Small mammals from barn owl *Tyto alba* pellets in a Mediterranean agroforestry landscape of central Italy

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Abstract - In order to investigate diversity patterns and similarities in the small mammal communities of an agroforestry landscape in western central Italy (Maremma of Lazio), we analyzed, in a multivariate setting (Cluster analysis, DCA-Detrended Correspondence Analysis), the prey content of barn owl *Tyto alba* pellets collected along one year in five sampling sites. Small mammal communities were composed by guilds typical of habitats included in agroforestry landscapes (croplands and mosaics, forests and ecotones, wet habitats and synanthropic ones). Since landscape matrices were characterized almost everywhere by croplands, typical agro-ecosystem species (*Apodemus* cfr. *sylvaticus, Microtus savii, Mus domesticus* and Soricidae) dominated in the majority of the collecting sites. The statistical analyses show how small changes in land use and cover can explain the faunal differences between sites, with the occasional presence of *Arvicola italicus* in wet habitats, and of *Muscardinus avellanarius* and *Sorex samniticus* in sites dominated by forest or agroforestry ecotones.

Communities recorded in sites characterized by wet and forest habitats showed a higher distance from the others, dominated by croplands. Communities occurring in landscapes with the lowest habitat diversity showed also the lowest species diversity.

Key words: Agroforestry landscapes, equitability, habitat diversity, species diversity, species richness.

Riassunto - Piccoli mammiferi da borre di barbagianni (*Tyto alba*) in un paesaggio agroforestale mediterraneo dell'Italia centrale.

Per indagare i pattern di diversità e di somiglianza nelle piccole comunità di mammiferi di un paesaggio agroforestale dell'Italia centro-occidentale (Maremma del Lazio), abbiamo analizzato, in un contesto di analisi multivariata (Cluster analysis, DCA-Detrended Correspondence analysis), il contenuto di prede in borre di barbagianni *Tyto alba* raccolte durante un anno in cinque siti di campionamento.

Le comunità di piccoli mammiferi erano composte da gilde tipiche degli ambienti compresi nei paesaggi agroforestali (terreni colti-

vati e mosaici, foreste ed ecotoni, zone umide e habitat sinantropici). Poiché le matrici paesaggistiche erano caratterizzate quasi ovunque da terreni coltivati, le specie tipiche dell'agroecosistema (*Apodemus* cfr. *sylvaticus, Microtus savii, Mus domesticus* e Soricidae) dominano nella maggior parte dei siti di raccolta. Le analisi statistiche evidenziano come piccoli cambiamenti nella copertura o nell'uso del suolo possono spiegare le differenze faunistiche tra siti, con *Arvicola italicus* occasionalmente presente negli ambienti umidi e *Muscardinus avellanarius* e *Sorex samniticus* nei siti dominati dalla foresta o negli ecotoni agro-forestali. I siti con la più bassa eterogeneità ambientale (=diversità di habitat) hanno mostrato anche la più bassa diversità di specie. La composizione e la dominanza di specifiche tipologie d'uso/copertura del suolo nelle aree limitrofe ai siti di campionamento spiegano le somiglianze tra le comunità di piccoli mammiferi. In particolare, due siti (uno inserito in un paesaggio a matrice forestale dominante, l'altro caratterizzato da bassa diversità ambientale) hanno evidenziato una maggiore dissimilarità strutturale dagli altri.

Parole chiave: Diversità ambientale, diversità di specie, equiripartizione, paesaggio agroforestale, ricchezza di specie.

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INTRODUCTION

Worldwide, communities of small mammals are indirectly studied using owl's (Aves: Strigiformes) pellets including remnants (mainly skulls and jaws) of these preys (e.g., Contoli & Sammuri, 1978; Bonvicino & Bezerra, 2003). Using this technique, a large amount of data is quickly available, although some limitation of this approach has been highlighted (Yom-Tov & Wool, 1997; Andrade *et al.*, 2016). For example, since some owl species are stenoecious, inhabiting only specific habitats (as in the case of barn owl *Tyto alba* foraging mainly in open-mosaic landscapes; Martínez & Zuberogoitia, 2004), information about small mammal assemblages may be obtained only for these contexts. Moreover, this type of sampling is highly opportunistic since it was indirectly obtained from the predators, therefore not following a standardized sampling protocol). However, excluding some weaknesses, there are many points of strength in this technique (Andrade *et al.,* 2016): for example, once selected a roosting or nesting barn owl site, we can obtain representative samples with a low research effort and, consequently, we may obtain a large set of data about prey species and their frequencies, richness, diversity, and other univariate metrics. Other methods (e.g. capture-mark-recapture by trapping) allow to obtain fewer representative samples (often with a high research effort) and diversity metrics

