Ann. Limnol. - Int. J. Lim. 52 (2016) 365–377
© EDP Sciences, 2016
DOI: 10.1051/limn/2016023

Available online at:
www.limnology-journal.org

Sediment chemistry and flooding exposure: a fatal cocktail for *Phragmites australis* in the Mediterranean basin?

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Received 20 March 2016; Accepted 5 September 2016

Abstract – The common reed die-back syndrome was formerly reported mostly in Central Europe and more recently in the Mediterranean basin. This study investigates a case of reed die-back in Central Italy through a Geographic Information System (GIS) diachronic survey and the analysis of macromorphological and ecological parameters. Data were recorded during field activities on ten plots randomly distributed according to two ecological statuses, permanently and temporarily flooded. Culm density, height and diameter, clumping habit, flowering head and dead apical bud number and rate were measured; for each plot, chemical parameters of sediments were analyzed. Information on interstitial waters for the flooded plots was also provided. Floristic-vegetational features of the reed-bed community such as the total vegetation cover, the species composition and abundance were estimated. Univariate and multivariate analyses were used to demonstrate differences between morphological traits of flooded and emerged stands and their relationships with the chemical features of sediment. Macromorphological traits differed according to the ecological status, with flooded stands showing patterns related to poor health status of *Phragmites australis*, such as high rates of clumping habit and dead apical bud rate, high culm density and, to a lesser extent, low culm diameter and flowering head rate. Sulfates were relatively abundant in the sediment of flooded stands and, together with some heavy metals, resulted in some of the mentioned traits. Our results showed a relationship between reed die-back and prolonged flooding and highlighted the potential role of some chemical parameters in affecting the growth of permanently flooded reeds.

Key words: Die-back / sediment / macromorphological traits / reed bed / wetland

Introduction

Phragmites australis (Cav.) Steud. is a sub-cosmopolitan species widely diffused in wetland ecosystems of several parts of the world (Haslam, 2010). This species grows in many habitat types, such as marshlands, riverbanks, lake shores and salty-marshes tolerating very different trophic conditions and colonizing from oligotrophic to eutrophic and hypertrophic waters and soils (van der Werff, 1991; Čížková *et al.*, 2001). Extensive reed-dominated communities play an important structural and functional role in wetland ecosystems, providing habitats for fauna and many other important ecosystem services (Ostendorp, 1993; Kiviat, 2013). The species exhibits high variability at the ploidy level and several different haplotypes (Clevering and Lissner, 1999; Lambertini *et al.*, 2012; Meyerson *et al.*, 2012). From a taxonomic point of view, several subspecies or varieties have been described (Saltonstall *et al.*, 2004; Lambertini *et al.*, 2006, 2012). In Europe, two subspecies are recognized (Lambertini *et al.*, 2012): *P. australis* subsp. *australis* and *P. australis* subsp. *chrysanthus* (Mabille) Soják [= *P. australis* subsp. *altissimus* (Benth.) Clayton]. Both species occur in Italy (Conti *et al.*, 2005; Lucarini *et al.*, 2015), although the latter seems to be rare and localized. Eurasian and, to a lesser extent, Mediterranean haplotypes of *P. australis* have been

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recognized as introduced in North America (Meyerson *et al.*, 2012). There, the Eurasian genotypes are nowadays considered invasive, being in competition with the native populations referred to as *P. australis* subsp. *americanus* Saltonstall, P.M. Peterson & Soreng (Saltonstall, 2002; Saltonstall *et al.*, 2004; Tulbure *et al.*, 2007). Even in Europe, although in most cases explicit indications of infraspecific ranks are lacking, *P. australis* is often considered as an expansive species (Próchnicki, 2005; Foggi *et al.*, 2011), and its control is considered an important aspect for the conservation of local biodiversity (Tomei *et al.*, 2000; Foggi *et al.*, 2014). Nevertheless, in the last decades, more and more cases of reed decline have been recorded in Europe, leading to the description of a syndrome known as common reed die-back (Den Hartog *et al.*, 1989; Ostendorp, 1989; van der Putten, 1997). Recently, this phenomenon was also detected in the Mediterranean basin, in both brackish and freshwater habitats (Fogli *et al.*, 2002; Gigante *et al.*, 2011, 2014). Symptoms related to reed die-back are relatively variable and range from macromorphological features, such as reduced culm diameter and height, high incidence of bud or rhizome death and flowering delay, to cyto-anatomical aspects such as abnormal lignification, suberization in the adventitious roots or presence of a *callus* that prevents the internal aeration of aerenchyma (Gigante *et al.*, 2011; Reale *et al.*, 2012). A typical and easily detectable trait of reed die-back is the clumping habit, an uncontrolled outgrowth of dormant buds caused by breaking of apical dominance (van der Putten, 1997).

P. australis decline has also been related to specific environmental conditions such as depth of water, concentration of chemicals in water or sediment geochemical features (Rea, 1996; van der Putten, 1997; Gigante *et al.*, 2011, 2013, 2014). Increasing eutrophication, often associated with other factors such as artificial alterations of the water levels, anoxic condition and methanogenesis in the sediment have been reported as important causes of die-back (Boar and Crook, 1985; Ostendorp, 1989; Weisner, 1996; Kubín and Melzer, 1997; Gigante *et al.*, 2011, 2014).

The detection of the die-back phenomenon in two large wetlands of Central Italy, lakes Trasimeno and Chiusi (Gigante *et al.*, 2011, 2014), has prompted new surveys in other lakes (Reale *et al.*, 2014), to better understand the extent of this syndrome in the Mediterranean basin and investigate possible relationships between die-back, macromorphological traits, ecological features of the stands and chemical parameters of waters and sediments.

The aims of this paper are to:

- investigate a new case of reed decline in Central Italy, by surveying Lake Montepulciano (Tuscany), where evident episodes of reed retreat have been recorded in the last 2 decades;
- evaluate quantitatively the retreat of reed beds in this lake, through a diachronic analysis in the GIS environment;
- confirm the existence of relationships between some macromorphological traits measured in the field and the die-back phenomenon in the Mediterranean area;
- evaluate whether some ecological and sediment chemical features of *P. australis* subsp. *australis* (hereafter *P. australis*) stands might be related to both the die-back syndrome and some macromorphological traits.

Materials and methods

Study site

The study was carried out at Lake Montepulciano (Fig. 1), in Southern Tuscany (Central Italy), located at a mean altitude of 248 m a.s.l. Only one emissary is present. From a geological point of view, the study area belongs to the Neogenic basin of Chiana Valley – Middle Tiber Valley and is characterized by Pliocene sediments and Quaternary deposits (Carmignani and Lazzarotto, 2004). The climate is humid Mediterranean (Barazzuoli *et al.*, 1993) with a mean annual temperature of 13.8 °C.

