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Drivers of Distribution and its Changes in two Closely Related Species with Contrasting Elevational Distributions Over two Decades

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Research Article

Keywords: Anthoxanthum odoratum, Anthoxanthum alpinum, climate change, alpine species, the Krkonoše Mts

Posted Date: April 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-345036/v1

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Drivers of distribution and its changes in two closely related species with contrasting elevational distributions over two decades

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TŠ and ZM designed the research, TŠ conducted the field work and analyzed the data. TŠ wrote the manuscript and both authors contributed to the final version of the manuscript.

Key words

Anthoxanthum odoratum, *Anthoxanthum alpinum*, climate change, alpine species, the Krkonoše Mts

Abstract

Climate change has an enormous impact on species and communities, especially those in the alpine and arctic environments. Even though the reactions of species to climate change have been widely studied, their responses are not straightforward, and it is necessary to focus on them in more detail. In this study, we assessed the distribution of two closely related grass species *Anthoxanthum odoratum*, an allotetraploid species of lower altitudes and *A. alpinum*, a diploid occurring in higher altitudes, in the Krkonoše Mts., the Czech Republic. We explored the drivers of their current distribution and its changes over the past two decades during the ongoing climate change. The results indicate that distribution of these two species has not considerably changed, as there is only a weak evidence of a wider distribution of *A. odoratum* compared to the past. Surprisingly, *A. alpinum* has newly appeared at some localities at lower altitudes. Changes in the distribution of the two species over time were significantly related to a range of local habitat characteristics such as vegetation or bryophyte cover, nutrient level, moisture, or species composition, but were largely independent of altitude, a variable expected to be a proxy of climatic conditions of the localities. This indicates that the environmental characteristics of the localities, play more important role in species distribution and its changes than global climate change.

Introduction

Mountains are exceptional environments as they host climatically and topographically heterogeneous environments and are spatially isolated. They are known as 'biodiversity hotspots' which are extremely fragile and host a high number of endemic species (Hassan et al. 2005). Especially semi-natural mountain grasslands that originated by human activities (mowing or livestock grazing) but are affected by the specific habitat conditions of the mountains, are an important source of biodiversity (Dijk 1991).

Mountain plant species are adapted to extreme conditions and are easily handling stress. On the other hand, they are weak competitors for light (Kikvidze et al. 2006) and are able to survive only under conditions of lower productivity (Grime et al. 2007). It has been predicted that climate change might promote migration of more competitive lowland species into higher elevations (Alexander et al., 2015), possibly leading to competitive exclusion of the mountain species (Easterling et al. 2000; Thomas 2010; Alexander et al. 2015; Petitpierre et al. 2016).

The effects of climate change are likely the strongest in the mountains as the climate in the mountains is rapidly changing and mountain ecosystems are predicted to have the fastest decreasing rate per decade (Thuiller et al. 2005, Beck and Goetz 2011, Fan 2019, Niskanen 2019). Plant extinctions in these areas may thus be large (Easterling et al. 2000, Thuiller et al. 2005). Another potential danger to the mountain ecosystems is the increasing impact of human activities, such as construction of infrastructure, downhill courses (Franke et al. 2019) and increased levels of tourism (Pignatti 1993) leading to changes in environmental conditions and increasing levels of disturbance subsequently affecting species distributions (Lenoir et al. 2010).

The changes in plant species distribution in the mountains have already been largely studied, yet the authors mainly focus on changes in vegetation composition by re-surveying

vegetation plots (e.g. Walther et al. 2005, Felde et al. 2012, Gritsch et al. 2016, Czortek et al. 2018, Rumpf et al. 2018, Rumpf et al. 2019a, Rumpf et al. 2019b). The studies often indicate that changes in a vegetation composition are driven by a change in moisture (Czortek et al. 2018) or both moisture and temperature (Felde et al. 2012) and the species are mostly shifting upwards (Walther et al. 2005, Wang et al. 2019, Rumpf et al. 2018, Rumpf et al. 2019a, Rumpf et al. 2019b). The upward shift is in most cases caused by increasing temperature allowing the lowland and more competitive species to move upwards. This upward migration may in fact also lead to increased species richness on mountain tops (Steinbauer et al. 2018). The patterns of upward migration are, however, largely modified by other environmental factors and their changes (Rumpf et al. 2019a).

In some cases, the species do not shift at all or are even shifting downwards (Lenoir et al. 2010; Crimmins et al. 2011). The downward shift of species could be caused by land-use-related habitat modification such as increased grazing intensity, and increased frequency of disturbance caused by climate change (e.g. insect outbreaks, wildfires, windthrows) allowing the species to find new habitats in the lower parts of the mountains (Lenoir et al. 2010). However, a decrease of land-use intensity in the higher altitudes might cause that the match between potential climatic ranges and appropriate habitat types increases for alpine plants (Hulber et al. 2020).

The studies based on re-surveying vegetation plots are useful to provide an overall picture of vegetation development. Due to limited size of the vegetation plots, these data are, however, not suitable for observing detailed changes in distribution of single species and thus do not allow to identify the specific mechanisms of species responses to changing climates (Kapfer et al. 2017).

Studies on detailed changes in distribution of single species, not the vegetation plots, and its drivers are, however, rare, probably mainly because of a lack of high-quality data on

past species distributions. Using such an approach, Crimmins et al. (2011) found that certain species may shift their distribution due to climate change downwards despite previously well documented uphill shifts and that it is caused by climatic water balance. In another study, Kopp and Cleland (2014) found that sagebrush moved upwards as expected, however, other alpine herbs declined in the lower areas of their distribution range without migrating upwards and bunchgrass species even declined in the upper parts of its range. The authors ascribe such changes to an increase in temperature and decrease in precipitation. The above-mentioned studies indicate that the reaction of the plant species to the environmental change is not as clear as it may be expected. The species differ in their responses and increasing temperature is not the only driver of the shift in species distribution. There is mostly a net elevation gain, though there is a huge noise.

Here, we studied change in distribution of two closely related grass species, *Anthoxanthum odoratum* and *A. alpinum*. *Anthoxanthum odoratum* is an allotetraploid, clonal autochorous species, that has probably originated by crossing of diploid *A. alpinum* and different unknown, probably Mediterranean and already extinct, diploid species (Chumova et al., 2015). The species differ mainly in their distribution. *Anthoxanthum odoratum* is a lowland species, whereas *A. alpinum* is a species of high latitudes and altitudes while they meet at the contact zones in intermediate elevations. Flegrova and Krahulec (1999) suggested that *A. odoratum* performance is limited by low temperatures in higher altitudes and *A. alpinum* performance is limited by high competition in the lower parts of the mountains.

Exploring changes in distribution of species from this pair is interesting as it offers an opportunity to compare species differing in their distribution, but also in their ploidy level. Species of higher ploidy levels are expected to be stronger competitors (Te Beest et al. 2012; Eliášová and Münzbergová 2017); that is another reason for *A. odoratum* to have likely high potential to profit from the ongoing climate change and outcompete *A. alpinum* at its previous

locations. However, the polyploids may still coexist due to for example different phenology, reproductive isolation (e.g. Petit et al. 1997; Rieseberg and Willis 2007) or infertility between cytotypes (e.g, Castro et al., 2011; Trávníček et al. 2011). On the other hand, the cytotype that is rare, might be eliminated from the population due to its reduced fitness and a production of new infertile hybrids (minority cytotype exclusion; Kolář et al. 2017).

Here, we take advantage of a unique opportunity of re-visiting of *A. odoratum* and *A. alpinum* localities after 17-19 years, previously explored by Filipová and Krahulec (2006) in the Krkonoše Mountains. Krkonoše Mts. are a part of Sudetes and are the highest mountains in the Czech Republic. In the period 2001-2016 (compared to 1961-2000) the annual mean temperature in Krkonoše Mts. increased by 0.8-1 °C (Kliegrová and Kašičková 2019). In 2000-2002, Filipová and Krahulec (2006) mapped distribution of *A. alpinum* and *A. odoratum* throughout the Krkonoše Mts. using 351 well localized sampling points. Altogether, they found 170 localities with *A. odoratum* (up to 1290 m a. s. l.), 109 localities with *A. alpinum* (from 745 m a. s. l. and mainly above the tree line, up to 1500 m a. s. l., but also in the areas without contact with alpine or subalpine vegetation) and 72 localities with both species present (800-1290 m a. s. l.).

In this study, we aim to describe how these two plant species react to climate change by shifting their distribution and what are the specific factors driving the distribution. We hypothesize that (i) due to globally increasing temperatures, more competitive lowland tetraploid *A. odoratum* moved upwards and suppressed alpine diploid *A. alpinum.* Further, we hypothesize that (ii) their zone of co-occurrence has extended due to increased level of human disturbance due to higher intensity of transportation and creation of new suitable habitats for species spread and growth. (iii) The occurrence of both species is driven by altitude, vegetation composition and cover with *Anthoxanthum odoratum* occurring in lower altitudes and more productive environments (higher vegetation and bryophyte cover, higher amount of

nutrients and higher temperatures) and *A. alpinum* occurring in higher altitudes and less productive environments (lower vegetation and bryophyte cover, lower amount of nutrients and lower temperatures). (iv) *Anthoxanthum alpinum* will more likely be replaced by *A. odoratum* at localities with characteristics favoring *A. odoratum*, while it will be more likely stable at localities with characteristics favoring *A. alpinum*.

