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Benthic macroinvertebrates in Italian rice fields

Daniela LUPI,* Anna ROCCO, Bruno ROSSARO

University of Milan, Department of Food, Environmental and Nutritional Sciences (DeFENS), Via Celoria 2, 20133 Milano, Italy.
*Corresponding author: daniela.lupi@unimi.it

ABSTRACT

Rice fields can be considered man-managed temporary wetlands. Five rice fields handled with different management strategies, their adjacent channels, and a spring were analysed by their benthic macroinvertebrate community to i) evaluate the role of rice agroecosystem in biodiversity conservation; ii) find indicator species which can be used to compare the ecological status of natural wetlands with rice agroecosystems; and iii) find the influence of environmental variables on biodiversity. Different methods of data analysis with increasing degree of complexity – from diversity index up to sophisticated multivariate analysis – were used. The investigation provided a picture of benthic macroinvertebrates inhabiting rice agroecosystems where 173 taxa were identified, 89 of which detected in rice paddies. Among them, 4 phyla (Mollusca, Annelida, Nematomorpha, and Arthropoda), 8 classes (Bivalvia, Gastropoda, Oligochaeta, Hirudinea, Gordioida, Insecta, Branchiopoda, and Malacostraca), 24 orders, 68 families, 127 genera and 159 species have been found. Ten threatened and 3 invasive species were detected in the habitats examined. The information obtained by the different methods of data analysis allowed a more comprehensive view on the value of the components of rice agroecosystems. Data analyses highlighted significant differences between habitats (feeding channel and rice field), with higher diversity observed in channels, and emphasised the role of the water chemical-physical parameters. The period of water permanence in rice fields resulted to be only one of the factors influencing the community of benthic macroinvertebrates. The presence of rare/endangered species allowed characterising some stations, but it was less informative about management strategies in rice paddies because most of these species were absent in rice fields.

Key words: biodiversity indices, faunistic composition, water parameters, wetlands, rice fields, self-organising map.

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INTRODUCTION

Wetlands are habitats suitable for many species of benthic macroinvertebrates (Biggs *et al.*, 1994). The communities are influenced by many environmental factors; the surrounding landscape and the duration of flood are of special importance, allowing the maintenance of a well-diversified fauna (Minelli, 2001; Della Bella *et al*., 2005). In the past, Northern Italy was characterised by large alluvial areas, but at present, the development of densely colonised, industrialised, and cultivated areas has strongly reduced the presence of natural temporary ponds (Fasola, 2003; Stoch, 2005). In this context, the widespread occurrence of rice agroecosystem in the Po river basin could surrogate the loss of natural wetlands (Lawler, 2001; Angelini *et al*., 2008) and could be evaluated as a hot spot of diversity in a highly impacted plain. Moreover, we must consider that rice paddies are not isolated but they are connected by channels, which could be of particular interest in supporting and enhancing rice field biodiversity.

Rice agroecosystem is subject to different anthropogenic effects that can vary significantly within the country and can have effect on macroinvertebrate composition. They are principally due to the source and the regularity of water supply, soil permeability and different environmental factors (Moormann and van Breemen, 1978). It must not be overlooked that the natural water cycle in rice fields is strongly altered because paddies are flooded during the rice growing period and dries up in autumn and winter, while natural ponds are generally richer in water during colder seasons. The absence of water in rice field in this period is unfavourable to the development of many species which overwinter in the preimaginal stage in wetlands (Suhling *et al.*, 2000). Besides, one must consider that the flooding period is strongly influenced by management techniques (*e.g.* seeding technique or water removal to allow operations such as weed and eventually insecticide treatments). The shortage of the period of water permanence can affect the survival of species with longer cycle (Bazzanti *et al.*, 2003; Caramujo and Boavida, 2010). Water in rice fields is also subject to quick physical and chemical changes (Panizzon *et al.*, 2012). Thermal fluctuations are directly affected by the height of rice plants, with the consequent creation of a microclimate with aspects of tropical and subtropical areas with small thermal excursions also at northern latitude (Confalonieri *et al.*, 2005). Al-Shami *et al.* (2010) reported the influence of agronomic practices on dissolved oxygen, pH, conductivity, phosphate and nitrate. As a result, the rice agroecosystem is a very complex environment and all small modifications can have direct effects on macroinvertebrate communities.

One must consider that Italian literature on macroinvertebrate community in rice fields is richer on rice pests than on the overall biodiversity. Furthermore, most papers are now outdated (Supino, 1932; Moroni, 1961) and remarkable information is restricted to some aquatic insects [Del Guercio (1911), Cavazza (1914), Supino (1916), Moretti (1932, 1934), Goidanich (1939), Zangheri (1956), Moroni (1961), Cocchi (1966), Corbetta (1973), Ferrarese (1992), Pasini and Ferrarese (1998), Bellini *et al.* (2000), and Caldara (2004)]. Apart from information on macroinvertebrates, an insight into other components of rice field communities is available (*e.g.* Ostracoda) (Rossi *et al.*, 2003).

The principal aim of this work is to evaluate the role of rice agroecosystem in biodiversity conservation in Northern Italy, also considering the effect of different management practices on the benthic macroinvertebrate communities. Different methods of data analysis with increasing degree of complexity – from simple Shannon diversity index up to sophisticated unsupervised neural networks and between-class co-inertia analysis (BCA) – are used to test how the different components of the community are affected. The role of management techniques and water chemistry on agroecosystem functioning will be evaluated emphasising the relation among environmental factors and species richness. This investigation will also provide a more comprehensive picture of benthic macroinvertebrates inhabiting rice agroecosystems with a detailed taxonomic study.

METHODS

Study area

Benthic macroinvertebrates were sampled between June 2009 and March 2011 in six different stations in Pavia province (Italy), in an area belonging to the Po river basin. The area, characterised by different crops and a prevalence of rice cultivation, has a temperate subcontinental climate (Pinna, 1978), and an altitudinal range of 100 m a.s.l. Rice fields in this area can be classified into two different groups on the basis of rice seeding: water seeded and dry seeded with delayed flooding after 40 days. These paddies are connected by a dense network of irrigation channels and ditches.

The habitats considered were the rice fields handled with different management strategies (Stations 1-5), the adjacent channels (Stations 1-4), and a spring (Station 6) (Tab. 1). Stations 1 and 2 were sampled from 2009 to 2011; Station 5 was sampled in 2009 for 7 months; Stations 3, 4, and 6 were sampled for 12 months in 2010-2011; some samples could not be collected because of the absence of water. In each station, 4 separate sample replicates were collected monthly in the presence of water. Different management strategies in rice paddies regarded seedling technique and water management, while fertilisation and weed management were conducted similarly in all rice paddies. No insecticide was used during the whole period of observation.

Sampling method

Benthic macroinvertebrates were sampled with a pond net (30×30 cm=0.09 m^2, 300-µm mesh size) used for 30 seconds in the substrate. For chironomids, extra samples of pupae, pupal exuviae, and pharate adults were collected with drift nets (Brundin's net) to confirm species identification. Samples were collected at random along a transect through the habitat considered.

The samples were transported to DeFENS laboratory (University of Milan), where the whole sediment was rinsed using a 0.5 mm mesh sieve to separate the specimens. The specimens were then stored in 75% ethyl alcohol and identified to the lowest taxonomic level (species, genus, family)

Tab. 1. Stations description.

