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Wetland Plant Diversity in a Coastal Nature Reserve in Italy: Relationships with Salinization and Eutrophication and Implications for Nature Conservation

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Abstract

Wetlands are important centers of biodiversity. Coastal wetlands are subject to anthropogenic threats that can lead to biodiversity loss and consequent negative effects on nature conservation. We investigated relationships between wetland vegetation and habitat conditions in a coastal Nature Reserve in Northern Italy that has undergone seawater intrusion and eutrophication for several decades. The wetland vegetation in the Nature Reserve consisted of nine communities of hygrophytic and helophytic vegetation and five communities of waterplant vegetation. The hygrophytic and helophytic communities were arranged according to a salinity gradient, from salt-free habitats to strongly saline habitats. The saline habitats had high nutrient levels, due to the influx of nitrate-rich saltwater from an adjacent lagoon. The waterplant communities were all typical of freshwater habitats. Water-table depth and concentration of dissolved nutrients in the water were the main factors structuring waterplant vegetation. The main driver of future changes in the wetland vegetation of the Nature Reserve is the ongoing increase in salinity levels which may enhance expansion of halophilic species and communities, thus outcompeting locally rare freshwater species. If nutrient, especially nitrate, load further increases in the next future, this may exert negative effects on wetland species and communities preferring nutrient-poor habitats.

Keywords Aquatic plants · Biodiversity · Nitrate · Nutrient load · Saline wedge · Vegetation

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Introduction

Wetlands represent important centers of biodiversity, hosting specialized plant and animal species. Wetlands generally exhibit high level of functional diversity (Bedford et al. 2001) and thus assume a central role for nature conservation (García-Madrid et al. 2014; Angiolini et al. 2017). Wetlands are prone to a number of threats which mine their survival: over-exploitation, habitat fragmentation, water eutrophication and pollution, human alteration of natural water cycles and alien species invasion are only some of the main threats and pressures which affect wetlands worldwide (Bedford et al. 2001; Dudgeon et al. 2006). Coastal wetlands are not immune from anthropogenic threats. Natural events such as erosion, hydrological and hydrochemical alterations, sea-level rise and subsidence may specifically affect these wetland types (Turner 1990). In particular, the Mediterranean basin represents one of the regions most prone to loss of coastal wetlands in the future (Nicholls et al. 1999).

Wetland plant diversity is scale-dependent and varies across regions, showing a decreasing trend from more humid toward less humid climatic regions (see Hrivnák et al. 2014 and

references therein). A number of environmental variables play an important role in driving plant species diversity in wetlands, such as size of the site, ground morphology, water-table depth and water chemistry (Edvardsen and Økland 2006; Lacoul and Freedman 2006; Hrivnák et al. 2014). Salinity is paramount for shaping the distribution of plant species in coastal wetlands (Watt et al. 2007). Salinity becomes a critical factor when sea-level rise and/or subsidence increase salinity levels, thus altering the composition and the distribution of wetland plant communities (Brock et al. 2005; Spalding and Hester 2007). Indeed, stress resulting from increased salinity has been reported as a cause of biodiversity loss in terrestrial to riparian and aquatic habitats (see Brock et al. 2005 and references therein). Ecological modifications due to increased salinity levels can also trigger dynamic processes which can eventually lead to dramatic changes in the vegetation of coastal wetlands (Donnelly and Bertness 2001). Water eutrophication is considered one of the most important causes of degradation of many types of wetlands, from inland to marine ecosystems. Zaldivar et al. (2008) focused on direct and indirect effects of eutrophication of transitional waters, showing a complex net of drivers and pressures brought about by eutrophication which in turn reverberate in complex socio-economic and ecological aspects. The consequences of nutrient enrichment include development of algal blooms which can lead to anoxic conditions with negative impact on waterfowl, fish, and invertebrates (Davis and Froend 1999). Water chemistry is also important in determining abundance, composition, and distribution of wetland communities. Consequently, several types of plant communities have been identified as indicators of water quality (Klosowski and Jablońska 2009; Ceschin et al. 2010; Sakurai et al. 2017). Anthropic activities such as urbanization and agriculture produce deep modifications in the chemical and physical features of the water ecosystems, inducing significant changes in the spatial pattern of wetland vegetation (Ceschin et al. 2010).

Vegetation dynamics, i.e., changes in vegetation composition over time, represents one of the most frequent causes of loss of habitats of conservation interest (Viciani et al. 2014). In Europe, plant and habitat conservation benefits from a powerful tool provided by the European Union, i.e., the Habitats Directive (Commission of the European Community 1992). The identification of habitat types and the selection of sites are supported by the implementation of the Interpretation Manual of European Union Habitats (Commission of the European Community 2013). Another important document is the European Red List of Habitats that provides an overview of the degree of endangerment of terrestrial and freshwater habitats in the European Union (Janssen et al. 2016). At the regional level, Biondi and Blasi (2009) produced the Italian Interpretation Manual of European Union Habitats and, more recently, Angelini et al. (2016) produced a handbook for monitoring habitats of community interest in Italy. This handbook is based on European guidelines (Evans and Arvela 2011) and

provides tools for an effective monitoring activity of Annex I Habitats occurring in Italy (Gigante et al. 2016). These documents not only show that wetlands host habitats of conservation importance but also report that these habitats are often subject to serious threats and pressures. Several vegetation types strictly related to wetlands, often widespread in Central Europe but very rare in Southern Europe, do not fall in any habitat type of conservation interest listed in the Habitat Directive (Angiolini et al. 2017). Analyzing relationships among species, plant communities, and ecological drivers provides important and useful information that can be used for the protection and monitoring of plants and habitats of conservation interest either listed in environmental protection laws or not.

The Nature Reserve "Bosco della Mesola" represents a relict of ancient coastal forests. Most of these forested areas disappeared in more or less recent times, while others underwent deep changes. Consequently, very few coastal forests still occur in relatively pristine conditions in this region (Stampi 1966; Piccoli et al. 1983; Pellizzari and Piccoli 2001). The Nature Reserve is rich in wetlands that host different plant communities, including both freshwater and brackish vegetation types. This area has undergone subsidence and eutrophication for several decades, which can potentially trigger significant changes in wetland vegetation. In this study, we analyzed relationships between wetland vegetation and environmental conditions in the Nature Reserve. Our main objective was to analyze environmental factors affecting wetland vegetation, with special focus on habitats and species of conservation interest. We also aimed at forecasting future changes in wetland vegetation and defining appropriate guidelines directed to implementing and updating the Management Plan of the Nature Reserve.

