Original Articles

Indicators of biodiversity in an intensively cultivated and heavily human modified landscape

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A B S T R A C T

Nowadays, the loss of biodiversity in agroecosystems due to the intensification of farming practices is happening very fast, and therefore, stopping or slowing it down should be a priority for conservation. To detect changes in these environmental contexts, one approach contemplates focusing on a limited set of indicator species that can alert us to ongoing changes in progress. In this research, we aimed to measure the biodiversity of vertebrates using a multi-taxa approach in an intensively cultivated and highly inhabited area located in northern Italy. We investigated the relationships between biodiversity and environmental characteristics and we identified the taxonomic groups that can be used as indicators of biodiversity. Data collection was carried out in 2016 with different methods depending on the taxonomic group, in 131 sampling units chosen using a Tessellation Stratified Sampling. Then we calculated for each sampling unit a standardized Biodiversity Index, which was related to environmental variables concerning the land use and the landscape configuration using Multiple Linear Regression Analysis and Information-Theoretic approach. We used correlation analyses and the Indicator Species Analysis (IndVal) to identify the taxonomic groups and species that can be used as indicators of biodiversity. Biodiversity was positively related to the number of patches of natural vegetation, whereas it was negatively affected by the number of patches of artificial surfaces and by habitat diversity. Our findings agree with those obtained by many other researchers, which pointed out that agroecosystems provide adequate shelters, suitable foraging habitats and nesting sites. The negative effect of habitat diversity was explained by the area-heterogeneity trade-off. Therefore, sites with high heterogeneity will not contain enough cover of residual natural vegetation, essential to maintain high biodiversity, because increasing compositional heterogeneity within a fixed area simultaneously reduces the surface of each cover type. The analyses showed that birds and reptiles might be used as biodiversity indicators of vertebrates. Eurasian Magpie and Green Whip Snake, both generalist species, were associated with sites of low biodiversity, whereas seven birds, both generalists and farmland specialists, were associated with sites of medium biodiversity. In high biodiversity sites there were not indicator species. To conclude, in less natural environments, such as urban and agricultural landscapes, a combination of specialist and generalist indicator species seems adequate to monitor biodiversity changes. Our findings increase the knowledge of these very dynamic ecosystems, being important both to plan strategies for biodiversity conservation and to guarantee ecosystems services useful for humans.

1. Introduction

Nowadays, the loss of biodiversity is hundreds to thousands of times faster than it would be because of human actions (Battisti et al., 2016). These anthropogenic processes (e.g. urbanization, resource use, pollution), properly called *threats*, interfere with ecosystems or their biotic components, being causes of ecological stresses, such as risks to survival or restrictions in growth or reproduction (Parker et al., 1999; Salafsky et al., 2008). Consequently, a specific field of conservation biology, known as *threat analysis*, has developed in the last few years and now represents an important step in the programs implemented by many institutions and organizations (Battisti et al., 2016; Salafsky et al.,

2008). The loss of biodiversity also affects agroecosystems, which are characterized by a diverse biological structure, with many species well adapted to human-modified habitats (Collins and Qualset, 1999; Paoletti and Pimentel, 1992). Moreover, agroecosystems are important for the services they provide to humans; indeed, they may be critical factors for the socio-economic and cultural fabrics, also playing an important role in community health (Bernués et al., 2014; Giller et al., 1997; Tscharntke et al., 2012; Tscharntke et al., 2005). In this context, what is challenging is to preserve biodiversity in environments that are under the pressure from continuous changes, necessary to increase yields and economic values of agroecosystems. In the past decades, the intensification of farming through the use of chemical fertilizers, pesticides, and mechanization, as well as the loss of semi-natural habitat features (e.g. fallows, hedgerows, small wetlands, small woodlands) from the agricultural landscape represented concrete risks for biodiversity. These changes have led to the environmental deterioration of farmlands (Geiger et al., 2010; Matson et al., 1997; Matson and Vitousek, 2006; Phelps et al., 2013). Consequently, the biodiversity in agroecosystems has decreased and its loss is still increasing. For instance, high agricultural intensification is negatively related to richness and diversity of plant and soil taxa (Dorrough and Scroggie, 2008; Geiger et al., 2010; Giller et al., 1997), as well as of arthropods and insects in general (Attwood et al., 2008; Habel et al., 2019). In particular, bees and other pollinators, which provide an essential ecosystem service to human survival, are threatened in many countries (Kremen et al., 2002; Van-bergen and the Insect Pollinators Initiative, 2013). Alteration of water bodies in agricultural lands, both physical and chemical, has caused the decline of native fish and amphibians (Arntzen et al., 2017; Beja and Alcazar, 2003; Lange et al., 2014) and their frequent substitution with alien invasive species (Genovesi et al., 2015). Besides, several species of farmland birds, which represent a large proportion of European avifauna, have suffered a dramatic decline in recent decades, especially in Western Europe (Donald et al., 2006; 2001; Newton, 2004), with a decrease of 57% since 1980 (PECBMS, 2019).

In this view, to halt and reduce the loss of biodiversity, surveys are fundamental, both to monitor the trend of this decline and to improve the gap of knowledge concerning this topic (Given, 1993; Morrison and Mathewson, 2015; Sinclair et al., 2006; Sutherland et al., 2009). However, the collection and management of data across time and space are both expensive and difficult, therefore, it is reasonable to ask whether data could be collected more efficiently (Morrison et al., 2001; Morrison and Mathewson, 2015). One approach to limit the cost of biodiversity surveys is to focus on a limited set of indicator species or taxonomic groups that can act as a proxy for wider patterns of biodiversity and environmental changes (McKenzie et al., 1992a; 1992b). Indeed, indicator species should be sensitive to the phenomenon under study (Amici and Battisti, 2009

Battisti and Luiselli, 2011). Currently, the indicator species approach is widely used in conservation biology, environmental resource management, and restoration ecology (Landres et al., 1988; Niemi and McDonald, 2004; Noss, 1990; Siddig et al., 2016). They are used in ecotoxicology to control pollution (Parmar et al., 2016; Torres et al., 2008), to monitor the quality and to plan the management of forests (Brooks et al., 1998; Cantarello and Newton, 2008), aquatic environments (Harig and Bain, 1998; Lasne et al., 2007), pastures (Pärt and Söderström, 1999a; 1999b), agricultural lands (Birkhofer et al., 2018; Rousseau et al., 2013) and urban areas (Dennis et al., 2017; Godefroid and Koedam, 2003). Besides, indicator species are used to quantify restoration success of terrestrial and aquatic environments (Gatica-Saavedra et al., 2017; González et al., 2014; Thompson et al., 2008) and to monitor climate changes (de Groot et al., 1995; Ellis et al., 2009; Reis et al., 2019). Furthermore, indicator species are also used as a proxy of the diversity of other species, taxa or communities (Niemi and McDonald, 2004; Noss, 1999; 1990; Rodrigues and Brooks, 2007). On the other hand, the indicator species approach has some limitations. The degree to which a single taxon can faithfully represent the status and