Station	Coordinates	Habitat	Water presence (months)	Details
1 Trovo	45°14'86 N;	R1	2	Dry seeded and flooded after 40 days
	9°01'34 E	C1	12	Smooth flow
2 Bereguardo	45°16'09 N;	R2	4	Water seeded
	9°02'16 E	C2	12	Smooth flow
3 Zeme Loja	45°12'42 N;	R3	4	Water seeded
	8°38'44 E	C3	12	Smooth flow
4 Zeme Zanaglia	45°11'74 N;	R4	11	Water seeded
	8°38'25 E	C4	12	Smooth flow
5 Vigna del Pero	45°14'27 N; 9°2'23 E	R5	2	Dry seeded and flooded after 40 days
6 Zeme Raina	45°12'8 N; 8°38'52 E	S6	12	Spring forming a channel; rippled flow

N, north; E, east; R1, rice field 1; C1, channel 1; R2, rice field 2; C2, channel 2; R3, rice field 3; C3, channel 3; R4, rice field 4; C4, channel 4; R5, rice field 5; S6, spring 6.

using a stereomicroscope (Leica MZ 12.5, Leica Microsystems GmbH, Wetzlar, Germany; and Wild Heerbrugg M5A, Leica Geosystems GmbH, Heerbrugg, Switzerland). The identification of many taxa, chironomids and oligochaetes in particular, required dissection of the specimens, the preparation of slides, and examination with an optical microscope (Leica DM LS B2, Leica Microsystems GmbH) connected to a Leica DFC320 camera to obtain measurements. Slides were prepared in Faure medium or Canada balsam. The following taxonomic keys were used in species identification: Olmi (1978), Tamanini (1979), Castagnolo (1980), Girod *et al.* (1980), Giusti and Pezzoli (1980), Ferrarese and Rossaro (1981), Pirisinu (1981), Rossaro (1982), Belfiore (1983), Ferrarese (1983), Moretti (1983), Wiederholm (1983, 1986, 1989), Nocentini (1985), Friday (1988), Timm (1999), Heidemann and Seidenbush (2002), Cham (2007, 2009). Scientific nomenclature was then updated according to the Fauna Europea inventory (de Jong, 2011).

Environmental variables

Nine environmental variables, chosen among those routinely measured in biological studies, were considered. Water temperature, pH and conductivity were measured with a multi-probe field meter (Geotech WTW 3400i Multi-Parameter Field Meter; Geotech Environmental Equipment Inc., Denver, CO, USA) in each locality at each sampling date to characterise the different environments. Water samples were collected at the same sampling date in acid-cleaned graduated bottles for chemical analysis (dissolved O_2, alkalinity, hardness, total phosphorus, nitrate nitrogen) by standard methods (APHA, 2005). The grain-size composition of the substrate was evaluated by visual assessment as percentage of stones/rocks (>20 cm), cobbles (5±20 cm), gravel (0.2±5 cm), sand (0.01±0.2 cm), and silt/mud (<0.01 cm).

Data analysis

Diversity

Diversity is a simple measure which synthesises the community and was selected to have a preliminary measure of the ecological status. The number of species and Shannon diversity index were calculated for all sites (Legendre and Legendre, 2012). Grouped values of diversity according to spatial and temporal factors were also calculated. A factorial ANOVA using the Shannon diversity index as dependent variable and stations, habitats, and months as factors was carried out to estimate the principal sources of variation of diversity.

Indicator values

The indicator values (IndVal) – a method combining the species mean abundance and its frequency of occurrence in the groups (Borcard *et al.*, 2011; Legendre and Legendre, 2012) – were calculated to select indicators species in each station and habitat.

The IndVal is the product of two terms: the first (specificity), referring to the performance of species as abundance over all groups, and the other (fidelity), referring to the performance of the same species as presence-absence within site group. These two terms are multiplied and then scaled to express the indicator value of one species with respect to cluster in terms of percentages. Finally, for each species, the higher value is chosen in order to express its indicator value with respect to the habitat examined (Podani and Csányi, 2010).

Self-organising map

An unsupervised artificial neural network, also known as a self-organising map (SOM) (Kohonen *et al.*, 1995; Vesanto *et al.*, 1999) which is able to visualise and explore linear and non-linear relationships in high-dimensional data sets, was applied (Lek and Guegan, 2000). The purpose was to i) classify all stations, habitats and temporal distribution according to macroinvertebrate assemblages; ii) evaluate differences among rice fields on the basis of the management strategy adopted; and iii) interpret the variability of the observed community in relation to the environmental variables measured.

The structure of the SOM consists of two layers of neurons connected by weights: the input layer consists of a data matrix with n sampling sites (rows) and p taxa (columns); the output layer is a matrix with the same column number (p), but a reduced number of rows (rc). The values of the output (codebook matrix) were calculated starting from a principal component solution of the input matrix as initial configuration. The number of rc elements was selected before the calculations began, minimising a quantisation error that measures the distortion of the final configuration compared to the initial one, to select the optimal rc map dimension. This number is a compromise between a too-detailed and too-approximate representation. The SOM aims to minimise the distances between the points in the original input matrix and the ones in the output matrix. To provide a visual geometrical representation, the output neurons were visualised as hexagonal cells in a two-dimensional space, with r rows and c columns (where rc=r×c). Sites with a similar species composition were clustered in the same output cell. In the training process, each output column vector (neuron) of the codebook matrix was moved from the initial configuration to a new position so that the sum of the distances between the input and output neurons was minimised. The iteration process began moving the codebook neuron having the lowest distance to an input neuron toward the input neuron itself, and continued moving all the other codebook neurons with the aim of minimising the global distance.

In this sense, the method was similar to nonmetric multidimensional scaling and to other multivariate ordination methods (Legendre and Legendre, 2012). The distribution according to macroinvertebrate assemblages and frequencies of the stations, the habitats and rice fields were plotted in different SOM maps. Each hexagon represented one of the map units [rc (r×c)=48 (6×8)]. Each element of the output neuron was a cluster of sites, and the values of the output (codebook) matrix quantify the frequencies of each species in the same cluster.

To measure the distance between units of SOM, the Unified distance matrix (U-matrix) was then used. This is a representation of the distances between the sites depicted in a grey scale on a 2-D image. The distance between the adjacent neurons was calculated and presented with different colours between the adjacent nodes: white areas represented compact clusters, while darker areas the gap between different clusters.

The measured environmental variables were subsequently introduced into the SOM map in the attempt to provide a visual representation of the value of each environmental variable in each clustered site.

The species with the highest codebooks and the ones of particular interest (rare, vulnerable, endangered, invasive) were then selected and plotted separately in different maps which could be superimposed to the station and the habitat distribution maps.

Co-inertia

Co-inertia analysis is a symmetrical approach which associates an environmental variable with a faunistic data set (the taxa) and extracts a simpler data structure from both sets, which are well correlated (Borcard *et al.*, 2011; Legendre and Legendre, 2012). All the taxa detected were included in the co-inertia analysis to relate them with the 9 environmental variables. The between-class analysis (BCA) (Dolédec and Chessel, 1994) was then applied to investigate differences among habitats in the different stations. The BCA is a co-inertia analysis with instrumental variables. In the present case, the instrumental variable was a factor with 10 levels: the habitats in the different stations. First, a correspondence analysis was carried out on the fitted variables of interest [the log(x+1)-transformed species matrix] after the regression on the instrumental variable; then, a principal component analysis (PCA) was carried out on the fitted environmental variables after the regression on the same instrumental variable. The results of the two analysis were entered in a new co-inertia analysis (Dray *et al.*, 2007). The BCA also allowed to have a joint plot of the instrumental variable: the habitats in the different stations could be represented by an arrow joining the score derived from the environmental set with the score derived by the biological set; a short arrow meant a good agreement between the two sets. The BCA was preferred to other constrained ordination methods (as redundancy analysis), because it allows to plot the instrumental variable in the plane of the principal axes directly.

The R Project for Statistical Computing® (Version 2.15.1) was used to calculate Diversity, IndVal and co-inertia. A toolbook of Matlab® (Version R2012A) modified (Lencioni *et al.*, 2007) was used to perform the SOM calculation.

RESULTS

The 173 taxa captured belong to 4 phyla (Mollusca, Annelida, Nematomorpha, and Arthropoda), 8 classes (Bivalvia, Gastropoda, Oligochaeta, Hirudinea, Gordioida, Insecta, Branchiopoda, and Malacostraca), 24 orders, 68 families, and 127 genera (Tabs. 2, 3, 4, and 5).