Material and methods

Study system

Anthoxanthum odoratum is a lowland meadow species that is widely distributed all over temperate and alp-arctic Europe, and north-west Africa. Secondarily, it is distributed all over the world (Americas, Australia, New Zealand) except of high latitudes and altitudes (Hedberg 1967). *Anthoxanthum alpinum* is present in extreme arctic and alpine environments of Europe and Asia (Conert 1985; Hedberg 1986). The two species can co-occur in intermediate locations (Drapikowska et al. 2012; Filipova and Krahulec 2006). The altitude of their contact zone differs between regions likely due to difference in climatic conditions of the regions and possibly also due to differences in other environmental conditions in the areas. In Polish Carpathians, Babia Góra Massive, authors collected 11 samples only and found that the zone of overlap is ranging between 1153 and 1166 m a. s. l. (Drapikowska et al., 2012). In the Czech Republic in Krkonoše Mountains, the contact zone was indicated between 750 and 1290 m a. s. l. (Filipova and Krahulec, 2006). The position of the contact zone in other regions is unknown, only one study describes that in southeastern Europe, *A. alpinum* was found at the altitude of 1400 m a. s. l. and higher (Stančić 2005).

Theoretically, the two species may be distinguished based on the hairiness of palea and lemma. The hairs are, however, hard to see and this trait cannot be used in vegetative plants. Thus, the most reliable and easiest approach is to distinguish the two species based on

different ploidy levels using flow cytometry (Pimentel and Sahuquillo, 2008). The species nomenclature in this paper is unified according to Kaplan et al. (2019).

Study area

The field research was carried out in the Krkonoše Mountains National Park, the highest mountain range in the Czech Republic. The ridge is approx. 35 km long, with the altitude ranging from 400 to 1600 m a. s. l. The climate is cold and wet with mean annual temperature ranging from 0 to 6 °C and the mean annual precipitation ranging from 800 to 1400 mm. Snow cover is usually present from November to March, in the highest positions it stays over 180 days a year. At the altitude of 1250 m a. s. l., which is the mean altitude of a tree line in Krkonoše Mts., the mean annual temperature increased by 0.8-1°C in the period 2001-2016 (compared to 1961-2000). The temperature increased the most in April (by 1.6°C) and December, July, and January (by more than 2°C). The mean annual precipitation has increased only slightly, by 2 mm, over 56 years (from 1961 to 2016, Kliegrová and Kašičková 2019). However, a decrease in precipitation in April and May and an increase in July was detected (Kliegrová and Kašičková 2019). The tree line in Krkonoše Mts. shifts upwards by 0.43 m per year (Treml and Chuman 2015). Because of the warmer winters, the snow amount as well as snow cover duration have decreased (Urban et al. 2018; Urban et al. 2019). The nitrogen deposition that influences vegetation productivity has not changed between 1994 and 2005, though the deposition exceeded the critical load of nitrogen during this period (Hošek et al. 2007). The nitrogen deposition generally decreased by about 70% between 2005 and 2017 in the Czech Republic, still exceeding critical load (Bäumelt et al. 2017).

Krkonoše Mts. are characterized by species rich semi-natural grasslands that were managed by humans since the 12th or 13th century. These grasslands are connecting the alpine environment to lowland grasslands and are thus allowing the species to migrate and create

exceptionally species rich mountain grasslands (Krahulec et al. 1997). Krkonoše Mts. are amongst the most visited central European mountains and even European national parks. According to the mobile phone services, in 2018, the Krkonoše Mts. were visited by 3.79 million of visitors who spent 11.87 million person- days, whereas in 2017, it was 4.8% less (WEB 1). The data from the beginning of the millennium are not available, but the tourism in the area is rapidly increasing. For example, from 1963 to 2015, the area of downhill courses increased from 65 to 672 ha (Flousek 2019). Such an increase of visitors may have an immense impact on ecosystems by disturbing living organisms, accumulating rubbish, increasing transport and development of infrastructure (Stursa 2002).

Data collection

In August 2019, 243 localities were re-visited out of 350 localities visited in 2000-2002 by Filipova and Krahulec (2006) in the Krkonoše Mountains. In 2000-2002, the species were collected mainly at the sites where they were likely to grow in mixtures. In 2019, we focused on localities where either *A. alpinum* or mixture were present in the past omitting some of the lowland *A. odoratum* localities, which prevailed in the original dataset (Supporting information 1 and 2).

At each locality, the presence of *Anthoxanthum* species was surveyed. If present, a 0.5×0.5 m phytosociological relevé was made. Note that Filipova and Krahulec (2006) did not collect vegetation relevés at all localities, and the new vegetation relevés thus could not be compared to the old ones. Such relevé size is sufficient as the patches with *Anthoxanthum* occurrence were often small and a relevé was made in its center. If *Anthoxanthum* was absent, the relevé was not made, especially because we did not have any evidence where the species has been exactly found at the site in the past. When the locality was completely destroyed (new construction of a chalet, parking place), it was excluded from the analyses. Braun-

Blanquet scale was used for estimating the species cover. In 2019, the slope, aspect and cover of all vascular plants, bryophytes and litter within each relevé and tree canopy cover above the relevé were estimated (as the relevés, these data were not available in the older dataset. While slope and aspect are static parameters and thus did not change over time, the other data represent the conditions at the localities in 2019 only.

Leaves of 5 *Anthoxanthum* individuals were collected, if possible, within the relevé. Leaves of additional 5-10 individuals, if present, were collected outside of the relevé evenly covering the area about 10 m around the relevé. The leaves were stored in plastic bags, for a maximum of one week. Afterwards, the species were distinguished by flow cytometry (DAPI; Otto 1990) with *Solanum pseudocapsicum* used as a standard. Our approach to species identification was a bit different from Filipova and Krahulec (2006) who distinguished the species by examination of hairiness of palea and lemma by a binocular magnifying glass (magnification 20-40x). Only when they were unsure, they used flow cytometry as well.

For each locality, we calculated topographic wetness index (TWI) for each locality in ArcMap software (ESRI 2012) by using a digital elevation model (DEM) layer with 1×1 m resolution. From this, the raster layers of flow direction and flow accumulation were made, and TWI was calculated according to Zimmerman and Shallenberger (2016). This is also a static parameter and thus did not change over time.

All localities were also checked in current orthophoto maps and compared to those from 2003 with a focus on tree canopy cover. The tree canopy cover was visually estimated and when the cover increased, it was coded as 1, if it decreased or remained the same, it was coded as 0. While we initially planned to use more detailed classification of the degree of canopy cover change, we eventually used this simple classification as only about 5.3% of localities showed any increase in cover and most localities did not change. In this way, we obtained a new predictor expressing change in canopy cover over time.

Data analyses

We used Chi-square test of 2 × 2 contingency table with Yates correction for low number of observations to compare probability that the two species differ in appearance or disappearance from the locality since 2000 using localities with both species present in 2000 (mixed localities) and using all the localities.

Data from vegetation relevés were summarized in two ways. First, Braun-Blanquet scale was expressed as percent. Ellenberg indicator values (EIV; Ellenberg et al., 1991) were assigned to each species, weighted by species cover and used to calculate weighted mean value per plot for nutrients, pH, moisture, light, temperature and continentality. Second, the data were used to run detrended correspondence analysis (DCA) excluding all species with less than 5 occurrences in the whole dataset and both *Anthoxanthum* species. The *Anthoxanthum* species were included in the resulting plot as supplementary variables. Site scores on the first two axes from this analysis were used as summarized vegetation composition. Vegetation data were analyzed in vegan package (Oksanen et al. 2015) in R version 3.1.2.

Position of plots along the first and second DCA axes were used as predictors of change in *Anthoxanthum* distribution as described below. The first DCA axis likely represents gradient of pH; on the positive side of the axis are acidophilous species such as *Avenella flexuosa* or *Luzula luzuloides* and on the negative side are species of more neutral pH such as *Lotus corniculatus* or *Dactylis glomerata*. The second DCA axis represents larger uncommon species of higher elevations, wet and nutrient rich environments on the positive side of the gradient, such as *Cirsium canum*, *Angelica sylvestris* and species of mountain grasslands, such as *Homogyne alpina*, *Avenella flexuosa*. The negative side of the axis is represented by species that are common grassland species, are stronger competitors and grow in relatively

dry and poor conditions, such as *Lotus corniculatus*, *Dactylis glomerata*, *Plantago lanceolata* and *Leontodon hispidus* (see Fig. 1).

We used the data on past and current distribution of *A. odoratum* and *A. alpinum* to express 6 different dependent variables. Current occurrence (2019) of *A. odoratum* and *A. alpinum*, new appearance of *A. odoratum* and *A. alpinum* and disappearance of *A. odoratum* and *A. alpinum*, all in 2019. For the analyses of the changes in their occurrence, we used only the subsets, where the change was possible. For example, in the case of the appearance of *A. odoratum*, the subset consisted of localities where *A. odoratum* was absent in the past.