trends in other taxa is the main matter of debate (Carignan and Villard, 2002; Niemi and McDonald, 2004). For instance, in the "guild-indicator" approach (Block et al., 1987; Jansson, 1998; Verner, 1984), extrapolating the changes on any species in a guild to every other species in the guild, is difficult because each species has a specific ecology (Landres et al., 1988). Nevertheless, the "guild-indicator" approach allows defining more reliable spatio-temporal patterns when compared to the abundance of single species, an aspect particularly important when a rapid assessment of a phenomenon is required. A related question is that the ability to detect responses to environmental changes may depend on the selected taxon; taxa with short generation times may react quicker than others, which can show delayed responses to the same disturbance (Niemëla et al. 1993; Carignan and Villard, 2002). This is true especially in urban lands and agroecosystems because of the rapid changes both at the field (e.g. due to ploughing, harvesting, spraying) and at the landscape scale (e.g. due to crop rotations). However, it is undoubted that good indicator species should have some characteristics: (i) to provide rapid warning of natural responses to environmental changes, (ii) to indicate directly the cause of the change rather than the existence of it, (iii) to provide assessment over a wide range and intensity of ecological stresses. Moreover, indicator species should be cost-efficient, not rare and easy to survey, and appealing to people (Carignan and Villard, 2002; Mandelik et al., 2010; McKenzie et al., 1992a; 1992b; Niemi and McDonald, 2004; Noss, 1990).

The first aim of this research was to measure the biodiversity of vertebrates using a multi-taxa approach in an intensively cultivated area located in a landscape subject to high human pressure, as well as to investigate its relationships with the environment. Secondly, we aimed to assess if there were taxonomic groups that can be used as biodiversity indicators and, lastly, we specifically looked for indicator species. We expected that the highest level of biodiversity to be linked to areas more heterogeneous and with residual semi-natural features, such as hedgerows, shrublands, and small woodlands. In fact, this is a general rule in agroecosystems and urban contexts (Benton et al., 2003; Biaggini and Corti, 2015; Chiatante et al., 2017; Fahrig et al., 2015; Shwartz et al., 2008). Also, we expected that species inhabiting urban landscapes and benefitting from human proximity to be identified as indicators of low biodiversity, since heavy urbanization has a clear negative effect on biodiversity (McKinney, 2008; 2002). Besides, species adapted to live in green urban areas and rural areas, where human pressure is lower, should be indicators of sites with higher biodiversity (Angold et al., 2006; Knapp et al., 2008; Van Nuland and Whitlow, 2014). On the other hand, species completely avoiding anthropization, if present, should be very rare and therefore not useful as indicator species, because of the reasons previously described. The importance of this study lies in the fact that just because agroecosystems and human-modified landscapes are very dynamic environments, to increase the knowledge in these contexts is fundamental both to plan strategies for biodiversity conservation and to guarantee ecosystems services essential for humans. Indeed, given the dominant role of urbanization and agriculture as drivers of environmental change, it is important to establish indicators of their impact on biodiversity in these landscapes, also in the view of sustainable development and ecosystem services (Paoletti 1999; Hagan and Whitman, 2006; Birkhofer et al. 2018). Furthermore, this study is important because it is essential to identify indicator species site-specific because planning in one place based on indicators developed in other geographical context is tricky (Hess et al., 2006; Landres et al., 1988; Remme et al., 2016). Besides, indicators are needful to monitor biodiversity status, also in the view of the Post-2020 Global Biodiversity Framework, whose basis was posed by the Convention on Biological Diversity (opened for signature in Rio de Janeiro on 5 June 1992), which is a roadmap toward the 2050 Vision for Biodiversity of "Living in Harmony with Nature". Indeed, the Biodiversity Targets adopted (also known as Aichi Biodiversity Targets) underlined the need to find practical, measurable and coordinated actions addressed towards the reduction of biodiversity loss. Finally, many species living in

agricultural landscapes are of conservation concern, for instance, many farmland birds, therefore our findings may offer new insights to better understand the ecology of these species.

2. Methods

2.1. Study area

The study area was located in northern Italy, at the southeast of Milan, in the western Po Plain (Lombardy Region; 9.283° E, 45.457° N). In particular, we collected data in four protected areas, which comprised natural, semi-natural, and agricultural areas, extending for 148 km² (Fig. 1). Specifically, we selected the eastern part of 'South Milan Agricultural Park' (140.3 km², encompassing the Site of Community Importance IT2050009 'Sorgenti della Muzzetta') and three Parks of Local Interest (PLIS 'Parco Est delle Cave', 3.2 km²; PLIS 'Parco delle cascine di Pioltello', 2.1 km²; PLIS 'Parco della Media Valle del Lambro', 2.1 km²). We chose this study area, because, despite its level of protection, it is an intensively cultivated and highly inhabited area, where the landscape is subject to high human pressure. Indeed, the land cover was characterized by built-up areas (9.2%), green urban areas (2.6%), arable lands (68.0%), permanent grasslands (9.7%), natural vegetation areas (i.e. woodlands and shrublands, 3.6%), and water bodies (2.9%). The data used to measure the environmental variables were obtained from the regional land use map DUSAF 4.0 (Ente Regionale per i Servizi all'Agricoltura e alle Foreste [ERSAF], 2014). The agroecosystem of the study area is not a High Nature Value (HNV) farmland (i.e. agricultural land with a great diversity of species and habitat, or with a high number of species of conservation concern, or both; Paracchini et al. 2008;

Makarewicz et al. 2012) and it is among the areas with the lowest relative farmland species richness in Europe (Overmars et al., 2014).

