

GRASSLANDS MANAGEMENT AND BIODIVERSITY CONSERVATION:
AN EXAMPLE WITH REFERENCE TO ABOVE GROUND ARTHROPOD COMMUNITIES
IN LIGURIA (NW ITALY)

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ABSTRACT

In 2016 the species richness of arthropod communities was investigated in two grasslands overlying limestone substrate in order to obtain management information useful for the conservation of biodiversity of a protected area within the Antola Regional Park in Liguria, North-Western Italy. During the summer season arthropods with different ecological and behavioural traits were collected using sweep nets, butterfly nets and pitfall traps from an extensive pasture in Bavastrelli and an irregularly mown, unfertilised meadow in Garaventa. Individual- and sample-based rarefaction analyses were performed to estimate the expected species richness detectable for each collection method. Concurrently, an expeditious study of the plant assemblages for both sites was also undertaken in order to characterize their central area and margins. Differences between the expected and observed number of arthropod species for the two study areas were influenced by collection methodology and associated statistical analysis; however, the Bavastrelli pasture resulted in higher values of species richness both in terms of the number of estimated and observed taxa and species. Plant species richness was slightly greater in Garaventa (totally 90 species, 63 of which in the core area) than in Bavastrelli (80 species overall, including 54 in the central part of the pasture) with a greater number of *Festuco-Brometea* species showing an evolutionary tendency towards more xeric formations. The observed herbaceous formations characteristic of the *Molinio-Arrhenatheretea* were richer in species of the *Plantaginetalia majoris* complex when animals grazed and trampled for longer periods. Also, there seems to be an inverse relationship between overall plant and arthropod species richness in the two study areas. Overall, these results highlight the importance of a middle-low level of grazing in maintaining high arthropod biodiversity level at a local scale and suggest the opportunity to diversify grassland management in order to increase, and conserve, the biodiversity on a larger scale.

KEYWORDS: Insects, Meadow mowed, Pasture, Species richness, Spiders.

INTRODUCTION

Grasses cover the highest percentage of the earth's land surface (Tscharntke & Greiler, 1995; Wang & Fang, 2009). For example, in the Palearctic region, there are about 10.0 million km² of grasslands, corresponding to approximately 18% of its territory (Dengler et al., 2020). The importance of semi-natural and natural grasslands for biodiversity conservation and for ecosystem services globally has been highlighted by Bengtsson et al. (2019) and Habel et al. (2013), respectively. Moreover, calcareous grassland is one of the most species rich and diverse habitat in Europe, characterized by many rare and specialized plants and insects (Lyons et al., 2018; Steffan-Deventer & Tscharntke, 2002). Most European grasslands are anthropogenic habitats, established in historical times, and they often appear to be the only habitat remnants for several species of invertebrates and plants, since many other open habitats have been modified or destroyed (Habel et al., 2013; Steffan-Deventer & Tscharntke, 2002). In recent times, calcareous grasslands and their insect communities continue to decline as a consequence of their destruction,

fragmentation and succession due to agricultural intensification and abandonment, and through the loss of traditional extensive animal husbandry related to low-moderate grazing regimes (Lyons et al., 2018; Steffan-Deventer & Tschardtke, 2002;). Tschardtke & Greiler (1995) provide a useful overview on the relationship between insect communities and different grassland types.

This study focuses on two grasslands on a limestone substrate in a Ligurian Regional Park (North-Western Italy) that are subjected to different management regimes: grazing and mowing. The aim of the research was to assess the relative impact of grazing and mowing to plant and insect biodiversity by sampling and analyzing these communities using a range of collection methods and statistical analyses so as to inform and improve current management plans for the conservation of local biodiversity, which is the primary objective of a protected area.

MATERIALS AND METHODS

Study site

Antola Regional Park is located in Liguria, North-Western Italy, in the Genoa Province on the Apennine. Inside the Park two grasslands rather close to each other, and with the same exposure and elevation range were specially chosen to exclude any effects that the local geological substratum, location and relative climatic differences may have on arthropod communities in situ (Figure 1). The first sampling site was a meadow mowed located close to Garaventa at 925 m elevation on the South-East slope. The second one was a pasture near Bavastrelli at 1000 m elevation on the South/South-East slope. Both the sites overlay the limestone formation of “Monte Antola”.



Figure 1. Study area: the central part of Antola Regional Park. Asterisks indicate the sampling localities. At the top right of the figure, is the Liguria Region with the localization of the shown area. The regional border is shown in black and the Park's southern border is shown in grey.

