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GENERAL ABSTRACT

For the most part, there is no doubt that human impact on biodiversity has been negative: habitat loss and degradation, pollution, overexploitation, and introduction of invasive species had played significant roles in biodiversity decline. However, human impact on Earth is not always so detrimental and its effects are not the same for all taxa. The aim of this thesis was to assess the anthropogenic impact on one of the most endangered and often unnoticed groups - terrestrial molluscs - firstly focusing on natural and then urban context. In Chapter I, effects of forest age as a surrogate for forest continuity on land snail richness and composition in Tuscan evergreen and deciduous woods were assessed. Chapter II explored the role of dispersal and local environment in urban land snail assemblages comparing different human-made habitat types in three cities in central Italy (Grosseto, Siena and Arezzo). Finally the link between man and urban environment has been further elaborated in Chapter III, where a fully synanthropic land snail species (*Papillifera papillaris*) has been used to assess atmospheric pollution from human activities, especially vehicular traffic, in the urban area of Siena. Concerning results obtained, Chapter I revealed that successional age as surrogate for forest continuity parameter seemed not to be a key factor in controlling land snail species richness and abundances but habitat structure more than forest age was the factor mostly involved in affecting communities; furthermore, no difference was found between evergreen and deciduous oak litter in terms of land snail richness, abundance and diversity. Focusing on urban environment, Chapter II demonstrated that cities can show relatively high land snail richness with values comparable to natural areas. After having accounted and later removed spatial structures influence, urban gradient effect turned out to be the principal component structuring urban land snail assemblages. This finding proved a likely human-mediated transport, which enhances dispersal abilities of organisms normally considered scarcely mobile. However, no establishment there would be in the absence of suitable microhabitat conditions. In particular, "vegetation cover" and "distance from the city centre" were the environmental variables that explained most of the variation in species composition. In Chapter III, results elected *Papillifera papillaris* a promising species to monitor the metal distribution and bioavailability in urban environments and to evaluate the possible transfer of pollutants to higher levels in food chains. Mollusc soft tissues showed very low concentrations of lithophile elements, and much higher concentrations of anthropogenic metals such as Cd, Cu, Pb, and Zn while excreta and traditional biomonitors such as mosses were the matrices most involved in lithophile contamination. Surprisingly, *Papillifera papillaris* tissues showed extreme Mn values. These concentrations, for their magnitude, have no comparison with those reported in literature for other species of terrestrial gastropods. Excreta showed a high concentration of Pb, demonstrating the scarce bioavailability and absorption of this element and probably its limited impact on urban food chains.

INTRODUCTION

How do humans affect the environment?

All species shape and interact with environment, adapting themselves to the conditions of the habitats in which they live and transforming in turn their surrounding to suite their necessities. However, no species has been as thorough or as global about it as humans. At least at the beginnings of agriculture more than 10,000 years ago, humans have altered the face of the Earth more than any other species, using more complex instruments and technologies. Humanity's impact on the Earth's atmosphere, oceans and biodiversity has been so strong and pervasive that pushed the world into a new geological epoch – the Anthropocene – ending the Holocene which began around 12,000 years ago (Carrington, 2016). According to the common belief, the impact of man on Earth has had consequences for the most negative and devastating: oceans, lakes, and rivers suffer from our pollution and overfishing; industrial activity and resources exploitation cause habitat and biodiversity loss. But humans' impact on Earth is really always so harmful? And are its effects the same for all groups of organisms?

All about land snails

To give an answer to these complex questions I tried to assess anthropogenic impact effects on one of the most endangered and often unnoticed groups: terrestrial molluscs. Land snails are widespread organisms, occurring virtually everywhere on all continents; they are cornerstone species that contribute to the ecosystem functionality, living primarily in the upper leaf litter of forests, old fields, and wetlands, but also in more disturbed sites such as gardens, suburbs and cities. Terrestrial molluscs feed upon a wide variety of organic material, mainly herbaceous plants, rotting wood and fungi, bark and algae, being among the most active soil and litter detritivorous; in turn they are eaten by a wide range of invertebrate and vertebrate predators. Land snails do not move far over their lifetime, so they can be excellent indicators of site

history and site conditions (Hotopp, 2005). For this reason, due to their relative sedentary and vagile nature, land snails could be extremely susceptible to anthropogenic disturbance.

Objectives of this thesis

To assess the type of human impact as well as its effects on land snail communities' parameters such as richness and composition, I first focused on natural environment. Because of multiple cycles of deforestations and reforestations, most forest areas in southern Europe have been suffering a strong human impact. This is being reflected into an almost complete absence of primary forests towards the formation of secondary forest succession patches at different age. In the first chapter I explored the effects of forest age as a surrogate for forest continuity on land snail richness and composition in Tuscan evergreen and deciduous woods, trying also to partitionate the pure effect of the forest age on species variation respect to spatial and environmental variables (Chapter I).

Anthropogenic impact has been then evaluated where the human presence reaches its highest values: cities and urban matrices. In this regard, in order to demonstrate the persistence of human impact on Earth, Ellis and Ramankutty (2008) proposed a new characterization of terrestrial biomes based on the assumption that the world is no longer "a natural ecosystem with human disturbance" but more precisely the result of interaction between human and ecosystems. Among the 18 anthropogenic biomes identified, great relevance is represented by urbanization, since 54% of the world's population already lived in densely populated urban and village biomes in 2014 (United Nations, 2014). Despite the common belief that urbanization only negatively affects communities as a consequence of fragmentation and habitat loss as well as biotic homogenization, urban ecology is becoming an actual and interesting subject. New species to science have been recently discovered in urban matrices and the same richness values were found in cities as in natural areas. Urban environments contain a large number of widely different habitats at small scales such as gardens, parks and wasteland. Each of these

may provide very different habitats and thus support a wide variety of species. In this chapter, I analysed the effect of dispersion and local environment on land snail communities in three cities in central Italy. The main aim was to describe changes in richness and diversity in four human altered habitats as well as the impact of alien species (Chapter II).

Lastly, to testify the tight relationship between human and ecosystems, I evaluated the use of a synanthropic land snail species (*Papillifera papillaris*), commonly dwelling on stonewalls and monuments in Italian and Mediterranean urban environments, as new biomonitor of atmospheric pollution due to vehicular traffic in the city of Siena. To do that, I analyzed the elemental composition of soft tissues and excreta, comparing these matrices to the accumulation capacity of traditional biomonitors such as mosses (Chapter III).

The results of Chapter III were the subject of one paper published by Elsevier:

Rota E, Barbato D, Ancora S, Bianchi N, Bargagli R (2016) *Papillifera papillaris* (O.F. Müller), a small snail living on stones and monuments, as indicator of metal deposition and bioavailability in urban environments. *Ecological Indicators* 69:360-367. doi: 10.1016/j.ecolind.2016.04.024

The results of chapter II were the subject of one manuscript published by Springer:

Barbato D, Benocci A, Caruso T, Manganelli G (2017) The role of dispersal and local environment in urban land snail assemblages: an example of three cities in central Italy. *Urban Ecosystems*, pp 1-13. doi: 10.1007/s11252-017-0643-8

The results of chapter I constitute an original unpublished manuscript.

CHAPTER I

Effects of forest age, as surrogate for forest continuity, on land snail richness and composition in a Mediterranean secondary forest succession

Abstract

As a result of gradual agricultural land abandonment in favour to a sequential natural development of vegetation, most forested areas in southern Europe consist of secondary woodlots at different successional stage.

Based on data already used by Amici et al., 2013 for vascular plants, the effect of forest age as surrogate for forest continuity on land snail richness, abundance and composition in some Sites of Community Importance (SCIs) in southern Tuscany, using descriptive and quantitative statistics, has been assessed. Possible differences in land snail assemblages between evergreen and deciduous oak forest litter have been analysed too. A total of 25 species and 2518 individuals were recorded. Box plots showed that forest age as surrogate for forest continuity parameter did not seem to be a key factor in controlling land snail species richness and abundances; further, no difference between evergreen and deciduous forests was found. Considering species composition, NMDS demonstrated the presence of an assemblage of generalist species widespread in all forest age classes and no species was found as perfect indicator for each age category as shown by Indicator Species Analysis. This testifies that a process is acting but still not sufficient to differentiate the assemblages.

Habitat structure more than forest age may be the factor mostly involved in affecting communities: suitable microhabitat (just a few old/very old trees serving as refuges), are sufficient prerequisites to sustain diversity of land snail communities ensuring survival and protection, irrespective to age classes. These findings highlight the importance of structural complexity of forestal habitat for preserving land snail diversity.

INTRODUCTION

Old-growth forests (i.e. "later stages in forest development that are often compositionally and always structurally distinct from earlier successional stages"; Franklin and Spies, 1991) with an age range between 150 (boreal forests) and 300 years (temperate rain-forests) (Hunter and White, 1997) are becoming very rare in Europe. This is even more emphasized in the Mediterranean region (Gilg, 2004), where forests have been exploited since pre-Roman times (Blondel, 2006), either through direct periodic destruction or by different management activities as well as habitat transformation (Motta, 2002).

Nowadays most forested areas in southern Europe consist of secondary woodlots, as a result of gradual agricultural land abandonment in favour to a sequential natural development of vegetation. Since Roman times, land use in Europe has included alternate cycles of deforestation, land abandonment and reforestation (Kaplan et al., 2009). The most recent modification in land use was after World War II, a radical socio-economic revolution that caused significant transformations in most European landscapes. This breakpoint towards secondary succession has deeply changed the physiognomy of temperate forests, often leading to marked variation in habitat characteristic as well as homogenization and biodiversity loss in traditional landscapes of Mediterranean Europe (Amici et al., 2013). One of the most evident ecological consequences of secondary succession is the creation of closed-canopy stands (Van Breugel et al., 2007; Rancka et al., 2015), characterized by a marked shift in vegetation composition, with the increase in shade-tolerant species and the reduction of open-habitat and more generalist species (Wulf, 2004; Amici et al., 2013); furthermore closed-canopy may favor 1) a decrease in species richness, as a result of microhabitat heterogeneity loss, biotic interactions and niche specialization (Bonet and Pausas, 2004), 2) the introduction of invasive plant species (Flory and Clay, 2009).

Among a number of factors involved in explaining secondary succession effects, forest age is one of the ecological parameters that can most easily be obtained (Moning and Müller, 2008). Most studies tested forest stand age,

often considered as the stand age of the oldest tree within each sampling plot, (1) as a surrogate parameter for habitat continuity (Moning and Müller, 2008) and (2) as an estimate of forest disturbance regimes since in managed forest stands tree age relates to the frequency of cutting and therefore could reflect the human impact intensity (Erdmann et al., 2006).

However, a forest age assessment solely based on the age of the oldest tree seems to be a reductive estimation, a purely numeric information that may turn into an unrepresentative measure of global ecological dynamics in forest habitats. In this regard, Amici et al. (2013) proposed the duration of continuous forestation (so called "forest continuity", i.e the time in years since a land patch has become a real forest patch) as a measure of continuity of forest characteristics in a study about influence of secondary forest succession on plant diversity in some protected areas in Mediterranean region. Respect to tree age values, the measure of continuous forestation furnishes the estimate of the age of forest habitat, regardless of the management and harvesting regime (Amici et al., 2013). Forest continuity has been identified as a crucial factor influencing the structure and diversity of forest vegetation: a study about epiphytic lichens in coniferous forests in Estonia revealed a great influence of forest history and continuity on diversity and richness (Marmor et al., 2011).

The use of forest successional age as surrogate for forest continuity may have a higher significance for soil organisms. In this context, land snails, due to their very low mobility and close dependence on stable and specific microhabitat characteristics for their survival, are much more sensitive bioindicators than other soil animals (Douglas et al., 2013). Molluscs mainly live in the litter, but also use stems and foliage; they contribute to forest litter decomposition, nutrients recycle, soil formation and productivity, provide food for birds and other predators, and join other essential ecological functions (Baur and Baur, 1993). In forest ecosystems, numerous studies reported snail diversity and community composition to be closely linked to different biotic and abiotic factors, both at local (percentage of forest cover, shadiness and humidity, presence of suitable shelter and refuges, sufficient amount of woody debris, available food, soil chemistry and pH) and wider scale (e.g

forest type, size and age) (Müller et al., 2005).

Considering leaf litter effect as a consequence of forest type, it is generally accepted that majority of snails and slugs show a preference for deciduous forests (Abele, 2010). Coniferous forests host in fact lower snail density due to acid and inhospitable soil and leaf litter, which translates into reduced calcium bioavailability. Even leaves from different non-coniferous tree species differ in their chemical composition and nutritive value to gastropods. For example, oak (*Quercus* spp.) and beech (*Fagus sylvatica*) leaves are rich in oxalate-bound calcium, while ash (*Fraxinus* spp.), maple (*Acer* spp.), and elm (*Ulmus* spp.) leaves are dominated by calcium citrate and other more soluble forms of calcium (Wareborn, 1979) and this addition of different calcium salts influences snail reproductive success. Apart from this, little is known about differences between snail assemblages of co-occurring woody Mediterranean species of trees such as evergreen oak forests (dominated by *Quercus ilex*) and deciduous oak forests (dominated by *Quercus cerris* and *Quercus pubescens*).

Furthermore, excluding one research about significant forest age threshold in beech forests in Central Europe, no study took into account the effect of forest continuity (in this paper henceforward called "forest age") on land snail assemblages.

Here we investigate the effects of forest age on land snail communities through the examination of land snail richness and community composition in Mediterranean evergreen and deciduous oak forests during a secondary succession. The study took place in four Sites of Community Importance (SCIs) of Siena Province, Tuscany, Italy.

The main aims of this research are:

1. to explore possible differences in land snail composition as well as richness and abundance between the two oak forest types of different stages of secondary forest succession;
2. to test the hypothesis that recent forest patches host more alien/generalist/ecotonal species (such as *Rumina decollata*, *Monacha cantiana*, *Cornu aspersum*) than older patches;

3. to partitionate the pure effect of the forest successional age on species variation respect to spatial and environmental variables;

I expect to find differences in the forest age classes in terms of species richness and composition: younger patches, which should not offer stable habitat characteristics (i.e coarse woody debris, large broadleaved trees, estivation sites and refuges) will probably host generalist species, which do not exhibit a high degree of habitat specialization. Instead, older forest patches will host true forest species, strictly associated with certain micro-habitat characteristics and also more vulnerable to habitat changes. In other words, it is hypothesized that forest age closely matches the increase of land snail habitat specialization.

MATERIALS AND METHODS

Study sites

The province of Siena, Italy, due to various historical and biogeographical reasons, detains one of the highest naturalistic values in central Italy: it hosts habitats and species of great scientific and conservation interest, protected by European, National and Regional legislation.

Seventeen SCIs are present in the Siena province, ranging in size from 483 ha (Lago di Montepulciano) to 13,744 ha (Montagnola Senese), for a total of 58,969 ha, and accounting for more than 15% of the whole province territory. SCIs are established under the European Union Habitats Directive (92/43/EEC) as sites considered to highly contribute towards the conservation of European species and habitats of particular importance. Since our main aim was to test the effect of forest continuity on land snail assemblages, only SCIs with the highest forest percentage were chosen and used as study areas, for a total of 4 out of 17 SCIs (Fig.1): SIC "Montagnola Senese" IT5190003; SIC "Alta Val di Merse" IT5190006; SIC "Basso Merse" IT5190007; SIC "Val di Farma" IT51A0003.

Information about SCIs is published in Servizio Aree Protette Provincia di Siena (2013).

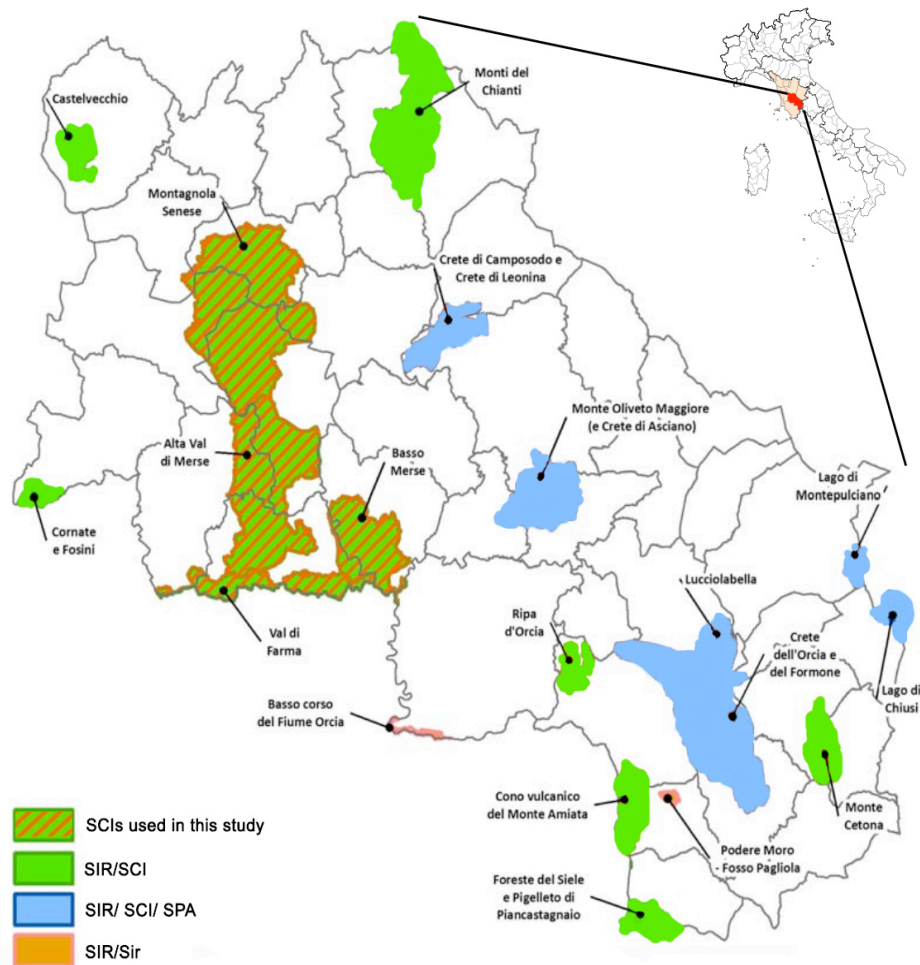


Fig.1 Study area. Crossed areas represent the 4 Sites of Community Importance in the Province of Siena used in this research (Montagnola Senese, Alta Val di Merse, Basso Merse, Val di Farma). Map modified from Servizio Aree Protette della Provincia di Siena (2013).

SIC Montagnola Senese

The site (137.44 km²) includes a wooded dorsal that reaches a maximum altitude of 658 meters a.s.l. (Monte Maggio). Prevailing forest type comprises holm oak and thicket forests, mixed oak forests (dominated by *Quercus cerris* and *Quercus pubescens*), timber and fruit chestnut groves. The site hosts many species of community interest including, among birds, the short-toed snake eagle (*Circaetus gallicus*), the red-backed shrike (*Lanius collurio*), the nightjar (*Caprimulgus europaeus*) and the woodlark (*Lullula arborea*); among reptiles, the four-lined snake (*Elaphe quatuorlineata*). Remarkable is the presence of seven bat species related to karst caves: serotine bat (*Eptesicus serotinus*), Savi's pipistrelle (*Hypsugo savii*), long-fingered bat (*Myotis*

capaccinii), greater mouse-eared bat (*Myotis myotis*), Mediterranean horseshoe bat (*Rhinolophus euryale*), greater horseshoe bat (*Rhinolophus ferrumequinum*) and lesser horseshoe bat (*Rhinolophus hipposideros*). Among amphibians, the main species are the Italian crested newt (*Triturus carnifex*), the Italian stream frog (*Rana italica*), endemic of Apennines, and the spectacled salamander (*Salamandrina terdigitata*), endemic to the Italian peninsula.

SIC Alta Val di Merse

The site (94.85 km²) includes hills carved by one of the best preserved sections of the Merse River and its tributaries. Hills, in particular those where the siliceous rocks belonging to the Verrucano group crop out, are mostly covered by deciduous woodlands dominated by *Quercus cerris*. In the lower and cooler part of these slopes, sessile oak woods, chestnut woods, riparian forests of willows, poplars and alders and conifers can be found. Other portions are occupied by open areas, consisting of cultivated fields, fallow and shrubs, including heath portions with *Calluna vulgaris*.

Overall the site shows a high environmental heterogeneity, which reflects into a rich wildlife characterized by many species of Community interest. Among birds, we can find the hen harrier (*Circus cyaneus*), the short-toed snake eagle, the red-backed shrike, the woodlark, the nightjar and the kingfisher (*Alcedo attis*); remarkable is the presence of three bat species: the greater horseshoe bat, the lesser horseshoe bat and Geoffroy's bat (*Myotis emarginatus*). The high wilderness of the site and the quality of water courses is confirmed by the presence of rare predators such as the European pine marten (*Martes martes*) and the wild cat (*Felis silvestris*) and nine species of amphibians, such as the alpine newt (*Triturus alpestris*), the Italian stream frog (*Rana italica*), the spectacled salamander (*Salamandrina terdigitata*), the yellow-bellied toad (*Bombina variegata*), threatened in the province of Siena, and the Italian crested newt.

SIC Basso Val di Merse

The site (41.40 km²) includes hills mostly covered by Mediterranean forests dominated by the holm oak and its stages of degradation. The site was set up mainly for the presence of widespread forest ecosystems, for some rare or endemic species typical of ophiolites and in general, for the high wilderness of the area and the presence of river ecosystems with well-preserved riparian vegetation. The fauna includes many species of conservation interest such the short-toed snake eagle, the black kite (*Milvus migrans*), the European honey buzzard (*Pernis apivorus*), the woodlark, the nightjar; among mammals, we can find the wild cat and the marten, among the amphibians the salamander (*Salamandra salamandra*), one of the less common amphibians in Tuscany, the Italian stream frog and the spectacled salamander. There are also several species of regional and Community interest such as, among invertebrates, the white-clawed crayfish (*Austropotamobius fulcisanus*).

SIC Val di Farma

Located between the provinces of Siena and Grosseto, the site (26.29 km²) took his name by the presence of Farma river. The shape of the valley, deeply carved and with east-west orientation, has led to a remarkable diversity of micro-climates and environments. The area is mainly woodland, with the presence of thermophilic and mesophilic deciduous forests; remarkable is the presence of beech, birch and sycamore, at much lower heights than usual. The European yew, the laurel and the boxwood, are also present as relics in point stations. The site includes species of Community interest which rely on high quality of river environments, such as the Italian stream frog, the spectacled salamander, the yellow-bellied toad, and the white-clawed crayfish; very interesting is the presence of relict populations of alpine newts. Among mammals the wild cat, the marten, the polecat (*Mustela putorius*) and seven species of bats. The site was also set up for the conservation of the otter (*Lutra lutra*), although there are no recent reports.

Sampling design

Background data were collected within a project of plant diversity assessment in the 17 SCIs in the Province of Siena (Chiarucci et al., 2012) and later used by Amici et al. (2013) to evaluate the influence of successional age on vascular plants in the same network. The original sampling design was based on a grid of 1 x 1 km with one random point selected within each cell. A 10 x 10 m plot was then centred in each sampling point, located with a high-precision GPS, for a total of 604 plots.

Only plots classified on the basis of the dominant tree species into evergreen and deciduous oak forests and falling in the selected SCIs (Montagnola Senese, Alta Val di Merse, Bassa Val di Merse, Val di Farma) were considered, for a total of 110 plots. In order to maximize differences in the forest age categories, only those belonging to 3 out of the original five forest age classes were retained (age class 1 $\leq$ 22 years; age class 3 between 38 and 56 years; age class 5 $>$ 77 years), restricting the number of eligible plots to 79 (Amici et al., 2013). Forest age variable identifies the age of continuous forest cover regardless any activity of forest coppicing or management; namely it provides time from which each plot can be classified as forest, giving an indication of the age of forest habitat and a measure of forest continuity (for further information on methods and classification of forest successional age see Amici et al., 2013). Plots have been then scattered through the 4 SCIs in order to obtain a good representative distribution of all forest age classes both in evergreen and deciduous oak forests, obtaining a final subset of 18 plots: 9 in deciduous oak forests and 9 in evergreen oak forests, 3 for each forest age class (Fig. 2).

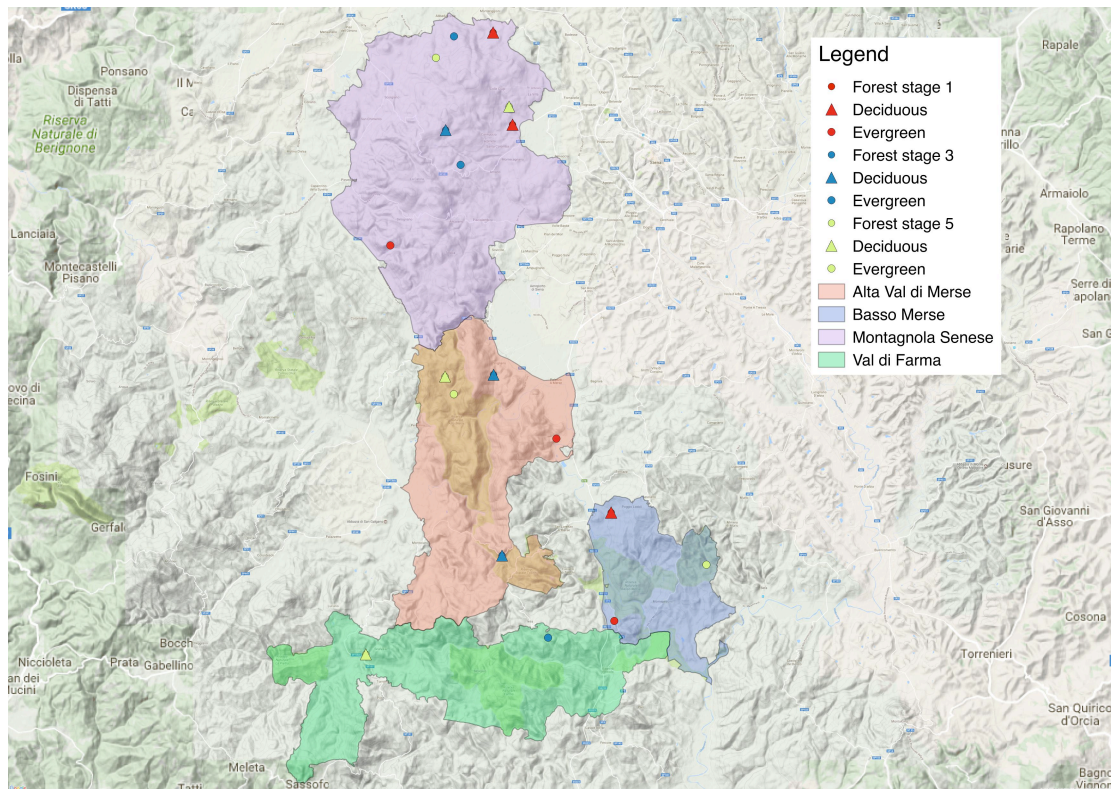


Fig. 2 Map of the 18 sampling units in four SCIs (Alta Val di Merse, Basso Merse, Montagnola Senese, Val di Farma) according to the forest type (deciduous/evergreen) and class age (forest age class 1 $\leq$ 22 years; age class 3 between 38 and 56 years; age class 5 $>$ 77 years).

To highlight the pure effects of forest age on land snail communities taking out other environmental influence, variables most commonly involved in affecting land snail species diversity and composition have been homogenized in all chosen plots. In particular, all sites characterized by acid and inhospitable geological substrate and extreme slope values have been excluded, since these factors could negatively affect species dynamics: it is generally accepted, in fact, that soil pH is one of the strongest predictors of both gastropod species richness and composition while steep slope values could inhibit land snail presence. At each sampling plot the following environmental variables were finally considered for statistical analysis: elevation above sea level (m), terrain slope (degrees), annual average rainfall (mm), annual average temperature ($^{\circ}\text{C}$), tree cover (%) and forest age (age of continuous forest cover according to the following classes: age class 1 $\leq$ 22 years; age class 3 between 38 and 56 years; age class 5 $>$ 77 years, data from Amici et al., 2013) (Tab. 1).

Tab. 1 Summary table of descriptive statistics (mean values, standard deviation, min-max range) of explanatory variables collected in each sampling plot.

