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The carabids (Coleoptera Carabidae) of the new established "Foresta Carpaneta" (Italy, Lombardy) - the first 14 years (2006/2007 and 2020/2021) of a long term monitoring project

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Abstract

Between 2003 and 2006, the Regional Agency for Services to Agriculture and Forestry of Lombardy planted a new forest in the province of Mantua (San Giorgio Bigarello), the "Foresta Carpaneta". One aim for this new forest is to associate the conservation of biodiversity and recreational activities. To investigate the evolution of biological diversity of the "Foresta Carpaneta", carabids were one of the groups chosen, because they are good biological indicator for forest habitats. Pitfall traps were placed in two sites in the years 2006/07 and 2020/21. In the first sampling period, when the forest was still very young and open, an average of 23.5 species were caught at each site. All species caught were typical for open grasslands, ecotones, pioneer vegetation and fields. In 2020/21, in the now more mature forest an average of only 9 species were collected in each site. This decrease in species richness was accompanied by a considerable difference in the composition of the carabid fauna. During the second sampling period two forest generalists were caught for the first time: *Notiophilus rufipes* Curtis 1829 and *Pterostichus* (*Platysma*) *niger niger* (Schaller 1783). However, carabid species that depend on mature forest stands were absent. These species, which generally disperse poorly, can only arrive from old forests. However, the "Foresta Carpaneta" is separated from the nearest old forest by fields, streets, urban centres and industrial areas, which are barriers for the dispersal of forest specialist carabids. These results suggest that complete forest communities will not autonomously establish in the "Foresta Carpaneta" in the foreseeable future.

Key words: Carabidae, Po Plain, lowland forest, monitoring, reforestation.

Introduction

During most of the early Holocene, the landscape of the Po River Plain was covered by a thick, deciduous forest, with major deforestation starting from the Bronze Age (Starnini *et al*., 2018). Over the centuries the human impact has led to a reduction of tree cover and today only small and isolated forest remnants remain, surrounded by a matrix typically composed of towns and arable crops (Ruffo, 2002; Buffa and Villani, 2012). Forest loss and fragmentation are major threats to biodiversity and ecosystem functioning through their effects on habitat availability, quality and connectivity (Fahrig, 2003; Haddad *et al*., 2015, Marrec *et al*., 2021). Generally, ancient forest patches (i.e. patches that have continuously existed for centuries) harbour a high number of forest-specialist species with limited dispersal abilities (Desender *et al*., 2005; Brunet, 2007; Marrec *et al*., 2021). The few remnants of the ancient forest of the Po Valley conserve much of the remaining biodiversity and protect relict populations of typical forest species (Brandmayr, 1975; Brandmayr and Brunello Zanitti, 1986; Vigna Taglianti, 2004; Buffa and Villani, 2012; Bonato *et al*., 2014) and are thus of vital importance for the conservation of silvicolous species in the fragmented, agricultural landscape of the Po Valley (Buffa and Villani, 2012). They also represent potential source populations for the colonization of new forests, which have been planted in recent years.

Biodiversity loss is a global threat and appears to be of particular concern for insect communities. The proportion of insect species in decline (41%) has recently been estimated to be twice as high as that of vertebrates (Sánchez-Bayo and Wyckhuys, 2019) and insects have declined in Europe in recent decades (Hallmann *et al*., 2017; Wagner, 2020; Warren *et al*., 2021). Important declines of insect biomass and species richness have also been reported in nature protection areas (Hallmann *et al*., 2017; Rada *et al*., 2019). Carabid beetles are one of the groups that have decreased significantly during investigated time spans (33-100 years) in Europe, particularly specialist species of large body size (Kotze and O'Hara, 2003; Skarbek *et al*., 2021), and species numbers of carabids declined even in old nature reserves (Homburg *et al*., 2019).

Carabidae constitute one of the most species-rich beetle families worldwide with over 40,000 species (Brandmayr *et al*., 2005); of these, about 1646 taxa belong to the Italian fauna (Casale *et al*., 2021). They also readily react to abiotic and biotic variation and they respond predictably to small-scale and landscape-level phenomena and therefore are considered good biological indicators (Brandmayr *et al*., 2005; Gobbi, 2008; Kotze *et al*., 2011; Langraf *et al*., 2019), also for forest habitats (Brandmayr *et al*., 2005; Gobbi, 2008; Kotze *et al*., 2011; Langraf *et al*., 2019; Marrec *et al*., 2021). Importantly, the stable taxonomy, well-known biology, knowledge of species-specific dispersal ability and habitat preferences makes carabids useful indicators of the severity of habitat fragmentation (Niemelä, 2001).

