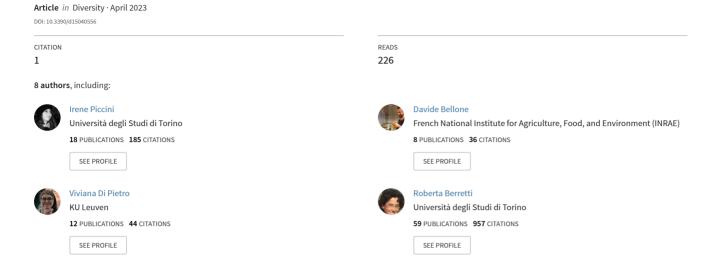
# Saproxylic Beetle Community in the Expansion Site of a Megaproject and in the Surrounding Area in the Western Italian Alps







Article

# Saproxylic Beetle Community in the Expansion Site of a Megaproject and in the Surrounding Area in the Western Italian Alps

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Abstract: Beetles are one of the most diverse and often highly specialized groups among saproxylic organisms and play a key role in forest dynamics. To develop conservation plans in forests threatened by human activities, such as construction sites, it is crucial to identify key parameters characterizing forest structure in turn influencing saproxylic beetle diversity and abundance. Here, we investigate the difference in forest structure parameters and their cascading effect on saproxylic beetle communities between a forest site affected by the construction site expansion of the Turin–Lyon High–Speed Railway Line and a nearby second forest site. Our study showed differences in forest structure parameters between the two sites, in particular in the overall volume and diameter of coarse woody debris and in standing dead tree abundance. Even saproxylic beetle community structure was different between the two sites and this difference was linked to the different abundance and species richness of standing dead trees. Our findings provide information for the development of a local conservation plan for the saproxylic beetle community within forest habitats. Increasing the volume of deadwood and enriching tree diversity can locally sustain abundant and diverse communities and, eventually, support those species that are threatened by the building site expansion.

**Keywords:** chestnut forest; conservation plan; construction site; deadwood; forest; forest management

Citation: Piccini, I.; Bellone, D.; Di Pietro, V.; Berretti, R.; Cristiano, L.; Caprio, E.; Biscaccianti, A.B.; Bonelli, S. Saproxylic Beetle Community in the Expansion Site of a Megaproject and in the Surrounding Area in the Western Italian Alps. *Diversity* 2023, 15, 556. https://doi.org/10.3390/ d15040556

Academic Editor(s): Viktor Brygadyrenko and Luc Legal

Received: 23 February 2023 Revised: 6 April 2023 Accepted: 8 April 2023 Published: 14 April 2023



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#### 1. Introduction

Megaprojects are those projects that have a large-scale impact rapidly and radically changing the landscape. They usually last several decades and are generally funded by international finance capitals, typically 1 billion US dollars or more [1,2]. Beyond their importance, megaprojects have raised major concerns for the high environmental impact on vulnerable habitats such as forests; for instance, 80% of the project area of the third airport in Istanbul was a forest [3]. Another example was the construction of the Panama Canal where a large forest area was cut and, consequently, the surrounding forested areas have also been degraded [4]. Forests are habitats rich in biodiversity, among them species linked to wood or deadwood, such as saproxylic fauna and flora (beetles, flies, moths, and fungi). The strong link with the woody substrate makes these biodiversity components strongly affected by habitat subtraction, reduction, or deterioration [4,5]. Despite efforts

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to reduce and/or mitigate impacts on natural and seminatural habitats, it is difficult to completely avoid the detrimental effects of those projects. Thus, nowadays some management plans for compensation and mitigation of the effects of megaprojects on biodiversity have been already developed and applied [5].

Saproxylic beetles represent an important component of forest biodiversity related to deadwood and dead parts of living trees [6,7]. Deadwood provides a favorable environment and substrate from which saproxylic beetle communities benefit in terms of food resources, nesting or breeding sites, and shelters against natural enemies or adverse abiotic conditions [8]. In addition to biodiversity enrichment per se, saproxylic beetles are providers of several ecosystem services, being among the main factors contributing to the decomposition process of deadwood and subsequently to the organic matter recycling in forest habitats [9]. This key role has attributed to saproxylic beetles the status of bioindicators of forest sustainability and their conservation represents an important task in preserving forest health conditions [10]. The threat to saproxylic beetles represented by the increase in anthropic activities within forest areas requires specific conservation plans for which it would be crucial to identify the components of forest structure that can be managed to mitigate saproxylic beetle community loss [11,12].

Several components of forest structure and their management have been shown to shape the communities of different taxa including saproxylic beetles [13]. For instance, deadwood is an essential structural component of forests and its overall volume [14,15] along with its heterogeneity and decay stage have been suggested to be indicators of saproxylic beetle species richness, abundance, and diversity [16–18]. Other evidence has highlighted the importance of microhabitat occurrence, such as tree hollows, dead branches, cracked bark, saprophytic fungi growing on trees, and so on [8], which provide saproxylic beetles communities with a favorable and safer environment [14,17]. Further, other studies have also linked the type of forest management with the amount of deadwood influencing saproxylic fungi communities, in turn, correlated with the species richness of saproxylic beetles [18,19]. It results that to carry out monitoring activities and subsequently a conservation plan for saproxylic beetle communities several parameters of forest management and forest structure need to be considered.