Species belonging to different ecological niches were identified. Among Oligochaeta, the abundance of Tubificidae *Limnodrilus hoffmeisteri* Claparède, 1862 is noteworthy as this species is generally tolerant to low levels of oxygen and organic pollution (Martins *et al.*, 2008). Five families and 6 species of Gastropoda were detected. Among them, the species *Bithynia tentaculata* (Linnaeus, 1758), *Gyraulus albus* (Müller, 1774) and *Planorbis planorbis* (Linnaeus, 1758), considered good indicators of water quality (Dussart, 1979), were very common and widespread. *Valvata piscinalis* (Müller, 1774) is a relatively tolerant organism, and generally lives in slow or steady waters, mildly polluted (Kalyoncu *et al*., 2008). Among insects, Odonata, Hemiptera, Coleoptera, and Diptera were well represented: Odonata with 15 species belonging to 7 families, Hemiptera with 10 species from 6 different families, and Coleoptera with 30 species from 8 families. Among Diptera, 15 families were detected. The richest was the family of Chironomidae, with 62 species, including 8 Tanypodinae, 25 Orthocladiinae, 8 Tanytarsini, and 17 Chironomini. Except for the predators Tanypodinae, *Cardiocladius fuscus* Kieffer, 1924 and *Cryptochironomus defectus* (Kieffer, 1913), all the other species were grazers (mostly Orthocladiinae), or detritus or filterer feeders (mostly Chironominae). Among Insecta, endangered, vulnerable and rare species were also detected: the Odonata *Gomphus flavipes* (Charpentier, 1825) and *Ophiogomphus cecilia* (Fourcroy, 1785) are endangered species (European Commission, 2006; IUCN, 2012); the Coleoptera Hydrophilidae *Berosus frotifoveatus* Kuwert, 1888*, Hydrophilus piceus* (Linnaeus, 1758) and *Hydrochara caraboides* (Linnaeus, 1758) are vulnerable species (Ruffo and Stoch, 2005); the Hemiptera *Gerris lateralis* Schummel, 1832, the Coleoptera *Hydaticus grammicus* (Germar, 1830) are rare species (Ruffo and Stoch, 2005). The Diptera *Odontomesa fulva* (Kieffer, 1919), *Diamesa tonsa* (Haliday, 1856), and *Sympotthastia spinifera* Serra-Tosio, 1969 are rare species in lowlands (Ferrarese and Rossaro, 1981). All these species have been detected in channels, while only two Hydrophilidae – the predator *H. piceus* and the vegetarian *B. frontifoveatus* – were detected in both rice fields and channels. *Berosus frontifoveatus* was detected in all stations at different sampling dates, while *H. piceus* was occasionally found only in few.

Few species of Trichoptera (5) and Ephemeroptera (4) were collected. Among Trichoptera, specimens in the family of Hydropsychidae and Limnephilidae are considered good indicators of water quality in natural ponds and rivers (Higler and Tolkamp, 1983; Briers and Biggs, 2003), but here, only the first was abundantly represented by the species *Hydropsyche pellucidula* (Curtis, 1834), which were found in channels and rarely in rice fields. Among Ephemeroptera, the family of Baetidae and Caenidae are also considered bioindicators in wetlands (Menetrey *et al.*, 2008), but they were poorly detected in the investigated sites.

Some invasive species were identified as well: the Coleoptera *Lissorhoptrus oryzophilus* Kuschel, 1952 was detected in all sites in both rice fields and channels, the Decapoda *Procambarus clarkii* (Girard, 1852) and the Bivalvia *Corbicula fluminalis* (Müller, 1774), was collected only in channels.

In rice paddies, 89 of the 173 taxa sampled have been found. Precisely: 1 Bivalvia, 5 Gastropoda, 6 Oligochaeta, 2 Hirudinea, 74 Insecta (2 Ephemeroptera, 5 Hemiptera, 3 Odonata, 19 Coleoptera, 43 Diptera, 2 Trichoptera) and 1 Malacostraca.

Diversity

The diversity indices were significantly different with respect to all the factors considered (stations, habitats, and months), with the exception of the number of species between habitats (Tab. 6). The ANOVA results in Tab. 6 show

Tab. 2. List of the taxa found (excluding Insecta).

Class	Order	Family	Species
Bivalvia	Unionoida	Unionidae	*Unio pictorum* (Linnaeus, 1758)
	Veneroidea	Curbiculidae	*Corbicula fluminalis* (Müller, 1774)
		Sphaeriidae	*Musculium lacustre* (Müller, 1774)
			Pisidium casertanum (Poli, 1791)
			Sphaerium corneum (Linnaeus, 1758)
Gastropoda	Ectobranchia	Valvatidae	*Valvata piscinalis* (Müller, 1774)
	Neotaenioglossa	Bithyniidae	*Bithynia tentaculata* (Linnaeus, 1758)
	Pulmonata	Lymnaeidae	*Lymnaea stagnalis* (Linnaeus, 1758)
		Physidae	*Physa fontinalis* (Linnaeus, 1758)
		Planorbidae	*Gyraulus albus* (Müller, 1774)
			Planorbis planorbis (Linnaeus, 1758)
Oligochaeta	Haplotaxida	Haplotaxidae	*Haplotaxis gordioides* (Hartmann, 1821)
	Lumbriculida	Lumbriculidae	*Rhynchelmis limosella* Hoffmeister, 1843
			Stylodrilus heringianus Claparède, 1862
	Opisthopora	Lumbricidae	*Eiseniella tetraedra* (Savigny, 1826)
	Tubificida	Naididae	*Nais communis* Piguet, 1906
			Ophidonais serpentina (Müller, 1774)
		Tubificidae	*Branchiura sowerbyi* Beddard, 1892
			Limnodrilus claparedianus Ratzel, 1869
			Limnodrilus hoffmeisteri Claparède, 1862
			Psammoryctides barbatus (Grube, 1861)
			Tubifex tubifex (Müller, 1774)
Hirudinea	Arhynchobdellida	Erpobdellidae	*Erpobdella octoculata* (Linnaeus, 1758)
	Rhyncobdellida	Glossiphoniidae	*Alboglossiphonia heteroclita* (Linnaeus, 1761)
			Glossiphonia complanata (Linnaeus, 1758)
Gordioida	Gordea	Gordiidae	*Gordius tirolensis* Heinze, 1937
Branchiopoda	Diplostraca	Cyzicidae	*Cyzicus tetracerus* (Krynicki, 1830)
		Limnadiidae	*Limnadia lenticularis* (Linnaeus, 1761)
	Notostraca	Triopsidae	*Triops cancriformis* (Bosc, 1801)
Malacostraca	Amphipoda	Gammaridae	*Echinogammarus stammeri* (Karaman, 1931)
	Decapoda	Cambaridae	*Procambarus clarkii* (Girard, 1852)
	Isopoda	Asellidae	*Asellus aquaticus* (Linnaeus, 1758)

that the habitat is the poorest predictor of the diversity indices. The most significant differences were among months, with the highest diversity indices found in March, April and September (Tab. 6 and Fig. 1a). The number of species and the Shannon diversity index were highest in Station 4, where the feeding channel (C4) contributed more to diversity than the rice field itself (R4) (Figs. 1b and 1c). The same analysis applied to rice fields subject to different management strategies highlighted that the lowest diversity was observed in Station 5, one of the two stations with the shorter period of flooding. Diversity was always higher in feeding channels than in rice fields (Figs. 1c and 1d).

Indicator values

Using the 10 habitats in the different stations as factor, the IndVal analysis allowed to detect 18 taxa (Tab. 7). At least one indicator species was detected in each station. The analysis pointed out that all the channels have at least one species with $P<0.05$. As far as rice paddies are concerned, R1 was characterised by *Triops cancriformis* (Bosc, 1801), *Enochrus quadripunctatus* (Herbst, 1797), *Chironomus plumosus* (Linnaeus, 1758), *Alboglossiphonia heteroclita* (Linnaeus, 1761), *Serratella ignita* (Poda, 1761), R3 by *Sigara lateralis* (Leach, 1817), *Orthetrum albistylum* (Sélys, 1848), *G. albus*, *Helochares lividus* (Forster, 1771), Stratiomyidae and Ephydridae, and R5 by *Cryptochironomus defectus* (Kieffer, 1913) (Tab. 7). No indicator species were found in R2 and R4. Only the species *Microtendipes pedellus* (De Geer, 1776) was indicator of the spring (S6).

Tab. 3. List of the species of insects detected (excluding Diptera and Coleoptera).

