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Spatio-temporal analysis of beetles from the canopy and ground layer in an Italian lowland forest

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Abstract

Canopies of temperate forests are still largely unexplored and the biodiversity they harbor is still poorly known, even for Coleoptera, the most studied insect group. In a lowland forest in northern Italy, Malaise traps were set in the canopy (n = 7) and ground layer (n = 7) and eleven beetles families (Buprestidae, Eucnemidae, Throscidae, Elateridae, Cantharidae, Dasytidae, Malachiidae, Tenebrionidae, Cerambycidae, Anthribidae, and Scolytidae) were compared for species richness and similarity of assemblages. Additionally it was investigated if species were associated with the forest layers studied and which of the families best qualified as bioindicator taxon. Finally, it was analyzed if similarity between the two layers was affected by season. The beetle assemblages in the two layers were significantly different, but species richness was similar. Eight species were significantly associated with the canopy and 15 species with the ground layer; for 16 of these this association is reported for the first time. Cerambycidae characterized the differences between ground and canopy best, as assemblages of this taxon were well separated; and these were significantly related to the composition of all families considered. Season had a strong influence on the trapping results and the dissimilarity of beetle assemblages between canopy and ground layer was most pronounced between late May and late July. This study showed that the distribution of beetles in the forest is structured in time and space and that season influences the capacity to distinguish between beetle assemblages sampled in the canopy and at the ground.

Key words: Coleoptera, canopy, Malaise trap, temperate forest, season.

Introduction

The beetle fauna of Europe and its forests is generally well known (e.g. Müller and Goßner, 2010; Alonso-Zarazaga, 2012; Audisio, 2012), but the ecological requirements and habitat preferences of many beetle species are still poorly documented. Especially the beetles in the canopy of forests, being difficult to sample, remain mostly unexplored and the number of species living here is still largely unknown (Ulyshen and Hanula, 2007; Bouget *et al.*, 2011). Few community-level entomological studies have been carried out in the canopy of temperate forests and here species composition per stratum is still poorly documented (Bouget *et al.*, 2011). Generally the composition of insect assemblages differs between the ground and canopy layers (Stork and Grimbacher, 2006; Bouget *et al.*, 2011; Birtele and Hardersen, 2012) and, in the few forests so far investigated, habitat preferences for horizontal strata have been established for some species (Wermelinger *et al.*, 2007; Vodka *et al.*, 2009; Bouget *et al.*, 2011; Birtele and Hardersen, 2012; Stiremann *et al.*, 2012). Considering that insects form a hyper-diverse taxon, our knowledge on vertical distribution of species and assemblages in temperate forests is still in its infancy.

Studying insect assemblages in temperate forest is further complicated because their composition changes across seasons (Ulyshen and Hanula, 2007; Leksono *et al.*, 2005a; Choi *et al.*, 2010; Birtele and Hardersen, 2012). Published sampling regimes of beetles in temperate forests range from continuous sampling for more than 6 months (e.g. Hutcheson, 1990; Leksono *et al.*, 2005a; Audisio *et al.*, 2008; Müller and Goßner, 2010) to trapping over 1-2 months (e.g. Hutcheson and Kimberley, 1999). A few studies sampled separated time periods spread over several months (e.g. Vance *et al.*, 2003). Therefore it is important to investigate the influence of sampling period on trapping results and to identify optimal sampling regimes, which could reduce the cost for taxonomic experts, while providing reliable information on forest biodiversity. A further shortcut might be to focus on biodiversity indicators (e.g. a group of taxa (e.g. genus, tribe, family or order), or functional group, the diversity of which reflects some measure of the diversity of other taxa in a habitat (McGeoch, 1998) which can evaluate forest biodiversity at only a fraction of the cost of complete inventories (cfr. Sebek *et al.*, 2012), but so far no consensus has been reached on these issues.

Beetles (Coleoptera) are an enormously species rich taxon, including more than 400,000 known species (Hammond, 1992) and representing almost 25% of all known life-forms (Hunt *et al.*, 2007). Species belonging to this hyper-diverse taxon cannot be easily identified to species level and many species belonging to certain families can only be determined by a few experts and are sometimes omitted (e.g., families Staphylinidae and Eucnemidae; Müller and Goßner, 2010; Sebek *et al.*, 2012; Vodka and Cizek, 2013). Three general approaches exist to overcome this limitation and to allow a biodiversity analysis: 1) identification is carried out at family level (e.g. Leksono *et al.*, 2005b; Ulyshen and Hanula, 2007); 2) assigning specimens to morphospecies (e.g. Ulyshen *et al.*, 2010a); 3) a sub-set of families, for which taxonomic expertise is available, is identified to species level (e.g. Wermelinger *et al.*, 2007; Audisio *et al.*, 2008). In this paper, the third approach was followed, by selecting eleven beetle families which contain species that are saproxylic, i.e. any species that depends, during some part of its life cycle, upon wounded or decaying woody material from living, weakened or dead trees (Stokland *et al.*, 2012). These beetles constitute a large proportion of the biodiversity in forests (Siitonen, 2001) and have been proposed as indicators of forest biodiversity (e.g. Müller and Bussler, 2008; Lachat *et al.*, 2012).

The aims of this study were: 1) to compare species richness and similarity of beetles assemblages between canopy and ground layer and to analyze species associations of the selected beetle families with these layers; 2) to evaluate the suitability of the selected beetle families as indicators of differences in beetle diversity between canopy and ground layer; 3) to study the phenology of the trapping results and to analyze how season influences the similarity of community composition between canopy and ground layer.

Methods

Study site

The study was carried out in the nature reserve Bosco Fontana (45°12'04"N 10°44'32"E, 25 m a.s.l.), about 5 km NW of Mantua (Lombardy, Italy). The reserve covers an area of about 233 ha, 199 ha of which is a mature mixed-deciduous forest (*Carpinus betulus* L., *Quercus robur* L., *Quercus cerris* L.), and 33 ha are meadows. The forest is one of the best preserved lowland forests of Italy as it had continuous woodland cover since at least 1600 A.D. (Mason *et al.*, 2002). The reserve is an isolated forest patch, as the surrounding landscape is highly modified by human activities, including agricultural fields, rural settlements, industrial buildings (for further details see Mason *et al.*, 2002). The climate is a relatively cool, midlatitude version of the humid subtropical climate. The annual average rainfall is 658 mm, average maximum temperature during the warmest month is 30 °C and average minimum temperature during the coldest month is −1 °C. The soil types of the reserve are mainly Mollic gleysols.

