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Elemental composition and fungal colonisation of decomposing *Phragmites australis* (Cav.) Trin. ex Steud. litter at different water regimes

Elementna sestava in glivna kolonizacija razpadajočega opada trsta *Phragmites australis* (Cav.) Trin. ex Steud. pri različnih vodnih režimih

Matevž Likar*, Nataša Dolinar, Katarina Vogel-Mikuš, Alenka Gaberščik, Marjana Regvar

Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia

*correspondence: matevz.likar@bf.uni-lj.si

Abstract: Plant litter decomposition in intermittent dry and wet habitats share decomposition mechanisms of both dry land and submerged habitats. The aims of the present study were therefore to compare fungal communities on the decomposing plant material regarding the water regime of the location. Furthermore we wanted to evaluate the effects of the water regime on the decomposition in combination with fungal decomposers. Litter decomposition was followed on selected sites of Lake Cerknica with different hydrological regimes, using the litterbag method. The elemental composition of the decomposing plant tissues of *Phragmites australis* and fungal communities developing on the decomposing plant material were analysed. The hydrological regime has an important role in defining the fungal community of *P. australis* leaf litter. Water regime affected the fungal communities, which exhibited higher diversity under more stable dry or submerged conditions (in contrast to intermittent). Decomposition rates were more affected by the environment as by the fungal community diversity or composition. But, despite differences in the fungal communities the elemental composition showed similar patterns of enrichment due to decreases in the organic fraction of the plant tissue.

Keywords: litter decomposition, fungal community, elemental composition, intermittent habitat, wetland

Izvleček: Razgradnja rastlinskega materiala v presihajočih habitatih kaže lastnosti razgradnje značilne tako za suha kot vlažna okolja. Namen naše raziskave je bil primerjati glivne združbe na razkrajajočem rastlinskem materialu glede na vlažnostne razmere v okolju in oceniti vplive vodnega režima na razgradnjo v kombinaciji z glivnimi združbami v presihajočih ekosistemih. Na izbranih lokacijah ob Cerkniškem jezeru, ki so se razlikovale v vodnem režimu, smo spremljali razgradnjo listov in stebel navadnega trsta (*Phragmites australis*) in ovrednotili razlike v elementni sestavi in glivnih združbah, ki so se razvile na razkrajajočem rastlinskem materialu. Hidrološki režim lokacije se je izkazal kot pomemben dejavnik pri oblikovanju glivnih združb na razkrajajočih listih trsta. Vodni režim je močno vplival na glivne združbe, ki so imele večjo diverziteto pri stabilnejših razmerah tj. suhih ali potopljenih razmerah v primerjavi s presihajočimi. Naši rezultati kažejo, da je okolje samo močneje vpli-

valo na hitrost razgradnje kot glivna združba, ki se je oblikovala na razkrajajočem se rastlinskem materialu. Kljub razlikam v glivnih združbah, je elementna sestava rastlinskega materiala sledila podobnim vzorcem koncentriranja elementov zaradi razgradnje organske matrice.

Ključne besede: razgradnja opada, glivna združba, elementna sestava, presihajoči habitat, mokrišče

Introduction

Wetlands with prevailing helophyte vegetation have high primary productivity of biomass (Cronk and Fennessy 2001), which is subjected to decomposition processes (Longhi et al. 2008). The decomposition of wetland plants starts when senescent leaves are still attached to the plant (Bärlocher 1997, Van Ryckegem et al. 2007), with colonisation of the dead plant parts by fungi and bacteria (Komínková et al. 2000, Van Ryckegem et al. 2006). Microbial colonisation is followed by fragmentation of the material, due to different physical factors (Wallis and Raulings 2011), which can include currents, wave action, and water-level fluctuations. In addition, macro-invertebrates that favour leaves colonised by microorganisms have important roles in the decomposition of this plant material (Webster and Benfield 1986).

During the decomposition processes, much of the carbon is released, as CO_2 or CH_4, depending on oxygen availability, although it is also incorporated into microbial biomass. In contrast, P, N and other elements such as Fe, Zn and Ni, are built into the microbial biomass, which results in increased nutrient content of the decomposing litter (Cleveland and Liptzin 2007, Bridgham and Lamberti 2009). During the decomposition, some element concentrations can reach more than 100-fold, and some even 1000-fold, their concentrations in the original plant tissue (Zawislanski et al. 2001, Windham et al. 2004, Du Laing et al. 2006). This element accumulation primarily occurs in the microbial biofilm that is formed on the decomposing plant material (Purchase et al. 2009), and less so in the actual matrix of the decomposing materials (Schaller et al. 2011). Ryckengem et al. 2006 concluded that fungi decomposing the plant litter are active immobilizers of nutrients from the external environment as in their study fungi often contained increased element concentration when compare to the original plant material. Therefore, fungi have a considerable impact on the organic matter dynamics and should be incorporated in any study trying to understand nutrient cycling in wetland ecosystems.