The sampling sites were located in the transition zone between the climax of the *Fagetalia sylvaticae* Pawlowski 1928 and that of the *Quercetalia pubescenti-petraeae* Klika 1933. This area, characterized (especially in the past) by anthropic activities linked mainly to forestry, pastoralism and agriculture, presents a composite mosaic of vegetational aspects. Beech woods alternate to mixed broad-leaved woods (with dominant species that differ according to altitude and exposure (*Quercus cerris* L., *Quercus pubescens* Willd., *Ostrya carpinifolia* Scop. and so on), chestnut groves (whose cultivation is currently largely abandoned), herbaceous formations, largely evolving towards shrublands, some still used as pastures or as mowing meadows and crops (mixed crops, in sharp decline) (Ente Parco Naturale Regionale, 2015; Orsino & Dameri, 1998). Garaventa meadow (lawn for mowing) is part of an environmental mosaic with former crops transformed into mowing meadows, crops characterized by mixed cultures (in the vicinity of settlements) and coppice woods. These are mainly characterized by *Quercus pubescens*, *Fraxinus ornus* L. and *Corylus avellana* L. (the last one sometimes dominant). Bavastrelli pasture meadow is located in an area dominated by shrub vegetation, surrounded by beech woods and mixed woods dominated by *Quercus cerris* and *Q. pubescens*.

Habitat analysis

The habitats within the sampling sites were studied as follows. Within each site, one transect about 200 m long was drawn. Four equidistant sampling points, two at the ends and two in the middle of the transect, were selected (Figure 2). At the middle points the Braun-Blanquet (1937) sampling method was adopted for the phytosociological characterization of the vegetation. In order to evaluate the minimum surface at which a new increase in area does not correspond to the detection of any new species, sampling was carried out by area increase. It can therefore be considered that the investigated area is sufficiently representative of the vegetation of the homogeneous site studied. At the ends of the transect seven “closed chain” circular plots of 40 cm in diameter were analyzed in order to assess the variations of ecotones at the margins of the area, using the Raunkiaer (1918) method.

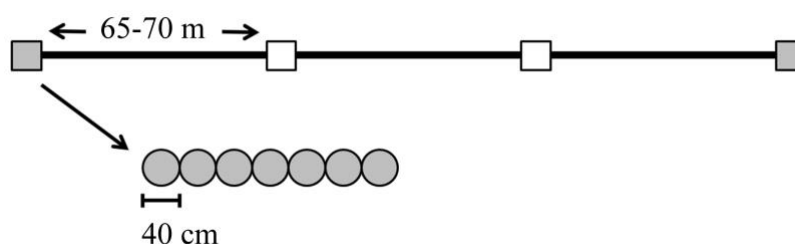


Figure 2. Sampling methods: 200 m transect with two ecotone and two central sampling sites. The seven “closed chain” circular plots are shown in detail in the lower part of the figure (see Materials and methods).

The surveys were carried out in June and July 2016. The non-flowering species at the time of the surveys were identified on the basis of knowledge of the vegetative characteristics of the species known in the local flora or on the basis of previous and subsequent floristic explorations.

Arthropods collection and identification

Above ground arthropods were collected using pitfall traps, a sweep net and a butterfly nets. Four pitfall traps (diameter 9 cm, depth 12 cm) containing a 50% blend of water and vinegar were placed at the corners of each sampling area. This in relation to the purpose of census of the ground species both typical of the ecotonal belt and of the prairie, avoiding placing the traps in the open areas most subject to the passage of men and animals. During each sampling session, pitfall contents were transferred into jars containing 70% ethanol. A sweep net was used to make ten sweeps at each of the four equidistant points along the same transects used for estimating plant species abundance and diversity (two at the ends and two in the middle). At the same sampling points, butterfly nets were used to catch butterflies within a ten-minute time interval. The collected specimens were preserved in 70% ethanol or with ethyl acetate vapour depending on the taxa. Samples were taken every ten days at each site from the beginning of June to the end of September 2016 between 08:00–15:00.

Arthropods were classified to the order or family level, as appropriate, and sent to taxonomic specialists for the identification to genus or, where possible, species level. Since the collected specimens were identified to different taxonomic levels based on the availability of specialists, the term species when used should be interpreted to mean ‘to the best possible level of identification’.

Statistical analyses

Differences between the phytosociological composition at the two sampling localities (Garaventa and Bavastrelli) was assessed by one-way ANOSIM (Bonferroni corrected P values) based on the Bray-Curtis similarity index to data expressed both as abundance classes, and as mean abundance percentage within each class (+ = 0.5%, 1 = 2.5%, 2 = 12.5%, 3 = 37.5%, 4 = 62.5%).

Ecotones of the studied meadows were compared by applying the χ^2 test to the frequencies of the identified plants in the seven circular plots at each locality.

Differences in the arthropod assemblages collected by sweep nets and pitfall traps from the two areas were investigated by means of Non-metric Multidimensional Scaling and one-way ANOSIM (Bonferroni corrected P values) based on the Bray-Curtis similarity index. SIMPER analysis enabled the identification of taxa responsible for the occurrence of significant differences. Butterfly assemblages were compared using the χ^2 test applied to the frequencies of eight dominant species representing about 70% of individuals in both localities (*Melanargia galathaea*, *Kanetisa circe*, *Plebejus argus*, *Coenonympha pamphilus*, *Pieris napi*, *Polyommatus icharus*, *Maniola jurthina* and *Lasiommata maera*).

