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Consistent successional trajectories of ant species richness, composition, and functional traits on post-mining sites in European deciduous forest

Marie Hovorková, Michal Holec, Ondřej Mudrák & Jan Frouz

Abstract

Since ants are a prominent taxon of terrestrial food webs and significant ecosystem engineers, understanding ant community succession is important to understand ecosystem recovery after disturbance. We studied the succession of ant communities on brown coal mine spoil heaps in mixed deciduous forests of NW Czech Republic by censusing multiple sites in which assemblages differed in age between 1 and 50 years since initial spoil heap formation. We compared the species composition, ant traits, and habitat characteristics of chronosequences of reclaimed and unreclaimed sites and undisturbed reference sites in the surrounding landscape in the years 2001 and 2020. Although habitat characteristics such as canopy and bare soil cover were important, successional age was the single strongest predictor of ant species richness and composition, suggesting a similar successional trajectory of sites. The number of ant species, but not their total abundance, increased during succession. The composition of the ant community changed towards that of the referential surrounding landscape, where less aggressive species were common. During the early successional stages, the common species had queens which establish colonies independently by enclosing themselves in a founding chamber and relying on stored resources (claustral mode). During later successional stages, the common species had queens which establish colonies by leaving the founding chamber to forage for food (semi-claustral mode). Species of social parasites were also more common in late versus early successional stages. These results suggest that resource availability to ants increases during succession, which may enhance species coexistence. The rate of change in ant species richness and composition was high at young sites and slowed down with site age. As forest stands aged through the successional sequence, the abundance of forest ant species increased. We did not record differences in species richness between reclaimed and unreclaimed sites. Eurytopic species were the most numerous, but their abundance did not change significantly during succession. Collectively, these results suggest that as forest stands age and canopies close, ant species richness and composition consistently change, with potentially more resources and more complex species interactions in later stages.

Key words: Ants, chronosequence, Formicidae, functional traits, Hymenoptera, post-mining site, succession.

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Marie Hovorková (contact author), Institute for Environmental Studies, Faculty of Science, Charles University, Benátská 2, 12801 Prague 2, Czech Republic. E-mail: hovorkoma@natur.cuni.cz

Michal Holec, Faculty of Environment, Jan Evangelista Purkyně University in Ústí nad Labem, Pasteurova 3632 / 7, 40096 Ústí nad Labem, Czech Republic.

Ondřej Mudrák & Jan Frouz, Institute for Environmental Studies, Faculty of Science, Charles University, Benátská 2, 12801 Prague 2, Czech Republic; Institute of Soil Biology and Biogeochemistry, Biology Centre of the Czech Academy of Sciences, Na Sádkách 7, 37005 České Budějovice, Czech Republic.

Introduction

Ants are an important component of ecosystems. They are referred to as ecosystem engineers for the strong potential to alter the environment they inhabit (FOLGARAIT 1998, JOUQUET & al. 2006, ROCHA & al. 2024). They are sensitive indicators of ecosystem conditions and are also abundant, widespread, and reasonably easy to sample (ANDERSEN & al. 2004, ANDERSEN & MAJER 2004, OT-TONETTI & al. 2006, TIEDE & al. 2017). They interact with numerous other organisms in their surroundings. Ants are important predators of arthropods (Hölldobler & Wilson 1990) and affect vegetation either directly by dispersing seeds (HANDEL & BEATTIE 1990, SCHÜTZ & al. 2008) or indirectly by influencing soil fertility (Cul-VER & BEATTIE 1983, KOVÁŘ & al. 2001, FARJI-BRENER & WERENKRAUT 2017). It is therefore important to know how well their communities can re-establish in disturbed



habitats and what the mechanisms of succession of their communities are.

Chronosequences are a commonly used method for studying temporal dynamics of development of different communities. When applying this method, sets of various sites of different age of succession since initial conditions are studied, and by comparing them at one point in time, changes over time can be assessed. Chronosequences often allow to study successional processes over time periods longer than direct observation of real-time changes would allow. There are however constraints limiting the appropriate use of this method. Importantly, it relies on the assumption that each site in a chronosequence only differs in age, while abiotic as well as biotic conditions have remained the same for all sites throughout their history, that is, sites should follow the same trajectory (JOHNSON & MIYANISHI 2008). If these assumptions are not met, which is often the case to some extent, results obtained from studies using chronosequences may give a blurred idea of the ongoing succession. Repeated sampling which verifies the predictions based on a chronosequence is therefore needed (WALKER & al. 2010).