The element enrichment however also depends on environmental conditions and on the properties of the original plant material (Du Laing et al. 2006, Batty and Younger, 2007). Given the plant species richness and assuming equally chemically diversity of plant material, which can affect the decomposition (Loranger et al. 2002, Kasurinen et al. 2006), it is critical to further investigate the importance of litter quality in order to increase our ability to understand and predict the decomposition process in different environments.

The decomposition in intermittent ecosystems is highly affected by the periodical flooding (Bedford 2005, Dolinar et al. 2016), which change the availability of oxygen and moisture that are needed for aerobic decomposition. The most important factors affecting the decomposition process seem to be the frequency and the duration of the submergence (Neckles and Neill 1994, Langhans and Tockner 2006, Dolinar et al. 2016). At drier locations where oxygen is readily available, flooding accelerates decomposition by increasing moisture, whereas longer submergence creates anoxic conditions that slow decay (Neckles and Neill 1994). Anoxic conditions represent one of the possible reasons for low decomposition rates in permanently flooded wetlands (Ryder and Horwitz 1995). High flood frequency also supports high decomposition rates (Brinson et al. 1981), as it also affects the fungal communities that colonise plant litter (Capps et al. 2011).

Lake Cerknica (Slovenia) is an intermittent wetland that undergoes pronounced water-level fluctuations (Dolinar et al. 2010, 2011), which makes it an ideal model situation for studies of plant decomposition. The vegetation of Lake

Cerknica is characterised by wetland communities with *Phragmites australis* (Cav.) Trin. ex Steud. common reed and *Carex elata* All. tufted sedge as the dominant plant species. Both species contribute significantly to the primary production (Martinčič 2003, Martinčič and Leskovar 2003), and consequently also to the production of litter in this ecosystem.

The main aim of the present study was to assess the influence water regimes in intermittent environment on elemental composition of decomposing *P. australis* tissues and fungal communities forming on the litter during the decomposition process. We hypothesised that differences in water regimes during the decomposition will affect formation of fungal communities on the decomposing *P. australis* material, and directly or indirectly (through fungal community) also influence the decomposition rate and elemental composition of decomposing *P. australis* material and therefore change the litter quality.

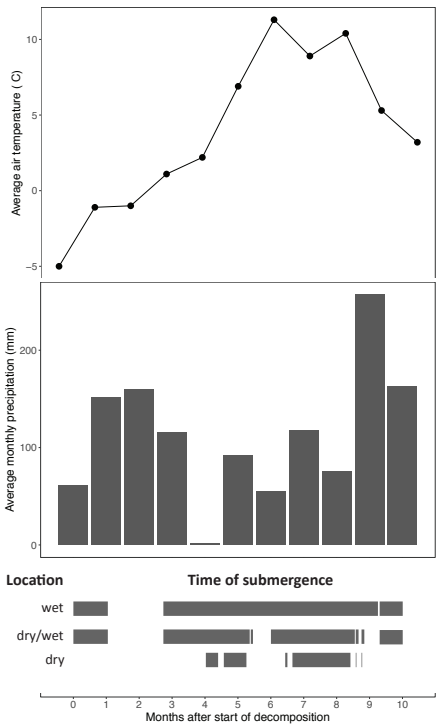

Figure 1: Average temperatures, precipitation and time of submergence for the locations with decomposing *Phragmites australis* material.

Slika1: Povprečne temperature, padavine in čas potopljenosti lokacije, na katerih je prihajalo do razgradnje materiala vrste *Phragmites australis*.

Material and methods

Site description

Lake Cerknica covers an area of 38 km^2 as an intermittent wetland that lies at the bottom of the Cerkniško polje depression (Slovenia). The annual precipitation for the locality is around 2000 mm and the average temperature is 10 °C. The monthly average temperatures and precipitation data is represented in Fig. 1.

In spring and usually in late autumn, Lake Cerknica reaches its normal level at 550 m above sea level, and covers the area of 26 km^2. The floodplain is flooded for 9 months to 10 months of the year, and dry for about 2 months, usually in summer. This provides a variable ecosystem that changes in both time and space. Its vegetation patterns show clear zonal distribution that depends on the extent and frequency of the flooding (Martinčič 2003, Martinčič and Leskovar 2003).