PAST Software (Paleontological Statistics version 4.02, last access May, 2020; Hammer et al., 2001) was used to perform the statistical analyses mentioned above.

Both sample- and individual-based species rarefaction curves (Gotelli & Colwell, 2011) were computed to compare the between-site species richness estimated through the sampling methods adopted. According to the sampling design, both sweep-nets and pitfall-trap samples have been analysed through the sample- and individual-based approach, while only the individual-based approach was followed for data on Lepidoptera.

The number of species observed in a sample is not only known to be sensitive to the number of samples but also to the number of individuals collected. The introduction of linked rarefaction and extrapolation curves by Colwell et al. (2012) allows the comparison between surveys based on samples of different sizes. Thus, species richness can be more meaningfully compared between sites by rescaling the sample-based rarefaction and extrapolation curves to individuals, as suggested by Butler and Chazdon (1998) and Gotelli and Colwell (2001). In this study, to compare the insect diversity of the two sampling sites, the extrapolation was performed by doubling the size of the smaller sample. Rescaling was not carried out for pitfall-trap data due to the different sampling effort at the two study sites.

When rarefaction curves do not reach the asymptote (corresponding to the estimated total species richness of a sampled assemblage, according to the sampling method), the completeness of the inventory can be assessed through species richness estimators (Hortal et al., 2006). Rarefaction curves as well as seven non-parametric species richness estimators were calculated using EstimateS (Version 9.0) (Colwell, 2013), with 10000 randomizations. The percentage of maximum estimated richness (to the asymptote) detected in our samples has been calculated according to the formula: $(\text{Species Observed}/\text{Species Estimated}) \times 100$.

The sampling effort necessary to reach asymptotic values was calculated according to the algorithm proposed by Chao et al. (2009) for abundance data (Chao 1 estimator). Different values were set to calculate further additional samples needed to identify certain proportions of the estimated maximum species number (100%, 90% and 80%).

RESULTS

Habitats

From a phytosociological point of view (see *Supplementary Material Plants - Reliefs*) the vegetation of both sites is attributable to the *Molinio-Arrhenatheretea* Tüxen 1937 with a robust component of *Festuco-Brometea* Br.-Bl. & Tüxen ex Br.-Bl. 1949, but with different distinctive elements.

Garaventa mowed lawn, currently mown irregularly and no longer fertilized, has a greater number of *Festuco-Brometea* species, showing an evolutionary tendency towards more xeric formations.

Bavastrelli meadow it is currently used as pasture but it was probably once cultivated, as the presence of bands supported by dry stone walls (now deteriorated) seems to indicate. However, pasture shows a certain heterogeneity in the vegetation, with areas more subject to trampling and with discontinuous turf, others with richer and more compact turf and others with the presence of shrubs and small scattered trees. The herbaceous formations are dominated by species of the *Molinio-Arrhenatheretea* and, in areas where the animals stay longer, the pasture is richer in species belonging to the *Plantaginietalia majoris* Tüxen ex Von Rochow 1951 as well as in synanthropic and ruderal species.

At both sites the marginal areas (see *Supplementary Material Plants - Borders*) are affected by the influence of the surrounding vegetation: wooded in Garaventa and anthropic or shrubby in Bavastrelli; although, in both sites, the marginal strip has a floristic composition quite similar to that of the central part of the meadow. In general, it should be noted that the Bavastrelli

pasture is characterized by the presence of dry-stone walls that are partly still standing, others partly collapsed where plant species, not reported in the reliefs, have been found: *Adiantum capillus-veneris* L., *Achillea millefolium* L., *Anagallis arvensis* L., *Asplenium trichomanes* L., *Rosa arvensis* Hudson, *Sedum dasyphyllum* L., *Verbascum blattaria* L. This area also shows signs of trampling due to grazing cattle. This is confirmed by the presence of plants related to trampling such as *Lolium perenne* L., *Plantago major* L. and *Polygonum aviculare* L. Both these factors result in a discontinuity in the turf and a reduction in plant diversity, with a higher abundance of nitrophilic species in the area (*Picris hieracioides* L., *Sisymbrium officinale* (L.) Scop., *Capsella rubella* Reut., *Artemisia vulgaris* L. and *Potentilla reptans* L.).

Both One-way ANOSIM approaches (the one based on abundance classes and that based on percentages of coverage) to assess differences between the core vegetation of Garaventa and Bavastrelli did not show any statistically significant differences; however, it was clearly possible to differentiate them based on the plant species richness (Figure 3).

