Seasonal patterns of *Phragmites australis* breakdown in a karstic freshwater system (Doberdò Lake, Northeast Italy) in relation to water level fluctuations, environmental features, and macrobenthic invertebrate communities

Marco Bertoli · Filippo Franz · Paolo Pastorino · Marino Prearo · Elisabetta Pizzul

Abstract We investigated the main seasonal drivers of *Phragmites australis* decomposition dynamics in a freshwater karst lake subject to water level fluctuations. Physical and chemical parameters were monitored every two weeks for one year and *P. australis* decomposition rates were measured using the leaf bag technique during two seasons (autumn and spring). Decomposition dynamics were analyzed for the contribution of macrobenthic invertebrate colonizers and the influence of intermittent water level variations. Seasonal trends for changes in physical and chemical parameters were observed in relation to the underground origin of the water supply, which also affected the macrobenthic invertebrate communities. Decomposition rates k differed significantly between seasons ($k = 0.0131$ in autumn and $k = 0.0115$ in spring) but remained within a narrow range, reflecting the trend for changes in water temperature. Our findings show that the variability in decomposition rates was largely due to water level fluctuations (46.7% relative importance), rainfall (12.4% relative importance) and macrobenthic invertebrate communities dominated by shredders (23.9% relative importance; 40.9–93.7% of the collected samples). The influence of physical and chemical changes, particularly water temperature, was less important (relative importance 1.82%).

Keywords Decomposition rates; Karstic lake · Leaf bag; Shredders

M. Bertoli · F. Franz · P. Pastorino · E. Pizzul (✉)
Department of Life Science, University of Trieste, Via Giorgieri10, 34127 Trieste, Italy
e-mail: pizzul@units.it

P. Pastorino · M. Prearo
Experimental Zooprofilactic Institute of Piedmont, Liguria and Valle d'Aosta, Via Bologna 148, 10154 Torino, Italy

Introduction

Karst systems are distinct freshwater environments that form an interface between underground and surface waters (Smith et al., 2003; Tanaka et al., 2006). Wetlands and/or lakes are uncommon in karst areas. They occur at the discharge of the groundwater continuum near springs and resurgent systems that do not usually form by the accumulation of surface runoff in areas of recharge where precipitation rapidly sinks into the ground (Euliss et al., 2004; Pipan & Culver, 2019). Water movements to and away from the surface supply "disappearing" lakes and ponds through connections between surface and underground aquifers and result in temporary/intermittent freshwater ecosystems (Pipan & Culver, 2019). Water level fluctuations provide for the formation of a variety of habitats that support diverse communities (Dolinar

et al., 2011) and constitute a driving force and a limiting factor for ecosystem processes (Gaberščik et al., 2003) by maintaining these environments in an early, relatively productive stage of development (Dolinar et al., 2010).

Water level variations, together with the intensity, timing, and extent of flood/drought, affect sedimentation and erosion of alluvial sediments, primary production, vegetation, animal life cycles (i.e., spawning of fish and nesting of birds), mineralization, turnover rate, interaction among species, and decomposition dynamics (Boulton & Brock, 1999; Gaberščik et al., 2003; Dinka et al., 2008; Dolinar et al., 2016). Particularly interesting among these factors is organic matter decomposition: plant breakdown is a fundamental process in the metabolism of all wetlands (Webster et al., 1995; Wallace et al., 1997; Eid et al., 2014; Bertoli et al., 2016) as it provides for the maintenance of nutrient status and constitutes a basis for defining wetland properties (Fennessy et al., 2008; Dolinar et al., 2016). In this setting, reedbed detritus decomposition has been widely studied. The common reed, *Phragmites australis* (Cav.) Trin. ex Steud, is one of the most widespread freshwater species and it often forms extensive stands (Gessner, 2000) in detritus-based systems (Cowie et al., 1992; Komínková et al., 2000). These systems can be considered among of the most productive (Bedford, 2005).

Moreover, plant breakdown rates in aquatic ecosystems are affected by internal factors, such as leaf characteristics (Kok et al., 1990; Canhoto & Graça, 1996), and by numerous external environmental factors including: water temperature and salinity (Carpenter & Adams, 1979; Reice & Herbst, 1982; Sangiorgio et al., 2008a; Quintino et al., 2009); pH (Thompson & Bärlocher, 1989); nutrient concentrations (Webster & Benfield, 1986; Sangiorgio et al., 2008b; Woodward et al., 2012; Ferreira et al., 2014; 2015); regional characteristics (Sangiorgio et al., 2008a) such as climate (Murphy et al., 1998) and solar radiation (Denward & Tranvik, 1998). Among biotic factors, microbial activity contributes to litter decomposition through bacterial (Kuehn et al., 2000; Jackson & Vallaire, 2007) and fungal processes (Gessner & Chauvet, 1994; Findlay et al., 2002). Also, macrobenthic invertebrates can significantly affect litter breakdown (van Dokkum et al., 2002; Bedford, 2005; Karádi-Kovács et al., 2015), as shredders generally accelerate litter decomposition

(Hieber & Gessner, 2002; Cornut et al., 2010; Raposeiro et al., 2016).

Litter breakdown has been widely studied in lotic environments (Diez et al., 2002; Menéndez et al., 2003; Pinna et al., 2004; Sangiorgio et al., 2006), lakes (Hietz, 1992; Gupta et al., 1996; Karádi-Kovács et al., 2015; Raposeiro et al., 2016), and transitional aquatic ecosystems such as coastal lagoons and river mouths (Rossi & Costantini, 2000; Sangiorgio et al., 2004; Bayo et al., 2005; Sangiorgio et al., 2008a, b). However, increasing attention has also been directed at decomposition dynamics in temporary freshwater bodies with seasonally fluctuating water levels (Bedford, 2005; Völlm & Tannenberger, 2014; Gingerich et al., 2015; Bertoli et al., 2016). Studies on litter breakdown in karst systems have been reported: several investigated lotic environments (Tanaka et al., 2006; Martínez et al., 2015) and the relationship between calcite deposition and litter breakdown in travertine systems (Casas & Gessner, 1999; Carter & Marks, 2007; Belančič et al., 2009; Miliša et al., 2010). Kelley & Jack (2002) carried out the first study within a karst lake in Kentucky (USA) and Dolinar et al. (2016) investigated the influence of water level fluctuations on the primary productivity litter decomposition of *Phragmites australis* and fungal root colonization in an intermittent karst wetland (Cerknica Lake, Slovenia).

In this context it was deemed of interest to investigate plant detritus decomposition dynamics in a karst lentic system subject to intermittent hydrometric level variations and to examine seasonal patterns in relation to abiotic (physical and chemical characteristics) and biotic features (macrobenthic invertebrate communities involved in decomposition processes), both of which are affected by water level fluctuations. The study area was Doberdò Lake (northeast Italy). Of karstic origin, the lake is a typical surface formation in the Italian sector of the Classic Karst (Cucchi et al., 2010) and presents one of the most notable examples of karst hydrology (Altobelli et al., 2016). The lake is located in the Regional Natural Reserve of Doberdò and Pietrarossa Lakes, in a Special Area of Conservation (SAC IT3340006) and in a Special Protection Area (SPA IT3341002).

