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Survey of saproxylic beetle assemblages at different forest plots in central Italy

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Abstract

Saproxylic beetles from coarse deadwood debris found on the forest floor were documented for the first time at four permanent monitoring plots in central Italy that are part of the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests). The plots consisted of unmanaged vegetation communities representing typical beech forest, mixed broadleaf and conifer forest, Turkey oak forest, and cork oak forest respectively. With the present study, we identified beetle assemblages to species level and investigated whether the type of vegetation affects beetle communities. In order to detect more of the species present and perform a better comparison among study sites, samples were collected with two types of traps: flight interception traps hanging from tree branches (n = 1 per plot) and emergence traps mounted on deadwood like fallen branches or trunks (n = up to 8 per plot, depending on the availability of deadwood pieces). A total of 1372 individual beetles, belonging to 133 species of 36 families were captured, identified and enumerated. Considering all beetles caught in both trap types, alpha-diversity values indicated high beetle diversity at all of the four forest sites, while measured species richness, accumulation curves and species richness estimators agreed that the highest species density was at Rosello. Monte Rufeno had the highest abundance of beetle individuals. Monte Rufeno and Monte Circeo had the highest numbers of saproxylic species, even though Rosello had the highest total number of beetle species. Ninety species (67.7% of the species found in all plots combined) were caught in only one plot, while only three species, representing 2.3% of the total of species, were collected at all of the four plots; nine saproxylic species were collected exclusively at Monte Circeo, among them rare singletons like *Agrilus convexicollis mancini* Obenberger (Buprestidae) and *Nematodes filum* (F.) (Melasidae), the latter recorded in central Italy for the first time. Environmental variables having the strongest correlations with the assemblage composition were plot-scale variables (slope, stand age, amount of deadwood). The only trap-scale variable that showed up as related to assemblage composition was wood decay-class. The study highlighted that the diversity in saproxylic beetle communities reflects the different tree communities at the four study plots. The research also showed that even at the small and very small scale of forest plots, traits of beetle assemblages can be revealed on coarse deadwood debris.

Key words: biodiversity, Coleoptera, deadwood, emergence traps, ICP Forests.

Introduction

Saproxylic organisms depend, at some stage of their life cycle, upon deadwood of old trees or fallen timber, or upon other saproxylics (Speight, 1989; Mason *et al.*, 2003). Several authors have further analyzed the microhabitat requirements and feeding ecology of saproxylic beetles (Bouget *et al.*, 2005; Alexander, 2008; Brin *et al.*, 2013). The relevance of deadwood as suitable breeding substrate for several organisms has thus been widely recognized during the last decades (Speight, 1989; Mason *et al.*, 2003; Jabin *et al.*, 2004; Castagneri *et al.*, 2010), but consequences of modern exploitation of forests have indeed given rise to increasing concern regarding current availability of suitable environmental conditions for several animal groups and among them for saproxylic beetles (Similä *et al.*, 2003; Alinvi *et al.*, 2007; Hjältén *et al.*, 2007; Vodka *et al.*, 2009). In northern boreal countries, modern intensive forest management, including short rotation periods and clear-cutting, have created mono-specific, even-aged stands (Johansson *et al.*, 2007; Gibb *et al.*, 2006), consequently reducing the availability of deadwood for saproxylic organisms. In central and southern Europe, exploitation of forests has followed different historical course. In the Mediterranean basin, woods have been overexploited by man since prehistoric ages (Castagneri *et al.*, 2010) and through Roman and Byzantine times, resulting in degraded forms of woodlands and widespread regions cleared of mature woody vegetation (Cappelli, 2000).

Disturbances such as grazing, fire management and agricultural techniques have influenced the external shape and type of these woodlands, though the abandonment of grazing during the 20th century (Franc and Götmark, 2008) and the decline of extensive agriculture in the late 1970s produced a new propagation of trees (De Natale and Gasparini, 2011).

Decreased economic interest in harvesting woody debris for energy production but also the increased number of unmanaged stands devoted to protection, research or monitoring purposes can lead to the accumulation of deadwood materials on the forest floor, which may be important for the conservation of saproxylic beetles (Travaglini *et al*., 2006; Jonsell and Hansson, 2007; Jonsell, 2008), even at the very small scale of single deadwood pieces (Johansson *et al*., 2007; Jonsell and Hansson, 2007; Sirami *et al*., 2008).

Saproxylic beetles are subject to a growing interest in research on forests detritus-based food chain, as they are clearly involved in soil fertility and productivity (Jabin *et al*., 2004), decomposition, nutrient cycling (Dollin *et al*., 2008) and carbon storage functions (Castagneri *et al*., 2010). Several studies from northern Europe (e.g. Økland, 1996; Grove, 2002; Gibb *et al*., 2006; Alinvi *et al*., 2007; Franc *et al*., 2007; Johansson *et al*., 2007; Victorsson and Jonsell, 2013) have recently investigated the interactions between forest structure, stand age, availability and quality of deadwood and diversity of forest dwelling and saproxylic beetles at different scales. On the contrary, studies in central and southern Europe are relatively few (Kappes and Topp, 2004; Sirami *et al*., 2008; Lamperiere and Marage, 2010; Buse *et al*., 2010; Brin *et al*., 2011; Bouget *et al*., 2011; Lassauce *et al*., 2011; Russo *et al*., 2011; Lassauce *et al*., 2013; Redolfi De Zan *et al*., 2014).

For the present study, we considered monitoring plots belonging to the ICP Forests programme (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests) (Lorenz, 1995; Ferretti *et al*., 2006). The ICP Forests programme was launched in 1985 under the Convention on Long-range Transboundary Air Pollution of the United Nations Economic Commission for Europe (UNECE). It is based on a network of permanent sites which were installed across European forests, into the most representative forest ecosystems in each region. Forest conditions have been monitored at these sites using a set of selected parameters (including environmental variables) and protocols agreed at international level (ICP Forests, 2010). The programme was instituted in Italy in 1995 and it is still in progress. Sampling of invertebrates and lists of insect species have been produced at several ICP Forests plots in Italy (Mason *et al*., 2006). We selected four ICP forest plots in central Italy to characterize their saproxylic beetle faunas at the selected plots and to study the relationship between saproxylic beetle communities and tree communities. We addressed specifically the following questions:

1- what is the beetle diversity at the four considered study plots? How dissimilar is beetle community composition at these plots?
2- are diversity and typology of the tree communities reflected by correspondingly different saproxylic beetle faunas inhabiting each forest plot?
3- what environmental factors can affect saproxylic beetle diversity and community composition at small and very small scale (plot and single deadwood piece scale)?