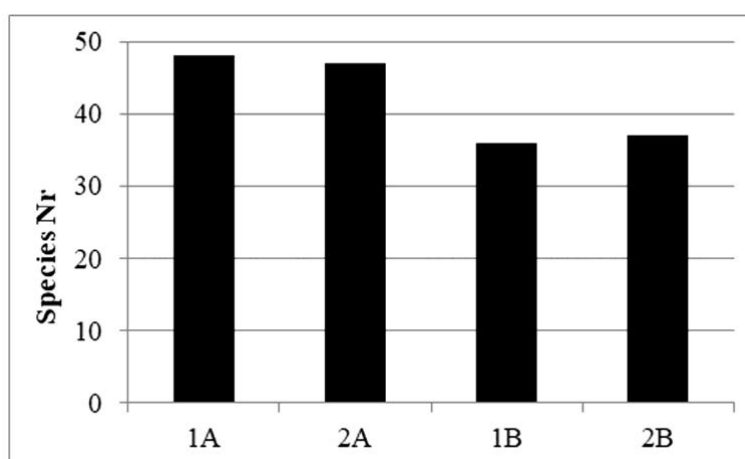


Figure 3. Plant species richness in the core-plots analysed based on the phytosociological approach of Braun-Blanquet (1937) at the sampling localities: A = Garaventa, B = Bavastrelli.

Furthermore, the analysis of frequencies of identified plants in the seven circular plots within each locality using the χ^2 test highlighted a statistically significant difference between Garaventa and Bavastrelli ecotonal strips ($\chi^2 = 260.12$; 83 d.f.; Monte Carlo $p = 0.0001$). In this case the species richness is significantly higher at the former locality (58 vs 52 species) but the number of species in the 14 plots of both areas was distributed over a rather wide range, with a large overlap between them (Figure 4).

In total, the number of plant species detected in Garaventa mowed meadow and Bavastrelli pasture was 90 and 80, respectively.

Arthropod assemblages

Sweep-net samplings

Use of a sweep-net caught 843 individuals belonging to 135 taxa and 694 individuals in 120 taxa at the Bavastrelli pasture and from the mown meadow at Garaventa, respectively (see *Supplementary Material Arthropods – Sweeping net* and *Sweeping summary*). Non-metric

Multidimensional Scaling produced a clear separation of the point clouds corresponding to the sampling sessions in the studied localities (Figure 5). Indeed, One-way ANOSIM of samples at the two localities demonstrated a highly significant difference between the insect communities both at the species ($p_{(\text{same})} = 0.0002$; $R = 0.2903$) and at the order level ($p_{(\text{same})} = 0.0001$; $R = 0.3836$). SIMPER analysis identified that two orders, Hemiptera and Coleoptera, were each responsible for more than a 26% dissimilarity (Table 1). At the “species” level an unidentified species of Cicadellidae, *Longitarsus* spp. and *Stenodema laevigatum* accounted for between 9 and 10% dissimilarity.

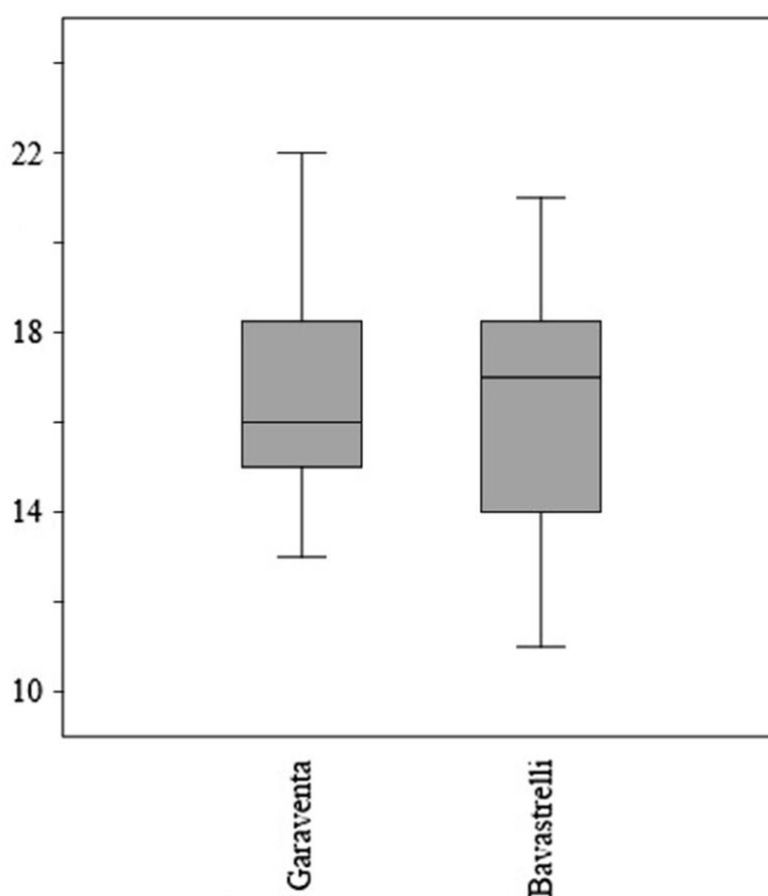


Figure 4. Box-plots showing the richness of plant species found in 14 circular plots along the border of the Garaventa and Bavastrelli grasslands.

