

Research article

Submitted: November 13th, 2021 – Accepted: September 20th, 2022 – Published: December 15th, 2022
DOI: 10.13133/2284-4880/582

Saproxylic weevils and edaphic beetles as indicators of environmental quality of relict forests in Piedmont lowlands (Coleoptera)

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Abstract

Saproxylic weevils and edaphic beetles of some relict forests of north-western Italy were analysed to determine the role of the age of the forests, their isolation and the degree of naturalness of the patches within each forest on the composition of litter-associated communities. Ten species of saproxylic weevils were found. The communities showed variation among the various forests, and were mainly dependent on the age of the forest and their connectivity; the degree of naturalness did not influence species richness or abundance, but apparently had an effect on some more specialized taxa that were only present in the patches with highest degree of naturalness. No saproxylic weevils were found in a relatively recently established forest, demonstrating their difficulty in colonizing newly formed habitats isolated from other forests. The edaphic beetles (usually predators belonging to Carabidae and Staphylinidae) proved to be more abundant, were also present in the recently established forest and occurred in quite large numbers in some deteriorated patches too. Saproxylic weevils and edaphic predatory beetles thus provide different information on the environmental quality of the forests.

Key words: Bioindicators; forest naturalness; litter Coleoptera; lowland relict forests; species richness.

Introduction

The woodlands of the Po Plain are one of the rarest and most endangered ecosystems in Europe. These forests were present in ancient times throughout northern Italy and covered almost the entire Po Valley (Camerano et al. 2010; Matonti 2011). Over the last two millennia, ever larger portions of land have been deforested in favour of crops, settlements and related infrastructures. In addition to drastically reducing wooded areas, human activity has also modified to varying degrees the composition and structure of the few remnants, which were seen as a valuable reserve of fuel or building materials (Camerano et al. 2009, 2010). The relict forests that have survived the radical anthropic transformation of the territory represent the last, albeit partially altered, examples of the ancient woodlands of the Po Plain. Hence, their conservation is particularly important, not only from an ecological perspective, but also in terms of their historical interest and educational role. Moreover, it is a fundamental tool to

have as a model for the reconstruction of lowland forests (Taffetani 2008; Camerano et al. 2009) and their management (Della Rocca et al. 2014).

More than a half of the entire surface of the lowland forests of northern Italy is located in the Piedmont plain (over 90,000 ha out of 175,000), which corresponds to a woody index of 10% (Camerano et al. 2009, 2017). This value is rather high when compared with other regions of the Po Valley (Camerano et al. 2010). In Piedmont, the maintenance of some lowland forests is linked to their use for past hunting activities near residences of the House of Savoy royal family, often on soils largely unsuitable for agriculture (Camerano et al. 2009, 2010). Unfortunately, most of these woods present a high level of anthropization. About 45% of them are dominated by allochthonous black locust woods (*Robinia pseudoacacia*), originally planted to improve forest productivity, but which are naturalized almost everywhere. Substitution of the original trees by other alien species, especially black cherry (*Prunus serotina*) and red oak (*Quercus rubra*), is also common (Camerano et al.

2009, 2010). Other problems include habitat fragmentation, limited extension of some sites, and deterioration due to excessive water extraction for irrigation. These alterations have caused two fundamental changes in forest ecosystems, spatial discontinuity and temporal discontinuity (Cateau et al. 2016, 2018), resulting in serious consequences for biodiversity (Assmann 1999; Beiroz et al. 2014).

Several studies have shown that bioindicators allow the assessment of human impact on ecosystems (Nitzu et al. 2008; Gerlach et al. 2013; Ghannem et al. 2017). Some species or groups of species seem to be more affected by such discontinuities than others. In particular, saproxylic beetles of lowland forests have been impacted strongly by human-induced forest changes and are thus today considered as threatened (Eckelt et al. 2017).

Species with a limited dispersal capability and restricted trophic or ecological requirements are those that can be more severely affected by habitat alteration. Saproxylic litter weevils (Coleoptera: Curculionoidea) can be considered good indicators of environmental quality since they display two key traits (Anderson & Ashe 2000; Buse 2012; Maioglio et al. 2022 Cateau et al 2016, 2018): 1) limited dispersal, due to their lowered motility and inability to fly (low-vagility species, often highly specialised in terms of humidity, substrate and micro-habitat); 2) restricted trophic requirements, resulting from their saproxylic diet (dead and decaying wood can be removed in managed forests). Therefore, saproxylic weevils can be used as a valid biological model for studying the impact of human disturbance and to evaluate forest conservation actions. Nevertheless, the few studies that have investigated these beetles in temperate forests have reported conflicting results (Buse 2012; Horák et al. 2013). Based on both field work and species records in collections and literature, Buse (2012) analysed Curculionidae Cryptorhynchinae and Molytinae in 29 forests (15 ancient and 14 recent) in north-west Germany, in order to examine spatial distribution and phenology of saproxylic weevils and their status as relict species. The beetles were sampled by sieving the litter at the base of 10 trees per forest and extracted by inspecting in situ the sieved litter. Nine individuals belonging to five species of Cryptorhynchinae [*Acalles camelus* (Fabricius, 1792), *Acalles echinatus* (Germar, 1824), *Acalles fallax* (Boheman, 1844), *Echinodera hypocrita* (Boheman, 1837), *Kyklioacalles navieresi* (Boheman, 1837)] and 18 individuals of one species of Molytinae [*Trachodes hispidus* (Linnaeus, 1758)] were collected. Since none of these species were found in recent forests, the author concluded that these six species were associated with ancient woodlands.

With similar aims to Buse's study, Horák et al. (2013) used 62 window traps in five study areas in the Czech Republic, collecting 50 specimens of four Cryptorhynchinae species [*Acalles camelus*, *Acalles fallax*, *Echinodera hypocrita*, *Kyklioacalles roboris* (Curtis, 1835)] and 20 individuals of *Trachodes hispidus*. As the five species

were found in secondary forests, the authors suggested that these weevils are typical of secondary woodlands. The contradictory results of these two studies highlight the need to improve knowledge of these beetles, and of their role in characterizing relict forests.

