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IRRIGATION AND DRAINAGE CANALS ROLE FOR PLANT DIVERSITY AND NATURE CONSERVATION

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Preface

This thesis is the result of the industrial doctorate that the Bigea Department of the University of Bologna activated together with Arpae Emilia-Romagna, based at the research group of Plant Diversity, Ecology and Conservation. The thesis has been supervised by Prof. Alessandro Chiarucci.

The theme of the PhD was also developed with the support of the Consorzio della Bonifica Renana (here called Consortium) that manages the canals of the Bologna plain. They are necessary for irrigation and drainage of agricultural areas. The Consortium provided the structural data of the canals and the analysis of the water quality of the canals carried out by the Faculty of Agriculture of the University of Bologna.

The thesis is divided into two parts. The first part, named "Overview", develops in three chapters describing the study area, the methods used for the collection of data in the field and for their analysis. The second part consists of three papers (one published, one reviewed in an international journal and the third being completed). In the first paper we described the geographical patterns of the study area in term of hydrographic features, water quality, land use, flora and plant communities (Chapter II). In the second one we characterized the plant communities in relation to depth of the monitored canals (Chapter III). The third paper is being elaborated: it concerns some functional traits of the species detected in the field compared with environmental parameters (some geographical patterns and water quality data, Chapter IV).

During the three-year period I contributed to the organization of the 2nd International Conference on Community Ecology which was held in Bologna in June 2019, being part of the Local Organizing Committee in support of Akademiai Kiado. I was the tutor of three Bachelor thesis (Nicola De Bernardini, Luigi Spiezia, Ilenia Castellari), of two master thesis (Natalia Sacchetti e Gina Gizzi), of two internship (Luigi Spiezia e Ilenia Castellari) and of one international interniship with the partnership of the University of Toulouse (Louise Campione). Furthermore I assisted the field activities for the course of Vegetation Ecology (graduate level, year 2018).

Facing the three-year doctorate after many years of work was a very significant personal and professional challenge. It was also a great opportunity to compare myself to the scientific world highly renewed in techniques and tools, very different from those that I usually use in my work.

Irene Montanari
Bologna, Italy, May 2021

Abstract

Aims: With this research we wanted to investigate and promote the conservation of biodiversity in the network of drainage canals of the Po Valley. In particular, we wanted to increase the knowledge about the relationship that exists between the plant community and some characteristics of the territory in order to verify the contribution of artificial lowland aquatic ecosystems in supporting plant diversity. Furthermore, on the basis of the research results, we wanted to identify the main management guidelines to be suggested to the Consorzio della Bonifica Renana in order to create better environmental conditions in which the most fragile plant species and habitats can find an adequate refuge.

Study area: The canal network of Bologna plain, long more 1150 km (Po Valley, North Italy)

Methods: In Chapter II we analized the geographical patterns that characterize our transects (slope, exposure of the sampled bank, proximity to protected areas), the land use of their upstream basins, the water quality at the closure points of their river basins. In Chapter III we described the plant communities with some ecological information (species groups, common/rare species, alien /invasive species, Ellenberg’s Index, beta-diversity) and we also tested the effect of the canal size on the plant communities. In Chapter IV we described the relation beetween some functional traits of the plant species sampled and some environmental parameters (the same geographical patterns of Chapter II and water quality where was possible)

Results: A total of 272 species were sampled in 118 transects. On the plant communities of the drainage canals has been found to have a significant influence: the geographical pattern "proximity to protected areas", the class of land use "agrozootechnical settlements" and water parameters “pH”, “Sodium”, “Sodium uptake ratio”, “Orthophosphates”, “Chlorine” and “Ammonia”. The analysis of the parameter "canal depth" indicated a significant distinction between small and large canals in the sorting of sites based on plant communities. The functional composition of the plant community was affected by the bank aspect, the inclusion and exclusion from the protected areas and the upstream basin land uses. Moreover was highlighted that the phanerophyte life form was related to the presence of aquatic environments upstream the transect, while nitrophilia was related to “artificial” land use upstream of the transect. Finally, the functional groups of species responded differently to environmental drivers, water quality gradients and was influenced by a combination of environmental stresses.

Conclusions: This research confirms the key role of the canals network in sustaining the plants richness in oversimplified landscapes. The drainage canals are also the first receptors of polluting loads of widespread agricultural origin; for this reason they are essential for the improvement of the quality of the water. Considering the fragility of the floodplains and the global warming that is taking place, it will be necessary to rethink the role of irrigation canals and their plant communities in the near future. This work reinforces the belief that long-term sampling plans and greater knowledge about canal management practices are needed

CHAPTER I

Overview

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Introduction

This doctoral work stems from the desire of the Consorzio della Bonifica Renana to know the state of the vegetation in its remediation canals and understand whether the quality of the water flowing in these canals affects the environment, on nature conservation and surrounding agricultural production.

Globally the inland freshwaters are the most threatened ecosystems (e.g. Cantonati et al. 2020; Guareschi et al. 2020) and the wetland plant species the most threatened by the hard alteration of habitats and ecosystem dynamics (Bolpagni and Piotti 2016). The impacts could be strongly negative due to production factors or riparian management. In fact, it could both encourage the development of invasive species by increasing the dispersion of seeds in water (hydrochorus), and improve the shelters (Nilsson et al, 2010).

The current ecological fragility of humid environments is not only the result of their own nature, but also depends on their history that has led to a progressive artificialization of habitats (Alessandrini et al., 2010).

The reclamation works and the successive transformations of the land dried up in arable fields, started by the Etruscans and then by the Romans, made habitable vast unhealthy and marshy areas of the Po Valley, satisfying the needs of liveability of the territory, but at the expense of pre-existing naturalness. The technological evolution of the last two hundred years and, in particular, of the Twenties of the Twentieth century intensified the activity of reclamation. This caused the disappearance of large valleys and the transformation of the landscape. Subsequently, the agricultural development, industrial and demographic increase of this territory have led to a landscape consisting of a highly anthropized matrix with small areas of greater naturalness. Therefore, in this context, the canals also assume importance as artificial ecological corridors, connecting more natural relict areas through a territorial matrix deeply transformed by the man (Dallai et al., 2015).

Before these massive changes in spatial planning, wetlands guaranteed geographical continuity to hygrophilous species. Nowadays, these species survive laboriously in small isolated populations, threatened by pollution, by the eutrophication of the waters, by the presence of invasive vegetal and faunal species whose containment is not very effective. To these are added the effects of the reshaping of the banks of canals and rivers that modify the transition habitat between wetlands and areas only temporarily submerged (Alessandrini & Manzini, 1997). There is no lack of indirect effects of the urbanization of the low plain: in fact, the waterproofing of the soil and the alteration of the pluviometric regimes require the Consortium to keep free the canals to ensure the outflow in case of emergency (Dallai et al., 2014). In addition to the loss of available surface area, other anthropogenic changes such as the lowering of groundwater and the deterioration of water quality should be added.

Anyway, biodiversity plays a key role within those canals and the ecosystem services they provide. Drainage canals can support aquatic biodiversity such as natural rivers (Chester and Robson, 2011), although there are few studies showing that canals could play a key role in the conservation of hygrophytes and not just aquatic species (Harvolk et al, 2014).

In addition, some plants of riparian banks are useful to retain phosphorus and nitrogen and thus improving water quality and acting asrural hotspots and qualifying the agricultural landscape (Hulina 1998; Pedullà and Garbari 2001; Bonafede et al. 2003; Goulder 2008; Herzon and Helenius 2008; Bolpagni et al. 2013, 2018; Dorotovičová 2013), others improve bank stability or provide aquatic habitats for many species (Parkyn et al, 2003). Canals provide habitats and corridors for biodiversity and thus enhance regulating services. Drainage canals provide different sorts of ecosystem services such as provisioning service (water to irrigate crops), regulating services (water regulation, ways for seed dispersal, sediment transport and deposition, nutrient exchanges with groundwater…) (Harvolk et al, 2014)

Being aware that the physical characteristics of the canals, together with the water regime and the density of macrophytes, are determining factors on the quality and quantity of water outflow that enters the water reclamation system (Bouldin et al., 2004) we wanted to investigate the determinants that affect the species-specific and structural composition of the plant communities of the canals of the studied territory. Many other studies have explained what relationship exists between the riparian vegetation and the physical and chemical characteristics of water and sediments in the remediation canals (Bouldin et al., 2004; Boedeltje et al., 2001), but the drainage canals of the Po Valley constitute a very particular environmental system for extension and management methods, and are not easily comparable with the canals studied in the North Europe that have a direct exchange with the sea.

It is important to improve the status of the biodiversity present both in order to increase its potential in terms of nature conservation and to improve the environmental quality of this area through its ecological functions.

This project focused mainly on human impacts through the effects of morphology of drainage canals, land uses and chemical inputs in water on the plant communities.

For all these reasons it was important, first, study and describe the system from a geographical point of view and spatial relations between the different elements present in the territory, investigating their interactions with plant communities in addition to environmental factors that affect them such as the land use and water quality (Chapter II). Moreover it was important understand the impacts of human activities on hygrophilous plants and to suggest a better way of management if needed (Chapter III). Finally, it was important to interpret the plant community through its functional traits. This has made it possible to understand the chemical and structural characteristics of the plant species that are most related to the different water quality parameters. Even this information, when it will be more investigated, can give important indications on the most effective ways to manage canals to maintain a high level of biodiversity and ecological functionality in the environmental system of remediation canals (Chapter IV).

Structure of the thesis

The thesis was based on independent works. Each work is in the form of a scientific article and the purpose of this chapter I "Overview" is to communicate to the reader, in a synthetic way, the essence of the research carried out and to guide him in the reading of the three subsequent papers produced. The "Overview" presents the logical thread that connects the three papers through a graphic diagram of the structure of the thesis, a graphic diagram of the methods used in each paper, and the essence of the overall results obtained. The Overview has the meaning of introducing the reader to the thesis work and to present the structural characteristics. Moreover, the Overview presents the general Conclusions of the thesis that propose to the reader the most important reflections that have arisen from all the work as a whole. The reader can then deepen the individual studies made through the three chapters that follow (chapters II, III and IV) that are scientific papers that live each one of their own life. It follows that each paper provides a description of the Study area and the Methods that have been used for the analysis of the data in more detail according to the objectives of the paper. The first paper (chapter II) is been submitted to international journal, the second paper (chapter III) was published from an international journal, the third one is in elaboration (chapter IV). All the work was carried out focusing on the plant communities of the banks of the irrigation and drainage canals of the Bologna plain.

The structure of the thesis is summarized in Figure 1 where it's also highlighted the main questions of the chapters.

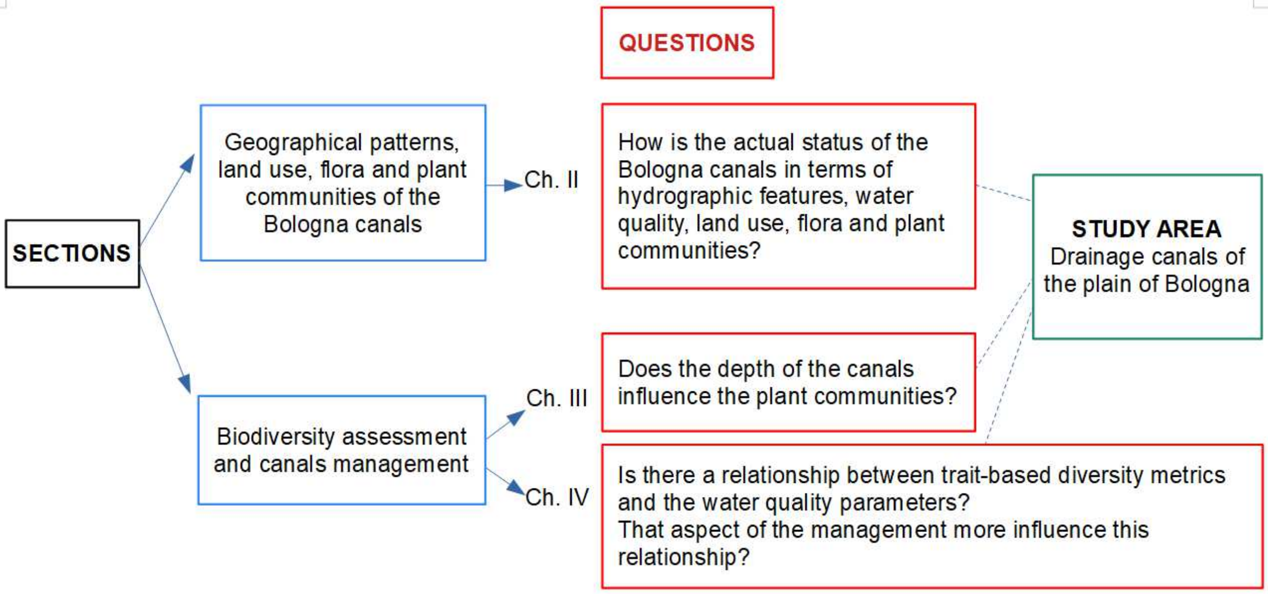


Figure 1 - Structure of the thesis. For each chapter the main questions and the study area are shown.

Study area

The research activity of my PhD has developed in the plain of the Bologna province (1787 km2, PTCP/CittaMetropolitanaBologna/norme_allegato_A.pdf). Here there is a dense network of canals (2075 km long, Report 2019 of the Consortium) managed by the Consorzio della Bonifica Renana.

These canals act as irrigation, drainage and hydraulic protection for a territory of about 1400 km^2. Overall, throughout the Po Valley (41,850 km^2), in which the study area falls, there are 15 reclamation consortia of 1st degree and 2 reclamation consortia of 2nd degree.

In Bologna plain there is a strict division in "highlands" and "lowlands" (Fig. 2). In the first, the waters drain by gravity, in the second the waters drain by the use of the water-pumping stations. These drain over 56·000 hectares of land in artificial courses and drainage canals. This is a very important function because some municipalities of the "lowlands" are located below the level of the Reno river waters (Fig. 3). All the waters of the Bologna plain flow into the Reno river near Valle Campotto.

Climatic, pedological, orographic and biotic factors are part of the autoecology of plant species. They are the ones that most influence the presence and the growth of plants in a territory. For this reason if you study the vegetation of an area it is important to know the climate (di Luzio, 2015). The Po Valley is characterized by a continental climate. This is due to the atmospheric circulation influenced by the presence to the north and west of the Alps, to the east of the Adriatic Sea and to the south of the Apennines. Their ridge is oriented mainly in the NW-SE direction. This geophysical barrier is a major obstacle to warm winds from the south so the climate of the region is mostly influenced by Nordic factors. In addition, the low temperature regulation of the Adriatic Sea, as shallow, contributes to the establishment of these conditions. The winter is therefore long and cold while the summer is hot and humid. The thermal regime of the Emilia-Romagna region can be classified as "temperate subcontinental".

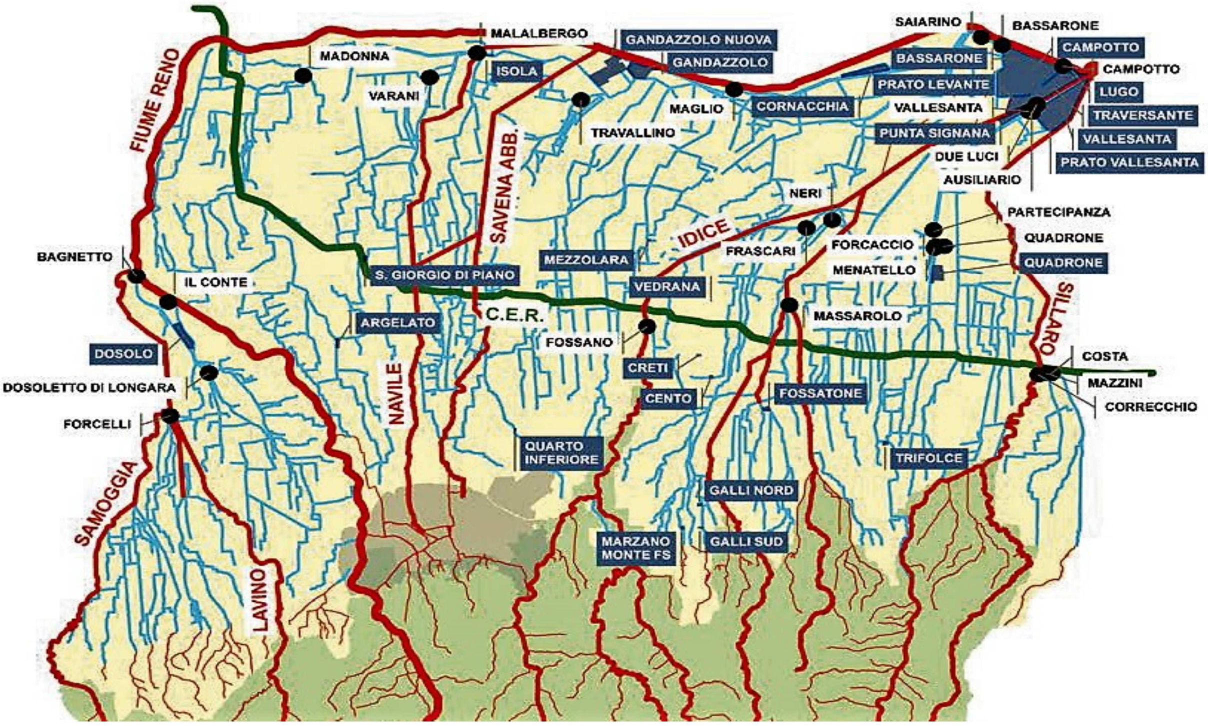


Figure 2 - Plain district of the Consorzio della Bonifica Renana. High lands (green); low lands (yellow); network of canals managed by the Bonifica Renana (blue); dewatering systems (black); Canale Emiliano Romagnolo (dark green); natural waterways of regional competence (red). (*Report 2017 of Consorzio della Bonifica Renana*)

Figure 3 - Altitude of the Bologna plain with respect to the banks of the Reno river (Report 2017 of Consorzio della Bonifica Renana)

Based on data from the period 1981-2010, the average annual temperature is 12.4 °C; the warmest month is July (average temperature 31.4 °C) and the coldest is January (average temperature -1.1 °C). Annual precipitation is 684 mm, with maximum in October and minimum in July (Arpae Emilia-Romagna 2019, https://simc.arpae.it/dext3r/).

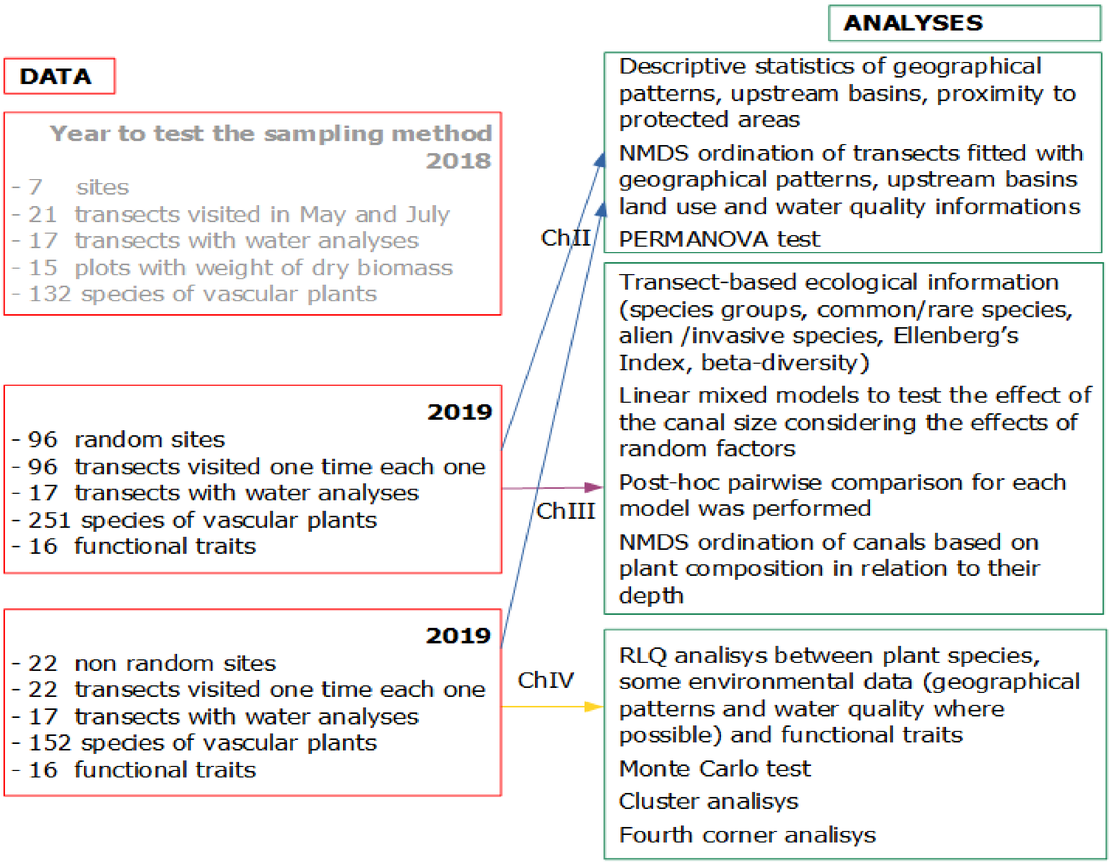
The pluviometric regime is of Apennine sublitoranean type with an absolute minimum in summer, and an annual maximum usually higher than the spring one (di Luzio, 2015; Zucaro and Furlani, 2009).

The canals studied have both irrigation and drainage functions. The irrigation season usually starts in April and ends in the first days of October. The most abundant crops in this area are the polyphyte meadow, maize and sugar beet, however the vine and vegetables are also widespread (Zucaro and Furlani, 2009).

Diagram of the Methods applied in Chapters II, III and IV

Below is a graphical summary of the methods applied in each paper that follow for ease of reading. Each paper has its own detailed paragraph regarding the methods used for the analysis of the data carried out in that paper.

The methods used during the sampling phases are described in detail in chapter III. Analytical methods and results are described and discussed in chapters II, III and IV (Fig. 4). All the analyses were performed with the R statistical software (R Core Team, 2017).



Year to test the sampling method 2018
- 7 sites
- 21 transects visited in May and July
- 17 transects with water analyses
- 15 plots with weight of dry biomass
- 132 species of vascular plants

2019
- 96 random sites
- 96 transects visited one time each one
- 17 transects with water analyses
- 251 species of vascular plants
- 16 functional traits

2019
- 22 non random sites
- 22 transects visited one time each one
- 17 transects with water analyses
- 152 species of vascular plants
- 16 functional traits

Descriptive statistics of geographical patterns, upstream basins, proximity to protected areas
NMDS ordination of transects fitted with geographical patterns, upstream basins land use and water quality informations
PERMANOVA test

Transect-based ecological information (species groups, common/rare species, alien /invasive species, Ellenberg's Index, beta-diversity)
Linear mixed models to test the effect of the canal size considering the effects of random factors
Post-hoc pairwise comparison for each model was performed
NMDS ordination of canals based on plant composition in relation to their depth

RLQ analisys between plant species, some environmental data (geographical patterns and water quality where possible) and functional traits
Monte Carlo test
Cluster analisys
Fourth corner analisys

Figure 4 - Summary of data, sampling methods and analytical methods presented in each chapter of the thesis

Conclusions

In all chapters, thanks to the stochastic sampling done that ensured that the data collected were representative of the entire hydrological network and its plant community (Chiarucci, 2007), it has been confirmed that the hydrological and eutrophic conditions of the remediation canals significantly influence the plant communities of the banks with particular regard to the periods of dry winter and the lack of water oxygenation in addition to high concentrations of nitrates and orthophosphates. However, the canals network is one of the few semi-natural elements remaining in the Po Valley characterized by a landscape completely artificialized by human uses as demonstrated in Chapter II with the analysis of the land use of the upstream basins of transects.

Irrigation and drainage canals role for plant diversity and nature conservation

Moreover, thanks to their widespread diffusion throughout the Po Valley, the reclamation canals are the first receptors of polluting loads of widespread agricultural origin; for this reason they are essential for the improvement of the quality of the water distributed there. For this reason it is necessary to continue to investigate these environments in order to define really effective and long-term action aimed at the conservation of the biodiversity asset (in terms of flora, vegetation and habitat) of the artificial hydrographic network and, more generally, HMWB.

There is a balance between the flow of water into the canals network and the surrounding agricultural environment which must be maintained with rational and efficient management. The Reclamation Consortia are now increasingly involved in projects aimed at improving the ecology of the territory as a prerequisite for the conservation of biodiversity. In fact, in addition to providing water for wetlands, they create wooded areas along the canals and engage in the protection of stretches of canals in which there are still rare species in the waterways (Dallai et al., 2014).The Reclamation Consortia, however, suffer a serious lack of knowledge related to the ecology of the systems that govern and their ecosystem functions. It is well known that pesticides used in intensive agriculture can spread into drainage water and reach surface and groundwater bodies. This type of pollution endangers the ecological quality and health of these basins. It would therefore be necessary to plan a management that optimizes the whole range of ecosystem services that the vegetation of the canals can offer as, for example, the creation of meandriform canal sections to increase the capacity of water retention and absorption of fertilizers and pesticides (Herzon and Helenius, 2008; Vymazal and Březinová, 2018). In particular, it is well known that small drainage canals have the potential to mitigate excessive nitrate loads due to punctual and diffuse pollution. This absorption capacity is much more consistent along the vegetated canals (mainly with *Phragmites australis* and *Typha latifolia*) than those without vegetation: in the former, an average absorption level of 50% of the total N can be reached for each km of canal (Pierobon et al., 2013). In Chapter II it emerges that the presence of *Phragmites australis* is also very related to the Sodium Adsorpion Ratio: this shows that this species tolerates better than other high contents of salts that can be caused both by the evapotranspiration of crops in arid climates as well as by the hardness of water and the use of pesticides and fertilizers.

It is widely recognized that biodiversity is the main element of the richness and functionality of ecosystems and that its erosion is the most important cause of the decrease of their functionality (Floris and Ruggeri, 2018). Our study confirmed that the network of irrigation channels, given the richness of the species identified, has a central role for the biodiversity it hosts and, consequently, for the ecological functions it supports. It is also true, however, that ecological functions are influenced not only by the number of species that make up a community, but rather by the characteristics and traits of the species present in it (Harvolk et al., 2014). We can therefore affirm that a great vegetal diversity does not necessarily indicate a good state of the ecosystem, especially if it is given by an important diffusion of ruderals and, in part, of aliens that do not guarantee the functionality of the ecosystem. However, in general it is important that an ecosystem has the highest possible biodiversity and that it is complex enough to ensure the survival of those species that are essential for the ecological functions it performs. Finally, it is important to underline that the greater the biodiversity of an ecosystem, the greater its resilience, as often the functional redundancy of the species is able to fill the loss of some key taxa for the ecosystem.

Among the management practices, in accordance with the objectives of preserving biodiversity, it is suggested to the Consorzio della Bonifica Renana to adopt practices for the repopulation of canals by floating macrophytes because their presence is very low (chapters II, III e IV). In fact, they promote the oxygenation of the water column, reduce the load of organic matter through the assimilation and accumulation in biomass and provide oxygen to the sediment where oxidative processes take place (Caraco et al., 2006). Also important is the indirect support of the microbial communities associated with phanerogams: the root hairs of the plants, releasing oxygen in the interstitial waters, create, in fact, micro-oxygenated niches inside otherwise anoxic and reducing sediments. These micro-niches can accommodate bacterial communities that, in their part, implement oxidative processes and promote the decomposition of organic substance and the segregation of nitrogen and phosphorus (Longhi et al., 2011). In addition, in the light of recent studies, best practices to preserve biodiversity and ensure a rapid outflow of water are:

- management with different approaches in order to maximize the metabolism of emerging vegetation with consequent improving purification efficiency of the water (Pierobon et al, 2013). The mowing, for example, generates dense covering of litter that together with the ash produced by the burn improve the retention of hydrophobic herbicides. These operations, if carried out in a suitable manner and in the appropriate time, can significantly contribute to improve the purification capacity of the canals. In the Mediterranean context, for example, burning in winter and mowing at the end of summer is the optimal combination of maintenance and timing for the seizure of herbicides and water outflow (Dollinger et al., 2017),

- ensure the timing of reproduction of riparian species avoiding mowing in spring and early summer (Dallai et al, 2011),
- build meanders within the canal this would ensure high water retention and good uptake of nitrates and other substances (Herzon and Helenius, 2008).
- realize buffer zones designated exclusively to phytopurification,
- further studies in relation to the spread of alien species in order to reduce their presence. Since alien species take advantage of running water environments to colonize new territories (e.g., Pyšek and Prach 1994; Pyšek et al. 1998), management practices to reduce the spread of alien and invasive species are needed. In relation to this issue it is important to underline that the effort should be made starting from the sampling phases, in particular for endangered species (but the same is true for invasive and alien species). In our case, finding the transects randomly did not allow us to investigate in particular the places where there are environmental conditions more favourable to their settlement (Palmer et al., 2002). This limit did not affect the elaborations of Chapters II and III in which these three groups of species were analyzed from various points of view within the plant community of the network of canals (richness, relationship with the goegraphical patterns of the territory and with the water quality of 16 transects in which we had this information).

