University of South Bohemia in České Budějovice
Faculty of Science

THE EFFECT OF P ENRICHMENT ON EXUDATE QUANTITY AND BIOAVAILABILITY – A COMPARISON OF TWO MACROPHYTE SPECIES –

RNDr. Thesis

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Annotation

A study on rhizodeposition rates and rhizodeposits bioavailability (microbial respiration, N mineralization and phosphatase activity) of two macrophyte species with different life strategies (stress-tolerator and competitor) was conducted. Research was carried out in tropical marshes of Belize; results from the field were supported by ^{13}C partitioning mesocosm study. The stress-tolerant *Eleocharis* spp. released more C from roots than *Typha domingensis* and this C was more biodegradable. The two species responded to P enrichment differently. While *Eleocharis* spp. invested more assimilated C to the belowground (roots, rhizomes and rhizodepositions) after P fertilization, in *T. domingensis* the belowground C investment decreased. The effect of plant species was larger than the effect of P enrichment. *Eleocharis* spp., adapted to growth under low nutrients, invests more carbon into exudation a promotion of its microbial communities in the rhizosphere while competitive *T. domingensis* spends more fixed C on its own growth and metabolism.

Declaration [in Czech]

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Tímto potvrzuji, že mgr. Jaroslava Kubešová provedla s prof. Eliškou Rejmánkovou pokusy zaměřené na kvantifikaci exsudace rostlin *in situ*, pomáhala s pokusy s izotopovým značením exsudátů, statisticky vyhodnotila výsledky a významným způsobem se podílela na psaní rukopisu.

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Poděkování [in Czech]

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ORIGINAL RESEARCH

The Effect of P Enrichment on Exudate Quantity and Bioavailability - a Comparison of Two Macrophyte Species

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Abstract We compared exudation and rhizosphere microbial activity of two macrophytes growing in tropical marshes. *Eleocharis* spp. are adapted to low nutrient level in phosphorus limited conditions, while *Typha domingensis* is a strong competitor in nutrient enriched areas. In situ measurements of carbon fluxes from roots to interstitial water and ^{13}C partitioning after pulse-labelling of the plants in a mesocosm experiment were used to estimate root-derived C fluxes to rhizosphere under P limited and enriched conditions. Root-released compounds collected in the field were analysed for dissolved organic C, dissolved nitrogen and their biodegradability was characterized through microbial respiration, N mineralization and phosphatase activity. Independent of P loading, *Eleocharis* released more C from roots than *T. domingensis*, and the released compounds were more biodegradable. The two species responded to P enrichment differently. While *Eleocharis* invested more assimilated ^{13}C to the belowground (roots, rhizomes and rhizodepositions) after P fertilization, in *T. domingensis* the belowground investment decreased. The effect of plant species on belowground C allocation was larger than the effect of P enrichment. Low nutrients adapted *Eleocharis* invested more carbon into exudation and promotion of its rhizosphere microbial community while competitive *T. domingensis* spent more fixed carbon on its own growth and metabolism.

Keywords Biological availability · C partitioning · Eutrophication · Herbaceous marshes · Mineralization · P limitation · Plant life strategy · Rhizodeposition/exudation

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Introduction

The relationship between plants and soil microbiota influences wetland productivity and biogeochemical cycling (Reddy and DeLaune 2008). Plants as the main primary producers support soil microbiota with organic carbon (C) from litter fall and rhizodeposition while microbiota help plants to cover their nutrient demands (Fig. 1). Litter and its further decomposition have a larger-scale effect, which is particularly important in the bulk soil (Personeni and Loiseau 2005; Brüggemann et al. 2011), while rhizodeposition directly influences only the minute part of soil volume in the closest root vicinity – known as the rhizosphere. Despite the relatively small volume of rhizosphere soil compared to bulk soil, the rhizosphere processes impact ecosystem nutrient cycling (Calvaruso et al. 2006; Lambers et al. 2009) and potentially alter global biochemistry through greenhouse gas emissions (Phillippot et al. 2009).

Amount and chemical composition of rhizodepositions is plant species specific (Ström et al. 2003; Berg and Smalla 2009) and its quantity and quality further varies depending on plant physiological status and environmental factors (Kuzyakov and Domanski 2000; Warembourg et al. 2003; Jones et al. 2004; Sauer et al. 2006; Badri and Vivavco 2009). Even though nutrient availability is one of the most important environmental factors, the effect of nutrient

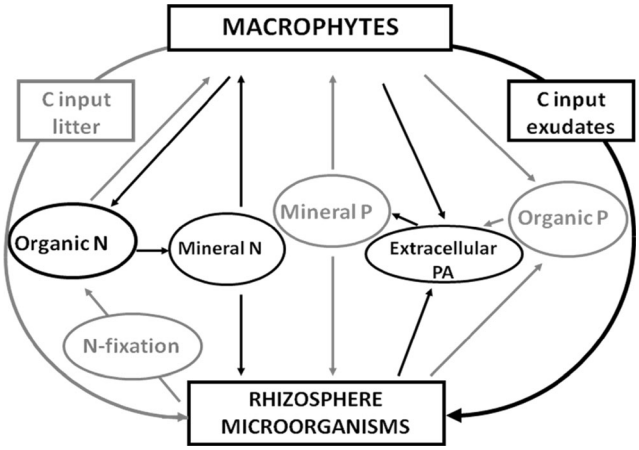

Fig. 1 Scheme of plant C input into the sediment and its impact on nutrient fluxes in the marshes. *Black signs* – fluxes studied in our experiment, *grey signs* – other fluxes and processes

enrichment on the rhizodeposition flux remains virtually unexplained (Kuzyakov 2010). While change in belowground dynamics caused by N addition has been addressed (Nguyen 2003; Jones et al. 2009; Sillen and Dieleman 2012), an information on the effect of P enrichments is scarce.

