



Does forest age affect soil biodiversity? Case study of land snails in Mediterranean secondary forests

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ARTICLE INFO

Keywords:

Molluscs
Soil fauna
Forest age
Mediterranean
Microhabitat

ABSTRACT

Based on a sampling design used for vascular plants, the effect of forest age (i.e. the time in years since a land patch became a real forest patch as a measure of temporal continuity of forest characteristics) on land snail richness, abundance and composition was assessed in 18 plots in Mediterranean evergreen and deciduous oak forests, belonging to four Sites of Community Importance in Italy. Most examined plots belong to young forests; in particular, three age classes were considered (class 1, < 22 years; class 3, 38–56 years; class 5, > 77 years). Twenty-seven species and 2433 individuals were recorded. Box plots showed that forest age did not seem to be a key factor for land snail species richness and abundance at least in the time range of examined forests. However, this may not be true, if real old-growth forests are considered. No differences between evergreen and deciduous forest were found too. With regard to species composition, Non-metric multidimensional scaling demonstrated an assemblage of species widespread in all forest age classes, while Indicator Species Analysis failed to identify any species as indicator for any age category. This shows that a process is underway but has had insufficient time to differentiate assemblages and to accumulate the majority of rare species, due to the young nature of examined forests. Habitat structure may affect communities more than forest age: suitable microhabitats (a few old/very old trees for shelter) are a sufficient prerequisite for land snail diversity, ensuring community survival and protection, irrespective of forest age class. These findings highlight the importance of habitat structural complexity at local scale for preserving invertebrate diversity in Mediterranean secondary forests.

1. 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) are rare in Europe, especially in the Mediterranean region (Gilg, 2004), where woodland has been exploited since pre-Roman times (Blondel, 2006), or cleared for agriculture (Motta, 2002). Indeed, nowadays most forested areas in southern Europe are secondary woodlots resulting from alternating cycles of deforestation and reforestation (Kaplan et al., 2009). These processes have profoundly modified the physiognomy of temperate forests, often leading to sharp variations in habitat characteristics, homogenization and biodiversity loss in traditional landscapes of Mediterranean Europe (Amici et al., 2013). One of the most evident ecological consequences is the creation of closed-canopy stands (Van Breugel et al., 2007; Rancka et al., 2015), characterized by a radical shift in vegetation composition, with increase in shade-tolerant species and reduction of open-habitat and more generalist species (Wulf, 2004;

Amici et al., 2013).

Forest age is the principal ecological parameter invoked to explain the effects of change (Moning and Müller, 2008). Most studies have tested forest stand age, often taken to be the age of the oldest tree in each sampling plot: 1) as a surrogate parameter for habitat temporal continuity (Moning and Müller, 2008) and 2) as an estimate of forest disturbance, since tree age in managed forest stands is related to cutting frequency and may therefore reflect the intensity of human impact (Erdmann et al., 2006).

In a study on the influence of forest changes on plant diversity in certain Mediterranean protected areas, Amici et al. (2013) proposed the duration of continuous forestation (i.e. the time in years since a land patch became a real forest patch) as a measure of temporal continuity of forest characteristics. Compared to the parameter tree age, duration of continuous forestation gives an estimate of the age of forest habitat, irrespective of the management and harvesting regime (Amici et al., 2013).

The use of this forest age concept may have more significance for

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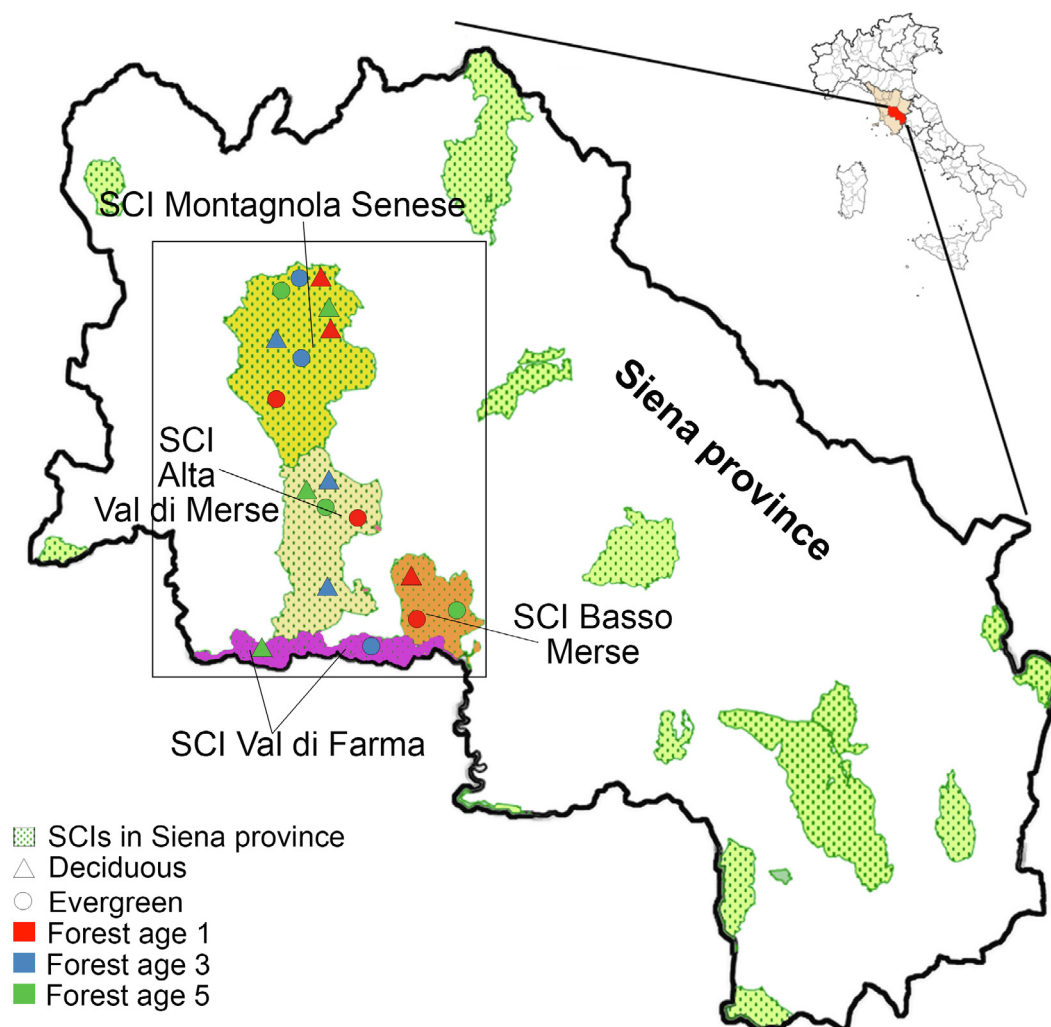