at community level are more difficult to achieve (Heisler *et al.,* 2016). Data on small mammal communities, obtained from owl' pellets, may be also useful to assess change in local owl diets (e.g. Catalisano & Massa, 1987; Love *et al.,* 2000; Veselovský *et al.,* 2017), composition, diversity and temporal trends in prey assemblages (e.g., Clark & Bunck, 1991; Bonvicino *et al.,* 2003; Avenant, 2005; Celauro & Battisti, 2006; Meek *et al.,* 2012; Stefke & Landler, 2020), multi-level trophic systems (e.g., Contoli, 1986; Contoli *et al.,* 2002), ecological value and environmental quality at different spatial/temporal scales (e.g., Prete *et al.,* 2012; Torre *et al.,* 2015; Mancini *et al.,* 2019), until to altitudinal (e.g. Milana *et al.,* 2019), latitudinal (e.g. Leveau *et al.,* 2006), biogeographical (e.g. Contoli *et al.,* 1987; Battisti *et al.,* 1997, 2019, 2020a) and genetic analyses (e.g. Taberlet & Fumagalli, 1996). Finally, also information about the presence or absence of specific, cryptic or threatened taxa can be obtained using this approach (e.g., Contoli, 1986; Contoli *et al.,* 1992; McDonald *et al.,* 2014; Biedma *et al.,* 2019; Kiamos *et al.,* 2019; Battisti *et al.,* 2020b).

Among the Strigiformes, the barn owl is widely used as source of data. Barn owls may be selective in habitat use, but are described by many as opportunistic predators that do not exhibit any food preferences; therefore, the species present in their pellets should provide a quite accurate representation of local prey abundance or accessibility (Taylor, 1994; Salvati *et al.,* 2002; Heisler *et al.,* 2016).

Generally, the foraging habitat of barn owls is represented by open landscapes, including grasslands, orchards, newly planted coniferous plantations and recently felled woodlands (Taylor, 1994). The unmanaged grassland edges provided by agricultural crops, woodland ecotones, riverbanks, ditches and hedgerows also have value in more intensively managed landscapes (Salvati *et al.,* 2002). In the wild, barn owls are generally sedentary. Nests are abandoned only if the surrounding habitat becomes devoid of prey, due to changes in habitat quality and structure, or if the nests or roosts are altered (Taylor, 1994). Therefore, a good knowledge of the species' feeding habitat and nesting/roosting sites allows to rapidly collect a large number of pellets containing a significant representation of their small-mammal preys (Contoli, 1975).

In this work, by using barn owls' pellets, we collected data about the small mammal guilds living in five sites located in an agroforestry landscape of Tyrrhenian central Italy (Maremma of Lazio). With the statistical analysis of these data, we aim to obtain an arrangement of small mammal frequency, richness, equitability and diversity, also in relation with the local landscape features and heterogeneity (habitat diversity).