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CHAPTER II

Flora and plant communities across a complex network of heavily modified water bodies: local geographical patterns, land use and hydrochemical drivers

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Irrigation and drainage canals role for plant diversity and nature conservation

Abstract

Freshwater vegetation disappearance is among the most relevant problems in the conservation of biological diversity, for the alteration or reclamation of inland wetlands. It is also known that the contribution of artificial lowland aquatic ecosystems in preserving plant diversity is notable, but the underlying ecological mechanisms still are not completely explored. To contribute to this debate, a canal network of about 1150 km (Po valley, northern Italy) was analysed to study geographical patterns of riparian plant communities, land use and hydrochemical drivers. A systematic sampling procedure was adopted by randomly selecting 96 transects (1 m × 10 m) along 79 different canals, classified as large, medium and small according to water depth. 22 non-random transects were also sampled for water quality evaluation. Flora was characterized based on species richness and presence of threatened, alien and invasive species. NMDS and PERMANOVA were used to study the relative contribution of land use and water quality in explaining plant richness and composition along the canal network. Our results show that slope and aspect of canals do not significantly affect plant species composition, whereas the proximity to protected areas seems to have, surprisingly, a negative influence. Among land uses, only agro-zootechnical settlements influence plant species composition, favoring nitrophilous species. Water parameters significantly affect the floristic items. This work confirms the key role of heavily modified water bodies in sustaining plant species diversity in oversimplified landscapes; some water quality parameters and some land use categories are good proxies for the alien or ruderal species patterns.

Key words

drainage canals, plant diversity, threatened plant species, alien plant species, Po valley

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Introduction

Inland waters are one of the most endangered ecosystems globally (Reid et al. 2019; Dudgeon 2019), and the loss of native freshwater species is one of the hot topics in biological conservation (e.g., Bolpagni et al. 2019; Cantonati et al. 2020; van Rees et al. 2020). These issues are especially relevant for lowland alluvial areas in so-called developed countries, that are disturbance-dominated landscapes mainly devoted to intensive agriculture and scattered urban and industrial sites (Kadoya et al. 2009; Verhoeven and Setter 2009; Bolpagni et al. 2020). In Europe, for example, from the Industrial Revolution onwards, two centuries of drainage and simplification of lowland aquatic ecosystems have led many wetland plant species to a strong decline, or even at the brink of extinction (e.g. *Aldrovanda vesiculosa* L., *Leucojum aestivum* L., *Marsilea quadrifolia* L., *Nymphoides peltata* (S.G. Gmel.) Kuntze, *Oenanthe fistulosa* L., *Salvinia natans* (L.) All., *Trapa natans* L., *Viola elatior* Fr., *V. pumila* Chaix), whereas in the recent past they often were much more frequent, so that their presence was hardly noteworthy for many botanists until the mid-twentieth century (Schnittler and Günther 1999; Dorotovičová 2013; Buldrini et al. 2013a, 2014). The process of «ecosystem simplification» of lowlands has been so intense that, at the European level, from 1945 onwards, 66% of the disappeared plant species is composed solely by amphibian and aquatic taxa (Denny 1994; Sager and Clerc 2006). Thus, it is generally acknowledged that, at least at mid-latitudes, wetland plants are among the most threatened species (Preston and Croft 1997; Moser et al. 2002) due to habitat destruction or deterioration (Riis and Sand-Jensen 2001; Buldrini et al. 2013b; Bolpagni and Piotti 2016).

In Italy, and especially in the Po valley, the above-mentioned issues are even more relevant than in other parts of the Northern Hemisphere. As is known, Italy is a biodiversity hotspot for macrophytes at a global level (Bolpagni et al. 2018; Murphy et al. 2019) and the reclamation works began in much more ancient times than elsewhere in Europe. The first drainage works date back to the I millennium B.C., then they continued with ups and downs until the Renaissance and the Enlightenment, ending only in 1970 (Bondesan 1990; Tinarelli and Tosetti 1998; Dallai et al. 2014, 2015). Today, the Po valley is a territory almost completely dominated by intensive farming and widespread urbanization (Romano and Ciabò 2008), with obvious deleterious consequences for the biological diversity (Guareschi et al. 2020).

In zones where human impact is so pervasive, artificial water bodies like ditches and drainage canals may play a fundamental role in offering artificial habitats to preserve plant richness and diversity, often behaving as true rural biotopes (Buldrini and Dallai 2011; Bolpagni et al. 2013, 2018; Dorotovičová 2013). In many cases, canals and other heavily modified water bodies (HMWB) are among the very few (if not the unique) places that can host wetland species, sometimes rare or endangered with extinction (Gentili et al. 2010; Buldrini et al. 2015; Buldrini and Santini 2016; Bolpagni et al. 2019), and often show a higher biological and environmental richness with respect to the neighbouring habitats (Tölgyesi et al. 2020). Nevertheless, it must be borne in mind that drainage canals, due to their typical physiognomy (steep banks and trapezoidal cross section of variable dimensions, proximity to cultivated areas, management necessary to ensure the hydraulic function), are particularly sensitive to the impact of agriculture. Runoff and soil erosion imply the transport of a multitude of nutrients and chemicals (e.g., pesticides, fertilizers) with negative effects on riparian and aquatic biota (Herzon and Helenius 2008). Once the submerged macrophytes have disappeared, they are replaced by pleustophytes like *Lemna* pp., *Spirodela* spp. or *T. natans*, able to reduce the oxygen availability in water and sediments (Janse and Van Puijenbroek 1998; Bolpagni et al. 2007).

Even if the potential support of HMWB to plant diversity and lowlands environmental quality has been widely recognized, drainage canals are generally managed thinking only to their primary hydraulic and irrigation functions (e.g. Bolpagni 2020). In addition, for a long time the canals were considered scarcely interesting from a biological viewpoint, being them artificial environments, therefore data about flora composition, distribution and abundance (especially from a geographical and land use perspective) are rare and often published in grey literature (Dorotovičová 2013; Montanari et al. 2020), despite, as for example in Italy, HMWB networks can reach impressive dimensions (up to 100,000 km in Northern Italy; cfr. e.g. Bartoli et al. 2003; Bischetti and Chiaradia 2009; Longhi et al. 2010, 2011; Dallai et al. 2015).

With this work, we investigated the role of HMWB in supporting the conservation of wetland flora and vegetation, taking as a case study the network of canals of the Bologna plain (northern Italy). The main aim of this study is to provide an updated overview of the current conservation status of the HMWB of Bologna lowlands in terms of hydrographic features, water quality and land use as drivers of flora and plant communities.

We expect: *a*) a reduction of species richness along with the increase of average slope of canals (H1); *b*) no change in the plant composition of the transects as their aspect changes (H2); *c*) a reduction in endangered species richness as the distance from protected areas increases (H3); *d*) an opposite response for alien and invasive species, with their richness increasing as the distance from protected areas increases (H4); *e*) a non-negligible role of the water quality of canals in driving flora and plant communities, with a progressive reduction of plant richness and an increase of communities' simplification as the canal water quality worsens (H5).

In our study area, the largest HMWB are characterized by relatively lower variations in water level compared to what is expected for natural lowland lotic systems. This limits the environmental heterogeneity of riparian ecotones and reinforces the need to increase knowledge on the roles that drainage canals can play in the conservation of flora.

Materials and methods

Study area

The study area includes the lowland sectors of the province of Bologna (Emilia Romagna region, Italy; Fig. 1) that are situated in the south-eastern part of the Po Valley. The province of Bologna is one of the most productive agricultural and industrial areas of the Po basin and, consequently, one of the most impacted plain areas of Europe (Guareschi et al. 2020). The climate of the study area is continental, with cold winters and hot summers (Köppen climate classification Cfa, Peel et al. 2007), because the Apennines stop the southern warm winds, and the thermal regulation of the Adriatic Sea is scarce depending on its shallowness. Atmospheric circulation is normally limited, thus relative humidity is high. According to 1981-2010 data, the average annual temperature is 12.4 °C; the hottest month is July (average temperature 31.4 °C) and the coldest month is January (average temperature -1.1 C). Annual precipitation is 684 mm, with a maximum in October and a minimum in July (Arpae Emilia-Romagna 2019, https://simc.arpae.it/dext3r/).

The River Reno (212 km) and its tributaries (Samoggia, 60 km; Idice, 78 km; Sillaro, 66 km; Santerno, 103 km; Senio, 92 km) are the principal natural water courses of the study area. They rise from the Tuscan-Emilian Apennines and have a torrential regime. However, as normally happens in alluvial plains, the most important water courses of the study area are artificial (i.e. canals built for land drainage and/or irrigation), organised in a hydrological network and administered by a reclamation consortium called Consorzio della Bonifica Renana (hereafter Consortium). The total area managed by the Consortium is 3419 km² wide, whereas the lowland sector (coinciding to the study area) extends for 1439 km². The canal network is 1154 km long and is used for both drainage and irrigation; for this reason, all canals undergo periodic changes in their hydrological regime, with higher flows from May to September and a lean period from November to March, apart from exceptional rainy events. Smaller canals are frequently dry during autumn and winter (Montanari et al. 2020). All the canals in the study area were excavated between 1911 and 1925 (Piccoli 1983; Furlani 2009; Zampighi 2009), therefore they have a certain stability from an environmental and ecological viewpoint, given their age (see also Dorotovičová 2013).

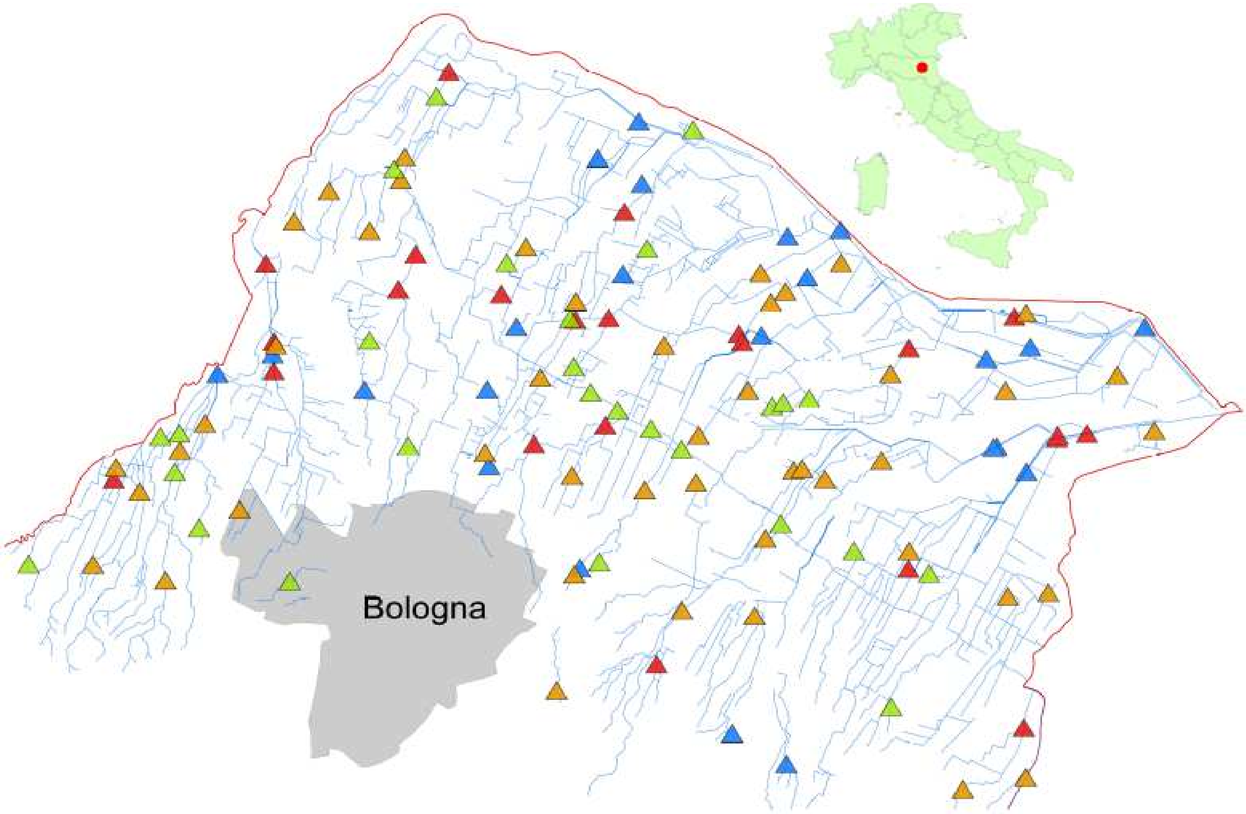

Figure 1 - Map of the study area (Bologna plain, northern Italy) with the indication of the sampling sites (red: large canals; orange: medium canals; green: small canals, blu: canals where the water quality was sampled too). The position of the study area within Italy (red dot) is also shown.

Soils are generally calcareous, characterised by a prevailing clay-silty texture (Carta dei Suoli dell'Emilia-Romagna, 1:50.000, https://geo.regione.emilia-romagna.it/cartpedo/). The potential climax vegetation is considered to be a mixed deciduous forest, with prevailing *Quercus robur* L. and *Carpinus betulus* L., whereas river courses would be characterised by a riparian forest dominated by *Populus alba* L. and *Salix alba* L. (Puppi et al. 2010). In fact, however, this vegetation has disappeared almost everywhere because of the intensive agriculture and farming, and only a few small remnants still persist (Tinarelli and Tosetti 1998). In many cases, riverbanks are characterised by a dense and intricate bush, dominated by allochthonous woody species such as *Amorpha fruticosa* L. and *Robinia pseudoacacia* L., accompanied by nitrophilous (*Rubus ulmifolius* Schott, *Rumex obtusifolius* L., *Urtica dioica* L. etc.) and disturbance-tolerant species (*Avena fatua* L., *Bromus hordeaceus* L., *Daucus carota* L. etc.). Along the canals, on the contrary, woody species are practically absent, because the banks are periodically mowed to permit a better water runoff in case of rainfall. Here the vegetation is composed by banal species, that are frequent in the entire continental Europe, whereas species that are protected or more demanding from an ecological viewpoint are extremely rare (< 3% ot the total floristic list, cfr. Montanari et al. 2020).

Sampling design

A preliminary inspection of the artificial hydrological network of Bologna plain was carried out by a GIS approach to identify the prevalent types of drainage canals. Ancillary information was provided by the Consortium and Emilia-Romagna Agency for Environmental Protection (Arpae). Based on this survey, 79 canals and 96 transects have been randomly selected to be subjected to field investigation.

The transect selection was achieved using the random points function of QGIS. The number of transect was defined based on the total length of the canals grouped in three different classes according to their depth (large, medium, and small), to be representative of the entire hydrological network (Chiarucci 2007). Additionally, we imposed a minimum spacing between sites equal to 2 km if two or more transects were selected along the same canal. This action generated 23 large canals (L), 46 medium canals (M), 27 small canals (S). In addition, 22 sampling sites were selected according to opportunistic criteria, as they coincide with the closure points of the hydrological sub-basins (here referred to as H2O). Therefore, the total number of transects considered is 118.

In each sampling point, a transect of 10 m × 1 m (that is 10 contiguous plots 1 m × 1 m) was positioned along the canal bank, parallel to the water flow. Starting from the water-bank interface, the transect was placed to include in the analysis the first 10 cm of the canal water body and the first 90 cm of the bank slope.

Plant species were identified and named following Pignatti et al. (2017-2019). More details of the sampling design and the sampling method are described in Montanari et al. (2020).

Spatial data

For this study we used:

1) the Land Use Map 2017 (reference scale 1:10.000) by the Emilia-Romagna Region. It is characterised by a minimum patch size of 0.16 ha and a minimum size of 7 m for linear elements. This map was realized by the Region using color (RGB) and infrared orthophotographs TeA of 2017 (20 cm cell resolution). The classification system used for the legend is Corine Land Cover IV and identifies the categories shown in Table 1 and Supplementary material 1. We merged the aforementioned land use categories into the following macro-categories: «Artificial surface», «Water bodies and inland wetlands», «Wood», «Meadows», «Agricultural area», «Agro-zootechnical settlements»

2) The map of the water catchment areas of all drainage canals in the territory of the Renana Reclamation Consortium

3) The map of protected areas in the area studied (regional parks, Natura 2000 sites, ecological rebalancing areas)

4) The regional map of Military Geographical Institute (IGM) altitude points, grid of 200 meters

Ground data

For the study we also used:

- vegetation data (collected by the Canal Bank Research Group as described in "*Sampling design*"),

- water quality data collected by a team of the DISTAL Department of the University of Bologna, from April to October 2019 to provide baseline data for the quality of the water supply to the farms of the area. The selected sampling points coincide with the closure points of 22 sub-basins of the drainage network examined (Fig. 2).

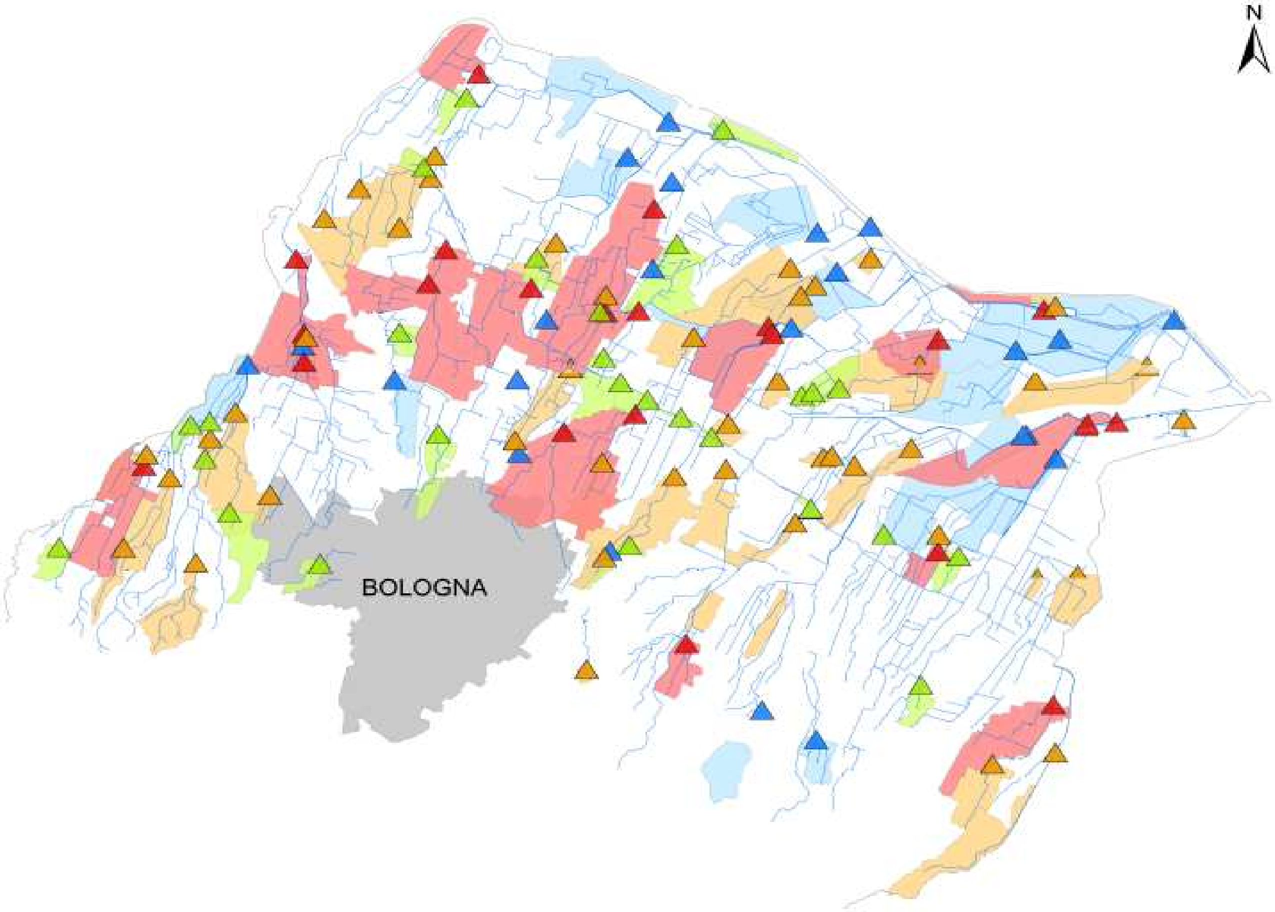

Figure 2 - Map of the sampled transects (triangles) and their upstream basins (colored areas. The following colors are referred to triangles and areas. Red: large canals; orange: medium canals; green: small canals, blu: canals where the water quality was sampled too).

Data analyses

To achieve the purpose of the study, the analysis of the plant community was carried out in relation to both geographical patterns in each transect (slope, bank aspect, land use of upstream basin of each transect, proximity to protected areas) and water quality.

Geographical patterns and species analysis

To calculate the slope of the different canal types (L, M, S, H2O), from the regional map of IGM altitude points, we extracted the farthest points within a 2 km radius area around each transect.

Assuming that the air-line distance between the two selected altitude points was 2 km, the slope was calculated as a percentage of the stretch of canal into which each transect falls.

Moreover, where we sampled the flora, we detected the bank aspect to check if this parameter affects the plant community. This was possible for 104 out of 118 sampled transects, the other lacking properly collected field information.

We also detected the land use in the upstream basin of each transect using the Land Use Map 2017. We considered an upstream stretch of canal of 2 km at least (up to the entire basin where the stretch of canal was not longer than 10 km). In case of several transects along the same canal, the upstream basin of each transect was identified excluding what was already attributed to the upstream transect. We normalized the areas of each land use in each upstream basin to compare them. The upstream basin could not be identified for 5 transects (NA_01, NA_02, NA_03, SAM_01, SAV_01) because they insist on canals that do not receive water from the surrounding agricultural area, but only from the urban area of Bologna distant even tens of kilometres (rainwater and city water treatment plant). Therefore, data about the upstream basins are available for 113 transects only.

To calculate the proximity of the transects to protected areas (parks, Natura 2000 sites, local protected areas) we considered four thresholds: a) within the protected areas; b) within a buffer of 100 m from the protected area; c) within a buffer of 100-500 m from the protected area; d) more than 500 m from protected areas. In addition, the analysis of the presence of alien, invasive and threatened species in the transects according to the four thresholds of distance from protected areas was performed. The alien and invasive species were identified according to the classification proposed by Galasso et al. (2018) for the regional distribution of Emilia-Romagna. They defined as alien the species whose presence in Italy is due to human activities, intentional or not, or that have spread to an area where they were not native. Aliens included the naturalized allochthonous species and the allochthonous casual species. The invasive species, a subgroup of the aliens, can reproduce quickly and at considerable distances from the mother plant and this gives them a huge potential as colonizers of vast areas (Pyšek et al. 2004; Galasso et al. 2018). According to Jalas et al. (1972-), cryptogenic taxa and archaeophytes (the alien species present in Europe since before 1492) were grouped together with native species. Threatened species were attributed to a IUCN category according to Regione Emilia-Romagna (2017).

Inference on geographical patterns

The analysis of transect plant composition in relation to the geographical patterns were obtained first performing an NMDS on transect species composition data, then overlaying to the ordination scatterplot the data of the three above mentioned geographic variables. Separate analyses were performed for all species and alien species to verify possible significant effect of the geographic variables on species composition. A PERMANOVA was subsequently applied to verify the significance of each relationships investigated with the NMDS. Only to verify significant effects of the land use of the upstream basins (data grouped into macro-categories) on species composition was applied the fitting function of R with 999 permutation to the NMDS results.

Water quality

The analysis of plant community in relation to water quality was performed applying the NMDS to the species composition of the 17 H2O transects for which it was possible to identify the upstream basin, separately using presence/absence and abundance data. The PERMANOVA was subsequently applied to verify the significance effect of the water chemical variables on plant community.

For the study of geographical patterns QGIS version 3.10 was used. All the shape files were aligned to the ETRS 1989 UTM Zone 32N geographical reference system. The shape files with the regional system called UTMRER were converted with the program Conver_2013 version 2.05.07.

For the comparison of plant species data with geographical patterns, water quality and land use, the software R version 3.6.0 was used (R Core Team 2019).

Results

Geographical patterns

The slope of the canals in the surroundings of each transect varied from 0% to 0.50% and only one canal had a slope of 0.75% (Supplementary material 2). The most frequent slope values were those ranging from 0% to 0.05% involving the stretches of canal in which 50 transects fell, while only 5 transects fell into stretches of canal with a slope between 0.40% and 0.50%. The average slope in the different canal type was very similar, ranging from 0.12% (large, medium and H2O canals) to 0.14% (small canals; see Supplementary material 3).

Considering the bank aspect of the sampled transects (Table 1), among large canals the highest number of transects was exposed to north (6), south (5) and west (4). Between medium canals, transects were exposed to south-east (13) and north-west (10), and between small canals to south-west (5), north-west (4) and east (4). In H2O canals, the largest number of transects was exposed to south-east (6) and north-west (4). Overall, the major part of the transects was exposed to south-east (21), north-west (18) and south (15). In 14 out of 118 sampled transects, this information was not recorded in the field for a human error.

Table 1 - Aspect of the sampled banks (where L=large canals, M= medium canals, S= small canals, H2O= canals where we have both vegetation and water quality)

Canal type	**Not recorded**	**E**	**N**	**NE**	**NO**	**O**	**S**	**SE**	**SO**
L	2	3	6	1	0	4	5	1	1
M	5	3	1	4	10	2	5	13	3
S	6	4	2	2	4	1	2	1	5
H2O	1	3	0	1	4	1	3	6	3
Total	14	13	9	8	18	8	15	21	12

The land use classes were fairly uniformly distributed between large, medium and small canals. The most common class was «Irrigated plain arable land», ranging from 69.5% in large canals to 75.2% in small canals, followed by «Fruit trees», ranging from 2.9% in large canals to 4.2% in small canals, and «Isolated residential facilities», ranging from 3.5% in large canals to 4.7% in small canals. The land-use classes «canals» and «inland wetlands» varied from 0.5% in medium canals to 1.5% in large canals and from 0.1% to 4.3% respectively in small canals and H2O canals (Supplementary material 4). The analysis of land use according to macro-categories (Table 2) shows that «agricultural areas» was, by far, the most widespread macro-category of land use ranging from 75.1% in large canals to 81.3% in small canals. The subsequent land use macro-category in term of importance was «artificial surfaces», which varied from 13.5% in small and medium canals to 15.8% in large ones. The macro-category «water bodies and inland wetlands» varied between 2.2% in the upstream basins of the small canals and 5.2% of the upstream basins of the large canals.

Table 2 - Percentage of macro-categories of land use in the upstream basin of each type of canal sampled (L = large canals, M = medium canals, S = small canals, H2O = canals for which both vegetation and water quality data are available). For the abbreviations of land use categories see Supplementary material 1

	Canal type			
Land use macro-categories	**H2O**	**L**	**M**	**S**
Artificial surfaces (Ec,Ed, Er, Ia, Ic, Io, Is, It, Qa, Qc, Qi, Qq, Qr, Qs, Qu, Ra, Re, Rf, Ri, Rm, Ro, Rs, Va, Vd, Vg, Vi, Vm, Vs, Vt, Vv, Es)	7,6	15,8	13,5	13,5
Water bodies and inland wetlands (Ac, Aa, Ax, Af, Ar, Av, Zi)	7,9	5,2	3,4	2,2
Wood (Bm, Bp, Bq, Br, Bs, Ta, Tn)	2,5	1,5	2,7	0,9
Meadows (Fs, Pp, Rv, Vp, Vx)	0,7	1,8	1,4	1,6
Agricultural area (So, Se, Sv, Ze, Zo, Zt, Sn, Cf, Co,Cv, Cl, Cp)	80,6	75,1	78,3	81,3
Agro-zootechnical settlements (Iz)	0,6	0,7	0,7	0,5
Total percentage	100	100	100	100

In relation to the parameter «proximity to protected areas», we verified that only 4 sites fall within regional parks. Focusing on Natura 2000 sites, 12 transects were included in these sites, 3 and 8 in a buffer of 100 meters and of 100 to 500 meters from their borders, respectively. Only one site fell within a local protected area.

Considering the presence of alien, invasive and threatened (according to IUCN criteria) species, we found that 56 transects had no alien or invasive or threatened species, whereas in the other 62 transects their presence was subdivided as follows: 21 transects had 1 threatened species, 1 transect had 2 threatened species, 31 transects had 1 alien species, 19 transects had 1 invasive species, 13 transects had 2 invasive species, 3 transect had 3 invasive species, 1 transect had 4 invasive species, 1 transect has 5 invasive species, 1 transect had 6 invasive species. Out of 272 total species sampled, 5 were alien, 9 invasive and 7 threatened species (Table 3); the alien represented 2.2%, the invasive 3.3% and the threatened 2.6% of the total richness. In the 96 random transects, on a total of 251 species the alien species represented 2.0%, while the invasive and the threatened 2.8% each of the relative floristic lists. In the 17 H2O transects, of 153 total species the aliens represented 2.6%, the invasive 3.3% and the threatened 0.6% of the relative floristic list.