Decomposition analysis

The decomposition analysis was performed using the litterbag method (Webster and Benfield 1986). Here, 120 litterbags (15 × 30 cm) made from plastic mesh with 1 × 1 mm openings were filled with either 5 g leaves of *P. australis*, or 10 g culms of *P. australis*, that were collected at Lake Cerknica at the end of the vegetative period. Dry *P. australis* culms were cut to 25-cm-long pieces, while leaves were used as whole. In December 2007, the litterbags of *P. australlis* were placed at three experimental locations at Lake Cerknica (N45° 43’ 40’’, E14° 24’ 17’’) that differed according to the extent of water-level fluctuations, from habitats that were almost completely dry (termed dry), across intermittent (dry/wet), to almost always submerged (wet). Litterbags were attached to wooden poles that positioned them

5 cm above the ground, and they were collected five times as four replicates during the following year (for details see Dolinar et al. 2016). The selected locations were in the middle of the reed stand where sediment was stabilised by numerous rhizomes. In addition, the samples were fixed above the soil level to avoid the influence of sediment. During the decomposition study, the *P. australis* material at the wet location was submerged for the majority of the study time (Fig. 1). Material at dry/wet location was submerged 7-times: twice for longer than 60 days, and five times for under 30 days. At the dry location the material was submerged 6-times, with an average submergence time under 7 days.

The original plant material (termed 'Initial') was analysed prior to the decomposition experiment. After four months, samples for elemental composition analysis and analysis of fungal communities were collected. The material for elemental composition analysis was dried at 30 °C prior the analysis, whereas material for molecular analyses was frozen at – 20 °C until processed.

Elemental composition analysis

The composition of the plant materials was determined using energy dispersive X-ray fluorescence spectrometry, according to the protocol of Nečemer et al. (2008). In brief, 0.5 g to 1.0 g powdered sample material was used to form the sample pellets using a pellet die and hydraulic press. The primary excitation sources were [^{55}Fe] (10 mCi), [^{109}Cd] (25 mCi) and [^{241}Am] (20 mCi) annular radioisotope excitation sources (Isotope Products Laboratories, USA). The emitted fluorescence radiation was measured using an energy dispersive X-ray spectrometer that comprised a Si(Li) detector (Canberra), a spectroscopy amplifier (Canberra M2024), an analogue-to-digital converter (Canberra M8075) and a PC-based multichannel analyser (S-100, Canberra). The spectrometer was equipped with a vacuum chamber. The energy resolution of the spectrometer was 175 eV at 5.9 keV. The analysis of the X-ray spectra was performed using the analysis of X-ray spectra by an iterative least squares programme (Van Espen and Janssens 1993), as included in the quantitative X-ray analysis system software package (Vekemans et al. 1994). Element quantification from the measured spectra was performed using the quantitative analysis of environmental samples based on fundamental parameters (Kump et al. 2007). Quality assurance for the element analyses was performed using standard reference material NIST SRM 1573a (tomato leaves as a homogenised powder).

PCR amplification and temporal temperature gel electrophoresis

For the temporal temperature gel electrophoresis (TTGE) analysis, the protocol described by Likar et al. (2009) was followed, using GenElute Plant Genomic DNA miniprep kits (Sigma), according to the manufacturer instructions. The DNA was extracted from three subsamples from each plant (as 30 mg dry weight) that were combined prior to the amplification step.

All of the PCR reactions were carried out in a thermal cycler (MJ Research), using Taq DNA polymerase (Promega). The 25 µL reaction mixtures contained: 2.5 µL 10× PCR buffer, 2.5 mM $MgCl_2$, 200 µM of each nucleotide, 500 nM of each primer, 0.75 U DNA polymerase, and 12.5 µL diluted template. The PCR conditions for the ITS1F-ITS4 and ITS3-ITS4 primer pairs (Gardes and Bruns 1993; White et al. 1990) were 1 min at 94 °C, followed by 35 cycles of 35 s denaturation at 94 °C, followed by 53 s annealing at 55 °C, and 30 min elongation at 72 °C. The duration of the elongation step was increased by 5 s per cycle. A final elongation was performed at 72 °C for 10 min. A nested PCR approach was used, as a second PCR was performed with the ITS3-ITS4 primer pair after amplification of the DNA with the ITS1F-ITS4 primers.

The TTGE analysis of the fungal ITS region was carried out on a Dcode electrophoresis apparatus (BioRad, Hercules, CA, USA), as described by Likar et al. (2009). The polyacrylamide gel was composed of 8% (v/v) acrylamide-bisacrylamide mixture, 8 M urea, 0.25% 50× TAE buffer, 400 µL ammonium persulphate, and 40 µL TEMED. Electrophoresis was performed at a constant voltage of 100 V for 18 h, with a temperature gradient from 52 °C to 68 °C. A 100-bp ladder (Fermentas) was included in the first and last lanes on every gel, as the standards for the normalisation across the gels. The gels were stained with

ethidium bromide and photographed under a UV transilluminator.