Objectives of the study

Since no data were available for any relict forest in north-western Italy, we investigated the role of the age of the forests, their isolation and the degree of naturalness of the patches within each forest in determining the composition of the saproxylic weevil communities. We also tried to recognize the contribution of the weevil species as bioindicators of the different types of forest in northern Italy. Furthermore, we evaluated the role of some predatory edaphic beetles collected together with the weevils for the characterization of these habitats.

Materials and Methods

Study areas

The field work was carried out in four protected areas, all located in central-western Piedmont, north-western Italy (Figs 1, 2): Bosco del Merlino, Bosco delle Sorti della Partecipanza di Trino (N45°13'35", E8°15'45", 167 m asl), Parco di Stupinigi (N44°58'50", E7°35'15", 244 m asl), Bosco del Vaj (N45°09'10", E7°53'20", 487 m asl). They were selected to reflect the diversity of relict woodlands in central and western Piedmont, and also with the perspective of comparing them to other central European woodlands subject to a continental climate. All areas were Natura 2000 sites and each of them included or coincided entirely with the respective SCI (Sites of Community Importance); moreover, all four SCIs have been designated as SACs (Special Areas of Conservation). All the study areas were lowland, except Bosco del Vaj which was situated on the hills above Turin.

In each area, two sampling sites were chosen: one that was as close as possible to natural forest and one in a patch that had been more or less severely altered by human activity, characterised by deforestation and/or the introduction of allochthonous plants and/or heavy recent management.

- **Bosco del Merlino** (SAC IT1160010, N44°47'40", E7°42'50", 252 m asl). This is a small (351 ha) remnant of the western Piedmont plain forest, representing lowland *Quercus-carpinetum*, surrounded by agro-ecosystems with meadows and *Zea mays* cultivation. Before its protection, it was subject to considerable management with cutting of trees (IPLA 2017). The close-to-natural patch was characterised by an oak (*Quercus robur*) – hornbeam (*Carpinus betulus*) wood,

with fairly old trees (possibly more than one century old), partly in deteriorated condition. Close by, there was an old abandoned poplar (*Populus x canadensis*) plantation which was used as the altered forest site.

- **Bosco delle Sorti della Partecipanza di Trino** (SAC IT1120002, N45°13'35", E8°15'45", 167 m asl). This is probably a remnant of the primary lowland forest; records of its presence date back to the 13th century. Since that time, it has been continuously managed with tree cutting carried out with a turnover of the various patches (IPLA 2006). The forest in its entirety was preserved, but at the same time there are no very old trees and a large part of the dead branches on the ground are continuously removed. The aquifer is quite superficial. The area of the site is 570 ha and it is surrounded by intensive rice cultivation. The close-to-natural forest site, deep inside the forested area, was an alder (*Alnus glutinosa*) – ash (*Fraxinus excelsior*) wood; the altered site, at the margin of the forest, near the rice fields, was an oak (*Quercus robur*) – hornbeam (*Carpinus betulus*) wood with black locust (*Robinia pseudoacacia*); all plants here were of small size, with a sparse litter layer.
- **Parco di Stupinigi** (SAC IT1110004, N44°58'50", E7°35'15", 244 m asl). This is a forested area that was established in the Middle Ages by the royals of Piedmont, derived from woodland expansion over previous humid areas after they were reclaimed; it has since been used as a hunting ground (Sindaco et al. 2008; Ente di gestione delle aree protette dei Parchi Reali 2020). It was not originally part of the lowland primary forest, and we chose it as a comparison with the remnants of the primary forest. It is 1611 ha in area and is surrounded by industrial areas, some meadows and pastures, and the town of Turin. The close-to-natural forest site was an oak (*Quercus robur*) – hornbeam (*Carpinus betulus*) wood with hazel (*Corylus avellana*); the altered forest site was a nearby plantation of white pine (*Pinus strobus*).
- **Bosco del Vaj** (SAC IT1110009, N45°09'10", E7°53'20", 487 m asl). This area, of slightly higher altitude, includes several beech trees that are considered to represent glacial relicts (IPLA 2016). The origin of the forest is therefore antique and the present-day woods surely derive from the primary forest of the Turin hills; it is not isolated like the other studied areas, but is connected with other woodland zones that are nowadays quite heavily impacted by human activities and usually managed as coppice woods. The close-to-natural forest site is a beech (*Fagus sylvatica*) wood; an oak (*Quercus robur*) – chestnut (*Castanea sativa*) wood is the nearby altered forest site.

Taxa considered

We primarily took into account flightless saproxylic weevils (Coleoptera: Curculionoidea), whose larvae develop

in dead branches, and in the adult stage remain closely associated with the forest habitat. We also included other taxa of the forest litter coenoses that are predators of small invertebrates, such as Carabidae and Staphylinidae, and their larvae, that were collected together with the weevils.

Sampling method

Saproxylic weevils are active and present all year round, with minimal inter-annual variations in their abundance (Heijerman 2004; Buse 2012; Cateau et al. 2016); however, autumn (mid-September to mid-December) and spring (mid-March to mid-June) are the most suitable periods for a comprehensive evaluation (Cateau et al. 2016). In this work, sampling in each area was performed in two periods: April-July and September-November 2019. During both sampling periods, meteorological and environmental conditions were constantly monitored and sampling was performed a few days after rains, to ensure moderate conditions of humidity in the substrate; sampling after long periods of drought was avoided, since in these conditions the beetles tend to shelter in the lower part of the litter layer or move towards more humid spots, and therefore they are often less accessible.

All specimens were sampled by sieving litter. This method was chosen based on its documented good performance (Angelini et al. 2002; Heijerman 2004; Bosmans 2008; Buse 2012; Cateau et al. 2016, 2018), as also previously experienced by one of the authors (M.M.).

In each sampling site, five plots of approximately 1 m² (squares of 1 m × 1 m) were delimited: one central area and four other areas at about 10 m distance from the central plot. Two litres of sieved litter were collected per plot using a Winkler sifter with a 10 mm square mesh. Whenever possible, squares were selected to include parts of decaying wood adjacent to large trees. During the second sampling event, we avoided the same plots sampled in the previous period. These were easily distinguishable due to the lower amount of litter present, but sampling was done nearby anyway. A total of 16 litter samples were collected, eight in the first sampling event and eight in the second one.