MATERIALS AND METHODS

Study area and sample collection

We carried out a broad-scale sampling of barn owl pellets (summer 2018-summer 2019), in five collection sites distributed throughout the Tyrrhenian territory of the province of Viterbo, in habitats referable to the Maremma of Lazio (Fig. 1), the southernmost part of the Maremma, a region situated across the border between southern Tu-
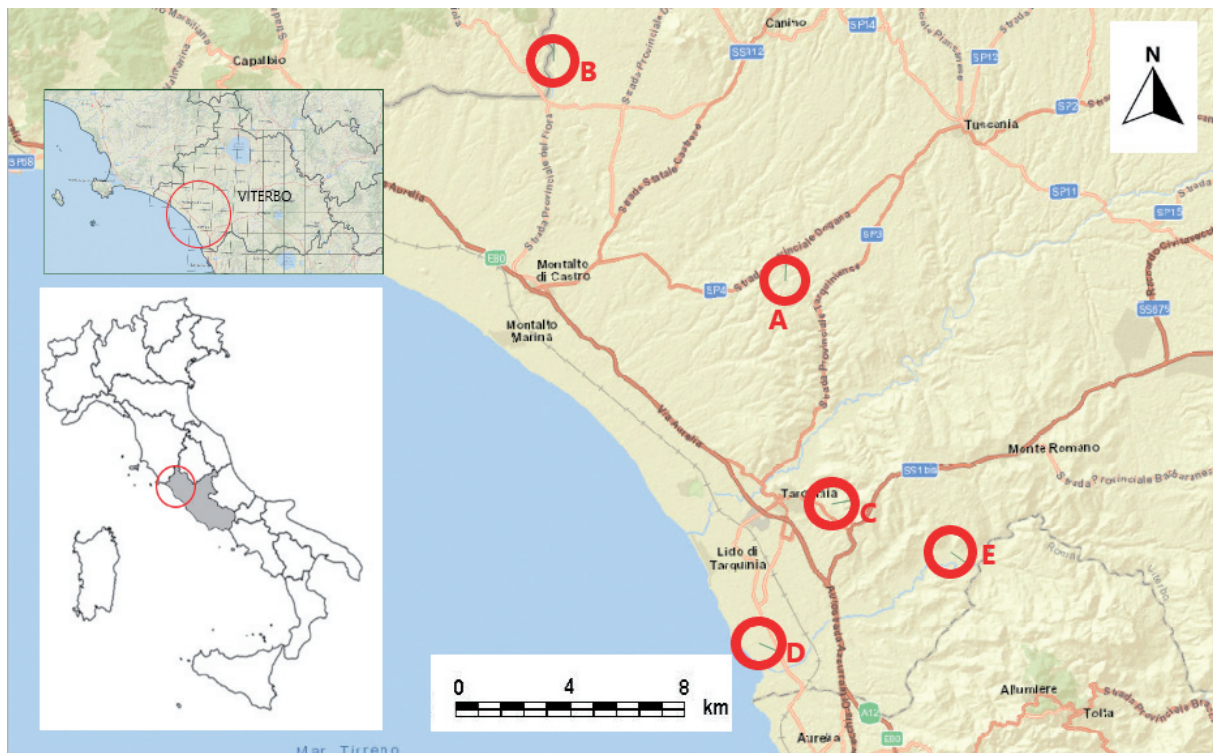

Fig. 1 - The study area. Circles and letters (A-E) show the five investigated sites. / Area di studio. I cerchi e le lettere (A-E) indicano i cinque siti studiati.

scany and northern Lazio, in central Italy. The study area includes the Tyrrhenian coastal strip and the corresponding plain delimited to the north-west by the final course of the Chiarone River, which marks the border between the Maremma di Grosseto, in Tuscany, and Tuscia, in Lazio (Olmi & Zapparoli, 1992), and to the south, up to Cerveteri, by the headland of Capo Linaro which represents the southernmost offshoot of the Tolfa Mountains (De Zuliani, 2011). The area was described as a transitional zone from a Mediterranean to a Temperate bioclimate (Blasi, 1994) and has distinctive geological and vegetation features (Marchetti *et al.*, 2014; Cutini *et al.*, 2010).

Sampling sites

Nest Site A. Roccaccia (42°21'0.50" N; 11°45'31.75" E; 149 m a.s.l.). The area is characterized by fodder crops, interspersed with coppices, composed of mixed Mediterranean oaks dominated by Turkey oaks *Quercus cerris* and downy oaks *Quercus pubescens*. Some sectors have been reforested with coniferous species (*Pinus* spp. and *Cupressus* spp.). Both cultivated areas and coppices are used in rotation or seasonally as a pasture for free-ranging cattle.

Nest Site B. Riminino (42°27'10.57" N; 11°37'9.57" E; 90 m a.s.l.). The area is characterized by a limestone escarpment irregularly covered by Mediterranean arboreal and shrub vegetation, overlooking a large grassy plain grazed by free-ranging cattle. The plain is crossed by the Fiora River, with hygrophilous vegetation growing along the banks. Above the escarpment there are plots bounded by hedges and crossed by ditches edged by hygrophilous vegetation, which are cultivated with fodder and cereals and successively used as pastures for sheep or as olive groves.

