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ORIGINAL RESEARCH

The impact of abandoned kaolin quarries on macromycetes (Fungi: Basidiomycota, Ascomycota), carabid beetle (Coleoptera: Carabidae), and spider (Araneae) assemblages

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Abstract
Post-mining sites represent important secondary refuges for invertebrates as well as for fungal species, often providing biodiversity hotspots in homogenous landscapes. Our study focuses on assemblages of carabid beetles (Coleoptera: Carabidae), spiders (Araneae), and macromycetes (Fungi: Basidiomycota, Ascomycota) in abandoned kaolin quarries and their immediate surroundings in the Pilsen region, Czech Republic. We studied mined and unmined sites and the impact of past mining, the vegetation composition of the sites, cover of herb and tree layers, and the amount of dead wood on the target groups. In total, we confirm the occurrence of 54 carabid beetle, 147 spider, and 139 macromycetes species, including several Red-listed species across the given groups. Carabid beetles and spiders, as well as the all Red-listed species, tend to prefer early successional open habitats. The fungal species displayed affinity to dead wood. Our results indicate that not only the invertebrates, but also macromycetes species prefer open post-mining sites, which are a substitute for endangered habitats such as natural wetlands or xerophilic grasslands.

Keywords Post-mining sites · Biodiversity · Threatened species · Czech Republic

Introduction

Rapid agriculture and forestry intensification exerted a crucial impact on temperate biodiversity (McLaughlin and Mineau 1995, van Swaay and Warren 1999). This change contributed to the loss of landscape heterogeneity (Beckmann et al. 2019; Mupepele et al. 2019) due to the rearrangement of fragmented land allotments into large and uniformly managed land cover units (Ntihinyurwa and de Vries 2021). This was augmented by change in the management of remaining habitat fragments, principally abandonment of traditional land uses (Ellis et al. 2012). As a result, landscape homogenization caused fundamental decline of species richness, and species occurrences were restricted to specific habitats or niches

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(Thomas et al. 2001), sometimes referred to as secondary refuges (Tropek et al. 2013). The attractivity of secondary refuges stems from several biotic and abiotic aspects which, in total, participate in co-creating suitable conditions for considerable numbers of species and may play a significant role for practical conservation (Reeves and Daniels 2020). These aspects correlate with habitats under primary succession, extreme abiotic conditions such as specific microclimates (wet or dry), shifting sands, nutrient deficiency or excess nutrients, or rugged reliefs (Novák and Konvička 2006). Nowadays, these habitats substitute for the original biotopes where considerable numbers of plants, fungi, and animal species could prosper; moreover, they substitute for endangered habitats such as wetlands or xerophilic grasslands (Janssen et al. 2016; Chytrý et al. 2019), where rare species can occur (Sucháčková et al. 2021). Among them, there are anthropogenic sites, particularly post-mining sites, which are known as important secondary refuges for invertebrates (Tropek et al. 2010). In particular, such sites often host species requiring disturbance regimes that vanished from cultural landscapes, including declining and endangered taxa. This phenomenon has been repeatedly demonstrated for higher plants (e.g., Prach and Pyšek 1994, Novák and Prach 2010), insects including Lepidoptera (e.g., Beneš et al. 2003, Tropek et al. 2013), Hymenoptera (e.g., Heneberg et al. 2013, Hendrychová and Bogusch 2016), Coleoptera and Araneae (Eyre et al. 2003; Tropek and Konvička 2008; Hula and Šťastná 2010a, b; Moradi et al. 2018), and even fungi (e.g., Zíbarová and Lepšová 2013, Kałucka et al. 2016, Adamo et al. 2022).

Carabid beetles and ground-dwelling spiders represent established bioindicators of environmental quality (Pearce and Venier 2006; Avgin and Luff 2010). They are readily surveyed in sufficient numbers, thus allowing meaningful conclusions to be drawn (Pearce and Venier 2006). Application of these two groups of invertebrates as environmental indicators has been surveyed in many cases (Churchill 1997; Dufrene and Legendre 1997; Rainio and Niemelä 2003; Pearce and Venier 2006; Kowal and Cartar 2012; Lehmitz et al. 2020; Ludwiczak et al. 2020), and also for post-industrial areas, due to their strong indicative value (Marc et al. 1999; Schwerk and Szyszko 2006; Tropek and Konvička 2008; Kędzior et al. 2018; Růžičková and Hykel 2019).