In 2003 the Regional Agency for Services to Agriculture and Forestry of Lombardy (ERSAF), started a forestry project at the Azienda Agroecologica Carpaneta

(Mantua, San Giorgio Bigarello) and the planting of the "Foresta Carpaneta" was completed in 2006. This project is part of the regional programme "Dieci grandi foreste di pianura", which aims to create new multifunctional forest areas in the Po Valley that combine the conservation of biodiversity and recreational activities (Assini and Sartori, 2009). Newly established forests on set-aside farmlands that were previously under intensive agricultural management do not create ecologically valuable forest habitats in the short term (Buffa and Villani, 2012). Even after 30 years, newly planted forests are colonized mainly by extremely vagile carabids, but not by species typical for forests (Baini *et al*., 2012), since most of those are apterous or brachypterous (Casale *et al*., 1982; Brandmayr *et al*., 2005). Given that the newly established "Foresta Carpaneta" is situated in a predominantly agrarian landscape, with the nearest ancient forest located at approximately 10 km, the colonization of the study area by forest-specialist carabid species is likely to be a long-term process. Here we present the results of two monitoring campaigns conducted within a long-term project in the "Foresta Carpaneta": a first one in 2006/07 and the second in 2020/21. The aims of this study are to document the evolution of the carabid assemblage of the new forest over a time span of 14 years, and to evaluate the colonization of "Foresta Carpaneta" by carabid species typical of lowland forests.

Materials and methods

Study area

In 2003 ERSAF, started a forestry project at the Azienda Agroecologica Carpaneta, municipality of San Giorgio Bigarello in the province of Mantua, close to the village of Gazzo (figure 1). This site is located at approximately 27 m a.s.l.. Here the soil has been classified as Haplic Cambic Calcisol (Gleyic, Hypercalcic, Loamic) (Class. WRB 2014) and the water table is always present at a depth of 70 cm. The new "Foresta Carpaneta", a total of 55 ha, was planted between 2003 and 2006 and aims also to protect the genetic heritage of the English oak of the wider Po Valley (Ducci, 2007). The main tree species planted were *Quercus robur*, *Carpinus betulus*, *Ulmus minor*, *Fraxinus angustifolia* and *Acer campestre* (Emma Minari, personal communication). Some parts of this forest were thinned between 2011 and 2013 (area S2 in figure 1), while in the remaining areas a first thinning was carried out in 2022. The trees cut were mainly the fast-growing species (*Populus alba* and *Ulmus minor*).

Trapping

Pitfall traps were placed in two sites: S1 (45.180845N 10.891687E) and S2 (45.18586N 10.891064E) (figure 1). In each site, 4 traps were placed at the corners of a square with the sides of 10 m. Each pitfall trap consisted of a plastic cup with a diameter of 10 cm at the top, 6 cm at the base and a height of 13 cm. These were filled three quarters with a solution of white wine vinegar (acidity 6%) saturated with table salt to attract and preserve insects (Allegro and Sciaky, 2003). The traps were buried in the soil and covered with an inverted plant saucer (diameter 20 cm) for protection and the border of the saucer was raised to about 1-2 cm above the ground. The traps were checked and emptied fortnightly. Monitoring of the carabid fauna lasted an entire year and was carried out from 16.II.2006 to 16.II.2007 and from 16.X.2020 to 27.X.2021. The begin of the second trapping period had to be delayed due to the restrictions imposed during the COVID-19 pandemic.

After sorting, the adults of carabids were identified by A. Vigna Taglianti (material collected in 2006/07) and by N. Pilon (material collected in 2020/21). Nomenclature and systematics follow Löbl and Löbl (2017).