2.2. Survey design and data collection

We collected data in 2016 following a Tessellation Stratified Sampling (TSS; Sutherland 2006; Barabesi and Fattorini, 2013). In particular, a 500 × 500 m grid was superimposed on the study area and 10% of the grid (131 cells, hereafter sampling units; Fig. 1) were randomly chosen to make the samples more representative. Then, inside each sampling unit, the surveys were carried out with different methods depending on the taxon (Table 1). Considering both the survey design and the different sampling techniques used, we tried to collect unbiased and representative multi-taxa data maximizing the detection by using standard field methods. Specifically, to collect data concerning medium-sized mammals, we walked a random linear transect within each sampling unit looking for species signs of presence (Krebs, 1999). First, transects were randomly placed using a GIS platform (QGIS v.2.14.12), then they were modified in situ especially because of the presence of inaccessible locations and to avoid damaging crops. Scats, droppings, footprints, feeding signs, dens and carcass remains were identified taking into consideration their size, shape, and location (Bang and Dahlstrøm, 2006). To collect data concerning small mammals, we deployed 10 baited Sherman live-traps (Hoffman et al., 2010; Torre and Arrizabalaga, 2009) along a 100 m strip within each sampling unit; trapping periods lasted three nights per sampling unit and captured animals were identified, aged, sexed and measured. Moreover, we placed 5 baited hair-tubes along a 50 m strip within each sampling unit to collect data about arboreal rodents (Gurnell et al., 2004; Zapponi
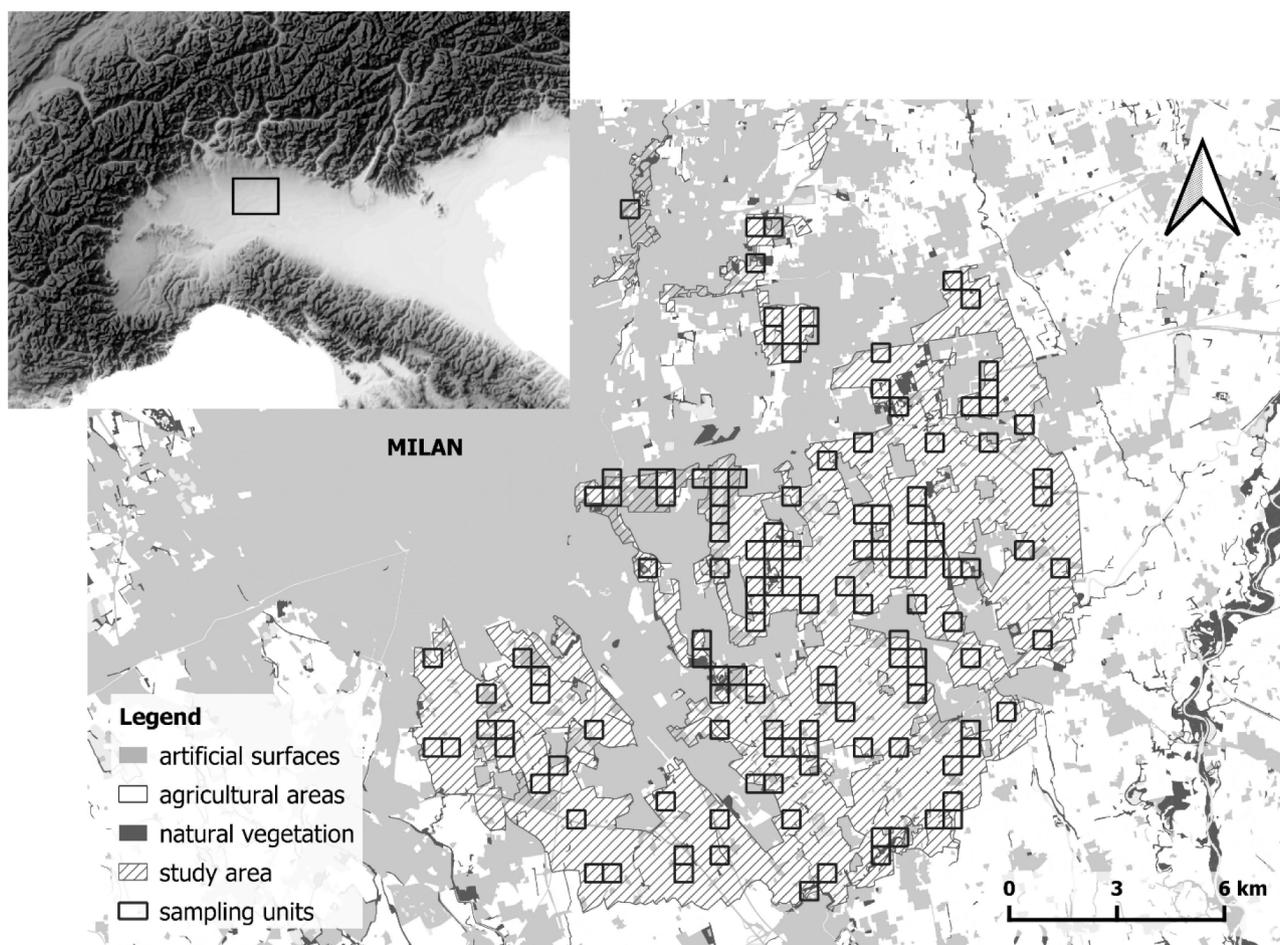

Fig. 1. The study area. The study area located southeast of Milan, in the western Po Plain (northern Italy). The sampling units surveyed are shown.

Table 1
Sampling methods used to survey different taxonomic groups in an intensively cultivated and heavily human-modified landscape of the western Po Plain (northern Italy).

Taxon	Method	Data collected	Total effort	Average effort for sampling units	Number of visits	Period of visits	Average duration of surveys
Mammals	Linear transects	Specimen observation,scats, footprints, feeding signs, dens, carcass remains	26.5 km	456 m	1	April-September	45 min
	Sherman live-traps	Specimen observation	13.1 km	100 m	1	April-September	Dependent on captures
	Hair-tubes	Hairs	6.6 km	50 m	2	April-September	30 min
Birds	Point counts	Specimen observations	1310 min.	10 min	1	April-May	10 min
Reptiles	Linear transects	Specimen observations	78.6 km	300 m	2	April-September	45 min
Amphibians	Visual census in breeding sites	Specimen observations	24.0 km	200 m	2	April-September	30 min
Fish	Electric fishing	Specimen observations	4.5 km	300 m	1	September-November	1.5 h
	Linear transects	Specimen observations	1.5 km	100 m	1	September-November	30 min

et al., 2013). Collected hairs were removed twice from the tubes every 15 days for species identification based on the characteristics of root, medulla and cortex (Teerink, 1991). We used point counts with unlimited distance to survey birds (Bibby et al., 2000; Blondel et al., 1981). Each point, randomly placed inside the sampling units (one point per each unit), was surveyed once from April to May, between dawn and 10:30. A count lasted ten minutes (Chamberlain and Rolando, 2014) and the surveys started five minutes after the observer arrived at the survey point to allow birds to settle down. Birds flying over, that had not taken-off from or landed in the site, were not included (Bibby et al., 2000; Sutherland et al., 2004). Data concerning reptiles were collected along linear transects (one transect for each sampling unit) located in potentially suitable areas (e.g. field margins, edge woodlands/ shrublands, hedgerows, rock piles, ponds) by observing and hand capturing individuals for species identification (Sutherland, 2006). Amphibians were searched walking around 60 potential breeding sites (e.g. ponds, artificial lakes, ditches; Sutherland, 2006), generally 1–3 per sampling units or absent at all. Species were identified by their calls or by observing adults, tadpoles or egg masses. Surveys of both reptiles and amphibians were carried out twice (in April-June and July-September) to encompass all the species' phenologies. Finally, we collected ichthyological data by daytime electric fishing (Sutherland, 2006), using an engine powered electrofisher ELT60II, 300/500 V max, 1300 W (Hans-Grassl Inc., Germany). A total number of 15 sampling sites were chosen opportunistically in the sampling units to cover a wide variety of aquatic habitats and environmental conditions. Electrofishing was applied according to appropriate water depth (not deeper than 1.2 m) and river conditions, following the APAT (2007) protocol for fish sampling in shallow waters. Particularly, in each sampling site were surveyed two transects (150–200 m long in small streams, 100 m long in larger streams) to collect data by sampling as many habitat types as possible (e.g. pools, riffles, meanders, ripraps, sandy littorals). All captured fish were identified, measured, and counted by species; after handling, the fish were released. In addition, we carried out both surface and underwater visual census to collect more fish data in deep water (Sutherland, 2006). More precisely, 100 m wide transects were assessed by scuba diving using an automatic rebreather system Castoro C96 Pro (OMG Italia) with no bubble emission. Photographs of fish were taken using a Nikon D750 camera inside a Subal tank equipped with Ikelite DS160 flash light. All surveys were carried out excluding days with bad weather conditions (e.g. rain, strong wind, fog) to maximize detectability of animals (Sutherland, 2006). For the common and scientific names we used the IUCN's nomenclature (www.iucnredlist.org).