Field work

A total of 14 Malaise traps (B&S Entomological Services, Northern Ireland, UK) were placed in the reserve: seven on the ground and seven in the tree canopy. All 14 sampling sites were selected independently by generating random numbers corresponding to a square grid placed over a detailed map of the reserve and were in forest sections classified as belonging to the association *Asparago tenuifolii-Quercetum roboris* (Lausi 1964) Marinček 1994. In order to minimize the influence of ecotones and edge-effect, the minimum distances allowed for the placement of the traps were: 40 m from the forest border, 30 m from small artificial clearings (with a diameter of approximately 40 m) and 10 m from forest tracks. Sections where *Quercus rubra* (L.), an allochthonous tree species, had been eradicated (Cavalli and Mason, 2003) were also excluded. The Malaise traps on the ground were erected in the standard manner, with pickets and thin guide ropes, whereas canopy traps were fixed to a solid frame of welded steel (Faulds and Crabtree, 1995), suspended at heights between 15 m and 21 m in the canopy using pulleys and ropes attached to big branches in the crown of a large *Q. robur* trees, at maximum of 15 m from the centre of the square grid. Average distance between traps was 698 m (span 93-1491 m). The traps were operated from 1 April to 25 November 2008 and collecting bottles were replaced every two weeks (17 sampling periods). Each collecting bottle, a 500 ml flask, contained 70% ethanol as preservative. A few incidents interrupted the functioning of single traps: Trap G4: on 29th of April the collection bottle of the Malaise trap was found empty on the ground. It was replaced on the same day; Trap C4: The trap could not be lowered on 24th of June (due to mechanical failure) and the collecting bottle was only changed on 26th of June; Traps C2, C3 and C4: on 4th of November a thunderstorm resulted in the loss of the collection bottles, which were replaced on 6th of November; Trap C6: on 14th of October the trap was found lowered to the ground and the rope was missing (presumably stolen). The trap was activated again on 20th of October.

Sorting and identification

The samples collected were sorted at the Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale “Bosco Fontana” and the families considered were identified by the following specialists: Enzo Gatti (Scolytidae), Gianfranco Curletti (Buprestidae), Giuseppe Platia (Elateridae), Gianfranco Liberti (Cantharidae, Dasytidae, Malachiidae), Lucien Leseigneur (Eucnemidae, Throscidae), Paolo Cornacchia (Cerambycidae, Anthribidae), Piero Leo (Tenebrionidae). All specimens are deposited in the collection of the Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale “Bosco Fontana”.

Statistical analyses

The data of the sampling periods were considered separately for the analysis of species richness over time, and were pooled for all the other analysis. If not specified otherwise, analysis were carried out using R (version 2.13.2, http://www.r-project.org/). The assemblage compositions of the two layers were analyzed by two-dimensional Nonmetric Multidimensional Scaling (NMDS) of the abundance data employing the function metaMDS, which is incorporated in the statistical package *vegan* (Oksanen *et al.*, 2010). Bray-Curtis similarity was used as the pair- wise distance among samples.

A non-parametric MANOVA (Anderson, 2001) was used to assess the similarity of beetle assemblages between canopy and ground layer by employing Bray-Curtis similarities and 999 permutations of the data. In order to study the association of a single species caught within the two forest layers, the package *indicspecies* for R was used, employing the function *multipatt* (De Cáceres and Legendre, 2009).

Comparing species richness without reference to a taxon sampling curve is problematic as measured species richness may differ because of differences in underlying species richness, differences in the shape of the relative abundance distribution, or because of differences in the number of individuals collected. Rarefaction methods, both sample-based and individual-based, allow for meaningful comparison of data sets but these two approaches may produce very different results (Gotelli and Colwell, 2001). Therefore both methods for standardizing data sets were calculated using the program EstimateS 8.20. The similarity of the selected beetle families between the two layers was evaluated with two approaches: 1. a non-parametric MANOVA (Anderson, 2001) was calculated as indicated above; 2. procrustes analysis (Oksanen *et al*., 2010) was used to assess the indicator functioning of different beetle families in reflecting variation in the whole beetle community in respect to vegetation layer, as reflected in the NMDS described above. Procrustes uses uniform scaling (expansion or contraction) and rotation to minimize the squared differences between two ordinations (Oksanen, 2006).

Species richness and the number of specimens collected in the canopy and at the ground layer were compared for all families using the Wilcoxon rank sum test. Phenology of species richness of all Coleoptera families and that of the individual families was plotted for the 17 sampling periods. To assess the influence of season on species composition, the sampling period was divided into 4 time periods spanning each 8 weeks (a: 1 Apr-27 May, b: 27 May-22 Jul, c: 22 Jul-16 Sep, d: 16 Sep-11 Nov). To create time periods of the same length, the last two week period (11-25 Nov) was omitted for this analysis, and a total of 8 individuals (0.2% of the total) was not considered. To assess similarities between the eight beetle assemblages (two heights, four time periods), NMDS was employed as described above. However, the average dissimilarities were calculated using the zero-adjusted Bray-Curtis coefficient (Clarke *et al*., 2006). This measure was used because one of the practical problems that can arise with the classical Bray-Curtis coefficient is its increasingly erratic behavior as values within samples become vanishingly sparse (Clarke *et al*., 2006). This statistical problem occurred with the data from time periods c and d, where some traps had caught no beetles or only a single species. In order to assess if species composition corresponded to sampling period and height, these parameters were fitted on the NMDS ordination of the community compositions with the function envfit from the *vegan* package. Non-parametric MANOVA was used to distinguish assemblages of the two layers (canopy, ground) and the four sampling periods (a-d).

Results

A total of 4375 beetles belonging to 88 species were identified (table 1). Considering the assemblage composition of all selected Coleoptera families, the NMDS analysis (Stress = 0.07) showed that the two assemblages (canopy and ground) were well separated (figure 1) and the non-parametric MANOVA confirmed this difference to be significant ($F_{1, 12} = 8.56$, $P < 0.01$). Eight species were significantly associated with the canopy and 15 species were significantly associated with the ground layer (table 1). Sixty-one species were recovered from the canopy traps and 74 species from those on the ground. The number of species collected at the ground was approximately 20% higher than in the canopy (Wilcoxon rank sum test, P = 0.01) (figure 2). The individual-based rarefaction curves (figure 3) showed that species richness was very similar at both layers if richness is plotted against number of individuals caught.

The non-parametric MANOVA, carried out for the families investigated, showed that the beetle assemblages of the following families were significantly different for the two layers considered: Throscidae, Cantharidae, Dasytidae, Cerambycidae, Anthribidae and Scolytidae (table 2). The Procrustes analysis revealed that assemblage composition of the same families was significantly related to the composition of all families included, with the exception of Throscidae and Anthribidae. The number of individuals and the number of species differed between the two layers in the following families: Eucnemidae, Throscidae, Dasytidae, Anthribidae and Scolytidae; while for the Cerambycidae only the number of individuals caught at the two layers was significantly different (table 3). A total of 46 species (52%) were shared between the strata, while 16 species (18%) were only caught in the canopy and 26 species (30%) were exclusive to the ground layer.

