Summary of the Ph.D. thesis

The effect of habitat heterogeneity and human disturbance on ant and plant assemblages of karst dolines

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1 Introduction

Karst areas cover 20% of the earth's terrestrial surface. The dissolution processes on limestone surfaces contributed to the formation of karst surface and subsurface features such as caves, limestone pavements, valleys, and enclosed depressions (dolines, sinkholes or tiankengs). Karst areas provide important ecosystem services, one of which is the habitat function. Karst depressions (dolines), due to their topographically intricate features and microclimatic inversions, may support several habitats where species composition and diversity vary with environmental heterogeneity. Dolines may have the potential to act as safe havens for a high diversity of valuable species. However, nowadays these important doline habitats are altered by various man-made activities that made them highly vulnerable and prone to degradation.

Topographic parameters (e.g., geometry, size, and slope aspect) and related environmental conditions (e.g., air temperature, air humidity and soil moisture), which contributed to heterogeneity of doline habitats, are known to play a major role in determining their biodiversity patterns. These topographic and biological attributes can create microhabitats with unique microclimates which species may depend on for their survival. Microhabitats may be warmer, drier, cooler and/or moister than the prevailing regional climate. As such, they create a mosaic of microclimates that can allow species to survive changes in their environment by migrating short distances between these microhabitats. These fine-scale mosaics improve species' chances also to persist in a certain landscape. Various studies have shown that dolines may provide microhabitats for unique species that are rare or absent from the surrounding landscape. The heterogenous habitats of dolines may serve as *refuges* for decades or *refugia* for millennia for vulnerable taxa such as endemic, montane, and relict species. Many of these species can be under threat because of global warming and anthropogenic activities.

Dolines have been cultivated extensively, providing sites for various traditional activities such as arable farming, gardening, pasturing and water extraction. The

degradation of dolines is among the most pressing issue in karst environments worldwide. Human-induced activities may compromise the capacity of dolines to act as safe havens for species during future climate oscillations, and, as a result, may also reduce the naturalness of their habitats. In Hungary, for centuries, intensive deforestation and grazing drastically have been affecting doline habitats.

Ants and plants were selected as focal taxa because ant colonies and plants share many similarities. Members of both groups usually 'nest' in or on the ground and use their modules (e.g., plant roots and ant workers) to forage in the surrounding habitat. In addition, due to the relatively fixed location of ant colonies and plants, competition in both groups is confined to well-defined zones. Similarities also exist in terms of their functional roles (e.g., subordinate, specialist, and cryptic species). Also, ant foundresses (i.e., colony-founding queens) can be considered analogous to dispersing plant seeds. Ants are particularly sensitive to changes in resources, moisture, and temperature, which makes them good indicators of environmental changes, ecosystem health and functioning. Generally, the species richness of ants is positively associated with habitat diversity, which may also affect the functional diversity of their assemblages. Topographically complex areas may contain a higher diversity of microhabitats providing suitable nesting sites and a higher amount of exploitable resources for different functional groups of ants. Plants have long been recognized as bioindicators and can indicate the naturalness of their habitat. The approach of naturalness indicator values is like the original Ellenberg-type indicator values and has been used successfully to study disturbance. Some species indicate disturbed sites, while others prefer less disturbed or natural habitats. For instance, the presence of many endemics, habitat specialists and endangered species within a habitat may indicate higher naturalness and higher conservation value.

Although recent investigations have indicated that dolines serve as important habitat islands for various taxa, data on influence of these heterogenous microhabitats on the functional diversity of these taxa are lacking. In addition,

since man-made activities are known to alter these habitats, the actual effect on naturalness of doline vegetation is sparse.

2 Aims

The general aim of the doctoral study was to assess the effect of habitat heterogeneity and human disturbance on ant and plant assemblages in two Hungarian karst areas. Specifically, the following questions were addressed:

Study 1: How microhabitats in dolines (i.e., south-facing slopes, north-facing slopes, bottoms, and plateau) determine the species distribution patterns of ant and plant assemblages?