Few studies have investigated beetle communities in forests dominated by sweet chestnut (Castanea sativa Mill.) [20]. In Northern Italy, sweet chestnut forests (habitat 9260; listed in Annex I of Habitats Directive 92/43/EEC) host a large community of saproxylic beetles [21]. Here, we investigate and compare the forest structure parameters and their influence on the saproxylic beetle communities between a forest site that will be affected by the expansion of the building site of the megaproject of the Turin-Lyon High-Speed Railway Line and a nearby forest site that will not be influenced by the expansion. More specifically, we aim to (a) evaluate the saproxylic beetle species abundance and richness in the two different forest sites both included in a sweet chestnut forest in Northern Italy; (b) characterize the forest complexity of the two sites; (c) identify which forest parameters determine saproxylic beetle species abundance and richness. In the threatened site and in its surroundings, we previously investigated the possible effects of the construction site expansion on several biodiversity components [22–24] to develop an ad hoc conservation plan [5]. Also in this case, we investigate the beetle communities and their link with forest components to understand which possible management interventions would make the surrounding forest suitable for those species that would suffer from habitat subtraction due to the expansion of Turin-Lyon Railway Lines.

# 2. Materials and Methods

#### 2.1. Study Area

Clarea valley is part of Susa Valley in the Cottian Alps, within the system of the Southwestern Alps (Piedmont, NW Italy) and belongs to the municipalities of Chiomonte and Giaglione in the Turin province. Within Clarea valley, we selected an area of about

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43 ha characterized by young mesoneutrophilic chestnut forests, with ash, cherry tree, Scots pine, downy oak, and other species locally more or less abundant (Table S1 in Supplementary Material). These are established on hay meadows dominated by *Festuca rubra* s.l., *Brachypodium rupestre* (Host) Roem. & Schult., and *Bromopsis erecta* (Huds.) Fourr., belonging to *Festuco-Brometea* and *Molinio-Arrhenatheretea* classes [5]. Originally, these woods were cultivated for both fruit and timber and the management practice were mainly coppicing [25]. Inside this area, we identified two different sites: (i) Site 1, that will be affected by the expansion site of the Turin–Lyon High-Speed Railway Line. It has an extension of about 10 ha situated at 750 m a.s.l. and the area has not been managed since 1960s, when most of the traditional coppices have been abandoned [25]; (ii) Site 2, which is located in the surrounding of Site 1, is representative of the surrounding forest, and it would not be affected by the construction site. It has an extension of around 10 ha at 1100 m a.s.l. and it is about 2 km west from Site 1. This portion of the forest was recently abandoned or managed at low intensity [26].

## 2.2. Sampling Design, Identification of Beetles and Plants

To collect saproxylic beetles, cross-vane window traps have been set up using ethanol as a lure. Ethanol is a lignin precursor monomer and it has been proven to be a chemical indicator of weakened, dying, or recently felled trees with compromised defenses that, therefore, are vulnerable to attack [27,28]. In each of the two sites, we placed 7 cross-vane window traps spaced at least 150 m apart (a total of 14 traps). Traps remained active from June to October 2019 and they were checked once a day approximately every 20 days. As a caveat, we did not collect beetles in May and we have no samples in June because the lure amount was not sufficient to capture insects. To have a more precise and broader sampling of the saproxylic community residing within each site, traps were placed on mature trees with evident necrosis or on large dead trunks. In 2020, the megaproject included part of Site 1 in its construction site and another expansion is expected in the next years. Each trap was hung on one branch of the selected trees [29]. All beetle specimens have been identified or checked by specialists (as listed in the Acknowledgments and by one of us: ABB). Systematic arrangement of families follows [30], while genera and species are alphabetically listed. Zoological nomenclature follows [31–36], in a few cases modified according to the opinion of the specialists involved [21,37-40]. Trophic and IUCN risk categories, at both the Italian and European level, are also reported in Table 1 for the species considered strictly saproxylic according to [21,41]. Plant species have been identified by botanists (as listed in the Acknowledgments); for deadwood, the identification at the species level was performed by visual examination of the characteristics of the bark and wood tissue. Specifically, trunks and trees on the ground and dead standing plants have been identified through traits such as residual bark and dead leaves (if still present) or from the appearance of wood (to distinguish conifers and deciduous) and from the composition of the ramifications when the bark is completely gone. Botanical nomenclature follows [42].

**Table 1.** Species checklist with the abundance of each species in Site 1, Site 2, and total. Trophic and IUCN categories of risk are reported for saproxylic beetles according to [21]. Trophic categories are CO commensal of SX/XY or of other saproxylic insects, HW saprophagous in small water pools inside hollow trees, MB mycetophagous, MY mycophagous, PR predator, SF feeding on fermented sap, SP saprophytophagous on rotting vegetal matter, SX saproxylophagous, and XY xylophagous. Risk categories (IUCN) are LC least concern, NT near threatened, VU vulnerable, EN endangered, NA not applicable, and DD data deficient. Species names marked with an asterisk [\*] follow the nomenclature of [21,37–40].

	Trophic Categories	Red List IT	Red List EU	Site 1	Site 2	Total
Scirtidae				1	1	2
Prionocuphon serricornis (Müller, 1821)	HW	NT		1	1	2