Because we obtained many predictor variables, (EIV for nutrients, pH, moisture, light, temperature and continentality, TWI, DCA axis 1, DCA axis 2, canopy cover, vegetation cover, litter and bryophyte cover, change in canopy cover), we calculated variance inflation factor (VIF) with the 'vifstep' function in the R package usdm (Naimi 2015). Based on this, EIV for pH has been excluded since its VIF value was larger than 3 as advised by Zuur et al. (2010). All the other variables have been used in the subsequent tests.

We used all the selected predictors to identify the optimal model explaining each of the six dependent variables. This was done using a generalized linear model (GLM) with binomial distribution selecting model with the lowest AIC by stepwise selection (function step in Hmisc package, Harrell 2016). All analyses were done only for localities, in which we found at least one *Anthoxanthum* species, testing the effects of all the predictors. Afterwards, the analyses of species disappearance included also localities at which *Anthoxanthum* occurred in the past but was not found at present. At these localities, we did not record a phytosociological relevé, so only the effects of TWI, elevation, slope, aspect and change in a canopy cover could be tested. Because this additional analysis did not provide any new insights, it is only briefly mentioned in the results. Besides testing the above-mentioned models, effect of each predictor on each explanatory variable was tested separately.

Results

A total number of re-visited localities was 243. At 71 of them, none of the study species was found and at 2 of them, the locality was destroyed by newly constructed chalet or parking place. At these localities, both *Anthoxanthum* species were present equally in the past (see Table 1 and supporting information 1 and 2, Chi = 17.97, p = 0.24). *Anthoxanthum alpinum* disappeared from 24 previously mixed populations of 61, while *A. odoratum* disappeared from 12 of them. Such a proportion is not significantly different from expected random disappearance of both species (Chi = 1.41, p = 0.23).

Distribution of *A. alpinum* along the elevation gradient (775-1445 m a. s. l.) has not changed (Fig. 2). At its lowest locality, it has been joined by *A. odoratum*. The elevational distribution of mixtures has also not changed significantly (Fig. 2). However, their distribution became narrower and the number of localities with mixtures has decreased (see also Table 1). *Anthoxanthum odoratum* distribution (526-306 m a. s. l.) along the elevation gradient has changed only subtly; the distribution is wider, and the species has been found in higher elevations compared to the past (Fig. 2).

Seven predictors have been chosen as significant in at least one of the models (elevation, vegetation, and bryophyte cover, EIV for nutrients, moisture, temperature and the second DCA axis). *Anthoxanthum odoratum* occurred significantly more likely at localities in lower altitudes, with higher bryophyte cover, nutrient level and temperatures, and lower moisture levels (Fig. 3). Occurrence of *A. odoratum* was also significantly affected by DCA2. It occurred together with species that have higher stature (such as *Phleum pratense*, *Dactylis glomerata*, *Nardus stricta*, *Hypericum maculatum*, *Polygonum bistorta*, *Leontodon hispidus* and *Trifolium repens*, Fig. 1). *Anthoxanthum alpinum* occurred significantly more likely at higher altitudes and at localities with lower temperature, vegetation and bryophyte cover,

lower nutrients and slightly higher moisture (Fig. 3, Table 1). The effect of DCA2 was marginally significant. *A. alpinum* tended to be present with species of poor habitats with lower pH (Fig. 1). For boxplots showing differences between both species and their mixture in bryophyte cover, elevation, EIV for nutrients and temperature and vegetation cover, see Supporting information 6-10.

Anthoxanthum odoratum newly appeared (joined or replaced *A. alpinum*) at 16 localities (Table 1). Such a change occurred at localities with lower moisture and higher temperature and bryophyte cover (Table 2). Surprisingly, *A. odoratum* was replaced by *A. alpinum* at 8 localities (Table 1). Such a replacement happened at colder and drier localities (Table 2, Fig. 3). Moreover, by comparing the points of their occurrence in the orthophoto maps from 2003 to present, it seems that at one locality, the original grassland with *A. odoratum* was ploughed into the field that allowed *A. alpinum* to grow on its edges. *Anthoxanthum alpinum* replaced *A. odoratum* also at localities were new forest has grown up or densified. The effect of canopy cover change has, however, been non-significant due to a small number of such localities. *Anthoxanthum alpinum* newly appeared (joined or replaced *A. odoratum*) at 18 localities with higher moisture and with typical species of mountain grasslands that also prefer lower temperatures. In total, it disappeared from 34 localities with higher temperature, nutrients level, vegetation, and bryophyte cover (Tables 1 and 2, Fig. 3).

Discussion

Despite the ongoing climate change and general expectations (e.g. Fan 2019; Niskanen 2019), we detected almost no effects of elevation (i.e. environmental factors linked to the elevation) on change in distribution of the two *Anthoxanthum* species. This may indicate that local conditions play more important role in species distribution than large-scale processes. It has been shown that alpine species may be decoupled from macroclimatic processes and find refugia in the microclimatically suitable habitats (Scherrer and Koerner, 2010). In line with this, Pointing et al. (2015), suggested that microclimate is more important driver of distribution of photoautotrophs in the arctic (and mountain) areas compared to macroclimate.

Overall, distribution of our two model species has not changed considerably over time. One could argue that 19 years is a short period of time as extinction or establishment of a new population needs long periods of time. However, *Anthoxanthum* species have high population turnover (Antonovics 1972; Herben et al. 1993) that could shorten these periods. Lesica (2014) showed that dicots restricted to mountain environments declined in their abundance and lowland species shifted upwards during the period of two decades, whereas monocots, especially graminoids, tend to remain stable or increase in their abundance. In contrast, (Hoeglind et al. 2013) pointed that the abundance of grasses will increase with increasing temperature. Cannone and Pignatti (2014), Lesica (2014) also show that the community interactions and interactions between species play a large role in a change of species abundance or distribution. This is in line with our findings.

Temperature anomaly globally increased by 0.5°C from 2000 to 2019 (NOAA 2020) that is the increase that could have the impact on communities already. The impacts are even more likely in the mountains including Krkonoše Mts., where in the period 2001-2016 (compared to 1961-2000, Kliegrová and Kašičková 2019) the mean temperature increased by 0.8-1°C. In contrast to the expectations, the changes in the distribution were not significantly

associated with altitude. Despite this, comparison of the primary data indicates that *A. odoratum* has slightly wider elevational distribution and occurs at higher altitudes than it used to 19-17 years ago. *Anthoxanthum alpinum* disappeared from some of its localities, but the rate of disappearance was comparable to the rate of disappearance of *A. odoratum* and this disappearance does not seem to be associated with expansion of *A. odoratum* and is independent of altitude. Unfortunately, we were unable to detect potential upward shift of *A. alpinum* since we did not have any evidence of the localities where the species were absent in the past and the Krkonoše Mts. have limited altitude (the highest peak reaches 1602 m a. s. l.) so it does not have any space where to expand. In contrast to expectations, *Anthoxanthum alpinum* spread to localities in lower altitude (in 7 cases). Other studies found a similar trend of species descending to lower altitudes (Cannone and Pignatti 2014; Crimmins et al. 2011). Crimmins et al. (2011) suggest that this change may be driven by climatic water balance. It partly corresponds with our results indicating that moisture plays a role in distribution of *Anthoxanthum* species. Specifically, *A. alpinum* newly appeared at the localities with species with higher EIV for moisture.

Generally, change in moisture may be another important factor of climate change affecting species distributions. It has been predicted that climate change will cause higher amounts of precipitation in the mountains (IPCC 2017). Precipitation indeed slightly increased in Krkonoše Mts. over the studied period, especially in the autumn and in the summer, whereas March, April and December were drier in 2001-2016 compared to 1961-2000. The number of days with summer temperatures (maximum daily temperature 25°C or higher) increased by up to 11.1 days in 2001-2016 compared to 1961-2000 in the region (Kliegrová and Kašičková 2019). Even though the precipitation amount is the same or increased in some parts of Krkonoše, higher evaporation causes the dry periods to be longer and more intensive (Flousek 2019). Because *A. odoratum* was found to prefer drier sites,

increased drought may support spread of *A. odoratum*. However, we did not find any effects of moisture on the new appearance of *A. odoratum*, nor did we find any clear increase in abundance in *A. odoratum*. Moisture thus likely did not play a role in changes of *A. odoratum* distribution.

The tree canopy cover in the Krkonoše Mts. is increasing and it is generally known that the tree line is shifting upwards (Treml and Chuman 2015; Franke et al. 2019; Gatti et al. 2019). Despite this, canopy cover increased only at 5% of the localities studied here and the increases were relatively small. Disappearance of any *Anthoxanthum* species was marginally significantly affected by this increase, but the data were too limited to show any differences among the species. The result is in line with the fact that the species are species of open habitats and are not able to persist under denser canopy.

The changes in the distribution of the two species can also be compared to studies looking at changes in distribution of different coexisting cytotypes. In such systems, the minority cytotype may be suppressed due to high loads of pollen of the other cytotype siring the plants (minority cytotype exclusion, Kolář et al. 2017). Such an exclusion can be possibly expected also in our system as the two species flower simultaneously and have the same stature. Due to minority cytotype exclusion principle, it may be hard for one cytotype to invade a stand of the other cytotype. Theoretically, this may stand behind the relative stability of our system. In line with this, Travnicek et al. (2010) did not find significant changes in distribution of two cytotypes of *Vicia cracca* over four decades over central Europe but found that the diploid cytotype disappeared from southern Bohemia. This indicates that even within a single species pair, the changes in distribution of two cytotypes may be region specific.