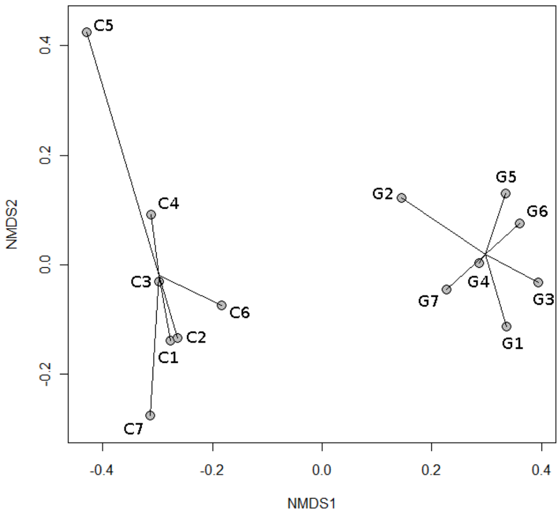

Figure 1. Nonmetric Multidimensional Scaling (NMDS) of the abundance data, using Bray-Curtis similarity as the pair wise distances among samples. (C: canopy trap, G: ground trap, numbers indicate trap numbers). Groups are connected to the cluster centroids by a line using the function 'ordispider' (statistical package Vegan).

Table 1. List of beetles collected with 14 Malaise traps in the canopy (C) and at ground layer (G) in the nature reserve Bosco Fontana between 1st April and 25th November 2008. Species marked with asterisk were significantly associated with one of the habitats (* < 0.05, ** < 0.01). Bold numbers indicate significant associations.

	C1	C2	C3	C4	C5	C6	C7	G1	G2	G3	G4	G5	G6	G7
Buprestidae														
Agrilus angustulus (Illiger)													5	
Agrilus convexicollis L. Redtenbacher										1				1
Agrilus hastulifer Ratzeburg		1	7	75	1	1			1			1		
Agrilus laticornis (Illiger)						1								
Agrilus olivicolor Kiesenwetter	1			7		1	1	4		6			5	2
Agrilus viridis (L.)												1		
Anthaxia nitidula (L.)	1							1			1			
Chrysobothris affinis (F.)	2	1	1	4										
Dicerca alni (Fischer von Waldheim)			1											
Eucnemidae														
Melasis buprestoides (L.)**								**2**	**5**	**1**	**12**	**3**	**1**	
Rhacopus sahlbergi (Mannerheim)													1	
Throscidae														
Aulonothroscus brevicollis (Bonvouloir)**			1		2	2	1	**10**	**60**	**19**	**44**	**80**	**39**	**13**
Trixagus dermestoides (L.)*								**1**		**57**	**65**	**6**		**2**
Trixagus elateroides (Heer)	5		1	4		4	3	3	1	3	6	18	3	5
Trixagus gracilis Wollaston											2			
Elateridae														
Agrypnus murimus (L.)						1			1	1				1
Ampedus glycereus (Herbst)			1								1	1	1	
Ampedus querciola (Buysson)		2	2	2		3	1	1	1	8	1	1	15	5
Athous haemorrhoidalis (F.)			1		2	2	2			1			3	2
Athous vittatus (Gmelin)		2	8	14		3	1	4	1	27	7	5	14	3
Dicronychus cinereus (Herbst)								1						
Lacon punctatus (Herbst)						1								
Melanotus crassicollis (Erichson)	2						1							
Melanotus villosus (Geoffroy)			1		1						2			1
Cantharidae														
Cratosilis laeta (F.)												3		
Malthinus flaveolus (Herbst)				1		1			3		2	5		2
Malthinus seriepunctatus Kiesenwetter**	4		3	1		5		**89**	**20**	**60**	**16**	**48**	**31**	**56**
Malthodes facetus Kiesenwetter					2						1	74	42	
Malthodes lobatus Kiesenwetter														12
Malthodes marginatus (Latreille)	3	1	9	6	36	4			2	1	1	3	1	4
Malthodes minimus (L.)						1								
Malthodes siculus Kiesenwetter	48	85	13	9		36	131	15	12	12	30	1	13	29
Malthodes umbrosus Kiesenwetter	1		1		5							37	2	
Rhagonycha fulva (Scopoli)**	**26**	**12**	**20**	**17**	**4**	**6**	**1**	3			5			
Rhagonycha fuscitibia Rey		2	9	8	1	1	1	1		1	1	2		3
Rhagonycha lignosa (O.F. Muller)	29	23	72	1		10	1	1	27					2
Rhagonycha lutea (O.F. Muller)													1	
Dasytidae														
Aplocnemus jejunus Kiesenwetter	4	1				3	4		1			1		
Aplocnemus nigricornis (F.)	48	40	35	21	7	22	29	4	7	5	7	4	1	4
Dasytes aeratus Stephens*	**7**	**5**	**1**		**1**	**1**	**1**				1			
Dasytes plumbeus (O.F. Muller)**	**5**	**3**	**9**	**4**	**1**	**1**	**10**			2		2		
Malachiidae														
Sphinginus lobatus (Olivier)			1				2					1		
Troglops albicans (L.)		2												
Troglops silo Erichson							3							
Tenebrionidae														
Nalassus dryadophilus (Mulsant)									11			3	1	
Scaphidema metallicum (F.)	1					1								
Stenomax aeneus (Scopoli)										1		1		

(Continued)

(Table 1 continued)