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Histeridae				0	1	1
Platysoma (Cylister) elongatum elongatum (Thunberg, 1787)	PR	LC		0	1	1
Leiodidae				4	2	6
Agathidium (Neoceble) nigripenne (Fabricius, 1792)	MY	LC		4	2	6
Staphylinidae				6	0	6
Aleochara (Xenochara) sparsa Heer, 1839				1	0	1
Lordithon lunulatus (Linnaeus, 1760)				1	0	1
Omaliinae sp. 1				1	0	1
Omaliinae sp. 2				3	0	3
Geotrupidae				15	0	15
Anoplotrupes stercorosus (Scriba, 1791)				15	0	15
Lucanidae				0	2	2
Sinodendron cylindricum (Linnaeus, 1758)	SX	LC	LC	0	2	2
Scarabaeidae	571	LC	LC	5	8	13
Cetonia (Cetonia) aurata pisana Heer, 1841	SX (SP)	LC		0	1	1
Gnorimus nobilis nobilis (Linnaeus, 1758)	SX	NT	LC	1	2	3
Gnorimus variabilis (Linnaeus, 1758)	SX	VU	NT	3	1	4
Onthophagus (Palaeonthophagus) fracticornis (Preyssler, 1790)	5A	VO	111	1	0	1
Onthophagus (Palaeonthophagus) verticicornis (Laicharting, 1781)				0	1	1
Protaetia (Cetonischema) speciosissima (Scopoli, 1786)	SX	LC	NT	0	1	1
Protaetia (Netocia) morio morio (Fabricius, 1781)	3A	LC	111	0	2	2
Throscidae				2	1	3
Aulonothroscus brevicollis (Bonvouloir, 1859)	SX	DD		1	0	1
	SX SX				-	_
Trixagus carinifrons (Bonvouloir, 1859)	5.7.	DD		1	1	2
Elateridae				6	20	26
Athous (Athous) haemorrhoidalis (Fabricius, 1801)	DD.	X 77 T	) III	0	1	1
Brachygonus megerlei (Lacordaire, 1835)	PR	VU	NT	1	2	3
Brachygonus ruficeps (Mulsant & Guillebeau, 1855)	PR	EN	NT	0	2	2
Cardiophorus (Cardiophorus) anticus Erichson, 1840	PR	NT		1	0	1
Dicronychus cinereus (Herbst, 1784)	P.D.	* * * *		0	3	3
Elater ferrugineus ferrugineus Linnaeus, 1758	PR	VU	NT	3	0	3
Hemicrepidius nigerrimus (Desbrochers des Loges, 1869)*	PR	EN		1	1	2
Nothodes parvulus (Panzer, 1799)				0	11	11
Lampyridae				0	3	3
Lamprohiza boieldieui Jacquelin du Val, 1859				0	3	3
Dermestidae				1	2	3
Anthrenus (Helocerus) fuscus Olivier, 1790				1	1	2
Globicornis (Globicornis) nigripes (Fabricius, 1792)	SX	LC		0	1	1
Ptinidae				11	11	22
Anobium punctatum (DeGeer, 1774)	XY	LC		1	0	1
Hadrobregmus denticollis (Creutzer, 1796)	XY	LC		4	2	6
Hadrobregmus pertinax (Linnaeus, 1758)	XY	LC		0	1	1
Hemicoelus fulvicornis (Sturm, 1837)	XY	LC		0	2	2
Mesocoelopus niger (Müller, 1821)	XY	LC		4	0	4
Ptilinus pectinicornis (Linnaeus, 1758)	XY	LC		1	5	6
Ptinus (Pseudoptinus) rufolimbatus Pic, 1908				0	1	1
Ptinus (Ptinus) subpillosus Sturm, 1837				1	0	1
Teredidae				2	0	2
Teredus cylindricus (Olivier, 1790)	PR	LC		2	0	2
Latridiidae				15	17	32
Cartodere (Aridius) nodifer (Westwood, 1839)	MY	LC		1	0	1
Enicmus atriceps Hansen, 1962	MY	DD		0	1	1
Enicmus brevicornis (Mannerheim, 1844)	MY	LC		5	2	7
· · · · · · · · · · · · · · · · · · ·						0.1
Enicmus rugosus (Herbst, 1793)	MY	LC		7	14	21
Enicmus rugosus (Herbst, 1793) Enicmus testaceus (Stephens, 1830)	MY MY	LC LC		7 2	14 0	21

