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Seasonal and multi-annual patterns of *Phragmites australis* decomposition in a wetland of the Adriatic area (Northeast Italy): a three-years analysis

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Abstract – Wetlands are one of the most biologically productive ecosystems, in which reedbeds of *Phragmites australis* are an essentially detritus-based system where litter decomposition is a fundamental process. This study represents a three year dataset describing cyclic trends of *Phragmites australis* leaf litter breakdown in a temporary and managed environment. For three years, 45 days field experiments were seasonally performed, using leaf bag technique within a large managed temporary pond (Natural Reserve of the Isonzo River Mouth, Northeast Italy), in order to analyze spatial and temporal variation in decomposition processes, to search for patterns on seasonal and inter-annual time scale and to infer the relevance of the main environmental features (physical and chemical) on decomposition dynamic. During the three years of analysis, decay rates (k) ranges were 0.0066–0.0075 days^{-1} in autumn, 0.0108–0.0158 days^{-1} in spring and $k = 0.0168$ days^{-1} in summer. Average mass loss per day % range was 0.97–1.31% in autumn, 1.12–2.04% in spring and 1.79–2.06% in summer. A well-defined seasonal cycle was observed through the study years as result of the drought-reflooding dynamic and the highest percentage (91.1%) of the average mass loss per day % variability was explained above all by rainfall, temperature and conductivity.

Key-words: Leaf bag / wetland / seasonal dynamic / temporary pond / decomposition rate

Résumé – Schémas saisonnier et pluriannuel de décomposition de *Phragmites australis* dans une zone humide de la région adriatique (Italie du Nord) : une étude de trois ans. Les zones humides sont parmi les écosystèmes les plus productifs, dont les roselières à *Phragmites australis* constituent un système basé essentiellement sur les détritus, où la décomposition de la litière représente un processus fondamental. Cette étude analyse un ensemble pluriannuel de données qui décrit les tendances cycliques relatives à la décomposition de *Phragmites australis* dans un milieu temporaire d'eau douce sujet à la gestion. Pendant trois ans, 45 jours d'expériences sur le terrain ont été effectués de façon saisonnière, en utilisant la technique du sac de feuilles dans un grand étang temporaire géré (Réserve Naturelle de l'embouchure de la rivière Isonzo, Italie du Nord), afin d'analyser les variations spatiales et temporelles dans les processus de décomposition, à la recherche de modèles à l'échelle temporelle saisonnière et inter-annuelle et d'en déduire la pertinence des principales caractéristiques environnementales (physiques et chimiques) sur la dynamique de la décomposition. Au cours des trois années d'analyse, les taux de décomposition (k) étaient 0,0066–0,0075 jours^{-1} à l'automne, 0,0108–0,0158 jours^{-1} au printemps et $k = 0{,}0168$ jours^{-1} en été. La perte moyenne de masse par jour était de 0,97 à 1,31 % en automne, 01,12 à 02,04 % au printemps et de 1,79 à 2,06 % en été. Un cycle saisonnier bien défini a été observé au cours des années d'études résultant de l'alternance assec-mise en eau. Le pourcentage le plus élevé (91,1%) de variabilité de la perte de masse moyenne par jour a été expliqué surtout par les précipitations, la température et la conductivité.

Mots-clés : Paquets foliaires / dynamique saisonnière / milieu provisoire / taux de décomposition

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1 Introduction

Wetlands are one of the most biologically productive ecosystems (Dixon and Wood, 2003; Rolon and Maltchik, 2006; Mereta *et al.*, 2012), performing many ecological functions, such as nutrient cycling (Bunn *et al.*, 1999) and carbon storage (Adhikari *et al.*, 2009). These environments are often dominated by macrophytes such as *Phragmites*, *Typha*, and *Juncus* (Kuehn and Suberkropp, 1998) which produce a considerable part of the organic material in these areas, and play an important role in the detritus food chain supplying an important nutrient source (van Dokkum *et al.*, 2002; Eid *et al.*, 2014). Reedbeds of *Phragmites australis* (Cav) Trin. Ex. Steud, especially, represent an essentially detritus-based system (Cowie *et al.*, 1992; Komínková *et al.*, 2000), which can be considered one of the most productive (Bedford, 2005). In this contest, litter breakdown appears as a fundamental process in the metabolism of wetlands (Webster *et al.*, 1995; Wallace *et al.*, 1997; Eid *et al.*, 2014).

Plant breakdown rates in aquatic ecosystems have been found to be affected by internal factors, such as distinctive characteristics of the leaves (Kok *et al.*, 1990; Canhoto and Graça, 1996), and by external environmental factors such as water temperature and salinity (Carpenter and Adams, 1979; Reice and Herbst, 1982; Vought *et al.*, 1998; Sangiorgio *et al.*, 2008a; Quintino *et al.*, 2009), pH (Thompson and Bärlocher, 1989), nutrients (Elwood *et al.*, 1981; Sharma and Gopal, 1982; Sangiorgio *et al.*, 2008b), or regional characteristics (Sangiorgio *et al.*, 2008a), such as climate (Murphy *et al.*, 1998) and solar radiation (Denward and Tranvik, 1998). Litter breakdown has been widely studied in streams and rivers (Graça and Pereira, 1995; Diez *et al.*, 2002; Menéndez *et al.*, 2003; Pinna *et al.*, 2003; Pinna *et al.*, 2004; Sangiorgio *et al.*, 2006), lakes (Gupta *et al.*, 1996; van Dokkum *et al.*, 2002) and transitional aquatic ecosystems, such as coastal lagoons or river mouths (Rossi and Costantini, 2000; Menéndez *et al.*, 2004; Sangiorgio *et al.*, 2004; Bayo *et al.*, 2005; Sangiorgio *et al.*, 2008a, 2008b). Otherwise, the decomposition of biomass on temporary freshwater wet sites with seasonally fluctuating water levels has received relatively little attention (Bedford, 2005; Völlm and Tannenberger, 2014). The water level in wetlands fluctuates seasonally due to natural processes, and at managed sites it can be deliberately manipulated to allow the access of personnel for vegetation cutting (Bedford, 2005), to enhance the mineralization of nutrients and to avoid conditions of hypoxia or anoxia (Street, 1982; Pizzul *et al.*, 2008). In this context, it was deemed of interest to investigate the reed decomposition in a managed wetland carrying out analyses which were seasonally repeated over a long time period (three years), to search for cyclic temporal patterns on a large timescale.

