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Assessing the efficacy of conservation measures for the rare endemic *Gentianella bohemica*

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TABLE OF CONTENT

SUMMARY

Despite strong conservation efforts during the last decades, the decline of the endemic Bohemian gentian *(Gentianella bohemica*), an endemic species of the Bohemian Massif in Austria, Bavaria, and the Czech Republic, appears to be unstoppable. My master thesis analyses historic and current land use and the impacts of climatic parameters on population size development. Further, I established 126 plots (0.25 m^2) in 21 sites of *G. bohemica*, of which one half was placed around a plant of the study species, while the other half was randomly located where the study species was absent, to analyze micro-habitat preferences (by using Ellenberg Indicator Values, EIVs) and associated plant species of the study species. Based on the results of my thesis, I found that population size of *G. bohemica* was fluctuating strongly and in a synchronized way across populations, and that the climate in preceding years – notably average temperature and precipitation in the growing season (May to August) – were important factors. As dry and warm temperatures during the growth seasons had a negative influence on population size, climate change will most likely emerge as another threat for *G. bohemica* in the future. The study species preferred micro-sites characterized by nutrient poor vegetation, and this preference was indicated by several species that were highly overrepresented in plots with *G. bohemica*. A dense moss layer in the plots was found to be negatively associated with *G. bohemica*, likely because it prevented germination and seedling establishment. Data on land use from 2003 onwards revealed that dedicated conservation measures had a positive influence on population size development of the study species. The results of my thesis shall serve as a contribution for improving conservation activities and ensuring the long-term survival of this highly threatened grassland specialist.

Conservation measures, Ellenberg Indicator Values, Gentianella bohemica, habitat preferences, threat parameters

ZUSAMMENFASSUNG

Trotz reger naturschutzfachlicher Schutzmaßnahmen der Länder Österreich, Deutschland und Tschechien konnte dem drastischen Populationsschwund des böhmischen Endemiten *Gentianella bohemica* noch nicht Einhalt geboten werden. In dieser Masterarbeit werden historische und gegenwärtige Landnutzungen und Klimaparameter, welche die Populationsentwicklung des Böhmischen Enzians beeinflussen, analysiert. Zusätzlich dazu wurde eine Analyse der Micro-Habitat Präferenzen (Verwendung der Ellenberg-Zeigerwerte (EIVs)) auf insgesamt 126 Kleinflächen (0,25m^2) durchgeführt. Die Hälfte dieser Kleinflächen waren mit der Zielart belegt und die andere Hälfte der Flächen, wurde ohne der Zielart zufällig ausgewählt. Basierend auf die in der Masterarbeit produzierten Ergebnisse, wurde festgestellt, dass die Populationsgröße von *G. bohemica* starken Schwankungen unterläuft. Diese Schwankungen verlaufen synchron mit dem Klima vorangegangener Jahre, vor allem der jährlichen Durchschnitts-Temperatur und dem jährlichen Durchschnitts- Niederschlag in der Wachstumsphase (Mai bis August). Der negative Einfluss auf die Populationsgröße der Pflanze, von trockenen und warmen Temperaturen während der Wachstumsperiode, lassen darauf schließen, dass in Zukunft der Klimawandel *G. bohemica* stark gefährden wird. Die untersuchte Art bevorzugt Mikrostandorte, die durch eine Vegetation gekennzeichnet ist, welche nährstoffarme Standorte bevorzugt. Außerdem wurde eine negative Assoziation zwischen einer dichten Moosschicht und dem Auftreten von *G. bohemica* gefunden, da es das Aufkommen von Keimlingen verhindern kann. Daten zur Landnutzung ab dem Jahr 2003 zeigten, dass spezielle Erhaltungsmaßnahmen einen positiven Einfluss auf die Entwicklung der Populationsgröße der untersuchten Art hatten. Die Ergebnisse meiner Masterarbeit sollen als Beitrag zur Verbesserung der Naturschutzmaßnahmen und zur Sicherung des langfristigen Überlebens dieses stark bedrohten Grünlandspezialisten dienen.

INTRODUCTION

More than 50 % of European endemic plant species are declining and considered to be threatened (Planta Europa 2002). The word endemism is derived from the Greek adjective “endemos”, which means indigenous (Essl & Rabitsch 2008). Endemic species are restricted to spatially restricted ranges (Essl & Rabitsch 2008, Hobohm & Bruchmann 2009). Due to their limited distribution, the responsibility for ensuring species survival is concentrated on one, or few countries (Tschudin et al. 2017). Besides harboring a very high biodiversity (Briemle 2003), Europe’s grasslands are habitats for the second highest number of endemic species (Hobohm & Bruchmann 2009). However, species-rich grassland ecosystems are severely declining in response to land-use changes such as intensification and abandonment (Hobohm & Bruchmann 2009, Bucharova et al. 2012). Consequently, many endemic species that are associated with semi-natural grassland ecosystems are strongly declining (Hobohm & Bruchmann 2009). This master thesis focuses on one charismatic endemic flagship species of extensively used grasslands – i.e. *Gentianella bohemica*, a species that is restricted to the Bohemían Massif.

This formerly wide-spread species currently is only known from 58 sites in the Czech Republic, Germany and Austria. Most populations (36) are located in the Czech part of the Bohemian Massif (Engleder 2017), while 16 populations are known from Austria, and 6 sites from Bavaria (Engleder 2017). In southern Poland, a few further sites are known (Plenk et al. 2015). Population sizes of the short-lived *G. bohemica* is known to fluctuate heavily between years (Dolek 2010, Engleder 2012, Königer et al. 2012, Bucharova et al. 2012, Plenk et al. 2015).

Gentianella bohemica is associated with nutrient-poor mesic acidophilic grasslands dominated by *Nardus stricta* (Centauro-Nardetum) and with the Festuca rubra-Agrostis capillaris-association (Engleder 2006, Dolek et al. 2010, Königer et al. 2012). Less frequently, the rare Bohemian gentian also grows in “moderately nutrient-rich or partially wet grasslands” (alliances Arrhenatherion and Molinon), as well as in “drier basal bromids” and “Koeolerio-Phleion meadows” (Dolek et al. 2010, Königer et al. 2012, Brabec 2012, Krenova et al. 2019). The plant is dependent on a mutualistic relationship with mycorrhizal fungi (Zillig et al. 2010), which is not yet well studied (Dolek et al. 2010). *G. bohemica* is a short-lived hemicryptophyte, which forms a rosette in spring, while the inflorescence is developed in late summer (Dolek et al. 2010). In autumns, the plant dies after the seeds have ripened (Dolek et al. 2010, Plenk et al. 2015). The seeds of the Bohemian gentian can germinate in the first year or stay dormant for several years, whereby the probability of germination after four years is about 20 % (Bucharova et al. 2012, Schiebold 2013). Thus*,* *G. bohemica* has a persistent seed bank (Bucharova et al. 2012).

In spring (late April to early May) seed germination as crucial life cycle stage determines further population survival (Brabec 2012). Extreme droughts or heavy precipitation during germination leads to lower germination and survival probabilities (Plenk et al. 2015). In terms of positive germination probability and plant development, disturbed areas with small vegetation gaps facilitate gentian establishment (Dolek et al. 2010).

G. bohemica possesses no special mechanisms for seed dispersal, other than bending the stiff stem to catapulting the small globular seeds (diameter 0.7 mm) close by the mother plant (Dolek et al. 2010). The plant is pollinated by insects, mainly bumblebees (*Bombus* spp.), honeybees (*Apis mellifera*) and hoverflies (*Syrphidae* spp.), butterflies (*Lepidoptera*) and flies (*Muscidae*) (Fischer 2015; Königer et al. 2012) Short-distance seed dispersal by wind is also possible (Plenk et al. 2015).

During the last decades, *G. bohemica* has strongly declined and remaining populations are highly threatened. Several interacting and mutually enforcing causes of threat have contributed to this dire conservation status. Most importantly, the transformation of land-use in the second half of the 20th century has caused a severe decline of extensively used grasslands (Königer et al. 2012, Lennartsson 2000), and this has greatly contributed to the loss of many populations. In the peripheral Bohemian Massif this land-use intensification started somewhat latter than in more agriculturally favorable regions of Central Europe (Bicik & Kupova 2007, Kurz 2010), but still the loss of once widespread nutrient-poor grasslands was extreme. While most previously extensively used grasslands were fertilized, others that were difficult to use intensively (e.g. steep slopes) were abandoned and afforested. While Germany and Austria still are characterized by private family-owned farms, farms in the Czech Republic were collectivized in the late 1940s in the early communist era (Kurz 2010, Bicik & Kupova 2007).

As a result of the dramatic land-use changes, remaining populations of *G. bohemica* became highly isolated, and many populations declined to small population sizes (Brabec 2005, Engleder 2006, Königer et al. 2012), or eventually, became extinct. Small population size may lead to genetic erosion, which may result in reduced fitness and low genetic diversity (Ellstrand & Elram 1993; Oostermeijer et al. 1994; Fischer & Matthies 1998a; Leimu et al. 2006;

Jacquemyn et al. 2009a). Thus, many of the extant *G. bohemica* populations likely suffer from reduced genetic diversity (Königer et al. 2012).

Finally, climate change has emerged as another novel threat for *G. bohemica* in recent years. Isolated small populations of habitat specialists are generally sensitive to emerging threats such as climate change (Bucharova et al. 2012, Thomas et al. 2004, Zipp 2016). Finally, as *G. bohemica* is a short-lived species, it is plausible that climate change may rapidly negatively affect remaining populations (Bucharova et al. 2012)

Given the poor conservation status of *G. bohemica*, it has been included into the Annex II of the Habitats Directive of the European Union as a priority species Habitat Directives 92/43/EEC). Further, the species is listed as "vulnerable" in the European Red List (IUCN 2020, Bilz et al. 2011). In the national Red Lists of the Czech Republic, Austria and Bavaria, the plant is listed as critically endangered and is subject to dedicated conservation measures (Königer et al. 2012). In particular, action plans for conservation were generated (Schiebold 2012) to improve the status of extant populations (Brabec 2010). In general, these measures consist of population monitoring, optimizing land-use and the collection of species-specific information on species biology and ecology (Brabec 2010). Further, measures such as seed collection and establishing ex-situ populations have been implemented (Zehm et al. 2017). Finally, adaptive management has been developed. For instance, at the Bavarian locations, the management measures are re-assessed annually to flexibly respond rapidly to unforeseen developments (Zipp 2016).