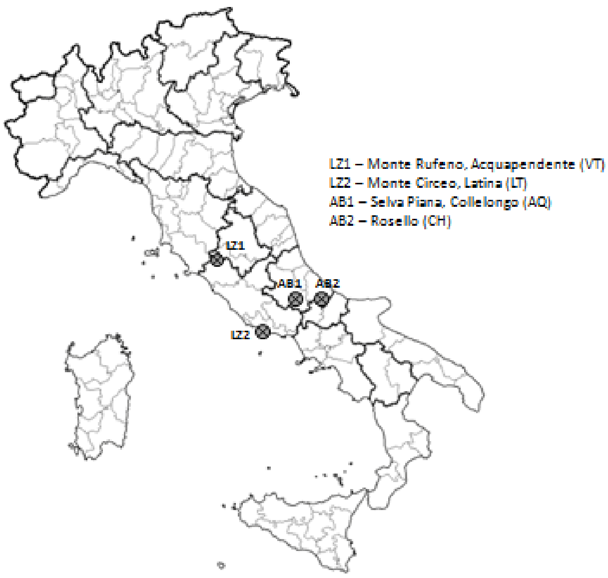


Figure 1. Map showing the location of study areas.

Materials and methods

Study areas

The four ICP Forests permanent plots chosen for the present study are representative of four different forest ecosystems in central Italy, three on the Apennines Range of Abruzzo (AB1, AB2) and Lazio Regions (LZ1) and one near the Mediterranean coast in Lazio Region (LZ2) (figure 1). Selva Piana (Collelongo-L'Aquila, 41°50'58.30"N 13°35'21.8"E; AB1) is a beech (*Fagus sylvatica* L.) high stand (EUNIS code G1.68), about 125 years old, located at 1550 m asl. Rosello (Rosello-Chieti, 41°53'1.96"N 14°21'11.48"E; AB2) is a high stand (about 95 years old) situated at 960 m asl, including *Carpinus betulus* L., *Acer campestre* L., *Tilia platyphyllos* Scop. with a significant presence of *Abies alba* Mill (EUNIS code G1.A). Monte Rufeno (Acquapendente-Viterbo, 42°49'25.07"N 11°54'6.21"E; LZ1), 690 m asl, is a thermophilous deciduous forest (EUNIS code G1.7), which has been cut intensively until as recently as 1970 and is now unmanaged. The dominant tree species is *Quercus cerris* L. Monte Circeo - Peretto (San Felice Circeo-Latina, 41°14'9.10"N 13°4'47.95"E; LZ2) is a Mediterranean evergreen oak woodland with *Quercus ilex* L., *Quercus suber* L. and *Arbutus unedo* L. (EUNIS code G2.1), located at 190 m asl on the northern slope of a promontory looking over the central Tyrrhenian Sea. It is a coppice wood subject to cut and grazing until 1950, then left unmanaged (EEA, 2007). Two of the sites in our study (Monte Rufeno and Selva Piana) are also included in the Italian Long Term Ecological Research Network (LTER Italy; Cocciufa *et al*., 2011). A permanent ICP Forests plot is made up by two North-oriented close 2500 m^2 areas: one established to carry out data collection and fenced, the other one chosen as control area and not fenced (the latter being used only to gather data on vegetation according to the ICP Forests protocol). The whole monitoring plot is located within a forest area, as large as 100,000 m^2 as a minimum, represented by the same type of ecosystem; it means that the area around the plot is supposed to be ecologically homogeneous (Allavena *et al*., 2000). For the present study, beetles were only sampled inside the fenced 50 × 50 m area of each monitoring plot.

Table 1. ICP Forests data set available at the four considered study plots.

	Topography	Tree growth	Tree Phenology	Soil solution chemistry	Leaves chemistry	Ground vegetation	Atmospheric deposition chemistry	Meteorology	Crown conditions	Ozone damage	Deadwood
AB1	×*	×*	×	×	×	×	×	×*	×	×	×*
AB2	×*	×*	×	-	×	×	×	×*	×	×	×*
LZ1	×*	×*	×	×	×	×	×	×*	×	×	×*
LZ2	×*	×*	×	-	×	×	×	×*	×	×	×*

* Sources of environmental data at plot scale used for the present research.

Table 2. Environmental variables at plot scale and at trap scale for the investigation of correlations between beetle assemblages and habitat and micro-habitat characteristics.

Plot scale			Trap scale		
Variable	Unit	Explanation	Variable	Unit	Explanation
Elevation	m asl	Elevation of the plot	Species	-	Tree species
Exposure	N,E,S,W	Main exposure of the plot	Type	-	Type of deadwood (log or branch)
Slope	(°)	Inclination of the plot	Decay class	Class 1-5	Decay stage of deadwood (Hunter, 1991)
Precipitation	mm/year	Mean daily precipitation	Volume	m^3	Volume enclosed in Et
Temperature	°C year	Mean daily temperature	Ants	1/0	Presence/absence of ants inside the Et
Stand age	years	Age of the stand	Canopy closure	%	Percentage of sky covered by canopies above Et
Basal area	m^2	Stand basal area in the plot	Sun exposure	1/0	Exposure of Et during at least one sampling
Basal area increment	m^2	Increment of stand basal area (2005-09)	Basal area	m^2	Basal area around each Et (three trees)
Deadwood	m^3/ha	Amount of deadwood within the plot			