Modified Berlese-Tullgren funnel extractors were used for the extraction of the fauna. As the studied species are not known to be lucifugal (Cateau et al. 2016), no lights were put on the top of the funnels. For each single replica, the sieved litter was placed in the extractors for at least 10 days, until it dried completely. After five days from the first day of extraction, the litter inside each funnel was turned by hand to ensure that it dried homogeneously. Since, according to Cateau et al. (2016), beetles become more active after the soil is disturbed, turning litter facilitated their descent towards the container with the preserving liquid (70% ethyl alcohol).

The extraction phase was followed by the sorting phase and finally the preparation and identification of the beetles. Specimens of edaphic beetles were identified at family or subfamily rank, whereas the Curculionidae were identified at species level. Their nomenclature was based on Alonso-Zarazaga et al. (2017), Stüben (2014–2020), as well as online catalogues WTaxa (Alonso-Zarazaga & Lyal 2020) and Catalogue of Life (Roskov et al. 2020).

Georeferencing, environmental variables and other collected data

Each sampling plot was characterised by the following parameters: GPS coordinates of the central square (WGS 84); type of forest (dichotomous variable, close-to-natural/ altered forest), corresponding to the age of forest (dichotomous variable, antique/recent forest); and litter thickness (cm). Variables (area, forest and litter) were combined to produce six different models used as environmental parameters for the following analyses concerning species richness and community composition.

Data analyses

Variations in species richness (only for litter weevils) and abundance (for all beetles) were analysed in relation to environmental variables, to assess the role that close-to-natural vs altered habitat, or antique vs recent forest, has for the beetle communities. In the case of the edaphic beetles, only the four taxa with the largest number of specimens were examined: larvae (considered globally, without taxonomical subdivisions), Carabidae, Staphylinidae: Staphylininae and Staphylinidae: Pselaphinae. A model with a Poisson distribution was specified for Curculionidae and Carabidae, whereas a negative binomial distribution was specified for larvae, Staphylininae and Pselaphinae. The analysis was performed by comparing six models related to various combinations of the starting environmental variables, corresponding to different biological hypotheses (Table 1). Based on the AIC (“Akaike Information Criterion”, Akaike 1973) value, the model with the lowest AIC for each group considered was considered as the best model upon which inference was based.

The IndVal method (Dufrene & Legendre 1997; De Caceres 2020) was used to identify potential indicator species of a certain type of forest within the four study areas. This approach involved the division of the plots in the two types of forest, in the sampling sites of the various study areas, and in the four areas themselves.

In order to highlight variations in community composition among sites and forest types, we applied the permutational multivariate analysis of variance “PERMANOVA” (Anderson 2001), based on distance matrices calculated by the Jaccard index. The analysis was carried out through

Model	Parameters	Biological hypothesis
Model 1	Area	Variations in species richness and abundance in relation to the four study areas (higher in more antique areas)
Model 2	Area + litter	Variations in species richness and abundance in relation to study areas and litter (higher in more antique areas with a large presence of litter)
Model 3	Area + forest	Variations in species richness and abundance in relation to study areas and forest type (higher in more antique areas and close-to-natural forests)
Model 4	Area + forest + litter	Variations in species richness and abundance in relation to study areas, forest type and litter (higher in more antique areas and close-to-natural forests with a large presence of litter)
Model 5	Area * forest	Variations in species richness and abundance in relation to the type of forest present in the sampling sites of the study areas, i.e. the habitat (higher in close-to-natural habitats)
Model 6	Area * forest + litter	Variations in species richness and abundance in relation to the litter present in each habitat (higher in close-to-natural habitats with a large presence of litter)

Table 1 – Explanation of the six models used for analysis of variations in species richness and abundance.

the “adonis” function contained within “vegan” package, version 2.5 (Oksanen et al. 2019).

To highlight differences in variability among communities, we applied the permutation analysis of multivariate dispersion “PERMDISP” (Anderson 2004). The centroids of each group and the distance of each plot from the reference centroid were calculated with the “betadisper” function, also from the vegan package, version 2.5 (Oksanen et al. 2019).

All statistical analyses were carried out with R 3.6.2 software (R Core Team 2020).

Results

Weevils – A total of 78 specimens of saproxylic beetles were collected, belonging to 10 different species (Table 2). The study area with the highest number of species and individuals was Bosco del Vaj, with 7 species and 59 individuals (70% of the total species sampled and about 77% of the specimens collected). The next highest was Bosco della Partecipanza (3 species, 11 individuals), followed by Bosco del Merlino (2 species, 8 individuals). Finally, all weevils collected in Parco di Stupinigi belonged to floricolous winged species, whose occurrence in the litter is occasional, and therefore they were excluded from the analysis.

Species	Merlino		Partecipanza		Stupinigi		Vaj		Σ	
	N	A	N	A	N	A	N	A	N	A
<i>Acalles echinatus</i> (Germar, 1824)	0	0	0	0	0	0	5	1	5	1
<i>Acalles lemur</i> (Germar, 1824)	0	0	1	5	0	0	0	0	1	5
<i>Acalles micros</i> (Dieckmann, 1982)	0	0	3	0	0	0	0	0	3	0
<i>Aparopion chevrolati</i> (Jacquelin du Val, 1855)	2	5	0	0	0	0	11	14	13	19
<i>Baromiamima concinna</i> (Boheman, 1834)	0	0	0	0	0	0	3	4	3	4
<i>Dryophthorus corticalis</i> (Paykull, 1792)	0	0	0	0	0	0	1	0	1	0
<i>Echinodera hypocrita</i> (Boheman, 1837)	0	0	0	0	0	0	4	0	4	0
<i>Kykliaacalles aubei</i> (Boheman, 1837)	0	0	0	0	0	0	2	0	2	0
<i>Kykliaacalles navieresi</i> (Boheman, 1837)	0	0	1	1	0	0	4	10	5	11
<i>Trachodes hispidus</i> (Linnaeus, 1758)	0	1	0	0	0	0	0	0	0	1
Σ specimens per sampling site	2	6	5	6	0	0	30	29	37	41

Table 2 – Number of individuals recorded for each species of litter weevils in the sampling sites of the study areas; species are listed in alphabetical order. N = close-to-natural habitat; A = altered habitat; Σ = total number of individuals per species collected in each sampling site.