The intensity of minority cytotype exclusion is also likely to affect the frequency of mixed cytotype populations. Studies dealing with mixed cytotype populations indicate whole gradient of outcomes from systems with hardly any cytotype mixing (e.g. Castro et al. 2012),

to system with high frequency of mixed cytotype populations (Čertner et al. 2017, Čertner et al. 2019). Interestingly, the localities of *Anthoxanthum* species co-occurrences (mixed populations) are common and seem to have fast dynamics. Their total number decreased by more than a half and *A. alpinum* disappeared from 40% of the mixed populations, while *A. odoratum* disappeared from 20% of such populations, but the rate of disappearance of the two species from these mixed populations was non-significantly different. This is in line with the expectations of the minority cytotype exclusion and may depend on the initial frequency of the two cytotypes. Unfortunately, we do not have data on the initial frequency of the two cytotypes and cannot test this assumption.

Generally, the results correspond with the hypothesis that *A. odoratum* occurs more likely at drier, warmer and nutrient richer localities with higher vegetation and bryophyte cover. *Anthoxanthum odoratum* occurrence is also influenced by vegetation composition. It was present within communities that are comparable to those detected by Filipová and Krahulec (2006). In both studies, the co-occurring species are mainly from *Polygono bistortae-Trisetion falvescentis* and *Cynosurion cristati* (Chytrý et al. 2001), such as *Phleum pratense*, *Dactylis glomerata*, *Hypericum maculatum*, *Polygonum bistorta*, *Leontodon hispidus*, *Alchemilla vulgaris* and *Trifolium repens*. In line with this, *A. odoratum* disappeared from localities where these vegetation types were absent, possibly due to changes in vegetation dominating these localities. Additionally, it newly appeared at the localities with higher temperature. When the moisture was lower and bryophyte cover higher, *A. odoratum* appeared at the expense of *A. alpinum*.

Anthoxanthum alpinum occurs more likely at localities with lower nutrient levels, bryophyte and vegetation cover and at colder and wetter localities. It was more likely to disappear from localities with higher productivity (higher bryophyte and vegetation cover) and nutrient levels. The results indicate that *A. alpinum* survive or even spread at localities

with lower vegetation cover. These findings support the findings of Flegrova and Krahulec (1999) that *A. alpinum* performance is limited by high competition in the lower altitudes and the performance of *A. odoratum* is limited by low temperatures at higher altitudes. Occurrence of *A. alpinum* is also marginally affected by species composition. In line with Filipová and Krahulec (2006), it is more likely to occur with species of *Nardion strictae* and *Nardo strictae-Agrostion tenuis* such as *Avenella flexuosa*, *Homogyne alpina*, *Potentilla erecta* or *Galium saxatile*. However, some species from these communities (such as *Polygonum bistorta*, *Nardus stricta*) also co-occur with *A. odoratum*. Species composition also plays a role in a change of studied species distribution. It influenced the disappearance of *A. odoratum* and new appearance of *A. alpinum*.

Unfortunately, the previous study (Filipova and Krahulec 2006) only recorded occurrence of the two species at each locality but did not record any habitat conditions that may have changed over time. Thus, we can only reckon that the certain conditions at the localities have changed and therefore, the species distribution has changed. The reasons why the conditions at the localities have changed may be indeed driven by climate change or too intensive management or its complete absence. Besides, the spread of all species, in this case especially the downward shift of *A. alpinum*, may be enhanced by the transport of hay from the mown grasslands. Similarly, Čertner et al. (2019) found that the distribution of *Tripleurospermum inodorum* cytotypes was influenced by transport and disturbance by cattle. Unfortunately, the exact history of infrastructure, management and increase of tourists is unknown and therefore we are unable to test the influence of such factors on a change of species distribution. The change in a canopy cover is also important, when it increased, both species often disappeared from the locality. It is thus crucial to continue with trying to keep the forests where they are, even though the tree line is moving upwards and the forests are spreading (Franke et al. 2019).

One of the possible caveats of this study could be the fact that we were unable to capture both species at the localities since they have slightly different phenology. This could explain the fact that we observed a lower number of mixed occurrences of both species than the previous study. However, we did not identify the species by morphology, but by flow cytometry using plant leaves (Supporting information 3). In all cases, we also paid attention to collect samples of both flowering and non-flowering plants. We are thus confident that the reduced number of mixed populations is not caused by a sampling bias.

Conclusions

Despite general expectations, the only significant effects of altitude, an expected proxy of macroclimate of the localities, on changes in distribution of *Anthoxanthum odoratum* and *A. alpinum* was loss of *A. odoratum* from lower altitudes likely linked to destruction of the habitats in these locations. In contrast, both appearance and disappearance of both species was significantly related to local habitat conditions. This indicates that it is predominantly the local conditions at the localities rather than the general climate change that drive *A. odoratum* and *A. alpinum* occurrence and migration.

Acknowledgements

We thank F. Krahulec and L. Filipová for the opportunity to consult our research and providing their data, M. Šimák for help with field data collection and Z. Líblová for help with flow cytometry analyses. The Administration of Krkonoše Mountains National Park provided permission to perform the research and provided us with a high-resolution DEM layer for ArcGIS. The study was supported by GAČR 19-00522S.

Declarations

The study was supported by project Grant Agency of Czech Republic 19-00522S.

This study is based on re-visiting the sites with occurrence of two related grass species, the similar research was previously conducted only on re-surveying vegetation plots.

All authors declare no conflict of interest.

All authors agree with this submission.

We followed the ethical standards.

Consent to participate not applicable.

Consent for publication: not applicable.

The data and material are on reasonable request from the corresponding author.

Code availability is on reasonable request from the corresponding author.

References

Alexander, J. M., Diez, J. M., & Levine, J. M. (2015) Novel competitors shape species' responses to climate change. Nature 525/7570: 515-518.

Allison I, Bindoff N. L. & Bindoff R. A. (2009) The Copenhagen Diagnosis 2009: Updating the World on the Latest Climate Science. The University of New South Wales Climate Change Research Centre (CCRC), Sydney, Australia.

Antonovics J. (1972) Population dynamics of the grass *Anthoxanthum odoratum* on a zinc mine. The Journal of Ecology: 351-365.

Bäumelt V., Hůnová I., Kurfürst P. (2017) Atmosférická depozice na území České republiky. In: Grafická ročenka 2017, Portál ČHMÚ. Available on web: http://portal.chmi.cz/files/portal/docs/uoco/isko/grafroc/17groc/gr17cz/Obsah_CZ.htm

Beck P. S. A. & Goetz S. J. (2011) Satellite observations of high northern latitude vegetation productivity changes between 1982 and 2008: ecological variability and regional differences. Environmental Research Letters 6/4: 045501.

Cannone N. & Pignatti S. (2014) Ecological responses of plant species and communities to climate warming: upward shift or range filling processes? Climatic Change 123/2: 201-214.

Castro S, Münzbergová Z, Raabová J, Loureiro J. (2011) Breeding barriers at a diploid-hexaploid contact zone in *Aster amellus*. Evolutionary Ecology, 25: 795-814.

Castro S., Loureiro J., Procházka T. & Münzbergová Z. (2012) Cytotype distribution at a diploid–hexaploid contact zone in *Aster amellus* (*Asteraceae*). Annals of Botany 110/5: 1047-1055.

Čertner M., Fenclová E., Kúr P., Kolář F., Koutecký P., Krahulcová A. & Suda, J. (2017) Evolutionary dynamics of mixed-ploidy populations in an annual herb: dispersal, local persistence and recurrent origins of polyploids. Annals of Botany 120/2: 303-315.

Čertner M., Kúr P., Kolář F. & Suda J. (2019) Climatic conditions and human activities shape diploid–tetraploid coexistence at different spatial scales in the common weed *Tripleurospermum inodorum* (*Asteraceae*). Journal of Biogeography 46/7: 1355-1366.

Conert, H. J. (1985) *Anthoxanthum*. In: HEGI G. (Ed.), Illustrierte Flora von Mitteleuropa, Band 1, Teil 3. Verlag Paul Parey, Berlin–Hamburg.

Chown S. L., Huiskes A. H. L., Gremmen N. J. M., Lee J. E., Terauds A., Crosbie K., Frenot Y., Hughes K. A., Imura S., Kiefer K., Lebouvier M., Raymond B., Tsujimoto M., Ware C., Van de Vijver B. & Bergstrom D. M. (2012) Continent-wide risk assessment for the establishment of nonindigenous species in Antarctica. Proceedings of the National Academy of Sciences of the United States of America 109: 4938-4943.

Chumova Z., Krejcikova J., Mandakova T., Suda J. & Travnicek P. (2015) Evolutionary and Taxonomic Implications of Variation in Nuclear Genome Size: Lesson from the Grass Genus *Anthoxanthum* (*Poaceae*). Plos One 10: e0133748.

Chytrý M., Tomáš K. & Kočí M. (2001) Katalog biotopů České republiky: interpretační příručka k evropským programům Natura 2000 a Smaragd. Praha: Agentura ochrany přírody a krajiny ČR.