The area selected for the present study was the Regional Reserve of the Isonzo River Mouth (Northeast Italy), which represents the northernmost wetland in the Mediterranean area and it is included in a Site of Community Importance (SCI IT3330005) and in a Special Protection Area (SPA IT3330005). A seasonal work including a first characterization of *P. australis* leaf litter breakdown was already done in this area (Ruzič *et al.*, 2013) and the analysis was carried on for another two years.

The aims of the present study are: (i) to analyze the spatial and temporal variation of *P. australis* decomposition processes in a large managed temporary pond subject to summer drought events; (ii) to search for temporal patterns on seasonal and annual timescale; (iii) to infer the relevance of the main extrinsic (environmental) chemical and physical features on plant detritus decay rates.

2 Material and methods

2.1 Study area

The study was carried out in a large temporary pond (30 ha surface) partially resulting from environmental recovery actions (Figure 1). The pond is enclosed by an embankment and it is supplied mostly by rainwater and by an artesian well. The western portion of the area is damp pasture, while the eastern portion is entirely constituted of a reedbed (*Phragmites australis*). The pond was of autumnal origin (temporary autumnal pools, *sensu* Wiggins *et al.*, 1980), with a dry phase occurring from the late June until September, and a wet phase which begins in early autumn. The area features limnic to oligohaline basin waters (Stoch, 1995) with maximum depth ranging from 1.7 to 2.0 m (Stoch, 1995; Perco *et al.*, 2006). As this area represents a wintering and stopover site for many bird species, management policy limits human access only to working personnel. However, the vegetation growth control is performed both passively (grazing of Camargue horses and periodically cattle) and actively (using machines). After mowing, plant material is usually removed in order to avoid anoxia conditions and reduce the amount of nutrients. The water level is controlled only with a flap sluice gate placed at the northwest side of the basin. This gate is occasionally open for a few days during summer (usually late July/mid-August) to ease the drought. The water level is monitored and managed for ecological reasons to avoid anoxic condition in the bottom sediments and to favor the remineralization of the nutrients (Street, 1982; Pizzul *et al.*, 2008). A detailed description of the former hydrological condition is provided by Perco *et al.* (2006).

Five sampling sites were chosen for the present study (Figure 1) after considering characteristics such as water depth, vegetation coverage on the bottom and presence of vegetation nearby. The first was placed near the sluice side gate and close to the reedbed; the second site beside an islet without vegetation and the third beside a wooded islet, the fourth site was placed at the center of the basin and the fifth close to the reeds, where vegetation cover was observed.

2.2 Leaf bag experiment

Organic matter decomposition processes were studied by experimental field work on leaves of *Phragmites australis*, using the leaf bag technique (Petersen and Cummins, 1974). Leaf bags were prepared according to Basset *et al.* (2006) and as reported by Ruzič *et al.* (2013) and Bertoli *et al.* (2015) in previous investigations using leaf bags in the same area.

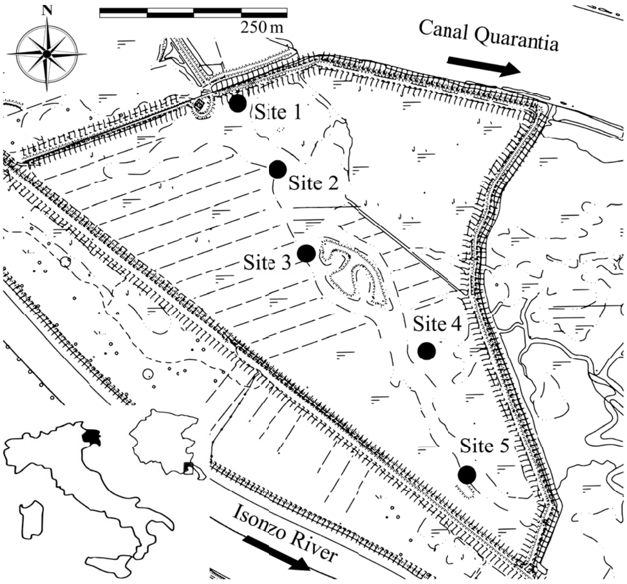

Fig. 1. The study area in the Natural Reserve of the Isonzo River Mouth (NE Italy) and position of the sampling sites (UTM coordinates: site 1 N 33T5067699.47 – E 383832.23; site 2 N 33T5067605.64 – E 383897.48; site 3 N 33T5067474.89 – E 383955.56; site 4 N 33T5067349.38 – E 384063.44; site 5 N 33T5067140.24 – E 384187.07).

