

# The relationship between abiotic conditions, habitat structure, reproductive success and the population size of eight endangered orchids in Switzerland

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# 1 Abstract

To understand the ongoing species loss and improve species conservation, many studies have focused on the drivers land-use intensification and climate change. To protect species of the family Orchidaceae, a plant family, which is amongst those with the highest number of endangered species, has many specialists, a complex life history and many species that are considered umbrella species, better understanding on the importance not only of land use and climatic conditions but also of other abiotic factors, habitat heterogeneity and reproduction potential is crucial to improve the efficiency of any conservation action.

We studied the importance of three potential drivers of population decline: abiotic conditions (nutrients, light, temperature, humidity, pH), habitat heterogeneity and reproductive success, for eight red listed orchid species in Switzerland in two habitats: dry grassland (*Mesobromion*) and open forest (*Quercion pubescenti-petraeae* and *Cephalanthero-fagenion*).

We found that in general, abiotic conditions, modelled with community weighted means of Landolt indicator values, are important drivers of the population size. In particular, we found that a temperature mismatch decreased the population size in both habitats, that a nutrient excess decreased the population size of our four grassland species and that forest species needed optimal light conditions to establish large populations. Moreover, we found larger populations in sites with higher habitat heterogeneity (modelled with beta-diversity) compared with sites with low habitat heterogeneity and that larger populations had higher germination rates and percentage of fruits than small populations. We therefore conclude on the importance of paying particular attention to the amount of nutrient in grasslands and light conditions in forests when protecting orchid populations or planning reintroductions. Additionally, we stress the importance of considering the reproductive success especially of small populations and its important role in the survival of a population.

## 2 Introduction

Nowadays there is a large agreement that biodiversity loss is happening on a global scale (IPBES, 2019). Therefore, one of the major challenges of the present is to protect threatened species from extinction (Butchart et al, 2010). There are many different drivers of species decline and they all need the right attention (IPBES, 2019). In the last couple years, climate change has received a lot of consideration for its detrimental effects on biodiversity (Parmesan, 2006). But other drivers, like habitat change, loss and fragmentation are also major drivers of species decline on their own (Malvasi et al, 2018) or in combination with climate warming (Travis, 2003). To prevent species loss at local scales, different potential drivers must be studied to be able to understand the precise mechanisms behind their decline, which can be very specific for each species.

To encourage and give guidelines for organized protection, each country has a Red List of species, which are or will be threatened by extinction in the future and a priority list of species for whose nations should make their main focus (www.iucn.org). Most species in the family Orchidaceae show a general decline worldwide (Wraith, 2019); in Switzerland all species are protected (Lauber et al, 2014) and the majority are red-listed and with national priority (Bornand et al, 2016; Pearson et al, 2010). Orchids have a very complex habitat specialization and life history and therefore have adapted to very narrow ecological niches and for this reason are very sensitive to any habitat change (Keppel et al, 2016). Due to this, they are considered umbrella species whose decline can indicate changes in the habitat (Nicolè et al, 2005) and potential loss of other species of this habitat. It is therefore important to put a large focus on the protection of orchid species. For these reasons, there are many ongoing monitoring projects in Switzerland providing important information like location and size of the populations. All of this makes orchids particularly important and very interesting study species.

For orchids, and many other threatened species, there are several aspects that are potentially important for a population's fitness and size. Due to the often narrow niche of orchids already, small changes in abiotic conditions can strongly influence the fitness of the populations (Keppel et al, 2016). To detect changes in these conditions, Landolt indicator values are an important tool widely used to calculate a reliable proxy of abiotic conditions in ecological studies (Diekmann, 2003). By calculating a weighted mean of the Landolt indicator values for nutrients, soil moisture, light, pH and temperature it is possible to receive information about the abiotic conditions for the whole community and to calculate any deviation from the ideal abiotic conditions for the study species. Further, based on the heterogeneity-diversity relationship, a diversified habitat structure increases the probability for certain species to find the necessary conditions for their survival (Lundholm, 2009). A commonly used proxy of habitat heterogeneity is beta-diversity, which mirrors underlying variation of a habitat in the changes of species composition (Wilson et al, 1984).

Another important aspect of species decline is the reproductive potential of a population. It is well demonstrated that inbreeding depression and pollination limitation can have detrimental effects on small populations and even drive them to local extinction (Lennartson, 2002). When comparing drivers of population decline or extinction, these two factors, measured by the percentage of flowers pollinated and germination rates, as well as other factors mentioned previously should be considered. To understand the mechanisms behind the decline of some orchids in Switzerland we chose eight red-listed orchid species (Table 1); five of them are also on the list of priority species. To get a more general overview of the drivers for population decline of different orchid species, we further chose orchids from two different habitats. Four species are found in dry grasslands (*Mesobromion*) and four in open forests (*Quercion pubescenti-petraeae* or *Cephalanthero-fagenion*). This allowed us to gain broader insight and therefore more general information for the future conservation of orchids in Switzerland.