Crimmins S. M., Dobrowski S. Z., Greenberg J. A., Abatzoglou J. T. & Mynsberge A. R. (2011) Changes in Climatic Water Balance Drive Downhill Shifts in Plant Species' Optimum Elevations. Science 331: 324-327.

Czortek P., Kapfer J., Delimat A., Eycott A. E., Grytnes J. A., Orczewska A., Ratynska H., Zieba A. & Jaroszewicz B. (2018) Plant species composition shifts in the Tatra Mts as

a response to environmental change: a resurvey study after 90 years. Folia Geobotanica 53: 333-348.

van Dijk, G. (1991) The status of semi-natural grasslands in Europe. The conservation of lowland dry grassland birds in Europe: 15-36.

Drapikowska M., Szkudlarz P., Celka Z. & Jackowiak B. (2012) Morphological variability of the two altitude vicariants, *Anthoxanthum odoratum* L. s.s. and *Anthoxanthum alpinum* in the Babia Gora Massif (Western Carpathian Mountains - Zywiec Beskid, Poland). – Acta Botanica Gallica 159: 433-442.

Easterling D. R., Meehl G. A., Parmesan C., Changnon S. A., Karl T. R. & Mearns L. O. (2000) Climate extremes: Observations, modeling, and impacts. Science 289: 2068-2074.

Eliášová A, Münzbergová Z. (2017) Factors influencing distribution and local coexistence of diploids and tetraploids of *Vicia cracca*: inferences from a common garden experiment. Journal of Plant Research.

Ellenberg H., Weber H. E., Düll R., Wirth V., Werner W. & Paulißen D. (1991): Zeigerwerte von Pflanzen in Mitteleuropa. Scr. Geobot. 18: 1–248.

Environmental Systems Research Institute (ESRI). (2012). ArcGIS Release 10.1. Redlands, CA.

Fan Z. M., Fan B. & Yue T. X. (2019) Terrestrial ecosystem scenarios and their response to climate change in Eurasia. Science China-Earth Sciences 62: 1607-1618.

Felde, V. A., Kapfer, J., & Grytnes, J. A. (2012) Upward shift in elevational plant species ranges in Sikkilsdalen, central Norway. Ecography 35/10: 922-932.

Filipova L, Krahulec F. (2006) The transition zone between *Anthoxanthum alpinum* and *A.odoratum* in the Krkonose Mts. Preslia, 78: 317-330.

Pimentel M, Sahuquillo E. (2008) Relationships between the close *congeners Anthoxanthum odoratum* and *A-alpinum* (*Poaceae*, *Pooideae*) assessed by morphological and molecular methods. Botanical Journal of the Linnean Society, 156: 237-252.

Flegrova M. & Krahulec F. (1999) *Anthoxanthum odoratum* and *A. alpinum*: Life history parameters at two different altitudes. Folia Geobotanica 34: 19-31.

Flousek, J. (2019) Krkonoše a klimatická změna. Fórum ochrany přírody 4/2019: 12-15

Frank E Harrell Jr (2016) Hmisc: Harrell Miscellaneous. R package version 3.17-4. URL [http://CRAN.R-project.org/package=Hmisc]

Franke A. K., Feilhauer H., Brauning A., Rautio P. & Braun M. (2019) Remotely sensed estimation of vegetation shifts in the polar and alpine tree line ecotone in Finnish Lapland during the last three decades. Forest Ecology and Management: 454.

Grime J.P., Hodson J.G. & Hunt R., Comparative Plant Ecology: A Functional Approach to Common British Species, 2nd ed., Chichester: Castlepoint Press 2007.

Gritsch A., Dirnbock T. & Dullinger S. (2016) Recent changes in alpine vegetation differ among plant communities. Journal of Vegetation Science 27: 1177-1186.

Hassan R., Scholes R. & Ash N. (2005) Ecosystems and human well-being: current state and trends. Washington, DC: Island Press, 2005: xxi + 917 p.

Hedberg I. (1986) The genesis of tetraploid *Anthoxanthum odoratum*. Acta Univ. Ups., Symb. Bot. Ups. 27/2: 147–154.

Herben T., Krahulec F., Hadincová V. & Skálová H. (1993) Small-scale variability as a mechanism for large-scale stability in mountain grasslands. Journal of Vegetation Science, 4/2: 163-170.

Hoeglind M., Thorsen S.M. & Semenov M.A. (2013) Assessing uncertainties in impact of climate change on grass production in Northern Europe using ensembles of global climate models. Agricultural and Forest Meteorology 170: 103-113.

Hosek J., Schwarz O. & Svoboda T. (2007) Vysledky desetileteho mereni atmosfericke depozice v Krkonosich [Results of ten-year measurements of atmospheric deposition in the Giant Mountains]. Opera Corcontica 44: 179.

Hulber K., Kuttner M., Moser D., Rabitsch W., Schindler S., Wessely J., Gattringer A., Essl F. & Dullinger S. (2020) Habitat availability disproportionally amplifies climate change risks for lowland compared to alpine species Global Ecology and Conservation: 23.

Intergovernmental Panel on Climate Change (IPCC) (2007) Climate change 2007: the physical science basis summary for policy makers. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC WGI 4th Assessment Report

Kapfer J., Hedl R., Jurasinski G., Kopecky M., Schei F. H. & Grytnes J. A. (2017) Resurveying historical vegetation data-opportunities and challenges. Applied Vegetation Science 20: 164-171.

Kaplan Z., Danihelka J., Chrtek J. jun., Kirschner J., Kubát K., Štech M. & Štěpánek J. (eds) (2019) Klíč ke květeně České republiky [Key to the flora of the Czech Republic]. Ed. 2. 1168 p., Academia, Praha.

Kikvidze, Z., Khetsuriani, L., Kikodze, D., & Callaway, R. M. (2006) Seasonal shifts in competition and facilitation in subalpine plant communities of the central Caucasus. – Journal of Vegetation Science, 17/1: 77-82.

Kliegrová S. & Kašičková L. (2019) Změny teploty vzduchu a úhrnů srážek v období 1961–2016 v Krkonoších. Meteorologické zprávy 72: 88–93.

Kopp C. W. & Cleland E. E. (2014) Shifts in plant species elevational range limits and abundances observed over nearly five decades in a western North America mountain range. Journal of Vegetation Science 25: 135-146.

Kolář, F., Čertner, M., Suda, J., Schönswetter, P. & Husband, B. C. (2017) Mixed-ploidy species: progress and opportunities in polyploid research. Trends in Plant Science, 22/12: 1041-1055.

Krahulec F., Blažková D., Balátová-Tuláčková E., Štursa J., Pecháčková S. & Fabšičová M. (1997) Louky Krkonoš: rostlinná společenstva a jejich dynamika. Opera Corcontica 33: 3–250.

Lenoir, J., Gégout, J. C., Guisan, A., Vittoz, P., Wohlgemuth, T., Zimmermann, N. E., ... & Svenning, J. C. (2010). Going against the flow: potential mechanisms for unexpected downslope range shifts in a warming climate. Ecography, 33/2: 295-303.

Lesica, P. (2014) Arctic-Alpine Plants Decline over Two Decades in Glacier Motional Park, Ariontana, USA. Arctic, Antarctic and Alpine Research 46: 327-332.

Naimi B. (2015) usdm: Uncertainty Analysis for Species Distribution Models. R package version 1.1-15. URL [http://CRAN.R-project.org/package=usdm].

Niskanen A. K. J., Niittynen P., Aalto J., Vare H. & Luoto M. (2019) Lost at high latitudes: Arctic and endemic plants under threat as climate warms. Diversity and Distributions 25: 809-821.

NOAA National Centers for Environmental Information, State of the Climate: Global Climate Report for Annual 2019 (2020). URL [https://www.ncdc.noaa.gov/sotc/global/201913].

Oksanen J., Blanchet G., Kindt R., Legendre P., Minchin P. R., O'Hara R. B., Simpson G. L., Solymos P, Stevens M. H. H. & Wagner H. (2015) Vegan Community Ecology Package. R package version 2.3-2. URL [http://CRAN.R-project.org/package=vegan].

Otto F. (1990) DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. Methods in cell biology 33: 105-110.

Petit C, Lesbros P, Ge X, Thompson J. D. (2007) Variation in flowering phenology and selfing rate across a contact zone between diploid and tetraploid *Arrhenatherum elatius* (*Poaceae*). Heredity, 79: 31–40.

Petitpierre B., McDougall K., Seipel T., Broennimann O., Guisan A. & Kueffer C. (2016) Will climate change increase the risk of plant invasions into mountains? Ecological Applications 26: 530-544.

Pignatti S. (1993) Impact of tourism on the mountain landscape of central Italy. Landscape and Urban Planning 24: 49-53.

Pimentel M. & Sahuquillo E. (2008) Relationships between the close congeners *Anthoxanthum odoratum* and *A. alpinum* (*Poaceae*, *Pooideae*) assessed by morphological and molecular methods. Botanical Journal of the Linnean Society 156: 237-252.

Pointing S. B., Buedel B., Convey P., Gillman L., Koerner C., Leuzinger S., & Vincent W. F. (2015) Biogeography of photoautotrophs in the high polar biome. Frontiers in plant science 6: 692.