2.3. Biodiversity Index

We calculated the biodiversity index using a method proposed by Rey Benayas and de la Montaña (2003) and used in other studies (Chiatante and Meriggi, 2016; Marfil-Daza et al., 2013; Rodeles et al., 2020). In particular, for each sampling unit and each taxon separately, we calculated four parameters: 1) the species richness S_r, i.e. the number of species occurring in the sampling units r, 2) the rarity index R_r, defined by the species range, measured as the inverse of the number of sampling units where it was present $(1/n_i)$; for a unit r, the rarity index was $\sum_{i=1}(1/n_{ri})/S_r$, where S_r was the species richness in the sample r, 3) the vulnerability index V_r, quantified using the categories of the Red List of Italian Vertebrates (Rondinini et al., 2013). A score was assigned to every species related to its degree of vulnerability: 3 for Endangered (EN), 2 for Vulnerable (VU) and Near Threatened (NT) species, 1 for Least Concern (LC) species, 0 for Data Deficient (DD) species. For alien species a score of 0 was assigned. Moreover, if the species was listed in Annex I of the Birds Directive 2009/147/CE or in the Annexes II and IV of the Habitat Directive 92/43/CEE a value of 1 was also added. For a unit r, the vulnerability index was $\sum_{i=1}V_{ri}/S_r$, where V_{ri} was the vulnerability score of the species i present in the sampling unit r, 4) the combined index of diversity C_r, which summarized the species richness, the rarity index, and the vulnerability index, calculated for the sample r as $\sum_{i=1}(1/n_{ri})V_{ri}$. In this index, the species richness is implicit in $\Sigma_{i=1}$. Then, we calculated the Biodiversity Index BI_r that measures the biodiversity of all five taxa together in every unit r. We first standardized by dividing the combined index of biodiversity of each taxon in every sampling unit by its mean, and then summed the five standardized combined indices. The formula of this index was the following

$$\sum_{j=1}^{5} 1/m_j \sum_{i=1}^{jS} (1/n_{ji})V_{ji}$$

where m_j is the mean combined index of biodiversity of the taxon j across sampling units.

Finally, we investigated how the environment affected the Biodiversity Index. Therefore, we measured 28 environmental variables (Table 2) in each sampling unit, concerning the land use cover and the landscape configuration. Among the configuration variables, we calculated one metric of habitat diversity (i.e. Shannon Index, equals 0 when the sampling unit contains only one patch of the considered habitat type), two metrics of density and patch size (i.e. Number of Patches, NP, and Mean Patch Size, MPS), one edge metric (i.e. Edge Density, ED, equals to 0 when there is no edge of the considered habitat type in the sampling unit) and one shape metric (i.e. Area Weighted Mean Shape Index, AWMSI, equals to 1 when all patches of the considered habitat

Table 2

Summary statistics of the environmental variables used to investigate how the Biodiversity Index was related to the landscape. Artificial surfaces comprised built-up areas, industrial units, roads/rails and mineral extraction sites. The AIC_c values of the model with the environmental variable are shown, to compare with the AIC_c of the model with only the intercept ($AIC_c = 315.86$). In bold are marked the variables retained for the Multiple Linear Regression Analysis.

Variable	Abbreviation	Mean	±	SE	Range	AIC_c
Built-up areas (%)	**BU**	**1.9**	**±**	**0.24**	**0.0–11.7**	**313.53**
Industrial units (%)	IND	3.5	±	0.81	0.0–63.6	317.66
Roads and rails (%)	RR	1.5	±	0.39	0.0–28.1	317.82
Mineral extraction sites (%)	MIN	2.3	±	0.81	0.0–67.2	317.77
Green urban areas (%)	**GRU**	**3.9**	**±**	**1.02**	**0.0–70.3**	**315.27**
Arable lands (%)	**ARA**	**61.5**	**±**	**2.80**	**0.0–100.0**	**315.70**
Vegetable gardens and greenhouses (%)	**VEG**	**2.4**	**±**	**0.86**	**0.0–62.7**	**315.81**
Rice fields (%)	RICE	4.7	±	1.55	0.0–100.0	315.91
Poplar plantations (%)	POP	0.5	±	0.31	0.0–31.3	317.84
Permanent grasslands (%)	GRASS	8.7	±	1.36	0.0–69.1	316.45
Woodlands (%)	WOOD	3.7	±	0.60	0.0–34.3	317.86
Shrublands (%)	SHR	1.2	±	0.37	0.0–29.9	316.62
Rivers (%)	RIV	0.5	±	0.18	0.0–15.5	317.79
Water bodies (%)	WAT	3.3	±	1.03	0.0–62.1	317.71
Hedgerows (m/ha)	HEDGE	22.1	±	1.65	0.0–89.8	316.79
Habitat Diversity (Shannon Index)[1]	**SHAN**	**0.32**	**±**	**0.02**	**0.0–0.76**	**311.89**
Number of Patches of artificial surfaces[2]	**NP_A**	**2.76**	**±**	**0.22**	**0.0–14.0**	**313.06**
Mean Patch Size of artificial surfaces (ha)[3]	**MPS_A**	**0.78**	**±**	**0.08**	**0.0–3.90**	**314.38**
Edge Density of artificial surfaces (m/km²)[4]	ED_A	0.06	±	0.007	0.0–0.41	316.68
Area Weighted Mean Shape Index of artificial surfaces[5]	**AWMSI_A**	**1.38**	**±**	**0.07**	**0.0–3.95**	**314.99**
Number of Patches of agricultural areas[2]	NP_AG	3.03	±	0.16	0.0–10.0	316.74
Mean Patch Size of agricultural areas (ha)[3]	MPS_AG	8.26	±	0.59	0.0–25.0	317.86
Edge Density of agricultural areas (m/km²)[4]	**ED_AG**	**0.03**	**±**	**0.002**	**0.0–0.15**	**315.42**
Area Weighted Mean Shape Index of	AWMSI_AG	1.43	±	0.03	0.0–2.50	317.37

Table 2 (*continued*)

Variable	Abbreviation	Mean	±	SE	Range	AIC_c
agricultural areas[5]						
Number of Patches of natural vegetation[2]	**NP_NV**	**1.19**	**±**	**0.14**	**0.0–8.00**	**313.64**
Mean Patch Size of natural vegetation (ha)[3]	MPS_NV	0.53	±	0.80	0.0–5.78	316.33
Edge Density of natural vegetation (m/km²)[4]	**ED_NV**	**0.09**	**±**	**0.01**	**0.0–0.97**	**315.06**
Area Weighted Mean Shape Index of natural vegetation[5]	**AWMSI_NV**	**1.12**	**±**	**0.09**	**0.0–3.21**	**313.48**

[1] Shannon Index = minus the sum, across all habitat types, of the proportional abundance of each habitat type multiplied by that proportion; calculated as $-\sum_{i=1}(P_i \cdot \ln P_i)$.