Sampling design

Two types of insect traps were used: window interception traps and emergence traps (WT and ET respectively in this paper). WT were made following Mason *et al.,* 2006. WT are suitable to sample flying forest beetles, which are intercepted by the hanging panels (Økland, 1996; Ranius and Jansson, 2002; Mason *et al*., 2006); they are also efficient in catching rare beetles in forests and show less variability than other sampling methods (Martikainen and Kouki, 2003; Hardersen *et al*., 2012). ET were made according to Alinvi *et al*., 2007. The size of the fabric cloth was 50 × 70 cm; the collector bottle was located beneath the trap, near the ground. Boring insects emerging from deadwood are not able to escape from the closed envelope and fall into the collector bottle, attracted by daylight (Owen, 1989; 1992; Økland, 1996; Wikars *et al*., 2005). In both types of traps, collector bottles were filled with 70% ethanol. One WT was located in the middle of each study plot, hanging from a tree branch, 1.50 m above ground. At each study plot, nine random points were selected using the random number generator in Excel (as random coordinates inside the 50 × 50 m plot). Potential deadwood pieces for emergence trapping were those in a circular area with a 10 m radius, centered at each random point. The criteria adopted to select deadwood were the following: 1) only logs were taken into consideration (no stumps or snags); 2) deadwood pieces of the same tree species and decay stage in each trap; 3) logs with diameter > 10 cm were primarily selected (one log per trap); 4) where logs > 10 cm were not available, logs with smaller diameters were also accepted, but at least three branches were included in the trap. Because suitable deadwood pieces were not always present at random points, number of emergence traps varied at each study plot (6 emergence traps at Selva Piana, 8 at Rosello and Monte Rufeno, 5 at Monte Circeo). Locations of traps were georeferenced. Traps were set in the field in spring and summer 2010, from early May until the end of August. Samples were collected every fifteen days.

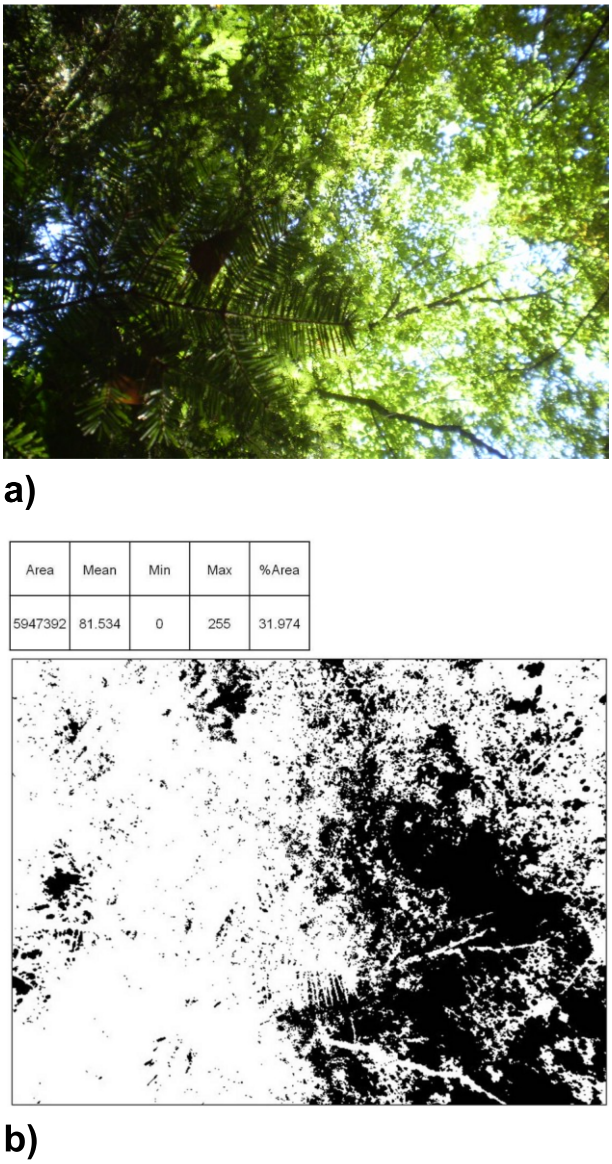
Environmental variables

Environmental variables at plot scale were extracted from the ICP Forests long term monitoring dataset while those at single trap scale were recorded as new data for the present study (tables 1 and 2). Percentage of canopy closure was measured by digital pictures of the canopy taken from the ground above each ET. Photographs were analyzed with the program ImageJ, (two pictures for each trap, one at the beginning and one at the end of the field campaign) (figure 2). Basal area of trees around each trap was calculated from circumferences of three trees shading the trap. Volume of deadwood inside emergence traps was calculated by the Huber formula ($V = \pi/4d^2*l$).

Statistical analysis

Diversity of beetle assemblages was investigated by Alpha-diversity indices (Shannon-Wiener, Simpson and Evenness) and rarefaction curves. Alpha-diversity indices were also measured for the tree community at each study plot. Diversity indices were calculated with PAST (Hammer *et al.*, 2001). Sample-based rarefaction curves (Mao's τ) were calculated with the software EstimateS (version 8.2.0, Colwell 2006) and displayed using Excel.

To estimate the total species number at each plot, estimators of total species richness based on different algorithms (Chao2, first and second order Jackknife and Bootstrap) were calculated with PAST. Spearman's rank correlation coefficient was used to detect relationships between indices measured for beetle assemblages and tree communities at each study plot. Multivariate dissimilarities of beetle assemblages were calculated by the Bray-Curtis Index. Because we assumed there were differences in trapping efficiencies of WTs and ETs and there were more ETs than WTs, we used presence-absence data to examine the beetle species dissimilarities in the four plots and in the different trap-types within these plots. Using only the ET data, non-metric multidimensional scaling (NMDS) ordination with log (x + 1) species abundance data and Bray-Curtis dissimilarity was used to illustrate the dissimilarities of beetle assemblages in traps and plots and to look for correlations between beetle assemblage composition in ETs and environmental variables. The ordination and Bray-Curtis index calculations were performed in PC-ORD (version 5.19, McCune and Mefford, 2006). NMDS was chosen for the ordination because it is one of the best methods for exploring biological data that rarely meet assumptions required for many other types of ordinations (McCune and Grace, 2002). A 3-dimensional NMDS solution was recommended using the medium auto-pilot setting in PC-ORD (version 5.19, McCune and Mefford, 2006); the final stress was 14.2 with about equal amounts of variation explained by each of the 3 axes (R^2: axis 1 = 0.27, axis 2 = 0.23, axis 3 = 0.27).