Post-mining sites may be viewed as wastelands, but they also provide a great opportunity for natural processes and for successional studies. Mining activities cause large disturbances and substantially modify the landscape. Original ecosystems are either excavated or overlaid by spoil, which results in the formation of unproductive areas which become overgrown in primary succession (Wor-LANYO & JIANGFENG 2021). During the mining process, post-mining sites with similar characteristics are formed repeatedly, and their history is well-documented (FROUZ & al. 2008). Different stages of succession from bare soil to forest can be found here (SIPOS & al. 2017). The widespread presence of various succession stages which have the same or a contrasting but well-documented history makes post-mining sites an excellent model for studies of ecosystem succession.

This study aims to investigate the development of ant communities on post-mining sites and to assess changes in functional traits during succession. We studied the composition of ant communities on spoil heaps near the town of Sokolov (NW Czech Republic, Central Europe) using chronosequences of reclaimed and unreclaimed series and also of the abandoned landscape with ongoing secondary succession surrounding them. The ant communities were studied initially in the year 2001 and then again after 19 years in the year 2020. Many of the initial sites were resampled in 2020, which allowed us to make a legacy comparison between the samples. We aimed to answer the following questions. (i) How did the ant communities change in the chronosequence and which environmental parameters influenced the succession? (ii) Do the ant communities develop along the chronosequence towards more species-rich, more specialised, and functionally more complex communities of the surrounding landscape? (iii) Do the successional changes observed by means of a chronosequence in the year 2020 correspond with changes indicated by 2001 sampling?

Material and methods

Study area

The study was carried out on spoil heaps formed by open-cast brown coal mining in the north-western part of the Czech Republic (Central Europe). The area is located at an altitude of 500 - 600 m above sea level, with a mean annual temperature of 6.8 °C and mean annual precipitation of 600 - 650 mm. Spoil heaps are formed mostly of tertiary clay material called Cypris facies. The soil reaction of this material is slightly alkaline with a pH value of about 8 and is formed mostly by kaolinite, illite, and montmorillonite (FROUZ & al. 2001). An unreclaimed spoil heap named Pastviny and a reclaimed spoil heap named Vintířovská, which are both part of the largest Czech spoil heap by the name of Velká podkrušnohorská spoil heap (50° 14' 09" N, 12° 39' 27" E) near the town of Sokolov, were sampled. A referential area in the surrounding of these spoil heaps (50° 12' 8" N, 12° 38' 12" E) in an area which belongs to the mining company and where future coal excavation is planned was also sampled. Human management was ceased there when mines were opened, so that secondary succession processes are taking place in the area.

Most of the area of spoil heaps was reclaimed by planting trees. The surface of sites chosen for reclamation was levelled by earthmoving machinery before trees were planted. Alder (*Alnus* spp.), oak (*Quercus* spp.), and spruce (*Picea* spp.) were the main trees planted at the sites that were chosen for this study.

Unreclaimed sites left to spontaneous succession become overgrown by forest stands mostly formed of European white birch (*Betula pendula*), European aspen (*Populus tremula*), goat willow (*Salix caprea*), and Norway spruce (*Picea abies*). Young unreclaimed sites are covered by very sparse herbaceous vegetation mostly formed of coltsfoot (*Tussilago farfara*) and wood smallreed (*Calamagrostis epigejos*), and by young trees of *S. caprea* and *P. tremula*. As a result of the heaping process, parallel rows of depressions and elevations were formed, creating a heterogeneous surface. Due to this, litter is concentrated in depressions, and a thick layer of humus is developing here. The surface of elevations at younger and intermediate sites is mostly covered by bare soil (FROUZ & al. 2008, MUDRÁK & al. 2016).

The area in the vicinity of the spoil heaps contained both mixed stands with dominant oak (*Quercus* spp.), maple (*Acer* spp.), *Betula pendula*, *Populus tremula*, and patches covered by grasses and scattered shrubs, which are habitually close to abandoned meadows with scattered shrubs. At the time of study, the age of these sites was 60 -80 years since management cessation.

Sampling

Ants were collected in three successional series: (1) a series of reclaimed sites (3 - 36 years old in 2001 and 22

Tab. 1: Numbers of repeatedly sampled sites and sites sampled in just one year. Numbers are shown for each successional series and year separately.