Fig. 1. Study area. Map of the 18 sampling units in four Sites of Community Importance (SCIs; Montagnola Senese, Alta Val di Merse, Basso Merse, Val di Farma) in Siena province (Tuscany, Italy), according to forest type (deciduous oak forest/evergreen oak forest) and age class (class 1, < 22 years; class 3, 38–56 years; class 5, > 77 years).

soil organisms. In this context, land snails, due to their very low active mobility and dependence on specific stable microhabitat characteristics for their survival, are much more sensitive bioindicators than other soil animals (Douglas et al., 2013). Molluscs live mainly in litter, but also use stems and foliage. They contribute to the decomposition of forest litter, nutrient recycling, soil formation and productivity, provide food for predators, and take part in other essential ecological functions (Baur and Baur, 1993). Many studies have reported snail diversity and community composition in forest ecosystems to be closely linked to different biotic and abiotic factors, both at local (forest cover, shadiness, humidity, suitable shelter and refuges, sufficient woody debris, available food, soil chemistry and pH) and at other scales (e.g. forest type, size and age) (Müller et al., 2005; Baur et al., 2014).

Considering leaf litter in relation to forest type, it is generally accepted that most snails and slugs prefer deciduous forests (Abele, 2010). Indeed, coniferous forests host lower densities of snails due to inhospitable leaf litter and acid soil, which translates into reduced calcium bioavailability. Even leaves from different non-coniferous tree species differ in their chemical composition and nutritional 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 tend to contain calcium citrate and other more soluble forms of calcium that influence snail reproductive success (Wäreborn, 1979). Except for this, little is known

about how snail assemblages differ in Mediterranean forest types such as evergreen oak forests (dominated by *Quercus ilex*) and deciduous oak forests (dominated by *Quercus cerris* and *Quercus pubescens*). While significant forest age thresholds have been studied in central European forests (see for example Moning and Müller, 2008), to our knowledge no research has considered the effect of forest age on land snail assemblages in Mediterranean area.

Here we investigated the effects of forest age on land snail communities by studying richness, abundance and community composition in Mediterranean evergreen and deciduous oak forests, varying in age from < 22 years to > 77 years. The study took place in four Sites of Community Importance (SCIs) in southern Tuscany, Italy. The main aims of the research were:

1. to explore any differences in land snail composition, richness and abundance in two forest types (deciduous and evergreen oak forests) at different ages;
2. to test the hypothesis that recent forest patches host more alien/ecotonal/generalist species (such as *Rumina decollata*, *Monacha cantiana*, *Cornu aspersum*) than older patches;
3. to partition pure effects of forest age on species variation with respect to spatial and environmental variables.

We expected to find differences in species richness and composition

Table 1

Summary of descriptive statistics (mean, standard deviation (SD), min–max range) of explanatory variables (elevation, slope, mean annual temperature, mean annual rainfall, tree cover) from each sampling plot (n = 18).

	Elevation (m)	Slope (degrees)	Mean annual temperature (°C)	Mean annual rainfall (mm)	Tree cover (%)
Mean values ± SD	331.67 ± 83.46	6.69 ± 2.66	13.69 ± 0.27	919.57 ± 75.84	68.61 ± 17.55
Min-max ranges	197–464	1.27–11.29	13.3–14.23	803.60–1060.50	30–95

in different forest age classes: younger patches, which should not offer stable habitat characteristics (i.e. coarse woody debris, large broadleaf trees, aestivation sites and refuges) would presumably host generalist species with low habitat specialization, whereas older forest patches would host true forest species, closely associated with certain micro-habitat characteristics and more vulnerable to habitat changes. In other words, our hypothesis was that increasing forest age closely matches increasing land snail habitat specialization.

2. Materials and methods

2.1. Study sites

The province of Siena (Tuscany, Italy) hosts 17 Sites of Community Importance (SCIs) from the European Commission Habitats Directive (92/43/EEC), that range in size from 483 ha (Lago di Montepulciano) to 13,744 ha (Montagnola Senese) and total 58,969 ha, which is more than 15% of the area of the province. Since our main aim was to test the effect of forest age on land snail assemblages, only the four with the highest forest percentages were chosen as study areas (Fig. 1): SCI “Montagnola Senese” IT5190003; SCI “Alta Val di Merse” IT5190006; SCI “Basso Merse” IT5190007; SCI “Val di Farma” IT51A0003 (for information on these SCIs, see [Servizio Aree Protette Provincia di Siena, 2013](#)). The most common forest types are holm oak forests (evergreen forests dominated by *Quercus ilex*) and mixed oak forests (deciduous forests dominated by *Quercus pubescens* and *Quercus cerris*) (De Dominicis, 1993). The prevailing method of forest management is coppicing with standards, but high forests are increasingly frequent (Amici et al., 2013).

2.2. Sampling design

Background data was collected in the framework of a plant diversity assessment project in the 17 SCIs of the province of Siena (Chiarucci et al., 2012; Amici et al., 2013) to evaluate the influence of forest age on vascular plants in the same network. The original sampling design was based on a 1 × 1 km grid with one random point selected in each cell. A 10 × 10 m plot was then centred on each sampling point, located by high-precision GPS, in a total of 604 plots. Four forest maps of different years (1933, 1954, 1972, 1988) were used to determine the age of present-day forest cover, regardless of the management and harvesting regime (Amici et al., 2013).

Only plots classified as evergreen and deciduous oak forests on the basis of the dominant tree species in the four SCIs were considered in this study, making a total of 110 plots. In order to maximize differences in the forest age categories, only those belonging to three out of the original five forest age classes were considered (class 1, < 22 years; class 3, 38–56 years; class 5, > 77 years), restricting the number of eligible plots to 79 (for further details about definition of forest age classes, see Amici et al., 2013). Finally, to obtain a good representative distribution of all forest age classes in the two types of oak forest, 18 plots (9 in deciduous oak and 9 in evergreen oak forest, 3 for each forest age class) were chosen across the four SCIs (Fig. 1).

To highlight the pure effects of forest age on land snail communities, excluding other environmental effects, plots were chosen so that variables most commonly affecting land snail species diversity and composition were homogeneous. In particular, all sites with very acid

geological substrate and extreme slope values were excluded, since these factors could have a negative effect on species dynamics. Indeed, it is generally accepted that soil pH is a major predictor of richness and composition, while steep slopes may inhibit presence (Valovirta, 1968; Kappes et al., 2006; Juříčková et al., 2008). The following environmental variables were considered for statistical analysis in each sampling plot: elevation above sea level (m), slope of terrain (degrees), annual average temperature (°C), annual average rainfall (mm), tree cover (%) and forest age (age of continuous forest cover, according to the following age classes: class 1, < 22 years; class 3, 38–56 years; class 5, > 77 years) (Table 1).