Class	Order	Family	Species
Insecta	Ephemeroptera	Baetidae	*Baetis rhodani* (Pictet, 1843)
		Caenidae	*Caenis horaria* (Linnaeus, 1758)
		Ephemerellidae	*Serratella ignita* (Poda, 1761)
		Oligoneuriidae	*Oligoneuriella rhenana* (Imhoff, 1852)
	Odonata	Aeshnidae	*Aeshna cyanea* (Müller, 1764)
		Calopterygidae	*Calopteryx splendens* (Harris, 1782)
		Coenagrioniidae	*Coenagrion puella* (Linnaeus, 1758) *Ischnura elegans* (Vander Linden, 1820)
		Gomphidae	*Gomphus flavipes* (Charpentier, 1825) *Gomphus vulgatissimus* (Linnaeus, 1758) *Onychogomphus uncatus* (Charpentier, 1840) *Ophiogomphus cecilia* (Fourcroy, 1785)
		Lestidae	*Lestes sponsa* (Hansemann, 1823)
		Libellulidae	*Libellula quadrimaculata* Linnaeus, 1758 *Orthetrum albistylum* (Sélys, 1848) *Orthetrum cancellatum* (Linnaeus, 1758) *Orthetrum coerulescens* (Fabricius, 1798) *Sympetrum pedemontanum* (Allioni, 1766)
		Platycnemididae	*Platycnemis pennipes* (Pallas, 1771)
	Hemiptera	Corixidae	*Sigara dorsalis* (Leach, 1817) *Sigara italica* Jaczewski, 1933 *Sigara lateralis* (Leach 1817)
		Gerridae	*Aquarius najas* (De Geer, 1773) *Aquarius paludum* (Fabricius, 1794) *Gerris lateralis* Schummel, 1832
		Hydrometridae	*Hydrometra stagnorum* (Linnaeus, 1758)
		Naucoridae	*Ilyocoris cimicoides* (Linnaeus, 1758)
		Nepidae	*Nepa cinerea* Linnaeus, 1758
		Notonectidae	*Notonecta maculata* Fabricius, 1794
	Trichoptera	Hydropsychidae	*Hydropsyche pellucidula* (Curtis, 1834)
		Hydroptilidae	*Hydroptila aegyptia* Ulmer, 1963
		Lepidostomatidae	*Lepidostoma hirtum* (Fabricius, 1775)
		Leptoceridae	*Ceraclea dissimilis* (Stephens, 1836)
		Limnephilidae	*Limnephilus flavicornis* (Fabricius, 1787)
	Lepidoptera	Crambidae	*Cataclysta lemnata* (Linnaeus, 1758)

Self-organising map

The SOM analysis allowed to detect major differences among stations (Fig. 2a) and habitats (Fig. 2b). The analysis of the different rice management strategies allocated only the rice field characterised by 11 months of submersion (R4) in a separated cluster (Fig. 2c). The U-matrix (Fig. 3a) shows that well separated clusters of stations cannot be defined according to species distribution.

In Fig. 4, the 12 species with the highest codebook are presented with their distribution in the SOM map. Most species were mapped in the bottom left part of the map evidencing a preference for channels (Fig. 2b) characterised by a larger granulometry (Fig. 3b). Among them, *Cricotopus sylvestris* (Fabricius, 1794)*, Calopteryx splendens* (Harris, 1782)*, H. pellucidula, Lymnaea stagnalis* (Linnaeus, 1758)*, Eiseniella tetraedra* (Savigny, 1826)*, L. hoffmeisteri, B. tentaculata, Chironomus riparius* Meigen, 1804 were found (Fig. 4). Interestingly, *C. riparius*, *B. tentaculata*, *L. hoffmeisteri* and *E. tetraedra* were detected in stations with high total phosphorus (TP) concentration (Fig. 3c).

Hydroglyphus geminus (Fabricius, 1792) and *Sigara dorsalis* (Leach, 1817), were most represented in a cluster of sites in the bottom right side of the SOM map; this cluster included both rice ponds and channels with lower granulometry and temperature (Figs. 3b and 3c).

Gyraulus albus and *Physa fontinalis* were mapped in the bottom part of the SOM map where both rice paddies and channels were clustered, regardless of the environmental variables and the agronomic management.

In Fig. 5, endangered, vulnerable, rare and invasive species are given. The endangered species *G. flavipes* and *O. cecilia* were respectively mapped in the bottom left and in the central right. Both areas were representative of channels but were characterised by a different granulometry. Among vulnerable species, *H. caraboides* was mapped in the higher part of the SOM map showing a clear preference for the spring. The rare *G. lateralis* and *H. grammicus* were clustered in the bottom left part of the SOM map with a clear preference for channels (Figs. 2, 3 and 5). The invasive species *L. oryzophilus* was mapped in different areas corresponding to channels and rice fields regardless of the management strategies adopted and the environmental variables. *C. fluminalis* was mapped in the

Tab. 4. List of the species of Coleoptera detected.

Class	Order	Family	Species
Insecta	Coleoptera	Brachyceridae	*Lissorhoptrus oryzophilus* Kuschel, 1952
		Dryopidae	*Dryops luridus* (Erichson, 1847)
		Dytiscidae	*Hydaticus grammicus* (Germar, 1830) *Hydroglyphus geminus* (Fabricius, 1792) *Hydroporus marginatus* (Duftschmid, 1805) *Hydrovatus cuspidatus* Kunze, 1818 *Hygrotus impressopunctatus* (Schaller, 1783) *Hygrotus inaequalis* (Fabricius, 1776) *Laccophilus hyalinus* De Geer, 1774 *Laccophilus minutus* (Linnaeus, 1758) *Laccophilus poecilus* Klug, 1834 *Rhantus suturalis* (MacLeay, 1825)
		Elmidae	*Elmis maugetii* Latreille, 1798 *Limnius volckmari* (Panzer, 1793)
		Gyrinidae	*Aulonogyrus concinnus* (Klug, 1834)
		Haliplidae	*Haliplus fulvus* (Fabricius, 1801) *Haliplus laminatus* (Schaller, 1783) *Haliplus lineaticollis* (Marsham, 1802) *Haliplus flavicollis* Sturm, 1834 *Haliplus heydeni* Wehncke, 1875 *Peltodytes caesus* (Duftschmid, 1805)
		Helophoridae	*Helophorus brevipalpis* Bedel, 1881
		Hydrophilidae	*Berosus signaticollis* Charpentier, 1825 *Berosus frontifoveatus* Kuwert, 1888 *Enochrus melanocephalus* (Olivier, 1792) *Enochrus quadripunctatus* (Herbst, 1797) *Helochares lividus* (Forster, 1771) *Hydrochara caraboides* (Linnaeus, 1758) *Hydrophilus piceus* (Linnaeus, 1758) *Laccobius minutus* (Linnaeus, 1758)

Tab. 5. List of the Diptera detected.

Class	Order	Family	Subfamily	Tribe	Species
Insecta	Diptera	Ceratopogonidae			
		Chironomidae			
			Tanypodinae		
				Tanypini	*Tanypus punctipennis* Meigen, 1818
				Procladini	*Procladius choreus* (Meigen, 1804)
				Pentaneurini	*Ablabesmyia longistyla* Fittkau, 1962
					Ablabesmyia monilis (Linnaeus, 1758)
					Arctopelopia griseipennis (van der Wulp, 1858)
					Conchapelopia pallidula (Meigen, 1818)
					Thienemannimyia carnea (Fabricius, 1805)
					Zavrelimyia punctatissima (Goetghebuer, 1934)
			Diamesinae		*Diamesa tonsa* (Haliday, 1856)
					Sympotthastia spinifera Serra-Tosio, 1969
			Prodiamesinae		*Prodiamesa olivacea* (Meigen, 1818)
					Odontomesa fulva (Kieffer, 1919)
			Orthocladiinae		*Brillia bifida* (Kieffer, 1909)
					Brillia longifurca Kieffer, 1921
					Cardiocladius fuscus Kieffer, 1924
					Corynoneura scutellata Winnertz, 1846
					Cricotopus annulator Goetghebuer, 1927
					Cricotopus bicinctus (Meigen, 1818)
					Cricotopus fuscus (Kieffer, 1909)
					Cricotopus trifascia Edwards, 1929
					Cricotopus sylvestris (Fabricius, 1794)
					Eukiefferiella claripennis (Lundbeck, 1898)
					Limnophyes minimus (Meigen, 1818)
					Orthocladius luteipes Goetghebuer, 1938
					Orthocladius rivicola Kieffer, 1911
					Orthocladius thienemanni Kieffer, 1906
					Orthocladius excavatus Brundin, 1947
					Orthocladius oblidens (Walker, 1856)
					Orthocladius rhyacobius Kieffer, 1911
					Orthocladius rubicundus (Meigen, 1818)
					Paracladius conversus (Walker, 1856)
					Parametriocnemus stylatus (Spaerck, 1923)
					Paratrichocladius rufiventris (Meigen, 1830)
					Psectrocladius sp. Kieffer, 1906
					Rheocricotopus chalybeatus (Edwards, 1929)
					Rheocricotopus fuscipes (Kieffer, 1909)
					Smittia pratorum (Goetghebuer, 1927)
			Chironominae		
				Tanytarsini	*Cladotanytarsus atridorsum* Kieffer, 1924
					Micropsectra atrofasciata (Kieffer, 1911)
					Paratanytarsus lauterborni (Kieffer, 1909)
					Tanytarsus brundini Lindeberg, 1963
					Tanytarsus ejuncidus (Walker, 1856)
					Tanytarsus eminulus (Walker, 1856)
					Tanytarsus volgensis Miseiko, 1967
					Virgatanytarsus triangularis (Goetghebuer, 1928)
			Chironomini		*Chironomus dorsalis* Meigen, 1818
					Chironomus plumosus (Linnaeus, 1758)
					Chironomus riparius Meigen, 1804
					Cryptochironomus defectus (Kieffer, 1913)
					Dicrotendipes nervosus (Staeger, 1839)
					Endochironomus tendens (Fabricius, 1775)
					Harnischia fuscimanus Kieffer, 1921
					Microtendipes pedellus (De Geer, 1776)
					Paratendipes albimanus (Meigen, 1818)
					Pentapedilum tritum (Walker, 1856)
					Phaenopsectra flavipes (Meigen, 1818)