2001								
	Repeatedly sampled	Sampled once	Total of sites					
Unreclaimed	11	5	16					
Reclaimed	28	10	38					
Surrounding	8	-	8					
Total of sites	47	15	62					
2020								
	Repeatedly sampled	Sampled once	Total of sites					
Unreclaimed	11	9	20					
Reclaimed	28	6	34					
Surrounding	8	-	8					
Total of sites	47	15	62					

- 46 years old in 2020), (2) a series of unreclaimed sites (3 - 31 years old in 2001 and 18 - 50 years old in 2020), and (3) a series of sites in the surrounding landscape (~ 60 years old in 2001 and ~ 80 years old in 2020). However, not all the sites could be resampled due the continuing mining activity, so that new ones had to be chosen in 2020. These new sites were selected so as to be close to and as similar as possible to the original ones. The newly selected sites originally mostly formed the continuous habitat with the one excavated. The ant community they hosted did not differ from sites sampled repeatedly (for further information, see Appendix S1, as digital supplementary material to this article, at the journal's web pages). In total, 47 sites were sampled repeatedly in both years, but each year, 62 sites were sampled in total (see summary of the numbers of sites in each series and each year in Tab. 1).

Quadrat sampling was the main method used to study the composition of ant communities in both years (2001 and 2020). Ants were sampled within a rectangular-delimited area (50 m \times 2 m) randomly placed at each site. At first, visible ant nests were looked for in the whole area, and then 12 quadrat subplots ($1 \text{ m} \times 1 \text{ m}$ each) were randomly placed inside the sample area. Each subplot was carefully examined for the presence of individual ants. Small or subterranean colonies were searched for by turning stones and disrupting tufts of grass and moss. The time invested into sampling was two hours per site. Sampling was performed in May - June, only during sunny and warm days (> 15 °C) when ant activity peaks. Easily identifiable ant species, namely Formica pratensis, Formica sanguinea, Lasius fuliginosus, and Manica ru*bida* were identified in the field with the help of a simple $10 \times -30 \times$ hand lens. In other cases, surface-active ants found in subplots were collected, and a sample of five workers was taken from nests for identification in the laboratory. Additionally, ants were sampled with pitfall traps, but pitfall captures represented less than 10% of sampled individuals in the year 2001 (HOLEC & FROUZ 2005). In the year 2020, most pitfall traps (± 90%) were destroyed by wild boars (Sus scrofa), so that only quadrat sampling was used to collect ants. All collected ant specimens were preserved in 70% ethanol. Colony size was estimated with a non-invasive method based on the number of active ants. Upon finding a nest, the surrounding area was disrupted to provoke ants into defending their nest so that the size of the colony could be estimated. In the case of ants building mounds, the mound's height and diameter was measured. Colony size was then calculated using linear correlation between number of ants and nest diameter (for further information, see HOLEC & FROUZ 2005). The abundance of workers present in subplots was converted to number per 100 m² for each species. Ant species sampled in the year 2001 were determined using the identification keys by CZECHOWSKI & al. (2002) and SEIFERT (1996), while ant species sampled in the year 2020 were identified using the keys by CZECHOWSKI & al. (2012) and SEIFERT (2018).

Environmental parameters

The environmental parameters measured at every site included surface heterogeneity (wavy or levelled) and presence of Cypris clay material and deadwood, which were recorded as categorical data (present / absent). Tree canopy cover and the percentage cover of bare soil, moss, litter, herbs, shrubs, and trees were estimated visually at each site, and the distance from the surrounding landscape was measured. Time since the last major disturbance (heaping of waste substrate at unreclaimed sites and reclamation at reclaimed sites) was used as site age. Information on time since dumping and reclamation were provided by the Sokolovská Uhelná Mining Company. The depth of fermentation and humus layers were measured using a soil profile and then assigned to the following categories (1) <1 mm, (2) 1 - 5 mm, (3) 5 - 10 mm, (4) 10 - 20 mm, and (5) >20 mm for the fermentation layer, while categories (1) < 5 cm, heterogeneous and weak layer, and (2) >5 cm, continuously developed layer, were distinguished for the humus layer. Soil samples (0 - 5 cm) were taken from five different places at each site using sample rings. If the surface was heterogeneous, three soil samples were taken from a ridge and another three from a depression. The pH, conductivity, bulk density, and loss on ignition were measured for each separate sample. The mean value of these parameters for each site was then used in statistical analyses.

Functional traits and functional groups

Ants were divided into functional groups based on their habitat requirements. They were classified into the following three categories: (1) eurytopic, (2) forest species, and (3) open-habitat species. The classification was based on ant occurrence and habitat requirements as provided in SEIFERT (2018) and CZECHOWSKI & al. (2012). Eurytopic species were defined as those occurring in a broad range of habitats, showing a high overall adaptability. Regarding forest and open-habitat species, ants occurring in more types of habitat were divided based on the more preferred or main habitat. For example, xerothermophilous species, whose main habitat is dry grasslands but which can also occur in light forests, were classified as open-habitat species. Similarly, species occurring in woodlands but also in grasslands with shrubs and solitary trees were classified as forest species. Note that this classification is valid for Central Europe. In other regions, the ecology of ant species might be different.