Materials and Methods

Study Area The study was carried out at Bosco della Mesola, North-Eastern Italy (44°50′ N, 12°15′ E, 1088 ha, 0–2.8 m above sea level, Fig. 1), a National Nature Reserve hosting Special Areas of Conservation, Special Protection Areas and a strict Nature Reserve area in the Po Delta Park. The study area lies on a dune system originated during the eleventh to fifteenth centuries, consisting of sand dunes and dune slacks with parallel, approximately North-South orientation. This area is prevalently covered with woodlands that represent a relict of ancient coastal forests (Stampi 1966; Piccoli and Gerdol 1984; Piccoli et al. 1983). Most of these areas disappeared in more or less recent times, while others underwent deep changes (Stampi 1966). The woodlands in the Reserve present transitional features between Mediterranean and Central European forest types (Gerdol et al. 1985). A xerophilic *Quercus ilex* community is situated on high, more recent arid dunes. A mesophilic *Carpinus betulus–Quercus robur* community is situated on flat, more

Fig. 1 Map of the study area with location of the sampling sites in the five wetland types
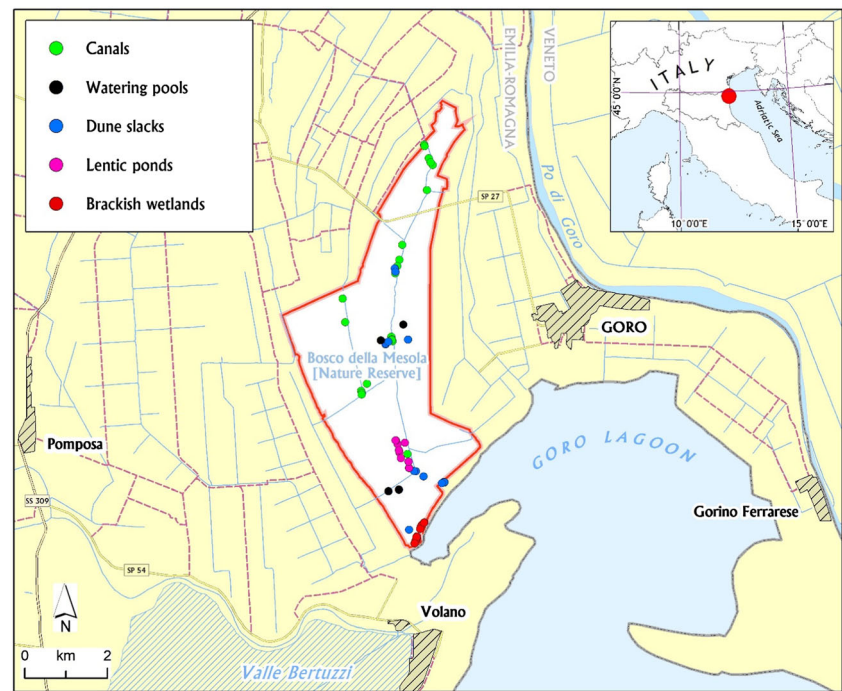
ancient and less arid dunes. The dune slacks are colonized by a *Populus alba–Fraxinus angustifolia* community typical of wet habitats (Gerdol et al. 1985). Hydrologically, this area is characterized by a coastal aquifer system in hydraulic continuity with the sea. This determines saltwater inflow which enables the development of halophilic vegetation in the marginal south-eastern part of the area (Piccoli et al. 1983; Fig. 1). Three main soil types occur in this area: typic xeropsamments, aquic xeropsamments and psammaquents, considerably differing from each other in depth and structure. However, all of these soils are rich in sand and have A-C profile and moderately alkaline pH (Gerdol et al. 1985). The soil pore-water is enriched in salt at the south-eastern border of the area (Piccoli et al. 1983).

Wetlands and their Management The study area hosts a rich variety of freshwater and brackish wetlands. Natural freshwater wetlands in the inland part of the area are mainly situated in temporarily flooded dune slacks not covered with forest vegetation. Therefore, dune slacks are prevalently fed by rainwater, and secondarily by water flowing from canals so that water-table depth in these wetlands is conditioned by precipitation and by the water level in the canal system. Natural brackish wetlands are concentrated in the south-eastern marginal part of the area and are fed with saltwater from an adjacent lagoon (Fig. 1). These brackish wetlands usually are flooded throughout the year, except during prolonged dry periods in summer. Three types of artificial wetlands occur in the study area: (1) a network of canals, (2) a number of pools used for watering wildlife, and (3) a lentic pond permanently fed by discharging water table. The watering pools have a more or less regular circular shape and usually are flooded, except

during periods of prolonged drought. A dense network of canals represents the main source of water supply to the Nature Reserve. This is of fundamental importance for supporting basic ecological processes that eventually guarantee correct functioning of the forest ecosystem. The canals provide freshwater from the surrounding areas, whereas a number of locks limit the intrusion of saltwater from the sea (the so-called saline wedge). Indeed, the Nature Reserve is situated in a reclamation area so that meteoric water must be drained in winter, while oxygen-rich freshwater must be supplied to the forest in summer. Over time, these canals gradually turned from simple hydraulic systems to complex ecosystems, representing preferential habitats for aquatic plants, fish, and herpetological species. The management of the protected area follows the guidelines stated in the Reserve Management Plan, as well as a series of conservation actions defined in specific LIFE projects (e.g., LIFE00NAT/7147). Major objectives are the conservation of priority habitats and species of the Habitats Directive. Most of the relevant conservation actions are closely related to the hydraulic management of the water supply through the canal systems.

Sampling and Analysis Wetlands in the study area were surveyed several times during the growing season (May–September) of 2016. A total of 84 phytosociological relevés were carried out at the peak of the growing season in all of the wetland types occurring in the Nature Reserve (Table 1; Fig. 1). Alluvial forests of the *Populus alba–Fraxinus angustifolia* community, although inhabiting wet habitats, were not considered in our survey. The choice of the sampling sites was subjective, but the number of relevés approximately

Table 1 List of the wetland types, with cover area and number of vegetation relevés

Wetland type	Area (ha)	No. of relevés
Canals	10.0	22
Watering pools	1.2	7
Dune slacks	14.9	25
Lentic ponds	7.1	16
Brackish wetlands	9.2	14

mirrored frequency and area covered by each wetland type (Table 1), partially calibrated by visual inspection of vegetation diversity in each wetland type.

Plant communities were sampled according to the phytosociological method (Braun-Blanquet 1932; Biondi 2011; Pott 2011). A phreatimeter was placed and the following data were recorded at each relevé site: GPS coordinates (UTM33, WGS84), relevé area (m^2), and vegetation cover (%). At the end of the growing season, water-table depth was measured and a 50-mL water sample was collected at each of the relevé sites. All water-table measurements and water collections were carried out on a single day (25 October 2016) in order to make the data comparable. The day after collection, electrical conductivity and salinity were measured with a Crison CM 35 conductivity-meter (Hach-Lange, L'Hospitalet de Llobregat, Spain), and pH was measured with a Hanna Jenway 3510 pH-meter (Hanna Instruments, Villafranca Padovana, Italy). The water samples were immediately filtered with Whatman 25-mm GD/X syringe filters (pore size 0.45 μm) and colorimetrically analyzed for ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) concentrations using a continuous-flow autoanalyzer (Systea Flowsys, Anagni, Italy). Ammonium was determined by the salicylate method in the presence of hypochlorite, nitrate by the cadmium-reduction method through a column, and phosphate by the molybdenum-blue method. Alkalinity (HCO_3^-) was determined by the double indicator method with phenolphthalein and methyl orange.

