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ORIGINAL RESEARCH PAPER

Bryophyte diversity in karst sinkholes affected by different degrees of human disturbance

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Abstract

The diversity of bryophytes in karst sinkholes has received little attention, and these habitats probably play a crucial role as refugia. In this study, bryophyte diversity affected by different levels of human disturbance in five karst sinkholes was compared. A total of 132 species of bryophytes (17 liverworts and 115 mosses) that belong to 64 genera and 30 families were recorded. The richness of the bryophytes in the natural sinkholes was significantly higher than that of the bryophytes in the sinkholes affected by tourism and used as farmland. Canonical correspondence analysis showed that soil moisture is one of the most important factors that affect the abundance of bryophyte distribution in the five sinkholes. Human activities, including agriculture, animal husbandry, and tourism development, reduce the bryophyte coverage of sinkholes and lead to soil moisture loss. Therefore, effective protection of karst sinkholes is required to maintain their original value for biodiversity conservation.

Keywords

negative terrain; microhabitat; liverworts; environmental factors; artificial interference; Southwestern China

Introduction

A sinkhole is a typical negative ground terrain; it is a small-to-large bowl-shaped depression on the surface of a karst [1]. The thermal inversion caused by the inverted terrain often keeps the interior of the sinkhole cold [2]. As a result, deep sinkholes may serve as microclimate refugia, especially for cold-adapted species vulnerable to climate changes [3]. Under continuous climate changes, the distribution patterns of many cold-adapted species have undergone tremendous changes [4]. In Europe, the refuge areas of cold-adapted species are primarily found at high elevations and lower latitudes and low to high elevations and higher latitudes [1]. Because of the narrow distribution of cold-adapted species, their survival depends to a large extent on the existence of appropriate microhabitats; therefore, their emergence is often limited to specific habitats (for example, gully forests and northerly rock groups) that can provide a place suitable for the sustainable survival of cool plant taxa outside the large climatic range [1,5,6]. Species distribution models indicate that, in response to persistent climate change, many cold-adapted plant species in Eastern and Central Europe will undergo range changes and may disappear from low-altitude areas [7]. Previous studies have shown that sinkholes are important refugia for many cold-adapted plant taxa, which could become space-for-time substitutions, and the buffering capacity of shelters increases in colder climates (higher elevations and latitudes) and colder (northward) slopes [1]. Bátori et al. [8] found that the bottom of a sinkhole is usually inhabited by very rare species or those unable to survive in the surrounding habitats. Significant floristic differences exist between the bottom of a sinkhole and a higher slope. Many plants, especially cool-adapted species, are restricted to the bottom of the sinkhole, where more water is available; therefore, the bottom of the sinkhole has higher humidity and soil moisture than at the top of the sinkhole [9]. For example, *Dracocephalum ruyschiana* from Eurasia may have maintained a viable population in a large sinkhole in northern Hungary, which is far from its main distribution [10]. The beech forests on Mecsek Mountains have a high chance of survival in deep sinkholes for longer periods [11]. As a result, sinkholes are also considered natural habitat islands, and they play an important role in reducing the rate of plant species extinction [11].

The development of sinkholes determines to some extent the high diversity and endemism of local biota; however, at the same time, they may also become endangered habitats, as they may lose their restoration characteristics because of changes in surface conditions [2]. The karst environment is extremely sensitive to human activities, and human interference and use of sinkholes to varying degrees may lead to the degradation of its environmental value, that is, the biological shelters will disappear [12]. In Slovenia, where sinkholes have been developed to meet agricultural needs or become landfills, such anthropogenic land degradation can have a significant negative effect on the fragile sinkhole environment [13]. Similarly, in some parts of Southwestern China, sinkholes have undergone tourism development or manmade destruction, followed by rapid degradation of the original landscape. The degradation of sinkholes is leading to the homogenization of vegetation, which reduces regional biodiversity [13]. Therefore, protection of the ecological environment of sinkholes is of great significance for the protection of plants, especially endangered species.

Bryophytes are widely distributed in nature. Most bryophytes, especially liverworts, are generally considered to be shade plants [14,15], which are very sensitive to changes in the environment [16]. As a result, bryophytes are often used as indicators of environmental changes [17–19]. The distribution of bryophyte species can reflect the environmental conditions of sinkholes [20], especially humidity. Pericin et al. [21] studied bryophytes at different levels in an Istrian karst doline and found that the distribution of bryophytes in the sinkhole showed a gradual change. Differences in relative humidity may have a greater effect on the distribution of bryophyte species, in which the influence of the microclimate in the location seems to be very important [21]. Often, bryophyte species that are rare outside the sinkhole, such as *Pedinophyllum interruptum*, *Lophocolea bidentata*, *Plagiomnium undulatum*, and *Thamnobryum alopecurum*, are found at the bottom of a sinkhole because of the presence of more water and nutrition.

Few studies have been performed on the diversity of bryophytes in sinkholes, and these were mostly based on a single independent sinkhole. In addition, the microhabitats of dolines are not yet considered to be valuable hot spots of karst diversity, and so they are not specifically protected. In this study, we analyzed the bryophyte data from a karst sinkhole group in Southern China. The sinkholes with different levels of human interference were compared. Our results provide a basis for determining the protection of karst sinkhole resources. The following issues were addressed:

- What is the overall diversity and distribution pattern of bryophyte species at various sampling points along the microclimate gradient in a sinkhole?
- What is the distribution of bryophytes in sinkholes affected by differing levels of human interference?
- What are the main environmental factors that affect the distribution of bryophytes in the sinkholes in the study area?

Material and methods

Study area

Our study area is located in a karstificated plateau in Guizhou Province, Southwestern China (26°46′21.83″–26°46′49.21″ N and 105°52′46.35″–105°54′33.03″ E). This area has a subtropical monsoon climate. The average annual temperature is 14.1°C, rainfall – 1,436

Tab. 1 Basic characteristics of the five karst sinkholes.