Despite dedicated conservation efforts since several decades, the long-term survival of *G. bohemica* is not secured (Königer et al. 2012). Similarly, there is only insufficient knowledge on (micro)habitat preferences (Zehm et al. 2017). Thus, this master thesis studies the following research questions: 1) What is the historical population development in the extant populations of *G. bohemica*? 2) Which environmental (e.g. climate) and anthropogenic (e.g. land use, conservation measures) factors explain differences in population developments of different sites? 3) What are the (micro)habitat preferences of *G. bohemica*? Based on the findings of these research questions, I assess the current conservation status of the study species and provide recommendations for future conservation measures.

MATERIAL AND METHODS

Description of the study species *Gentianella bohemica*

Gentianella bohemica Skalický (*G. praecox* A. and J. Kerner ssp. *bohemica* [Skalicky] Holub) belongs to the Gentianaceae family (Schiebold 2013). Species delineation in the genus Gentianella is difficult due to strong genetic similarities caused by hybridizations, crossings and backcrossings (Jang et al. 2005). Further, high morphological variability, seasonal dimorphisms and habitat-dependent polymorphisms causes further taxonomic uncertainties (Jang et al. 2005, Greimler et al. 2004, Schiebold 2013). Greimler et al. (2004) divides the European *Gentianella* genus into a total of 21 species, of which five species are considered to be currently insufficiently taxonomically supported and are therefore assigned to 3 groups (*G.amarella agg*., *G.campestris agg* and *G.germanica agg*.) (Greimler et al. 2004, Jang et al. 2005). According to this classification, a large part of the species in the genus are associated with *Gentianella germanica* agg.

Because of its poor morphological features and strong genetic similarities, the exact assignment of *G. bohemica* to one of these aforementioned groups is challenging, and the taxon seems to represent a transitional taxon between *G. austriaca* and *G. germanica* (Greimler et al. 2004, Jang et al. 2005). In addition to morphological variations, the Bohemian gentian has also two genetically distinct variants in time of flowering (Fischer 2015). The early flowering aestivale type blooms in June and the late flowering autumnal type blooms from August to September (Fischer 2015, Wettstein 1896, Janchen 1960, Skalicky 1969, Fischer et al. 2008, Plenk et al. 2015). In this work, data of the late flowering autumnal type were generated and processed.

Adult plants of *G. bohemica* reach heights of 5-50 cm, depending on the site conditions (Dolek et al. 2010). In late summer, the stem is often reddish with opposite, narrow-egg-shaped leaves (Dolek et al. 2010). The stalked pentamerous flowers are 20-35 mm long and the stem has a length of 2-6 mm (Dolek et al. 2010). The reddish-violet petals are partially fused together, creating a radial symmetric, winged corolla tube (Dolek et al. 2010). On the inside of the bell-shaped corolla tube throat scales (fringes on the throat) are positioned (Dolek et al. 2010). The linear to narrow triangular sepals are 1.5 times as long as the corolla tube (Fischer et al. 2008) The U- to V-shaped subangular calyx gaps are partially provided with papillae (Dolek et al. 2010, Fischer et al. 2008).

Description of the study area

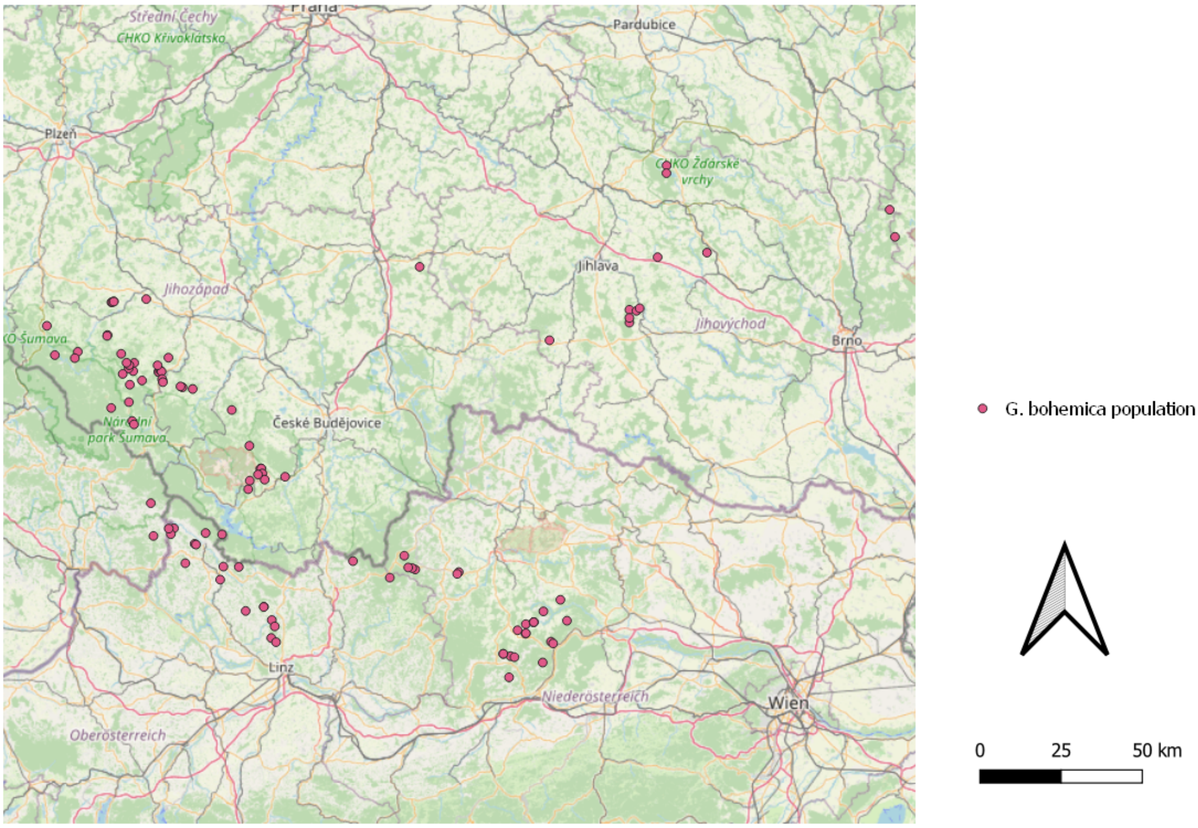

Figure 1: The locations of extant populations of *G. bohemica* (red dots) in the Bohemian Massif.

Geology

The study area consists of selected grassland areas of the Czech Republic (Sumava-Vorgebirge, Böhmisch Mährisches Hügelland) of Austria (Mühlviertel und Waldviertel) and Germany (Bayrischer Wald) (Rösler 2001). The study area extends from the southwestern part of the Czech Republic over a small Bavarian part up to Austria. The Bohemian Massif is geologically a very old mountain range that forms the largest bedrock in Central Europe (Ajathi & Krumme 2002), evolved from the Variscian folding in the Paleozoic era (Bettinger 1995). For a long time, the unfolded rock masses were exposed to strong erosion processes which gave the basement mountains its typical gentle hilly landscape (Bettinger 1995). The Bohemian Massif is geologically classified and divided into the parts Moldanubikum, Moravikum and Bavarikum (Ajathi & Krumme 2002, Fuchs 1976). Granites, Diorites and Gneisses are characteristic rock types for the area (Ajathi & Krumme 2002). The nutrient poor soils favor processes of Podsolization (Pleschke 1977). This acidic crystalline starting material promotes the formation of acidic oligotrophic soils which, depending on site and specific parental material, forms brown earths, pseudogleyes, gleyes and raised bog soils (Bettinger 1995; Bassler et al. 2003).

The high proportion of brown-earth soils in this area originates from the high occurrence of granite (Laister 2013). Soils in the Bohemian Massif are largely free of carbonate. Consequently, the soils of the grassland sites of the Bohemian Massif have pH values of <5 (BMLFUW 2015).

Climate

The described area has shares in the Bavarian Forest and Sumava-region as noted above. Both parts are situated in the Atlantic continental climatic impact area (Ajathi & Krumme 2002). Typical characteristics of this climate range are small differences between summer and winter precipitation rates and mean annual temperatures between 6.5 ° C in valleys and 3.5 ° C in high altitudes (Ajathi & Krumme 2002). The climate of the Bohemian Massif is characterized by Fink (1993) as cool boreal with increasing continental impact from west to east, resulting in decreasing average precipitation and rising annual average temperatures (Wrbka 1994). Thus, in the western part of the Mühlviertel, annual precipitation reaches up to 900 mm (Fink et al. 1989). The wind-exposed highlands and their undulating shape promote the formation of local cold-air lakes (Kropik 2014). Further, the "Bohemian Wind" coming from the north, reduces the average mean air temperature by 0.5 to 1 ° C (Wrbka 1994) and causes a climate depression (Kropik 2014). The climate of the Waldviertel, for example, as part of the Bohemian Massif, is characterized by low average winter temperatures and numerous frost days (Kropik 2014) and compared to other regions of similar altitudes in Austria, has substantially lower average annual temperatures (Harflinger & Knees 1999).

Data on population size and site attributes

Data of 122 surveyed sites monitored from 1993 to 2017 were used for this thesis, of which 68 are located in the Czech Republic, 44 in Austria and 10 in Bavaria. All in all, at 58 locations no gentians were found during field work in 2017, of which 34 are located in the Czech Republic, 21 in Austria and 3 in Bavaria. This large number of declining and (near) extinct populations causes a total decrease of 41.9% of flowering gentians from 2003 to 2017

Study design

Site selection

Due to the large variety of "favorable" and "unfavorable" gentian locations, the selection of sites for field data sampling was done based on several criteria:

- Locations have to be accessible
- Only those *G. bohemica* sites were selected, which had at least seven flowering individuals in the preceding years.
- In Austria and Bavaria, the consent of the land owners had to be obtained.

In order to be able to make a comprehensive analysis on micro-site preferences of the Bohemian gentian, sites in all three countries were selected for field data sampling. As number of *G. bohemica* populations differ among countries, 14 sites were located in the Czech Republic, 5 in Austria and 2 in Bavaria. Further information of T. Engleder, T. Zipp and J. Brabec – i.e. the persons in charge for conservation measures in each study country – were included.