The main aims of the present study were: (i) to characterize the seasonal trends of physical and chemical parameters and macrobenthic invertebrate communities in relation to water level fluctuations; (ii)

to analyze the seasonal variations in breakdown rates using leaves of *Phragmites australis*; and (iii) to infer the effect of the main extrinsic (environmental) physical and chemical features and the macrobenthic invertebrate communities on plant detritus decomposition. We assumed that water temperature would contribute less to the breakdown rates than water level fluctuations and macrobenthic invertebrate community structures. In addition, we assumed that by affecting other system features the underground origin of the water supply would have indirect effects on the decomposition dynamics in the environment.

Materials and methods

Study area

The present study was carried out at Doberdò Lake (Fig. 1) (Municipality of Doberdò, Friuli Venezia Giulia, northeast Italy). The lake covers the bottom of a base-level polje and is part of a spring system extending approximately 20 km^2 in surface area. The system is located in a low elevation area and contains numerous springs and several temporary lakes covering base-level poljes (Cucchi et al., 2010; Cucchi & Finocchiaro, 2017). Water is supplied by springs in the western and northwestern portion of the Doberdò polje. Some estavelles discharge water as springs or shallow-holes, while true shallow-holes are found at the eastern end of the lake. Inlets and/or outlets are absent. Annual mean and maximum water levels are 3.74 m and 8 m above sea level, respectively. Wide variations can lead to significant changes in the extension of the wet surface area, from 200 m^2 during dry periods to 400.000 m^2 during wet periods (Cucchi et al., 1999; Samez et al., 2005). Throughout the low-level phases, a small stream flows across the bottom of the polje from northwest to southeast, where shallow-holes drain the basin. Water supply depends mainly on groundwater inflow from the Soča River and the Vipava Stream and on rainfall; despite proximity to the sea, tidal influence has never been documented (Samez et al., 2005). Doctor (2008) estimated that the Soča River supplies 75% of the flow to the smaller springs and water supply wells of the resurgence karst zone. More recently, Calligaris et al. (2018) described the Soča River as one of the main sources of supply for
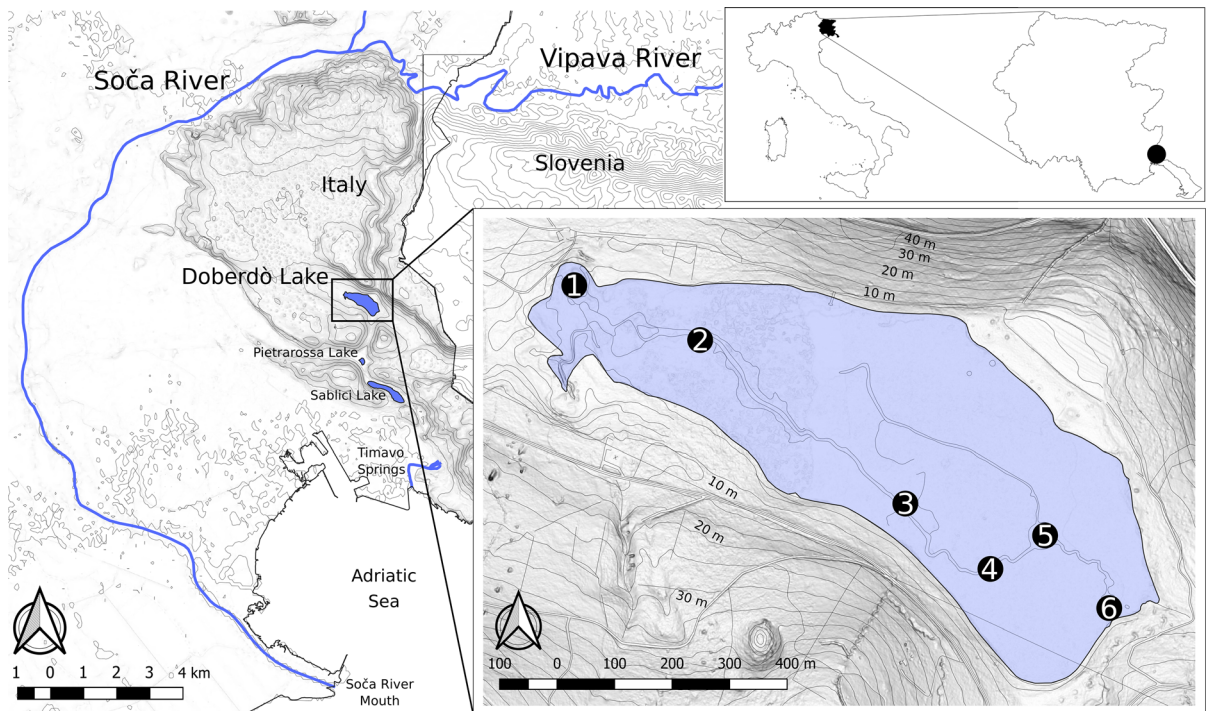

Fig. 1 Study area and sampling sites at Doberdò Lake (source: www.regione.fvg.it, modified)

the Doberdò waters. Small variations in water level during the dry period are linked to hydrometric fluctuations of the Soča River caused by its regulation in Slovenia (Samez et al., 2005; Zini et al., 2014).

Despite its small size, the lake is noted for its variety of features, such as exposition, vegetation shading, water presence, and permanence within the lake zones. Collectively, these features create different and well–diversified habitats where a rich vegetation assemblage thrives in diversified environmental and morphological conditions: *Nymphaea alba* Linnaeus, *Stuckenia pectinata* (L.) Börner, *Ranunculus trichophyllus* Chaix, and *Ceratophyllum demersum* Linnaeus in the wet area, while *Carex vesicaria* Linnaeus, *Carex elata* All., and especially *Phragmites australis* dominate the riparian zones; *Agrostis stolonifera* Linnaeus, *Rorippa Sylvestris* (L.) Besser, *Ranunculus repens* Linnaeus, and *Potentilla reptans* Linnaeus can also be found, while the external portion is characterized by *Salix cinerea* Linnaeus, *Salix alba* Linnaeus, *Ulmus minor* Mill., and *Quercus pubescens* Willd. (Liccari 2015). The lake is subject to swamping, though management activities, such as reedbed cut, could slow this phenomenon (Altobelli et al., 2016). During the present study, we observed that the wet portion of the lake hosts a rich fish community composed of *Scardinius hesperidicus* Bonaparte, 1845, *Squalius squalus* Bonaparte, 1837, *Leucos aula* Bonaparte, 1841, *Esox cisalpinus* Bianco & Delmastro, 2011, *Cobitis bilineata* Canestrini, 1865, *Padogobius bonelli* Bonaparte, 1846, and *Anguilla Anguilla* Linnaeus, 1758.

Six sampling sites (Fig. 1, Table 1) were selected for their environmental features (depth and aquatic vegetation composition on the lakeshore and the bottom) and on the basis of water permanence in the central portion of the lake, which is not subject to total drought even during low-level phases. For this study, water level fluctuations were considered only in terms of water depth. Site 1 was at the main spring, where the influence of groundwater and water depth are highest. Site 2 was a shallow water area along the course of the small central stream, where *Carex* sp. and *Phragmites australis* grow along the banks. Site 3 was located within the stream near a small shallow-hole where *Salix cinerea* affords some shade. Site 4 was located in the stream near the reedbed. Site 5 was at the center of a large pool characterized by a deposit area with soft substrates and reduced presence of aquatic macrophytes. Finally, site 6 was at the main shallow-hole where floating hydrophytes were abundant. Table 1 presents the data on vegetation cover (%) at maximum development (July) at each sampling site.