Other taxa – A total of 461 specimens of other edaphic beetles were sampled, belonging to 6 families, 2 subfamilies and their larvae (Table 3). Larvae of Coleoptera were the most abundant group with 153 specimens; they were mainly Staphylinidae larvae and were considered as belonging to a single group. Other taxa were adults of Staphylinidae: Staphylininae with 88 individuals, Staphylinidae: Pselaphinae with 125 individuals and Carabidae (60 specimens). All four study areas had a similar number of taxa: 7 for Merlino, Stupinigi and Vaj, and 6 for Partecipanza. The area that counted the highest number of individuals was Merlino (150 specimens), compared to 130 specimens in Stupinigi, 97 specimens in Partecipanza and 84 specimens in Vaj.

Based on the results of the model selection (Table S1 in Supplementary Files), species richness and abundance of litter Curculionidae varies according to study area, both being significantly higher in Bosco del Vaj (p-value < 0.001).

For variations in Carabidae abundance, the model including study area, forest type and litter was found to be the best (Table 5). Stupinigi had significantly higher estimates

than the other sites, whereas that abundance was lower in altered forest and positively related to leaf litter.

In the variation in abundance of larvae (mainly of Staphylininae and Pselaphinae), the best model included the type of forest in a certain study area, i.e. the habitat that characterises a particular sampling site within the area (Tables S3, S4, S5 in Supplementary files). It is notable that, especially for Staphylininae and Pselaphinae, there were marked differences between close-to-natural and altered forest in Stupinigi and Vaj areas, which represented opposite situations.

Applying the IndVal method, five of the 10 litter Curculionidae species were significantly associated (p-value < 0.05) with at least one grouping (Tables 4 and 5). Only one species was associated with a single category: *Baromiamima concinna* for the Vaj area. Each of the remaining four species were associated with two groups: area and type of forest present. The only species associated with the close-to-natural forest was *Acalles echinatus*, for Bosco del Vaj (corresponding to the beech wood habitat). The

Taxon	Merlino		Partecipanza		Stupinigi		Vaj		Σ	
	N	A	N	A	N	A	N	A	N	A
Carabidae	12	6	0	8	9	13	6	6	27	33
Coccinellidae	0	0	0	0	0	1	0	1	0	2
Coleoptera larvae	18	63	6	20	26	3	4	13	54	99
Elateridae	1	3	1	1	1	0	3	12	6	16
Leiodidae: Cholevinae	2	1	0	1	2	0	3	1	7	3
Silphidae	1	0	0	0	0	0	0	0	1	0
Staphylinidae: Pselaphinae	10	11	7	21	43	1	4	28	64	61
Staphylinidae: Staphylininae	9	13	16	16	28	3	3	0	56	32
Σ specimens per study area	53	97	30	67	109	21	23	61	215	246

Table 3 – Number of individuals recorded for each taxon of edaphic beetles in the study areas; species are listed in alphabetical order. N = close-to-natural habitat; A = altered habitat; Σ = total number of individuals per taxon collected in each sampling site.

other two species associated with the same area (*Aparopion chevrolati* and *Kykliaocalles navieresi*) were instead linked to altered forest (chestnut wood habitat). The only species associated with Partecipanza (*Acalles lemur*) was also linked to altered forest. No species was found to be associated exclusively with close-to-natural or altered forest categories (i.e. the total of the two forest types in the four areas). In addition, the results of the IndVal analysis did not identify any species linked to Bosco del Merlino, either for the grouping of study areas or for the type of forest.

The average value of the distance of each point from the centroid in the dispersion analysis was not significant regarding the study areas (F value = 3.013, p-value = 0.06): the average value was 0.341 for Merlino, 0.369 for Partecipanza, 0.447 for Stupinigi and 0.262 for Vaj. For the two types of forest, the difference in the dispersion around the centroid was not significant (F value = 0.126, p-value = 0.71), with an average value of 0.447 for the close-to-natural forest and 0.433 for the altered forest.

The PERMANOVA analysis was first carried out taking into account the abundance data of the families and subfamilies divided by plots belonging to the different study areas (Fig. 3). In this case, significant differences were observed between the four areas (p-value < 0.01), and the value of R² was relatively high (R² = 0.299). This analysis was also applied to groupings of plots according to forest type to test the differences between close-to-natural and altered forest (Fig. 4). In this case, there were no significant values for the abundance data (p-value = 0.497). PERMANOVA was significant (p-value < 0.01) for the area-forest type interaction (Figs 3, 4).

Species	Forest-area	IndVal	p-value
<i>Acalles echinatus</i>	Close-to-natural forest of Vaj	0.500	0.022
<i>Acalles lemur</i>	Altered forest of Partecipanza	0.666	0.005
<i>Aparopion chevrolati</i>	Altered forest of Vaj	0.437	0.018
<i>Kykliaocalles navieresi</i>	Altered forest of Vaj	0.500	0.015

Table 4 – List in alphabetical order of the weevil species associated with the grouping of forest-area combinations, with IndVal and p-value indicated.

Species	Area	IndVal	p-value
<i>Acalles echinatus</i>	Vaj	0.400	0.025
<i>Acalles lemur</i>	Partecipanza	0.500	0.005
<i>Aparopion chevrolati</i>	Vaj	0.703	0.002
<i>Baromiamima concinna</i>	Vaj	0.500	0.006
<i>Kykliaocalles navieresi</i>	Vaj	0.612	0.003

Table 5 – List in alphabetical order of the weevil species associated with the grouping of the study areas, with IndVal and p-value indicated.