Sinkholes name	Abbreviation	No. of quadrats	Area (ha)	Altitude (m)	Max depth (m)	Disturbance grade (scores)				
						Extent	Soil	Time	Frequency	Total scores
Dachaokou	DCK	40	22.68	999	330	HM (1.5)	L (0)	H (2)	M (1)	4.5
Xiaochaokou	XCK	15	4.59	1,075	220	ML (0.5)	L (0)	H (2)	M (1)	3.5
Jiayandong	JYD	30	0.59	1,200	65	ML (0.5)	H (2)	M (1)	H (2)	5.5
Dachilong	DCL	55	8.02	1,271	200	M (1)	L (0)	L (0)	L (0)	1.0
Xiaochilong	XCL	45	0.69	1,265	67	ML (0.5)	L (0)	L (0)	L (0)	0.5

The disturbance scoring was obtained from the archival information in the scenic area. Each index was scored 0, 0.5, 1, 1.5, and 2: low (L), medium/low (ML), medium (M), high/medium (HM), and high (H) disturbance, respectively. The interference intensity of each sinkhole is the sum of the scores assigned for each index [32].

mm, and sunshine – 1,172 h. Abundant rainfall and suitable heat conditions provide favorable natural conditions for the development of karst sinkholes [22]. We investigated five sinkholes developed in the 42-km^2 region with a distribution density of 0.17/km^2, which is one of the highest in the world. The basic characteristics and habitats of studied sinkholes are listed in Tab. 1 and Fig. 1, respectively.

The Xiaochilong sinkhole (XCL) is located in a remote foothill; the top of the sinkhole is closed, and it is surrounded by steep potholes, and only a narrow and steep slope to the southeast is available for entering the sinkhole. The entrance to the bottom is rich with shrubs and trees, such as *Viburnum chinshanense*, *Mallotus philippensis*, *Trachycarpus fortunei*, and *Cunninghamia lanceolata*. Under the forest, abundant herbs and ferns are present. Because of the closure of the sinkhole, obvious changes were observed in temperature, humidity, and illumination along the slope from the top toward the bottom. The Dachilong sinkhole (DCL) is located at the top of the mountain and not far to the northeast of XCL; it is oval, the diameter of the mouth is 390 × 262 m, the top of the sinkhole is open, and the wall is upright and towering with only a gentle slope to the south for entering the sinkhole. The vegetation in the sinkhole is mostly shrubs, medium-sized trees, and herbs, such as *Viburnum chinshanense*, *Cinnamomum parthenoxylon*, *Holboellia latifolia*, *Eremochloa ciliaris*, *Pennisetum alopecuroides*, and *Lolium perenne*. No sign of river activity was detected at the bottom of both DCL and XCL.

The Dacaokou (DCK) and Xiaocaokou (XCK) sinkholes are located in a canyon, and they are a part of the local tourist attractions. They developed as a pair, separated by a natural bridge, and surrounded by steep cliffs downstream of the Qijiehe River. They can only be entered along both sides of the river, where vegetation grows well and many tall trees, shrubs, and vascular plants can be observed, such as *Pteroceltis tatarinowii*, *Pterocarya stenoptera*, *Swida macrophylla*, *Musa basjoo*, and *Begonia grandis*. The top of XCK is oval, 325 × 180 m in diameter, and DCK can be found northeast of XCK, through the natural bridge. The top of DCK is a long strip, and it is one of the largest sinkholes in the world [22].

The Jiayandong sinkhole (JYD) is a single sinkhole and ellipsoid at the top. The sinkhole is seriously degraded, with no sign of river activity at the bottom and a rock wall on the west side. The bottom of the sinkhole has been used as arable land by the local residents for a long time. Natural vegetation can be found only in the uncultivated area at the top of the sinkhole. A large cave opens under the eastern wall and narrows inward.

Sample collection

The fieldwork and sampling were performed in September and October 2017, and we collected bryophyte samples from the five sinkholes. DCL and XCL were located in more remote areas with low accessibility, and we struggled to enter DCL along the gentle southern slope with the help of the locals. Taking into account the terrain and actual distribution of vegetation, we set up a sample point every 10 m for bryophyte collection. Finally, a total of 11 sample sites were set up in DCL, including the top, middle, and bottom (sample settings are shown in Fig. 2). A narrow stone slope road exists southeast of XCL, along which we smoothly entered the sinkhole. Bryophyte samples were collected from the top, middle, and bottom, with a total of nine sampling sites. In contrast, the sampling of DCK and XCK was much easier. To develop tourism, a 104 m vertical elevator has been built at the bottom of DCK; we took the elevator directly to the bottom. Because these two sinkholes (DCK and XCK) are surrounded by stone walls, we collected samples only from the bottom. In DCK, we set up four sampling sites on both sides of the river to collect bryophytes. The bottom of XCK is divided into north and south banks according to