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL [http://www.R-project.org/].

Rieseberg L. H. & Willis J. H. (2007) Plant speciation. Science, 317: 910–4.

Rumpf S. B., Hulber K., Klonner G., Moser D., Schutz M., Wessely J., Willner W., Zimmermann N. E., Dullinger S. (2018) Range dynamics of mountain plants decrease with elevation. Proceedings of the National Academy of Sciences of the United States of America 115: 1848-1853.

Rumpf S. B., Hulber K., Wessely J., Willner W., Moser D., Gattringer A., Klonner G., Zimmermann N. E., Dullinger S. (2019a) Extinction debts and colonization credits of non-forest plants in the European Alps. Nature Communications: 10.

Rumpf S. B., Hulber K., Zimmermann N. E., Dullinger S. (2019b) Elevational rear edges shifted at least as much as leading edges over the last century. Global Ecology and Biogeography 28: 533-543.

Scherrer D. & Koerner C. (2010). Infra-red thermometry of alpine landscapes challenges climatic warming projections. Global Change Biology, 16/9: 2602-2613.

Stancic Z. (2005) *Anthoxanthum alpinum* (*Poaceae Aveneae*) in the mountains from Croatia to Macedonia. Nat. Croat. 14/2: 87-9.

Steinbauer M. J., Grytnes, J. A., Jurasinski, G. et al. (2018). Accelerated increase in plant species richness on mountain summits is linked to warming. Nature 55: 231–234.

Stursa J. (2002) Impacts of Tourism Load on the Mountain Environment (A Case Study of the Krkonoše Mountains National Park-the Czech Republic). Proceedings International Conference on Monitoring and Management of Visitor Flows in Recreational and Protected Areas: 364-370.

Te Beest M., Le Roux J. J., Richardson D. M., Brysting A. K., Suda J., Kubešová M. & Pyšek, P. (2012) The more the better? The role of polyploidy in facilitating plant invasions. Annals of botany 109/1: 19-45.

Thomas C. D., Cameron A., Green R. E., Bakkenes M., Beaumont L. J., Collingham Y. C., Erasmus B. F. N., de Siqueira M. F., Grainger A., Hannah L., Hughes L., Huntley B., van Jaarsveld A. S., Midgley G. F., Miles L., Ortega-Huerta M. A., Peterson A. T., Phillips O. L. & Williams S. E. (2004) Extinction risk from climate change. Nature 427: 145-148.

Thuiller W., Lavorel S., Araujo M. B., Sykes M. T. & Prentice I. C. (2005) Climate change threats to plant diversity in Europe. Proceedings of the National Academy of Sciences of the United States of America 102: 8245-8250.

Trávníček P., Dočkalová Z., Rosenbaumová R., Kubátová B., Szeląg Z. & Chrtek J. (2011) Bridging global and microregional scales: ploidy distribution in *Pilosella echioides* (*Asteraceae*) in Central Europe. Ann Bot., 107: 443–54.

Trávníček P., Eliášová A. & Suda J. (2010) The distribution of cytotypes of *Vicia cracca* in central Europe: the changes that have occurred over the last four decades. Preslia, 82/1: 149-163.

Treml V. & Chuman, T. (2015) Ecotonal dynamics of the altitudinal forest limit are affected by terrain and vegetation structure variables: an example from the Sudetes Mountains in Central Europe. Arctic, Antarctic, and Alpine Research, 47/1: 133-146.

Urban G., Richterová D., Kliegrová S., Zusková I. & Pawliczek P. (2018) Winter severity and snowiness and their multiannual variability in the Karkonosze Mountains and Jizera Mountains. Theor. Appl. Climatology 134: 221–240.

Urban G., Richterová D., Kliegrová S. & Zusková I. (2019) Durability of snow cover and its long-term variability in the Western Sudetes Mountains. Theor. Appl. Climatology 137: 2681–2695.

Walther G. R., Beissner S. & Burga C. A. (2005) Trends in the upward shift of alpine plants. – Journal of Vegetation Science 16: 541-548.

Wang L., Wang W. J., Wu Z. F., Du H. B., Zong S. W. & Ma S. (2019) Potential distribution shifts of plant species under climate change in Changbai Mountains, China. Forests 10/6: 498.

WEB 1 [https://www.krnap.cz/aktuality/tz-v-roce-2018-mely-krkonose-o-pul-milionu-vic-navstev/].

Zimmerman Jr, J. L. & Shallenberger, J. P. (2016) GIS Topographic Wetness Index (TWI) Exercise Steps. Harrisburg, - Susquehanna River Basin Commission 16 p.

1 Zuur A. F., Ieno E. N. & Elphick, C. S. (2010) A protocol for data exploration to avoid
2 common statistical problems. Methods in ecology and evolution, 1/1: 3-14.

Tables

Table 1. A total number of localities with the occurrence of studied species, and their mixture, rows represent the number of localities in 2019 and column represent the number of localities in 2000-2002. A. odo: *Anthoxanthum odoratum*, A. alp: *Anthoxanthum alpinum*, mix: co-occurrence of both species, ABS: both species were absent. Grey fields indicate the localities where the species occurrence did not change.

		Past			
		A. odo	A. alp	mix	total
Present	A. odo	43	10	24	77
	A. alp	8	49	12	69
	mix	11	6	9	26
	ABS	27	28	16	71
	total	83	93	61	243

Table 2. The impact of chosen variables on the studied species occurrence and whether they disappeared from the localities or appeared at the localities. These results are from single tests testing each dependent variable at each predictor separately combined with results from the analyses of the model with the best fit. The arrows or n.s. indicate significance of each variable from the single tests, whereas S indicates a variable that was significant in a model with the best fit. A.a.: *A. alpinum*, A.o.: *A. odoratum*, Veg cover: vegetation cover, Bryophytes: bryophytes cover, nutrients, moisture and temperature are obtained from Ellenberg indicator values (EIV), DCA2: site scores of the second DCA axis (Fig. 1). Arrows indicate whether higher (↑) or lower (↓) amount of the certain variable influenced the occurrence/change in the occurrence of the species. The number of arrows indicates the significance, 3 arrows: p <0.001, 2 arrows: p <0.01, 1 arrow (black): <0.05, 1 arrow (grey): 0.05 0.1., *ns*. non-significant. Only variables with significant effect for at least one dependent variable are shown. Detailed results of these tests are shown in the Supporting information 4 and 5.

	Appeared		**Disappeared**		**Occurrence**	
	A. alpinum	***A. odoratum***	***A. alpinum***	***A. odoratum***	***A. alpinum***	***A. odoratum***
Veg cover	*ns.*	↑	↑↑ S	*ns.*	↓↓ S	↑↑ S
Bryophytes	*ns.*	↑↑ S	↑	*ns.*	↓↓ S	↑↑ S
Nutrients	*ns.*	↑	↑ S	*ns.*	↓ S	↑↑↑ S
Moisture	↑ S	*ns.*	*ns.*	*ns.* S	↑ S	↓ S
Temperature	↓ S	↑ S	↑↑↑ S	*ns.* S	↓↓↓ S	↑↑↑ S
DCA2	*ns.*	*ns.*	*ns.* S	↓ S	↓ S	↑ S
Altitude	*ns.*	*ns.*	*ns.*	↓↓↓	↑↑	↓↓