The following life-history traits were selected for ant species: competitive hierarchy and mode of colony founding. Ant species were divided into three categories according to their aggression and position in dominance hierarchy based on CERDÁ & al. (2013) and PISARSKI & VEPSÄLÄINEN (1989). The categories were: (1) subordinates - species which avoid physical contact with other ant colonies and only defend their nest when attacked, (2) subdominants - non-territorial ants, which are however aggressive when defending their nest or food resources, and (3) dominants - highly aggressive and territorial ants. For mode of colony founding, ant species were divided into the following three categories: claustral independent (meaning that the queen confines herself in a founding chamber and rears the first brood while relying on her own fat reserves and energy provided by histolysis of large flight muscles to nurture herself and the brood; KELLER & PASSERA 1989), semi-claustral independent (queens of ants which leave their nest during its establishment to forage for food as they have relatively weakly developed reserve organs; Keller & Passera 1989), and social parasites. Social parasites were defined sensu SEIFERT (2018) as ant species which are at least temporarily dependent on other ant species in order to establish a colony. Information on traits and groups in individual species is provided in Table S3.

Data analysis

The number of species found at each site, total ant abundance, and the abundance of ants in each functional group (eurytopic, forest, and open habitat) was calculated. To test changes in functional traits in ant communities during succession, the community-weighted mean was calculated for each trait as relative abundance of species with a given trait. Ant community parameters were then explained by site age, type of successional series (unreclaimed, reclaimed, surrounding landscape), year of sampling, and their interactions with the general linear model using the "lm" function in the R statistical software (R CORE TEAM 2021). Abundance of ant functional groups was log-transformed prior to analyses. To visualise the effect of age on individual community parameters, the general linear model testing the main effects of successional series, sampling year, and age was used. The estimate of the standardised regression coefficient of age and the standard error of its estimate were then plotted.

To express the differences in species composition between the sampled communities, Bray-Curtis dissimilarity

(ŠMILAUER & LEPŠ 2014) was used. Two arrangements of Bray-Curtis dissimilarity were computed on log-transformed data of species abundances. In one, the numbers of ant individuals in the surrounding landscape were averaged over the year 2001 and the year 2020. These two averaged communities were the reference with which the ant communities were compared. Communities sampled in 2001 were compared with the 2001 reference, and accordingly, communities sampled in 2020 were compared with the 2020 reference. In the other arrangement, Bray-Curtis dissimilarity was computed as the dissimilarity between the communities sampled in 2001 and 2020 for each sampling site separately. This dissimilarity represents the change in species composition over 19 years. Because of the loss of pitfall traps in 2020, the original sampling methodology could not be replicated, which might have affected the estimation of the numbers of individuals of ant species, and so also Sørensen dissimilarity was computed. This index is similar to Bray-Curtis dissimilarity but is based on presence / absence only and is not affected by abundance (ŠMILAUER & LEPŠ 2014). It was used in the same arrangements as in Bray-Curtis dissimilarity. The general linear model was used to assess the relationship between both Bray-Curtis and Sørensen dissimilarities (of both arrangements), successional age, and type of successional series (unreclaimed series vs. reclaimed series) using the "lm" function. To test whether the Bray-Curtis dissimilarity of reclaimed and unreclaimed series had changed between 2001 and 2020, two-way Analysis of Variance was performed, where year of sampling and reclamation status (reclaimed vs. unreclaimed) were used as categorical predictors. The test was again performed using the "lm" function. Only repeatedly sampled sites were used in these univariate analyses. The difference between individual categories was further tested with the Tukey post-hoc test.

Redundancy Analysis (RDA) with forward selection was used to select environmental variables responsible for ant community succession in both sampling years. For this purpose, RDA was performed for each sampling year separately (using all sites sampled in a particular year). This approach allowed to compare the significance of variables in relation to the time when the chronosequence was sampled. All quantified environmental variables were used to explain the species composition of the ant communities. Further, RDA was used to test whether there was significant dependence between particular series and their age. Site age and series characteristics were chosen as independent variables. The variability explained by these variables was partitioned to identify which part of data variability can be explained by site age and which by series identity (unreclaimed, reclaimed, surrounding landscape). Tests were applied to reclaimed and unreclaimed series on spoil heaps and the surrounding landscape separately. Significance of variables in all RDAs was tested by means of the Monte-Carlo permutation test with 499 permutations. Principal component analysis (PCA) was used to visualise the relationship between ant species abundance and

environmental parameters. For this analysis, the whole dataset, including sites sampled only one year, was used. Abundances of ant species were log-transformed prior to all multivariate analyses, which were computed using the CANOCO 5 software (TER BRAAK & ŠMILAUER 2012). In all tests, the used level of statistical significance (α) was 5% (i.e., p = 0.05).