Physical and chemical parameters

The main physical and chemical water parameters were monitored every two weeks at each sampling site from October 2016 to October 2017. Water conductivity (mS cm^{-1}), pH, temperature (°C), and dissolved oxygen (mg l^{-1}) were recorded using field meters (HI 9033 conductivity meter; HI 9125 pH/ORP meter; HI 9147 dissolved oxygen meter; Hanna Instruments Inc., Woonsocket, Rhode Island, USA). Parameters were measured at approximately mid-depth in the water column. Water depth was measured with a graduated rope. In order to determine the influence of the underground water supply, Soča River water level data were retrieved from the Regional Meteorological Observatory System of Friuli Venezia Giulia (http://omnia.meteo.fvg.it). The data were recorded about 72 hours before monitoring the physical and chemical features of the water at the sampling sites. This was done in line with previous studies (Cucchi et al., 1999; Samez et al., 2005) that reported a 72-hour delay for the influence of Soča flood events at Doberdò Lake. Rainfall data for the study area were retrieved from the same data source.

Water samples were collected in sterile containers from the water column (from approximately mid-depth to the bottom), taking care not to include sediment particles. Samples were frozen and brought to the laboratory where they were analyzed on a benchtop multi-parameter spectrophotometer (HI 83200-02, Hanna Instruments Inc., Woonsocket,

Table 1 Geographical coordinates and total vegetation cover (%) at each sampling site monitored at Doberdò Lake

Site	Latitude	Longitude	Vegetation cover (%)
1	45°49′59.05″N	13°33′15.05″E	90
2	45°49′55.84″N	13°33′27.69″E	70
3	45°49′47.87″N	13°33′40.79″E	95
4	45°49′44.92″N	13°33′48.14″E	100
5	45°49′46.72″N	13°33′52.40″E	10
6	45°49′43.03″N	13°33′57.92″E	80

Rhode Island, USA) according to the manufacturer's instructions. The concentration of nitrates (NO_3^-, mg l^{-1}) was calculated using an adaptation of the cadmium reduction method and absorption was measured at 525 nm; the ammonia content (NH_4^+, mg l^{-1}) was calculated by adaptation of the Nessler method and absorption was measured at 420 nm (ASTM, 2015); phosphate concentration (PO_4^{3-}, mg l^{-1}) was calculated by adjusting the ascorbic acid method (APHA, 1998) and measuring the absorption at 610 nm. Three replicates of measurement were done for each parameter at each sampling site.

Leaf bag experiment

The leaf bag technique (Petersen & Cummins, 1974) was used to study organic matter decomposition processes. Leaf bags were prepared by adapting the protocol reported by Basset et al. (2006) and used in previous studies in other freshwater environments (Ruzič et al., 2013; Bertoli et al., 2015; Bertoli et al., 2016; Bertoli et al., 2018). Leaves of *Phragmites australis* were chosen as it is one of the most abundant plant species in the Doberdò polje and dominates the riparian vegetation. Senescent leaves were collected at the beginning of autumn 2016, removing them from the shoots at least 30 cm above the water surface, avoiding apical leaves and any that had come in contact with water. Only intact leaves were collected, rejecting any with evident signs of decomposition from the top layer. The leaves were air-dried after collection and then stored in the dark in a dry laboratory room. Before use, the leaves were cut into 10-cm-long fragments (excluding basal and apical parts) and oven-dried to constant weight (60° C for 72 h). Lots (3.0000 ± 0.0001 g dry weight) were placed inside cotton net bags of 5 mm mesh. This mesh size allows colonization of macrobenthic invertebrates and limits leaf material loss (Basset et al., 2006). The oven-dried leaves served to achieve initial conditions as uniform as possible (Bärlocher, 1997), to obtain standardized samples, and to facilitate comparison. Sampling was performed during autumn (late October-early December 2016) and spring (late April-early June 2017): 12 leaf bags were submerged at each sampling site, by placing them gently near the bottom and tethering them with strings to stones to prevent loss. The leaf bags were then collected after 15, 30, and 45 days of submersion. At each sampling time point, four leaf bags were retrieved from each sampling site, placed in separate polyethylene boxes containing lake water, and rapidly brought to the laboratory, where the leaves were gently washed to remove sediments and macroinvertebrate colonizers. The invertebrates were stored in 70% ethanol solution until further analyses. The leaves from each bag were oven-dried at 60 °C for 72 h and weighed (x ± 0.0001 g dry weight). The remaining leaf mass at t = 15, 30, and 45 days of submersion is expressed in percentage, whereby the initial weight at t = 0 days (3.0000 ± 0.0001 g dry weight) is 100%. Macrobenthic invertebrate colonizers were identified to the genus level (if possible). Finally, each taxon was assigned to a Functional Feeding Guild (FFG) according to Merritt & Cummins (2006).

Statistical analysis

Two-way ANOVA and honestly significant difference (HSD) Tukey post-hoc test were used to check spatial and temporal differences in physical and chemical parameters by the two factors site and season. Data were checked for conformity to normality assumptions with a Shapiro–Wilks test and transformed where necessary ($\log(x + 1)$). Conformity to assumptions of variance homogeneity was checked using the Bartlett test. Principal coordinate analysis (PCA) was performed on a standardized data matrix to examine patterns of seasonal changes in physical and chemical parameters. Pearson product-moment correlation coefficient (r) was used to test correlations between variables.

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

$$M_t = M_0 e^{-kt}$$

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 remaining mass were natural log transformed and ANCOVA was used to compare seasonal values of k as slopes of linear regression equations using time as covariate (Zar, 1984; Bärlocher, 2005).

Two-way PERMANOVA (Anderson, 2001; McArdle & Anderson, 2001) was performed to test significant differences in taxa composition between macrobenthic invertebrate communities by site and season as factors. Pairwise comparisons were performed to test significant differences between sampling sites. Data were transformed ($\log(x + 1)$) prior to analysis to reduce the influence of very abundant taxa (Clarke & Gorley, 2006). The subsequent resemblance matrix was obtained using the Bray–Curtis measure. SIMPER analysis (Clarke, 1993) was applied to identify the taxa that contributed most to the significant differences highlighted by PERMANOVA.

Finally, the relevance of abiotic and biotic features as potential sources of variation in leaf decay rates was investigated using forward multiple stepwise regression analysis with organic matter decomposition rate (k) as the dependent variable. To avoid multicollinearity, a non-redundant subset of variables was used as predictors from the initial list of parameters when evaluating the results of PCA and correlation analyses. If two or more variables were strongly correlated (threshold value $r > |0.4|$ and $p < 0.001$), they were excluded from the analyses. In addition, the number of the most frequent FFG per leaf bag was defined as a biotic variable. Biotic and abiotic data were log transformed ($\log(x + 1)$) to fulfill assumptions of normality, which was checked with the same test as mentioned above. The relative importance of significant predictors to the variability explained by multiple stepwise regression was checked using the LMG method (Grömping, 2006) and quantified as a percentage for significant regressors. All statistical analyses were performed using RStudio version 3.5.3. Figures are produced with RStudio and processed with Inkscape version 0.92.