Plants can acquire P solubilising poorly soluble inorganic P forms by releasing protons, OH^-, CO_2 and organic acids from roots or producing phosphatases to gain P from organic compounds (Marschner et al. 2011). In addition, available C compounds in rhizodeposition enhance microbial decay of more complex organic material, increasing the gain of P from more complex organic matter, which is inaccessible to plants. The root exudates are thus vital for the rhizosphere microflora as they increase its ability to decompose complex organic matter (Grayston et al. 1996). After P addition, only small proportion of added P remains available in pore water and the major part is bound in insoluble inorganic forms in the soil and, accordingly, higher release of organic acids from roots after P addition has been detected in various species (Hunter et al. 2014). Phosphorus enrichment is also accompanied with improvement of plant P stoichiometry (Rejmánková and Snyder 2008) and P released during plant litter decay by rhizosphere microbial community can further enhance plant P nutrition. Thus both, the increase in exudation and support of rhizosphere microflora development can be expected in P enriched wetlands.

We studied belowground C allocation of two macrophyte species with different life strategies and their subsequent impact on the rhizosphere microbial community. The aim of the study was to complement other components of a larger project in wetlands of northern Belize focused on ecosystem processes and community structure following nutrient additions. In summary: unimpacted marshes are dominated by sparse macrophytes, namely sedges *Eleocharis* spp., (from now on *Eleocharis*) interspersed with floating mats of cyanobacteria. Nutrient enrichment, specifically by phosphorus (P), leads to replacement of these communities by dense monocultures of *Typha domingensis* Pers. (from now on *Typha*). Of these two macrophytes, *Eleocharis* is adapted to grow under oligotrophic conditions, while *Typha* acts as a strong competitor (Macek et al. 2010). Plant life strategy is known to influence the efficiency of nutrient acquisition and storage (Rejmánková and Snyder 2008) as well as C partitioning and exudation (Warembourg et al. 2003; Lambers et al. 2006). Indeed, soil analyses performed in the studied wetlands revealed that P addition increased microbial biomass and N_2 fixation through increase of root biomass and the effect was consistently higher in *Eleocharis* than in *Typha* dominated plots (Černá et al. 2009). It remained to be elucidated whether the positive effect of roots on soil microbial activity was related only to the increase of root biomass, or also to the enhancement of rhizodeposition.

In this study, we focused on the assessment of quantity and availability of rhizodeposits of *Eleocharis* and *Typha* and on the effect of plant life strategy and P fertilization on belowground C allocation. We hypothesized that:

(1) The low nutrient adapted and slow growing *Eleocharis* invests more C belowground to enhance organic matter decomposition and microbial activities. Fast growing *Typha* utilizes the assimilated C for its own growth and releases less C to the rhizosphere.
(2) P enrichment enhances the belowground C allocation of both plant species, but the effect of different plant life strategy will be stronger than effect of P enrichment.

To compare species response to P loading, we collected rhizodeposits from plants grown in situ in control (P limited) and P enriched experimental plots in two marshes. Quantity of rhizodeposits (concentrations of dissolved organic carbon, DOC, and nitrogen, DON) and their biodegradability (C and N mineralization rates and alkaline phosphatase activity, APA) were further examined. $^{13}CO_2$ pulse-labelling of plants grown in mesocosms in P limited and P enriched conditions was used to determine C partitioning belowground and to estimate exudation rates.

This study aims to fulfil a missing piece of information regarding the relationship between plants and their belowground C fluxes in tropical oligotrophic and P enriched marshes. To our best knowledge, it is the first attempt to characterize tropical wetland plant exudation fluxes in situ.

Methods

Study Site

Oligotrophic, P limited marshes are widespread in the lowlands of northern Belize, Yucatan peninsula (within a 50 km

radius of 18°9'58" N and 88°31'28"W). The region is characterized by a tropical wet-dry climate in Koeppen's classification. The bedrock is formed by a 2–3 km thick uplifted marine platform of limestone (Weidie 1985). Water level fluctuates according to precipitation, but, despite the regime of wet and dry seasons, marshes seldom dry out completely. Sediments with sufficient N availability but very low bioavailable P range from peaty clays underlain by alluvial sands to marls with limestone marls bedrock. Unimpacted marshes are dominated by a few species of emergent macrophytes and by very diverse cyanobacterial mats (Rejmánková et al. 2004). Intensifying sugar cane agriculture has resulted in wetland eutrophication, when P-rich agricultural run-off causes a change in plant communities, with the elimination of sparse *Eleocharis* and cyanobacterial mats and shift towards dense monocultures of *Typha* (Johnson and Rejmánková 2005). A long-term experiment across marshes of different salinity was set up in 2001 to study the ecosystem response to P additions (for details see Rejmánková et al. 2008). From this experiment, two low salinity (<0.5 ppt) marshes were selected for our study: F10 with sand-based peaty clay sediment and F12 with limestone-based peaty clay sediment. For detailed characteristics of interstitial water and sediment see Table 1.

^{13}C Partitioning within Plant and Exudation Rate

To estimate the proportion of assimilated C released from plant roots, pulse labelling with $^{13}CO_2$ was performed. *Eleocharis* and *Typha* plants were collected from the control (P limited) and P enriched treatments of the marsh F12 in February 2011 (middle of dry season, when both marshes were flooded and plants were actively growing) and transplanted to the outdoor mesocosms. For ^{13}C pulse labeling, individual plants were placed in aluminium wrapped jars (900 ml volume) with 500 ml of interstitial water collected from F12 marsh control plots. Nitrogen was kept non-limiting by addition of 2 mg N l^{-1} to all treatments, while P was added only to high P treatments (0.2 mg P l^{-1}). The jars with plants (three plant replicates for each treatment and harvest) were placed into gastight plexiglass chambers with the top opening fitted with rubber septa and equipped with two small fans and two valves (one at the top and one at the lower end of the chamber). The open end of the chamber was always immersed in water to prevent air leakage. The air cooled by ice was pumped through this closed system. The CO_2 concentration in the chamber was lowered to 20–40 ppm by its absorption to soda lime (checked by LiCOR 6400XT), and $^{13}CO_2$ (99.9 %, Cambridge Isotope laboratories, GB) was injected through the septum to reach the final concentration of 800 ppm. The labelling lasted from 2.5–3.5 h, until CO_2 concentration decreased to 200 ppm. Afterwards, plants were kept in an open system until harvested. To test for autotrophic CO_2 fixation in the interstitial water, two unplanted wrapped jars with interstitial water were kept in ^{13}C atmosphere for 3.5 h. No autotrophic CO_2 fixation was found.