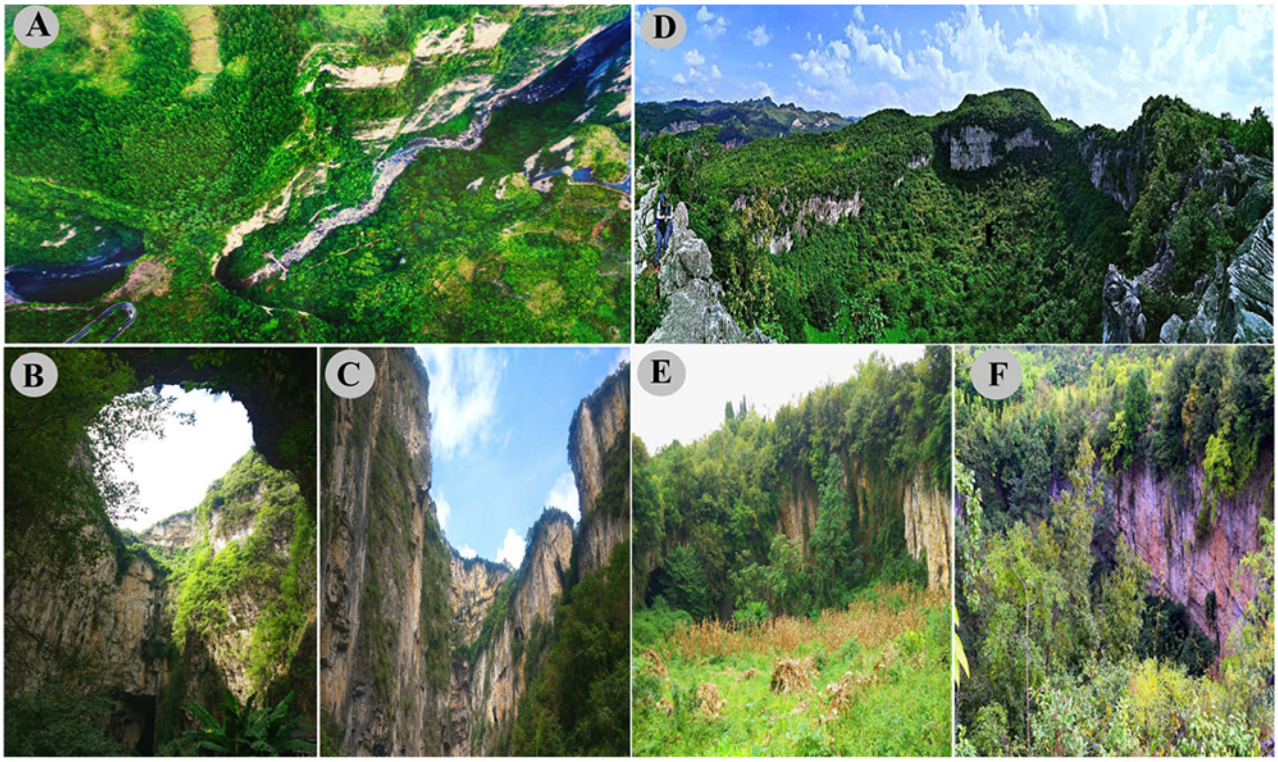

Fig. 1 The habitats of the five karst sinkholes. (**A**) The overlook of DCK (right) and XCK (left) sinkholes (photo credit: Zhijindong Cave Scenic Spot); (**B**) XCK sinkhole; (**C**) DCK sinkhole; (**D**) DCL sinkhole; (**E**) JYD sinkhole; (**F**) XCL sinkhole.

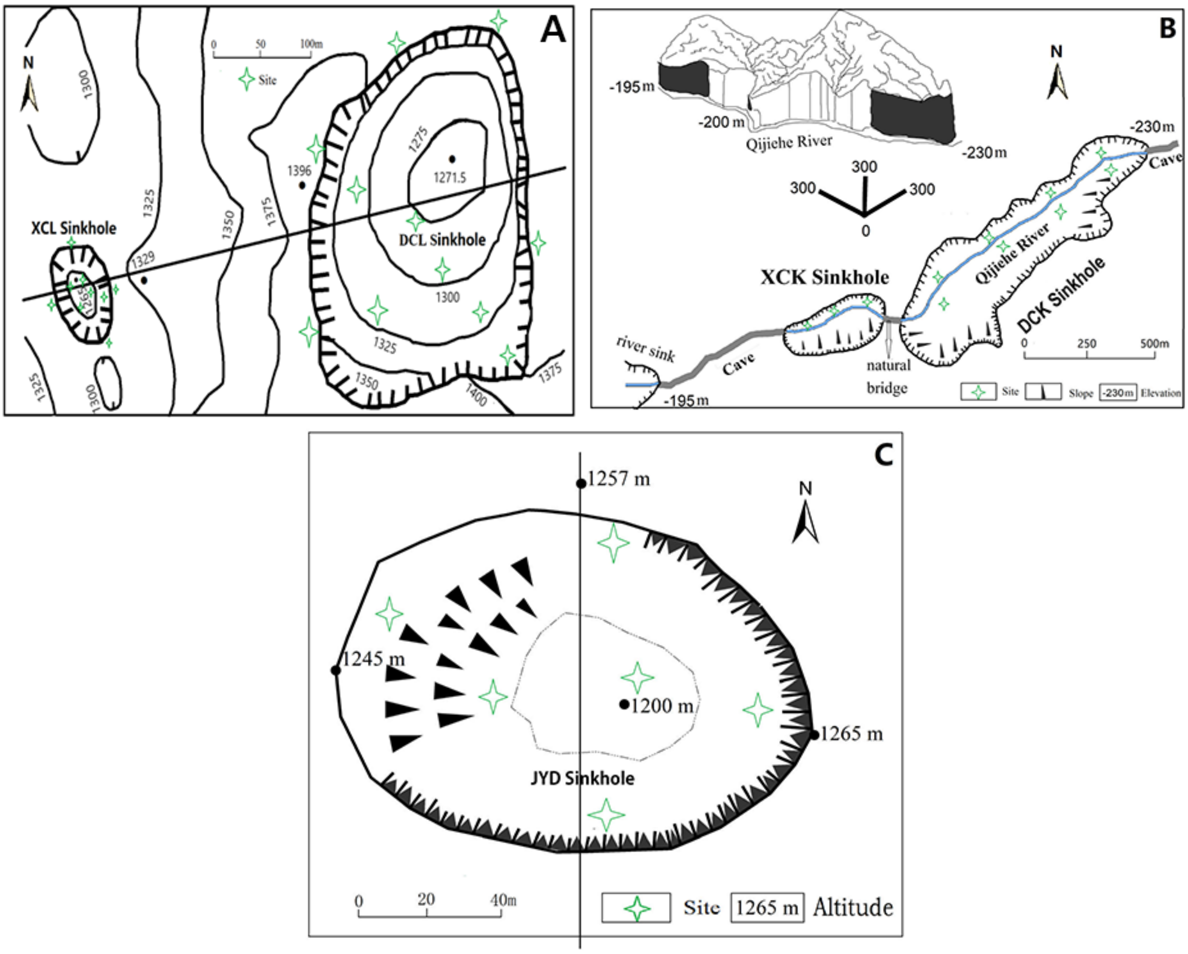

Fig. 2 Diagram of the sinkholes in Zhijin County of Guizhou Province. (**A**) after [22]; (**B**) after [22,56]; (**C**) after [22].

the location of the river. Because the south bank is cut off by the river, only the north bank was used to collect samples, and we set up three sampling sites. The rock wall on the west side of JYD is missing, and, thus, the sinkhole is easily accessible; in addition, this slope is used as cultivated land by the local people. We set up six sampling points at the top, middle, and bottom. All five sinkholes were analyzed, and 185 bryophyte samples were collected.