The landscape of this area has been affected by intensive anthropogenic modifications since Roman times due to the presence of fertile lands (Alexander, 1984). The location was designated as a Site of Community Importance (SCI) and a Special Protection Area (SPA) according to European Directives 92/43/EEC and 79/409/EEC, respectively. Lake Montepulciano also represents a Natural Reserve of Siena Province. From a botanical point of view, previous studies on flora and vegetation showed the presence of species and vegetation types of high conservation interest (Arrigoni and Ricceri, 1982; Lastrucci *et al.*, 2014).

The maximum depth of the lake is *ca.* 2.7 m (A.S.T.R.A., 2009). The lake has been affected over the years by burial phenomena, leading to a strong reduction of the lake surface (Arrigoni and Ricceri, 1982; A.S.T.R.A., 2009) and to significant fluctuations of the water level, dropping down up to few centimetres (F. Boschi, *personal communication*). The only available data about the water levels (kindly provided by the Province of Siena) are relative to five months (April–August) of four observation years (from 1997 to 2000). Although very partial, these data confirm the strong level variation of the lake, both for the maximum (from 177 cm in April 1997 to 122 cm in April 1999) and the minimum value (from 73 cm in August 1997 to 47 cm in August 2000). In order to minimize sediment supply to the lake, the excavation of five artificial ponds (2.5 m mean depth), collecting the waters of the main tributaries of Lake Montepulciano, was designed between 1999 and 2002 with a total surface of 40 ha (A.S.T.R.A., 2009). The water level is currently controlled by a small dam located in the northern part of the lake.

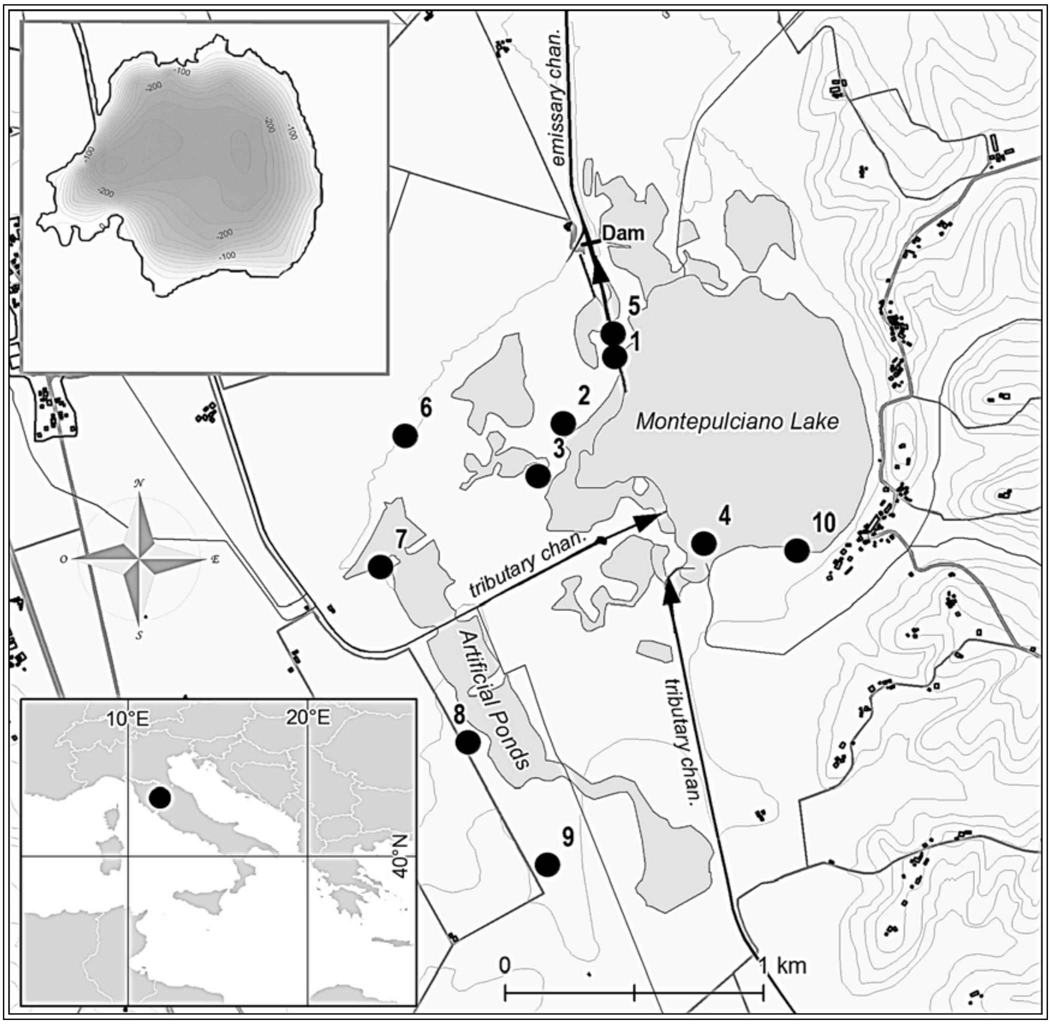

Fig. 1. Study area and location of the sample sites. Flooded plots (1–5) and emerged plots (6–10) are reported as black dots. At the top left a bathymetric map (kindly provided by the Siena Province) is shown.

Reed bed mapping and diachronic analysis

The quantification of reed bed retreat was carried out by a diachronic analysis of the reed-dominated vegetation surface.

The mapping was performed with the Open Source Geographic Information System Quantum GIS (QGIS 2.12), licensed under the GNU General Public License (GPL).

On the basis of digitized orthophotos (referring to years 1988, 1996, 2002, 2007 and 2013) available at the WMS Service on the Tuscany Region Online Service (http://www.regione.toscana.it/-/geoscopio-wms), five maps of the reed beds were performed by manual 1:2000 digitization for vectorizing raster data. The measured surfaces of the maps were compared with quantify reed bed spatial variation over the considered years.

Ten randomly distributed transects were placed from the center of the lake toward the lake shores, orthogonally to the reed bed front. Along each transect, retreat/progress of the reed bed was evaluated and the overall measures were used to evaluate the shift of the reed bed water-front in the study area.

Sampling sites

Preliminary field surveys were carried out in June and July 2013 to identify areas of reed bed with or without symptoms of die-back. On the basis of the results of the field surveys and the orthophoto analysis, ten sample sites were set in the study area (Fig. 1).