At the beginning of autumn 2009 (last week of September) senescent leaves were collected in the pond under investigation, removing them from the shoots at least 30 cm above the water surface, avoiding apical leaves and those that had come in contact with water. We were careful to take only intact leaves without clear sign of decomposition from the top layer. After collection, leaves were air dried and stored in the dark at room temperature and low humidity until needed. Before use, leaves were cut into 10 cm-long fragments (excluding the basal and apical parts), oven-dried to constant weight (60 °C for 72 h) and then lots of 3.0000 ± 0.0001 g dry weight were placed in 0.5 cm mesh bags. Oven-dried leaves were used in order to achieve initial conditions as uniform as possible (Bärlocher, 1997), to obtain standardized samples and to facilitate comparisons. Eventual bias as a result of the procedures used may have likely affected the absolute value of decay rates, as a systematic bias, but it will not have influenced the spatial and temporal patterns of reed decay rates, the analysis of which represents the principal objective of this study.

With the intention to reduce handling losses, leaf bags were kept separately in plastic containers and only the intact ones were used for the experiments, while those that showed a loss of material were immediately excluded. Thereby, in good approximation, we considered negligible the loss of material due to handling. During autumn (10/27/2009; 10/19/2010; 10/28/2011), spring (4/1/2010; 4/1/2011; 3/27/2012) and at the beginning of June (6/1/2010; 6/1/2011; 6/1/2012), 12 leaf bags were submerged at each sampling site, placing them gently on the bottom sediment and tethering them to stones with strings, in order to prevent the loss. Leaf bags were then collected after 15, 30 and 45 days after submersion: at each sampling time, four leaf bags were retrieved from each sampling site, placed in separate polythene boxes containing pond water and rapidly brought to the laboratory. Here, leaves were gently washed to remove sediments and macroinvertebrate colonizers. Leaves from each bag were dried in an oven at 60 °C for 72 h and weighed ($x \pm 0.0001$ g dry weight). Values of remaining mass at $t = 15$, 30 and 45 days of submersion were expressed as percentage, considering the initial weight at $t = 0$ days (3.0000 ± 0.0001 g dry weight) as 100%. Winter sampling campaigns were not performed due to the presence of ice, which does not allow regular access to the study area. During summer, if drought occurred before the end of the experiment (45 days), leaf bags were considered lost and data were not included in the study.

We designed this multi-annual series of experiments with the aim to investigate spatial and temporal effects at seasonal scale, and to compare results obtained at this temporal scale along a three years period. The duration of the experiments (45 days) was chosen in order to limit analysis at seasonal level, and in order to allow comparisons among seasonal results.

2.3 Physical and chemical parameters

Depth was measured at each sampling time with a graduated rod. Conductivity (mS cm^{-1}), pH, temperature (°C) and dissolved oxygen (mg dm^{-3}) were recorded using field meters (HI 9033 conductivity meter; HI 9125 pH/ORP meter; HI 9147 dissolved oxygen meter; all instruments are manufactured by Hanna Instruments Inc., Woonsocket, Rhode Island, USA). Values were measured approximately at mid-depth in the water column surrounding the leaf bags. Data were not collected during the drought. Seasonal rainfall data (mm of fallen rain) were obtained by a weather station placed nearby the Reserve (Davis Vantage Pro 2 Plus, manufactured by Davis Instruments, Hayward, USA).

From October 2011 to July 2012, water samples were collected in sterile containers from the water column surrounding the leaf bags (approximately from mid-depth to the bottom), paying attention to avoid inclusion of sediment particles. Samples were filtered, frozen and brought to the laboratory. Here, concentrations of NH_4^+ (mg dm^{-3}) was obtained measuring the absorbance at 625 nm obtained by reaction with phenol and hypochlorite in order to give an intensely blue solution of the so formed indophenols (Bolleter *et al.*, 1961); NO_2^- (mg dm^{-3}) was evaluated by spectrophotometric measurement at 543 nm of colored derivative obtained from reaction with p-aminobenzene sulphonamide and N-(1-naphtil)-etylendiamine (APHA, 1998); NO_3^- (mg dm^{-3}) concentration were measured at 220 nm with a correction for organic matter absorbance (Armstrong, 1963); finally, in order to obtain PO_4^{3-} (mg dm^{-3}) concentrations, samples were allowed to react with a composite reagent containing molybdic acid, ascorbic acid, and trivalent antimony. The resulting complex polyacid is reduced to give a blue solution with the maximum extinction measured at 885 nm (Strickland and Parsons, 1972).

2.4 Statistical analysis

The percentage of the original mass remaining at the sampling times (15, 30 and 45 days) were estimated and the decomposition rate was modeled as a negative exponential decay function, frequently used to describe decomposition (Olson, 1963; Petersen and Cummins, 1974):

$$M_t = M_0 e^{-kt} \quad (1)$$

where M_t is the percent mass remaining at time (t), M_0 is the initial percent mass and k is the decomposition rate. Percentage values of mass remaining were natural log-transformed (ln[%mass remaining]) and ANCOVA was used to compare k-values as slopes of linear regression equations using time as covariate (Bärlocher, 2005; Zar, 1984). Data were checked for conformity to normality assumption with Shapiro-Wilks test and for conformity to assumption of variance homogeneity using C-Cochran's test (Cochran, 1941).

Percentage mass loss per day were estimated using the equation reported by Petersen and Cummins (1974). The two-way ANOVA and the Fisher Least Significant Difference (LSD-Fisher) post-hoc test were used to check spatial and temporal differences in the mass loss per day % values (factors site-season and factors site-year; data were square root transformed: $x_{\text{transf}} = \sqrt{x}$). Conformity to normality and variance homogeneity assumption were tested as mentioned above for percentage of original mass remaining data.

Finally, mean seasonal values of environmental abiotic factors were calculated for each sampling site and the relevance of abiotic characteristics as potential sources of variation in leaf decay rates was analyzed for each year using forward multiple stepwise regression analysis. Correlations among physical and chemical parameters were investigated using the Pearson product-moment correlation coefficient (r). Abiotic data were transformed ($x_{\text{transf}} = \sqrt{x}$) to fulfill assumptions of normality. Conformity to normality and variance homogeneity assumption were tested with the same tests mentioned above.