	C1	C2	C3	C4	C5	C6	C7	G1	G2	G3	G4	G5	G6	G7
Cerambycidae														
Aegomorphus clavipes (Schrank)				3										
Aegosoma scabricorne (Scopoli)									1					
Alosterna tabacicolor (De Geer)	5	14	5	6		6	3	3	3	14	59	15	38	24
Anaesthetis testacea (F.)	1											1		
Anaglyptus mysticus (L.)							1					1		
Chlorophorus glabromaculatus (Goeze)	1		1			3	3							
Deroplia genei (Aragona)*	**4**	**2**	**1**		**2**		**3**							
Exocentrus adspersus Mulsant*	**1**	**3**	**5**	**9**	**8**	**1**	**2**		2	1	3			
Grammoptera ruficornis (F.)	4		1		9	2	3	9	9	4	2	7	6	5
Leiopus nebulosus (L.)*	**4**	**7**	**2**	**11**	**2**	**1**		2	1	1				
Leptura aurulenta F.**		1					1	**10**	**12**	**42**	**10**	**9**	**18**	**10**
Mesosa nebulosa (F.)			2	1		3			1			1		1
Neoclytus acuminatus (F.)	5	3	4	3	3	4	5	4				7	1	4
Phymatodes testaceus (L.)								1				1		1
Plagionotus detritus (L.)				1										
Poecilium alni (L.)*	**2**	**5**	**2**		**2**	**4**	**4**					3	1	1
Pogonocherus hispidus (L.)									1					
Pyrrhidium sanguineum L.	1													
Ropalopus femoratus (L.)*		**3**	**3**	**2**		**3**	**2**							
Stenurella melanura (L.)**		1	1	2				**6**	**7**	**43**	**26**	**8**	**17**	**5**
Xylotrechus stebbingi Gahan**		1		2			1	**9**	**4**	**6**	**2**	**7**	**9**	**9**
Anthribidae														
Anthribus nebulosus Forster	1			2	1	1	2		1		1	1		
Choragus sheppardi Kirby								1	5		1			3
Dissoleucas niveirostris (F.)**							1	**7**	**8**	**7**	**4**	**9**	**6**	**17**
Eusphyrus vasconicus Hoffmann et Tempere										1				1
Noxius curtirostris (Mulsant et Rey)	2					1	1		1					1
Phaeochrotes cinctus (Paykull)						1								
Platystomos albinus (L.)**		1				1		**2**	**1**	**5**	**10**	**2**	**4**	**13**
Tropideres albirostris (Schaller)												1		
Ulorhinus bilineatus (Germar)									1				1	
Scolytidae														
Anisandrus dispar (F.)	4	5	8	1	1	4		15	1	14	10	14	28	18
Hypothenemus eruditus (Westwood)									1		1			
Kissophagus vicinus (Comolli)*								**3**	**1**		**1**	**1**	**1**	
Scolytus intricatus (Ratzeburg)*		1		7				**4**	**14**	**2**	**2**	**6**	**3**	**8**
Scolytus multistriatus (Marsham)										1			1	
Scolytus rugulosus (P.W.J. Muller)	27	1		3				1			1			
Taphrorychus bicolor (Herbst)*								**2**	**2**			**2**	**7**	**4**
Xyleborinus saxesenii (Ratzeburg)	6	7	5	3		6	3	184	9	58	30	24	12	66
Xyleborus dryographus (Ratzeburg)**								**4**	**1**	**35**	**36**	**8**	**6**	**13**
Xyleborus monographus (F.)**								**7**	**5**	**49**	**29**	**16**	**2**	**26**
Xylocleptes bispinus (Duftschmid)								1						
Xylosandrus germanus (Blandford)**								**12**		**35**	**47**	**28**	**66**	**15**

Species richness of all coleopteran families peaked between 29 April and 24 June (figure 4). However, some families showed specific phenologies, which often deviated from this pattern (figure 5). Buprestidae peaked in June and early July, while Elateridae and Cantharidae were caught exclusively from April to the beginning of July and at the ground layer. Throscidae were always present with two or three species, from early April to early November.

When assessing the influence of season on species composition, each of the eight assemblages (canopy and ground divided into four time periods each) occupied a distinct space in the NMDS plot (figure 6), with the exception of the time period d (16 Sep-11 Nov), when a total of only 106 specimens of the selected families were trapped. Here the NMDS returned overlapping spiderplots. The difference between canopy and ground was highest for the time period b (27 May-22 July), when a total of 1415 beetles were caught. Season and height of traps were both significantly correlated with assemblage composition (season: $r^2 = 0.84$, $P < 0.001$; height: $r^2 = 0.75$, $P < 0.001$) (figure 6). Season influenced the capacity to distinguish the assemblages collected at the two layers. For the periods a, b and c the non-parametric MANOVA showed that the beetle assemblages caught in the canopy and at ground layer were significantly different (period a: $F_{1, 12} = 6.82$, $P < 0.001$, period b: $F_{1, 12} = 8.91$, $P < 0.002$, period c: $F_{1, 12} = 4.38$, $P < 0.001$). In the periods a and b the relative variances explained were 36% and 42%, respectively. This value decreased to 27% for period b (22 Jul-16 Sep). For the period c (16 Sep-11 Nov) the composition of the trapped beetles was not significantly different for the two layers investigated (period d: $F_{1, 12} = 1.19$, $P < 0.273$).

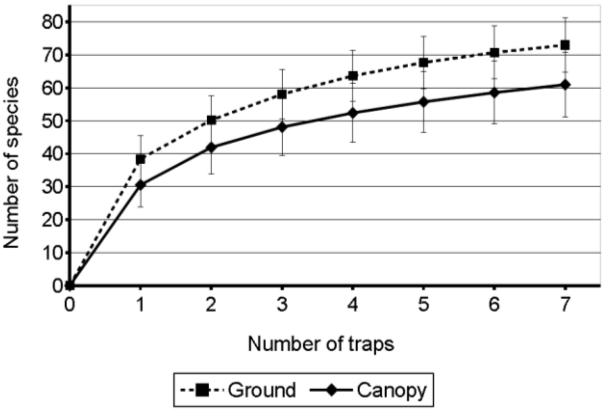

Figure 2. Sample-based rarefaction curves for canopy traps and ground traps.

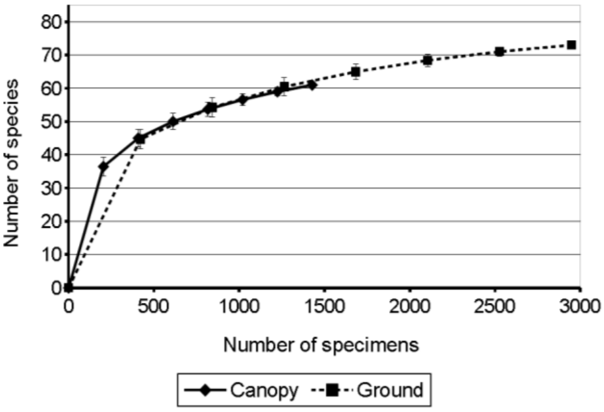

Figure 3. Individual based rarefaction curves for canopy traps and ground traps.

Table 2. Non-parametric MANOVA for a comparison of beetle families between canopy and ground layer, and procrustes analysis to evaluate the similarity of family-specific NMDS configurations with the NMDS configuration of the whole beetle community.

	non-parametric MANOVA		Procrustes analysis	
	$F_{1, 12}$	P	Correlation	P
Elateridae	1.27	0.291	0.50	0.059
Eucnemidae	-	-	-	-
Throscidae	6.93	**0.003**	0.24	0.656
Buprestidae	1.55	0.188	0.45	0.124
Cantharidae	5.72	**0.001**	0.90	**0.001**
Dasytidae	13.46	**0.001**	0.67	**0.008**
Malachiidae	-	-	-	-
Tenebrionidae	-	-	-	-
Cerambycidae	11.50	**0.001**	0.82	**0.001**
Anthribidae	8.64	**0.002**	0.25	0.612
Scolytidae	7.50	**0.002**	0.90	**0.001**

Table 3. Differences in the number of specimens and in species richness between canopy (C) and ground layer (G). The numbers in bold were significantly different (Wilcoxon Rank sum test).