Results

Physical and chemical parameters

Two-way ANOVA (Table 2) revealed significant seasonal effects for all parameters except for water depth, which showed only spatial variation between sampling sites. The *P*-value for dissolved oxygen concentration approached significance for the site factor (Table 2), with a certain degree of variability.

No spatial variation in the other parameters was observed and interaction between the two factors season and site was not significant for physical and chemical parameters.

Depth trends were similar for the seasons (Fig. 2a). Values were significantly higher at sites 1 and 6 (seasonal mean from 5.1 ± 0.5 m to 5.8 ± 0.6 m at site 1 and from 3.5 ± 0.9 m to 4.3 ± 1.3 m at site 6). Lower depths were recorded at sites 2, 3, and 4, for which the mean was $< 1.8 \pm 1.7$ m. Depth at site 5 ranged from 1.8 ± 1.0 m to 2.7 ± 1.3 m. All six sampling sites were always found submerged during the study period, despite substantial variations in water level, with depth extremely reduced at some sampling sites (especially site 2).

The mean temperature ranged between $9.2 \pm 0.7°C$ and $15.6 \pm 3.4°C$ and was significantly higher in summer than the other seasons and lower in winter (Fig. 2b). No significant differences between autumn and spring were detected. The mean dissolved oxygen concentration generally ranged between 6.5 ± 0.7 mg l^{-1} and 10.0 ± 1.4 mg l^{-1} and was significantly lower in autumn than in the other seasons (Fig. 2c). Oxygen levels were increased during winter and remained fairly constant throughout the other seasons. An exceptional minimum (2.9 mg l^{-1}) was recorded at site 6 (main shallow-hole) in July 2017: the temperature reached 21.7°C when the site became isolated from the main water body due to exceptionally low water levels. The pH was always significantly lower in autumn and summer than in winter and spring (Fig. 2d), while conductivity was significantly higher in autumn than in summer and significantly higher in winter than in spring and summer (Fig. 2e). NH_4^+ concentrations were lower in spring than during other seasons (Fig. 2f), while PO_4^{3-} levels were slightly albeit not significantly higher in autumn than in winter and summer and were significantly higher in autumn than in spring (Fig. 2g). Finally, there were no significant differences in seasonal NO_3 concentrations, except in summer when they were significantly lower than in spring (Fig. 2h).

PCA showed that the first two axes explained 54.10% of observed variability (Fig. 3) and the first three axes explained 66.63%. Water depth, conductivity, rainfall, and hydrometric level of the Soča River were negatively correlated with the first axis (PCA 1), while some data groupings (autumn, spring, and winter) were located at the left side of the plot

Parameter	Figure code	Source	d.f.	SS	MS	F	p-level
Depth	Depth	Season	3	0.134	0.045	1.924	0.131
		Site	5	4.459	0.892	38.543	**<0.001**
		Season × Site	15	0.044	0.003	0.127	1.000
Temperature	Temp	Season	3	0.279	0.093	57.810	**<0.001**
		Site	5	0.003	0.001	0.370	0.868
		Season × Site	15	0.030	0.002	1.229	0.264
Dissolved oxygen	DO	Season	3	0.159	0.053	15.730	**<0.001**
		Site	5	0.058	0.012	3.435	0.070
		Season × Site	15	0.071	0.005	1.400	0.163
pH	pH	Season	3	0.041	0.014	108.329	**<0.001**
		Site	5	0.001	0.000	0.881	0.497
		Season × Site	15	0.001	0.000	0.438	0.963
Conductivity	Cond	Season	3	0.034	0.011	10.237	**<0.001**
		Site	5	0.001	0.000	0.105	0.991
		Season × Site	15	0.002	0.000	0.147	1.000
NH_4^+	NH_4^+	Season	3	0.062	0.021	42.480	**<0.001**
		Site	5	0.001	0.000	0.566	0.725
		Season × Site	15	0.002	0.000	0.239	0.998
PO_4^{3-}	PO_4^{3-}	Season	3	0.014	0.005	14.177	**<0.001**
		Site	5	0.001	0.000	0.623	0.682
		Season × Site	15	0.002	0.000	0.372	0.983
NO_3^-	NO_3^-	Season	3	0.861	0.287	16.810	**<0.001**
		Site	5	0.080	0.016	0.941	0.458
		Season × Site	15	0.232	0.015	0.907	0.559

Table 2 Two-way ANOVA of chemical and physical parameters by site and season

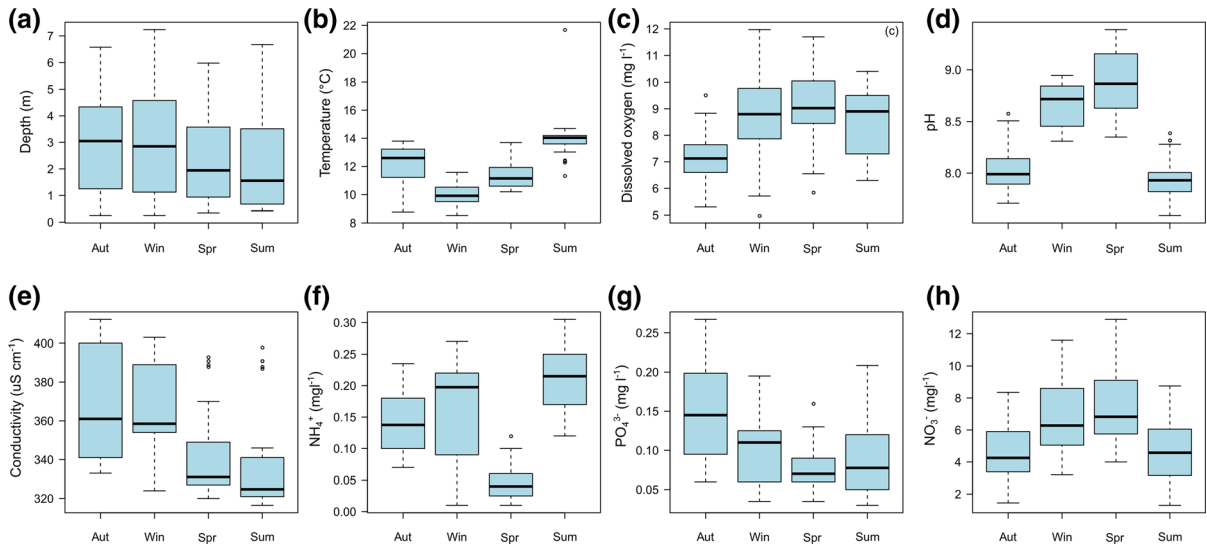Significance is highlighted in bold ($n = 120$ for all comparisons)