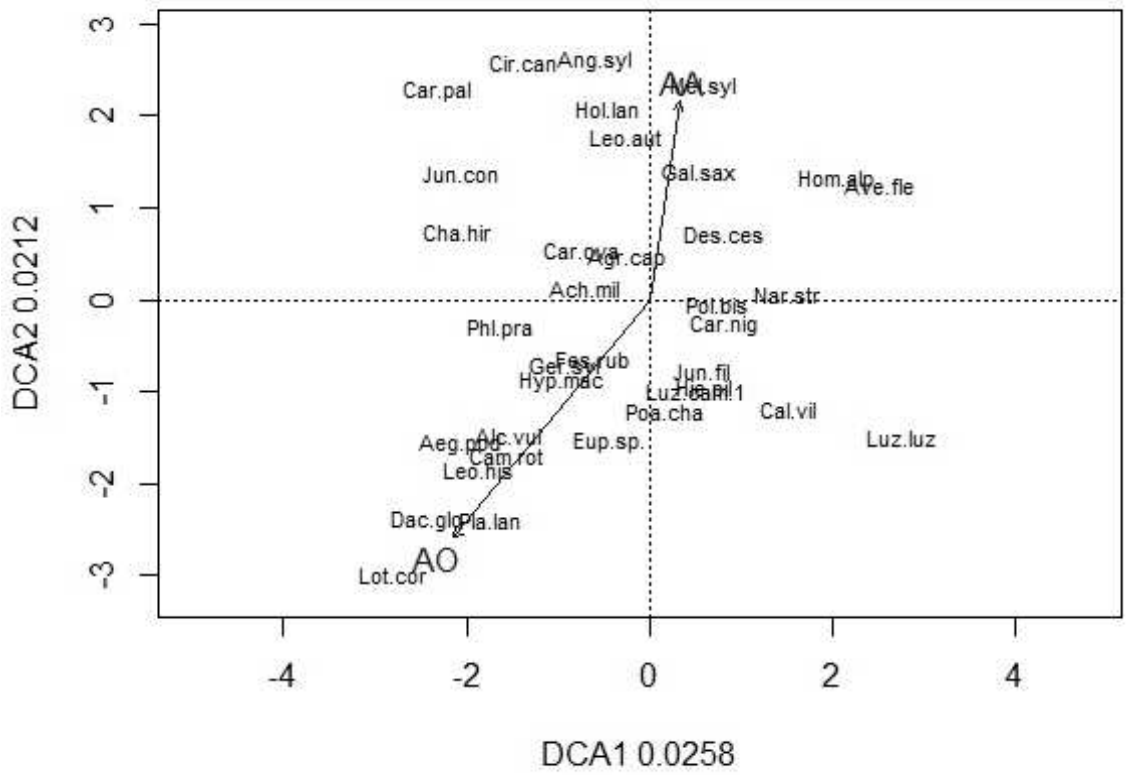
Figure legends

Fig. 1. The DCA analysis of a species composition in the plots of the species that were present in more than 5 relevés. DCA1 explains 2.58% of variability, while DCA2 explains 2.12% of variability. AA and AO were added as supplementary variables. Positions of the samples on the two DCA axes were used as predictors of species distribution and its changes. **AO**: *A. odoratum*, **AA**: *A. alpinum*, **Hol.lan**: *Holcus lanatus*, **Leo.aut**: *Leontodon autumnalis*, Jun.con: *Juncus conglomeratus*, Gal.sax*: Galium saxatile*, **Hom.alp**: *Homogyne alpina*, **Ave.fle**: *Avenella flexuosa*, **Cha.hir**: *Chaerophyllum hirsutum*, Car.pal: *Carex pallescens*, **Car.ova**: *Carex ovalis*, **Agr.cap**: *Agrostis capillaris*, **Ach.mil**: *Achillea millefolium* agg., **Nar.str**: *Nardus stricta*, **Pol.bis**: *Polygonum bistorta*, **Phl.pra**: *Phleum pratense*, **Fes.rub**: *Festuca rubra*, **Ger.syl**: *Geranium sylvaticum*, **Jun.fil**: *Juncus filiformis*, **Hyp.mac**: *Hypericum maculatum*, **Hie.pil**: *Hieracium pilosella*, **Luz.cam**: *Luzula campestris*, **Cal.vil**: *Calamagrostis villosa*, Poa.cha: *Poa chaixii*, Alc.vul: *Alchemilla vulgaris* agg., Eup.sp.: *Euphrasia* sp., **Luz.luz**: *Luzula luzuloides*, **Cir.can**: *Cirsium canum*, **Ang.syl**: *Angelica sylvestris*.

Fig. 2. Boxplot showing altitudinal distribution of the studied species and their mixture in the past (2000-2002) and at present (2019) along altitudinal gradients in the Krkonoše Mts.

Fig. 3. Boxplots of three chosen variables (Ellenberg indicator values for nutrients, temperature, moisture) and how they differ among the species and their absence (0) and presence (1). *: $p<0.05$, ***: $p<0.001$ indicate significant differences between localities with species presence and absence for each species separately.

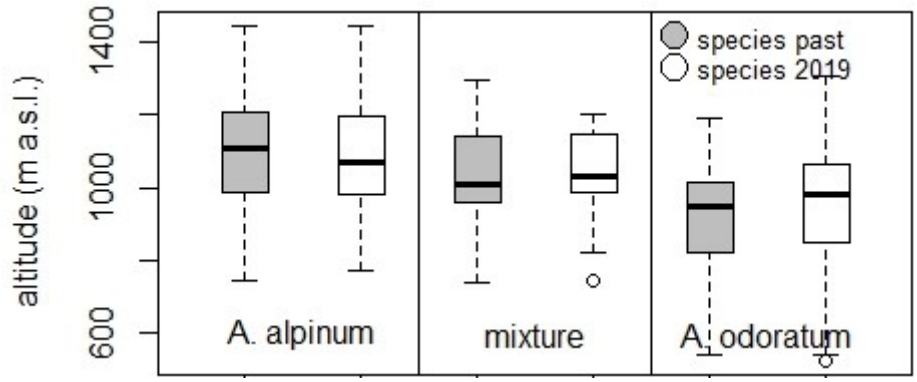
1 **Figures**



2

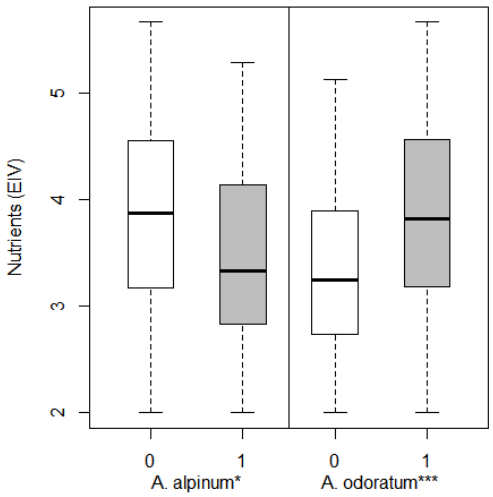
3 Fig. 1

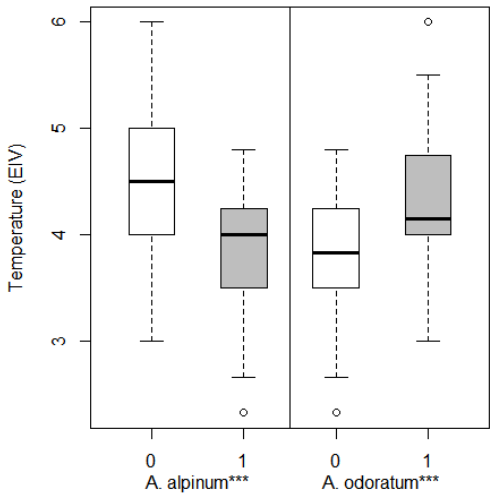
4

1 
altitude (m a.s.l.)
600
1000
1400
A. alpinum
mixture
A. odoratum
species past
species 2019