Table 3 - Occurrences of alien, invasive and threatened plant species among the canals of Bologna plain (L = large canals, M = medium canals, S = small canals, H2O = canals for which both vegetation and water quality data are available; CR = critically endangered, EN = endangered, VU = vulnerable)

	Species	**Canal type**			
		L	**M**	**S**	**H2O**
Alien taxa	*Acer negundo* L.	-	1	-	1
	Erigeron annuus (L.) Desf.	-	2	1	1
	Paspalum distichum L.	-	-	1	-
	Populus deltoides Marshall	-	-	-	1
	Veronica persica Poir.	2	6	10	4
	Total Occurrences	2	9	12	7
Invasive taxa	*Ailanthus altissima* (Mill.) Swingle	-	-	-	3
	Amaranthus retroflexus L.	1	-	-	-
	Amorpha fruticosa L.	4	2	-	1
	Artemisia verlotiorum Lamotte	2	-	-	-
	Erigeron canadensis L.	-	2	-	-
	Robinia pseudoacacia L.	-	1	1	4
	Rumex cristatus DC.	-	-	-	1
	Sorghum halepense (L.) Pers.	1	3	1	2
	Xanthium orientale L. ssp. *italicum* (Moretti) Greuter	1	1	-	-
	Total Occurrences	9	9	2	11
Threatened taxa	*Allium angulosum* L. - EN -	-	1	-	-
	Euphorbia palustris L. - EN -	4	2	3	1
	Juncus subnodulosus Schrank - CR -	-	1	-	-
	Nuphar lutea (L.) Sm. - VU -	1	-	-	-
	Samolus valerandi L. - EN -	-	-	1	-
	Schoenoplectus tabernaemontani (C.C. Gmel.) Palla - VU -	-	-	1	-
	Sium latifolium L. - EN -	-	-	1	-
	Total Occurrences	5	4	6	1

Irrigation and drainage canals role for plant diversity and nature conservation

Considering all types of protected areas present in the studied area, we verified that only 4 invasive and 2 threatened species have been surveyed inside to them. The largest number of threatened species have been recorded outside the buffer of 500 meters from protected areas. It should be considered that only 28 transects of the 118 sampled fell within or around 500 meters from protected areas, this limiting the possible inference of the data

Inference on geographical patterns

The NMDS analysis applied to our sampled data shew a wide dispersion of transect in relation to the species composition and a few transects were well separated because they are characterized by a monospecific plant composition of *Phragmites australis* (Cav.) Trin. When the data about slope of the canals and the bank aspect were fitted to the NMDS it was clear that these parameters had not an influence on the plant composition of the transects in our study area. This is consistent with the very small differences in slope between transects (from 0% to 0,50%), that do not allow to detect a gradient for this parameter. The PERMANOVA analysis confirmed that slope and bank aspect did not influence the composition of the transects vegetation (P = 0.1 and 0.2, respectively).

When the information about the proximity to protected areas of each transect was fitted to the NMDS, it emerged that some transects were less dissimilar to each other (Fig. 3). These transects were ZI_03, Zi_04, FD_01, AIV_04, PR_01 and SB_04, that had in common the species *Alopecurus myosuroides* Huds., *Elymus repens* (L.) Gould, *Geranium columbinum* L., *Galium aparine* L., *Lythrum salicaria* L., *Phragmites australis*, *Urtica dioica* L. The PERMANOVA analysis confirmed that proximity to protected areas influenced the composition of the transects vegetation (P ≤ 0.05).

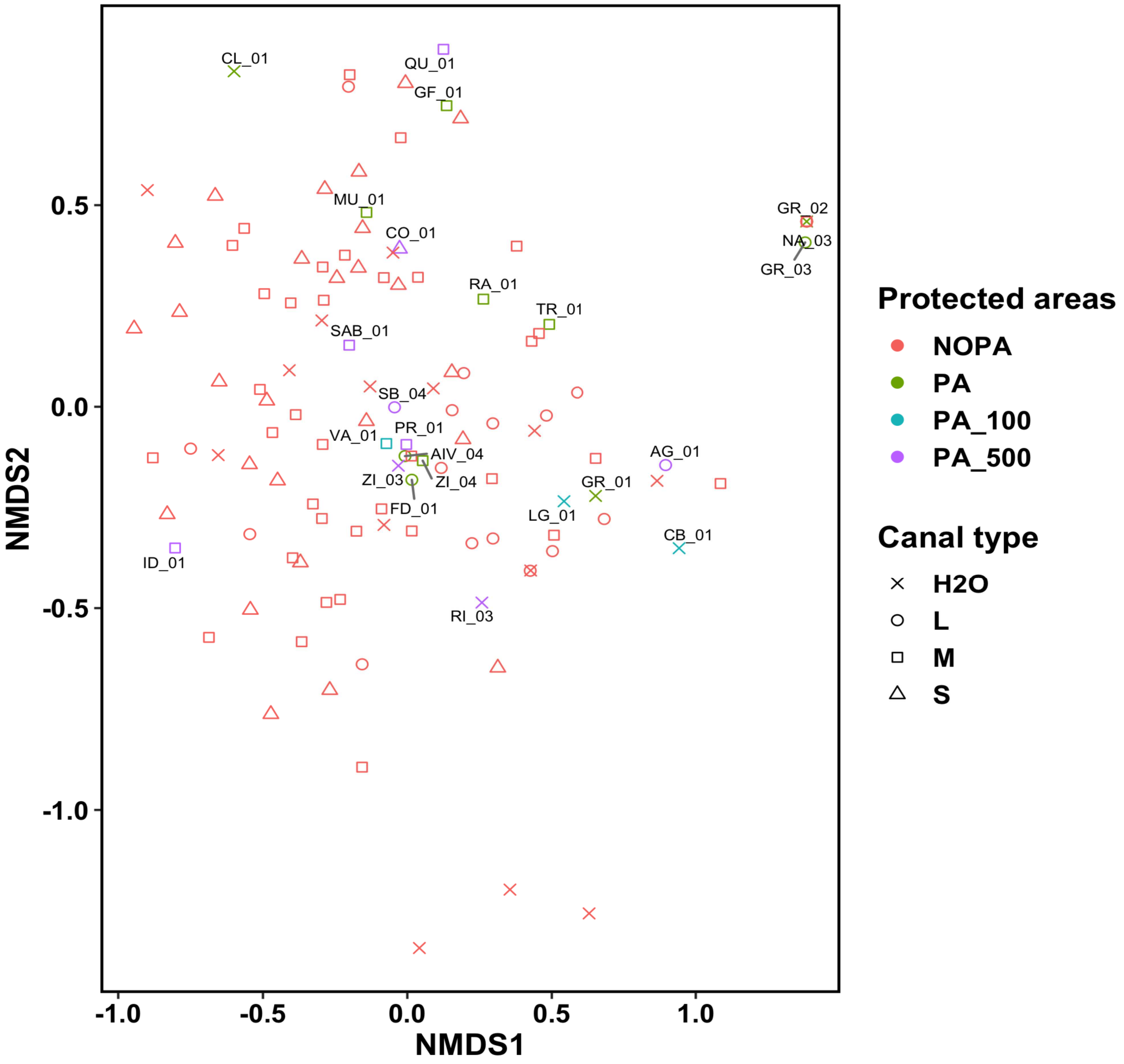

Figure 3 - Non-metric multidimensional scaling plot, in which each point represents a single transect of the 118 sampled (stress = 0.18). The distances from protected areas (PA) were grouped in 4 classes: inside PA (green), outside in a buffer of 100 m (blue), outside in a buffer from 100 to 500 m (purple), outside more than 500 m (red)

Irrigation and drainage canals role for plant diversity and nature conservation

When the data about proximity to protected areas of the canals were fitted to the NMDS applied to 62 transects considering only the presence of alien or invasive or threatened species (AIT), two transects emerged as different (Supplementary material 5):

RD_01 where there is *Juncus subnodulosus* Schrank that is the only AIT species of the transect,

RC_01 where there is *Allium angulosum* L. that is the only AIT species of the transect.

Moreover the transects MA_02, SAB_01, SS_01, GL_01, BR_01 and FS_01 were characterized by *Schoenoplectus lacustris* (L.) Palla and formed a cluster with:

DC_01 with *Schoenoplectus lacustris* and *Paspalum distichum* L.,

MO_02 with *Schoenoplectus lacustris* and *Acer negundo* L.,

SB_03 with *Acer negundo* that was the only AIT species.

Other transects were less strongly grouped because they shared *Veronica persica* Poir (22 sites), *Euphorbia palustris* L. (10 sites), *Sorghum halepense* (L.) Pers. (7 sites), *Amorpha fruticosa* L. (7 sites), *Robinia pseudoacacia* L. (6 sites). The PERMANOVA analysis has shown that proximity to protected areas does not influence the presence of alien, invasive and threatened species in the transects (P-value > 0.1)

When the data about the macro-categories of land use of the upstream basin of the 113 transects were fitted to the NMDS, it emerges that, in relation to presence/absence data, no land use macro-category significantly affects the plant composition of the transects, while in relation to abundance data (Fig. 4) only the macro-category «Agro-zootechnical settlements» significantly fitted the plant composition of the transects onto NMDS ordination, highlighting a significant relationship with the presence of two nitrophilous species (*Lysimachia nummularia* L. and *Setaria pumila* (Poir.) Roem. et Schult.).

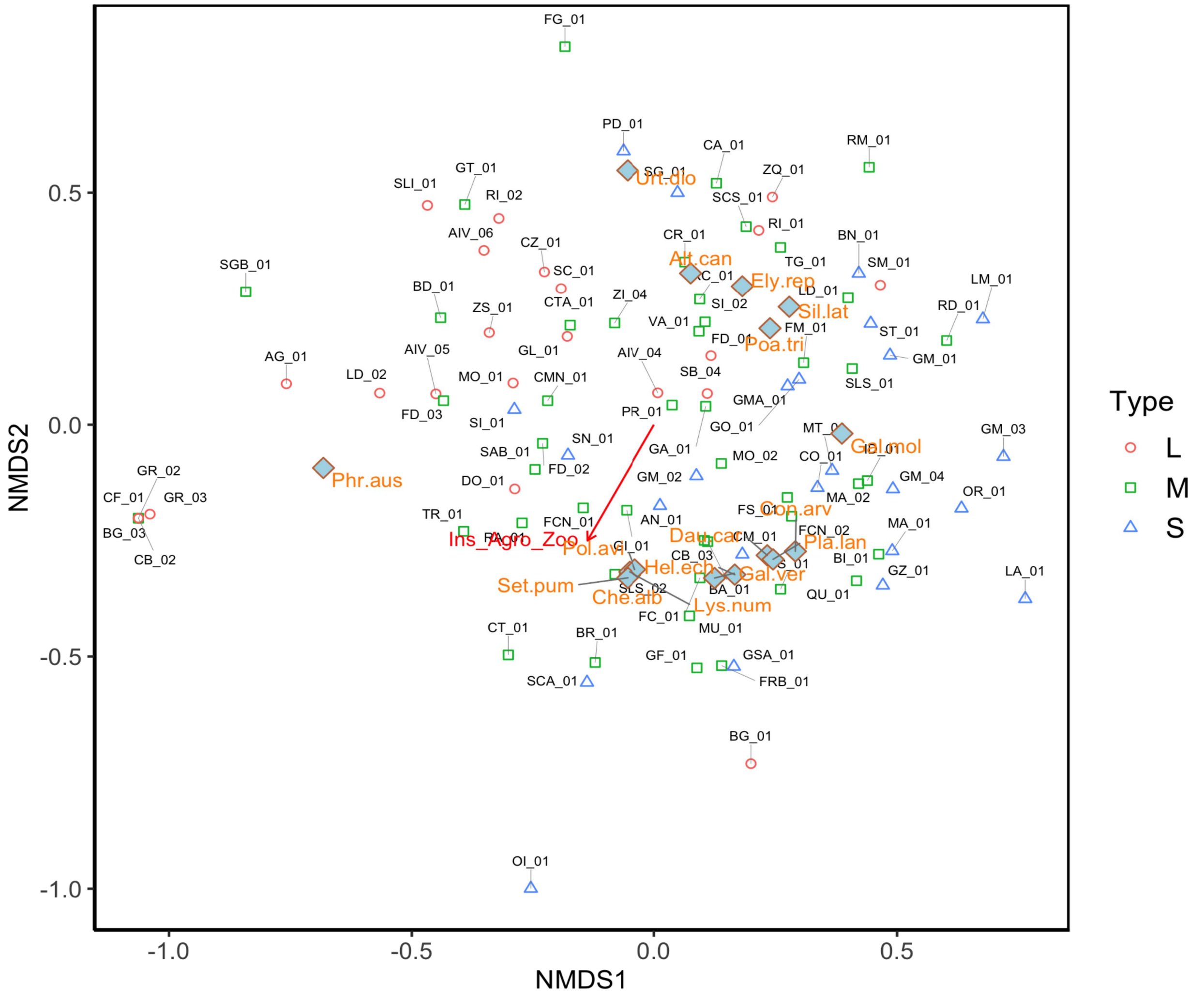

Figure 4 - Non-metric multidimensional scaling plot in which each point represents a single transect (stress = 0.20); occurrence data were used applying the Bray-Curtis method. The macro-category of land use upstream of transects significant for the plant composition is indicated by the arrow (Ins_Agro_Zoo: Agro-zootechnical settlements; P ≤ 0.05). The species that mainly characterize the groups of transects are also shown (P ≤ 0.05): Alt.can: *Althaea cannabina*, Che.alb: *Chenopodium album*, Con.arv: *Convolvulus arvensis*, Dau.car: *Daucus carota*, Ely.rep: *Elymus repens*, Gal.mol: *Galium mollugo*, Gal.ver: *Galium verum*, Hel.ech: *Helminthotheca echioides*, Lys.num: *Lysimachia nummularia*, Phr.aus: *Phragmites australis*, Pla.lan: *Plantago lanceolata*, Poa.tri: *Poa trivialis*, Pol.avi: *Polygonum aviculare*, Set.pum: *Setaria pumila*, Sil.lat: *Silene latifolia*, Urt.dio: *Urtica dioica*

Water quality

Focusing on the water quality of the 17 H2O transects, our analysis confirm that pH, sodium, sodium uptake ratio, orthophosphates and chlorine are significantly related with the composition of the plant community (Fig. 5). In relation to the abundance data of the transects vegetation (Fig. 6), in addition to the above-mentioned parameters ammonia emerged as a key predictive feature, being associated with canals characterized by *Robinia pseudoacacia*, *Rubus ulmifolius*, *Sambucus nigra* L. and *Urtica dioica*.

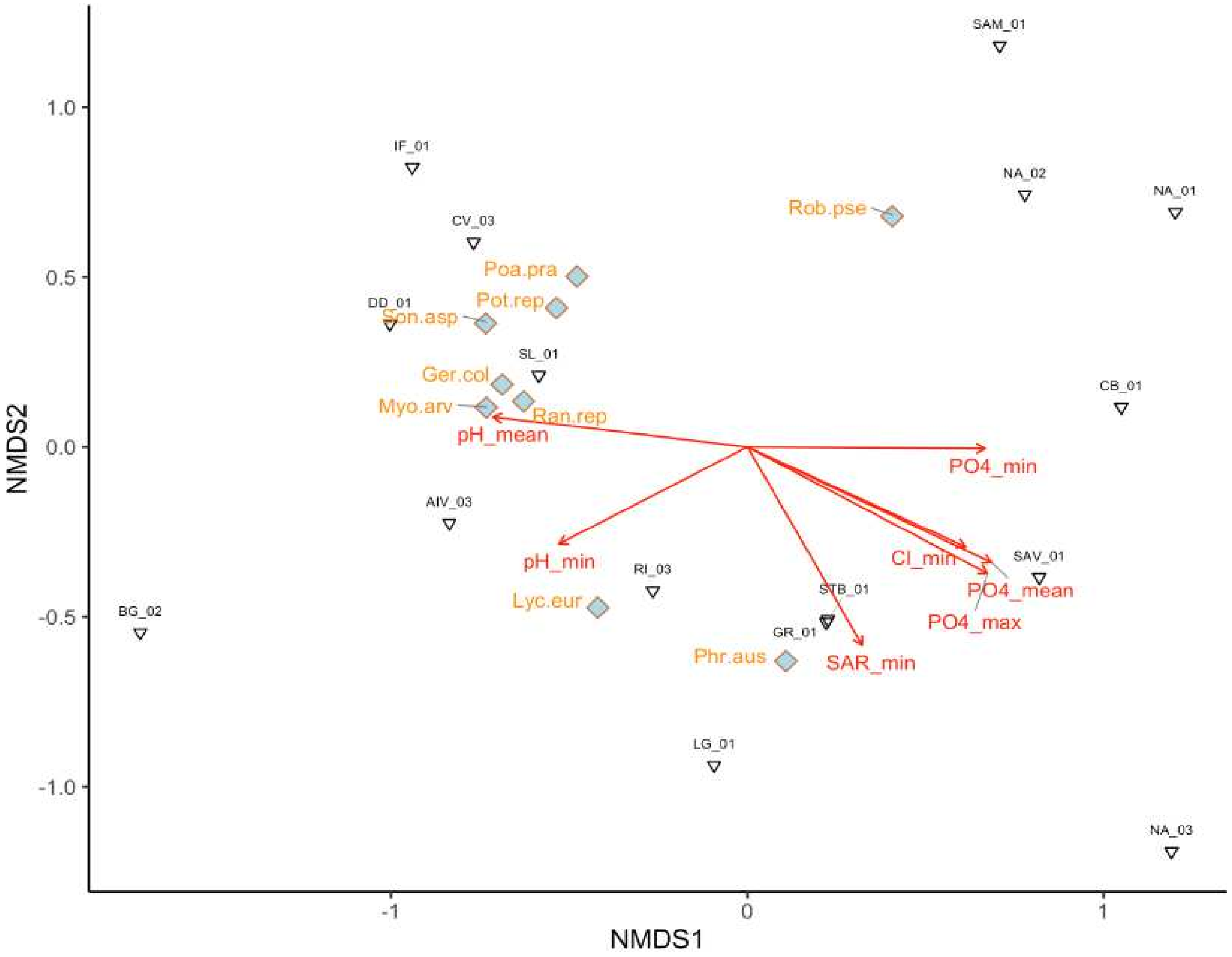

Figure 5 - Non-metric multidimensional scaling plot in which each point represents a H2O transect (stress = 0.13); presence/absence data were used applying the Jaccard method. The arrows represent the water quality parameters whose correlation with the plant composition of the transects is statistically significant (P ≤ 0.05): Cl_min: chlorines (minimum), pH_min/mean: minimum and mean pH, PO4_min/mean/max: orthophosphates (minimum, mean and maximum), SAR_min: minimum sodium absorption ratio. The plant species that most characterize the groups of transects are also shown (P ≤ 0.05): Ger.col: *Geranium columbinum*, Lyc.eur: *Lycopus europaeus*, Myo.arv: *Myosotis arvensis*, Phr.aus: *Phragmites australis*, Poa.pra: *Poa pratensis*, Pot.rep: *Potentilla reptans*, Ran.rep: *Ranunculus repens*, Rob.pse: *Robinia pseudoacacia*, Son.asp: *Sonchus asper*

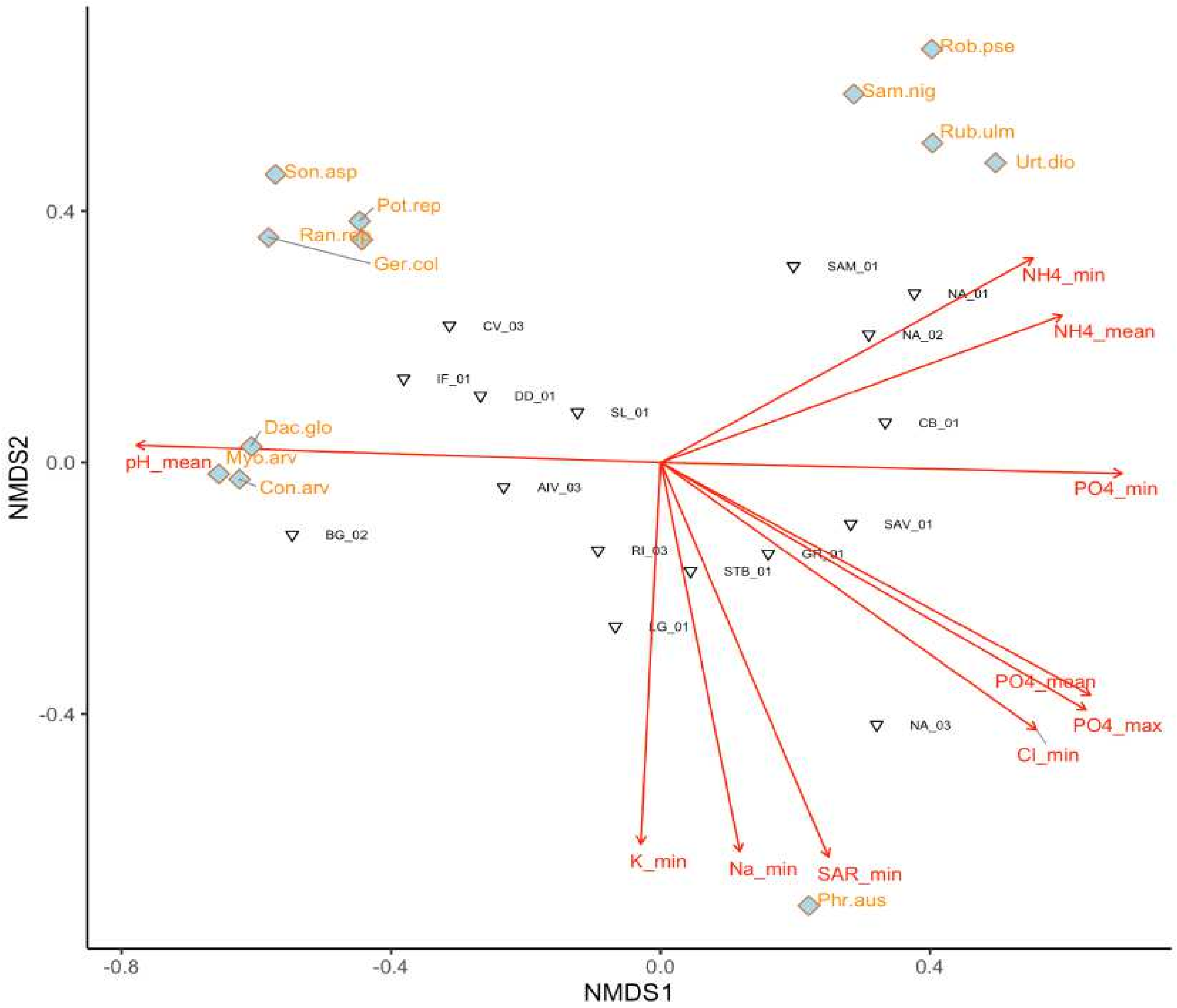

Figure 6 - Non-metric multidimensional scaling plot in which each point represents a H2O transect (stress = 0.134); occurrence data were used applying the Bray-Curtis method. The arrows represent the water quality parameters whose correlation with the plant composition of the transects is statistically significant (P ≤ 0.05): Cl_min: chlorines (minimum), K_min: potash (minimum), Na_min: sodium (minimum), NH4_min/mean: ammonia (minimum and mean), pH_mean: mean pH, PO4_min/mean/max: orthophosphates (minimum, mean and maximum), SAR_min: minimum sodium absorption ratio. The plant species that most characterize the groups of transects are also shown (P ≤ 0.05): Con.arv: *Convolvulus arvensis*, Dac.glo: *Dactylis glomerata*, Ger.col: *Geranium columbinum*, Myo.arv: *Myosotis arvensis*, Phr.aus: *Phragmites australis*, Pot.rep: *Potentilla reptans*, Ran.rep: *Ranunculus repens*, Rob.pse: *Robinia pseudoacacia*, Rub.ulm: *Rubus ulmifolius*, Sam.nig: *Sambucus nigra*, Son.asp: *Sonchus asper*, Urt.dio: *Urtica dioica*

Discussion

The present results contribute to better understand the role of HMWB and land uses in supporting flora and vegetation in a highly anthropized environment such as the Po Valley. The high number of species found confirms that HMWB can mimic natural environments, representing habitat-refugia for some endangered species (e.g., *E. palustris, J. subnodulosus, S. lacustris*). This is relevant considering that, as remarked by Cantonati et al. (2020), freshwater environments are key ecosystem service providers. Therefore, reclamation consortia, farmers, environmental managers and politicians must know the biological, ecological and conservation importance of drainage canals as one of the last aquatic habitats and refuge available in agricultural areas and, wherever appropriate, protect the most valuable sites (Buldrini et al. 2013c; Hill et al. 2016).

In our study area, we verified that both geographical patterns characterizing the transects (slope, aspect, proximity to protected areas) and land use behave as weak drivers with respect to the plant composition of the transects (even the most significant have never returned a significance lesser than $P \leq 0.1$, apart from the proximity to protected areas that has $P \leq 0.05$). It emerges that a significant proportion of variability among transects is not explained by the drivers we considered, because the forces acting on the different sites are multiple and random events such as floods, sudden dry, variations in management practices, crop changes in upstream basins etc. probably modify the spatial patterns of the species along the canals.

A more significant role can be attributed to the quality of water. Some of the monitored parameters (pH, orthophosphate, ammonia and sodium absorption ratio) resulted significant with respect to the HMWB plant richness. It is confirmed in this study that the concentration of ammoniacal nitrogen in water affects the composition of aquatic communities and banks, as the ammonium ion is the best source of nitrogen for plant growth (Bornette and Puijalon 2011). Our results agree with the study by Baláži and Hrivnák (2016), which stated that the plant communities of the Pannonian Lowland (South Slovakia) are significantly correlated with the water parameters of the water flowing there, especially with the chemical oxygen demand, the electrical conductivity, the ammonia and the pH. Moreover, the absence of submerged macrophytes can be associated to the low levels of oxygenation of the canals (Sukhodolova et al. 2017) observed through chemical oxygen demand (COD) and biochemical oxygen demand (BOD) parameters. This condition depends on the use of pesticides in crops and on civil and industrial discharges that cause organic pollution (Baláži and Hrivnák 2016) but, in our study area, a key role is played also by the fact that, during autumn and winter, most canals are partially emptied to prevent flood events resulting from strong and prolonged precipitations.

Our results partially confirm the initial assumptions: a) the hypothesis that there is a reduction of species richness along with the increase of average slope of canals was not verified, as it was impossible to define a slope gradient in the environmental context investigated (H1); b) the hypothesis that there are no changes in the plant composition of the transects in relation to their aspect has been confirmed, demonstrating that in a plain poor of trees the factor «aspect» turns out to be not influent on the plant composition of the transects (H2); c) the hypothesis that there is a reduction in endangered species richness as the distance from protected areas increases has been completely discarded, since endangered species increased by increasing the distance from the protected areas (H3; for this point, however, we remark that our sampling design was not focused on this specific goal); d) the hypothesis that there is an opposite behaviour for alien and invasive species, with their richness increasing as the distance from protected areas increases has been fully confirmed (H4); e) the hypothesis that there is a not negligible role of the water quality of canals in driving flora and plant communities, with an increase of community simplification as canal waters worsened, is confirmed, since the transects carried out in the canals where the highest level of ammonia were recorded are characterized by the presence of nitrophilous and ruderal species, in some cases with a monospecific community (H5).

This work reinforces the belief that long-term sampling plans and greater knowledge about canal management practices are needed. In fact, it is important to act now on management to reduce the biodiversity loss before critical levels difficult to restore are reached. In this regard, Tickner et al. (2020) identified the following priority actions: accelerating implementation of environmental flows, improving water quality, protecting and restoring critical habitats, managing the exploitation of freshwater ecosystem resources (especially species and riverine aggregates), preventing and controlling alien species invasions, and safeguarding and restoring river connectivity.

Final remarks

Over the centuries, extensive artificial hydrological networks have been created and maintained to reclaim the floodplains considered unhealthy and, subsequently, to irrigate crops and protect the inhabitants from floods. The original uses of HMWB have always exposed them to multiple anthropogenic pressures, both through the frequent remodelling of the hydraulic sections and embankments, and through the discharge of wastewater from agricultural activities and urban settlements into surface waters, with important consequences on the flora of the banks. Over time, the awareness that this surface hydrological network is a refuge for species of conservation interest and improves the environmental quality has become increasingly widespread. These functions would be even more relevant if the management of the canals - and HMWB more generally - paid more attention to sustain their natural features. In view of the susceptibility of floodplains to external perturbations, and the global warming that is taking place, it will be necessary to rethink the role of irrigation canals in the near future, considering them not only as an irreplaceable infrastructure for the hydraulic management of catchments, but also the cornerstone to enhance the environmental quality of crossed areas, so as to transform them into a powerful green infrastructure able to mitigate the impacts of human action and climate change and to connect the residual wetlands of the Po Valley.