	Elevation (m)	Slope (m)	Mean annual temperature (°C)	Mean annual rainfall (mm)	Tree cover (%)
Mean values $\pm$ SD	331.055 $\pm$ 83.57	6.184 $\pm$ 2.60	13.50 $\pm$ 0.85	946.44 $\pm$ 143.19	65.94 $\pm$ 23.04
Min-max ranges	197 - 457	1.27 - 9.98	10.26 - 14.23	803.6 - 1434.1	7-95

The quantitative research was carried out through visual search for a standardized time of 1 h at each 10 x 10 m plot; in each site, a representative sample of debris and leaf litter (5 l) in all microhabitats considered suitable was also collected in order to detect small and very small species (Cameron and Pokryszko, 2005). All live snails, as well as empty shells with intact periostracum, have been considered in order to obtain data on species' relative abundance.

Despite the huge investment in terms of effort and time, the use of this methodology instead of traps, provides a higher amount of information on small land snail species (Menez, 2007; Benocci et al., 2015). Collected material was sieved in a mesh of 10 mm, then dried and finally sieved again with meshes of decreasing size up to 0.5 mm to detect, through the use of a binocular microscope, small and very small specimens. Taxonomy and nomenclature of gastropods mainly follow Manganelli et al. (1995).

Statistical analysis

Species richness and abundance were compared to assess any differences between habitat types as well as forest age classes separately, through the use of box-plots and descriptive statistics. The significance of differences in mean richness and abundances between groups was performed using non-parametric Kruskal-Wallis rank sum test.

Spatially Explicit Rarefaction curves (SERs, Bacaro et al., 2016), initially described by Chiarucci et al. (2009) as Spatially Constrained Rarefaction, were used to compare species richness as a function of the number of sample plots in each habitat type and forest age class separately. Traditional rarefaction

curves (RCs) were firstly introduced for a direct comparison of species richness among data set giving the expected number of species and its variance to an equal-effort basis; furthermore, they enabled to estimate the complete richness of the assemblage, including species that were not detected by the sampling activity, extrapolating forward along a hypothetical projection the accumulation curve and testing the efficiency of sampling design (Colwell et al., 2004). Respect to RCs, SERs are able to also include the autocorrelation structure of the samples, accumulating species of the nearest plots for a given n number of plots sampled (Bacaro et al., 2012). In practice, SERs curves are built by ordering plots according to their minimum geographical distance and then performing an accumulation curve for each ordered sequence. In nature, in fact, individuals are far to be randomly distributed and the same number of sampling units with the same size could determine great difference in species diversity just as a consequence of more environmental complexity of larger areas (Bacaro et al., 2012). Compared case studies demonstrated that the exclusion of spatial autocorrelation could greatly affect results and consequently SERs should be always preferred in rarefaction techniques (Bacaro et al., 2016). R code (*poinpattern* and *SCR* functions) proposed by Bacaro et al. (2012) was used for SERs construction while *specaccum* function in package *vegan* for classic rarefactions.

Non-metric multidimensional scaling (NMDS; McCune and Grace, 2002), based on Jaccard (presence/absence data) and Bray-Curtis (abundance data) dissimilarity matrices, was used to analyse species composition in relation to habitat types and forest age classes. NMDS is an unconstrained ordination technique able to reveal variance structure among species, irrespective of any environmental gradients, producing an ordination based on a distance or dissimilarity matrix. Respect to other ordination methods, the goal of NMDS is to produce a plot in which different objects are placed far apart in ordination space, while similar objects are placed close to one other (Gotelli and Ellison, 2013). In other words, it guarantees the preservation of ordering relationship among objects. NMDS is based on an iterative algorithm that provides for a gradual adjustment of the position of points (representing samples or taxa) in an ordination space until it resembles the observed

dissimilarity matrix as closely as possible. As a result, an NMDS ordination is not a unique solution and a subsequent NMDS analysis on the same set of data and following the same methodology will likely result in a somewhat different ordination.

The iterative algorithm works calculating distances among samples in this starting configuration, typically with an Euclidean metric. These distances are then regressed against the original distance matrix and the predicted ordination distances for each pair of samples are calculated. In a perfect ordination, all ordinated distances would fall exactly on the regression, that is, they would match the rank order of distances in the original distance matrix. The goodness of fit of the regression (also called stress value) is measured based on the sum of squared differences between ordination based distances and the distances predicted by the regression. Stress value is usually used as criterion of goodness of fit in the construction of the optimal representation (the smaller the stress value, the better adaptation of reproduced distances to the original matrix) (Kruskal, 1964a,b). Clarke (1993) suggests the following guidelines for acceptable stress values: <0.05 = excellent, <0.10 = good, <0.20 = usable, >0.20 = not acceptable. Abundance data was $\log(x+1)$ transformed before NMDS.

A multi-response permutation procedure (MRPP) was then conducted to test any differences between groups obtained in NMDS analysis. MRPP nonparametric procedure provides a test of whether there is a significant difference between two or more groups of entities based on permutation test of among and within group dissimilarities (Mielke, 1984; 1991). This test calculates distance matrix (Euclidean default or dissimilarity matrix used in NMDS), it defines average distance in each group and finally calculates delta (A). The statistic A is given as a descriptor of within group homogeneity compared to the random expectation: thus, if two groups of sampling units are really different (e.g. in their species composition), then the average of the within-group compositional dissimilarities ought to be less than the average of the dissimilarities between two random collections of sampling units drawn from the entire population (Mielke and Berry, 2001).

Indicator species i.e species that have a high indicator value for a group of

sites and consequently used as ecological indicators of community or habitat types, environmental conditions, or environmental changes, were determined by analysis of the relationship between abundance and frequency values from a set of sampled plots and classification of the same sites into site groups (habitat types; forest age classes), for each species independently. The method of detecting Indicator Species, firstly described by Dufrêne and Legendre (1997), could be summarized as follow:

1. assignment of indicator value index (IndVal) between a species and each group; for each species j in each cluster of sites k , one computes the product of two values, A_{kj} and B_{kj} . A_{kj} is a measure of specificity based on abundance values whereas B_{kj} is a measure of fidelity computed from presence data:

$$INDVAL_{kj} = A_{kj}B_{kj}$$

2. identification of the group with the highest association value;
3. use of randomization methods (permutation test) to test statistical significance of value.

Indicspecies package in R (de Cáceres and Jansen, 2009) was used for indicator species analysis purpose.

Finally, variation partitioning (ter Braak, 1986; Borcard et al., 1992; Legendre and Legendre, 1998; Borcard and Legendre, 2002; Borcard et al., 2004) was used to quantify the relative effect of environmental (elevation, slope, mean annual rainfall, mean annual temperature, forest cover) and spatial variables (X-Y coordinates and PCNM) as well as forest age effect in shaping variation in community composition. Also the shared fraction has been evaluated. Variation partitioning was assessed using redundancy analysis (RDA) (Legendre and Legendre, 1998) considering all species checklist. For further information about PCNM and variation partitioning method see chapter II, pp. 46-47.

Vegan package (Oksanen et al., 2016) in RStudio 0.99.473 version (RStudio Team, 2015) was used for processing.

RESULTS

A total of 2518 individuals and 25 species were recorded in 18 selected forest plots. Of these species, 15 were classified as true forest species and 10 as generalist non-forest species (Tab. 2)

Tab.2 Mean change in abundances between forest age classes. P-values were calculated using Kruskal Wallis test (in bold the nearly statically significant ones for $\alpha=0.05$). * indicates True forest species (TFS). Only species occurring in ≥ 4 plots were considered.

Species	Mean change $\pm$ SE			n° plot	p-value ($\alpha=0.05$)
	Forest age class 1	Forest age class 3	Forest age class 5		
1) <i>Acanthinula aculeata</i> *	18.2 $\pm$ 17.8	24.8 $\pm$ 12.5	25.5 $\pm$ 20.7	17	0.6157
2) <i>Argna biplicata</i> *	0.8 $\pm$ 2	0	0.2 $\pm$ 0.4	2	
3) <i>Candidula unifasciata</i>	0	0.2 $\pm$ 0.4	0	1	
4) <i>Ceciloides acicula</i>	0.3 $\pm$ 0.5	0	0.2 $\pm$ 0.4	3	
5) <i>Cepaea nemoralis</i> *	2.2 $\pm$ 3.5	1.8 $\pm$ 3.3	1 $\pm$ 1.1	9	0.8995
6) <i>Chilostoma planospira</i> *	1.5 $\pm$ 2.3	0.7 $\pm$ 1.2	0.2 $\pm$ 0.4	6	0.3985
7) <i>Cochlodina bidens</i> *	6.2 $\pm$ 5.3	9.2 $\pm$ 11.6	11.8 $\pm$ 13	13	0.8245
8) <i>Discus rotundatus</i> *	9.7 $\pm$ 10.7	0.7 $\pm$ 1.6	18 $\pm$ 33.3	11	0.05121
9) <i>Granaria illyrica</i>	0.2 $\pm$ 0.4	0.2 $\pm$ 0.4	0.2 $\pm$ 0.4	3	
10) <i>Helicodonta obvoluta</i> *	4.3 $\pm$ 7.4	2.2 $\pm$ 2.6	8 $\pm$ 10.5	11	0.6676
11) <i>Hygromia cinctella</i>	1.7 $\pm$ 1.6	2 $\pm$ 4	0.3 $\pm$ 0.8	7	0.2486
12) <i>Merdigera obscura</i> *	1.3 $\pm$ 2.8	0.2 $\pm$ 0.4	0	3	
13) <i>Monacha cantiana</i>	0.5 $\pm$ 0.5	0.7 $\pm$ 0.5	0	7	0.05669
14) <i>Monacha cartusiana</i>	0	0.2 $\pm$ 0.4	0	1	
15) <i>Oxychilus clarus</i> *	0	0.5 $\pm$ 0.8	0	2	
16) <i>Oxychilus cf. draparnaudi</i> *	2.8 $\pm$ 3.2	1 $\pm$ 0.9	2.7 $\pm$ 2.3	12	0.45222
17) <i>Oxychilus uzielli</i> *	5.3 $\pm$ 13.1	2.2 $\pm$ 4.8	5.7 $\pm$ 12.9	5	0.8546
18) <i>Pomatias elegans</i>	36.5 $\pm$ 48.9	5 $\pm$ 5.3	15.7 $\pm$ 34.2	11	0.3351
19) <i>Punctum pygmaeum</i> *	44 $\pm$ 53.2	38.5 $\pm$ 35.4	13 $\pm$ 9	17	0.2763
20) <i>Retinella olivetorum</i> *	2.5 $\pm$ 3.7	1.3 $\pm$ 2.8	0.5 $\pm$ 1.2	6	0.4532
21) <i>Solatopupa juliana</i>	0	2.2 $\pm$ 4.4	0	2	
22) <i>Trochoidea pyramidata</i>	0.2 $\pm$ 0.4	0	0	1	
23) <i>Truncatellina callicratis</i>	7.3 $\pm$ 11	5.2 $\pm$ 12.7	0	4	0.1635
24) <i>Vitrea contracta</i> *	0	0	5.7 $\pm$ 12.7	1	
25) <i>Vitrea subrimata</i> *	19 $\pm$ 14.4	25.5 $\pm$ 20.7	23.2 $\pm$ 7	17	0.6747

The number of individuals recorded per site ranged from 21 to 370 and the number of species from 4 to 15. Most common land snail species were *Acanthinula aculeata*, *Punctum pygmaeum* and *Vitrea subrimata* (17 out of 18

plots). Four singleton were recorded: *Candidula unifasciata*, *Monacha cartusiana*, *Trochoidea pyramidata* and *Vitrea contracta*. No proper alien species was found and ten species are considered as Least Concern in IUCN Red List.

SERs concerning variation in species richness among habitat types (D deciduous oak forest; E evergreen oak forest), showed a lower amount of accumulated species given the same number of sampled plots with respect to the traditional exact-based method (Fig. 3); considering patterns inside SERs, no appreciable differences emerged between vegetational types; however, while deciduous forests showed an asymptotic pattern, evergreen reached no saturation indicating that the total number of species likely exceeded the number sampled.

SERs for forest age classes suggested some contrasts in cumulative species richness: despite an initial steep climb, forest age class 1 reached an asymptote after just four sampled plots, supporting a comparable richness to intermediate age class 3, which reversed its relative ranking, overcame forest age class 1 and reached itself an asymptotic pattern; lowest species richness was found in the the oldest age class 5. As for habitat types, SERs increased less steeply than the RCs curves, resulting in lower estimates of richness at a given extent.

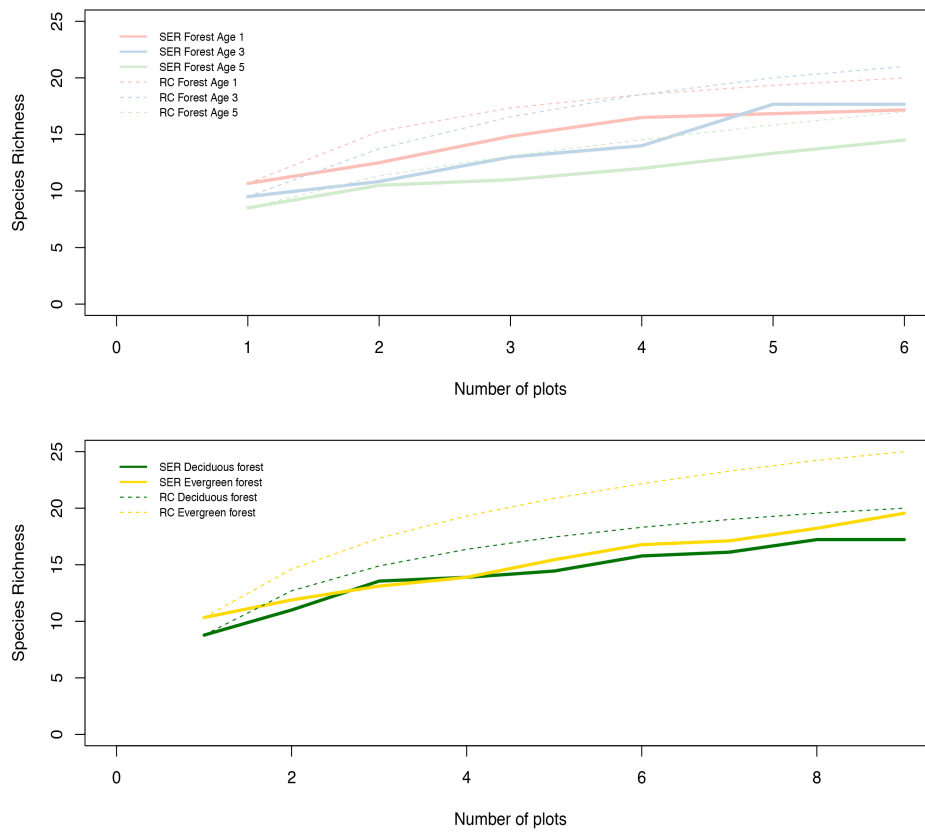


Fig. 3 SERs and RCs curves for habitat types (a, above) and forest age classes (b, below).

Considering descriptive box-plots, a marked decrease in total species with increasing forest age was observed (Fig. 4). However, despite the trend, a Kruskal-Wallis test for total richness showed no significant difference between forest age classes.

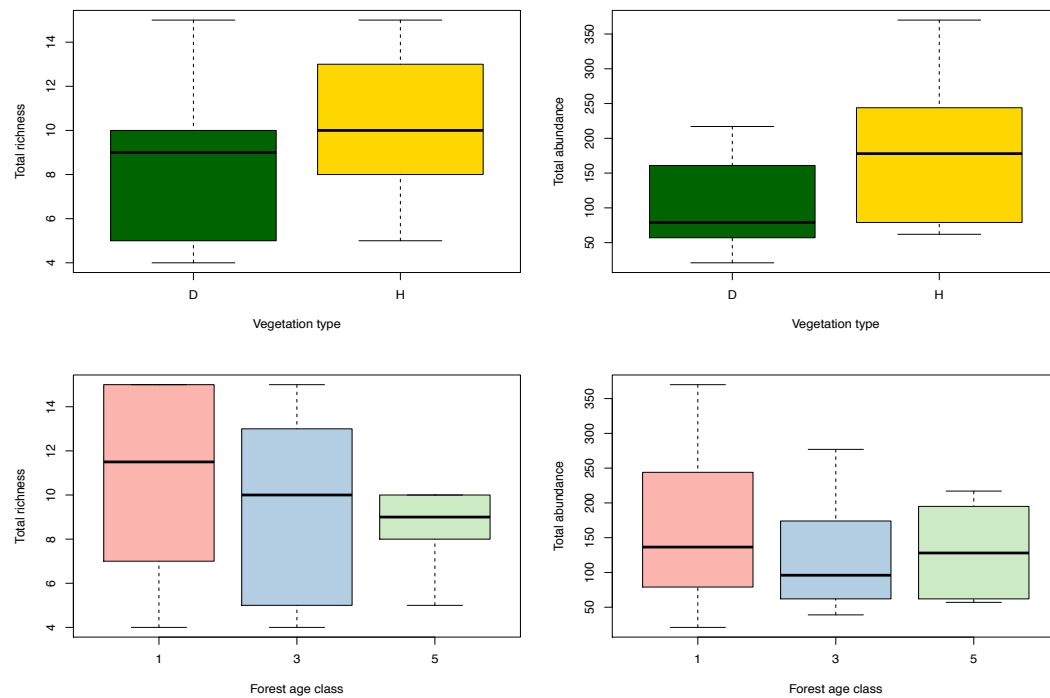


Fig. 4 Differences in the number of total species (total richness) and specimens (total abundance) between forest types (D deciduous oak forest, H holm oak forest) and forest age classes (1 <22; 3 38<x<56; 5 >77).

No distinction was also found between evergreen and deciduous oak, both considering total species richness and abundance (Fig. 4). Even examining forest species and generalist species separately, some trends were evident but not significant (Fig. 5), as confirmed by Kruskal-Wallis test: while forest species richness and abundance showed a homogeneous trend between age classes, a decrease in the richness and abundances of generalist non-forest species with increasing forest age was rather evident although not significant.

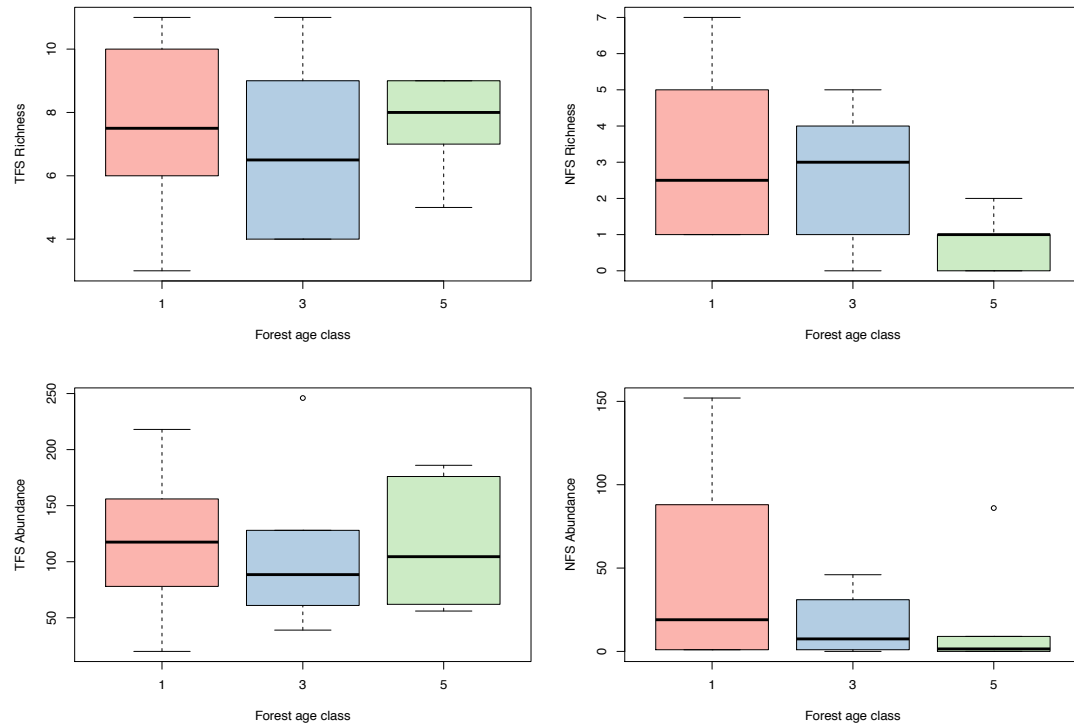


Fig. 5 Differences in the number of total species (total richness) and individuals (total abundance) of true forest species (TFS) and non-forest species (NFS) between the three classes of forest age. Boxplots show median, interquartile range and outliers.

Despite the partial overlap between ellipses with 95% confidence interval for habitat types, no clear differences in species composition were found in NMDS ordination (Fig. 6) and MRPP analysis as well (Tab. 3), both considering presence/absence and abundance data. NMDS analysis did not reveal a clear separation in community structure between forest age classes either. No qualitative differences were found both using occurrence and abundance data: the youngest forest age class (forest age class 1) showed the highest variability, clustering partially the other two age classes (forest age class 3 and 5) inside the 95% confidence ellipse boundaries. MRPP analysis revealed no differences between multidimensional groups (Tab. 3).

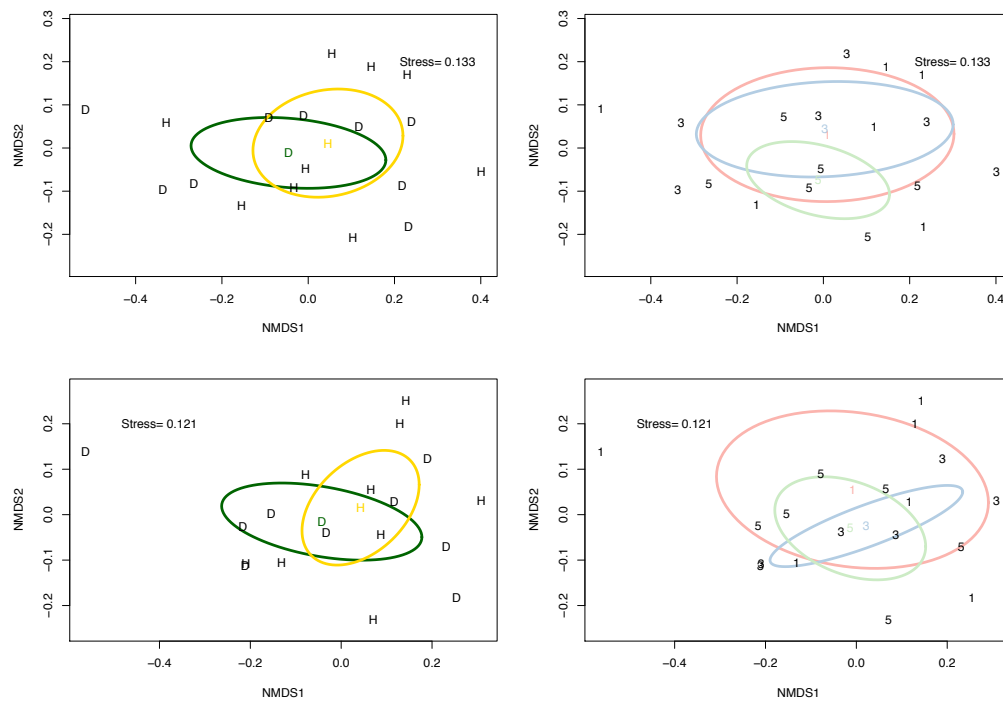


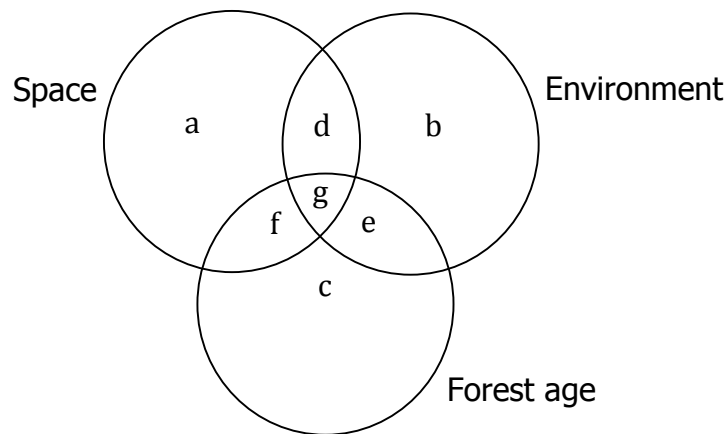
Fig. 6 Non metric multidimensional scaling for presence/absence data (above) and abundances (below) considering habitat type (first column) and forest age classes (second column).

Tab. 3 Multi-response permutation procedure output for habitat type and forest age classes considering both presence/absence (Jaccard dissimilarity index) and abundance (Bray-Curtis dissimilarity index) data.

	Delta	Chance corrected within-group agreement A	Significance of delta	Number of permutation
p/a				
-deciduous	0.6115	-0.02671	0.988	999
-holm oak	0.5828			
Abundance				
-deciduous	0.4786	-0.002616	0.476	999
-holm oak	0.4369			
p/a				
-Age class 1	0.6108	0.02553	0.167	999
-Age class 3	0.5954			
-Age class 5	0.4941			
Abundance				
-Age class 1	0.5464	0.004289	0.367	999
-Age class 3	0.406			
-Age class 5	0.4114			

Also IndVal analysis showed no species as perfect indicator for any of the three classes and just two out of 25 showed marginally not significant mean changes in abundances between age categories after Kruskal Wallis rank sum test analysis: *Discus rotundatus* and *Monacha cantiana* (Tab. 2).

Fig. 7 Variation partitioning in terms of variation explained by: space (X1) (X-Y coordinates and PCNM), environment (X2) (elevation, slope, mean annual temperature, mean annual rainfall, tree cover) and forest age (X3) (age class 1 $\leq$ 22 years; age class 3 between 38 and 56 years; age class 5 $>$ 77 years). In Venn diagram, a, b and c are unique effects of space and environment and forest age, respectively; while d, e, f and g are fractions indicating their joint effects. No one of these fractions were significant for $p < 0.05$.



	Df	R ²	Adjusted R ²	Testable
[a+d+f+g] = X1	9	0.57	0.07	TRUE
[b+d+e+g] = X2	5	0.26	-0.05	TRUE
[c+e+f+g] = X3	1	0.07	0.01	TRUE
[a+b+d+e+f+g] = X1+X2	14	0.83	0.04	TRUE
[a+c+d+e+f+g] = X1+X3	10	0.60	0.02	TRUE
[b+c+d+e+f+g] = X2+X3	6	0.33	-0.03	TRUE
[a+b+c+d+e+f+g] = All	15	0.90	0.15	TRUE
Individual fractions				
[a] = X1 X2+X3	9		0.18	TRUE
[b] = X2 X1+X3	5		0.13	TRUE
[c] = X3 X1+X2	1		0.11	TRUE
[d]	0		-0.17	FALSE
[e]	0		-0.15	FALSE
[f]	0		-0.09	FALSE
[g]	0		0.15	FALSE
[h]=Residuals			0.85	FALSE

Concerning variation partitioning results (Fig. 7), the largest fraction of the explained variation was accounted for by pure space (18%), followed by the shared variation (15%) and the pure effect of environment (13%), while forest successional age accounted for just 11%. Negative values of adjusted R^2 should be interpreted as zeros: they correspond to cases where the explained variables explain less variation than random normal variables would (Legendre, 2008). None of these fractions retains a statistical significance ($p > 0.05$).

DISCUSSION

Damesin et al. (1998) worked about key factors distinguishing functions of deciduous and evergreen oaks in South France: although evident divergences in terms of lignin and cellulose (with higher concentrations in *Q. ilex*) and in proteins (higher concentrations in *Q. pubescens*), authors revealed no significant difference in biochemical compositions of *Q. ilex* and *Q. pubescens* mature leaves. Also in our results the two types of oak litter do not produce significant differences in terms of richness, abundance and species composition according to the descriptive box-plots and the NMDS results.

Forest age as surrogate for forest continuity parameter seemed not to be a key factor in controlling species richness and abundances: total richness effectively decreased as a possible result of competitive interactions, habitat specializations and loss of microhabitats heterogeneity imposed by the closure of forest canopies but this result has no statistical meaning. Even considering forest species and generalist/non forest species separately, some trends are still evident but not significant, as confirmed by Kruskal-Wallis test.