Three plants of each species and treatment were harvested immediately after labelling to assess the amount of ^{13}C fixed in the system. One unlabelled plant of each species and treatment served as natural abundance control. Subsequent harvests of labelled plants followed in the evening after one, two, three and 4 days after labelling. Each harvest consisted of the shoot, root and interstitial water. Samples of interstitial water were divided into two fractions: a subsample (A) filtered through 45 μm filters to remove root hairs and plant cells. This subsample represented total organic ^{13}C released from roots ($TO^{13}C$), present in the form of dissolved ($DO^{13}C$) and particulate organic ^{13}C ($PO^{13}C$) mainly of microbial origin (Höfle 1990). The subsample (B), filtered through a glass fibre filter (bacterial, 0.2 μm), represented only $DO^{13}C$ released from roots. Both subsamples were acidified to pH 2 by

Table 1 Basic characteristics of interstitial water and sediment in the two investigated marshes. Mean values from 2–3 years of sampling and standard deviations are given. DON and DOC values correspond to the in situ measurements of exudation rates

		Interstitial water					Sediment			
		pH	SRP ppb	NH_4-N ppb	DOC mg l^{-1}	DON mg l^{-1}	BD[a] g cm^{-3}	TN %	TC %	TP %
F10	Ele LP	6.99 ± 0.37	16.8 ± 2.0	516.22 ± 219.6	8.23	0.23	0.50 ± 0.12	0.71 ± 0.27	7.97 ± 3.58	0.031 ± 0.029
	Ele HP	6.86 ± 0.41	15.7 ± 5.8	157.1 ± 129.7	8.67	0.19	0.42 ± 0.09	0.91 ± 0.04	9.03 ± 0.47	0.048 ± 0.043
	Ty LP	6.93 ± 0.40	12.0 ± 1.7	516.2 ± 219.6	7.92	0.16	0.51 ± 0.13	0.71 ± 0.27	7.97 ± 3.58	0.036 ± 0.006
	Ty HP	6.90 ± 0.44	17.1 ± 3.7	93.4 ± 55.1	7.81	0.20	0.46 ± 0.11	1.06 ± 0.82	18.31 ± 1.14	0.040 ± 0.023
F12	Ele LP	7.08 ± 0.23	27.5 ± 4.8	884.6 ± 876.4	7.89	0.23	0.42 ± 0.14	0.76 ± 0.15	9.32 ± 1.92	0.020 ± 0.014
	Ele HP	7.15 ± 0.15	21.9 ± 4.5	48.4 ± 24.5	9.64	0.19	0.16 ± 0.07	1.24 ± 0.43	15.60 ± 5.21	0.081 ± 0.081
	Ty LP	7.13 ± 0.13	25.6 ± 13.1	429.7 ± 234.8	8.00	0.22	0.42 ± 0.14	0.76 ± 0.15	9.32 ± 1.92	0.016 ± 0.001
	Ty HP	7.24 ± 0.08	18.6 ± 7.7	57.2 ± 22.7	7.72	0.22	0.23 ± 0.06	1.60 ± 0.39	20.12 ± 5.15	0.115 ± 0.097

[a] Bulk density

sulfuric acid after sampling and frozen. Shoot and root tissues were dried immediately after harvesting and homogenized on a ball mill, frozen liquid samples were freeze dried before analyses. Total C and ^{13}C (in ^{13}C at.%) contents were conducted on an NC Elemental analyzer (ThermoQuest, Germany) connected to an isotope ratio mass spectrometer (IR-MS Delta X Plus, Finnigan, Germany).

A binary mixing model with ^{13}C atomic percent was used to calculate the amount of the pulse-derived ^{13}C in various studied C pools:

$$^{13}\mathrm{C}\left(\mu\mathrm{g\ g^{-1}}\right) = \left[\left(\text{at. \%sample} - \text{at. \%control}\right) \Big/ \left(99.9 - \text{at. \%control air}\right)\right] \times \text{C pool size}\left(\mu\mathrm{g\ C\ g^{-1}}\right),$$

where the control is the natural abundance in control samples and 99.9 is at.% of ^{13}C pulse.

Total amount of assimilated ^{13}C was calculated as the sum of ^{13}C in shoots, roots and interstitial water immediately after labelling. In the following samplings, ^{13}C lost by respiration was calculated by subtracting the sum of ^{13}C in shoots, roots and interstitial water from the total amount of assimilated ^{13}C. Content of ^{13}C in microbial POC was calculated as a difference between subsample (A) and (B). Contents of ^{13}C in shoot, root, POC and DOC in individual harvests were expressed relatively (%) to the total amount of assimilated ^{13}C. The exudation rate was estimated from temporal increase of ^{13}C amounts in interstitial water using linear regression.