More precisely, this study focused on the following questions:

- Can changes in the abiotic conditions of the habitat reduce the population size of eight endangered orchid species in Switzerland?
- How important are the structure and the heterogeneity of the habitat for the population fitness?
- How does the population size relate to reproductive success?

### 3 Methods and Materials

#### 3.1 Species and Site selection

The study was conducted on eight red-listed orchid species in Switzerland, five of them with a national priority (Bornand et al, 2016; Pearson et al, 2010). Four species grow in grassland (*Mesobromion*) and four in forest habitats (*Cephalanthero-Fagenion* or *Quercion pubescenti-petraeae*) (Delarze et al, 2008). For each species, we selected ten populations, with the exception of *O. apifera*, *O. pallens* and *O. purpurea*, which are represented with eight, nine and nine populations respectively (Table 1). This resulted in a total of 76 replications, 38 for grasslands and 38 for forests. Pseudoreplication has been avoided by considering only one study species in each site. The selections were made in order to create a gradient in population size for each species. Information about population size and sites was based on expert knowledge (personal communication with Christian Gnagi, coordinator of the implementations of the masterplan orchid conservation for the canton of Bern, Switzerland) and the national data and information center on Swiss flora (infoflora). Most populations are located in the canton of Bern (55) and some in the cantons of Neuchatel (6), Vaud (4), Zurich (4), Schaffhausen (4), Aargau (2), and Ticino (Figure S1).

Table 1: *Summary of the species and their populations included in the study.*

Species	Habitat	Priority	Nr. Pop	Pop. Size
<i>Aceras anthropophorum</i>	<i>Mesobromion</i>	-	10	4-201
<i>Ophrys apifera</i>	<i>Mesobromion</i>	4	8	3-18
<i>Ophrys holosericea</i>	<i>Mesobromion</i>	4	10	3-89
<i>Orchis militaris</i>	<i>Mesobromion</i>	-	10	3-127
<i>Cypripedium calceolus</i>	<i>Cephalanthero-Fagenion</i>	4	10	11-500
<i>Limodorum abortivum</i>	<i>Quercion pubescenti-petraeae</i>	-	10	1-67
<i>Orchis pallens</i>	<i>Cephalanthero-Fagenion</i>	4	9	3-19
<i>Orchis purpurea</i>	<i>Quercion pubescenti-petraeae</i>	4	9	11-100

#### 3.2 Collection of field data

We visited each site at least once between May and July 2019. We counted the number of individuals present in the site (including non-flowering plants) and we made four vegetation records of 1x1 m for each population. We placed the plots at spots where the study species was present and we chose them in a way that the whole area of the population was covered. For each plot, we identified all species present and estimated their abundance (in %). We also estimated the abundance (%) of bryophytes, lichens, bare ground, stones, deadwood, litter and total vegetation cover (%). Additionally, we estimated the proportion of flowers producing fruits for four individuals of the study species per site (percentage of fruits) and collected one capsule from each for a germination test. In grasslands, we also measured the vegetation height in each plot (average of four random spots in the plot). In forests, we set a 10x10 m plot in the center of the site. In this plot we counted the number of trees and estimated canopy cover, total cover of understory vegetation and litter (in %).

We are aware that using the number of orchids counted may raise some questions, since the number of flowering orchids in one population can oscillate greatly between years. However, we think that our counts are reliable, since we made the effort to look actively for rosettes. Moreover the number of flowering individuals is proportional to the size of the population and despite oscillations in flowering individuals, big populations stay more or less big over time and vice versa (Wartmann, 2006), meaning that our overall gradient of population size would most likely still be the same in another year even though it might shift as a whole.

### 3.3 Abiotic conditions

To model the abiotic conditions, which our populations are experiencing, we used Landolt indicator values (Landolt, 2010), since they are recognized as a valid method for this purpose (Diekmann, 2003). For each site, we calculated community weighted means for each of the six indicators (nutrients, light, temperature, pH, humidity and continentality) using the program Vegedaz. We then used the difference between the literature value of the species (Table 2, Lauber et al, 2014) and the calculated value in each site as an explanatory variable ( $\text{deltaL} = L_{\text{literature}} - L_{\text{site}}$ ).

As a support for the Landolt indicator values, we used other variables to create more reliable results whenever possible. To model light conditions in the forest, canopy cover on the 10x10m plot (correlates with light values:  $r = -0.86$ ) was used as an explanatory variable in a separate model. To model nutrient conditions in the grassland, vegetation height (correlates with nutrients:  $r = 0.57$ ) was as an additional explanatory variable.