[2] Number of Patches (NP) = total number of patches of the habitat type.

[3] Mean Patch Size (MPS) = total area of the habitat type (ha), divided by the total number of patches.

[4] Edge density (ED) = sum of lengths (m) of all edge segments involving the habitat type, divided by the total area (km²).

[5] Area Weighted Mean Shape Index (AWMSI) = the sum, across all patches, of each patch perimeter (m) divided by the square root of patch area (m²), adjusted by a constant, multiplied by the patch area (m²) divided by total area; calculated as $\sum_{i=1}\sum_{j=1}\left[\left(\frac{p_{ij}}{2\sqrt{\pi \cdot a_{ij}}}\right) \cdot \left(\frac{a_{ij}}{A}\right)\right]$.

type in the sample unit are circular, increasing without limit as the patch shape becomes more irregular). For further details on the landscape configuration metrics see McGarigal and Marks (1994) and Elkie et al. (1999).

Before computing the analyses, considering the non-normality of the Biodiversity Index (Kolmogorov-Smirnov test, $D = 0.176$, $P < 0.001$) we log-transformed it reaching the normality (Kolmogorov-Smirnov test, $D = 0.055$, $P = 0.827$). As the number of measured variables was high, it was necessary to reduce collinearity and avoid model overfitting (Dormann et al., 2013; Lever et al., 2016). Particularly, we selected only the variables with a remarkable effect on the Biodiversity Index, with a pairwise comparison of the second-order Akaike Information Criterion (AIC_c; Akaike 1973) of two Linear Regression Analysis (Legendre and Legendre, 1998); the first one with only the intercept and the other one with each variable (Burnham et al., 2011). When the AIC_c value of the regression with the variable was greater than the one with the intercept only, that variable was retained (Burnham and Anderson, 2002). After this procedure, we retained 12 environmental variables for the next analyses (Table 2).

Once the number of variables was reduced, we ran a Multiple Linear Regression Analysis using *a priori* sets of models built with all combinations of the 12 environmental variables selected in the first step. Then, for each model, the AIC_c was calculated and the model with the lowest AIC_c was selected (Anderson et al., 2001; 2000; Burnham and Anderson, 2002). For this analysis, all the variables considered were standardized by normalization; that is, each variable had a mean of zero and a standard deviation of one (Quinn and Keough, 2002; Zuur et al., 2007). The goodness-of-fit of the model was measured by Pearson's correlation coefficient between observed and predicted values of the Biodiversity Index. We also tested the residual normality by the Kolmogorov-Smirnov test and the spatial autocorrelation by the Moran I test (Bivand et al., 2008; Zuur et al., 2007), and we used the variance inflation factor (VIF) with a threshold of three to exclude highly

correlated variables (Fox and Monette, 1992; Guisan et al., 2002). The coefficient of determination R^2 was used as a measure of the variation explained by the model (Legendre and Legendre, 1998). The data used to measure the environmental variables were obtained from the regional land use map DUSAF 4.0 (Ente Regionale per i Servizi all'Agricoltura e alle Foreste [ERSAF], 2014) and processed by the software QGIS v.2.14.12. All analyses were performed using the statistical software R v.3.3.2 (R Core Team, 2019) and the packages *MuMIn* (Bartoń, 2018), *car* (Fox and Weisberg, 2011), *sp* (Pebesma and Bivand, 2011), and *spdep* (Bivand et al., 2015).

2.4. Indicator of biodiversity

We explored the usefulness of each taxon as indicator of the overall biodiversity of vertebrates with Pearson's correlation coefficient. In particular, we compared both the species richness and the combined index of each taxonomic group with the overall Biodiversity Index. We used only the species richness and the combined index because they were often used as a proxy of overall biodiversity (Gotelli and Colwell, 2001; Hill et al., 2016; Rey Benayas and de la Montaña, 2003). To preserve independence between the correlates, we recalculated the overall Biodiversity Index excluding the considered taxon before testing the correlations (Kati et al., 2004; Leal et al., 2010; Ricketts et al., 1999). Then, we investigated which species of the indicator group can be used as indicators of overall biodiversity. Therefore, we carried out an Indicator Species Analysis (IndVal; Dufrêne and Legendre, 1997) a widely used method in ecology (Peck et al., 2014; Schiegg, 2000; Siddig et al., 2019; van Halder et al., 2008). Indeed, it is important for conservation biology because it allows researchers to identify biological indicators based on an indicator score. Furthermore, being the IndVal index for a species independent to that of other species in the assemblage, comparisons can be made between different taxa or taxa in different functional groups or communities (De Cáceres and Legendre, 2009; Dufrêne and Legendre, 1997). The IndVal method gives an indicator score combining measurement of the specificity A of a species j to a particular ecological state k (maximum when only found at a given type of site) with its fidelity B to that state (maximum when always found at a given type of site). Specifically, it is calculated following the formulas

$$IndVal_{kj} = A_{kj}B_{kj}$$

$$A_{kj} = Nindividuals_{kj}/Nindividuals_{+k}$$

$$B_{kj} = Nsites_{kj}/Nsites_{k+}$$

where N individuals$_{kj}$ is the average abundance of species j across the sites belonging to ecological state k and N individuals$_{+k}$ is the sum of the average abundances of species j within the various states. N sites$_{kj}$ is the number of sites in the ecological state k where species j is present and N sites$_{k+}$ is the total number of sites in that ecological state. In particular, we used the square root of IndVal (sqrtIV) because prevents double-zero problem when comparing species composition between sites (De Cáceres et al., 2010). For further details see Dufrêne and Legendre (1997), De Cáceres and Legendre (2009), and De Cáceres et al. (2010). In our case, the ecological state was represented by the Biodiversity Index, which was used to group the sampling units into three classes of biodiversity (low, medium, and high biodiversity). These groupings were performed with the *k*-mean cluster analysis, which was validated for differences with the non-parametric Kruskal-Wallis test (Legendre and Legendre, 1998). To assess the significance of each species as indicator in each class of biodiversity, we performed a restricted permutation test with 999 replicates (De Cáceres and Legendre, 2009). Considering the characteristics of indicator species (see the Introduction), we used only the species not rare in the study area, which were species occurring in at least the 25th quantile of the frequency distribution in the sampling units (Gaston, 1994; Raphael and Molina, 2007).

Moreover, as for indicator groups, to preserve the independence of analyses, for each species we recalculated the Biodiversity Index excluding it before running the IndVal (Halme et al., 2009). This prevented one of the major limitations of IndVal: when the classification of sites was obtained from the same species composition data used for analysis, the p-value is not genuine and must be taken with caution (De Cáceres et al., 2010). The analyses were performed using the statistical software R v.3.3.2 (R Core Team, 2019) and the package *indicspecies* (De Cáceres et al., 2020).