Likewise, the fungal taxa represent suitable environmental indicators (Peintner and Moser 1996; Christensen et al. 2004; Bai et al. 2018; Gupta 2020) and may reflect the different stages of succession (Suzuki 2002). However, they are rarely studied in the context of post-industrial areas in comparison with invertebrates (Kałucka et al. 2016), even though the disrupted habitats represent suitable conditions for some fungi species (Kałucka and Jagodziński 2017), which often are not known in the surrounding cultural landscape and tend to be rare or even protected.

In this study, we present the species assemblages of carabid beetles, spiders, and macromycetes recorded in the disused kaolin quarries and their immediate surroundings. Our study aimed to answer the following main questions: How species richness of carabid beetles, spiders, and macromycetes, and the numbers of Red-listed species, react to (1) the status of the sites regarding past mining; (2) the vegetation composition of the sites; (3) the cover of herb (E1) and tree (E3) layers; and (4) the amount of dead wood. We expected that the majority of species would prefer late successional habitats, which prevail in wider environs; whereas the Red-listed species would prefer the early successional open habitats, which are regionally rare. We also expected that the wooded areas with a high presence of dead wood will favour the occurrence of macromycetes. Our study is novel in targeting a type of post-mining habitat with presumably low species diversity, and by integrating patterns from two routinely studied arthropod groups with patterns observed on macromycetes, rarely targeted at post-mining habitats.

Methods

Study area

The study was conducted near the town of Horní Bříza, Pilsen Region, Czech Republic (49°52’6"N, 13°22’33"E, 455 m a.s.l.). The region is mildly warm (Quitt 1971), with mean annual temperature 7–8 °C and mean annual precipitation 500–550 mm (Tolasz et al. 2007). It is an important urban centre affected by industrial activity, including mining, with abundant occurrence of post-mining sites (Demek et al. 2006). An industrial zone was built near deposits of kaolin, a silicate mineral and raw material for ceramics and paper industries (Langhammer and Kaplická 2005). The disused kaolin quarries (Fig. 1) are steep-banked pits situated amidst pine-dominated conifer (*Picea abies* (L.) H. Karst., *Pinus sylvestris* L.) plantations with admixture of birch (*Betula pendula* Roth) and oak (*Quercus petraea* (Matt.) Liebl.) (≈50 years old), 1 km from a still operating quarry and the factory that produces kaolinite (Starý et al. 2017). In the quarry pits targeted here, excavation was terminated in 1931, owing to the poor quality of kaolin. The pits were left to spontaneous succession, and gradually flooded by stormwater (Wild 1977). The water level fluctuates with precipitation, creating periodically exposed muddy banks. In the 1970s, they were contaminated with chemical substances, such as arsenic, chrome, nickel, zinc, and polychlorinated biphenyls from a nearby chemical plant. Since then, the pits are considered an environmental hazard with restricted public access and regular pollutants monitoring (Čáslavský et al. 2012).

Sampling design

We selected 24 sites within the disused quarry pits (“mined”, n = 12) and their immediate surroundings (“non-mined”, n = 12), to sample carabid beetles (hereinafter “carabids”), ground-dwelling spiders (“spiders”) and macromycetes’ fruiting bodies in 2019 and 2020, each year from April to October, to cover entire seasons.

At each site, we recorded a standard phytosociological relevé in June 2020, listing all vascular plant species and their semiquantitative covers (Braun-Blanquet 1964; Walter et al. 2022). Further, we recorded the total % cover of herb (E1), and tree (E3, including rarely occurring shrubs) layers, and the amount of lying dead wood (perimeter > 3 cm, %) within a 10 m radius surrounding pitfall traps.