Table 2: Literature values of the Landolt indicator values for the eight study species

Species	Humidity	pH	Nutrients	Light	Temperature	Continentality
<i>Aceras anthropophorum</i>	2.5	4	2	4	4.5	2
<i>Ophrys apifera</i>	2.5	5	2	3	4.5	2
<i>Ophrys holosericea</i>	2.5	5	2	4	4.5	3
<i>Orchis militaris</i>	2.5	4	2	4	3.5	4
<i>Cypripedium calceolus</i>	3	4	2	3	3.5	3
<i>Limodorum abortivum</i>	2	4	2	3	4.5	3
<i>Orchis pallens</i>	3	4	3	3	4.5	4
<i>Orchis purpurea</i>	3.5	4	3	3	4.5	2

#### 3.3.1 Habitat heterogeneity

As a proxy for habitat heterogeneity we used beta-diversity, since previous studies have shown that this measure, intended as "the variability in species composition among sampling units for a given area" (Heino et al, 2015), is linked to the heterogeneity in the habitat (Anderson et al, 2006). To calculate it, we divided the total number of species present in each site (gamma-diversity) by the mean number of species in each plot (alpha-diversity). Again, for these calculations, the program Vegedaz was used.

### 3.4 Reproduction

With the collected seeds we performed an in-vitro germination test, following a specific protocol for asymbiotic germination (Figure S2). After six weeks from the start of the test, we estimated germination rates using the first 50 seeds counted in each capsule (under binocular, Figure S3). We used germination rate, percentage of fruits and "reproduction potential" (multiplication between germination rate and percentage of fruits) as response variables in the analysis. We excluded *C. calceolus* from all the analyses which include the variable fruits, since it builds either zero, one or rarely two capsules and therefore cannot be used as a continuous variable.

### 3.5 Data analysis

The statistical analysis was performed with Rstudio 3.6.1. As the first step of the analysis, we built a Correlation Matrix, with both Spearman's and Pearson's correlation coefficients, to check for collinearity between the variables. We considered two variables to be co-linear if their correlation coefficient exceeded 0.7 (Weaver, 2018). We never used two co-linear variables in the same model. To model abiotic conditions we used the natural logarithm of the population size (transformed to match a normal distribution) as the explanatory variable and the Landolt indicator values as response variables. Each indicator was modelled individually and we further used the sum of the absolute difference for each

Landolt indicator value (including the ones excluded from the individual analysis) as an explanatory variable in a subsequent model (sum deltaL). The same model was used for heterogeneity, with beta-diversity as the explanatory variable and the natural logarithm of the population size as the response variable. To model reproduction we used the natural logarithm of the population size as an explanatory variable (to keep the results consistent and comparable throughout the whole manuscript) and the indicators of reproduction (germination rate, fruits and reproduction potential) as response variables, each analyzed in a separate model.

For each of the considered explanatory variables, a linear model was built for each species individually, using the function `lm` (R 3.6.1) and an overall linear mixed-effect model with species as random factor was built using the function `lme` (from the package `nlme`, version 3.1-142). Linear and quadratic relationships were tested for each variable and the best relationship was selected based on the significance of the quadratic term. In the analysis, the two habitats *Cephalanthero-Fagenion* and *Quercion pubescenti-petraeae* were pooled in one habitat type called forest. To test for different relationships in the two habitats, a model including the habitat as a categorical and explanatory variable was built for each variable. If the interaction between the explanatory variables resulted to be significant, separate models were built for each habitat; this was only the case for the model including the Landolt indicator value of nutrients (Figure S5, C). Based on the correlation matrix, the modeling and the calculation of the AICs (Bozdogan et al, 1987) we excluded the following variables from the analysis: bryophytes, bare ground, deadwood, lichens, stones, litter and vegetation cover. Moreover, we performed a variance analysis for the Landolt indicator values with ANOVA. Based on its results (Figure S4) we did not include in the results the indicators explaining less than 10% of the variance in the response variable (pH and humidity). We also excluded continentality since it is a parameter, which summarizes all the other Landolt indicator values.

## 4 Results

In the 76 sites visited during the fieldwork, the orchid populations found ranged from one up to 500 individuals, with a mean population size of 36 individuals (41 in forests and 32 in grasslands) and a standard deviation of 65. The biggest population was a population of *C. calceolus* in the Gasterental (canton of Bern), where 500 individuals were counted. The vegetation records had a mean alpha diversity of 14 species (11 in forests and 17 in grasslands) and a mean gamma diversity of 27 species per site (22 in forests and 31 in grasslands).