In Situ – Quantity and Biodegradability of Root-Released Compounds

Root released compounds were collected in situ to asses their quantity and biodegradability. Five plants of *Eleocharis* (Ele) and *Typha* (Ty) were chosen in P limited (LP) and P enriched (HP) plots (Ele LP, Ele HP, Ty LP, Ty HP) in both (F10 and F12) marshes. Tissue P content averaged 0.203, 0.683, 0.365 and 0.812 mg g^{-1} for Ele LP, Ele HP, Ty LP and Ty HP, respectively, and there were no differences between marshes. The corresponding C/P ratios were 2142, 637, 1232 and 554 for Ele LP, Ele HP, Ty LP and Ty HP, respectively. Each plant was gently loosened from the sediment, placed back and left there for the following 2 days to let the root system recuperate. *Eleocharis* plants were then placed into jars (900 ml volume, one plant per jar) with 200 ml of non-filtered interstitial water from the same plot, tops of the jars were covered with parafilm and jars were placed back into the marsh. For *Typha*, three large intact roots (connected to the plant) were placed into vials filled with 50 ml of non-filtered interstitial water from the same plot, vials were gently, but firmly, covered by parafilm and the whole plant was placed back into the marsh. After the incubation lasting 36 to 48 h, the volume of water lost by evapotranspiration from the jars/vials during the experiment was refilled by distilled water, mixed thoroughly and used for further analyses. In the case of *Typha*, roots were gently removed from vials and stored for biomass and surface area determination. Contents of vial triplets were mixed together to gain sufficient volume of the sample from one plant. All liquid samples, together with samples of interstitial waters used in the experiment, were cooled immediately and transported to the laboratory for further analyses. Whole plants were harvested to examine their shoot and root biomass and tissue nutrient contents (C, N, P). From each root sample, three representative subsamples (0.075–0.15 g) were washed in distilled water, towel-dried and used for a measurement of root length, surface area and diameter using WINMac RHIZO interactive image capture and analysis software (Regent Instruments Inc., Ottawa, Canada).

Chemical and Biochemical Characterization of Root-Released Compounds

Samples of interstitial water were filtered through the glass fibre filter (0.2 μm, bacterial), acidified by sulfuric acid to pH 2 to release inorganic C and stabilize DOC, and stored in a dark cold place until analysis. Dissolved organic carbon (DOC) and total nitrogen (TN) contents were analysed on LiquiTOCII (Elementar Germany). Total P (TP) was measured by ascorbic acid reduction of phosphomolybdenate complex after acid digestion (McNamara and Hill 2000). Ammonium and nitrates were analysed using flow injection analyser (FIAstar 5012, Foss Tecator, Sweden). Organic nitrogen (DON) was calculated as a difference between the total dissolved N and mineral N (NH_4^+ + NO_3^-). Dissolved organic carbon and DON fluxes from roots were estimated from the difference between their concentrations in the interstitial water before and after incubation with plants.

Biodegradability of root-released compounds was estimated from C and N mineralization rates during incubation of samples in laboratory conditions. For the assessment of C mineralization rate, samples were incubated in hermetically closed flasks in a dark place at 28–29 °C for 5 days. Released CO_2 was trapped into NaOH and its amount was measured daily by backward titration with HCl on phenolftalein. At the beginning and the end of the incubation, samples were analyzed for the concentration of mineral N forms and their difference was used to calculate N mineralization rate. Both C and N mineralization rates were expressed

on DOC basis. Phosphorus mineralization potential was described by a phosphomonoesterase activity (APA), measured in filtered samples as an increase in fluorescence following the enzymatic hydrolysis of the substrate, methylumbeliferon phosphate (MUFP; 1 mM), to methylumbeliferon (MUF); for details see Sirová et al. 2013). Fluorescence changes were measured on a Fluoroskan (excitation and emission wavelengths of 355 nm and 460 nm, respectively).

Statistical Analyses

Effects of plant species and P loading on plant C partitioning, their exudation fluxes and biochemical characteristics of root-released compounds were analyzed by two-way ANOVA (Statistica 10), followed by Post-hoc Tukey HSD tests. Data from the F10 and F12 marshes were analyzed separately.

Results

Labelling Experiment: ^{13}C Allocation and Belowground Partitioning

Eleocharis fixed about double the amount of ^{13}C g^{-1}dry weight than *Typha* and the higher ^{13}C fixation efficiency was accompanied by higher nitrogen use efficiency (NUE) (Table 2). The significant portion of assimilated ^{13}C, representing 22–55 % of the fixation for *Eleocharis* and 10–13 % for *Typha* was quickly moved below ground already within the first day after fixation and partitioned between roots and exudation (Table 2). During this period, *Eleocharis* transported significantly more ^{13}C to roots and interstitial water than *Typha*. Both species responded to P enrichment by higher ^{13}C transport to the roots but only *Eleocharis* also released more ^{13}C from roots to interstitial water (Table 2). In the following period (from 1 to 4 days) initial effect of plant species on amount of rhizodeposition disappeared in unfertilized treatment. In the fertilized system, however, the initial positive effect of *Elleocharis* on amount of ^{13}C in roots and rhizopeposition lasted till the end of the experiment (Table 2). Altogether, under P limitation both species transported similar proportion of fixed ^{13}C below ground (to roots + rhizodepositions). *Eleocharis* responded to P fertilization by an increase of belowground ^{13}C allocation and by a decrease of $^{13}CO_2$ loss from the system via plant and microbial respiration, contrary to *Typha* which displayed opposite trend: it partitioned less ^{13}C below ground and lost more ^{13}C by respiration (Table 2). The relative proportions of $DO^{13}C$ in $TO^{13}C$ were not significantly affected either by plant species or by P fertilization. *Eleocharis* released more $TO^{13}C$ per gram of roots (daily specific ^{13}C flux) than *Typha* and the effect of P loading was less important (Fig. 2). Proportion of $DO^{13}C$ in $TO^{13}C$ remained within the 29–33 % fraction range for *Eleocharis* and 34–36 % for *Typha* (Fig. 2).