Discussion

1. Weevils richness and distribution

The weevil species that were sampled are typical of the forest litter (Fig. 5). Their preimaginal development occurs in dead wood and adults are found in the litter, often near decaying wood. The sampled species are relatively widespread in western Europe, even though *Acalles echinatus* and *A. micros* are quite scarce south of the alpine chain (Stüben 2014–2020). As previously indicated, faunistic studies on the forests of northern Italy are scarce and no data on the weevil species composition have ever been published. Based on personal observations by one of the authors (M.M.) in some areas of Piedmont, usually no more than 5-6 species are simultaneously present in a single site.

Influence of environmental variables

The communities of saproxylic weevils showed variations among the various forests that were mainly dependent on forest age and connectivity; the degree of naturalness did

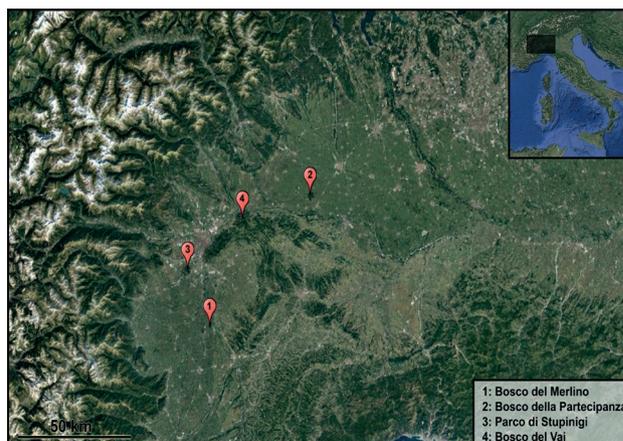


Fig. 1 – The collecting sites. Map data: Google Earth, Maxar Technologies, used according to Google Earth Terms of Service.



Fig. 2 – Map of Parco della Partecipanza, completely surrounded by agroecosystems. Map data: Google Earth, Maxar Technologies, used according to Google Earth Terms of Service.

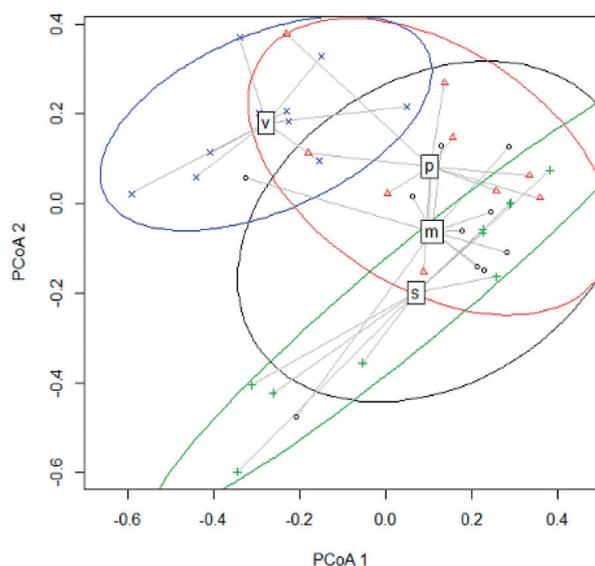


Fig. 3 – Scatter graph of study areas, relative to abundance data; × = sampling plots of Vaj (v), △ = sampling plots of Partecipanza (p), ○ = sampling plots of Merlino (m), + = sampling plots of Stupinigi (s).

not influence species richness and abundance, but apparently had an effect on some more specialized taxa, that were present in the close-to-natural patch, but not in the altered patch.

Age of the wooded tree formation

The age of the forests is undoubtedly a key-factor for the communities of saproxylic weevils. Our study confirms the observation of Cateau et al. (2018), who noted that species richness was related to the age of the forest formations.

Species richness (7 species) and number of specimens (59 specimens) were much higher in Bosco del Vaj, a glacial relict with beech formations, than in the other areas (Table 2). Conversely, no saproxylic weevils were found in Stupinigi forest which developed in historical times over a previous humid landscape, and has always been isolated from other wooded areas. Even though we sampled in patches of apparently good close-to-natural conditions, with old oaks and a large quantity of dead branches on the ground, the absence of saproxylic weevils indicates their difficulty in colonizing new habitats isolated from residual antique forests, even after centuries since forest establishment.

1.1. Connectivity

The connectivity of wooded tree formations also seemed to be of fundamental importance for the presence of weevil species (Buse 2012; Horák et al. 2013; Cateau et al. 2016, 2018). It has been reported that species of the genus *Acalles* depend on forest continuity (Buse 2012), but this can be applied to the generality of the saproxylic weevils, not only in temperate, but also in tropical areas (Anderson & Ashe 2000; Maioglio et al. 2022).

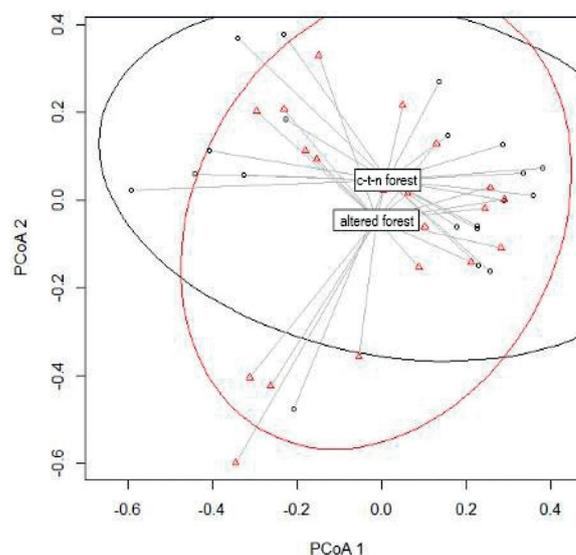


Fig. 4 – Scatter graph of forest types, relative to abundance data; △ = sampling plots of close-to-natural forest (c-t-n forest), ○ = sampling plots of altered forest.

The only study area among those examined to be part of a broader wooded area was Bosco del Vaj, that had good connectivity with a forested strip in the Turin hills, and was the richest habitat in terms of number of species and abundance of specimens.

1.2. Naturalness

We did not find differences between the close-to-natural and the altered patches in terms of abundance (37 vs 41 specimens), excepting in Stupinigi, where no saproxylic weevils were found. Five species were found in both habitats, whereas three were only found in the close-to-natural patches (*Acalles micros*, *Kyklioacalles aubei* and *Echinodera hypocrita*) (Table 2). These species appear therefore to be less tolerant to habitat alterations.