Nest Site C. Ripagretta (42°14'50.82" N; 11°47'4.60" E; 122 m a.s.l.). The site is characterized by slightly elevated limestone cliffs, partly covered by Mediterranean vegetation with prevalent arboreal and shrubby holm oak *Quercus ilex* and some downy oaks. The cliffs overlook a valley with vineyards, pastures and grasslands, with hedges and ecotonal habitats. In the above plateau, the westernmost sector is urbanized, while the rest is occupied by the Necropolis of Tarquinia, where the prevailing herbaceous cover is periodically mown, and by cereal crops.

Nest Site D. San Giorgio (42°11'4.00" N; 11°44'17.75" E; 6 m a.s.l.). Mosaic of croplands (mainly forage, cereal or horticultural crops), interspersed with uncultivated areas and sheep pastures. There are isolated oaks *Quercus* spp., hedges, reservoirs and drainage channels with hygrophilous and riparian vegetation, as well as a pine *Pinus* sp. and Italian stone pine *P. pinea* forest.

Nest Site E. Montericcio (42°13'26.52" N; 11°51'24.96" E; 26 m a.s.l.) Not far from the Mignone River, the area is mainly characterized by herbaceous (cereals) and horticultural crops, which are used in succession as sheep pastures. There are slopes with Mediterranean and sub-Mediterranean arboreal and shrubby vegetation, hedges and tree-lined rows and drainage channels with associated hygrophilous vegetation.

The landscape composition was assessed using Google Earth® (last accessed: October 2020) and landscape elements were analysed in detail within a 1 km radius area around each nest site, which approximates to the home range size (3 km²) of a barn owl during the breeding season (Taylor, 1994; Horvàth *et al.*, 2018). The five nest sites were described based on land-use types and the percentages for the following categories calculated: 1) agricultural fields (annual and perennial crops); 2) extensive land use (grasslands, pastures, orchards, vineyards); 3) wet habitats (including river banks, streams, artificial lakes, etc.); 4) forests (all forest habitats), and 5) urbanized areas (Tab. 1).

Pellet analysis

Pellets were collected from the nest sites between July 2018 and September 2019. Pellets were processed using the dry technique, with single pellets disassembled by hand and the prey items identified at the lowest possible taxonomic level.

Osteological parts (mainly skulls and jaws) were macroscopically identified, while teeth and feathers were identified under a stereo microscope at 25× or 50× magnifications. Analyses have been carried out in a private laboratory. The identification was carried out at the species level, where possible, by comparing undigested remains with a reference collection (see Contoli, 1980) and following Amori *et al.* (2008).

Prey numbers were estimated as the minimum number of individuals (MNI) obtained by counting the most

Tab. 1 - Percentages of land use/cover and habitat diversity index (Hh) in the five studied sites. / Percentuali di uso/copertura del suolo e diversità ambientale (Hh) nei cinque siti studiati.

	Roccaccia (A)	Riminino (B)	Ripagretta (C)	San Giorgio (D)	Montericcio (E)
Cropland	8.53	87.62	96.4	94.71	93.61
Orchards and vineyards	0.1	2.64	0.02	0	0.6
Wet habitats	0.77	8.03	0.45	2.3	1.35
Forests and wood mosaics	90.54	1.14	1.2	0.78	4.03
Urbanized areas	0.06	0.57	1.93	2.21	0.41
Hh index	0.349	0.495	0.191	0.26	0.303

common diagnostic osteological element of each species of small mammal in each pellet (McDowell & Medlin, 2009).

Nomenclature followed Loy *et al*. (2019). Since the determination of the remnants of *Apodemus* to the species level is difficult for this genus in Italy, we used *Apodemus* cfr. *sylvaticus* for the individuals assigned to this taxon.

The number of pellets collected from each site varied considerably, but cumulative occurrence plots demonstrated that collections >10 pellets were adequate to assess the main species present in the diet (Bond *et al.*, 2004).