Statistics

Compositions of the assemblages collected in 2006/07 and 2020/21 were compared by two-dimensional Non-metric Multidimensional Scaling (NMDS). The beetles collected in each trap and during each year were pooled,

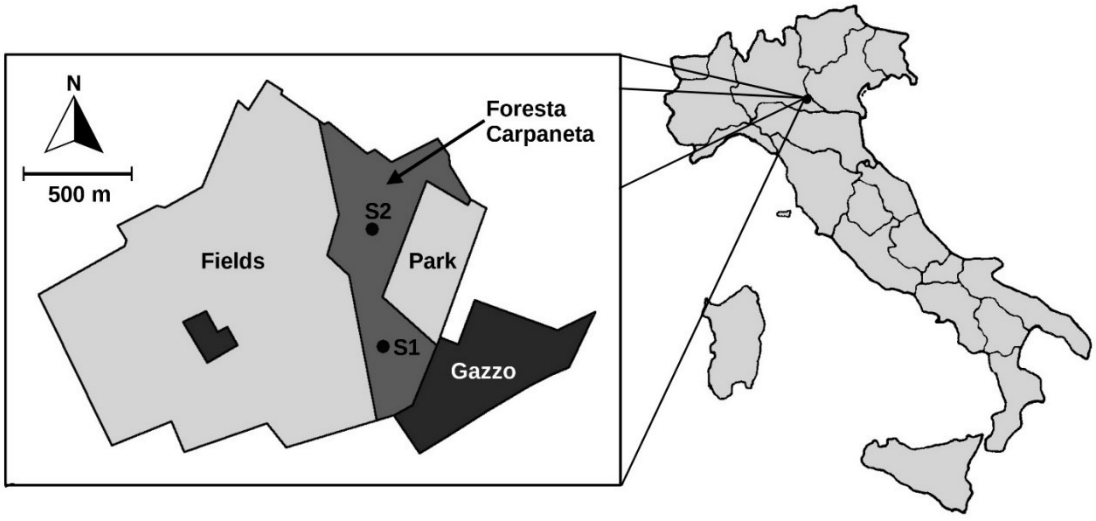

Figure 1. Location of the Azienda Agroecologica Carpaneta, with the "Foresta Carpaneta" and indications on the position of site 1 (S1) and site 2 (S2). Gazzo is the nearest village.

resulting in a total of 8 datasets. The NMDS was carried out using the abundance data and employing the function metaMDS, which is incorporated in the statistical package vegan (Oksanen *et al*., 2020) using R (version 4.2.0 http://www.r-project.org/). The zero-adjusted Bray-Curtis coefficient (Clarke *et al*., 2006) was used as the pairwise distance among samples. In the resulting plot the groups were connected to the cluster centroids by a line using the function 'ordispider' present in the package vegan (Oksanen *et al*., 2020).

Results and discussions

A total of 3259 carabids were collected in the years 2006/07 and 2020/21, which belonged to 36 species (table 1). In 2006/07 an average of 1988 individuals were caught in each site; this number decreased to an average of 88.5 individuals in 2020/21. The number of species collected was 31 in 2006/07 (average number per site: 23.5) and 12 in 2020/21 (average number per site: 9) (table 2).

The NMDS comparing the assemblage compositions returned a stress value of 0.037, which indicates that the configuration obtained is excellent and allows for a detailed inspection of the graphical representation (Zuur *et al*., 2007). The result of the NMDS, using the zero-adjusted Bray-Curtis coefficient (Clarke *et al*., 2006) is shown in figure 2. Within each survey year, the assemblages of individual sites were homogeneous and distinct because the various clusters did not overlap. The greater distance of the clustered groups between the years 2006/2007 and 2020/2021 showed that the assemblages captured at the two sites in the same year were more similar than the assemblages sampled at the same site 14 years apart.

Table 1. List of the carabid species captured in 2006/07 and 2020/21 with pitfall traps in the stations S1 and S2 of "Foresta Carpaneta". The number of individuals captured is indicated for each station and each year.