Fieldwork was carried out by visual search for a standardized time of 1 h in each 10 × 10 m plot. In each site, a representative sample of debris and leaf litter (5 l) was also collected in all microhabitats considered suitable (i.e. dead wood, tree bark, sheltered places under stones) in order to find small and very small species (Cameron and Pokryszko, 2005). This method combined with visual search provides the most information on small land snail species (Menez, 2007; Coppolino, 2010; Benocci et al., 2015). The material collected was sieved in a mesh of 10 mm, then dried and finally sieved again with meshes of decreasing size down to 0.5 mm. All live snails and shells were used to obtain data on species relative abundance. Taxonomy and nomenclature of gastropods are according to MolluscaBase, also available online at <http://molluscabase.org/>.

2.3. Data analysis

Snail species richness and abundance were compared separately for differences between forest types and forest age classes by box-plots and descriptive statistics. The significance of differences in mean richness and abundances between groups was assessed by the non-parametric Kruskal-Wallis rank sum test.

Spatially Explicit Rarefaction curves (SERs, Bacaro et al., 2016), first described by Chiarucci et al. (2009) as Spatially Constrained Rarefaction curves, were used to compare species richness separately in relation to the number of sample plots in each forest type and forest age class. Unlike traditional rarefaction curves (RCs), i.e. species accumulation curves (Gotelli and Colwell, 2001), SERs also include the spatial autocorrelation structure of samples, accumulating species of the nearest plots for a given number *n* of plots sampled (Bacaro et al., 2012). In practice, SERs are built by ordering plots according to their minimum geographical distance and then constructing an accumulation curve for each ordered sequence. Indeed, in nature individuals are far from randomly distributed and the same number of sampling units with the same size could give great differences in species diversity due merely to the greater environmental complexity of larger areas (Bacaro et al., 2012). Case studies demonstrate that exclusion of spatial autocorrelation can greatly affect results, so that SERs should always be preferred in rarefaction techniques (Bacaro et al., 2016). R code (*pointpattern* and *SCR* functions) proposed by Bacaro et al. (2012) was used for SER construction; the *specaccum* function in the *vegan* package was used for traditional RCs.

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 forest types and forest age classes. The stress value was used as criterion of goodness of fit in constructing the optimal

representation (the smaller the stress value, the better the adaptation of reproduced distances to the original matrix). Abundance data was $\log(x + 1)$ transformed before NMDS.

A multi-response permutation procedure (MRPP) was then run (999 permutations) to test any differences between groups (habitat type and age classes) obtained by NMDS analysis (Mielke, 1984, 1991), considering both presence/absence (Jaccard dissimilarity index) and abundance (Bray-Curtis dissimilarity index) data.

Indicator species (i.e. species having high indicator value for a group of sites and therefore used as ecological indicators of community or habitat types) and environmental conditions were determined by analysis of the relationship between abundance and frequency values on a set of sampled plots and by classification of the sites into site groups (forest types; forest age classes) for each species independently. *Indicspecies* package in R (De Cáceres and Jansen, 2016) was used for indicator species analysis.

Finally, variation partitioning (ter Braak, 1986; Borcard et al., 1992; Legendre and Legendre, 1998; Borcard and Legendre, 2002; Borcard et al., 2004), based on Hellinger-transformed land snail data, was used to quantify the relative effect of environmental (elevation, slope, mean annual temperature, mean annual rainfall, forest cover) and spatial variables (XY geographical coordinates and the spatial structures at different scales detected by principal coordinates of neighbour matrix analysis, PCNM; Borcard et al., 2004), as well as forest age effect, in shaping variation in community composition. The shared fraction was also evaluated. Partitioning was based on the adjusted R^2 statistic as recommended by Peres-Neto et al. (2006); 999 permutations were used to assess the significance of constraints. Quantification of the variance components was based on the function *varpart* of the R package *vegan* (Oksanen et al., 2016). Before variance partitioning analysis, we minimized multicollinearity by performing a Principal Component Analysis (PCA) separately in spatial and environmental predictor sets, extracting the first two axes of each PCA (PC1 and PC2 site scores) to use as explanatory variables in the next analysis.

All statistical analysis was performed using R version 3.4.4 (R Development Core Team, 2018).

3. Results

A total of 2433 individuals and 27 species were recorded in the 18 forest plots. Of these, 16 species were classified as true or mainly forest species (FS) and 11 as generalist species (GS) (Table 2).

The number of individuals recorded per plot ranged from 20 to 361 and the number of species from 4 to 15. The most common and abundant species were *Acanthinula aculeata*, *Punctum pygmaeum* and *Vitrea subrimata* (17/18 plots). Four singletons were recorded: *Candidula unifasciata*, *Monacha cartusiana*, *Trochoidea pyramidata* and *Vitrea contracta* (1/18 plots). No true alien species were found, whereas 25 out of 27 species are classified Least Concern, one (*Oxychilus clarus*) Near Threatened and one (*Campylaea planospira*) Data Deficient in the IUCN Red List.

The descriptive box-plots showed a decrease in total species richness and a constant pattern in abundances with increasing forest age (Fig. 2 below); however the Kruskal-Wallis test for total richness and abundances did not detect any significant difference between forest age classes. Nor was any distinction found between evergreen and deciduous oak forest types for total species richness and abundance (Fig. 2 above): evergreen forests showed higher values of species richness and abundance but differences with respect to deciduous forests were not significant.

When we examined true or mainly forest species (FS) and generalist species (GS) separately, some trends were evident but they were not significant (Fig. 3), as confirmed by the Kruskal-Wallis test: while FS richness and abundance showed a homogeneous trend between age classes, with more variability in class 1 and 3 (Fig. 3, left column), a decrease in the richness and abundance of GS with increasing forest age

was evident but not significant (Fig. 3, right column).

SERs for forest age classes (Fig. 4a) suggested some contrasts in cumulative species richness: despite an initial steep climb, forest age class 1 reached an asymptote after only four sampled plots, while the intermediate age class 3 reached the same species richness value as forest age class 1 more steeply; the lowest value was found in the oldest age class 5.

SERs for variations in species richness between forest types (Fig. 4b) showed an approximately equivalent number of accumulated species for the same number of sampled plots when compared with the traditional exact-based method. With regard to patterns within SERs, while deciduous forests showed lower species richness and an asymptotic pattern, the evergreen forests curve failed to reach saturation, suggesting that the total number of species exceeded the number sampled.

As shown by the partial overlap of 95% confidence ellipses, no clear differences in species composition were found by NMDS ordination or MRPP analysis (Table 3) of presence/absence and abundance data for forest types (Fig. 5, left column).