The spatial distribution of electrical conductivity was graphically represented by a Geographical Information System (GIS), using the software ArcView GIS 3.2 for Windows and IDW interpolation. The resulting map was compared with a similar map obtained using data on electrical conductivity measured in 2003 at 60 sites, most of which coincided with the sampling sites of the present study.

Statistics The matrix of the vegetation data (i.e., the cover of each species in the 84 relevés) was statistically treated by a hierarchical numerical classification method after transforming the ordinal scale used for assessing species cover. Scale transformation was performed using the van der Maarel (1979) numerical scale. A cluster analysis (i.e.,

numerical classification) was performed by the Ward method based on the matrix of between-relevé Euclidean distances (Orlóci 1978). Relationships between species composition and environmental variables were statistically analyzed by a multivariate ordination method (detrended canonical correspondence analysis, DCCA). Significance level of the environmental variables was assessed by Monte Carlo permutations based on forward selection of the variables. Occasional species, i.e., those recorded in one or two relevés only, were excluded from the cluster analysis and the multivariate ordinations as well.

Species composition of the plant communities defined by the numerical classification was assessed in terms of species rarity. Species rarity was calculated based on rarity indices of all plant species, i.e., also including the occasional species not considered in the multivariate analyses. The rarity indices (Table 2) were derived from estimates of species frequency in the local plant species checklist (Piccoli et al. 2014). The cluster analysis and the DCCA were performed with the statistical software CANOCO 5.0 (ter Braak and Šmilauer 2012). Univariate statistics were computed with the statistical software STATISTICA 7.0 (StatSoft©; Version 7; StatSoft Inc., Tulsa, OK, USA).

Results

Vegetation Classification The vegetation relevés were clustered into two big groups (Fig. 2). The first group (rel. 1–56) included relevés of hygrophytic or helophytic vegetation, while the second group (rel. 57–84) included relevés of waterplant communities (sensu Den Hartog and Segal 1964). Fourteen clusters were recognized at a lower hierarchical level in the dendrogram, i.e., at a Euclidean distance of 25–30 (Fig. 2). These clusters represented the main wetland vegetation types in the Nature Reserve. A synthetic description of these communities, as well as their correspondence with the Natura 2000 habitat codes and the Red List Habitat EUNIS codes, is given in Table 3. Details on the full species composition can be found in Supplementary Table 1.

Table 2 List of the rarity indices derived from estimates of species abundance in the local plant species checklist

Species frequency in the checklist	Rarity index
Very rare	6
Rare	5
Uncommon	4
Widespread	3
Common	2
Very common	1
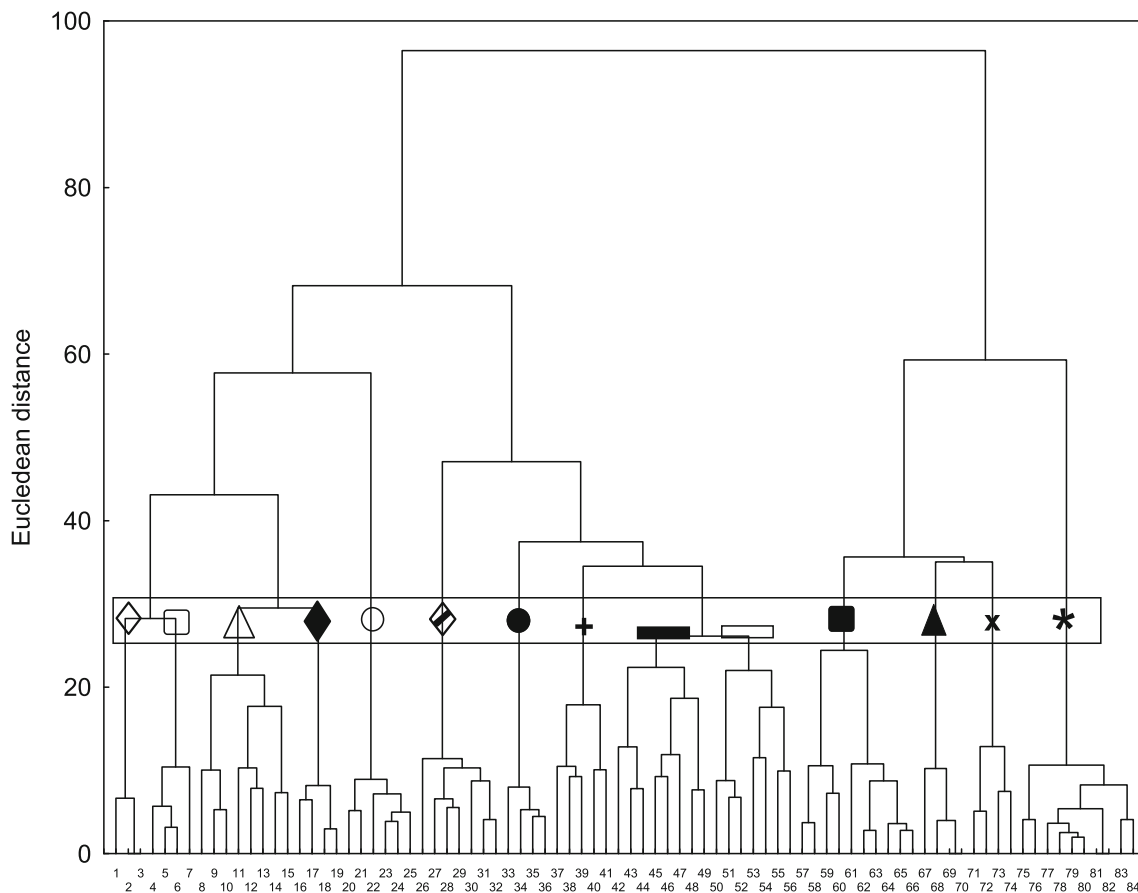
Fig. 2 Classification dendrogram of the 84 vegetation relevés. The rectangle indicates the range of Euclidean distance (ca. 25–30) in which the clusters corresponding to the 14 plant communities were recognized (symbols as in Fig. 3)

In brief, nine hygrophytic and helophytic communities were recognized based on the cluster analysis (Fig. 2; Table 3). Two of these communities (c. of *Spartina anglica* and c. of *Tripolium pannonicum*) were composed of few halophytes, exclusive of brackish wetlands. Three communities (c. of *Juncus acutus* and *J. littoralis*, c. of *J. subnodulosus* and *Phragmites australis*, and c. of *J. acutus* and *J. maritimus*) had different rush species and/or common reed as dominant species. These three communities were richer in species and were composed of more or less strongly salt-tolerating hygrophytes and helophytes. They were found in brackish wetlands, lentic ponds, and dune slacks. The community of *Cladium mariscus* was found only in dune slacks and had *Cladium mariscus* as the dominant species with scattered occurrence of a few other, moderately salt-tolerating species. Three further communities (c. of *Schoenus nigricans* and *Tripidium ravennae*, c. of *Juncus maritimus* and *Galium palustre*, and the wet meadows) were composed of several species generally possessing low levels of salt tolerance. These communities usually occurred in lentic ponds and dune slacks, and occasionally in watering pools with poor if any saltwater influx. The waterplant communities were overall poorer in species compared with the hygrophytic and helophytic communities. Five waterplant

communities were recognized based on the cluster analysis (Fig. 2; Table 3). Four of them were characterized by dominance of an only waterplant species each. These dominating waterplant species were both pleustophytes (Den Hartog and Segal 1964), i.e., waterplants floating on the water surface (*Spirodela polyrrhiza* and *Lemna minuta*) or floating freely between the bottom and the surface (*Ceratophyllum demersum*) and rhizophytes (Den Hartog and Segal 1964), i.e., waterplants with their basal parts penetrating into the bottom (*Myriophyllum spicatum*). These four communities were usually found in canals. The fifth waterplant community (c. of *Chara* sp.) was prevalently comprised of rhyzophytes, with *Chara vulgaris* or *C. intermedia* (not separated in the field during the relevés) usually dominating. This community was found only in watering pools.