Species	(2006/07)		(2020/21)	
	S1	S2	S1	S2
Acinopus (*Acinopus*) *picipes* (Olivier 1795)	1	0	0	0
Amara (*Amara*) *aenea* (De Geer 1774)	15	1	3	0
Anchomenus dorsalis dorsalis (Pontoppidan 1763)	9	40	0	0
Anisodactylus (*Pseudanisodactylus*) *signatus* (Panzer 1796)	0	1	0	0
Badister (*Badister*) *bullatus* (Schrank 1798)	1	1	0	0
Brachinus (*Brachinus*) *elegans* Chaudoir 1842	0	1	0	0
Brachinus (*Brachynidius*) *crepitans* (F. 1792)	0	52	0	0
Brachinus (*Brachynidius*) *explodens* Duftschmid 1812	1	12	0	0
Brachinus (*Brachynidius*) *sclopeta* (F. 1792)	17	419	0	0
Calathus (*Calathus*) *fuscipes punctipennis* Germar 1823	110	894	52	3
Calathus (*Neocalathus*) *melanocephalus melanocephalus* (L. 1758)	3	0	26	9
Calosoma (*Calosoma*) *maderae maderae* (F. 1775)	1	0	0	0
Cylindera (*Cylindera*) *germanica germanica* (L. 1758)	13	8	0	0
Diachromus germanus (L. 1758)	3	0	0	0
Dinodes (*Dinodes*) *decipiens* (L. Dufour 1820)	0	5	0	0
Harpalus (*Cryptophonus*) *tenebrosus tenebrosus* (Dejean 1829)	1	0	0	0
Harpalus (*Harpalus*) *affinis* (Schrank 1781)	11	3	0	0
Harpalus (*Harpalus*) *dimidiatus* (P. Rossi 1790)	35	1	0	0
Harpalus (*Harpalus*) *distinguendus distinguendus* (Duftschmid 1812)	446	167	1	1
Harpalus (*Harpalus*) *oblitus oblitus* Dejean 1829	5	10	0	0
Harpalus (*Harpalus*) *tardus* (Panzer 1796)	0	0	1	0
Harpalus (*Pseudoophonus*) *griseus* (Panzer 1796)	5	0	0	0
Harpalus (*Pseudoophonus) rufipes* (De Geer 1774)	190	446	0	3
Lebia (*Lamprias*) *cyanocephala cyanocephala* (L. 1758)	0	1	0	0
Microlestes fissuralis (Reitter 1901)	0	1	0	0
Microlestes maurus maurus (Sturm 1827)	0	0	0	19
Notiophilus rufipes Curtis 1829	0	0	15	27
Notiophilus substriatus G. R. Waterhouse 1833	0	0	1	3
Ophonus (*Hesperophonus*) *azureus* (F. 1775)	6	4	0	0
Parophonus (*Parophonus*) *maculicornis* (Duftschmid 1812)	10	0	0	0
Poecilus (*Poecilus*) *cupreus cupreus* (L. 1758)	9	7	0	0
Pterostichus (*Adelosia*) *macer macer* (Marsham 1802)	0	13	0	0
Pterostichus (*Feronidius*) *melas italicus* (Dejean 1828)	0	1	0	0
Pterostichus (*Morphnosoma*) *melanarius melanarius* (Illiger 1798)	1	97	2	0
Pterostichus (*Platysma*) *niger niger* (Schaller 1783)	0	0	1	0
Trechus (*Trechus*) *quadristriatus* (Schrank 1781)	1	3	8	2

Table 2. Number of individuals and number of species captured in 2006/07 and 2020/21 with pitfall traps in the stations S1 and S2 of "Foresta Carpaneta".

	(2006/07)		(2020/21)	
	S1	S2	S1	S2
N. of individuals	894	2188	110	67
N. of species	23	24	10	8

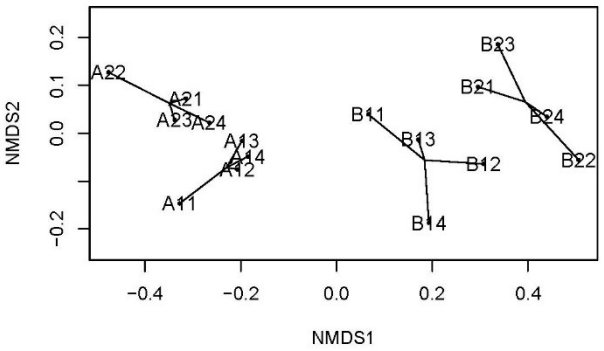

Figure 2. Graphical representation of the carabid assemblages collected in 2006/07 and 2020/21, using twodimensional Nonmetric Multidimensional Scaling. Groups are connected to the cluster centroids (for more details see main text). (Code abbreviations: A = 2006/2007; B = 2020/2021; first number = site; second number = trap).

The large majority of the species trapped in 2006/2007 were typical for open grasslands, ecotones, pioneer vegetation and fields (Klaiber *et al*., 2017) and are common in the Po Valley (Magistretti, 1965; Degiovanni, 2015). Also, the species caught in 2020/21 were generally typical for open grasslands, ecotones, pioneer vegetation and fields (Klaiber *et al*., 2017).