To be continued on next page.

Tab. 5. Continued from previous page.

Class	Order	Family	Subfamily	Tribe	Species
			Chironomini		*Polypedilum convictum* (Walker, 1856)
					Polypedilum laetum (Meigen, 1818)
					Polypedilum nubeculosum (Meigen, 1804)
					Polypedilum scalaenum (Schrank, 1803)
					Stenochironomus ranzii Rossaro, 1982
					Synendotendipes impar (Walker, 1856)
Insecta	Diptera	Culicidae			
		Dolichopodidae			
		Ephydridae			
		Limoniidae			
		Muscidae			
		Psychodidae			
		Rhagionidae			
		Sciomyzidae			
		Simuliidae			
		Stratiomyidae			
		Syrphidae			
		Tabanidae			
		Tipulidae			

Tab. 6. Analysis of variance carried out on Shannon diversity index and the number of species found.

Shannon	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Stations	5	5.607	1.121	3.214	0.0106	*
Habitats	2	2.076	2.076	5.949	0.0169	*
Months	11	9.683	0.880	2.523	0.0086	**
Number of species	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	
Stations	5	1183	236.56	4.104	0.0022	**
Habitats	2	158	158.17	2.744	0.1014	
Months	11	2566	233.27	4.047	0.0001	***

Df, degree of freedom; Sum Sq, sum of squares; Mean Sq, mean square.

Tab. 7. Indicator species with the higher indicator value using habitats as factor.

Species	IndVal	Station/Habitat	P
L. hoffmeisteri	0.449	C1	0.026
T. cancriformis	0.490	R1	0.006
E. quadripunctatus	0.511	R1	0.008
C. plumosus	0.432	R1	0.013
A. heteroclita	0.433	R1	0.014
H. pellucidula	0.510	C2	0.015
Simuliidae	0.421	C2	0.022
L. stagnalis	0.450	C3	0.018
S. lateralis	0.518	R3	0.009
Stratiomyidae	0.390	R3	0.012
O. albistylum	0.421	R3	0.021
Ephydridae	0.438	R3	0.027
G. albus	0.355	R3	0.035
H. lividus	0.274	R3	0.036
E. stammeri	0.330	C4	0.022
V. piscinalis	0.423	C4	0.027
C. defectus	0.148	R5	0.042
M. pedellus	0.237	S6	0.013

IndVal, indicator values; C1, channel 1; R1, rice field 1; C2, channel 2; C3, channel 3; R3, rice field 3; C4, channel 4; R5, rice field 5; S6, spring 6. Only species with P<0.05 are listed.

upper part of the SOM map with channels and spring prevailing and *C. clarkii* mostly in the bottom left part of the SOM map where channels were represented.

Co-inertia

The results of co-inertia analysis highlighted a good agreement between the two sets of variables, as emphasised by their high correlations (Tab. 8). The scatter plot of the BCA results is provided in Fig. 6. According to the eigenvalues barplot, more than 70% of the variance of the co-inertia analysis was accounted for by the first two co-inertia axes and thus presented a good initial summary of the co-structure between the two datasets. In the plot of X canonical weights (environmental variables), the first axis separated sites with high dissolved oxygen from those with high conductivity, hardness, and alkalinity; the second axis separated sites with high temperature, granulometry, and TP from those with high nitrate and pH. The result is that differently managed rice fields were characterised by different environmental variables and species: low oxygen content and high TP characterised rice fields (R1) and channels (C1 and C3); high granulometry and water temperature typified the channel C2; high conductivity and alkalinity characterised rice fields R3, R4 and S6; and high oxygen and nitrate content identified R2 and C4. A joint plot of sites scores, calculated starting from both environmental and species variables, emphasises the good agreement between the 2 sets, which is especially evident in C1 and C4.

In the plot of Y canonical weights (species), some species were able to characterise different habitats: in the area with high TP concentration, *L. hoffmeisteri* characterised channel C1; *Peltodytes caesus* (Duftschmid, 1805) channel C3; *C. plumosus* and *T. cancriformis* rice field R1. In the area with high conductivity and alkalinity, *B. frontifoveatus* and Ephydridae were characteristic of R3; *Hydrometra stagnorum* (Linnaeus, 1758), *Aulonogyrus concinnus* (Klug, 1834), *H. caraboides* and *M. pedellus* were typical of the spring. In the area with high oxygen and nitrate content (C4), there were 15 taxa superimposed: among them, *Lestes sponsa* (Hansemann, 1823), *Aquarius najas* (De Geer, 1773), *Haliplus laminatus* (Schaller, 1783), *Cataclista lemnata* (Linnaeus, 1758), *Limnadia lenticularis* (Linnaeus, 1761), Rhagionidae, and 9 Chironomidae with the rare species *D. tonsa* and *S. spinifera*. The remaining species were not separated in specific clusters as they were not correlated with the environmental variables and consequently with the different habitats. As a result, they were allocated in the central part of the plot. Among them, the invasive and the most abundantly detected species were present.

DISCUSSION

The current study highlighted the high species richness of rice agroecosystems in agreement with Della Bella *et al*. (2005) on the conservation status of natural temporary ponds in Italy. The number of species found (173) was high if we consider that the ecosystems examined are subject to intense anthropogenic impact, and this emphasises the importance of rice cultivation areas in supporting wetland conservation. Water in paddies and channels favours the development of species competitive to those damaging rice; these competitors belong to different ecological niches: phytophagous, predators, saprophagous, and phytosaprophagous.

In rice paddies the number of species found (89) is similar to the results obtained in other researches in Europe (Portugal) and in Asia (Sri Lanka) (Bambaradeniya

Tab. 8. Co-inertia analysis results: eigenvalue decomposition of the matrix of co-inertia, eigenvalues, covariance, and standard deviation of the two sets of sites scores on the co-inertia axes and correlations between the two sets of site scores. The inertia of the cumulated projections of the X and Y tables as projected in the co-inertia analysis (CoIA) compared with the maximum inertia of the axes of the separate ordinations, the ratio of these values measures the concordance between the 2 projections.

		Eig	Covar	sdX	sdY	Corr	
	1	0.393	0.627	0.489	1.619	0.792	
	2	0.345	0.588	0.581	1.230	0.823	
	3	0.209	0.457	0.553	1.063	0.778	
	4	0.136	0.369	0.490	1.051	0.716	
X	Inertia	Max	Ratio	Y	Inertia	Max	Ratio
1	0.239	0.581	0.412	1	2.622	2.746	0.955
12	0.576	1.150	0.501	12	4.134	4.413	0.937
123	0.882	1.641	0.538	123	5.264	5.610	0.938
1234	1.122	2.119	0.530	1234	6.369	6.481	0.983

Eig, eigenvalues; Covar, covariance; sdX, standard deviation of the environmental variables of sites scores on the co-inertia axes X; sdY, standard deviation of the species of sites scores on the co-inertia axes Y; Corr, correlations between the two sets of site scores; X, X axes; Y, Y axes; Max, maximum inertia of the axes of the separate ordinations.