With respect to conservation value, the community of *Chara* sp. stood out among all other communities because of the presence of three locally very rare species: *Chara fragilis*, *C. intermedia*, and *Ranunculus peltatus* ssp. *baudotii* (Supplementary Table 1). Hence, this community presented the highest rarity index (Table 4). The community of *Chara* sp. presented considerable importance with respect to habitat type because this community is quite rare in the lowland

Table 3 Synthetic description of the vegetation types with reference to Habitat Natura 2000 codes and European Red List of Habitats—EUNIS codes

Community type	Species composition	Wetland type	Natura 2000 code	EUNIS code
C. of *Spartina anglica*	Almost pure *Spartina anglica* stands	Brackish wetlands	1320	A2.5d
C. of *Tripolium pannonicum*	Very species-poor community with *Tripolium pannonicum* as the dominant species and scattered occurrence of few other helophytes	Brackish wetlands	1410	A2.5d
C. of *Juncus acutus* and *J. littoralis*	Dominated by halophytes or salt-tolerant species often with high frequency of *Phragmites australis*	Brackish wetlands, dune slacks, lentic ponds	1410	A2.5d
C. of *Juncus subnodulosus* and *Phragmites australis*	Co-dominated by *Juncus subnodulosus* and *Phragmites australis* with scattered occurrence of salt-tolerant species	Lentic ponds	–	C5.1a
C. of *Juncus acutus* and *J. maritimus*	Co-dominated by *Juncus acutus* and *J. maritimus* with low frequency of *Phragmites australis* and some other salt-tolerant species	Dune slacks	1410	A2.5d
C. of *Cladium mariscus*	Species-poor community strongly dominated by *Cladium mariscus*	Dune slacks	7210	B1.8b (D4.1b)
C. of *Schoenus nigricans* and *Tripidium ravennae*	Species-rich community co-dominated by *Schoenus nigricans* and *Tripidium ravennae* together with several moderately salt-tolerating species	Lentic ponds	6420	E3.1a
C. of *Juncus maritimus* and *Galium palustre*	Species-rich community co-dominated by *Juncus maritimus* and *Galium palustre* with modest, if any, saltwater influx	Dune slacks	6420	B1.8b (E3.1a)
Wet meadows	Species-rich community having *Mentha aquatica*, *Molinia arundinacea*, and *Scirpoides holoschoenus* as the most abundant species associated with several poorly salt-tolerating species	Dune slacks, watering pools, lentic ponds	6420	E3.1a (B1.8b)
C. of *Spirodela polyrrhiza*	Species-poor community dominated by the small pleustophyte *Spirodela polyrrhiza*	Canals	3150	C1.2b
C. of *Ceratophyllum demersum*	Species-poor community dominated by the freely-floating pleustophyte *Ceratophyllum demersum*	Canals	3150	C1.2b
C. of *Lemna minuta*	Extremely species-poor community dominated by the small pleustophyte *Lemna minuta*	Canals, watering pools	3150	C1.2b
C. of *Chara* sp.	Species-poor, prevalently rhizophytic community with *Chara vulgaris* and/or *C. intermedia* as the dominant species and rather high frequency of *Potamogeton pectinatus* and *Ranunculus peltatus* ssp. *baudotii*	Watering pools	3140	C1.2a
C. of *Myriophyllum spicatum*	Species-poor community dominated by the rhyzophyte *Myriophyllum spicatum* usually associated with *Ceratophyllum demersum*	Canals	3150	C1.2b

regions of northern Italy. Other communities were important in terms of species rarity, although presenting lower rarity index (Table 4; Supplementary Table 1). Firstly, the community of *Juncus acutus* and *J. littoralis* hosted one of the very few populations of *Kosteletzkya pentacarpos* in Italy. This species is listed both in the 92/43/CEE Habitat Directive (Annex II) and in the Bern Convention (Annex I) and is considered threatened with extinction in Italy and in Europe, where *Kosteletzkya pentacarpos* is classified as "critically endangered" and "vulnerable," respectively (Bilz et al. 2011; Rossi et al. 2016). Secondly, the community of *Juncus maritimus* and *Galium palustre* hosted three locally very rare species, i.e., *Euphorbia lucida*, *Hydrocotyle vulgaris*, and *Teucrium scordium* (Table 4; Supplementary Table 1). The community of *Juncus subnodulosus* and *Phragmites australis*

and the community of *Cladium mariscus* had quite high rarity indices for being characterized by high abundance of the locally very rare species *Juncus subnodulosus* and *Cladium mariscus*, respectively (Table 4; Supplementary Table 1). The species-poor community of *Lemna minuta* had a high rarity index owing to the high abundance of the locally uncommon, but invasive alien species *Lemna minuta*. Therefore, the rarity index seemed to overemphasize the real conservation value of this community. All of the other communities did not exhibit remarkable conservation value based on the rarity index (Table 4).

Ecological Gradients As the hygrophytic and helophytic vegetation on one side and the waterplant vegetation on the other side differed very strongly from each other in terms of species

Table 4 Mean (± SE) values of the rarity index and of hydrochemical variables in nine communities of hygrophytic and helophytic vegetation and five communities of waterplant vegetation. For each variable in each of the two vegetation groupings, the means followed by the same letter do not differ significantly ($P < 0.05$) based on Tukey's HSD post hoc tests (capital letters for hygrophytic and helophytic vegetation; small letters for waterplant vegetation)