3. Results

3.1. Biodiversity Index

During the fieldwork, we collected data of 24 mammals, 57 birds, seven reptiles, seven amphibians, and 23 fish (Electronic Supplementary Material, ESM Table S1), which were used to calculate all the indices (Table 3). The Biodiversity Index was positively related to the number of patches of natural vegetation (e.g. small woodlands, shrublands), whereas it was negatively affected by the number of patches of artificial surfaces (e.g. built-up areas, roads) and the habitat diversity (Table 4, Fig. 2). The expected values were significantly correlated to the observed ones ($r = 0.322$, $df = 129$, $P < 0.001$) and there was no collinearity among the variables (Table 4). Model residuals were normally distributed ($D = 0.042$. $P = 0.974$) and not spatially correlated ($I = 0.905$, $P = 0.183$). The explained variance was quite low ($R^2 = 0.104$).

3.2. Indicator of biodiversity

The Biodiversity Index was correlated with the combined index of birds ($r = 0.181$, $P = 0.039$) and both the species richness ($r = 0.262$, $P = 0.002$) and combined index of reptiles ($r = 0.176$, $P = 0.045$). There was no correlation between the Biodiversity Index and the species richness of birds ($r = 0.105$, $P = 0.143$), as well as with mammals (for species richness, $r = 0.098$, $P = 0.266$; for combined index, $r = 0.144$, $P = 0.100$), amphibians (for species richness, $r = 0.104$, $P = 0.236$; for combined index, $r = 0.321$, $P = 0.087$), and fish (for species richness, $r = 0.167$, $P = 0.056$; for combined index, $r = 0.162$, $P = 0.065$).

In the study area, there were 38 out of 57 species of birds not considered rare (i.e., the 25th quantile of the frequency distribution in the sampling units; see Table S2). Only the Eurasian Magpie (*Pica pica*) was associated with low biodiversity sites (Table 5), even though six species were nearly significant ($P < 0.10$) indicator species of low biodiversity (ESM Table S2). On the other hand, seven species were associated with sites of medium biodiversity (Table 5). Two species were nearly significant indicator species of medium biodiversity (ESM Table S2). No bird species indicated high biodiversity sites. Among reptiles, five out of seven species were not considered rare; Green Whip Snake (*Hierophis viridiflavus*) was associated with sites of low biodiversity (Table 5) whereas Western Green Lizard (*Lacerta bilineata*) was a nearly significant indicator of medium biodiversity (ESM Table S3). Similarly, as for birds, no reptiles were associated with sites of high biodiversity.

4. Discussion

This research aimed firstly to measure the biodiversity of vertebrates in an intensively cultivated agroecosystem surrounded by heavy anthropization and to investigate how it is related to the environment, in particular to land use and landscape configuration. Our results showed that sites with the highest biodiversity were positively related to the number of patches of natural vegetation (i.e. small woodlands and shrublands). This finding is not surprising, given that many researches concerning agricultural lands showed the same pattern (Bäckman and Tiainen, 2002; Duelli and Obrist, 2003a; Pita et al., 2007; Stoate et al., 2009). Indeed, in the farmed landscape, patches of natural vegetation

Table 3

Statistics of the indices used to calculate the overall Biodiversity Index using five taxa.

	Richness (S)			Rarity (R)			Vulnerability (V)			Combined (C)		
	Mean	±	SE	Mean	±	SE	Mean	±	SE	Mean	±	SE
Mammals	1.73	±	0.12	0.23	±	0.03	1.64	±	0.12	1.07	±	0.18
Birds	8.41	±	0.26	0.19	±	0.02	2.03	±	0.03	3.41	±	0.38
Reptiles	0.96	±	0.96	0.12	±	0.01	1.73	±	0.12	0.44	±	0.07
Amphibians	0.60	±	0.80	0.06	±	0.01	0.51	±	0.09	0.48	±	0.17
Fish	0.70	±	2.18	0.07	±	0.03	0.25	±	0.08	0.56	±	0.22

Table 4

The best model selected explaining the relationship between the Biodiversity Index and the environmental variables in the study area. We show also the standard error (SE) of the estimate, the 95% confidence intervals (LCI lower confidence interval, UCI upper confidence interval) and the variance inflation factor (VIF).

Variable	Coefficient	SE	LCI 95%	UCI 95%	VIF
intercept	−0.32	0.07	–	–	–
Habitat Diversity (Shannon Index)	−0.15	0.07	−0.29	−0.01	1.12
Number of Patches of artificial surfaces	−0.12	0.07	−0.26	0.02	1.01
Number of Patches natural vegetation	0.17	0.07	0.03	0.30	1.11

and hedgerows provide refuges, foraging habitat and nesting sites for many birds (Hinsley and Bellamy, 2000; Nagy et al., 2017; Vickery et al., 2009) and reptiles (Biaggini and Corti, 2015; Wisler et al., 2008). In agroecosystems, amphibians are strongly linked both with woodlands (Collins and Fahrig, 2017; Pellitteri-Rosa et al., 2008) and with ponds and other small wetlands (Arntzen et al., 2017; Beja and Alcazar, 2003; Hartel and von Wehrden, 2013), which in our study area are often located in sites with natural vegetation. Also, streams with wooded riparian zones have higher diversity and more structured communities of fish than streams with open riparian zones (Stauffer et al., 2000; Talmage et al., 2002; Wichert and Rapport, 1998). However, in intensive agroecosystems, the suitability for fish of irrigation ponds and canals is usually low because of altered hydrological regime, water temperature fluctuations, inadequate management and pollution (Walser and Bart, 1999; Wang et al., 2000; Zimmerman et al., 2003). Nonetheless, irrigation ponds and canals can be very important for the conservation of endangered species particularly during periods of drought (Casas et al., 2011). The importance of natural features in the agricultural matrix is highlighted also by the fact that they play an important role as dispersal corridors and stepping stones, enabling individuals to move through a wider and unsuitable landscape (Gehring and Swihart, 2003; Hinsley and Bellamy, 2000). Our results showed that artificial surfaces (e.g. built-up areas, industrial units, and roads) negatively affected biodiversity. This is undoubted (Mcdonald et al., 2008; McKinney, 2002) and many researchers have concluded that biodiversity increases along an urban-to-rural gradient, with species classified as "urban-avoiders", "urban-adapters" and "urban-exploiters" reflecting their response to human activities (Clergeau et al., 1998; Conole and Kirkpatrick, 2011; Sandström et al., 2006). Urban forests, parks, and other urban green areas have often a positive effect on biodiversity in heavily human-modified landscapes, primarily on birds (Canedoli et al., 2017; Cornelis and Hermy, 2004; Goddard et al., 2009), also playing a role as corridors (Bolger et al., 2001; Fernández-Juricic and Jokimäki, 2001). For instance, in Britain, common frog (*Rana temporaria*) populations have increased in urban parks and gardens despite their decline in rural areas (Carrier and Beebee, 2003). In these contexts, however, the landscape configuration is of great interest, especially the patch size, because biodiversity could be enriched also by urban-avoider species (i.e. forest interior specialists) (Canedoli et al., 2017; Fernández-Juricic and