### 4.1 Abiotic conditions of the sites

In general we found that the orchid populations frequently grow in habitats outside their optimal abiotic niche (Figure 1). In particular, all meadow sites have excess nutrient availability, many orchid populations in forests (28/38) do not receive enough light and almost all populations (grasslands and forests) are experiencing too low temperatures. The pH is too low in many cases, but since it correlates with nutrients ( $r = -0.64$ ) this result is probably a consequence of the nutrient content on the site. Humidity does not deviate strongly from the optimal value, with most of the sites (69/76) located between 0.5 and -0.5.

Summing together the differences of all Landolt indicator values between measured and optimal conditions (sum deltaL) we find that for some species the habitat mismatch is larger than for others (Figure 2A). For example, while *O. apifera* has a mean sum deltaL of 5.4, *O. militaris* only has a mean sum deltaL of 1.6. Even though the individual species do not show a significant

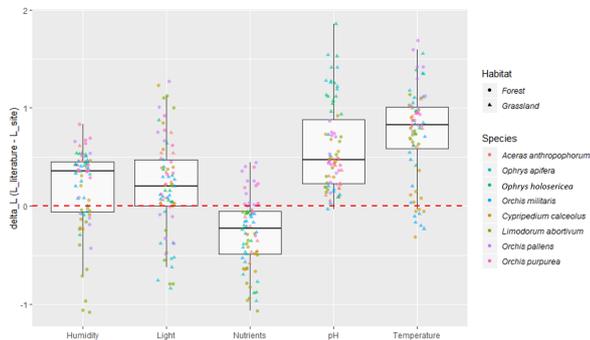


Figure 1: *Differences between measured and optimal conditions for each Landolt indicator value and population. The red horizontal line indicates the optimal conditions, the different colors indicate the different species and the different symbols indicate the two habitats, see legend.*

change in population size with sum deltaL, they show a relatively consistent trend: 5 out of 8 species decrease in population size when the mismatch of the abiotic condition increases. If we analyze all species together, we find a significant decline in population size when the sum deltaL increases ( $p = 0.002$ , slope = - 0.4), indicating a problem of statistical power for the analysis of individual species.

While all grasslands show an excess of nutrients, forests are more centered around optimal values for the respective orchid species (Figure 2B). We find that the population size of orchids in forests and in grasslands tends to be largest if the nutrient conditions approach the optimal values of the orchids. Two species (*C. calceolus* and *O. militaris*) show significant relationships, with an increase in population size as the nutrient value approaches the optimum. The separate analysis (Figure S5, A) for the two habitats shows a positive relationship between nutrients and population size in grassland ( $p = 0.0003$ , slope = 2.395), meaning that we found bigger populations in grassland sites with fewer nutrients. In the forest we found the biggest populations around zero, meaning when the difference between optimal and measured nutrient availability was smallest (quadratic relationship,  $p = 0.004$ ). As higher nutrient availability increases plant growth, we also tested the influence of the vegetation height on population size in grasslands. We found, consistent to the analysis of the Landolt nutrient values, that orchid populations tended to be smaller in grasslands with higher vegetation ( $p = 0.046$ , slope = -0.056, Figure S6, B).

Across all species we find that populations seem to be smaller with declining light availability (Figure 2C). This general trend is however not significant over all species and for the individual species it is significant only for *O. holosericea* ( $p = 0.03$ , slope = -2.54). If we use canopy cover as a proxy for light availability for the forest plots (Figure 2D) we find that orchid populations are largest in forests with roughly 50% canopy cover above the plots and smaller if the canopy cover deviated from 50% ( $p$

= 0.023).

For the Landolt indicator value of temperature we find that the majority of the populations (67/79) are situated in sites with too low temperature (Figure 2E). In general we find that population size is smaller at lower temperatures ( $p = 0.038$ ). If analyzed individually, only population sizes of *O. pallens* and *C. calceolus* show a significant relationship with temperature. While *O. pallens* follows the general pattern ( $p = 0.012$ , slope = -2.5495), *C. calceolus* shows an opposite behavior, with increasing population size when temperature is lower ( $p = 0.001$ , slope = 3.083).