Field data collection

Field data collection was done from July to September 2017. In the field, the search for gentian plants, the sampling of the accompanying vegetation, the determination of the degree of coverage of the accompanying species, the collection of additional site-specific data such as inclination, open vegetation gaps, moss density and land use were done. For vegetation sampling, plots (0.25 m^2) were established in areas with and without gentians. A total of 21 sites were sampled and 126 plots with and without gentian plants were examined to assess differences in accompanying plant species composition. Three plots were established around gentian plants and the other three plots were established randomly in sites without gentians. The determination of plant species was carried out with the help of special literature such as Fischer, Adler & Oswald (1994), Rothmaler (2007), Dietl & Jorquera (2007), Lauber & Wagner (2001). In addition, during field work, indeterminable plant species were collected and herbarized. The correct species identification was done with the help of Professor Franz Essl.
For each species, its cover in the plots was assessed visually using the Braun-Blanquet-categories (Traxler et al.2005).:

- The species is very rare (r)
- The species has low cover (+)
- 1 (Species with a coverage of up to 5%)
- 2 (Species with a coverage of 5-25%)
- 3 (Species with a coverage of 26-50%)
- 4 (Species with a coverage of 51-75%)
- 5 (Species with a coverage of 76-100%)

Further, the percentage of open soil, moss cover and total vegetation cover was recorded. All species were assigned to indicator values according to Ellenberg (1992), which provide information about the ecological preferences of a plant (Ellenberg 1992). For the allocation of the EIVs an online program (https://statedv.boku.ac.at/zeigerwerte/) was used created by Gerhard Karrer and Robert Wiedermann of the University of Natural Resources and Life Sciences of the Institute of Botany (BOKU).

Calculation of Ellenberg indicator values

The use of Ellenberg indicator values has been proved to be an appropriate method in vegetation science. It is essential to know that the Ellenberg indicator values are not measured values, but an instrument to determine indirectly ecological values based on expert-derived indicators (Ellenberg 1996). Based on this approach, Ellenberg (1992) produced a list of about 2700 plant species in Central Europe and assigned numbers to each species based on the factors light, temperature, humidity, nitrogen supply, continentality and soil reaction. In this thesis analysis, I used the indicator values light (L), humidity (F), temperature (T), nitrogen/nutrient supply (N), and soil reaction (R).

Land use data acquisition

Alongside the representation of population dynamics and the investigation of possible influencing factors of the study species, land use data at the extant site was collected. Due to long-term monitoring and conservation activities, land use data were made available by colleagues from 2000 onwards. Data on land-use before 2000 was largely not available and was thus not used for analysis.

Data assessment and statistical analysis

Statistical analysis was conducted in RStudio (RStudio core team 2016). Graphical data processing was done using the software Microsoft excel and the R package ggplot2 (Wickham 2016). To analyze the relationship between population fluctuation patterns, precipitation and temperature a Generalized Linear Mixed Model (GLMM) using the following packages MCMCglmm (Hadfield 2010), sqldf (Grjothendieck 2017), lme4 (Bates et al., 2015) dismo (Hijmans et al. 2017) rgdal (Bivand et al. 2018) and sp (Pebesma & Bivand 2005) was used.

Increase or decrease of population size was calculated by averaging the numbers of recorded flowering specimens of each population from the most recent three years (2015 to 2017) and

subtracting the average numbers of the first three years (2003 to 2005) for which data were available To correlate population fluctuations patterns between the three countries a Sperman's rank correlation test was conducted.
Probability of occurrence of *G. bohemica* according to Ellenberg-indicator values (EIVs) is described by Generalized linear modeling. To calculate EIVs for each plot, the cover-weighted EIV of the plant community within the plot was calculated by the following formula:

$$EIV_{wa} = \frac{a_1 * EIV_1 + a_2 * EIV_2 + a_n * EIV_n}{a_1 + a_2 + a_n}$$

EIV_{wa}= weighted average of Ellenberg-indicator value of the respective plot
a_n=coverage of species n
EIV_n= Ellenberg-indicator value of species n

A Dufrene-Legendre Indicator Species Analysis was performed to test for significant co-occurrences of plant species with *G. bohemica* in the 126 plots. I used the function which is based on permutation, and which is part of the R-package labdv (Roberts 2016).

RESULTS

The number of flowering plant individuals per population differs greatly among sites and years. For example, in 2011 – which was a year with large population sizes – 8300 flowering plant individuals were recorded at Chvalsiny (Czech Republic), while only few individuals were found at other locations. Another example is the site Fuchsgraben in Austria, where 4000 gentians were counted in 2007. In the same year at the Bavarian site Teufelsbachwiese another local maximum value with 420 blooming plants was recorded. In the years 2003 to 2005, the total mean number of gentians counted per site is 141 ± 641 SD and decreases to 81 ± 286 SD in the years 2015 to 2017. For the Czech Republic, the average number of gentians recorded per site is 142 ± 744 SD between 2003 and 2005, and about 72 ± 286 SD individuals for 2015 and 2017. Slightly lower is the number for Austria with 140 ± 400 SD individuals between 2003 and 2005, which also decreases to 98 ± 278 SD for 2015 and 2017. Substantially lower numbers of gentians with only 52 ± 112 SD gentians per site between the years 2006 and 2008 and 5 ± 6 SD flowering plants per site for the years 2015 and 2017 were recorded in Bavaria. These significant differences in population sizes results partially from the large differences in spatial extent of the sites which ranges from 5 m^2 to 30000 m^2. Therefore, population density of 0.19 ± 0.41 SD individuals per m^2 showed is highly variable. Significant differences in population densities over the years could not be observed (t = -0.35741, df = 78.065, p-value = 0.7218).

Population trend of *G. bohemica*

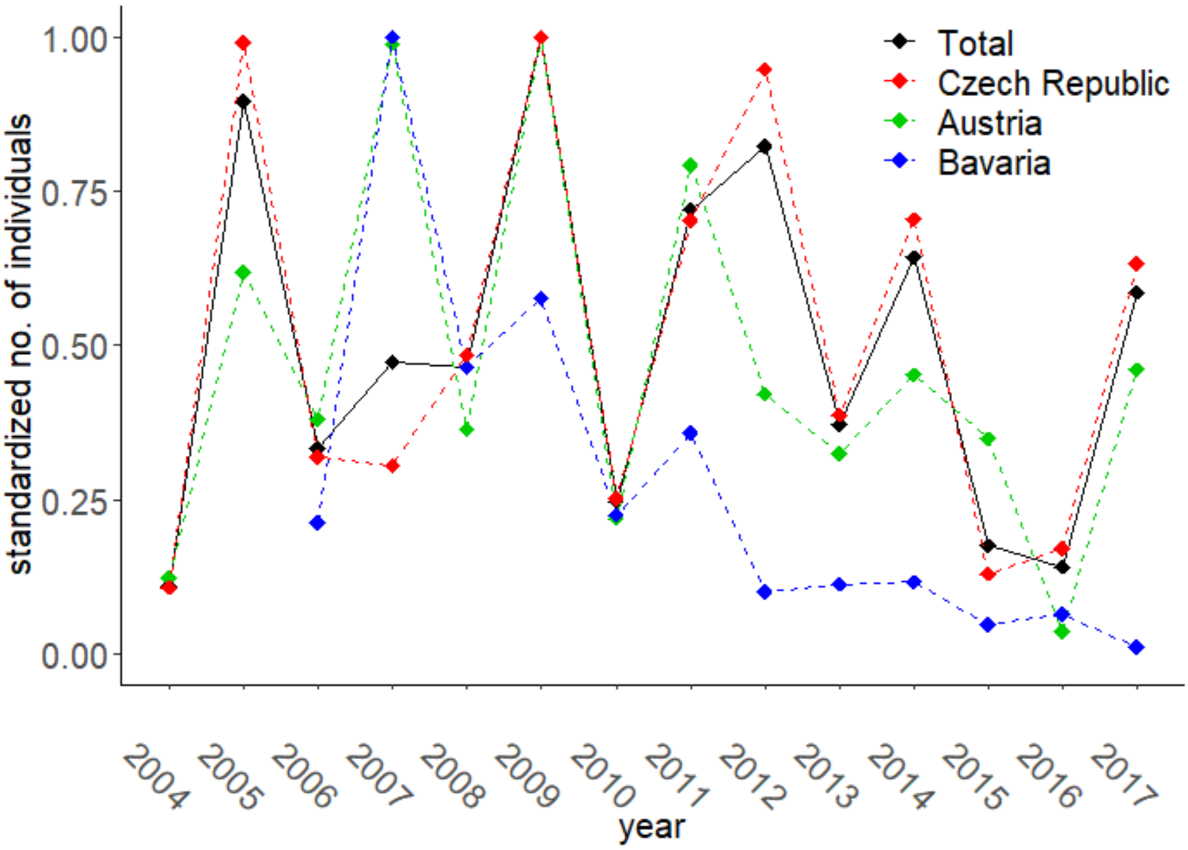

Figure 2: Standardized total annual number of individuals of *G. bohemica* in the Czech Republic, Austria and Bavaria from 2003 to 2017.

The Bohemian Gentian shows substantial fluctuations in the annual population size for the period 2003-2017, which is the time-span when populations were closely monitored (Fig. 2). In 2009 the highest number with a total of 29200 blooming Gentian individuals was documented, of which 22427 plants were recorded in the Czech Republic, 6297 individuals in Austria, and 476 in Bavaria. In the year 2005 and 2007 high values were also recorded in Bavaria and Austria. Populations of Bavaria were largest in 2007 with a total of 825 individuals, but there was a strong decline thereafter. In the Czech Republic and Austria, the decline is less pronounced, but also evident (Fig. 2). Smallest individual numbers in all three countries were observed in 2016 with a total amount of only 4084 plants. In the following year 2017 the numbers of gentian individuals rose strongly to a total of 16064 individuals (Fig. 2).

Interestingly, temporal fluctuations were synchronized between the countries, i.e. between the Austrian and Czech populations (rs [128] = 0.77, p<0.01) (Fig.2). However, no significant correlation was found between annual population sizes of the Bavarian and Czech and the Bavarian and the Austrian sites, respectively (Appendix 1).