Data analysis

The differences in the individual elements were compared using ANOVA. All tests were performed in R v3.3.2 statistical programme (R core team, 2016).

The effects of the hydrological regimes of the locations on the elemental contents of the decomposing *P. australis* material were visualised using non-metric multidimensional scaling with the *metaMDS* function of the Vegan v2.4-1 library (Oksanen et al. 2016).

Using the function *heatmap.2* in R package Gplots 2.10.1, a heatmap was generated for the z-scores of elemental composition data. Z-scores were obtained by subtracting the mean of all data points from each individual data point and then dividing those points by the standard deviation of all points with *scale* function. Using the function *hclust* in R package 2.15.1, the dendrograms of rows or columns were added on the margins of the heatmap. Hierarchical clustering with the complete linkage method was used for the dendrograms, based on the Euclidean distances among clusters in rows or among elements in columns.

The Euclidean distance matrices obtained from the elemental composition datasets were then analysed according to a hierarchical experimental design (with location of decomposing material as the grouping factor), using permutational multivariate analysis of variance (PERMANOVA; see Anderson 2001). Analysis of variance was performed with the *adonis* function of the R Vegan v2.4-1 library (Oksanen et al. 2016), with 999 permutations. The possibility of significant effects arising due to differences in multivariate dispersion rather than compositional changes was tested by calculation of the multivariate homogeneity of the group dispersions (variances), using the *betadisper* function of the Vegan package.

The TTGE gels were normalised, and similarity levels were calculated using the GelManager software package, version 1.5 (BioSystematica). GelManager 1.5 uses the Pearson product moment coefficient to generate the band similarity matrix using information about the apparent molecular weights of the bands and the band spacing. It should be noted that there is not necessarily a one-to-one correspondence between the number of bands and the number of unique sequences or fungal species, therefore we use term operational taxonomic units (OTUs). Chao Richness estimator (Chao 1984; 1987), Shannon diversity (Chao et al. 2013) and Gini-Simpson index (Good 1953, Chao et al. 2014) were calculated using package iNEXT 2.0.12 (Chao et al. 2014).

To calculate the unique and shared effects of water regime and fungal diversity, as well as how much variation in loss of mass of *P. australis* material during the decomposition was explained by each set of these variables, a variation partitioning analysis (partial RDA) was performed. Variation partitioning analysis by using *varpart* function and the significance of each testable fraction in variation partitioning analysis was obtained from the functions *rda* and *anova.cca*. The functions *varpart, rda* and *anova.cca* are all found in the package Vegan (Oksanen et al. 2016).

Results

Elemental analysis

The elemental analysis of the senescent plant material before the plant litter was exposed to decomposition was compared to the corresponding plant material after 4 months of decomposition. The initial concentrations of the elements K, Ca, Mn, Fe, Zn and Br in the plant material prior to decomposition are given in Tab. 1 (‘Initial’). The contents of the individual elements across *P. australis* culms and leaves were significantly different. Indeed, *P. australis* leaves showed K, Ca, Mn and Zn concentrations that were several times higher than for *P. australis* culms.

Table 1: Element concentrations in *Phragmites australis* culms and leaves before decomposition and at differ ent micro-locations after four months of decomposition.
Tabela 1: Elementna sestava poganjkov in listov trsta (*Phragmites australis*) pred poskusom in štiri mesece po začetku dekompozicije na različnih mikrolokacijah.

Organ	Location	Concentrations of elements [mg kg^{-1}]					
		K	**Ca**	**Mn**	**Fe**	**Zn**	**Br**
culms	Initial	166 ± 21	630 ± 104^{a}	83 ± 9	121 ± 27^{b}	20 ± 2	15 ± 2^{ab}
	dry	193 ± 93	1638 ± 273^{b}	100 ± 21	678 ± 112^{a}	14 ± 6	11 ± 2^{a}
	dry/wet	LOD	1490 ± 362^{b}	114 ± 8	228 ± 52^{b}	16 ± 4	15 ± 1^{ab}
	wet	193 ± 15	1913 ± 178^{b}	721 ± 111	416 ± 21^{a}	LOD	19 ± 4^{b}
leaves	Initial	1370 ± 193^{a}	10145 ± 942	474 ± 111^{a}	295 ± 67^{a}	36 ± 13^{b}	19 ± 2^{a}
	dry	2633 ± 705^{b}	10895 ± 1952	721 ± 111^{b}	5120 ± 269^{b}	77 ± 16^{a}	36 ± 2^{b}
	dry/wet	1998 ± 396^{ab}	9805 ± 597	743 ± 57^{b}	4230 ± 625^{b}	56 ± 9^{ab}	49 ± 6^{b}
	wet	1708 ± 494^{b}	10618 ± 2004	739 ± 157^{b}	3047 ± 618^{b}	46 ± 7^{b}	73 ± 15^{c}

Numbers represent averages ± SD (n = 4). Data not sharing the same letter in superscript are statistically significantly different at $p < 0.05$. LOD, limit of detection.
Številke predstavljajo povprečno vrednost ± SD (n = 4). Rezultati z različnimi črkami so statistično značilno različnio pri $p < 0,05$. LOD, meja detekcije.