Results

In total, we found 24 ant species belonging to 2 subfamilies, Formicinae and Myrmicinae, on spoil heaps and in the surrounding landscape (Tab. S2). The number of ant species significantly increased between the sampling years as well as with site age (Fig. 1). In the year 2001, we recorded 18 species on the spoil heaps, while 22 species were found in 2020. The most common species were Lasius niger, Formica fusca, Myrmica rubra, and Myrmica ruginodis, which were also the most numerous ones, except for F. fusca. Lasius flavus had numerous colonies but was not widespread. One species (Formica sanguinea) found in 2001 was not discovered again in 2020. Three species (Myrmica lobicornis, Myrmica schencki, and Lasius platythorax) found only in the surrounding landscape in 2001 were discovered on spoil heaps in 2020 together with two new species (Camponotus ligniperda and Tetramorium caespitum) which had not been present in 2001. In both years, we found a comparable number of ant species in the surrounding landscape. The number of species found per site was higher there than on the spoil heaps (Tab. 2).

The total abundance of ant species differed marginally significantly (p = 0.056) between successional series and showed interaction of successional series and site age. The lowest abundance of ants was at unreclaimed sites, the highest in the surrounding landscape (Tab. 2, Fig. S1). Eurytopic species (dominated by *Lasius niger* and *Myrmica rubra*) were the most numerous group of ants, but their abundance did not change significantly during succession



Fig. 1: Summary of standardised regression coefficients of successional age obtained from multiple regression models, where individual ant community parameters were explained by year of sampling, site, and age. Error bars indicate the standard error of the regression coefficient estimates. Significant correlations (p < 0.05) are in black, non-significant correlations (p > 0.05) are in grey.

(Tab. 2). They were more abundant on spoil heaps, more specifically at reclaimed sites. The abundance of both forest species (*Camponotus ligniperda, Formica rufa*, and

	Sampling year (Y)	Series (S)	Age (A)	$\mathbf{Y} \times \mathbf{S}$	Y×A	$\mathbf{S} \times \mathbf{A}$	$\mathbf{Y} \times \mathbf{S} \times \mathbf{A}$
S	18.7***	1 2.9 ***	14.2***	0.7	0.4	3.6*	1.3
Total abundance	0.75	3.0^	1.3	1.1	2.8	3.4*	2.5
Eurytopic	1.2	0.4	2.04	0.9	3.6	2.05	1.8
Forest	8.9**	25.4***	20.7***	1.4	3.1	2.4	1.7
Open-habitat	0.05	7.4***	1.6	2.1	13.9***	4.2*	0.3
Hierarchy	0.2	30.6***	0.8	0.8	0.3	0.3	1.0
Claustral independent	1.6	7.4***	21.9***	0.6	0.7	2.5	1.4
Semi-claustral independent	2.9	11.8***	19.8***	0.14	0.77	2.9	1.7
Social parasites	1.4	5.0**	2.1	2.2	0.01	0.04	0.1

Tab.2: Results of the general linear model testing changes in numbers of species and in functional traits and groups in ant communities during succession. Significant F values are marked in bold and by asterisks (***p < 0.001, **p < 0.01, *p < 0.05, ^ p = 0.056).



Fig. 2: Comparison of Bray-Curtis dissimilarity to the surrounding landscape between unreclaimed and reclaimed sites in individual sampling years (upper panel) and correlation between this Bray-Curtis dissimilarity and site age in individual sampling years (lower two panels). Diamonds denote unreclaimed sites, circles denote reclaimed sites. Different letters in the upper panel mark significant (p < 0.05) difference in Analysis of Variance and the Tukey post-hoc test.

Lasius platythorax) and open-habitat species (Formica rufibarbis, Manica rubida, and Myrmica sabuleti) (further examples in Tab. S3) was significantly higher in the surrounding landscape in comparison with the spoil heaps (Tab. 2, Fig. S1). Forest species were the least numerous, but their abundance significantly increased with site age. It also differed between the sampling years. There was a numerous colony of *Formica rufa* at one site in 2001, otherwise forest species were more abundant in the year 2020. The abundance of open-habitat species increased in the year 2001 and remained relatively constant in 2020. In both years, however, they were most numerous at 20 - 30 year old sites (Fig. S2).

More competitive species (subdominants and dominants) such as *Formica rufa* and *Lasius niger* were more prevalent on spoil heaps, whereas less aggressive ones (subordinates) such as *Myrmica scabrinodis* and *Myrmica schencki* (more examples in Tab. S3) were rather found in the surrounding landscape (Fig. S1). The proportion of ants with a claustral independent mode of colony founding decreased with site age and was higher on spoil heaps, while the proportion of ants with a semi-claustral independent mode of colony founding increased with site age and was higher in the surrounding landscape (Fig. S1). The proportion of social parasites was higher at unreclaimed sites (Tab. 2, Fig. S1).