ANOVA, multiple stepwise regression and correlations analysis were performed using StatSoft STATISTICA 7.1 (StatSoft, 2005); ANCOVA was performed using PAST 3.0 application (Hammer *et al.*, 2001). Sites 3 and 5 were excluded from the comparisons involving autumn 2009 and summer 2012 respectively, because leaf bags were lost due to interference of animals (coypus and/or horses) living in the study area and to the summer drought, respectively. Finally, a summer dataset could only be obtained for 2012 owing to drawdown that interrupted the summer sequence of field experiments during the first and second years.

3 Results

3.1 Leaf bag experiment

Phragmites australis processing in the study area fits with negative exponential models in all the monitored seasons and temporal patterns were observed (Figure 2): reed leaves decay rates k (days^{-1}) differ significantly between autumn and spring every year, with lower values in autumn and higher in spring (Table 1, Figure 2a; $n = 144$, $p < 0.001$; Figure 2b, $n = 160$, $p < 0.001$; Figure 2c, $n = 160$, $p < 0.001$); the highest k-value ($k = 0.0168 \pm 0.0006$ days^{-1}) was recorded during the summer of 2012. Inter-annual comparisons highlight that there are no differences among autumnal decay rates ($n = 224$, $p = 0.163$), which ranged from 0.0066 ± 0.0004 days^{-1} to 0.0075 ± 0.0003 days^{-1} (Figure 2d); on the other hand, spring k-values differ significantly from year to year (Figure 2e, $n = 240$, $p < 0.001$): the highest spring k-value was recorded during 2011 ($k = 0.0158 \pm 0.0004$ days^{-1}) and the lowest during 2012 ($k = 0.0108 \pm 0.0004$ days^{-1}). In addition, we observed that, even though the difference was significant, in the 2011–2012 period the gap between autumn and spring values was lower than the one observed for previous years (Figure 2c). It was observed that in autumn mean daily percentage of reed detritus weight loss ranged from 0.97% to 1.31%, while in the spring periods values ranged between 1.12% and 2.04% (Figure 3). During summer 2012 daily mass loss range was 1.79–2.06%. Spatial variation of reed decay among sampling sites was never significant, while decomposition of *P. australis* leaf bags always differed significantly on a temporal scale between autumn and spring (two-way ANOVA, $p < 0.001$; Table 2) with the exception of the third year (Figure 3; LSD post hoc test; $p > 0.77$ for the autumn-spring comparisons).

Values observed during the summer of 2012 were significantly higher than those recorded in the other seasons of the same year (Figure 3; Table 2) (LSD post hoc test; $p < 0.05$ for all comparisons). Autumnal values of percentage mass loss per day do not differ among the years, while spring values always differ significantly (two-way ANOVA, $p < 0.001$; Table 3), as observed for k-value.

3.2 Physical, chemical data and nutrients

Mean seasonal values of abiotic parameters and relative standard deviations are shown in Table 6. Variation of reed decomposition processes during the three-year period was analyzed taking into account the physico-chemical parameters measured during the whole study period (nutrients excluded) and during the last year (nutrient included).

Considering the whole study period, stepwise regression showed that 91.1% of the variability observed for the reed mass loss per day % was explained by three abiotic characteristics (Table 5) (Stepwise multiple analysis: $r^2 = 0.911$; $F_{3,29} = 99.208$; $p < 0.001$): rainfall ($\beta = -0.864$), water temperature ($\beta = 0.268$) and water conductivity ($\beta = -0.222$). Considering the last year dataset, which includes nutrients, stepwise regression showed that 91.9% of variance was explained by only two variables (Table 5) (Stepwise multiple analysis: $r^2 = 0.919$; $F_{4,9} = 45.513$; $p < 0.001$): rainfall ($\beta = -0.660$) and water temperature ($\beta = 0.289$). It was not possible to highlight significant contribution of the external nutrient concentration to the variation of daily mass loss. Rainfall was correlated with all physical and chemical monitored parameters, positively with depth ($r = 0.423$; $n = 33$; $p < 0.02$) and dissolved oxygen concentration ($r = 0.449$; $n = 33$; $p < 0.01$) and negatively with water temperature ($r = -0.663$; $n = 33$; $p < 0.001$), pH ($r = -0.458$; $n = 33$; $p < 0.01$) and conductivity ($r = -0.542$; $n = 33$; $p < 0.001$).