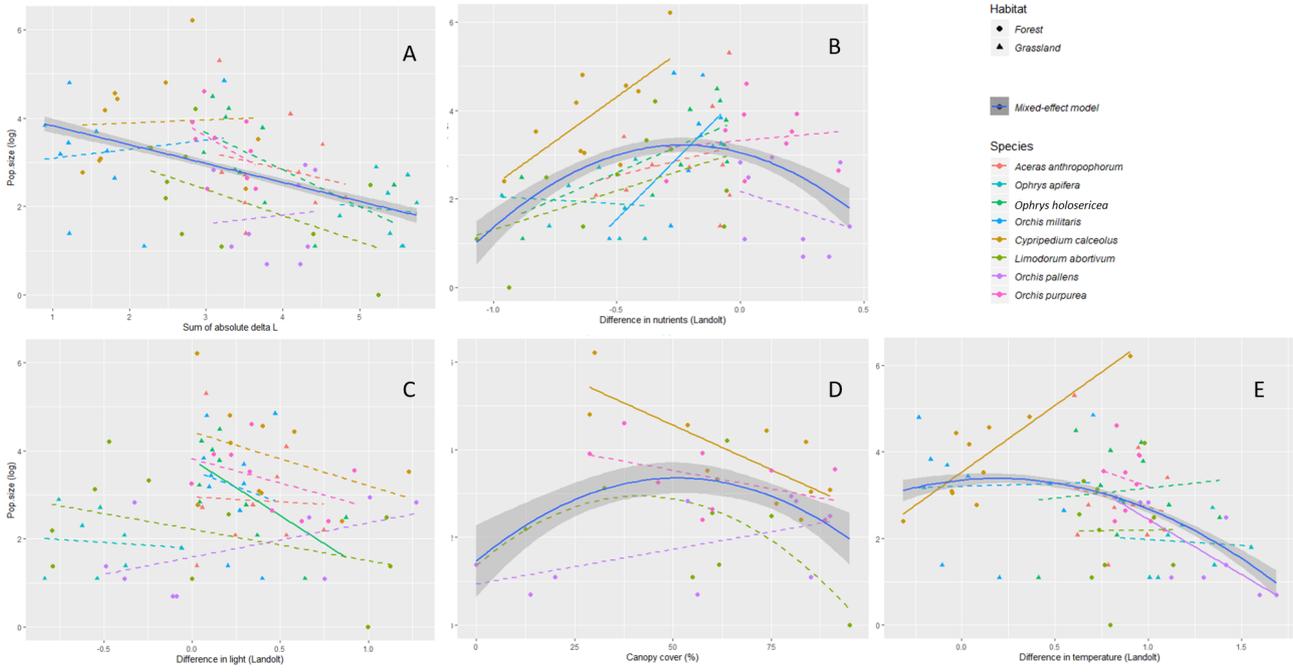


Figure 2: Results of the linear models with population size (log) against the deviation of the measured versus the optimal abiotic conditions. For the plot of the sum of deltaL (summed difference of all six Landolt indicator values, A) the difference between the measured and the optimal conditions increases towards the right of the plot. For the other plots showing the individual Landolt indicator values (nutrients, light, temperature; B, C and E) negative values mean that abiotic parameter exceeds the optimal conditions; positive values mean that the measured value is lower than the optimal value. Panel D shows the analysis of the canopy cover. Dashed lines indicate that the relationship is not significant whereas full lines indicate a significant relationship ( $p < 0.05$ ), different colors indicate the different species and the different symbols indicate the two habitats, see legend.

## 4.2 Heterogeneity

Five out of eight species have larger populations in sites with higher beta-diversity. A trend which is not significant for any of the study species (Figure 3). However, by analyzing all species together, we find a significant relationship between beta-diversity and population size, meaning that orchid populations are larger in sites with higher beta-diversity ( $p = 0.046$ , slope = 0.886).

## 4.3 Reproduction

In seven out of eight species we find that larger populations have higher germination rates (Figure 4A). The relationship is significant for three species (*L. abortivum*:  $p = 0.006$ , slope = 0.19; *O. militaris*:  $p = 0.03$ , slope = 0.116; *A. anthropophorum*:  $p = 0.05$ , slope = 0.75). The general trend of having higher germination rates in larger populations is supported by finding this significant relationship when analyzing all species together ( $p = 0.001$ , slope = 0.083).

Looking at the models for the percentage of fruits (Figure 4B), we find a similar trend: six out of seven species produce more fruits in larger populations. However, this relationship is not significant over all species and for the individual species it is only significant for *O. pallens* ( $p = 0.019$ , slope = 0.187). The reproduction potential (multiplication of percentage of fruits and germination rate) again, like the previous two reproduction parameters, increases with increasing population size (Figure S7). The models show positive relationships for six out of seven species. These are significant for *L. abortivum* ( $p = 0.008$ , slope = 0.173), *O. militaris* ( $p = 0.043$ , slope = 0.061), *O. pallens* ( $p = 0.026$ , slope = 0.16) and over all species ( $p = 0.001$ , slope = 0.077).