At every sampling site, the light intensity was measured using a digital illuminometer (PM6612L; Huayi United, Shenzhen City, Guangdong Province, China); air temperature and humidity were measured with a handheld air temperature and humidity meter (HT-635; Guangzhou Hongcheng, Guangzhou City, Guangdong Province, China); soil temperature and humidity were measured using a soil temperature and humidity analyzer (TR-6; Beijing Shunkeda Technology Co., Ltd, Beijing, China); and the length and depth of the five sinkholes were measured using a handheld laser rangefinder (SW-1500A; SNDWAY, Dongguan City, Guangdong Province, China). All measurements were performed for a week from September 25 to October 1, 2017. Each index was determined by repeatedly measuring five times to obtain an average value.

Specimen identification

Individual species were identified using traditional morphological identification techniques, using an HWG-1 anatomical lens and a SMARTe-320 microscope, and reference to Flora Bryophytarum Sinicorum [23–31]. Voucher specimens were deposited in the bryophyte herbarium of Guizhou Normal University Bryophyte Research Laboratory (GZNUB; Appendix S1).

Data analysis

To quantify the effects of human disturbance on bryophyte diversity in the five karst sinkholes, we used the evaluation system of Kimberling et al. [32]. It mainly consists of four evaluation indexes, namely, areal extent, soil profile disturbance, time since disturbance, and frequency of disturbance. To understand the distribution structure and geographic composition of the bryophytes in the analyzed sinkholes, we classified them according to Wu's distribution of Chinese plant genera [33]. The aspect to the north was recorded as 0 degrees and clockwise. The application formula $TRASP = 1 - \cos[(\pi/180)(aspect - 30)]/2$ converts the aspect from 0 to 1 [34]. The larger value after conversion indicates that the habitat conditions are hotter. Slope positions 0.4, 1.0, and 0.8 were assigned to the bottom, middle, and top positions, respectively [35]. The canopy was evaluated by processing the sample photos with Photoshop software [36]. In the formula $\varepsilon = 1 - d/D$, ε represents canopy, d represents the pixel value of the sky part of the selected area, and D represents the pixel value of the selected region.

The correlation between bryophytes and heterogeneous environmental factors was analyzed using canonical correspondence analysis (CCA). The significance of the environmental variables was determined using a forward selection with the Monte Carlo permutation test (999 permutations, $p \leq 0.05$) [37]. The website Draw Venn Diagram [38] was used to prepare Venn diagrams of the bryophytes species in the five sinkholes. One-way ANOVA in SPSS 10.0 (IBM, Armonk, NY, USA) was used to determine the differences between the species abundances in the different sinkholes. A regression model for human disturbance and bryophytes species was established using PAST 3.2 (Palaeontologia Electronica, UK) [39]. A rank abundance curve was established to compare the relative species abundance in the different sinkholes by using ORIGIN 9.0 (OriginLab, Northampton, MA, USA). The relationship between the environmental factors (shown in Tab. 3) and bryophyte distribution was analyzed using CCA in CANOCO for Windows 5.0 (Cambridge University Press, University of Cambridge, UK).

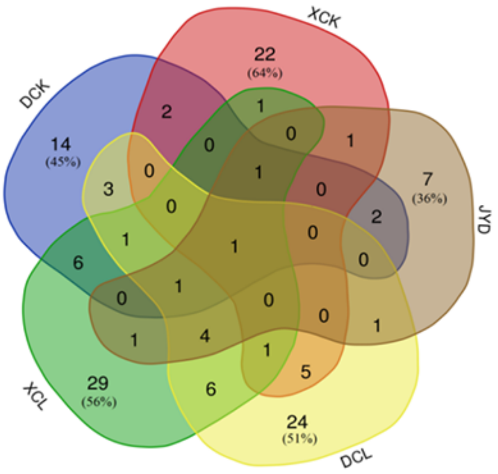

Fig. 3 Venn diagram of bryophytes species in the five sinkholes.

Results

Bryophytes in five sinkholes

A total of 132 species of bryophytes (17 liverworts and 115 mosses) that belong to 64 genera and 30 families were recorded in five sinkholes (Appendix S1). The number of bryophytes corresponding to DCK, XCK, JYD, DCL, and XCL was 31, 34, 19, 47, and 52, respectively. As shown in Fig. 3, XCL has the highest number of species shared with the other sinkholes: 14 species with DCL, 10 with DCK, eight with JYD, and four with XCK. XCL also maintains a high number of bryophyte species, with 29 species specific to this sinkhole and accounting for 42% of the total number of recorded bryophytes. XCK has the highest percentage of specific species, accounting for 64% of the total. JYD has the lowest number of specific species (only seven), accounting for 36% of the total. The bottoms of the five sinkholes have the highest species richness, and the abundance of bryophytes among sinkholes differed gradually: XCL > DCL > XCK > DCK > JYD. Among the five karst sinkholes, most of the liverworts, such as *Chiloscyphus horikawanus*, *Heteroscyphus argutus*, *Marchantia polymorpha*, *Porella perrottetiana*, and *Porella campylophylla*, are distributed at the bottom of the sinkholes.

Analysis of the floristic composition of the bryophytes

The bryophyte species found in the five sinkholes represent 10 types of geographical elements (Tab. 2). Most species represent the East Asia, Tropical Asia, and north temperate distribution types (22.72%, 21.97%, and 19.7%, respectively). In general, geographical composition reflects the subtropical characteristics of bryophyte flora in the karst sinkholes in the analyzed area.