Area	Mean	Min	Max	%Area
5947392	81.534	0	255	31.974

Figure 2. Example of crown digital picture (a; AB2, trapE6, final picture) above emergence trap and processing by the Software ImageJ (b) for the calculation of canopy closure.

Results

We collected a total of 1372 individuals, belonging to 133 species of 36 families (table 3). The highest numbers at all taxonomic levels (families, genera and species) were sampled at Rosello, the only site where ET were able to catch more species and individuals than WT (figure 3). Monte Rufeno showed the highest total abundance (405 individuals). Alpha diversity indices of tree communities at the four study areas revealed remarkable differences among plots, reflecting the actual difference in tree species composition and abundance: Selva Piana and Monte Rufeno scored a Simpson and Shannon Index equal to 0, with only one tree species present at each site, beech and Turkey oak, respectively; Monte Circeo had a Simpson Index = 0.69 and a Shannon Index = 1.35, while Rosello had higher values (Simpson Index = 0.75, Shannon Index = 1.74). Surprisingly, values of the same metrics for beetle assemblages were particularly high at all of the four study plots (table 4). Measures of evenness of beetle assemblages were similar at Rosello and Monte Circeo, but differed between Selva Piana and Monte Rufeno (table 4). No significant correlation was detected between indices measured for tree layers and beetle assemblages at each study plot (table 4). The number of beetle species collected at each plot varied as follows: 34 species at Selva Piana, 64 at Rosello, 50 at Monte Rufeno and 41 at Monte Circeo. Observed sample-based rarefaction curves showed a positive slope at the maximum number of samples (figure 4). Selva Piana, Monte Rufeno and

Table 3. List of beetle families and species collected by interception traps and emergence traps at Italian ICP Forests plots: SelvaPiana (AB1), Rosello (AB2), Monte Rufeno (LZ1), Monte Circeo (LZ2).

Family	Species	AB1	AB2	LZ1	LZ2	Tot
		No. of individuals				
Anthribidae	*Choragus sheppardi* Kirby 1819	0	0	0	2	2
	Rhaphitropis oxyacanthae (Brisout 1863)	0	0	2	0	2
Biphyllidae	*Diplocoelus fagi* Guerin-Meneville 1844	7	59	18	71	155
Buprestidae	*Agrilus convexicollis mancinii* Obenberger 1927	0	0	0	1	1
Carabidae	*Calathus montivagus* Dejean 1831	0	2	0	0	2
Cerambycidae	*Arhopalus syriacus* (Reitter 1895)	0	0	0	3	3
	Callimus abdominalis (Olivier 1795)	0	0	0	2	2
	Leptura aurulenta F. 1792	0	0	1	0	1
	Mesosa nebulosa (F. 1781)	0	0	1	0	1
	Nathrius brevipennis (Mulsant 1839)	0	0	0	1	1
	Obrium brunneum (F. 1792)	0	1	0	0	1
	Prionus coriarius (L. 1758)	0	0	1	0	1
	Pseudosphegesthes cinerea (Castelnau et Gory 1836)	0	0	1	1	2
	Xylotrechus arvicola (Olivier 1795)	0	0	1	0	1
Cetoniidae	*Cetonia aurata* (L. 1761)	0	0	1	0	1
Ciidae	*Cis* (*Orthocis*) *pygmaeus* (Marsham 1802)	0	0	21	0	21
	Cis quadridentulus Perris in Abeille 2011)	0	0	0	3	3
	Cis sp.	0	0	0	2	2
	Ennearthron palmi Lohse 1966	0	0	1	0	1
Cryptophagidae	*Cryptophagus cylindrellus* Johnson 2007	0	7	0	0	7
	Cryptophagus dentatus (Herbst 1793)	1	1	0	0	2
	Cryptophagus punctipennis C. Brisout de Barneville 1863	0	1	0	0	1
	Cryptophagus reflexus Rey 1982	0	2	0	0	2
	Cryptophagus scanicus (L. 1758)	6	4	0	0	10
Cucujidae	*Pediacus dermestoides* (F. 1792)	0	2	0	0	2
Curculionidae	*Acalles camelus* (F. 1792)	0	1	0	0	1
	Acalles lemur cisalpinus Stuben 2003	1	1	0	0	2
	Acallocrates minutesquamosus (Reiche 1860)	0	0	0	1	1
	Aparopion chevrolati (Jacquelin du Val 1854)	0	5	0	0	5
	Brachysomus hirtus (Boheman 1845)	0	1	0	0	1
	Echinodera aspromontensis Stuben 2008	0	2	0	0	2
	Echinodera hypocrita (Boheman 1837)	0	9	0	0	9
	Gasterocercus depressirostris (F. 1792)	0	0	4	0	4
	Mecinus pascuorum (Gyllenhal 1813)	0	1	0	0	1
	Orchestes fagi (L. 1758)	5	0	0	0	5
	Orchestes pilosus (F. 1781)	0	1	14	0	15
	Orchestes quercus(L. 1758)	0	0	23	0	23
	Otiorhynchus duinensis Germar 1824	0	2	0	0	2
	Otiorhynchus pseudoligneoides Magnano 1996	0	4	0	0	4
	Phyllobius argentatus (L. 1758)	1	0	0	0	1
	Phyllobius etruscus Desbrochers 1873	0	2	4	0	6
	Phyllobius oblongus (L. 1758)	0	2	0	0	2
	Phyllobius romanus Faust 1890	0	1	0	0	1
	Polydrusus cervinus (L. 1758)	0	2	1	0	3
	Polydrusus elegantulus (Boheman 1840)	0	0	0	8	8
	Polydrusus frater Rottenberg 1871	0	0	2	1	3
Dasyceridae	*Dasycerus sulcatus* Brongniart 1800	0	3	0	0	3
Drilidae	*Drilus flavescens* Olivier 1790	0	1	0	0	1
Dryophthoridae	*Dryophthorus corticalis* (Paykull 1792)	0	2	0	0	2
Elateridae	*Agriotes infuscatus* Desbrochers des Loges 1870	0	1	0	0	1
	Ampedus pomorum (Herbst 1784)	0	1	0	0	1
	Ampedus quercicola (Buysson 1887)	0	0	1	0	1
	Athous (Haplathous) subfuscus (O.F. Muller 1764)	3	0	0	0	3
	Athous limoniiformis sensu stricto Candeze 1865	0	0	2	0	2
	Athous vittatus sensu stricto (Gmelin 1790)	1	1	0	0	2
	Dalopius marginatus (L. 1758)	2	0	0	0	2

