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Evaluation of macrophytes suitable for agriculture drainage treatment with respect to their carbon sequestration potential

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ABSTRACT

Relationship between the phenolics content and the decomposition rate of *Carex nigra*, *Scirpus sylvaticus*, *Phalaris arundinacea* and *Phragmites australis* was evaluated in this study. The study was aimed at the selection of plants growing in the Czech Republic, that can be used for constructed wetlands treating agricultural runoff and that can also contribute to carbon sequestration. Plant decomposition was studied using the litterbag experiments lasting 365 days. One set of samples (each plant from three various location in four replicates) were taken after 81, 173, 301 and 365 days and decomposition rate was evaluated. The phenolics concentrations in aboveground biomass of selected macrophytes were varied in narrow range from 6.73 ± 1.26 $g.kg^{-1}$ (leaves of *P. arundinacea*) to 14.09 ± 3.52 $g.kg^{-1}$ (*C. nigra*). On the other hand, decomposition rate significantly varied among different plants and different plan parts as well. The close relationship between the concentrations of phenolics and decomposition rates was found for *C. nigra, S. sylvaticus* and leaves of *P. arundinacea* and *P. australis* in this study – the higher the phenolics concentration, the lower decomposition rate. The study also revealed a very strong relationship between total phenolics/total nitrogen ratio in the biomass, and therefore, the plants with high phenolics content would be more efficient in carbon sequestration. It is necessary to extend the research with the aim to find other plants naturally occurring in the Czech Republic characterized by high concentration of phenolics in the aboveground biomass, thus, having the potential of slow decomposition and high carbon sequestration.

1. Introduction

Constructed wetlands for treatment of agricultural drainage waters have been used for decades (Vymazal, 2017). Most such constructed wetlands were built with the only aim of nutrient removal despite early recommendation to build such wetlands with multiple objectives such as biodiversity enhancement or wildlife habitat creation (Mitsch, 1992). Arheimer and Pers (2017) pointed out that many constructed wetlands built in agricultural landscape of southern Sweden were originally built for various reasons such as bird watching or hunting, biodiversity or landscape reconstruction. Indeed, at the same time these constructed wetlands remove nutrients and suspended solids from drainage waters.

The wetlands are known as major carbon sink on the Earth and provide an optimal natural environment for the sequestration and long term storage of carbon dioxide from the atmosphere (Mitsch et al., 2013; Mitsch and Mander, 2018). The main reason of carbon accumulation in wetland soils is the incomplete decomposition of plant biomass. Rates of decomposition vary in wetlands and the fate of material released and adsorbed during decomposition depends on the physical and chemical composition of decomposing materials such as toughness, nutrient content or the presence of the inhibitory chemicals (Brinson et al., 1981; Rejmánková and Houdková, 2006; Nelson, 2011; Lan et al., 2012; Eid et al., 2014), environmental conditions such as water chemistry and temperature at the site of decomposition (Lopes et al., 2011; Balasubramania et al., 2012; Flury and Gessner, 2014; Hines et al., 2014; Qu et al., 2014) and decomposing organisms (Van Ryckegem et al., 2006). For example, plants in nutrient poor environments are often characterized by high nutrient resorption resulting in poor litter quality and slow decomposition (Rejmánková, 2005). In their study, Rejmánková and Houdková (2006) evaluated what is more important for decomposition - the initial litter quality, or site differences. They described the positive effect of P-enrichment on the decomposition rate due to both, litter and site quality, where the site effect was stronger. They also showed at the C/P mass ratio of > 4000 decomposition processes are extremely slow.

It has also been reported that phenolics which are usually resistant to decomposition, can slow down the decomposition rate and potential carbon sequestration. Phenolics are the most widely distributed class of

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plant secondary metabolites and have many functions. They play important role in plant growth, pigmentation, reproduction and pathogen resistance (Bravo, 1998; Giada, 2013; Vermerris and Nicholson, 2006). There are currently more than 8000 known phenolic structures found in plants, ranging from the simple molecules such as carbolic acid which is often referred to as phenol, to the highly polymerized substances such as tannins (Dai and Mumper, 2010).

It has been shown that the phenolics inhibit the soil respiration as well as plant litter decomposition and nutrient recycling (Horner et al., 1988; Xu et al., 2013). Freeman et al. (2001) described the inhibitory effects of phenolics on extracellular hydrolase enzymes controlling decomposition and nutrient cycling. Dunn and Freeman (2018) reported that phenolics inhibitors slow the rate of decomposition to below that of photosynthetic production in the peats, primarily due to suppressed activity of phenol oxidase in flooded areas. In the study they described importance of phenolics molecular weight in inhibiting effects. They found that the higher the molecular weight of phenolic compounds, the greater its inhibitory effect on the breakdown of organic matter.

The objective of the study was to evaluate whether the concentration of phenolics in senescent influence the decomposition rate of four common wetland macrophytes which are used in constructed wetlands for municipal wastewater treatment in the Czech Republic and could be used for constructed wetlands treating agricultural drainage.