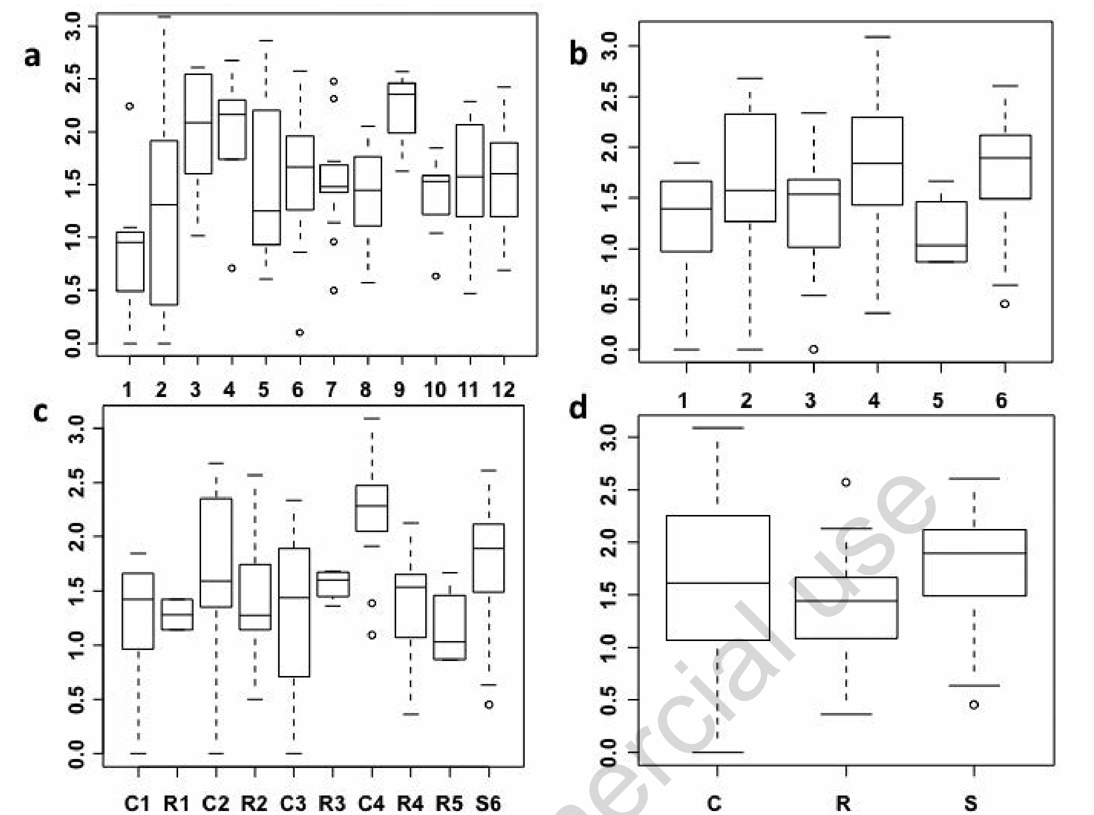

Fig. 1. Box and whisker plots for the Shannon diversity index. Grouping factors are: a) months, b) stations, c) habitats/stations, and d) habitats. [The box lines are median values, while the box ends are quartiles; whiskers show the ranges (non-outliers within an interval of 1.5×height of the box), and the circles indicate outliers].

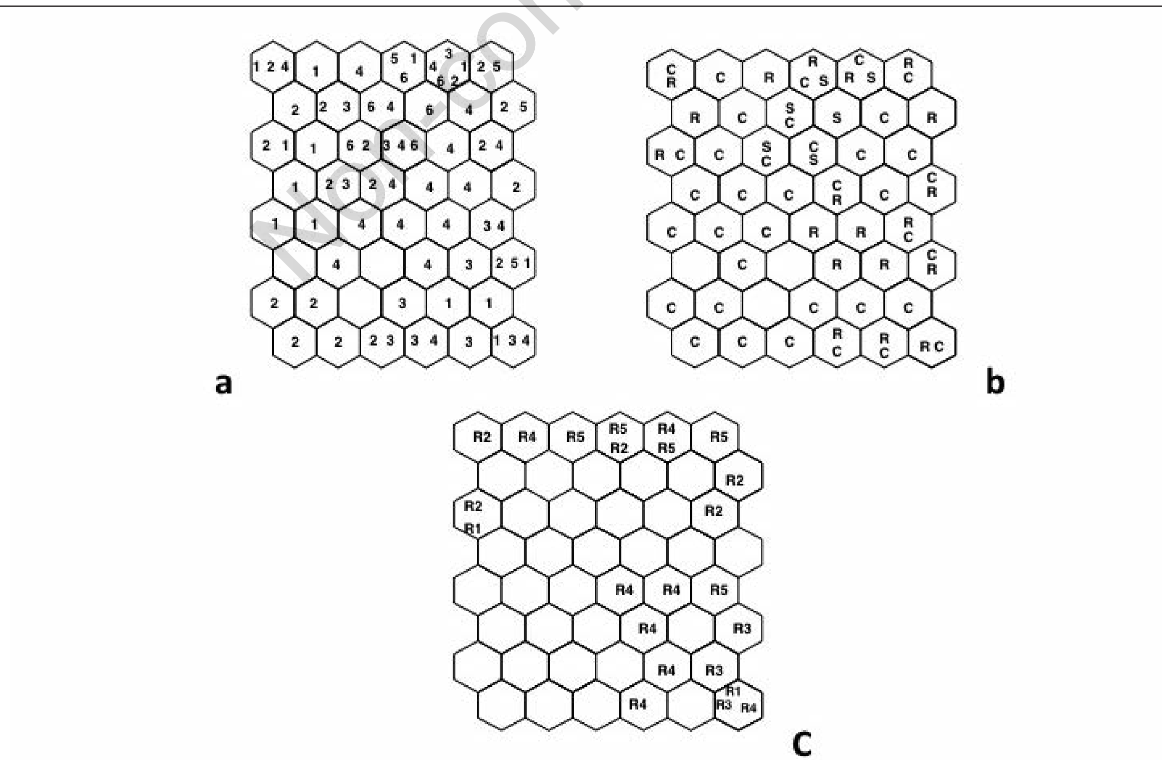

Fig. 2. Self-organising maps (SOMs): a) six stations; b) rice fields and channels; c) rice fields in different habitats.

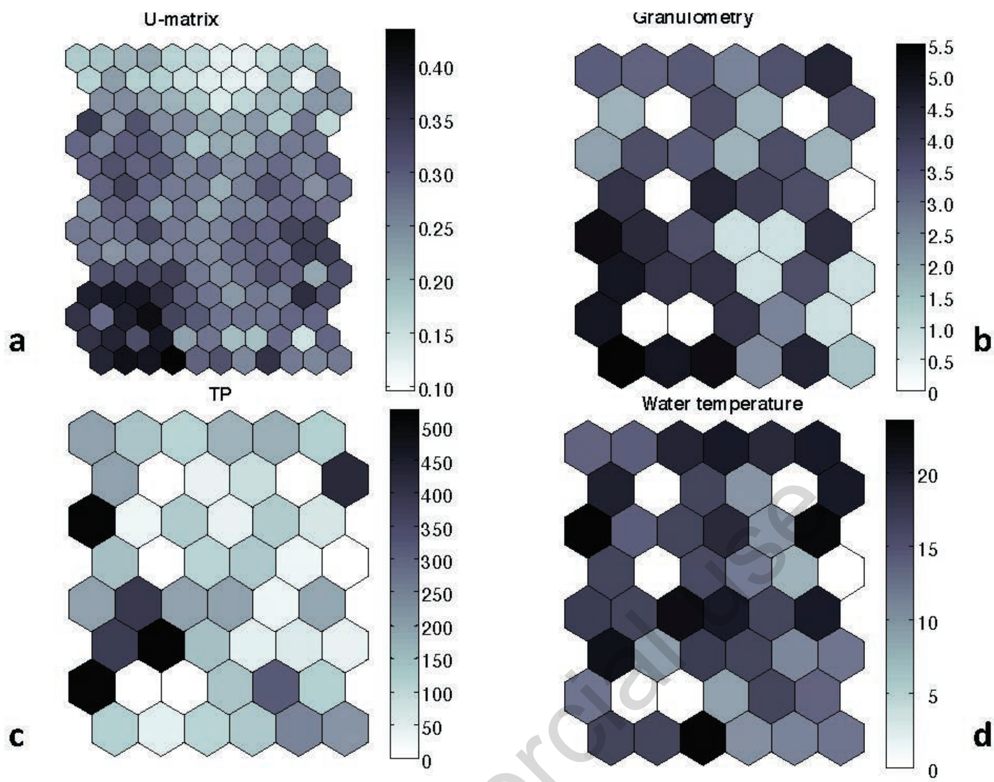

Fig. 3. Self-organising maps (SOMs): a) U-matrix (the scale bar on the right side is the distance between clusters); b) granulometry; c) total phosphorus (TP); d) water temperature with visualisation in shading scale (dark=high value, light=low value). The scale bars on the right side of each map show the value of the environmental variable.