In both sampling years, older spoil heap sites were more similar to the referential surrounding landscape than the younger sites were. The Bray-Curtis dissimilarity to the reference sites was negatively correlated with site age, and there was no significant difference between reclaimed and unreclaimed series (Tab. 3; Fig. 2, panel A). The dissimilarity to the reference significantly decreased between the years 2001 and 2020, which reflects the progress in succession over the 19 years between the samplings. The decrease at reclaimed series was comparable with that of unreclaimed series. The changes which occurred between the years 2001 and 2020 were stronger in early successional stages than in late succession (Tab. 3; Fig. 2, panels B and C). The Bray-Curtis dissimilarity between ant communities in 2001 and 2020 was negatively correlated with site age at the first sampling, and the difference between reclaimed and unreclaimed series was non-significant (Tab. 3, Fig. 3). Changes in the surrounding landscape were smaller than those on the spoil heaps. The Bray-Curtis dissimilarity between 2001 and 2020 in the surrounding landscape was on average 0.41. Similar results were

Tab. 3: Results of the general linear model explaining Bray-Curtis dissimilarity by site age, reclamation status (unreclaimed vs. reclaimed), and interaction between both variables. Significant F values are marked in bold and by asterisks (***p < 0.001, **p < 0.01, *p < 0.05).

	Site age	Reclamation	Interaction
Dissimilarity to reference in 2001	6.03*	0.03	3.6
Dissimilarity to reference in 2020	17.23***	1.5	0.29
Dissimilarity between 2001 and 2020	4.18*	0.02	1.13

Tab. 4: Results of variation partitioning in Redundancy Analysis showing contribution of site age and site identity to explained data variability for reclaimed and unreclaimed sites on spoil heaps and for the surrounding landscape, "ns" means non-significant (p < 0.05). The "Total variability" row shows which part of data variability was explained by both site age and site identity among other factors. The other rows show which part of this variability was explained by site age, site identity, and its interaction. The sum of these contributions is always 100% because they are relative to the two chosen factors.

Succession	Total		Reclaimed		Unreclaimed		Surrounding landscape	
	%	р	%	р	%	р	%	р
Age	100	0.02	100	0.03	91	0.04	19	ns
Identity	0	ns	0	0	9	ns	81	0.03
Interaction	0	ns	0	0	0	ns	0	ns
Total variability	16	0.01	11	0.06	10	0.05	56	0.02

Dissimilarity between 2001 and 2020



Fig. 3: Bray-Curtis dissimilarity between the 2001 and 2020 sampling years (computed at the same site) correlated with site age (in 2001). Diamonds denote unreclaimed sites, circles denote reclaimed sites.

observed when only presence / absence data of ant species were considered, that is, when Sørensen dissimilarity was used. The only difference was that in the year 2001, the Sørensen dissimilarity to the surrounding landscape did not correlate with site age (Figs. S3 and S4, Tab. S4).

The results of variation partitioning in RDA (Tab. 4) revealed that site age was a much stronger predictor than series identity. In the case of reclaimed series, age was responsible for 100% of variability explained by the full model, while age explained 91% of data variability in unreclaimed series. Series identity and its interaction with succession age were in both cases non-significant. An opposite pattern was observed in the surrounding landscape. Series identity, which explained 81% of data variability, was the only significant parameter, while successional age of the sites had no significant effect. This indicates that the age of the chronosequence sites was more important for the species composition of ant communities than local environmental factors and landscape context.

The ant communities were affected by similar parameters in both sampling years, as indicated by RDA. The most important environmental parameters, significant **Tab. 5:** Environmental parameters responsible for ant succession selected with forward selection in Redundancy Analysis.

2001								
Parameter	Explains %	Pseudo-F	р					
Site age	19	14.1	0.002					
Bare soil cover	7.6	6.1	0.002					
Canopy	6.3	5.5	0.002					
Humus layer depth (cat.)	4.3	3.9	0.002					
Fermentation layer depth (cat.)	2.9	2.7	0.002					
Herb cover	2.7	2.6	0.004					
Distance to surrounding landscape	2.4	2.4	0.006					
Humus layer depth	2.3	2.4	0.012					
Succession x reclamation	2	2.1	0.022					
Bulk density	2.1	2.2	0.022					
2020								
Parameter	Explains %	Pseudo-F	р					
Site age	18.7	13.8	0.002					
Canopy	8.3	6.7	0.002					
Bare soil cover	5.9	5.1	0.002					
Succession x reclamation	4	3.7	0.002					
pH	3.1	2.9	0.008					
Presence of deadwood	2.3	2.1	0.03					

in the succession of ant communities in both years, were site age, bare soil cover, and canopy cover, which together explained 32.9% of data variability. The difference between unreclaimed series and reclaimed series was also