(Continued)

(Table 3 continued)

Family	Species	AB1	AB2	LZ1	LZ2	Tot
		No. of individuals				
Elateridae	*Harminius spiniger* (Candeze 1860)	0	0	0	2	2
	Nothodes parvulus (Panzer 1799)	0	5	2	0	7
Geotrupidae	*Anoplotrupes stercorosus* (Scriba 1791)	0	0	6	0	6
Laemophloeidae	*Cryptolestes duplicatus* (Waltl 1834)	0	0	7	2	9
	Cryptolestes ferrugineus (Stephens 1831)	0	0	3	1	4
	Laemophloeus nigricollis Lucas 1849	0	0	1	1	2
	Placonotus testaceus (F. 1787)	0	1	1	0	2
Languridae	*Cryptophilus integer* (Heer 1841)	0	0	0	1	1
Latridiidae	*Cartodere* (*Aridius*) *nodifer* (Westwood 1839)	8	1	0	1	10
	Corticarina similata (Gyllenhal 1827)	1	0	0	0	1
	Cortinicara gibbosa (Herbst 1793)	0	2	0	0	2
	Dienerella clathrata (Mannerheim 1844)	0	1	8	0	9
	Dienerella vincenti Johnson 2007	1	5	1	0	7
	Enicmus atriceps Hansen 1962	1	0	0	0	1
	Enicmus brevicornis (Mannerheim 1844)	22	2	19	0	43
	Enicmus rugosus (Herbst 1793)	0	12	5	3	20
	Enicmus testaceus (Stephens 1830)	2	1	0	0	3
	Enicmus vincenti (Johnson 2007)	2	0	0	0	2
	Latridius consimilis (Mannerheim 1844)	3	0	0	0	3
	Latridius minutus (L. 1767)	5	0	0	0	5
Melandryidae	*Abdera biflexuosa* (Curtis 1829)	0	0	1	0	1
	Abdera quadrifasciata (Curtis 1829)	0	0	19	1	20
	Phloiotrya vaudoueri (Mulsant 1856)	0	0	8	1	9
	Serropalpus barbatus (Schaller 1783)	0	11	0	0	11
Melasidae	*Hylis simonae* (Olexa 1970)	0	9	0	1	10
	Nematodes filum (F. 1801)	0	0	0	27	27
Mycetophagidae	*Litargus connexus* (Geoffroy 1785)	2	14	139	11	166
	Mycetophagus atomarius (F. 1787)	2	0	0	0	2
	Mycetophagus quadripustulatus (L. 1761)	1	0	0	0	1
	Typhaea stercorea (L. 1758)	0	0	0	4	4
Nitidulidae	*Epuraea fuscicollis* (Stephens 1835)	0	0	0	2	2
	Epuraea guttata (Olivier 1811)	0	1	0	0	1
	Epuraea marseuli Reitter 1872	0	1	0	0	1
	Epuraea ocularis (Fairmaire 1849)	0	0	0	16	16
	Epuraea silacea (Herbst 1784)	18	0	0	0	18
	Epuraea unicolor (Olivier 1790)	0	1	0	0	1
	Omosita discoidea (F. 1775)	0	1	0	0	1
Omalisidae	*Omalisus* sp.	1	1	0	0	2
Platypodidae	*Platypus cylindrus* (F. 1792)	0	0	0	1	1
Ptiliidae	*Acrotrichis intermedia* (Gillmeister 1845)	0	1	0	0	1
	Pteryx suturalis (Heer 1841)	0	1	0	0	1
	Ptiliolum fuscum (Erichson 1845)	1	0	0	0	1
	Ptiliolum schwarzi (Flach 1887)	4	0	0	0	4
	Ptinella aptera (Guerin-Meneville 1839)	0	0	1	5	6
	Ptinella denticollis (Fairmaire 1857)	0	0	12	0	12
Ptinidae	*Ptinus corsicus* Kiesenwetter 1877	0	2	1	0	3
	Ptinus lichenum Marsham 1802	0	0	0	2	2
	Ptinus sexpunctatus Panzer 1789	0	0	1	1	2
Rhizophagidae	*Monotoma longicollis* (Gyllenhal 1827)	0	0	0	1	1
	Rhizophagus fenestralis (L. 1758)	1	12	0	0	13
	Rhizophagus parallelocollis Gyllenhal 1827	0	1	0	0	1
Salpingidae	*Lissodema denticolle* (Gyllenhall 1813)	0	1	0	4	5
	Salpingus planirostris (F. 1787)	8	44	11	0	63
	Salpingus ruficollis (L. 1761)	1	0	0	0	1
	Vincezellus viridipennis (Panzer 1794)	0	1	0	0	1
Scaphidiidae	*Scaphidium quadrimaculatum* Olivier 1790	0	1	0	0	1
Scarabaeidae	*Onthophagus verticicornis* (Laicharting 1781)	0	0	4	0	4
	Pachypus candidae (Petagna 1786)	0	0	0	1	1

(Continued)