The influence of weather on *G. bohemica* population size

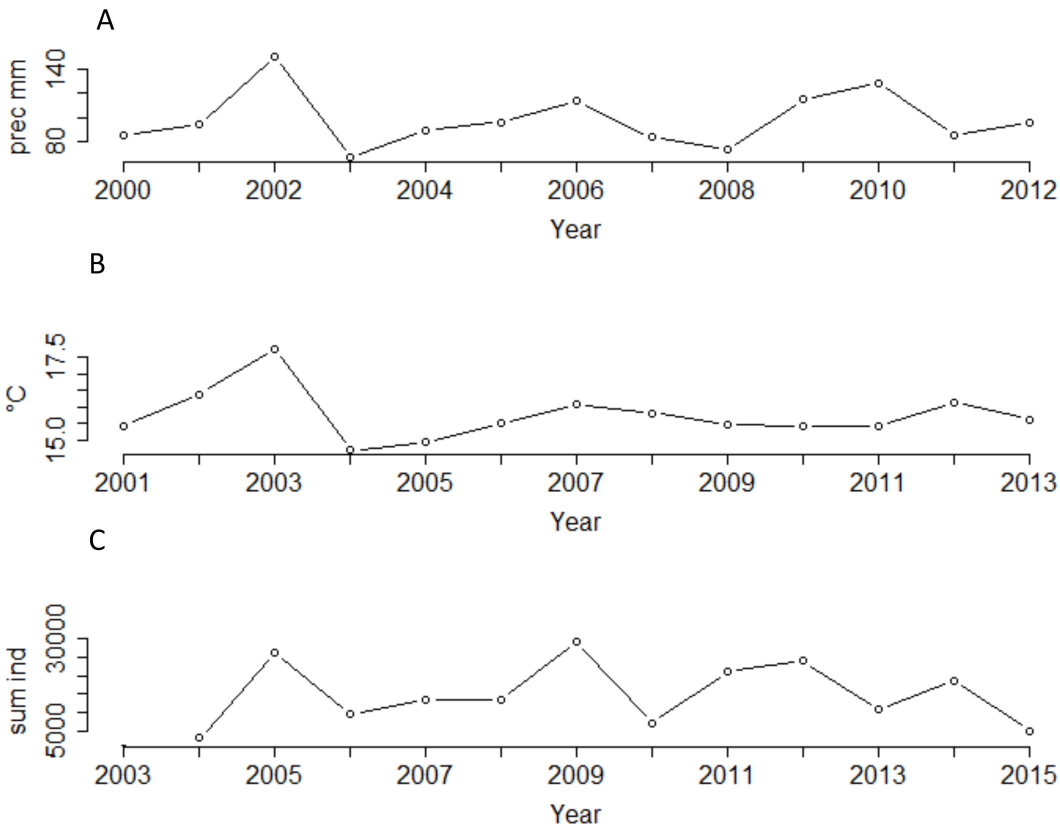

Figure 3: Mean precipitation values (mm) of 2000 to 2013 in the growing season (May to August) for the *G. bohemica* sites (A). Mean annual temperature (°C) from 2001 to 2013 in the growing season (May to August) for the *G. bohemica* sites (B), and total number of gentian individuals (2004 to 2015) (C).

Table 1: Generalized linear mixed model of population size (numbers of flowering plants) from the year 2004 to 2015 of *G. bohemica* populations (n = 114), on size of the location (area), mean precipitation and mean temperature in the vegetation period (May to August). Note that only the best model is shown, and this model includes the explanatory variables "precipitation three years preceding the response variable" and "temperature two years preceding the response variable".

Fixed effects	Estimate	SE	z	p-Value
Area	0.8174	0.4631	1.765	0.078
Precipitation	0.3061	0.1165	2.627	<0.01
Temperature	0.3774	0.1465	2.576	<0.01

The generalized linear mixed model shows that total size of *G. bohemica*-populations is significantly influenced by mean precipitation and temperature during the growing season (Tab. 1). Interestingly, the best model has a lag time of three (precipitation) and two (temperature) years between explanatory and response variable, indicating that weather conditions in previous years are important for population size of *G. bohemica*. The size of the locations had a near-significant influence on population size *of G. bohemica*.

Micro-habitat preference of *G. bohemica*

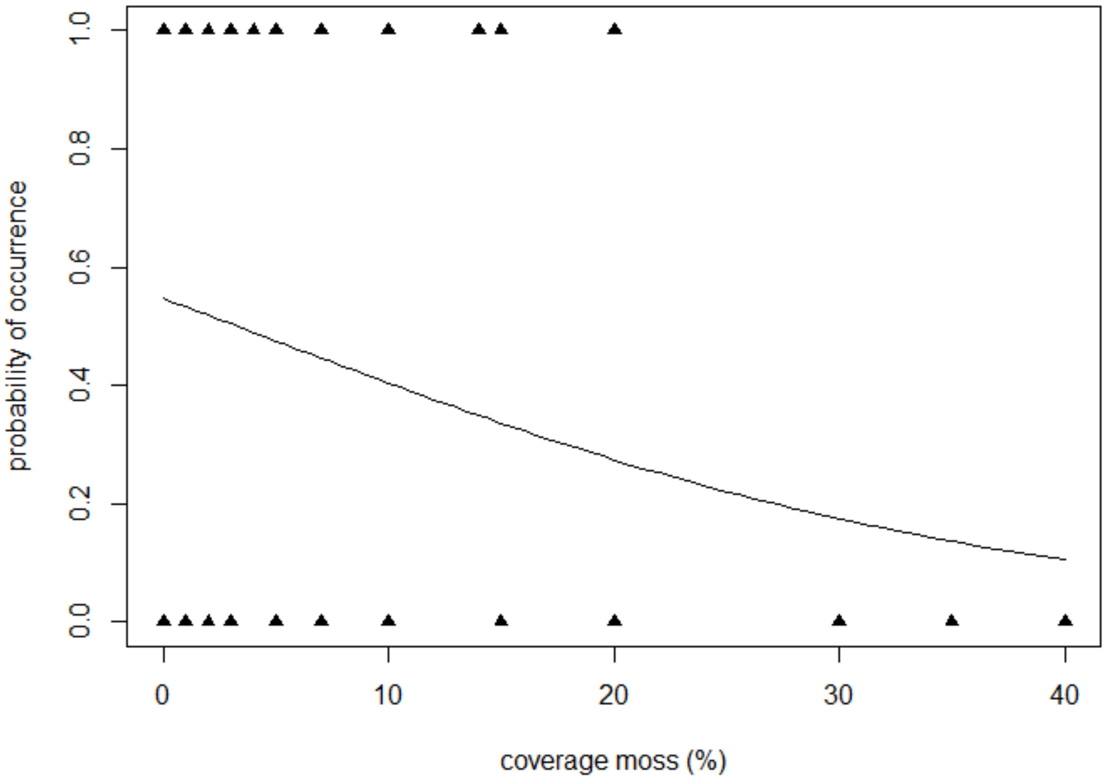

Figure 4: Probability of occurrence of *G. bohemica* depending on moss layer cover in 126 plots (0.25 m^2) (GLM: D^2=0.033; $p<0.05$).

I found that *G. bohemica* shows distinct preferences to specific micro-habitats in the 126 plots established in 26 sites. First, the probability of occurrence is significantly declining with increasing moss layer cover (Fig. 4). Gentians were only found in plots with a moss layer cover of at most 20 %.

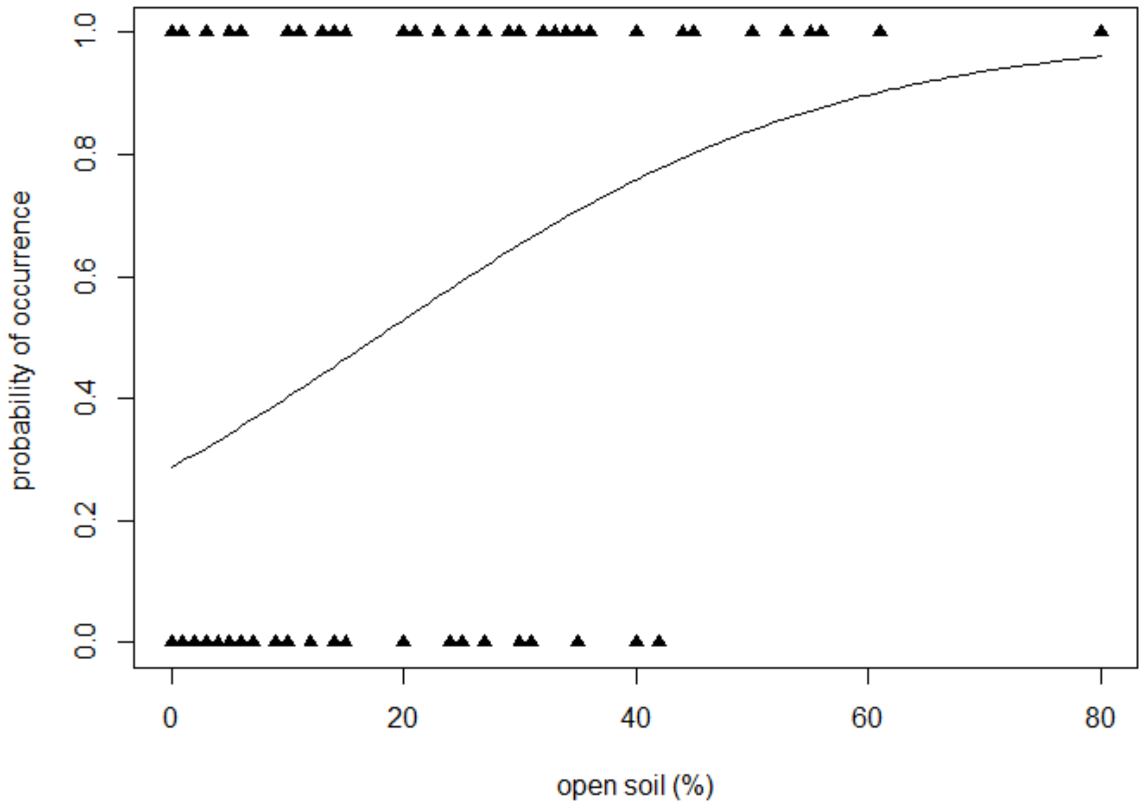

Figure 5: Probability of occurrence of *G. bohemica* depending on the proportion of open soil in 126 plots (0.25 m^2) (GLM: D^2=0.092; p<0.01).

Further, the proportion of open soil in the plots had a significant positive influence on *G. bohemica* occurrence (Fig.5).