Fig. 4: Principal component analysis of species composition of ant communities. Grey arrows represent measured environmental variables, dark blue arrows represent ant species. Abbreviations of ant species: Fcuni – Formica cunicularia, Fprat - Formica pratensis, Lbrun - Lasius brunneus, Lflav - Lasius flavus, Lfulig - Lasius fuliginosus, Lniger - Lasius niger, Lplaty – Lasius platythorax, Mrubid – Manica rubida, Mrubra - Myrmica rubra, Mrugi - Myrmica ruginodis, Mscab - Myrmica scabrinodis, Mschen - Myrmica schencki; environmental parameters: A layer - humus layer depth, Age - site age since last major disturbance, Bulkd - bulk density, Canopy% - canopy cover, Dist - distance from surrounding landscape, Dwood - presence of deadwood, Herbs% - herb cover, Moss% - moss cover, Oe layer - fermentation layer depth, pH - soil pH, Soil% - bare soil cover, Wavy - surface heterogeneity.

significant in both years but explained only 2 - 4% of data variability. Distance of the site to the surrounding landscape was significant only in 2001. Other variables, selected as significant by forward selection, were depth of humus and fermentation layers, herb cover, and bulk density in 2001, and soil pH and presence of deadwood in 2020. Each of these parameters explained less than 5% of data variability (Tab. 5).

The main gradient in ant species composition distinguished by the first PCA ordination axis can be characterised as a gradient of successional development from sites with a large proportion of bare ground towards sites with thick litter, fermentation, and humus layers, and with a higher proportion of herbaceous layer cover. The one located along axis 2 was characterised as a gradient between open habitats and forest (indicated by canopy cover and deadwood presence). PCA also showed that the presence of forest and dendrophilous species such as *Lasius fuliginosus* and *Lasius platythorax* corresponds with canopy cover and presence of deadwood. Presence of species preferring open habitats, such as *Lasius flavus*, *Formica pratensis*, *Myrmica scabrinodis*, and *Myrmica schencki*, corresponded with moss and herb cover (Fig. 4).

Discussion

Over the course of succession, ant communities developed from species-poor communities, characterised by a few species of open early-successional habitats, towards more species-rich communities similar to those of the surrounding landscape formed by meadows with scattered shrubs and forest stands. The communities of late successional stages were characterised by a higher proportion of ants with a semi-claustral mode of colony founding and higher abundance of forest species. Our results also showed that the chronosequence method represented a reliable way of studying succession of ant communities. This is mainly supported by the fact that successional age was found to be the most important variable determining ant species composition and exceeded the effect of site alone.

Of the environmental parameters measured, we found the key factors driving ant community succession to be related to soil and vegetation development (bare soil cover and tree canopy cover). Both of these affect soil temperature and humidity, which are crucial factors for the occurrence of ants. Ants are a thermophilic insect group, and the richness as well as biomass of their community is strongly influenced by temperature and soil moisture (SEIFERT 2017). Vegetation development and canopy closure create shade, and differences in soil shading together with plant transpiration can create gradients in soil temperature, humidity and therewith different microclimatic conditions, which can then affect the occurrence of certain ant species (LUBERTAZZI & TSCHINKEL 2003). The only parameter important in the historical survey but insignificant in the recent survey was distance to the surrounding landscape. This can be explained by the fact that over time, the development of vegetation on spoil heaps has created suitable habitats for ants to inhabit. As ants began to colonise spoil heaps, the surrounding landscape was no longer the only place with potential colonisers. The distance between spoil heaps and the surrounding landscape has therefore become less significant and was no longer an important migration barrier. A previous study by VELE & al. (2011) also showed that migration distance can contribute to variation between chronosequences, particularly in initial stages of succession. This suggests that, despite the very good predictive power of chronosequences, one should beware of the effect of migration distance.

The number of ant species increased both between the sampling years and with site age. The rate of change in ant community composition was higher at young sites and slowed down with site age. The faster change at younger sites may be explained by the way ants colonise

new habitats. Ant species capable of founding colonies independently are the first pioneering species to colonise a newly formed habitat and are followed by ants founding nests through temporary nest parasitism (PUNTTILA & HAILA 1996). This order of nest founding may slow down the rate of change within an ant community because species founding colonies through parasitism are dependent on the presence of an already established colony of their hosts. This aspect is important as more than a quarter of ant species living in Central Europe are social parasites (SEIFERT 2018). In our case, we also observed changes in the proportion of species with different modes of independent colony founding. The proportion of ants with a claustral mode of colony founding decreased with site age while the proportion of ants with a semi-claustral mode of colony founding increased. The latter group was represented mostly by ants of the genus Myrmica in our study. This shift probably corresponds with the development of vegetation cover during succession, which provides a suitable environment to ants with a semi-claustral mode of colony founding, offering both shelter from predators and food sources (Hölldobler & Wilson 1990).