Study 2: How microhabitat heterogeneity and vegetation structure in dolines influence the occurrence of specific functional groups and traits of ant assemblages?

Study 3: How different types of human activities, i.e., human-mediated invasion of reed grass (*Calamagrostis epigeios*) and intensive forestry management affect the naturalness of doline vegetation?

3 Materials and Methods

Study 1

The first study was conducted in the karst plateau of the Bükk Mountains, Northern Hungary with an altitude between 780 m and 950 m. The plateau has a cool and humid climate with a mean annual temperature of 6.3 °C and a mean annual precipitation of 800 mm. A mountain range considered as an important refugial area in Hungary. Solution dolines are the typical karst landform features in this area with bowl-shaped geometry and unique microclimate.

Three large solution dolines were selected for the study. Six sampling sites were established for each doline. Microclimate measurements and sampling (ants and plants) were carried out in August 2017 under clear weather conditions at the peak of the growing season. For the microclimate measurements, air temperature (T) and relative air humidity (RH) were recorded every minute for 24 hours using Voltcraft DL-121 TH data loggers. Sensors were set-up 10 cm above the ground to measure actual air temperature and humidity. For the sampling of ants, non-destructive sampling methods such as baiting, and hand collecting were used to assess the species diversity and relative abundance of ants. At each study site, five bait stations were placed in a cross-shaped pattern at 5-m intervals. Baits were plastic discs (8 cm in diameter) with a quarter teaspoon of a combination of tuna and honey as food reward. The foraging activity on baits were observed every 40 minutes from 7:00─10:20 am, overlapping with the daily period of the peak activity of ants. We recorded the presence and absence of workers of each ant species on the bait. Hand collecting was also done to sample those ant species that may have not visited the baits during the baiting. We visually searched the ground surface in each site for 5 minutes collecting any individuals (workers, incipient queens, etc.) found. Ants were identified to morphospecies or genus level in the field, and representatives were collected and preserved in 95% ethanol for later species determination. For plants, five randomly selected plots of 1m × 1m were established in each site. We recorded the presence/absence of all vascular plant species in all plots. Nomenclature for plants follows 'The Plant List'.

All ant and plant species sampled were classified into six functional groups following the methods of Czechowski et al. (2012) for ants and Borhidi (1995) for plants. The functional groups are (1) species adapted to warmer conditions, (2) species adapted to cooler conditions, (3) species adapted to intermediate temperature conditions, (4) species adapted to drier conditions, (5) species adapted to moister conditions, and (6) species adapted to intermediate moisture. For the data analysis, temperature and relative humidity data were averaged over 10-minute intervals across all sites of south-facing slopes, bottoms, north-facing

slopes, and the plateau, respectively, and plotted using a line graph. From the site-averaged data, we calculated the mean daily temperature (T_{24}) and relative humidity (RH_{24}), mean daytime temperature (T_d) and relative humidity (RH_d), and mean night temperature (T_n) and relative humidity (RH_n). We also calculated these microclimate variables separately for each site and used them in multivariate analyses. The diagnostic ant and plant species of the microhabitats were determined by calculating the phi coefficient (Φ) of association between species and habitat. To test the effect of microhabitats (i.e., south-facing slopes, bottoms, north-facing slopes, and the plateau) on the species composition of ant and plant assemblages, we used permutational multivariate analysis of variance (PERMANOVA). To visually illustrate compositional differences, we prepared non-metric multidimensional scaling (NMDS) ordinations. To assess the relationships between microclimate variables and species assemblages, we fitted environmental vectors into the ordination space and calculated correlations between ordination values and fitted vectors. To illustrate the distribution of the various functional groups in different microhabitats, we used mean-and-whisker plots. To test if these differences were significant, we used generalized linear mixed-effects models (GLMMs). Full models were tested for significance with analysis of variance (ANOVA). Pairwise comparisons of factor levels were done, and the false discovery rate (FDR) method were used for multiple comparisons. For the microclimate, temperature and relative humidity data were averaged over 10-minute intervals across all sites of south-facing slopes, bottoms, north-facing slopes, and the plateau, respectively, and plotted using a line graph.