For each site, we obtained the number of species (S) and the normalized species richness (Margalef index) as Dm=(S-1)/ln(N-1) (Margalef, 1958), where N was the total number of prey items. For each site we obtained the relative frequency of each species from the number of individuals (fr_i, as number of ind. of the i-th species/total number of individuals). Finally, we obtained two univariate metrics of diversity: the Shannon-Wiener diversity index (Shannon & Weaver, 1949), as $H' = -\Sigma\ fr_i \times lnfr_i$ and the equitability index, as $e=H'/H'_{max}$ where $H'_{max} = ln(S)$ (Pielou, 1966; details in Magurran, 2004). To assess the level of environmental heterogeneity (Hh), in each site we calculated an index of habitat diversity, following the analogous Shannon-Wiener formula.

We compared H' to Hh using the non-parametric Spearman rank correlation test (2 tail).

From a species/site matrix, we performed both a Cluster analysis (Algorithm: Paired group - UPGMA, Euclidean similarity index) obtaining a dendrogram, in order to assess the similarity among communities, and a Detrended Correspondence Analysis (DCA) in order to test for the linkage among sites (i.e. assemblages) and species (Hill & Gauch, 1980).

For statistical analyses we used the computer software PAST 4.01 (Hammer *et al.,* 2001). The statistical tests were considered significant at the alpha level of 0.05 as standard in all analyses (Dytham, 2011).

RESULTS

Totally, we examined 373 pellets, identifying 687 remnants belonging to 11 terrestrial small mammal species: *Apodemus* cfr. *sylvaticus* (39%), *Microtus savii* (19.5%), *Mus musculus* (15.3%), *Crocidura suaveolens* (7.9%), *Crocidura leucodon* (7.4%) and *Suncus etruscus* (5.1%) being dominant (total frequency > 5%) on the whole (Tab. 2). *Arvicola italicus* is the only species of conservation concern at national scale (Near Threatened; Rondinini *et al*., 2013). We obtained also data on bats (Chiroptera: *Pipistrellus* sp., *Eptesicus serotinus*), and on other taxonomic groups (birds: *Passer italiae*, and undetermined beetles), not included in the analyses.

The sites of Riminino (B), San Giorgio (D) and Monte Riccio (E) showed the highest Shannon-diversity and equitability indices, while Ripagretta (C) showed the lowest values. San Giorgio showed also the highest normalized species richness (Tab. 3). Species diversity H' was not significantly correlated directly to habitat diversity Hh (r_s=0.743, p=0.20; Spearman rank correlation test, 2 tail).

Dendrogram indicated two similarity groups: the less diversified A-C vs. other sites (correlation: 0.9851; Fig. 2). Detrended Correspondence Analysis (DCA; Eigenvalue: axis 1: 0.1675, axis 2: 0.060; Fig. 3) displayed an analogous pattern, highlighting the general similarities and showing the faunal peculiarity of two sites (Riminino - B, the only site with *Arvicola italicus*, and San Giorgio - D, with the only record of *Rattus norvegicus*) and the low evenness and diversity of the sites A (Roccaccia) and C (Ripagretta) with a high concentration of dominance (frequency) of synanthropic species (*Apodemus* cfr. *sylvaticus* and *Mus musculus*).

Tab. 2 - Small mammal species, number of records (n) and relative frequency (fr_i) in the five studied sites. / Specie di piccoli mammiferi, numero di reperti (n) e frequenza relativa (fr_i) nei cinque siti studiati.

Species	Roccaccia (A) n	Roccaccia (A) fr_i	Riminino (B) N	Riminino (B) fr_i	Ripagretta (C) n	Ripagretta (C) fr_i	San Giorgio (D) n	San Giorgio (D) fr_i	Montericcio (E) N	Montericcio (E) fr_i	Total n	Total fr_i
Suncus etruscus (Savi, 1822)	9	0.054	3	0.014	4	0.040	3	0.083	16	0.094	35	0.051
Sorex samniticus Altobello, 1926	4	0.024	0	0	3	0.030	0	0	3	0.018	10	0.015
Crocidura suaveolens (Pallas, 1811)	9	0.054	26	0.123	6	0.059	4	0.111	9	0.053	54	0.079
Crocidura leucodon (Hermann, 1780)	11	0.065	16	0.076	6	0.059	0	0	18	0.105	51	0.074
Muscardinus avellanarius (Linnaeus, 1758)	1	0.006	0	0	1	0.010	0	0	4	0.023	6	0.009
Arvicola italicus Savi, 1839	0	0	6	0.028	0	0	0	0	0	0	6	0.009
Microtus savii (de Sélys Longchamps, 1838)	7	0.042	75	0.355	4	0.040	4	0.111	44	0.257	134	0.195
Apodemus cfr. *sylvaticus* (Linnaeus, 1758)	86	0.512	54	0.256	66	0.653	12	0.333	50	0.292	268	0.390
Mus domesticus Schwarz and Schwarz, 1943	35	0.208	25	0.118	10	0.099	9	0.250	26	0.152	105	0.153
Rattus rattus (Linnaeus, 1758)	6	0.036	6	0.028	1	0.010	3	0.083	1	0.006	17	0.025
Rattus norvegicus (Berkenhout, 1769)	0	0	0	0	0	0	1	0.028	0	0	1	0.001
Total	168	1	211	1	101	1	36	1	171	1	687	1