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Symbiotes gibberosus (Lucas, 1846)	MB	LC		2	1	3
Corylophidae	3.07			2	0	2
Arthrolips obscura (Sahlberg, 1833)	MY	DD		2	0	2
Mycetophagidae	MV	N T A		<b>686</b> 49	27	713
Litargus (Alitargus) balteatus LeConte, 1856	MY	NA	I.C		1	50
Litargus (Litargus) connexus (Geoffroy, 1785)	MY	LC LC	LC LC	632	26 0	658
Mycetophagus (Mycetophagus) quadripustulatus (Linnaeus, 1760)	MY MY	LC	LC	2 1	0	2 1
Mycetophagus (Ulolendus) atomarius (Fabricius, 1787)	MY	NT	LC		0	1
Mycetophagus (Ulolendus) piceus (Fabricius, 1777) Triphyllus bicolor (Fabricius, 1777)	MY	LC	LC	1	0	1
Tetratomidae	IVI I	LC	LC	1		
Hallomenus (Hallomenus) binotatus (Quensel, 1790)	MB	NT		0	1 1	1 1
Melandryidae	IVID	111		1	0	_
Phloiotrya (Phloiotrya) rufipes (Gyllenhal, 1810)	MY	NT		1	0	<u>1</u>
Mordellidae	IVI I	1 1 1		5	0	_
				<u>5</u>	0	<b>5</b> 5
Mordellaria aurofasciata (Comolli, 1837) <b>Zopheridae</b>				3	0	3
Colydium filiforme Fabricius, 1792	PR	NT		1	0	1
Synchita undata Guérin-Méneville, 1844	SX	NT		2	0	2
Tenebrionidae	571	111		10	18	28
Allecula (Allecula) morio (Fabricius, 1787)	SX	LC		4	2	6
Cteniopus (Cteniopus) sulphureus (Linnaeus, 1758)	SP (SX)	LC		1	1	2
Gerandryus aetnensis (Rottenberg, 1871)	SX	EN		0	1	1
Gonodera luperus luperus (Herbst, 1783)	571	2.1		1	0	1
Hymenalia (Hymenalia) rufipes (Fabricius, 1792)	SX	LC		2	1	3
Isomira (Isomira) hypocrita Mulsant, 1856	571	LC		1	0	1
Isomira (Isomira) marcida Kiesenwetter, 1863				0	2	2
Isomira (Isomira) murina murina (Linnaeus, 1758)				0	1	1
Lagria (Lagria) hirta (Linnaeus, 1758)				0	1	1
Mycetochara (Ernocharis) thoracica (Gredler, 1854)	SX	NT		0	5	5
Pentaphyllus testaceus (Hellwig, 1792)	SX	EN		1	1	2
Prionychus ater (Fabricius, 1775)	SX	NT		0	2	2
Pseudocistela ceramboides (Linnaeus, 1758)	SX	NT		0	1	1
Oedemeridae	0.71	- 1 -		0	1	1
Nacerdes (Xanthochroa) carniolica carniolica (Gistel, 1834)	SX	LC		0	1	1
Salpingidae	0.71	20		17	7	24
Salpingus planirostris (Fabricius, 1787)	SX	LC		6	6	12
Salpingus ruficollis (Linnaeus, 1760)	SX	NT		11	1	12
Anthicidae				0	1	1
Microhoria fasciata fasciata (Chevrolat, 1834)				0	1	1
Scraptiidae				3	0	3
Anaspis (Anaspis) lurida Stephens, 1832	SX	LC		2	0	2
Anaspis (Silaria) brunnipes (Mulsant, 1856)	-			1	0	1
Biphyllidae				90	22	112
Biphyllus frater (Aubé, 1850)	SX (MY, PR)	LC		3	0	3
Diplocoelus fagi (Chevrolat, 1837)	SX (MY, PR)	LC		87	22	109
Cleridae	, ,			2	2	4
Clerus mutillarius mutillarius Fabricius, 1775	PR	NT		1	1	2
Opilo mollis (Linnaeus, 1758)	PR	LC		1	0	1
Thanasimus formicarius formicarius (Linnaeus, 1758)	PR	LC		0	1	1
Melyridae				4	4	8
Clanoptilus (Clanoptilus) emarginatus (Krauss, 1902)				0	1	1
Danacea (Danacea) nigritarsis alpina Pic, 1894				2	0	2
· ·				1	0	1
Danacea (Danacea) pallipes (Panzer, 1793) Dasytes (Mesodasytes) plumbeus (Müller, 1776)	PR	LC		1 1	0 3	1 4

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Rhizophagus (Rhizophagus) ferrugineus (Paykull, 1800)	MY (PR)	LC		0	1	1
Erotylidae				4	0	4
Cryptophilus propinquus Reitter, 1874*	MY	[LC]		2	0	2
Dacne (Dacne) bipustulata (Thunberg, 1781)	MB	LC	LC	1	0	1
Triplax russica (Linnaeus, 1758)	MB	LC	LC	1	0	1
Cryptophagidae				377	1232	1609
Caenoscelis sibirica Reitter, 1889	MY	[DD]		1	0	1
Cryptophagus dentatus (Herbst, 1793)	MY	LC		5	0	5
Cryptophagus micaceus Rey, 1889	MB	DD		2	2	4
Cryptophagus quadridentatus Mannerheim, 1843*				10	0	10
Cryptophagus reflexus Rey, 1889				120	199	319
Cryptophagus scanicus (Linnaeus, 1758)	MY	LC		239	1031	1270
Laemophloeidae				89	2	91
Cryptolestes duplicatus (Waltl, 1839)	MY	NT		1	0	1
Cryptolestes ferrugineus (Stephens, 1831)	SX	LC		9	0	9
Laemophloeus monilis (Fabricius, 1787)	MY	LC		7	2	9
Leptophloeus hypobori (Perris, 1855)	CO	LC		1	0	1
Notolaemus unifasciatus (Latreille, 1804)	MY	NT		4	0	4
Placonotus testaceus (Fabricius, 1787)	SX	LC		67	0	67
Nitidulidae				168	133	301
Carpophilus (Ecnomorphus) sexpustulatus (Fabricius, 1792)	MY	NT		3	0	3
Epuraea (Epuraea) fuscicollis (Stephens, 1835)	SF	LC		131	131	262
Epuraea (Epuraea) marseuli Reitter, 1873	MY	LC		3	0	3
Haptoncus ocularis (Fairmaire, 1849)*	SF (SP)	NA		25	2	27
Stelidota geminata (Say, 1825)	SF (SP)	NA		6	0	6
Cerambycidae	01 (01)			12	4	16
Chlorophorus glabromaculatus (Goeze, 1777)	XY	LC		1	0	1
Leiopus nebulosus nebulosus (Linnaeus, 1758)	XY	LC		1	1	2
Morimus asper (Sulzer, 1776)*	XY	LC		2	0	2
Pachytodes cerambyciformis (Schrank, 1781)	SX	LC		1	0	1
Pachytodes erraticus erraticus (Dalman, 1817)	SX	LC		1	0	1
Parmena balteus (Linnaeus, 1767)	XY	LC		0	1	1
Phymatodes (Phymatodes) testaceus (Linnaeus, 1758)	XY	LC	LC	0	1	1
Rutpela maculata maculata (Poda von Neuhaus, 1761)	XY	LC		4	1	5
Stenurella bifasciata bifasciata (Müller, 1776)	SX	LC		1	0	1
Stenurella melanura (Linnaeus, 1758)	SX	LC		1	0	1
Chrysomelidae				0	2	2
Gonioctena (Goniomena) quinquepunctata quinquepunctata (Fabricius,						
1787)				0	1	1
Luperini sp.				0	1	1
Anthribidae				1	1	2
Noxius curtirostris (Mulsant & Rey, 1861)	XY	LC		1	0	
Tropideres albirostris (Schaller, 1783)	XY	LC		0	1	1
Curculionidae	χī	LC		1008	210	1218
Acalles (Acalles) parvulus parvulus Boheman, 1837	SX	LC		1	0	1
Anisandrus dispar (Fabricius, 1792)	MY	LC		20	19	39
Hylastinus fankhauseri Reitter, 1895	XY	LC		20	2	4
Hylesinus toranio (Danthoine, 1788)	XY	LC		1	2	3
Magdalis (Magdalis) phlegmatica (Herbst, 1797)	Λ1	LC		0	1	1
Phyllobius (Dieletus) argentatus argentatus (Linnaeus, 1758)				0	1	1
Scolytus intricatus (Ratzeburg, 1837)	XY	LC		0	1	1
	MY	LC		984	183	1 1167
Xyleborinus saxesenii (Ratzeburg, 1837)	1 <b>V1 1</b>	LC		704	103	110/
Xyleborus monographus (Fabricius, 1792)	MY	LC		0	1	1