The carabids and spiders were sampled using pitfall traps consisting of two brown plastic cups (upper diameter/depth: 9/15 cm). The first cup was perforated 2 cm under the upper rim around the perimeter, the second had a hole in the bottom; this prevented flooding the traps by rainwater (Hradská and Těťál 2017). Two pitfall traps were placed at each site three meters apart. We used 8% acetic acid, filling two-thirds of the inner cup as a conservation fluid. The traps were emptied at three-week intervals; the catch was transferred into 70% ethyl alcohol. The carabids and spiders were identified to the species level (Heimer and Nentwig 1991; Roberts 1995; Hůrka 1996; Kůrka et al. 2015).

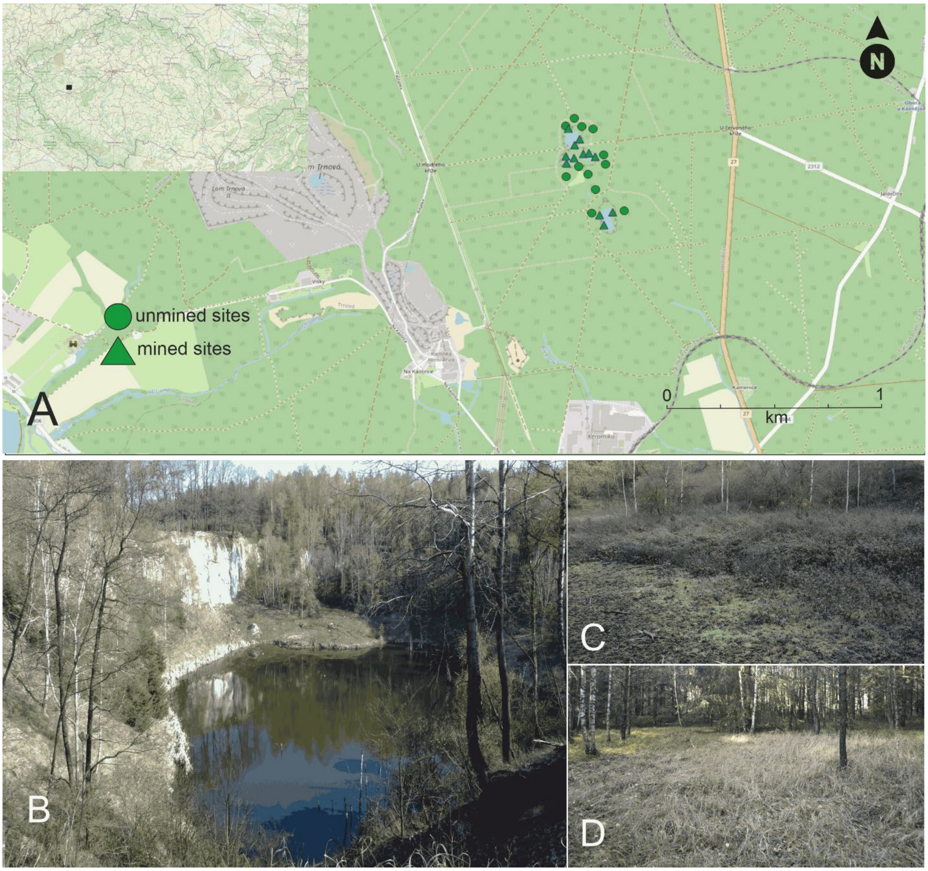

Fig. 1 Location (A) of the study sites in surroundings of kaolin quarries (B) near the town of Horní Bříza (Central Europe, Czech Republic, western Bohemia, Pilsen Region). The early successional mined sites (C) and unmined site (D) are documented

The macromycetes (Ascomycota, Basidiomycota) were recorded within the 10 m radius surrounding the pitfall traps (Horák et al. 2018), during four visits per site (early spring, mid-summer, and early and late autumn). Each visit to a site lasted ≈ 15 min. The taxonomically difficult species were identified in the laboratory, using standard methods (Bernicchia and Gorjón 2010; Knudsen and Vesterholt 2018).