Fig. 2 Seasonal trends of physical and chemical parameters monitored during the study period in Doberdò Lake ($n = 30$ for each season)
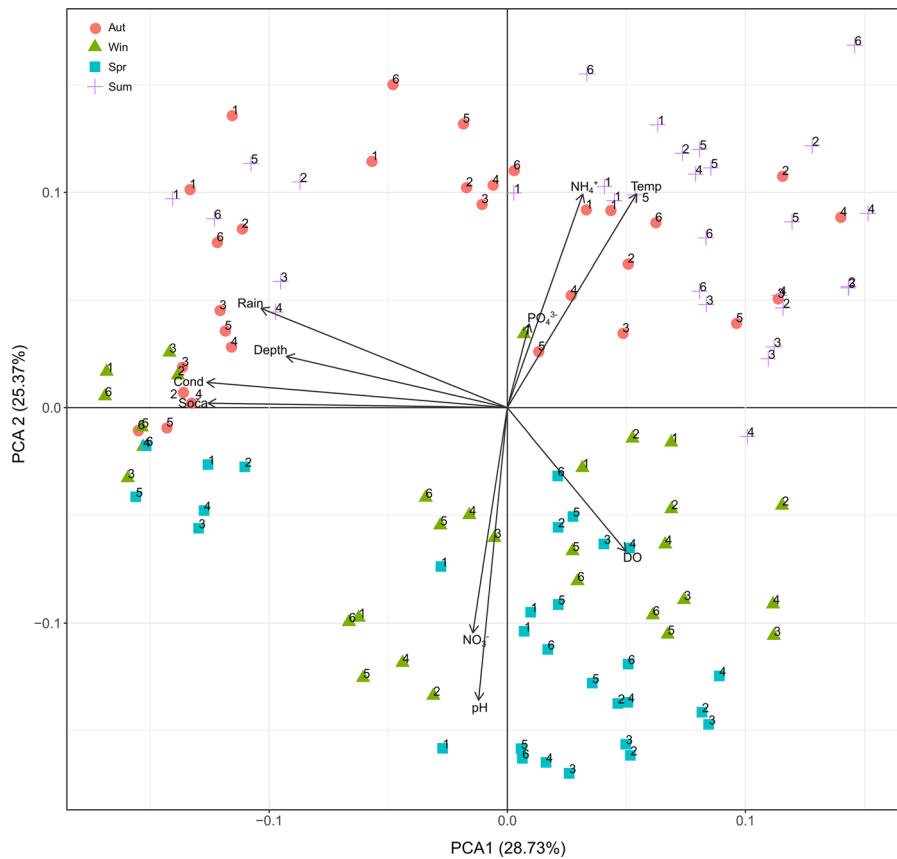
Fig. 3 Principal component analysis (PCA) applied to physical and chemical parameters (*Temp* water temperature; *Cond* conductivity; *Soča* Soča river hydrometric level; *DO* dissolved oxygen concentration; *Rain* total rainfall) ($n = 30$ for each season)

regardless of seasonality, close to the axis; the data were related to greater water depth, conductivity, rainfall, and hydrometric level of the Soča River. These findings suggest that the first axis (PCA 1)

primarily shows the influence of flooding events and water level variations in Doberdò Lake. Water temperature and NH_4^+ concentrations were positively correlated with the second axis (PCA 2), while pH,

Table 3 Significant correlations (Pearson coefficient in bold, $p < 0.001$) between parameters and PCA axes (first three axes are shown)

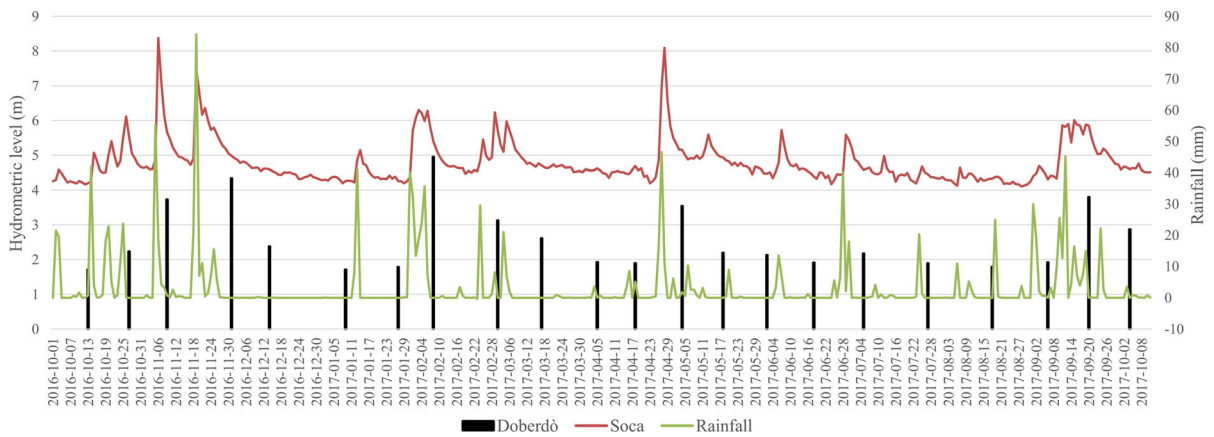| Parameter	Figure code	PCA 1	PCA 2	PCA 3
Depth	Depth	**− 0.654**	0.158	− 0.020
Temperature	Temp	0.383	**0.658**	0.477
Dissolved oxygen	DO	0.351	**− 0.441**	0.369
pH	pH	− 0.085	**− 0.899**	− 0.056
Conductivity	Cond	**− 0.889**	0.078	− 0.139
NH_4^+	NH_4^+	0.223	**0.657**	− 0.212
$PO_4 3^-$	PO_4^{3-}	0.065	0.258	**− 0.835**
NO_3^-	NO_3^-	− 0.102	**− 0.692**	− 0.110
Rainfall	Rain	**− 0.729**	0.306	0.290
Hydrometric level of the Soča River	Soča	**− 0.885**	0.014	0.171
Explained variability (%)		28.73	25.37	12.53

Fig. 4 Doberdò Lake mean depth (m) measured during the study period (black bars) compared to total rainfall (mm) and hydrometric level of the Soča River (m). Soča River levels recorded to 72 hours before measurement of the water depth of Doberdò Lake

dissolved oxygen, and NO_3^- were negatively correlated, as was dissolved oxygen concentration (Fig. 3, Table 3). Data groupings on the plot suggest that PCA 2 represent a seasonal gradient since the autumn and summer data are located in the upper plot section, while the winter and spring samples are located in the lower section. The PO_4^{3-} concentration appeared to be related to the third axis (PCA 3) (Table 3).

Pearson's coefficient showed a strong and significant positive correlation between conductivity, rainfall, and the Soča River level ($r > 0.625$, $n = 120$, $p < 0.001$ for all comparisons). Depth was positively correlated with conductivity and Soča River level ($r > 0.433$, $n = 120$, $p < 0.001$ for all comparisons), while the correlation with rainfall was weaker albeit significant ($r = 0.354$, $n = 120$, $p < 0.001$). Figure 4 illustrates the relationship between Doberdò Lake depth, Soča River level, and rainfall. Water temperature was positively correlated with NH_4^+ concentrations ($r = 0.401$, $n = 120$, $p < 0.001$) and negatively with NO_3^- and pH levels ($r < -0.472$, $n = 120$, $p < 0.001$ for all comparisons). Dissolved oxygen and PO_4^{3-} concentration showed weaker correlation with other variables ($r < |0.035|$ for all comparisons): dissolved oxygen was negatively correlated with water depth. Therefore, water temperature (Temp), Soča River level (Soča), rainfall (Rain), dissolved oxygen (DO), and PO_4^{3-} concentration were entered in stepwise regression analysis. Rainfall (Rain) was entered, despite the positive correlation with the other variables (Soča River level, conductivity and depth), due to the

contributing effect of local runoff. pH was also entered because of the seasonal trends separating the autumn and summer from the winter and spring samples.