Mean Ellenberg indicator values and occurrence of *G. bohemica*

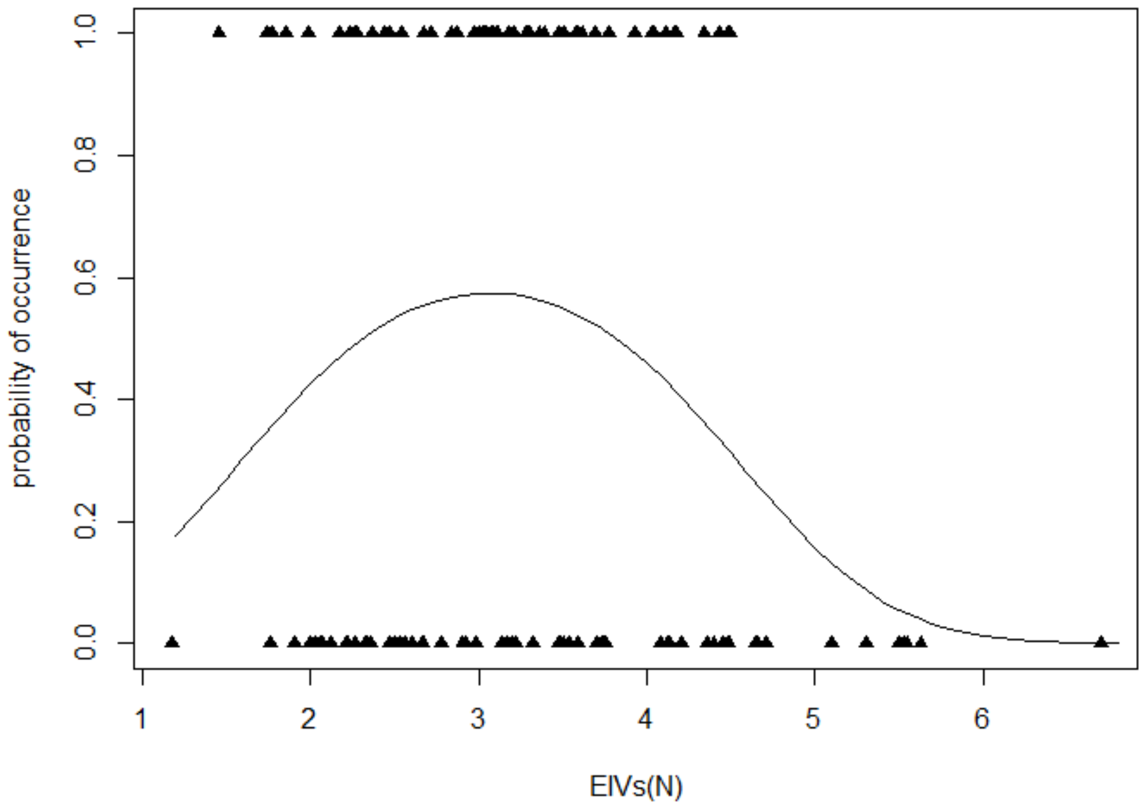

Figure 6: Probability of occurrence of *G. bohemica* based on the mean Ellenberg-indicator value nitrogen (EIVs(N)) of the vegetation in 126 plots (0.25 m^2) (GLM: D^2=0.060; $p<0.05$).

I found that the Ellenberg-indicator value of vegetation in the plots revealed a unimodal response of the occurrence of *G. bohemica* in response to nutrient availability (Fig. 6). The optimum is located at nutrient poor sites, but it declines strongly at very nutrient poor and rather nutrient rich sites. Gentian individuals were limited to plots with a mean nitrogen value from 1.5 to 5.

Co-occurrence analysis of species

Table 2: Dufrene-Legendre Indicator Species Analysis. Shown are species that associated in 126 plots (0.25 m^2) with the occurrence of *G. bohemica* (A), and species that are significantly overrepresented in plots without *G. bohemica*-occurrence (B).

A

Co-occurring species	*indicator value*	*P-value*
Alchemilla vulgaris agg.	0.1794	<0.05
Festuca ovina agg.	0.3873	<0.05
Hieracium pilosella	0.1815	<0.05
Leontodon hispidus	0.3829	<0.01
Linum catharticum	0.2026	<0.05
Lotus corniculatus	0.4273	<0.01
Nardus stricta	0.3714	<0.001
Pimpinella saxifraga	0.4712	<0.01
Trisetum flavescens	0.1699	<0.05

B

Gentianella-avoiding species		
Dactylis.glomerata	0.3274	<0.05
Taraxacum.officinalis agg.	0.1667	<0.001

The co-occurrence analysis shows that the occurrence of nine species is closely affiliated with the presence of *G. bohemica* in the 126 plots (Tab. 2); the majority of these species are typical for nutrient poor grasslands. On the contrary, two species that are typical for nutrient rich grasslands are significantly overrepresented in plots without *G. bohemica*-occurrence.

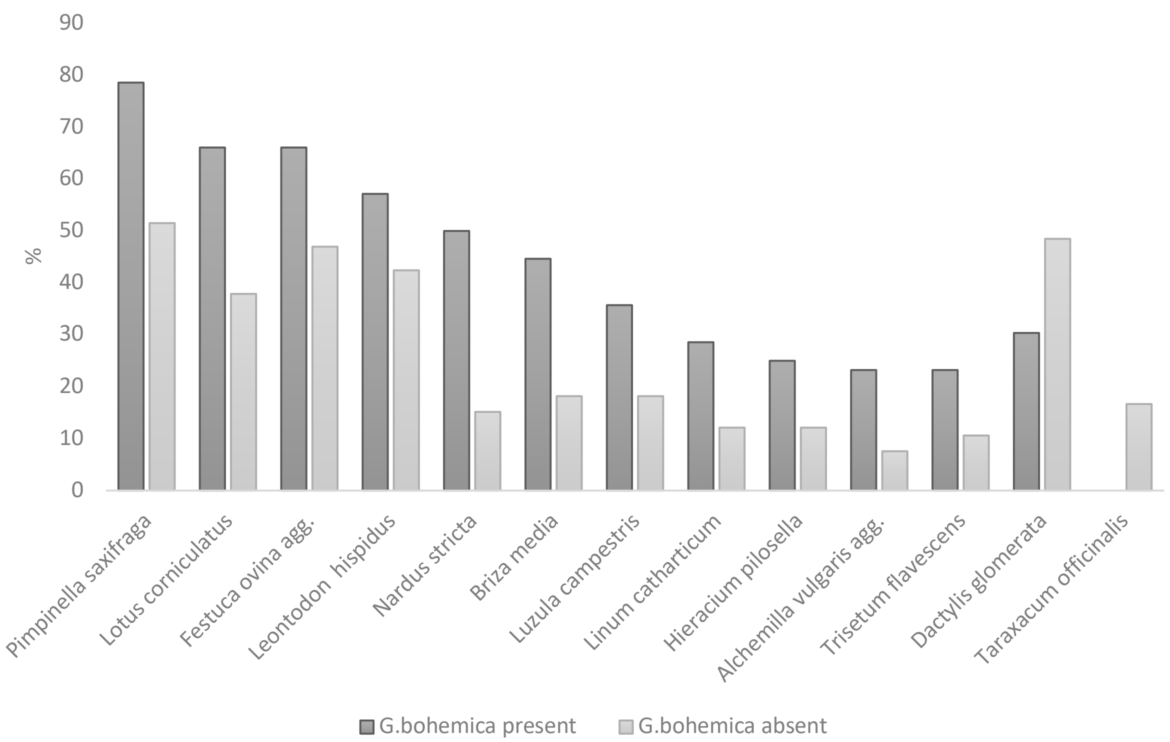

Figure 7: The most frequent species shown as percentage of colonized plots with (dark- grey) and without (light-grey) *G. bohemica*.

From the nine positively correlated species, five species (*Pimpinella saxifraga, Lotus corniculatus, Festuca ovina* agg.*, Leontodon hispidus and Nardus stricta*) occurred in > 50 % of the plots where *G. bohemica* was present. *Dactylis glomerata* a species negatively correlated with *G. bohemica* was found in almost 50% of the surveyed plots without gentian occurrence. *Taraxacum officinalis,* the second plant negatively correlated with *G. bohemica* was rarely found in the plots without the endemic plant being present (~15%), while it was absent in the recorded plots where *G. bohemica* was present (Fig.7).

The role of land use and land-use history

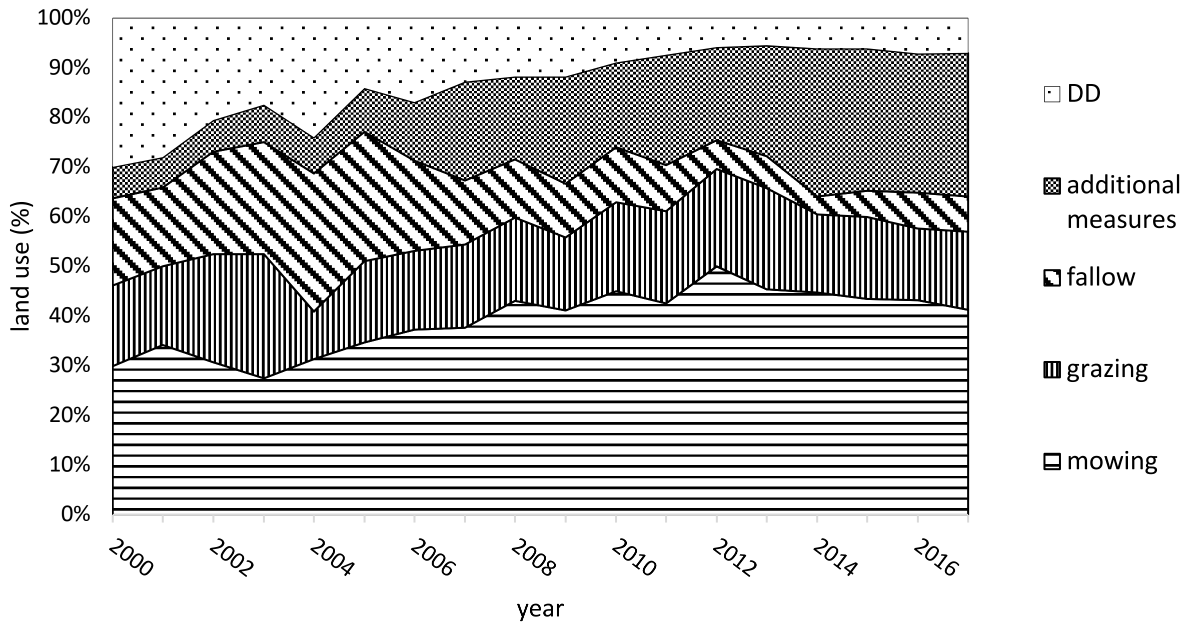

Figure 8: Percentage of three different land use types (fallow, mowing, grazing) and additional measures (gentian-specific management), such as clearance, cutting, mechanical scarification, harrowing, seed dispersal) at 81 sites for the years 2000 to 2017. DD = Data deficient.