Study 2

The second study was conducted in the karst plateau of the Bükk Mountains, Northern Hungary. Four doline types used for sampling were grouped based on vegetation cover (grassland vs. forest): type 1 – grassland dolines surrounded only by grasslands (forest cover in their surroundings: 0%), type 2 – grassland dolines surrounded by low amounts of forest (30–40%), type 3 – grassland dolines surrounded by large amounts of forest (90–100%), and type 4 – forested

dolines surrounded only by forests (100%). Ant sampling were adapted from Study 1 with slight modifications. Three large solution dolines were selected from each doline type for the ant assessment. Six sampling sites were selected for each doline types. Methods for baiting and hand collecting, as well as ant identification and preservation methods were the same as in Study 1. Data collection was done in August 2018 on good weather condition.

All ant species sampled were classified into four main functional groups that relate to (1) temperature preference, (2) moisture preference, (3) habitat preference, and (4) habitat plasticity following Czechowski et al. (2012) and Seifert (2018). Species were also classified according to three main functional traits that relate to (1) dispersal ability, (2) aggressiveness, and (3) social structure (i.e., monogynous or polygynous colonies). For the data analysis, the diagnostic ant species of microhabitats (doline vs. plateau) and doline types (types 1-3) were determined by fidelity calculations using the phi coefficient (Φ) of association. To analyze the effects of microhabitat (doline vs. plateau) and doline type (types 1-3) on the functional groups and traits of ants visiting the baits, we used generalized linear mixed-effects models (GLMMs). Full models were tested for significance with analysis of variance (ANOVA). We used the false discovery rate (FDR) method to account for multiple comparisons when performing pairwise comparisons.

Study 3

The third study was conducted in the karst plateaus of Bükk Mountains, Northern Hungary (700–800 m a.s.l.; mean average temperature: 6.3 °C; mean average precipitation: 800 mm) and of Mecsek Mountains, Southern Hungary (300–500 m a.s.l.; mean average temperature: 9.5 °C; mean average precipitation: 750 mm). Natural dolines in the Bükk Mountains are covered by semi-dry, dry, and wet grasslands, while disturbed dolines can be characterized with a high cover of *C. epigejos*. Natural dolines in the Mecsek Mountains are covered with 90–120-year-old trees, while disturbed dolines, which are characterized by a markedly

higher intensity of forest management, are covered mostly with 40–50-year-old trees.

Six dolines, three for ‘natural’ and three for ‘disturbed’ were sampled in the Bükk and Mecsek Mountains, respectively. For plant sampling, transects consisting of 1 m × 1 m plots spaced at 2 m intervals from north to south orientation across each doline traversing their deepest point were established. We recorded the presence/absence of all herbs, shrubs and tree seedlings and saplings in each plot. Plant nomenclature follows ‘The Plant List’. Fieldwork was carried out from June to August at the peak of the growing season.

All the plant species sampled were classified according to their social behavior types. We used the “Social Behavior Types” (SBT) of Borhidi, (1995) which are defined for the Pannonian biogeographical region to characterize the naturalness of dolines. Four main functional groups of species were analyzed: (1) specialist species, (2) competitor species, (3) generalist species, and (4) species of disturbed habitats (disturbance tolerant species, natural pioneers, weeds, and ruderal competitors). For the data analysis, to identify species that prefer one specific habitat type (natural or disturbed dolines) diagnostic species analysis was performed. To assess the differences in the vegetation composition of the two habitat types (natural vs. disturbed dolines), non-metric multidimensional scaling (NMDS) ordinations and one-way analysis of similarities (ANOSIM) were done. To compare the social behavior types of the habitats, generalized linear mixed models (GLMMs) were used. The percentage frequency of each species was determined both for the natural and disturbed dolines.