Tab. 2 Geographical elements of bryophyte flora in the five karst sinkholes.

		The number of species in different sinkholes				
		Tourist		Farmland	Natural	
Areal types	Percentage (%)	DCK	XCK	JYD	DCL	XCL
North temperate	19.70	4	4	4	8	11
North temperate – south temperate disjuncted	1.52	-	-	-	2	-
East Asia i North America disjuncted	1.52	-	-	1	-	1
East Asia	22.72	4	4	2	16	11
Pantropic	8.33	4	5	1	3	4
Tropical Asia	21.97	3	5	5	9	10
Cosmopolitan	7.58	1	3	0	4	3
Temperate Asia	3.03	1	-	-	-	4
Temperate Asian – tropical Asian elements	2.27	2	-	-	-	1
Chinese endemic species	11.36	1	3	2	5	6
Total	100.00	20	24	15	47	51

Relationship between microenvironment and bryophyte distribution

The biplot (Fig. 4) clearly shows that altitude and soil humidity are the most important variables that affect the distribution of bryophytes in the karst sinkholes. The

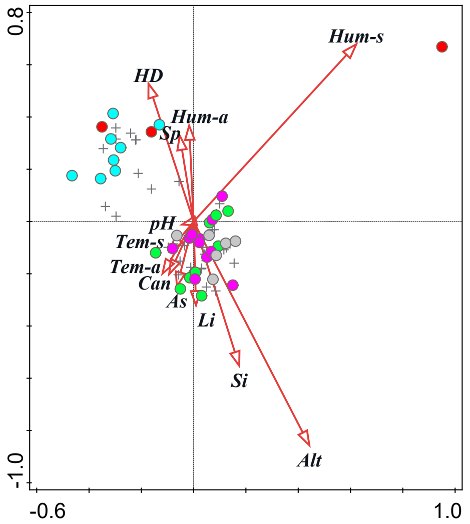

Fig. 4 The biplot of CCA showing the relationship among species distribution, sites, and environmental factors. The color of the circle represents the sinkhole: blue – DCK; red – XCK; grey – JYD; purple – DCL; green – XCL. The arrow represents environmental factors, and the cross represents bryophytes. Abbreviations for the environmental factors are listed in Tab. 3.

Tab. 3 Summary of the effects of the explanatory variables used in the canonical correspondence analysis.

Variable (abbreviation)	Explains (%)	Pseudo *F*	*p*
Altitude (Alt)	5.3	2.0	0.002
Soil humidity (Hum-s)	5.3	2.0	0.002
Canopy (Can)	4.2	1.6	0.002
Light (Li)	3.9	1.4	0.004
Human disturbance (HD)	3.8	1.4	0.002
Slope inclination (Si)	3.7	1.4	0.006
Air humidity (Hum-a)	3.6	1.4	0.072
Slope position (Sp)	3.4	1.3	0.006
Aspect (As)	3.1	1.2	0.058
Soil temperature (Tem-s)	3.0	1.1	0.162
Air temperature (Tem-a)	2.6	1.0	0.658
pH	2.5	0.9	0.690

explanatory content for the environmental factors is shown in Tab. 3. The bryophyte distribution in DCL, XCL, and JYD was positively correlated with canopy density, light, and slope inclination and negatively correlated with human disturbance. The bryophyte distribution in DCK and XCK was negatively correlated with slope inclination and positively correlated with position on the slope (bottom, middle, and top) and human disturbance to some extent.

Relationship between human disturbance and bryophytes

The rank abundance curve visually depicts both bryophyte species richness and species evenness in the five sinkholes. In the horizontal direction, the species abundance is reflected by the width of the curve. Higher the abundance of the species, larger the range of the curve on the horizontal axis. The smoothness of the curve reflects the average degree of species in the sample; a smoother curve indicates higher evenness [19]. As expected, the sinkhole within the cultivated land (JYD) showed low relative abundance and diversity (Fig. 5). Furthermore, the evenness index showed a decreasing trend from the natural sinkholes to the farmland sinkhole.

In addition, we established a regression model to explain the relationship between human interference and abundance of bryophytes in the five sinkholes, and it showed a significantly decreasing trend between the number of bryophyte species and human disturbance (Fig. 6).

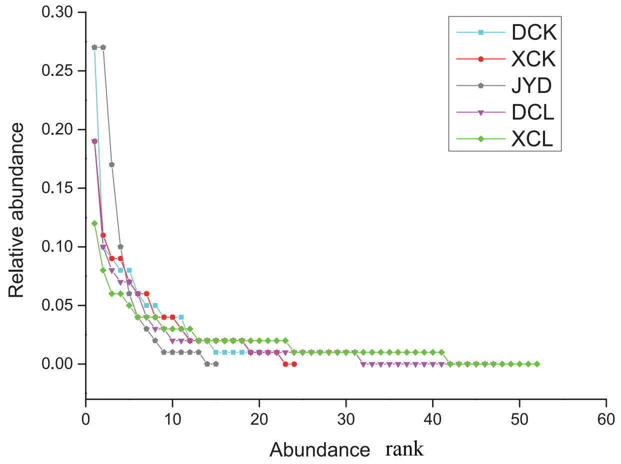

Fig. 5 Rank abundance distribution curve of bryophyte species in the five sinkholes.

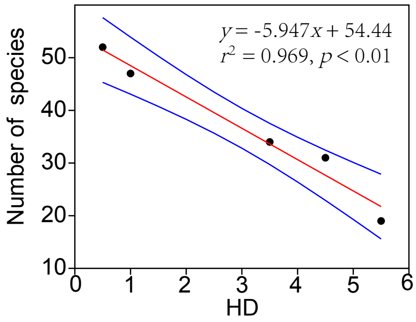

Fig. 6 Relationship between human disturbance (HD) and number of species in the five sinkholes.