(Table 3 continued)

Family	Species	AB1	AB2	LZ1	LZ2	Tot
		No. of individuals				
Scarabaeidae	*Sisyphus schaefferi* (L. 1758)	0	0	2	0	2
Scolytidae	*Crypturgus mediterraneus* Eichhoff 1871	0	0	0	1	1
	Dryocoetes villosus minor Eggers 1903	0	0	1	0	1
	Ernoporicus fagi (F. 1798)	3	2	0	0	5
	Hylesinus toranio (Danthoine 1788)	0	0	0	121	121
	Scolytus rugulosus (Muller 1818)	0	0	2	0	2
	Trypodendron domesticum (L. 1758)	18	0	0	0	18
	Xyleborinus saxesenii (Ratzeburg 1837)	7	0	5	7	19
	Xyleborus dispar (F. 1792)	58	108	3	9	178
	Xyleborus monographus (F. 1792)	0	1	14	24	39
Scraptiidae	*Anaspis (Anaspis) lurida* Stephens 1832	0	0	5	31	36
	Anaspis (Nassipa) flava (L. 1758)	0	1	3	0	4
	Scraptia ferruginea Kiesenwetter 1861	0	0	8	0	8
Silvanidae	*Silvanus bidentatus* (F. 1792)	0	1	0	0	1
Tenebrionidae	*Enoplopus dentipes* (Rossi 1790)	0	4	11	0	15
Trogositidae	*Nemozoma elongatum* (L. 1761)	2	0	1	0	3
Zopheridae	*Corticus celtis* Germar 1824	0	11	0	1	12
	Coxelus pictus (Sturm 1807)	1	2	0	0	3
	Synchita undata Guerin-Meneville 1844	0	3	1	0	4
Total		200	388	405	379	1372

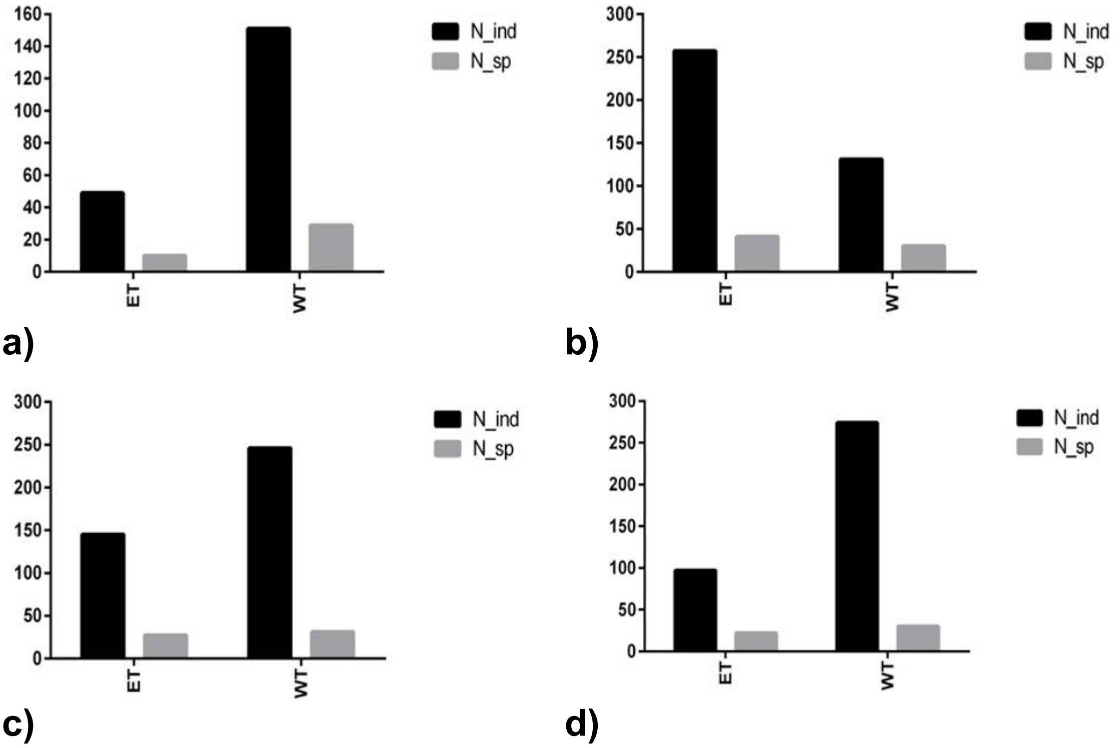


Figure 3. Number of individuals and species captured by emergence (Et) and interception (Wt) traps at each study plot: a) AB1 SelvaPiana, b) AB2 Rosello, c) LZ1 Monte Rufeno d) LZ2 Monte Circeo.

Table 4. Alpha diversity indices (Shannon, Simpson and Evenness) of tree communities (_t) and beetle communities (_b) and their correlation indices, at each study plot.

	Shannon_t	Shannon_b	Simpson_t	Simpson_b	Evenness_b	Correlation_txb (n = 2)	
						P	rs
AB1	0	2.7	0	0.87	0.43	0.66	−0.86
AB2	1.74	2.91	0.75	0.87	0.28	0.33	1
LZ1	0	2.85	0	0.86	0.34	1	−0.5
LZ2	1.35	2.47	0.69	0.84	0.28	0.33	1

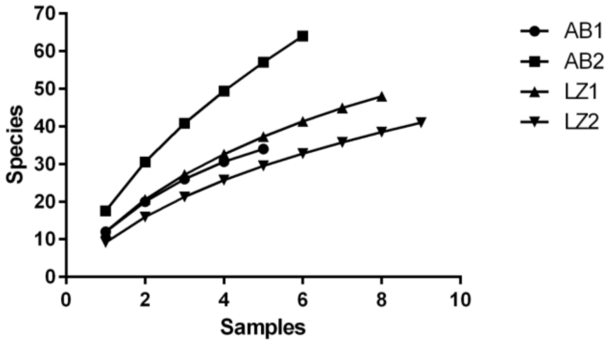


Figure 4. Comparison of sample-based rarefaction curves of beetle assemblages at Selva Piana (AB1), Rosello (AB2), Monte Rufeno (LZ1), Monte Circeo (LZ2).