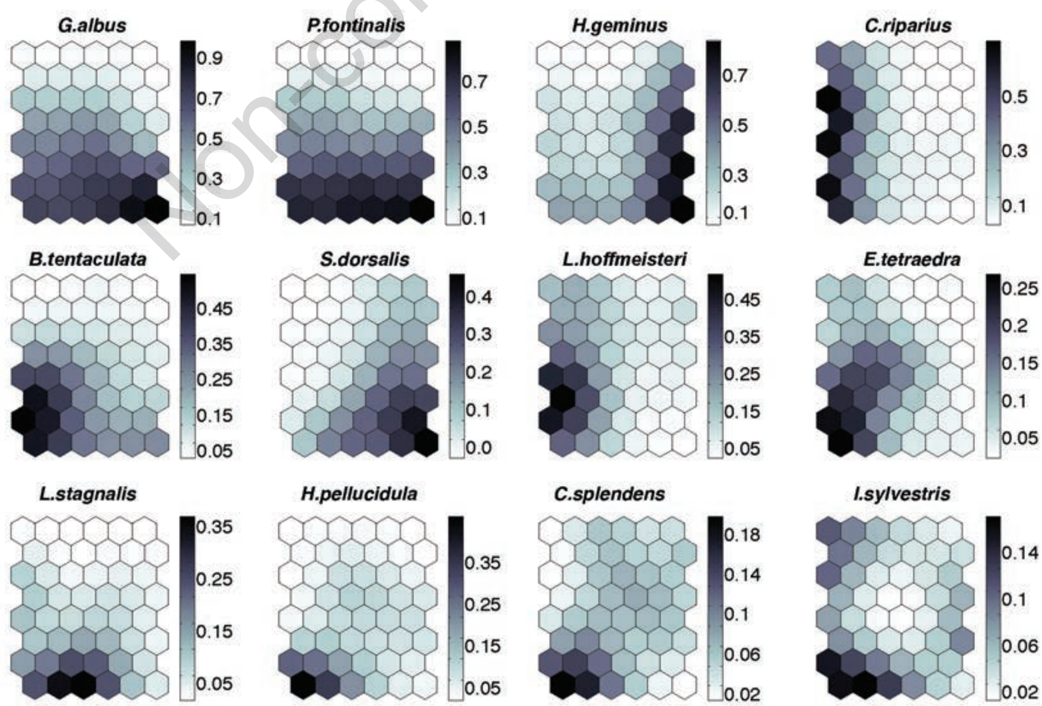

Fig. 4. Self-organising maps (SOMs) of the 12 species with the highest codebook, with visualisation in shading scale (dark=high codebook values, light=low codebook values). The scale bars on the right side of each map show the coded abundance of each taxon.

et al., 2004; Leitão *et al*., 2007). More than the half of the species of Gastropoda and Coleoptera and at least half of the species of Oligochaeta, Hirudinea, Ephemeroptera, Hemiptera, Diptera and Trichoptera have been detected. Among them, some Coleoptera (Dytiscidae, Haliplidae, and Hydrophilidae) and many Oligochaeta are known as very resistant taxa (Lafont, 1984; Smith and Golladay, 2011). Despite showing low resistance, different taxa show high resilience recovering rapidly from different disturbances; among them, Baetidae and many Chironomidae (Viera *et al.*, 2004). Species with longer cycle like some dragonflies were localised mostly in channels. Dragonflies such as *C. splendens* were found in rice fields, but as they develop over two years and overwinter buried in mud it is doubtful whether they can complete their development in this habitat. No Odonata was captured in rice paddies with shorter water period (Stations: R1 and R5). On the contrary, rice fields resulted to be valuable places for small water beetles able to fly in channels when the condition in paddies were unfavourable (*e.g., H. geminus* and *Laccophilus* spp.).

The detection of rare species, with no apparent contribution to community stability or ecosystem functioning, draws attention to the role these environments play in biodiversity conservation, emphasising the value of less abundant species which may be at higher risk of extinction. Conversely, the presence of exotic invasive species such as the rice water weevil *L. oryzophilus* and *P. clarkii* may become a serious threat for biodiversity (Jucker and Lupi, 2011). Most importantly, in the study period no insecticide was registered for the control of the rice water weevil, but further researches are needed to evaluate the impact on biodiversity of either agronomic or chemical treatments applied in the control of this pest.

The interaction of the different methods of data analysis allowed a more comprehensive view on the value of the components of rice agroecosystems. All the data analyses highlighted significant differences between habitats (feeding channel and rice field), with higher diversity observed in channels; this is in agreement with the well-known differences generally observed between fauna living in lentic and lotic habitats (Maroneze *et al.*, 2011). Biodiversity indices stressed a significant influence of the season, which is in line with the variation of the community composition during the year according to the life cycles of different species (Barbone *et al.*, 2012). According to management practices, lower biodiversity values were found in one of the rice fields characterised by a shorter

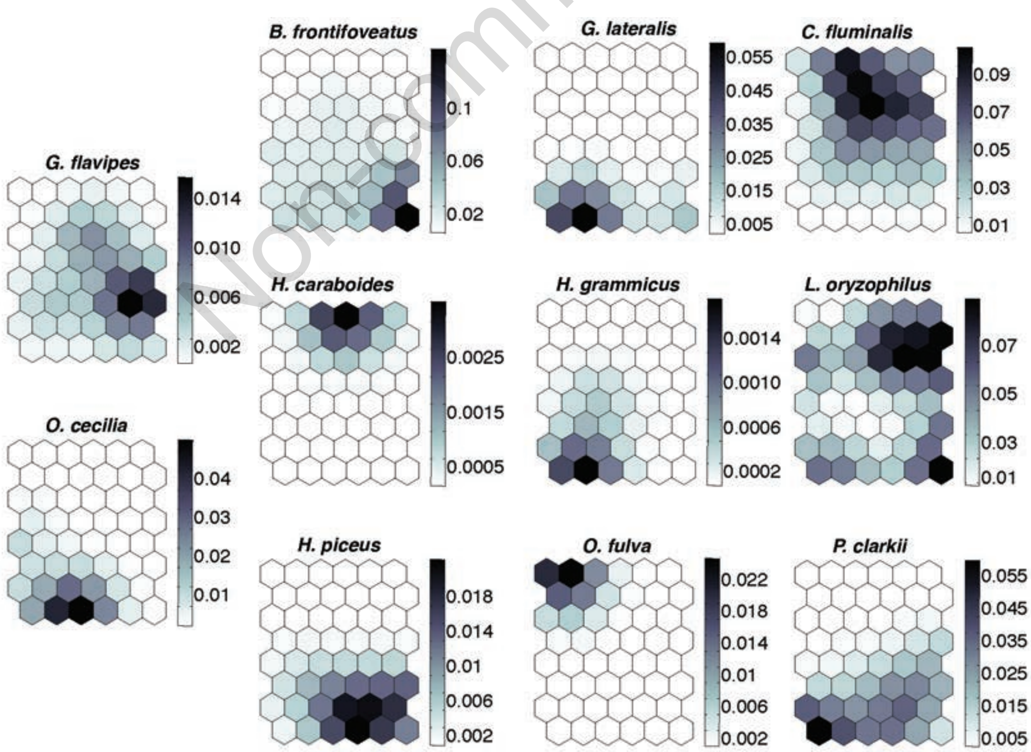

Fig. 5. Self-organising maps (SOMs) of, respectively, from the left to the right: endangered, vulnerable, rare and invasive species with visualisation in shading scale (dark=high codebook values, light=low codebook values). The scale bars on the right side of each map show the coded abundance of each taxon.

period of flooding (R5), underlining the role of water permanence on species biodiversity. Contrary to our expectations, the spring (S6), which was not subject to any impact bound to rice cultivation, gave biodiversity indices only a bit higher than in other agroecosystems (*e.g.* Station 4). These results point out that diversity may be useful to measure stress determined by rice-crop management, but it is a rough index not well suited to detect subtle impacts.