	Median n. of specimens		Median n. of species	
	C	G	C	G
Elateridae	5	11	2	4
Eucnemidae	**0**	**2**	**0**	**1**
Throscidae	**4**	**61**	**1**	**3**
Buprestidae	3	3	3	2
Cantharidae	111	90	6	6
Dasytidae	**44**	**7**	**4**	**2**
Malachiidae	0	0	0	0
Tenebrionidae	0	1	0	1
Cerambycidae	**28**	**60**	10	8
Anthribidae	**2**	**13**	**1**	**4**
Scolytidae	**13**	**150**	**2**	**8**

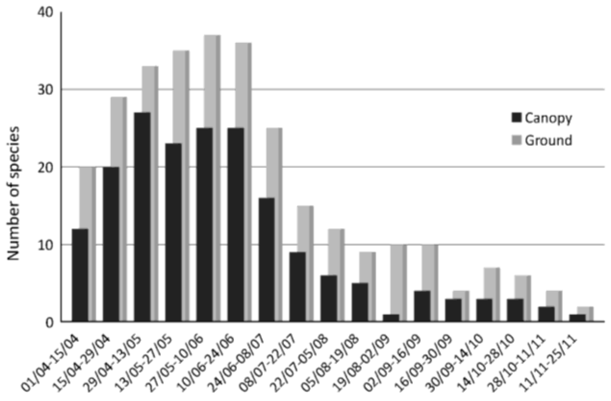

Figure 4. Total beetle species richness of all selected Coleoptera for 17 sampling periods.

Discussion

We showed that the vertical and temporal distribution of different beetle families varied considerably and were clearly distinctive. This finding corroborates beetle studies in temperate forests (Vance *et al*., 2003; Ulyshen and Hanula, 2007; Bouget *et al*., 2011) as well as on other insect taxa (Ulyshen *et al*., 2010b; Stireman *et al*., 2012; Birtele and Hardersen, 2012). These differences in assemblage composition are likely to be determined by a range of factors including resource availability, micro-climate preferences and predator avoidance (Stork and Grimbacher, 2006; Ulyshen, 2011).

At Bosco Fontana, eight species were significantly associated with the canopy, but for only three of these (*Dasytes aeratus*, *Exocentrus adspersus*, *Poecilium alni*) this is indicated in the literature (Vodka *et al*., 2009;

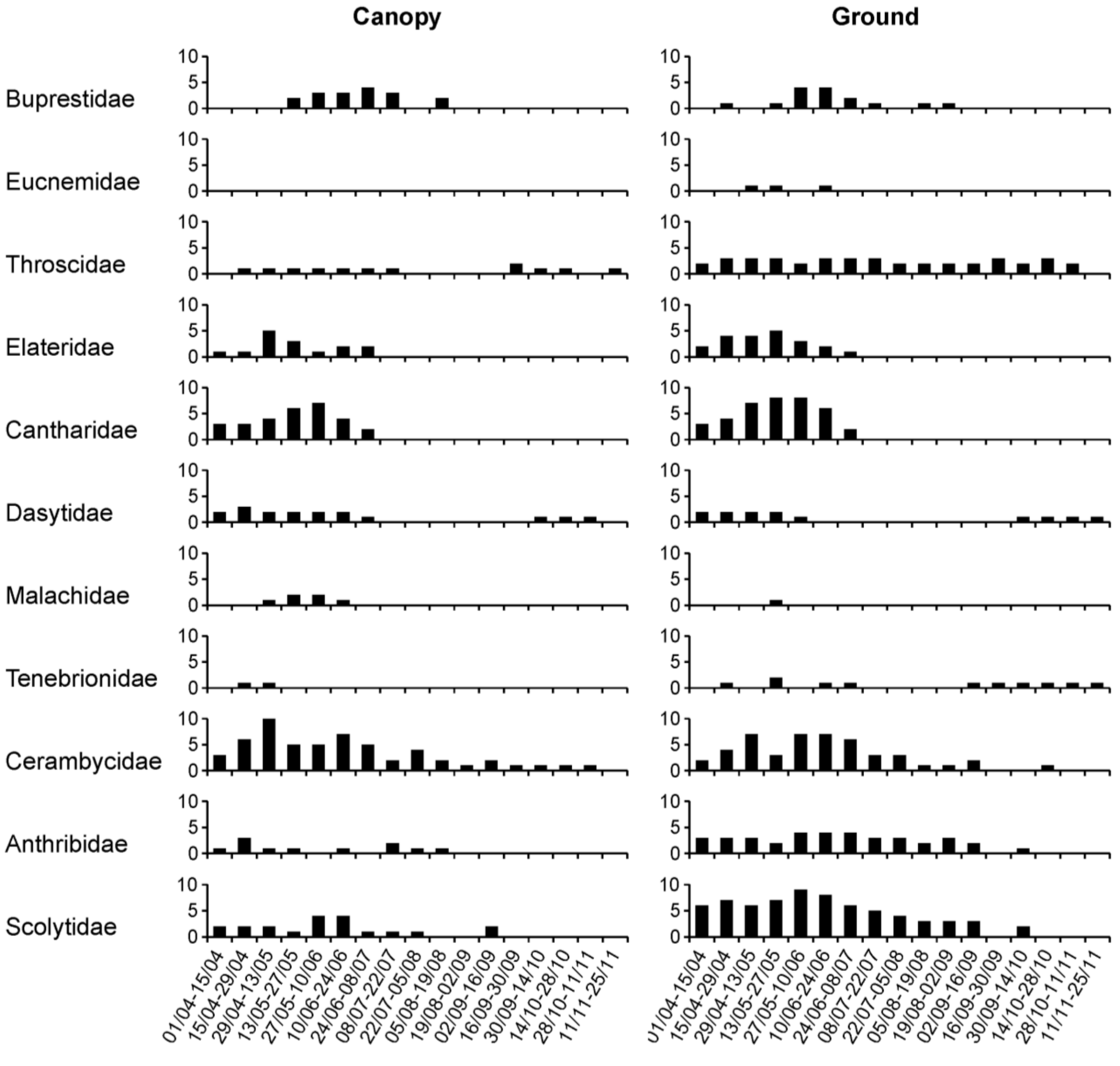

Figure 5. Total species richness of different beetle families for 17 sampling periods.

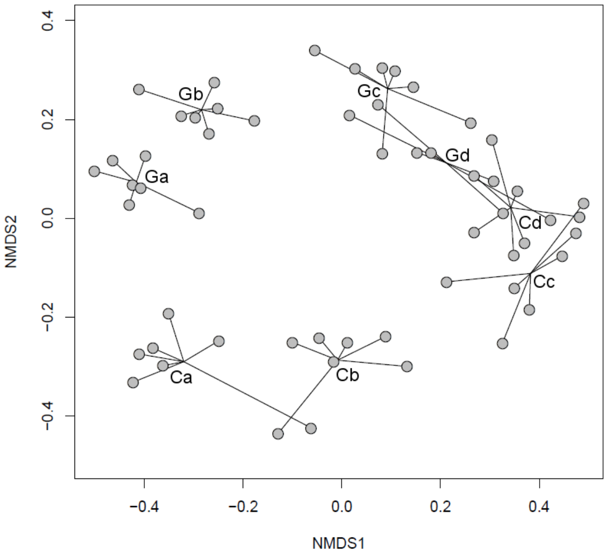

Figure 6. Nonmetric Multidimensional Scaling (NMDS) of the abundance data, using the zero-adjusted Bray-Curtis coefficient (Clarke *et al.*, 2006) The catches of the canopy traps (C) and ground traps (G) were divided into 4 time periods (a: 1 Apr-27 May; b: 27 May-22 Jul; c: 22 Jul-16 Sep; d: 16 Sep-11 Nov). Groups are connected to the cluster centroids by a line using the function ‘ordispider’.