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Supplementary material 1 - Land use categories cited in this study (classification follows Corine Land Cover IV)

Acronym	Description	Acronym	Description
Aa	Aquaculture in continental env.	Ro	Photovoltaic systems
Fs	Airports for sports flights and heliports	It	Technological systems
Cl	Other wood crops	Iz	Agro-zootechnical settlements
Av	Riverbeds of rivers and streams with abundant vegetation	Ic	Commercial settlements
Af	Riverbeds of rivers and streams with poor vegetation	Is	Service establishment
Ze	Areas with important agricultural crops and natural areas	Io	Hospital settlements
Qa	Active extractive areas	Ia	Production sites
Qi	Inactive extractive areas	Vi	Racecourses
Vx	Uncultivated urban areas	Co	Olive groves
Vs	Sport areas	Vp	Parks
Rv	Green areas associated with roads	Vd	Leisure parks
Ar	Banks	Cp	Poplar crops
Va	Racetraks	Pp	Meadows
Ra	Highways and expressways	Rf	Rail networks
Ax	Artificial basins	Re	Energy distribut./production networks
Br	Ruderal thickets	Ri	Networks for water distribution
Bq	Oak, hornbeam, chestnut forests	Rs	Road networks
Bs	Predominantly willow and poplar forests	Ta	Recent reforestation
Bm	Mixed coniferous and broad-leaved forests	Sn	Non-irrigated arable land
Bp	Plain forests with a prevalence of farnies and ash trees	Se	Irrigated plain arable land
Vt	Camping/tourist accommodation	Zo	Complex crop and particle systems
Vg	Golf courses	Es	Isolated residential facilities
Ac	Canals and waterways	Qs	Modified soils and artifacts
Qc	Construction sites and excavations	Er	Sparse residential fabric
Vm	Cemeteries	Ec	Compact and dense residential fabric
So	Horticultural crops	Ed	Urban residential fabric
Zt	Temporary crops combined with permanent crops	Tn	Evolving shrubby and arboreal vegetation
Qr	Salvage yards	Cv	Vineyards
Qu	Landfill of municipal solid waste	Vv	Villas
Qq	Landfills and deposits of quarries, mines & industries	Sv	Nurseries
Cf	Fruit trees	Ui	Inland wetlands
Rm	Marshalling yards		

Supplementary material 2 - Slope (in percentage) of each stretch of canal where we sampled vegetation (L = large canals, M = medium canals, S = small canals, H2O = canals for which both vegetation and water quality data are available)

Transect	Canal type	Slope %
AG_01	L	0.15
AIV_04	L	0.05
AIV_05	L	0.05
AIV_06	L	0.15
BG_01	L	0.00
BG_03	L	0.40
CB_02	L	0.00
CZ_01	L	0.05
DO_01	L	0.00
FD_01	L	0.10
GL_01	L	0.30
GR_02	L	0.05
GR_03	L	0.15
LD_02	L	0.15
MO_01	L	0.05
RI_01	L	0.10
RI_02	L	0.10
SB_04	L	0.10
SC_01	L	0.25
SLI_01	L	0.05
SM_01	L	0.05
ZQ_01	L	0.20
ZS_01	L	0.20
BA_01	M	0.05
BD_01	M	0.05
BI_01	M	0.10
BR_01	M	0.10
CA_01	M	0.05
CB_03	M	0.05
CGB_01	M	0.05
CMN_01	M	0.05
CR_01	M	0.15
CT_01	M	0.25
CTA_01	M	0.30
FC_01	M	0.10
FCN_01	M	0.05
FCN_02	M	0.00
FD_02	M	0.30
FD_03	M	0.30
FG_01	M	0.30
FM_01	M	0.10
FRB_01	M	0.05
FS_01	M	0.20
GA_01	M	0.00
GF_01	M	0.10
GI_01	M	0.05
GT_01	M	0.05
ID_01	M	0.40
LD_01	M	0.50
MA_02	M	0.00
MO_02	M	0.05
MU_01	M	0.20
PR_01	M	0.00
QU_01	M	0.00
RA_01	M	0.20
RC_01	M	0.05
RD_01	M	0.00
RM_01	M	0.35
SAB_01	M	0.10
SCS_01	M	0.25
SGB_01	M	0.20
SI_02	M	0.05
SLS_01	M	0.10
SLS_02	M	0.05
SS_01	M	0.05
TG_01	M	0.20
TR_01	M	0.00
VA_01	M	0.05
ZI_04	M	0.10
AN_01	S	0.15
BN_01	S	0.40
CF_01	S	0.10
CM_01	S	0.20
CMT_01	S	0.05
CO_01	S	0.05
DC_01	S	0.10
GM_01	S	0.05
GM_02	S	0.10
GM_03	S	0.15
GM_04	S	0.25
GMA_01	S	0.05
GO_01	S	0.45
GSA_01	S	0.10
GZ_01	S	0.10
LA_01	S	0.25
LM_01	S	0.15
MA_01	S	0.00
MT_01	S	0.10
OI_01	S	0.00
OR_01	S	0.10
PD_01	S	0.20
SCA_01	S	0.10
SG_01	S	0.35
SI_01	S	0.05
SN_01	S	0.10
ST_01	S	0.15
AIV_03	H2O	0.10
BG_02	H2O	0.00
CB_01	H2O	0.05
CL_01	H2O	0.05
CV_03	H2O	0.05
DD_01	H2O	0.25
GR_01	H2O	0.20
IF_01	H2O	0.10
LG_01	H2O	0.10
NA_01	H2O	0.10
NA_02	H2O	0.10
NA_03	H2O	0.05
RI_03	H2O	0.10
RR_01	H2O	0.75
SA_03	H2O	0.10
SAM_01	H2O	0.05
SAV_01	H2O	0.00
SB_03	H2O	0.10
SL_01	H2O	0.25
SQ_03	H2O	0.00
STB_01	H2O	0.05
ZI_03	H2O	0.05

Supplementary material 3 - Average slope for each type of canal (L = large canals, M = medium canals, S = small canals, H2O = canals for which both vegetation and water quality data are available)

Canal type	Average slope (%)	Standard deviation (σ)
L	0,12	0,10
M	0,12	0,12
S	0,14	0,11
H2O	0,12	0,16

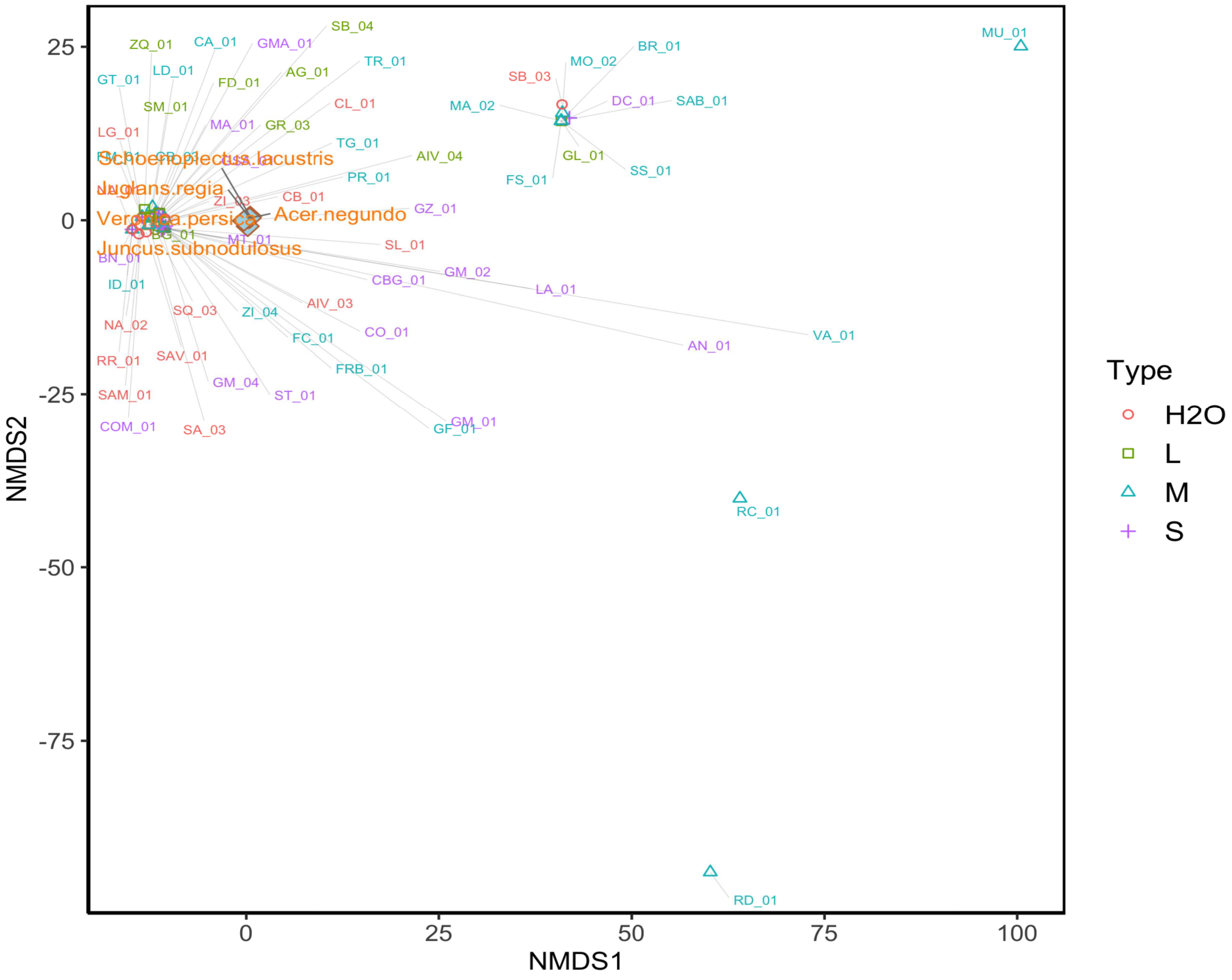
Supplementary material 4 - Percentage of land use classes in the upstream basin of each type of sampled canal (L = large canals, M = medium canals, S = small canals, H2O = canals for which both vegetation and water quality data are available)

		Canal type			
Land use classes	**Acronym**	**H2O**	**L**	**M**	**S**
Aquaculture in the continental environment	Aa	0,1	0,4	0,0	0,0
Airports for sports flights and heliports	Fs	0,1	0,1	0,1	0,0
Other wood crops	Cl	0,2	0,1	0,2	0,1
Riverbeds of rivers and streams with abundant vegetation	Av	0,0	0,0	0,1	0,2
Riverbeds of rivers and streams with poor vegetation	Af	0,1	0,0	0,3	0,2
Areas with important agricultural crops and natural areas	Ze	0,0	0,0	0,0	0,0
Active extractive areas	Qa	0,0	0,0	0,0	0,2
Inactive extractive areas	Qi	0,0	0,0	0,0	1,0
Uncultivated urban areas	Vx	0,2	0,6	0,5	0,3
Sport areas	Vs	0,1	0,3	0,3	0,4
Green areas associated with roads	Rv	0,1	0,5	0,2	0,3
Banks	Ar	0,5	0,2	0,2	0,0
Racetraks	Va	0,0	0,0	0,0	0,0
Highways and expressways	Ra	0,0	0,3	0,1	0,4
Artificial basins	Ax	0,4	0,4	0,5	0,5
Ruderal thickets	Br	0,0	0,1	0,1	0,1
Oak, hornbeam and chestnut forests	Bq	0,4	0,0	1,2	0,0
Predominantly willow and poplar forests	Bs	0,2	0,0	0,0	0,1
Mixed coniferous and broad-leaved forests	Bm	0,0	0,0	0,0	0,0
Plain forests with a prevalence of farnies and ash trees	Bp	0,1	0,2	0,0	0,2

Camping and tourist accommodation	Vt	0,0	0,0	0,0	0,0
Golf courses	Vg	0,0	0,0	0,0	0,0
Canals and waterways	Ac	2,5	1,5	0,5	1,1
Construction sites and excavations	Qc	0,1	0,5	0,3	0,6
Cemeteries	Vm	0,0	0,1	0,1	0,1
Horticultural crops	So	0,1	0,3	0,3	0,2
Temporary crops combined with permanent crops	Zt	0,2	0,1	0,1	0,1
Salvage yards	Qr	0,0	0,0	0,1	0,0
Landfill of municipal solid waste	Qu	0,1	0,0	0,2	0,0
Landfills and deposits of quarries, mines & industries	Qq	0,0	0,0	0,0	0,0
Fruit trees	Cf	4,2	2,9	3,3	4,2
Marshalling yards	Rm	0,0	0,8	0,0	0,0
Photovoltaic systems	Ro	0,1	0,2	0,1	0,1
Technological systems	It	0,0	0,0	0,0	0,0
Agro-zootechnical settlements	Iz	0,6	0,7	0,7	0,5
Commercial settlements	Ic	0,0	0,1	0,1	0,1
Service establishment	Is	0,2	0,5	0,4	0,2
Hospital settlements	Io	0,0	0,0	0,0	0,0
Production sites	Ia	1,0	2,9	2,1	1,0
Racecourses	Vi	0,1	0,4	0,1	0,1
Olive groves	Co	0,1	0,0	0,0	0,0
Parks	Vp	0,2	0,3	0,3	0,5
Leisure parks	Vd	0,0	0,0	0,0	0,0
Poplar crops	Cp	0,4	0,1	0,3	0,4
Meadows	Pp	0,3	0,3	0,4	0,5
Rail networks	Rf	0,1	0,4	0,3	0,2
Energy distribution and production networks	Re	0,1	0,1	0,2	0,1
Networks for water distribution	Ri	0,0	0,0	0,0	0,0
Road networks	Rs	0,8	2,0	1,4	1,4
Recent reforestation	Ta	1,4	0,8	0,4	0,2
Non-irrigated arable land	Sn	0,5	0,0	1,6	0,0
Irrigated plain arable land	Se	74,6	69,5	70,3	75,2
Complex crop and particle systems	Zo	0,1	0,1	0,2	0,4
Isolated residential facilities	Es	2,3	3,5	4,2	4,7
Modified soils and artifacts	Qs	0,3	0,4	0,2	0,3
Sparse residential fabric	Er	1,3	2,0	1,9	1,0
Compact and dense residential fabric	Ec	0,0	0,0	0,0	0,0
Urban residential fabric	Ed	0,4	0,8	0,8	0,5

Evolving shrubby and arboreal vegetation	Tn	0,4	0,4	0,9	0,3
Vineyards	Cv	0,3	1,4	1,9	0,5
Villas	Vv	0,2	0,6	0,5	1,0
Nurseries	Sv	0,1	0,6	0,1	0,3
Inland wetlands	Ui	4,3	2,6	1,8	0,1

Supplementary material 5 - Non-metric multidimensional scaling plot in which each point represents a transect with alien, invasive or threatened species (stress = 0.001); Jaccard's method was applied. In this analysis we did not consider the other species to highlight the influence of these three categories



CHAPTER III

Role of irrigation canal morphology in driving riparian flora in over-exploited catchments

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Irrigation and drainage canals role for plant diversity and nature conservation

Abstract

Freshwater plants loss is one of the preeminent issues concerning biodiversity conservation, due to the alteration of inland waters by water regulation and agricultural intensification. At the same time, data suggest a relevant contribution of artificial, lowland aquatic ecosystems in supporting plant diversity. However, the underlying ecological mechanisms remain to be fully understood. To add knowledge to this subject, a wide canal network in the Bologna area (~1400 km², northern Italy) was investigated to analyse the riparian flora in relation to canal morphology. A systematic sampling procedure was adopted by randomly selecting 96 transects (1 m x 10 m) along 79 different canals, classified as small, medium, and large in terms of water depth. Flora were characterized based on the Ellenberg's humidity and nitrophily indices, life forms, chorotypes, and alien species. The distribution of the number of species and floristic categories between transects, and the role of canal depth were explored using linear mixed models and nMDS. 251 plant species were recorded, characterized by a broad ecology in terms of soil moisture (71% of the list) and nutrient availability (59%). Wetland and alien species – including invasive ones – were a marginal presence (< 5%, < 6%, respectively) – and canal depth showed a significant effect on compositional dissimilarity between canals, with larger canals characterized by lower diversity rates. This work reinforces the pivotal contribution of heavily modified water bodies in supporting plant richness in oversimplified landscapes, confirming the role of canal depth in driving local flora.

Key words

hydro-hygrophilous flora, plant diversity, threatened plant species, alien plant species, Po valley, heavily modified water bodies

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Introduction

The biodiversity loss in freshwaters is a critical issue globally, particularly in inland waters, which are among the most threatened ecosystems on a world scale (e.g. Cantonati et al. 2020; Guareschi et al. 2020). Aquatic and wetland plant species are among the most affected in this regard (Preston and Croft 1997; Moser et al. 2002), because of the severe alteration of habitats and ecosystem dynamics (Riis and Sand-Jensen 2001; Bolpagni and Piotti 2016). Most of the agricultural landscapes across Europe have been dramatically subjected to intense human-mediated impacts in the last two centuries, resulting in a severe over-simplification of lowland watersheds with strong cascading effects on related biota (Palov 1985; Dorotovičová 2013). As a consequence, several aquatic and wetland plants, which were widespread and common until a few decades ago, underwent a steep decline and today are generally endangered and at risk of extinction in most of their pristine range (e.g. Schnittler and Günther 1999; Riis and Sand-Jensen 2001; Eckstein et al. 2004; Gentili et al. 2010; Beretta et al. 2012). In Europe, for example, from 1945 onwards, about 70% of the extinct plant species concerns amphibian and aquatic taxa (Denny 1994; Sager and Clerc 2006).

In Italy, the above-mentioned issues are even more critical, since this country is a recognized hotspot of plant diversity at a European and global level (Bartolucci et al. 2018; Bolpagni et al. 2018; Murphy et al. 2019). Indeed, urbanization and land use changes resulted in an intensely fragmented, isolated aquatic landscape with distinctive relict features (Buldrini et al. 2013a). In addition, human-mediated land transformation processes are extremely relevant, being much older than in other parts of Europe (the first drainage works date back already to the I millennium B.C. – Dallai et al. 2014a). Specifically, in Emilia-Romagna region the decrease in wetland extent was equal to 89% of the pristine situation (Tinarelli and Tosetti 1998), leading to a generalized loss of the typical floristic facets they hosted (Dallai et al. 2014a).

Inland waters, under oligo- to meso-trophic conditions, were largely interconnected before the massive land reclamation policy of the Po valley, stopping the regular genesis of new marginal aquatic ecosystems which formerly ensured the survival of local ecotypes of many aquatic and wetland plants characterised by wide distributive ranges (central European, Eurasian or Palaeo-temperate chorotypes), such as *Hydrocharis morsus-ranae* L., *Nuphar lutea* (L.) Sm., *Nymphaea alba* L., *Nymphoides peltata* (S.G. Gmelin) Kuntze, *Trapa natans* L., *Viola elatior* Fr. and *V. pumila* Chaix. Currently, such plants are confined to small, relict and isolated populations, often hosted by artificial lotic habitats that, in some respects, mimic the characters of natural counterparts (Buldrini and Dallai 2011; Buldrini et al. 2013a,b,c, 2015; Dallai et al. 2014b). In this regard, artificial and heavily modified water bodies (HMWB), such as ditches and drainage canals, seem to have a great importance to preserve plant richness and diversity, acting as rural plant hotspots and qualifying the agricultural landscape (Hulina 1998; Pedullà and Garbari 2001; Bonafede et al. 2003; Goulder 2008; Herzon and Helenius 2008; Bolpagni et al. 2013, 2018; Dorotovičová 2013). Specifically, drainage canals thanks to their micro-environmental heterogeneity have shown to host a higher number of species than the surrounding areas (Tölgyesi et al. 2020). Furthermore, in several cases, HMWB are the unique available habitats for aquatic and wetland plants, often of great conservation concern due to their rarity or the phytogeographical importance (Goulder 2008).

Despite the great potential to support plant diversity and improve lowland environmental quality, HMWB are not normally managed to this purpose, but only to ensure flood protection and provide water for irrigation for crossed agricultural landscapes (e.g. Bolpagni 2020). Furthermore, since the irrigation canals are artificial environments, for a long time they have been commonly believed to be of little interest from a biological viewpoint, thus data about habitats, plant communities and possible factors reducing plant biodiversity are scarce (see also Dorotovičová 2013) and often confined to grey literature. In addition, even if it has been demonstrated that, in drainage canals and wetlands, the structure of the plant communities is correlated to physical and chemical water characteristics and management methods of the canals and their embankments (Güsewell and Le Nédic, 2004; van Belle et al. 2009; Fraaije et al. 2019), much still remains to be learnt about the natural and anthropogenic factors regulating species diversity in such environments. For all these reasons, we investigated the riparian herbaceous vegetation of some drainage/irrigation canals of the Po plain, taking the sector pertaining to the province of Bologna (northern Italy) as a case study. The main objectives of this study are: 1) to analyse the floristic composition of riparian herbaceous plant communities that colonize the banks of the canals; 2) to quantify the role of canal depth as driver of floristic facets; 3) to assess the conservation value of canal banks in terms of rare and threatened plant species; 4) to analyse the local presence of alien and invasive species.

The general hypothesis is that a decrease in plant richness along with the increase of the depth of canals (as a proxy of their physical dimensions) is expected (h_1), with deeper canals subjected to higher potential external impacts. Indeed, since larger canals connect territories far away from each other, they are inferred to be more prone to pollution by nutrients and chemicals from arable lands, and to colonisation by alien and invasive plant species (h_2). Further, unlike what is expected for lowland natural lotic ecosystems, larger HMWB are characterized by lower water level variations reducing the expected complexity of bank ecotone. All this reinforces the need to fill the significant knowledge gaps regarding the roles of drainage canals in flora conservation.

Materials and methods

Study area

The study area encompasses the lowland sector of the province of Bologna (Emilia Romagna region, northern Italy; Fig. 1), which is one of the most productive sectors of the Po River basin, and in turn of the European irrigated plains and, at the same time, one of the most impacted by agricultural activities (Guareschi et al. 2020).

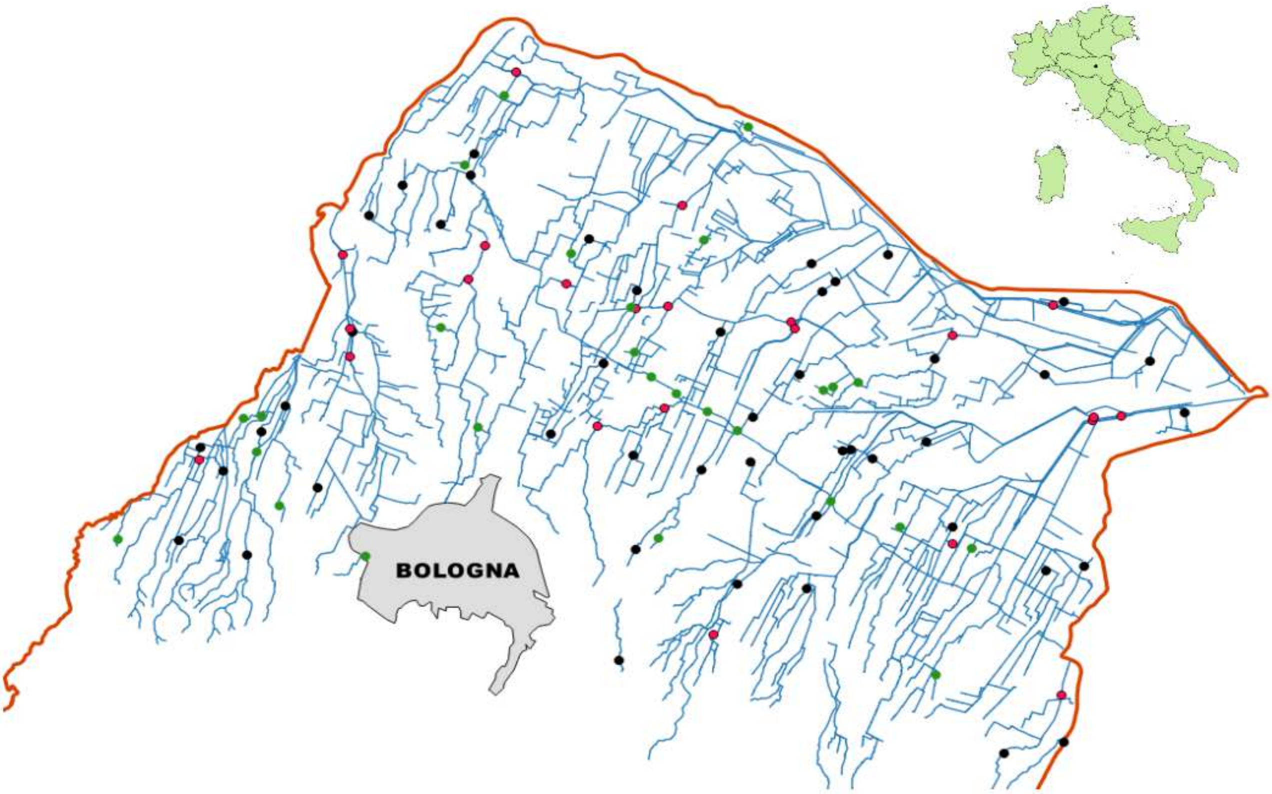

Figure 1 - Map of the study area (Bologna plain, northern Italy) with the indication of the sampling sites (black: large canals; red: medium canals; green: small canals). The position of the study area within Italy (black dot) is also shown.

Since the Apennines are a barrier for the southern warm winds, and the thermal regulation of the Adriatic Sea is poor due to its scarce depth, the climate of the area typically has cold winters and warm summers (Köppen climate classification Cfa, Peel et al. 2007). The atmospheric circulation is generally scarce, therefore atmospheric humidity is high and, during autumn and winter, fog is quite frequent. Based on data from the period 1981-2010, the average annual temperature is 12.4 °C; the warmest month is July (average temperature 31.4 °C) and the coldest is January (average temperature -1.1 °C). Annual precipitation is 684 mm, with a maximum in October and a minimum in July (Arpae Emilia-Romagna 2019, https://simc.arpae.it/dext3r/).

The Reno River (total length of 212 km) and its tributaries are the main natural watercourses of the study area, all of them having a torrential regime. As is typical in an irrigated plain, the most relevant watercourses are represented by the artificial hydrological network, here managed by a land-reclamation consortium called Consorzio della Bonifica Renana (hereafter referred to as "Consortium"). This Consortium manages a total area of 3419 km² and its lowland sector (i.e. the present study area) has an extent of 1439 km². The hydrological network made up by the canals is 1154 km long and is used both for drainage and irrigation purposes; for this reason, all canals are subjected to periodic variations of their hydrologic regime, with higher flows from May to September and a lean period from November to March, apart from exceptional rainfall events. Such variations are proportionally much more pronounced in smaller canals, which can even be dry during autumn and winter.

Soils are generally characterised by a prevailing clay-silty texture (Carta dei Suoli dell'Emilia-Romagna, 1:50.000, https://geo.regione.emilia-romagna.it/cartpedo/), and the mature vegetation is composed of a mixed deciduous broad-leaved forest, dominated by *Quercus robur* L. and *Carpinus betulus* L., turning into a riparian forest with *Populus alba* L. and *Salix alba* L. along river courses (Puppi et al. 2010). Presently, such vegetation has practically disappeared due to the land use transformation by intensive farming, and only a few remnants still exist. Indeed, riverbanks are generally characterised by oversimplified vegetation dominated by nitrophilous (e.g. *Aristolochia clematitis* L., *Rubus ulmifolius* Schott, and *Rumex obtusifolius* L.), disturbance-tolerant (e.g. *Avena fatua* L., *Bromus hordeaceus* L., *Daucus carota* L., and *Rumex crispus* L.) and alien plant species (*Robinia pseudoacacia* L., *Morus alba* L., *Sambucus nigra* L., *Amorpha fruticosa* L., and *Reynoutria* spp.). Drainage canals, on the contrary, are nearly always devoid of tree cover, to assure a rapid water runoff in case of rainfall, and to allow easier periodic mowing of their banks.

Sampling design

A preliminary analysis of the artificial hydrological network was carried out by a GIS approach to identify the prevalent types of drainage canals. Ancillary information (mainly concerning the water depth of canals) was provided by the Consortium and Arpae Emilia-Romagna. The canals are dredged every 10-15 years on average, unless the appearance of hydraulic problems of primary relevance (exceptional flood events), whereas bank vegetation is regularly mowed thrice a year in the period May-October. The canals were classified based on their depth as small (S), medium (M) and large (L), with section depths respectively below 2 m, between 2 m and 4 m, and more than 4 m, considering depth as the distance between the top of the embankments and the bottom of the canal bed.