Regarding species composition, the initial hypothesis that recent forest patches host more alien/generalist/ecotonal species (such as *Pomatias elegans*, *Monacha cantiana*, *Rumina decollata*, *Cornu aspersum*) than older patches was just partially supported: the older plots were not characterized by a well defined cluster of exclusive species but rather by the presence of a mixed assemblage of generalist species widespread in all forest age classes. Forest age may have an effect on the number of forest and open habitat specialist species since open habitat species can easily colonize stands after disturbance episodes (clear cutting or forest succession), persisting in young plantations (Gallé et al., 2014). NMDS showed in fact a trend with a higher presence of generalist and open habitat specialists in younger forest classes but this assemblage persists in older ones being widely distributed along all age classes, not showing statistically significant differences in species composition between forest age categories. Despite the common belief that successional age is linked to species invasions (Roser and Montserrat, 2006; Flory and Clay, 2009), checklist did not comprehend alien species. Alien

invasive mollusc species in Italy are usually linked to freshwater and ruderal environments rather than pure forestal habitats (Cianfanelli, 2009); nevertheless alien entities such as the land snail *Paralaoma servilis* are nowadays well widespread in all forest environments, even those with high habitat naturalness, and the presence of these species in the regional pool is robust. In the case of some forests in Siena Province, a certain degree of isolation and the absence of disturbance together with local environmental factors, could act as filter to the invasion from alien snail species from other areas, especially surrounding mosaic environments. A separate issue concerned slug alien species: slugs were not collected since the sampling method used was not suitable for complete inventories of this group of molluscs (Cameron and Pokryszko, 2005). However, the Mediterranean *Deroceras invadens* is nowadays one of the most widespread alien entities all over Italy, occurring both in disturbed sites and natural habitats such as woodland and grassland. Its presence, although not considered, was recorded in most sampling sites in Siena province.

The issue around the age concept is still very controversial: a deeper analysis of the state of the art of age effect on forestal biodiversity revealed no common response pattern. In addition to having considerable inconsistency of terminology in the literature, many researchers failed to give a clear definition of what age is as well as how it is defined and measured. This fact entails high ecological implications since tree age and forest age (or continuity) are very different and not interchangeable ecological concepts, which interact each other in a multiple way. Trying to separate these effects is therefore really challenging. Gallé et al. (2014), for example, did not detect a direct effect of forest age (however, no information is furnished about what forest age really is) on the spider assemblages in lowland poplar forests in Hungary; on the contrary, Koivula et al. (2002) found higher species richness and diversity of carabid beetles in young forest stands due to the invasion of open-habitat species, while many forest species were scarce in the young forests and gradually became more abundant towards the older forest age classes. Also Moning and Müller (2008) found differences in species richness and composition considering diverse organisms (lichens, molluscs, breeding

birds) in young vs. unmanaged old-growth forests in Central Europe; however in their study the stand ages ranged up to around 300-400 years, an extremely wide time range.

Dittrich et al. (2013) worked on the ground epiphyte vegetation in old growth and secondary forests with similar tree age but different forest continuity values in central Europe founding no difference between the two stands types: the authors' attempt to separate the effect of forest age on plant diversity from that of tree age resulted in no effect of forest continuity, showing that a period of 200 years was sufficient for the secondary forest to be colonized by the same assemblages of plant species found in older forests. Although doubts arise to which extent results could be generalized, Dittrich and colleagues did not reject a priori the possibility that forest continuity beyond a time span of 200 years may have a remarkable effect on diversity. Later, Amici et al. (2013) confirmed this hypothesis revealing that forest successional age was an important factor affecting both plant richness and composition at least in Mediterranean landscape, despite a reduced time span of <80 years.

Our research on land snail communities, though conducted on the same environmental framework of Amici et al. (2013), demonstrated the presence of a process acting but non sufficient to highlight differences in land snail assemblages between age classes. Concerning soil organisms, the general framework is even more complex compared to vegetation and much more time may be necessary to led land snails to differentiation: Moning and Müller (2008) reported threshold values for the diversity of molluscs range from 100 to 170 years in sub-montane forests and from 160-220 years in mixed montane forests of oak and coniferous trees. However, they did not take into account forest continuity but rather stand age of oldest tree as surrogate factor for habitat persistence in an attempt to identify significant forest age threshold ranges for the occurrences of lichens, molluscs and breeding birds. According to Moning and Müller (2008) forest continuity and habitat continuity are not synonyms: the distinction is fundamental since the first one does not necessarily entail continuity of habitat structure, especially for those organisms relying on specific habitat characteristics.

The loss of forest continuity effect on land snail assemblage could reveal a greater importance of habitat structure at local scale rather than the continuity of forest cover in shaping diversity, and this was confirmed by variation partitioning results too, where pure forest successional age fraction described the lowest portion of compositional variation. The lack of significance in all the analysed elements of variability could be explained by two phenomena: 1) a natural low variation of parameters (climate, topography) across the studied area (as showed by low standard deviations in summary table of explanatory variables, Tab. 1), and a mediated homogenization of all variables involved in affecting land snail communities (especially geological substrate and therefore soil pH and slope) in order to extrapolate the real effect of forest continuity. For all these reasons, the absence of a pure forest continuity effect detained higher ecological meaning. As a consequence of low mobility and habitat specialization, determinants of gastropod richness and abundances may vary strongly over few metres: the presence of a single old/very old tree in the middle of a highly disturbed matrix could be a sufficient prerequisite to guarantee ecological conditions required for the maintenance and survival of well defined populations.

Furthermore, land snails are not xylophage organisms neither rely on fresh plants for certain stages of development as it happens for other groups such as butterflies; rather, the diet of land snail is based on vegetables, stems, fungi, algae. For this reason, since the unique well documented relationship is the preference for certain tree species or forest types due to leaf-litter requirements (coniferous vs. deciduous trees), there is not a direct relationship to plant species composition but more likely an indirect effect in terms of forest coverage, presence of coarse woody debris, refuges, holes and shelters.

A study about forest spiders in lowland poplar forests in Hungary revealed that assemblage composition and diversity were determined uniquely by structural changes of the habitat while age and canopy closure did not show significant influence (Gallé et al., 2014). Results obtained were comparable with findings of this research since the age of the forests investigated by Gallé et al. (2014) ranged between 6 and 48 years. Authors revealed that the

typical structure of old growth forests may occur in young stands independently of stand age and this justifies the absence of significant effect of forest continuity on diversity over a short time scale, less than 50 years in Gallé et al. (2014) and <80 years in our case.

So far we highlighted the importance of structural complexity of forests as well as the presence of a defined habitat structure for maintaining forest biodiversity without taking into account clear-cut practices. In general, natural forests and low impacted forests host more species than managed ones (Økland et al., 2003), but the effect is highly variable and taxon-specific (Paillet et al., 2010; Gallé et al., 2014). A study about short-term conservation thinning effect on land molluscs in mixed oak forest in Sweden revealed that conservative thinning reduces both species richness and overall abundance of molluscs; some mollusc species (5 out of 53) respond negatively but anyway the negative effect of thinning should be interpreted as mild, and probably transient. One reason may be that many trees, shrubs and other forest structures (fallen logs, stumps) remained after the treatment, providing refuges, holes and shelters and maintaining habitat structure continuity (Rancka et al., 2015). Furthermore, land snails could benefit from higher pH in regenerating forests (Ström et al., 2009).

In our case it was not possible to trace back with certainty the age of the last clearing cut for all the investigated plots, which is on average around 50 years ago. However, since decline in richness and abundance is recovered within a few decades by a quick increase, the impact of clear-cut could be considered as negligible.

In conclusion, our time range was not narrow enough to show a clear colonization pattern characterized by higher molluscs abundance and richness in regenerating and young plantations and at the same time not large enough to show a clear differentiation in the species composition as it usually happens between young and old growth forests.

CONCLUSION

- While the effect of acidic litter on land snail assemblages is well known, no difference between evergreen and deciduous oak litter in terms of richness, abundance and species composition occurred;
- Despite results obtained by Amici et al. (2013) for vascular plants, forest age as surrogate for forest continuity parameter seemed not to be a key factor in controlling land snail species richness and abundance;
- Regarding species composition, the presence of an assemblage of generalist species widespread in all forest age classes likely contributed to the absence of a perfect indicator, showing that a process is acting but still not sufficient to differentiate the assemblages;
- Habitat structure more than forest age is the factor mostly involved in affecting communities: due to the very high specialization and poor mobility of molluscs, suitable environmental conditions and the presence of just few old/very old trees serving as shelters, are sufficient conditions to sustain land snail communities ensuring survival and protection.
- In conclusion, different aged forests may have similar land snail assemblages if they have the same habitat structure.

CHAPTER II

The role of dispersal and local environment in urban land snail communities: the example of three cities in central Italy

Abstract

Ecologists increasingly appreciate the central role that urban biodiversity plays in ecosystems, however much urban biodiversity is neglected, especially some very diverse groups of invertebrates. For the first time in southern Europe, land snail communities are analysed in four urban habitats along a geographical gradient of three cities, using quantitative methods and assessing the relative roles of local environmental conditions ("distance from sea", "distance from city centre", "vegetation cover") and spatial effects by principal coordinate analysis of neighbour matrices, redundancy analysis and variation partitioning.

A total of 53 species was recorded, a richness similar to that of natural areas. At habitat level, species richness did not show a clear increasing trend from more to less urbanized habitats, but rather a homogeneous pattern. At city level, study areas hosted rather heterogeneous species assemblages and biotic homogenization did not seem to have any impact; indeed, only three species could be considered alien.

Variation partitioning showed that land snail communities were mostly structured by environmental factors, even when spatial structures independent of measured environmental variables were included: "vegetation cover" and "distance from city centre" were the environmental variables that explained most of the variation in species composition. The lack of strong spatial structure also unexpectedly suggested that transport by humans aids dispersal of organisms with low mobility, which are usually limited by spatial constraints in natural environments. These results provide ecological and conservation implications for other invertebrate groups, suggesting to set priorities in management strategies that include habitat conservation at local scale.

INTRODUCTION

Nowadays more than half of the world's population lives in large urban settlements and this trend is going to increase since by 2050 66 % of the world's population will be urban (United Nations, 2014). However, despite the status of most common habitat worldwide (Grimm et al., 2008), urban environment has been traditionally neglected by researchers (Maurer et al., 2000; Beninde et al., 2015; Hartop et al., 2015,) as considered unnatural place poor in biodiversity (Grimm et al., 2008; Paul and Meyer, 2008; Güneralp and Seto, 2013,)), subjected to biotic homogenization due to increasing number of non-native species (Lockwood, 2004; McKinney, 2006; La Sorte et al., 2007; Williams et al., 2009) as well as to habitat fragmentation (McKinney, 2002; Cadenasso et al., 2007). Although urbanization is often considered one of the major causes of biodiversity and habitat loss (Czech et al., 2000; McKinney, 2006; Grimm et al., 2008; Hahs et al., 2009), the influence of urban land use on biodiversity is more complex than expected (McKinney, 2008).

In last years the common belief that cities only negatively affect surrounding environment has been challenged. Research investigating biodiversity relationships at a finer scale is rich and constantly growing (Beninde et al., 2015), covering different taxonomic groups such as birds, invertebrates, plants and more recently even zooplankton (Mimouni et al., 2015). What researchers are discovering is surprisingly incredible: cities can protect vulnerable and particular ecosystems (UNEP, 2005) as well as high levels of biodiversity (Aronson et al., 2014), even including native species (Barratt et al., 2015), sometimes showing the same dynamic interactions as in nature (Baldock et al., 2015, Beninde et al., 2015). Cities may also host species new to science or of biogeographical interest: the recent BioSCAN Project (Hartop, 2015) in the city of Los Angeles, has discovered thirty new fly species and one first reported from the Nearctic Region.

Further studies have pointed out the extreme ecological importance of particular urban habitats in relation to their function in species dispersal (Angold et al., 2006). These transient habitats work as corridors or stepping

stones, provide ecosystem services, increase urban wildlife habitat and can include gardens (Thompson et al., 2003; Loram et al., 2008), abandoned areas (Knapp et al., 2008) as well as completely artificial and human made habitats such as city walls or living roofs (Oberndorfer et al., 2007; Lundholm and Richardson, 2010; Francis and Lorimer, 2011). Finally, urban green areas could have a positive influence on the quality of human life (Fuller et al., 2007; Mitchell and Popham, 2008; Carrus et al., 2015; Taylor and Hochouli, 2015), in terms of recreational aspects and public participation in scientific activities. In that regard, many projects (i.e BIMBY "Biodiversity in my (Back) Yard", Beumer and Martens, 2015) have been developed to promote citizen engagement in exploring biodiversity in urban habitats (i.e citizen science).

Although urban ecology is becoming a field of growing interest, less charismatic invertebrates such as land snails are still poorly known. It is quite surprising since, according to the IUCN Red List, molluscs represent the group most impacted by (often completely unnoticed) extinctions (Lydeard et al., 2004; Régnier et al., 2009). The current lack of scientific documentation together with a low mobility, could easily lead to land snails being one of the most susceptible organisms to anthropogenic disturbance and habitat fragmentation (Douglas, 2011). At this time worldwide malacofauna consists of several rare and endangered species resulting in 20% of all threatened animals, and 37% of known animal extinctions since 1600 A.D. (Seddon, 1998). Land snails are numerous and diverse, they live in almost all terrestrial environments serving a key role in worldwide trophic webs (Baur and Baur, 1993; Douglas et al., 2013): snails are preyed and consumed by a great diversity of animals, from insects to salamanders and toads, lizards and snakes, birds and mammals. Some species of fireflies (i.e *Pyrocoelia pectoralis*, *Cratomorphus spp.*) mainly feed on land snails (Viviani, 2001; Wang et al., 2007); many birds require a huge quantity of calcium carbonate for the formation of eggshells, relying on land snails for *Ca* supplementation (Mänd et al., 2000). Besides low mobility makes these organisms perfect bioindicators for environmental quality (Cuttelod et al., 2011; Rota et al., 2016) together with a good bioaccumulation capacity (Pauget et al., 2013).

In central Europe, Horsák et al. (2009) and Lososová et al. (2011) first

compared plant and land snail diversity in different urban habitats across several cities, using a standardized sampling protocol. Later, Chytrý et al. (2012) highlighted the importance of not only the environmental but also the space effect in shaping urban communities structure across groups of different size and with different dispersal ability (i.e. subaerial cyanobacteria and algae, vascular plants, land snails, grass, animal-dispersed trees and shrubs and wind-dispersed trees and shrubs).

In ecology, space in fact detains a key role in shaping species assemblages distribution; spatial heterogeneity is often not a result of a single cause but it may be driven by a wide range of different factors, which interact each other in a multiple way (Borcard et al., 1992): discovering all the hidden spatial structures at each scale as well as all the processes involved in their creation is therefore a crucial step to well understand ecological patterns underlying natural communities. The traditional niche based model assumes that the local environment itself controls for the spatial structures found in species assemblages: in other words the interaction between each species and its physical environment may model species distribution as a result of adaptation and interaction processes to different habitat characteristics (Borcard et al., 2004). According to this model, it is the environment itself, through the provision of several resource types and habitat conditions, that determines how similar / dissimilar the communities are, independently of spatial proximity or distance of assemblages (Caruso et al., 2012). However, spatial structures may sometimes be generated by the species assemblages themselves and can persist irrespective of environmental variation (neutral model) (Hubbell, 2001). In this case space effect is directly described by community dynamics through dispersal limitations, demographic stochasticity or competitive/ predatory processes without any environmental influence (Legendre et al., 2009).

Advanced and nowadays well established multivariate techniques such as principal coordinates of neighbour matrix (PCNM) and variation partitioning (ter Braak, 1986; Borcard et al., 1992; Legendre and Legendre, 1998; Borcard and Legendre, 2002; Borcard et al., 2004) have allowed to disentangle and quantify the relative and pure effect of both environment and space in

shaping variation in community composition (beta diversity) as well as the fraction of species variation explained by space and environment together, providing an useful tool to decompose the amount of variation uniquely attributable to measured environmental factors and separate it from spatial structures potentially created by unmeasured environmental factors but also population and dispersal dynamics independent of environmental factors (Legendre et al., 2009).

In this context, research papers relating to any aspect of urban land snails biodiversity in a quantitative and spatially explicit way, are missing in southern Europe. It is a major gap in scientific knowledge since Mediterranean is traditionally known as one of the world's richest areas in terms of species diversity, but also one of the most threatened, mainly by anthropogenic impact, especially habitat loss and degradation (Cuttelod et al., 2008). In this geographic context, thanks to an extremely complex biogeographical framework, Italy is a huge biodiversity sink: concerning molluscs, it is the second one in Europe just after Greece in terms of species and subspecies richness (Cuttelod et al., 2011).

Here for the first time we also take into account the spatial variation component in urban land snail communities in different habitats along a gradient of three cities in Tuscany (Italy). The aims of this chapter are (1) to analyse and compare the land snail biodiversity in four different human made habitats along a gradient of three cities in central Italy in terms of richness and potential differences in species composition (alpha α , beta β and gamma γ diversity) (2) to evaluate the presence of species considered to be of Conservation Concern to EEC as well as the impact of alien species, if any; (3) to detect processes involved in shaping land snails community structure by partitioning the pure effects of "environment" (measured environmental factors) and "space" (unmeasured environmental variation, biotic interactions that structure population spatially even within an environmentally homogeneous habitat and also dispersal limitations) as well as the shared effect on the distribution of urban land snail communities by means of multivariate variance partitioning; (4) to understand which environmental variables are most significantly involved in explaining variation in species

composition; (5) to evaluate and compare obtained results to those of previous studies in central Europe in order to detect major biogeographical differences in study areas, similitudes in species composition, any difference in variation in communities structure associated to purely spatial or environmental effects as well as new research ideas.

MATERIALS AND METHODS

Study area

Three cities in central Italy, along a geographical gradient of about 100 km from the Tuscan west coast to the Northern Apennine chain, were investigated. Cities differ under several aspects (i.e altitude, population, date of foundation) but all share the presence of historical city walls.

Grosseto (10 m a.s.l., 82.284 inhabitants, 474 km²) is located in southwestern Tuscany, 10 km away from the Tyrrhenian coast; it lies on the alluvial plain of the Ombrone river, in Maremma, the largest plain area in southern Tuscany. Grosseto was founded in High Middle Ages while the Medicean Walls were begun in 1574, replacing those from the 12th-14th centuries. It has a Mediterranean climate, characterized by very mild wet winters and very hot and dry summers.

Siena (322 m a.s.l., 52.774 inhabitants, 118 km²) is located in the central part of Tuscany; it is the highest one above sea level, with the lowest number of inhabitants, founded in Roman times, surrounded by naturalized and well preserved green areas which still lie inside the city perimeter. As well as Grosseto, Siena historical city center, declared by UNESCO a World Heritage Site, is surrounded by medieval walls which include Fortezza Medicea, a fort built in the city between 1561 and 1563.

Arezzo (296 m a.s.l, 99.232 inhabitants, 386 km²) is located further to eastern Tuscany border, near Apennines. It is the oldest city concerning the date of foundation, believed to have been one of the twelve most important Etruscan cities. It has a continental climate due to its location straddling the Arno Valley and Val di Chiana with the Apennine nearby. In the upper part of the town are the cathedral, the town hall and the Medici Fortress together with Medicean Walls.

Sampling design and data collection

Sampling design included four urban habitat types over a gradient of human impact, i.e from heavily affected and disturbed sites to quite well preserved

naturalized habitats (Lososová et al., 2011); aerial Google Earth and city maps were used as guides in order to choose two well-scattered and spatially independent sites for each habitat type in the three areas, for a total of 24 sites (Fig. 8).

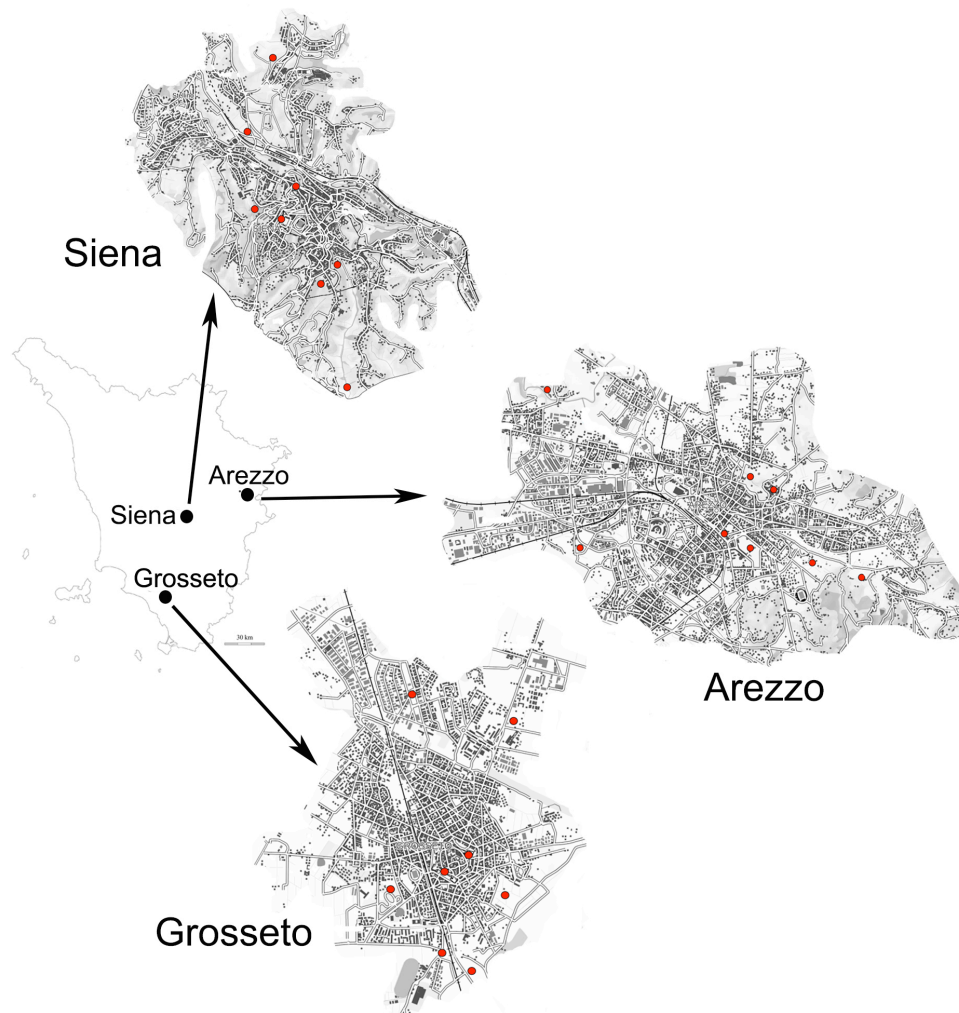


Fig. 8 Map of the study area and location of sampling sites (red dots) in each city.

Habitat types are in part taken and adjusted from the standardized protocol of Lososová et al. (2011): 1. WALLS: historical walls in the city center; 2. PARKS: city parks and public gardens with old deciduous trees (tree cover 10–50%) and frequently mown lawns; 3. E-SUC: early successional sites, strongly disturbed 1–3 years earlier, with prevailing bare ground and sparse vegetation cover, usually in or around construction and industrial sites; 4. WOOD: quite preserved wood patches, with well-structured vegetation (old and young trees, especially holm oak with scattered shrubs).

A qualitative research (presence/absence) was carried out through visual search for a standardized time of 2 h at each site; the choice of using temporally standardized sampling strategy allows maximizing species number lists through the possibility to include a huge number of heterogeneous microhabitats and environmental conditions. This methodology also enables to avoid the logistic problems related to sampling activity on vertical surface (i.e city walls), whereon a common standard size plot could be hard to arrange. Sampling strategy followed method described in Chapter I page 19.

Explanatory variables

Two groups of explanatory variables were calculated for each site and used to model land snail species richness and composition: (1) environmental variables; and (2) spatial variables. Environmental properties of each site were quantified by three variables: 1) distance from city center ("*Dist_center*") measured as linear distance from the city cathedral to each sampling site (Horsák et al., 2009); 2) distance from sea ("*Dist_sea*") assessed as minimum linear distance from each site to the nearest coastline, and 3) vegetation cover ("*Tree_cover*"), ranging from 0 to 100 and expressed as percentage of trees respect to the total area of the sample plot. Spatial variables comprised X-Y geographical coordinates and all the spatial structures at different scales detected through PCNM method.

Statistical analysis

Observed and estimated species richness

Alpha, beta and gamma diversity were used to assess richness and diversity patterns at both habitat and city level. Alpha diversity was defined as the species richness in each of 24 sites while gamma diversity the species richness found in each urban habitat or city. Beta diversity, concerning turnover in species composition within and among habitats as well as cities, was examined using Simpson dissimilarity index (β_{sim} ; Baselga et al., 2007), which only takes into account dissimilarity due to spatial turnover without being influenced by species richness values (Baselga, 2010). Analysis of

Variance (ANOVA) and Bonferroni p-value adjustment method were applied to test statistically significant differences. *Chao2* richness estimator and its standard error *Chao.se* (Chao, 1987) were also used to estimate the minimum asymptote of species richness for both habitat and city in order to detect number of species unseen or undetected in relation to observed species richness and to sampling effort efficiency.

Environmental and spatial effects on land snail assemblages: PCNM and Variance Partitioning

To evaluate the effects of environment and space on species composition, Principal Coordinate Analysis of Neighbor Matrices analysis (PCNM, Borcard et al., 2004) was used to detect space structure in the data. The real utility of PCNM method compared to the traditional multivariate trend-surface analysis is that it enables to detect, decompose and model any type of hidden spatial structure inside the data (fine vs broad scales) and that can be perceived by the sampling design limit (Borcard and Legendre, 2002). PCNM method allows selecting the best linear combination of eigenvectors maximizing correlation with the data and minimizing number of vectors (Dray et al., 2006). First of all a matrix of Euclidean distances among the 24 sites using latitude and longitude values associated with each sampling site was computed. Before running the proper PCNM analysis, data were detrended by regressing all variables on the geographical coordinates (X-Y) in order to detect linear trend and retaining the residuals. The Euclidean distances were then truncated: PCNM analysis is based on the assumption that the only meaningful spatial signal for each plot site is that one circumscribed to its neighbourhood site (Dray et al., 2006). The truncation distance, which defines the neighbourhood thresholds, is considered as the maximum distance connecting all plots based on a minimum spanning tree criterion (Rangel et al., 2006). Function *give.thresh()* in package *spacemakerR* was used.

PCNM analysis was performed through a principal coordinates analysis on the modified (i.e truncated) distance matrix in order to extract eigenvectors (PCNM variables) to be used as new spatial explanatory variables. Since

PCNM are orthogonal, each PCNM variable represents an independent and different spatial gradient. The higher the eigenvalue of a given PCNM, the broader the spatial scale represented, while PCNM variables with low eigenvalues are able to explain only small fractions of the total spatial structure and they are related to finer spatial gradients (Duarte et al., 2012). Model selection procedures were based on a multivariate extension of the AIC criterion using *ortho.AIC* function in package SpacemakeR. Six PCNM vectors obtained from the X-Y coordinates of each sampling site were then incorporated as explanatory variables in a Redundancy analysis (RDA) to account for the space effect on urban land snail matrix. In order to reveal the urban gradient effect after partialling out the effect of space (i.e pure spatial patterns that cannot be related to any measured variable + the unmeasured spatially structured environmental variation), the relative contribution of three environmental variables (distance from city center, distance from sea, % vegetation cover) from a partial RDA (pRDA) that excluded the effect of spatial influence from the community matrix, was then computed.

Variation partitioning method was later conducted to detect the fractions of the total variance explained exclusively by the environmental predictors [a], the variance explained by the environment shared with space [b], the variance explained exclusively by spatial variables [c] as well as the residual variation (Borcard et al., 1992; Peres-Neto et al., 2006), which may be attributable to internal biological variation, survey error, or missing variables not comprised in the analysis (Legendre and Legendre, 1998). The quantification of the variance components was based on the function *varpart* of the R package vegan (Oksanen et al., 2016).

All multivariate statistical analyses, based on Hellinger-transformed land snails presence/absence data, were performed with RStudio 0.99.473 version (RStudio Team, 2015)

RESULTS

Observed and estimated species richness

Tab. 4 Land snail species in the sampled cities (Grosseto, Siena, Arezzo) and total number of species in each urban area.