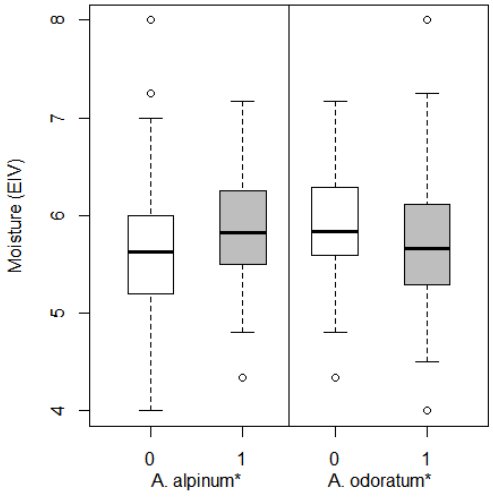
2 Fig. 2

3


Nutrients (EIV)
2
3
4
5
0
1
0
1
A. alpinum*
A. odoratum***


Temperature (EIV)
3
4
5
6
0
1
0
1
A. alpinum***
A. odoratum***

1


Moisture (EIV)
4
5
6
7
8
0
1
0
1
A. alpinum*
A. odoratum*

2

3 Fig. 3

Figures

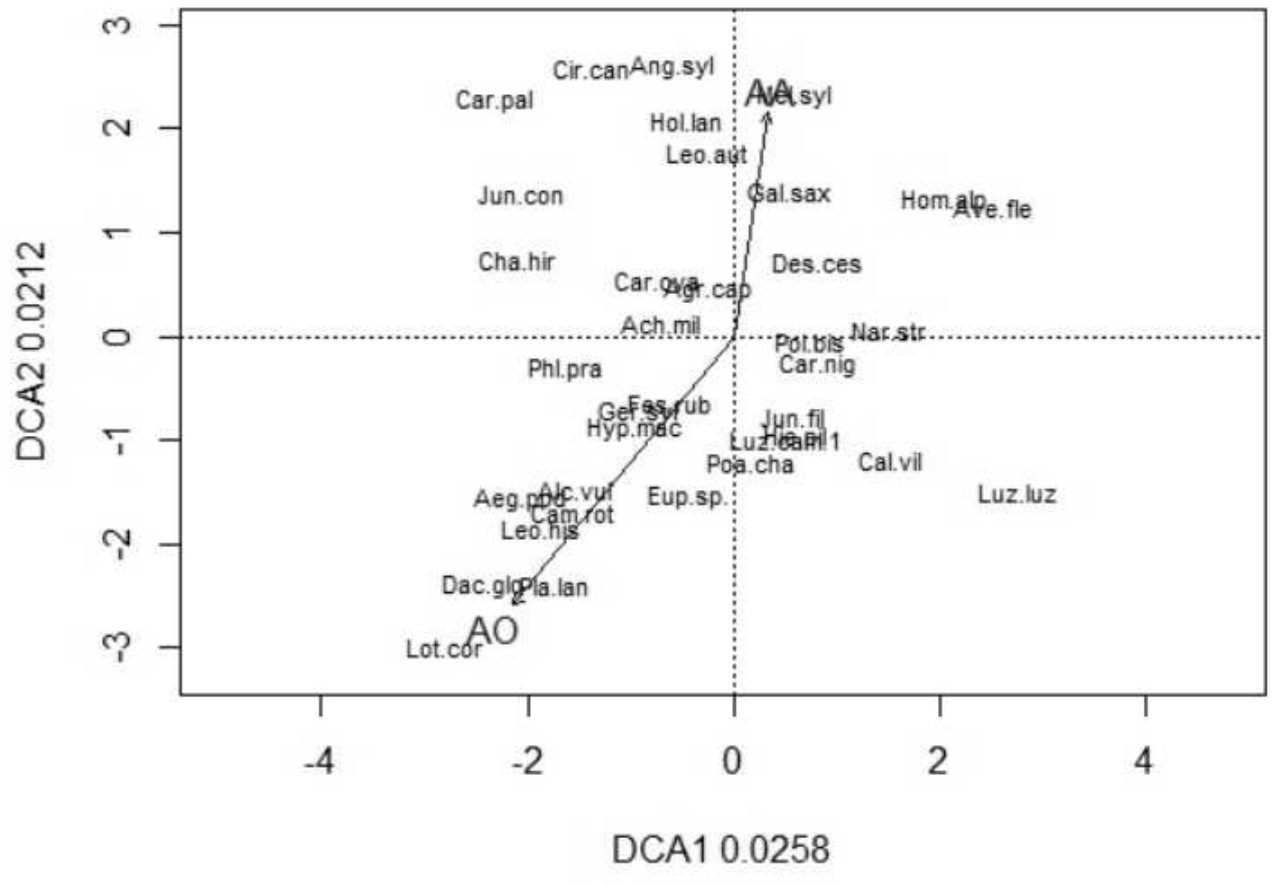


Fig. 1

Figure 1

The DCA analysis of a species composition in the plots of the species that were present in more than 5 relevés. DCA1 explains 2.58% of variability, while DCA2 explains 2.12% of variability. AA and AO were added as supplementary variables. Positions of the samples on the two DCA axes were used as predictors of species distribution and its changes. AO: A. odoratum, AA: A. alpinum, Hol.lan: Holcus lanatus, Leo.aut: Leontodon autumnalis, Jun.con: Juncus conglomeratus, Gal.sax: Galium saxatile, Hom.alp: Homogyne alpina, Ave.fle: Avenella flexuosa, Cha.hir: Chaerophyllum hirsutum, Car.pal: Carex pallescens, Car.ova: Carex ovalis, Agr.cap: Agrostis capillaris, Ach.mil: Achillea millefolium agg., Nar.str: Nardus stricta, Pol.bis: Polygonum bistorta, Phl.pra: Phleum pratense, Fes.rub: Festuca rubra, Ger.syl: Geranium sylvaticum, Jun.fil: Juncus filiformis, Hyp.mac: Hypericum maculatum, Hie.pil: Hieracium pilosella, Luz.cam: Luzula campestris, Cal.vil: Calamagrostis villosa, Poa.cha: Poa chaixii, Alc.vul: Alchemilla vulgaris agg., Eup.sp.: Euphrasia sp., Luz.luz: Luzula luzuloides, Cir.can: Cirsium canum, Ang.syl: Angelica sylvestris.

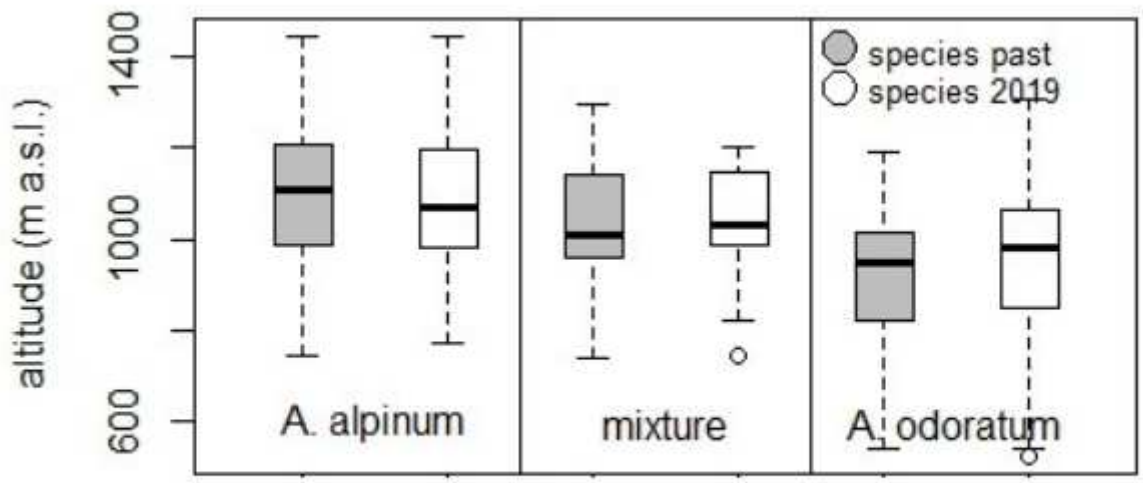


Fig. 2

Figure 2

Boxplot showing altitudinal distribution of the studied species and their mixture in the past (2000-2002) and at present (2019) along altitudinal gradients in the Krkonoše Mts.

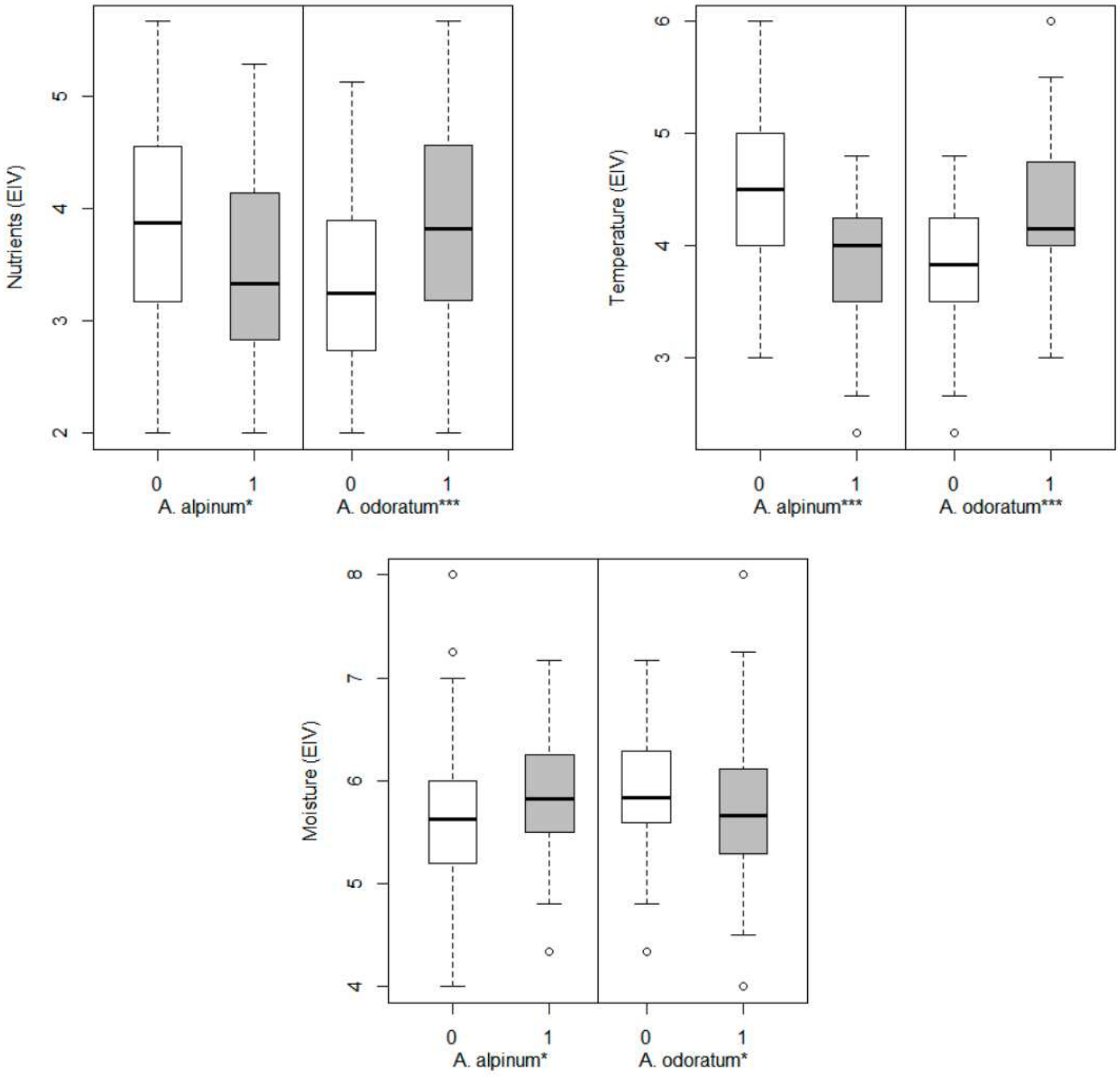


Figure 3

Boxplots of three chosen variables (Ellenberg indicator values for nutrients, temperature, moisture) and how they differ among the species and their absence (0) and presence (1). *: p<0.05, ***: p<0.001 indicate significant differences between localities with species presence and absence for each species separately

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supportinginfooecologia.pdf

- data.xlsx