These data were used to select 79 canals, representative of the main types of canal in the area, with a total length of 1154 km. Sampling points were consequently selected based on the total length of each type of canal (325 km for S, 551 for M, and 278 for L), by adopting a random selection (achieved by the *random points* function of QGIS). Therefore, we created the buffers of the canal polylines (*buffer* function of QGIS), imposing a minimum distance of 2 km between two points within the same canal. This approach resulted in 96 sampling sites (23 for L canals, 46 for M canals, 27 for S canals), representative of the entire hydrological network (Chiarucci 2007). The sampling sites were chosen to be representative of the three canal types, also in consideration that these canals are fed by different sub-catchments. Within each sampling point, a transect of 10 m x 1 m was established along the canal bank and parallel to the water course, starting from the water surface and posing the transect for 90 cm on the dry ground and the remnant 10 cm in the water. The transect was divided into 10 plots, each of 1 m x 1 m. The plant species within each plot were identified and recorded as presence/absence; their frequency at the transect level was calculated as the sum of the occurrences in the single 10 plots.

Data analyses

To characterise the canal flora, first we analysed the species richness subdivided by canal type (L, M, S) and, secondly, we explored the adaptive response of the plant species considering the ecological categories based on the Ellenberg's indicator values for moisture and nutrient availability (Pignatti et al. 2005). All plants were characterized as follows:

a) non-wetland taxa (NWT), including both tendentially meso-xerophilous or xerophilous species, or indifferent to soil moisture conditions (U ≤ 4), and mesophilous species (U = 5 and 6);

b) hygrophilous species (indicators of humid soils, U = 7 and 8; HYG);

c) hydrophilous (indicators of palustrine conditions, U = 9 and 10) and aquatic species (indicators of constant soil submersion, U ≥ 11) (WAT);

d) species tendentially meso-oligotrophic or oligotrophic, or indifferent to soil trophic conditions (N ≤ 3), and mesotrophic species (indicators of soils quite or well humified, N = 4 and 5; MOT);

e) eutrophic species (indicators of nutrient concentration in the soil, N = 6 and 7; EUT);

f) nitrophilous species (indicators of nutrient excess in the soil, N ≥ 8, NNT).

In addition to the above-mentioned ecological categories, each plant was classified also according to the life form type (CH = chamaephytes, G = geophytes, H = hemicryptophytes, HE = helophytes, HY = hydrophytes, P = phanerophytes, T = therophytes) and native-range distribution (BOR = boreal, COS = cosmopolite, EUR = Eurasian, MED = Mediterranean taxa) based on Pignatti et al. (2017-2019), and Poldini (1991) and Tomaselli and Gualmini (2000). Archaeophytes (i.e. those alien species that are present in Europe since before 1492) were grouped together with native species, according to Jalas et al. (1972-). The nomenclature of species follows The Plant List (2013).

The effect of the canal size on the above mentioned categories (moisture and nutrient indices, life forms and chorotypes), and their interaction were tested with linear mixed models to consider the effects of random factors. In this work, the site identity was set as the random effect as it was repeated for each category. Dependent variables were transformed with the square root to meet the normality assumption. A post-hoc pairwise comparison for each model was performed to test for the differences among canals within category. A non-metric multidimensional scaling (nMDS) was performed on the plant richness data to test the importance of canal depth in structuring the plant community. This analysis was carried out considering the Bray-Curtis as dissimilarity measure and the stress measure to evaluate the goodness of fit (Legendre and Legendre 1998). The measure of nMDS' stress – calculated over three axes – was 0.19, lower than the critical threshold for having a reliable ordination as reported by Clarke (1993).

All the statistical analyses were carried out in the R environment (R Core Team 2019), using the packages *lmerTest Package* (Kuznetsova et al. 2017), *emmeans* (Lenth 2020), *pbkrtest* (Halekoh and Højsgaard 2014), and *vegan* (Oksanen et al. 2019).

Results

General floristic features

Globally, 251 plant species were recorded in 96 transects (1 per sampling site), with a number of species per transect ranging from 1 to 38, and a mean species number of 19.1 ± 8.8 (standard deviation). Only very few species were recorded in more than 50% of transects: *Calystegia sepium* (L.) R. Br. in 69 transects, *Phragmites australis* (Cav.) Trin. ex Steud. in 56, *Ranunculus repens* L. in 55, *Potentilla reptans* L. in 52, and *Urtica dioica* L. in 50, respectively. A total of 12 alien species were recorded (4.8% of the total species list), of which 7 were invasive. The most frequent alien species was *Veronica persica* Poir. (18 transects); the others (*Acer negundo* L., *Erigeron annuus* (L.) Pers., *Juglans regia* L., *Paspalum distichum* L.) were extremely sporadic presences. The most frequent invasive species were *Amorpha fruticosa* L. (6 transects) and *Sorghum halepense* (L.) Pers. (5); the others (*Amaranthus retroflexus* L., *Artemisia verlotiorum* Lamotte, *Erigeron canadensis* L., *Robinia pseudoacacia* L., *Xanthium orientale* L. ssp. *italicum* (Moretti) Greuter) were extremely sporadic presences (Table 1). 7 species (2.8% of the total species recorded) are considered threatened: *Juncus subnodulosus* Schrank (critically endangered), *Allium angulosum* L., *Euphorbia palustris* L., *Samolus valerandi* L. and *Sium latifolium* L. (endangered), *Nuphar lutea* (L.) Sm. and *Schoenoplectus tabernaemontani* (C.C. Gmel.) Palla (vulnerable). *E. palustris* was recorded in 9 transects, the others threatened *taxa* in 1 transect only.

Table 1 - Occurrences of alien, invasive and threatened plants among the three canal types: L = large (with a section depth > 4 m), M = medium (with a section depth in the range 2 to 4 m), and S = small (with a section depth < 2 m)

Species	**Canal type** **L**	 **M**	 **S**
Alien *taxa*			
Acer negundo L.		1	
Erigeron annuus (L.) Pers.		2	1
Paspalum distichum L.			1
Veronica persica Poir.	2	6	10
TOTAL OCCURRENCES	2	9	12
Invasive *taxa*			
Amaranthus retroflexus L.	1		
Amorpha fruticosa L.	4	2	
Artemisia verlotiorum Lamotte	2		
Erigeron canadensis L.		2	
Robinia pseudoacacia L.		1	1
Sorghum halepense (L.) Pers.	1	3	1
Xanthium orientale L. ssp. *italicum* (Moretti) Greuter	1	1	
TOTAL OCCURRENCES	9	9	2
Threatened *taxa*			
Allium angulosum L.		1	
Euphorbia palustris L.	4	2	3
Juncus subnodulosus Schrank		1	
Nuphar lutea (L.) Sm.	1		
Samolus valerandi L.			1
Schoenoplectus tabernaemontani (C.C. Gmel.) Palla			1
Sium latifolium L.			1
TOTAL OCCURRENCES	5	4	6

Overall, the canal flora was largely composed by species with no particular ecological requirements in terms of soil moisture (71% of the total list: mesophilous, meso-xerophilous,

xerophilous and indifferent species = NWT) and nutrient availability (59% of the total list: mesotrophic, meso-oligotrophic, oligotrophic and indifferent species = MOT). Indeed, hydrophilous (HYG), aquatic and hygrophilous (WAT) and nitrophilous (NNT) plant species were of secondary importance, being limited to 11%, 2% and 14% of the total of the surveyed species, respectively (Fig. 2). The life form spectrum calculated on the whole set of species was dominated by hemicryptophytes (H, 41%, in the present case mostly grassland plants) and therophytes (T, 31%, in the present case mostly ruderal plants). Concerning the chorotypes, Eurasian species prevailed (EUR, 43%), followed by Mediterranean (MED, 20%), Boreal (BOR, 20%) and cosmopolite species (COS, 16%) (Fig. 3).

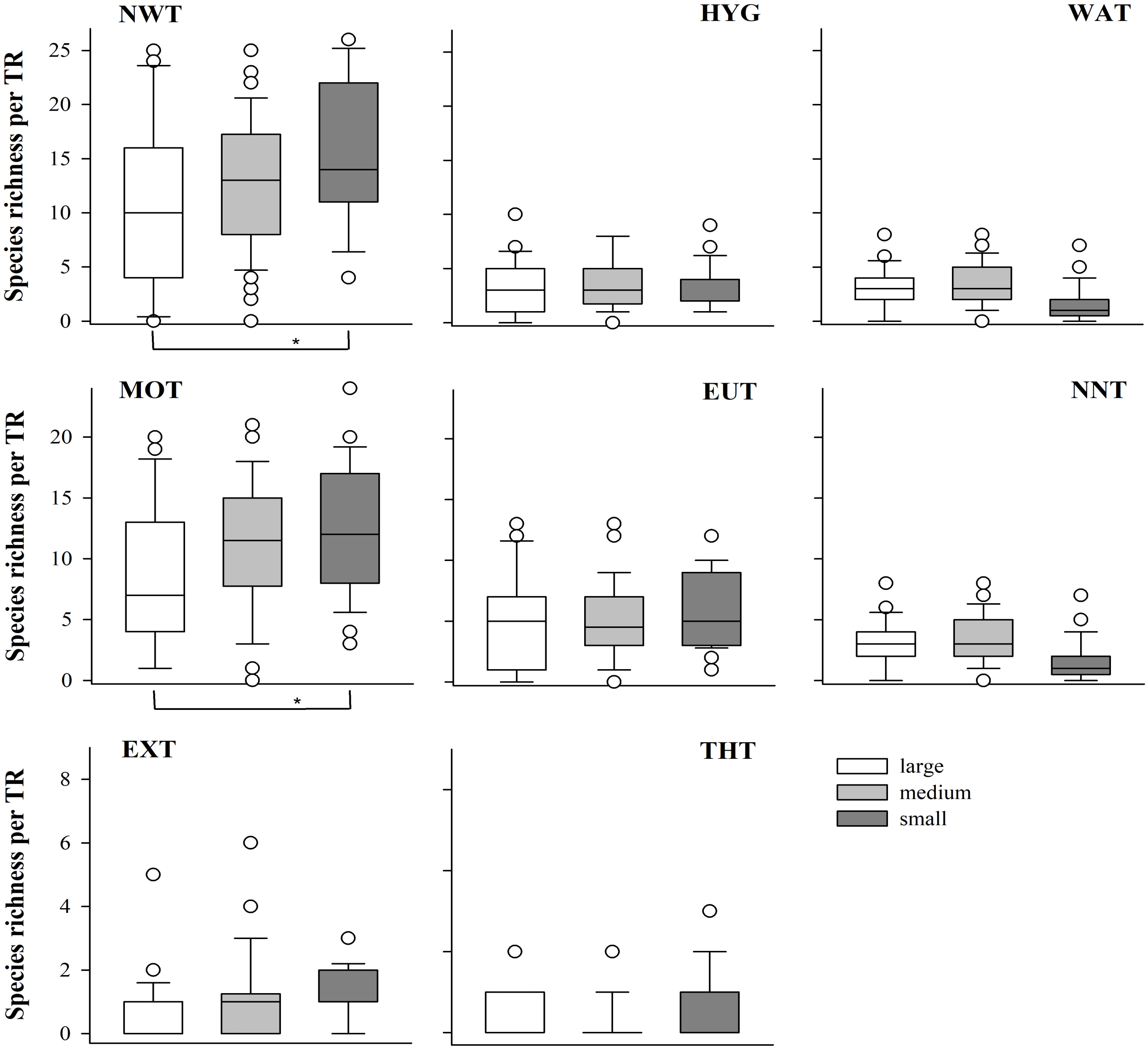

Figure 2 - Distribution of the Ellenberg's ecological categories for the humidity (non-wetland taxa = NWT, hygrophilous taxa = HYG, and aquatic + wetland taxa = WAT), and nitrophily (meso-oligotriphic taxa = MOT, eutrophic taxa = EUT, and nitrophilous taxa = NTT), and the status of the species (exotic taxa = EXT, and threatened taxa = THT) by canal type. Asterisks indicate the level of significance: *P < 0.05 obtained by the post-hoc pairwise comparison

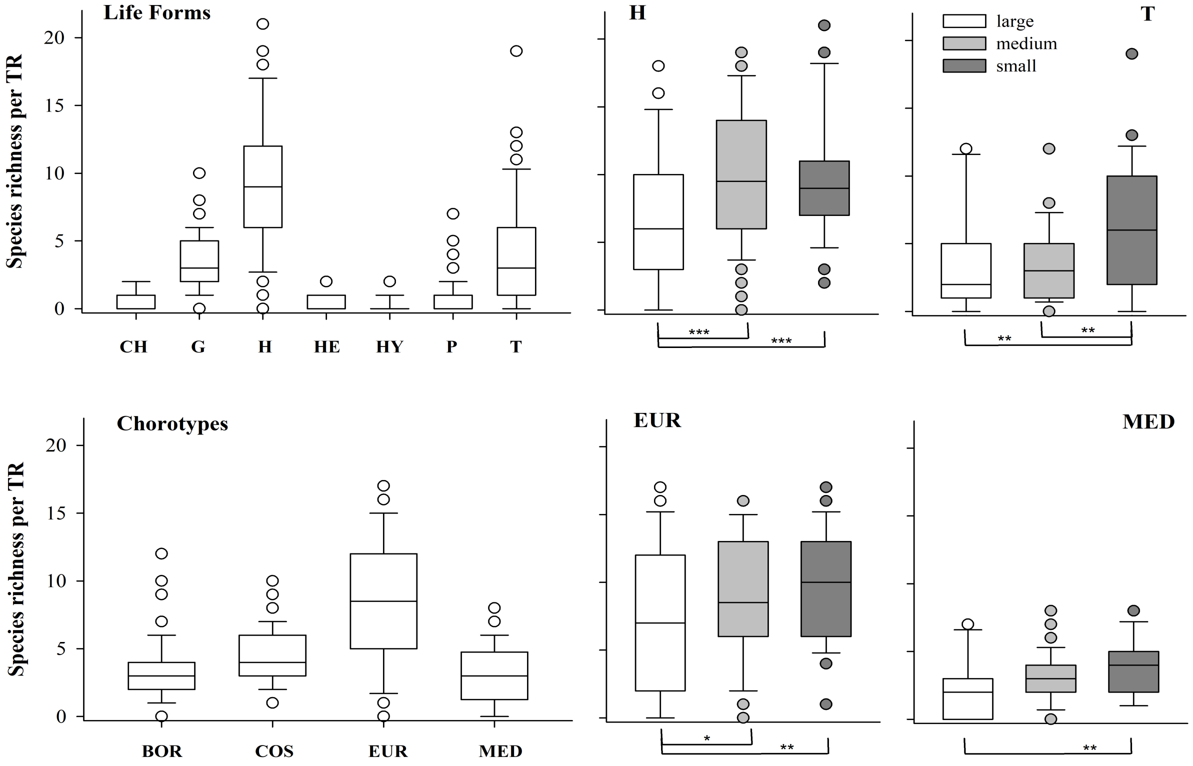

Figure 3 - Distribution of the life forms (CH = chamaephytes, G = geophytes, H = hemicryptophytes, HE = helophytes, HY = hydrophytes, P = phanerophytes, T = therophytes) and chorotypes (BOR = boreal, COS = cosmopolite, EUR = Eurasian, MED = Mediterranean taxa) by transects (TR) and canal type (exclusively for H and T). Asterisks indicate the level of significance: *P < 0.05, **P < 0.01, ***P < 0.001 obtained by the post-hoc pairwise comparison

Species richness facets across canal types

There were no significant differences in the total species richness among the transects by canal types, even if a slightly not significant increase in species number appeared passing from L to S canals. However, significant statistical differences were found for all the richness facets investigated, except for rare and threatened species (Tab. 2). Exploring the ecological categories, statistically significant values were obtained for non-wetland *taxa* ($U < 9$) and meso-oligotrophic plant species ($N \leq 3$), with a higher number of both species groups in S and M canals compared to L ones ($p < 0.001$ and $p = 0.017$ for NWT, $p = 0.021$ and $p = 0.035$ for MOT, respectively)

(Fig. 2; Tab. 2). Focusing on the life forms, statistically significant variations among canal types were found for hemicryptophytes (H) and therophytes (T), with a greater presence of H in M and S canals compared to L ones (both $p < 0.001$), and of T in S canals compared to M and L canals ($p = 0.003$ and $p = 0.005$, respectively; Fig. 3). Small canals show a greater number of EUR and MED plant species compared to larger ones ($p = 0.001$, and $p = 0.004$, respectively); similarly, medium canals show a greater numbers of EUR taxa than the larger canals ($p = 0.021$) (Fig. 3).

Table 2 - Results of the linear mixed models (CanDep = depth of canal)

Parameter	***numDf***	***denDf***	***F***	***P***
Moisture indicator (WET)				
CanDep	2	93	1.9228	0.1520
WET	2	186	195.8156	**<2.2e-16**
CanDep x WET	4	186	5.0095	**0.0007**
Nutrient indicator (NIT)				
CanDep	2	93	2.5568	0.0830
NIT	2	186	128.6662	**<2.2e-16**
CanDep x NIT	4	186	3.0063	**0.01961**
Life forms (LF)				
CanDep	2	93	2.1020	**0.1280**
LF	6	558	202.2143	**<2.2e-16**
CanDep x LF	12	558	3.3601	**9.5e-05**
Chorotypes (COR)				
CanDep	2	93	3.3338	**0.0400**
COR	3	279	96.0947	**<2.2e-16**
CanDep x COR	6	279	3.0992	**0.0060**

The structure of the riparian plant communities is severely affected by canal depth, as distinct clusters can be identified based on the nMDS ordination plot (Fig. 4). Indeed, this analysis highlighted the presence of a continuum gradient moving from S to L canal types, with the clouds of the S and L canals clearly separated and the cloud of M canals largely overlapping with the other two.

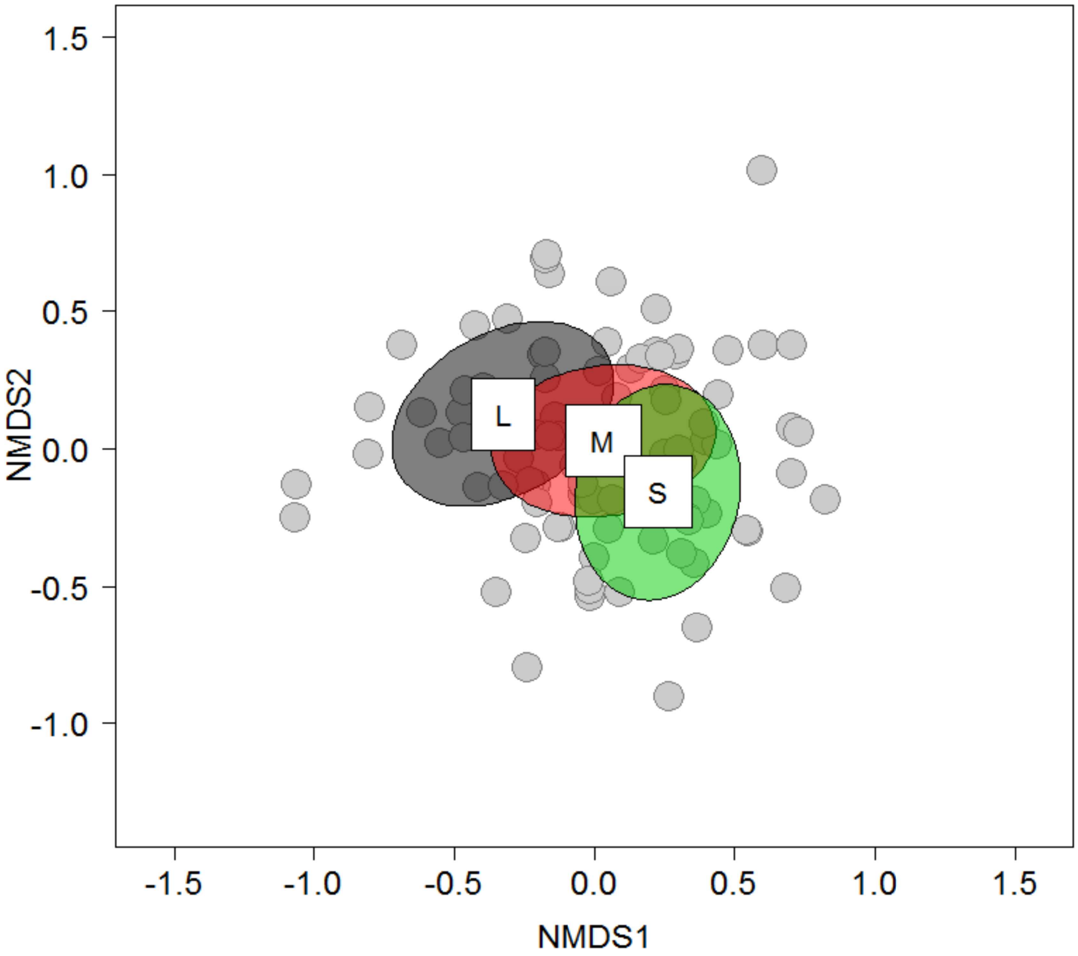

Figure 4 - Non-metric multidimensional scaling plot in which each point represents a single transect (stress = 0.19); 95% confidence ellipses were reported for S = small canals (green), M = medium canals (red), L = large canals (grey)

Discussion

The present results contribute to better understanding the role of the canal morphology in driving the riparian flora in overexploited watersheds, partially confirming the hypothesized increase (h_1) in plant richness along with the decrease in the section depth of canals. Indeed, only the number of non-wetland *taxa* and the meso-oligotrophic plant species were found to be statistically higher in smaller and medium canals compared to larger ones. At the same time, higher degrees of invasion by alien plants were recorded in smaller canals compared to larger ones, although the differences are not statistically significant (thus rejecting h_2). This agrees with the idea that the progressive deepening of canals strongly modulates the composition and structure of bank vegetation by increasing the flow velocity and the recurrence of flood events, as well as the possibility of a complete drought during winter times for small canals (Fraaije et al. 2019).

The total plant richness recorded in the system (251) is comparable, but higher, with what has been described in similar agricultural landscapes across Italy and Europe (Edvardsen and Økland 2006; Gołdyn 2010; Bolpagni et al. 2013). Indeed, the studied hydrological network is characterized by an exceptionally high range of hydrologic regime variations (see above in the study area section), mirroring the differences in the depth profile among canals (in the range < 2 m to > 4 m), that offers multi-dimensional niches to riparian plants. Further, the recorded plant diversity is substantially higher to those of mountain wetlands placed in a contiguous Apennine sector (Casentino), where a maximum of 159 "lacustrine and palustrine" vascular plants were recognized in 45 different wetland sites covering a wide range of habitats (Angiolini et al. 2019). At the same time, the number of plants per plot is relatively low (with an average species richness per site of 19.1 species), largely represented by common species, well adapted to disturbed areas (e.g. *Calystegia sepium*, *Urtica dioica*, *Potentilla reptans*), and widespread in most of the European lowlands. In addition, helophytes and hydrophytes are locally represented only by 11 species, a very small contingent compared to similar environments in Italy and Central Europe, where Bolpagni et al. (2013) and Hrivnák et al. (2014) found 41 and 72 species for a series of aquatic water bodies and riparian habitats (60 and 160 sites, respectively). On the other hand, Alahuhta (2015) recorded only 18 submerged macrophytes, and 21 helophytes from a comprehensive investigation of lakes in Minnesota (USA). These strong divergences are probably related to the much wider set of habitat types investigated by Bolpagni et al. (2013) and Hrivnák et al. (2014), and to the attention specifically directed to lakes carried out by Alahuhta (2015) compared to the case under consideration.

The evidence emerging from this study is in line with the general acknowledged decline in aquatic and wetland species across Europe and developed countries (Riis & Sand-Jensen 2001; Gołdyn 2010), reinforcing the role of human-mediated impacts in reducing the "spontaneophyte diversity" of wetlands across lowland agricultural landscapes. The life form spectrum of canals is coherent with high levels of human-mediated disturb, regulated by the periodic mowing of bank vegetation (mirrored by the grassland and ruderal species predominance, equal to 72% of the total plant diversity) and by extremely strong variations in flow regime (mirrored by the hydrophytes and helophytes rarity, < 5% each one). Güsewell and Le Nédic (2004), and van Belle et al. (2009)

have recognized similar results investigating the successional dynamics of fens. The former authors verified an increase of plant species richness with mowing, probably mediated by soil disturbance more so than periodic removal of biomass. The latter ones confirmed the key role of discharge and – in a lesser way – of the periodic mowing in stimulating plant richness, and that a slight eutrophication triggers the establishment of ruderal plant species, promoting *Phragmites australis* sere. This can further justify the high total number of species identified in the study area, but – at the same time – the relatively low plant diversity at site scale. This is also corroborated by the predominant plant ecological categories (*sensu* Buldrini et al. 2019), with very few hydrophilous (11%) and aquatic (2%) plant species. On the contrary, eutrophic and nitrophilous plant species are 41% of the total list: this is due to the intensive agriculture performed from decades in all the Po plain, that translates into turbid, nutrient-enriched waters and sediments with not negligible cascading effects on aquatic and wetland plants and vegetation (Bolpagni and Piotti 2015; Bolpagni and Pino 2016).

The number of alien species is low if compared to the extent of the territory examined, but these species were quite widespread, being present in 40% of sampled sites. Invasive species were 2.8% of the local flora, a slightly higher value if compared to that of the Italian flora (2.3%, Galasso et al. 2018). This is probably due to the artificial origin of drainage canals and to the active human interventions necessary to keep the network efficient. The preeminent role of lotic systems as privileged spread corridors of alien species is generally acknowledged (e.g. Pyšek and Prach 1994; Pyšek et al. 1998); however, the amount of invasive species detected in the study area is lower than expected, since in some central European areas they constitute 4.2% of the national flora (Pyšek et al. 2012). A possible explanation might be the peculiar hydro-morphology of the canals, which naturally makes them highly disturbed ecosystems. In riverine habitats, in fact, the presence of alien species can be estimated at 2.7% of the total species number and, more generally, in wetland vegetation aliens are 3.7% of the total, that is lower values than the general invasion rates (Pyšek et al. 2012). Of course, as clearly shown by Gotelli and Colwell (2001), the proportion of alien species is also dependent on the level of data aggregation, changing from the plot scale to the whole sample data (e.g. Chiarucci et al. 2012), but the comparisons here reported are within similar range of sampling intensity.

Generally, the presence of exotic plant species (alien + invasive) is higher – even if not in a statistically significant way – in S canals compared to the larger ones. This partially contrasts our hypothesis h_2 and could be related to substantial differences in the management of these types of canals, with smaller ones being more subjected to periods of low or no water flow. The occurrence of prolonged periods of reduced water supply, with consequent dry phase, can increase the local nutrients availability, as mirrored by the high numbers of non-oligotrophic *taxa* in S canals. In turn, such conditions may favour alien plant species and, more generally, pollution- and drought-tolerant ones. In this regard, considering the relevance of alien plants spread issues, further studies are needed to better explore the consequences of canal management expanding the study areas and deepening the quality status of the water and sediments of canals.

In addition, because alien species often take advantage of running water environments as a way for colonising new territories (e.g., Pyšek and Prach 1994; Pyšek et al. 1998), specific attention must be given to implement dedicated practices able to reduce the invasiveness of riparian vegetation and alien plant spreading. More shallow and narrow transversal canal dimensions, and much wider, more gradually sloping riparian zones are necessary to increase the spatial variability in flow and depth, enhancing the potential for plant species coexistence (Soons et al. 2017), as well as stimulating more favourable substrate conditions (Wood and Armitage 1997; Bolpagni and Pino 2016). Such interventions would be desirable at least in canals with reduced functionality, so to reconcile the necessary flood protection with nature conservation and environmental quality enhancement.

A small contingent of rare and threatened species (i.e., belonging to national or regional red lists; Regione Emilia-Romagna 2017) was also recognized. This, though it strengthens the awareness on the critical conservation status of HMWB in the Po plain, also highlights that drainage canals can host species of conservation concern (Bolpagni et al. 2013). Indeed, in highly modified floodplains, canals act as artificial ecological corridors, connecting the few relict natural or semi-natural areas, favouring the spread of wetland species among them. In addition, the periodical management interventions, like bank vegetation mowing with plant biomass removal (Hulina 1998; Güsewell and Le Nédic 2004), can favour the survival of species disappeared elsewhere – such as *Allium angulosum*, *Euphorbia palustris*, *Jacobaea paludosa* (L.) Gaertn., Mey. & Scherb. subsp. *angustifolia* (Holub) Nord. & Greuter, *Viola pumila*, and *V. elatior* – by reducing interspecific competition (Güsewell and Le Nédic, 2004; van Belle et al., 2009; Dallai et al. 2014b, 2015). In fact, it is recognized that drainage canals, and HMWB in general, can behave as secondary habitats for hydro-hygrophilous plants (i.e. aquatic and wetland plants) that can no longer find the natural habitat in the reclaimed alluvial plains. The embankments, in particular, are one of the most widespread secondary habitats in areas formerly occupied by wetlands or aquatic ecosystems and often host numerous vascular plant species nearly disappeared in the surrounding territories (Bátori et al. 2016). In such secondary habitats, threatened plant species (mainly wetland *taxa*) may find a refugium and survive even for long periods, particularly when the habitats are traditionally managed, despite the habitats themselves are potentially unsuitable from a reproduction viewpoint (see also Fekete et al. 2017). For all these reasons, the secondary habitats are fundamental for maintaining species that have become rare or endangered in agricultural environments (Láníková and Lososová 2009; Bátori et al. 2016; Fekete et al. 2017). However, drainage canals are managed with the general purpose of flood protection and irrigation, completely underestimating their ecological and functional roles (e.g., Bolpagni 2020). In this regard, rare and sensitive plant species seem to survive in populations along the canals with permanent water, as already observed by Dallai et al. (2015). Further, the chorological spectrum of the canals is coherent with a lowland area with continental climate, but the notable presence of microthermal species (20% of Boreal species, that is the double of the normal value for the Po valley; see Pignatti 1994) indicates that the drainage canals have a microclimate colder than the surrounding plain areas. Buldrini et al. (2013a) obtained similar results analysing the presence sites of three riverine plants endangered at the European level (*V. pumila*, *V. elatior*, and *J. paludosa* subsp. *angustifolia*).