In Situ Experiment – Quantity and Biodegradability of Root-Released Compounds

Specific DOC and DON fluxes from roots were higher for *Eleocharis* than *Typha* in both marshes both expressed per root dry weight and root surface area, independent of P loading (Table 3). Correspondingly, the DOC and DON fluxes from roots to sediment, expressed per square meter of monospecific macrophyte stand per day, were also markedly higher

Table 2 Total amount of ^{13}C assimilated during labelling (μg ^{13}C g^{-1}_{DW}; mean ± sd, n = 3), the final ^{13}C distribution and nitrogen use efficiency in P fertilized and non-fertilized systems with *Eleocharis* and *Typha* 4 days after labelling; means and standard deviations (n = 3) are shown. Results of a Two-Way ANOVA are also given, with plant species (P) and fertilization (F) as categorical predictors

	Ele LP	Ele HP	Ty LP	Ty HP	Plant (P)	Fert (F)	P × F
^{13}C net fixation mg C g^{-1} leaf dry weight	2.70 ± 0.64	2.64 ± 0.59	0.60 ± 0.23	1.48 ± 0.32	***	*	*
$^{13}C_{MAX}$ in roots (% ^{13}C net fixation)	7.75 ± 2.08	20.1 ± 5.98	4.34 ± 0.376	8.87 ± 2.97	**	**	ns
^{13}C in interstitial water in time of $^{13}C_{MAX}$ in roots (% ^{13}C net fixation)	14.1 ± 2.37	34.6 ± 9.53	5.33 ± 1.7	4.36 ± 1.48	***	(*)	**
^{13}C distribution in the system after 4 days incubation (% ^{13}C net fixation)							
^{13}C in shoots	8.70 ± 1.40	9.63 ± 2.55	10.43 ± 2.51	10.57 ± 2.39	ns	ns	ns
^{13}C in roots	2.83 ± 0.31	6.40 ± 1.68	2.90 ± 1.23	2.83 ± 1.10	*	(*)	*
^{13}C root/shoot	0.33 ± 0.08	0.66 ± 0.06	0.29 ± 0.15	0.26 ± 0.04	***	(*)	(*)
^{13}C in interstitial water	30.53 ± 6.54	48.73 ± 7.51	32.53 ± 3.55	14.67 ± 1.46	***	ns	***
Total ^{13}C belowground	33.37 ± 6.82	55.1 ± 7.2	35.43 ± 4.28	17.5 ± 2.5	***	ns	***
^{13}C-CO_2 released from the system	57.93 ± 7.02	35.23 ± 8.05	59.37 ± 6.34	71.93 ± 4.91	**	ns	**
NUE (mol $^{13}C_{fixed}$ mol N_{leaf}^{-1})	0.227 ± 0.054	0.237 ± 0.053	0.079 ± 0.030	0.137 ± 0.030	**	ns	ns
Root/shoot	0.67	0.60	0.44	0.46			

***$p > 0.001$; **$p > 0.01$, *$p > 0.05$, (*) $p > 0.1$

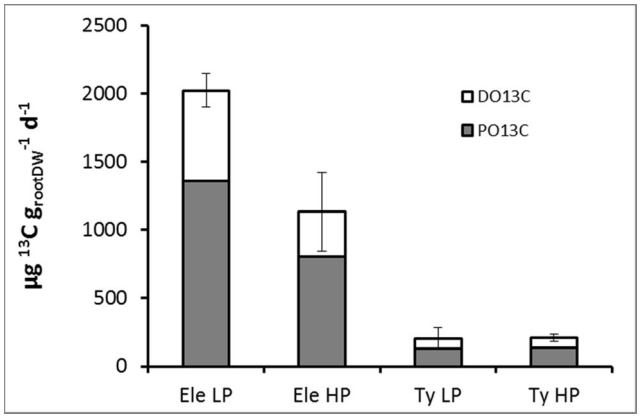

Fig. 2 Average release rate of ^{13}C to interstitial water during 4 days after ^{13}C labelling expressed on root dry weight basis and its distribution to dissolved organic fraction ($DO^{13}C$) and particulate organic fraction ($PO^{13}C$). Mean from 3 independent replicates and standard deviations are given

in *Eleocharis* than in *Typha* plots (Table 3). Both the specific and total DOC and DON fluxes were generally lower in the marsh with more alkaline pH (F12, marly clay) (Table 3). The DOC and DON release from roots was not related in *Eleocharis* and weakly correlated in *Typha* (Fig. 3a). While the plant species effect on specific DOC and DON fluxes from

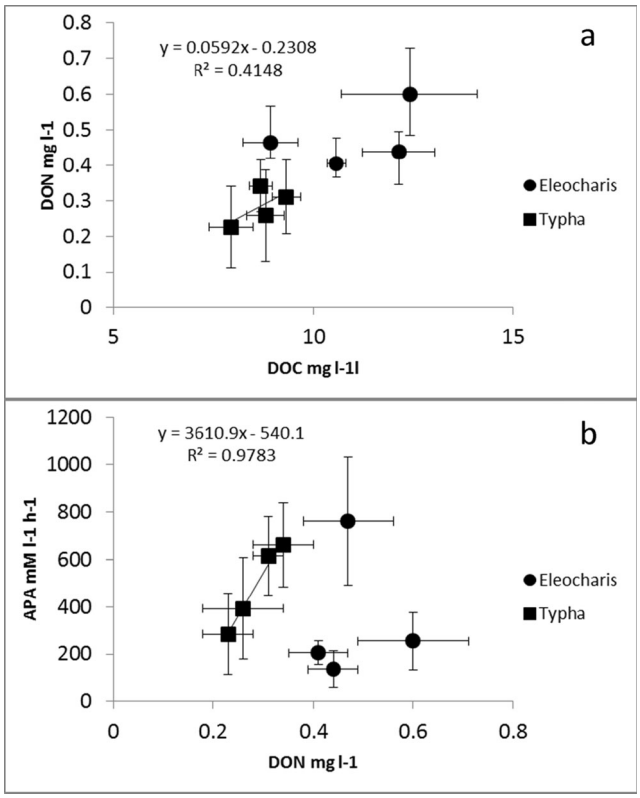

Fig. 3 Correlation between **a**) DOC and DON concentrations in exudates, **b**) DON concentration and phosphatase activity (APA) in exudates. White circles – *Eleocharis*, dark diamonds – *Typha*. Three extreme values in *Eleocharis* APA (section b), belong all to F12 CE treatment