In line with similar studies carried out in Central Italy (Gigante *et al.*, 2011, 2014), a stratified random sampling design was performed for site selection, considering two different strata, corresponding to the two main ecological conditions affecting the reed beds in the area: (i) permanent flooding ("F" sites), with the presence of a water column (at least 10 cm depth) even in the driest season of the year (end of summer, for the study area), and (ii) emersion or temporary flooding ("E" sites), with periodic emersion at least in the driest season.

Ten georeferred plots (1×1 m^2) were randomly placed, as follows: (i) five in the F sites (nos. 1–5) covered at the end of summer by 99.6 ± 21.3 cm water (mean ± standard deviation), and (ii) five in the E sites (nos. 6–10, see Fig. 1). The geographic coordinates of the surveyed plots are reported in Appendix 1. For each site, three different sets of parameters (macromorphological traits, species composition/abundance and geochemical features) were determined within the plots.

Macromorphological traits

Due to the clonal behavior of *Phragmites australis,* the functional unit of the reed populations refers to the "ramet", a potentially independent functional unit of a clonal plant commonly used in demographic studies (Ekstam, 1995; Canullo and Falinska, 2003).

In each 1×1 m^2 plot, the following macromorphological traits were measured: (1) culm height (cm), measured with a metric tape, including the portion between the substrate and the base of the inflorescence; (2) culm diameter (mm), measured with a calliper at about 120 cm from the base of the culm; height and diameter were measured on 10 reed culms randomly chosen for each plot and are reported here as mean values per plot; (3) culm density (number of culms per m^2), corresponding to the total number of culms in the plot; (4) absolute number and rate (% of the number of culms) of inflorescences; (5) absolute number and rate (%) of dead apical buds; and (6) clumping habit, expressed both as absolute number of clumped culms per m^2 and as a rate (%). As a quantitative evaluation of the clumping habit, we counted the number of culms belonging to clumps per square meter.

In accordance with other studies about reed bed decline in central Italy (Gigante *et al.*, 2011, 2014) all the field activities were carried out in early September (year 2013), *i.e.* at the end of the reed vegetative season, when the maximum standing crop was achieved.

Ecological parameters

For each plot, the surrounding 3×3 m^2 area was subjected to a vegetation analysis in order to analyze some community features such as: (i) total vegetation cover, estimated in percentage as the projection of the foliage on the ground; and (ii) the species composition and abundance, analyzed by the census and the relative percentage cover of the species. The nomenclature of the *taxa* is in accordance with Conti *et al.* (2005).

In each 1×1 m^2 plot, one sediment sample was collected from the permanent flooded sites (nos. 1–5) with a core driller, at the depth of 25–30 cm; in order to provide information about the interstitial water composition of the lake, interstitial water was collected in the flooded plots by means of water-intake by a slight pipe within the driller. It was filtered (2 aliquots were acidified with HCl and HNO_3 for further laboratory analysis) and transferred to PE bottles stored in a fridge box. Being relative only to the submerged plots, the data of interstitial water chemistry were used as background information and no further statistical correlation with other parameters was performed.

Sediment samples were also collected in the emerged plots (6–0) at the same depth.

In the laboratory, pH and bicarbonates were measured by a Crison pH-meter and acidimetric titration (0.01 M HCl and methyl-orange as the indicator), respectively. One filtered (0.45 μm) and two filtered-acidified (with suprapur HCl and HNO_3, respectively) water aliquots were collected in polyethylene bottles for the analysis of anions, cations and trace species, respectively, with the exception of samples F1 and F5 as not enough water was available for trace element analysis. Fluoride, Cl^-, Br^-, NO_3^-, NH_4^+, SO_4^{2-} and Ca^{2+}, Mg^{2+}, Na^+ and K^+ were determined by ion-chromatography (IC) (Metrohm 761 and Metrohm 861, respectively). Arsenic, Cd, Co, Cr, Cu, Fe_{tot}, Hg, Ni, Pb and Zn were analyzed at C.S.A. Ltd. Laboratories (Rimini, Italy) by inductively coupled plasma mass spectrometry (ICP-MS) with an Agilent 7500 spectrometer, on the filtered samples acidified with 1% suprapur HNO_3, according to the procedure reported in Tassi *et al.* (2014). The analytical errors for IC and ICP-MS were ≤ 5 and $\leq 10\%$, respectively.

The sediment samples were dried at 40 °C for at least 24 h and sieved to remove the > 2 mm fraction. Then, 10 g of each < 2 mm sediment sample were weighed in a 75 mL graduated beaker and 50 mL of MilliQ water saturated in CO_2 were added. The interaction between the sediment and the CO_2-saturated MilliQ water lasted for 24 h. This solution was obtained by bubbling for about 15 min pure CO_2 into a gas bubbler partly filled with 100 mL of MilliQ water and equipped at its end with a diffuser to finely reduce the CO_2 bubbles. After 15 min, pH was controlled in order to verify that a value of 4.5 was achieved. This solution was used to mimic the effect that the plants have on the sediments where they are growing (Venturi *et al.*, 2015).

Then, the solution was filtered at 0.45 μm and acidified with 1% of suprapur HNO_3 and analyzed for the same trace elements determined in the interstitial water.

The sediment samples were also analyzed for total SO_4^{2-} and S^{2-}. The sulfate content was determined after its extraction with a solution of 0.5 M of ammonium acetate and 0.25 M of acetic acid. The solution was separated from the solid phase by centrifugation. Sulfate was then precipitated as $BaSO_4$ by adding $BaCl_2$ and separated by paper filtering. $BaSO_4$ was solubilized using an $(NH_4)_4$-EDTA solution and sulfate was indirectly determined by measuring the concentration of Barium by atomic absorption spectrophotometry.

The concentration of sulfide was measured according to the British Environmental Agency (Environment Agency, 2010) procedure. The sediment samples were treated with phosphoric acid and steam-distilled into 20 mL of 0.1M NaOH. An aliquot of the distillate was treated with H_2SO_4 and then, a potassium dichromate solution was added. The absorbance of the blue color was measured by molecular spectrophotometry at about 670 nm. The analytical errors for sulfate and sulfide were $< 10\%$.

Acetic acid ($C_2H_4O_2$), propionic acid ($C_3H_6O_2$), butyric acid and isobutyric acid ($C_4H_8O_2$) were determined in the solid samples according to the EPA 8315A 1996 procedure by high performance liquid chromatography equipped with ultraviolet/visible (UV/vis) detectors.