Bavastrelli pasture shows higher values of species richness both in terms of number of observed taxa and of species estimated by all seven asymptotic estimators adopted. This statement is further supported by the fact that the proportion between observed and estimated diversity by each estimator is almost the same in both sites considered (*Supplementary Tables S1 to S4*). The individual-based rarefaction curves are shown in Figure 6. Also, in the case of the rescaled values for individuals as suggested by Colwell (2012), the species richness estimated for Bavastrelli was higher than that of Garaventa (*Supplementary Tables S3*).

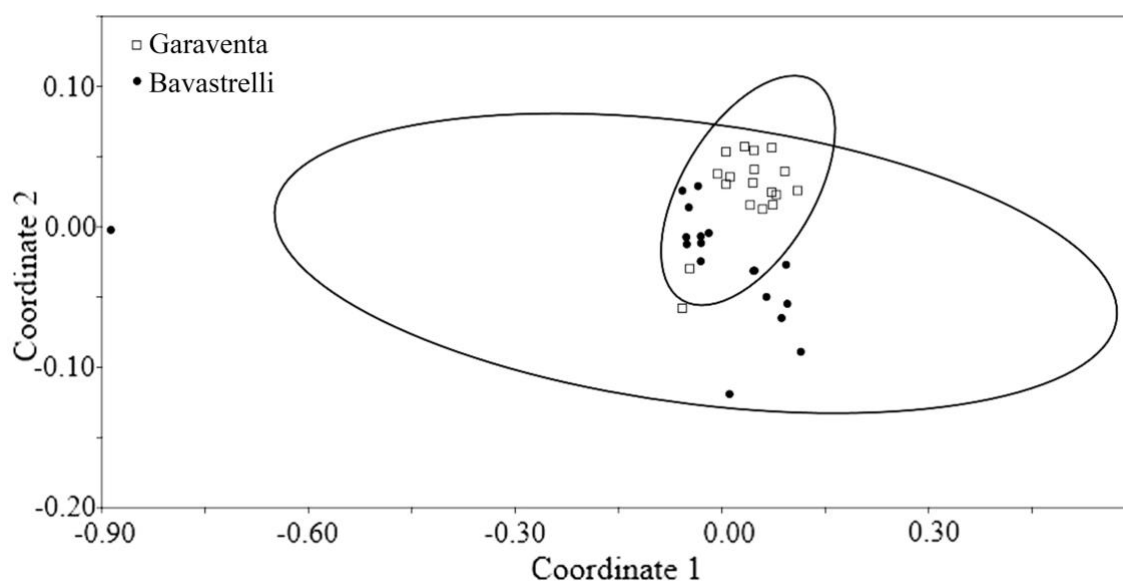


Figure 5. Non-metric Multidimensional Scaling (Bray-Curtis similarity index) on data of daily samplings of arthropods by sweeping-net in Garaventa mowed grassland and Bavastrelli pasture. 95% confidence ellipses are shown.

Table 1. Output of SIMPER analysis based on the comparison at the Order level of arthropods assemblages obtained from catches with sweep-net.

Taxon	Average dissimilarity	Contribution %	Cumulative %
Hemiptera	10.8	26.64	26.64
Coleoptera	10.73	26.46	53.1
Araneae	5.115	12.61	65.71
Diptera	4.157	10.25	75.96
Thysanoptera	3.244	7.998	83.96
Hymenoptera	2.189	5.397	89.35
Orthoptera	1.817	4.48	93.83
Lepidoptera larvae	0.5891	1.453	95.29

Lepidoptera

In total, 120 butterflies were collected (52 in Bavastrelli and 68 in Garaventa) and 117 of them were identified to species level. Butterfly assemblages for the two localities were analysed using the χ^2 test ($\chi^2 = 33.166$; 7 d.f., Monte Carlo $p = 0.0001$) and were shown to be statistically different. However, the recorded species richness was the same (19) in both localities (see *Supplementary Material Arthropods – Butterflies and Butterflies summary*) but the individual-based approach rarefaction analysis led to an estimate slightly higher in the number of species at Bavastrelli compared with Garaventa (Figure 7 and *Supplementary Tables S5 to S8*).

Pitfall Trapping

Samples taken from pitfall traps along the edge of the Bavastrelli pasture and the mown meadow at Garaventa yielded 234 individuals from 33 taxa, and 558 from 41 taxa, respectively (see *Supplementary Material Arthropods – Pitfall traps and Pitfalls summary*). One-way

ANOSIM of samples collected from the two localities showed a highly significant difference between their insect communities ($p_{(same)} = 0.0012$; $R = 0.3493$). Based on SIMPER analysis *Anoplotrupes stercorosus* alone accounted for almost 23% dissimilarity; being much more abundant in Garaventa where it represented 31.4% of the ground arthropods compared with only a representation of 9% of the ground arthropods at Bavastrelli.