These results indicate that the landscape has a greater importance in determining the richness of species and their abundance compared to the type of forest or litter. The communities of Curculionidae do not seem to suffer to a great extent from habitat alteration, provided that sufficient resources in terms of dead tree branches are available, even though some more sensitive species may be fairly intolerant of alterations to their optimal microhabitat. Our results agree with the data reported by Cateau et al. (2018), who noted that the presence of dispersal-limited species appears to be more greatly influenced by the landscape than by the forest characteristics and confirms the observations by Horák et al. (2013), who reported that the saproxylic weevils are not irretrievably affected by the partial removal of dead wood and may reappear in altered forests when these reach an acceptable structure, of course provided that connectivity with more natural habitats is conserved.

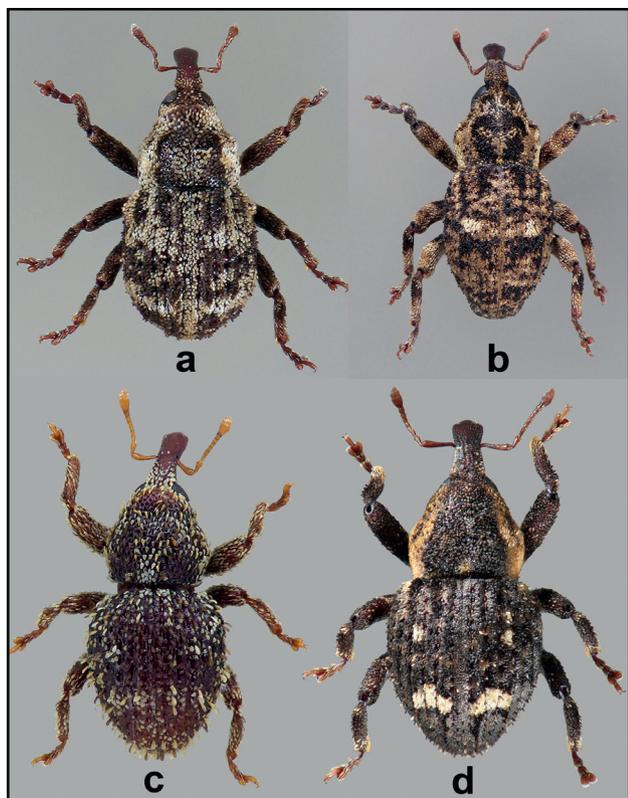


Fig. 5 – Some of the weevils collected in the research. **a**, *Kyklioacalles navieresi* (Boheman, 1837); **b**, *Kyklioacalles aubei* (Boheman, 1837); **c**, *Acalles echinatus* (Germar, 1824); **d**, *Echinodera hypocrita* (Boheman, 1837). From Stüben (2014–2020), used with permission.

1.3. Bioindicator species

All the saproxylic weevils that were sampled had rather low IndVal values, with an average of about 0.5. We decided to consider these species as indicative of the respective biocoenosis since these values are in agreement with the relatively low frequency of these species, that are often difficult to detect even with specialized research (Buse 2012; Horák et al. 2013; Cateau et al. 2016).

Four out of five IndVal species were found to be associated with the ancient relict forest of Bosco del Vaj (Tables 3 and 4); two of these species (*Aparopion chevrolati* and *Kyklioacalles navieresi*) were also associated with the altered forest present there (chestnut wood). *Aparopion chevrolati* was also found at Bosco del Merlino. This is quite a tolerant species that is widespread and not rare in broadleaved wooded areas of Piedmont, mainly in hills and the submontane region, provided that the habitat is not isolated and is not of relatively recent onset. *Acalles echinatus* was a close-to-natural forest indicator species for Vaj, the beech forest environment. This species is indeed known to be preferentially associated with beech wood (Stüben 2014–2020).

Although the IndVal analysis did not provide any results regarding *Echinodera hypocrita*, this species is potentially indicative of close-to-natural habitats. It was found

exclusively in the Vaj forest in both sampling periods and is positively associated with beech, as also indicated by Heijerman (2004), Horák et al. (2013), De Zan et al. (2014); Stüben (2019). *E. hypocrita* is also cited as a relict species in ancient woodland by Buse (2012). The presence of the other species in the chestnut wood supports the hypothesis that these weevils may also be present in altered environments, when these are not subject to a too intense modification and there is sufficient availability of dead branches.

Kyklioacalles navieresi was considered a species typical of ancient primary woodland by Buse (2012) in his study carried out in the forests of northwest Germany. However, we have found it in the altered forest sites of Partecipanza and Vaj, so it is identified by the IndVal analysis as an indicator species of altered forest. The same remarks apply to *Trachodes hispidus*, also considered by Buse (2012) as linked to ancient woodlands, but in our research it was collected only in the altered forest of Merlino, albeit only a single specimen. Such a difference between the Central European and the Italian forests may be determined by different autoecological aspects of the different populations, that have adapted to local environmental conditions during long historical periods. In particular, populations from the Central European forests are post-glacial re-colonizers that followed the post-glacial invasion of forests from refuge areas in south-eastern Europe, whereas, in northern Italy, these species may have also remained in local refuge areas during the last glacial. Moreover, climatic conditions to which the different populations of these species have adapted are relatively different between Central Europe and northern Italy. Their optimal habitat requirements may therefore be different. This confirms that a species is not an indicator of a specific habitat *per se*, but its autoecology must always be correlated with the biogeographical and ecological characteristics of the investigated area (Stüben & Bahr 2005; Müller et al. 2015; Eckelt et al. 2017).

2. Other taxa

2.1. Carabidae

Variations in Carabidae abundance in relation to various environmental parameters were associated with area, forest and litter (Table S2). It is indeed well known that the Carabidae community respond promptly to habitat variation (Assmann 1999; Desender et al. 1999; Rainio & Niemelä 2003; Sroka & Finch 2006).