For almost a third of the gentian locations no data on land use were available for land use for the first years of this study, but information on land-use increases subsequently (Fig. 8).
From the year 2000 onwards, following four land use types (grazing, mowing, fallow and gentian specific management) could be demonstrated on all areas with gentian occurrences. The land use type additional measures (gentian-specific management) includes all conservation methods (clearance, cutting, mechanical scarification, harrowing, seed dispersal) applied that aim to improve the conservation status of *G. bohemica* (Fig. 8).
In the year 2000 about 30 % of all gentian sites were mown. In all three countries this land use type was the most common form of land use. From the years 2000 to 2017, mowing increased to 41 %. Abandoned (fallow) sites had a share of 18 % in 2000, which declined to 11 % in 2017. Grazing was done at 16 % of the sites in 2000, and this value remained constant until 2017. In the early 2000s, gentian-specific management constituted only a small proportion with about 7 %, but this share increased substantially since 2006 and was done at almost 30 % of the sites in the year 2017 (Fig. 8).

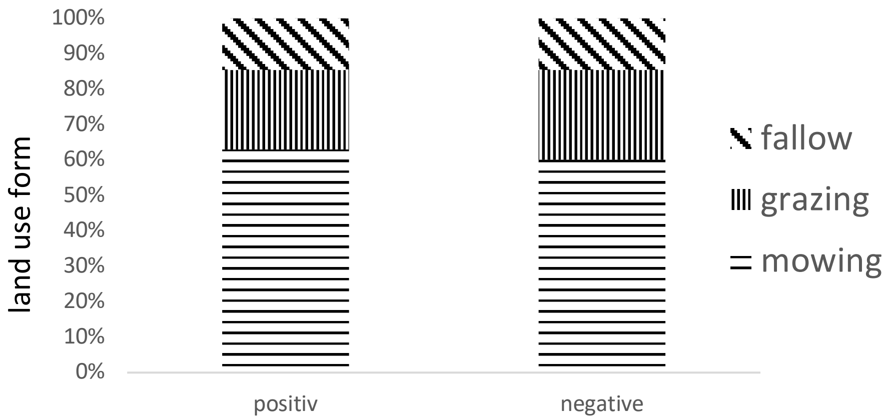

Figure 9: Percentage of the three land uses fallow, grazing, mowing from increasing and decreasing populations recorded from 2003 to 2017.

Sites with increasing population size of *G. bohemica* did not differ from those with decreasing population size in terms of land-use (Fig. 9). With about 60 % the land use type mowing has the highest share between 2003 to 2017. This management type is followed by grazing with a percentage close to 20 % and followed by the land use type fallow with a share of 10%.

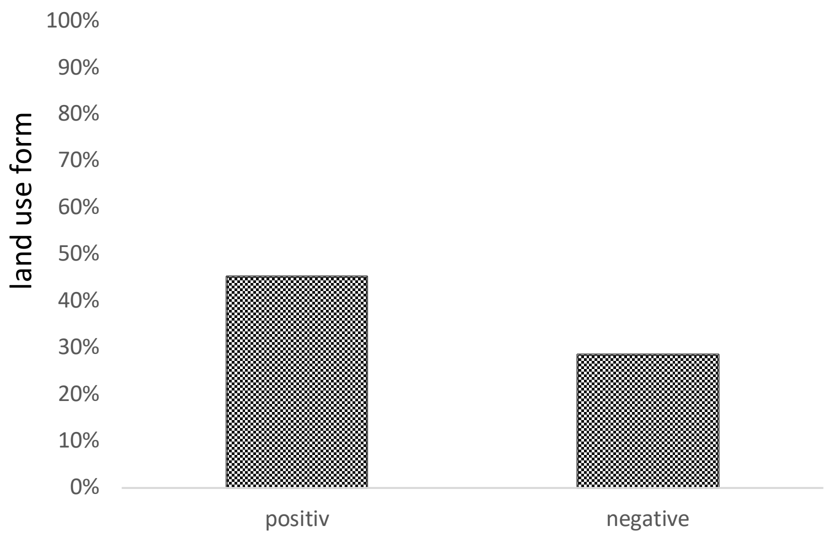

Figure 10: Percentage of gentian specific conservation measures from increasing and decreasing populations recorded from 2003 to 2017.

Gentian sites with increasing population size are characterized by a higher proportion of gentian beneficial measures such as harrowing, raking, clearance and seed dispersal. Sites with increasing populations received nearly twice as often gentian specific measures compared to declining ones (Fig.10).

DISCUSSION

Assessing the population dynamics of *G. bohemica* for the years 2003-2017 revealed that a drastic decline in individual numbers is apparent (Fig. 2). This drastic reduction in population-size is also evident in other European *Gentianella* species (Häupler& Schönfelder 1988, Korneck et al. 1996, Greimler & Dobes 2000, Stadler et al. 2010). Different causes for population decline are known as mentioned in the study's introduction. What is more, the population size of *G. bohemica* fluctuates strongly between years (Fig. 2). This was also previously detected in different *G. bohemica*-surveys (Dolek et al. 2010; Engleder 2017; Königer et al. 2012; Bucharova et al. 2012; Plenk et al. 2015; Kriechbaum et al. 2018, Křenová et al. 2019). I found that these fluctuations are synchronized among populations and countries due to the important role of prevailing climatic conditions (temperature, precipitation) in the vegetation period – interestingly with a time lag of three and two years on population size (Fig. 2, Appendix 1).

In short-lived plants like the study species, strong inter-annual fluctuations in population size are a common phenomenon (Runge 1963, Grubb 1985, Dierschke 1986), as generative regeneration depends on favorable conditions for germination and seedling establishment (Dolek et al. 2007). Křenová et al. (2019) attribute the yearly fluctuations in population size particularly to changes in agricultural use and weather conditions – especially a lack of precipitation in spring and early summer. Similar correlations between climatic conditions and population fluctuations were detected in this thesis (Fig. 3). Especially sufficient rainfall in late spring and early summer three years before had a positive impact on population size, while heavy precipitation two years earlier had a negative impact on population size (Fig. 3). In terms of mean annual temperature heat events 2 years earlier seems to positively influence population sizes (Fig. 3). Dierschke (1986) has already described a correlation between gentian development and weather conditions. He pointed out a typical three-year period pattern explained by an existing persistent seedbank to bypass dry years. Different monitoring programs of gentian population development reveals analogous correlations between population changes and weather conditions, whereas in terms of seedling development for the following year rainfall positively affects population growth and high temperatures have a negative impact on population development (Königer 2008). Seed germination as crucial life cycle phase for *G. bohemica* (Brabec 2012), need medium precipitation as an important factor for further plant survival but also partially dry summers seem to favor gentian blossoming notably (Rösler 2001). In the long term, the occurrence of several drought seasons between the years 2000 to 2007 actually triggered population growth of the rare plant (Bucharova et al. 2012). Bucharova et al. (2012) identified in their studied model of extinction probability of *G. bohemica* a positive link between high frequency of drought events and increased population survival in the following years. Repeated drought events by lack of precipitation and high temperatures indirectly promote germination for *G. bohemica* due to the creation of open gaps in the vegetation layer (Křenová et al. 2019). Besides the results of this study and the studies of Bucharova et al. (2012) and Křenová et al. (2019), a weather dependent improvement in germination and seedling establishment due to reduced competition caused by drought events and increased plant mortality in the previous year has already been reported in different other studies (Gross et al., 1998; Horvitz et al., 1998; Smith et al. 2005, Toräng et al., 2010).

In the study, I found a positive correlation between *G. bohemica* presence in plots and the proportion of open soil (Fig. 5). The gentian depends on vegetation gaps as a competitive weak and light-demanding species (Rösler 2001; Engleder 2006; Dolek et al. 2010; Plenk et al. 2015). Open gaps around the mother plant facilitate germination for *G. bohemica (*Dolek et al. 2003*)* given that the species possesses no further dispersal mechanisms (Dolek et al. 2010).

The preference of the Bohemian Gentian and other Gentian-species to occur preferably in open gaps in the vegetation layer has been reported in several studies, e.g. for *G. germanica* (Verkaar et al. 1984; Fischer et al. 1997), *Gentiana pneumonanthe* and *Gentiana punctata* (Kobiv 2018**).**

Further, I found a negative correlation between gentian occurrence and the proportion of moss layer (Fig. 4). A dense moss layer may prevent seedling development of *G. bohemica* (Bucharova et al. 2012). There is evidence of negative effects of dense moss layers on seedlings of vascular plants (Keizer et al. 1985, van Tooren 1990, Zamfir 2000), particularly reducing light availability (Ingerpuu et al. 2005, Keizer et al. 1985). The germination success especially of plant species with small seeds – such as *G. bohemica* – are often profoundly reduced (van

Tooren 1990). Further, germination of seeds is often stimulated by light input of a special wavelength (photosynthetically active radiation: PAR) (Fenner &Thompson 2005; Keizer et al. 1985, vanTooren 1990). High moss concentrations could modify that required PAR supply for seeds (Fenner & Thompson 2005; Jensen & Gutekunst 2002, Keizer et al. 1985, Jeschke & Kiehl 2008).

The analysis of habitat preferences of the Bohemian gentian based on EIVs (Fig.6) revealed that the species prefers nutrient-poor microhabitats. This finding supports empirical knowledge by experts of the species (Brabec 2012, Plenk et al. 2015). Further, Königer et al. (2008) carried out seed experiments and tested for nutrient availability in soil. This nutrient sensitivity may be also the case with other Gentianaceae species like *G. campestris* that mainly grow on nutrient-poor calcareous grassland (Oostermeijer et al. 2002).