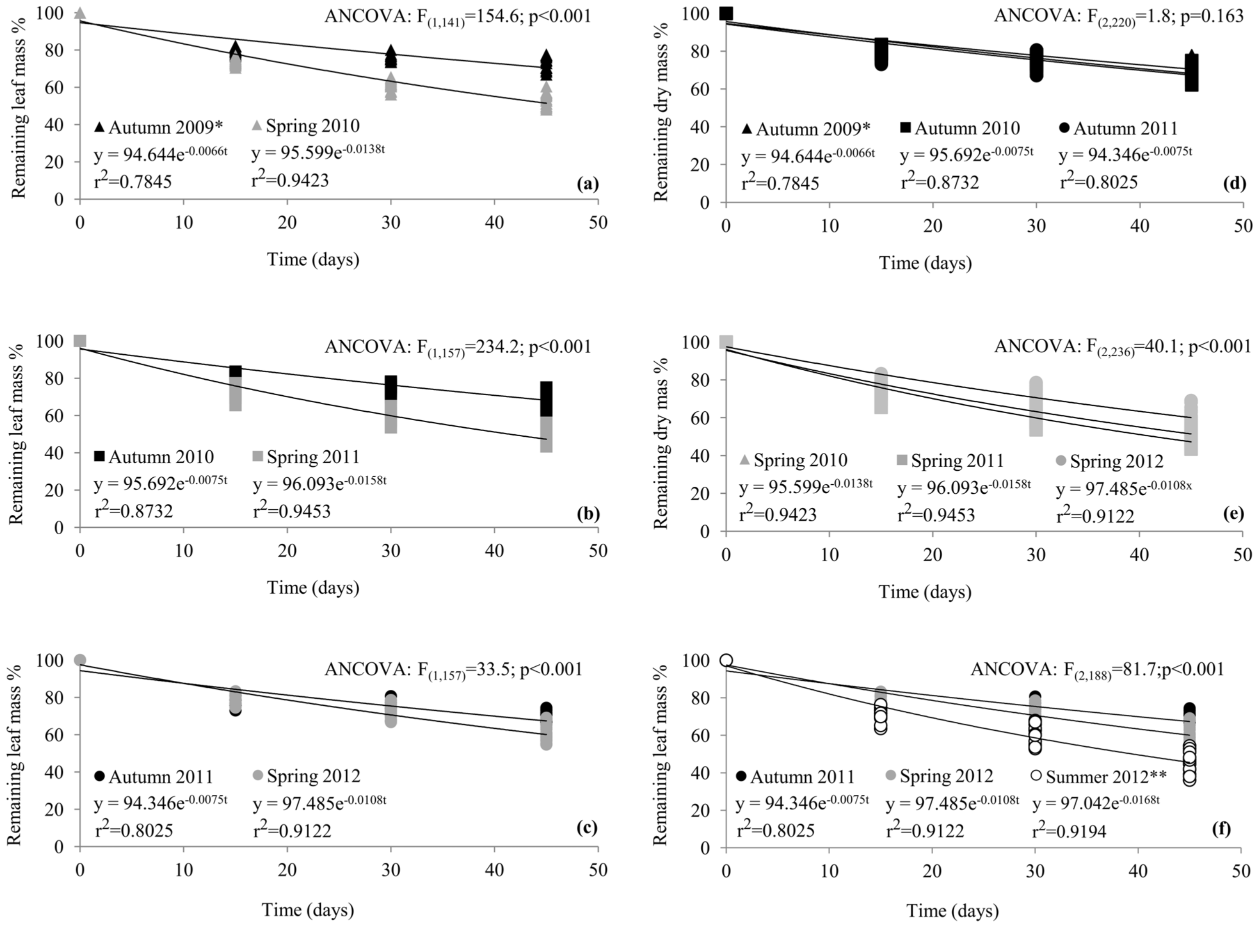

Fig. 2. Comparisons of *P. australis* leaf mass decay between successive seasons (a, b, c, f) and among homologous season of different years (d, e). The initial weight at t = 0 days (3.0000 ± 0.0001 g dry weight) was considered as 100%. (*): site 3 data not included; (**): site 5 data not included; (a): data and figure from Ruzič *et al.* (2013), modified.

Table 1. Mean values, standard deviations and sample sizes (n) relative to the decomposition rates (k) of the monitored seasons.

	2009–2010		2010–2011		2011–2012		
	Autumn	Spring	Autumn	Spring	Autumn	Spring	Summer
k	0.0066	0.0138	0.0075	0.0158	0.0075	0.0108	0.0168
S.D.	0.0004	0.0004	0.0003	0.0004	0.0004	0.0004	0.0006
n	64	80	80	80	80	80	64

4 Discussion

4.1 Leaf bag experiment

The present study reports a multi-year continuous series of seasonal data regarding decomposition processes of *Phragmites australis* in a managed freshwater temporary environment. Our dataset is the result of sampling plans seasonally repeated for three consecutive years.

Previous seasonal investigations about *P. australis* breakdown processes have already been performed (Menéndez *et al.*, 2001; Sangiorgio *et al.*, 2004; Pinna *et al.*, 2004; Sangiorgio *et al.*, 2006) but the seasonal analyses were carried out for only one year. Furthermore, sampling designs extended for more than one year were employed by other authors, but work designs were planned as a single prolonged experiment without replication of separated seasonal activities (Hietz, 1992; Gessner, 2000; Asaeda *et al.*, 2002; Bedford, 2005; Ágoston-Szabó *et al.*, 2006); Asaeda *et al.* (2002) reported decomposition measures (made by Hietz, 1992) and model simulations for two consecutive years but the activities were carried out for 12 or 24 months consecutively; however, the authors reported a similar pattern for the two periods of analysis.

The decomposition rates (k) of *Phragmites australis* leaf litter determined in the present study (k = 0.0066–0.0075 days^{-1} in autumn; k = 0.0108–0.0158 days^{-1} in spring; k = 0.0168 days^{-1} in summer) for a large temporary pond of the Isonzo River Mouth Reserve are consistent with literature data (Table 4). However, direct comparisons