The IndVal analysis gave a somewhat more informative result, highlighting variations among stations and habitats. Channels were characterised by many indicator species which brought to light differences in the quality of the water channels. The indicator species *L. hoffmeisteri* present in C1 was indicator of bad quality of the water, as highlighted also by BCA; in fact, *H. pellucidula* and *Echinogammarus stammeri* (Karaman, 1931), found respectively in C2 and C4, were indicators of good water quality (Galli *et al.*, 2001). Few species resulted to be able to characterise rice fields. Among them, taxa with very short life cycles as Chironomidae, Ephydridae, and *T. cancriformis* resulted to be indicators. Two Hydrophilidae characterised different rice fields: *E. quadripunctatus*, typical of smooth, astatic water, and of an environment

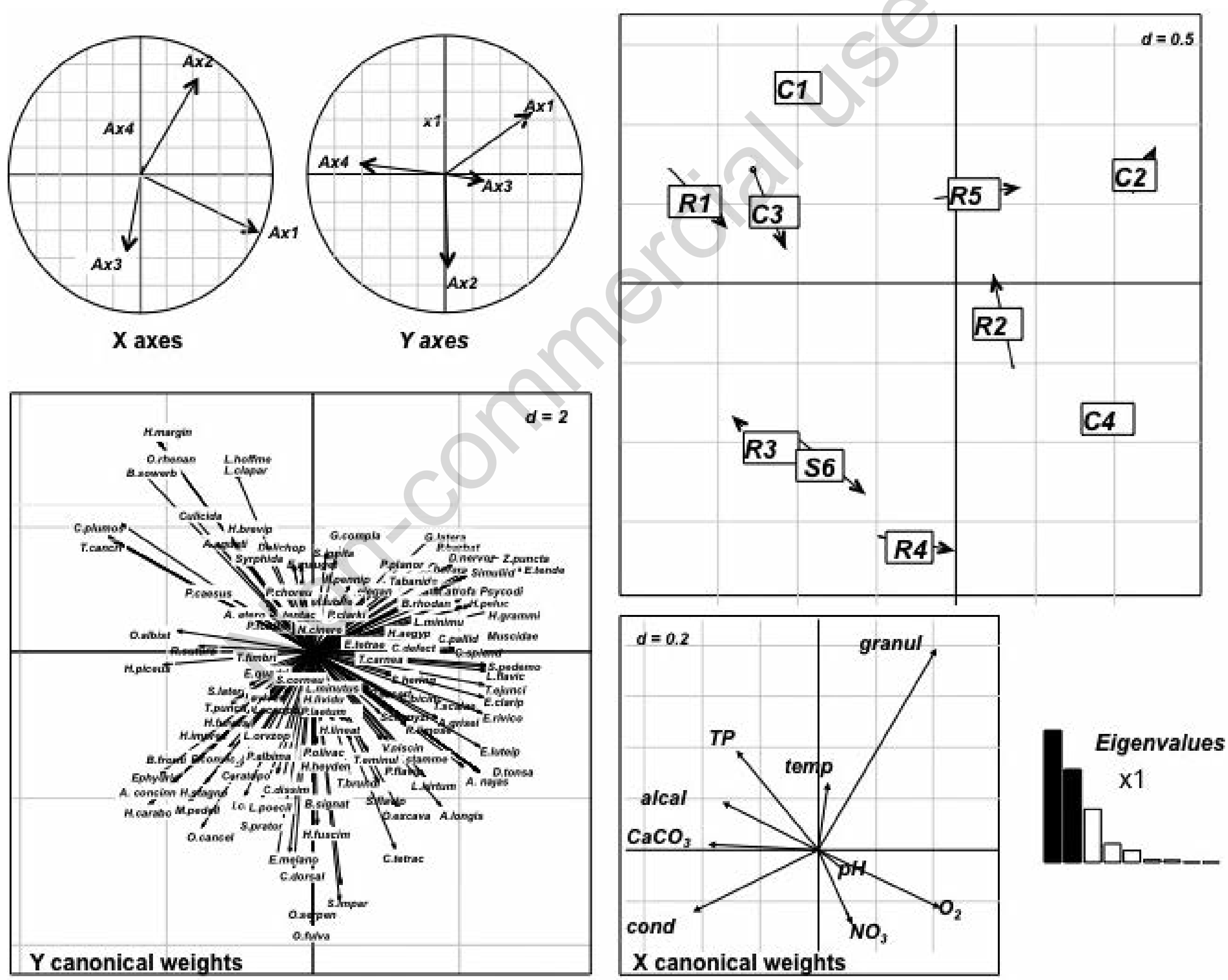

Fig. 6. Between class co-inertia analysis carried out with the habitats in the different stations as instrumental variables. The upper-left circular plots show the position of the principal component analysis (environmental variables) and CoA (species) axes with respect to the Co-inertia analysis (CoIA) axes. The lower-right barplot indicates the eigenvalues. The upper right-hand plot shows the position of the habitats in the different stations on the first two co-inertia axes using the environmental variables (origin of the arrows) and species (arrowheads) co-inertia weights (R=rice-fields; C=channels; S=spring). The lower central plot indicates the environmental variables' weights, and the lower left plot shows the species scores.

subject to quick variation of the hydrometric level and to total drainage (Ortmann-Ajkai and Kálmán, 2011); and *H. lividus* with a clear preference for waters with a low current velocity, high pH, high conductivity with a luxurious vegetation, clay- and sand-substrate (Cuppen, 1986). In the present study, *H. lividus* ecology is emphasised as the species is plotted in the BCA in a separate area representing a rice field characterised by high pH and conductivity. The species *O. albystilum,* which results indicator in R3, probably cannot complete its cycle as it overwinters as larva and cannot find the condition for its development in a habitat which is dry in colder seasons. Once again it is important to notice that the result of statistical analysis must be used with caution, if it cannot be related to the autoecology of the species.

The SOM described differences in fauna composition with a lot of detail, adding information on the ecology of the different species in the habitat considered. It also emphasised that the presence of rare/endangered species allows to characterise some stations, but it is less informative about management strategies in rice paddies because most of these species are absent in rice fields.

The BCA pointed out the role of environmental variables in separating the habitats in the different stations, and their importance in allowing the colonisation by some species.

Some Chironomidae resulted to be good indicators of different habitats. This conclusion was supported by statistical analysis and was in agreement with the knowledge about the ecology of the species (Rossaro, 1982; Pillot, 2009). The IndVal analysis showed that *M. pedellus* preferred S6 and *C. defectus* R5, while the BCA revealed that *O. fulva, Orthocladius excavatus* Brundin, 1947*, Paracladius conversus* (Walker, 1856)*, M. pedellus* and *Harnischia fuscimanus* Kieffer, 1921 preferred habitats with the lowest impact (R3, R4, C4, S6).

The present study confirmed the importance of different taxa of benthic macroinvertebrates in representing habitat quality in rice field wetlands. The different habitats chosen for the study, considered representative of Northern Italy rice agroecosystems, showed how these environments are very dynamic and complex, thus emphasising strict relations between benthic macroinvertebrates living in channels and rice fields. The period of water permanence in paddies resulted to be only one of the factors influencing the community of benthic macroinvertebrates, as evidenced in the analysis giving importance also to water chemical-physical parameters. These variables were influenced by human activities, but it is hard to separate the different sources of stress as they are strictly related. Although the natural ecosystem is in harmony with natural water quality, any significant changes in water will usually be disruptive to the ecosystem (Bartram and Ballance, 1996).

However, as arthropods play an important role in the structure and maintenance of the ecosystems status, the high number of taxa found in the present research in few rice agroecosystems indicates that more information is required about the distribution and the status of macroinvertebrates in similar environments in Italy. Once more information on the communities is available, these results will also be valuable for having a more comprehensive view and found proper indicator species and indices of rice agroecosystem conservation status.

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