Community	Rarity index	pH	Electrical conductivity ($\mu S\ cm^{-1}$)	Salinity ($mg\ L^{-1}$)	Water-table depth (cm)	Alkalinity ($mg\ L^{-1}$)	NO_3^- ($mg\ L^{-1}$)	PO_4^{3-} ($mg\ L^{-1}$)
Hygrophytic and helophytic vegetation								
C. of *Spartina anglica*	2.67 ± 0.17 B	7.57 ± 0.03 A	38,967 ± 524 A	23,833 ± 384 A	8.0 ± 0.6 AB	186 ± 11 B	0.540 ± 0.087 A	0.053 ± 0.003 B
C. of *Tripolium pannonicum*	2.69 ± 0.19 B	7.58 ± 0.03 A	34,375 ± 2226 A	10,342 ± 6088 B	4.5 ± 1.9 ABC	204 ± 16 B	0.349 ± 0.077 A	0.042 ± 0.004 B
C. of *Juncus acutus* and *J. littoralis*	2.93 ± 0.11 B	7.63 ± 0.06 A	12,801 ± 2932 B	7268 ± 1739 B	4.9 ± 4.1 AB	363 ± 48 B	0.110 ± 0.049 B	0.175 ± 0.106 B
C. of *Juncus subnodulosus* and *Phragmites australis*	3.31 ± 0.12 AB	7.45 ± 0.06 A	1196 ± 149 C	576 ± 73 C	14.0 ± 3.0 A	206 ± 13 B	0.007 ± 0.001 B	0.004 ± 0.001 B
C. of *Juncus acutus* and *J. maritimus*	2.66 ± 0.12 B	7.36 ± 0.04 A	16,539 ± 493 B	9269 ± 297 B	−0.7 ± 3.5 ABC	412 ± 31 B	0.030 ± 0.004 B	0.305 ± 0.155 B
C. of *Cladium mariscus*	3.04 ± 0.1 AB	7.53 ± 0.17 A	6207 ± 2987 BC	3363 ± 1671 BC	−19.8 ± 4.2 BC	358 ± 202 B	0.054 ± 0.016 B	0.562 ± 0.555 AB
C. of *Schoenus nigricans* and *Tripidium ravennae*	2.68 ± 0.2 B	7.50 ± 0.00 A	2140 ± 759 C	1087 ± 403 C	−27.6 ± 12.6 C	188 ± 17 B	0.018 ± 0.009 B	0.003 ± 0.001 B
C. of *Juncus maritimus* and *Galium palustre*	3.54 ± 0.15 A	7.11 ± 0.07 B	2918 ± 532 C	1491 ± 282 C	−14.9 ± 5.7 BC	907 ± 141 A	0.102 ± 0.029 B	1.990 ± 0.701 A
Wet meadows	3.07 ± 0.25 AB	7.49 ± 0.12 A	1876 ± 775 C	969 ± 414 C	−13.0 ± 8.2 BC	349 ± 62 B	0.070 ± 0.017 B	0.011 ± 0.002 B
Waterplant vegetation								
C. of *Spirodela polyrrhiza*	2.53 ± 0.21 b	7.60 ± 0.12 a	881 ± 147 b	434 ± 74 b	15.8 ± 6.8 ab	240 ± 17 b	0.154 ± 0.085 a	0.013 ± 0.006 a
C. of *Ceratophyllum demersum*	2.24 ± 0.15 b	7.73 ± 0.17 a	497 ± 31 c	239 ± 15 c	33.0 ± 5.5 a	170 ± 14 c	0.168 ± 0.096 a	0.006 ± 0.002 a
C. of *Lemna minuta*	3.75 ± 0.25 a	7.20 ± 0.11 b	1322 ± 211 a	661 ± 112 a	12.8 ± 3.3 b	357 ± 34 a	0.550 ± 0.005 a	0.012 ± 0.002 a
C. of *Chara* sp.	4.38 ± 0.13 a	7.48 ± 0.02 a	428 ± 20 c	356 ± 10 bc	10.8 ± 1.2 b	306 ± 27 ab	0.012 ± 0.001 a	0.006 ± 0.001 a
C. of *Myriophyllum spicatum*	2.24 ± 0.11 b	7.52 ± 0.04 a	474 ± 7 c	227 ± 3 c	33.3 ± 3.5 a	190 ± 7 bc	0.046 ± 0.007 a	0.004 ± 0.001 a

composition, we run two separate DDCAs for each of the two groups (Fig. 3). All environmental factors accounted significantly for the observed variation in the species composition of the hygrophytic and helophytic vegetation (Table 5). Electrical conductivity accounted for the largest part of the variance in the hygrophytic and helophytic vegetation. Salinity was significant as well but accounted for a smaller portion of variance because of its high collinearity with electrical conductivity (Fig. 3a; Table 5). The nine hygrophytic and helophytic communities were thus arranged according to a gradient of increasing salt tolerance, parallel to the vectors of electrical conductivity and salinity, from the community of *Juncus subnodulosus* and *Phragmites australis*, the wet meadows, the community of *Schoenus nigricans* and *Tripidium ravennae*, and the community of *Juncus maritimus* and *Galium palustre*, all characterized by mean salinity levels of about 1000 mg L^{-1} or lower indicating no saltwater influx, to the community of *Juncus acutus* and *J. littoralis*, the community of *Juncus acutus* and *J. maritimus*, the community of *Tripolium pannonicum*, and the community of *Spartina anglica*, all settled in more or less strongly saline habitats. The community of *Cladium mariscus* had intermediate position across the gradient (Fig. 3a; Table 4). Similarly, the species were arranged according to increasing salt-tolerance levels from *Hedera helix*, *Brachypodium sylvaticum*, and *Vincetoxicum hirundinaria* at the left end of the gradient to *Kosteletzkya pentacatpos*, *Carex extensa*, *Atriplex prostrata*, *Spartina anglica*, and *Tripolium*