Macrobenthic invertebrates

A total of 13306 macrobenthic invertebrate specimens were collected, 8855 during autumn and 4462 during spring. The number of taxa was 25 in autumn and 21 in spring. Crustaceans (genera *Gammarus* and *Asellus*) comprised the most abundant taxa during both seasons, ranging from 25.9 to 93.6% of the community, except at site 5, where the range was 2.0–25.9% (Table 4). In autumn, Hirudinea (genera *Erpobdella* and *Glossiphonia*) were the second most abundant taxa in frequency (5.9–56.5%) but were decreased in spring (1.9–11.5%). Hexapoda comprised mainly the Diptera family Ceratopogonidae and the Coleoptera genus *Haliplus* in autumn and the Diptera families Chironomidae and Ceratopogonidae in spring. Oligochaeta were generally absent from the leaf bags or were less frequent, except at site 5 where a higher number was generally observed in the samples (Table 4). PERMANOVA disclosed significant effects for the two factors season and site, while the interaction between these factors was not significant (Table 5). However, pairwise comparisons showed that only site 5 differed from the others in autumn and spring ($p < 0.05\%$ for both comparisons). The SIMPER test showed that the percentage contribution to the dissimilarity for the two factors season and site was

Table 4 Percentage frequencies of macrobenthic invertebrate taxa in the leaf bags during the sampling seasons at Doberdò Lake

Class/Order	Taxon	FFG	Autumn						Spring					
			1	2	3	4	5	6	1	2	3	4	5	6
Bivalvia	*Sphaerium*	FC		0.42	0.60	6.79	0.68	0.25	0.00	1.76	8.23	21.87	0.56	0.58
Gastropoda	*Bithynia*	SC	0.08	0.04		0.17	0.00	9.06	0.79					
	Lymnaea	SC	0.08											
	Physa	SC			0.03	0.66	3.40	0.25						
	Planorbis	SC		0.11	0.03	0.99	2.04	0.50				0.10		
	Valvata	SC	0.08		0.03				0.23		0.10			0.19
Malacostraca	*Asellus*	SH	78.05	63.38	70.11	26.49	2.04	45.04	17.25	32.80	38.92	53.94	19.22	68.46
	Gammarus	SH	11.36	21.76	8.37	8.11		14.39	76.32	20.35	13.21	6.71	6.69	2.88
Hydracarina		P					11.56			0.29			0.56	
Hirudinea	*Erpobdella*	P	4.92	6.38	15.75	37.25	45.58	17.25	1.69	2.05	1.93	2.53	4.46	9.62
	Glossiphonia	P	1.02	0.11	0.09	1.16	10.88	5.46	1.47	0.44		0.78	2.79	1.92
Oligochaeta	*E. tetraedra*	GC					0.68			0.29		0.10		
	Tubificinae	GC			0.23	1.32	2.72				6.20	2.62		0.58
	Limnodrilus	GC	0.51	0.08	0.80	1.99	7.48			1.61	8.54	3.98	56.82	1.92
	Psammorictides	GC				1.16						2.14		
Tricladida	*Polycelis*	P	0.51	0.65	0.14	1.82	0.68	0.12	0.34	0.15	0.20			0.58
	Dugesia	P	0.76	0.61	1.43	0.99		1.99	0.34	0.15	2.95	0.39		0.77
Hexapoda														
Coleoptera	*Elmis*	GC				0.17								
	Haliplus	SH	0.42	0.95	0.20	5.63	9.52	1.12	0.11	0.44	0.10	0.19	1.11	3.65
Diptera	Ceratopogonidae	P	2.12	4.96	2.06	2.65	2.72	3.23	0.11	4.98	1.73	2.14	7.52	8.46
	Chironomidae	GC		0.08					0.68	34.70	16.57	0.97	0.28	
Ephemeroptera	*Baetis*	GC						0.25		0.15				
Megaloptera	*Sialis*	P	0.08	0.42	0.06	1.32		0.00	0.11	0.15	0.10			
Odonata	*Coenagrion*	P						1.12						
Trichoptera	Leptoceridae	SC		0.04	0.09	1.32			0.56	1.61	1.22	1.55		0.38
Total number of observed invertebrates			1184	2619	3499	591	140	760	887	691	1034	1033	359	520

Functional feeding guilds (FFG) are assigned for each taxon in agreement with Merritt & Cummins (2006) and indicated as follows: *FC* filtering collectors, *GC* gathering collectors, *SC* scrapers, *SH* shredders, *P* predators

Table 5 Results of PERMANOVA based on macrobenthic invertebrate communities at the sampling sites monitored via the leaf bag technique in Doberdò Lake during the study period

PERMANOVA	Df	Sum of squares	Mean squares	F	p-level	Overall average dissimilarity (%)	
Source							
Season	1	0.207	0.207	5.036	**0.002**	34.05%	
Site	5	0.613	0.123	2.987	**0.001**	Aut	Spr
Interaction	5	0.278	0.056	1.355	0.161	34.99%	34.01%
Season						Site	
						Aut	Spr
SIMPER results: significant taxa and relative percentage contribution to dissimilarity							
Asellus		25.16	*Asellus*			33.76	21.56
Gammarus		15.54	*Gammarus*			16.71	19.47
Erpobdella		12.40	*Erpobdella*			11.92	4.95
Tubificinae		8.38	Ceratopogonidae			6.84	6.73
Chironomidae		6.36	Tubificinae			4.47	14.15
Ceratopogonidae		6.33	Chironomidae			0.18	12.07

Significant taxa detected by SIMPER test and showing a relative percentage contribution to observed dissimilarity of at least > 5% for the factor season and/or site are reported

Significance is given in bold

higher for *Asellus* and *Gammarus* than the other taxa (Table 5). *Erpobdella*, Tubificinae, and the Diptera families Chironomidae and Ceratopogonidae also showed significant contributions. The percentage of other taxa was always < 4%. With regard to FFG, the shredders (genera *Asellus*, *Gammarus* and *Haliplus*) were generally frequent at each sampling site (40.9–93.7%), except at site 5 in both seasons (11.6–27.0%). In autumn, the predators were the second most abundant group at all sites (9.4–46.0%), except for site 5 where they were predominant (71.5%); conversely, they were less frequent in spring. Predators comprised mainly *Erpobdella* and *Glossiphonia* (order Hirudinea), *Sialis* (order Megaloptera), *Dugesia* and *Polycelis* (class Plathelminthes), Ceratopogonidae (order Diptera), and Hydracarina. Collector-gatherers were mainly worms and midges (Oligochaeta and Chironomidae) and were more abundant in spring, especially at site 5, where the collector-gatherers were the most abundant in autumn. Filterers and scrapers were less frequent. Due to their higher abundance and their role in decomposition, only shredders were considered as a biotic factor in the stepwise regression.