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Around each of the 14 traps, we collected parameters of forest structure in a circular plot of 10 m radius (area per plot: 314 m<sup>2</sup>). Thus, the plots are 14, 7 per site. In each plot, we have counted the number of living and dead trees and identified the species; thus, we recorded the abundance and species richness of living and dead trees. Then we counted the number of downed logs (defined as pieces of stem or branch that have fallen and have at least 5 cm diameter and length > 1 m), standing dead trees or snags (dead standing trees, dbh > 5 cm, and taller than 1.3 m), and stumps (short, vertical pieces created by cutting or by windthrow, diameter at the top > 5 cm, and height < 1.3 m). Similarly to [43], we divided the total deadwood into coarse woody debris (CWD) such as the fraction of deadwood laying on the ground (including logs and dead downed trees that are not found in the study area) and the fraction of deadwood still standing (standing dead trees or snags and stumps; hereafter STD). For each standing dead tree or snag the minimum diameter at breast height was collected. The height of standing dead trees was estimated using the height-diameter relationship (hypsometric curve) available from local forest inventories [44]. Coarse woody debris (CWD) was surveyed, measuring their length/height and diameter, and recording the species. The volume of standing dead trees (SDT) was calculated by double-entry volume equations [45], while the volume of logs and stumps was calculated through the cone trunk formula. The decay stage of CWD was assigned using the five-class scale used by [46].

#### 2.4. Data Analysis

#### 2.4.1. Characterization of Forested Sites and Beetle Communities

To describe differences between the two forest sites, we applied Wilcoxon–Mann–Whitney and Principal Component Analysis (PCA) to forest parameters measured around the experimental mature trees. In order to compare saproxylic beetle communities between the two forest sites, we applied rarefaction and extrapolation sampling curves using the effective number of species collected [15,47]. Moreover, to investigate beetle communities, measured as Sorensen dissimilarity and species turnovers measured as Simpson dissimilarity, we used betapart package in R.

#### 2.4.2. Relationship between Beetle Diversity and Forest Variables

Nonmetric multidimensional scaling (NMDS) was used to identify plot similarities or dissimilarities in relation to species abundance, using the Bray–Curtis distance on abundance data (metaMDS function of the "vegan" package). Then to identify which forest variables influenced site species abundance dissimilarities, we tested all the forest variables using the *envfit* function of the "vegan" package, with 999 permutations (following [15]). Finally, to investigate which forest variables influenced saproxylic beetle communities, we performed a generalized linear model (GLM) using beetle species richness and abundance as dependent variables and the top four forest not-correlated variables that contributed most to the PCA analysis (eigenvalues > 1) as fixed factors. To account for overdispersion, tested with the "Dharma" package [48], we used a negative binomial distribution. Each PCA, NMDS, and GLM models were performed in R v. 4.1.3 [49].

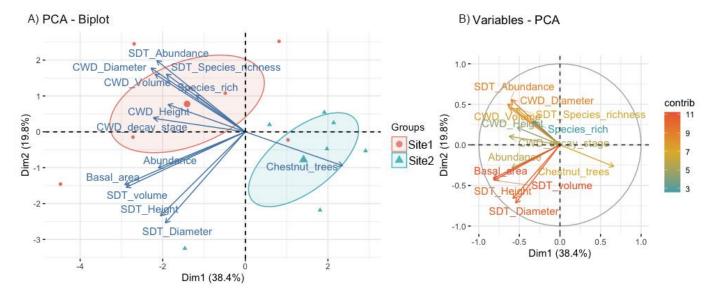
#### 3. Results

## 3.1. Characterization of Forested Sites

Dead downed trees (defined as a single tree—in one piece or more pieces unambiguously belonging to the same tree—dead and laying on the ground) were not found within plots in the study area. Site 1 and Site 2 presented differences in the forest structure parameters measured in each plot: CWD volume, SDT volume, CWD diameter, tree species richness (Table S1 in Supplementary Material), SDT richness, and abundance (Table 2). The two sites were significantly different in CWD volume (p-value = 0.047\*, R = -0.22; Table 2) and diameter (p-value = 0.016\*, R = -0.67; Table 2) and in STD abundance (p-value = 0.042\*, R = -0.389; Table 2).

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PCA results showed the first two axes explained 55.9% of the total variance (first axis 37.1% and second 18.8%; Figure S1 in Supplementary Material). The proportion of sweet chestnut trees was negatively correlated with the first axis while living trees species richness, SDT species richness, and SDT abundance. In relation to CWD diameter, height, decay stage, and volume were negatively correlated with the second axis (Figure 1). The two sites were clearly separated along both axis (Figure 1). The top four variables that describe most the forest are volume (PC1 –0.364, the diameter of STD (PC2 –0.446), CWD height (PC3 -0.511), and species richness (PC4 0.599) (Table S2 in Supplementary Material).