Another aspect affecting the rate of change may be the long lifespan of queens in certain species and their competitive ability. Queens of species forming large colonies can live for many years, for example, about 20 years in Lasius niger (KRAMER & al. 2016) and about 10 years in Formica species (Keller 1998), so their nests may persist at a site for many years (PECH 2011). Both genera (Lasius niger and Formica (Serviformica) fusca) were present and quite abundant at early successional stages. Moreover, the majority of ant species are omnivorous and can live in more than one type of habitat, so their ecological niches overlap, which may lead to competition. Aggressive species are likely to live in more profitable habitats (PISARSKI & Vepsäläinen 1989). The mortality of nest-founding queens is very high (PUNTTILA & HAILA 1996), as most of them are killed either by predators or by workers of other ant species before they establish a colony (WHITCOMB & al. 1973). It may thus be extremely difficult for a newly mated queen to establish a new colony in the territory of an already existing one, which can block the arrival of later species even if environmental conditions would be favourable for them. This might be the case here, since more aggressive species were found on the spoil heaps and probably became strong competitors.

Contrary to previous studies, we found the composition of ant communities at reclaimed and unreclaimed post-mining sites to be rather similar and developing towards that of the referential surrounding landscape. Other studies showed development of more diverse communities at unreclaimed sites (HENDRYCHOVÁ 2008, ŠÁLEK 2012, TROPEK & al. 2012). However, TROPEK & al. (2014), who studied differences in arthropod assemblages on reclaimed and unreclaimed fly ash deposits including ants, found ants to be the only group whose composition did not differ between habitats, discussing that interspecific competition was the main factor forming ant communities. In our case, another factor which might have contributed to the similar composition of ant communities on spoil heaps and in the surrounding landscape was the fact that our reference sites were originally managed by people and after their abandonment secondary successional processes started there. These sites thus had a history and vegetation structure similar to that on spoil heaps.

We did not observe differences in the rate of change in ant community composition between reclaimed series and series spontaneously revegetated despite their different characteristics. Ants are influenced by the structure of vegetation cover rather than vegetation diversity, but also by other factors such as presence of habitats suitable for founding nests (GALLÉ & al. 1998, LASSAU & HOCHULI 2004). Presence of woodland as a late successional stage may have been more important for the rate of change in ant community composition than differences in forest composition between particular sites. Also eurytopic species were the most numerous and their abundance did not change throughout our observation period, which contributed to this. However, aging of the forest stands and accumulation of deadwood allowed the spread of forest species. Similarly, SEIFERT & PROSCHE (2017), who also studied succession of ant communities on spoil heaps after opencast mining, found forest species to be prevalent at the oldest sites with forest stands. In their case however, forest ants occupied the majority of ant nests and essentially replaced ants preferring open habitats.

In conclusion, we found ant communities on spoil heaps to develop towards that of the surrounding landscape. Environmental parameters driving ant community succession were related to soil and vegetation development. Nevertheless, successional age was the single strongest predictor of ant species richness and composition, suggesting a similar successional trajectory of sites. Despite the very good predictive power of chronosequences, there was still some unexplained variability left in our data, which indicates certain variability in individual chronosequence trajectories. The number of species but not their total abundance increased during the succession. The rate of changes was higher at young sites, and we did not observe any differences in development of ant communities between reclaimed and unreclaimed sites. Aging of the forest stands and accumulation of deadwood allowed forest species to spread. The shift in ant community composition from ants with a claustral mode of colony founding at younger sites towards those with a semi-claustral mode and social parasites at older sites suggest an increase in resource availability during succession, which may then enhance species coexistence. Overall, our results suggest a consistent change in ant species richness and composition, with potentially more resources and more complex species interactions in later stages.

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Declaration on use of generative artificial intelligence tools

The authors declare that they did not utilize generative artificial intelligence tools in any part of the composition of this manuscript.

Author contributions

Jan Frouz conceived the major idea, Jan Frouz and Michal Holec designed the methodology; Michal Holec and Marie Hovorková collected the data and identified ant species; Ondřej Mudrák and Jan Frouz analysed the data; Marie Hovorková led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Conflict of interest

The authors have no conflict of interest to declare.

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