	Grosseto	Siena	Arezzo
1) <i>Acanthinula aculeata</i>		•	•
2) <i>Cantareus apertus</i>	•	•	•
3) <i>Carychium tridentatum</i>		•	•
4) <i>Cecilioides acicula</i>	•	•	•
5) <i>Cecilioides janii</i>	•	•	
6) <i>Cepaea nemoralis</i>			•
7) <i>Cernuella cisalpina</i>	•	•	•
8) <i>Cernuella virgata</i>	•	•	•
9) <i>Chilostoma planospira</i>		•	
10) <i>Cochlicella acuta</i>	•		
11) <i>Cochlicella barbara</i>	•	•	
12) <i>Cochlodina bidens</i>		•	•
13) <i>Cornu aspersum</i>	•	•	•
14) <i>Deroceras invadens</i>	•	•	•
15) <i>Deroceras reticulatum</i>		•	•
16) <i>Discus rotundatus</i>		•	•
17) <i>Ena obscura</i>		•	
18) <i>Eobania vermiculata</i>	•	•	
19) <i>Granaria illyrica</i>		•	•
20) <i>Helix straminea</i>			•
21) <i>Hohenwartiana hohenwartii</i>		•	•
22) <i>Hygromia cinctella</i>		•	•
23) <i>Lauria cylindracea</i>		•	•
24) <i>Lehmannia melitensis</i>		•	
25) <i>Limacus flavus</i>		•	
26) <i>Limax corsicus</i>		•	•
27) <i>Limax maximus</i>		•	•
28) <i>Marmorana serpentina</i>	•	•	
29) <i>Mediterranea hydatina</i>	•	•	•
30) <i>Milax nigricans</i>		•	
31) <i>Monacha cantiana</i>		•	
32) <i>Monacha cartusiana</i>	•	•	
33) <i>Monacha parumcincta</i>	•	•	•
34) <i>Oxychilus cf. draparnaudi</i>	•	•	•
35) <i>Papillifera papillaris</i>	•	•	•
36) <i>Papillifera solida</i>	•		
37) <i>Paralaoma servilis</i>	•	•	•
38) <i>Pomatias elegans</i>	•	•	•
39) <i>Punctum pygmaeum</i>		•	•
40) <i>Solatopupa juliana</i>		•	
41) <i>Succinella oblonga</i>			•
42) <i>Rumina decollata</i>	•	•	•
43) <i>Tandonia sowerby</i>	•	•	
44) <i>Theba pisana</i>	•		
45) <i>Trochoidea pyramidata</i>	•	•	
46) <i>Truncatellina callicratis</i>	•	•	•
47) <i>Truncatellina cylindrica</i>		•	•
48) <i>Vallonia costata</i>			•
49) <i>Vallonia pulchella</i>	•		•
50) <i>Vitrea etrusca</i>	•	•	•
51) <i>Vitrea subrimata</i>	•	•	•
52) <i>Xerotricha apicina</i>	•		
53) <i>Xerotricha conspurcata</i>	•	•	•
	29	44	35

Total land snails richness amounts to 53 species (Tab. 4). Communities were dominated by *Cornu aspersum* and *Xerotracha conspurcata* present in 19 and 18 out of 24 sites respectively, while rare species such as *Howenwartiana howenwartii*, *Marmorana serpentina*, *Cepaea nemoralis* and *Vitrea etrusca* occur in 2 or 3 out of 24 sites. Some species are ranked as LC (Least Concern) in IUCN Red List, while only three consist of alien entities (the Australasian *Paralaoma servilis*, the Sardo-Corsican *Marmorana serpentina* and the Mediterranean *Deroceras invadens*).

Numbers of land snail species found in sites ranged from 5 to 29 with a mean of 14 (Lososová et al., 2011: 0-21, mean 7.5). The lowest mean number of land snail species per site (α diversity) was found in early successional sites (E-SUC) and old city walls (WALLS) (9 and 13, respectively) while the highest mean α diversity was in wooded sites (WOOD) and urban gardens and parks (PARK) (17 and 16, respectively).

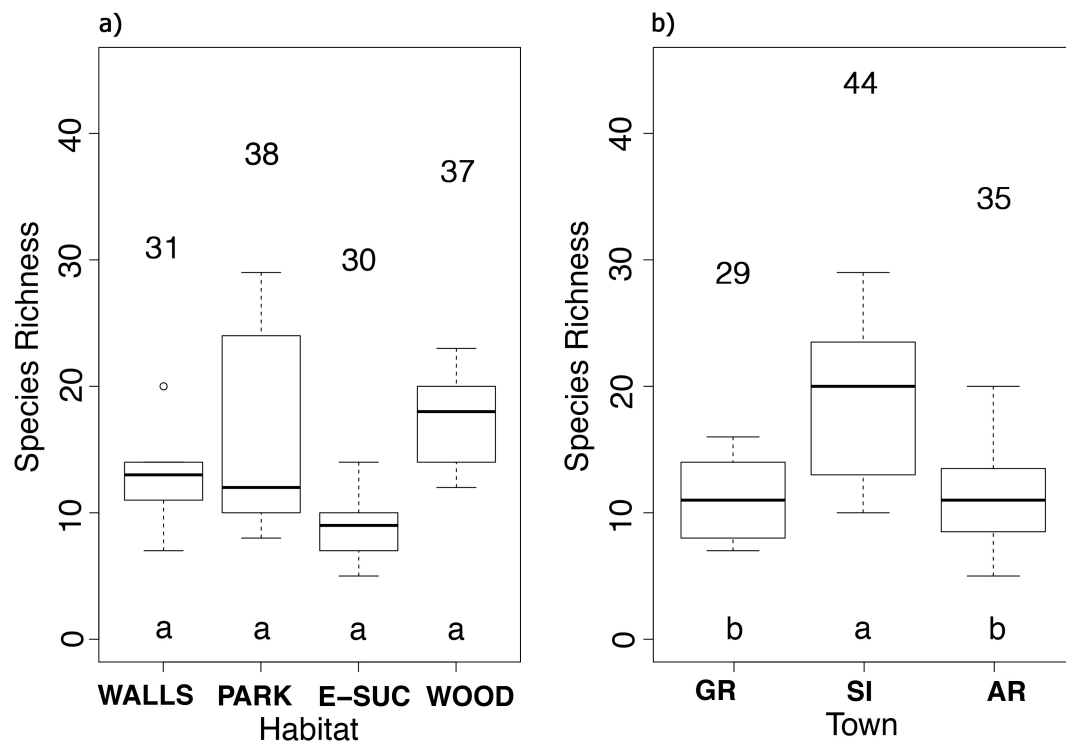


Fig. 9a,b Box plots of α diversity (species richness per site; box and whiskers) and γ diversity (total number of species, numbers outside box and whiskers) in habitat types (a, on the left) and cities (b, on the right). Different letters denote significant differences among groups (ANOVA with Bonferroni p-value adjustment, $p < 0.05$).

The total number of species found in each habitat type (γ diversity) was lowest in early successional sites (E-SUC: 30) and increased in old city walls (WALLS: 31), reaching its highest values in wooded suburban areas (WOOD: 37) and parks (PARK: 38) (Fig. 9a). Across habitats, γ diversity was correlated with mean α diversity ($r=0.94$, $p=0.05$). One-way ANOVA (df 3; F-value 2.793; P-value 0.067) revealed no significant difference among habitat types. β diversity was highest in E-SUC, lowest in all the other habitats (Fig. 10a). One-way ANOVA (df 3; F-value 12.25; P-value 2.87e-06) and Bonferroni p-value adjustment for multiple comparisons showed that E-SUC significantly differed with respect to all the other habitats in β diversity values (E-SUC-PARK $p=9.5e-05$; E-SUC-WALLS $p=3.7e-05$; E-SUC-WOOD $p=2.5e-05$). Pairwise β diversity values between habitat types were: WALLS-PARK 0.16; WALLS-E-SUC 0.37; WALLS-WOOD 0.32; PARK-E-SUC 0.20; PARK-WOOD 0.19; E-SUC-WOOD 0.17.

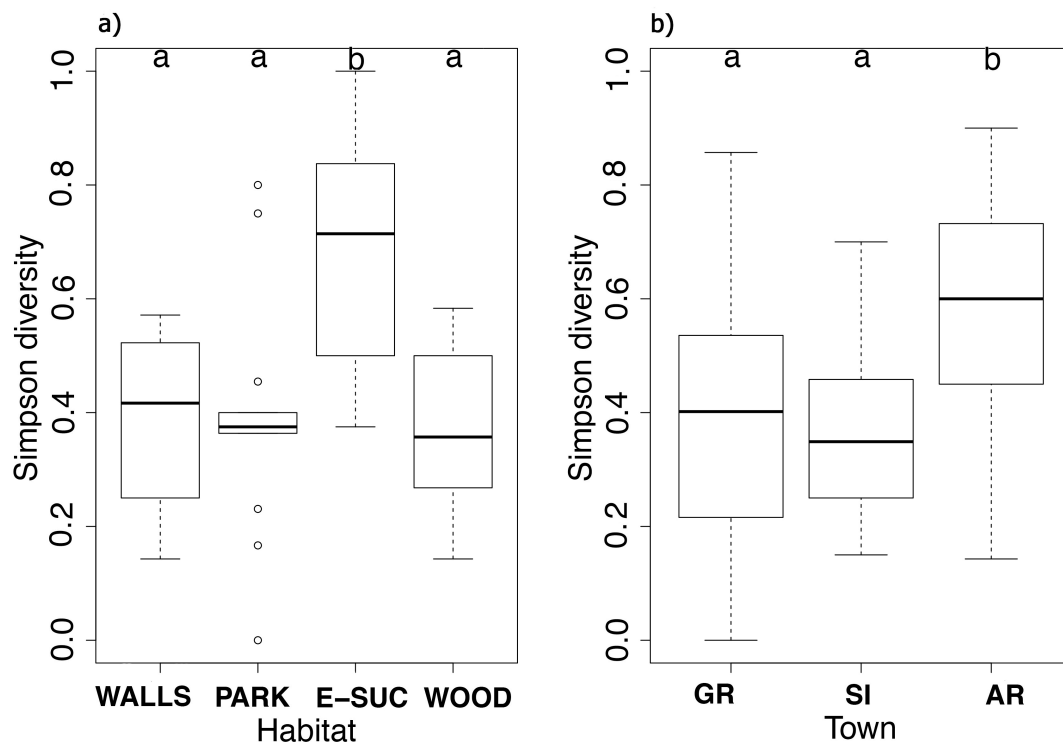


Fig. 10a,b Box plots of Simpson β diversity in habitat types (a, on the left) and cities (b, on the right). Different letters denote significant differences among groups (ANOVA with Bonferroni p-value adjustment, $p < 0.05$).

The lowest mean α diversity in the cities was recorded in Grosseto (GR) and Arezzo (AR) (11 in both cases) while the highest was recorded in Siena (SI) (19). The total number of species found in each city (γ diversity) was lowest in Grosseto (29), higher in Arezzo (35) and highest in Siena (44) (Fig. 9b). Across cities, γ diversity was not correlated with mean α diversity ($r=0.84$, $p>0.05$). After one-way ANOVA (df 2; F-value 6.439; P-value 0.007) and Bonferroni correction for multiple comparisons, the SI-GR and SI-AR pairs proved to be significantly different in α diversity (SI-GR $p=0.014$; SI-AR $p=0.018$). Arezzo showed the highest internal β diversity, while Grosseto and Siena showed similar values (Fig. 10b). After one-way ANOVA (df 2; F-value 9.739; P-value 0.0002) and Bonferroni p-value adjustment, the AR-GR and AR-SI pairs resulted to be significantly different in β diversity values (AR-GR $p=0.001$; AR-SI $p=0.001$). Pairwise β diversity values between cities were: SI-GR 0.17; SI-AR 0.14; AR-GR 0.38.

The minimum estimated richness *Chao2* and its standard error *Chao.se* differed both among habitat types and cities (Fig. 11). However, few species are missing (Tabs. 5-6): more or less all species have been detected with a reasonable degree of certainty through the sampling effort.

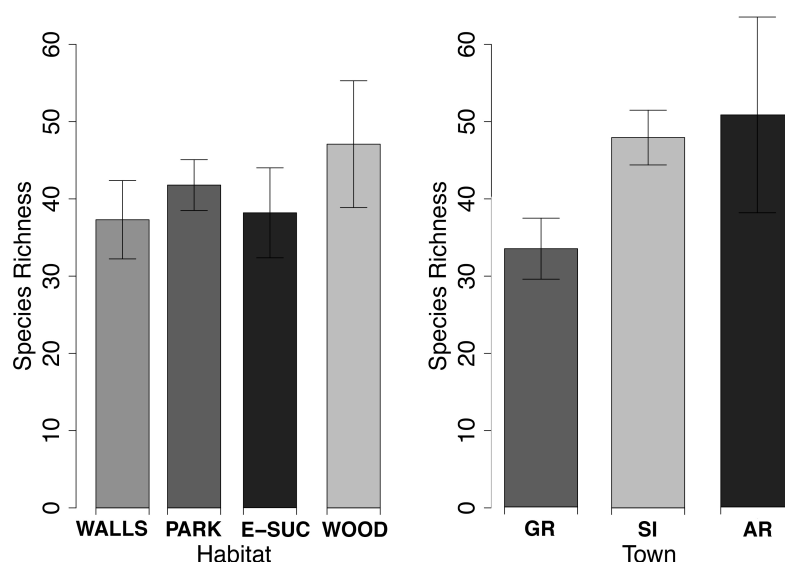


Fig. 11 Bar plots of species richness in each habitat types (a, on the left) and city (b, on the right) showing the minimum estimated richness *Chao2* and its standard error *Chao.se*.

Tab. 5 Asymptotic estimators summary statistics for city types

	Species	Chao2	Chao.se	Confidence interval
GR	29	33.4	3.9	30-37
SI	44	47.9	3.5	44-51
AR	35	50.7	12.7	38-63

Tab. 6 Asymptotic estimators summary statistics for habitats

	Species	Chao2	Chao.se	Confidence interval
WALLS	31	37.3	5.1	32-42
PARK	38	41.8	3.3	39-45
E-SUC	30	38.2	5.8	32-44
WOOD	37	47.1	8.2	39-55

Environmental and spatial effects on land snails assemblages: PCNM and Variance Partitioning

Total variation in species matrix (Tab. 7) can be portioned in four parts: (a) pure effects of space, (b) shared variation i.e. spatially structured environmental variation, (c) pure effects of environment, (d) residual variation (Borcard et al., 1992).

Tab. 7 Summary of the variation partitioning constituents for urban land snails using partial RDAs with environmental and spatial data as predictors.

Effect and main variables	Variation fraction	Explained variation Adjusted R ²	P (<0.05)
Total effect			
Environmental, spatial	[A + B + C]	0.35	0.001***
Partial effects			
Spatial	[A + B]	0.16	0.001***
Spatial (environmental)	[A]	0.13	0.001***
Environmental	[B + C]	0.22	0.001***
Environmental (spatial)	[C]	0.19	0.001***
Joint effect			
Environmental, spatial	[B]	0.03	
Residuals	[D]	0.65	

In our data matrix, total contribution of spatial structures (a + b) accounted for 16% of community structure whereas the variation attributed uniquely to pure spatial patterns i.e. that cannot be related to any measured environmental variable, is 13% (a). Environmental factors (c + b) are involved to explain a higher portion of the variation in species matrix (22%) than spatial structures do. Furthermore, almost this entire amount of variation (c) (19%), which is rather high, represents the pure effect of environment, i.e. species-environment relationship associated with local environment

independently to space. The percentage of residual variation (d) is very large: more than half of the total variation remains unexplained, while just 3% of the explained variation is shared (b) between both factors. All the variation components resulted to have a significant effect for $p < 0.05$.

Redundancy analysis (RDA) (Fig. 12) detected significant effect of two out of three local environmental variables in shaping urban land snail composition, "distance from the city center" and "vegetation cover". *Papillifera papillaris* was clearly negatively associated with *Dist_center* and it is the most characteristic species of WALLS assemblages followed by *Mediterranea hydatina*, which is negatively associated to *Tree_cover* variable as well; *Hohenwartiana hohenwartii* is most linked to E-SUCC habitat type, *Monacha parumcincta* is clearly positively associated to both *Dist_center* and *Tree_cover*, resulting to be the most characteristic species of WOOD assemblages while *Deroceras invadens* and *Cernuella virgata* to PARK habitat type.

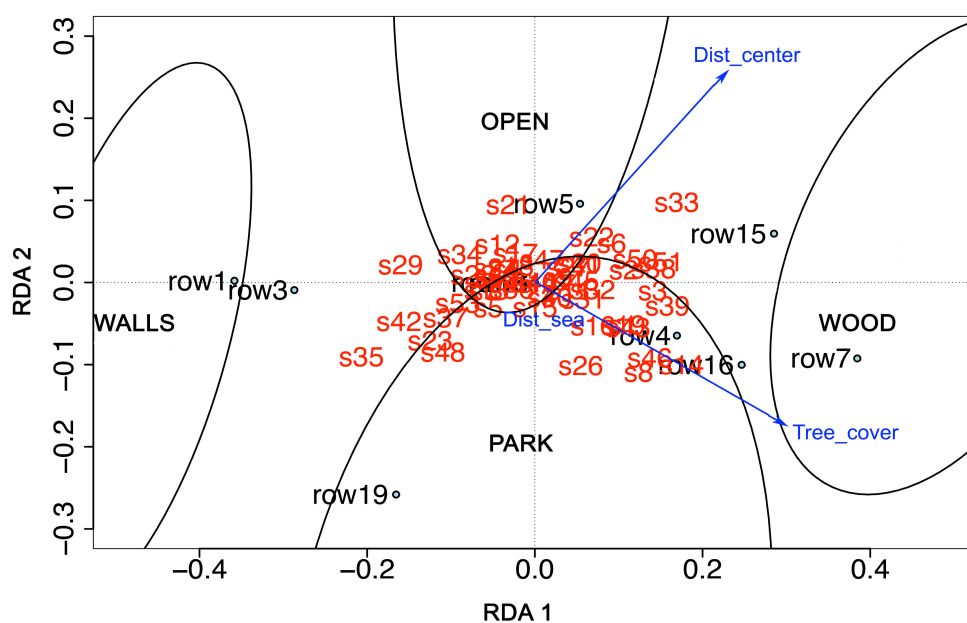
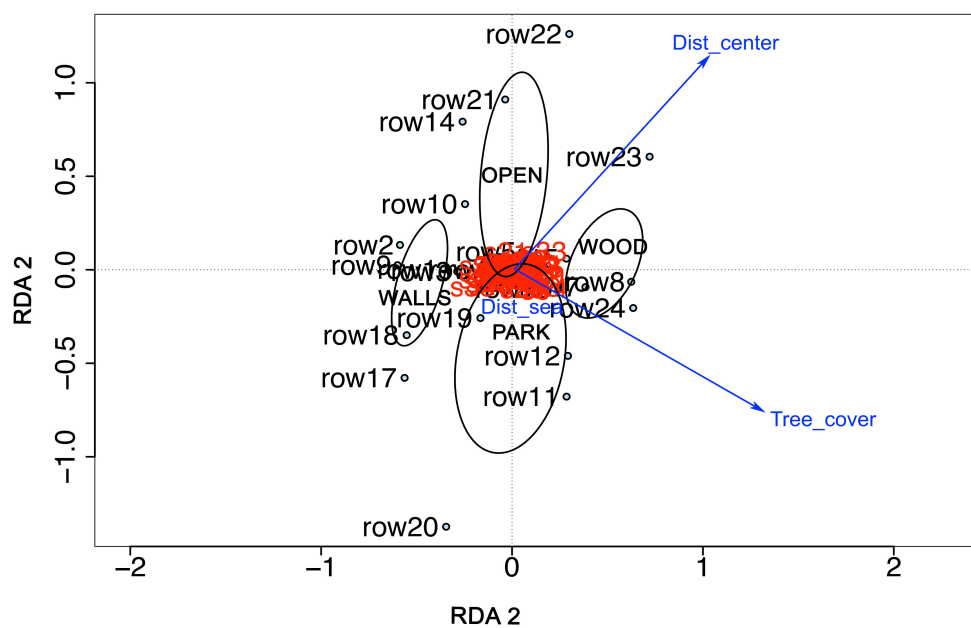


Fig. 12 Ordination plots of RDA showing the pure effect of environmental variables (arrows in blue) shaping land snail communities. Each species number (s1, s2, ..., s53) matches the numerical order in Tab. 4, while row number (row1, row2, ...row24) corresponds to each of 24 sampling sites. Ellipses show the 95% confidence intervals associated with each habitat type.

DISCUSSION

This study first accounts for the environmental and spatial control in urban land snail communities in southern Europe, providing with remarkable insights. In terms of land snail total species richness, 53 species were recovered over a 100 km transect, an interesting number compared to the overall 87 species detected through a much larger transect (over 1200 km) in central Europe by Chytrý et al. (2012) and the 55 forest entities found in three well preserved forest areas of Tuscany (Benocchi et al., 2015). Urban environments harbour different habitats and environmental conditions, as a sort of huge heterogeneous melting pot of unlimited ecological situations. It is well understandable why so many species, which rely on a wide variety of ecological requirements, look at urban environments as optimum places where to adapt, flourish and spread out, as well as it may happen in naturalized areas.

(1) Concerning habitat γ diversity, a clear decreasing pattern from most (WALLS and PARKS) to less urbanized habitats (E-SUC and WOOD) was not found, but rather a homogenous trend with just a significant decreasing peak in proximity to early successional sites. These sub-urban open habitats are typically placed in city peripheries; many of them consist of strongly disturbed places, often close to trafficked roads or industrial settlements, functioning as ecological corridors (or barriers?) to the open countryside or woods. City parks, public gardens and wood patches proved to be, instead, real islands of biodiversity inside the urban matrices, showing the highest values of both alpha and gamma diversity in our results; this finding agrees with other researches where parks represented the most diverse urban habitat for several groups of organisms (Lososová et al., 2011). Pairwise species composition differences among habitats show relevant beta diversity values, the highest difference being among WALLS and E-SUC habitats: while PARK and WOOD share a common more or less forestal matrix, WALLS and E-SUC are those with the strongest differences in terms of ecological conditions and environmental heterogeneity. At city level, Siena has the richest alpha and gamma diversity: it is the greenest one, characterized by many and well

preserved wooded and green patches still inside urban cores and relatively less impacted by vehicular traffic as well as urbanization effects.

(2) The checklist does not comprise species in danger of extinction and included in threat categories; xenodiversity is apparently very low and only three (5.7% of the total) are true aliens: *Paralaoma servilis* is an invasive species worldwide, probably of Australasian origin, living among litter and humus (Manganelli et al., 2015); *Deroceras invadens*, probably of central Mediterranean origin, is an invasive species worldwide (Reise et al., 2011); *Marmorana serpentina*, which has been first reported in the city of Grosseto during this research, occurs in Sardinia and Corsica and it has been introduced to some Tuscan cities (Livorno, Pisa, Siena) (Fiorentino et al., 2009). As regards the remaining species, at least two (*Lehmannia melitensis*, *Papillifera papillaris*) could have been introduced through anthropocore dispersion as well. These species are nearly always located in habitats at the edge of urban areas or used by man in historical periods. Finally, it is likely that the creation of vast open and built-up areas has facilitated the colonization of potentially indigenous but originally less widespread synanthropic species, such as the slug *Limacus flavus* and land snails *Xerotricha conspurcata*, *Eobania vermiculata* and *Cornu aspersum* (Manganelli et al., 2015). Therefore biotic homogenization does not seem to have remarkable ecological impact: the small Tuscan cities still maintain a considerable beta diversity both within and among study areas (despite the restricted geographical distance gradient), unlike other cosmopolitan European urban areas where the introduction of invasive species, together with a human mediated biotic interchange, resulted in a widespread floristic and faunistic homogenization (La Sorte et al., 2007; Ferenc et al., 2014).

(3) The attempt to discover the processes underlying land snails community structure by partitioning the pure effects of "environment" and that of unmeasured factors (dispersal, biotic interactions, unmeasured environmental variables) that create spatial structure (called "space"), provided the most interesting findings. Both environment and space can be equally important in shaping communities but their function should be always related to dispersal capacity in the analysed group (Heino, 2013). The relative role of

deterministic and stochastic determinants driving ecological metacommunities has been examined in different organisms in natural environments (i.e. oribatid mites, Caruso et al., 2012; oribatid mites and collembola, Ingimarsdóttir et al., 2012; microbial communities, Caruso et al., 2011; several groups of aquatic organisms, Rádková et al., 2014, Padial et al., 2014, Zhai et al., 2015) but few studies concern urban areas (i.e. spiders, bees and birds; Sattler et al., 2010; subaerial cyanobacteria and algae, vascular plants and land snails; Chytrý et al., 2012). According to general belief, high dispersers should be less influenced by spatial influence, since they are able to more easily reach the best suitable habitat than low dispersers can do and, consequently, species are sorted according to their ecological requirements (Martiny et al., 2006; Heino, 2013). Conversely, weak dispersers should show significant spatial structuring which is a direct consequence of their low mobility capacities and their distribution is often due to stochastic processes mediated by limited dispersal (Bell, 2000; Hubbell, 2001). Paradoxically, although land snails are widely recognised as low mobility organisms (Baur and Baur, 1993), they resulted to be most significantly shaped by pure and spatially independent environmental processes, which often turn to be irrelevant since the environment is usually spatially structured by various ecological processes (Legendre and Fortin, 1989). Namely, after having removed the effect of space (i.e. pure spatial patterns that cannot be related to any measured variables + the unmeasured spatially structured environmental variation), urban gradient turned out to be the main factor in modelling land snail communities structure.

Some exceptions to the rule were also found by Hájek et al. (2011), who described a much stronger environmental than spatial structure in bryophytes but not in high dispersers as diatoms in Western Carpathian fens, and similarly Astorga et al. (2011) confirmed a preponderant influence of niche-related factors respect to spatially limited dispersal in bryophytes and macroinvertebrates in Finnish streams. Sattler et al. (2010) first took seriously into account urban environments observing a higher environmental control together with a weak spatial structure in spiders within a gradient of three Swiss cities. According to Sattler et al. (2010), a higher environmental

influence together with a weaker or nearly absent spatial structure (i.e. concerning traditional limited dispersers) may be a feature typical of urban areas, where the high disturbance affecting the heterogeneous mosaic of urban habitats could inhibit the instauration of well defined spatial structures, selecting only the species able to adapt and coexist with human activities. Recent studies about land snail dispersal abilities have well confirmed the possibility of long distance passive dispersal (Ozgo et al., 2016) and consequently a human-mediated transport in urban environments may be the most reasonable explanation for the high pure environmental constraint detected in urban land snail data: molluscs can be passively dispersed but only in the presence of suitable environmental conditions they establish developing different and well defined assemblages. The presence of diverse microhabitat conditions as well as small scale habitat structures is in fact one of the necessary conditions for molluscs diversification together with a suitable geological substratum (Cuttelod et al., 2011). Therefore a pure environmental contribution could reveal a clear selection on species well adapted to anthropic environments while other species show a random distribution probably as a consequence of occasional dispersion events.

The hypothesis of a stronger niche-based control has been also confirmed by RDA analysis showing four well structured and differentiated species assemblages at habitat level, as proved by ellipses with 95% of confidence.

(4) "*Dist_centre*" and "*Tree_cover*" resulted to be the environmental variables most significantly involved in explaining variation in species composition. With the exception of parks and gardens still included in city limits, which can be easily considered real islands of richness and biodiversity inside the urban core (Dedov and Penev, 2004), bigger the distance from the urban center, higher the vegetation density degree. It is well known that the increase of the forest coverage provides better shelter for land snails during unfavourable conditions (Dedov and Penev, 2004). This has strong implications for species richness since the richest urban habitats are generally those with the highest trees and vegetation cover values (i.e. PARK and WOOD in our research). At the same time different forest cover values work as ecological filters, acting through the selection of different species, which rely on different

environmental conditions. It is true that poor assemblages, made up of a lower number of species, are usually more frequent close to the city centre due to the lower frequency of preserved natural habitat (Horsák et al., 2009) but even if poor in terms of richness, these completely human made assemblages often consist of surprisingly unique entities: WALLS harbour extremely specialist species (i.e. *Marmorana serpentina*, *Papillifera papillaris*, *Papillifera solida*), well adapted to seek refuge not in litter or vegetation, but in rock clefts and cracks and whereon pure environmental control acts in a preponderant way. Furthermore, subterranean species living in the ground behind walls, such as *Mediterranea hydatina*, which proved to be typical species in WALLS assemblages, can be included in this habitat.