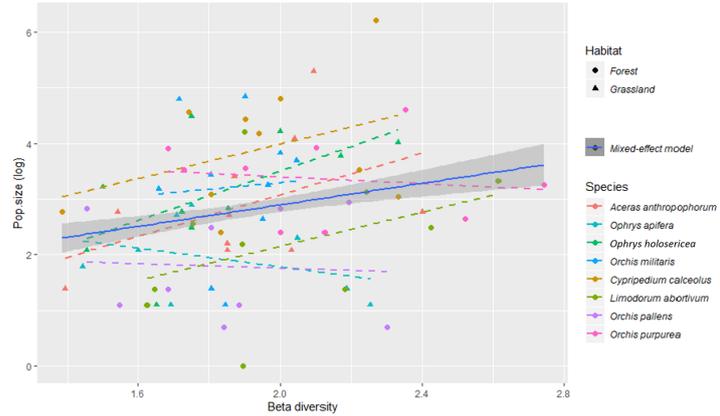


Figure 3: Results of the models with beta-diversity against the population size. Dashed lines indicate that the relationship is not significant whereas full lines indicate a significant relationship ( $p < 0.05$ ), different colors indicate the different species and the different symbols indicate the two habitats, see legend.

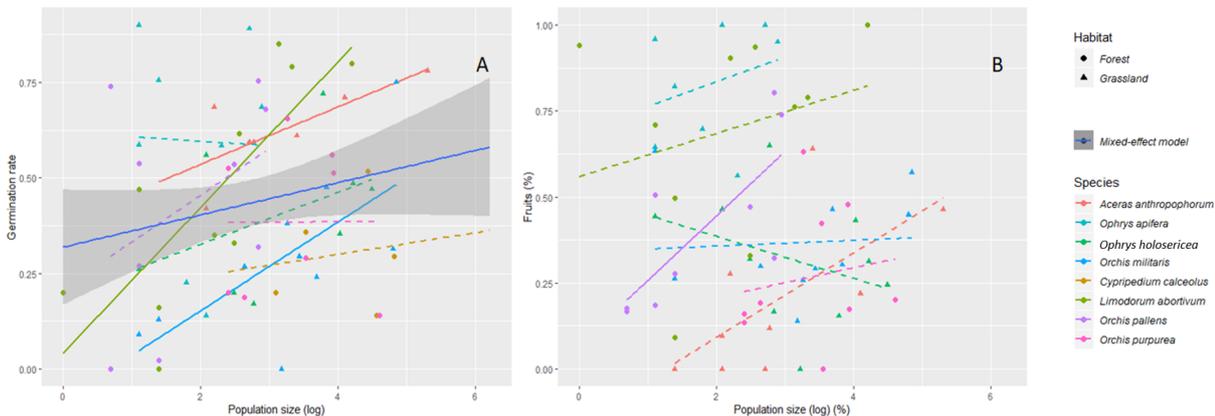


Figure 4: Models for population size germination rate (A) and percentages of fruits (B). Dashed lines indicate that the relationship is not significant whereas full lines indicate a significant relationship ( $p < 0.05$ ), different colors indicate the different species and the different symbols indicate the two habitats, see legend.

## 5 Discussion

### 5.1 Abiotic factors

In general, we find that abiotic conditions are important drivers of the population size, shown by the analysis of the sum of all differences between optimal and measured Landolt indicator values and by the analysis of the individual values. These results are in line with our hypothesis and with two other studies which considered the fitness of orchid populations in western Europe (Vogt-Schilb et al, 2015) and in Czech Republic (Hemrova et al, 2019). They both found a decline of orchids with deviations from optimal conditions; both in grasslands and in forests. It is interesting to underline, that some species (e.g. *O. apifera*) seem to suffer much more than others from poor abiotic conditions in their sites. This is not only the case for the sum of deltaL but also for the individual indicators. This indicates that each species can suffer because of different conditions and that the precise mechanisms behind the decline need to be disentangled to ensure successful conservation measures.

#### Nutrients

For both habitats, we found that both nutrient surpluses and deficits lead to smaller population sizes. As forests were more centered around the optimum, they suffered less than grasslands from this mismatch.

All grassland sites included in our study had too many nutrients for the orchid species considered. One reason for the higher amount of nutrients in the soils is increased atmospheric nitrogen deposition, mainly caused by human activities (Vitousek et al, 1997). It is a phenomenon which has already been reported to decrease species richness in grasslands (Stevens et al. 2004). Another explanation for the increased levels of nutrients in grasslands may be a legacy effect of old land-use practices. In particular, fertilization could still affect nutrient levels in soil today (Abraha et al, 2018) even though the studied grassland sites are not fertilized anymore. A possible mechanism for this negative effect on population size is increased dominance of species adapted to higher levels of nutrients resulting in stronger light competition (Hautier et al, 2009). This is supported by the positive correlation between the nutrient indicator and vegetation height and the decreased population sizes in sites with higher vegetation. Another possibility, which finds support in literature, is that the increase in nutrients may cause a decline in the diversity of mycorrhizal fungi associated with orchids (Mujica et al, 2016). This can reduce the possibility for orchids to find their fungal partner, consequently reducing the population size of the orchids by reducing rejuvenation. While we could not find any paper on the influence of too high nutrient levels on orchid species, the review of Humbert and colleagues (2016) shows that species richness in general decreases with increasing nutrient levels. On the other hand, increased availability of nutrients can promote biotic homogenization of communities, both in grassland and in forest, indicating that the species lost, most often are rare species (Ross et al, 2012; Durak & Holeska, 2015).