2.2. Larvae, Staphylinidae: Staphylininae, Staphylinidae: Pselaphinae

Rove beetles are reconfirmed to be the numerically most represented edaphic beetles that inhabit the forest litter (Cano & Schuster 2009). Their abundance was higher in altered forest habitats, probably due to their higher heterogeneity. Environmental heterogeneity is considered one of the most important factors capable of governing abundance

and species richness, particularly for not strictly stenoeious taxa, such as some generalist predators (Tews et al. 2004; Vanbergen et al. 2007; Stein et al. 2014; Ortega et al. 2018).

The only exception is represented by Parco di Stupinigi, where an inverse situation was observed (Tables S1, S3-S5). This is probably due to the fact that the altered habitat of Stupinigi is a white pine plantation, a fully artificial environment. Therefore, an allochthonous component in an artificial environment (such as Stupinigi) has a heavy impact on its biocoenoses, whereas a marginal component in a more natural environment allows a structured edaphic community to be conserved. Moreover, the abundance of these predators in the Stupinigi areas, where no sprox-

ylic weevils were found, highlights the different type of information that can be obtained from these taxa that usually have a good mobility and therefore can quite easily colonize new forested habitats, also reaching them from non-forested agroecosystems in the surroundings. Therefore, the edaphic and sproxylic beetle components must be analysed independently.

2.3. Alien species

Lissorhoptrus oryzophilus Kuschel, 1952

In Bosco della Partecipanza, we recorded the rice water weevil *Lissorhoptrus oryzophilus*, native to North America and detected for the first time in Italy in 2004 (Caldara et al.

Models	Parameter coefficients	Curculionidae species richness					Curculionidae abundance				
		AIC	Estim.	Std. Err.	z	Pr(> z)	AIC	Estim.	Std. Err.	z	Pr(> z)
Model 1 (area)		82.009					105.272				
	Intercept		-0.693	0.447	-1.550	0.121		-0.223	0.353	-0.631	0.528
	Partecipanza		0.587	0.557	1.054	0.291		0.318	0.464	0.685	0.493
	Stupinigi		-18.609	2980.958	-0.006	0.995		-19.079	2980.958	-0.006	0.995
	Vaj		1.791	0.483	3.709	0.000***		2.014	0.376	5.353	0.000***
Model 2 (area + litter)		84.603					107.835				
Model 3 (area + forest)		84.631					107.780				
Model 4 (area + forest + litter)		87.272					110.524				
Model 5 (area*forest)		90.938					114.523				
Model 6 (area*forest + litter)		94.293					117.878				

Table 4 – Results of model selection and parameter coefficients for Curculionidae species richness and abundance, based on the lowest AIC value. AIC = Akaike Information Criterion; Estim. = estimate; Std. Err. = standard error; z = z value; Pr(>|z|) = significance; *** p < 0.001

Models	Parameter coefficients	Carabidae abundance				
		AIC	Estim.	Std. Err.	z	Pr(> z)
Model 1 (area)		135.625				
Model 2 (area + litter)		133.497				
Model 3 (area + forest)		138.180				
Model 4 (area + forest + litter)		128.319				
	Intercept		-2.782	1.086	-2.560	0.010*
	Partecipanza		-0.050	0.475	-0.106	0.915
	Stupinigi		0.755	0.347	2.177	0.029*
	Vaj		-0.405	0.372	-1.088	0.276
	Altered forest		1.243	0.494	2.512	0.011*
	Litter		0.361	0.110	3.262	0.001**
Model 5 (area*forest)		131.907				
Model 6 (area*forest + litter)		132.520				

Table 5 – Results of model selection and parameter coefficients for Carabidae abundance, based on the lowest AIC value. AIC = Akaike Information Criterion; Estim. = estimate; Std. Err. = standard error; z = z value; Pr(>|z|) = significance; * p < 0.05, ** p < 0.01

2004). This species is considered one of worst pests of rice (*Oryza sativa*) in the world and is now widespread in northern Italy, mainly in the rice-growing areas between Piedmont and Lombardy (Chersi et al. 2010; Lupi et al. 2010). *L. oryzoophilus* was found in both sampling sites of Partecipanza, in an extremely large number of specimens: 729 individuals were counted (and some more escaped from the funnels), most of which were found in the close-to-natural forest site, the alder-ash wood. Since the species is linked to the wet environments of rice fields (Lupi et al. 2015), the

high number of *L. oryzoophilus* in this site is probably due to the high and rather constant humidity of the substrate, that makes it suitable as an area for shelter and hibernation (Caldara et al. 2004; Chersi et al. 2010). Indeed, in the September sampling period, when the rice weevil is active, very few specimens were found. *L. oryzoophilus* is a polyphagous pest, which feeds on rice and other herbaceous plants present in the agroecosystem (Lupi et al. 2009), therefore food competition with native edaphic weevil species, associated to decaying wood, can be excluded. However, the scarcity

Models	Parameter coefficients	Staphylinidae larvae abundance				
		AIC	Estim.	Std. Err.	z	Pr(> z)
Model 1 (area)		196.240				
Model 2 (area + litter)		196.454				
Model 3 (area + forest)		196.775				
Model 4 (area + forest + litter)		199.106				
Model 5 (area*forest)		182.333				
	Intercept		1.280	0.284	4.505	0.000***
	Partecipanza		-1.098	0.522	-2.103	0.035*
	Stupinigi		0.367	0.380	0.967	0.333
	Vaj		-1.504	0.596	-2.520	0.011*
	Altered forest		1.252	0.349	3.586	0.000***
	Altered forest of Partecipanza		-0.048	0.623	-0.078	0.937
	Altered forest of Stupinigi		-3.412	0.737	-4.625	0.000***
	Altered forest of Vaj		-0.074	0.706	-0.105	0.916
Model 6 (area*forest + litter)		184.918				

Table 6 – Results of model selection and parameter coefficients for Staphylinidae larvae abundance, based on the lowest AIC value. AIC = Akaike Information Criterion; Estim. = estimate; Std. Err. = standard error; z = z value; Pr(>|z|) = significance; * p < 0.05, *** p < 0.001