**Figure 1.** PCA results on forest variables: (**A**) Biplot and (**B**) contributions of each variable. (**A**) Red points (one point for each trap) represent Site 1; while blue triangles (one triangle per each trap) Site 2. The large red point and the large blue triangle represent centroids of the all traps per site. (**B**) All variables are presented with their distribution and contribution in the cartesian axes.

# 3.2. Characterization of Beetle Communities

In total, we collected 4304 specimens of which 4289 were determined at the species level. Overall, we identified 128 species of which 96 (corresponding to 3892 specimens) are considered [21] saproxylics (checklist in Table 1). Site 1 presents more species and higher abundance than Site 2. In Site 1, 2552 specimens have been collected belonging to 90 species, of which 2387 specimens belonging to 74 species are considered saproxylics. The most abundant species in Site 1 were *Xyleborinus saxesenii* (Ratzeburg, 1837) and *Litargus connexus* (Geoffroy, 1785), respectively, with 984 and 632 specimens collected. In Site 2, 1737 specimens have been collected belonging to 74 species, of which 1505 specimens belonging to 56 species are considered saproxylics. The most abundant species in Site 2 were *Cryptophagus scanicus* (Linnaeus, 1758) and *C. reflexus* Rey, 1889, respectively, with 1031 and 199 specimens collected. During our samplings we detected two species new to Italy: *Caenoscelis sibirica* Reitter, 1889 (Cryptophagidae) and *Ptinus rufolimbatus* Pic, 1908 (Ptinidae), the latter previously known only from Bulgaria [31]. Moreover, we confirm the presence of the firefly *Lamprohiza boieldieui* Jacquelin du Val, 1859 (Lampyridae) in Italy, apparently no longer collected since 1932 [50].

**Table 2.** Thirteen environmental variables collected in the field to characterize the plot area surrounding each window trap. CWD stands for Coarse Woody Debris, while SDT stands for Standing Dead Trees. Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1.

Forest Variables	Description	Site	Site 1 Site 2		2	Wilcoxon <i>p</i> -Size Effect		
	Description	Mean	SD	Mean	SD	Value	(R)	

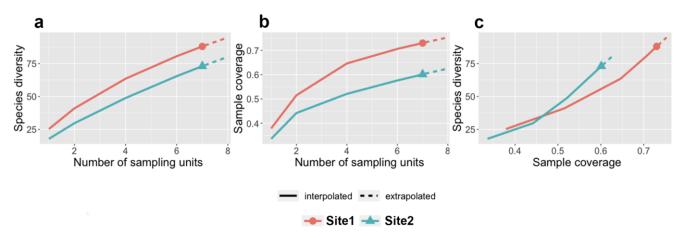
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Basal area	Sum of the cross-sectional area at breast height (1.3m aboveground) of standing dead trees or snags (m²/ha)	1112.04	1406.77	323.17	591.38	0.219	0
CWD volume	Volume of coarse woody debris and logs (CWD) (m³/ha)	46.308	43.280	2.658	3.752	0.047 *	-0.224
SDT volume	Volume of standing dead trees or snags and stumps (m³/ha)	374.238	505.998	112.630	221.915	0.219	0
SDT Height	Mean tree height of Standing dead trees or snags (m)	15.78	8.201	13.28	11.488	0.578	0.704
SDT Diameter	Mean tree diameter of standing dead trees or snags and stumps (cm)	91.272	83.506	92.488	142.996	0.688	0.704
CWD Height	Mean tree height of CWD (m)	4.60	1.918	3.31	3.154	0.687	-0.224
CWD Diameter	Mean of max-diameter of CWD (cm)	80.178	19.163	28.531	28.152	0.016 *	-0.67
CWD decay stage	Assigned using the five- class scale used by [29].	2.13	0.395	1.33	1.414	0.310	0.224
Species rich	Species richness of all living trees	5.43	2.225	3.57	1.512	0.142	-0.388
Abundance	Abundance of all living trees	36.43	16.672	34.57	10.753	0.937	0
SDT Species richness	Species richness of SDT	2	1	0.86	0.690	0.066	-0.204
SDT Abundance	Abundance of SDT	6.57	4.685	1.29	1.113	0.042 *	-0.389
Chestnut trees	Proportion of sweet chestnut trees	0.324	0.249	0.670	0.118	0.016	0.707

Among saproxylic beetles, 32 species (33%) are mycophagous and mycetophagous (MY and MB), 29 species (30%) are saproxylophagous (SX), 17 species (18%) are xylophagous (XY), 12 species (12%) are predators (PR), 3 species (3%) feed on fermented sap, and the other three species (each species 1% of the total saproxylic beetles) belong to other three different categories (saprophagous in small water pools, saprophytophagous, and commensal; Table 1). Other trophic categories are poorly represented. Moreover, 7 species are threatened in Italy [21]: *Brachygonus megerlei* (Lacordaire, 1835) (VU), *B. ruficeps* (Mulsant & Guillebeau, 1855) (EN), *Elater ferrugineus* Linnaeus, 1758 (VU), *Gerandryus aetnensis* (Rottenberg, 1871) (EN), *Gnorimus variabilis* (Linnaeus, 1858) (VU), *Hemicrepidius nigerrimus* (Desbrochers des Loges, 1869) (EN), and *Pentaphyllus testaceus* (Hellwig, 1792) (EN), while 5 species were assessed as near threatened (NT) in Europe [41]: *Brachygonus megerlei*, *B. ruficeps*, *Elater ferrugineus*, *Gnorimus variabilis*, and *Protaetia speciosissima* (Scopoli, 1786).