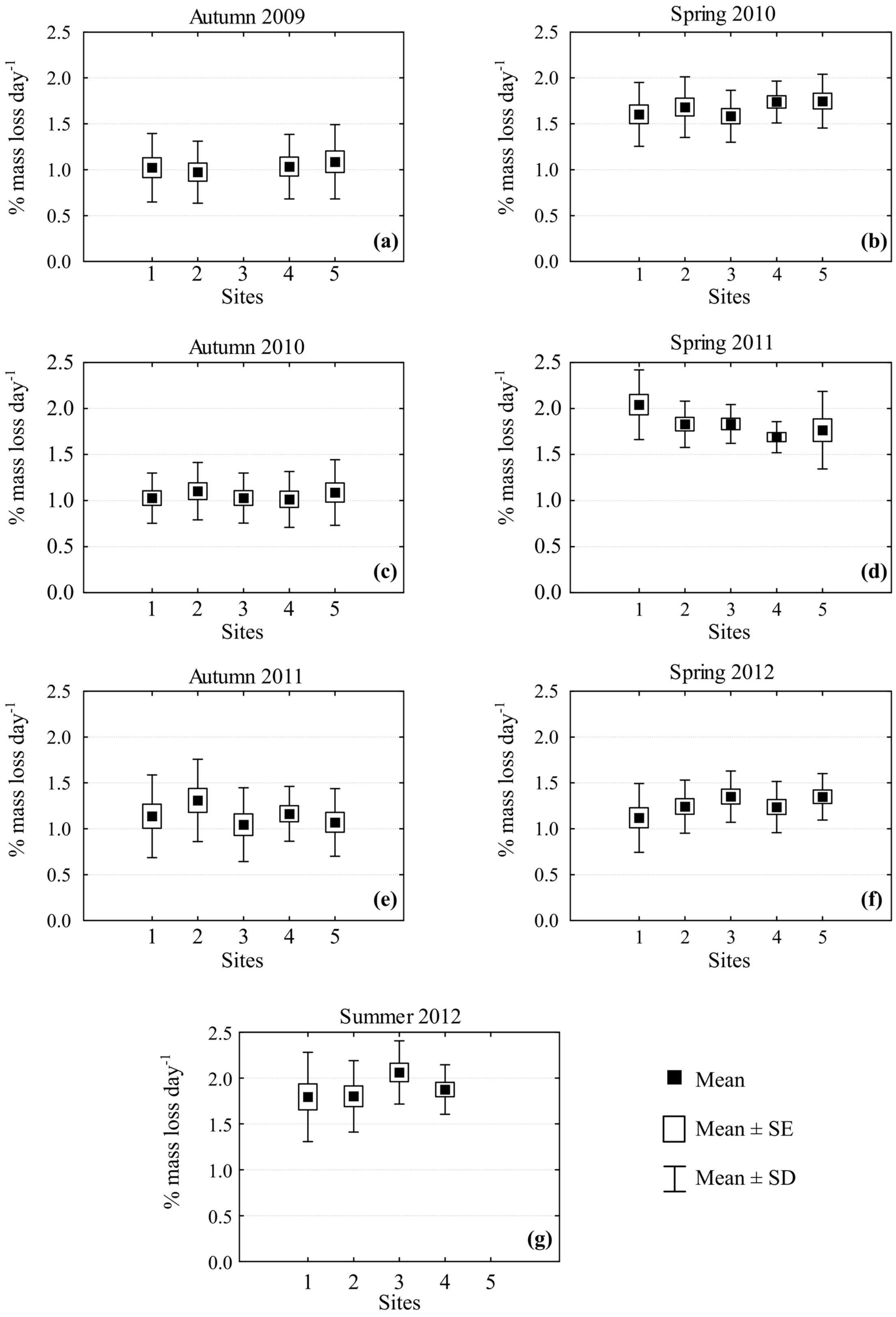

Fig. 3. Average mass loss per day (%) observed at each sampling site during every monitored season.

Table 2. Two-way ANOVA results for sites and seasonal periods of percentage loss per day values. Significance is highlighted in bold.

Year	Source	Sum of Squares	d.f.	Mean Squares	*F*	*p*-level
2009–2010	Season	2.644	1	2.644	18.820	**<0.001**
	Site	0.043	3	0.014	0.102	0.957
	Site × Season	0.016	3	0.005	0.038	0.990
2010–2011	Season	4.859	1	4.859	50.860	**<0.001**
	Site	0.140	4	0.035	0.366	0.830
	Site × Season	0.109	4	0.027	0.285	0.884
2011–2012	Season	5.498	2	2.749	18.015	**<0.001**
	Site	0.206	4	0.052	0.338	0.850
	Site × Season	0.472	8	0.059	0.387	0.919

are generally difficult because individual investigations are carried out in different conditions, such as environments, time periods, hydrologic regimes, depths and decomposition rates could depend on geographical context (Sangiorgio *et al.*, 2008a) and experimental conditions, such as different types of leaf bags mesh size (Bedford, 2005; Quintino *et al.*, 2009).

The results of the present work highlight the following points: (i) temporal patterns of reed decomposition were observed, resulting in a clearly defined cycle; (ii) a high percentage of the variability in reed decomposition within the studied pond is explained by rainfall and, to a lesser extent, by water temperature and conductivity.

Following Petersen and Cummins (1974) classification k-values are medium ($0.005 < k < 0.010$) during the autumnal seasons while becoming fast in the spring ($k > 0.010$), in agreement with the first observations reported by Ruzič *et al.* (2013) for the same studied pond (data included in the present study: Figure 2a); then k-values likely increase further in the summer (data obtained during the last year support this assessment) and, finally, every year summer drought events bring the system back to the same decay rate (in autumn $0.0066 < k < 0.0075$). No significant spatial variation was observed, therefore we can conclude that the leaf litter decomposition proceeds homogeneously over the whole pond.

4.2 Physical, chemical data and nutrients

Similar seasonal leaf litter breakdown patterns, with lower decay rates during the fall-winter season in opposition to higher values recorded in the spring-summer period, have been already observed (Menéndez *et al.*, 2003; Sangiorgio *et al.*, 2004; Pinna *et al.*, 2004). Temperature is one of the main driving factors in *P. australis* breakdown, positively influencing the decay rate values (Hanson *et al.*, 1984; Webster and Benfield, 1986; Bedford, 2005; Sangiorgio *et al.*, 2008a; Eid *et al.*, 2014): water temperature increases microbial processing during decomposition and leaves served as a major energy source for invertebrates in aquatic ecosystems (Dudgeon, 1982); the role of microfungi and invertebrates in decomposition is well-known (Gessner and Chauvet, 1994; Graça, 2001; Hieber and Gessner, 2002; Menéndez *et al.*, 2003).