pannonicum at the right end of the gradient (Fig. 3a). The vectors of water-table depth and pH on one side, and the vectors of alkalinity and phosphate on the other side presented similar trends but opposite orientation (Fig. 3a). Water-table depth was higher in the four communities having rushes (*Juncus subnodulosus*, *J. acutus*, and *J. littoralis*), tall helophytes (*Phragmites australis* and *Typha angustifolia*), or halophytes (*Tripolium pannonicum*, *Spartina anglica*, and *Atriplex prostrata*) as dominant or abundant species. Accordingly, the centroids of these four communities (c. of *Spartina anglica*, c. of *Tripolium pannonicum*, c. of *Juncus acutus* and *J. littoralis*, and c. of *Juncus subnodulosus* and *Phragmites australis*) were located in the lower-right part of the DCCA diagram (Fig. 3a). In all of these communities, mean water-table depth was positive indicating that the soil was submerged (Table 4). These communities also presented somewhat higher mean pH although there were very poor, if any, significant differences in terms of mean pH that ranged from 7.11 to 7.63 across all of the wetland communities (Table 4). Alkalinity and phosphate were both higher in less humid habitats, with highest values in the community of *J. maritimus* and *Galium palustre* (Fig. 3a; Table 4). The nitrate vector was oriented almost parallel to the first DCCA axis. This corresponded to highest nitrate concentrations in the community of *Tripolium pannonicum* and the community of *Spartina anglica*, both having halophytes as the dominant species (Fig. 3a; Table 4).
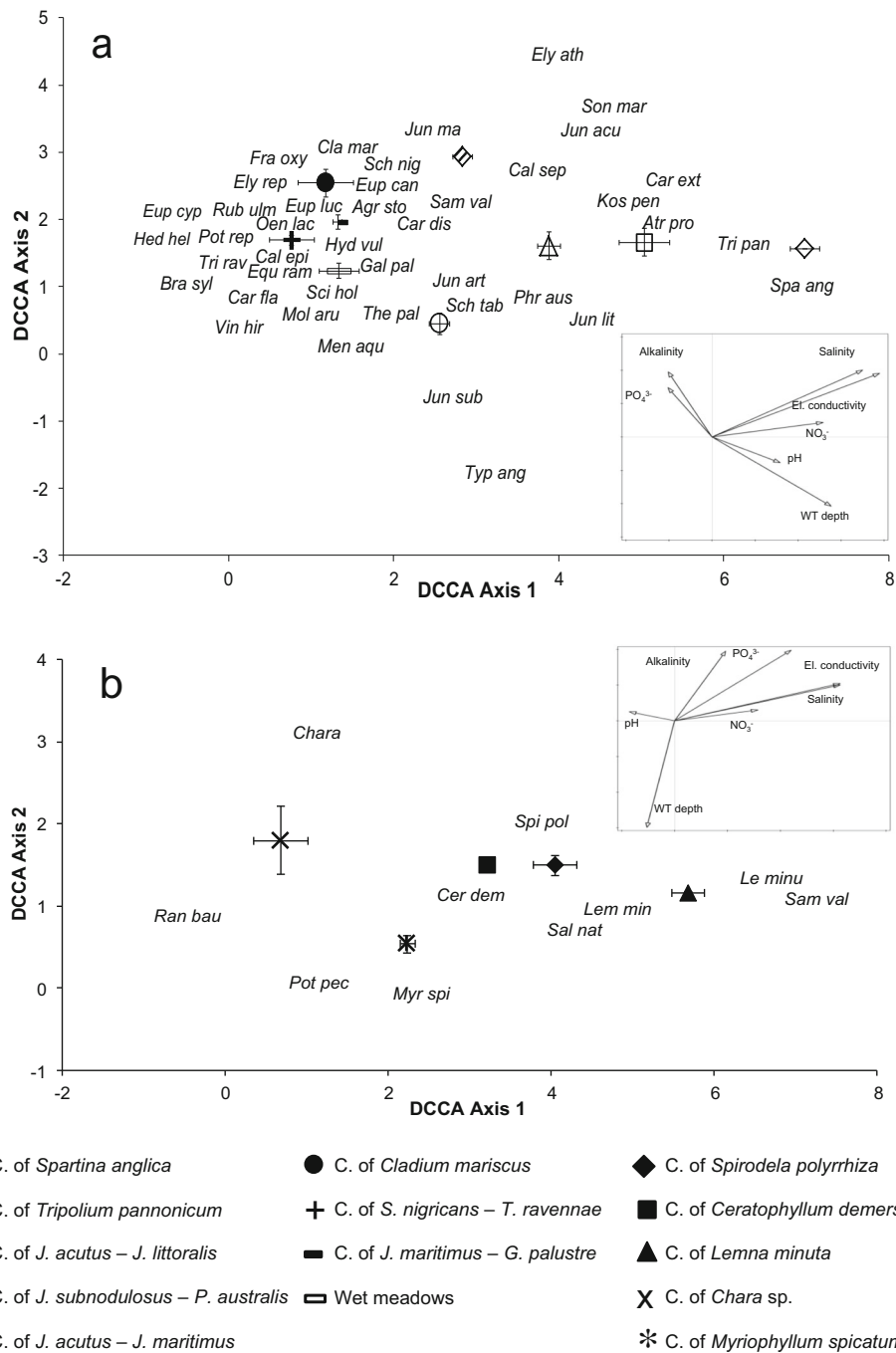
Fig. 3 Species scores and environmental variables scores on the first two DCCA axes for the hygrophytic and helophytic vegetation (**a**) and for the waterplant vegetation (**b**) Agr sto *Agrostis stolonifera*, Atr pro *Atriplex prostrata*, Bra syl *Brachypodium sylvaticum*, Cal epi *Calamagrostis epigejos*, Cal sep *Calystegia sepium*, Car dis *Carex distans*, Car ext *Carex extensa*, Car fla *Carex flacca*, Cer dem *Ceratophyllum demersum*, Chara *Chara* sp. (including *C. vulgaris* and *C. intermedia*), Cla mar *Cladium mariscus*, Ely ath *Elymus athericus*, Ely rep *Elymus repens*, Eup can *Eupatorium cannabinum*, Eup cyp *Euphorbia cyparissias*, Eup luc *Euphorbia lucida*, Gal pal *Galium palustre*, Hyd vul *Hydrocotyle vulgaris*, Jun acu *Juncus acutus*, Jun art *Juncus articulatus*, Jun lit *Juncus littoralis*, Jun mar *Juncus maritimus*, Jun sub *Juncus subnodulosus*, Kos pen *Kosteletzkya pentacarpos*, Lem min

Lemna minor, Le minu *Lemna minuta*, Men aqu *Mentha aquatica*, Mol aru *Molinia arundinacea*, Myr spi *Myriophyllum spicatum*, Oen lac *Oenanthe lachenalii*, Phr aus *Phragmites australis*, Pot pec *Potamogeton pectinatus*, Ran bau *Ranunculus peltatus* ssp. *baudotii*, Rub ulm *Rubus ulmifolius*, Sal nat *Salvinia natans*, Sam val *Samolus valerandi*, Sch tab *Schoenoplectus tabernaemontani*, Sch nig *Schoenus nigricans*, Sci hol *Scirpoides holoschoenus*, Son mar *Sonchus maritimus*, Spa ang *Spartina anglica*, Spi pol *Spirodela polyrrhiza*, The pal *Thelypteris palustris*, Tri rav *Tripidium ravennae*, Tri pan *Tripolium pannonicum*, Typ ang *Typha angustifolia*, Vin hir *Vincetoxicum hirundinaria*. The symbols indicate the centroids (mean ± SE) of the relevé scores for the 14 plant communities

Table 5 Summary of Monte Carlo statistics for the hygrophytic and helophytic vegetation (upper part) and for the waterplant vegetation (lower part). Significant ($P < 0.05$) P adjusted values in bold character

	Contribution (%)	Pseudo-F	P	P adjusted
Hygrophytic and helophytic vegetation				
Electrical conductivity	32.1	6.8	0.002	**0.014**
Water-table depth	18.8	4.2	0.002	**0.014**
Alkalinity	13.2	3.1	0.002	**0.014**
Nitrate	12.7	3.1	0.002	**0.014**
Phosphate	8.7	2.2	0.004	**0.02**
pH	7.6	1.9	0.004	**0.02**
Salinity	6.9	1.8	0.046	**0.046**
Waterplant vegetation				
Electrical conductivity	36.5	6.6	0.002	**0.014**
Water-table depth	16.4	3.4	0.004	**0.02**
Alkalinity	14.6	3.4	0.004	**0.02**
Salinity	12.2	2.3	0.056	0.224
pH	8.1	1.9	0.076	0.228
Nitrate	6.0	1.5	0.22	0.416
Phosphate	6.1	1.5	0.208	0.416