Nor did NMDS analysis reveal any clear separation in community structure between forest age classes (Fig. 5, right column). No qualitative differences were found with occurrence and abundance data: the youngest forest age class (class 1) showed the highest variability, clustering partially with the other two age classes (forest age classes 3 and 5) inside the boundaries of the 95% confidence ellipse. MRPP analysis did not reveal any differences between multidimensional groups (Table 3).

IndVal analysis did not highlight any species as a perfect indicator for any of the three age classes and just two out of 27 showed marginally non-significant mean changes in abundances between age categories by Kruskal Wallis rank sum analysis: *Discus rotundatus* and *Monacha cantiana* (Table 2).

Variation partitioning (Table 4) showed that predictors accounted for 37% of variability in land snail communities: the largest fraction of explained variation was accounted for by pure space (7%, a), followed by pure effect of forest age (3%, c). Negative values of adjusted R^2 should be interpreted as zeros: they indicate cases where the explained variables explained less variation than would normal random variables (Legendre, 2008). None of these fractions reached statistical significance ($p > 0.05$).

4. Discussion

Forest age (defined as the time in years since a land patch became a real forest patch as a measure of temporal continuity of forest characteristics) did not seem to be a key factor for land snail species richness and abundance in the time-range of examined forests: even considering forest species and generalist species separately, some trends were still evident but not significant, as confirmed by the Kruskal-Wallis test. Total richness effectively decreased with increasing forest age, possibly as a result of competitive interactions and/or habitat specializations, but the used time-span resulted to be inadequate for analysing the overall effect of forest age on snail diversity. In central Europe, Moning and Müller (2008) reported that threshold time values for the diversity of land molluscs in temperate forests are of the order of centuries: 170 years in sub-montane forests and from 160 to 220 years in mixed montane forests of oak and coniferous trees.

For this reason, although our study takes into consideration Mediterranean deciduous and evergreen oak forests, a higher time threshold could be necessary to appreciate real changes in land snail dynamics.

Regarding species composition, forest age may have an effect on forest and open habitat specialist species in many organism groups (e.g. ground dwelling spiders, insects), since open habitat species can easily exploit the abundance of certain resources persisting in young woodlots in the early stages of forest change after disturbance (i.e. clear cutting, forest fire) (Larrivé et al., 2005; Niemelä et al., 2006; Ström et al.,

Table 2

Total abundance in all plots (n = 18) for each species separately and mean and standard deviation (SD) in abundances per plot between forest age classes (class 1, < 22 years; class 3, 38–56 years; class 5, > 77 years). P-values were calculated by Kruskal-Wallis test (in bold those close to statistical significance at alpha = 0.05). * indicates true or mainly forest species (FS). Only species occurring in at least 4 plots were considered.

Species	Total abundance	Mean abundance ± SD			n° plots	p-value (α = 0.05)
		Forest age class 1	Forest age class 3	Forest age class 5		
<i>Acanthinula aculeata</i> *	387	17 ± 17	23.5 ± 11.3	24 ± 19.6	17	0.47
<i>Argna biplicata</i> *	10	1.5 ± 3.7	0	0.2 ± 0.4	2	
<i>Campylaea planospira</i> *	13	1.5 ± 2.3	0.5 ± 0.8	0.2 ± 0.4	6	0.38
<i>Candidula unifasciata</i>	1	0	0.2 ± 0.4	0	1	
<i>Cecilioides acicula</i>	3	0.3 ± 0.5	0	0.2 ± 0.4	3	
<i>Cepaea nemoralis</i> *	26	2 ± 3.1	1.5 ± 2.5	0.8 ± 0.7	9	0.90
<i>Cochlicella barbara</i>	2	0.2 ± 0.4	0	0.2 ± 0.4	2	
<i>Cochlodina bidens</i> *	160	6 ± 5.2	9 ± 11.4	11.7 ± 13.2	13	0.86
<i>Discus rotundatus</i> *	170	9.7 ± 10.6	0.8 ± 2	17.8 ± 31.7	11	0.06
<i>Granaria frumentum</i>	3	0.2 ± 0.4	0.2 ± 0.4	0.2 ± 0.4	3	
<i>Helicodonta obvolvata</i> *	86	4.2 ± 7	2 ± 2.4	8.2 ± 10.7	11	0.67
<i>Hygromia cinctella</i>	24	1.8 ± 1.8	1.8 ± 3.2	0.3 ± 0.8	7	0.25
<i>Merdigera obscura</i> *	9	1.2 ± 2.9	0.3 ± 0.8	0	2	
<i>Monacha cantiana</i>	9	0.7 ± 0.8	0.8 ± 0.7	0	7	0.07
<i>Monacha cartusiana</i>	1	0	0.2 ± 0.4	0	1	
<i>Oxychilus clarus</i> *	4	0	0.5 ± 0.8	0.2 ± 0.4	3	
<i>Oxychilus cf. draparnaudi</i> *	41	2.8 ± 3.1	1.3 ± 1.2	2.3 ± 2.2	12	0.55
<i>Oxychilus uzielli</i> *	73	5.3 ± 13.1	2 ± 4.4	4.8 ± 11.3	5	0.89
<i>Pomatias elegans</i>	334	35.5 ± 45.7	5.2 ± 5.5	15 ± 32.5	11	0.33
<i>Punctum pygmaeum</i> *	528	42.2 ± 51.1	38.8 ± 33.8	12 ± 7.9	17	0.31
<i>Retinella olivetorum</i> *	28	2.5 ± 3.5	1.3 ± 2.8	0.8 ± 2	6	0.45
<i>Solatopupa juliana</i>	11	0	1.8 ± 3.6	0	2	
<i>Testacella scutulum</i> *	9	0.2 ± 0.4	0.5 ± 0.8	0.8 ± 0.7	7	0.23
<i>Trochoidea pyramidata</i>	1	0.2 ± 0.4	0	0	1	
<i>Truncatellina callicratis</i>	72	7.2 ± 10.7	4.8 ± 11.8	0	4	0.16
<i>Vitrea contracta</i> *	29	0	0	4.8 ± 11.9	1	
<i>Vitrea subrimata</i> *	399	18.8 ± 14.3	24.8 ± 21.2	22.8 ± 7.1	17	0.76