The response of local land snail assemblages to urbanisation can not be described as a gradual and linear decline in species richness as the proximity to city centre increases (Horsák et al., 2009), but rather a clear change in species composition through an adaptation to a gradient of different ecological conditions, as a consequence of a likely passive human mediated dispersal and a following environmental selection on species more prone to coexist under human impact.

CONCLUSION

Urban environments can show relatively high richness of land snail similar to that found in natural areas; species richness does not show a clear increasing pattern from most to less urbanized habitats, but rather an irregular trend. While parks prove to be real islands of biodiversity inside the urban patchwork, only urban environments such as historical city walls provide an ideal environment for many specialist species strongly associated with particular ecological requirements (i.e. the presence of carbonate rocks, calcium enriched shaded walls as well as humid refuges).

Despite the reduced transect examined (100 km), gradient has comparable values to those obtained by Lososová et al. (2011) and Chytrý et al. (2012) studying a transect of over 1200 km in Central Europe, demonstrating how the Mediterranean area and especially the Italian peninsula are able to offer concerning diversity patterns.

At city level, study areas host rather heterogeneous species assemblages where the biotic homogenization seems very low: only three out of 53 species are to be considered proper alien species (*Paralaoma servilis*, *Marmorana serpentina* and *Deroceras invadens*).

After having accounted for and later removed spatial structures influence, urban gradient effect turns out to be the principal component in structuring urban land snail assemblages. A significant environmental control, reflecting niche-processes as well as environmental filtering, is an interesting result since a stronger spatially independent environmental constraint has never been detected before for a scarcely mobile organism inside urban matrices. This finding proves a likely human mediated transport, which enhances dispersal abilities of organisms normally considered scarcely mobile. However, no establishment there would be in the absence of suitable microhabitat conditions. In particular, two urban environmental variables have been detected as the main constraints in shaping urban land snail communities i.e. vegetation cover and distance from the city centre. The detection of a pure spatially independent environmental effect retains important conservation implications which go beyond the mere analysis of one single taxonomic

group: regardless the position of each study area, a sampling activity in different cities would likely reveal the similar species assemblages explained exactly by the same dataset of environmental variables. This finding discloses the importance of niche-based processes at a local level for traditionally scarcely mobile organisms usually structured by spatial constraints and it opens new scenarios in urban environment together with relevant ecological and conservation implications for other invertebrates groups.

CHAPTER III

***Papillifera papillaris* (Gastropoda: Pulmonata, Clausiliidae), a small snail living on stones and monuments, as indicator of metal deposition and bioavailability in urban environments**

Abstract

Although mosses, lichens and vascular plants are the most used organisms as biomonitors of metal deposition in urban environments, they also adsorb rock and soil dust particles, making difficult to assess the element contribution from anthropogenic sources and their bioavailability. Based on the assumption that a stationary, widespread and omnivorous animal species, scarcely interacting with soil and inhaling fine particles, could be a much more reliable indicator of metal deposition and bioavailability in urban environments, for the first time the elemental composition of soft tissues and excreta of a land gastropod, *Papillifera papillaris*, commonly dwelling on stone walls and monuments in Italian and Mediterranean urban environments was analysed. Snails and some moss species were collected in 2014 and 2015 from stone- and brick-walls at the side of roads with different traffic intensity and in control sites. Results showed that the soft tissues of *Papillifera papillaris*, purged of the gut contents, accumulated much higher values of typical anthropogenic elements such as Cd, Cu, Zn, Pb, than mosses from the same walls. Surprisingly, tissues also accumulated much higher Mn concentrations than those reported in literature for other species of terrestrial gastropods. Excreta and mosses appear to be more prone to contamination by soil and stone particles as indicated by high Al and Fe concentrations, the PCA analysis and boxplots of PC component scores. These elements, scraped from wall vegetation and crusts, fail to accumulate in soft tissues, pass unscathed in the excreta, where they are concentrated in high amounts as if they were not subjected to the action of digestive processes and assimilation.

Although preliminary, this study indicates that *Papillifera papillaris* is a very promising species to monitor metal deposition pattern and bioavailability in urban environments and to evaluate the possible metal transfer along food chains.

INTRODUCTION

In urban areas, atmospheric pollution represents the single largest environmental health risk in Europe today (EEA, 2015). The global situation is made even worse since the urbanization trend is going to increase and 66% of the world population will reside in urban areas in the next 50 years (United Nations, 2014).

Common air pollutants in urban matrix consist of a complex mixture of gases and other particles from several sources, both natural and anthropogenic. For their nature, these compounds may be transported over long distances, accumulating in the environment and in the food chain, with the possibility to interact with humans and animals via air, water and food intake. Air pollution is, therefore, a complex problem that poses multiple challenges in terms of management and mitigation (EEA, 2015).

Technologies and normative introduced in the last decade have resulted in a significant reduction of some air pollutants (as SO_2) compared with an increase of tropospheric ozone (O_3) and in number of compounds (organic and inorganic) associated with particles, so called Airborne Particulate Matter (PM). The term PM has a very complex meaning. It refers to a mixture of solid particles and liquid droplets found in the air, whose effects are largely dependent on the aerosol properties, including the number concentration, size, and chemical composition of its thousands of chemicals. According to size, PM includes PM_{10} : inhalable particles, with diameters that are generally 10 micrometers and smaller; and $\text{PM}_{2.5}$: fine inhalable particles, with diameters that are generally 2.5 micrometers and smaller (EPA, 2016). Ultrafine particles (UFPs) comprise instead particles with a diameter less than 100 nm: they are ubiquitous in urban air and an acknowledged risk to human health since they have a potential for lung deposition and translocation to other parts of the body (Oberdörster et al., 2002; 2004). For their abilities to pass undamaged through the defences offered by respiratory system, fine and ultrafine particles are in fact among the most problematic pollutants (EEA, 2015). For all these reasons, air quality assessment is becoming a topical and urgent subject of major concern.

Monitoring stations have been traditionally used for many years as most

common analytical instruments for this purpose; however, not considering the high costs of long term monitoring, most stations in urban environments only measure the concentrations of particles based on size (aerodynamic diameter of PM_{10} : $\leq 10 \mu m$; or $PM_{2.5}$: $\leq 2.5 \mu m$) and not their chemical composition (Bargagli, 2012). For this reason, bio-monitoring, i.e the use of living organisms, especially lower plants as an indicator of the environmental pollution, has been recently considered as alternative technique for monitoring trace-element air pollution. This technique exploits the natural and different sensitivity of living organisms to the complex mixture of air pollutants (bioindicators) or the capability of certain species to accumulate persistent atmospheric pollutants at easily detectable concentrations (bioaccumulators), achieving information about their biological effects or spatial deposition patterns (e.g., Bargagli, 1998). Lichens and mosses are among the most used bioaccumulators of potentially dangerous trace metals mainly released by vehicle exhausts and combustion processes since more than 50 years ago. Mosses have gained the attention of many researchers because in general, they are considered as independent of their substrate since they do not have real roots and so they can not take their nutrients from soil; they obtain most of their nutrients directly from precipitation and dry deposition and furthermore, slow growth rates let heavy metals accumulate over a long period of time (Szczepaniak and Biziuk, 2003). However, both moss and lichens also adsorb rock and soil dust particles, making difficult to assess the element contribution from anthropogenic sources and their bioavailability (e.g., Bargagli, 1995). For this reason researchers have been looking for more valid and accurate indicators.

In this context, the use of an animal organism, physiologically more complex, vagile, widely diffused in urban environments, able to inhale the polluting particles through the tissues and which slightly interacts with the ground, represents an undeniable advantage in the assessment of pollutants bioavailability and the evaluation of their potential transfer along terrestrial food chains (Wilcke, 2007), bypassing all the limits encountered with traditional biodindicators.

In the past, some surveys have already been performed with urban birds such as the house sparrow (*Passer domesticus*; e.g., Kekkonen et al., 2012) or the pigeon (*Columba livia*; e.g., Frantz et al., 2012), with lizards (e.g., Loumbourdis, 1997), and soil invertebrates such as terrestrial isopods (e.g., Gál et al., 2008), earthworms (Nahmani et al., 2007), springtails (e.g. Fiera, 2009; Fountain and Hopkin, 2004; Santorufo et al., 2012). Although molluscs (particularly bivalves) are among the most used organisms for large-scale and long-term monitoring of metals in aquatic ecosystems, in terrestrial ecosystems their use as biomonitors is rather limited to few researches (Regoli et al., 2006; Pignolo, 2010; Kowalczyk-Pecka and Czepiel-Mil, 2013; Kowalczyk-Pecka and Stryjecki, 2013). Several species of terrestrial land snails have wide ranges, a suitable biomass for the analysis of pollutants, are easy to sample and have strong ability to accumulate various kinds of environmental pollutants through different pathways (food, contact and, to a minor extent, inhalation) (e.g., Dallinger and Wieser, 1984; Rabitsch, 1996; Pihan and de Vaufleury, 2000; Scheifler et al., 2006; Boshoff et al., 2013; de Vaufleury, 2015), as well as tolerance to high levels of exposure (de Vaufleury and Pihan, 2000; Notten et al., 2005). The species of terrestrial molluscs commonly used for the monitoring of pollutants include *Eobania vermiculata* (Bertani et al., 1994; Pignolo, 2010), *Cornu aspersum* (Laskowski and Hopkin, 1996; Gomot and Pihan, 1997; Gomot-de Vaufleury, 2000; Regoli et al., 2006), *Helix pomatia* and *Cepaea nemoralis* (Notten et al., 2005).

These animals are herbivorous and detritivorous feeders and accumulate metals for ingestion, in particular accumulating them in the digestive gland (hepatopancreas) which is the most important organ used to detoxification. Molluscs are also able to inactivate metals like Cd and Cu, tying them to particular cytoplasmatic proteins called metallothionein proteins (Berger and Dallinger, 1993). In general, concentrations of some heavy metals in gastropods reflect the pollution levels in their environment, and being eaten by birds and mammals, land snails and slugs contribute to the metal transfer through terrestrial food chains. However, although land snails can represent reliable monitors of metal contamination in terrestrial ecosystems, most species live in direct contact and move on soil, eat plants and dirt and use the

ground for egg-laying, embryo development, shelter and hibernation; thus, their chemical composition mainly reflects the metal bioavailability in soil and plants, and only indirectly the amount of metals in atmospheric deposition.

Unlike other species of terrestrial molluscs hitherto used as bio-accumulators of metals, *Papillifera papillaris* does not live in soil but on limestone (walls, statues and other artifacts occurring in cities) and does not feed on plants or debris but on cyanobacteria, algae, lichens and mosses, that is completely exposed organisms to the accumulation of heavy metals in atmospheric deposition. The fact of living in a substrate free of litter and humus and directly exposed to atmospheric deposition and breathing those that are the main pollutants in urban environments (ultrafine particles and tropospheric ozone), would make this species a reliable biomonitor of air pollution in Italian cities.

Because there is evidence that mosses from stonewalls in urban areas are traditionally reliable biomonitors of metal deposition (e.g., Kosior et al., 2015), here we planned to assess the concentration of ten elements (Al, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) in mosses (*Hypnum cupressiforme*, *Homalothecium sericeum*, *Homalothecium lutescens*, and *Brachythecium rutabulum*) and *Papillifera papillaris* (soft tissues and excreta) dwelling on the same walls in order to:

1. determine the concentrations of metals attributable to atmospheric deposition (including the atmophile Hg) and those mainly occurring in soil and rock dust particles, such as Al, Fe, Cr, in sample sites (walls with mosses and *Papillifera papillaris*) with different degree of air pollution in the urban environment of Siena. Urban sites have been compared with those of a natural area used as a control.
2. assess the accumulation power of the land snail *Papillifera papillaris* (soft tissues and excreta) as new bioindicator in relation to traditional biomonitors (mosses) in urban environments.

MATERIALS AND METHODS

Target species: Papillifera papillaris

Papillifera papillaris (sometimes reported as *Papillifera bidens*) (Gastropoda, Pulmonata, Clausiliidae), is a species of terrestrial pulmonate gastropod with a clausilium, a calcareous anatomical structure, characteristic of the family Clausiliidae, the door snails. It has a small to medium sized, fusiform, sinistral shell, ash-grey to greyish-brown in colour, smooth or ribbed, with suture dark and strongly papillated and darker brown-red band below the suture running between the white dots (Fig. 13). The animal is light brownish with light tentacles; it differs from the similar *Siciliaria gibbula* in the absence of a lower palatal fold and in the colour between the dots at the suture being darker than the rest of the shell (Welter-Schultes, 2012). In Tuscany, another species of *Papillifera* can be found, *Papillifera solida*. However the latter has a Tyrrhenian distribution, being restricted to the Italian peninsula coastline from S Calabria to Liguria, SE France and the Tuscan Archipelago. Furthermore, a horny whitish shell, sometimes violet with a subsutural band characterized by small white dots well separates *Papillifera solida* from *Papillifera papillaris* (Manganelli et al., 2015).



Fig. 13 *Papillifera papillaris* (Gastropoda, Pulmonata, Clausiliidae)

Regarding its distribution, *Papillifera papillaris* is a southern European species,

native to central Mediterranean areas (Italian peninsula, Sardinia, Corsica, Sicily) (Giusti et al., 1995; Manganelli et al., 2015); in Malta it occurs in open rocky habitats mainly below small shrubs (Welter-Schultes, 2012). However, in recent times it has been likely introduced to many countries of the Mediterranean region (e.g., Albania, Spain, Balearic Islands, North Africa, Turkey) through passive dispersal along with plant material and stonework. In particular, through marble manufactures transported as construction materials from Rome in 1896, *Papillifera papillaris* was introduced to England (Ridout-Sharpe, 2010) and in the 1880s probably from Greece to Brownsea Island (Dorset, England).

Papillifera papillaris is a calciphile, draught resistant, termophilic species: in natural environments it lives in carbonate rocks of dry open limestone reliefs; however, it is also frequent, sometimes almost exclusively, in anthropogenic habitats (fortifications, churches, stone walls and ruins) (Fig. 14) which it uses as surrogate for natural habitats, presumably feeding on algae, lichens, mosses and other plants growing on stone surfaces. It is not globally threatened and apparently not negatively affected by human activities, its relationship with humans is so strong to be considered as real anthropophilic species.





Fig. 14 Land snail *Papillifera papillaris* and its urban habitat.

Sampling sites

The study was performed in the urban area of Siena (322 m a.s.l., 52.774 inhabitants, 118 km²) (Fig. 15), a small medieval town in the centre of Tuscany, Italy. Climate is sub-Mediterranean, with a mean annual temperature of 13.9°C and a mean annual rainfall of 813 mm concentrated in coldest months, while summer is fairly dry (Barazzuoli et al., 1993). Since historical city center is surrounded by medieval walls with narrow streets where limited or no traffic is permitted, traffic concentrates in ring-roads around the urban area. Due to its prevalent touristic vocation and very limited industrial activity, vehicular traffic and domestic heating resulted to be, in fact, the main sources of atmospheric pollution in the urban area (Loppi et al., 2002).

The sampling activities related to mosses and *Papillifera papillaris* began in Siena in May 2014 and continued during spring 2015, involving five plots distributed in different sites in the urban area at different pollution degree

and intensity of vehicular traffic. Each site, in order to be considered eligible, had to submit a wall (natural or artificial), placed near a road and with the simultaneous presence of moss and snails. In each plot, moss samples (each being a composite of 5-10 subsamples from a 20 m² area) of different species and 30 to 50 exx of *Papillifera papillaris* adult snails, were collected by using tweezers, latex gloves and paper bags, so as to reduce possible contaminations and at the same time to ensure a sufficient quantity of biomass (excreta and sof tissues) for the performance of chemical analysis. Two plots located outside the urban area (Abbazia di Monte Oliveto Maggiore) and one in the nearby suburbs of Siena, not affected by high degree of vehicular traffic, were used as control. For each plot wall features, vegetation cover degree (scarce, medium, abundant), distances from the road and traffic intensity (vehicles/h) were considered too.

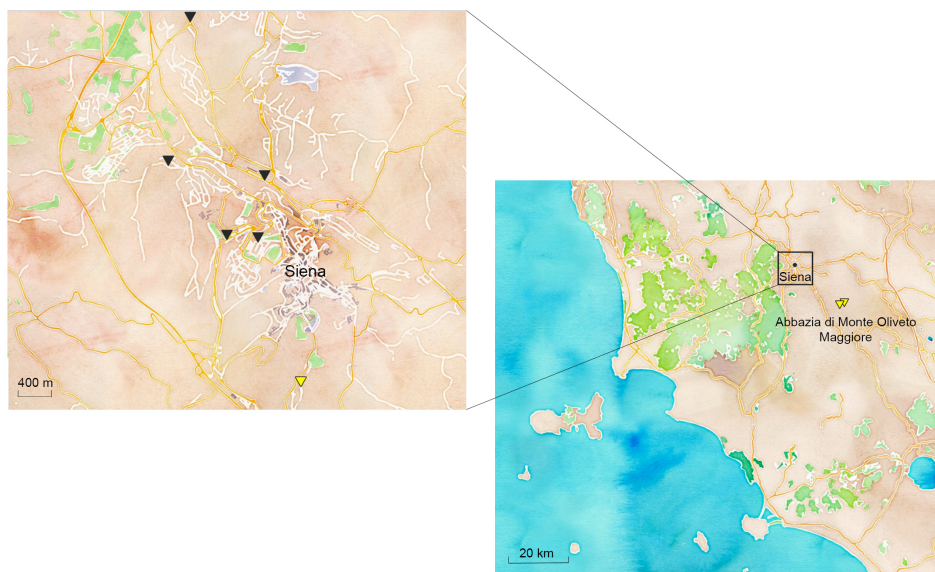


Fig. 15 Maps of southern Tuscany (Italy) and the city of Siena, showing the location of sampling sites. Yellow triangles represent control sites (from Rota et al., 2016).

Sample treatments

Sample treatments and chemical analyses followed the methodology proposed and described in Rota et al. (2016). In particular, snails were kept fasting in dry Petri dishes to help eliminating food residues until guts were

empty (3 weeks) and their excreta (feces) were periodically collected. Shells were cleaned to remove any materials adsorbed and then, for each sample, gently crushed all along and snail body accurately removed to a collective vial which was then stored at -20°C. The average fresh weight per individual without shell was 0.039 g.

Moss samples were prepared and cleaned by removing all dead material and any adhering substratum of lithogenic origin; only the upper green shoots were selected and brushed against a white paper background until clean, then left to dry for 3 weeks, avoiding the use of stoves at higher temperatures which could favor the partial loss of the more volatile elements (Bargagli et al., 1987). The moss species found were: *Brachythecium rutabulum*, *Homalothecium sericeum*, *Homalothecium lutescens* and *Hypnum cupressiforme*.

Analytical determination of metal concentrations

The selected moss green shoots, the snail soft tissues and the excreta were lyophilized and homogenized and subsamples of about 120 mg of each matrix type were digested in Teflon containers with HNO₃ (69 %) 2.5 ml and H₂O₂ (30%) 0.5 ml in a pressure digestion block system overnight. Concentrations of elements were determined in sample solutions (brought to 10 ml with ultrapure water) by atomic absorption spectrometry with graphite furnace (Perkin-Elmer AAnalyst800) for Al, Cd, Cr, Ni and Pb, a flow injection system (Perkin-Elmer FIMS-400) for Hg, and inductively coupled plasma emission spectrometry (Perkin-Elmer ICP-AES Optima 5300) for the other metals (Cu, Fe, Mn and Zn). Element concentrations were established by the method of internal standard additions and were expressed in µg/g on a dry weight basis. Procedural blanks were usually below the minimum detection levels. Whenever possible, to check the sample homogeneity and uncertainties related to mineralization and analysis of samples, the analyses were repeated. Accuracy was checked by concurrent digestion and analysis of standard reference materials (N°1577b Bovine liver and N°1573a Tomato leaves) from NIST, Gaithersburg, MD, USA. Depending on the element, the mean recovery

rate ranged from 87% (Al) to 115% (Cr) in moss samples, from 82% (Al) to 116% (Ni) in excreta and from 88% (Al) to 112% (Cr) in snail tissues.

Statistical analysis

Boxplots and descriptive statistics, such as mean and standard deviations, were used to evaluate the basic features of the data, providing simple summaries about elemental concentrations in the three matrices considered (mosses, mollusc excreta and soft tissues). Non-parametric Kruskal-Wallis rank sum test was then used to detect significant differences between matrices and elements concentrations. Kruskal-Wallis test is a non-parametric test used for comparing samples from two or more groups, not making assumptions about normality. If significant differences were detected, a post hoc Wilcoxon test with Bonferroni correction for multiple comparison was performed, trying to identify where differences were between groups (i.e. matrices). The Bonferroni correction is an adjustment made to P values when several dependent or independent statistical tests are being performed simultaneously on a single data set, since the probability of identifying at least one significant result due to chance increases as more hypotheses are tested. To perform a Bonferroni correction, the critical P value (α) is divided by the number of comparisons being made. The statistical power of the study is then calculated based on this modified P value. The Bonferroni correction is used to reduce the chances of obtaining false-positive results (type I errors) when multiple pair wise tests are performed on a single set of data.

Scatterplot matrix using function *chart.Correlation* in PerformanceAnalytics package produced a scatterplot matrix, with histograms, kernel density overlays, and correlations. On the diagonal are the univariate distributions, plotted as histograms and kernel density plots. On the right of the diagonal are the pairwise Spearman correlations coefficients between each element and the others, ranging from 1, perfect positive correlation to -1, perfect negative correlation: as the correlations get bigger the font size of the coefficient gets bigger. On the left side of the diagonal is the scatterplot matrix, with loess smoothers in red to help illustrate the underlying

relationship. Also red stars signifying significance levels corrected for multiple comparison with Bonferroni correction (*0.05, **0.01, ***0.001) are shown. A Principal Component Analysis (PCA) based on a correlation matrix was then performed in order to identify clusters of elements with similar variation pattern, getting a correlation between the studied elements and gaining insight into the potential origin, as well as a multivariate graphical representation of the overall metal distribution in relation to the three matrices (mosses, tissues and excreta). Principal component analysis (PCA) is the most straightforward way to ordinate data, having the main purpose to reduce the dimensionality of multivariate data, synthesizing and making patterns more obvious to the experimenter (Gotelli and Ellison, 2013). Its objective is to find the principal component underlying structure in the data, i.e the directions where there is most variance and where data are most spread out, identifying appropriate linear transformations of the observed variables, easy to interpret and able to highlight and summarize the information inside the initial matrix.

Based on the assumptions that the eigenvector with the highest eigenvalues is therefore the principal component and the amount of eigenvectors/values that exist equals the number of the data set, the first two most significant principal components (PC1 and PC2) out of a total of 7 PCA were used as x and y axis, respectively, in the PCA plot. In the resulting biplot, vectors that point in the same direction correspond to elements that have similar response profile, and can be interpreted as having similar meaning in the context set by the data. PCA was conducted using RStudio 0.99.1228 using the function *prcomp* (RStudio Team, 2015).

Interpolated map of anthropogenic metal concentrations (Cu, Cd, Pb, Zn) was elaborated in the Quantum Gis application version 2.12.1 (Lion). For visualization, three layers were applied: a map of the city of Siena, map of the sampling points (study and control plots), and interpolation results. Patterns of metals concentrations were visualized by interpolation methods based on Inverse Distance Weighting (IDW).

RESULTS

The average concentrations and ranges of 10 measured elements (Al, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) in samples of moss, excreta and soft tissues of *Papillifera papillaris* collected in 2014 and 2015 are listed in Tab. 8.

Absolute concentrations, wall characteristics, site description and traffic intensity in each plot are summarized in Tab. 9. The levels and patterns of detected trace metals in the three matrices were different. The averages for the trace metals in mosses were in the order of Fe > Al > Mn > Zn > Cu > Cr > Ni > Pb > Cd > Hg, while the order for excreta was Al > Fe > Mn > Pb > Cr > Zn > Cu > Ni > Cd > Hg and finally for soft tissues was Mn > Zn > Fe > Cu > Al > Pb > Cr > Cd > Ni > Hg.

Tab. 8 Range and mean ($\pm$ SD) concentrations ($\mu\text{g g}^{-1}$ d.w.) of metals in mosses and *Papillifera papillaris* excreta and soft tissues.