2. Materials and methods

2.1. Litter collection

The macrophytes involved in the study were *Phragmites australis* (Cav.) Trin. ex Steud., *Phalaris arundinacea* L., *Scirpus sylvaticus* L. and *Carex nigra* (L.). These species commonly occur in the Czech natural wetlands and *P. australis* and *P. arundinacea* are the dominant species used in constructed wetlands for municipal wastewater treatment. *S. sylvaticus* and *C. nigra* were selected because these two species were employed in a study aimed at removal of nutrients from a stream impacted by agricultural drainage in a semi-constructed wetland (Vymazal and Dvořáková Březinová, 2018). Freshly senescent standing aboveground biomass was collected in autumn 2015 from three different natural wetlands in the Czech Republic – Březnice (wet meadow), Chmelná (floodplain) and Pařez pond (pond littoral zone). The plants were cut into 10-cm long segments and dried at 40 °C until constant weight. *P. australis* and *P. arundinacea* were also separated into leaves and stems.

2.2. Decomposition experiment

Decomposition was carried out outdoor basin in the experimental area at the Czech University of Life Sciences in Prague. The basin was filled with about 10 cm of soil and flooded by a nearby stream water. Dry biomass of *C. nigra* and *S. sylvaticus* (about 10 g) were weighed and placed into nylon bags (15 × 10 cm) with mesh size 1 mm. In case of *P. australis* and *P. arundinacea*, leaves and stems were studied separately and litterbags were filled by dried stems and leaves, about 6 g and 4 g respectively. The litter bags were installed in the basin in spring 2016. Four bags of each plant were taken out after 81, 173, 301 and 365 days (until spring 2017). A longer period between the second and third sampling was caused by ice cover during that period.

The biomass from collected litter bags was divided into particular plant parts (if necessary), carefully cleaned, dried at 40 °C, weighted and homogenized in the cutting mill Pulverisette 15 (Fritsch, Idar-Oberstein, Germany) using the 0.5 mm mesh size screen.

2.3. Analytical methods

In the ground plant material, total phenolics content was determined using the spectrophotometric methods according to the Folin-Cioacalteau method (Bärlocher and Graca, 2005). The ground plant material (approximately 0.1 g) was firstly extracted in 5 ml of 70% acetone for one hour. Standards solutions ranging from 0 to 250 mg/l were prepared from tannic acid (Sigma Aldrich) dissolved in 70% acetone. Standard solutions and centrifuged extracts (14000 RCF, 15 min, Ø17 × 75 mm, centrifuge MPW-251/MPW Med. Instruments) were transferred to the test tubes, solution of 2% Na_2CO_3 in 0.1 NaOH and Folin-Cioacalteau reagent (Sigma Aldrich) diluted 1:2 with deionized water were added. Absorbance at 760 nm was measured after 120 min using Cary UV–Vis spectrophotometer (Agilent Technologies). The same analytical procedure was carried out in the original biomass samples before the decomposition experiment started.

Biomass used for the decomposition experiment was also analyzed before the study and during each sampling directly for total N and total C using a Skalar Primacs SNC analyzer (Breda, the Netherlands). For nitrogen and carbon, NIST 1547 Peach Leaves was used as the standard (National Institute of Standards and Technology, Gaithersburg, MD, USA). The triplicate measurements of the standard agreed to within 5% and NIST reference material recoveries were found in the range 92–98%.

2.4. Data processing

For each plant (or plant part) decomposition constant (d^{-1}) was calculated according to the first-order exponential decay model (Olson, 1963; Wider and Lang, 1982):

$$-\ k = (W_t/W_0)/t,$$

where W_t is dry matter weight at moment t, W_0 is initial dry matter weight, t is time in days. The constant k was determined for the whole period (365 days). The time necessary for 50% loss of dry biomass (t_{50}) was calculated by formula (Olson, 1963):

$$t_{50} = 0.693\,k^{-1}$$

Statistical analyses were performed with the software Statistica 12 (StatSoft, Tulsa, OK, USA). Statistical analysis of variance (ANOVA) followed by post-hoc Tukey HSD test was used to evaluate differences between phenolics content and t_{50} of various plants. The significance level was set at $p < 0.05$.

3. Results

3.1. Carex nigra

The results indicate that the decomposition of *C. nigra* was the fastest in the sample taken at Chmelná (Fig. 1). It was characterized by rapid decomposition between days 0 and 173 (dry weight loss of 81,3%). Thereafter, the decomposition process slowed down and after one year, 11.1% of the initial biomass remained. Similar decomposition curve was observed for samples taken at Březnice but the overall litter mass remaining after 365 days was higher (44.00%). Decomposition of *C. nigra* taken at Pařez pond was faster than sample from Březnice in the first stage (0–81 days) and then there was a gradual decline with 46.6% biomass remaining after 365 days of decomposition.

Mentioned results showed differences in decomposition rate of *Carex nigra* from different study sites. On the other hand, concentration of phenolics in litter before decomposition were very similar for all study localities (Fig. 2). The highest average concentration was observed at Březnice (16.5 ± 1.07 $g.kg^{-1}$ DW) and the lowest at Pařez (11.45 ± 4.2 $g.kg^{-1}$ DW). Concentrations in samples taken after one year of decomposition experiment ranged from 7.12 ± 0.93 $g.kg^{-1}$ DW in Březnice to 4.48 ± 0.54 $g.kg^{-1}$ DW in Chmelná (Fig. 2).