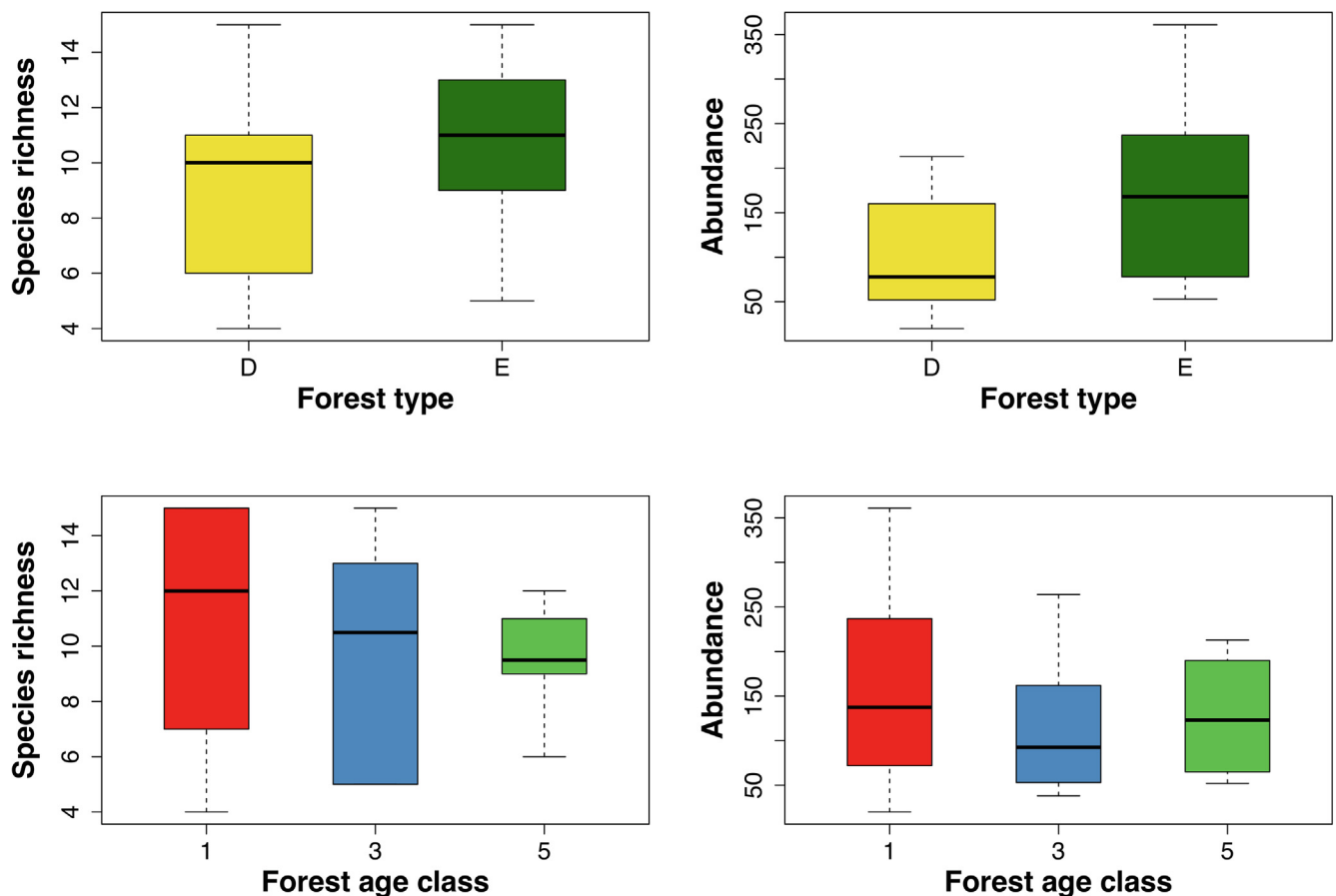


Fig. 2. Boxplots showing differences in the total number of land snail species (total richness) and specimens (total abundance) between forest types (D deciduous oak forest, E evergreen oak forest) and forest age classes (class 1, < 22 years; class 3, 38–56 years; class 5, > 77 years) in sampling units (n = 18).

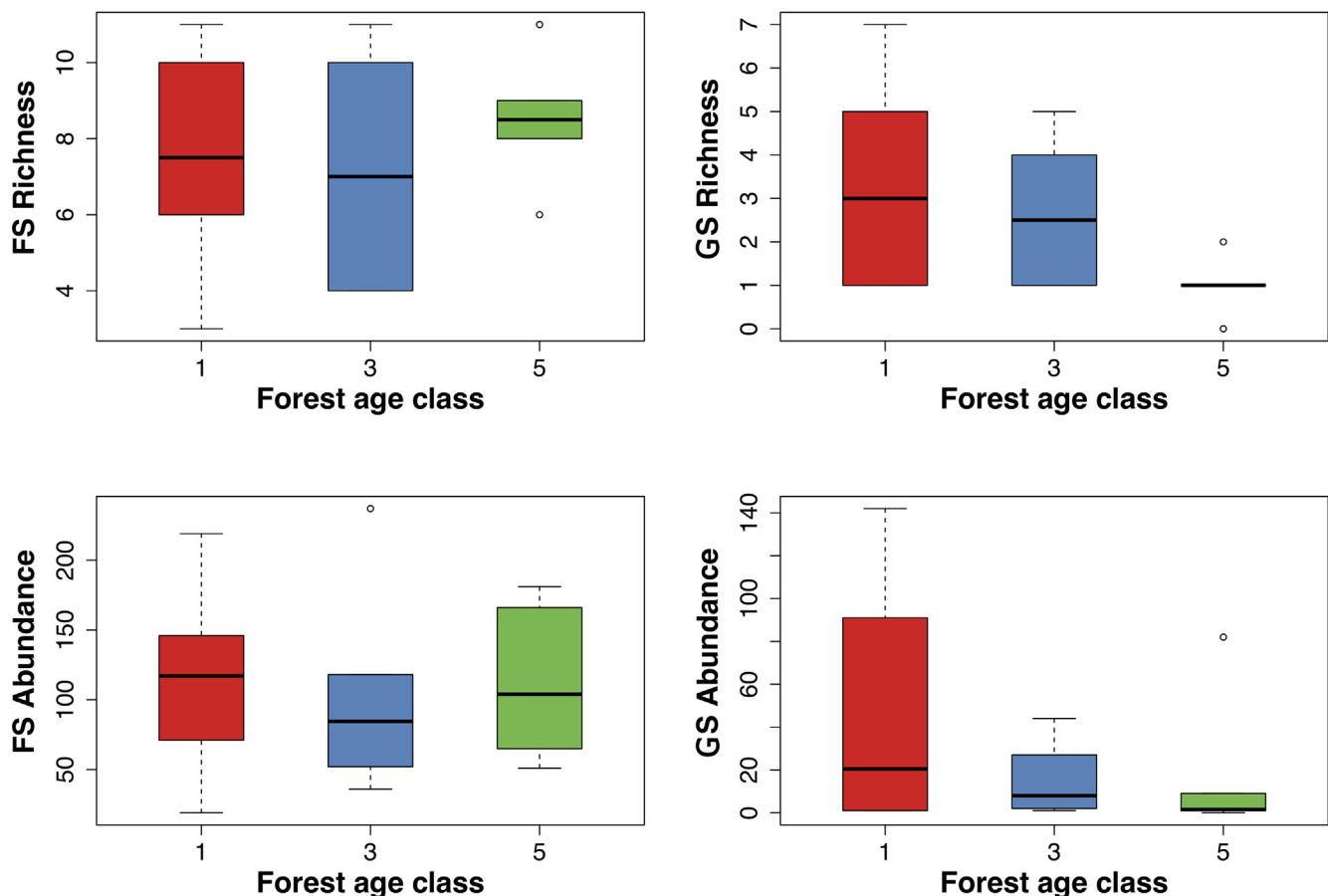


Fig. 3. Boxplots showing differences in the total number of land snail species (total richness) and specimens (total abundance) for true or mainly forest species (FS) and generalist species (GS) in the three forest age classes (class 1, < 22 years; class 3, 38–56 years; class 5, > 77 years) in sampling units (n = 18).