The elemental analysis of the samples after 4 months of decomposition showed statistically significant differences in individual measured elements (Tab. 1) and overall elemental composition of the samples (Tab. 2) across the different conditions (i.e., wet vs. wet /dry vs. dry). When element concentrations in culms from different locations were compared, however, lowest concentration of Fe was observed in culms on dry/wet location, whereas dry location had lowest Br concentration. (Tab. 1). Fe was concentrated in all of the decomposing *P. australis* samples, although to a greater degree in leaves (reaching 10-17-fold the initial values). In *P. australis* culms, the Fe concentrations did not differ significantly from the initial plant material if decomposition took place under the dry/wet conditions, but reached up to 6-fold the initial Fe concentrations under the dry conditions (Tab. 1). No statistically significant differences were seen for the Zn concentrations in the decomposing *P. australis* culms, whereas concentrations in the decomposing *P. australis* leaves increased with increasing dryness of the location (from wet through dry/wet to dry) (Tab. 1). The opposite was seen for the Br concentration in the decomposing *P. australis* culms, as it increased with increased duration of submergence, whereas no statistically significant differences were seen for the different conditions for Br concentrations in the decomposing *P. australis* leaves (Tab. 1).

Comparison of the element compositions of the decomposing *P. australis* culms and leaves prior to and after 4 months of decomposition was also analysed by the heatmap clustering of element composition z-scores (Fig. 2). This demonstrated clustering of the samples according to the plant organ. In contrast, the water status of location at which samples decomposed showed no clear trend on the elemental composition of decomposing material.

The non-metrical multidimensional scaling returned two-dimensional ordinations for element loss from the decomposing material with clear separation between the initial and decomposed samples for all of the plant tissues (Fig. 3). As decomposing *P. australis* culms showed less progress compared to leaves, there was more pronounced separation between the different culms conditions, whereas for decomposing *P. australis* leaves, the dry, dry/wet and wet conditions were overlapping, which again was particularly obvious for the wet and dry/wet condition.

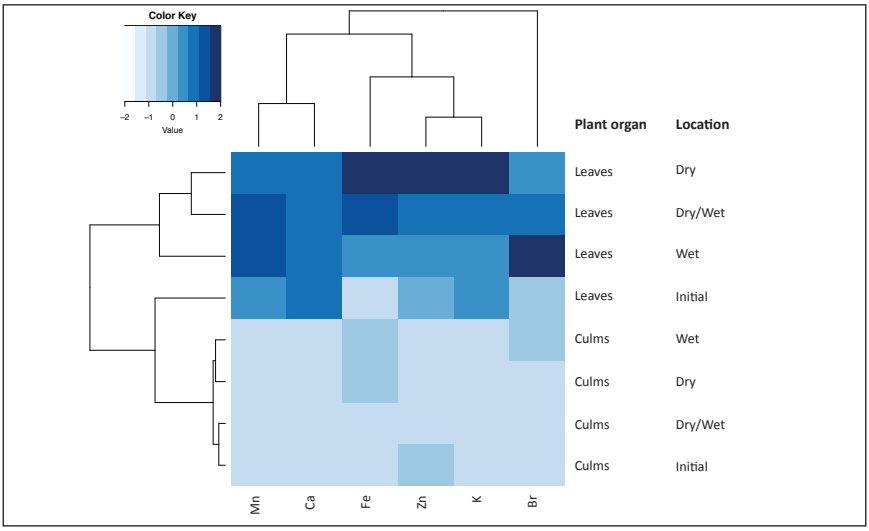

Figure 2: Z-scaled representation of element compositions in *Phragmites australis* culms and leaves after four months of decomposition under the different water conditions.

Slika 2: Z-lestvičena predstavitev elementne sestave stebel in listov vrste *Phragmites australis* po štirih mesecih dekompozicije pri različnih vodnih režimih.