roots was highly significant, the effect of P enrichment was negligible. Phosphorus addition increased only total DOC fluxes from roots, mainly due to the larger root biomass in P enriched plots (Table 3). Results about DOC fluxes from roots measured in situ were generally consistent with the results of the labelling experiment: *Eleocharis* released more DOC per gram of roots (daily specific C flux) than *Typha*, and the effect of P loading was less important. The acidity of interstitial water in the rhizosphere of *Eleocharis* was lower compared with *Typha* (Tables 3 and 4).

The compounds released from *Eleocharis* roots exudates exhibited higher C and N mineralization rates than those of *Typha*, while the P mineralization potential expressed by APA was lower (Table 4). Phosphorus loading did not affect C and N mineralization rates and tended to decrease APA for both plants species (Table 4).

Discussion

The present study shows that P enrichment increases plant C investment below ground and net DOC flux to the soil. While *Eleocharis* transported more of fixed C below ground and released higher proportion of the belowground C to the rhizoshere to support microbial development, *Typha* invested more C to build up root biomass and DOC flux to the soil was enhanced due to larger root system. The P loading effect, however, was less pronounced than the plant species effect indicating importance of plant life strategy for ecosystem functioning.

Recently assimilated ^{13}C (from 10 to 55 %) was rapidly (within 1 day) reallocated to roots (4 % - 20 %) and interstitial water (4 % - 35 %) of *Eleocharis* and *Typha*. During further incubation ^{13}C was gradually released from roots and its content in the interstitial water (15 % - 49 %) increased until the end of experiment. About two third of ^{13}C released from roots was found in particulate form ($PO^{13}C$), the fraction that can be considered to be composed mainly of bacterial cells (Höfle 1990). Similarly, Kaštovská and Šantrůčková (2007) observed 68 % of assimilated C to be immobilized in soil microbial biomass in wet meadows 6 days after labelling. Similar patterns of ^{13}C partitioning have been described for various perennial plants in wet meadows (Kaštovská and Šantrůčková 2007) and grasslands (Leake et al. 2006; Hill et al. 2007), as well as annual plants (Kuzyakov and Domanski 2000; Jones et al. 2009; Brüggemann et al. 2011). Daily specific $DO^{13}C$ fluxes from roots (Fig. 2) were within the same range as rates of DOC release by roots measured in the field (Table 3): from tens (*Typha*) to hundreds (*Eleocharis*) µg of DOC per g of root dry weight per day supporting the comparability of the two experiments. Namely results from the F12 marsh corresponded well to the trends observed during mesocosm incubations, possibly because the mesocosm study was done

Table 3 Exudation rates of dissolved organic carbon (DOC) and nitrogen (DON) released by roots, expressed per root dry weight (= DW), root surface area (= SA) and per the area of a monospecific plant stand per day (NET fluxes). Concentration values of interstitial water were subtracted, means and standard deviations (n = 4) are shown. Results of a Two-Way ANOVA are also given, with plant species (P) and fertilization (F) as categorical predictors

			Ele LP	Ele HP	Ty LP	Ty HP	Plant (P)	Fert (F)	P × F
F10	Root DW	g m^{-2}	88.2	74.5	9.3	135.4			
	DOC	μg g^{-1}_{DW} d^{-1}	571 ± 129	938 ± 358	290 ± 86	233 ± 79	***	ns	(*)
		μg cm^{-2}_{SA} d^{-1}	1.19 ± 0.30	1.82 ± 0.74	0.54 ± 0.15	0.42 ± 0.09	***	ns	ns
	DON	μg g^{-1}_{DW} d^{-1}	91.6 ± 42.6	61.0 ± 25.0	30.4 ± 17.1	24.9 ± 16.3	**	ns	ns
		μg cm^{-2}_{SA} d^{-1}	0.191 ± 0.095	0.117 ± 0.052	0.057 ± 0.032	0.042 ± 0.022	**	ns	ns
	Net flux DOC	mg m^{-2} d^{-1}	50.4 ± 11.4	69.8 ± 26.7	2.7 ± 1.8	31.6 ± 10.7	***	**	ns
	Net flux DON	mg m^{-2} d^{-1}	6.53 ± 2.36	4.54 ± 1.86	0.28 ± 0.16	3.37 ± 2.21	***	ns	*
	TN to TP ratio	mol mol^{-1}	16.1 ± 7.4	10.8 ± 1.5	24.6 ± 3.0	10.4 ± 2.8	(*)	***	(*)
	pH		7.76 ± 0.39	7.69 ± 0.09	7.15 ± 0.02	7.06 ± 0.13	***	ns	ns
F12	Root DW	g m^{-2}	31.1	81.9	11.4	99.7			
	DOC	μg g^{-1}_{DW} d^{-1}	388 ± 366	241 ± 127	137 ± 65	93 ± 71	***	ns	ns
		μg cm^{-2}_{SA} d^{-1}	0.83 ± 0.82	0.42 ± 0.26	0.22 ± 0.10	0.16 ± 0.12	**	ns	ns
	DON	μg g^{-1}_{DW} d^{-1}	51.9 ± 20.8	50.2 ± 24.1	38.4 ± 18.0	4.2 ± 17.7	**	ns	ns
		μg cm^{-2}_{SA} d^{-1}	0.104 ± 0.053	0.085 ± 0.043	0.059 ± 0.024	0.006 ± 0.029	**	ns	ns
	Net flux DOC	mg m^{-2} d^{-1}	12.1 ± 11.4	19.7 ± 10.4	1.6 ± 0.7	9.3 ± 7.1	**	(*)	ns
	Net flux DON	mg m^{-2} d^{-1}	1.61 ± 0.65	4.11 ± 1.97	0.44 ± 0.21	0.42 ± 1.76	***	ns	ns
	TN to TP ratio	mol mol^{-1}	21.0 ± 5.0	21.5 ± 3.7	14.7 ± 2.7	6.8 ± 2.3	***	(*)	(*)
	pH		8.42 ± 0.15	8.25 ± 0.02	7.39 ± 0.05	7.68 ± 0.21	***	ns	ns