2009; Hylander, 2011; Gallé et al., 2014). Concerning land snails, the initial hypothesis that younger forest patches host more alien/ecotonal/generalist species (such as *Pomatias elegans*, *Monacha cantiana*, *Rumina decollata*, *Cornu aspersum*) than older patches was only partially supported: the older plots were not characterized by a well-defined cluster of exclusive forest species but rather by a mixed assemblage of species widespread in all forest age classes.

Indeed NMDS showed a trend with a higher presence of generalist and open habitat specialists in younger forest classes but this assemblage persisted in older ones, being distributed among all age classes without statistically significant differences in species composition between categories.

Despite the common belief that early forest age is linked to species invasions (Roser and Monteserrat, 2006; Flory and Clay, 2009), no alien species were collected. Alien mollusc species in Italy are mostly linked to freshwater and ruderal environments rather than pure forest habitats (Hallgass and Vannozzi, 2010); nevertheless aliens such as the Australasian land snail *Paralaoma servilis* are nowadays widespread in all forest environments, even those with high wildness, and their presence in the regional pool is robust. In the case of the forests studied by us, a certain degree of isolation and the absence of disturbance together with local environmental factors could filter invasion by alien snail species from other areas, especially from surrounding mosaic environments.

Concerning differences in biochemical composition of oak litter, descriptive box-plots and NMDS results indicate that the two types of oak litter are not associated with significantly different land snail richness, abundance or species composition. This agrees with Damesin et al. (1998), who studied key factors that distinguish the functions of deciduous and evergreen oaks in southern France. Despite evident differences in lignin, cellulose (higher concentrations in *Q. ilex*) and

proteins (higher concentrations in *Q. pubescens*), they did not find any significant difference in biochemical composition of mature leaves of *Q. ilex* and *Q. pubescens*.

A review of the literature on the effect of age on forest biodiversity did not reveal any common response pattern. Apart from some conceptual inconsistency, researchers failed to give a clear definition of age and how they measured it. This has strong implications, since tree age and forest age are different, non-interchangeable ecological concepts that interact with each other in many ways. Separating these effects is therefore a real challenge.

One of the first attempts to disentangle the effect of forest age on plant diversity from that of tree age was made by Dittrich et al. (2013), who considered ground epiphyte vegetation in old-growth and secondary forests of similar tree age but different forest temporal continuity values in central Europe. They found no differences between the two stand types: the results highlighted the absence of any effect of forest temporal continuity, showing that a period of 200 years was sufficient for secondary forest to be colonized by the same assemblages of plant species as older forests. Although it is not clear to what extent the results can be generalized, Dittrich and colleagues did not reject *a priori* the possibility that forest temporal continuity beyond a time span of 200 years may have a remarkable effect on diversity. Indeed Amici et al. (2013) subsequently found that forest age was an important factor for plant richness and composition, at least in Mediterranean landscapes, even in a reduced time span of < 80 years.

Although our study on land snail communities was conducted in the same environmental framework as the study by Amici et al. (2013), it demonstrated that the age of examined forests was not sufficient to produce differences in land snail assemblages between age classes. Concerning soil organisms, the general framework is probably more