Species threatened in the Czech Republic were classified according to national Red lists (spiders: Řezáč et al. 2015; carabids: Veselý et al. 2017; macromycetes: Holec and Beran 2006), considering categories *EX?* (probably extinct); *CR* (critically endangered); *EN* (endangered); *VU* (vulnerable); *NT* (near threatened).

Data analysis

We used multivariate (ordination) analyses to relate species richness of the study groups and the numbers of Red-listed species to the environmental variables characterising the sampling sites using CANOCO 5 (Šmilauer and Lepš 2014).

As a first step, we subjected the composition of vascular plants per site to principal component analysis (PCA), a linear method which extracts major gradients of variation from multi-species data. We used the resulting PCA axes as predictors describing the vegetation composition; i.e., to assess the effects of the vascular plant assemblages' composition on the species richness of the targeted groups.

The effects of predictors of interest on species richness and the numbers of Red-listed species of the targeted groups were investigated using redundancy analysis (RDA), a multivariate variant of linear regression. The history of mining was a categorical (mined/not mined) predictor. Further predictors were vegetation (the PCA scores of samples from the vascular plants analysis), E1 and E3 covers, and dead wood amount. We used square root transformation of the response variables, centered the species data and used no centering for environmental predictors. We reflected the temporal aspects of the two seasons (2019, 2020) by treating the two years as a time series in the hierarchical split-plot permutation design for repeated sampling; the sites were permuted freely. The results of all RDAs were evaluated with 999 Monte Carlo permutations.

Results

We found 54 carabid species in 2838 individuals, 147 spider species in 1852 individuals, and 139 macromycetes species in the disused kaolin quarries and their surroundings (Appendix 1). The Red-listed included one near-threatened carabid (*Chlaenius tristis*) in five individuals, 22 spiders in 77 individuals (EN: *Ceratinella major*, *Gonatium paradoxum*, *Lophomma punctatum*, *Mecopisthes silus*; VU: *Coriarachne depressa*, *Haplodrassus soerenseni*, *Micaria dives*, *M. fulgens*, *Ozyptila brevipes*; NT: *Agyneta mollis*, *Alopecosa inquilina*, *A. schmidti*, *Antistea elegans*, *Arctosa leopardus*, *Bathyphantes setiger*, *Gnaphosa lugubris*, *Hygrolycosa rubrofasciata*, *Lepthyphantes nodifer*, *Pardosa paludicola*, *Pelecopsis elongata*, *Piratula knorri*, *Silometopus elegans*), and four macromycetes (EX?: *Arrhenia retiruga*; CR: *Hohenbuehelia cyphelliformis*; EN: *Psathyrella typhae*, *Steccherinum oreophilum*).

The PCA analyses of vascular plants species composition (eigenvalues of ordination axes 1–4: 0.160, 0.149, 0.083, 0.079) showed that the main gradient distinguished sites with vascular plants typical for nutrient-poor forest habitats (negative values of the ordination axis: *Picea abies* (L.) H. Karst., *Vaccinum myrtillus* L.) from unforested sites (positive values: *Cirsium arvense* (L.) Scop., *Typha latifolia* L., *Urtica dioica* L.); the secondary gradient distinguished dry (negative: *Betula pendula* Roth, *Cirsium arvense* L.) from moist (positive: *Glyceria fluitans* (L.) R. Br., *Juncus effuses* L.) conditions; the tertiary gradient differentiated open sward (negative: *Lupinus polyphyllus* Lindl, *Scrophularia nodosa* L.) from closed-sward (positive: *Arrhenatherum elatius* (L.) J. Presl et C. Presl, *Luzula luzuloides* (Lam.) Dandy et Wilmott); and the quaternary gradient differentiated sunny (negative: *Fragaria vesca* L., *Hieracium lachenalii* Suter) from shady (positive *Geranium robertianum* L., *Moehringia trinervia* (L.) Clairv.) conditions.