4 Results

Study 1

The first study showed that doline habitats which possess a wide range of microclimatic conditions may have the capacity to support diverse ant and plant assemblages. Karst dolines present wide variation in microclimates, in these dolines, mean daytime temperatures were more than 8 °C warmer on south-facing slopes than on north-facing slopes. The study demonstrated that the distributions of different functional groups of both ants and plants respond to the fine-scale microclimatic differences among the microhabitats in a similar manner. The cooler and moister north-facing slopes and bottoms of dolines acted as key habitats for plants adapted to cooler and/or moister conditions (e.g., *Bupleurum longifolium* and *Iris sibirica*) and for ants adapted to cooler conditions (e.g., *Myrmica ruginodis*). On the other hand, south-facing slopes acted as key habitats both for ants (e.g., *Myrmica sabuleti*) and plants (e.g., *Iris variegata*) adapted to warmer and/or drier conditions, while many ant (e.g., *Lasius niger*) and plant species (e.g., *Galium verum*) found on the plateau indicated intermediate temperature and/or moisture conditions. Since the recorded ant species are not known to form strict trophic relationships with plants, the main drivers of the observed patterns seem more of their temperature and moisture preferences. These salient findings underline the importance of considering fine-scale environmental variation when investigating the distribution patterns of biodiversity.

The study highlighted that enclosed depressions (dolines) in karst surfaces contain a broad spectrum of microclimatic habitats that have the capacity to support various taxa (ants and plants) in different phyla under various climatic trends.

Study 2

The second study showed that microhabitat heterogeneity within dolines has a positive effect on the functional diversity of ant assemblages by supporting specific functional groups and traits that are rare on the plateau and possibly could be eliminated during global warming. In addition, the study found out that the increasing cover of resource-poor environments such as the European beech (*Fagus sylvatica*) forests negatively influence the capacity of dolines to act as functional refugia for ants within karst landscapes. Considering that *F. sylvatica* forests provide unfavorable habitats for ants, some aggressive species associated with forest habitats like *Formica truncorum* and *L. platythorax* could find suitable habitats in these dolines for their foraging and nesting activities. The findings of the study underline the importance of dolines as local biodiversity hotspots in karst landscapes by supporting specific functional group patterns of ant assemblages. Grassland dolines in the study area acted as key habitats for ants adapted to cooler and/or moister conditions (e.g., *Myrmica lobicornis*, *Myrmica ruginodis*, and *Lasius platythorax*), while species on the plateau indicated intermediate temperature and/or moisture conditions (e.g., *Lasius bombycina*, *Lasius niger*, and *Myrmica scabrinodis*).

The study demonstrated that ants are reliable model organisms which can be indicators for identifying those locations that have the capacity to provide safe havens for different functional groups of species. Additionally, the study provided further evidence to the hypothesis that heterogeneous karst landscapes may maintain functionally diverse animal assemblages and play an important role in buffering the negative effects of global warming.

Study 3

The third study showed that anthropogenic activities may alter the conservation value of dolines. Furthermore, findings of the study suggest that valuable species in dolines are threatened by different human activities, such as the human-

mediated invasion of ruderal competitor species (e.g., the invasion of *C. epigejos*) and high levels of forest management (e.g., clear cutting and frequent logging), and one possible way to evaluate the impacts of such human activities on vegetation is the assessment of the naturalness of habitats. It was found that various disturbances decreased the number of specialist and competitor species in dolines, thus reducing the naturalness and conservation value of these special habitats. Also, results of the study suggest that the climatically diverse habitats of karst surfaces are extremely sensitive to anthropogenic disturbances and that their recovery from such disturbances may be slow or may not occur at all. On the other hand, dolines with natural vegetation continuously provide unique habitats for a high diversity of valuable species and indicate high naturalness.

The study indicated that human-mediated invasion of dominant grassland competitors and extensive forestry activities negatively influence the degree of naturalness in dolines and alter the capacity of dolines to support valuable species.