The most obvious difference observed between 2006/07 and 2020/21 is an important decrease in the number of individuals and species. Additionally, a clear difference was observed in the composition of the carabid fauna of the two sampling periods and many species that were common in 2006/07 were absent or rare in the samples of 2020/21 (table 1). For example, *Brachinus* (*Brachynidius*) *sclopeta* (F. 1792), a species that was common in both sites in 2006/07 and which is typical for pioneer habitats (e.g. fallow land) (Klaiber *et al*., 2017), was not captured in 2020/21. *Harpalus* (*Harpalus*) *distinguendus distinguendus* (Duftschmid 1812), which generally lives in in arable land, open and pioneer habitats (e.g. fallow land) (Brandmayr *et al*., 2005; Pilon *et al*., 2013; Klaiber *et al*., 2017): a total of 613 individuals of this species had been trapped in 2006/07 but only two specimens were caught in 2020/21. *Harpalus* (*Pseudoophonus*) *rufipes* (De Geer 1774), a species typical of arable lands (Brandmayr *et al*., 2005), was abundant in 2006/07 whereas fourteen years later only 3 individuals were caught.

Considerable difference in the composition of the carabid fauna between 2006/07 and 2020/21 had been expected because the "Foresta Carpaneta" was planted between 2003 and 2005 on former agricultural land and has been developing rapidly. At the time of the first sampling (2006/2007), the forest, 2-3 years after its plantation, had an average height of only 3.3 m and the canopy covered only approximately 53% (Emma Minari, personal communication). The structure of the young plantation did not resemble a forest and did not yet have a forest microclimate. These biotic conditions also explain that the most common species observed in 2006/2007 were typical for open grasslands, ecotones, pioneer vegetation and fields (Klaiber *et al*., 2017). These assemblages did not contain any species which are considered forest generalists or forest-specialists (Marrec *et al*., 2021). In the Po Plain, young poplar stands have carabid assemblages similar to crop fields (Allegro and Sciaky, 2003).

After 14 years the average height of the planted trees was 8.7 m and the tree crowns now covered approximately 87% (Emma Minari, personal communication). This more complex and stratified structure also created a more typical forest microclimate which is expected to strongly influence the composition of the carabid assemblages as many species typical for open habitats are unable to penetrate forests (Della Rocca *et al*., 2021). Therefore, the observed decrease in species richness had been expected, as open habitats show higher richness than forests (Allegro and Sciaky, 2003; Brandmayr *et al*., 2005; Pizzolotto *et al*., 2005; Gobbi and Fontaneto, 2008; Burgio *et al*., 2015). However, eurytopic species can use forests as alternative, temporary habitat, but are less competitive there than in more open habitats (Marrec *et al*., 2021). Only two species of forest generalists (Marrec *et al*., 2021) were collected, both in 2020/21. The first is 42 individuals of *Notiophilus rufipes* Curtis 1829, which is typical for ecotones (Klaiber *et al*., 2017), but also lives in forests (Müller-Kroehling *et al*., 2014) and is considered a forest generalist (Marrec *et al*., 2021). The second is *Pterostichus* (*Platysma*) *niger niger* (Schaller 1783), a species with a wide ecological niche, that typically lives in fields and among pioneer vegetation, but is also able to colonize forests (Pizzolotto *et al*., 2005; Klaiber *et al*., 2017; Della Rocca *et al*., 2021) and is considered a forest generalist (Marrec *et al*., 2021). A single individual of this species was collected in 2020/21. However, none of the species captured in 2020/21 are forest-specialists, i.e. a species limited to stable, mature forest stands (Marrec *et al*., 2021).