The studied orchid populations in forests seemed to be less affected by this nutrient mismatch, probably because of two reasons. Firstly, although forest ecosystems may be affected by land-use legacies as well, anthropogenic fertilization is not considered as one of those in many studies on this subject (Josefsson et al, 2009; Munteanua et al, 2015). Secondly, two out of four forest species considered here are naturally adapted to soils with higher levels of nutrients (Table 2) and therefore, do not suffer from higher nutrient levels. This indicates that for the population size of our forest species other abiotic factors might be more important and that the influence might be more species specific than in grasslands.

#### Light

While light indicator values did not influence the population sizes in either forests or grasslands, canopy cover, which can be used as a proxy of light availability for the forest understory vegetation (Cole and Weltzin, 2005), was important for the population size of orchid species. Forest species are known to be very susceptible to incorrect light conditions and may suffer from too much as well as not

enough light. On the one hand, excessive light may increase competition (Scanga, 2014) and cause a shift towards communities, which require more light (Tsai et al, 2018). On the other hand, not enough light, caused by canopy closure, may result in the disappearance of species adapted to living in open forests (Hårdtle et al, 2003). In our study we found results supporting both mechanisms. Three forest species adapted to intermediate light conditions (Table 2) performed best around 50% canopy cover. It therefore seems to be important to maintain the optimal light conditions to conserve these forest species.

## Temperature

Almost all populations grew in sites with lower temperatures than would be ideal for the study species and we found that populations decreased in size when the mismatch of temperature between the site and the literature values increased. Colder temperature have been shown to reduce fitness in species not adapted to it (Rodriguez et al, 2015). However, finding most populations in habitats with too low temperatures seemed surprising considering current climate warming. A possible explanation could be that the increase of temperature in the original habitats does not allow our study species to survive and, therefore, they are forced to move upwards and occupy only sites with lower temperatures (Walther, 2005). Different mechanisms could be behind this. Firstly, the interspecific interactions in the communities are altered and some dominant species profit more from higher temperatures, outcompeting weaker species (Partzsch, 2019; Niu, 2008). In particular, grasses have been shown to respond strongly to high temperatures by growing faster and higher, especially if the increase in temperature is combined with an increase in the availability of nutrients (Klanderud, 2005). Secondly the increase in temperature may promote the invasion of species from lower elevations, which modify the competition patterns in the community, resulting in the exclusion of some less competitive species (Walther, 2003). Therefore, the increase of temperature may accelerate the decline of orchids, through the different mechanisms explained before. This is supported by other studies, which found a general decline of specialists in favour of generalists under climate warming (Jimenez-Alfaro, 2014).

## General comments on abiotic factors

Landolt indicators are one amongst many tools for studying abiotic conditions and just like other tools they have their drawbacks. Especially in forests, where species number and plant cover are lower compared with grasslands, they may be less accurate and could give misleading results. Nevertheless, based on the reviews of Diekmann (2003) and of Bartelheimer & Poschlod (2016), we decided to use Landolt indicator values rather than direct measurements in the field for several reasons. Firstly, Landolt indicator values reflect the long term dynamics of a site, whereas direct measurements can fluctuate strongly over a short period and secondly, with Landolt indicator values we could cover a large variety of abiotic measurements, which would have needed more time and financial effort than floristic observations. Since we are aware of the problems related to the indicators, we complemented the analysis whenever possible with other measurements (i.e. canopy cover and vegetation height) to add more reliability to the results.

Summarized, we find that orchids can suffer from poor abiotic conditions in grassland and forest habitats. In particular, grassland species suffer from increased competition due to too high nutrient availability in the soil, whereas forest species were most susceptible to poor light conditions. Moreover, climate warming seems to indirectly impact orchid species of both habitats, which can, especially in combination with the two other factors, put great pressure on their populations.

## 5.2 Heterogeneity

Using beta-diversity as a proxy for habitat heterogeneity we found that orchid populations tended to be larger in sites with higher habitat heterogeneity. This indicates that a higher number of patches with different characteristics in the sites increases the chance of having the right conditions for the orchids (e.g. nutrients, light, fungi in the soil), which therefore have a higher probability to establish and grow. As found in previous studies and summarized in a review of Moeslund and colleagues (2013),

we can, therefore, state that habitat heterogeneity is an important characteristic of the habitat, which favours the presence of rarer species, in our particular case of orchids.