Bouget *et al.*, 2011). Expert knowledge and literature data indicated for one further species (*Deroplia genei*) a likely association with the canopy, as its larval habitat are dead branches, mainly of *Quercus* spp. (Sama, 1988). In contrast, for one species (*Leiopus nebulosus*) literature data would suggest a preference for the ground layer, as Wermelinger *et al.* (2007) found it to be a typical forest species. Vodka *et al.* (2009) found this species to be significantly associated with shady conditions; Barbalat (1998) stated that *L. nebulosus* prefers artificial clearings, where it was caught at the ground layer. For the remaining three species that significantly preferred the canopy (*Rhagonycha fulva*, *Dasytes plumbeus*, *Ropalopus femoratus*) this association could not have been predicted.

Out of the 15 species which were significantly associated with the ground layer, four (*Stenurella melanura*, *Taphrorychus bicolor*, *Xyleborus dryographus* and *Xylosandrus germanus*) are known to be ground specialists (Wermelinger *et al.*, 2007; Reding *et al.*, 2010; Bouget *et al.*, 2011). The biology of four further species (*Melasis buprestoides*, *Trixagus dermestoides*, *Leptura aurulenta*, *Dissoleucas niveirostris*) suggests that these might be specialists of the shady forest floor (Burakowski, 1975; Alexander, 2002; Leseigneur, 2004; Hardersen *et al.*, 2012). For the remaining seven species that significantly preferred the ground layer (*Aulonothroscus brevicollis*, *Malthinus seriepunctatus*, *Xylotrechus stebbingi*, *Platystomos albinus*, *Kissophagus vicinus*, *Scolytus intricatus*, *Xyleborus monographus*) expert knowledge and the literature data did not allow to predict this association. In conclusion, for seven species known associations with a forest layer were confirmed and for 16 species an association with a specific forest stratum is here reported for the first time.

Generally, species associations with the canopy or ground layer varies along a sliding scale, with some entirely associated with a particular stratum and others equally split between strata (Stork and Grimbacher, 2006). It is therefore common that a high proportion of species is shared by ground and canopy and a smaller proportion is generally strongly associated with either layer to qualify as specialist species (e.g. Stork and Grimbacher, 2006; Bouget *et al.*, 2011).

Segregation of beetles between the two strata is likely to be underestimated by flight-intercept traps, such as Malaise traps, as these might catch dispersing individuals far from their larval substrate (Bouget, 2011). On the other hand, Malaise traps are known to sample only part of the aerial fauna and like all interception traps, what it in fact measures is invertebrate activity/density, which is correlated to local population density around the trap (Nageleisen, 2009). A further fact to consider is that ethanol was used as preservative, a known attractant for several beetle families (Roling and Kearby, 1975; Montgomery and Wargo, 1983; Allison *et al.*, 2004; but see Bouget *et al.*, 2009).

Approximately 20% less species were trapped in the canopy than at ground layer, a significant difference. But rarefaction curves of canopy and ground were virtually identical when richness was plotted against the number of individuals. This indicates that species density was lower in the canopy and this is supported by the fact that here less than half the number of individuals were collected than at the ground layer. In a similar study Vodka and Cizek (2013) found that slightly (but not significantly) more saproxylic beetles species were trapped in the canopy of the forest interior. Thus it seems that species richness of saproxylic beetles is similar at ground and in the canopy in the temperate forests so far investigated. One likely reason why many of the saproxylic beetles were collected at the shady ground layer is that the majority of dead wood accumulates on the forest floor and therefore insects associated with this resource are more likely to be found here (Stork and Grimbacher, 2006). Similarly Birtele and Hardersen (2012) found that hoverflies species with saprophagous and saproxylic larvae were collected more commonly at the ground layer.

The families Cantharidae, Dasytidae, Cerambycidae and Scolytidae best reflected the differences between ground and canopy. Of these only the Cantharidae and the Cerambycidae contained species which were significantly associated with one of the layers investigated. While in the Cantharidae only a single species was associated with either stratum, a total of eight species of the Cerambycidae were specialists for one of the layers investigated. Thus of the beetle families analyzed, Cerambycidae best fulfilled the commonly cited feature for bio-indicators to be representative of the assemblage (Gerhardt, 2006). Using a similar analysis, Oshawa (2010) also suggested that Cerambycidae might be particularly suited as indicators in forest systems, an opinion shared by Holland (2007) and Gobbi *et al.* (2012) (but see Warriner *et al.*, 2004). Cerambycidae showed the highest correlation value for saproxylic beetle species richness, but explained more than 70% of the variation only in combination with other families (Sebek *et al*., 2012). Additionally, Cerambycidae also have a low sorting error (Majka and Bondrup-Nielsen, 2006), which qualifies this group further as a useful bioindiators. The results presented from this Italian mixed-deciduous forest extend the known specificity of species and community composition of Cerambycidae to vertical forest strata and indicate that this family might reflect the vertical assemblage composition of the whole beetle community. It is thus suggested to verify in other forest ecosystems if Cerambycidae qualify as indicators of canopy and ground layer beetle community as a whole.

Season had a strong influence on trapping results, as reported previously (e.g. Leksono *et al*., 2005a; Ulyshen and Hamula, 2007; Choi *et al*., 2010). In the data presented, species richness peaked in May-June and the beetle families investigated showed different phenological patterns, as also demonstrated by other authors (e.g. Wermeling *et al*., 2002). The distinct flight periods of the numerous species collected structured the assemblages temporally (figure 5) and this influenced the capacity to distinguish between the beetle assemblages sampled in the canopy and at the ground (figure 6). The samples from early summer showed the most pronounced separation of the assemblages. Also in New Zealand beetles sampled in early summer were most characteristic of site and most effectively discriminated beetle assemblages (Hutcheson, 1990; Hutcheson and Kimberley, 1999). During the final weeks of our trapping campaign in autumn the lowest number of beetles was caught and sampling could have stopped after mid-September, without losing important information.

In summary, saproxylic beetles in a temperate forest of northern Italy were vertically and temporally structured and if trapping needs to be restricted to a few months, it should be carried out from late May to late July, as the capacity to differentiate between assemblages is highest in this period.

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References

ALEXANDER K. N. A., 2002.- The invertebrates of living and decaying timber in Britain & Ireland - a provisional annotated checklist.- *English Nature Research Reports* No. 467.