	Al	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
MOSSES										
CONTROL (n=3)	512-696	0.07-0.22	2.80-62.5	8.71-23.9	360-834	0.06-0.14	30.8-55.0	1.30-5.35	0.60-2.11	6.40-34.3
mean$\pm$SD	625 $\pm$ 99	0.13 $\pm$ 0.08	41.4 $\pm$ 33.4	14.9 $\pm$ 7.95	573 $\pm$ 241	0.09 $\pm$ 0.04	42.2 $\pm$ 12.1	3.25 $\pm$ 2.03	1.25 $\pm$ 0.77	16.8 $\pm$ 15.2
URBAN (n=12)	245-1113	0.05-0.37	1.57-37.0	11.1-27.2	406-980	0.05-0.20	18.6-129	0.13-17.8	0.98-9.26	8.59-54.1
mean$\pm$SD	582 $\pm$ 232	0.19 $\pm$ 0.10	14.1 $\pm$ 12.7	17.7 $\pm$ 5.30	647 $\pm$ 177	0.09 $\pm$ 0.05	53.6 $\pm$ 35.7	4.07 $\pm$ 5.54	3.95 $\pm$ 2.68	27.0 $\pm$ 13.9
EXCRETA										
CONTROL (n=4)	25669-38871	0.09-0.30	37.0-54.7	51.1-147	17331-28181	0.09-0.90	387-1379	3.22-41.4	18.0-33.0	43.1-64.0
mean SD	32270 $\pm$ 9335	0.22 $\pm$ 0.09	45.8 $\pm$ 12.5	99.0 $\pm$ 67.8	22756 $\pm$ 7672	0.50 $\pm$ 0.33	883 $\pm$ 701	29.5 $\pm$ 18.0	23.1 $\pm$ 6.87	53.5 $\pm$ 14.8
URBAN (n=10)	8624-29389	0.04-2.63	25.0-332	80.0-184	8036-15.973	0.03-0.63	291-895	8.93-39.3	65.0-1022	30.0-333
mean$\pm$SD	15793 $\pm$ 6548	0.86 $\pm$ 0.85	139 $\pm$ 117	119 $\pm$ 36	11834 $\pm$ 3029	0.31 $\pm$ 0.24	538 $\pm$ 183	19.1 $\pm$ 9.5	300 $\pm$ 295	136 $\pm$ 108
TISSUES										
CONTROL (n=4)	31.4-236	1.27-2.67	2.47-22.10	154-230	122-457	0.10-0.44	744-1991	0.34-2.00	0.53-3.11	146-269
mean$\pm$SD	111 $\pm$ 92	2.04 $\pm$ 0.59	11.1 $\pm$ 9.60	189 $\pm$ 39.3	239 $\pm$ 149	0.23 $\pm$ 0.15	1530 $\pm$ 544	1.42 $\pm$ 0.75	1.76 $\pm$ 1.10	184 $\pm$ 57
URBAN (n=10)	7.80-283	1.78-6.43	1.25-27.4	123-264	101-379	0.06-0.69	540-2696	0.10-1.84	2.75-40.9	305-769
mean$\pm$SD	55.2 $\pm$ 81	4.10 $\pm$ 1.55	7.02 $\pm$ 7.82	171 $\pm$ 45	184 $\pm$ 92	0.20 $\pm$ 0.18	1289 $\pm$ 721	0.82 $\pm$ 0.62	12.2 $\pm$ 12.3	482 $\pm$ 174

Tab. 9 Absolute metal concentrations in pooled sampled of mollusc excreta, soft tissues and mosses of *Papillifera papillaris* collected in may 2014 and april 2015. Wall features, vegetation cover, distance from the road as well as traffic intensity in each plot are considered.

id/year	Site	Plot type	Matrix	Wall features	Vegetation cover	Moss species	Distance from road	Traffic Vehic h ⁻¹	Al	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
1/2014	MANDORLO	Control	EXCRETA	Retaining, 130 cm high, travertine stones and bricks, loosely cemented	Scarce (mainly mosses and herbs)	<i>Brachythecium rutabulum</i>	roadside	<20	25669	0.09	37	51.1	17331	0.09	387	3.22	19	64
1/2015	MANDORLO	Control	EXCRETA						38871	0.30	54.7	147	28181	0.49	1379	41.4	33	43.1
2/2014	MARCIANO	Study plot	EXCRETA	Fencing, 180 cm, smooth travertine stone, cemented	Scarce (mosses, under tree canopy)	<i>B. rutabulum</i> , <i>Homalothecium sericeum</i>	roadside	500-700	18361	0.82	280	93.1	13104	0.03	410	11.8	232	74.1
2/2015	MARCIANO	Study plot	EXCRETA						10658	0.05	36.8	80	8036	0.51	700	25.2	80.3	39
3/2014	ROSSELLI	Study plot	EXCRETA	Retaining, 200 cm high, rough travertine stones, cemented and plastered	Medium (mosses and herbs)	<i>Hypnum cupressiforme</i>	roadside	1000-1500	16467	2.63	201	129	14144	0.05	367	11.4	509	232
3/2015	ROSSELLI	Study plot	EXCRETA						11240	0.93	24.9	88	9150	0.43	665	21.8	114	69.2
4/2014	MONTARIOSO	Study plot	EXCRETA	Retaining, 110 cm high, vacuolar dolomitic limestone, loosely cemented	Scarce (lichens, mosses, herbs)	<i>Homalothecium lutescens</i>	8 m	800-1000	29389	0.62	233	111	15973	0.11	494	13.6	167	106
4/2015	MONTARIOSO	Study plot	EXCRETA						19558	0.42	61.8	101	13868	0.63	895	39.3	83.1	30
5/2014	PESCAIA	Study plot	EXCRETA	Retaining, 150 cm high, rough travertine stones, cemented	Medium (lichens, mosses, herbs)	<i>Hypnum cupressiforme</i>	roadside	1500-2000	12442	1.13	161	184	10403	0.13	291	8.93	1022	333
5/2015	PESCAIA	Study plot	EXCRETA						8624	0.06	31.4	101	8156	0.58	542	26.9	406	120
6/2014	FORTEZZA	Study plot	EXCRETA	Rampart of medieval fortress, 25 m high, red bricks, cemented	Abundant (mosses, lichens, herbs)	<i>Hypnum cupressiforme</i>	roadside	800-1000	21513	1.95	332	127	15535	0.11	407	12.3	329	287
6/2015	FORTEZZA	Study plot	EXCRETA						9680	0.04	31.1	176	9971	0.5	608	19.7	65	68.2
7/2015	MONTE OLIVETO	Control	EXCRETA	Outer wall of an old abbey, red bricks, cemented	Abundant (mosses, lichens)	<i>Homalothecium sericeum</i>	100 m	none	ND	0.21	ND	ND	ND	0.54	ND	41.2	18	ND
8/2015	MONTE OLIVETO	Control	EXCRETA	Retaining, 250 cm high, red bricks, cemented	Scarce (lichens)	-	200 m	none	ND	0.27	ND	ND	ND	0.9	ND	32.1	22.2	ND

id/year	Site	Plot type	Matrix	Wall features	Vegetation cover	Moss species	Distance from road	Traffic Vehic h ⁻¹	Al	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
1/2014	MANDORLO	Control	MOLLUSC	Retaining, 130 cm high, travertine stones and bricks, loosely cemented	Scarce (mainly mosses and herbs)	<i>Brachythecium rutabulum</i>	roadside	<20	236	1.98	22.1	154	457	0.1	1991	0.34	3.11	170
1/2015	MANDORLO	Control	MOLLUSC						53	1.27	2.47	230	167	0.25	1642	1.84	0.53	146
2/2014	MARCIANO	Study plot	MOLLUSC	Fencing, 180 cm, smooth travertine stone, cemented	Scarce (mosses, under tree canopy)	<i>B. rutabulum, Homalothecium sericeum</i>	roadside	500-700	283	4.5	27.4	203	379	0.12	1109	0.41	10.5	706
2/2015	MARCIANO	Study plot	MOLLUSC						35	1.78	4.78	204	113	0.06	540	1.29	2.85	351
3/2014	ROSSELLI	Study plot	MOLLUSC	Retaining, 200 cm high, rough travertine stones, cemented and plastered	Medium (mosses and herbs)	<i>Hypnum cupressiforme</i>	roadside	1000-1500	54.1	6.43	9.36	167	217	0.11	1737	0.18	10.6	671
3/2015	ROSSELLI	Study plot	MOLLUSC						35.2	3.92	2.15	127	116	0.09	620	0.88	3.95	323
4/2014	MONTARIOSO	Study plot	MOLLUSC	Retaining, 110 cm high, vacuolar dolomitic limestone, loosely cemented	Scarce (lichens, mosses, herbs)	<i>Homalothecium lutescens</i>	8 m	800-1000	35	5.21	10.2	191	194	0.16	2696	0.6	11.7	490
4/2015	MONTARIOSO	Study plot	MOLLUSC						50.3	2.62	1.89	264	103	0.32	2224	1.45	3.42	305
5/2014	PESCAIA	Study plot	MOLLUSC	Retaining, 150 cm high, rough travertine stones, cemented	Medium (lichens, mosses, herbs)	<i>Hypnum cupressiforme</i>	roadside	1500-2000	7.8	4.13	3.02	126	145	0.14	954	0.15	40.9	482
5/2015	PESCAIA	Study plot	MOLLUSC						13.1	2.52	3.27	123	101	0.12	745	1.84	26.6	342
6/2014	FORTEZZA	Study plot	MOLLUSC	Rampart of medieval fortress, 25 m high, red bricks, cemented	Abundant (mosses, lichens, herbs)	<i>Hypnum cupressiforme</i>	roadside	800-1000	17.4	6.23	6.92	169	298	0.16	1397	0.1	9.03	769
6/2015	FORTEZZA	Study plot	MOLLUSC						21	3.63	1.25	140	170	0.39	872	1.29	2.75	382
7/2015	MONTE OLIVETO	Control	MOLLUSC	Outer wall of an old abbey, red bricks, cemented	Abundant (mosses, lichens)	<i>Homalothecium sericeum</i>	100 m	none	122	2.67	16.2	158	212	0.14	1743	1.49	2.09	269
8/2015	MONTE OLIVETO	Control	MOLLUSC	Retaining, 250 cm high, red bricks, cemented	Scarce (lichens)	-	200 m	none	31.4	2.26	3.68	217	122	0.44	744	2	1.31	153

id/year	Site	Plot type	Matrix	Wall features	Vegetation cover	Moss species	Distance from road	Traffic Vehic h ⁻¹	Al	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
1/2014	MANDORLO	Control	MOSS	Retaininig, 130 cm high, travertine stones and bricks, loosely cemented	Scarce (mainly mosses and herbs)	<i>Brachythecium rutabulum</i>	roadside	<20	696	0.07	58.9	8.71	834	0.08	30.8	1.3	1.04	34.3
1/2015	MANDORLO	Control	MOSS						512	0.09	62.5	12.2	360	0.14	41	5.35	0.6	6.4
2/2014	MARCIANO	Study plot	MOSS	Fencing, 180 cm, smooth travertine stone, cemented	Scarce (mosses, under tree canopy)	<i>B. rutabulum</i> , <i>Homalothecium sericeum</i>	roadside	500-700	357	0.1	12.4	14.3	406	0.06	27.8	0.49	3.84	48.6
2/2015	MARCIANO	Study plot	MOSS						612	0.05	33.7	15.4	980	0.08	36.8	1.23	4.12	54.1
2/2014	MARCIANO	Study plot	MOSS						682	0.25	2.15	22.3	631	0.05	86	3.23	9.26	20
2/2015	MARCIANO	Study plot	MOSS						1113	0.23	3.01	27.2	935	0.2	129	2.71	7.41	23.9
3/2014	ROSSELLI	Study plot	MOSS	Retaining, 200 cm high, rough travertine stones, cemented and plastered	Medium (mosses and herbs)	<i>Hypnum cupressiforme</i>	roadside	1000-1500	245	0.13	26.1	11.1	641	0.06	18.6	0.74	1.15	25.8
3/2015	ROSSELLI	Study plot	MOSS						446	0.22	2.2	18.1	482	0.07	43.1	6.24	2.19	8.59
4/2014	MONTARIOSO	Study plot	MOSS	Retaining, 110 cm high, vacuolar dolomitic limestone, loosely cemented	Scarce (lichens, mosses, herbs)	<i>Homalothecium lutescens</i>	8 m	800-1000	542	0.06	37	11.6	693	0.09	29.4	0.69	0.98	38.1
4/2015	MONTARIOSO	Study plot	MOSS						845	0.19	4.32	18.7	700	0.19	99	17.8	1.31	15
5/2014	PESCAIA	Study plot	MOSS	Retaining, 150 cm high, rough travertine stones, cemented	Medium (lichens, mosses, herbs)	<i>Hypnum cupressiforme</i>	roadside	1500-2000	496	0.16	17.1	15.6	624	0.06	23.7	0.56	3.46	32.8
5/2015	PESCAIA	Study plot	MOSS						668	0.37	1.57	25.4	686	0.09	57	2.62	6.76	13.2
6/2014	FORTEZZA	Study plot	MOSS	Rampart of medieval fortress, 25 m high, red bricks, cemented	Abundant (mosses, lichens, herbs)	<i>Hypnum cupressiforme</i>	roadside	800-1000	407	0.17	10.1	12.4	414	0.07	20.7	0.13	2.11	24.1
6/2014	FORTEZZA	Study plot	MOSS						569	0.33	19.7	20.4	571	0.09	72	12.4	4.8	19.7
7/2015	MONTE OLIVETO	Control	MOSS	Outer wall of an old abbey, red bricks, cemented	Abundant (mosses, lichens)	<i>Homalothecium sericeum</i>	100 m	none	666	0.22	2.8	23.9	525	0.06	55	3.1	2.11	9.78

Boxplots of elements in the three matrices (mollusc soft tissues and excreta, mosses; Fig. 16) and Kruskal-Wallis rank sum test showed statistically significant differences in the power of accumulation of the three matrices for all elements analysed.

A post-hoc test using Wilcoxon tests with Bonferroni correction for multiple comparisons showed that excreta had significantly higher concentrations of Al, Cr, Fe, Ni and Pb than moss and soft tissues ($p < 0.05$).

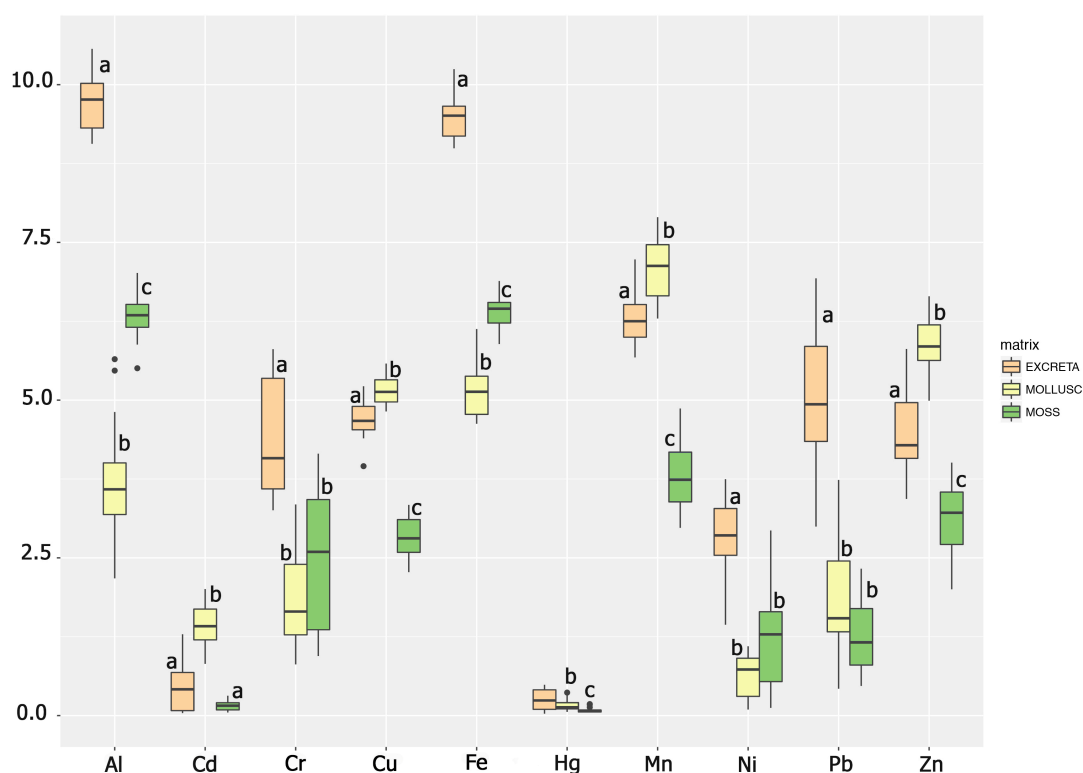


Fig. 16 Boxplot of Al, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn logarithmic concentrations in the three matrices (excreta, soft tissues and mosses) collected in sampled and control sites. Solid line within the box indicates the median value. First and third quartile ("hinges", box extremes) and 95% confidence interval of median ("notches", vertical lines) are illustrated too. Different letters denote statistical significant differences ($p < 0.05$).

Spearman test in scatterplot matrix revealed statistically significant positive correlation values between the fecal contents in lithophile elements (Al/Fe, Mn/Ni), as well as between Pb and Zn (Fig. 17a).

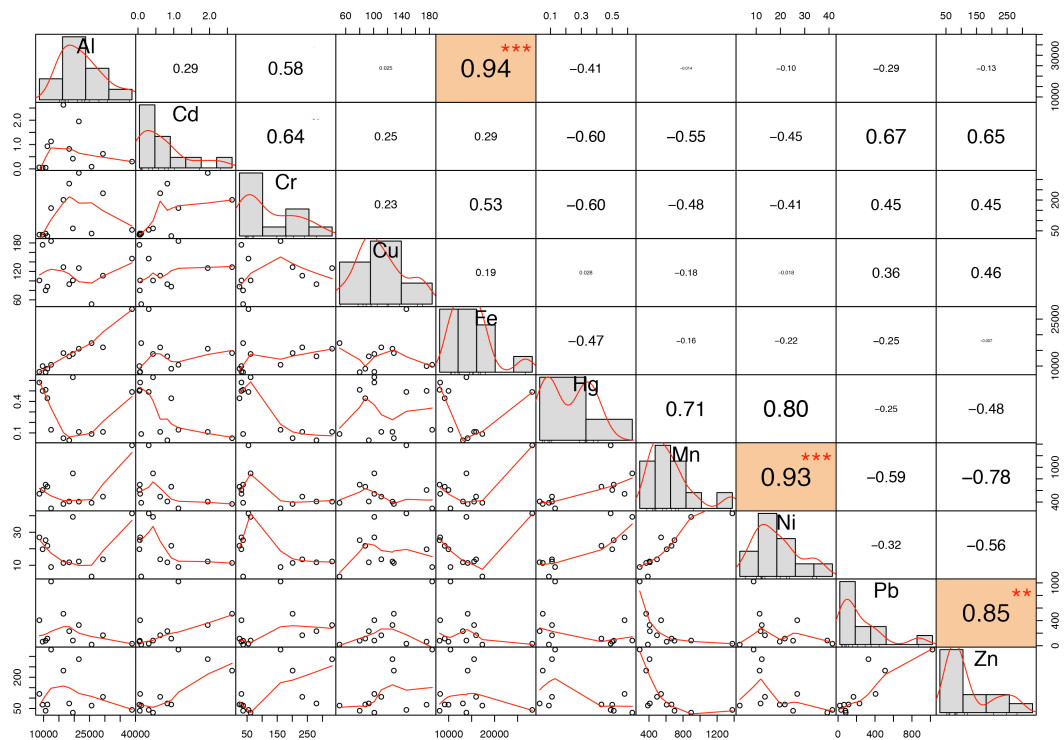


Fig. 17a Scatterplot matrix of the ten chemical elements (Al, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) in **mollusc excreta** with the corresponding Spearman correlation coefficients (top right) and the univariate distribution (diagonally downwards) plotted as histograms and Kernel density plot. On the left side of the diagonal is the scatterplot matrix, with loess smoothers in red illustrating the type of relationship between elements. Red stars are significance levels after Bonferroni correction (*0.05, **0.01, ***0.001).

Cu, Mn, Zn elements showed higher concentrations in mollusc tissues than the other two matrices ($p < 0.05$) (Fig. 16): the average concentrations of these latter elements in *Papillifera papillaris* soft tissues were one order of magnitude higher than in mosses and 1.3-3 times higher than those in the excreta. The anthropogenic element Cd showed significant differences between mollusc/moss and mollusc/excreta matrices; no difference between Cd concentrations in moss and *P. papillaris* excreta. Only one pairwise comparison was found significant for element Hg (mollusc soft tissues/moss). Litophile elements such as Al and Fe showed the lowest concentrations in mollusc soft tissues ($p < 0.05$).

Correlation matrix and Spearman test showed only one highly statistically significant correlation between Cd and Zn in soft tissues (Fig. 17b).

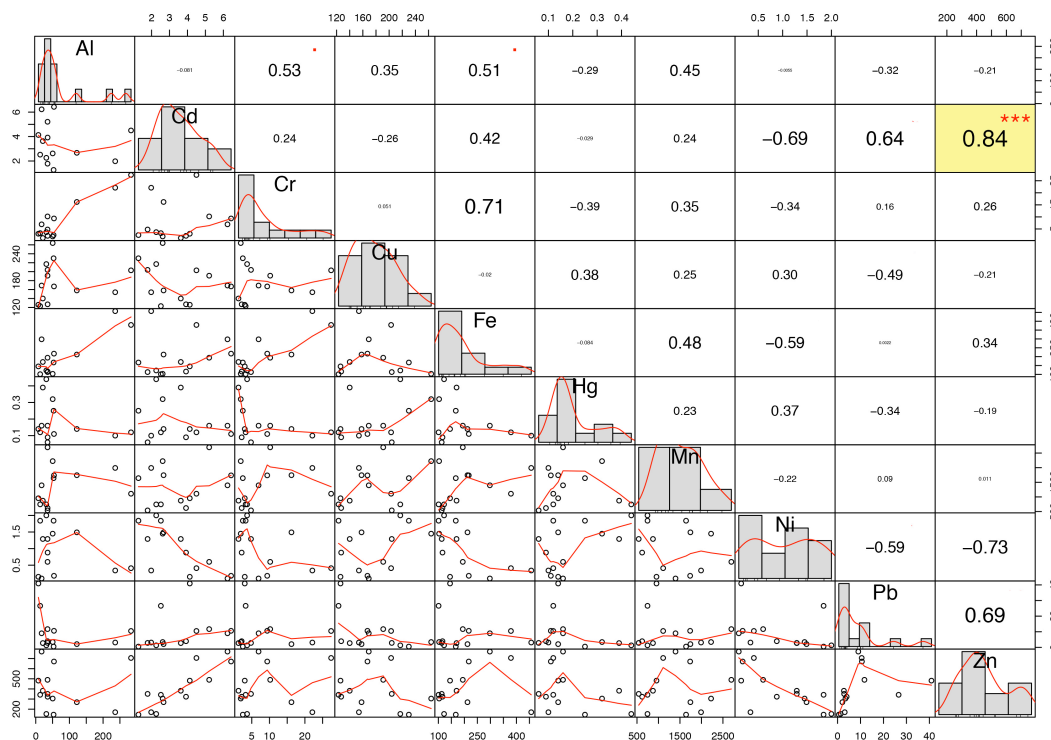


Fig. 17b Scatterplot matrix of the ten chemical elements (Al, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) in **mollusc soft tissues** with the corresponding Spearman correlation coefficients (top right) and the univariate distribution (diagonally downwards) plotted as histograms and Kernel density plot. On the left side of the diagonal is the scatterplot matrix, with loess smoothers in red illustrating the type of relationship between elements. Red stars are significance levels after Bonferroni correction (*0.05, **0.01, ***0.001).

Respect to mollusc matrices, moss samples in descriptive boxplots (Fig. 16) show intermediate concentration for almost all elements considered. Cu and Zn detain the lowest values than the other two matrices ($p < 0.05$).

Positive and very significant Spearman's correlation values even after Bonferroni correction for multiple comparison (Fig. 17c) were found between pairs of lithophile elements such as Al/Mn or Mn/Ni, and between those likely arising, at least in part, from anthropogenic sources, such as Cd, Cu and Pb. Concentrations of Cd and Cu were inversely and significantly related to those of Cr whereas Mn showed positive correlations with lithophile (Al) as well as anthropogenic elements (Cu).

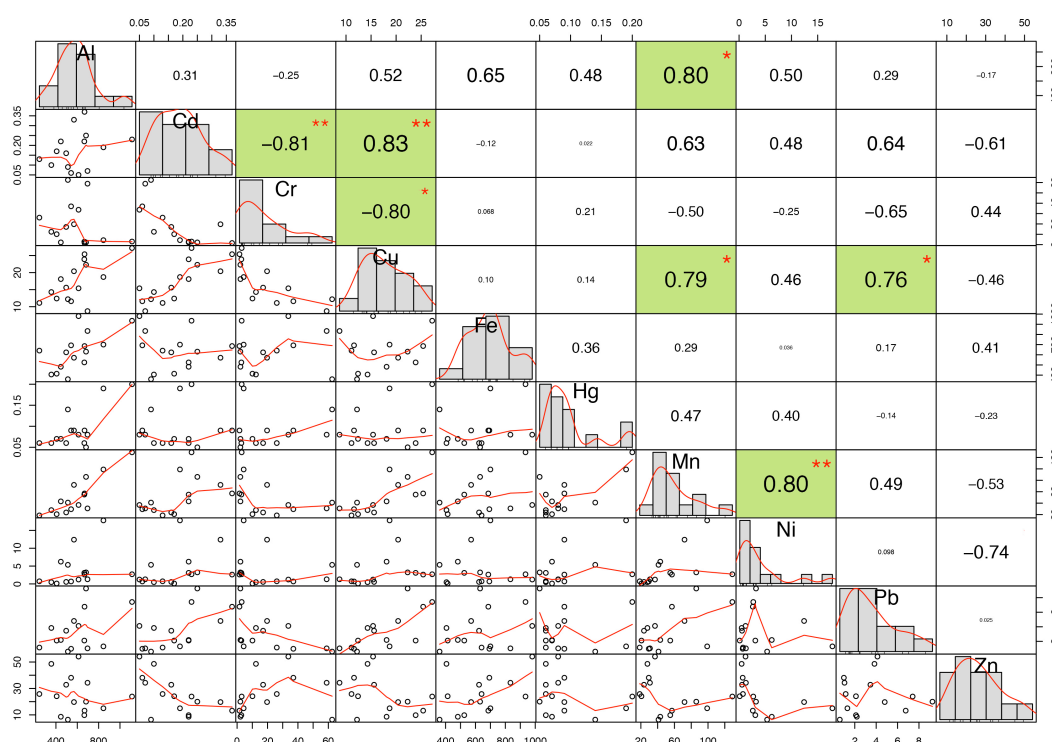


Fig. 17c Scatterplot matrix of the ten chemical elements (Al, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) in **mosses** with the corresponding Spearman correlation coefficients (top right) and the univariate distribution (diagonally downwards) plotted as histograms and Kernel density plot. On the left side of the diagonal is the scatterplot matrix, with loess smoothers in red illustrating the type of relationship between elements. Red stars are significance levels after Bonferroni correction (*0.05, **0.01, ***0.001).

The results of principal components analysis for the whole concentration data in moss, mollusc tissues and excreta are presented in Fig. 18. Based on the eigenvalue gained in this study, two principal components (PC1 and PC2) were observed to be significant, together accounting for 79% of the total data set variability. PC1 showed a contraposition between two groups of elements, showing negative loadings for crustal elements such as Al (-0.47), Fe (-0.45), Ni (-0.41), Cr (-0.35) while Cd (0.31) was the element with the highest positive loading; PC2 (36%) included high loadings for all the elements, especially Cu (0.50), Mn (0.49), Zn (0.42), Cd (0.35) and Pb (0.32). In the biplot, with respect to PC1 mosses have a neutral and intermediate position, while soft tissues and excreta occupy mutually opposite directions. With respect to PC2, mollusc soft tissues and excreta lie on the positive side, whereas mosses lie on the negative side.

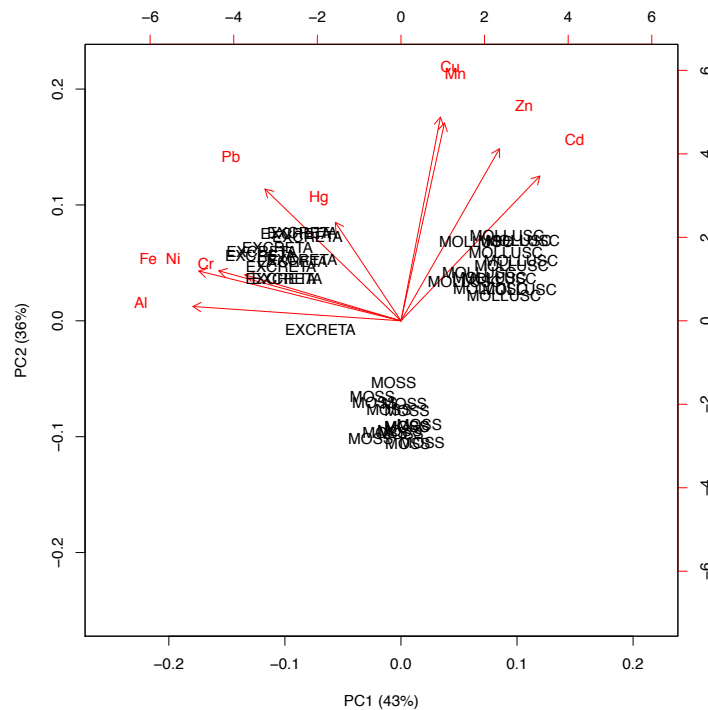


Fig. 18 PCA analysis of metal concentrations in mosses and *Papillifera papillaris* excreta and tissues at the urban and control sampling sites. The first two ordination axes account for 43 % and 36 % of the variance, respectively, and the relative length of vectors shows the influence of metals on separating samples, within and between the 3 matrices along these axes.

Boxplots of the scores of the first two PCs (Fig. 19a, b) plotted as index for the three matrices confirmed that PC1 is associated with crustal materials, representing a geochemical index led by Al element, while PC2 showed an association with metal pollutants bioaccumulation.

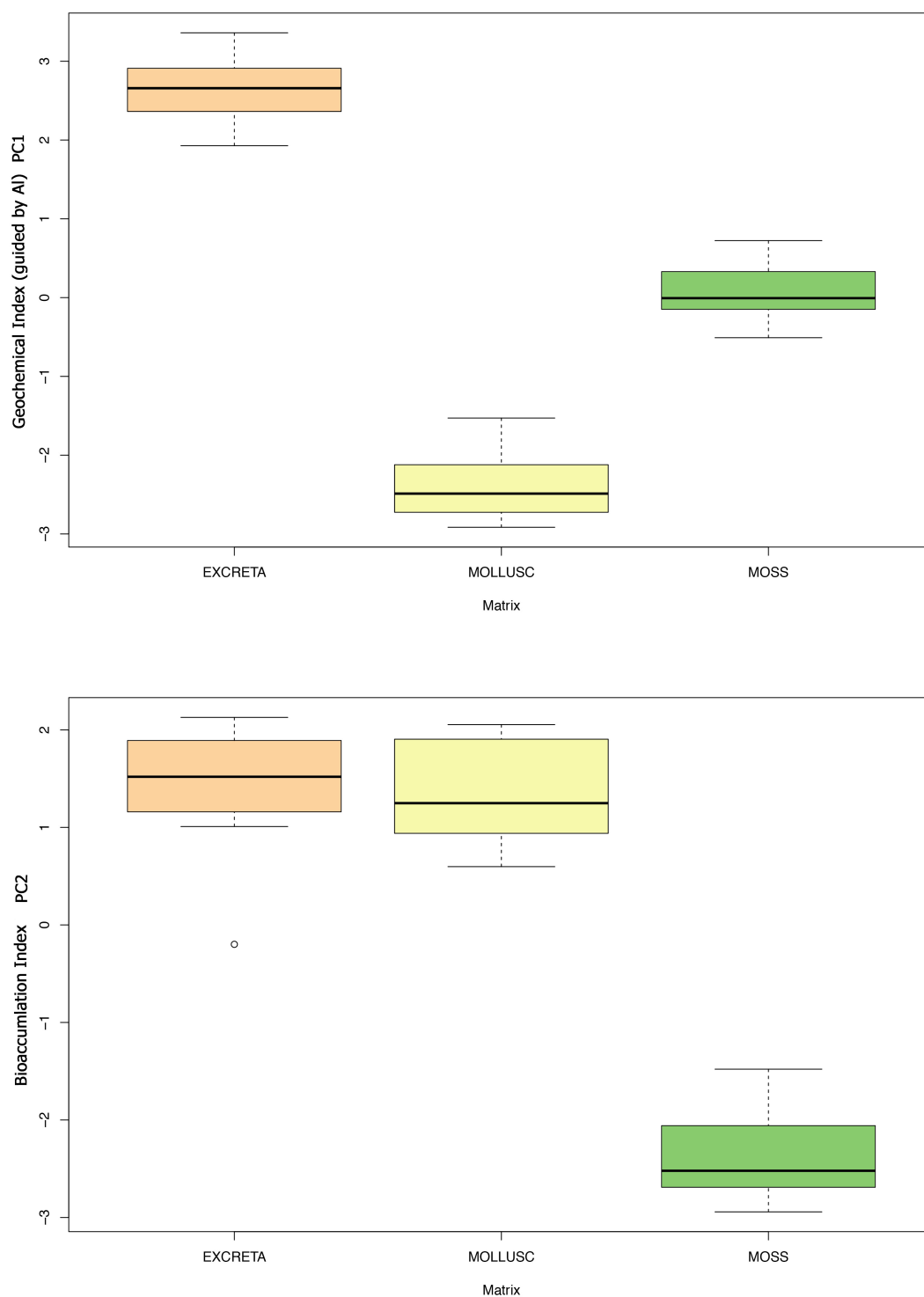


Fig. 19a,b Boxplot of PC1 and PC2 scores derived from PCA analysis of metals in mollusc soft tissues, excreta and mosses.