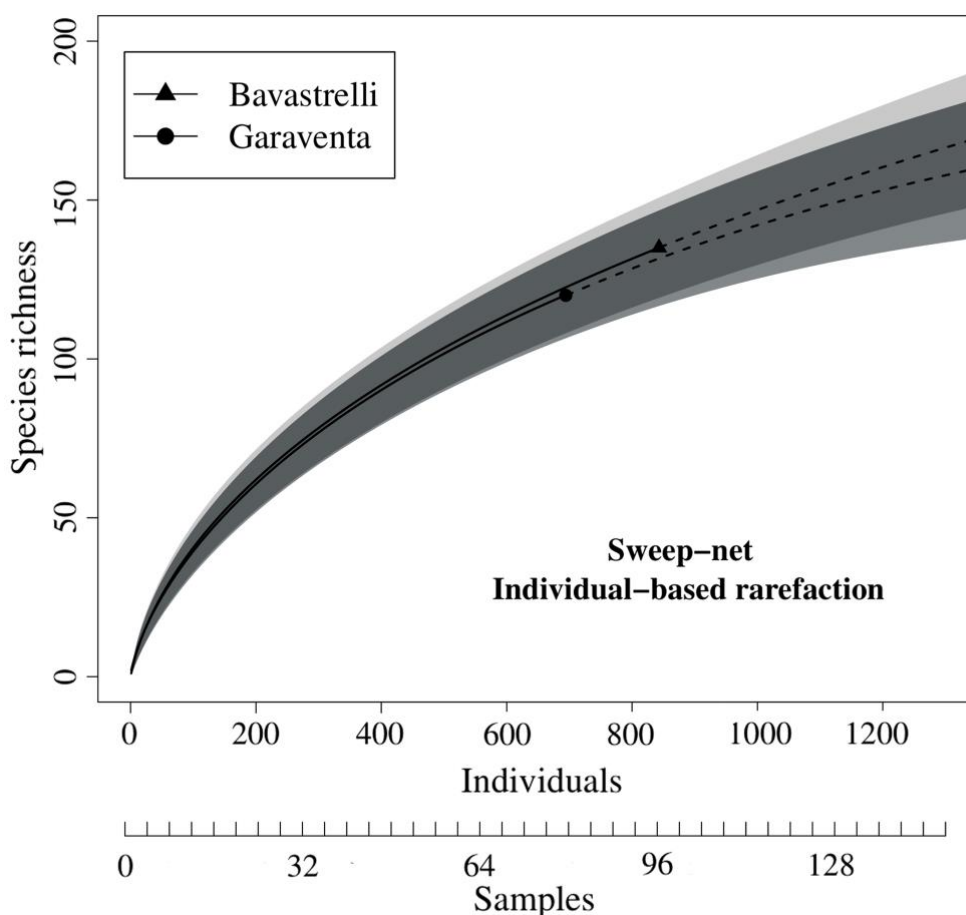


Figure 6. Individual-based rarefaction curves on arthropod assemblages collected by sweeping-net.

It seems incongruous that the abundance of this species is higher in Garaventa than in Bavastrelli, where the presence of grazing cattle should favour these dung beetles. This could be due to the abundant presence of rotting wood and its associated fungi and moulds at the edges of Garaventa meadow. This organic substrate is a food source for *Anoplotrupes* spp. The capture of a large number of *Nebria tibialis* and *Abax parallelepipedus contractus* in traps also confirms the influence of the forests on the entomological community of the grassy margin where pitfall traps were placed.