ALLISON J. D., BORDEN J. H., SEYBOLD S. J., 2004.- A review of the chemical ecology of the Cerambycidae (Coleoptera).- *Chemoecology*, 14 (3): 123-150.

ALONSO-ZARAZAGA M. A., 2012.- *Coleoptera 1. Fauna Europaea version 2.3*.- [online] URL: http://www.faunaeur.org. (accessed February 18, 2013).

ANDERSON M. J., 2001.- A new method for non-parametric multivariate ANOVA.- *Australian Journal of Ecology*, 26: 32-46.

AUDISIO P., 2012.- *Coleoptera 2. Fauna Europaea version 2.3*.- [online] URL: http://www.faunaeur.org. (accessed February 18, 2013).

AUDISIO P., CORNACCHIA P., FATTORINI L., FRANCESCHI S., GATTI E., HARDERSEN S., LESEIGNEUR L., NARDI G., PENATI F., PLATIA G., 2008.- Selected beetle families in natural forests and Norway spruce stands at Vincheto di Celarda Nature Reserve and the effects of conservation actions (Coleoptera), pp. 195-217. In: *Research on the natural heritage of the reserves Vincheto di Celarda and Val Tovanella (Belluno province, Italy). Conservation of two protected areas in the context of a LIFE Project*.- *Quaderni Conservazione Habitat*, 5 (HARDERSEN S., MASON F., VIOLA F., CAMPEDEL D., LASEN C., CASSOL M., Eds).- Arti Grafiche Fiorini, Verona, Italy.

BARBALAT S., 1998.- Importance of forest structures on four beetle families (Col: Buprestidae, Cerambycidae, Lucanidae and phytophagous Scarabaeidea in the Areuse Gorges (Neuchâtel, Switzerland).- *Revue Suisse de Zoologie*, 105: 569-580.

BIRTELE D., HARDERSEN S., 2012.- Analysis of vertical stratification of Syrphidae (Diptera) in an oak-hornbeam forest in northern Italy.- *Ecological Research*, 27: 755-763.

BOUGET C., BRUSTEL H., BRIN A., VALLADARES L., 2009.- Evaluation of window flight traps for effectiveness at monitoring dead wood-associated beetles: the effect of ethanol lure under contrasting environmental conditions.- *Agricultural and Forest Entomology*, 11: 143-152.

BOUGET C., BRIN A., BRUSTEL H., 2011.- Exploring the "last biotic frontier": are temperate forest canopies special for saproxylic beetles?- *Forest Ecology and Management*, 261: 211-220.

BURAKOWSKY B., 1975.- Development, distribution and habits of *Trixagus dermestoides* (L.), with notes on the Throscidae and Lissomidae (Coleoptera, Elateroidea).- *Annales Zoologici*, 32: 375-405.

CAVALLI R., MASON F., 2003.- *Techniques for re-establishment of dead wood for saproxylic fauna conservation. LIFE Nature project NAT/IT/99/6245 Bosco della Fontana (Mantova, Italy)*.- Gianluigi Arcari Editore, Mantova, Italy.

CHOI W. I., CHOI K.-S., LYU D.-P., LEE J.-S., LIM J., LEE S., SHIN S.-C., CHUNG Y.-J., PARK Y.-S., 2010.- Seasonal changes of functional groups in coleopteran communities in pine forests.- *Biodiversity and Conservation*, 19: 2291-2305.

CLARKE K. R., SOMERFIELD P. J., Chapman M. G., 2006.- On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages.- *Journal of Experimental Marine Biology and Ecology*, 330: 55-80.

DE CÁCERES M., LEGENDRE P., 2009.- Associations between species and groups of sites: indices and statistical inference.- *Ecology*, 90: 3566-3574.

FAULDS W., CRABTREE R., 1995.- A system for using a Malaise trap in the forest canopy.- *New Zealand Entomologist*, 18: 97-99.

GERHARDT A., 2006.- Bioindicator species and their use in biomonitoring, 10 pp. In: *Encyclopedia of life support systems (EOLSS). Environmental monitoring - Volume 2* (INYANG H. I., DANIELS J. L., Eds).- Oxford, EOLSS Publishers.

GOBBI M., PRIORE C., TATTONI C., LENCIONI V., 2012.- Surprising longhorned beetle (Coleoptera, Cerambycidae) richness along an Italian alpine valley.- *ZooKeys*, 208: 27-39.

Gotelli N. J., Colwell R. K., 2001.- Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness.- *Ecology Letters*, 4: 379-391.

Hammond P., 1992.- Species inventory, pp. 17-39. In: *Global biodiversity: status of the earth's living resources* (Groombridge B., Ed.).- Chapman & Hall, London, UK.

Hardersen S., Toni I., Cornacchia P., Curletti G., Leo P., Nardi G., Penati F., Piattella G., Platia G., 2012.- Survey of selected beetle families in a floodplain remnant in northern Italy.- *Bulletin of Insectology*, 65: 199-207.

Holland J. D., 2007.- Sensitivity of Cerambycid biodiversity indicators to definition of high diversity.- *Biodiversity and Conservation*, 16: 2599-2609.

Hunt T., Bergsten J., Levkanicova Z., Papadopoulou A., John O. S., Wild R., Hammond P. M., Ahrens D., Balke M., Caterino M. S., Gómez-Zurita J., Ribera I., Barraclough T. G., Bocakova M., Bocak L., Vogler A. P., 2007.- A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation.- *Science*, 318: 1913-1916.

Hutcheson J., 1990.- Characterization of terrestrial insect communities using quantified, Malaise-trapped Coleoptera.- *Ecological Entomology*, 15: 143-151

Hutcheson J., Kimberley M. O., 1999.- A pragmatic approach to characterising insect communities in New Zealand: malaise trapped beetles.- *New Zealand Journal of Ecology*, 23: 69-79.

Lachat T., Wermelinger B., Gossner M. M., Bussler H., Isacsson G., Müller J., 2012.- Saproxylic beetles as indicator species for dead-wood amount and temperature in European beech forests.- *Ecological Indicators*, 23: 323-331.

Leksono A. S., Nakagoshi N., Takada K., Nakamura K., 2005a.- Vertical and seasonal variation in the abundance and the species richness of Attelabidae and Cantharidae (Coleoptera) in a suburban mixed forest.- *Entomological Science*, 8: 235-243.

Leksono A. S., Takada K., Koji S., Nakagoshi N., Anggraeni T., Nakamura K., 2005b.- Vertical and seasonal distribution of flying beetles in a suburban temperate deciduous forest collected by water pan trap.- *Insect Science*, 12: 199-206.

Leseigneur L., 2004.- Throscidae (Coleoptera), pp. 145-148. In: *Invertebrati di una foresta della Pianura Padana, Bosco della Fontana, Secondo contributo*.- *Conservazione Habitat Invertebrati*, 3 (Cerretti P., Hardersen S., Mason F., Nardi G., Tisato M., Zapparoli M., Eds).- Cierre Grafica Editore, Verona, Italy.