***$p > 0.001$; **$p > 0.01$, *$p > 0.05$, (*) $p > 0.1$

using plants and interstitial water from F12 marsh. Lower DOC fluxes measured in F12 compared to F10 marsh could be related to calcareous character of sediment, higher N and P content in sediment and higher P content in interstitial water of fertilized treatments.

Only DOC was measured in situ and we have no data of total flux of C from roots to the interstitial water in the field. Based on similarity of the results from in situ and mesocosm labelling experiments and on the assumption that TOC to DOC ratio was comparable in the field and the system used for labelling, we made a rough estimate of TOC flux in the field. We used $TO^{13}C$ to $DO^{13}C$ ratio found in the labelling experiment, the measured root mass of monospecific macrophyte stands (g m^{-2}) and mass-specific flux of root-derived DOC from the in situ experiment. Estimated TOC flux from roots (TOC) of the monospecific macrophyte stands was much higher for *Eleocharis* (from 4 to 17 mg C m^{-2} d^{-1}) than *Typha* (from 0.6 to 1 mg C m^{-2} d^{-1}) in the unfertilized marshes. Fertilization increased TOC flux from the roots of both plants mainly due to positive effect of P addition on root

Table 4 Mineralization rates of C (respiration) and N (ammonification and nitrification), phosphatase activity (APA) and pH in exudates. p values of 2-way ANOVA for P level and plant species effect; means and standard deviations (n = 4) are shown. Results of a Two-Way ANOVA are also given, with plant species (P) and fertilization as categorical predictors

Marsh		Ele LP	Ele HP	Ty LP	Ty HP	Plant (P)	Fert (F)	P × F
F10	C mineralization (mg g^{-1}_{DOC} h^{-1})	2.23 ± 0.25	2.63 ± 0.49	1.92 ± 0.54	1.33 ± 0.86	(*)	ns	ns
	N mineralization (mg g^{-1}_{DOC} h^{-1})	0.053 ± 0.026	0.052 ± 0.032	0.018 ± 0.009	−0.030 ± 0.024	*	ns	ns
	APA (mM g^{-1}_{DOC} h^{-1})	20.6 ± 7.4	11.2 ± 3.0	66.3 ± 14.9	44.6 ± 21.1	**	(*)	ns
	pH	7.76 ± 0.39	7.69 ± 0.09	7.15 ± 0.02	7.06 ± 0.13	***	ns	ns
F12	C mineralization (mg g^{-1}_{DOC} h^{-1})	2.86 ± 0.57	2.71 ± 0.14	1.07 ± 0.31	2.44 ± 1.25	*	ns	ns
	N mineralization (mg g^{-1}_{DOC} h^{-1})	0.048 ± 0.009	0.044 ± 0.025	0.023 ± 0.009	0.029 ± 0.045	ns	ns	ns
	APA (mM g^{-1}_{DOC} h^{-1})	85.6 ± 26.1	19.3 ± 3.9	77.0 ± 18.2	35.6 ± 18.4	(*)	ns	ns
	pH	8.42 ± 0.15	8.25 ± 0.02	7.39 ± 0.05	7.68 ± 0.21	***	ns	**

***$p > 0.001$; **$p > 0.01$, *$p > 0.05$, (*) $p > 0.1$

growth. The trend of higher TOC release from *Eleocharis* roots (from 6 to 20 mg C m^{-2} d^{-1}) than from roots of *Typha* (from 3 to 11 mg C m^{-2} d^{-1}) persisted.

The results from in situ and ^{13}C labelling experiments showed that *Eleocharis* allocated a larger amount of recently fixed C to the interstitial water and also to the $PO^{13}C$ and the bioavailability of the released C was also higher for *Eleocharis* than *Typha*. These findings agreed with our expectations that *Eleocharis*, well adapted to low nutrient conditions, invests larger proportion of assimilates into promotion of its rhizosphere microbial community. Conversely, *Typha*, an efficient competitor, releases less C by exudation and its exudates are less biologically available.

Besides organic C, plants also release a variety of organic N compounds (Uren 2001). *Eleocharis* exuded more DON than *Typha* in the in situ experiment and also C/N ratio of *Eleocharis* exudates was lower. It was accompanied by higher N mineralization and larger DON losses (Table 3), which indicate more favorable composition of *Eleocharis* exudates for rhizosphere microbiota and the access of DON for microbial metabolism. According to Schimel and Bennett's (2004) concept, microbial N mineralization (ammonification and nitrifications) can be important only in N rich sites, where N is in excess and NH_4 remains available for plants and nitrifiers. In N limited sites, most N is directly immobilized into microbial cells. A closer link between DON exudation and microbial activity in *Eleocharis* rhizosphere is supported by the lack of correlation between DOC and DON (Fig. 3a). In exudates with the predominance of organic acids, amino acids and sugars, which are passively released from the plant cells, DOC should be to some extent correlated with DON on daily scale. Larger DON release and its faster and more efficient mineralization in *Eleocharis* than in *Typha* rhizosphere indicate a faster N turnover and a higher supply of easily degradable DON to rhizosphere microbial community. This supply is enhanced especially under P limitation, where the N_2 fixation is limited (Pivničková et al. 2010; Šantrůčková et al. 2010) and plant N demands are restricted by P deficiency (Rejmánková and Snyder 2008).