Regarding rarefaction and extrapolation curves on the community species richness, Site 1 has higher species richness and sample coverage than Site 2 (Figure 2) and the sample coverage is higher for Site 1 (Figure 2b,c).

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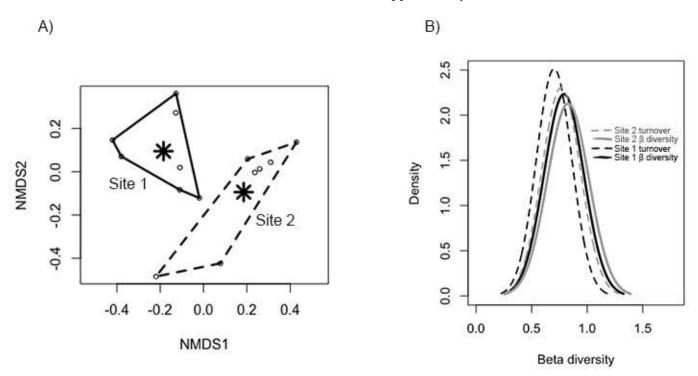


**Figure 2.** Rarefaction curves: (a) Sample-size-based rarefaction curves on species richness considering all the species; (b) Sample-size-based rarefaction curves on sample coverage considering all the species; (c) Sample coverage rarefaction curves on species richness considering all the species.

# 3.3. Relationship between Beetle Diversity and Forest Variables

We found that the communities differed between the two study sites (Figure 3a). However, the beetle communities between the two sites did not greatly differ in beta diversity and did not present large differences in terms of species turnover (Figure 3b).

The abundance and species richness of standing dead trees is the forest structure parameter that significantly affected the difference in beetle abundance and species richness between the two sites (Table S3 in Supplementary Material).



**Figure 3.** A) Nonmetric multidimensional scaling (NMDS) convex hulls on log-transformed abundance data of the most abundant species of the beetle community divided by the two sites: Site 1 filled line convex hull and Site 2 dashed convex hull. Within each convex hull the \* represents the centroid. The goodness of fit for Sites  $r^2 = 0.385$ , p-value = 0.004 \*\*. Signif. codes: 0.004 \*\*\* 0.001 \*\*\* 0.00

GLM results highlighted that the larger impact on both components of beetle diversity (species richness and abundance) was linked to the CWD volumes. Indeed, the larger

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volume promotes higher abundance (z value = 3.48, p-value < 0.001\*\*\*; Table S4 in Supplementary Material) and higher species richness (z value = 4.05, p-value < 0.001\*\*\*; Table S5 in Supplementary Material). Moreover, we found that the greatest species richness is found smaller diameter of standing dead trees (z value = -2.94, p-value = 0.003\*\*; Table S5 in Supplementary Material). Finally, we found that increasing living tree species richness significantly increases beetle species richness (z value = 2.00, p-value = 0.046\*; Table S5 in Supplementary Material).

#### 4. Discussion

Our study was performed to investigate the forest beetle community with a particular focus on two sites that differ in their previous and future management. We found a rich community that included 4289 specimens belonging to 128 beetle species. Of particular interest is the finding of two species new to Italy, namely *Caenoscelis sibirica* and *Ptinus rufolimbatus*, and some species considered threatened in Italy [21], such as the rare *Gerandryus aetnensis* [51].

Within the two sites studied, the abundance of beetle species is best determined by the volume of the coarse woody debris which also influences the beetle species richness (in accordance with [52]) together with the living tree species richness. In our study, the volume of dead-standing trees is slightly shaping beetle species richness and it is determined by a large number of trees with a small diameter. The two sites differ in their structure (e.g., CWD volume and diameter and higher tree species richness) which might be related to the different forest management applied in the last decade (in accordance with [6,11]). In addition, Site 1 hosted a higher abundance and richness of saproxylic beetle species in comparison with Site 2 (2252 vs. 1737 specimens and 90 vs. 74 species). As already proven in [14], different abundance and species richness of standing dead trees might determine the different abundance and species richness of beetles. Those differences between the sites resulted in differences in saproxylic beetle communities which need to be accounted for in planning species-specific conservation programs. Thus, now the two beetle communities in the sites are different (Figures 1 and 3), but appropriate management (e.g., [12]) that increases deadwood and species richness of trees can shape the forest in order to support the local community threatened by the expansion of the construction site of the Turin-Lyon High-Speed Railway Line (similarly to [5]).

The two sites investigated have been subjected to two completely different management histories during the last decades: Site 1 is an unmanaged forest that has been abandoned for more than 60 years, while Site 2 was recently abandoned or subjected to lowintensity management for production purposes. Therefore, Site 2 is characterized by a more abundant presence of sweet chestnut trees than Site 1 (172 vs. 85; Table S1 in Supplementary Material). Differences are also revealed in the parameters that characterize the structure of the two forest sites, where Site 1 showed a more abundant and larger amount of coarse woody debris and a higher number of standing dead trees (SDT). Generally, a lower amount of deadwood is found in managed rather than unmanaged forest habitats [53]. Managed forests are periodically subjected to maintenance that can involve deadwood and unwanted tree species removal to guarantee easier working conditions and facilitate the production purpose [54,55]. The removal of deadwood under managed conditions represents for saproxylic beetles a reduction in potential substrates that provide them with favorable microhabitats [13,56] and, therefore, explains a higher species richness and abundance of saproxylic beetles in Site 1 in comparison with Site 2. A potential solution suggests an enrichment of deadwood within managed forest habitats, thus increasing microhabitats availability that can result in higher diversity and abundance of saproxylic beetles [12].