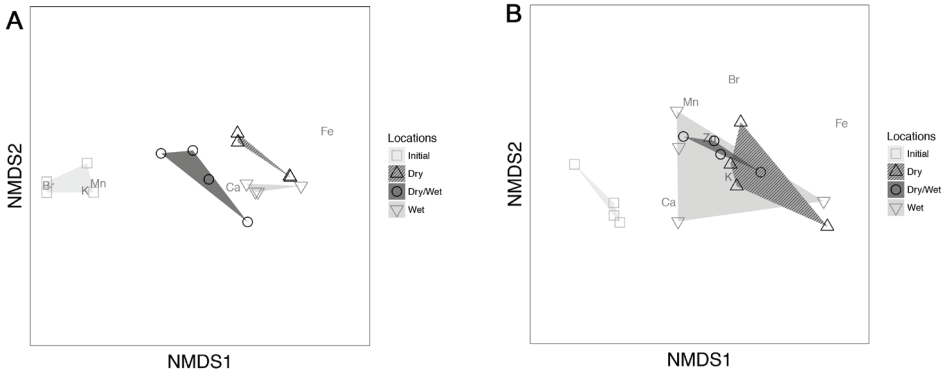

Figure 3: Ordination plot of the decomposing *Phragmites australis* culms (A) and leaves (B) along the dry, dry/wet and wet humidity gradient, based on the elemental contents, using non-metric multidimensional scaling.

Slika 3: Geometrična predstavitev elementne sestave razkrajajočih stebel (A) in listov (B) vrste *Phragmites australis* na lokacijah z različno vlažnostjo (suha, presihajoča in vlažna), pridobljen z nemetričnim večrazsežnostnimo lestvičenjem.

Relevance of fungal community and water regime for decomposition

Richness and diversity estimators showed that the highest richness/diversity of OTUs was observed in the initial *P. australis* material and fell during the decomposition process (Tab. 3). The lowest richness/diversity was observed in dry/wet location. Rank-abundance plots indicated that some of OTUs that were not present in the initial plant material were later found in all decomposing samples with high relative abundance (Fig. 4, marked with an asterisk). Also, some of the more abundant OTUs were observed to be missing from the samples decomposing in completely submerged conditions (Fig. 4, wet, marked with a cross) or dry/wet conditions (Fig. 4, dry/wet, marked with a double cross). In contrast, one of the OTUs was observed only in the initial material and was not found on any of the decomposing samples (Fig. 4, wet, marked with a double asterisk).

Table 3: Species richness and diversity estimators for fungal communities on *Phragmites australis* leaves prior to (initial) and after 7 months of decomposition at different locations (dry, dry/wet and wet). Values represent estimators ±SE (n=4).

Tabela 3: Vrstna pestrost in cenilci pestrosti za glivne združbe na listih trsta (*Phragmites australis*) pred in po 7 mesecih razgradnje na različnih mikrolokacijah. Vrednosti predstavljajo cenilko ±SN (n=4).

Location	Chao richness		Shannon diversity		Simpson diversity	
Initial	22.75 ±	20.72	23.79 ±	11.89	28.00 ±	9.08
Dry	13.53 ±	2.08	15.19 ±	2.53	18.07 ±	3.05
Dry/ Wet	8.00 ±	0.00	9.53 ±	1.29	11.77 ±	1.60
Wet	10.49 ±	2.03	12.79 ±	2.66	18.20 ±	2.87

Variation partitioning showed that water regime as average time of submergence together with frequency of submergence explained 96% of the total variation (with 52% of total variation shared by both factors), whereas fungal diversity explained only less than 1% and was not statistically significant.

Variation partitioning showed that variation in fungal communities was largely explained by the overall fungal diversity on the decomposing material (10% of total variation). Average time of submergence together with frequency of submergence together explained only 5% of total variation, but was not statistically significant.

Discussion

Periodical flooding can affect the decomposition in intermittent ecosystems as it changes the environmental conditions and thus affects the microbial communities and its activity (Bedford 2005, Dolinar et al. 2016).

In the present study, we observed differences in fungal communities on the decomposing *P. australis* leaves between locations with different water regimes. The majority of the OTUs on decomposing *P. australis* leaves was observed already in the initial leaf material collected from the plants, thus supporting observation that senescent leaves begin decomposition still attached to the plant (Bärlocher 1997, Van Ryckegem et al. 2007), when they are colonized by fungi and bacteria (Komínková et al. 2000, Van Ryckegem et al. 2006). Additional OTUs joined the fungal community of decomposers during the decomposition, while some disappeared. The later probably represented plant pathogens or less competitive