In general samples collected in control sites (< 20 vehicles h^{-1}) showed lower average concentrations of anthropogenic elements such as Cd, Cu, Pb and Zn (Fig. 20).

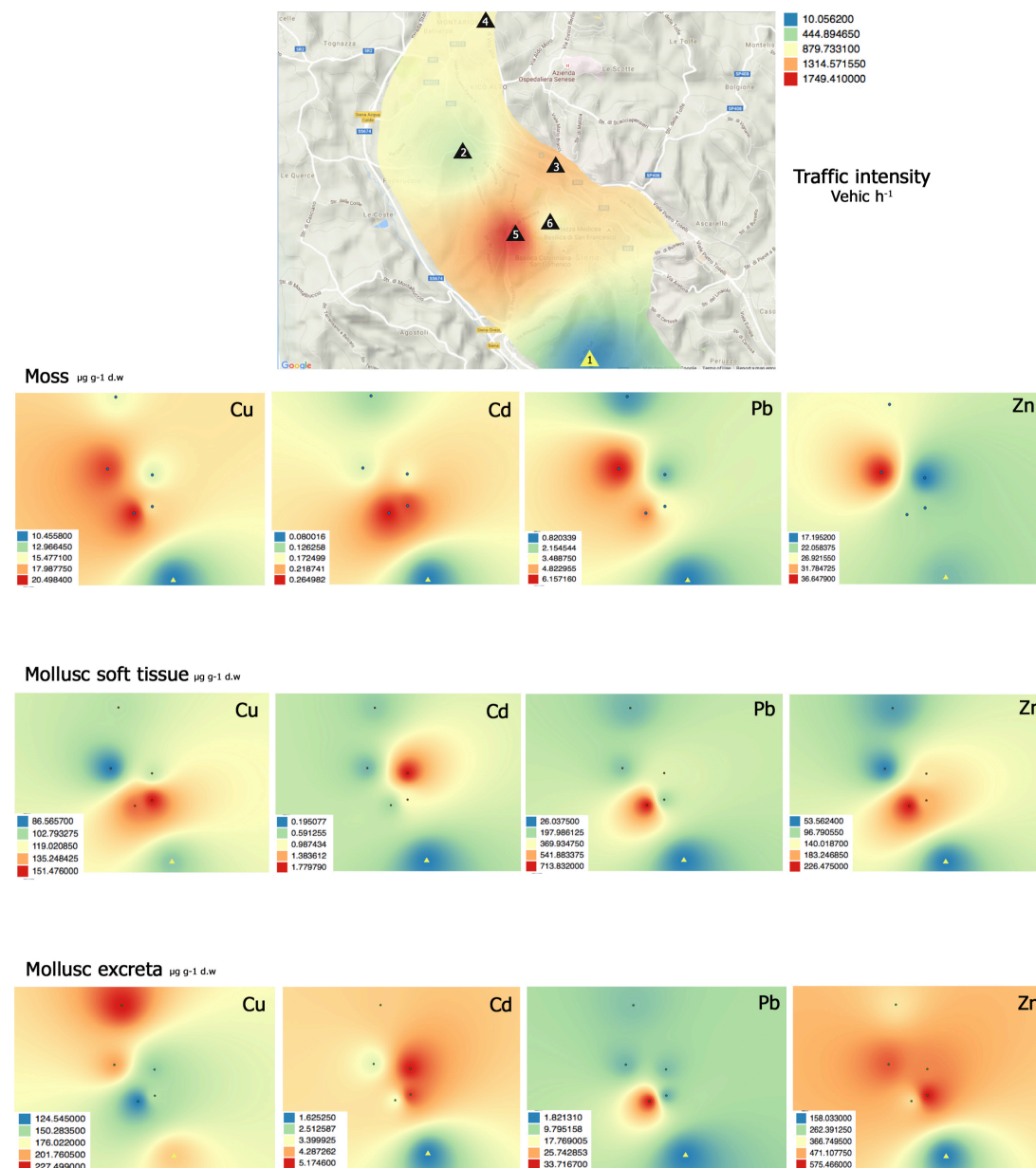


Fig. 20 Interpolated map of concentrations of anthropogenic elements Cu, Cd, Pb and Zn compared to intensity of vehicular traffic in the city of Siena. Black triangles represent study plots (for plot identification see Tab. 9), the yellow one the control site.

As a rule, the content of these elements in samples from alongside roads with a rather intensive vehicular traffic such as site 5 was 2-3 times higher.

Considering the feces of *Papillifera papillaris*, maximum values of lithophile

elements were measured in samples from sites 1 and 4 (retaining walls), whereas samples from roadsides with higher traffic intensity (sites 3, 5, and 6) had the highest Pb concentrations (from 114 to 1022 $\mu\text{g g}^{-1}$ d.w.).

Mn concentrations were highest in mollusc tissues from site 4 (a retaining wall with vacuolar dolomitic limestone), which contained low concentrations of lithophile elements.

The bioaccumulation of lithophile elements such as Al, Fe, Cr, Mn and Ni, was generally higher in mosses from retaining walls (with overhanging soil), whereas Hg concentrations were low and rather homogeneously distributed among analyzed samples.

DISCUSSION

The results of this preliminary survey on metal accumulation in mosses and *Papillifera papillaris* from different types of walls along urban and suburban roads showed a different ability of the matrices (mosses, mollusc excreta and soft tissues) to interact with pollutants and therefore to accumulate them. Excreta and mosses appeared to be more prone to contamination by soil and stone particles as indicated by high Al and Fe concentrations, the PCA analysis and boxplots of PC component scores. These elements, which are scraped with the radula from wall vegetation and crusts on travertine, stone or brick substrata, fail to accumulate in soft tissues and pass unscathed in the excreta, where they are concentrated in high amounts. Despite signs of contamination by soil particles as a consequence of the ingestion of considerable amount of lithophile elements, for some elements such as Ni, Cr and especially Pb, feces confirmed to be the best descriptor as confirmed by PCA analysis too. For instance, in the site with the highest intensity of vehicular traffic the mean Pb concentration in feces ($714 \mu\text{g g}^{-1} \text{ d.w.}$) was 21 times higher than that in soft tissues. This latter result, together with a low concentration of Pb in the *Papillifera papillaris* tissues, suggested that this metal might have a relatively small impact on urban food chains since only a small fraction of ingested Pb is likely bioavailable.

Also mosses showed some kind of lithogenic contamination: although adhering materials were accurately removed from moss shoots before the analysis, statistically significant correlations between lithophile elements likely suggest that entrapped soil and rock dust particles contributed to their concentrations. However, Kosior et al. (2015) found that there are no relationships between metal concentrations in mosses growing on walls and those in the underlying stone materials. The elemental composition of the different species of mosses used in our survey seems to confirm a negligible metal uptake from stones or black crusts. Besides the evident lithogenic contamination, the intermediate position in PC1 and PC2 suggests that mosses likely intercept metals from wet atmospheric deposition and airborne

particles (mostly soil and road dust) re-suspended by vehicles and wind. Therefore, it is quite difficult to assess the contribution from anthropogenic sources to the bioaccumulation of elements such as Fe, Cr or Ni and to evaluate their bioavailability to herbivores (Bargagli, 1995).

Surprisingly, molluscs soft tissues revealed to be a better biomarker for environmental contamination by metals compared with traditional biomonitors as confirmed by PCA and boxplot of PC scores: *Papillifera papillaris* tissues better accumulate strictly anthropogenic elements such as Cd, Cu and Zn which are among the most widespread airborne pollutants in urban environments (7.0 to 20.0 times higher in snail tissues than in mosses) (Table 2). These concentrations may be easily explained by the different physiological accumulation capacity of the two matrices: mosses accumulate metals in wet and dry atmospheric deposition through passive cation exchange processes and the interception (adsorption) of airborne particles (e.g., Adamo et al., 2008), whereas in *Papillifera papillaris* the metals interact with the animal through different pathways, such as the ingestion of algae, lichens, mosses, herbs, black crusts, soil and rock particles scraped from stones or bricks, by inhalation and/or by contact with wall surfaces. It has been demonstrated, in fact, that snails are able to well assume heavy metals orally or through skin absorption, due to the large moist surface which is in contact with the ground (Coeurdassier et al., 2002).

The majority of the contaminants are concentrated in the digestive gland which is the most important organ for both detoxification and accumulation of bioavailable non-essential elements such as Cd, Pb or Hg, or essential elements such as Cu or Zn assumed in excess. Their bioaccumulation is mainly due to the compartmentalization in intracellular granules or vesicles in the digestive gland or other organs such as the foot or the albumen gland (e.g., Dallinger et al., 1993; Beeby and Richmond, 2002; Regoli et al., 2006; Boshoff et al., 2013). Like the other terrestrial gastropods, even this species accumulates in its soft tissues high concentrations of Cd, Cu, Pb and Zn. The reference ranges for non-contaminated areas in the other land snail species are Cd= 0.6-6; Cu= 80-190; Pb= 0.7-6; Zn= 100-200. In *Papillifera papillaris*

the assessed minimum values are 1.27 for Cd, 123 for Cu, 170 for Pb and 12.53 for Zn; then in line with other biomonitoring surveys with molluscs (e.g., Berger and Dallinger, 1993; Pihan et al., 1995; Scheifler et al., 2006; Fritsch et al., 2011; de Vaufleury, 2015). Soft tissues from snails purged of their gut contents have low Al, Fe, Cr and Ni concentrations and in general they do not contain soil particles, as confirmed by the mean values and standard deviations and by PCA results. Essentially, the elemental composition of mollusc soft tissues is much more informative than that of cryptogams because well reflects the bioavailability of environmental pollutants.

In general, the analyses conducted so far support the hypothesis that molluscs (excreta and tissues) are better bioindicators than traditional mosses, making plausible the promotion of *Papillifera papillaris* as new bioindicator species of the effects of air pollution from vehicular traffic in urban environment. Extraordinary is the concentration of Mn, which was among the most amazing novelties of our survey: *Papillifera papillaris* accumulates in its tissues amounts of Mn (1530 ± 544) incredibly in excess to those reported in the literature for other species of molluscs.

Manganese is a relatively non-toxic and essential element, serving to animals as cofactor for a number of enzymes such as hydrolase, kinases, decarboxylases or transferases (Adriano, 2001). As a rule, Mn concentrations in the hepatopancreas of different species of terrestrial molluscs (e.g., Menta and Parisi, 2001; Regoli et al., 2006; Bordean et al., 2014) are always $< 730 \mu\text{g g}^{-1}$ d.w. (i.e., about one-half of those in *Papillifera papillaris* soft tissues). In the viscera of *Helix pomatia* from an industrial waste dump, Pihan and de Vaufleury (2000) measured from 89 to $538 \mu\text{g g}^{-1}$ d.w. of Mn, and in wild and cultured terrestrial gastropods Mn concentrations spanned from < 100 ppm (in animals from reference areas; Class I) to > 400 ppm (in those from very polluted environments, Class IV) (Pihan et al., 1995). According to this classification, all our *Papillifera papillaris* individuals, including those from reference stations, would fall in Class IV. Bordean et al. (2014) in a study about a 60-days exposure of the snail *Cornu aspersum* to neutral soils enriched with Mn, found that the metal uptake can occur independently of

food ingestion (i.e., by contact with soil) and the Mn accumulation in the hepatopancreas does not determine toxic effects. Since the Mn concentrations found in mosses showed the same concentration range of those usually measured in samples from reference areas, the exceptional amount of Mn in *Papillifera papillaris* soft tissues may be related to a physiological acting process. This finding is also supported by (1) the lack of statistically significant relationships between Mn and potentially toxic elements in the mollusc soft tissues; (2) the position of Mn close to essential trace elements such as Cu and Zn in the PCA plot; (3) the much higher Mn content in soft tissues than in the excreta.

Unlike elements such as Cd, Cu or Hg, which are removed from the blood by metallothionein proteins (Dallinger and Wieser, 1984), elements such as Mn, Ca or K are stored in granules with pyrophosphate ions and charged oxygen atoms acting as ligands (Hopkin, 1989). Interestingly, after intravascular injections of Mn, Mason and Simkiss (1982) found that this element induced the loss of granules from the basophil cells and their voiding in the feces. As *Papillifera papillaris* lives on travertine and limestone it can not be excluded that the Mn accumulation has a crucial role in Ca metabolism and excretion.

CONCLUSION

The analytical determination of Al, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn concentrations in different species of mosses and the soft tissues and excreta of the land snail *Papillifera papillaris* collected in 2014 and 2015 from walls at the side of roads with different intensities of vehicular traffic and from control sites showed that:

- mosses and excreta are the matrices most involved in lithophile contamination;
- excreta showed a high concentration of lithophile elements, scraped from walls and not absorbed through the digestive tracts, and Pb, demonstrating the scarce bioavailability and absorption of this element and probably its limited impact on urban food chains;
- when compared to the elemental composition of mosses growing on the same walls, mollusc tissues, following the accurate purge of gut contents, showed very low concentrations of lithophile elements, and much higher concentrations of metals mostly released by anthropogenic sources such as Cd, Cu, Pb, and Zn.
- Surprisingly, *Papillifera papillaris* tissues accumulated much higher Mn concentrations than those reported in literature for other species of terrestrial gastropods.

GENERAL CONCLUSIONS

Human impact on snail assemblages and their diversity resulted to be significant but not always detrimental. Overall sampling recovered 60 species, 26 found exclusively in urban areas, if slugs were omitted. Only three entities (*Paralaoma servilis*, *Marmorana serpentina* and *Deroceras invadens*) had to be considered proper alien species, confirming the minor impact of biotic homogenization and alien species invasion in study areas.

Results confirmed the huge importance of habitat structure in affecting land snail communities: in forestal context, suitable environmental conditions are sufficient requisites to sustain land snail communities ensuring survival and protection, more than other parameters analysed such as forest age as surrogate for forest continuity parameter.

Surprisingly, the main results concerned city context: urbanization resulted to greatly alter land snail community structure, especially concerning human-mediated transport, which improves dispersal abilities of organisms normally considered scarcely mobile. However, things are not always so negative: the extreme heterogeneity found in urban areas represents a rich mosaic of different environmental conditions, even enhancing biodiversity and encouraging species to colonize urban areas. Despite the small geographical gradient, Tuscan cities showed relatively high land snail richness with values comparable to natural areas, demonstrating the wealth of diversity patterns offered by the Mediterranean area and especially the Italian peninsula. Many species adapted to the new stresses, food sources, predators and threats in urban and suburban environments, where they thrive in close proximity to humans, leading to a strong adaption to artificial habitats. Not only but some land snail species developed such a strong relationship with humans to be found in urban ecosystems only outside their native range. This coexistence led to benefits for both parties: *Papillifera papillaris* revealed to be one of the most promising species to monitor the metal distribution and bioavailability in urban environments, providing researchers with valuable and unexpected

insights into heavy metal accumulation processes.

In conclusion, the impact of humans on biodiversity has clearly been harmful to groups or organisms, but the story is more complex than has been appreciated. According to John Marzluff, “we should be more positive about our relationship with the natural world, celebrating the creative aspects of our impact on biodiversity in addition to concerning ourselves with the negative effects”.

REFERENCES

1. Abele SE (2010) Gastropod diversity in the boreal mixedwood forest of northern Alberta – variation among forest types and response to partial harvesting. M.S. Thesis. Edmonton, Alberta: University of Alberta. 107 pp.
2. Adamo P, Giordano S, Naimo D, Bargagli R (2008) Geochemical properties of airborne particulate matter (PM₁₀) collected by automatic devices and biomonitors in a Mediterranean urban environment. *Atmospheric Environment*, 42: 346-357.
3. Adriano DC (2001) Trace Elements in Terrestrial Environments. Biogeochemistry, Bioavailability and Risks of Metals. II ed. Springer-Verlag, New York, 866 pp.
4. Amici V, Santi E, Filibeck G, Diekmann M, Geri F, Landi S, Scoppola A, Chiarucci A (2013) Influence of secondary forest succession on plant diversity patterns in a Mediterranean landscape. *Journal of Biogeography*, 40: 2335-2347.
5. Angold PG, Sadler JP, Hill MO, Pullin A, Rushton S, Austin K, Small E, Wood B, Wadsworth R, Sanderson R, Thompson K (2006) Biodiversity in urban habitat patches. *Science of the Total Environment*, 360(1): 196–204.
6. Aronson MF, La Sorte FA, Nilon CH, Katti M, Goddard MA, Lepczyk CA, Warren PS, Williams NSG, Cilliers S, Clarkson B, Dobbs C, Dolan R, Hedblom M, Klotz S, Louwe Kooijmans J, Kühn I, MacGregor-Fors I, McDonnell M, Mörtberg U, Pyšek P, Siebert S, Sushinsky J, Werner P, Winter M (2014) A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. *Proceedings of the Royal Society London B: Biological Sciences*, 281(1780): 20133330.
7. Astorga A, Oksanen J, Luoto M, Soininen J, Virtanen R, Muotka T (2012) Distance decay of similarity in freshwater communities: do

macro-and microorganisms follow the same rules? *Global Ecology and Biogeography*, 21(3): 365–375.

8. Bacaro G, Altobelli A, Cameletti M, Ciccarelli D, Martellos S, Palmer MW, Ricotta C, Rocchini D, Scheiner SM, Tordoni E, Chiarucci A (2016) Incorporating spatial autocorrelation in rarefaction methods: implications for ecologists and conservative biologists. *Ecological Indicators*, 69: 233-238.
9. Bacaro G, Rocchini D, Ghisla A, Marcantonio M, Neteler M, Chiarucci A (2012) The spatial domain matters: Spatially constrained rarefaction in a Free and Open Source environment. *Ecological Complexity*, 12: 63-69.
10. Baldock KC, Goddard MA, Hicks DM, Kunin WE, Mitschunas N, Osgathorpe LM, Potts SG, Robertson KM, Scott AV, Stone GN, Vaughan IP, Memmott J (2015) Where is the UK's pollinator biodiversity? The importance of urban areas for flower-visiting insects. *Proceedings of the Royal Society London B: Biological Sciences*, 282(1803): 20142849.
11. Barazzuoli P, Guasparri G, Salleolini M (1993) Il clima. In: Giusti F (ed) *La Storia Naturale della Toscana Meridionale*. Amilcare Pizzi, Cinisello Balsamo, pp. 141-171.
12. Barbato D, Benocci A, Caruso T, Manganelli G (2017) The role of dispersal and local environment in urban land snail assemblages: an example of three cities in central Italy. *Urban Ecosystems*, pp 1-13.
13. Bargagli R (1995) The elemental composition of vegetation and the possible incidence of soil contamination of samples. *Science of the Total Environment*, 176: 121-128.
14. Bargagli R (1998) *Trace Metals in Terrestrial Plants: an Ecophysiological Approach to Biomonitoring and Biorecovery*. Springer-Verlag, Berlin, 324 pp.
15. Bargagli R (2012) *Ecologia applicata. Per un uso consapevole dell'aria, dell'acqua e del suolo*. Amon Edizioni. 272 pp.

16. Bargagli R, Iosco FP, Barghigiani C (1987) Assessment of mercury dispersal in an abandoned mining area by soil and lichen analysis. *Water, Air, and Soil Pollution*, 36(1-2): 219-225.
17. Barratt BI, Dickinson KJ, Freeman C, Porter S, Johnstone PD, Wing J, Heezik Y (2015). Biodiversity of Coleoptera and other invertebrates in urban gardens: a case study in a New Zealand city. *Insect Conservation and Diversity*, 8(5): 428-437.
18. Baselga A (2010) Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, 19(1): 134-143.
19. Baselga A, Jiménez-Valverde A, Niccolini G (2007) A multiple-site similarity measure independent of richness. *Biology Letters*, 3(6): 642-645.
20. Baur A, Baur B (1993) Daily movement patterns and dispersal in the land snail *Arianta arbustorum*. *Malacologia*, 35: 89–98.
21. Beeby A, Richmond L (2002) Do the soft tissue of *Helix aspersa* serve as a quantitative sentinel of predicted free lead concentrations in soils? *Applied Soil Ecology*, 22: 159-165.
22. Bell G (2000) The distribution of abundance in neutral communities. *The American Naturalist*, 155(5): 606–617.
23. Beninde J, Veith M, Hochkirch A (2015) Biodiversity in cities needs space: a meta-analysis of factors determining intra-urban biodiversity variation. *Ecology Letters*, 18(6): 581–592.
24. Benocci A, Bacaro G, Manganelli G (2015) Local and regional scale biodiversity patterns of forest snail assemblages in Tuscany (central Italy). *Community Ecology*, 16(2): 147-155.
25. Berger B, Dallinger R (1993) Terrestrial snails as quantitative indicators of environmental metal pollution. *Environmental and Monitoring Assessment*, 25: 65-84.
26. Bertani R, Cosimi C, De Liso A, Ferrara R, Maserti BE, Trifoglio M, Zuccarelli D (1994) Mercury in a primary consumer (*Eobania*

- vermiculata*) collected near a chloralkali complex. Environmental Technology, 15(11): 1095-1100.
27. Beumer C, Martens P (2015) Biodiversity in my (back) yard: towards a framework for citizen engagement in exploring biodiversity and ecosystem services in residential gardens. Sustainability Science, 10(1): 87-100.
 28. Blondel J (2006) The 'design' of Mediterranean landscapes: a millennial story of humans and ecological systems during the historic period. Human Ecology, 34(5): 713-729.
 29. Bonet A, Pausas JG (2004) Species richness and cover along a 60-year chronosequence in old-fields of southeastern Spain. Plant Ecology, 174(2): 257-270.
 30. Borcard D, Legendre P (2002) All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. Ecological Modelling, 153: 51–68.
 31. Borcard D, Legendre P, Avois-Jacquet C, Tuomisto H (2004) Dissecting the spatial structure of ecological data at multiple scales. Ecology, 85: 1826–1832.
 32. Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. Ecology, 73(3): 1045–1055.
 33. Bordean DM, Nica DV, Harmanescu M, Banatean-Dunea I, Gergen II (2014) Soil manganese enrichment from industrial inputs: A gastropod perspective. Plos One, 9: e85384.
 34. Boshoff M, Jordaens K, Backeljau T, Lettens S, Tack F, Vandecasteele B, De Jonge M, Bervoets L (2013) Organ- and species-specific accumulation of metals in two land snail species (Gastropoda, Pulmonata). Science of the Total Environment, 449: 470-481.
 35. Cáceres MD, Legendre P (2009) Associations between species and groups of sites: indices and statistical inference. Ecology, 90(12): 3566-3574.

36. Cadenasso ML, Pickett ST, Schwarz K (2007) Spatial heterogeneity in urban ecosystems: reconceptualizing land cover and a framework for classification. *Frontiers in Ecology and the Environment*, 5(2): 80-88.
37. Cameron RAD, Pokryszko BM (2005) Estimating the species richness and composition of land mollusc communities: problems, consequences and practical advice. *Journal of Conchology*, 38(5): 529-547.
38. Carrington D (2016) The Anthropocene epoch: scientists declare dawn of human-influenced age. *The Guardian* 29 August 2016. Web
39. Carrus G, Scopelliti M, Laforteza R, Colangelo G, Ferrini F, Salbitano F, Agrimi M, Portoghesi L, Semenzato P, Sanesi G (2015) Go greener, feel better? The positive effects of biodiversity on the well-being of individuals visiting urban and peri-urban green areas. *Landscape and Urban Planning*, 134: 221–228.
40. Caruso T, Chan Y, Lacap DC, Lau MC, McKay CP, Pointing SB (2011) Stochastic and deterministic processes interact in the assembly of desert microbial communities on a global scale. *The ISME Journal*, 5(9): 1406–1413.
41. Caruso T, Taormina M, Migliorini M (2012) Relative role of deterministic and stochastic determinants of soil animal community: a spatially explicit analysis of oribatid mites. *Journal of Animal Ecology*, 81(1): 214–221.
42. Chao A (1987) Estimating the population size for capture-recapture data with unequal catchability. *Biometrics*, 43: 783–791.
43. Chiarucci A, Bacaro G, Filibeck G, Landi S, Maccherini S, Scoppola A (2012) Scale dependence of plant species richness in a network of protected areas. *Biodiversity and Conservation*, 21: 503-516.
44. Chiarucci A, Bacaro G, Rocchini D, Ricotta C, Palmer MW, Scheiner SM (2009) Spatially constrained rarefaction: incorporating the autocorrelated structure of biological communities into sample-based rarefaction. *Community Ecology*, 10(2): 209-214.
45. Chytrý M, Lososová Z, Horský M, Uher B, Čejka T, Danihelka J, Fajmon

- K, Hájek O, Juříčková L, Kintrová K, Láníková D, Otýpková Z, Řehořek V, Tichý L (2012) Dispersal limitation is stronger in communities of microorganisms than macroorganisms across Central European cities. *Journal of Biogeography*, 39(6): 1101–1111.
46. Cianfanelli S (2009) I Molluschi della Provincia di Pistoia: le specie da tutelare e quelle da combattere. Quaderni del Padule di Fucecchio n. 6. Centro di Ricerca, Documentazione e Promozione del Padule di Fucecchio. 112 pp.
 47. Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, 18: 117-143.
 48. Cœurdassier M, Gomot-de Vaufleury A, Lovy C, Badot PM (2002) Is the cadmium uptake from soil important in bioaccumulation and toxic effects for snails? *Ecotoxicology and Environmental Safety*, 53(3): 425-431.
 49. Colwell RK, Mao CX, Chang J (2004) Interpolating, extrapolating, and comparing incidence - based species accumulation curves. *Ecology*, 85(10): 2717-2727.
 50. Cuttelod A, García N, Abdul Malak D, Temple H, Katariya V (2008) The Mediterranean: a biodiversity hotspot under threat. In: Vié JC, Hilton-Taylor C. and Stuart SN (eds). *The 2008 Review of The IUCN Red List of Threatened Species*. IUCN Gland, Switzerland.
 51. Cuttelod A, Seddon M, Neubert E (2011) *European Red List of Non-marine Molluscs*. Publications Office of the European Union, Luxembourg. <https://portals.iucn.org/library/efiles/documents/RL-4-014.pdf>. Accessed 16 June 2016
 52. Czech B, Krausman PR, Devers PK (2000) Economic Associations among Causes of Species Endangerment in the United States Associations reflect the integration of economic sectors, supporting the theory and evidence that economic growth proceeds at the competitive exclusion of nonhuman species in the aggregate. *BioScience*, 50(7): 593-601.