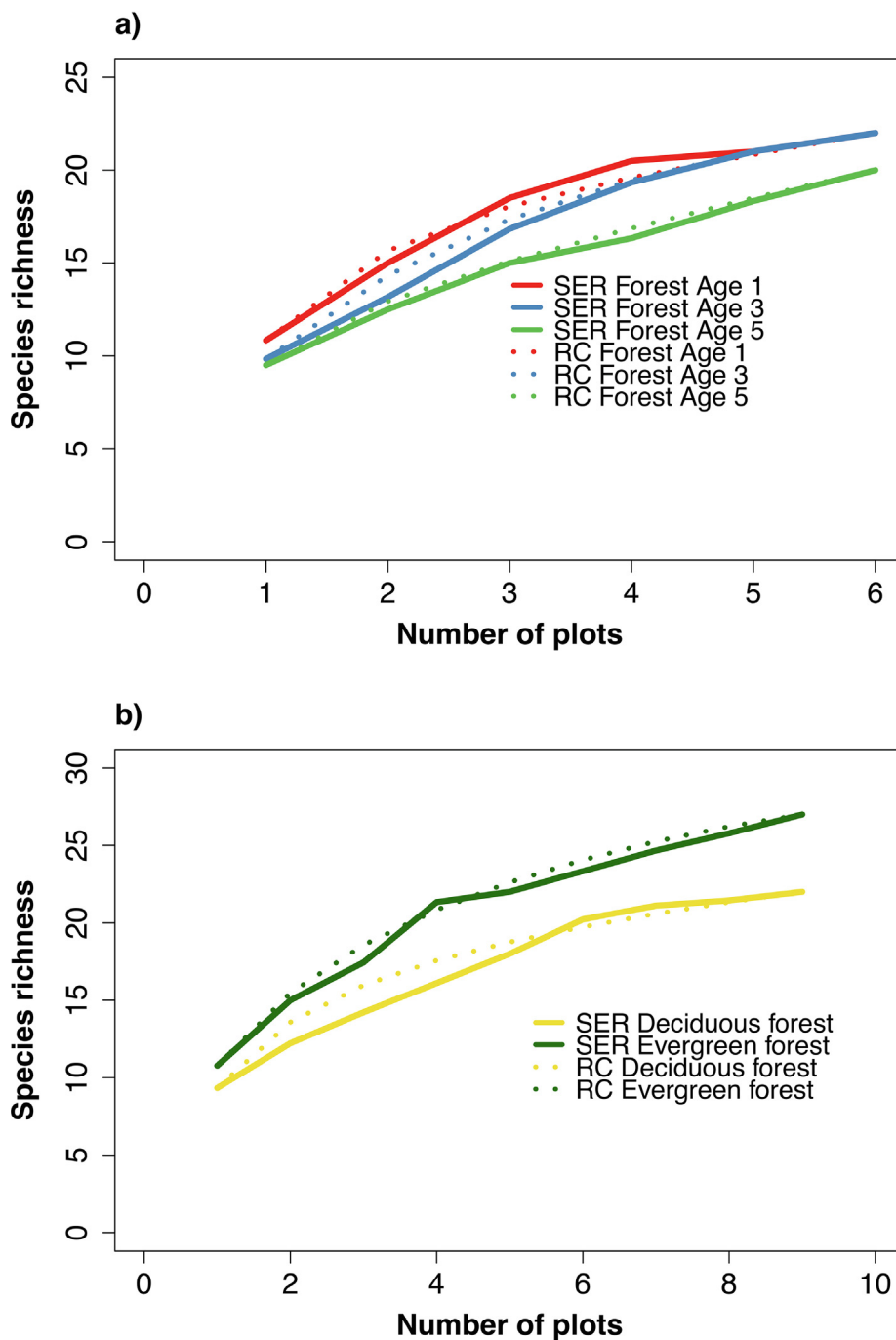


Fig. 4. Spatially explicit rarefaction curves (SERs) and traditional rarefaction curves (RCs) for forest types (a, deciduous oak forest/evergreen oak forest) and forest age classes (b, class 1, < 22 years; class 3, 38–56 years; class 5, > 77 years) in sampling units (n = 18).

complex than that of vegetation and much more time may be necessary for land snails to differentiate.

In the reduced time-span of the age of our plots, a lack of forest age effect on land snail assemblages could indicate that habitat structure at local scale is more important than forest age in shaping diversity, and this was indeed confirmed by the variation partitioning results, where the pure forest age fraction described a low portion of compositional variation. The lack of significance of all the elements of variability analysed may be explained by two phenomena: the natural limited variation of parameters (climate, topography) across the study areas (as shown by the low standard deviations in Table 1), and our homogenization of all variables affecting land snail communities (especially geological substrate and therefore soil pH and slope) in order to remove

background noise and bring out any real effect of forest continuity. Thus the absence of any effect of pure forest age has more ecological meaning.

A study on forest spiders in lowland poplar forests in Hungary revealed that assemblage composition and diversity were determined solely by structural changes in habitat, whereas forest age did not have significant direct influence (Gallé et al., 2014). In a range from 6 to 48 years, Gallé and colleagues showed that the occurrence of typical structure of older forests in young stands as well as the presence of different habitat types in the surrounding landscape acting as population-source for forest specialist species, may justify the absence of significant effect of forest age on ground dwelling spiders diversity in a short time scale.

Table 3

Multi-response permutation procedure (MRPP) output for habitat type (deciduous oak forest/evergreen oak forest) and forest age classes (class 1, < 22 years; class 3, 38–56 years; class 5, > 77 years) considering presence/absence (Jaccard dissimilarity index) and abundance (Bray-Curtis dissimilarity index) land snail data.

	Delta	Chance corrected within-group agreement A	Significance of delta	Number of permutations
p/a				
-deciduous	0.62	-0.03	0.60	999
-evergreen	0.61			
Abundance				
-deciduous	0.59	0.02	0.16	999
-evergreen	0.59			
p/a				
-Age class 1	0.63	0.02	0.15	999
-Age class 3	0.60			
-Age class 5	0.52			
Abundance				
-Age class 1	0.71	0.004	0.39	999
-Age class 3	0.49			
-Age class 5	0.58			

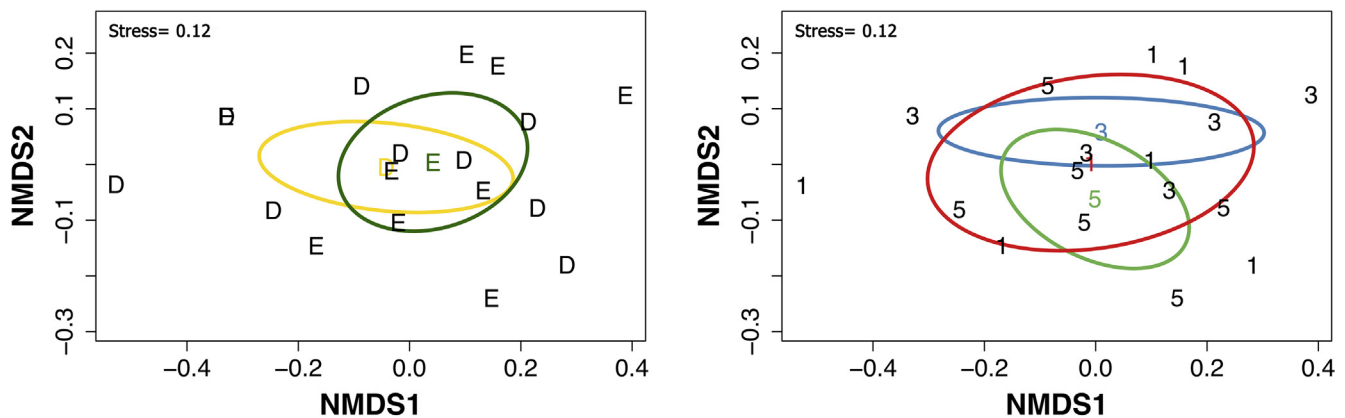
Because of the low active mobility of snails respect to other invertebrates, it could take a longer time until most rare snail species colonize and accumulate in recent patches (Moning and Müller, 2008).

For some species, the local survival in micro-refugia along with short-distance recolonization pathways may be more important factors than limitations in active dispersal (Ström et al., 2009). An example is *Oxychilus clarus*, a true rare and infrequent species, also classified as near threatened by IUCN red list (Neiber, 2017). Its presence, never recorded in the study area before, testified the occurrence of rare and scatterly distributed entities also in young and very young forests. We do not know if it represents a relict, locally survived during centuries to different management regimes or a species established from immediately adjacent forest patches. However, this finding well supports the importance of microhabitat structure also in young forests, which can possess environmental and ecological conditions similar to older forests for the sustainment of at least some rare species.

Due to dispersal limitations and habitat specialization of snails, determinants of gastropod richness and abundance may vary widely in the space of a few metres: some very old trees in the middle of a highly disturbed matrix could be sufficient to ensure the necessary ecological conditions for the survival and maintenance of well-defined populations (Hylander, 2011).