Statistical treatment of the data

Differences between macromorphological measurements and sediment composition of flooded and emerged reed stands were estimated by means of a non-parametric Mann–Whitney–Wilcoxon Test with the use of the function *wilcox.test* of R Software for Linux (R Core Team, 2015).

Relationships between macromorphological traits and chemical parameters of sediments and water were explored by the Spearman's rank correlation test using the function *cor.test* of R software, with *Phragmites australis* traits considered as the dependent variables and chemical traits used as individual explanatory variables.

Principal Component Analysis (PCA) was performed to investigate the major gradients in the macromorphological measures of all plots. The function *prcomp* of R software was used, scaling the data with the parameter *scale*.

According to Gigante *et al.* (2014), the diagram of PCA was passively projected with the total vegetation cover, species number and chemical parameters in order to highlight their variation across the main gradient. In order to prove the relationships between sample scores and ecological parameters on PCA axes 1 and 2 respectively, the Pearson Correlation test (*cor.test* function on R) was performed.

Results

Diachronic analysis

Diachronic analysis showed a retreat of the front of the common reed from the deeper waters toward the shores of the lake (Fig. 2; Table 1). A general retreat from 1988 to 2013 was observed in all transects, with a mean value of 124.3 m. The maximum retreat of the front of the reed bed was 441 m in transect no. 5.

The total measured surface of reed vegetation in 1988 was 195 ha. Although in the water-side of the study area reed bed retreat has been observed since the period between 1988 and 1996, a first stage of increase of the total surface can be noticed, due the expansion of the reed bed in the external (terrestrial) areas of the lake. Then the total surface was affected by a decline, reaching the value of 137.46 ha in 2013. The total decrease of the reed beds between 1988 and 2013 was estimated to be 57.54 ha, accounting for a total loss of 29.5% of the initial surface.

Macromorphological traits of the reed stands

The measured values of the macromorphological traits are shown in Table 2 where the mean values and the relative standard deviation, referring to the emerged and flooded groups of stands, are also reported.

The sampled plots showed a mean culm density of 95.6 culm.m^{-2}, ranging from a minimum of 31 to a maximum of 174 culm.m^{-2}.

The data were characterized by high variability for both the culm height (from 126 to over 400 cm) and diameter (from 1.5 to 9.6 mm). The presence of prematurely dead apical buds was observed in nine plots (five flooded and four emerged); the rate of dead apical buds varied from 0 to 52.9%. Inflorescences were present in all the plots; the flowering head rate was 17.09%, with a minimum of 4.25% and a maximum of 64.52%. The clumping rate varied from 0 to 100%. With the exception of the culm diameter and the flowering head number and rate, all the measurements were significantly different between flooded and emerged stands (Table 2). Flooded plots have a higher number of culms per m^2 than the emerged ones and the culms in those plots were also higher. Similarly, the flooded plots showed a higher number and rate of dead buds and clumped culms than the emerged ones.

Ecological features of reed stands

As far as reed community features are concerned, the mean of the total vegetation cover was 83% with a minimum of 45% and a maximum of 100%. The number of species in the reed bed communities appeared rather variable, with a mean value of 4.2. In some cases the presence of monospecific stands was observed. The floristically richest plot had nine species (Table 2).

Emerged plots had a significantly higher mean cover and a larger number of species than the flooded plots. The floristic analysis (Table 3) confirmed these trends, showing remarkable species poorness in the flooded plots. Only *Phragmites australis* and some sporadic hydrophytes (*Ceratophyllum demersum* and *Najas marina*) were growing there, while in the emerged plots, a large variability of species was recognized.

The mean values for each chemical parameter determined in the lake sediment are listed in Table 4. Acetic, propionic, butyric and isobutyric acids were below the instrumental detection limit. Setting aside a few exceptions, most chemical parameters showed higher concentrations in the permanent flooded sediments than the emerged ones.

Strong differences were observed for the sulfate contents in the bulk sediments, the flooded sediments