Only three out of the seven environmental variables (electrical conductivity, water-table depth and alkalinity) did significantly account for the observed variation in waterplant vegetation (Table 5). The five waterplant communities were arranged from left to right according to increasing values of electrical conductivity from the communities of *Ceratophyllum demersum*, *Chara* sp., and *Myrophyllum spicatum* to the community of *Spirodela polyrrhiza* and, especially, the community of *Lemna minuta* (Fig. 3b; Table 4). The ordination of the five waterplant communities along the vector of water-table depth reflected a gradient of increasing water-table depth from the communities of *Chara* sp. and *Lemna minuta* to the communities of *Ceratophyllum demersum* and *Myriophyllum spicatum*. Similar to the hygrophytic and helophytic vegetation, the vector of alkalinity was oriented in opposite direction to that of water-table depth. Accordingly, mean alkalinity levels were somewhat higher in the communities of *Chara* sp. and *Lemna minuta* than in the communities of *Ceratophyllum demersum* and *Myriophyllum spicatum*. The community of *Spirodela polyrrhiza* had intermediate levels of both water-table depth and alkalinity (Fig. 3b; Table 4). Contrary to helophytic and hygrophytic vegetation, waterplant vegetation was not affected by pH, nitrate or phosphate concentrations, and salinity (Table 5). Mean salinity levels always were < 1000 mg L^{-1}, indicating no saltwater influx in any of the waterplant communities.

Discussion

Conservation Values and Ecological Gradients The rich variety of plant communities recorded in the Nature Reserve reflected strong environmental differences among wetlands that ranged from natural to artificial, lentic to lotic, permanent to temporary, and freshwater to saline wetland types. The species composition of the plant communities reflected such environmental heterogeneity. Indeed, species diversity is strongly affected by niche differentiation, in turn depending on relationships between environmental heterogeneity and species diversity at different scale levels (Lundholm 2009; Lastrucci et al. 2015). The wetland vegetation in the study area was rather heterogeneous in terms of structural diversity as well, because it consisted of plant communities dominated by hygrophyes, helophytes, pleustophytes, or rhizophytes. Most of the plant communities in the Nature Reserve had good correspondence with Natura 2000 habitat types. Several of these habitats presented high conservation value based on the species rarity index, although none of them falls within the Community priority habitats. Interestingly, the community of *Juncus subnodulosus* and *Phragmites australis* presented one of the highest rarity values even if it does not fall under any of the Natura 2000 habitat types. This supports the results of recent studies showing that, especially in Mediterranean wetlands, plant communities not corresponding to Natura 2000 habitat types may be extremely important from the conservation viewpoint because they host locally rare and threatened species (Angiolini et al. 2017; Viciani et al. 2017).

The multivariate ordination pointed to electrical conductivity, closely related to salinity, as the main ecological factor structuring the hygrophytic and helophytic vegetation in the Nature Reserve. The three communities at the high end of the salinity gradient, i.e., the community of *Juncus acutus* and *J. littoralis* and especially the communities of *Tripolium pannonicum* and *Spartina anglica*, only occurred in brackish wetlands close to the adjacent lagoon where salinity levels were highest. The community of *Juncus subnodulosus* and *Phragmites australis*, the community of *Schoenus nigricans* and *Tripidium ravennae*, and the wet meadows were all situated at greater distance from the lagoon in habitats with poor if any saltwater influx. However, some of the species most frequently occurring in these communities, especially *Phragmites australis* and to a lesser extent *Schoenus nigricans*, were also found in moderately saline habitats owing to their rather broad ecological amplitude with respect to salinity level (Lissner and Schierup 1997; Bernhardt and Kropf 2006). The community of *Cladium mariscus* was located in the

mid part of the salinity gradient. *Cladium mariscus* also possesses high tolerance to salinity. Indeed, *Cladium mariscus*-dominated communities are quite frequent both in inland areas free from saltwater influx and in coastal habitats with quite high salt content (Géhu and Biondi 1988; Landucci et al. 2013). Nutrient levels also played a significant role in structuring the hygrophytic and helophytic vegetation. However, nitrate and phosphate appeared to affect differently the hygrophytic and helophytic communities in the Nature Reserve. The high nitrate levels detected in the saline habitats colonized by the communities of *Spartina anglica* and *Tripolium pannonicum* were determined by riverine N influx into the lagoon (Tappin 2002). The saltwater progressing landwards as an effect of saline wedge intrusion became progressively impoverished in dissolved N probably because the plant species in the communities of *Spartina anglica* and *Tripolium pannonicum* were effective in absorbing nitrate that was thus removed from the soil water (Sousa et al. 2008). Hence, the habitats with low to moderate salinity had lower nitrate concentrations and were characterized by species usually regarded as sensitive to high N load such as *Juncus subnodulosus* (Ceschin et al. 2010), *Cladium mariscus* (Landucci et al. 2013), and *Schoenus nigricans* (Bakker et al. 2005). Contrary to nitrate, phosphate was not associated with salinity but rather with alkalinity which resulted in highest phosphate concentration and highest alkalinity level in the salt-poor community of *Juncus maritimus* and *Galium palustre*. A possible explanation consists in a higher fraction of phosphate being incorporated on calcium phosphate in the soil of this community (Turner 2008).

The waterplant vegetation in the Nature Reserve was strongly conditioned by water-table depth. Water-table depth generally is a major driver in affecting species composition of waterplant communities (Edvardsen and Økland 2006; Klosowski and Jabłońska 2009; Hrivnák et al. 2014; Sakurai et al. 2017). The community of *Chara* sp. was located at the low end of the water-table depth gradient. In these habitats, the water table fluctuated strongly depending on precipitation, but the mean water-table depth was lower than in all other waterplant communities. The temporary character of this kind of wetlands represents a favorable condition for species such as *Ranunculus peltatus* subsp. *baudotii* or *Chara* sp. (see also Melendo et al. 2003; Florencio et al., 2014). Water bodies with relatively shallow water were colonized by small pleustophytes in the communities of *Spirodela polyrrhiza* and *Lemna minuta*. In contrast, bigger pleustophytes or rhyzophytes in the communities of *Ceratophyllum demersum* and *Myriophyllum spicatum* were found in deeper water in line with results of previous studies (Buchwald 1994; Lastrucci et al.