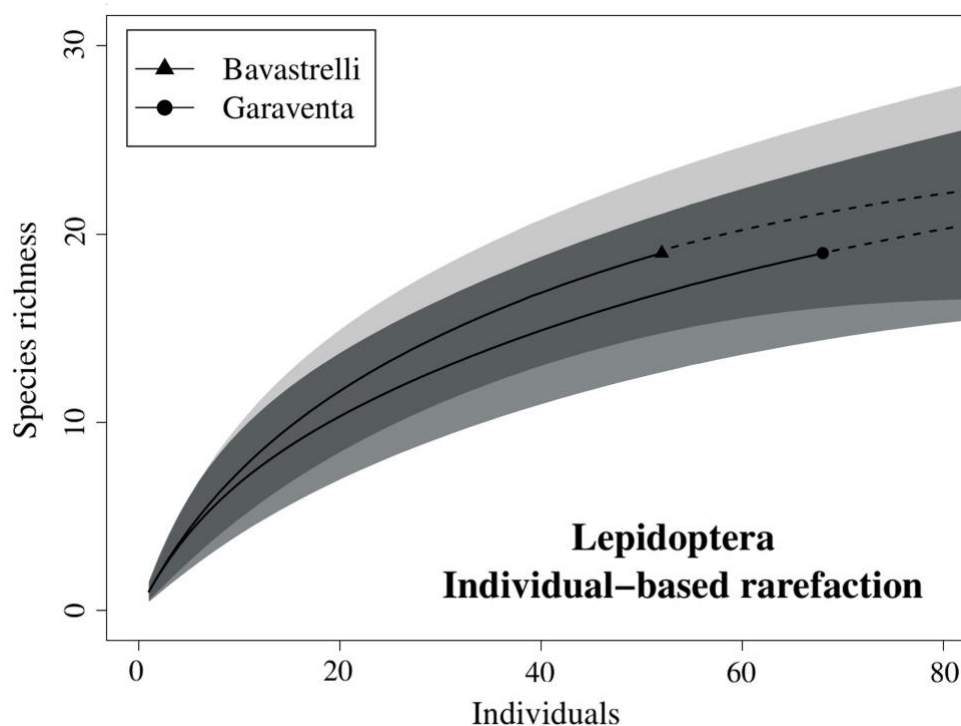


Figure 7. Individual-based rarefaction curves on butterfly assemblages.

Even though observed species richness is higher in Garaventa, the asymptotic estimators all converge towards greater diversity values for the Bavastrelli site. This aspect is also evident in the comparison of the two sites in terms of interpolation rarefaction both through the sample-based and individual-based approaches (*Supplementary Tables S9 to S14*). In accordance with this, rarefaction curves (Figure 8) show as in Bavastrelli the asymptote species richness detectable through pitfall-trapping should result higher than in Garaventa, even if the data seem to indicate otherwise. This turnaround of the species richness that emerges when comparing the sample-based rarefaction curves is due to the lower sampling effort in the Bavastrelli pasture than in the Garaventa mowed grassland (25 vs 30), which justifies a smaller number of species observed in the former locality in spite of its greater number of species expected.

DISCUSSION

There seems to be an inverse relationship between overall plant and arthropod species richness in the studied grasslands: the former is higher in Garaventa, the latter in Bavastrelli. This result contradicts previous observations of Albrecht et al. (2007) whereby a higher diversity of insect species and interactions between them related to a higher plant species richness in restored rather than in intensively managed meadows. Moreover, Hertzog et al. (2016) concluded that abundance and species richness of both herbivores and carnivores increases with increasing plant species richness. However, Bavastrelli pasture has a more complex conformation, which probably determines a greater ‘edge effect’. This is also reflected by the higher number of plant species observed along the borders of Bavastrelli. This effect, together with the lack of an estimate of the expected species richness for plants may partly explain the aforementioned apparent discrepancy.

Moreover, our results can be related to the complexity of relationships between species at different trophic levels in a single locality, and between contiguous ecosystems at a landscape level. For instance, Sundell (2021) showed that forests in the surrounding landscape have a positive effect on the abundance of species of flower-visiting insects in clear-cuts, while the presence of nearby clear-cuts had a negative effect.