Table 5. Bray-Curtis dissimilarity indices of plots, based on presence/absence of 133 species, detected by all traps.

	AB1	AB2	LZ1	LZ2
AB1	0	0.6735	0.8095	0.8667
AB2	0.6735	0	0,6842	0.8286
LZ1	0.8095	0.6842	0	0.6484
LZ2	0.8667	0.8286	0.6484	0

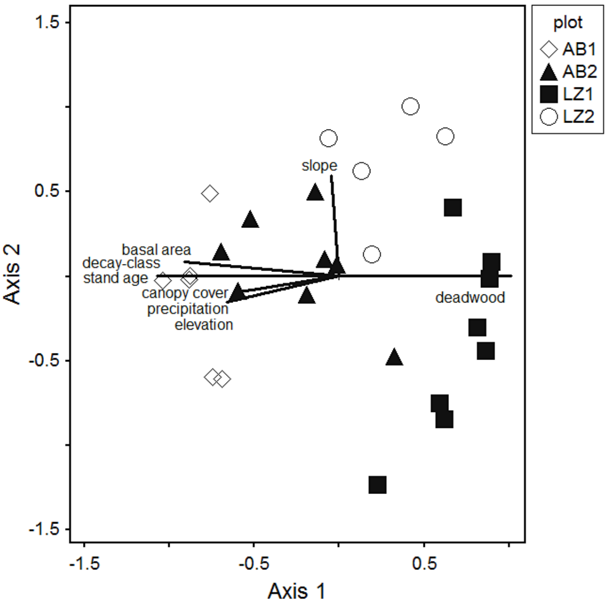


Figure 5. First 2 axes of a 3-dimensional NMDS ordination plot showing the dissimilarities of ET assemblages (log(x+1) abundance data with Bray-Curtis dissimilarity measures) at the four study areas. Vectors show the directions of positive correlations with environmental variables; vector lengths are proportional to correlation strengths. Only highly correlated environmental variables are shown ($r \geq 0.6$).

Monte Circeo curves exhibited similar trends, with fewer species being added with greater sampling effort, while the curve for Rosello confirmed a higher species density, rising steeply. Outputs of species richness estimators for all of the four sites differed based on the algorithm used, but they all listed a similar site ranking, with the highest species richness at Rosello and lowest at Monte Circeo, confirming the trends shown by rarefaction curves. The range of the estimators was wide: Bootstrap returned the smallest values, close to the actual numbers of species detected by sampling but, looking at the trends of rarefaction curves, second order Jackknife seemed to suggest the most correct number of beetle species present, also showing a clear peak value at Rosello. Considering Bootstrap and second order Jackknife, the following results may be suggested: between 44 and 77 beetle species at Selva Piana, 80 and 135 at Rosello, 61 and 100 at Monte Rufeno, 51 and 80 at Monte Circeo. Considering all types of captures to study beetle assemblage composition at the four study plots, a very high degree of dissimilarity was found (table 5). Ninety species (67.7% of the species found in all plots combined) were found in only one plot, while only three species (*Diplocoelus fagi*, *Litargus connexus* and *Xyleborus dispar*), representing 2.3% of the species, were collected at all of the four plots.

Among the seven species shared by three plots, 4 species belong to the family Latridiidae *Dienerella vincenti*, *Enicmus brevicornis*, *Enicmus rugosus*, *Cartodere* (*Aridius*) *nodifer*.

Comparing window and emergence traps captures within each plot, marked trap-type dissimilarities were also found, particularly at Rosello (Bray-Curtis Index = 0.80), where only 7 out of 64 species (10.9%) were sampled in both trap types. A lower degree of trap-type dissimilarity was exhibited at Monte Circeo, where Bray-Curtis Index was 0.57. A further analysis was performed considering only the specimens captured by ETs, assuming that they represent the subset of data including the higher proportion of true saproxylic species (eusaproxylic beetles). The results of the 3-dimensional NMDS are shown in figure 5. Axis 1 was primarily a gradient of traps from those that had high abundances of the scolytid, *Xyleborus dispar*, and none of the mycetophagid, *Litargus connexus*, to traps that had some *L. connexus* and no *X. dispar*. Axis 2 primarily represented a gradient of traps from those with some of the latridiid, *Dienerella clathrata*, and none of the scraptiid, *Anaspis lurida*, to traps with high abundances of *A. lurida* and no *D. clathrata*. Like Axis 1, Axis 3 distinguished sites with high abundances of *X. dispar* from those without this species, but Axis 3 did not exhibit a gradient related to *L. connexus*. The NMDS ordination plot showing the first 2 axes revealed that the ETs from each plot formed a cluster, indicating greater similarity of assemblages within plots than among them. A similar pattern was seen when axes 1 and 3 were plotted (not shown). From the same data subset, we looked at correlations between the assemblage composition (as shown in the ordination) and environmental variables. Relationships were evaluated for plot scale variables (elevation, slope, average precipitation, average temperature, stand age, basal area, and amount of deadwood) and trap scale variables (decay-class, wood volume, average canopy closure, and basal area near the trap). The variables that had the strongest correlations with the assemblage composition were plot-scale variables. The only trap-scale variable that showed up as related to assemblage composition was decay-class, which represents a relevant explanatory variable for saproxylic species, as saproxylic beetles use decayed wood as feeding substrate (figure 5). The majority of the environmental variables were related to the assemblage gradient along Axis 1, but slope was related to the assemblage gradient along axis 2; the assemblage gradient along Axis 3 had no strong correlations ($r \geq 0.6$) with environmental variables.