Jokimäki, 2001). Furthermore, biodiversity in urban green areas is related to the heterogeneity of the surrounding landscape (Kadlec et al., 2008; Melles et al., 2003). Surprisingly, our results showed that habitat heterogeneity had a negative effect on the biodiversity of vertebrates, despite what has been usually reported (Benton et al., 2003; Fahrig et al., 2011). In fact, many bird species are associated with heterogeneous farmland (Browne et al., 2000; Chamberlain et al., 1999; Robinson et al., 2001), both at field and landscape scale (Benton et al., 2003; Pickett and Siriwardena, 2011). At the field scale, a complex structure of vegetation guarantees the suitability for many species simply because it affects the accessibility and detectability of both prey and predators (Henderson et al., 2001; Josefsson et al., 2017; Perkins et al., 2000). At the landscape scale, increasing diversity can benefit birds because of the great availability of resources (Firbank et al., 2008; Lindsay et al., 2013). Likewise, Collins and Fahrig (2017) found that both configurational and compositional heterogeneity of farmland are positively related to anuran species abundance, which in turn is negatively related to mean field size (Collins and Fahrig, 2017). Still, Fahrig et al. (2015) suggested that farmlands with smaller fields have higher biodiversity because the access to field boundary, where there is a great proportion of seminatural features, is easier. In any case, in most agricultural landscapes, as in our study area, a great contribution to biodiversity comes from the residual natural and semi-natural habitats and is directly influenced by their extension (Billeter et al., 2008; Bruun, 2000; Kremen et al., 2004; Steffan-Dewenter et al., 2002). Yet, we need to consider the 'area-heterogeneity trade-off' (Kadmon and Allouche, 2007; Allouche et al. 2012), which claims that, within a fixed area (as our sampling units), increasing compositional heterogeneity simultaneously reduces the surface of each cover type. Therefore, in accordance with previous researches (Duelli, 1997; Fahrig et al., 2011), sites with high heterogeneity will not contain enough cover of residual natural vegetation, essential to maintain high levels of biodiversity, which explains the negative effect that we found. This is a pattern well explained by the Intermediate Disturbance Hypothesis (IDH), which posits that species richness and diversity at the local scale peaks at a specific threshold (Collins and Glenn, 1997; Connell, 1978; Yuan et al., 2016).

The second aim of this research was the identification of taxonomic groups useful as indicators of biodiversity and, in particular, of indicator species of biodiversity in a heavily human-modified landscape and in an intensively cultivated agroecosystem. Our results showed that birds and reptiles are the best indicator taxonomic groups for the biodiversity of vertebrates. Generally, birds are considered good indicators both for other taxa and for the overall biodiversity (Chase et al., 2000; Fraixedas et al., 2020; Kati et al., 2004; Leal et al., 2010; Yong et al., 2016), also in agricultural areas (Sauberer et al., 2004), even though this is not always the case (Moore et al., 2003). In the same way, reptiles could be used as indicators (Mazaris et al., 2008; Ricketts et al., 1999), even if sometimes they are inadequate (Reid, 1998; Yong et al., 2016). However, although many studies highlighted that some taxa can be used as indicators for others, both at global or continental scales (Pearson and Cassola, 1992; Ricketts et al. 1999) and finer scales (e.g. national or regional) (Reyers et al., 2000; Sauberer et al., 2004; Warman et al., 2004), this evidence in most cases is questionable (Carignan and Villard, 2002; Lombard, 1995; Williams and Gaston, 1998). Moreover, previous researches have shown that rare and threatened species are the ones most likely to be missed
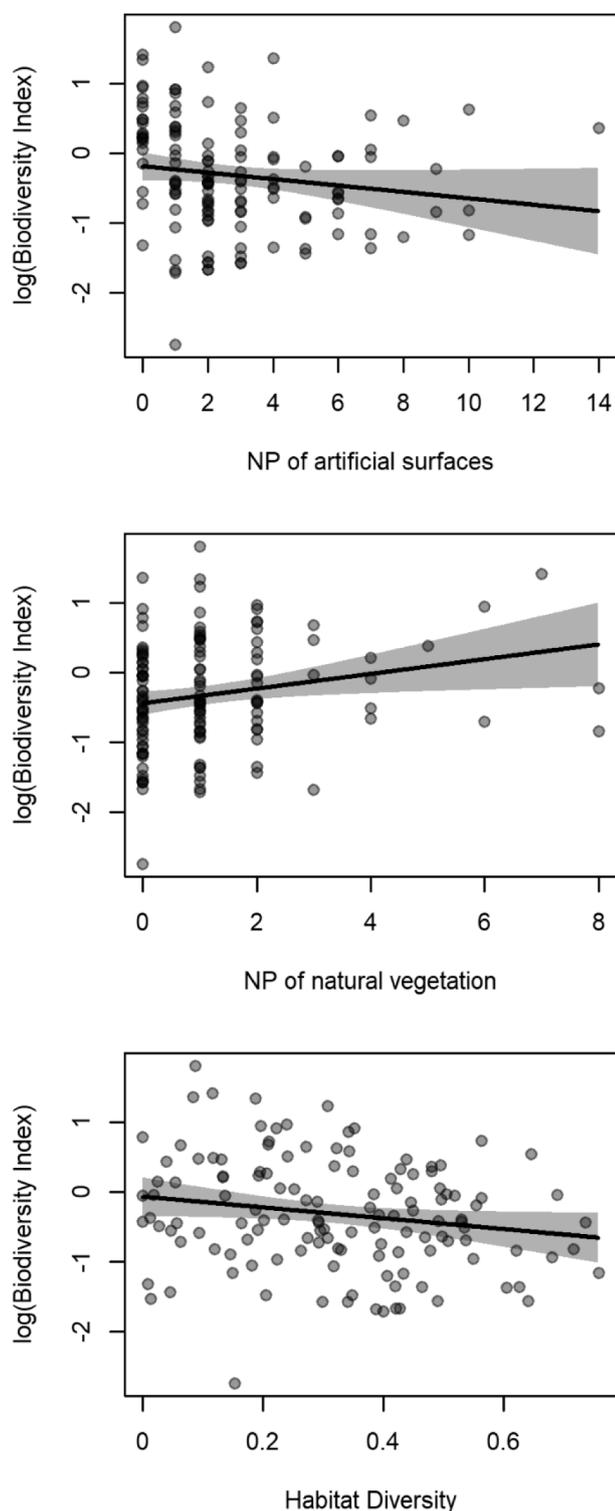
Fig. 2. Response curves of the best model. Response curves of the best model explaining the relationship between the Biodiversity Index and the environmental variables in the study area.

Table 5

Bird and reptile indicators of biodiversity. The specificity (A), sensitivity (B), indicator value (sqrtIV = square root of IndVal), and significance (*P*, α = 0.05, 999 permutations) are presented.