Discussion

Diversity characteristics of bryophytes in the sinkholes

After investigating the bryophytes in the five sinkholes in the study area, we found that the number and distribution of bryophytes were determined by large-scale and small-scale environmental factors. As predicted, the sites with low light and high humidity were more suitable for bryophyte growth. The bottom of the sinkhole is often the place where bryophyte species are concentrated to the greatest extent, and most species are limited to the bottom of the sinkhole, such as liverworts (*Porella perrottetiana*, *Plagiochila fruticosa*, and *Calypogeia arguta*) and mosses (*Climacium dendroides*, *Eurhynchium longirameum*, and *Duthiella wallichii*). However, at the top of the sinkhole, drought-tolerant, tenacious species are often found, for example, *Hypnum calcicolum*, *Campylopus hemitrichus*, and *Didymodon asperifolius*. Robinson and Wells [20], who studied bryophytes in limestone sinks of Michigan, found species not reported previously in Michigan, such as *Mannia siberica*, *Seligeria calcarea*, and *Tritomaria scitula*, and pointed out that the large distribution of bryophytes in sinkholes is related to low temperature, high relative humidity, and the matrix humidity. Usually, liverwort is better adapted to dark and humid environments than is moss [15,40]. In the five karst sinkholes, most of the liverworts are distributed at the bottom, where water is abundant and light intensity is low; this has a good conservation effect on the liverworts. Our results show that the bryophyte distribution is influenced by not only microhabitat factors such as temperature and humidity but also topographic factors. Toure et al. [41] believed that the effects of geomorphology on inversion intensity, diurnal temperature field, and vertical temperature gradient have a great influence on vegetation pattern and plant survival. Altitude gradient is one of the most common determinants for shaping the spatial pattern of species richness [42]. Our CCA results are consistent with these findings. In addition, we showed that the interactions among canopy density, light, and position at the slope together form a natural gradient that affects the distribution of bryophytes, resulting in their obvious vertical changes.

Value of a sinkhole as a refuge for bryophytes

Comparison of the local bryophyte flora characteristics in previous studies [43,44] showed that the bryophyte flora in sinkholes does not exist independently but is consistent with the division of the regional system. Although the sinkhole flora is isolated from the outside world, the bryophytes in the five sinkholes are elements of the local flora [45]. The study area is mainly a plateau and mountainous area (87%). Lenoir et al. [46] found that, in the last century, mountain and small grass species changed more at optimal altitudes than did widespread and large woody species, underscoring the particular sensitivity of mountain (adapted to cool) species to climate change [47]. Cool-adapted species tend to occur in climatically diverse regions, where they would survive climatic fluctuations by tracking their preferred habitat [1,6]. The microclimatic environment of a sinkhole provides shelter for these species to grow and develop well without external disturbance. Jian et al. [48] found that the microhabitat is a factor that affects the stability of the plant community. Isolation of a sinkhole by steep rocky walls and its depth provide unique microenvironmental conditions, which are suitable for the growth of endemic plant communities. Our study proves that the microclimate gradient in karst areas may serve as a source of exceptional diversity of bryophytes and provide important habitats for local and relict taxa.

Influence of human activities on the sinkhole environment

In the context of global climate change, many species in karst areas have degraded or disappeared [49,50], and sinkholes provide shelter for these species. However, with the increase in human interference, the value of sinkhole shelters is gradually declining. Human disturbance had a significant effect on bryophyte diversity in the karst sinkholes ($p < 0.01$). This is mainly reflected by human activities such as agriculture and tourism development, which reduce the coverage of sinkhole vegetation and lead to soil water loss. After the vegetation is destroyed, steep slopes will undergo soil erosion. The field study showed that the sinkhole with the cultivated land would undergo extreme drought if there is no rainfall for a long time. Almost no other vegetation, except crops, weeds, and mosses, is present in the cultivated land sinkhole. In addition, attention should be paid to the safety of crops in sinkholes. In Guangxi, China, many sinkholes have been proven to have a vertical enrichment of organic pollutants, which means that pollutants may accumulate at the bottom of the sinkhole [51–53]. Therefore, to protect the value of sinkholes as refugia for rare plants as well as for food safety, it is highly recommended to not use sinkhole areas as arable lands. After assessing the harmful effects of land-use change on the karst landscape of Slovenia, Kovačič and Barrage [12] found that 1/4 sinkholes disappeared because of population growth and settlement area increase over the past 40 years and more sinkholes are on the verge of extinction. This is because of the vulnerability of the karst environment, which is a nonrenewable natural resource. Intensive human interference not only destroys its unique esthetic value but also leads to the degradation of its environmental value [12]. Plant communities and biodiversity are considered to be highly spatial variabilities controlled by both abiotic and biotic factors [41]. Many types of environmental changes may affect processes that can increase or decrease diversity [54]. Thus, understanding the patterns and processes that affect the spatial distribution of species is a fundamental issue in ecological protection [55]. As a unique habitat space, karst sinkholes have a high protection value for biodiversity. Therefore, it is necessary to establish an appropriate protection strategy for sinkhole refuges by understanding the effect of human disturbance on the biodiversity and different ecological processes in karst environments.

Conclusion

The distribution of bryophyte species diversity in sinkholes shows obvious vertical changes, and the distribution is affected by not only microhabitat factors such as temperature and humidity but also topographic factors. The microclimate gradient in a karst area may serve as a source of exceptional diversity for bryophytes and provide an important habitat for local and relict taxa. The number and species of bryophytes decreased obviously with the increase in human disturbance intensity. Intensive land use and tourism development have seriously threatened the existence of these unique habitats. We hope that effective measures will be implemented to protect this unique karst landscape.

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Supplementary material

The following supplementary material for this article is available at http://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.3620/0:

Appendix S1 Occurrence of bryophyte species in five sinkholes sampling sites.