In the pond under exam, water conductivity reflects salinity levels which could negatively influence *P. australis* processing (Mendelsshon *et al.*, 1999; Sangiorgio *et al.*, 2008a) and this effect could likely be mediated by the activity of microorganisms and benthic macroinvertebrates (Quintino *et al.*, 2009). Several studies showed that increasing salinity correspond to decreasing microorganism activity (van Bruggen and Semenov, 2000; Rietz and Haynes, 2003; Sardinha *et al.*, 2003; Muhammad *et al.*, 2006; Wichern *et al.*, 2006). The results described herein are in line with these findings: in fact, daily percentages of mass loss recorded during the spring of 2012 in correspondence of higher levels of conductivity (Table 4) did not differ from the autumnal values and are significantly lower than those observed in the previous years (Figure 3) and the gap between autumn and spring k-values was lower than the one observed for previous years (Figure 2c) with the spring value close to the limit between medium and fast decay rate classes reported by Petersen and Cummins (1974).

Annual variations in conductivity could be partially related with possible inputs of brackish water through the sluice side gate and largely related to the reduced rainfall observed in the 201–2012 period, which prevented new freshwater supply to the pond.

However, the drought/reflooding dynamic has probably played a major role in the variance of litter bag decomposition in temporary environments, as observed by Sangiorgio *et al.* (2006) in a riverine ecosystem subjected to drawdown. *P. australis* leaf litter decomposition in the studied pond is heavily influenced by the precipitation regime, which explains 84.9% of daily mass loss% for the three year dataset and 87.2% of variation for the last year dataset which includes nutrients (Table 5). In fact, the monitored system was supplied by an artesian well and mostly by rainwater. Therefore, it is reasonable that significant differences in seasonal or annual rainfall could likely lead to variations in the temporal pattern of the drought-reflooding cycle and consequently to a change of the breakdown rates. Water level and inundation dynamics are known to have an influence on reed decomposition rates in wetlands (Völlm and Tannenberger, 2014) and the effects of extended drawdown periods were discussed by Bedford (2005), though using a different experimental leaf bag design. Metabolic activity of microbial decomposers is positively correlated with water availability and in presence of dry conditions the activity of decomposers is restricted, with consequent slowdown in the decay processes: flooding and/or drying events could be disruptive for invertebrates and their contribution to breakdown will therefore be limited (Bedford, 2005). In addition, colonization following reflooding is generally slow

Table 3. Two-way ANOVA results for sites and seasons of different years of percentage loss per day value. Significance is highlighted in bold.

Season	Source	Sum of Squares	d.f.	Mean Squares	*F*	*p*-level
Autumn	Year	0.133	2	0.066	0.402	0.673
	Site	0.024	3	0.008	0.047	0.986
	Year × Site	0.105	6	0.017	0.106	0.995
Spring	Year	2.853	2	1.427	15.463	**<0.001**
	Site	0.093	4	0.023	0.252	0.906
	Year × Site	0.311	8	0.039	0.421	0.899

Table 4. Mean seasonal values and standard deviations of the physicochemical parameters (2009–2012) and nutrients (2011–2012) for the whole study area (Aut = autumn; Spr = spring; Sum = summer; Temp = water temperature; DO = dissolved oxygen; Cond = conductivity; *n* = 30 in all cases, except for (*), when *n* = 28).

		2009–2010		2009–2011		2011–2012		
		Aut	Spr	Aut	Spr	Aut	Spr	Sum(*)
Depth (cm)	Mean	30.2	36.4	37.9	24.3	33.8	27.8	12.6
	St. Dev.	9.2	11.1	11.4	14.6	10.4	10.7	11.5
Temp (°C)	Mean	11.7	19.0	13.3	19.3	10.0	20.3	22.3
	St. Dev.	3.4	4.2	1.9	2.5	5.2	2.6	1.6
pH	Mean	7.2	7.8	7.7	8.5	7.7	7.8	8.3
	St. Dev.	0.5	0.6	0.6	0.4	0.3	0.3	0.7
DO (mg dm^{-3})	Mean	4.5	4.0	5.1	2.3	8.5	2.4	3.2
	St. Dev.	2.3	1.9	1.0	0.6	2.7	1.3	1.3
Cond (mS cm^{-1})	Mean	2.6	2.7	2.1	3.0	3.7	4.9	6.5
	St. Dev.	0.9	0.6	0.2	1.4	1.0	1.3	2.9
Rainfall (mm)	Mean	185.3	62.4	172.2	51.8	96.9	104.6	31.5
	St. Dev.	3.8	26.8	65.3	21.1	103.5	39.1	28.2
NO_3^- (mg dm^{-3})	Mean	–	–	–	–	0.32	0.31	0.24
	St. Dev.					0.04	0.03	0.23
NO_2^- (mg dm^{-3})	Mean	–	–	–	–	0.10	0.14	0.26
	St. Dev.					0.03	0.07	0.31
NH_4^- (mg dm^{-3})	Mean	–	–	–	–	0.17	0.61	0.30
	St. Dev.					0.23	0.35	0.03
PO_4^{3-} (mg dm^{-3})	Mean	–	–	–	–	0.02	0.03	0.19
	St. Dev.					0.01	0.02	0.26

(Bedford and Powell, 2005). Analyses on the macrobenthic communities within the study area carried out by Bertoli *et al.* (2015), simultaneously with the present work, showed how abundances of macrobenthic invertebrates were always lower during the fall than in the spring seasons. Our results are likely to be in line with these findings, considering also that observed *k*-values and daily mass loss % measured in autumn (immediately after or during the completion of reflooding phase) do not show significant differences from year to year.