Discussion and conclusions

Despite the small size of the surveyed area, the plot scale was able to reveal differences in beetle communities among the four study sites. The interception surface of WT is capable of collecting insects flying from all directions within a huge three-dimensional space inside the forest plot, compared to trapping sources of ET (about 3000 cm^3 deadwood per trap as average in the frame of the present study). Emergence trapping captured insects from a known substrate, providing information on habitat and microhabitat species requirements. Thus, as expected, number of species and abundance were higher in WT compared to ET samples in three out of four plots. Considering all plots, each WT collected an average of 22 species and 200 individuals during the whole sampling period, while each ET in the same period trapped as average of 3 species and 20 individuals. We confirmed that window flight traps could efficiently passively capture saproxylic Coleoptera flying inside the forest, especially around trunks (Mason *et al.*, 2006; Ranius and Jansson, 2002), providing more information on species richness and composition.

In the present study, for the first time, since the installation of the four targeted ICP Forests plots, the diversity of beetle assemblages was analyzed against the diversity of tree communities but no significant correlation was detected between them. Indeed, the diversity of beetles is not related to richness and abundance in the tree community but, as shown by the NMDS analysis, it seems to be affected by forest structure and vegetation typology (figure 5). Further investigations should be carried out to detect the ecological factors explaining the details of this correlations. Other researchers have already shown that species richness of saproxylic beetles can be affected by tree characteristics, like trunk girth and shape of tree hollows (Ranius, 2002; Redolfi De Zan *et al.*, 2014).We can hypothesize that the abundance of flying insects, and thus captures by WT, may be higher where the available flying space between the forest floor and the canopies is particularly open, being occupied only by tree stems, like in even-aged stands. If this hypothesis is correct, it may also explain why Rosello was the only site characterized by a lower efficiency of WT compared to ET: the vertical structure at this site is a complex three-dimensional mosaic with a continuous ground vegetation and shrubs coverage, and old and younger trees and snags among spots of natural regeneration. At the same time, a complex forest structure is likely to create different available microhabitat conditions for forest dwelling insects, which may account for a higher species richness. Actually, among several forest structure diversity indices (Neumann and Starlinger, 2001; Corona *et al.*, 2005), Rosello scored high values regarding vertical evenness (Vertical Evenness Index = 85.72) and horizontal dimensional structure (DBH Variation Coefficient = 0.73) (Bertini *et al.*, 2007). Several other metrics highlighted the Rosello study area. Considering all trap captures together, results concerning species richness all agree that Rosello has the highest number of species, followed by Monte Rufeno.

Estimations of total number of species returned similar ranges for Rosello and Monte Rufeno, both clearly differing from the other two study areas, which showed lower values of species richness. Nevertheless, the very steep accumulation curve for Rosello (figure 3) demonstrated that a longer and more intense sampling would be needed at this plot to reach the target of actual species richness. This evidence may be due to the difficulty of sampling within a more complex environment, including several different microhabitats and potential niches.

The investigation of beetle community composition at the four study plots revealed a significantly low species overlap and thus high dissimilarity among areas (figure 5). The few species shared by all plots show a wide geographic distribution and were very abundant. Two of them live under bark or on deadwood fungi, while *Xyleborus dispar* is a saproxylophagous species. A wider array of functions and feeding ecology traits can be recognized in species assemblages that exhibited a preference to selected plots. This specificity becomes clearer when considering only saproxylic species. Among them, *Latridius consimilis* and *Trypodendron domesticum* were collected only at Selva Piana; four species were only sampled at Rosello (*Obrium brunneum*, *Pediacus dermestoides*, *Ampedus pomorum*, *Silvanus bidentatus*); eleven species were found only at Monte Rufeno, among them *Ampedus quercicola*, *Xylotrechus arvicola*, *Leptura aurulenta*, *Rhaphitropis oxyacanthae*; nine saproxylics species were found exclusively at Monte Circeo, among them rare singletons like *Agrilus convexicollis mancinii* and *Nematodes filum*, the latter recorded in central Italy for the first time. The evaluation of species diversity at sites and dissimilarities among sites thus suggested that the four forest plots host four different highly diverse beetle faunas. This diversified pattern was confirmed by the subgroup of true saproxylic species, even though, unexpectedly, Monte Rufeno and Monte Circeo (in second order), showed a higher number of saproxylic species compared to the whole dataset at Rosello. This reversal may be explained by the amount of deadwood, one of the environmental variables that showed strong correlation with beetle assemblages at plot scale. In fact, according to recent studies (Travaglini *et al.*, 2006, Bertini *et al.*, 2010), the amount of deadwood on the ground is actually greater at Monte Rufeno (6.63 m^3/ha) and at Monte Circeo (6.53 m^3/ha) than at Rosello (2.66 m^3/ha). Quality of deadwood is also different: early decaying wood still retaining bark is frequent at Monte Rufeno and Monte Circeo, while late decaying wood was found at Rosello. These qualitative differences also account for the different saproxylic fauna, being exclusive species at Monte Rufeno and Monte Circeo mainly connected to early wood decay stages (*e.g.* Scolytidae, Cerambycidae), while saproxylic species specialized on later stages of wood decomposition were present at Rosello (*e.g.* Cucujidae, Silvanidae).

The assessment of dissimilarities of captures within plots further showed how the two types of traps were suitable to collect different beetle samples and thus how it was relevant to use both to catch complementary aspects of biodiversity. Even within each plot ET set, it may be difficult to define two identical traps, because they were set randomly, without choosing the appropriate substrate and uncontrolled microhabitat variables may affect trap efficiency (*e.g.* sun-exposure, soil moisture, interaction with other micro- and macro fauna individuals like ants, shrews etc.). Species exclusive of single ET were also detected: *e.g.* all specimens of *Nematodes filum* were collected in ET4 at Monte Circeo, set on *Arbutus unedo* dead branches.

Following the classification of keystone species categories given by Scott Mills *et al.* (1993), we may assume that saproxylic beetles are keystone modifiers, as their hypothetical removal may affect habitat type and energy flows. Nevertheless, the results indicate that this role of modifiers cannot be attributed to single species but to the whole assemblage detected in each plot, because no species is numerically dominant but many species co-operate in modifying deadwood. Further studies are recommended, based on increased number of spatial and temporal replicates within similar and homogeneous forest environments.

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