References

1. Bátori Z, Vojtkó A, Farkas T, Szabó A, Havadtői K, E. Vojtkó A, et al. Large- and small-scale environmental factors drive distributions of cool-adapted plants in karstic microrefugia. Ann Bot. 2017;119(2):301–309. https://doi.org/10.1093/aob/mcw233
2. Raschmanová N, Miklisová D, Kováč Ľ. A unique small-scale microclimatic gradient in a temperate karst harbours exceptionally high diversity of soil Collembola. Int J Speleol. 2018;47(2):247–262. https://doi.org/10.5038/1827-806X.47.2.2194

3. Bátori Z, Körmöczi L, Erdös L, Zalatnai M, Csiky J. Importance of karst sinkholes in preserving relict, mountain, and wet-woodland plant species under sub-Mediterranean climate: a case study from southern Hungary. Journal of Cave and Karst Studies. 2012;74(1):127–134. https://doi.org/10.4311/2011LSC0216
4. Galbreath KE, Hafner DJ, Zamudio KR. When cold is better: climate-driven elevation shifts yield complex patterns of diversification and demography in an alpine specialist (American pika, *Ochotona princeps*). Evolution. 2010;63(11):2848–2863. https://doi.org/10.1111/j.1558-5646.2009.00803.x
5. Stewart JR, Lister AM, Barnes I, Dalén L. Refugia revisited: individualistic responses of species in space and time. Proc Biol Sci. 2010;277:661–671. https://doi.org/10.1098/rspb.2009.1272
6. Ohlemüller R, Huntley B, Normand S, Svenning JC. Potential source and sink locations for climate-driven species range shifts in Europe since the Last Glacial Maximum. Global Ecology and Biogeography. 2012;21:152–163. https://doi.org/10.1111/j.1466-8238.2011.00674.x
7. Theurillat JP, Guisan A. Potential impact of climate change on vegetation in the European Alps. Clim Change. 2001;50:77–109. https://doi.org/10.1023/A:1010632015572
8. Bátori Z, Lengyel A, Maróti M, Körmöczi L, Tölgyesi C, Bíró A, et al. Microclimate–vegetation relationships in natural habitat islands: species preservation and conservation perspectives. Quarterly Journal of the Hungarian Meteorological Service. 2014;118(3):257–281.
9. Bátori Z, Galle R, Erdős L, Körmöczi L. Ecological conditions, flora and vegetation of a large doline in the Mecsek Mountains (south Hungary). Acta Bot Croat. 2011;70:147–155. https://doi.org/10.2478/v10184-010-0018-1
10. Lazarević P, Lazarević M, Krivošej Z, Stevanović V. On the distribution of *Dracocephalum ruyschiana* (Lamiaceae) in the Balkan Peninsula. Phytologia Balcanica. 2009;15:175–179.
11. Bátori Z, Csiky J, Farkas T, Vojtkó EA, Erdős L, Kovács D, et al. The conservation value of karst dolines for vascular plants in woodland habitats of Hungary: refugia and climate change. Int J Speleol. 2014;43(1):15–26. https://doi.org/10.5038/1827-806X.43.1.2
12. Kovačič G, Barrage N. Analysis of human induced changes in a karst landscape – the filling of dolines in the Kras plateau, Slovenia. Sci Total Environ. 2013;447(1):143–151. https://doi.org/10.1016/j.scitotenv.2013.01.002
13. Valjavec MB, Zorn M, Čarni A. Bioindication of human-induced soil degradation in enclosed karst depressions (dolines) using ellenberg indicator values (classical karst, Slovenia). Sci Total Environ. 2018;640–641:117–126. https://doi.org/10.1016/j.scitotenv.2018.05.294
14. Marschall M, Proctor M. Are bryophytes shade plants? Photosynthetic light responses and proportions of chlorophyll *a*, chlorophyll *b* and total carotenoids. Ann Bot. 2004;94(4):593–603. https://doi.org/10.1093/aob/mch178
15. Liu Y, Li Z, Cao T, Glime JM. The influence of high temperature on cell damage and shoot survival rates of *Plagiomnium acutum*. Transactions of the British Bryological Society. 2004;26(4):265–271. https://doi.org/10.1179/174328204X19432
16. Mölder A, Schmidt M, Schönfelder E, Engel F, Schulz F. Bryophytes as indicators of ancient woodlands in Schleswig-Holstein (northern Germany). Ecol Indic. 2015;54:12–30. https://doi.org/10.1016/j.ecolind.2015.01.044
17. Alatalo JM, Jägerbrand AK, Molau U. Testing reliability of short-term responses to predict longer-term responses of bryophytes and lichens to environmental change. Ecol Indic. 2015;58:77–85. https://doi.org/10.1016/j.ecolind.2015.05.050
18. Frego KA. Bryophytes as potential indicators of forest integrity. For Ecol Manage. 2007;242(1):65–75. https://doi.org/10.1016/j.foreco.2007.01.030
19. Santos NDD, Costa DPD, Shepherd GJ, Kinoshita LS. Windborne: can liverworts be used as indicators of altitudinal gradient in the Brazilian Atlantic forest? Ecol Indic. 2014;36(36):431–440. https://doi.org/10.1016/j.ecolind.2013.08.020
20. Robinson H, Wells J. The bryophytes of certain limestone sinks in Alpena County, Michigan. Bryologist. 1956;59(1):12–17. https://doi.org/10.2307/3240283
21. Pericin C, Hürlimann H. Beobachtungen zur vertikalen Verteilung der Moosarten in der Doline Sterna-Filaria im Karstgebiet von Buje/Buie in Istrien (Kroatien). Bauhinia. 2001;15:91–96.
22. He W, Li P, Qian Z. Tiankeng and formation process in Zhijin cave geopark. Guizhou

Science. 2011;29(3):1–7.