Majka C. G., Bondrup-Nielsen S., 2006.- Parataxonomy: a test case using beetles.- *Animal Biodiversity and Conservation*, 29: 149-156.

Mason F., Cerretti P., Tagliapietra A., Speight M. C. D., Zapparoli M., 2002.- Invertebrati di una foresta della Pianura Padana, Bosco della Fontana, Primo contributo.- *Conservazione Habitat Invertebrati* 1, Gianluigi Arcari Editore, Mantova, Italy.

McGeoch M. A., 1998.- The selection, testing and application of terrestrial insects as bioindicators.- *Biological Reviews*, 73 (2): 181-201.

Montgomery M. E., Wargo P. M., 1983.- Ethanol and other host-derived volatiles as attractants to beetles that bore into hardwoods.- *Journal of Chemical Ecology*, 9: 181-190.

Müller J., Bussler H., 2008.- Key factors and critical thresholds at stand scale for saproxylic beetles in a beech dominated forest, southern Germany.- *Revue d'Écologie - La Terre et la Vie*, 63: 73-82.

Müller J., Goßner M. M., 2010.- Three-dimensional partitioning of diversity informs state-wide strategies for the conservation of saproxylic beetles.- *Biological Conservation*, 143: 625-633.

Nageleisen L. M., Bouget C., 2009.- *Forest insect studies: methods and techniques. Key considerations for standardisation. An overview of the reflections of the "Entomological Forest Inventories" working group (Inv.Ent.For.)*.- Les Dossiers Forestiers 19, Office National des Forêts, Paris, France.

Ohsawa M., 2010.- Beetle families as indicators of Coleopteran diversity in forests: a study using Malaise traps in the central mountainous region of Japan.- *Journal of Insect Conservation*, 14: 479-484.

Oksanen J., 2006.- *Multivariate analysis of ecological communities in R: vegan tutorial*.- [online] URL: http://cc.oulu.fi/~jarioksa/opetus/metodi/vegantutor.pdf (accessed 11 March 2012).

Oksanen J., Blanchet F. G., Kindt R., Legendre P., O'Hara R. B., Simpson G. L., Solymos P., Stevens M. H. H., Wagner H., 2010.- *Vegan: community ecology package*. R package version 1.17-2.- [online] URL: http://r-forge.r-project.org/projects/vegan (accessed 12 March 2012).

Reding M., Oliver J., Schultz P., Ranger C., 2010.- Monitoring flight activity of ambrosia beetles in ornamental nurseries with ethanol-baited traps: influence of trap height on captures.- *Journal of Environmental Horticulture*, 28: 85-90.

Roling M. P., Kearby W. H., 1975.- Seasonal flight and vertical distribution of Scolytidae attracted to ethanol in an oak-hickory forest in Missouri.- *Canadian Entomologist*, 107: 1315-1320.

Sama G., 1988.- *Coleoptera Cerambycidae. Catalogo topografico e sinonimico*. Fauna d'Italia 26.- Edizioni Calderini, Bologna, Italy.

Sebek P., Barnouin T., Brin A., Brustel H., Dufrêne M., Gosselin F., Meriguet B., Micas L., Noblecourt T., Rose O., Velle L., Bouget C., 2012.- A test for assessment of saproxylic beetle biodiversity using subsets of "monitoring species".- *Ecological Indicators*, 20: 304-3015.

Siitonen J., 2001.- Forest management, coarse woody debris and saproxylic organisms: Fennoscandian boreal forests as an example.- *Ecological Bulletins*, 49: 11-41.

Stireman J. O., Cerretti P., Whitmore D., Hardersen S., Gianelle D., 2012.- Composition and stratification of a tachinid (Diptera: Tachinidae) parasitoid community in a European temperate plain forest.- *Insect Conservation and Diversity*, 5: 346-357.

Stokland J. N., Siitonen J., Jonsson B. G., 2012.- *Biodiversity in dead wood*.- Cambridge University Press, Cambridge, UK.

Stork N. E., Grimbacher P. S., 2006.- Beetle assemblages from an Australian tropical rainforest show that the canopy and the ground strata contribute equally to biodiversity.- *Proceedings of the Royal Society B: Biological Sciences*, 273: 1969-1975.

Ulyshen M. D., 2011.- Arthropod vertical stratification in temperate deciduous forests: Implications for conservation-oriented management.- *Forest Ecology and Management*, 261: 1479-1489.

Ulyshen M. D., Hanula J. L., 2007.- A comparison of the beetle (Coleoptera) fauna captured at two heights above the ground in a North American temperate deciduous forest.- *American Midland Naturalist*, 158: 260-278.

Ulyshen M. D., Horn S., Hanula J. L., 2010a.- Response of beetles (Coleoptera) at three heights to the experimental removal of an invasive shrub, Chinese privet (*Ligustrum sinense*), from floodplain forests.- *Biological Invasions*, 12: 1573-1579.

Ulyshen M. D., Soon V., Hanula J. L., 2010b.- On the vertical distribution of bees in a temperate deciduous forest.- *Insect Conservation and Diversity*, 3: 222-228.

Vance C. C., Kirby K. R., Malcolm J. R., Smith S. M., 2003.- Community composition of longhorned beetles (Coleoptera: Cerambycidae) in the canopy and understorey of sugar maple and white pine stands in south-central Ontario.- *Environmental Entomology*, 32: 1066-1074.

VODKA S., CIZEK L., 2013.- The effects of edge-interior and understorey-canopy gradients on the distribution of saproxylic beetles in a temperate lowland forest.- *Forest Ecology and Management*, 304: 33-41.

VODKA S., KONVICKA M., CIZEK L., 2009.- Habitat preferences of oak-feeding xylophagous beetles in a temperate woodland: implications for forest history and management.- *Journal of Insect Conservation*, 13: 553-562.

WARRINER M. D., NEBEKER T. E., TUCKER S. A., SCHIEFER T. L., 2004.- Comparison of saproxylic beetle (Coleoptera) assemblages in upland hardwood and bottomland hardwood forests, pp. 150-153. In: *Upland oak ecology symposium: history, current conditions, and sustainability* (SPETICH A., Ed.).- Gen. Tech. Rep. SRS-73. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station, USA.

WERMELINGER B., DUELLI P., OBRIST M. K., 2002.- Dynamics of saproxylic beetles (Coleoptera) in windthrow areas in alpine spruce forests.- *Forest Snow and Landscape Research*, 77: 133-148.

WERMELINGER B., FLÜCKIGER P. F., OBRIST M. K., Duelli P., 2007.- Horizontal and vertical distribution of saproxylic beetles (Col., Buprestidae, Cerambycidae, Scolytinae) across sections of forest edges.- *Journal of Applied Entomology*, 113: 104-114.

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