Taxon	Biodiversity	Species	A	B	sqrtIV	*P*
Birds	Low	*Pica pica*	0.885	0.253	0.473	0.013
	Medium	*Anas platyrhynchos*	0.478	0.324	0.393	0.015
		Luscinia megarhynchos	0.385	0.714	0.524	0.002
		Phoenicurus phoenicurus	0.833	0.143	0.345	0.006
		Hippolais polyglotta	0.727	0.229	0.408	0.003
		Oriolus oriolus	0.625	0.147	0.303	0.039
		Corvus corone cornix	0.348	0.914	0.564	0.001
		Passer italiae	0.400	0.514	0.454	0.012
Reptiles	Low	*Hierophis viridiflavus*	0.944	0.187	0.420	0.010

only Eurasian Magpie and Green Whip Snake as indicators of low biodiversity. The Eurasian Magpie is a generalist species breeding regularly in rural areas (Chiatante and Meriggi, 2019; Gregory and Marchant, 1996; Møller, 1982), even though many studies have demonstrated that this species is present at lower densities in the countryside than in urban areas (Antonov and Atanasova, 2003; Górska and Górski, 1997; Tucakov and Kucsera, 2008). Indeed, the urbanization of this species has been widely documented (Chiron et al., 2008; Gooch et al., 1991; Jokimäki et al., 2017). The Green Whip Snake occurs in grassy environments, shrublands, hedgerows, woodlands, and is very generalist (Capula et al., 1997; Lelièvre et al., 2011; Luiselli and Filippi, 2006; Meek, 2015). Certainly, it is able to reach high densities also in highly urbanized or suburban areas (Meek, 2015; Sindaco et al., 2006; Vignoli et al., 2009). Instead, seven birds (*A. platyrhynchos*, *L. megarhynchos*, *P. phoenicurus*, *H. polyglotta*, *O. oriolus*, *C. corone cornix*, *P. italiae*) were associated at sites with medium biodiversity. These species are both migrants and residents, inhabiting agroecosystems with farmhouses, gardens, and some natural features (Cramp, 1992; 1988; Cramp and Perrins, 1994; 1993; Cramp and Simmons, 1977). The reason why we did not find any species indicating high biodiversity is likely related both to the area-heterogeneity trade-off and to the fact that the species in our study area are mostly generalists (thus not associated with high biodiversity sites, such as *A. platyrhynchos*, *P. phoenicurus*, *H. polyglotta*; see Table S2) or farmland specialists (hence rare in our study area, such as *L. megarhynchos*, *O. oriolus*, *C. corone cornix*, *P. italiae*; see Table S2) (EBCC, 2019; Jennings and Pocock, 2009). Generally, it is preferable to select habitat specialists as indicator rather than habitat generalists (Gregory and Strien, 2010), likely because the latter are more tolerant to environmental conditions and changes (Jiguet et al., 2010). Contrarily, habitat specialists are considered better indicators (Amici and Battisti, 2009; Battisti and Luiselli, 2011), as they are more sensitive to changes (Landres et al., 1988), which imply that these species are rarer (Franklin and Miller, 2009; Raphael and Molina, 2007). Nonetheless, in less natural environments (such as urban and agricultural landscapes) a combination of specialist and generalist species seems to be adequate (Morelli, 2015). Our study confirms birds as good indicator species, also for biodiversity (Croonquist and Brooks, 1991; Fleishman et al., 2005; Gregory and Strien, 2010; Mekonen, 2017). As a matter of fact, they respond to environmental changes over many spatial scales, are relatively easy to survey also over large spatial scales, and their occurrence, abundance, and reproductive success are affected by surrounding habitats (Chiatante et al., 2019; Mazerolle and Villard, 1999; Villard et al., 1999). Nevertheless, some studies suggest that birds are not good indicators of biodiversity of other taxa (Ramírez, 2000) and in general should be used with caution (Beintema, 1983; Morrison, 1986; Temple and Wiens, 1989). Moreover, their mobility compared to other

when indicator species of biodiversity are used to design protected areas (Bonn et al., 2002; Dobson, 1997; Moore et al., 2003; Reyers et al., 2000). Yet, cross-taxonomic surrogacy can change over time (Yong et al., 2016). Therefore, indicators of overall biodiversity should be used cautiously, especially when they are used for conservation purposes (Hess et al., 2006; Landres et al., 1988; Lombard, 1995; Mazaris et al., 2008; Reid, 1998; Reyers et al., 2000). In this research, we identified

taxa could be a problem as their movements and migratory behaviours mean that their population dynamics can show mixed effects across different spatial scales and a decline in their abundance may be unrelated to conditions on the breeding grounds (Landres et al. 1988; Gregory and Strien, 2010). For this reason, a good indicator bird should be a resident species (Koskimies, 1989). Reptiles, due to their ecological and physiological traits (e.g. relatively low dispersal ability, small home ranges), are among the taxa that are primarily threatened by land use changes worldwide, even at local scale (Díaz et al., 2000; Driscoll, 2004; Glor et al., 2001; Ribeiro et al., 2009). Moreover, they constitute a high portion of the vertebrate fauna in terms of biomass and play a key role in the food web (Hastings et al., 2014; Padilla et al., 2007). For such reasons, reptiles can be particularly suitable to detect the consequences of environmental changes, such as human-induced habitat fragmentation (Battisti and Luiselli, 2011). Nonetheless, together with amphibians, are the least used group as ecological indicators (1%; Siddig et al. 2016).

5. Conclusion

Our results showed that in an intensively cultivated and heavily human-modified landscape, biodiversity is linked to remnant natural features, such as little woodlands, shrublands, and hedgerows. In addition, the higher the anthropization the lower the biodiversity. These findings are relevant in a management perspective, by showing that it is very important maintain remnant natural features within the anthropized matrix. Not only because they can help to support good levels of biodiversity, but also to guarantee ecosystems services essential for humans (Decocq et al., 2016; Ottewell et al., 2019; Robinson and Lundholm, 2012). Furthermore, we found that in these environments birds and reptiles can be used as indicators of biodiversity. Where biodiversity is low, indicators are generalist and human-related species (i.e. Eurasian Magpie and Green Whip Snake). When biodiversity increases, the indicators are mainly represented by common birds, sometimes classified as farmland species, therefore of conservation interests (EBCC, 2019; Rete Rurale Nazionale and LIPU, 2015). Generally speaking, given the complexity of ecosystems in terms of species richness, abundance and diversity, and ecosystem functioning, identifying a set of indicators that can well indicate these various aspects of biodiversity is complex (Landres et al. 1988; Duelli and Obrist, 2003b; Remme et al. 2016). For this reason, as confirmed by our results, the use of more than one indicator group is valuable for conservation goals (e.g. to design protected areas) and to monitor biodiversity (Maes and Van Dyck, 2005; Finch and Löffler, 2010; Escalante et al. 2020). Using more than one taxon is necessary even because no single indicator possesses all the desirable properties that make it valuable, therefore a set of complementary indicators is preferable (Noss, 1990; Remme et al., 2016).

CRediT authorship contribution statement

Gianpasquale Chiatante: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft. **Daniele Pellitteri-Rosa:** Data curation, Investigation, Writing - review & editing. **Elisa Torretta:** Data curation, Investigation, Writing - review & editing. **Francesco Nonnis Marzano:** Conceptualization, Data curation, Investigation, Methodology, Resources, Supervision, Writing - review & editing. **Alberto Meriggi:** Conceptualization, Methodology, Resources, Supervision, Writing - review & editing. Francesco Nonnis Marzano and Alberto Meriggi contributed equally to this paper.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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