Models	Parameter coefficients	Staphylinidae Staphylininae abundance				
		AIC	Estim.	Std. Err.	z	Pr(> z)
Model 1 (area)		155.641				
Model 2 (area + litter)		157.533				
Model 3 (area + forest)		156.295				
Model 4 (area + forest + litter)		159.235				
Model 5 (area*forest)		153.925				
	Intercept		0.470	0.423	1.109	0.267
	Partecipanza		0.693	0.544	1.273	0.203
	Stupinigi		1.216	0.520	2.336	0.019*
	Vaj		-0.980	0.753	-1.302	0.192
	Altered forest		0.559	0.552	1.013	0.311
	Altered forest of Partecipanza		-0.559	0.734	-0.762	0.446
	Altered forest of Stupinigi		-2.469	0.837	-2.948	0.003**
	Altered forest of Vaj		-19.351	4215.711	-0.005	0.996
Model 6 (area*forest + litter)		156.478				

Table 7 – Results of model selection and parameter coefficients for Staphylinidae Staphylininae abundance, based on the lowest AIC value. AIC = Akaike Information Criterion; Estim. = estimate; Std. Err. = standard error; z = z value; Pr(>|z|) = significance; * p < 0.05, ** p < 0.01

of native weevils found at Partecipanza, in a patch of apparently good habitat conditions, could be caused by spatial competition with specimens of *L. oryzophilus*.

Conclusions

Most of the research concerning the possible use of saproxylic weevils as indicators of environmental quality has been carried out in tropical regions, where it is often possible to make a clear distinction between primary and secondary forests (Anderson & Ashe 2000; Cano & Schuster 2009; Erwin & Geraci 2009; Maioglio et al. 2022).

In Europe, and particularly in the densely populated Po Valley, this distinction is more complicated, and indeed true primary forests are no longer present. All study areas have woodlands that have been continuously managed (Sindaco et al. 2008; Camerano et al. 2010). These anthropic changes, although nowadays reduced compared to the past, involve environmental alteration. The litter-associated saproxylic Curculionidae, together with the edaphic predator Coleoptera, enable an assessment of the degree of naturalness of woodlands remnants surrounded by strongly anthropic areas. Our study confirmed that connectivity between isolated patches of relict forest seems to be essential to maintain a high abundance in terms of species and specimens of saproxylic fauna. Moreover, the permanence of dead branches and fallen trees on the ground, in the best conserved patches as well as in those that were partially altered, is essential to the permanence of this fauna, not only to allow the presence of wood for larval development, that can also be completed on dead branches on the trees, but also because this habitat provides better microhabitat condition, with more stable

humidity conditions and a broader availability of food resources. These conditions also facilitate the slow movement of the beetles on the soil from the better conserved patches towards those partially altered, helping to improve the quality of the altered habitats connected with close-to-natural patches.

This study recorded the absence of saproxylic weevil species in a relatively recently established forested area, that had never been connected to any ancient forest. In these cases, the management of the forest could also take into account the possibility of introducing some taxa from other nearby forested areas, chosen among those that are more typical of that coenosis, such as, for Stupinigi, *Ap-aropion chevrolati* and *Kyklioacalles navieresi*, in order to improve the ecosystems integrity of these habitats and their biodiversity.

Our survey indicates that saproxylic weevils and predator edaphic beetles may give different information regarding the environmental quality of the woodlands, therefore the choice of the taxon to be sampled must be evaluated according to the object of the study.

Furthermore, some saproxylic weevils can be considered as good bioindicators, but information given by a certain species may vary depending on the geographical region where the ecosystem is located, as seen in our case for *Kyklioacalles navieresi* and *Trachodes hispidus*. Even more stenoeccious taxa can, in the long term, adapt to modifications of the ecosystems, therefore historical biogeographical and climatological aspects should always be taken into account when evaluating the information that a particular species can give for a particular habitat.

More complete research including more relict woodlands and surrounding areas will further clarify the overall

Models	Parameter coefficients	Staphylinidae Pselaphinae abundance				
		AIC	Estim.	Std. Err.	z	Pr(> z)
Model 1 (area)		191.898				
Model 2 (area + litter)		192.788				
Model 3 (area + forest)		194.504				
Model 4 (area + forest + litter)		195.259				
Model 5 (area*forest)		161.871				
	Intercept		0.693	0.322	2.149	0.031*
	Partecipanza		-0.356	0.500	-0.712	0.476
	Stupinigi		1.458	0.362	4.025	0.000***
	Vaj		-0.916	0.598	-1.531	0.125
	Altered forest		0.095	0.446	0.214	0.830
	Altered forest of Partecipanza		1.003	0.630	1.591	0.111
	Altered forest of Stupinigi		-3.856	1.109	-3.477	0.000***
	Altered forest of Vaj		1.850	0.701	2.636	0.008**
Model 6 (area*forest + litter)		165.114				

Table 8 – Results of model selection and parameter coefficients for Staphylinidae Pselaphinae abundance, based on the lowest AIC value. AIC = Akaike Information Criterion; Estim. = estimate; Std. Err. = standard error; z = z value; Pr(>|z|) = significance; * p < 0.05, ** p < 0.01, *** p < 0.001

picture, allowing the most significant sites to be recognized, that are currently not always identifiable from a simple visual approach of the tree component, and to more precisely identify IndVal species that can be used as bioindicators. This, in turn, will allow the optimization of strategies management for the conservation of relict forest habitats in otherwise strongly altered environments in the temperate region.

Acknowledgments – We are grateful to the personnel of the study areas for their assistance during the field work: Sandro Ferregutti from Parco di Stupinigi, Ivano Fossarello and Paola Palazzolo from Bosco delle Sorti della Partecipanza di Trino. We also thank Luca D’Angelo, Andrea Samorè and Roberto Sindaco for indications on the research material and Alberto Selvaggi, Consolata Siniscalco, Alfredo Vizzini for help with the botanical aspects. Special thanks to Oscar Maioglio, for sharing his expertise and frequent suggestions. Dan Chamberlain (Dept. Life Sciences, University of Turin) kindly checked the English text.

Contributions to the paper: M.M. and C.C.: general structuring of the research; P.P. and M.M.: field sampling; P.P. and C.C.: statistical analyses; P.P., C.C., M.M.: preparation of the paper.

The authors declare no conflicts of interest

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