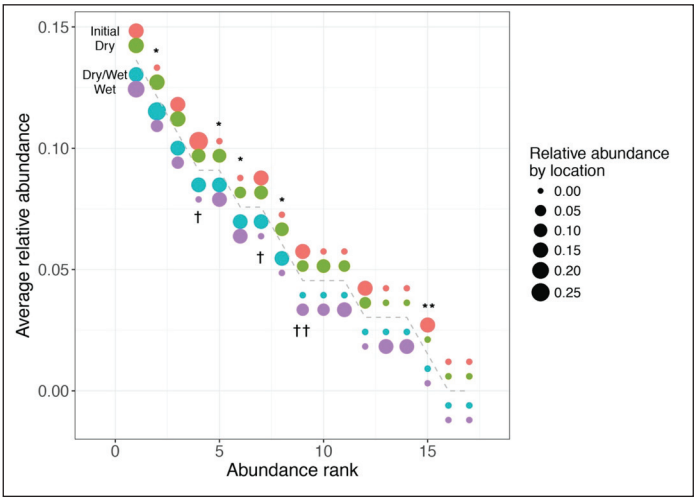

Figure 4: Rank abundance plot with average relative abundance for OTUs (dashed line) and relative abundance for individual OTUs from initial *Phragmites australis* leaves or leaves decomposing at dry, dry/wet and wet location (circles). The size of average relative abundance for OTUs is represented on the y-axis, whereas the relative abundance of the individual OTU at different locations is represented by the size of the circle. Legend: asterisks mark the OTUs that were not present in the initial plant material, double asterisks mark the OTUs that were missing at dry location, double crosses mark OTUs missing at the dry/wet location, and crosses mark OTUs that were absent in material decomposing at wet location.

Slika 4: Graf ranžirane številčnosti in povprečne relativne številčnosti operacijskih taksonomskih enot (črtkana črta) in relativna številčnost individualnih operacijskih taksonomskih enot iz izvornega materiala vrste *Phragmites australis* ali listov, ki so se razkrajali na suhi, presihajoči in vlažni lokaciji (krogci). Velikost povprečne relativne številčnosti operacijskih taksonomskih enot je predstavljena na y-osi, medtem ko je velikost relativne številčnosti posameznih operacijskih taksonomskih enot na različnih lokacijah predstavljena z velikostjo kroga. Legenda: zvezdica označuje operacijske taksonomske enote, ki niso bile prisotne v izvornem materialu, dvojna zvezdica označuje operacijske taksonomske enote, ki manjkajo na suhi lokaciji in križ označuje operacijske taksonomske enote, ki manjkajo na vlažni lokaciji.

saprophytes, which disappear with progression of decomposition (Berg and McClaugherty 2003). Our findings suggest that the contrasting environmental conditions to which these decomposing leaves were exposed might have lead to changes in the composition of fungal communities. The changes in fungal community composition were accompanied by difference in diversity, with the lowest diversity observed for *P. australis* leaves decomposing at dry/wet location. This suggests that the combination of submergence times and frequency of submergences at this location created an unstable community or at least the least favourable conditions for the development of a diverse community of fungal decomposers. This is in line with the observations of Kaisermann et al. (2015), who reported rapid changes in the fungal communities after changes in the soil moisture content. The reason for this might be the permanent disturbance and the variable environmental conditions in the shallower water, which might negatively affect the establishment of biotic communities on the decomposing plant parts (Collinson et al. 1995).

Differences in fungal communities could lead to changes in the metabolic performance of fungal community (Webster and Benfield 1986), resulting in changed decomposition rates. In line with this, Dolinar et al. (2016) reported that decomposition rate for *P. australis* leaves was faster under the wet conditions in comparison to the intermittent dry/wet conditions. But our results suggest that the main factor for differences was the environment (e.g. water regime), which

explained the majority of variation in weight loss during the decomposition. Fungal communities as an indirect effect of the environment were not a very strong factor, thus suggesting that changes in fungal communities did not heavily affect the decomposition efficiency.

The concentrations of elements in plant tissues depend on the properties of their habitat, as primarily on the amount of individual elements in the water and sediment, and also on environmental factors, and in particular, pH (Batty and Younger 2007). The initial values of the elements measured in these *P. australis* tissues in the present study were comparable to those in *P. australis* material collected from an acidic (pH 3) spoil heap discharge in Northumberland, UK (Batty and Younger 2007), and an urban district in Sicily (Bonanno 2011). This would suggest the importance of the plant species characteristics on their ionome independently from the growing locations and expand the comparability between different studies on *P. australis* elemental composition.