Final remarks

The artificial hydrological network is a landmark of primary importance in the context of irrigated lowland plains, offering multiple services to guarantee high agronomic productivity rates (e.g., flood protection, irrigation). Their conventional use translates into high levels of disturbance, both hydrological and trophic, with relevant consequences on bank flora. Despite this, at ever increasing frequencies, these systems – and HMWB more in general – are the only semi-natural sites in oversimplified landscapes. The present study reinforces the awareness of the key contribution of irrigation canals in supporting not negligible plant richness levels. However, in the next few years, it will be mandatory to identify and implement management practices as more compatible as possible with the ecological requirements of aquatic and wetland plants. This is also of primary importance considering the pivotal contribution of aquatic and riparian plant communities in regulating the quality of surface waters, or in modulating the stability of the banks of the canals themselves, significantly reducing the loss of soil, which is one of the main problems of lowlands in the light of current climate change.

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CHAPTER IV

Functional trait analysis of riparian plant along a complex network of lowland heavily modified water bodies

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Abstract

Heavily modified water bodies proved to be a fundamental place for the conservation of wetlands, that are among the most threatened environments in the world. In order to contribute to their conservation and restoration we investigated the functional structure of the plant communities of the canal network of the Bologna plain. An analysis based on the functional traits of the species was carried out to investigate their relationship with both the environmental variables and the water quality. The RLQ and the fourth-corner analysis were adopted. In addition, the hierarchical clustering was used to discriminate groups of plants with similar functional traits and to assess how they varied along environmental and water quality gradients.

Our study showed that the main environmental drivers that affect the functional composition of the plant community were the bank aspect, the inclusion and exclusion from protected areas and the upstream basin land uses. Furthermore, two significant associations were found: one binds phanerophyte life form and presence of aquatic environments upstream of the transect, and one combines nitrophily to "artificial" land use upstream of the transect. In addition, functional groups of sampled species respond differently to environmental drivers and water quality gradients and are influenced by a combination of environmental stresses. The latter should be further investigated, individually and cumulatively, to better understand the role of canal management and its potential developments, with the aim of an ever-closer integration of hydraulic-agronomic purposes and protection and restoration of the ecological functionality of the canals.

Key words

Functional composition, plant functional traits, Leaf Area, Leaf Dry Mass, Leaf Dry Matter Content, Specific Leaf Area, Po valley, heavily modified water bodies

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Introduction

Heavily modified water bodies (HMWB) may act as refuges for aquatic and wetland species and vegetation (Bolpagni et al. 2019, Cantonati et al. 2020, van Rees et al. 2020), which are among the most threatened biodiversity facets globally (Reid et al. 2019, Dudgeon 2019). Drainage canals can ecologically mimic lowland wetlands which practically disappeared in most of agricultural areas, besides allowing the connection among small, isolated and relict wetlands (Deane et al. 2017). Indeed, connectivity is one of the main positive drivers able to explain the spatial patterns of biodiversity associated to inland waters within agroecosystems (Bolpagni et al., 2020).

To contribute to an effective management of HMWB, and restoration where possible, it is crucial to overcome the knowledge gaps that exist on biodiversity and functioning of HMWB, especially within hyper-exploited agricultural plains (Bátori et al., 2016; Bolpagni and Piotti, 2016; Bolpagni, 2020). For that reason, intense sampling efforts have been carried out to investigate the functional structure of plant communities present along the drainage canal network focusing on the Bologna plain (northern Italy).

It is generally known, in fact, that functional traits can be used to assess the adaptive strategies of plant species, allowing to compare communities across geographical, environmental and pressure gradients (Pierce et al., 2017; Dalla Vecchia et al., 2020). Functional traits are increasingly studied in community ecology and have allowed a better understanding of the ecological functionality of the ecosystems studied, more than classical biodiversity approaches such as those based on the abundance of species (Ricotta et al., 2015, 2020). In Italy, functional traits have been widely applied in several studies, from forest management to land-use change studies, but very few functional studies have investigated wetland or aquatic habitats or ecological processes (Chelli et al., 2019; Dalle Fratte et al., 2019).

To this regard, one of the most commonly functional approach applied in the analysis of plant communities is based on the ways plants invest into biomass. Among others, Cerabolini et al. (2010) clarified the three main ways: (1) architectural extension of individual ramets (tall canopies, large leaves), (2) durable tissues (high leaf dry matter contents and carbon contents, low specific leaf areas), or (3) regenerative development (early, extensive flowering with delicate, nitrogen-rich leaves). The same authors also highlighted how these three modalities are significantly correlated with the extent of competitive ability, stress-tolerance and ruderalism strategies as defined by Grime in the frame of the CSR ecological theory (Grime, 2001).

The aim of this research is to implement the knowledge on riparian vegetation across HMWB, offering new insights on how the i) geographical drivers (bank aspect, land use of the hydrological basins that feed the canals, and proximity to protected areas), and ii) the water quality can modulate the functional structure of plant communities along the riparian banks of HMWB.

Based on the strong relations between functional traits and plant adaptive strategies as defined by Grime (2001) classification, we expect: 1) no compositional functional changes in the riparian plant communities as the canal bank aspect changes (e.g., cardinal orientation) (H1); 2) the predominance of perennial species and phanerophytes within protected areas due to the least human-made disturbance (H2); 3) a not negligible role of land use intensity, with a progressive increase in ruderal species along with anthropic uses increase (H3); 4) a non-negligible role of water quality of canals, with a progressive increase of species adopting more productive strategies (high diffusion and annual/biennial growth) to the increase of the availability of nitrogen and phosphorus (H4).

Materials and methods

Study area

The study area includes the lowlands of the province of Bologna (Emilia Romagna region, Northern Italy; Fig. 1) that are situated in the south-eastern part of the Po River basin. In this geographical context, the province of Bologna is one of the most productive agricultural and industrial areas and, consequently, it is one of the most impacted plain areas of Europe (Guareschi et al., 2020). Its climate is continental, with cold winters and hot summers (Köppen climate classification Cfa, Peel et al., 2007), because Apennines stop the southern warm winds, and the thermal regulation mediated by Adriatic Sea is scarce depending on its shallowness. Atmospheric circulation is normally limited, thus relative humidity is high. According to 1981-2010 data, the average annual temperature is 12.4 °C; the hottest month is July (average temperature 31.4 °C) and the coldest month is January (average temperature -1.1 °C). Annual precipitation is 684 mm, with a maximum in October and a minimum in July (Arpae Emilia-Romagna 2019, https://simc.arpae.it/dext3r/).

The Reno River (total length of 212 km) and its tributaries, having all of them a torrential regime, are the main natural watercourses of the study area. Since it is a typical irrigated plain, the most relevant watercourses are locally represented by artificial ones, here managed by a land-reclamation consortium called Consorzio della Bonifica Renana (hereafter referred to as "Consortium"). This Consortium manages a total area of 3419 km² and its lowland sector (i.e., the present study area) has a spatial extent of 1439 km². Here, the HMWB cover a total length of 1154 km and are used both for drainage (i.e., hydraulic safety) and irrigation purposes. For these reasons, all canals are subjected to huge seasonal variations of their hydrologic regime, with higher flows from May to September (irrigation period) and a lean period from November to March, apart from exceptional rainfall events. Such variations are proportionally much more pronounced in smaller canals, which can even be dry during autumn and winter periods. Instead, the largest canals are characterized by lower variations in water level compared to what is expected for natural lowland lotic ecosystems, reducing the expected complexity of bank ecotones. Based on this evidence, the depth dimension of canals was used as a proxy of their hydrological functioning (i.e., regime) allowing us to separate the studied canals into dimensional classes: large, medium and small (for details see Montanari et al., 2020).

Soils are generally characterised by a prevailing clay-silty texture (Carta dei Suoli dell'Emilia-Romagna, 1:50.000, https://geo.regione.emilia-romagna.it/cartpedo/), and the climacic vegetation is composed of a mixed deciduous broad-leaved forest, dominated by Quercus robur L. and Carpinus betulus L., turning into a riparian forest dominated by Populus alba L. and Salix alba L. along river courses (Puppi et al., 2010). Currently, such vegetation has practically locally disappeared due to the land use transformation by intensive farming, and only a few remnants still exist. Drainage canals are almost always free of tree cover, to ensure a rapid water runoff in case of rainfall, and to allow easier periodic mowing. The canals are dredged every 10-15 years on average, unless the appearance of hydraulic issues of primary relevance (exceptional flood events), whereas bank vegetation is regularly mowed thrice a year in the period May-October.

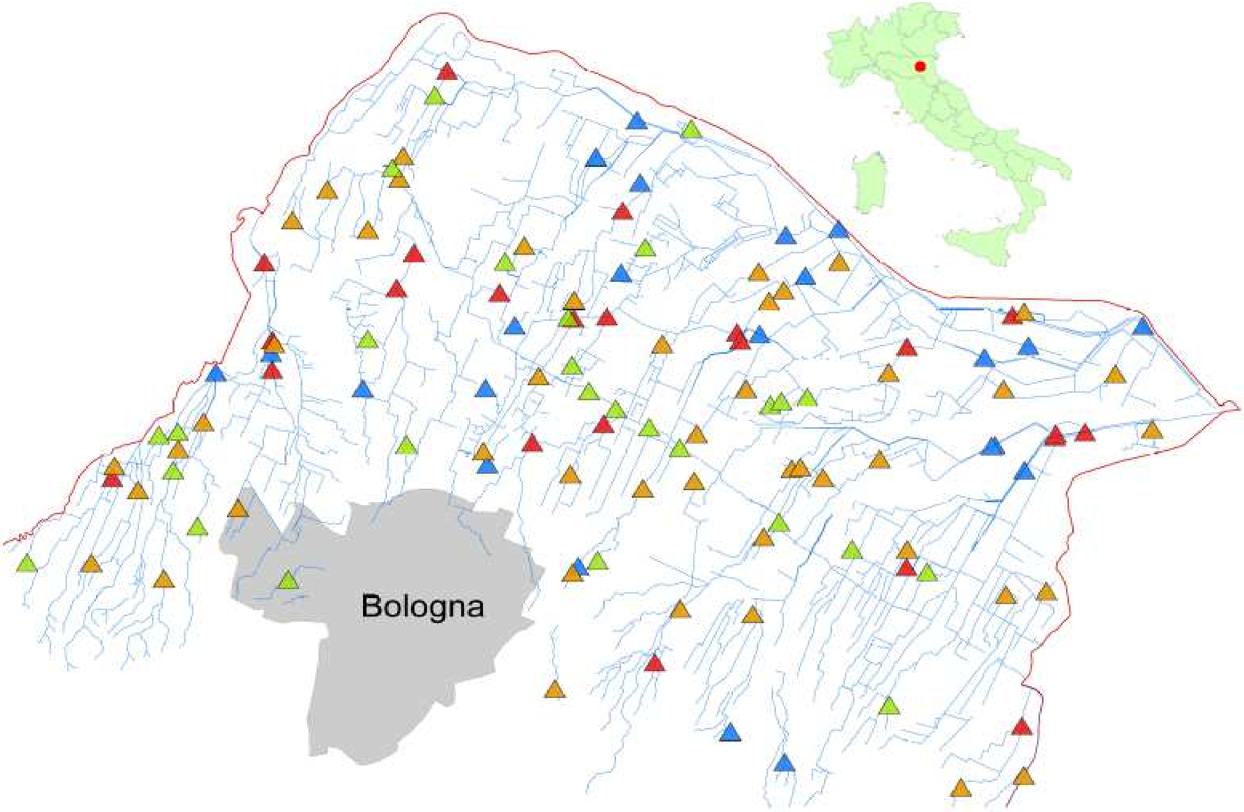

Figure 1 - Map of the study area (Bologna plain, northern Italy) with the indication of the sampling sites (red: large canals; orange: medium canals; green: small canals, blu: canals where the water quality was sampled too). The position of the study area within Italy (red dot) is also shown.

Sampling design

A preliminary survey of the HMWB in the study area was carried out by a GIS approach to identify the prevalent types of drainage canals present. Ancillary information (mainly concerning the water depth of canals) was provided by the Consortium and Arpae Emilia-Romagna. Based on this survey, and specifically on the total length of the canals grouped in three different classes (large, medium and small), 96 transects were randomly selected as representative of the entire local canal network – 23 large canals (L), 46 medium canals (M), and 27 small canals (S) – using the random points function of QGIS (Chiarucci, 2007). Additionally, we forced a minimum distance of 2 km between the transects that, in case, were randomly located along the same canal. Finally, 17 transects were selected according to opportunistic criteria, as they coincide with the closure points of some hydrological sub-basins (referred to here as "H2O") to be subjected to chemical-physical characterization of waters. For each transect, a plot of 10 m x 1 m was positioned along the canal bank, parallel to the flow. Starting from the water-bank interface, the plot was placed including in the analysis the first ten centimetres of the canal water body, and the first 90 centimetres of the bank. All plant species present in the plots were identified and their coverage estimated. For details see Montanari et al. (2020).

Spatial data

The geographical drivers considered were the “bank aspect” at the transects (slope of the bank), the proximity of each transect to nearest protected areas (expressed in m from the nearest border of protected areas) and the upstream basins land use (determined by data provided by the Consortium and the Emilia-Romagna region database with the support of the Land Use Map 2017, reference scale 1:10.000, made in 2019/20 by the Emilia-Romagna Region. It is characterised by a minimum patch size of 0.16 hectares, a minimum size of 7 m for linear elements and it’s realized based on Corine Land Cover classes; https://www.regione.emilia-romagna.it/la-regione/archivi-1/archivio-cartografico) (Montanari et al., 2021; see Supplementary material 1, 2, 3, and 4 respectively).

Ground data

For the study we also used:

- vegetation data (collected by the Canal Bank Research Group as described in "Sampling design"),

- water quality data collected by a team of the DISTAL Department of the University of Bologna, from April to October 2019 on a mandate of the Consortium, to provide baseline data for the quality of the water supply to the farms of the area. The selected sampling points coincide with the closure points of 17 sub-basins of the drainage canals network examined.

Water quality data

Water quality data were collected by a team of the DISTAL Department of the University of Bologna, from April to October 2019, to provide baseline data for the quality of the water supply to the farms of the area. The selected sampling points coincide with the closure points of 17 sub-basins of the drainage network examined (17 triangles of the 22 shown in blue in Fig. 1; was impossible use the data collected in all the 22 because some mistakas appened during the sampling).

Functional trait data

Plant communities have been characterized in terms of Ellenberg's indicator values for moisture and nutrient availability (Pignatti et al. 2005; Montanari et al. 2020), life form (F-BIO, Pignatti et al. 2017), flowering start (FLO ST), flowering period (FLO PER), leaf area (LA, mm2), leaf dry mass (LDW, mg), specific leaf area (SLA, mm2 mg-1), leaf dry matter content (LDMC, %). Functional trait data (LA, LDW, SLA, LDCM) were obtained from the TRY database (Kattge et al., 2020) for the greatest number of species, integrated by data from Varese University Archive. For six species (Aristolochia rotunda L., Cerastium sylvaticum Waldst. & Kit., Crepis pulchra L., Populus deltoides W. Bartram ex Marshall, Ranunculus velutinus Ten., and Schoenoplectus tabernaemontani C.C. Gmel.), lacking specific data, we used values regarding close congeners (see Supplementary material 5). Conversely, for Lycopus exaltatus L. and Silybum marianum Gaertn., even applying the previous approximation, it was impossible to assign "reliable" values.

Consequently, we decided to exclude these species from the analyses, also considering their scarce relevance (< 5% of the transects).

Data analyses

According to Stefanidis and Papastergiadou (2019), we applied the RLQ analysis combined with the fourth-corner and the Cluster Analyses with the aim of identifying the relationships between environmental gradients (R) and functional traits (Q) mediated by the abundance of species (L) (Dray and Legendre, 2014; Stefanidis and Papastergiadou, 2019). First, a correspondence analysis was applied to the species abundance matrix (L), then the Hill-Smith ordination was applied to the functional matrix (Q; Hill and Smith, 1976) and, finally, to the principal component analysis (PCA) ordination of environmental variables (R). The RLQ analysis identifies, through a simultaneous ordination, linear combinations of environmental variables and functional traits such that their squared covariance is maximum. The significance of these relationships was tested using the Monte Carlo test with 49999 permutations. The contribution of each trait and environmental parameter to the total inertia has been used as a measure of relative importance and criterion to identify the most important functional traits and environmental variables that have a major influence on the distribution of species in HMWB studied (Stefanidis and Papastergiadou, 2019, Duflot et al., 2014). Afterwards, the fourth-corner analysis explored the relationship between each functional trait and each environmental variable (Duflot et al., 2014) which allowed us to elaborate more detailed and precise interpretations of their associations (Baattrup-Pedersen et al., 2015). To obtain a test with a correct type of error I, the results of model 2 (site permutation) and the results of model 4 (species permutation) were combined by setting model 6, as suggested by Dray et al. (2014). In addition, to conduct the analysis we used 10,000 permutations and the method of false discovery rate (FDR) to adjust the p-value (Dray et al., 2014). The clustering analysis was conducted on the scores of the species obtained from the RLQ analysis to identify clusters of plants that have common functional traits; the dissimilarity matrix on which the analysis is based was created using the Euclidean method and the hierarchical cluster tree was produced using the Ward.D2 method. We considered 5 clusters because it corresponds to the minimum number that guarantees a more balanced division of the species within the functional groups. Finally, we combined the functional groups of the species with the environmental drivers through a biplot to show how the latter influence the distribution of the species according to their functional traits.

In this study we applied the above methodology to two different combined datasets: between functional traits and environmental variables (1), and between functional traits and water quality data (2). In the first case, the RLQ analysis was carried out considering 113 transects (those in which we had all the geographical patterns of interest). Since in many of them the plant species sampled were very sporadic, we considered for the analysis only the species with a presence at least in 4% of the 113 transects sampled (equal to 5 transects). We made an exception for six endangered species (as defined by IUCN criteria: Allium angolosum L., Euphorbia palustris L., Juncus subnodulosus Schrank., Nuphar lutea (L.) Sm., Schoenoplectus tabernaemontani (C.C. Gmel.) Palla, Samolus valerandi L.). In the second case, the RLQ analysis was carried out on a small number of transects (17) for which water quality parameters were available. Considering the very small number of transects, all the species surveyed were considered regardless of their spatial representativeness.

All analyses were carried out with the "ade4" package in the working environment R 3.6.0.

Results

Relationships between environmental variables, functional traits and species

Focusing on the environmental variables, the first two axes of the RLQ analysis explained 64% and 11% of the total variance, respectively. The Axis 1 mainly discriminated sampling transects with a north orientation (N) and close to protected areas (PA.Yes, PA.B100) from those with north-west (NW) and west (W) orientation, and lacking connections with protected areas (PA.No). The Axis 1 also turned out to be associated with specific land uses in the underlying catchments: "forest", "aquatic environments", "agricultural areas", and "meadows". However, the bank aspect was found to be linked to both RLQ axes: Axis 2 clearly differentiated the south (S)-oriented banks from those oriented east (E), north-east (NE) and south-east (SE), besides to distinguish transects in terms of proximity to protected areas (PA.Yes) compared to those influenced by land uses “artificial soil” (Artif) and “meadows” (Meadows), and by location into buffers of 100 m all around the protected areas (PA.B100) (Figure 2a).

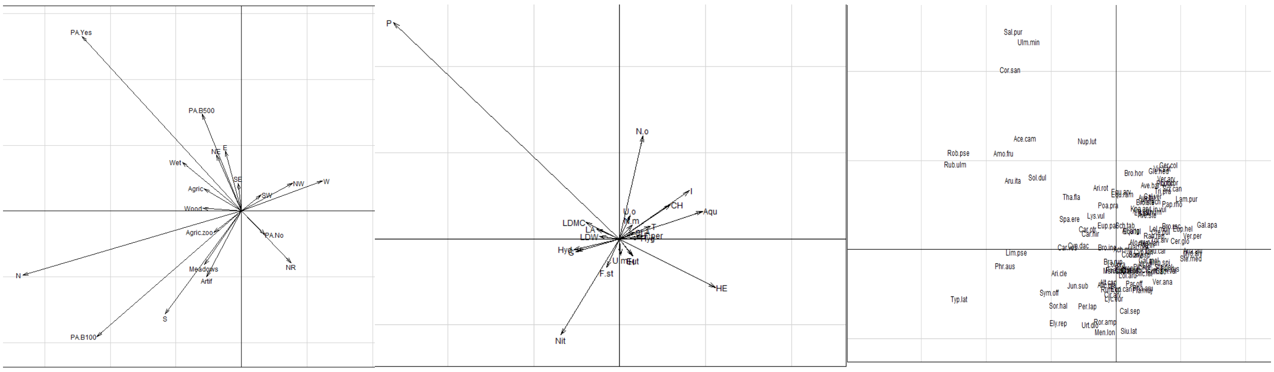

Figure 2: Results of the first two axes of RQL analysis applied to the complete HMWB dataset: a) coefficients for the environmental variables, b) coefficients for the traits and c) scores of species (Bank aspect: E = East, N = North, NE = North-East, NW = North-West, NR = not detected, W = West, S = South, SE = South-East, SW = South-West; proximity to protected areas: PA.B100 = in a buffer of 100 m from protected areas, PA.B500 = in a buffer between 100 and 500 m from protected areas, PA.No = absence of protected areas, PA.Yes = inside protected areas; Land use: Agric = agricultural area, Agric.zoo = agro-zootechnical settlements, Artif = artificial surface, Meadows = meadows, Wet = water bodies and inland wetlands, Wood = forest; Functional traits: Ellenberg U indices (Aqu = aquatic plants, Hyd = hydrophilous, Hyg = hygrophilous, U.m = mesophilous, U.o = meso-xerophilous or xerophilous), Ellenberg N index (Eut = eutrophic, Nit = nitrophilous, N.m = mesotrophic, N.o = meso-oligotrophic or oligotrophic); Life forms: CH = chamaephyte, G = geophyte, H = hemicryptophyte, HE = helophyte, I = hydrophyte, P = phanerophyte, T = therophyte; F.per = flowering period, F.st = flowering start, LA = leaf area, LDW = leaf dry weight, LDMC = dry matter content of the leaf, SLA = specific leaf area; for species abbreviations we used the R function "spCodes").

In terms of functional traits, the first RLQ axis mainly discriminated the phanerophyte life form (P) from the other ones, but it was also linked to the leaf functional traits (LDMC, LA, LDW and SLA). The axis 2, instead, discriminated the ecological preference of plants for nitrogen-rich soils (Nit) and, in a weaker way, the flowering start (FLO.ST) by the preference of plants for nitrogen-poor soils (N.o) (Figure 2b).

The Monte Carlo test confirmed a high significance (p-value < 0.05) of the relationship between environmental variables (R) and the functional structure of the sampled plant community (Q). The fourth-corner analysis demonstrated that the ecological preference for soils with an excess of nutrients (N.n) was positively associated with the artificial land use in the underlying catchments (Artif). In the same way, a significant positive association was observed between the phanerophyte life form (P) and the presence of water bodies and inland wetlands (Wet) in the underlying catchments (Supplementary material 6).

Through the cluster analysis, 5 functional groups of species were identified (Supplementary material 7). The relationships between them and the environmental variables (Figure 3) indicated that groups 1, 5 and 4 were associated to transects placed in proximity to protected areas, whereas group 4 showed a preference for land uses "artificial" and "meadows" in the underlying catchments. Conversely, groups 1 and 5 were more related to the presence of aquatic environments upstream. In addition, groups 2 and 3 showed a preference for west, north-west and south-west bank orientation, and were correlated to the distance from the protected areas. Group 2 showed greater association with "artificial" and "meadow" land uses in underlying catchments than group 3. Finally, group 4, associated with north and south bank aspect, differed from group 5, which preferred the east, north-east and south-east bank orientation.

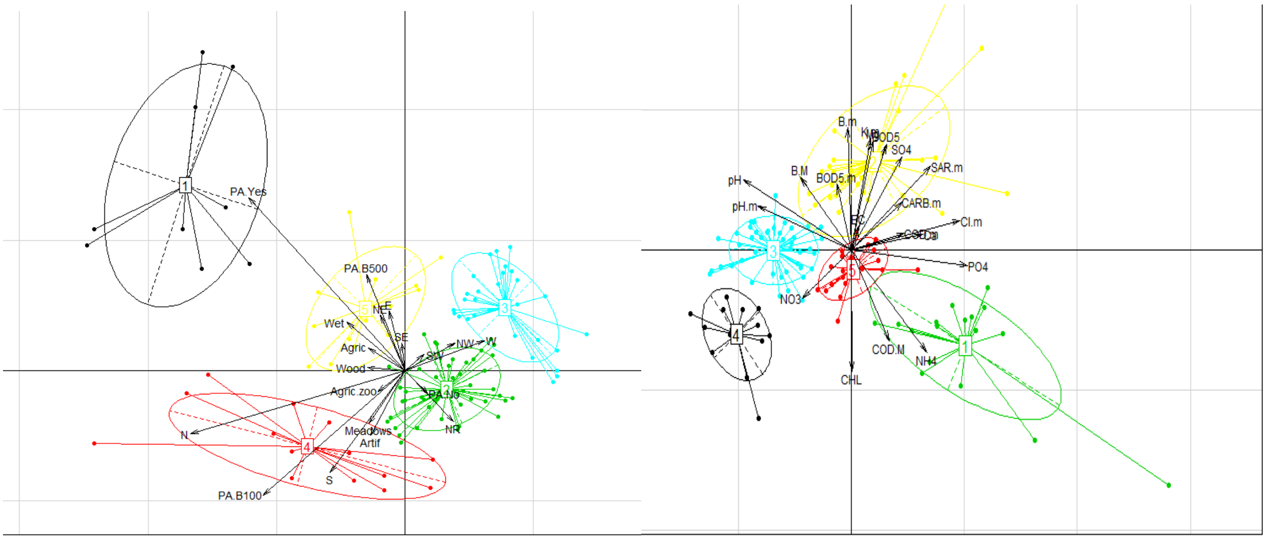

Figure 3: Bitplots showing the relationships between the functional macrophyte assemblages derived from the hierarchical clustering and the vectors of environmental variables: a) complete HMWB dataset; b) sub-set (17 transects) related to the sampling sites for which the chemical-physical data of the waters were collected.

Relationships between water quality, functional traits and species

By relating the functional traits (Q) to the water quality parameters (R) it emerged that the first two RLQ axes explained respectively 76% and 13% of the total variance that links the distribution of species (L matrix) with R and Q tables. For water parameters, the axis 1 was positively correlated with phosphate (PO4), ammonia (NH4), minimum chlorine (Cl.m) and minimum sodium absorption ratio (SAR.m), and it was negatively correlated with pH, minimum pH (pH.m) and nitrates (NO3). The second axis was positively associated with minimum boron (B.min) and maximum boron (B.M), potassium (K), magnesium (Mg), biochemical oxygen demand (BOD5) and sulphate (SO4), and it was negatively associated with chlorophyll a (CHL) and the maximum chemical oxygen demand (COD.M) (Figure 4).