Among the different forest parameters, the difference in the abundance and species richness of standing dead trees (SDT) explains the most difference in saproxylic beetle abundance between the two Sites (Table S3 in Supplementary Material). Previous evidence has shown that managed forests, such as Site 2, are generally lacking in the presence

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of standing dead trees and reduction of deadwood [57], thus reducing the number of favorable microhabitats [8,56] which can host deadwood dependent communities [58]. Indeed, standing dead trees might offer a higher number of potential microhabitats [8], providing specific favorable conditions and substrates supporting saproxylic beetles through shelter, forage, or breeding. Diverse species of standing dead trees can also host more microhabitats and can be able to be a hotspot for an increased abundance of saproxylic beetles as measured in our sites. The importance of standing dead trees is also given by their role as reservoirs of rare saproxylic beetle species [59,60]. For these reasons, they should be placed at the center of the discussion for designing conservation plans in forest habitats to increase their number within forest habitats [12].

Some potential caveats should, however, be considered when interpreting our results. The first caveat involves the forest structure parameters that we did not measure and that have shown to be a potential cause of the difference in saproxylic beetle community between and within forest sites. These parameters might involve a measure of the number of microhabitats present in deadwood [58], the canopy openness [17,61], the presence of deadwood-related food resources such as saproxylic fungi [18], and the living tree species composition surrounding our cross-wane traps [14]. A second potential caveat involves the scale effects of the parameters measured to explain differences in saproxylic beetle communities between and within forest sites. Even if sites support different beetle communities, beta diversity and turnover did not greatly differ between sites. Indeed, tree species richness might affect the species turnover [62]. However, other factors such as microhabitat presence also might affect beetle community and turnover [63]. Moreover, differences in the saproxylic beetle community might have emerged by measuring forest structure parameters on a larger spatial scale instead of a single plot around experimental mature trees [64]. However, the study was conducted to understand the saproxylic biodiversity that might be compromised because of the expansion site of the Turin-Lyon High-Speed Railway Line and if a close forest site might support those species. Despite these caveats, we are confident that the beetle species and their abundance are representatives of the two Sites. In addition, our study is framed as a preliminary work to explore the available saproxylic beetle fauna to which more detailed studies to organize and plan conservation projects (e.g., [5]) will follow.

#### 5. Conclusions

Our study is framed in a context that places the protection of chestnut-dominated forests as a priority for the development of effective conservation of the saproxylic biodiversity residing in these habitats. Chestnut-dominated forest importance has indeed been highlighted for a long time by European regulations, i.e., the EU Habitats Directive and Natura 2000, but unfortunately concrete actions of protection and maintenance are far from being an established reality, especially in fragile areas characterized by a high human pressure or adverse climatic [20]. The preservation of chestnut-dominated forests in the landscape cannot disregard the identification and management of the habitat traits fostering biodiversity, i.e., saproxylic beetles, richness, abundance, and diversity. We indeed revealed that the differences in structure between the two forest sites in Clarea valley are likely linked to the different management to which they are subjected, in turn influencing saproxylic beetle species communities. Standing dead trees (abundance and species richness) have shown to be a main reservoir to preserve saproxylic beetles and conservation plans should strongly consider their maintenance in forest habitats. However, we also highlighted the necessity of including more forest structure features that can support beetle saproxylic communities. This information can be crucial in future potential local conservation plans, chiefly in understanding how and on which parameters management actions should be applied in the areas not affected by the expansion of the Turin-Lyon High-Speed Railway Line. Potential interventions include the local increase in deadwood volume and the enrichment of living tree species which will support larger and richer beetle communities. The increases in forest ground complexity can provide benefits not only to Diversity 2023, 15, 556

saproxylic beetles but to several other woody-related taxa, thus representing a multiplewin solution essential for conservation plans in forest habitats.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15040556/s1. Table S1: List of tree species and their abundance. Table S2: Forest variable influence on each PCA axis. Table S3. Forest variables that affect differences between beetle communities in the two sites 1 and 2. Table S4: Results of GLM analysis on species abundance. Table S5: Results of GLM analysis on species richness. Figure S1: PCA results: PCA screen.

**Author Contributions:** Conceptualization, I.P., S.B., A.B.B., D.B. and V.D.P.; Formal analysis, I.P. and E.C..; Investigation, I.P., V.D.P., D.B., A.B.B. and R.B.; Methodology, I.P. and L.C.; Supervision, A.B.B.; Visualization, I.P.; Writing—original draft, I.P., A.B.B., D.B., V.D.P. and R.B..; Writing—review and editing, I.P., A.B.B., D.B., V.D.P., R.B., S.B., E.C. and L.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the company TELT sas.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: In Table 2.

Acknowledgments: We are thankful to Elisa Di Marco for her support during fieldwork. We are grateful for their contribution to species identification to Paolo Audisio (Nitidulidae), Dario Cioppa (Dermestidae), Enzo Colonnelli (Curculionoidea), Silvio Cuoco (Staphylinidae), Michael Eifler (Ptinidae), Jens Esser (Cryptophagidae), Fabrizio Fabbriciani (Scarabaeidae), Fabrizio Fanti (Lampyridae), Gabriele Franzini (Melyridae Malachiinae), Gianfranco Liberti (Melyridae Dasytinae), Gianluca Nardi (Anthicidae), Giuseppe Platia (Elateridae), Enrico Ruzzier (Mordellidae; Scraptiidae), Zdenek Svec (Leiodidae), Michael Lonati and Marco Pittarello (plants).

**Conflicts of Interest:** The authors declare no conflict of interest.

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