The element concentrations measured in the present study in *P. australis* leaves were much higher than in culms, which is in agreement with data from other studies (Vymazal et al. 2009, Bonanno 2011). Comparison of the contents of the elements K, Ca, Mn, Fe, Zn and Br in the dried plant tissues initially and the decomposition showed increases in most of the elements measured. Similarly to our observation, Windham et al. (2004) reported Zn and Cr concentration in *P. autralis* leaves increased 10-fold after 1 year of decomposition. In a similar study, Du Laing et al. (2006) reported approximately 5-fold higher Zn, Pb and Cu concentrations in leaves and about 10-fold higher Cd, Cr and Ni concentrations after 9-16 months of decomposition, while there was a much smaller accumulation of these elements in the culm. In the present study, decomposing materials differed depending on the conditions where they are decomposing, although the accumulation pattern of the individual elements showed no clear trend connected to the decomposition conditions. This suggests that the location where the element is incorporated in plant cell and tissue is more important for the resulting changes in its concentration during decomposition than environmental conditions at which the plant material is decomposing. The reasons for this accumulation of elements in decomposing plant tissues are numerous. One of the important reasons is that the dried tissue might retain some fine particles of sediment (Zawislanski et al. 2001, Windham et al. 2003, Du Laing et al. 2006), and another is the accumulation in the microbial biofilm that covers decomposing plant tissues (Batty and Younger 2007). As such, accumulation of individual element is greatly dependent on its availability in water, sediment and needs of the microbial communities in the biofilm. The greatly increased Fe concentrations during decomposition might also be a result for the formation of an oxalate complex. Oxalate crystals can adhere to fungal hyphae and chelate Fe and Al ions (Cromack et al. 1979), and consequently they increase these concentrations during decomposition of the plant matrix.

In conclusion, decomposition of *P. australis* tissues in intermittent environment showed differences in microbial communities and elemental concentrations, connected to the frequency and time of the submergence. The combination of submergence frequencies and submergence times at both extremes (longer submergence times or drier periods) seemed to form more stable conditions that promoted formation of more diverse communities. But, despite differences in the fungal communities the elemental composition showed similar patterns of enrichment due to decreases in the organic fraction of the plant tissue, which suggests that in different microbial communities, the same function during degradation of plant material can be performed by different representatives of the community.

Povzetek

Mokrišča s prevladujočo helofitsko vegetacijo imajo visoko produkcijo rastlinske biomase (Cronk and Fennessy 2001), ki se počasi razkraja (Longhi et al. 2008). Pri tem se razgradnja prične že na rastlinah samih, ko odmirajoče organe kolonizirajo bakterije in glive (Komínková et al. 2000, Van Ryckegem et al. 2006). Med mokrišči si posebno mesto zaslužijo presihajoči ekosistemi, saj je v njih razgradja močno odvisna od periodičnih poplav (Bedford 2005, Dolinar et al. 2016), med katerimi pride do večjih sprememb v vlagi in prezračenosti okolja. Razgradnja rastlinskega

materiala v presihajočih habitatih ima tako lastnosti razgradnje značilne za suha kot tudi vlažna okolja.

Glavni namen naše raziskave je bil primerjati glivne združbe na razkrajajočem materialu navadnega trsta (*Phragmites australis*) glede na vlažnostne razmere v okolju in oceniti vplive vodnega režima v kombinaciji z glivnimi združbami na razgradnjo. Predpostavili smo, da bodo različni vodni režimi vplivali na oblikovanje glivnih združb na razkrajajočem rastlinskem material in posredno ali neposredno (preko gliv) vplivali na hitrost razgradnje ter elementno sestavo ostankov trsta.

Na izbranih lokacijah ob Cerkniškem jezeru, ki so se razlikovale v vodnem režimu, smo spremljali razgradnjo rastlinskega materiala in ovrednotili razlike v elementni sestavi in glivnih združbah, ki so se razvile na razkrajajočem rastlinskem material. Elementno sestavo smo določili z metodo XRF (X-ray fluorescence spectrometry). Glivne združbe smo karakterizirali s pomnoževanjem ITS rDNA in elektroforezo v temperaturnem gradientu (TTGE, temporal temperature gel electrophoresis).

Hidrološki režim lokacije se je izkazal kot pomemben dejavnik pri oblikovanju glivnih združb na razkrajajočih listih navadnega trsta (*Phragmites australis*), pri čemer sta bila elementna sestava in profil glivnih združb odvisna od pogostosti in časa potopljenosti lokacije. Naši rezultati so pokazali, da so glivne združbe pestrejše v bolj stabilnih razmerah vodnega režima v ekostistemu tj. suhih ali potopljenih razmerah v primerjavi s presihajočimi. Kljub razlikam v glivnih združbah pa je elementna sestava pokazala podobnosti v koncentriranju nekaterih elementov zaradi razgradnje organske matrice, kar nakazuje, da lahko različne skupine gliv prevzamejo podobno funkcijo pri razgradnji rastlinske biomase v okoljih z izrazito različnimi vodnimi režimi.

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