Regarding other environmental factors such as moisture, light and soil reaction, EIVs showed less distinct preferences of *G. bohemica* (Fig.s 4-6) (see Appendix 1). One main reason for this finding likely is that only micro-habitats in sites of *G. bohemica* were sampled, thus the above-mentioned environmental parameters allow in principle for the occurrence of the study species – this may mask habitat preferences because unsuitable sites have not been sampled. For instance, all sites are characterized by EIVs that indicate high light values, which is in line with previous studies (Rösler 2001, Engleder 2006, Dolek et al. 2010). Further, EIVs indicate a moderate preference for moderately acidic sites, while highly acidic and neutral sites are less suitable; this finding support results from Dolek et al. (2003). The analysis of species co-occurrence showed that species that are overrepresented in plots with *G. bohemica*, are characteristic species of nutrient-poor grasslands, while species that are overrepresented in plots where the study species is absent, are species with high nutrient demands. (Table 2, Fig.7). On the other side, the two species that significantly overrepresented in plots without *G. bohemica* are typical species of fertilized grasslands.

The data on land-use of *G. bohemica* sites from 2003 and 2017 showed that – particularly in response to targeted species conservation activities – land use has changed over time, i.e. the proportion of abandoned grasslands has declined, while mowing and the implementation of targeted conservation measures has increased (Brabec 2012, Křenová et al. 2019). Conservation measures focus on removing litter, creating open gaps and on adjusting the timing of land use to *G. bohemica* development (Brabec 2012, Zehm et al. 2017). Such intense management measures in spring and autumn may increase seedling mortality, but this is compensated by reduced competition and improved development of surviving *G. bohemica* plants (Brabec 2012, Bucharova et al. 2012). Křenová et al. (2019) had studied the effects of long-term conservation efforts and they found that intensive conservation measures positively affected population under current climatic condition (Křenová et al. 2019). My study confirmed the importance of dedicated management measures for population survival (Fig. 9, Fig. 10).

I conclude that the long-term survival of *G. bohemica* critically depends on dedicated and ambitious conservation measures, and that it is by no way secured. Protecting this species also protects many associated species of nutrient-poor grasslands, a habitat that has declined severely in the last decades (Schweighofer 2006).

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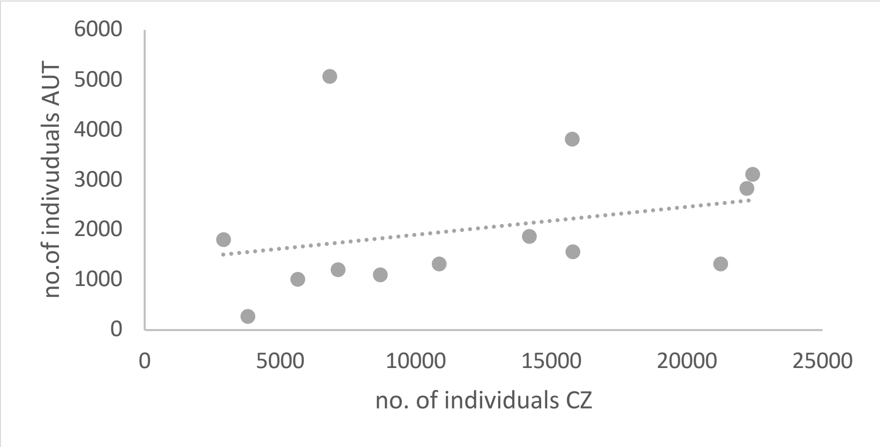
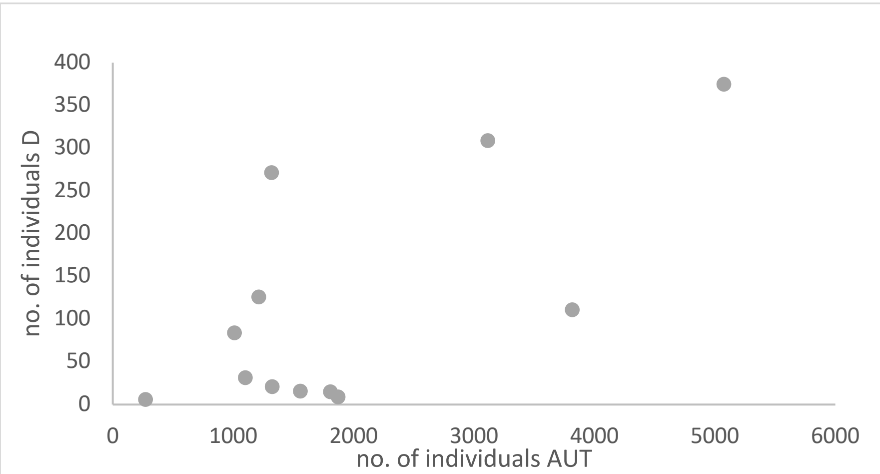
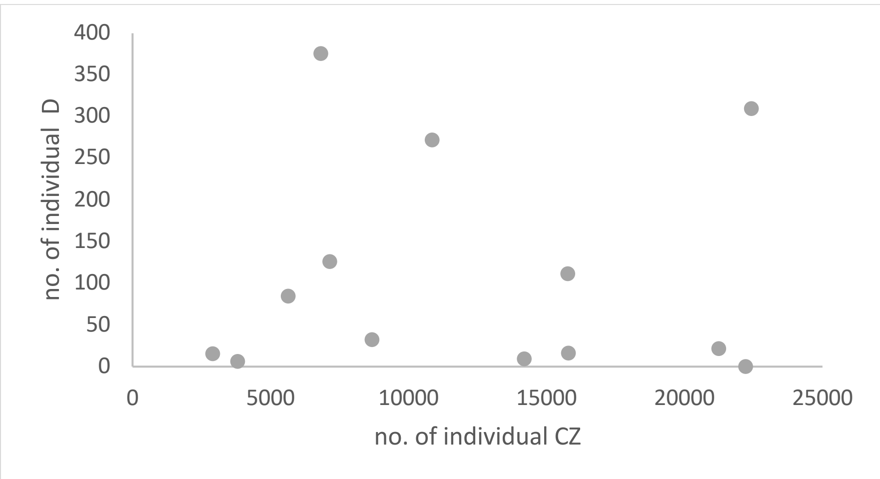
APPENDIX 1



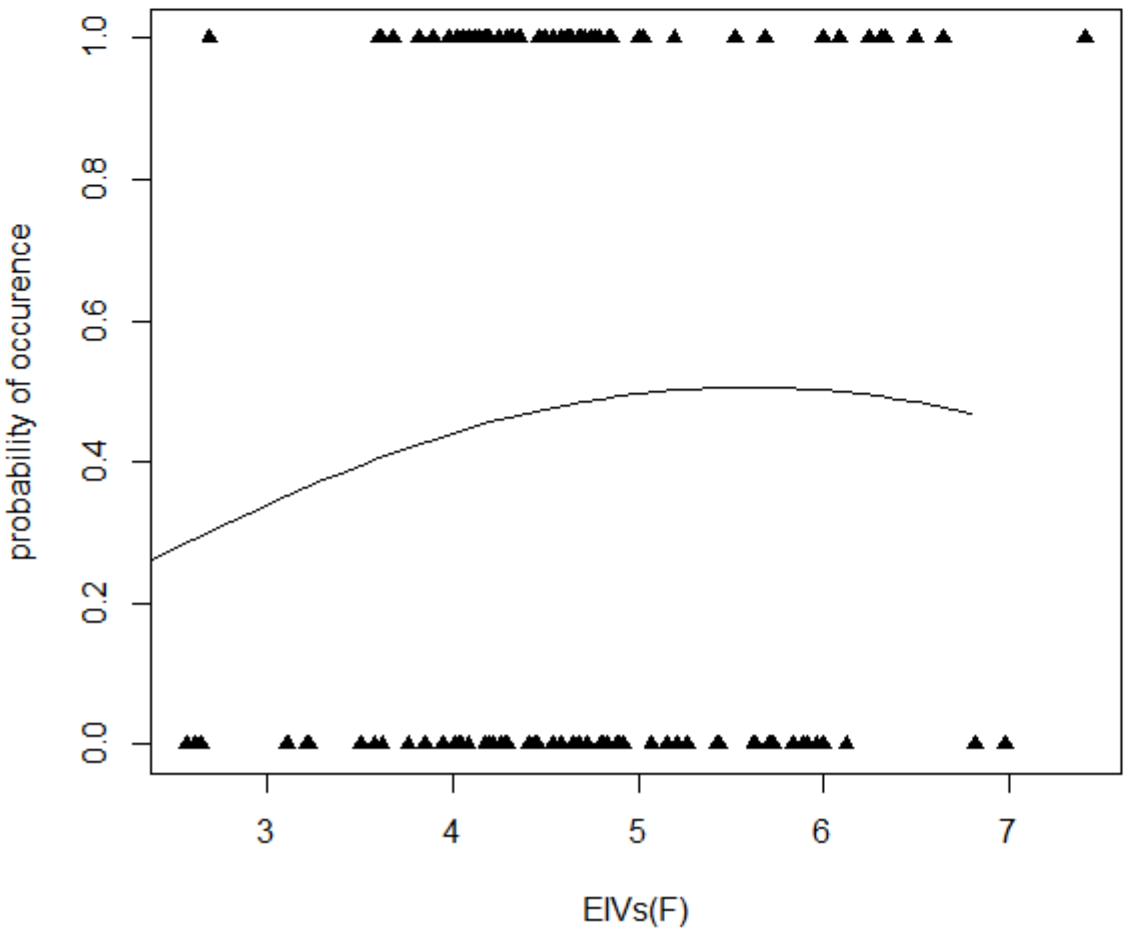
Supplementary Figure 1: Correlation analysis between number of individuals of the Czech and Austrian (rs [128] = 0.77, p<0.01).