Visualisation of all the predictors using PCA (Fig. 2) showed that mined sites had low cover of E3, vegetation typical for non-wooded conditions, and relatively high representation of hygrophilous vegetation. Unmined sites, in contrast, contained high representation of E3, vegetation typical for forest plantations on nutrient-poor soils, and a high amount

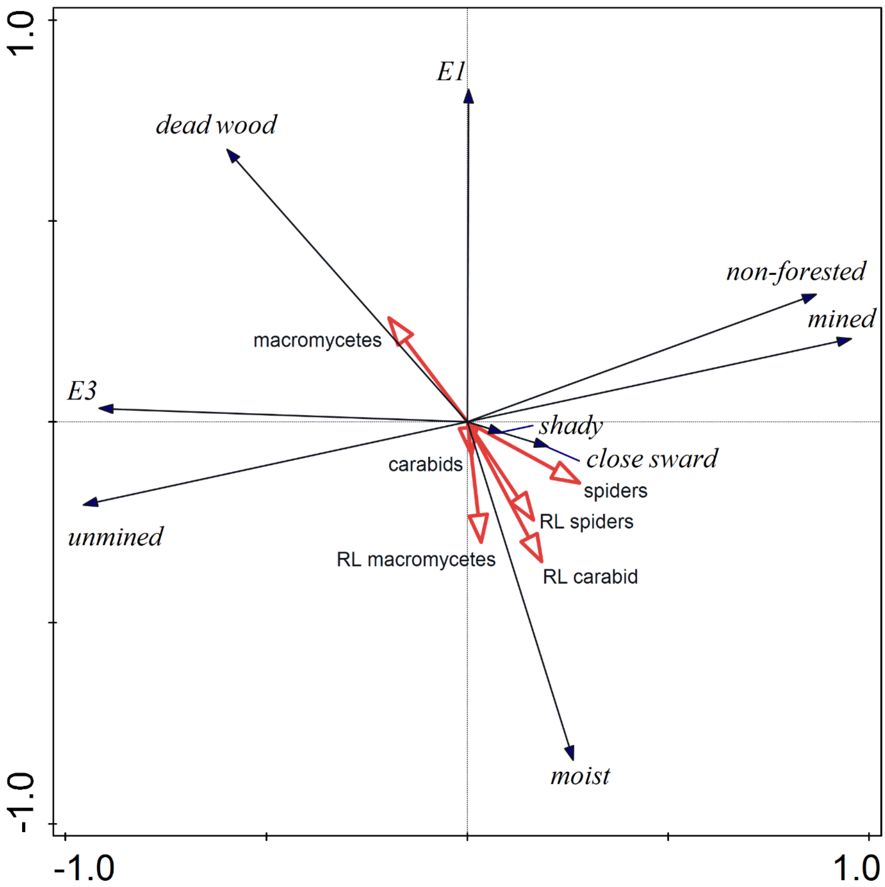

Fig. 2 Ordination plot PCA analysis for the environmental variables and their impact on carabids, spiders, macromycetes, and the Red-listed species of these group surroundings at the mined and unmined sites. Eigenvalues of Axes 1–4 were 0.433, 0.227, 0.127, 0.111. E1 – herb layer; E3 – tree layer; RL – Red-listed species

of dead wood. The representation of E1, and humidity gradient, were perpendicular to the main PCA axis.

Past mining significantly affected only the numbers of spiders, positively so. Vegetation composition had no effect on carabids, whereas the single Red-listed carabid occurred at sites with moist conditions. Richness of spiders positively responded to nutrient-poor, humid and sunny vegetation, and Red-listed spiders were positively influenced by moist conditions only. Richness of macromycetes increased in nutrient-poor and open-sward vegetation, while Red-listed macromycetes increased marginally significantly with moisture. E1 cover had a significant (positive) effect on the Red-listed carabid beetle, whereas E3 cover had negative effect on spiders' species richness. Finally, the amount of dead wood had a significant negative effect on the single Red-listed carabid, richness of spiders, and Red-