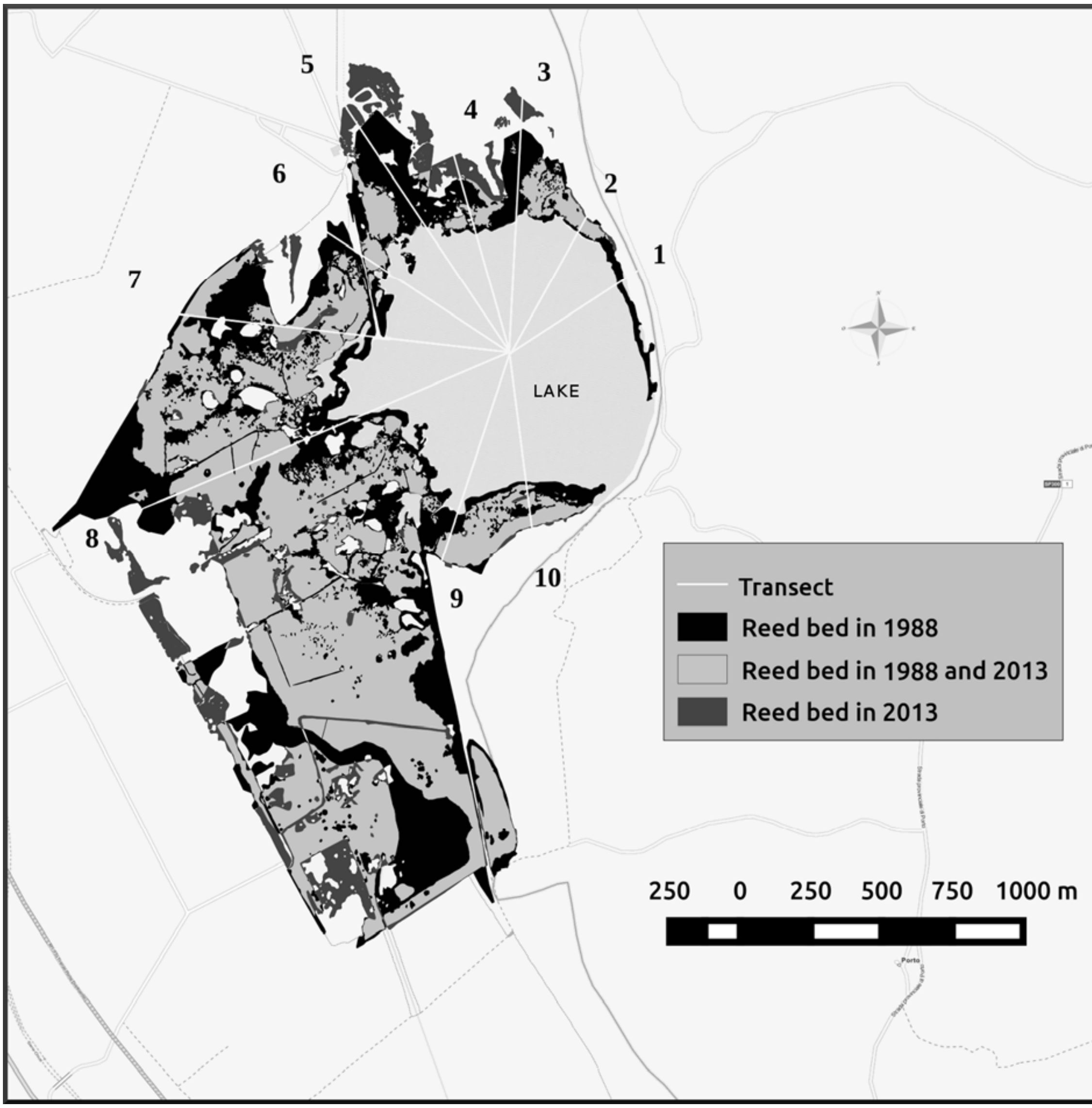

Fig. 2. Surface of the reed bed at Lake Montepulciano: former areas only present in 1988 (black); new areas only present in 2013 (dark grey); stable areas present both in 1988 and 2013 (light grey); location of the ten transects used to estimate reed bed retreat from the center of the lake towards the lake shores.

Table 1. Shift of the water-front of the reed bed (m) along the ten transects (tr) and changes of the total reed bed surface (ha), in each considered year and over the whole 25-year period.

Year	tr1	tr2	tr3	tr4	tr5	tr6	tr7	tr8	tr9	tr10	Total surface
1988	–	–	–	–	–	–	–	–	–	–	195.00
1996	0	− 3	− 100	− 9	− 1	− 4	− 57	− 51	− 62	0	208.07
2002	− 20	− 6	7	− 1	− 26	− 5	0	− 115	16	− 8	172.10
2007	0	6	− 23	1	0	− 18	− 2	− 56	− 21	0	136.50
2013	0	2	− 238	− 9	− 414	− 8	0	− 12	− 3	− 3	137.46
1988–2013	− 20	− 1	− 354	− 18	− 441	− 35	− 59	− 234	− 70	− 11	− 57.54

Negative values indicate a retreat of the reed bed front along the transects; positive values indicate an increase of the reed bed front along the transects.

being enriched (mean = 5240 $mg.kg^{-1}$) when compared with those of the emerged stands (mean = 331.6 $mg.L^{-1}$). Similarly, the As contents in the flooded and emerged sediment leachates were relatively distinct, since they showed mean values of 20.44 and 2.88 $mg.L^{-1}$ in the permanent flooded and emerged sediments, respectively. Also for Cd and Co, significant differences between flooded and emerged plots were found (Table 4).

Table 2. Macromorphological traits of *Phragmites australis* (referring to 1×1 m^2 plots), vegetation cover and species number of the reed bed community (referring to 3×3 m^2 plots) for the whole data set and for the flooded (F) and emerged (E) plots.

	Whole data set			F	E	*P*
	mean	min	max	mean ± sd	mean ± sd	
Culm density (n.m^{-2})	95.60	31	174	**144 ± 20.31**	**47.20 ± 14.86**	**
Culm height (cm)	262.40	126	425	**287 ± 18.38**	**237.10 ± 22.92**	**
Culm diameter (mm)	5.60	1.50	9.60	4.90 ± 1.47	6.20 ± 1.73	
Dead apical buds (n.m^{-2})	34.70	0	92	**63.80 ± 20.76**	**5.60 ± 3.78**	*
Dead apical bud rate (%)	27.49	0	52.90	**43.97 ± 11.20**	**10.99 ± 7.04**	**
Clumping (n.m^{-2})	56.70	0	174	**111.2 ± 62.73**	**1.20 ± 2.68**	**
Clumping rate (%)	39.43	0	100	**76.30 ± 38.41**	**2.55 ± 5.71**	*
Flowering heads (n.m^{-2})	12.90	2	26	16.20 ± 8.76	9.6 0 ± 7.57	
Flowering head rate (%)	17.09	4.25	64.52	11.19 ± 5.96	23.00 ± 24.27	
Total vegetation cover (%)	83.03	45	100	**66.20 ± 20.92**	**99.86 ± 0.31**	**
Number of species	4.20	1	9	**1.60 ± 0.55**	**6.80 ± 2.28**	*

min, minimum; max, maximum; sd, standard deviation.
The values in bold indicate statistically significant differences among the groups according to the results of the Mann–Whitney–Wilcoxon Test. * = $P < 0.05$; ** = $P < 0.01$.

Table 3. Floristic composition of flooded (F) and emerged (E) plots with species mean cover (%) and number of occurrence plots.

	F	E	Occurrence
Atriplex prostrata Boucher ex Dc.	0	0.04	2
Bidens tripartita L.	0	0.02	1
Calystegia sepium (L.) R. Br. subsp. *sepium*	0	1.9	5
Carex elata All. subsp. *elata*	0	3.2	2
Carex riparia Curtis	0	4	1
Ceratophyllum demersum L.	4.2	0	2
Cirsium creticum (Lam.) d'Urv. subsp. *triumfetti* (Lacaita) Werner	0	0.02	1
Cornus sanguinea L.	0	0.2	1
Crataegus monogyna Jacq.	0	0.1	1
Cyperus longus L. subsp. *longus*	0	0.1	1
Galega officinalis L.	0	0.02	1
Hypericum tetrapterum Fries	0	0.02	1
Lythrum salicaria L.	0	0.44	3
Najas marina L. subsp. *marina*	2	0	1
Phalaris arundinacea L. subsp. *arundinacea*	0	2.8	3
Phragmites australis (Cav.) Steud. subsp. *australis*	60	90	10
Rubus caesius L.	0	16.1	2
Sorghum halepense (L.) Pers.	0	0.02	1
Stachys palustris L.	0	0.02	1
Urtica dioica L. subsp. *dioica*	0	0.2	1
Veronica anagalloides Guss.	0	0.02	1

The chemical composition of the interstitial water is shown in Table 5. Among the anions and cations, HCO_3^- and Ca^{2+} were the most abundant solutes showing that the geochemical classification of Lake Montepulciano interstitial waters is Ca(Mg)-HCO_3. Importantly, the chemical composition of the interstitial waters differed from that of the lake surface measured in 2005 since the content of sulfate was up 255 mg.L^{-1} (Nisi, 2005), even higher than that measured by Cortecci and Dinelli (1999) in 1971 (234 mg.L^{-1}).