23. Gao Q. Flora Bryophytarum Sinicorum. Vol. 1. Beijing: Science Press; 1994.
24. Gao Q. Flora Bryophytarum Sinicorum. Vol. 2. Beijing: Science Press; 1996.
25. Li XJ. Flora Bryophytarum Sinicorum. Vol. 3. Beijing: Science Press; 2000.
26. Wu PC. Flora Bryophytarum Sinicorum. Vol. 6. Beijing: Science Press; 2002.
27. Wu PC, Jia Y. Flora Bryophytarum Sinicorum. Vol. 8. Beijing: Science Press; 2004.
28. Li XJ. Flora Bryophytarum Sinicorum. Vol. 4. Beijing: Science Press; 2004.
29. Hu RL, Wang YF. Flora Bryophytarum Sinicorum. Vol. 7. Beijing: Science Press; 2005.
30. Gao Q, Wu YH. Genera *Hepaticopsida* et *Anthocerotopsida* Sinicorum. Beijing: Science Press; 2010.
31. Wu PC, Jia Y. Flora Bryophytarum Sinicorum. Vol. 5. Beijing: Science Press; 2011.
32. Kimberling DN, Karr JR, Fore LS. Measuring human disturbance using terrestrial invertebrates in the shrub-steppe of eastern Washington (USA). Ecol Indic. 2001;1(2):63–81. https://doi.org/10.1016/S1470-160X(01)00009-7
33. Wu ZY. The areal-types of Chinese genera of seed plants. Acta Botanica Yunnanica. 1991;4:1–139.
34. Roberts DW, Cooper SV. Concepts and techniques of vegetation mapping. In: Land classifications based on vegetation: applications for resource management. Ogden, UT: U.S. Department of Agriculture; 1989. p. 90–96.
35. Liu C, Li C, Shi M, Liang HY. Multivariate statistical analysis techniques applicated in differentiation of soil fertility. Acta Ecologica Sinica. 1996;16:444–447.
36. Qi YX, Luo H, Zhao TN. Simplified approach to measure canopy closure based on fish lenses. Journal of Beijing Forestry University. 2009;31(6):60–66.
37. Skuja A, SpuņGis V. Influence of environmental factors on the distribution of caddisfly (*Trichoptera*) communities in medium-sized lowland streams in Latvia. Estonian Journal of Ecology. 2010;59(3):197–215. https://doi.org/10.3176/eco.2010.3.03
38. Bioinformatics and Evolutionary Genomics. Calculate and draw custom Venn diagrams [Internet]. 2019 [cited 2019 May 5]. Available form: http://bioinformatics.psb.ugent.be/webtools/Venn/
39. Hammer Ø, Harper DAT, Ryan PD. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica. 2001;4(1):[9 p.].
40. Li CY, Zhang ZH, Wu J, Li XF. Distribution pattern of liverwort community in relation to environmental factors of caves in karst tiankeng: a case study of Monkey-Ear Tiankeng of Guizou Province. Chinese Journal of Ecology. 2019;38(3):744–752.
41. Toure D, Ge J, Zhou J. Spatial patterns of tree species number in relationship with the local environmental variations in karst ecosystems. Applied Ecological and Environment Research. 2015;13(4):1035–1054. https://doi.org/10.15666/aeer/1304_10351054
42. Zhang Z, Gong DJ, Sun CX, Li XJ, Li WJ. Altitudinal patterns of species richness and species range size of vascular plants in Xiaolongshan Reserve of Qinling Mountain: a test of Rapoport's rule. Chinese Journal of Applied Ecology. 2014;25(9):2477–2485.
43. Peng T, Zhang ZH. Study on bryoflora in Xiangzhigou karst area. Guizhou Province. Guizhou Science. 2009;27:56–62.
44. Zhang ZH, Chen JK. Floristic characteristics of aquatic bryophytes and their biokarst deposition types at waterfalls in central Guizhou, China. Carsologica Sinica. 2007;26(2):170–177.
45. Xiong YX. Bryophyte flora of Guizhou China. Guiyang: Guizhou Science and Technology Press; 2014.
46. Lenoir J, Gégout JC, Marquet PA, de Ruffray P, Brisse H. A significant upward shift in plant species optimum elevation during the 20th century. Science. 2008;320:1768–1771. https://doi.org/10.1126/science.1156831
47. Thuiller W, Lavorel S, Araújo MB, Sykes MT, Prentice IC. Climate change threats to plant diversity in Europe. Proc Natl Acad Sci USA. 2005;102:8245–8250. https://doi.org/10.1073/pnas.0409902102
48. Jian XM, Shui Wei, Wang YA, Wang QF, Chen YP, Jiang C, et al. Species diversity and stability of grassland plant community in heavily-degraded karst tiankeng: a case study of Zhanyi tiankeng in Yunnan, China. Acta Ecologica Sinica. 2018;38(13):4704–4714. https://doi.org/10.5846/stxb201706281163

49. Zhang ZH, Hu G, Zhu JD, Ni J. Stand structure, woody species richness and composition of subtropical karst forests in Maolan, South-West China. Journal of Tropical Forest Science. 2012;24(4):498–506.

50. Wei YW, Su YR, Chen XB, He XY. Effects of human disturbance on profile distribution of soil organic C, total N, total P and microbial biomass in karst region of northwest Guangxi. J Soil Water Conserv. 2010;24(3):164–169.

51. Kong XS, Qi SH, Sun Q, Huang BJ. Transport and differentiation of polycyclic aromatic hydrocarbons in air from Dashiwei karst sinkholes in Guangxi, China. Environmental Science. 2012;33(12):4212–4219.

52. Kong XS, Qi SH, Huang BJ, Zhang Y, Li J. Atmospheric deposition of PAHs in Dashiwei karst tiankeng group in Leye, Guangxi. Environmental Science. 2012;33(3):746–753.

53. Kong XS, Qi SH, Jian ZC, Huang BJ. Environmental factors on distribution of polycyclic aromatic hydrocarbons in soils from Dashiwei karst giant doline (tiankeng) in Guangxi, China. Environmental Science. 2012;33(11):3905–3915.

54. Sagar R, Raghubanshi AS, Singh JS. Tree species composition, dispersion and diversity along a disturbance gradient in a dry tropical forest region of India. For Ecol Manage. 2003;186(1–3):61–71. https://doi.org/10.1016/S0378-1127(03)00235-4

55. Moritz C. Strategies to protect biological diversity and the evolutionary processes that sustain it. Syst Biol. 2002;51(2):238–254. https://doi.org/10.1080/10635150252899752

56. Zhu X, Tony W. Tiankeng: definition and description. Speleogenesis and Evolution of Karst Aquifers. 2006;4(1):8.