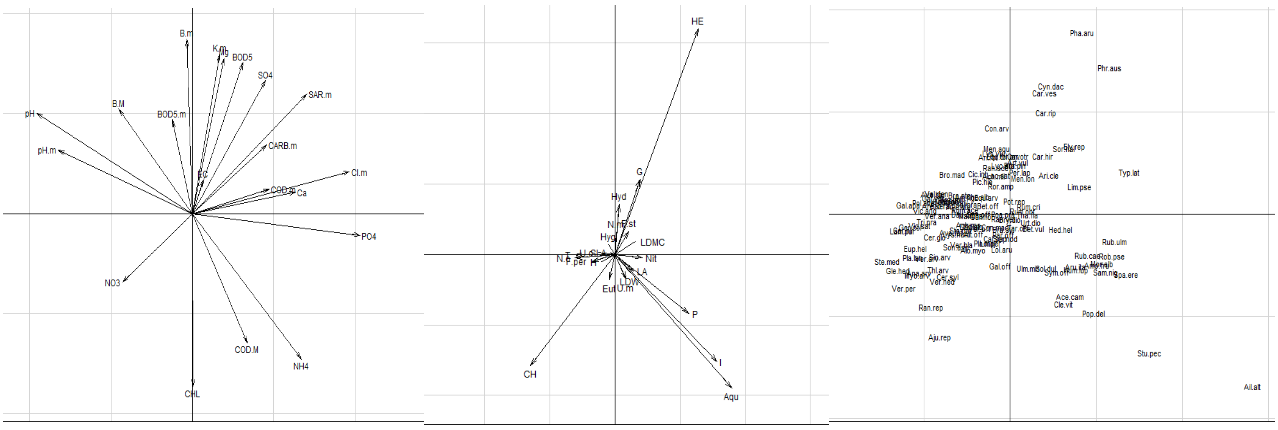

Figure 4: Results of the first two axes of RQL analysis applied to the water HMWB dataset (17 transects): a) coefficients for the environmental variables, b) coefficient for the traits, c) scores of species (B.m = minimum boron concentration, B.M = maximum boron concentration, BOD5 = biochemical oxygen demand, BOD5.m = minimum biochemical oxygen demand, Ca = calcium, CARB.m = minimum carbonate concentration, CHL = chlorophyll a, Cl = chlorine, COD.M = maximum chemical oxygen demand, COD.m = minimum chemical oxygen demand, EC = electrical conductivity, K.m = minimum potassium concentration, Mg = magnesium, NH4 = ammonium, NO3 = nitrate, pH.m = minimum pH, PO4 = phosphate, SO4 = sulphate, SAR.m = minimum sodium uptake ratio; Arrangement of functional traits along the first two axes of RLQ analysis:Ellenberg U indices: Aqu = aquatic plants, Hyd = hydrophyle, Hyg = hygrophyle, U.m = mesophile, U.o = mesoxerophilous or xerophilous; Ellenberg N index: Eut = eutrophic, Nit = nitrophile, N.m = mesotrophic, N.o = meso-oligotrophic or oligotrophic; Life forms: CH = chamaephyte, G = geophyte, HE = helophyte, H = hemicryptophyte, I = hydrophyte, P = phanerophyte, T = therophyte; F.per = flowering period, F.st = flowering start, LA = leaf area, LDW = leaf dry weight, LDMC = dry matter content of the leaf, SLA = specific leaf area; for species abbreviations we used the R function “spCodes”).

Concerning the functional traits, the first RLQ axis had a positive correlation with the hydrophyte (I) and phanerophyte (P) life forms, the ecological preference of aquatic species (Aqu) and soils rich in nitrogen (Nit), the LDMC and LA, while it was negatively correlated with the chamaephyte (CH) and therophyte (T) life forms, the flowering period and the preference of soils poor in nitrogen (N.o). The second axis, instead, showed a positive association with the helophyte (HE) and geophyte (G) life forms and the ecological preference of very humid soils (Hyd), while it was negatively associated with the ecological preference for aquatic species (Aqu) and, less, nutrient-rich soils (Eut), the hydrophyte (I), chamaephyte (CH) and phanerophyte (P) life forms, and LDW (Figure 4).

The Monte Carlo test confirmed a high significance ($p\text{-value} < 0.05$) of the relationship between environmental gradients (R) and the functional structure of the sampled plant community (Q).

The result of the fourth-corner analysis showed no significant bivariate associations between functional traits and water quality parameters. This means that no significant influence ratios were found between singular water parameter and singular functional trait.

Through the cluster analysis, 5 functional groups of species were identified (Supplementary material 9).

Our research confirmed that some environmental drivers affected the functional composition of the plant community studied (Figure 9):

- Groups 1 and 4 were linked to high chlorophyll a (CHL) values, but while group 1 showed a preference for water with high phosphate (PO4) and ammonia (NH4), group 4 preferred water with high nitrate content (NO3);

- Group 3 was negatively correlated with the concentration of nitrogen in water in all its forms, and it preferred water rich in boron (B.m, B.M), potassium (K), magnesium (Mg) and sulphates (SO4). Groups 3 and 4 were positively associated with higher pH values than the others, while group 1 was associated with lower pH values;

- Group 5 was linked to nitrates (NO3), ammonia (NH4) and chlorophyll a (CHL), but to a lesser extent than groups 1 and 4, and to intermediate pH values.

Discussion

Environmental variables, functional traits and plants relationships

Our data showed that the geographic patterns and land uses had an influence on the functional composition of the bank plant communities. In particular, the bank aspect and the proximity to protected areas are the key variables explaining the total variance of the RLQ analysis. Concerning functional traits, life form, nitrophily, oligotrophy, and LDMC are the greatest contributors to the total inertia of the observed distributions. The first RLQ axis clearly separates the plant groups 1 and 2, which were characterized by being respectively clearly associated and not associated to the presence of protected areas in the surrounds. Group 1 consisted of phanerophytes with high LA, LDW and LDMC, while group 2 consisted of predominantly hemicryptophytic species with low LA, LDW and LDMC and high SLA. Herbaceous species with high SLA and short leaf lifespan (as recorded for group 2) may be associated to early stages of primary succession (Kazakou et al. 2007, Navas et al. 2003), while taller woody species with longer-lasting leaves and high LDMC (as recorded for group 1) were associated with late ecological succession stages (Garnier et al. 2004; Navas et al. 2010). Chai et al. (2015) and Ciccarelli (2015) showed that plant communities present in the early stages of succession had a predominantly ruderal strategy; on the contrary, the plant communities typical of the late stages mainly had a stress tolerant or competitive strategy, or a strategy mixed with the ruderal one. In line with those considerations, in our study the species of group 1, found in transects close to protected areas, are typical of later succession phases, while the species of group 2, recorded in transects at significant distances from protected areas, are typical of earlier succession phases and show greater potential resistance to external perturbations. Moreover, it was observed that the woody species of group 1 bloom before the companion herbaceous species, in agreement with Chai et al. (2015). Indeed, and in according to Duflot et al. (2014), it is postulated that open environments tend to select early flowering species, so that they can reproduce before the start of the major agricultural disturbances (e.g., mowing, grazing, pesticides application and harvesting), resulting in greater reproductive success and persistence. Moreover, a significant positive association was found between the phanerophyte life form and the presence of water bodies or wetlands in the underlying catchments, in agreement with Lopez et al. (2002) who found an increase in the richness of shrubby species in depressional wetlands as the distance between wetlands decreased. Furthermore, the creation, maintenance and recovery of dispersion corridors for aquatic environments, increasingly marginal and isolated, represented one of the main actions for conserving the wetland and aquatic plant richness in a purely agricultural landscape (Bolpagni et al. 2020, Chester and Robson 2013, Schopke et al. 2019).

Our analysis also clearly distinguished the species of group 1 from those of group 4, which were typical of transects laid within a 100 m buffer from protected areas and characterized by artificial land uses and meadows in the underlying catchment area. Similarly, Assani et al. (2007) found that nitrophilous species indicate pollution of the canals by urban effluents and agricultural fertilizers. This is also supported by the fact that the RQL axis 2 distinguished nitrophilous species of group 4 from meso- or oligotrophic species of group 3, which were found in transects located relatively far from human settlements or impacted areas. However, in contrast to what was expected, group 3, characterized by less artificial or impacted land uses in the respective underlying basins, showed typical ruderal functional traits, such as a therophyte life form and long flowering periods. According to the CSR classification (Grime 2006), ruderal species are typical of environments with high disturbance level. In this respect, Montanari et al. (2020) explained that conventional canal management determined high levels of human-mediated disturbance, such as mowing and very strong variations in the water regime, so that regardless the land use planning in underlying catchments, all the sampled transects were however affected by not negligible levels of disturbance.

Water quality, functional traits and plants relationships

Although the number of sites analysed is relatively small (17), this study showed that pH, PO43-concentration, chlorine and minimum SAR may strongly influence the plant community responses, giving the greatest contribution to the total variance of the RLQ analysis and resulting in a strong relationship with its first axis. These results are in agreement with Pan et al. (2017) and Stefanidis and Papastergiadou (2019), which showed how the total phosphorus concentration represents the environmental variable that most influences the distribution of species, even more than nitrogen in aquatic ecosystems. However, in contrast to Stefanidis and Papastergiadou (2019), our study found that pH is influent as phosphate concentration. It is negatively correlated with the concentration of phosphate in water, in accordance with Silva Cerozi and Fitzsimmons (2016), and positively correlated with nitrates, although to a lesser degree. Concerning functional traits, it was observed that the life form and the Ellenberg index U provide the greatest contribution to total inertia. In particular, hydrophytes, helophytes, chamaephytes and aquatic plants were strongly correlated to the RLQ axes, although they represent only a small group of species in our sample. Phalaris arundinacea L. is, for example, the only helophyte that was sampled in the 17 transects and is associated with waters with high concentrations of sulphate and other elements; Stuckenia pectinata (L.) Börner and Sparganium erectum L. are the only hydrophytes sampled and are associated with waters with a higher concentration of NH4+.

Irrigation and drainage canals role for plant diversity and nature conservation

Our study shows that the species of group 1 (with phanerophyte life form, high leaf area and a preference for soils rich in nutrients and nitrogen) are associated with waters rich in PO43- and NH4+. This may be since plants are more productive in environments with greater availability of nitrogen and phosphorus (Grime 1988, Baattrup-Pedersen et al. 2015). Many studies use the Ellenberg index N, the morphological index (which includes the height and lateral extension of the canopy) and the leaf area as indicators of greater productivity and competitiveness of plants (Baattrup-Pedersen et al. 2015, Grime 1998, Stefanidis and Papastergiadou 2019, Zervas et al. 2019). According to the work of Baattrup-Pedersen et al. (2016), the PO43- levels showed a direct and positive correlation with the functional traits associated with the production strategy of the plants. In fact, along a phosphate concentration gradient, it was observed that group 3, consisting mainly of therophytes and hemicryptophytes with low leaf area and oligo- or mesotrophic species, prefer water poor in phosphate unlike group 1. Group 5, on the other hand, mainly consisting of hemicryptophytes with a high leaf area and eutrophic species, occupies the central position of the gradient. It is known that phosphorus is an essential element of many molecules and plants cannot grow without an adequate supply of this nutrient (Schachtman et al. 1998), but the baseline availability of this element is relatively limited in not perturbed ecosystems (Smith and Smith 2015). At least an excess of macronutrients in the water resulting from human activity gives rise to the phenomenon of eutrophication, that causes an excess of growth of aquatic plants and the exclusion of less competitive species (O'Hare et al. 2018). This is in accordance with our work, in which the only hydrophytes sampled are associated with waters rich in nitrogen and phosphorus, as verified in several other cases. Moreover, NH4+ is preferred as a nitrogen source over nitrates. This aspect has been highlighted several times, such as by Walstad (2017), in which it was shown that most aquatic plant species prefer ammonium to nitrate. In our study, the abundance of nitrogen and phosphorus, the increase in turbidity (expressed through the value of chlorophyll a) and organic matter (expressed through the value of the chemical oxygen demand) showed a positive correlation with eutrophic species representativeness.

In contrast to Stefanadis and Papastergiadou (2019), in our study chlorophyll a does not show to be one of the fundamental environmental drivers in guiding the functional composition of the plant community: this is probably because we studied lotic systems instead of lentic ones, where the competitive role of phytoplankton is greater compared to non-biological turbidity (e.g., suspended matter) in running waters.

With respect to the gradient of pH and PO43-, the five groups of plants here defined on the basis of functional traits are distributed in the following order: groups 3 and 4 are placed in waters with low PO43- and high pH, group 1 prefers waters with high PO43- and low pH, while groups 2 and 5 are in intermediate positions. This is explained by the fact that the pH of a solution can affect the absorption of nutrients (White 2012), in particular that of phosphorus (Silva Cerozi and Fitzsimmons 2016).

We observed that the LDMC is negatively correlated with the SLA, similarly to what was observed by Pan et al. (2017), where the LDMC was strongly correlated with the thickness of the leaf which contributes to add strength to the plant. The carbonates dissolved in the water showed a weak positive correlation with group 2, mainly consisting of hemicryptophytes and geophytes that have LDMC as high as the species of group 1 which are predominantly phanerophytes. This is consistent with the high availability of carbonates in water, which favours the allocation of carbon in secondary compounds. In fact, the dry matter content is positively correlated with the leaf starch content, the LDMC and the leaf starch content increase as the carbon availability increases (CO2 and HCO3-) and this effect is even more pronounced when nitrogen is limited (Dülger et al, 2017).

Our results partially confirmed the initial assumptions: 1) the hypothesis that the functional composition of the species does not change with the variation of the bank aspect of the canals has not been confirmed, since the exposure to the north is consistent with the presence of more hydrophilous plants and that to the south is consistent with the presence of more mesophilous plants (H1); 2) the hypothesis according to which the canals close to protected areas host plant species with perennial life cycle and phanerophyte life form has not been confirmed, while it has been highlighted that the presence of water bodies within the underlaying catchments can guarantee the dispersion of phanerophytes and therefore support the plant richness of interconnected wet areas, including HMWB (H2); 3) the land use of the underlying catchment of the transects has a not negligible role in driving the functional composition of the plant communities with a progressive increase of ruderal species in the more man-made areas, was only partially confirmed, since we found a greater component of nitrophilous species in the canals closest to anthropic settlements, but, contrary to what was expected, we found ruderal species even in the less directly impacted areas. We therefore believe that the widespread diffusion of ruderal species in the embankment plant community is mainly associated with the management of the canals of the Bologna plain, in particular with the periodic mowing and leaching from surrounding agricultural lands (H3); 4) our hypothesis which foresees a not negligible role of the water quality of the canals in driving the functional composition of the plant community, and assumes a progressive increase of species with more productive strategies as the availability of nutrients increases in the water, was verified as we found plants with functional traits more linked to productive strategy with the increase of the concentration of PO43- and NH4+ in water.

This work reinforces the belief that, even for anthropized floodplains, the study of plant communities through a functional traits-based approach is very useful to understand the ecological functions of habitats, especially with respect to the pressures exerted at the catchment scale.

Final remarks

Our results encourage a functional approach to the study of riparian plant communities. It can be a useful tool to support an effective plan of recovery and restoration of HMWB through the selection of resilient plant communities that persist in a variety of environmental conditions, such as those found in heavily exploited lowlands in which climate change phenomena are a further factor of imbalance and threat to the local biodiversity.

In this study, we verified that the functional composition of the plant community of drainage canals in a temperate context responded clearly to geographic patterns, land use and water quality gradients. Overall, the main environmental drivers that explained an important variance in the composition of sampled community traits were the bank orientation, the being part of a protected area or being far away from it, the artificial or aquatic land use in the underlying catchment and the PO43- concentration in the water, as well as the pH. The plant community of the canals differentiated mainly based on their responses to human disturbance and nutrient availability in soil and water.

Although in the first part of the study we founded only two significant associations between functional traits and environmental variables and in the second none, the functional groups of our species responded differently to environmental drivers. They were influenced by a combination of environmental stresses that need to be further investigated, singularly and cumulatively, to better understand the role of canal management and its potential developments, having as its objective the ever-closer integration of the hydraulic-agronomic aims to those of protection and restoration of the ecological functionality of the canals, for a general improvement of the environmental quality and the conservation of the most endangered plant species.

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Supplementary material 1 - Frequency of each bank aspect type in 104 of the sampled transects (L = large canals, M = medium canals, S = small canals, H2O = canals for which both vegetation and water quality data are available)

Canal type	Not recorded	E	N	NE	NO	O	S	SE	SO
L	2	3	6	1	0	4	5	1	1
M	5	3	1	4	10	2	5	13	3
S	6	4	2	2	4	1	2	1	5
H2O	1	3	0	1	4	1	3	6	3
Total	14	13	9	8	18	8	15	21	12

Supplementary material 2 - Number of transects falling within or around protected areas

Canal type	Inside Regional park	Inside Natura 2000	Inside Ecological rebalancing areas	Outside but in 100m buffer from Regional park	Outside but in 100m buffer from Natura 2000	Outside but in 100m buffer from Ecological rebalancing areas	In 100-500m buffer from Regional park	In 100-500m buffer from Natura 2000	In 100-500m buffer from Ecological rebalancing areas
H2O	1	3	0	0	2	0	0	2	0
L	1	4	0	0	0	0	0	2	0
M	2	5	0	0	1	0	0	3	1
S	0	0	0	0	0	0	0	1	0
Total	4	12	0	0	3	0	0	8	1

Supplementary material 3 - Land use categories (Corine Land Cover IV)

Acronym	Description	Acronym	Description
Aa	Aquaculture in the continental environment	Ro	Photovoltaic systems
Fs	Airports for sports flights and heliports	It	Technological systems
Cl	Other wood crops	Iz	Agro-zootechnical settlements
Av	Riverbeds of rivers and streams with abundant vegetation	Ic	Commercial settlements
Af	Riverbeds of rivers and streams with poor vegetation	Is	Service establishment
Ze	Areas with important agricultural crops and natural areas	Io	Hospital settlements
Qa	Active extractive areas	Ia	Production sites
Qi	Inactive extractive areas	Vi	Racecourses
Vx	Uncultivated urban areas	Co	Olive groves
Vs	Sport areas	Vp	Parks
Rv	Green areas associated with roads	Vd	Leisure parks
Ar	Banks	Cp	Poplar crops
Va	Racetraks	Pp	Meadows
Ra	Highways and expressways	Rf	Rail networks
Ax	Artificial basins	Re	Energy distribution and production networks
Br	Ruderal thickets	Ri	Networks for water distribution
Bq	Oak, hornbeam and chestnut forests	Rs	Road networks
Bs	Predominantly willow and poplar forests	Ta	Recent reforestation
Bm	Mixed coniferous and broad-leaved forests	Sn	Non-irrigated arable land
Bp	Plain forests with a prevalence of farnies and ash trees	Se	Irrigated plain arable land
Vt	Camping and tourist accommodation	Zo	Complex crop and particle systems
Vg	Golf courses	Es	Isolated residential facilities
Ac	Canals and waterways	Qs	Modified soils and artifacts
Qc	Construction sites and excavations	Er	Sparse residential fabric
Vm	Cemeteries	Ec	Compact and dense residential fabric
So	Horticultural crops	Ed	Urban residential fabric
Zt	Temporary crops combined with permanent crops	Tn	Evolving shrubby and arboreal vegetation
Qr	Salvage yards	Cv	Vineyards
Qu	Landfill of municipal solid waste	Vv	Villas
Qq	Landfills and deposits of quarries, mines & industries	Sv	Nurseries
Cf	Fruit trees	Ui	Inland wetlands
Rm	Marshalling yards		

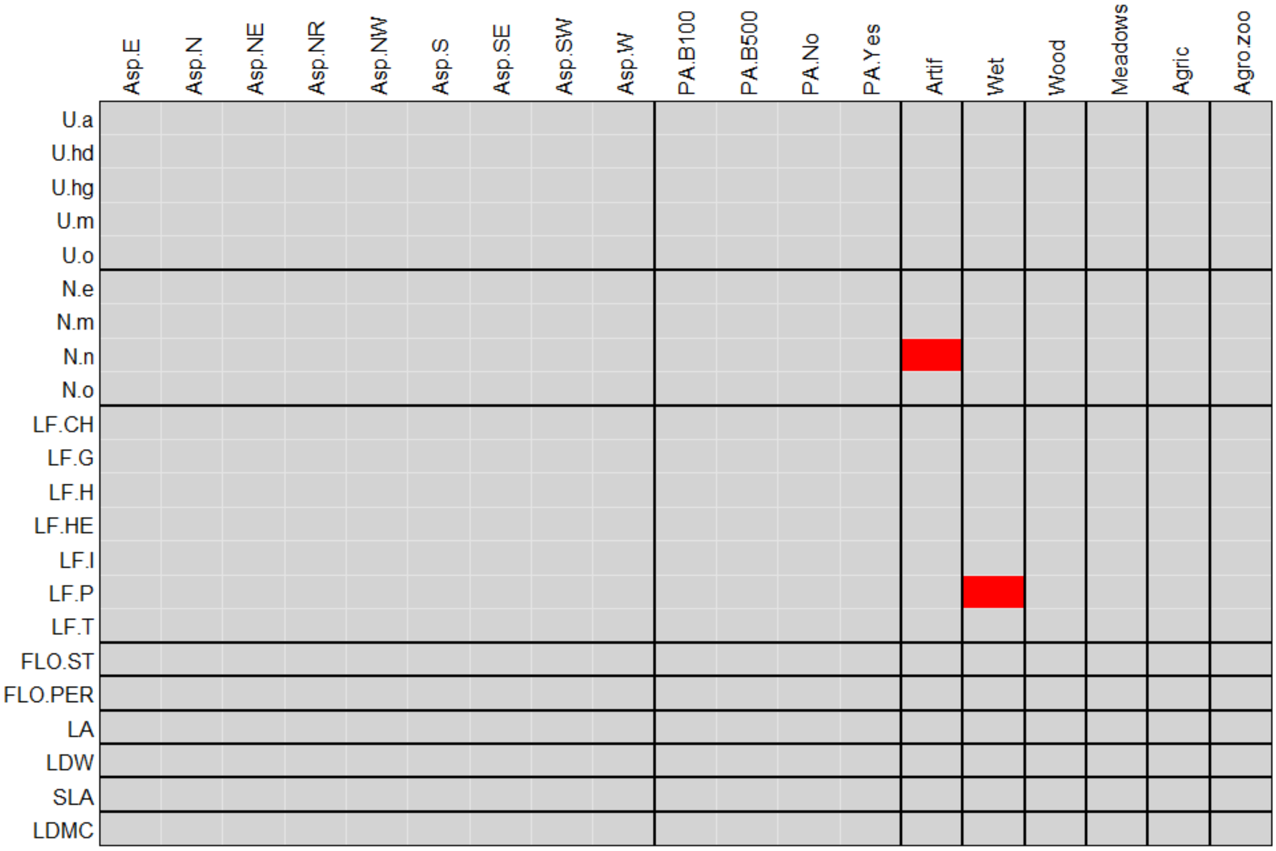
Supplementary material 4 - Percentage of macro-categories of land use in the upstream basin of each type of canal sampled (L = large canals, M = medium canals, S = small canals, H2O = canals for which both vegetation and water quality data are available). For the abbreviations of land use categories see Supplementary material 1

	Canal type			
Land use macro-categories	**H2O**	**L**	**M**	**S**
Artificial surfaces (Ec,Ed, Er, Ia, Ic, Io, Is, It, Qa, Qc, Qi, Qq, Qr, Qs, Qu, Ra, Re, Rf, Ri, Rm, Ro, Rs, Va, Vd, Vg, Vi, Vm, Vs, Vt, Vv, Es)	7.1	16.0	13.7	13.5
Water bodies and inland wetlands (Ac, Aa, Ax, Af, Ar, Av, Zi)	10.6	5.1	3.1	2.1
Wood (Bm, Bp, Bq, Br, Bs, Ta, Tn)	2.8	1.5	2.6	0.9
Meadows (Fs, Pp, Rv, Vp, Vx)	0.7	1.8	1.5	1.6
Agricultural area (So, Se, Sv, Ze, Zo, Zt, Sn, Cf, Co,Cv, Cl, Cp)	78.2	74.9	78.4	81.4
Agro-zootechnical settlements (Iz)	0.6	0.7	0.7	0.5
Total percentage	100	100	100	100

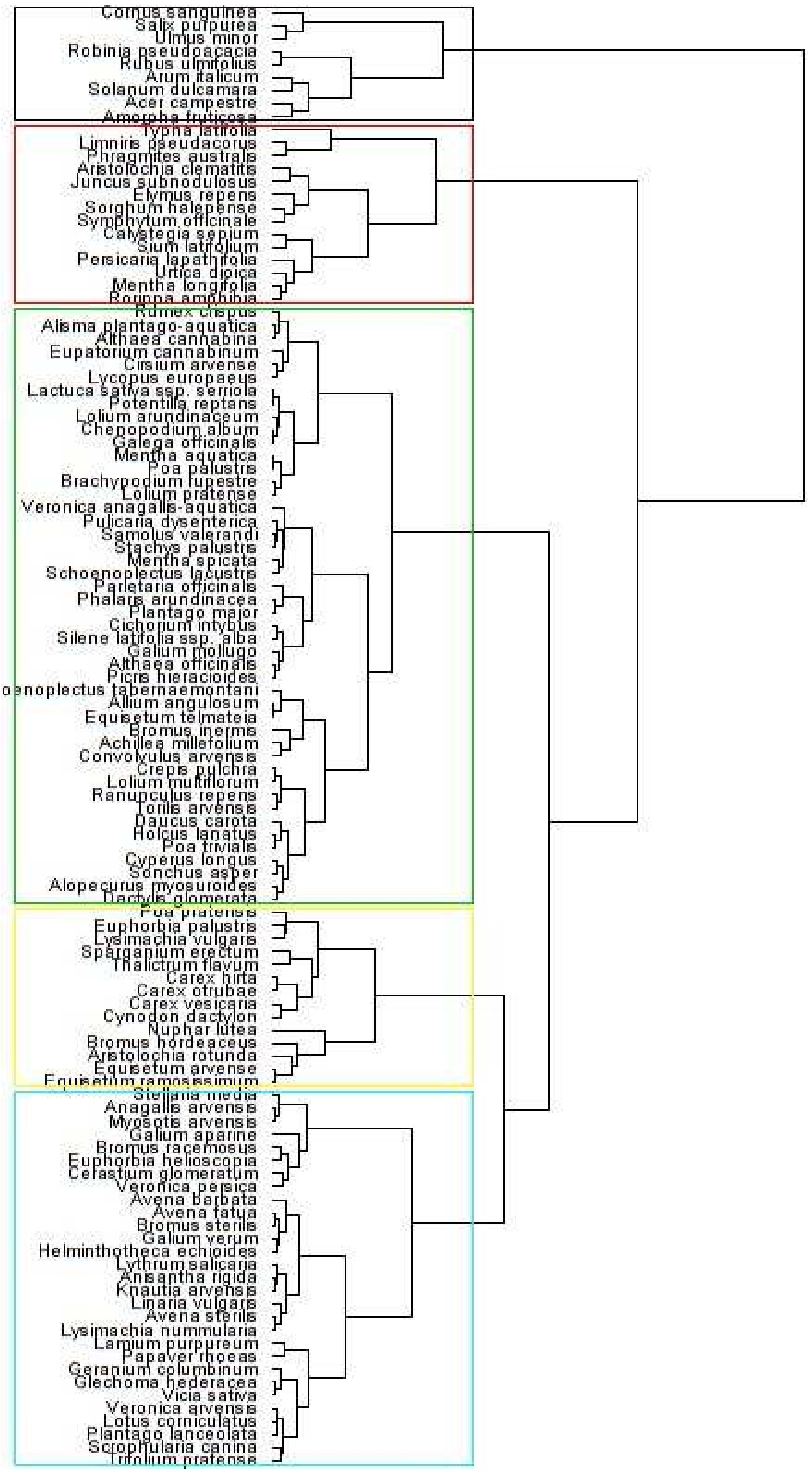
Supplementary material 5 - Species for which we have not found the functional traits and congener species of which we have used the functional traits to fill the information gaps

Species lacking functional traits	**Missing functional traits**	**Species of which we have used the average of the single functional traits**
Aristolochia rotunda	LA, LDW	*Aristolochia clematitis*
Cerastium sylvaticum	LA, LDW, LDMC	*C. capillaris, C. conyzifolia, C. santa, C. setosa, C. vesicaria*
Crepis pulchra	LA, SLA, LDW, LDMC	*C. capillaris, C. conyzifolia, C. santa, C. setosa, C. vesicaria*
Populus deltoides	LDMC	*P. canadensis, P. alba, P. nigra*
Ranunculus velutinus	LA, SLA, LDW, LDMC	*R. repens, R. sceleratus*
Schoenoplectus tabaernemontani	LDW	*Schoenoplectus lacustris*

Supplementary material 6 – Bivariate associations between environmental variables and functional traits (Bank aspect: Asp.E = east, Asp.N = north, Asp.NE = north-east, Asp.NW = north-west, Asp.NR = not detected, Asp.W = west, Asp.S = south, Asp.SE = south-east, Asp.SW = south-west. Proximity to protected areas: PA.B100 = within a buffer of 100 m from a protected area, PA.B500 = within a buffer between 100 and 500 m from protected areas, PA.No = outside protected areas, PA.Yes = inside a protected area. Land uses: Artif = artificial surfaces, Wet = water bodies and inland wetlands, Wood = forest, Meadows = meadows, Agric = agricultural areas, Agric.zoo = agro-zootechnical settlements), (Ellenberg index U: U.a = aquatic plants, U.hd = hydrophilous, U.hg = hygrophilous, U.m = mesophilous, U.o = xerophilous. Ellenberg index N: N.e = eutrophic, N.m = mesotrophic, N.n = nitrophilous, N.o = oligotrophic. Life forms: LF.CH = chamaephytes, LF.G = geophytes, LF.H = emicriptophytes, LF.HE = helophytes, LF.I = hydrophytes, LF.P = phanerophytes, LF.T = therophytes; FLO.ST = flowering start, FLO.PER = flowering period, LA = leaf area, LDW = dry weight leaf, LDMC = dry matter content leaf, SLA = specific leaf area). The significant positive associations (p-value < 0.05) was represented by red cells. Non-significant associations were in gray.