DISCUSSION

The study revealed small mammal communities composed by typical species of the agroforestry landscapes of central Italy (e.g. Contoli, 1986; Aloise *et al.*, 1990; Capizzi & Luiselli, 1998; Battisti *et al.,* 2019) that host different ecological guilds, as species strictly linked to croplands and mosaics (e.g. *Microtus savii, Apodemus* cfr. *sylvaticus* and Soricidae), to forests and ecotones (*Muscardinus avellanarius, Sorex samniticus*), to wet habitats (*Arvicola italicus*) and finally, synanthropic rodents (*Mus domesticus*, *Rattus* spp.).

Tab. 3 - Number of pellets (N pel), number of records (n), number of species (S), Shannon-Wiener diversity index (H'), equitability (e), normalized richness (Margalef index, Dm). / Numero di borre (N pel), numero di reperti (n), numero di specie (S), indice di diversità di Shannon-Wiener (H'), equiripartizione (e), ricchezza normalizzata (indice di Margalef, Dm).

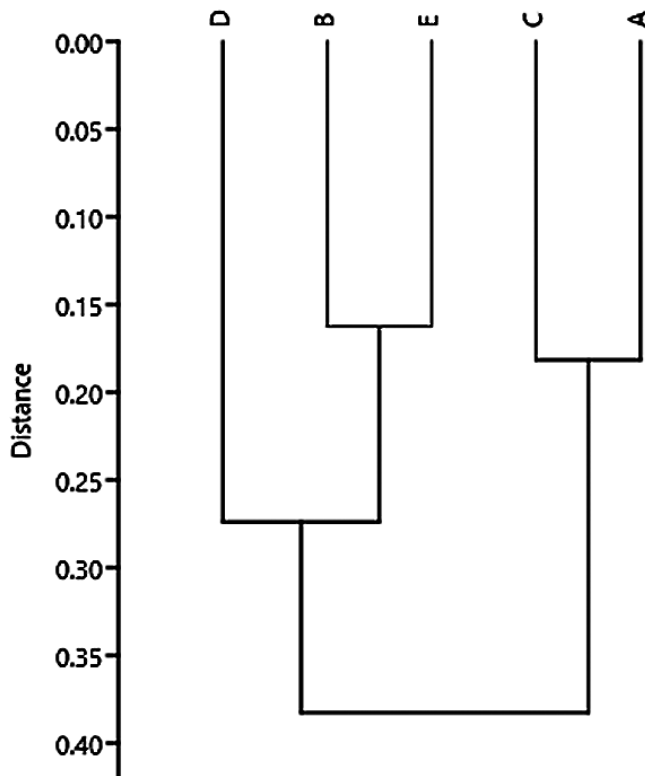| Site	N pel	n	S	H'	e	Dm
Roccaccia (A)	88	168	9	1.533	0.698	1.561
Riminino (B)	92	211	8	1.686	0.811	1.308
Ripagretta (C)	46	101	9	1.294	0.589	1.733
San Giorgio (D)	20	36	7	1.715	0.881	1.674
Montericcio (E)	127	171	9	1.798	0.818	1.556