Furthermore, land snails are not xylophagous, nor do they rely on fresh plants for certain stages of their development, as do butterflies, for example. Rather, land snail diet is mainly based on decomposing plant matter, fungi and algae (Herbert and Kilburn, 2004). Since the only well documented relationship is a preference for certain tree species or forest types due to leaf-litter requirements (coniferous vs. deciduous trees), there cannot be any direct relationship to plant species composition, but more likely an indirect effect through forest cover and

Presence/absence data



Abundance data

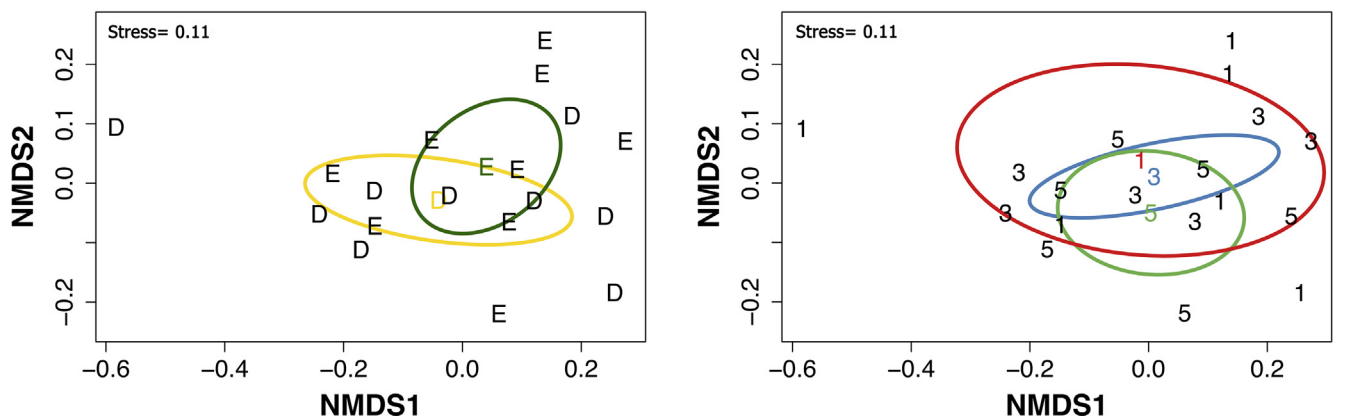
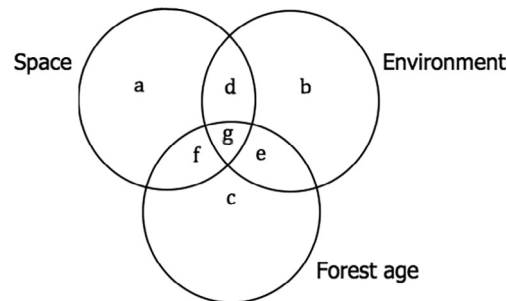


Fig. 5. Non-metric multidimensional scaling (NMDS) for presence/absence land snail data (above) and abundances (below) considering forest type (first column; deciduous oak forest/evergreen oak forest) and forest age classes (second column; class 1, < 22 years; class 3, 38–56 years; class 5, > 77 years) in sampling units (n = 18). Ellipses represent ordination confidence intervals (95%).

Table 4

Variation partitioning of total variation in land snail data in terms of: (a) pure effect of space (X-Y coordinates and PCNM); (b) pure effect of environment (elevation, slope, mean annual temperature, mean annual rainfall, tree cover); (c) pure effect of forest age (class 1, < 22 years; class 3, 38–56 years; class 5, > 77 years); (d, e, f) shared variation explained by the overlap of two sets of predictors; (g) total joint effect of all variables considered. None of these fractions was significant at $p < 0.05$.



Effect and main variables	Variation fraction	Explained variation Adjusted R ²	P-value
Total effect			
Environment, space, forest age	[A + B + C + D + E + F + G]	0.37	
Partial effects			
Space	[A + D + F + G]	0.19	
Space (environment, forest age)	[A]	0.07	0.09
Environment	[B + D + E + G]	0.13	
Environment (space, forest age)	[B]	0.01	0.34
Forest age	[C + E + F + G]	0.07	
Forest age (environment, space)	[C]	0.03	0.17
Joint effect			
Environment, space	[D]	0.02	
Environment, forest age	[E]	- 0.01	
Space, forest age	[F]	- 0.01	
Environment, space, forest age	[G]	- 0.00	
Residuals	[H]	0.89	

availability of coarse woody debris, refuges, holes and shelters (Evans, 1972).

We therefore highlight the importance of the structural complexity of forests and defined habitat structure for maintaining forest biodiversity without taking clear-felling into account. In general, natural forests and forests with low human impact 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). Many studies show that gastropod communities seem to be highly resilient to different disturbance regimes, from clear-cutting (Hylander, 2011) to fires (Kiss et al., 2004) but trends in response patterns of land snail communities are rarely clear and do not follow a common model (Nekola, 2002; Kiss and Magnin, 2003). Species can persist and survive locally as many trees, shrubs, fallen logs and stumps can remain after disturbance episodes, providing refuges, holes and sheltered places and maintaining habitat structural continuity (Kiss and Magnin, 2003; Kiss et al., 2004; Rancka et al., 2015).

In our case it was not possible to precisely determine the interval since the last clear cut for all plots, but the average was about 50 years, a shorter time range than that assumed by Moning and Müller (2008) who stated that the recovery process takes centuries for an almost complete restoration of the original community, at least in sub-montane and mixed montane forests of oak and coniferous trees in Central Europe. Moreover, we suppose that the investigation of forests for most part young may constitute a stronger limitation in the interpretation of results than the impact of management practices: many other factors are likely to explain the malacofauna composition after some anthropogenic episodic changes (i.e., biogeographical or historical factors) (Kiss et al., 2004).

5. Conclusions

- While the effect of acidic litter on land snail assemblages is well known, no differences in mollusc richness, abundance or species composition between evergreen and deciduous oak litter were detected;
- In contrast with the results of Amici et al. (2013) for vascular plants, forest age did not emerge as a key factor for land snail species richness and abundance in the time-span of the examined forests;
- Regarding snail species composition, an assemblage of species widespread in all forest age classes presumably contributed to the absence of a perfect indicator, suggesting that the age range of the forests investigated in this study could be inadequate for analysing the overall effect of forest age on snail diversity in Mediterranean area;
- Habitat structure, more than forest age, was the factor that most affected snail communities in the time-range of the analysed forests: due to specialization and limited active mobility of land snails, suitable environmental conditions and just a few old trees serving as shelters are sufficient to sustain land snail communities, ensuring their survival and protection. However, it is not excluded that forest age variable may be more important if true old-growth forests are considered.
- In conclusion, in the time range of examined woodlots, different aged forests may have similar land snail assemblages if they have the same habitat structure. Nevertheless, different results could be reached if young forests are compared with old-growth forests.

Declaration of Competing Interest

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

Acknowledgements

Alessandro Chiarucci (Alma Mater Studiorum University of Bologna) and Sara Landi provided access to the original dataset and gave precious advice on sampling design; Gianmaria Bonari and Paolo Cantiani gave useful suggestions during manuscript revision; Leonardo Favilli, Tommaso Mori and Sandro Piazzini helped during field work; Helen Ampt revised the English.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2019.117693>.

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