Supplementary material 7 – Functional groups obtained based on RLQ species scores. The colors of the rectangles correspond to the five functional groups (Group 1 = black, Group 2 = green, Group 3 = blue, Group 4 = red, Group 5 = yellow).



Through the cluster analysis 5 functional groups of species were identified:

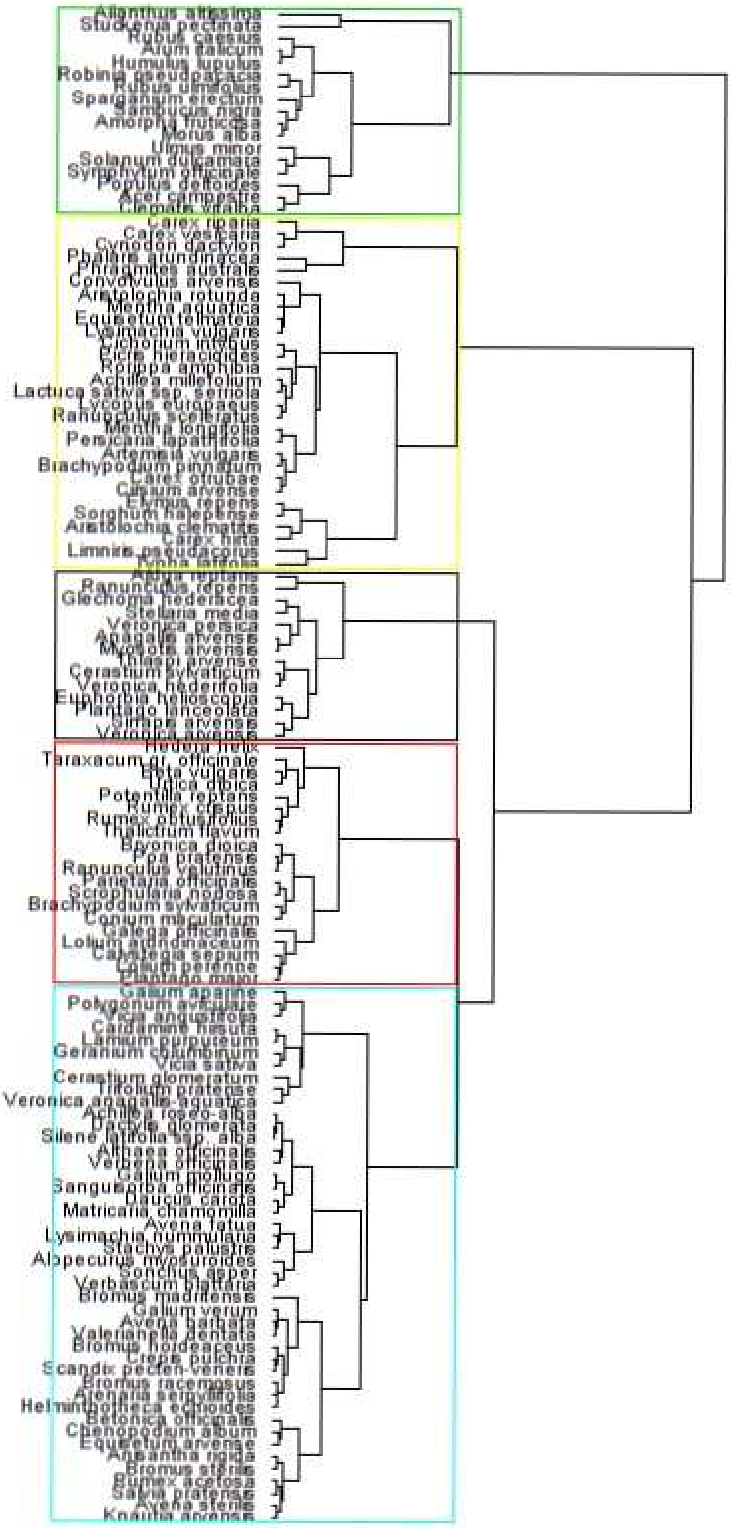
- Group 1 (black) is characterized by species with an ecological preference for soils not humid, requirements in terms of availability of nitrogen very variable, phanerophyte life form (except for the geophyte *Arum italicum*). These species begin to bloom between March and June and tend to have short flowering periods. They also have leaf area (LA), leaf dry weight (LDW) and leaf dry matter content (LDMC) averagely higher than the other groups, while the specific leaf area (SLA) is very low, second only to group 5;
- Group 2 (green) brings together species that prefer soils with medium to high nutrient availability and variable (tendentially medium to high) soil moisture. Here are included numerous species needing very humid soils, such as *Cyperus longus* and *Schoenoplectus tabernaemontani* which usually live in continuously submerged soils. It includes various life form, such as hemicryptophytes, helophytes and, marginally, hydrophytes. Group 2 species begin to bloom mainly from May to June and have flowering periods ranging from 2 to 4 months. They also present LA and LDW in-between at groups 1, 4 and 3, very low LDMC and high SLA (but less than group 3);
- Group 3 (blue) is composed of species that prefer soils with low to medium amounts of nitrogen (except for *Anagallis arvensis, Euphorbia helioscopia, Myosotis arvensis* and *Veronica persica*, which are eutrophic) and a low to medium soil moisture (except *Bromus racemosus*, *Glechoma hederacea* and *Lythrum salicaria* which prefer damp soils). The prevalent life form are the terophytes and, to a much lesser extent, the hemicryptophytes. Group 3 species begin to bloom mainly between April and May and have relatively long flowering periods (2 to 7 months), such as *Stellaria media* and *Veronica persica*. They also have lower LA, LDW and LDMC than other groups, while SLA is the highest;
- Group 4 (red) is characterised by species with an ecological preference for soils with high nitrogen content and high moisture content. The prevalent life form are the geophytes and, to a lesser extent, the hemicryptophytes. The species of this group begin to bloom between May and July and have flowering periods ranging from 1 to 3 months. They also have a relatively high LA and similar to group 1, LDW is very variable but higher than group 1, LDMC remains in-between groups 1, 5 and 3, but higher than 2 and SLA is low;

- Group 5 (yellow) contains species that show an ecological preference for soils with medium to low nitrogen availability and variable humidity. Inside this group are however numerous the species which prefer very humid soils, such as *Nuphar lutea* which is an aquatic plant and lives almost completely submerged. The prevalent life form are the geophytes, with a not negligible percentage of hydrophytes. The species of this group begin flowering between May and June and have relatively short flowering periods (1 to 2 months). They also have an intermediate LA between groups 1, 4 and 3, but higher than group 2, intermediate LDW between groups 1, 4 and 3, high LDMC while the SLA is the lowest.

Supplementary material 8 - Water parameters chosen as "independent" and water parameters strongly related to them.

Indipendent water parameters	**Strongly related water parameters**
pH mean	pH max
PH min	
CHL mean	CHL min, CHL max, NT min, NT mean, NT max, NOR min, NOR mean, NOR max
EC mean	Ec min, EC max, CARB mean, CARB max, K mean, Na max, SAR mean, SAR max
BOD5 mean	BOD5 max
BOD5 min	COD mean
COD min	
COD max	
NO3 mean	NO3 min, NO3 max
NH4 mean	NH4 min, NH4 max
CARB min	SO4 min, Ca min, Na min
PO4 mean	PO4 min, PO4 max
SO4 mean	SO4 max, CARB mean, CARB max, B mean
Cl min	
Ca mean	Ca min, Ca max, Na mean
Mg mean	Mg max
K min	EC min, B mean
SAR min	Na min, SO4 min
B min	
B max	

Supplementary material 9: Functional groups obtained based on RLQ species scores. The colors of the rectangles correspond to the five functional groups (Group 1=green, Group 2=yellow, Group 3=blue, Group 4=black, Group 5=red).



Through the cluster analysis 5 functional groups of species were identified:

- Group 1 (green) included species that preferred soils with high nitrogen and nutrient availability and medium to low humidity values. The prevalent life form are the phanerophytes, although it is important to note that this group includes the only hydrophytes sampled, that are *Stuckenia pectinata* and *Sparganium erectum*. Group 1 species tend to bloom between April and June, have relatively short flowering periods and higher LA, LDW and LDMC values than other groups, while SLA is the lowest;
- Group 2 (yellow) contains species with variable humidity and nitrogen soil availability, geophyte and hemicryptophyte life forms. Within this group falls the only helophyte sampled in the 17 transects: *Phalaris arundinacea*. Group 2 species begin to bloom between May and July and have rather short flowering periods (1-2 months). They also have an LA between groups 3, 4 and 5, the intermediate LDW between groups 3, 4 and 1, LDMC and SLA similar to Group 1;
- Group 3 (blue) contains species that have an ecological preference for soils that are not humid (even though it includes a few species notably hygrophilous, namely *Althaea officinalis*, *Betonica officinalis*, *Bromus hordeaceus*, *Stachys palustris* and overall *Veronica anagallis-aquatica*) and poor in nutrients or fairly humified. Life forms are predominantly therophytes and hemicryptophytes. Group 3 species begin to bloom between April and June and tend to have long flowering periods (3-7 months), such as *Lamium purpureum* and *Cardamine hirsuta*. They also have very low LA, LDW and LDMC values, second only to group 4, and high SLA;
- Group 4 (black) contains species with preference for soils with high nutrient concentration and that do not show particular needs in terms of humidity (apart from *Glechoma hederacea* and *Ranunculus repens*). Group 4 species tend to bloom early (March and April) and have relatively long flowering periods, such as *Stellaria media* and *Veronica persica*. They also have lower LA, LDW and LDMC than the other groups, and high SLA;
- Group 5 (red) contains species that have no particular preferences for soil moisture. The eutrophic species are numerous and the prevalent life form are the hemicrytophytes. Group 5 species tend to bloom between May and June and have relatively short flowering

periods (1-3 months). They also have a very variable but high LA, LDW similar to group 2, intermediate LDMC between groups 3-4 and 2-1 and high SLA.

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Annex I – Floristic list 2019

1. *Acer campestre* L. – P – Europeo-Causasica
2. *Acer negundo* L. – P – Nord Americana – ALL. NAT.
3. *Achillea millefolium* L. – H – Eurosiberiana
4. *Achillea roseoalba* Ehrend. – H – Centro Europeo
5. *Agrimonia eupatoria* L. – H – Euroasiatica
6. *Ailanthus altissima* (Mill.) Swingle – P-Asiatica – INV.
7. *Ajuga reptans* L. – CH – Europeo-Causasica
8. *Alisma plantago-aquatica* L. – I – Subcosmopolita
9. *Allium angulosum* L. – G – Eurosiberiana – EN.
10. *Alopecurus myosuroides* Huds. – T – Subcosmopolita
11. *Althaea cannabina* L. – H – Sud Europeo
12. *Althaea officinalis* L. – H – Sudest Europeo
13. *Amaranthus retroflexus* L. – T – Cosmopolita – INV.
14. *Amorpha fruticosa* L. – P – Nord Americana – INV.
15. *Anagallis arvensis* L. – T – Eurimediterranea
16. *Anisantha madritensis* (L.) Nevski – T – Eurimediterranea
17. *Anisantha rigida* (Roth) Nevski – T – Subtropicale
18. *Anisantha sterilis* (L.) Nevski – T – Eurimediterranea
19. *Arenaria serpyllifolia* L. – T – Subcosmopolita
20. *Aristolochia clematitis* L. – G – Eurimediterranea
21. *Aristolochia rotunda* L. – G – Eurimediterranea
22. *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl et C. Presl – H – Euroasiatica
23. *Artemisia verlotiorum* Lamotte – H – Asia orientale – INV.
24. *Artemisia vulgaris* L. – H – Circumboreale
25. *Arum italicum* Mill. – G – Stenomediterranea
26. *Arundo donax* L. – G – Subcosmopolita – ALL. NAT.
27. *Asparagus officinalis* L. – G – Eurimediterranea
28. *Atriplex patula* L. – T – Circumboreale
29. *Avena barbata* Pott ex Link – T – Eurimediterranea
30. *Avena fatua* L. – T – Euroasiatica
31. *Avena sativa* L. – T – Avventizia naturalizzata
32. *Avena sterilis* L. – T – Mediterraneo-Turaniano
33. *Ballota nigra* L. – H – Eurimediterranea
34. *Bellis annua* L. subsp. *annua* – T – Stenomediterranea
35. *Berula erecta* (Huds.) Coville – G – Circumboreale
36. *Beta vulgaris* L. – H – Eurimediterranea

37. *Brachypodium cespitosum* (Host) Roem. et Schult. – H – Subatlantico
38. *Brachypodium pinnatum* (L.) P. Beauv. – H – Euroasiatica
39. *Brachypodium sylvaticum* (Huds.) P. Beauv. – H – Paleotemperato
40. *Bromopsis inermis* (Leyss.) Holub – H – Euroasiatica
41. *Bromus arvensis* L. – T – Eurosiberiana
42. *Bromus hordeaceus* L. – T – Subcosmopolita
43. *Bromus racemosus* L. – T – Europeo-Causasica
44. *Bryonia dioica* Jacq. – G – Eurimediterranea
45. *Calepina irregularis* (Asso) Thell. – T – Eurimediterranea-Turan.
46. *Calystegia sepium* (L.) R. Br. – H – Euroasiatica
47. *Cardamine amara* L. – H – Euroasiatica
48. *Cardamine hirsuta* L. – T – Stenomediterranea
49. *Carduus acanthoides* L. – H – Europeo-Causasica
50. *Carduus pycnocephalus* L. – H – Eurimediterranea-Turan.
51. *Carex cuprina* (Sandor ex Heuffel) Nendtwich ex A. Kern. – H – Eurimediterranea-Atlantica
52. *Carex elata* All. – H – Europeo-Causasica
53. *Carex hirta* L. – G – Europeo-Causasica
54. *Carex riparia* Curtis – G – Euroasiatica
55. *Carex vesicaria* L. – G – Circumboreale
56. *Centaurea nigrescens* Willd. – H – Europeo
57. *Cerastium glomeratum* Thuill. – T – Eurimediterranea
58. *Cerastium ligusticum* Viv. – T – Mediterraneo occidentale
59. *Cerastium sylvaticum* Waldst. et Kit. – H – Centro Europeo
60. *Chenopodium album* L. – T – Subcosmopolita
61. *Cichorium intybus* L. – H – Cosmopolita
62. *Cirsium arvense* (L.) Scop. – G – Euroasiatica
63. *Cirsium vulgare* (Savi) Ten. – H – Euroasiatica
64. *Clematis vitalba* L. – P – Europeo-Causasica
65. *Clematis viticella* L. – P – Sud Europeo-Sudsiberiano
66. *Clinopodium nepeta* (L.) Kuntze – H – Mediterraneo montano
67. *Conium maculatum* L. – H – Paleotemperato
68. *Convolvulus arvensis* L. – G – Paleotemperato
69. *Cornus sanguinea* L. – P – Euroasiatica
70. *Crataegus monogyna* Jacq. – P – Euroasiatica
71. *Crepis pulchra* L. subsp. *pulchra* – T – Eurimediterranea
72. *Crepis setosa* Haller f. – T – Eurimediterranea orientale
73. *Crepis vesicaria* L. – T – Eurimediterranea-Subatl.
74. *Cruciata glabra* (L.) Ehrend. – H – Euroasiatica
75. *Cruciata laevipes* Opiz – H – Euroasiatica
76. *Cuscuta cesattiana* Bertol. – T – Nord Americana
77. *Cynodon dactylon* (L.) Pers. – G – Cosmopolita

78. *Cyperus longus* L. – HE – Paleotemperato
79. *Dactylis glomerata* L. – H – Paleotemperato
80. *Daucus carota* L. – H – Paleotemperato
81. *Digitaria sanguinalis* (L.) Scop. – T – Subcosmopolita
82. *Dipsacus fullonum* L. – H – Eurimediterranea
83. *Echinochloa crus-galli* (L.) P. Beauv. – T – Subcosmopolita
84. *Eleocharis palustris* (L.) Roem. et Schult. – G – Cosmopolita
85. *Elymus repens* (L.) Gould – G – Circumboreale
86. *Epilobium hirsutum* L. – H – Euroasiatica
87. *Epilobium tetragonum* L. – H – Paleotemperato
88. *Equisetum arvense* L. – G – Circumboreale
89. *Equisetum fluviatile* L. – G – Circumboreale
90. *Equisetum palustre* L. – G – Circumboreale
91. *Equisetum ramosissimum* Desf. – G – Paleotemperato
92. *Equisetum telmateia* Ehrh. – G – Circumboreale
93. *Erigeron annuus* (L.) Desf. – T – Nord Americana – ALL. NAT.
94. *Erigeron canadensis* L. – T – Nord Americana – INV.
95. *Euonymus europaeus* L. – P – Euroasiatica
96. *Eupatorium cannabinum* L. – H – Paleotemperato
97. *Euphorbia helioscopia* L. – T – Cosmopolita
98. *Euphorbia palustris* L. – G – Eurosiberiana – EN.
99. *Festuca ligustica* (All.) Bertol. – T – Stenomediterraneo occid.
100. *Ficaria verna* Huds. – G – Euroasiatica
101. *Fumaria officinalis* L. – T – Paleotemperato
102. *Galega officinalis* L. – H – Sudest Europeo-Pontica
103. *Galium aparine* L. – T – Euroasiatica
104. *Galium mollugo* L. – H – Eurimediterranea
105. *Galium verum* L. – T – Euroasiatica
106. *Geranium columbinum* L. – T – Sud Europeo-Sudsiberiano
107. *Geranium dissectum* L. – T – Euroasiatica
108. *Geranium lucidum* L. – T – Eurimediterranea
109. *Glechoma hederacea* L. – CH – Circumboreale
110. *Glyceria fluitans* (L.) R.Br. – I – Subcosmopolita
111. *Glyceria maxima* (Hartm.) Holmb. – I – Circumboreale
112. *Hedera helix* L. – P – Eurimediterranea
113. *Heliotropium europaeum* L. – T – Mediterraneo-Turaniano
114. *Helminthotheca echioides* (L.) Holub – T – Eurimediterranea
115. *Holcus lanatus* L. – H – Circumboreale
116. *Humulus lupulus* L. – P – Europeo-Causasica
117. *Hypericum perforatum* L. – H – Paleotemperato
118. *Inula britannica* L. – H – Europeo-Causasica

119. *Inula conyzae* (Griess.) Meikle – H – Europeo-Causasica
120. *Iris pseudacorus* L. – G – Euroasiatica
121. *Juglans regia* L. – P – Asia occidentale – ALL. NAT.
122. *Juncus articulatus* L. – G – Circumboreale
123. *Juncus inflexus* L. – H – Paleotemperato
124. *Juncus subnodulosus* Schrank – G – Europeo-Causasica – CR.
125. *Kickxia spuria* (L.) Dumort. – T – Euroasiatica
126. *Knautia arvensis* (L.) Coult. – H – Euroasiatica
127. *Lactuca saligna* L. – T – Mediterraneo-Turaniano
128. *Lactuca serriola* L. – H – Sud Europeo-Sudsiberiano
129. *Lamium purpureum* L. – T – Euroasiatica
130. *Lathyrus latifolius* L. – H – Sud Europeo
131. *Lathyrus ochrus* (L.) DC. – T – Stenomediterranea
132. *Lathyrus pratensis* L. – H – Paleotemperato
133. *Lathyrus sylvestris* L. – H – Europeo-Causasica
134. *Leucanthemum ircutianum* DC. – H – Eurosiberiana
135. *Linaria arvensis* (L.) Desf. – T – Submediterranea
136. *Linaria vulgaris Mill.* – H – Euroasiatica
137. *Linum catharticum* L. – T – Eurimediterranea
138. *Lolium multiflorum* Lam. – T – Eurimediterranea
139. *Lolium perenne* L. – H – Circumboreale
140. *Loncomelos brevistylus* (Wolfner) Dostál – G – Sudest Europeo
141. *Lotus corniculatus* L. – H – Cosmopolita
142. *Lycopus europaeus* L. – H – Circumboreale
143. *Lycopus exaltatus* L.f. – H – Eurosiberiana
144. *Lysimachia nemorum* L. – H – Europeo-Causasica
145. *Lysimachia nummularia* L. – H – Europeo-Causasica
146. *Lysimachia punctata* L. – H – Sudest Europeo
147. *Lysimachia vulgaris* L. – H – Euroasiatica
148. *Lythrum salicaria* L. – H – Subcosmopolita
149. *Malva sylvestris* L. – H – Eurosiberiana
150. *Matricaria chamomilla* L. – T – Asiatica
151. *Medicago lupulina* L. – T – Euroasiatica
152. *Mentha aquatica* L. – H – Subcosmopolita
153. *Mentha longifolia* (L.) Huds. – H – Euroasiatica
154. *Mentha spicata* L. – H – Eurimediterranea
155. *Microthlaspi perfoliatum* (L.) F.K.Mey. – T – Paleotemperato
156. *Morus alba* L. – P – Asia orientale
157. *Myagrum perfoliatum* L. – T – Asia occidentale
158. *Myosotis arvensis* (L.) Hill – T – Euroasiatica
159. *Nasturtium officinale* R.Br. – H – Cosmopolita

160. *Nuphar lutea* (L.) Sm. – I – Euroasiatica – VU.
161. *Oxalis corniculata* L. – CH – Eurimediterranea
162. *Paliurus spina-christi* Mill. – P – Sudest Europeo
163. *Papaver rhoeas* L. – T – Mediterraneo orientale
164. *Parietaria officinalis* L. – H – Sud Europeo
165. *Paspalum distichum* L. – H – Subcosmopolita – ALL. NAT.
166. *Pastinaca sativa* L. – H – Eurosiberiana
167. *Persicaria hydropiper* (L.) Delarbre – T – Circumboreale
168. *Persicaria lapathifolia* (L.) Delarbre –T – Cosmopolita
169. *Phalaris paradoxa* L. – T – Stenomediterranea
170. *Phalaroides arundinacea* (L.) Rauschert – HE – Circumboreale
171. *Phragmites australis* (Cav.) Trin. ex Steud. – G – Subcosmopolita
172. *Picris hieracioides* L. – H – Eurosiberiana
173. *Plantago lanceolata* L. – H – Euroasiatica
174. *Plantago major* L. – H – Euroasiatica
175. *Poa palustris* L. – H – Circumboreale
176. *Poa pratensis* L. – H – Circumboreale
177. *Poa trivialis* L. – H – Euroasiatica
178. *Polygonum aviculare* L. – T – Cosmopolita
179. *Populus alba* L. – P – Paleotemperato
180. *Populus deltoides* Marshall – P – Nord Americana – ALL. CAS.
181. *Populus nigra* L. – P – Paleotemperato
182. *Potamogeton pectinatus* L. – I – Subcosmopolita
183. *Potentilla reptans* L. – H – Subcosmopolita
184. *Prunella vulgaris* L. subsp. *vulgaris* – H – Circumboreale
185. *Prunus domestica* L. – P – Asia sudoccidentale
186. *Prunus spinosa* L. – P – Europeo-Causasica
187. *Pulicaria dysenterica* (L.) Bernh. – H – Eurimediterranea
188. *Quercus robur* L. – P – Europeo-Causasica
189. *Ranunculus bulbosus* L. – H – Euroasiatica
190. *Ranunculus repens* L. – CH – Euroasiatica
191. *Ranunculus sceleratus* L. – T – Paleotemperato
192. *Ranunculus velutinus* Ten. – H – Nord Eurimediterranea
193. *Reichardia picroides* (L.) Roth – H – Stenomediterranea
194. *Robinia pseudoacacia* L. – P – Nord Americana
195. *Rorippa amphibia* (L.) Besser – H – Eurosiberiana
196. *Rorippa palustris* (L.) Besser – T – Subcosmopolita
197. *Rubus caesius* L. – P – Euroasiatica
198. *Rubus ulmifolius* Schott – P – Eurimediterranea
199. *Rumex acetosa* L. – H – Circumboreale
200. *Rumex conglomeratus* Murray – H – Euroasiatica

201. *Rumex crispus* L. – H – Subcosmopolita
202. *Rumex cristatus* DC. – H – Nordest Meditettraneo – INV.
203. *Rumex obtusifolius* L. – H – Europeo-Causasica
204. *Rumex pulcher* L. – H – Eurimediterranea
205. *Salix alba* L. – P – Euroasiatica
206. *Salix purpurea* L. – P – Euroasiatica
207. *Salix viminalis* L. – P – Eurosiberiana
208. *Salvia pratensis* L. – H – Eurimediterranea
209. *Salvia verbenaca* L. – H – Stenomediterranea
210. *Sambucus nigra* L. – P – Europeo-Causasica
211. *Samolus valerandi* L. – H – Subcosmopolita – EN.
212. *Sanguisorba minor* Scop. – H – Subcosmopolita
213. *Sanguisorba officinalis* L. – H – Circumboreale
214. *Scandix pecten-veneris* L. – T – Eurimediterranea
215. *Schedonorus arundinaceus* (Schreb.) Dumort. – H – Paleotemperato
216. *Schedonorus pratensis* (Huds.) P. Beauv. – H – Euroasiatica
217. *Schoenoplectus lacustris* (L.) Palla – HE – Subcosmopolita
218. *Schoenoplectus tabernaemontani* (C.C.Gmel.) Palla – HE – Eurosiberiana – VU.
219. *Scrophularia canina* L. – H – Eurimediterranea
220. *Scrophularia nodosa* L. – H – Circumboreale
221. *Scutellaria galericulata* L. – G – Circumboreale
222. *Senecio viscosus* L. – T – Eurimediterranea
223. *Setaria pumila* (Poir.) Roem. & Schult. – T – Subcosmopolita
224. *Silene latifolia* Poir. – H – Stenomediterranea
225. *Silene vulgaris* (Moench) Garcke – H – Subcosmopolita
226. *Silybum marianum* (L.) Gaertn. – H – Mediterraneo-Turaniano
227. *Sinapis arvensis* L. – T – Stenomediterranea
228. *Sium latifolium* L. – HE – Centro Europeo – EN.
229. *Solanum dulcamara* L. – P – Eurosiberiana
230. *Solanum nigrum* L. – T – Euroasiatica
231. *Sonchus arvensis* L. –H – Eurosiberiana
232. *Sonchus asper* (L.) Hill – T – Euroasiatica
233. *Sonchus oleraceus* L. – T – Euroasiatica
234. *Sorghum halepense* (L.) Pers. – G – Cosmopolita – INV.
235. *Sparganium erectum* L. – I – Euroasiatica
236. *Stachys annua* (L.) L. – T – Eurimediterranea
237. *Stachys officinalis* (L.) Trevis. – H – Europeo-Causasica
238. *Stachys palustris* L. – H – Circumboreale
239. *Stellaria media* (L.) Vill – T – Cosmopolita
240. *Symphytum officinale* L. – H – Europeo-Caucasica
241. *Taraxacum officinale* Weber – H – Circumboreale

242. *Thalictrum aquilegiifolium* L. – H – Eurosiberiana
243. *Thalictrum flavum* L. – H – Euroasiatica
244. *Thlaspi arvense* L. – T – Asia occidentale
245. *Tordylium maximum* L. – T – Eurimediterranea
246. *Torilis arvensis* (Huds.) Link – T – Subcosmopolita
247. *Torilis japonica* (Houtt.) DC. – T – Subcosmopolita
248. *Torilis nodosa* (L.) Gaertn. – T – Eurimediterranea
249. *Tragopogon porrifolius* L. – H – Eurimediterranea
250. *Trifolium medium* L. – G – Euroasiatica
251. *Trifolium pratense* L. – H – Subcosmopolita
252. *Trifolium repens* L. – H – Subcosmopolita
253. *Triticum aestivum* L. – T – Avventizia naturalizzata
254. *Typha angustifolia* L. – G – Circumboreale
255. Typha latifolia L. – G – Cosmopolita
256. *Ulmus minor* Mill. – P – Europeo-Causasica
257. *Urtica dioica* L. – H – Subcosmopolita
258. *Valeriana officinalis* L. – H – Est Europeo
259. *Valerianella coronata* (L.) DC. – T – Eurimediterranea
260. *Valerianella dentata* (L.) Pollich – T – Subatlantico
261. *Verbascum blattaria* L. – H – Euroasiatica
262. *Verbena officinalis* L. – H – Euroasiatica
263. *Veronica anagallis-aquatica* L. – H – Cosmopolita
264. *Veronica arvensis* L. – T – Subcosmopolita
265. *Veronica hederifolia* L. – T – Euroasiatica
266. *Veronica persica* Poir. – T – Asia occidentale – ALL. NAT.
267. *Veronica serpyllifolia* L. – H – Circumboreale
268. *Vicia sativa* L. – T – Stenomediterranea
269. *Vicia sativa* subsp. *angustifolia* (Grufb.) Gaudin – T – Mediterranea
270. *Viola odorata* L. – H – Eurimediterranea
271. *Vitis vinifera* L. – P – Coltivata
272. *Xanthium italicum* Moretti – T – Sud Europeo – INV.