Dissolved organic N concentrations in exudates responded to P enrichment negatively. This trend might be caused by higher N demands for growth and metabolism once plant and microbial P limitation was relieved (co-limitation, Arrigo 2005) and P limitation can turn to N limitation. This assumption is supported by enhancement of NUE, especially that of *Typha*. Higher N demand in P enriched treatments could be only partly covered by increased N_2 fixation (Šantrůčková et al. 2010). On the other hand, higher DON exudation under P limited conditions could be related to the enhanced secretion of N-rich extracellular enzymes, phosphatases. A correlation between DON and APA appeared only in *Typha* treatment (Fig. 3b).

Enhanced extracellular phosphatase activity (APA) under P deficiency has been already described for root APA of both plant species (Rejmánková and Snyder 2008; Rejmánková et al. 2011) as well as for the microbial APA in the sediments (Sirová et al. 2006). *Eleocharis* consistently displays higher root phosphatase activities than *Typha*. In our study, we observed a positive trend between APA and N/P in exudates, which might be caused by substantial release of N from roots as phosphatases (Güsewell 2004).

Typha has been reported to exhibit higher saturated P uptake, higher affinity for P uptake and stronger response of P uptake kinetics to P availability than sawgrass (*Cladium jamaicense*,), another common macrophyte of P limited calcareous marshes of the Caribbean similar to *Eleocharis* in its growth strategy (Brix et al. 2010). Yet, *Eleocharis* is markedly more efficient in P uptake and P resorption efficiency than *Typha*. In addition, *Eleocharis* is able to adjust P acquisition and P resorption rates depending on P availability better than *Typha* (Rejmánková and Snyder 2008). Differing ability to adapt to a wider range of P levels favors *Eleocharis* under P deficiency while more "self-centered" C use strategy promotes *Typha* under P sufficiency.

The pH of *Eleocharis* exudates from all treatments was about one half a pH unit higher compared to pH measured in interstitial water from the same plot. In contrast, pH values of *Typha* exudates did not differ from related interstitial water. We suggest several explanations for the increased pH in *Eleocharis* exudates: *i*) *Eleocharis* releases lower amounts of acid/acidic compounds (e.g. organic acids) and higher amounts of compounds of alkaline character (alkaline aminoacids, OH^-). This can be supported by the higher DON concentrations, lower C/N ratio and higher biodegradability of *Eleocharis* exudates, *ii*) *Eleocharis* roots acquire cations and anions in proportion, which causes a relative increase of pH compared to interstitial water. *Iii*) Microbial processes in *Eleocharis* rhizosphere alter pH conditions. Ammonification increases pH, while nitrification acidifies the environment. We have data showing ammonification being much higher (30 μg N g^{-1} h^{-1} – 56 μg N g^{-1} h^{-1}) than nitrification (9 μg N g^{-1} h^{-1} – 14 μg N g^{-1} h^{-1}) in the *Eleocharis* rhizosphere.

Both plant species and the sediment characteristics are reported to significantly influence rhizosphere microbial community (Marschner et al. 2001; Berg and Smalla 2009). It is thus not surprising that plant species play the major role in our rhizosphere study. In contrast, research on sediment microbial activities showed a stronger effect of P addition compared to the effect of plant species (Pivničková et al. 2010). Pivničková and her co-workers (2010) found higher concentrations of TOC, TN and available C in the sediment of P enriched plots. Despite the less evident effect of P enrichment on rhizosphere processes, we also found higher DOC and DON fluxes in high P treatment in most cases. In the sediment, the P impact on microbial processes is sustained more by plant litter input than by exudation. Accordingly, the litter of *Typha* (P enriched)

was reported to have more favourable C/P and N/P ratios than *Eleocharis*, which is able to resorb larger proportions of N and especially of P from its senescing tissues (Rejmánková and Snyder 2008). The concept of (especially P) low nutrients – adapted *Eleocharis* and competitive *Typha* corresponds with the results of Personeni and Loiseau (2005) who related the plant life strategy (N conservative and N competitive) to nitrogen cycles and distinguished the different roles of rhizosphere and bulk soil microbial activity in SOM and litter mineralization of two grassland species. Similarly, Oelmann et al. (2011) described the effect of plant functional types on P cycling in grasslands. *Eleocharis* promotes faster nutrient cycling between the plant, its litter and microbes, while *Typha* enables the nutrient cycling also between SOM pools.

Conclusions

Typha domingensis grows from tropical to temperate zones worldwide. This macrophyte possesses a great potential to expand into recently eutrophied areas, such as the Everglades (Brix et al. 2010), wetlands of northern Belize (Macek et al. 2010), or marshes of Palo Verde in Costa Rica (McCoy and Rodriguez 1994). *Typha* replaces oligotrophic-adapted species *Eleocharis* and *Cladium jamaicense*. We suggest that it is not primarily N cycle or P use efficiency that enables *Typha* to outcompete these species (as *Eleocharis* is much more efficient in these characteristics), but the C use strategy. Compared to *Eleocharis*, *Typha* invests higher proportion of C to above ground and in the conditions of high P availability decreases investments into roots and rhizosphere. Moreover, it develops robust rhizomes for expansion and accumulation of assimilates. *Typha* does not markedly support the rhizosphere community as it usually grows in eutrophic conditions where the microbes are also less limited. On the other hand, *Eleocharis* supports the microbial community, which enables it to mobilize nutrients more efficiently, which is favorable in oligotrophic marshes.

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