Table 1 The results of Redundancy analyses between environmental variables and the carabid beetle, spider and macromycetes species sampled surroundings of kaolin quarries near the town of Horní Bříza (Central Europe, Czech Republic, western Bohemia, Pilsen Region). Significant values are in **bold**. Almost significant values are in (parentheses). + – positive effect to the group of species; - – negative effect to the group of species; n. s. – not significant

	All species (RDA)				Red-listed species (RDA)			
	R^2	F	P	Effect	R^2	F	P	Effect
Carabid beetles								
Mining	0	0.3	0.71	n.s.	0	0.3	1.00	n.s.
Vegetation	n.s	n.s	n.s	n.s.	8.45	**5.3**	**0.024**	+PCA2
E1	0	0.3	0.65	n.s.	7.17	**4.6**	**0.033**	-
E3	0	0.2	0.65	n.s.	0.61	1.3	0.29	n.s.
Dead wood	0	0.7	0.50	n.s.	10.77	**6.7**	**0.013**	-
Spiders								
Mining	2.43	**2.2**	**0.047**	+	0	0.5	0.31	n.s.
Vegetation	10.5	**2.8**	**0.001**	+PCA1+2+4	7.20	**4.6**	**0.02**	+PCA2
E1	0	0.1	0.62	n.s.	0	0.7	0.22	n.s.
E3	7.13	**4.6**	**0.006**	-	0.47	1.2	0.098	n.s.
Dead wood	3.61	**2.8**	**0.03**	-	7.21	**4.7**	**0.001**	-
Macromycetes								
Mining	0	0.7	0.48	n.s.	0	0.1	1.00	n.s.
Vegetation	12.38	**4.3**	**0.03**	-PCA2+PCA3	3.62	2.8	0.075	(+PCA2)
E1	1.85	1.9	0.23	n.s.	5.02	3.5	0.065	(-)
E3	3.24	2.6	0.15	n.s.	0	<0.1	0.91	n.s.
Dead wood	8.93	**5.6**	**0.038**	+	4.23	3.1	0.064	(-)

listed spiders; it had significant positive effect on richness of macromycetes, and marginally significant negative effect on Red-listed macromycetes (Table 1).

Discussion

Although the landforms affected by kaolin excavation so far have received much less attention than other types of post-mining localities (e.g., Mrzljak and Wiegleb 2000, Beneš et al. 2003, Tropek et al. 2013, Tizado and Nunez-Perez 2016, Twerd et al. 2019), some similarities are evident. Disused kaolin quarries in western Czech Republic host higher species richness of carabids, spiders, and macromycetes than their immediate environs, covered by plantation forests. The conservation potential of abandoned kaolin quarries is illustrated by the relatively high percentage of Red-listed species (8%), agreeing with the claims that post-industrial areas may develop into secondary refuges harbouring high numbers of rare species (Rebele and Dettmar 1996, Beneš et al. 2003, Tropek et al. 2013).

The assemblage of carabid beetles was relatively species-poor (54 species with a single Red-listed), if compared with studies from such habitats as arable fields in Central Europe; Veselý and Šarapatka (2008): 91 spp. or Knapp et al. (2022): 61 spp. The low species richness at our study site may reflect both the secondary character of the plantation woodlands, and the past chemical contamination of soils caused by the inorganic and organic substances of sites (Čáslavský et al. 2012). Due to their sensitivity, carabids represent an important model group for bioindication of environmental pollution (Butovsky 2011). Detailed study of pollutants contamination of carabid samples is highly warranted.

Our results revealed that more species of spiders, as well as Red-listed species of all three groups, tend to prefer open canopy moist biotopes, represented in our system by sparsely vegetated banks of the flooded kaolin quarries. The association of high numbers of declining species with frequently disturbed early successional conditions, noted by many authors (Thomas et al. 1994; Niemelä 2001; Tropek and Konvička 2008; Kałucka and Jagodziński 2017), is due to decline of such habitats, and changes of their spatial distribution, in European landscapes (Janssen et al. 2016; Chytrý et al. 2019). In a preindustrial landscape, small-scale but intensive disturbance was a common side effect of farming, local extraction of raw materials and other human activities; with modern economics, such disturbance became more concentrated, causing mutual isolation of early successional sites. Near-barren surfaces typical of opencast mine spoils seem to be particularly important not only for carabid beetles (Schwerk and Szyszko 2011) but also for spiders (Tropek and Konvička 2008) and for fungi (Kałucka and Jagodziński 2017).