The absence of forest-specialist carabids, also in the assemblages collected in 2020/21, is most probably due to the fact that "Foresta Carpaneta" is completely isolated from other mature forests, which host populations of these species. The State Reserve Bosco Fontana is the nearest ancient forest and is located 10.5 km away. Here, populations of forest specialists, such as *Carabus* (*Carabus*) *cancellatus emarginatus* Duftschmid 1812 are present (Vigna Taglianti, 2004). The area between these two sites is occupied by fields, streets, urban centres, industrial areas and a railway line. These man-made areas make the colonization of the new forest very unlikely, particularly for carabid forest specialists, as these often have a low dispersal capacity, being apterous or brachypterous (Casale *et al*., 1982; Brandmayr *et al*., 2005; Gnetti *et al*., 2015; Gobbi *et al*., 2017) and open habitats constitute a dispersal barrier for these species (Della

Rocca *et al*., 2021). The arrival of forest species at the "Foresta Carpaneta" is even less likely because of the lack of ecological corridors (Niemelä, 2001). Hedgerows support populations of some forest carabid species (Petit and Burel, 1993), particularly hedgerows consisting of autochthonous species are effective corridors for many forests species (Niemelä, 2001; Šustek, 2008). A further indication that the absence of forest-specialist carabids in 2020/21 was mainly due to a lack of connectivity is provided by the observation that some hoverfly species typical for mature forests, which are often good flyers (Burgio *et al*., 2015), were already present at "Foresta Carpaneta" in 2020/21. One example is *Caliprobola speciosa* (Rossi 1790), a hoverfly species (Diptera Syrphidae) that typically lives in deciduous forests with mature and/or senescent trees (Daniele Birtele, personal communication). Thus, the "Foresta Carpaneta", that had matured for almost 20 years with very limited interventions, already developed some ecological niches typical for forests. However, these were not occupied by forest-specialist carabids, as these were probably unable to reach this new and isolated habitat. This allowed euryecious species, capable of living in a nemoral context, to populate this forest, however, with a limited number of species and individuals.

Conclusions

The isolation of the "Foresta Carpaneta", which is particularly severe due to the lack of ecological corridors, seems to have made it impossible for forest-specialist carabids to colonize this new forest over a period of 14 years. As this taxonomic group is a good biological indicator for forest habitats (Brandmayr *et al*., 2005; Gobbi, 2008; Kotze *et al*., 2011; Langraf *et al*., 2019; Marrec *et al*., 2021) and for the severity of habitat fragmentation (Niemelä, 2001), the results probably also apply to other forest specialists which disperse poorly, such as saproxylic weevils (Perone *et al*., 2022) and molluscs (Moning and Müller, 2009). Patch isolation and matrix composition both determine landscape connectivity and reduced connectivity between forest patches represents a major threat to species that are forest specialists, which often exhibit low dispersal abilities. As a result, colonization rate of forest patches can be very low (Marrec *et al*., 2021). This situation is very similar for herbaceous forest species, which disperse poorly over long-distances. As a consequence, forest plant species richness in plantations decreases with increasing distance from ancient woodlands and plantations isolated by open land accumulate forest plants very slowly (Brunet, 2007; Hermy and Verheyen, 2007). Similarly, Perone *et al*. (2022) found that saproxylic weevils were unable to colonize an isolated forest even after centuries since forest establishment.

This very slow colonization of isolated, planted forests by carabids, as observed in the present study, and by many other taxonomic groups that disperse poorly (Brunet, 2007; Hermy and Verheyen, 2007; Dekoninck *et al*., 2008; Perone *et al*., 2022), suggests that complete forest communities will not autonomously establish in the "Foresta Carpaneta" in the foreseeable future. If the long-term aim is to develop a forest that contains the communities of old forests, it is probably necessary to aid the establishment of a more complete ecosystem in the "Foresta Carpaneta" by active introductions, particularly of those taxonomic groups which disperse slowly or not at all. For example, the introduction of forest plant species into recent forests usually proved successful (Hermy and Verheyen, 2007). Also, introductions of carabid species with a low dispersal ability have already been carried out (de Vries, 1996; Busato, 2022). Possible source populations of forest specialist species are the few remaining ancient forests in the Po Plain (Cerretti *et al*., 2004; Allegro *et al*., 2016). It seems advisable to select source populations which are geographically close to the planted forest; in the case of "Foresta Carpaneta", the Nature Reserve Bosco Fontana is the nearest ancient forest, located at a distance of 10.5 km. However, it is worth considering that ancient forest patches exhibit more heterogeneous environmental conditions than recent patches and therefore provide more potential niches (Marrec *et al*., 2021). For example, in ants, forest species arrived in isolated new forests from nearby mature forest, but were apparently unable to establish, probably due to inappropriate vegetation structure and litter characteristics (Dekoninck *et al*., 2008). Thus, currently the young "Foresta Carpaneta" is unlikely to allow for the establishment of all carabid species typical for old forests.

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