Breakdown decomposition rate could be also influenced by dissolved oxygen concentration (Chauvet, 1997), pH (Thompson and Bärlocher, 1989) or dissolved nutrients (Bärlocher and Corkum, 2003). In particular, concerning this last point, the contribution of the observed nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}) to the daily mass loss % variation was expected, due to extensive presence of migratory and non-migratory birds in the study area, especially during the fall-winter seasons (Bertoli *et al.*, 2015), and to the presence of horses and periodically cattle used for vegetation growth control. As part of a wetland, nutrient cycle is very complex within environments like the study area, and the changing redox potential largely influence the cycling of sensitive nutrients. The variability in concentration of nutrients and their availability could be influenced by the drying/re-flooding cycle. Moreover, management practices have to be considered, because water level control can be implemented through the opening of the sluice gate during summer, in order to favor mineralization of nutrients, avoiding hypoxia/anoxia (Street, 1982; Pizzul *et al.*, 2008; Bertoli *et al.*, 2015). As observed by Sangiorgio *et al.* (2008a), the daily reed mass loss could positively covary with reduced inorganic nitrogen compounds, while in the eutrophic system of the Danube Delta (Sangiorgio *et al.*, 2008b), *P. australis* decay rates covaried positively with total phosphorus. Nevertheless, our results do not highlight the influence of nutrient concentrations in the breakdown dynamics for the studied area, even though the contribution of NH_4^+ to the daily mass loss variability tended to be significant ($\beta = -0.188$; $p = 0.064$). It is reasonable to assume that, on this temporal scale, the influence of rainfall was stronger than the influence of other abiotic features and it represented the main driving factor for the dynamic of the system under exam, together with temperature. Considering the spatial variability in decomposition dynamics, climate characteristics (expressed as evapotranspiration) is the best predictor of *k*-values at the global scale, while chemistry parameters explained a high percentage of

Table 5. Stepwise multiple regression analyses between leaf mass loss per day (%) and physico-chemical features measured over the three-year period (2009–2012; nutrients are excluded) and over the last year (2011–2012; nutrient are included). Significance is highlighted in bold (Rain = Rainfall; Temp = water temperature; Cond = conductivity).

Physico-chemical dataset 2009–2012 (nutrient excluded)				Physico-chemical dataset 2011–2012 (nutrient included)			
Variable	r^2	F	p-level	Variable	r^2	F	p-level
Rain	0.849	174.87	<**0.001**	Rain	0.872	81.83	<**0.001**
Rain, Temp	0.877	6.90	**0.013**	Rain, Temp	0.919	6.47	**0.027**
Rain, Temp, Cond	0.911	10.97	**0.002**				

Table 6. Leaf litter breakdown rates (k, days^{-1}) for submerged litter of *Phragmites australis* observed in the study pond (Isonzo River Mouth Natural Reserve) compared with those reported in other studies.

Ecosystem	Length of the experiment (days)	Mesh size (mm)	Period	$-k$ (days^{-1})	Reference
Freshwater pond (North East Italy)	45	5	Autumn 2009–2011 Spring 2011–2012 Summer 2012	0.007–0.008 0.014–0.016 0.017	Present study
Transitional waters (Mediterranean area)	30–40	5	Spring 2005	0.014–0.029	Sangiorgio *et al.* (2008a)
River basins subjected to summer drought (Sardinia, Italy)	90	5	Spring–Summer 1998 Winter–Fall 1998-99	0.028 0.009	Pinna *et al.* (2004)
Freshwater lake (Southern Italy)	90	5	Autumn 1998 Spring 1999 Summer 1999	0.007 0.013 0.011	Sangiorgio *et al.* (2004)
Salt-marsh lake (Southern Italy)	90	5	Autumn 1998 Spring 1999 Summer 1999	0.006 0.009 0.010	
River delta (Danube)	45	5	Autumn 2005	0.009–0.0024	Sangiorgio *et al.* (2008b)
Coastal lake (Egypt)	150	5	Apr–Sept 2003	0.0117	Eid *et al.* (2014)
Reedbed wetlands (North East England)	599	5	Jan 1995–Aug 1997	0.004	Bedford (2005)
Natural fen (Northern Germany)	248–257	0.3	Sept 2010–May 2011	0.003	Völlm and Tannenberger (2014)
Monomictic lake (Netherlands)	38	5	Summer 1999 Winter 1999	0.055 0.012	Van Dokkum *et al.* (2002)

variance in k-values within a particular climatic region (Aerts, 1997; Sangiorgio *et al.*, 2008a). Similarly, it is possible that climate factors, such as precipitation regime and temperature variations, are responsible for the pattern of leaf litter decomposition on large timescale.

5 Conclusions

The above study reports the seasonal data series from multiple years regarding breakdown processes of *Phragmites australis* within a temporary environment. This represents the principal value of this body of work, which results are consistent with the following conclusions: (i) *P. australis* reed leaf breakdown in the monitored large freshwater pond of Natural Regional Reserve of the Isonzo River Mouth shows clear seasonal patterns closely linked with the drought-reflooding cycle; (ii) these patterns repeat every year with some variations, depending chiefly on local climate factors (rainfall, temperature) and on conductivity variations due to structural features of the system.

The investigated pond provided an interesting scenario where reed decomposition processes could be more accurately studied and where the resilience of the system could be analyzed.

Finally, the results reported in the present work represent important information for the planning of management actions: regulation of the water level could be carried out to favor or slow down drought in managed ponds with direct consequences both on biotic and abiotic compounds, and indirect consequences on leaf litter decomposition rates in systems such as those monitored in the present study.

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