53. Dallinger R, Berger B, Gruber A (1993) Quantitative aspects of zinc and cadmium binding in *Helix pomatia*: differences between an essential and a non essential trace element. In: Dallinger R, Rainbow PS (eds) *Ecotoxicology of Metals in Invertebrates*. Lewis Publishers, Boca Raton, pp. 315-332.
54. Dallinger R, Wieser W (1984) Patterns of accumulation, distribution and liberation of Zn, Cu, Cd and Pb in different organs of the land snail *Helix pomatia* L. *Comparative Biochemistry and Physiology*, 79: 117-124.
55. Damesin C, Rambal S, Joffre R (1998) Co-occurrence of trees with different leaf habit: a functional approach on mediterranean oaks. *Acta Oecologica*, 19: 195–204.
56. Dedov I, Penev L (2004) Spatial variation in terrestrial gastropod communities (Gastropoda, Pulmonata) along urban-rural gradients in Sofia City, Bulgaria. In: Penev L, Niemelä J, Kotze J, Chipev N (eds) *Ecology of the city of Sofia. Species and communities in an urban environment*. Pensoft Publishers, Moscow, pp 307–318.
57. Dittrich S, Hauck M, Schweigatz D, Dörfler I, Hühne R, Bade C, Jacop M, Leuschner C (2013) Separating forest continuity from tree age effects on plant diversity in the ground and epiphyte vegetation of a Central European mountain spruce forest. *Flora-Morphology, Distribution, Functional Ecology of Plants*, 208(4): 238-246.
58. Douglas DD (2011) Land snail species diversity and composition between different forest disturbance regimes in central and eastern Kentucky Forests. Dissertation, Eastern Kentucky University.
59. Douglas DD, Brown DR, Pederson N (2013) Land snail diversity can reflect degrees of anthropogenic disturbance. *Ecosphere*, 4(2): 1-14.
60. Dray S, Legendre P, Peres-Neto PR (2006) Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecological Modelling*, 196(3): 483–493.

61. Duarte LD, Prieto PV, Pillar VD (2012) Assessing spatial and environmental drivers of phylogenetic structure in Brazilian Araucaria forests. *Ecography*, 35(10): 952-960.
62. Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, 67(3): 345-366.
63. EEA (2015) Air Quality in Europe – 2015 Report. European Environment Agency, Luxembourg, 64 pp.
64. Ellis EC, Ramankutty N (2008) Putting people in the map: anthropogenic biomes of the world. *Frontiers in Ecology and the Environment*, 6(8): 439–447.
65. EPA (2016) Particulate Matter (PM) Basics. <https://www.epa.gov/pm-pollution/particulate-matter-pm-basics#PM>. Accessed 25 September 2016.
66. Erdmann G, Floren A, Linsenmair KE, Scheu S, Maraun M (2006). Little effect of forest age on oribatid mites on the bark of trees. *Pedobiologia*, 50(5): 433-441.
67. Ferenc M, Sedláček O, Fuchs R, Dinetti M, Fraissinet M, Storch D (2014) Are cities different? Patterns of species richness and beta diversity of urban bird communities and regional species assemblages in Europe. *Global Ecology and Biogeography*, 23(4): 479–489.
68. Fiera C (2009) Biodiversity of Collembola in urban soils and their use as bioindicators for pollution. *Pesquisa Agropecuária Brasileira*, 44(8): 868-873.
69. Fiorentino V, Caruso T, Manganelli G, Giusti F (2009) Population dynamics of an urban population of the land snail *Marmorana serpentina* (Gastropoda: Pulmonata). *Malacologia*, 51(1): 201–209.
70. Flory SL, Clay K (2009) Effects of roads and forest successional age on experimental plant invasions. *Biological Conservation*, 142(11): 2531-2537.

71. Fountain MT, Hopkin SP (2004) Biodiversity of Collembola in urban soils and the use of *Folsomia candida* to assess soil 'quality'. *Ecotoxicology*, 13(6): 555-572.
72. Francis RA, Lorimer J (2011) Urban reconciliation ecology: the potential of living roofs and walls. *Journal of Environmental Management*, 92(6): 429–1437.
73. Franklin JF, Spies TA (1991) Ecological definitions of old-growth Douglas-fir forests. US Department of Agriculture Forest Service, Pacific Northwest Research Station, Portland, Oregon. General Technical Report PNW GTR, 285: 61-69.
74. Frantz A, Pottier MA, Karimi B, Corbel H, Aubry E, Haussy C, Gasparini J, Castrec-Rouelle M (2012) Contrasting levels of heavy metals in the feathers of urban pigeons from close habitats suggest limited movements at a restricted scale. *Environmental Pollution*, 168: 23-28.
75. Fritsch C, Coeurdassier M, Gimbert F, Crini N, Scheiffer R, de Vaufleury A (2011) Investigations of responses to metal pollution in land snail populations (*Cantareus aspersus* and *Cepaea nemoralis*) from a smelter- impacted area. *Ecotoxicology*, 20: 739-759.
76. Fuller RA, Irvine KN, Devine-Wright P, Warren PH, Gaston KJ (2007) Psychological benefits of greenspace increase with biodiversity. *Biology Letters*, 3(4): 390–394.
77. Gál J, Markiewicz-Patkowska J, Hursthouse A, Tatner P (2008) Metal uptake by woodlice in urban soils. *Ecotoxicology and environmental safety*, 69(1), 139-149.
78. Gallé R, Maák I, Szpisjak N (2014) The effects of habitat parameters and forest age on the ground dwelling spiders of lowland poplar forests (Hungary). *Journal of Insect Conservation*, 18: 791–799.
79. Gilg O (2004) Forêts à caractère naturel: caractéristiques, conservation et suivi. *Cahiers Techniques de l'ATEN*, 74. Montpellier.
80. Giusti F, Manganelli G, Schembri PJ (1995) The non-marine molluscs of the Maltese Islands. *Monografie Mus. Reg. Sc. Nat., Torino* 15: 1-607.

81. Gomot A, Pihan F (1997) Comparison of the bioaccumulation capacities of copper and zinc in two snail subspecies (*Helix*). *Ecotoxicology and Environmental Safety*, 38(2): 85-94.
82. Gomot-de Vaufleury A (2000) Standardized growth toxicity testing (Cu, Zn, Pb, and Pentachlorophenol) with *Helix aspersa*. *Ecotoxicology and Environmental Safety*, 46(1): 41-50.
83. Gotelli NJ, Ellison M (2013) A primer of ecological statistics. Sinauer Associates, Inc. Sunderland, MA, USA, pp 406-414.
84. Grimm NB, Faeth SH, Golubiewski NE, Redman CL, Wu J, Bai X, Briggs JM (2008) Global change and the ecology of cities. *Science*, 319: 756–760.
85. Güneralp B, Seto KC (2013) Futures of global urban expansion: uncertainties and implications for biodiversity conservation. *Environmental Research Letters*, 8(1): 014025.
86. Hahs AK, McDonnell MJ, McCarthy MA, Vesik PA, Corlett RT, Norton BA, Clemants SE, Duncan RP, Thompson K, Schwartz MW, Williams NS (2009) A global synthesis of plant extinction rates in urban areas. *Ecology Letters*, 12(11): 1165-1173.
87. Hájek M, Roleček J, Cottenie K, Kintrová K, Horsák M, Pouličková A, Hájková P, Fránková M, Dítě D (2011) Environmental and spatial controls of biotic assemblages in a discrete semi-terrestrial habitat: comparison of organisms with different dispersal abilities sampled in the same plots. *Journal of Biogeography*, 38(9): 1683–1693.
88. Hartop EA, Brown BV, Disney RHL (2015) Opportunity in our Ignorance: Urban Biodiversity Study Reveals 30 New Species and One New Nearctic Record for *Megaselia* (Diptera: Phoridae) in Los Angeles (California, USA). *Zootaxa*, 3941(4): 451–484.
89. Heino J (2013) Does dispersal ability affect the relative importance of environmental control and spatial structuring of littoral macroinvertebrate communities? *Oecologia*, 171(4): 971–980.

90. Hopkin SP (1989) *Ecophysiology of Metals in Terrestrial Invertebrates*. Elsevier Applied Science Publ., New York, 366 pp.
91. Horsák M, Juříčková L, Kintrová K, Hájek O (2009) Patterns of land snail diversity over a gradient of habitat degradation: a comparison of three Czech cities. *Biodiversity and Conservation*, 18(13): 3453-3466.
92. Hotopp K (2005) Land Snail Ecology. Retrieved from <http://www.carnegiemnh.org/science/mollusks/landsnailecology.html>
93. Hubbell SP (2001) The unified neutral theory of biodiversity and biogeography (MPB-32) (Vol. 32). Princeton University Press.
94. Hunter ML, White AS (1997) Ecological thresholds and the definition of old-growth forest stands. *Natural Areas Journal*, 17:292–296.
95. Ingimarsdóttir M, Caruso T, Ripa J, Magnúsdóttir ÓB, Migliorini M, Hedlund K (2012) Primary assembly of soil communities: disentangling the effect of dispersal and local environment. *Oecologia*, 170(3): 745–754.
96. Kaplan JO, Krumhardt KM, Zimmermann N (2009) The prehistoric and preindustrial deforestation of Europe. *Quaternary Science Reviews*, 28: 3016-3034.
97. Kekkonen J, Hanski IK, Väisänen RA, Brommer JE (2012) Levels of heavy metals in house sparrows (*Passer domesticus*) from urban and rural habitats of southern Finland. *Ornis Fennica*, 89: 91-98.
98. Knapp S, Kühn I, Mosbrugger V, Klotz S (2008) Do protected areas in urban and rural landscapes differ in species diversity? *Biodiversity and Conservation*, 17: 1595–1612.
99. Koivula M, Kukkonen J, Niemelä J (2002) Boreal carabid-beetle (Coleoptera, Carabidae) assemblages along the clear-cut originated succession gradient. *Biodiversity and Conservation*, 11(7): 1269-1288.
100. Kosior G, Prell M, Samecka-Cymerman A, Stankiewicz A, Kolon K, Kryza R., Brudzińska-Kosior A, Frontasyeva M, Kempers AJ (2015) Metals in

- Tortula muralis* from sandstone buildings in an urban agglomeration. *Ecological Indicators*, 58: 122-131.
101. Kowalczyk-Pecka D, Czepiel-Mil K (2013) The effect of accumulation of metals on selected physiological biomarkers in *Cochlodina* (*Cochlodina*) *laminata* (Pulmonata: Clausiliidae) inhabiting urban biocenoses/Wpływ akumulacji metali na wybrane biomarkery fizjologiczne *Cochlodina* (*Cochlodina*) *laminata* (Pulmonata: Clausiliidae) zasiedlających urbicenozy. *Ochrona Środowiska i Zasobów Naturalnych-Environmental Protection and Natural Resources*, 24(2): 45-49.
 102. Kowalczyk-Pecka D, Stryjecki R (2013) Use of selected aspects of *Cepaea nemoralis* (Gastropoda: Pulmonata) polymorphism in environmental biomonitoring on Lublin area. *Teka Komisji Ochrony i Kształtowania Środowiska Przyrodniczego*, 10.
 103. Kruskal JB (1964a) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika*, 29: 1-27.
 104. Kruskal JB (1964b) Nonmetric multidimensional scaling: A numerical method. *Psychometrika*, 29: 115-129.
 105. La Sorte FA, McKinney ML, Pyšek P (2007) Compositional similarity among urban floras within and across continents: biogeographical consequences of human-mediated biotic interchange. *Global Change Biology*, 13: 913–921.
 106. Laskowski R, Hopkin SP (1996) Accumulation of Zn, Cu, Pb and Cd in the garden snail (*Helix aspersa*): implications for predators. *Environmental Pollution*, 91(3): 289-297.
 107. Legendre P (2008) Studying beta diversity: ecological variation partitioning by multiple regression and canonical analysis. *Journal of Plant Ecology*, 1(1): 3-8.
 108. Legendre P, Fortin MJ (1989) Spatial pattern and ecological analysis. *Vegetatio*, 80(2): 107–138.
 109. Legendre P, Legendre L (1998) *Numerical Ecology*. Elsevier, Amsterdam.

110. Legendre P, Mi X, Ren H, Ma K, Yu M, Sun IF, He F (2009) Partitioning beta diversity in a subtropical broad-leaved forest of China. *Ecology*, 90(3): 663–674.
111. Lockwood JL (2004) How do biological invasions alter diversity patterns? In: Lomolino MV, Heaney LR (eds) *Frontiers of Biogeography: New Directions in the Geography of Nature*. Sinauer, Sunderland, MA, pp 297–309.
112. Loppi S, Ivanov D, Boccardi R (2002) Biodiversity of epiphytic lichens and air pollution in the town of Siena (Central Italy). *Environmental Pollution*, 116(1): 123–128.
113. Loram A, Thompson K, Warren PH, Gaston KJ (2008) Urban domestic gardens (XII): the richness and composition of the flora in five UK cities. *Journal of Vegetation Science*, 19(3): 321–330.
114. Lososová Z, Horsák M, Chytrý M, Čejka T, Danihelka J, Fajmon K, Hájek O, Juříčková L, Kintrová K, Láníková D, Otýpková Z, Řehořek V, Tichý L (2011) Diversity of Central European urban biota: effects of human-made habitat types on plants and land snails. *Journal of Biogeography*, 38: 1152–1163.
115. Loumbourdis NS (1997) Heavy metal contamination in a lizard, *Agama stellio stellio*, compared in urban, high altitude and agricultural, low altitude areas of North Greece. *Bulletin of Environmental Contamination and Toxicology*, 58: 945–952.
116. Lundholm JT, Richardson PJ (2010) Habitat analogues for reconciliation ecology in urban and industrial environments. *Journal of Applied Ecology*, 47: 966–975.
117. Lydeard C, Cowie RH, Ponder WF, Bogan AE, Bouchet P, Clark SA, Cumming KS, Frest TJ, Gargominy O, Herbert DG, Hershler R, Perez KE, Roth B, Seddon M, Strong E, Thompson FG (2004) The global decline of nonmarine mollusks. *BioScience*, 54(4): 321–330.
118. Mänd R, Tilgar V, Leivits A (2000) Calcium, snails, and birds: a case study. *Web Ecology*, 1(1): 63–69.

119. Manganelli G, Benocci A, Giusti F (2015) Chioccioline e lumache dell'Arcipelago Toscano. I Quaderni del Parco, volume 6.
120. Manganelli G, Bodon M, Favilli L, Giusti F (1995) Gastropoda Pulmonata. In: Minelli A, Ruffo S and La Posta S (eds) Checklist delle specie della fauna d'Italia, 16. Edizioni Calderini, Bologna, pp 1–60.
121. Marmor L, Tõrra T, Saag L, Randlane T (2011) Effects of forest continuity and tree age on epiphytic lichen biota in coniferous forests in Estonia. *Ecological Indicators*, 11(5): 270-276.
122. Martiny JBH, Bohannan BJ, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Adams Krumins J, Ruske CR, Morin PJ, Naeem S, Øvreås L, Reysenbach A, Smith VH, Staley JT (2006) Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology*, 4(2): 102–112.
123. Mason AZ, Simkiss K (1982) Sites of mineral deposition in metal-accumulating cells. *Experimental Cell Research*, 139: 383-391.
124. Maurer U, Peschel T, Schmitz S (2000) The flora of selected urban land-use types in Berlin and Potsdam with regard to nature conservation in cities. *Landscape and Urban Planning*, 46(4): 209–215.
125. McCune B, Grace JB (2002) Analysis of ecological communities. MjM Software Design, USA, Glenden Beach, OR.
126. McKinney ML (2002) Urbanization, Biodiversity, and Conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *BioScience*, 52(10): 883–890.
127. McKinney ML (2006) Urbanization as a major cause of biotic homogenization. *Biological Conservation* 127(3): 247–260.
128. McKinney ML (2008) Effects of urbanization on species richness: a review of plants and animals. *Urban Ecosystems*, 11(2): 161–176.
129. Menez A (2007) A new approach to studying and sampling land molluscs: habitat structure and the effects of scale on land molluscs. *Journal of Conchology*, 39: 321–327.

130. Menta C, Parisi V (2001) Metal concentrations in *Helix pomatia*, *Helix aspersa* and *Arion rufus*: a comparative study. *Environmental Pollution*, 115: 205-208.
131. Mielke PW (1984) 34 Meteorological applications of permutation techniques based on distance functions. *Handbook of Statistics*, 4: 813-830.
132. Mielke PW (1991) The application of multivariate permutation methods based on distance functions in the earth sciences. *Earth-Science Reviews*, 31(1): 55-71.
133. Mielke PW, Berry KJ (2001) *Permutation Methods: A Distance Function Approach*. Springer Series in Statistics. Springer.
134. Mimouni EA, Pinel-Alloul B, Beisner BE (2015) Assessing aquatic biodiversity of zooplankton communities in an urban landscape. *Urban Ecosystems*, 18(4): 1353-1372.
135. Mitchell R, Popham F (2008) Effect of exposure to natural environment on health inequalities: an observational population study. *The Lancet*, 372(9650): 1655–1660.
136. Moning C, Müller J (2008) Critical forest age thresholds for the diversity of lichens, molluscs and birds in beech (*Fagus sylvatica* L.) dominated forests. *Ecological Indicators*, 9: 922-932.
137. Motta R (2002) Old-growth forests and silviculture in the Italian Alps: the case-study of the strict reserve of Paneveggio (TN). *Plant Biosystems- An International Journal Dealing with all Aspects of Plant Biology*, 136(2): 223-231.
138. Müller J, Strätz C, Hothorn T (2005) Habitat factors for land snails in European beech forests with a special focus on coarse woody debris. *European Journal of Forest Research*, 124(3): 233-242.
139. Nahmani J, Hodson ME, Black S (2007) A review of studies performed to assess metal uptake by earthworms. *Environmental Pollution*, 145(2): 402-424.

140. Notten MJM, Oosthoek AJP, Rozema J, Aerts R (2005) Heavy metal concentrations in a soil-plant-snail food chain along a terrestrial soil pollution gradient. *Environmental Pollution*, 138: 178-190.
141. Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Cox C (2004) Translocation of inhaled ultrafine particles to the brain. *Inhalation Toxicology*, 16(6-7): 437-445.
142. Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Lunts A, Kreyling W, Cox C (2002) Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *Journal of Toxicology and Environmental Health Part A*, 65(20): 1531-1543.
143. Oberndorfer E, Lundholm J, Bass B, Coffman RR, Doshi H, Dunnett N, Gaffin S, Köhler M, Liu KKY, Rowe B (2007) Green roofs as urban ecosystems: ecological structures, functions, and services. *BioScience*, 57(10): 823–833.
144. Økland T, Rydgren K, Økland RH, Storaunet KO, Rolstad J (2003) Variation in environmental conditions, understory species number, abundance and composition among natural and managed *Picea abies* forest stands. *Forest Ecology and Management*, 177(1): 17-37.
145. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner HH (2016) *vegan*: Community Ecology Package. R package version 2.3-3. <http://CRAN.R-project.org/package=vegan>
146. Özgo M, Örstan A, Kirschensten M, Cameron R (2016) Dispersal of land snails by sea storms. *Journal of Molluscan Studies*, 82(2): 341-343.
147. Padial AA, Ceschin F, Declerck SA, De Meester L, Bonecker CC, Lansac-Tôha FA, Rodrigues LC, Train S, Velho LFM, Bini LM (2014) Dispersal ability determines the role of environmental, spatial and temporal drivers of metacommunity structure. *PloS one*, 9(10): e111227.
148. Paillet Y, Bergès L, Hjältén J, Ódor P, Avon C, Bernhardt-Römermann M, Bijlsma RJ, De Bruyn L, Fuhr M, Grandin U, Kanka R, Lundin L, Luque S, Magura T, Matesanz S, Mészáros I, Sebastiá MT, Schmidt W,

- Standovar T, Tóthmérész B, Uotila A, Valladares F, Vellak K, Virtanen R. (2010) Biodiversity differences between managed and unmanaged forests: meta-analysis of species richness in Europe. *Conservation Biology*, 24(1): 101-112.
149. Pauget B, Gimbert F, Coeurdassier M, Crini N, Pérès G, Faure O, Douay F, Richard A, Grand C, de Vaufléury A (2013) Assessing the in situ bioavailability of trace elements to snails using accumulation kinetics. *Ecological Indicators*, 34: 126-135.
 150. Paul MJ, Meyer JL (2008) Streams in the urban landscape. In: Marzluff JM, Shulenberger E, Endlicher W, Alberti M, Bradley G, Ryan C, Simon U, ZumBrunnen C (eds), *Urban ecology*. Springer, US, pp. 207-231.
 151. Peres-Neto PR, Legendre P, Dray S, Borcard D (2006) Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology*, 87(10): 2614–2625.
 152. Pignolo G (2010) Studio su gasteropodi terrestri come potenziali bioaccumulatori per metalli pesanti ed idrocarburi policiclici aromatici nella provincia di Trieste. Doctoral dissertation. Retrieved from https://www.openstarts.units.it/dspace/bitstream/10077/4584/1/Pignolo_phd.pdf
 153. Pihan F, de Vaufléury A (2000) The snail as a target organism for the evaluation of industrial waste dump contamination and the efficiency of its remediation. *Ecotoxicology and Environmental Safety*, 46: 137-147.
 154. Pihan JC, Morhain E, Promeyrat S, Pihan F (1995) Contamination métallique des escargot d'élevage et de la nature. Proposition de grilles de qualité (Cu, Zn, Fe, Mn, Cr, Pb). Journée National Hélicicole, ITAVI, GNPE, Lyon, 1-20.
 155. Rabitsch WB (1996) Metal accumulation in terrestrial pulmonates at a lead/zinc smelter site in Arnolstein, Austria. *Bulletin of Environmental Contamination and Toxicology*, 56: 734-741.

156. Rádková V, Bojková J, Křoupalová V, Schenková J, Syrovátka V, Horsák M (2014) The role of dispersal mode and habitat specialization in metacommunity structuring of aquatic macroinvertebrates at isolated spring fens. *Freshwater Biology*, 59: 2256–2267.
157. Rancka B, von Proschwitz T, Hylander K, Götmark F (2015) Conservation thinning in secondary forest: negative but mild effect on Land Molluscs in closed-canopy mixed Oak Forest in Sweden. *PloS one*, 10(3): e0120085.
158. Rangel TFL, Diniz-Filho JAF, Bini LM (2006) Towards an integrated computational tool for spatial analysis in macroecology and biogeography. *Global Ecology and Biogeography*, 15(4): 321–327.
159. Régnier C, Fontaine B, Bouchet P (2009) Not knowing, not recording, not listing: numerous unnoticed mollusk extinctions. *Conservation Biology*, 23(5): 1214–1221.
160. Regoli F, Gorbi S, Fattorini D, Tedesco S, Notti A, Machella N, Bocchetti R., Benedetti M, Piva F (2006) Use of the land snail *Helix aspersa* as sentinel organism for monitoring ecotoxicological effects of urban pollution: an integrated approach. *Environmental Health Perspectives*, 114: 63-69.
161. Reise H, Hutchinson J, Schunack S, Schlitt B (2011) *Deroceras panormitanum* and congeners from Malta and Sicily, with a redescription of the widespread pest slug as *Deroceras invadens* n. sp. *Folia Malacologica*, 19(4): 201-223.
162. Ridout Sharpe J (2010) *Papillifera papillaris*: a second colony is discovered in England. *Archaeo+Malacology Group Newsletter*, 18: 4-6.
163. Roser D, Monteserrat V (2006) The role of successional stage, vegetation type and soil disturbance in the invasion of the alien grass *Cortaderia selloana*. *Journal of Vegetation Science*, 17: 591-598.
164. Rota E, Barbato D, Ancora S, Bianchi N, Bargagli R (2016) *Papillifera papillaris* (O.F. Müller), a small snail living on stones and monuments,

- as indicator of metal deposition and bioavailability in urban environments. *Ecological Indicators*, 69: 360-367.
165. RStudio Team (2015) RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL <http://www.rstudio.com/>
 166. Santorufo L, Carotenuto R, Rocco A, Picione FDL, Maisto G (2012) *Orthonychiurus pseudostachianus* (Collembola) as a toxicity test organism and selection of an ecotoxicological test battery to assess soil quality. *Applied Soil Ecology*, 54: 49-54.
 167. Sattler T, Borcard D, Arlettaz R, Bontadina F, Legendre P, Obrist MK, Moretti M (2010) Spider, bee, and bird communities in cities are shaped by environmental control and high stochasticity. *Ecology*, 91(11): 3343–3353.
 168. Scheifler R, de Vaufleury A, Coeurdassier M, Crini N, Badot PM (2006) Transfer of Cd, Cu, Ni, Pb, and Zn in a “soil-plant-invertebrate” food chain: a microcosm study. *Environmental Toxicology and Chemistry*, 25: 815-822.
 169. Seddon MB (1998) Red Listing for Molluscs: a tool for Conservation? *Journal of Conchology*, (2): 27–44.
 170. Servizio Aree Protette della Provincia di Siena (2013) Realizzazione dei Piani di gestione di 13 SIR della Provincia di Siena. Retrieved from <http://www.provincia.siena.it/var/prov/storage/original/application/a2068ec701cc48bd0642a3a8059be443.pdf>
 171. Ström L, Hylander K, Dynesius M (2009) Different long-term and short-term responses of land snails to clear-cutting of boreal stream-side forests. *Biological Conservation*, 142(8): 1580-1587.
 172. Szczepaniak K, Biziuk M (2003) Aspects of the biomonitoring studies using mosses and lichens as indicators of metal pollution. *Environmental Research*, 93(3): 221-230.

173. Taylor L, Hochuli DF (2015) Creating better cities: how biodiversity and ecosystem functioning enhance urban residents' wellbeing. *Urban Ecosystems*, 18(3): 747–762.
174. Ter Braak CJ (1986) Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology*, 67(5): 1167–1179.
175. Thompson K, Austin KC, Smith RM, Warren PH, Angold PG, Gaston KJ (2003) Urban domestic gardens (I): Putting small-scale plant diversity in context. *Journal of Vegetation Science*, 14: 71–78.
176. United Nations (2014) World Urbanization Prospects: The 2014 Revision. United Nations Department of Economic and Social Affairs/Population Division (ST/ESA/SER.A/352).
177. United Nations Environment Programme (UNEP) (2005) Ecosystems and Biodiversity The Role of Cities. <https://esa.un.org/unpd/wup/Publications/Files/WUP2014-Report.pdf>. Accessed 20 June 2016
178. Van Breugel M, Bongers F, Martínez - Ramos M (2007) Species dynamics during early secondary forest succession: recruitment, mortality and species turnover. *Biotropica*, 39(5): 610-619.
179. Vaufleury de A (2015) Landsnail for ecotoxicological assessment of chemicals and soil contamination - ecotoxicological assessment of chemicals and contaminated soils using the terrestrial snail, *Helix aspersa*, at various stage of its life cycle: a review. In: Armon RH, Hänninen O (eds), *Environmental Indicators*. Springer, Netherlands, pp. 365-391.
180. Vaufleury de AG, Pihan F (2000) Growing snails used as sentinels to evaluate terrestrial environment contamination by trace elements. *Chemosphere*, 40(3): 275-284.
181. Viviani VR (2001) Fireflies (Coleoptera: Lampyridae) from Southeastern Brazil: habitats, life history, and bioluminescence. *Annals of the Entomological Society of America*, 94(1): 129–145.

182. Wang Y, Fu X, Lei C, Jeng ML, Nobuyoshi O (2007) Biological Characteristics of the Terrestrial Firefly *Pyrocoelia pectoralis* (Coleoptera: Lampyridae). *Coleopterists Bulletin*, 61(1): 85–93.
183. Wareborn I (1979) Reproduction of two species of land snails in relation to calcium salts in the foena layer. *Malacologia*, 18: 177-180.
184. Welter-Schultes FW (2012) European non-marine mollusks, a guide for species identification. Planet Poster Editions, Göttingen.
185. Wilcke W (2007) Global patterns of polycyclic aromatic hydrocarbons (PAHs) in soil. *Geoderma*, 141(3): 157-166.
186. Williams NS, Schwartz MW, Vesik PA, McCarthy MA, Hahs AK, Clemants SE, Corlett RT, Duncan RP, Norton BA, Thompson K, McDonnell MJ (2009) A conceptual framework for predicting the effects of urban environments on floras. *Journal of Ecology*, 97(1): 4–9.
187. Wulf M (2004) Plant species richness of afforestations with different former use and habitat continuity. *Forest Ecology and Management*, 195(1): 191-204.
188. Zhai M, Nováček O, Výravský D, Syrovátka V, Bojková J, Helešic J (2015) Environmental and spatial control of ostracod assemblages in the Western Carpathian spring fens. *Hydrobiologia*, 745(1): 225–239.

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