5 Conclusions

Enclosed depressions (dolines) in karst surfaces possess a wide range of microclimatic habitats that have the capacity to support various taxa in different phyla such as Arthropoda and Tracheophyta under various climatic trends. Therefore, these dolines may be crucial in facilitating the in-situ persistence of numerous species under local and global climate oscillations. This implies that modelling of climate change impacts on the distribution of biodiversity will need to consider fine-scale topographic variation occurring within tens of meters to arrive at accurate predictions.

There is a strong relationship between environmental heterogeneity and the distribution of functional groups and traits among ant assemblages in karst ecosystems. Heterogeneous karst landscapes may maintain functionally diverse animal assemblages particularly of ants. Hence, proper management and

conservation of these safe havens (dolines) should be given more priority to strengthen their role as important environmental buffers against the effect of global warming to organism.

Human-induced invasion of grassland competitor species and intensive forestry activities may decrease the naturalness of vegetation of dolines. Thus, to maintain the naturalness of vegetation in these doline habitats, it is recommended that the populations of ruderal competitors should be eliminated by controlled mowing and grazing, and sustainable forest management should be adapted by protecting surrounding forests, maintaining old forest stands, rehabilitating young forest and homogeneous stands, and implementing nature-based forest management(e.g., continuous cover forestry). Further investigations are needed on the differential impacts of disturbance to these dolines in order to identify more appropriate conservation strategies for these disturbed doline habitats and its important species.

6 List of publications

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Publications related to the Ph.D. thesis

Aguilon, **D.J**., Vojtkó, A., Tölgyesi, C., Erdős, L., Kiss, P.J., Lőrinczi, G., Juhász, O., Frei, K., & Bátori, Z. (2020). Karst environments and disturbance: evaluation of the effects of human activity on grassland and forest naturalness in dolines. *Biologia*, *75*, 1529–1535**. IF_{2020}: 1.350 (Q3)**

Bátori, Z., Vojtkó, A., Maák, I.E., Lőrinczi, G., Farkas, T., Kántor, N., Tanács, E., Kiss, P.J., Juhász, O., Módra, G., Tölgyes, C., Erdős, L., **Aguilon**, **D.J**. & Keppel, G. (2019). Karst dolines provide diverse microhabitats for different functional groups in multiple phyla. *Scientific Reports*, *9*, 7176. **IF_{2019}: 3.998 (D1)**

Bátori, Z., Lőrinczi, G., Tölgyesi, C., Módra, G., Juhász, O., **Aguilon**, **D**.**J**., Vojtkó, A., Valkó, O., Deák, B., Erdős, L. & Maák, I.E. (2020). Karstic microrefugia host functionally specific ant assemblages. *Frontiers in Ecology and Evolution, 8*, 613738. **IF_{2020}: 4.171 (Q1)**

Other publications

Aguilon, **D**.**J**., Velasco, L.R.I, & Medina C.D. (2015). Effects of larval rearing temperature and host plant condition on the development, survival, and coloration of African armyworm, *Spodoptera exempta* Walker (Lepidoptera: Noctuidae). *Journal of Environmental Science and Management, 18*, 54–60. **IF_{2015}: 0.250 (Q3)**

Juhász, O., Bátori, Z., Trigos-Peral, G., Lőrinczi, G., Módra, G., Bóni, I., Kiss, P.J., **Aguilon**, **D**.**J**., Tenyér, A., & Maák, I. (2020). Large- and small-scale environmental factors drive distributions of ant mound size across a latitudinal gradient. *Insects, 11*, 350. **IF_{2020}: 2.769 (Q1)**

Juhász, O., Fürjes-Mikó, Á., Tenyér, A., Somogyi, A.Á., **Aguilon**, **D**.**J**., Kiss, P.J., Bátori, Z., & Maák, I. (2020). Consequences of Climate Change-Induced Habitat Conversions on Red Wood Ants in a Central European Mountain: A Case Study. *Animals, 10*, 1677. **IF_{2020}: 2.752 (Q1)**

Cumulative impact factor (IF): 15.290