The concentrations of trace elements, which were measured at three stations (F2, F3 and F4), were characterized by values of Cd, Co, Cr, Fe, Hg, Ni and Pb below or close to the instrumental detection limit, with the exception of As (mean value = 0.67 mg.L^{-1}), Cu (mean value = 1.67 mg.L^{-1}) and Zn (mean value = 7.83 mg.L^{-1}).

Significant relationships between sediment parameters and macromorphological measurements are reported in Table 6. The sulfate, As and Cd contents were positively correlated with reed culm density, culm height, dead bud rate and clumping rate. Cobalt concentration was also positively correlated with the dead bud rate. The content of sulfate, Cd and Co appeared negatively correlated with the culm diameter.

The PCA results (Fig. 3) substantially agree with the results of the univariate analysis (Tables 2 and 4) in terms of dissimilarities in the macromorphological characters. In particular, the first axis of PCA ordination diagram explained 66.5% of the total variance; the second axis explained 19.9% of the total variance.

Flooded plots appeared to be concentrated in the left quadrants of the PCA, while the emerged plots lie in the

Table 4. Mean values of the chemical parameters of the sediments measured respectively in the flooded (F, $n = 5$) and emerged (E, $n = 5$) plots.

	Units	F	E	*P*
SO_4^{2-}	$mg.kg^{-1}$	**5240.00**	**331.60**	**
S^{2-}	$mg.kg^{-1}$	29.60	96.80	
Acetic acid	$mg.kg^{-1}$	< 0.1	< 0.1	
Propionic acid	$mg.kg^{-1}$	< 0.1	< 0.1	
Butyric acid	$mg.kg^{-1}$	< 0.1	< 0.1	
Isobutyric acid	$mg.kg^{-1}$	< 0.1	< 0.1	
As	$\mu g.kg^{-1}$	**20.44**	**2.88**	**
Cd	$\mu g.kg^{-1}$	**0.14**	**<0.1**	**
Co	$\mu g.kg^{-1}$	**6.04**	**3.26**	*
Cr	$\mu g.kg^{-1}$	4.80	2.54	
Cu	$\mu g.kg^{-1}$	19.00	17.24	
Fe	$\mu g.kg^{-1}$	119.60	109.40	
Hg	$\mu g.kg^{-1}$	< 0.1	< 0.1	
Ni	$\mu g.kg^{-1}$	15.36	18.42	
Pb	$\mu g.kg^{-1}$	1.06	0.60	
Zn	$\mu g.kg^{-1}$	13.72	9.84	

The values in bold indicate statistically significant differences among the groups according to the results of the Mann–Whitney–Wilcoxon Test. * = $P < 0.05$; ** = $P < 0.01$.

Table 5. Mean values of the chemical parameters of the interstitial waters in the sampled plots (*n*, number of plots).

	Units	Mean	*n*
pH		7.48	5
HCO_3^-	$mg.L^{-1}$	496.20	5
Cl^-	$mg.L^{-1}$	64.48	5
SO_4^{2-}	$mg.L^{-1}$	68.90	5
F^-	$mg.L^{-1}$	0.63	5
NO_3^-	$mg.L^{-1}$	0.21	5
Na^+	$mg.L^{-1}$	57.04	5
K^+	$mg.L^{-1}$	8.49	5
Ca^{2+}	$mg.L^{-1}$	111.96	5
Mg^{2+}	$mg.L^{-1}$	33.36	5
NH_4^+	$mg.L^{-1}$	1.57	5
Total dissolved solids	$mg.L^{-1}$	842.84	5
Sum(cat.)	$meq.L^{-1}$	943.30	5
Sum(anio.)	$meq.L^{-1}$	246.18	5
As	$\mu g.L^{-1}$	0.67	3
Cd	$\mu g.L^{-1}$	< 0.1	3
Co	$\mu g.L^{-1}$	0.20	3
Cr	$\mu g.L^{-1}$	< 0.1	3
Cu	$\mu g.L^{-1}$	1.67	3
Fe	$\mu g.L^{-1}$	< 5	3
Hg	$\mu g.L^{-1}$	< 0.1	3
Ni	$\mu g.L^{-1}$	0.70	3
Pb	$\mu g.L^{-1}$	0.10	3
Zn	$\mu g.L^{-1}$	7.83	3

Table 6. Significant relationships between reed traits and chemical parameters of the sediment analyzed by Spearman's rank correlation coefficient.

	S	rho	*P*
Reed culm density			
SO_4^{2-}	34.00	0.7939	0.0061
As	28.00	0.8303	0.0029
Cd	15.75	0.9045	0.0003
Dead apical bud rate			
SO_4^{2-}	16.00	0.9030	0.0003
As	32.00	0.8061	0.0049
Cd	15.75	0.9045	0.0003
Co	46.00	0.7212	0.0186
Clumping rate			
SO_4^{2-}	44.00	0.7286	0.0169
As	48.97	0.7032	0.0233
Cd	25.17	0.8475	0.0020
Reed culm diameter			
SO_4^{2-}	281.35	− 0.7052	0.0227
Cd	272.01	− 0.6485	0.0425
Co	269.32	− 0.6322	0.0499
Reed culm height			
SO_4^{2-}	40.00	0.7576	0.0111
As	44.00	0.7333	0.0158
Cd	15.75	0.9045	0.0003

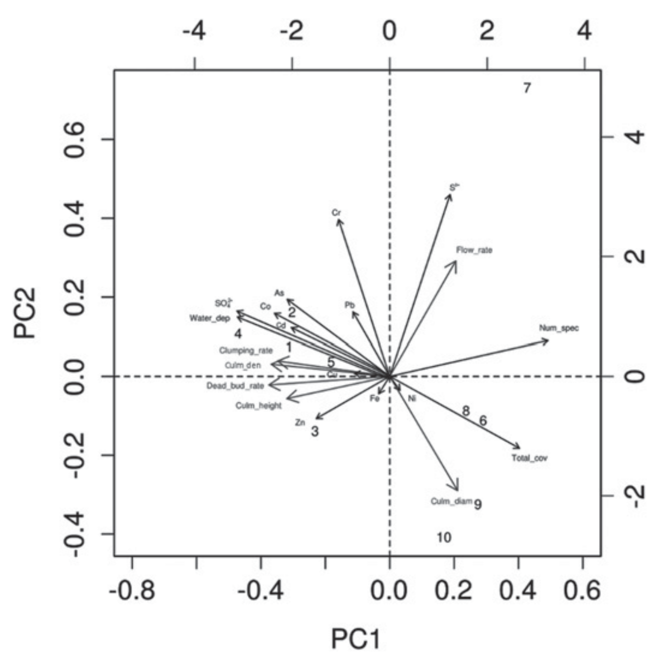

Fig. 3. Principal Component Analysis ordination diagram of plots and macromorphological traits with ecological features superimposed. Flooded plots = nos. 1–5; emerged plots = nos. 6–10.

right quadrants. This pattern can be explained by the water depth vector that significantly increases towards the negative values of the first axis. Along the first axis, we can note a gradient concerning clumping, culm height, dead bud rate and culm density, which increase along the negative side of the axis. The second axis showed a gradient concerning the culm diameters that were generally larger in the emerged stands than in the flooded stands; also the flowering rate showed a gradient along the second axis. The first axis of PCA (Table 7) appeared to be significantly and negatively correlated with sulfate, As and Co and positively correlated with the number of species and the total cover of the reed bed communities.

