release from integrated constructed wetland sediments, also found that sediments released substantially more organic matter than the incoming organic matter that could be degraded. The living

reed roots and rhizomes are also capable of emitting DOC to a greater degree during the active growth period [69]. The sulphate concentration of water in most cases decreased as a function of the sediment depth and displayed an inverse relationship to the vertical profile of sulphide concentrations. This happened because sulphate reduction processes can also precipitate heavy metals, where they occur, in the root-zones [16, 70].

The water slowly flowing through the outlet, leaves the parcel, eventually reaching the Virágosmajor Canal and subsequently Fertőrákos Bay. As a result of the dilution of the outflow water its Eh, pH and oxygen content is lower, while its nutrient content (PO_4^{3-}, NO_3^-) is higher than that of the open water at the reference site, showing a similar situation to the 6 km-wide reed stand which lies across the Bozi Canal [71].

The inhomogeneity of the reed stand area set aside for the subsequent cleaning of the pre-treated wastewater made it difficult to work out a sampling strategy which would allow the preparation of a mass balance with appropriate accuracy.

At the data evaluation stage, another circumstance urged caution, namely the quantity of wastewater discharged daily into the reed parcel. It did not usually inundate the parcel, partly due to the current water level fluctuation of Lake Fertő/Neusiedler See. Consequently, the pre-treated wastewater did not leave the parcel at all times as surface water, *i.e.* the flow of the wastewater was not continuous. Therefore the determination of the residence time became highly difficult and in most cases impossible.

Reeds

There are three main processes which interact and simultaneously influence each other: (i) nutrient uptake by *Phragmites* from both the sediment and the water, (ii) the mineral concentrations of the surrounding water and soil, and (iii) the biomass [12]. According to previous studies, nutrient enrichment of the water raises the N and P concentrations in the reed tissue [5, 7–9, 61, 72, 73]. In the present study, the N and P concentrations of the leaves were higher and the P concentration of the stem, senescence rhizomes and roots was also significantly higher after the introduction of pre-treated wastewater (in 2004) than before (2003). This indicated that the *P. australis* took up and stored more P from its nutrient-enriched environment (sediment interstitial water and surface water), which was caused by the introduction of the pre-treated wastewater. This is seems to be supported by the decrease in the N and P concentrations of the water from the inlet to the outlet. This result is in harmony with work of Meuleman *et al.* [20]. They also found that nutrient concentration and nutrient storage in *P. australis* vegetation in an infiltration wetland used for wastewater treatment was significantly higher than in the natural wetland. This statement is in harmony with the results of the study [74]. In previous studies, it has been pointed out that the uptake of nutrients by reeds is only of quantitative importance in low loaded systems [11].

The significant influence of the wastewater on the biometrical parameters of the reed was described by [13]. They found that the maximal density of shoots was higher, the biomass was twice as high and the shoot diameter was significantly greater in the treated water than in the natural reed stand. A higher shoot density was also recorded in our study, which, on the one hand can be attributed to the effect of wastewater, while on the reeds, and on the other hand to the result of the reeds' winter harvesting.

All the above findings were reflected in and in harmony with the multivariate results as well, specifically that the reed stand functioned as an effective filtering area for the pre-treated wastewater. It retained it, thus leaving the processes of the open water unaffected. This is why its samples remained in one group while those in the parcel separated in both time (before and after) and space (inlet and outlet) after the introduction of the pre-treated wastewater. This situation is similar to the one witnessed in a constructed wetland, the Kis-Balaton Water Protection System [75–77].

5 Conclusions

The nutrient concentrations of the reed organs, surface water, sediment interstitial water and the effect of pre-treated wastewater were analysed in a partly separated reed stand used for pre-treated wastewater treatment, near Fertőrákos Bay at the landward edge of a wide reed belt of Lake Fertő/Neusiedler See.

Based on the results it can be concluded that the *Phragmites australis* stand, is (i) able to fulfil an important role in efficiently and sustainably removing nutrients (NO_3^-, PO_4^{3-}, DN) from pre-treated wastewater, while (ii) conserving and protecting the natural processes of the open water. The investigation underlines the efficient applicability of "close-to-natural" reed parcels for the purification of pre-treated wastewater in a highly unstable hydrological system.

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Appendix

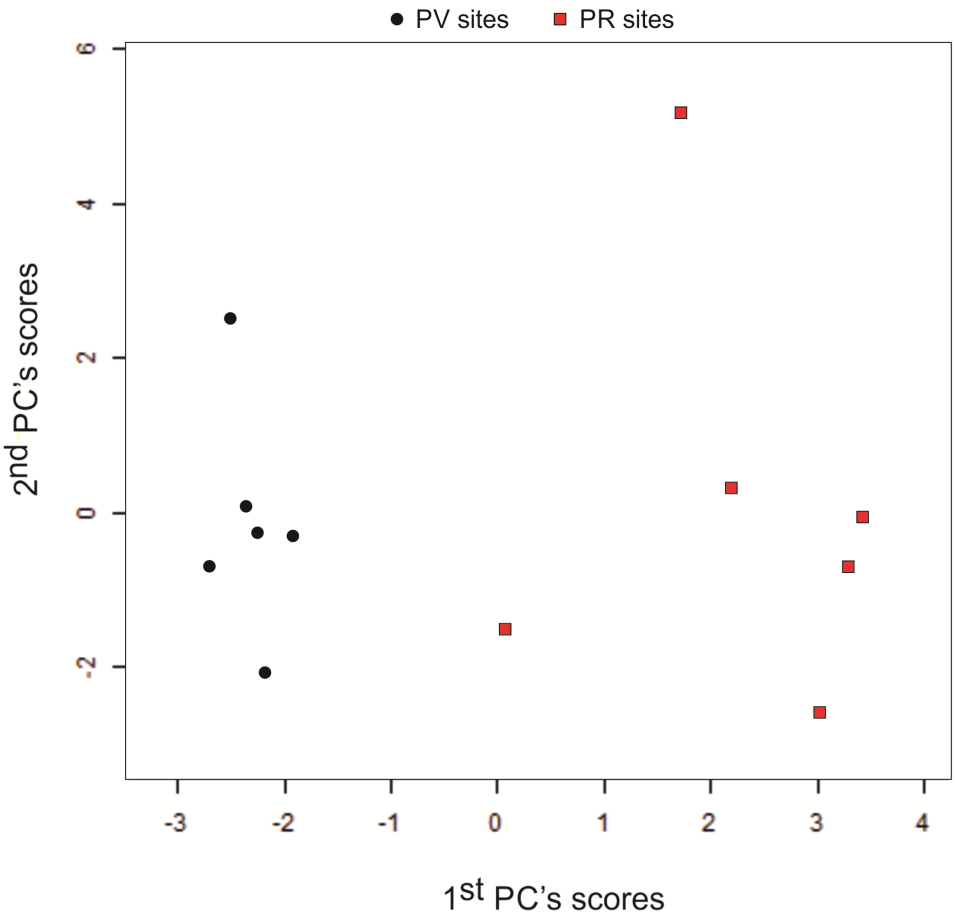

Figure A1: Plot of the first and second principal component scores obtained from the interstitial water samples.