Decomposition rates

Decomposition of *Phragmites australis* organic matter in the study area fit with negative exponential models for both seasons (Fig. 5). Decay rates k (days^{-1}) differed significantly between autumn and spring, with higher rates observed in autumn. Stepwise regression showed that 69.0% of the variability in leaf mass decay rates k was explained by five abiotic features and by one biotic parameter (Table 6). The Soča River level accounted for 32.7% of the variability and showed the highest relative importance, followed by the abundances of shredders, while rainfall was the third most important variable. Taken together, these regressors accounted for almost 60% of the variability observed for k values, while the other variables indicated only relative importance ≤ 10% and explained < 5.4% of the variability. Interestingly, water temperature, though still significant, had the lowest relative importance.
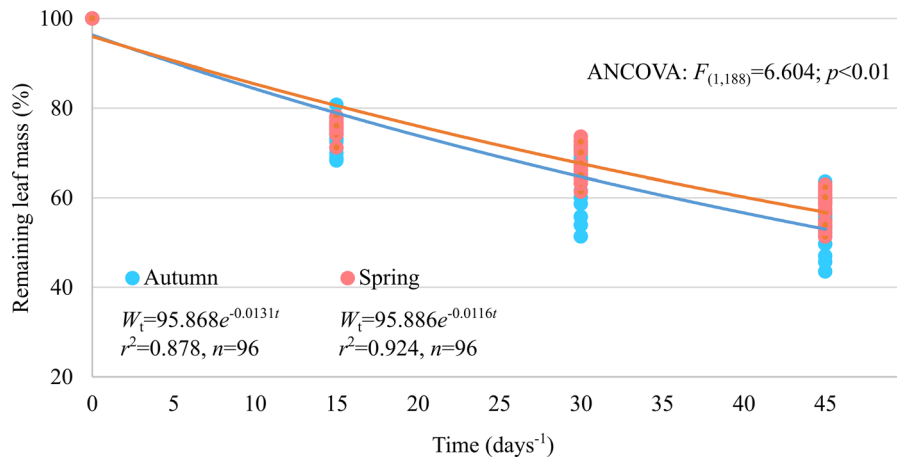
Fig. 5 Comparison of *P. australis* leaf mass decay calculated for Doberdò Lake between seasons. The initial weight at $t = 0$ days $(3.0000 \pm 0.0001$ g dry weight) was set at 100%

Table 6 Stepwise multiple regression analysis between organic matter decomposition rates (k) and biotic/abiotic features. Only significant regressors are reported

	β	St. error β	Estimate	St. error	t value	p-level	r^2	F	p-level	Relative importance (%)
Intercept	0.0000	0.0000	− 0.0001	0.0055	− 0.0170	0.9867				
Soča	− 0.6853	0.0533	− 0.0305	0.0024	− 12.8560	<0.001	0.3269	68.95	<0.001	46.70
SH	0.6080	0.0661	0.0028	0.0003	9.2000	<0.001	0.4962	69.44	<0.001	23.92
Rain	− 0.3402	0.0514	− 0.0008	0.0001	− 6.6160	<0.001	0.5924	67.84	<0.001	12.37
pH	0.4236	0.0712	0.0215	0.0036	5.9520	<0.001	0.6465	63.55	<0.001	10.01
PO_4^{3-}	0.2517	0.0587	0.0243	0.0057	4.2850	<0.001	0.6758	57.54	<0.001	5.19
Temp	0.1523	0.0600	0.0052	0.0020	2.5400	0.0122	0.6904	50.92	<0.001	1.82

Rain total rainfall; *Temp* water temperature; *Soča* Soča River hydrometric level, *SH* Shredders

Discussion

The present study reports seasonal data on the decomposition dynamics of *Phragmites australis* leaves in relation to pulsating water level fluctuations, physical and chemical parameters, and macrobenthic invertebrate communities in a freshwater karst lentic system. The data were obtained during an annual seasonal sampling campaign to describe seasonal *P. australis* litter breakdown in this environment.

The water temperature measurements are in line with those reported for Doberdò Lake by Cucchi et al. (1999) and Calligaris et al. (2018). The range of conductivity, pH, and NO_3^- concentration overlaps with the previous studies, though our ranges are

slightly wider overall. This difference could be due to slightly different sampling designs: while previous studies collected data during limited periods/seasons, we consistently carried out samplings throughout the year. The water temperature, pH, and the concentrations of dissolved oxygen, NH_4^+ and PO_4^{3-} are also in line with those reported for Cerknica Lake (Slovenia) by Gaberščik et al. (2003) and Krzyk et al. (2015). Cerknica Lake is a karst system similar to Doberdò Lake. It is located at the bottom of a polje and subject to intermittent water level variations but, unlike Doberdò Lake, it is subject to complete drought during dry phases (Kranjc, 1986; Gaberščik et al., 2003; Dolinar et al., 2010; Dolinar et al., 2016).

The present study highlights seasonal trends for several physical and chemical parameters: ANOVA revealed significant seasonal differences in all parameters, except for water depth, although these differences regarded only some seasons and seasonal ranges often overlapped (Fig. 2). ANOVA generally did not reveal significant spatial variation in the parameters (except for water depth), which indicates a certain degree of homogeneity of the sampling sites; a slightly higher though not significant spatial variability was noted for dissolved oxygen concentration. The seasonal overlap emerged on PCA (Fig. 3), particularly between summer and autumn (upper section of the plot) and between winter and spring (lower section), along a gradient that was positively correlated with water temperature and NH_4^+ and negatively correlated with NO_3^-, dissolved oxygen concentration, and pH.

It is reasonable to infer that water temperature is one of the main drivers determining the environmental conditions of Doberdò Lake: despite the observed seasonal trend, water temperature remained within a narrow range throughout the year (from $9.2 \pm 0.7°C$ to $15.6 \pm 3.4°C$), closely related to the underground origin of the water supply, with underground waters smoothing temperature fluctuations and effectively acting as a buffer to seasonal extremes, as observed in Cerknica Lake (Gaberščik et al., Gaberščik et al. 1994; Dolinar et al., 2010). Such conditions are favorable for the growth of aquatic plants, allowing them to prolong their vegetation period into wintertime (Gaberščik et al., 2003) and likely favoring macrobenthic invertebrate communities, which were more abundant in autumn than in spring in Doberdò Lake. This difference was highlighted by PERMANOVA and was mainly due to a small number of taxa (*Asellus*, *Gammarus*, *Erpobdella*, Tubificinae, and Diptera). Intraseasonal spatial variability was related to these taxa and regarded mainly site 5 which, unlike the other sites, has a softer substrate and reduced vegetation cover (Table 1). The communities were well structured and showed a high number of taxa in autumn and spring (Table 4), though dominated in both seasons by shredders, mainly *Gammarus* and *Asellus*.

Pulsating variations in the hydrological regime constitute the second main factor driving dynamics in Doberdò Lake. These typical variations, similar to those observed in Cerknica Lake, may also play a pivotal role in shaping floral and faunal communities

(Cronk & Fennessy, 2001; Gaberščik et al., 2003; Dolinar et al., 2011; Dolinar et al., 2016). Water level changes in Doberdò Lake strongly correlated with the changes in the hydrometric Soča River level (Figs. 3, 4). The influence of pulsating fluctuations can again be noted when analyzing the PCA, as some samples in the left portion of the biplot result from the increasing values of depth, conductivity, total rainfall, and Soča River level. Moreover, they are located closer to the first axis and average water temperatures, regardless of the sampling season. These samples were related to Soča River flooding events.