Table 7. Pearson's correlation among the sediment chemical measurements, the community floristic-vegetational features and the first two PCA ordination axes.

	Axis 1	Axis 2
SO_4^{2-}	– 0.8934***	0.1731
S^{2-}	0.5368	0.7187*
As	– 0.6392*	0.5368
Co	– 0.6882*	0.1635
Cr	– 0.4569	0.6226*
Water depth	– 0.8824***	0.1597
Number of species	0.8993***	0.0899
Total cover	0.7759**	– 0.1918

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

The second axis was positively correlated with sulfide (related particularly to plot No. 7) and Cr.

Discussion

Lake Montepulciano has shown a strong reduction of reed-dominated vegetation, as demonstrated by the diachronic analysis. This plant community has decreased in surface in less than 30 years, and in some sectors of the lake linear retreat of the reed front amounted to more than 400 m. Among the possible reasons for this trend, it should be noted that, as previously reported, in the study area several hydraulic works were carried out starting from the end of the 1990s in order to prevent silting of the lake (A.S.T.R.A., 2009). Some of these actions, such as excavation of five artificial ponds, have affected the marshy and semi-terrestrial areas externally to the lake shores, and caused variations of reed bed surface, which were not dependent on the die-back phenomenon. Thus, besides considering the variations of the total reed bed surface, we can observe retreat of the water-front of the reeds from the deepest areas of the lake toward the shores, where the works were not undertaken. Similar trends were reported for Lake Chiusi and Lake Trasimeno, where the disappearance of large areas of marsh vegetation was recorded, with evident impacts on these lake ecosystems (Gigante *et al.*, 2011, 2014).

The stands dominated by *Phragmites australis* play an important role for aquatic and wetland fauna and it is known that the decline of the reed-bed has repercussions on the associated fauna (*e.g.* Graveland, 1998; Velatta *et al.*, 2014). As also observed in several European wetlands (Ostendorp, 1989), the reduction of reed stands at Lake Montepulciano can affect the survival of some specialized *taxa* and, in particular, can disrupt at least part of the life cycle of some birds like *Botaurus stellaris*, *Ixobrychus minutus* and *Acrocephalus arundinaceus*, all present in the study area (A.S.T.R.A., 2009).

The analysis of the macromorphological traits of *P. australis* in the study area confirms the results obtained from previous studies in Europe (*e.g.* Ostendorp, 1989; Čížková *et al.*, 1996; Rea, 1996; Gigante *et al.*, 2011, 2014), which showed relationships between water depth, duration of the flooding period and *P. australis* die-back or retreat.

Strong differences in several macromorphological traits between stands subjected to a permanent vs. temporary flooding regime were detected. Some traits often associated with the die-back phenomenon (such as the clumping habit, high culm density, high number/rate of dead buds) appeared significantly more evident in the permanently flooded stands rather than in the emerged ones. The flooded stands also showed two more features correlated with the die-back syndrome, as reported in the previous studies from Central Italy (Gigante *et al.*, 2011): a lower culm diameter and a lower flowering rate, although differences are not statistically significant in this study.

The culm height of *P. australis* is the only trait that showed an unexpected pattern compared with previous studies, being higher in the permanently flooded stands than in the emerged ones. It is of note that in Lake Montepulciano, these stands were located in rather deep waters (mean water depth of 93.6 ± 21.3 vs. 37.2 ± 18.2 cm, for Lake Montepulciano and Lake Chiusi, respectively). The presence of reeds with signs of die-back in remarkable deep waters may be interpreted as an early stage of reed die-back, probably related to the recent water level changes due to hydraulic management. As a consequence of the current declining conditions, further retreat of reeds can be expected, leading in the short term to the complete disappearance of the reed beds from the deepest waters, as already observed in other lakes (*e.g.* Gigante *et al.*, 2014).

As previously mentioned, in the study area the water level was recently regulated by the restoration of a small dam. In other studies, the alteration and/or artificial regulation of water levels were reported as possible causes of the reed decline (Ostendorp, 1989; Rea, 1996). Several processes can be related to prolonged submersion, such as reduction of carbohydrate availability (van der Putten, 1993; van der Putten *et al.*, 1997) and an increase of eutrophication processes (Gaberščik *et al.*, 2003), both negatively affecting common reed growth (Rea, 1996). Prolonged submersion can lead to an extreme lack of oxygen in the rhizosphere, favoring anoxia, production of phytotoxines (Ponnamperuma, 1984) and infection by pathogens (Nechwatal *et al.*, 2008).

Strong differences between flooded and emerged stands are also due to bioecological features such as structure and composition of the reed-dominated communities. According to Gigante *et al.* (2013), the permanently flooded and declining stands are typically poor in species (often monospecific) and have a lower total cover when compared with the emerged stands. Conversely, Gigante *et al.* (2013) highlighted the species richness of the emerged reed bed, with a high presence of nitrophilous species but poor in marshland or hygrophilous plants present in the typical palustrine habitat of *P. australis*.

In the study area, nitrophilous elements species (*e.g.* *Sorghum halepense, Bidens tripartita, Calystegia sepium, Urtica dioica, Atriplex latifolia, Rubus caesius, Galega officinalis*) appeared numerically consistent in the emerged stands. Gigante *et al.* (2013) underlined the different conservation value of these so-called "pseudo-reed beds", which from a floristic and ecological point of view cannot constitute compensation for the retreat and loss of the flooded reed beds.

Gigante *et al.* (2014) showed the relationships between macromorphological traits related to reed die-back and some chemical parameters of waters and sediments in nearby Lake Chiusi.

Our data show that several sediment parameters are associated with the die-back symptoms, although as also pointed out by Gigante *et al.* (2014), some of these relations might be accidental.