The banks of the pools in the kaolin quarries are periodically drained, which supports combinations of species associated with xeric (e.g., carabids: *Amara aenea*, *Harpalus froelichii*, *Harpalus signaticornis*; spiders: *Alopecosa inquilina*, *A. schmidti*) or humid (carabids: *Acupalpus flavicollis*, *Chlaenius tristis*; spiders: *Bathyphantes setiger*, *Ozyptila brevipes*) conditions (cf. Hůrka 1996, Buchar and Růžička 2002, for habitat requirements of individual species). The presence of hygrophilous species is supported by the good water-binding capacity of this clay mineral (Lioa et al. 2019), thus the sites do not drain completely. The effect of periodical drying of anthropogenic habitats appears important both for plants (Prach et al. 1987) and terrestrial arthropods (Tropek 2012).

Not surprisingly, the presence of dead wood had a positive impact on fungal species, as the positive relationship between fungi and dead wood has been firmly established (e.g., Rypáček 1957, Stokland et al. 2012, Avis et al. 2016, Horák et al. 2018) (Fig. 2). On the other hand, the recorded Red-listed macromycetes tend to prefer the sites with a low supply of dead wood (a marginally significant pattern). For instance, the critically endangered *Arrhenia retiruga* grows on mosses, while the endangered *Psathyrella typhae* grows on dead parts of various aquatic plants, especially *Typha* ssp. The counterintuitive absence of Red-listed species in sites with a higher proportion of dead wood is explicable by the plantation character of the surrounding forests, composed by even-aged stands without ancient woodland elements. Woodlands-inhabiting endangered macromycetes tend to be associated with undisturbed conditions (Hofmeister et al. 2015), whereas in young secondary forests, a low number of species is expected (Horák et al. 2019).

Similarly counterintuitive associations were those between the amount of dead wood and spiders, Red-listed spiders, and Red-listed carabids. Ground-dwelling spiders utilise the dead wood habitat for feeding, mating, overwintering, or laying eggs (Lowrie 1948), and carabid beetles use it mainly for shelter and overwintering, and to a lesser extent for hunting (Kacprzyk et al. 2021). However, this preference does not apply for all species of these groups, as shown for spiders, e.g., by Buddle (2001). The lack of association with dead wood may also be due to the limitation of pitfall traps. Radiotelemetry studies of several species of the genus *Carabus* revealed that despite using dead wood for shelter, the beetles may be more active at open surfaces due to a higher occurrence of food resources, and hence are more likely captured there into pitfall traps (Bérces and Růžičková 2019; Růžičková et al. 2021). A similar consideration likely applies to ground dwelling spiders (e.g., those from the family Lycosidae), who use similar diet and hunting strategies as carnivorous carabids. Most importantly, and similarly to macromycetes, the carabids and spiders inhabiting the plantation woodlands (with some dead wood) were not the species of conservation concern associated with open surfaces (with low dead wood).

Conclusion

Despite being situated on acidic rocks, kaolin quarries perform as important secondary refuges for carabids, spiders, and macromycetes. They hence represent a conservation resource, similar to alkaline quarries in warm lowlands (Beneš et al. 2003) or post-mining coal heaps (Tropek et al. 2013). The presence of species of conservation concern is associated with open canopy and sparse vegetation, maintained by alternation of dry and moist conditions in our study system. Such environments have become rare in modern landscapes of Central Europe, emphasising both the scientific and biodiversity conservation value of the post-industrial sites.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Jan Walter], [Ivana Hradská], [Jiří Kout] and [Jan Bureš]. The first draft of the manuscript (text, figures, table) was written by [Jan Walter] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent All authors agreed to participate in the research.

Consent for publication All authors agree with the contents of the manuscript and its submission to the journal.

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