Fig. 2 - Dendrogram of similarity based on species frequency (algorithm: Paired group - UPGMA, Euclidean similarity index). / Dendrogramma di similarità basato sulla frequenza di specie (algoritmo: gruppi appaiati - UPGMA, indice di similarità euclidea). Sites: / Siti: A) Roccaccia. B) Riminino. C) Ripagretta. D) San Giorgio. E) Montericcio.
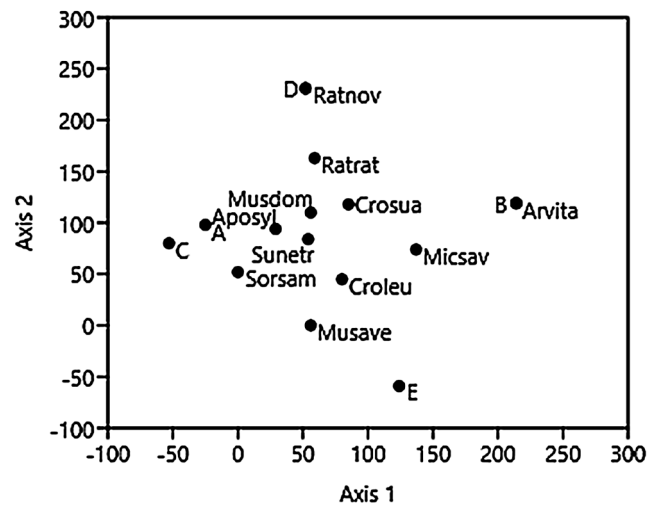

Fig. 3 - Detrended Correspondence Analysis. / Analisi delle Corrispondenze 'Detrended' Sites: / Siti: A) Roccaccia; B) Riminino; C) Ripagretta; D) San Giorgio; E) Montericcio. Species: / Specie: Sunetr: *Suncus etruscus*; Sorsam: *Sorex samniticus*; Crosua: *Crocidura suaveolens*; Croleu: *Crocidura leucodon*; Musave: *Muscardinus avellanarius*; Arvita: *Arvicola italicus*; Micsav: *Microtus savii*; Aposyl: *Apodemus* cfr. *sylvaticus*; Musdom: *Mus domesticus*; Ratrat: *Rattus rattus*; Ratnor: *Rattus norvegicus.*

Except for site A (Roccaccia), all the other sites are characterized by landscape matrices with dominance of extensive croplands and other open habitats. These features explain the almost general predominance of species linked to these environments. However, minor changes among sites about land cover and its use can explain the different faunal composition. *Muscardinus avellanarius* and *Sorex saminiticus* (forest and ecotone species) have been recorded in the site with higher forest cover (Roccaccia - A and, secondarily, Ripagretta - C and Montericcio - E). Moreover, in the site A forest-dominated the frequency of *Microtus savii*, a species linked to open agro-mosaic (Caroli *et al.*, 2000), is lowest than other sites. The localized presence of wet habitats (near the site B - Riminino) explains the occurrence of *Arvicola italicus* and the dissimilarity from the sites A (Roccaccia) and C (Ripagretta). These last sites showed a high similarity. This pattern could be assigned to a low evenness of both of them that, although different in land use cover, showed communities with a very high concentration of dominance in only two synanthropic species (*Apodemus* cfr. *sylvaticus* and *Mus musculus*) and the lower frequencies of in *Microtus savii*.

The limited number of sites and the different level of anthropization did not allow to obtain a significant correlation between habitat diversity index (a proxy of landscape heterogeneity) and species diversity. However, excluding the site D having a low prey sample, the sites with lowest habitat diversity also showed the lowest species diversity. The less environmental diversified site (Ripagretta - C) showed the lowest species diversity index and equitability.

Land use composition and habitat diversity can explain species frequency similarities among small mammal communities. In this regard, two sites (A and C) showed

a higher distance from the others. The first is included inside a forest-dominated landscape and the second is the less environmentally diversified. This finding would corroborate what stated by Varuzza *et al.*, (2001), which evidenced differences in prey diversity between sites located in forest *vs*. cultivated sites. This pattern of similarity is confirmed by DCA analysis, which highlights the faunal distinctiveness of sites B and D, the low diversity and uniformity of sites A and C, and suggests the existence of a relationship between species and sites. In this regard, axis 1 can represent the diversity/uniformity pattern. However, the same axis shows a low amount of overall information (eigenvalue about 17%) and the relative frequency of individual species may likely influence the observed pattern.

Data from owl's pellets provide a large amount of faunal, ecological and biogeographical information at site- and landscape-levels allowing in-depth analyses on diversity metrics of small mammal assemblages with a limited field effort. In this regard we would stimulate further research.

Acknowledgements

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