Favorable temperatures, high flooding frequency, and plant biomass with a high proportion of soluble substances generally promote high decomposition rates (Brinson et al., 1981; Dolinar et al., 2016). In addition, previous studies showed that macrobenthic invertebrates make a significant contribution to reed leaf decay (Hieber & Gessner, 2002; van Dokkum et al., 2002; Cornut et al., 2010; Karádi-Kovács et al., 2015). Following the classification established by Petersen and Cummins (1974), the decomposition rates (k) observed in the present study can be classified as "fast" ($k > 0.010$) for both autumn and spring. These rates are consistent with the literature on the decomposition of *P. australis* (Table 7). However, it is difficult to draw comparisons because each study was carried out in different conditions (i.e., environment, season, hydrologic regime, depth, different types of leaf bag mesh size) so that decomposition rates could be affected by a variety of factors such as geographical context and experimental conditions (Bedford, 2005; Sangiorgio et al., 2008a; Quintino et al., 2009; Bertoli et al., 2016).

Interestingly, although ANCOVA detected significant differences between k values in autumn and spring, these differences are less pronounced when compared to the rates reported by studies carried out in other systems which showed wider ranges between seasons (Table 7). This can also be observed for a nearby area, a managed freshwater temporary pond at the mouth of the Soča River (Fig. 1; Table 7), where Bertoli et al. (2016) used the same approach as we did. However, the k values for Doberdò Lake appear to agree with the measures for *P. australis* leaf decomposition reported by Dolinar et al. (2016) for Cerknica Lake, though they used a different methodological approach. The narrower range in decomposition rates we observed at Doberdò Lake is probably related to

Table 7 Comparison between leaf litter breakdown rates (k, days^{-1}) for submerged litter of *Phragmites australis* calculated in the present study and in previous studies

Ecosystem	Length of the experiment (days)	Mesh size (mm)	Period	k (days^{-1})	Reference
Doberdò Lake (Northeastern Italy Italia)	45	5	Autumn 2016	0.013	Present study
			Spring 2017	0.012	
Freshwater pond (Soča River Mouth Northeastern Italy)	45	5	Autumn 2009–2011	0.007–0.008	Bertoli et al. (2016)
			Spring 2010–2012	0.014–0.016	
			Summer 2012	0.017	
Karstic lake (Cerknica Lake—Slovenia)	79–249	1	December 2007–November 2008	0.013	Dolinar et al. (2016)
Balaton Lake (Hungary)	59	10	March 2012	0.016	Karàdi-Kovàcs et al. (2015)
			June 2012		
			September 2012		
Coastal lake (Egypt)	150	5	April–September 2003	0.012	Eid et al., (2014)
Natural fan (Northern Germany)	248–257	0.3	September 2010–May 2011	0.003	Völlm & Tannenberger (2014)
Transitional environments (Mediterranean Area)	30–40	5	Spring 2005	0.014–0.029	Sangiorgio et al. (2008a)
Danube delta	45	5	Autumn 2005	0.009–0.024	Sangiorgio et al. (2008b)
Reedbed wetland (Northeastern England)	599	5	January 1995–August 1997	0.004	Bedford (2005)
River basins subjected to summer drought (Sardinia, Italy)	90	5	Spring–Summer 1998	0.028	Pinna et al. (2004)
			Autumn–Winter 1998–1999	0.009	
Freshwater lake (Southern Italy)	90	5	Autumn 1998	0.007	Sangiorgio et al. (2004)
			Spring 1999	0.013	
			Summer 1999	0.011	
Salt-marsh lake (Southern Italy)	90	5	Autumn 1998	0.006	
			Spring 1999	0.009	
			Summer 1999	0.01	
Monomictic Lake (Netherlands)	38	5	Summer 1999	0.055	Van Dokkum et al. (2002)
			Winter 1999	0.012	

the water temperature as well, which is one of the main factors driving *P. australis* breakdown and positively influencing reed decay rate (Hanson et al., 1984; Webster & Benfield, 1986; Bedford, 2005; Eid et al., 2014; Bertoli et al., 2016): water temperature increases microbial processing during decomposition during which leaves serve as a major energy source for invertebrates in aquatic ecosystems (Dudgeon, 1982). As the temperature range become narrower, it is reasonable to assume that k values may follow a similar trend and/or that other factors become more important. Due to the nature of the study area, the water level fluctuations may strongly affect decomposition rates. The alternation of wet and dry periods can accelerate decomposition because it favors oxic conditions and growth of microbial communities (Battle & Golladay, 2001; Bedford, 2005; Dolinar et al., 2016).

Stepwise multiple regression showed that, albeit significant, water temperature contributed least to k variability, while variation in the Soča River hydrometric level contributed the most (Table 6). Contributions of the Soča River level and rainfall to variability were negative, while those of other

regressors were positive. This could be due to indirect effects related to the physical and chemical parameters influenced by the underground water supply. As can be observed in the PCA (Fig. 3) and correlation analysis, increasing Soča River levels are strongly correlated with higher conductivity, which can negatively affect decomposition rates (Webster & Benfield, 1986; van Dokkum et al., 2002). The influx of water masses into the lake, originating underground due to Soča River flooding events, dramatically increases the lake water level. Consequently, dissolved oxygen concentrations may likely decrease with increasing depth and negatively affect decomposition rates (Webster & Benfield, 1986; van Dokkum et al., 2002) since anoxic levels limit decomposition in permanently flooded sites (Ryder & Horwitz, 1995). The sampling sites were not subject to total drought and were always found flooded during sampling events.

Furthermore, the input from flooding events could be disruptive for macrobenthic invertebrates. Several factors influence the composition of macroinvertebrate assemblages in these systems, including migration patterns, flow permanence, and input of leaf litter (Smith et al., 2003; Barquin & Death, 2004; Tanaka et al., 2006). The contribution of macrobenthic invertebrates to leaf decomposition was positive due to the abundance of shredders in the communities. This FFG generally plays an important role in leaf litter decomposition and can accelerate litter breakdown (Hieber & Gessner, 2002; van Dokkum et al., 2002; Cornut et al., 2010; Raposeiro et al., 2016). Phosphates and pH can also positively affect decomposition rates (Webster & Benfield, 1986; Sangiorgio et al., 2008b) showing a non-negligible effect though less important than other regressors (relative importance $\leq 10\%$). Variations in pH levels can affect the fungal community composition, and mass loss is known to increase with higher pH (Thompson & Bärlocher, 1989). Our findings show a higher contribution of pH and phosphates to the decomposition rates than that of temperature. Indeed, water temperature ranked lowest among the variables, whereas the Soča River hydrometric level ranked highest. This finding is shared by Dolinar et al. (2016) who assumed that water regime could have a greater effect than temperature on the decomposition dynamics in Cerknica Lake. In fact, the water level of Doberdò Lake was heavily correlated with the Soča River

hydrometric level, since the river is one of the main water sources for the lake (Calligaris et al., 2018).

Conclusions

The present study highlighted the influence of abiotic and biotic features on decomposition rates of *Phragmites australis* in a lentic karst freshwater system subject to pulsating water level fluctuations. The underground water supply impacts lake system processes and living communities, creating a buffer effect on the lake's water temperature, which is one of the most important drivers in *P. australis* breakdown. Intermittent water level fluctuations may be both a driving force and a limiting factor. Pulsating phenomena, strong and relatively regular, such as water fluctuations at Doberdò and Cerknica Lakes, constitute a pivotal factor for organic matter turnover and energy fluxes (Gaberščik et al., 2003) and can maintain these systems in an early and relatively productive state of development (Odum, 1971; Gaberščik et al., 2003; Dolinar et al., 2010).

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