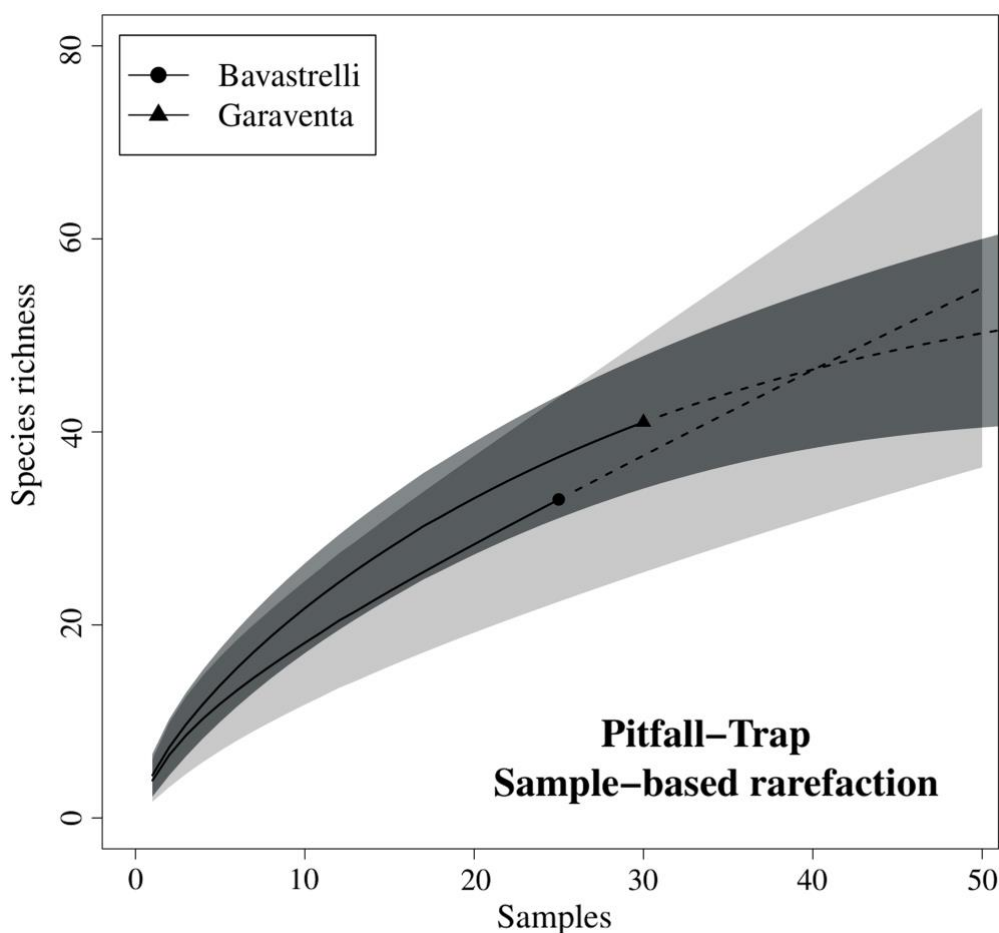


Figure 8. Sample-based rarefaction curve on ground arthropods assemblages collected by pitfall-traps.

A richer arthropod community in the Bavastrelli pasture should indicate low intensity grazing by herbivores. In fact, van Klink et al. (2015) observed that large herbivores at low densities or with spatio-temporal variation in densities caused an increase in biotic and abiotic heterogeneity large enough to compensate for the loss of resource abundance for arthropods, and their increased mortality rate. Similarly, Chisté et al. (2016) observed that various species of Orthoptera show different responses to land-use intensity, but that the overall orthopteran diversity was negatively affected by high levels of fertiliser and mowing, and grazing activity.

Based on the results from our observations and analyses we expected a slightly higher species richness for butterflies at Bavastrelli than at Garaventa. Steffan-Dewenter & Tschardtke (2002) observed that species richness of butterflies in general, and the proportion of monophagous species in particular, increase as the area and fragmentation of habitats increases. Butterfly

assemblages are also influenced by the extent of habitat connectivity, which increases inter-patch movements resulting in a corresponding positive effect on population density and a reduction in the risk of species extinction, locally (Baguette et al., 2000; Schultz et al., 2012; Steffan-Dewenter & Tschardtke, 2002). Similarly, the regular mowing of grasslands, increases population densities of grassland butterflies, which brings into question the exclusive practice of using uncut refuge sites for the conservation of grassland arthropods in agricultural landscapes and in natural reserves (Lebeau et al., 2015).

Conversely, spider diversity was higher at Garaventa (22 species) compared with the 18 species found at Bavastrelli. This result supports observations made by DeBano (2006), Dennis et al. (1998) and Farrell et al. (2015) that the different responses of various taxa to grazing corresponds with a higher biovolume of predatory arthropods in ungrazed plots. For an in-depth analysis of spider community responses to grassland management see Smith DiCarlo & DeBano (2019).

CONCLUSIONS

Land use and its intensity is well known to affect arthropod species abundance in grasslands (Simons et al., 2015). Our research highlighted the differences between the arthropod communities of two grasslands subject to different management practices. In general, the species richness of arthropod assemblages monitored using three sampling methods (sweep-nets, butterfly nets and pitfall-traps) seems to be higher in pasture than in mowed lawn. This result highlights the different impact of any management action and conservation strategy on grassland ecosystems at different trophic levels (Albrecht et al., 2007) and for different taxa (Chisté et al., 2016).

The goal of preserving arthropod diversity, especially important in a protected area like a Regional Park, presupposes the adoption of strategies aimed at avoiding habitat fragmentation and its detrimental effects on biodiversity (Steffan-Deventer & Tschardtke, 2002; Tschardtke, 2002a, 2002b; van Noordwijk et al., 2015). In particular, efforts should be made to maximize grasslands' heterogeneity, by implementing management strategies that regulate herbivore densities, and encourage the use of novel approaches to herd management and associated grazing regimes (Kruess & Tschardtke, 2002; Lyons et al., 2018; Piano et al., 2017; van Klink et al., 2015). Another aspect not to be underestimated is the influence that the invasion of exotic grasses could have on the biodiversity of grassland arthropods (Andersen et al., 2019; Farrell et al., 2015; Poniatowski et al., 2018). This emphasizes the importance of having in place an efficient network for monitoring the introduction of alien/exotic species. In Liguria the monitoring program is coordinated by the Regional Agency for Environment Protection (ARPAL).

In conclusion, further field research like this should be periodically undertaken so as to monitor not only the true effects of the management practices implemented but also to record and verify the impact of climate changes on arthropod communities; the prediction of which is far from unambiguous (Barnett & Facey, 2016).

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