Sulfate appears directly related to several traits indicating symptoms of decline. At Lake Montepulciano, remarkably high sulfate concentrations were measured in the sediment of the flooded stands. Cortecci and Dinelli (1999) suggested that the presence of sulfate in the waters of Lake Montepulciano was derived from the bottom springs recharged from carbonate-evaporitic outcrops and/or provided by the feeding stream. According to the low isotopic values of sulfur isotopes in sulfate (expressed as $d^{34}S\text{-}SO_{4(aq)}$ ‰ V-CDT), and the respective seasonal variations from 1968 to 1971, the authors supposed that the sulfate isotopic composition of Lake Montepulciano waters could likely be ascribed to biochemical processes related to the biological cycle of aquatic macrophytes, and in particular reed prairies. The strikingly high concentrations of sulfate in the sediments might be related to the presence of gypsum ($CaSO_4xH_2O$) possibly caused by its direct precipitation from the relatively sulfate-rich lake waters (see Cortecci and Dinelli, 1999; Nisi, 2005) during the periods of low waters of the past years. This was confirmed by modeling the chemical composition of Lake Montepulciano waters reported in Nisi (2005) and simulating the amount of water to be evaporated in order to precipitate gypsum by the geochemical code PHREEQC (Parkhurst and Appelo, 1999). The Montepulciano water is slightly saturated in carbonate minerals such as calcite and dolomite and undersaturated in gypsum. Gypsum is expected to precipitate when more than 80% of the lake water is evaporated, this amount being compatible with the lake oscillation reported in the past (F. Boschi, *personal communication*). Considering the high agricultural vocation of the surrounding areas, it is also conceivable that residues from fertilization are a significant source of sulfates (Cortecci *et al.*, 2002). Moreover, Di Giovanni *et al.* (1988) showed high presence of sulfates coming from thermal waters in one of the lake tributaries.

A certain release of oxygen can arise from plant roots by diffusion to the sediment, where it can support the re-oxidation of reduced compounds such as sulfide (Armstrong and Armstrong, 1988; Ágoston-Szabó, 2004). However, it must be noted that presently in the study area, the flooded stands are heavily affected by die-back and according to Armstrong *et al.* (1996), declining sites generally exhibit a low redox potential. Therefore, a substantial contribution by the rhizosphere of *P. australis* stands to the sulfate values measured in the flooded plots of Lake Montepulciano is rather implausible.

Although *P. australis* is considered a tolerant species to high concentrations of heavy metals, often acting as a bioaccumulator (*e.g.* Ye *et al.*, 1997; Bonanno and Lo Giudice, 2010), some authors have shown possible negative effects under certain circumstances (Boar and Crook,1985; Weisner, 1996; Gigante *et al.*, 2014).

Univariate analysis shows that As, Cd and Co in the sediment of Lake Montepulciano, although at relatively low concentrations in the sediment leachates, seem to be related to some traits associated with the die-back symptoms and with the occurrence of flooded conditions. Nevertheless, *P. australis* has a certain ability to accumulate As (Ghassemzadeh *et al.*, 2008; Afrous *et al.*, 2011). Besides that, no discernible differences in root, leaf or rhizome growth parameters were observed in Cd-treated *P. australis*, which is therefore considered as a plant with high potential for Cd detoxification (Iannelli *et al.*, 2002; Ederli *et al.*, 2004). Cobalt concentrations correlated negatively with culm diameter at Lake Chiusi (Gigante *et al.*, 2014) and this trend was also confirmed for Lake Montepulciano. In addition, for Cr an inverse correlation with the culm diameter can be observed from the multivariate analysis. Several studies have shown that *P. australis* is also able to absorb and accumulate Co and Cr (*e.g.* Bragato *et al.*, 2009; Ayeni *et al.*, 2012; Ranieri *et al.*, 2013), although some harmful effects of Cr on *P. australis* were recorded (Calheiros *et al.*, 2008).

Our results may suggest a possible synergy of this pool of sediment heavy metals with high sulfate values in reducing the viability of submerged stands of *P. australis*. According to Batty and Younger (2004), *P. australis* growth seems to be heavily affected by high metal concentrations associated with high levels of sulfate. In addition, comparing plots with different concentrations of heavy metals, Ayeni *et al.* (2012) showed effects such as decreasing chlorophyll concentrations and photosynthetic rates for *P. australis* at sites with high metal rates.

Conclusion

This study supports the occurrence of a new case of reed die-back, confirming an expanding trend of this phenomenon in the Mediterranean basin. Our results allowed us to reassess the relationships between some macromorphological traits and reed retreat. From an ecological point of view, permanent-artificially induced flooding conditions seem to be a key factor for the occurrence of die-back, as already observed in other studies in Europe. Lake sediments showed the presence of some chemical species such as sulfate, As, Co and Cd that might be correlated with macromorphological traits, indicating the declining condition of *Phragmites australis*. These results thus help to outline a pattern of co-causes for reed decline, mostly centered on the harmful combination of chemical traits co-occurring with permanent flooding, a fixed ecological condition often favored by human management of wetlands. Only by way of further investigations and case studies, at present still relatively scanty, particularly in the Mediterranean area, can a more comprehensive understanding of this complex phenomenon be achieved, and a robust interpretative model drawn.

Acknowledgements. This research was funded by the Siena Province Administration-Sector for Fauna Resources and Protected Areas (recently merged with the Tuscany Region Administration-Sector for the Protection of Nature and Sea). L. Giannini and M. Paolieri (Department of Earth Sciences – Florence) are warmly thanked for their assistance during the sediment and interstitial water sampling and PHREEQC geochemical modeling, respectively. F. Boschi (Hydraulic Official of the Tuscany Region Administration) provided useful information about Lake Montepulciano; his help is gratefully acknowledged. Particular thanks are due to D. Nonis (Tuscany Region Administration-Sector for the Protection of Nature and Sea) and B. Anselmi, D. Morrocchi and F. Sassetti (Provincial Agency for Energy, Environment and Sustainable Development, Siena), for supporting and promoting this research. The authors would like to thank the two anonymous reviewers for their useful suggestions.

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Appendix 1

Geographic coordinates of the ten plots (reference system WGS84; latitude and longitude expressed in decimal degrees)

1 (43.09353, 11.91900); 2 (43.09126, 11.91645); 3 (43.08946, 11.91516); 4 (43.08693, 11.92293); 5 (43.09433, 11.91895); 6 (43.09109, 11.9083); 7 (43.08706, 11.90762); 8 (43.08030, 11.91141); 9 (43.075944, 11.91496); 10 (43.08656, 11.92733).