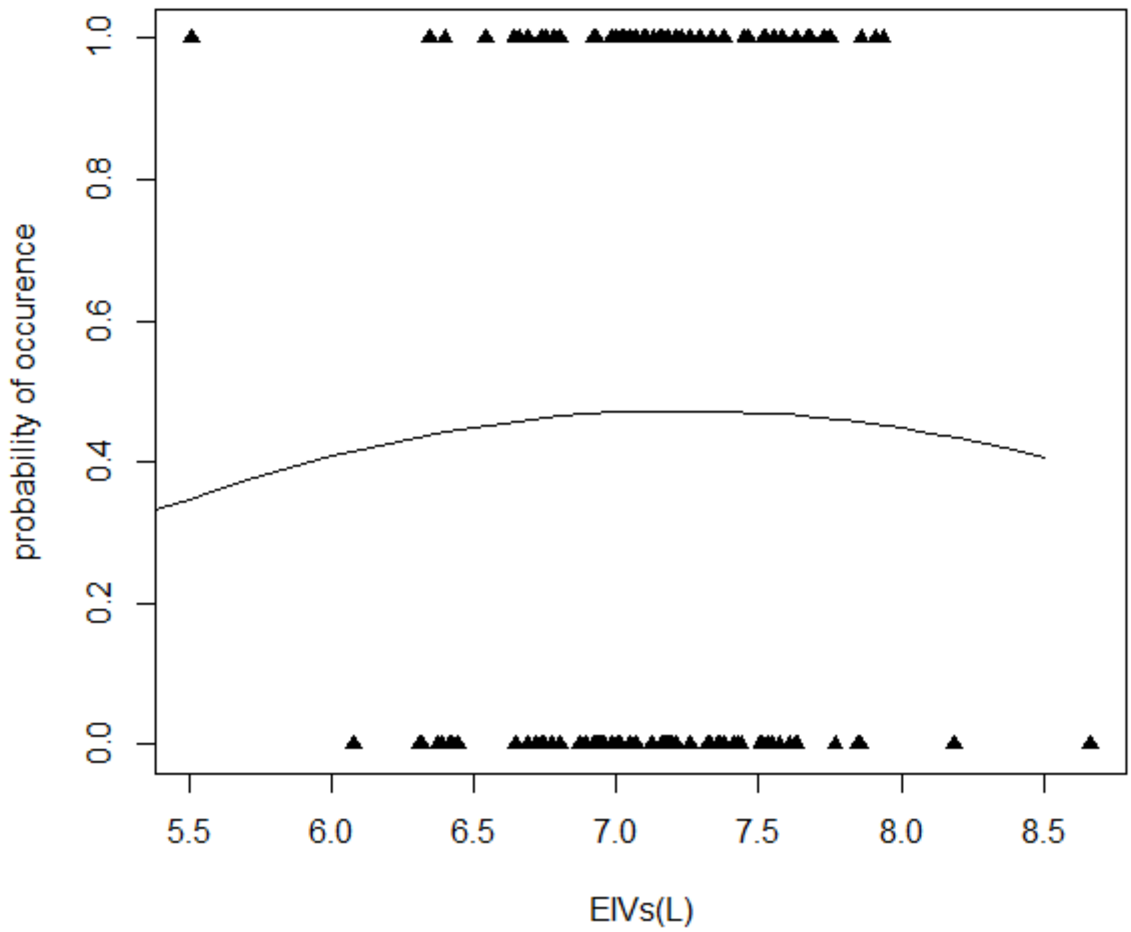
Supplementary Figure 2: Correlation analysis between number of individuals of the Bavarian and Austrian locations (rs[379] =0.32, p=0.24).



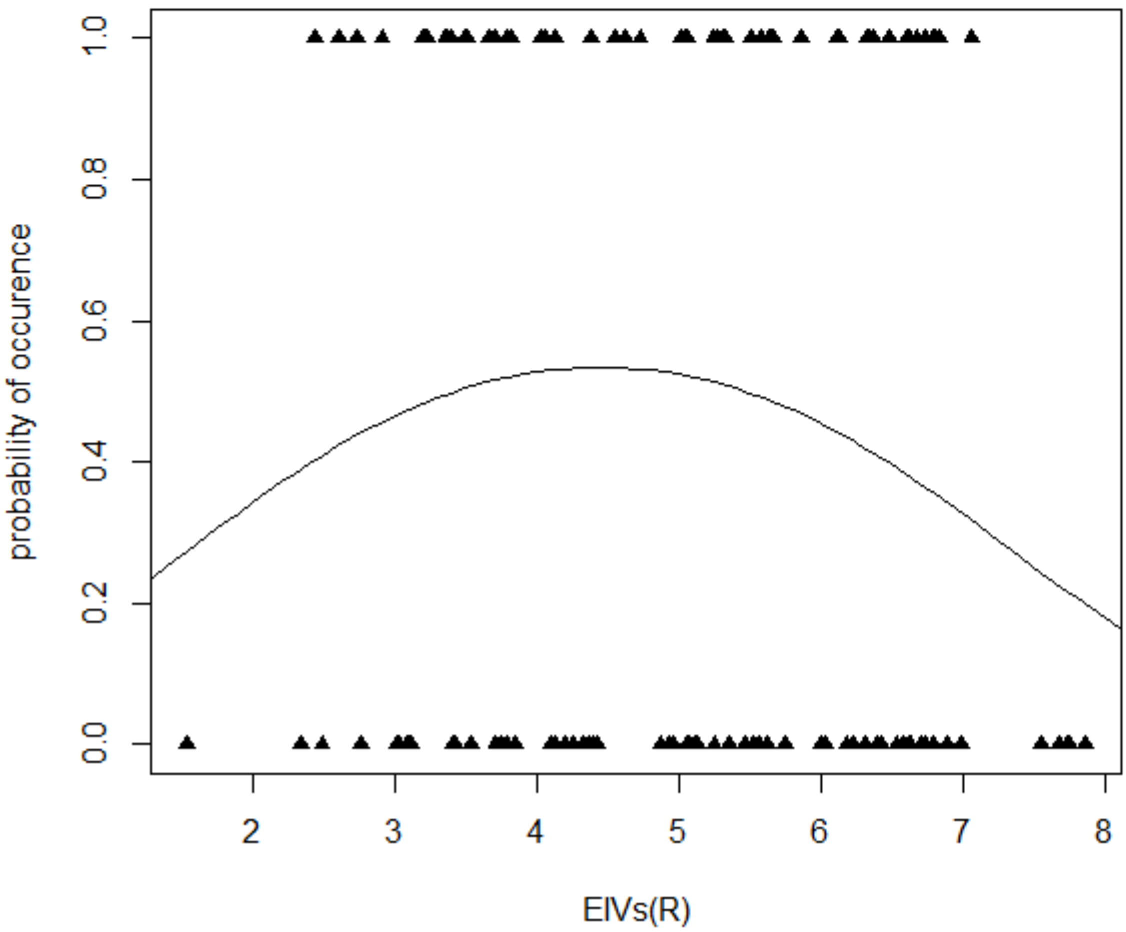
Supplementary Figure 3: Correlation analysis between number of individuals of the Bavarian and Czech locations (rs[283] =0.49, p=0.061).



Supplementary Figure 4: Probability of occurrence of *G. bohemica* based on the mean Ellenberg-indicator value moisture (EIVs(F)) of the recorded plots (GLM: D^2=0.006; p=0.491).



Supplementary Figure 5: Probability of occurrence of *G. bohemica* based on the mean Ellenberg-indicator value light (EIVs(L)) of the recorded plots (GLM: D^2=0.0007; p=0.735).



Supplementary Figure 6: Probability of occurrence of *G. bohemica* based on the mean Ellenberg-indicator value soil reaction (EIVs(R)) of the recorded plots (GLM: D^2=0.019; p=0.151).

List of species recorded in the vegetation plots

Achillea millefolium agg.
Acer pseudoplatanus
Aegopodium podagraria
Agrostis canina
Agrostis capillaris
Agrostis stolonifera
Ajuga reptans
Alchemilla cf. glabrescens
Alchemilla sp.
Alchemilla vulgaris agg.
Alopecurus pratensis
Angelica sylvestris
Anthoxantum odoratum
Anthriscus sylvestris
Anthyllis vulneraria
Arrhenatherum elatius
Bellis perennis
Betonica officinalis
Betula pendula
Brachypodium pinnatum agg.
Briza media
Bromus erectus
Bromus inermis
Calamagrostis epigeos
Calluna vulgaris
Campanula patula
Campanula rhomboides
Capsella pursa -pastoralis
Campanula rotundifolia
Carex brizoides
Carex caryophyllea
Carex pallescens
Carex cf. panicea
Carex pilulifera
Carlina acaulis
Centaurea jacea
Centaurea scabiosa
Cerastium holosteoides
Chaerophyllum hirsutum
Cirsium acaule
Cirsium arvense
Cirsium heterophyllum
Clinopodium vulgare
Corylus avellana
Cuscuta epithymum
Cynosurus cristatus
Dactylis glomerata
Danthonia decumbens
Daucus carota
Deschampsia cespitosa
Deschampsia flexuosa
Dianthus deltoides
Echium vulgare
Elmys repens
Equisetum arvense
Erigeron acris
Euphorbia cyparissias
Euphrasia officinalis
Festuca ovina agg.
Festuca pratensis
Festuca rubra
Filipendula ulmaria
Fragaria vesca
Galeopsis tetrahit
Galium aparine
Galium album
Galium sp.
Galium saxatile
Galium uluginosum
Galium verum
Gentianella bohemica
Gentiana cruciata
Glechoma hederacea
Helianthemum nummularium
Hepatica nobilis
Heracleum sphondylium
Hieracium lactucella
Hieracium pilosella
Holcus lanatus
Hypericum maculatum
Hypericum perforatum
Hypochaeris radicata
Hippocrepis sp.
Juncus effusus
Knautia arvensis
Lathyrus pratensis

Leontodon hispidus
Leucanthemum vulgare
Lilium martagon
Linaria vulgaris
Linum catharticum
Lollium perenne
Lotus corniculatus
Luzula campestris
Luzula cf. pilosa
Luzula luzuloides
Medicago campestris
Medicago lupulina
Molinia caerula
Nardus stricta
Origanum vulgare
Phleum pratense
Picea abies
Pimpinella major
Pimpinella saxifraga
Pinus sylvestris
Plantago lanceolata
Plantago medium
Parnassia palustris
Polygala amara
Polygala chamaebuxus
Polygala vulgaris
Potentilla erecta
Poa trivialis
Poa pratensis
Prunella vurlgaris
Quercus robur
Ranunculus acris
Ranunculus nemorosus
Rhinanthus angustifolius
Rhinanthus minor
Rosa cf. canina
Rubus caesius
Rumex acetosa
Sanguiosba minor
Sanguisorba officinalis
Scabiosa columbaria
Scirpus sylvatica
Scorzonera humilis
Senecio jacobea
Securigera varia
Sesleria varia
Silaum silaus
Silene vulgaris
Stellaria graminea
Succisa pratensis
Tanacetum vulgare
Taraxacum officinalis
Thesium cf. pyrenaicum
Thymus pulegoides
Trifolium arvense
Trifolium hybridum
Trifolium medium
Trifolium pratense
Trifolium repens
Trisetum pubescens
Trisetum flavescens
Vaccinium myrtilis
Vaccinium vitis-idea
Veronica chamaedrys
Trifolium campestre
Veronica officinalis
Vicia cracca
Vicia pisiformis
Viola arvensis
Viola canina
Viola sp.

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