2014; Sakurai et al. 2017). An additional factor responsible for variation in the waterplant vegetation of the Nature Reserve was electrical conductivity. Unlike what we observed for the hygrophytic and helophytic vegetation, relationships between electrical conductivity and nitrate or phosphate concentrations were not straightforward for the waterplant vegetation. Neither was electrical conductivity related to salinity as all of the waterplant communities were situated in freshwater habitats. This suggests that electrical conductivity in the waterplant habitats mirrored the total load of nutrients with no close association with specific components such as nitrate or phosphate. The community of *Chara* sp. presented rather low values of electrical conductivity which indicates quite low levels of dissolved nutrients in the water. However, the species occurring in the community of *Chara* sp. have been found to tolerate quite high levels of dissolved nutrients in the water. For example, Ceschin et al. (2010) considered *Potamogeton pectinatus* as an indicator of moderate eutrophication. *Chara vulgaris* has rather wide ecological amplitude with respect to nutrient levels, ranging from mesotrophic to eutrophic waters (Lambert-Servien et al. 2006). *Ranunculus peltatus* ssp. *baudotii* has also been frequently observed in mesotrophic to eutrophic waters (Brullo et al. 2001; Melendo et al. 2003). The low nutrient levels recorded in the community of *Chara* sp. probably depended on the peculiar hydrological features of the watering pools where water supply prevalently comes from precipitation rather than from the watertable. The communities dominated by bigger plants, either pleustophytes (*Ceratophyllum demersum*) or rhyzophytes (*Myriophyllum spicatum*), had lower mean values of electrical conductivity compared with the communities dominated by small pleustophytes (*Lemna minuta* and *Spirodela polyrrhiza*). This may suggest that these two types of waterplant communities were associated with waterbodies having differing levels of dissolved nutrients. As all of these communities were found in canals receiving nutrient-rich water from the surrounding arable land, a more likely explanation consists in greater amounts of dissolved nutrients being removed by plant uptake in the communities where plant biomass was higher.

Environmental Changes and Vegetation Dynamics The present state of the wetlands in the Natural Reserve is the result of the history and the hydraulic management of the Reserve and of the surrounding areas. Reclamation of freshwater wetlands that once surrounded the Nature Reserve has altered the balance of the aquifer, enhancing the intrusion of the saline wedge. Furthermore, increasing subsidence rates hamper the hydrological
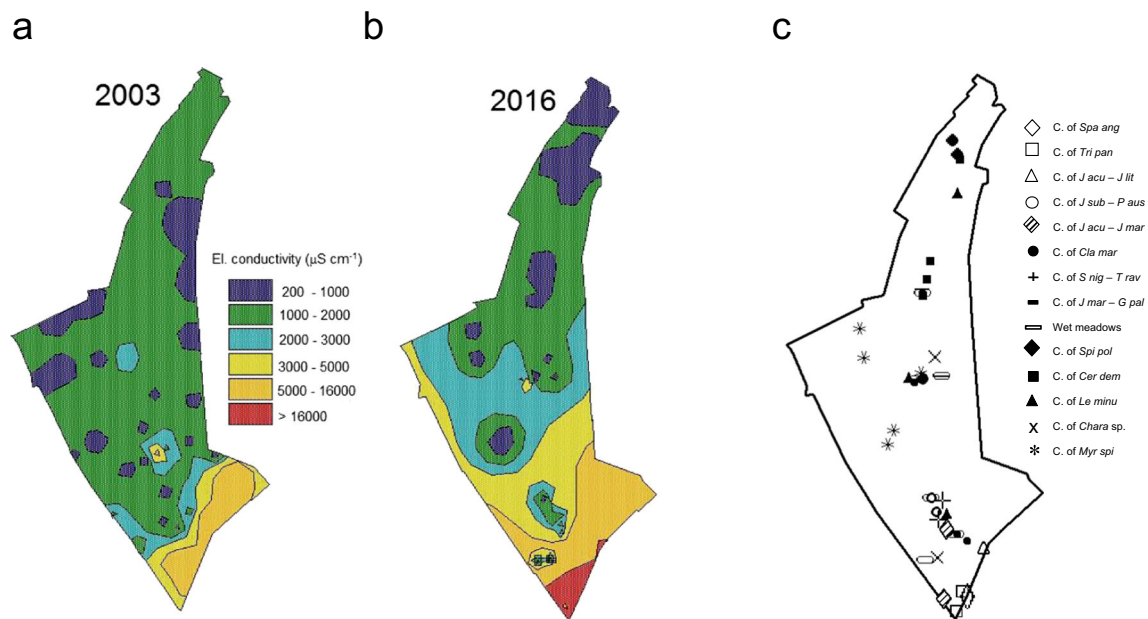
Fig. 4 Maps of the spatial distribution of electrical conductivity in 2003 (**a**) and in 2016 (**b**) and location of the relevés for the 14 plant communities (**c**) with abbreviated legend for the communities (full legend in Fig. 3)

turnover in the Natural Reserve (Caschetto et al. 2016), which is largely situated below the sea level. As a combined effect of these causes, electrical conductivity in the water is strongly increasing (2034 ± 343 μS cm^{-1} in 2003 vs. 7410 ± 1210 μS cm^{-1} in 2016; Fig. 4). Progressing saltwater intrusion may imply expansion of halophilic species and communities in the hygrophytic and helophytic vegetation. Hence, rare species occurring in poorly to moderately saline habitats, such as *Euphorbia lucida*, *Hydrocotyle vulgaris*, *Teucrium scordium* and, to a lesser extent, *Juncus subnodulosus* and *Cladium mariscus* may be out-competed by species preferring more strongly saline conditions. Saltwater may also impact waterplant species and communities if the intrusion of the saline wedge turns freshwater into brackish water. This may have negative effects on the locally rare species *Chara vulgaris*, *C. intermedia*, and *Ranunculus peltatus* ssp. *baudotii*.

Two sources of eutrophication may impact the wetlands in the Nature Reserve. The main source of eutrophication consists in influx of nutrient-rich saltwater by the intrusion of saline wedge from the adjacent lagoon. Our results show that the vegetation of brackish wetlands presently acts as an effective filter in removing nutrients, thus reducing the influx of nutrients to oligotrophic habitats. However, if the nutrient load in the lagoon increases, this may imply degradation of salt-tolerant vegetation (Deegan et al. 2012) which may hamper the filtering capacity of vegetation. An additional source of wetland eutrophication in the Nature Reserve is the network of canals conveying water from the surrounding arable land to the Nature Reserve. Agriculture is the

main source of nutrient, especially nitrate, load with agricultural activities accounting for about 60% of nitrogen (N) compounds influx in the waters of arable lands in Northern Italy (Castaldelli et al. 2013). Eutrophication may increase in the near future because of a derogation from European rules (Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources) allowing farmers to raise the amount of manure applied to arable farms up to 250 kg N ha^{-1} year^{-1}. If nitrate load increases in the next future, this may have negative effects on freshwater species preferring nitrate-poor habitats such as *Cladium mariscus* or *Juncus subnodulosus*. Both of these species are rather sensitive to eutrophication and may thus be out-competed by more tolerant species, especially *Phragmites australis* (see Viciani et al. 2017). On the other hand, the endangered species *Kosteletzkya pentacarpos* may benefit from future increases in nitrate levels (Abeli et al. 2017).

In conclusion, the wetlands of the Reserve are subject to a series of threats deriving from a complex interplay of environmental changes and anthropogenic alterations. Improved hydraulic management including more accurate maintenance and higher flow rate in the canals, with consequently increased drainage capacity, could counter the saline wedge intrusion (Antonellini et al. 2015). Furthermore, hydraulic management should be oriented to create a system of freshwater habitats around the area in order to reduce the influx of nitrate-rich water from canals draining agricultural land.

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