### 5.3 Reproduction

We found that smaller populations tended to have a lower percentage of flowers resulting in fruits and lower germination rates. The first indicates that orchids in small populations are facing pollination limitation. Since orchids have very specific insect pollinators, this is probably attributable to the fact that in smaller populations insects struggle to find individuals of the right species to pollinate (Pellegrino et al, 2005). Moreover, the fragmentation of habitat caused by human activities has been demonstrated to contribute to species decline via disruption of the pollination networks (Pellegrino et al, 2014). The latter indicates that inbreeding depression is affecting smaller populations, reducing their reproductive success and therefore also their fitness. Moreover, in these small populations, random genetic drift may increase the negative effect of inbreeding depression even more by further reducing the gene pool and the viability of the seeds (Hens et al, 2017).

However, with our observational study we cannot conclude on the causality between population size and lowered fruit production and germination rates. Both phenomena could be the cause or consequence of reduced population size but the most likely option is that they are linked by a circular relationship, where they are both cause and effect. Taking into account the abiotic factors as well, we could hypothesize, that a change in abiotic conditions or habitat fragmentation can reduce population size in the first place. Inbreeding depression and pollination limitation could subsequently limit the reproduction, resulting in a further decrease of population size and increased extinction risk of a population.

### 5.4 Conclusions and Outlook

This study underlines the important links between abiotic conditions, habitat heterogeneity, reproductive success and the population size of eight endangered orchids in Switzerland and the importance of their consideration when protecting populations or planning reintroductions. Our study supports that as a first priority for existing populations, the abiotic conditions must be improved, or, if suitable for the orchid species, maintained, for example by keeping forests open enough. For reintroductions, it is crucial to choose sites with suitable abiotic conditions to not compromise the success of the conservation action. Moreover, we could demonstrate the importance of also considering the reproductive aspects. In small populations where the reproduction appears to be problematic, genetic rescue should be considered, by breeding individuals in-vitro and transplanting them to other populations.

## 6 Acknowledgements

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## 7 References

- Abraham M, Gelfand I, Hamilton S.K, Chen J, Robertson G.P. 2018.** Legacy effects of land use on soil nitrous oxide emissions in annual crop and perennial grassland ecosystems. *Ecological Applications*. 28. 1362–1369.
- Anderson M.J, Ellingsen K.E, McArdle B.H. 2006.** Multivariate dispersion as a measure of beta diversity. *Ecology Letters*. 9. 683–693.
- Bartelheimer M, Poschlod P. 2016** Functional characterizations of Ellenberg indicator values – a review on ecophysiological determinants. *Functional Ecology*. 30. 506-516.
- Bornand C, Eggenberg S, Gygas A, Juillerat P, Jutzi M, Möhl A, Rometsch S, Sager L and Santiago H. 2016.** Lista Rossa Piante vascolari. *Infoflora*.
- Bozdogan H. 1987.** Model selection and Akaike’s information criterion (AIC): the general theory and its analytical extensions. *Psychometrika*. 52, 3. 345-370.
- Butchart S.H.M, Walpole M, Collen B, Van Strien A, J.P.W Scharlemann, Almond R.E.A, Baillie J.E.M, Bomhard B, Brown C, Bruno J, Carpenter K.E, Carr G.M, Chanson J, Chenery A.M, Csirke J, Davidson N.C, Dentener F, Foster M, Galli A, Galloway J,N, Genovesi P, Gregory R.D, Hockings M, Kapos V, Lamarque J-F, Leverington F, Loh J, McGeoch M.A, McRae L, Minasyan A Hernández Morcillo M, Oldfield T.E.E, Pauly D, Quader S, Revenga C, Sauer J.R, Skolnik B, Spear D, Stanwell-Smith D, Stuart S.N, Symes A, Tierney M, Tyrrell T.D, Vié J-C, Watson R. 2010.** Global Biodiversity: Indicators of Recent Declines. *Science*. 328. 1164-1168.
- Cole P.G and Weltzin J.F. 2005.** Light limitation creates patchy distribution of an invasive grass in eastern deciduous forests. *Biological Invasions*. Vol. 7. 477–488.
- Delarze R, Gonseth Y. 2008.** Lebensräume der Schweiz. *Ott Verlag*. 2. Auflage.
- Díaz S, Settele J, Brondízio E.S, Ngo H.T, Guèze M, Agard J, Arneth A, Balvanera P, Brauman K.A, Butchart S.H.M, Chan K.M.A, Garibaldi L.A, Ichii K, Liu J, Subramanian S.M, Midgley G.F, Miloslavich P, Molnár Z, Obura D, Pfaff A, Polasky S, Purvis A, Razaque J, Reyers B, Roy Chowdhury R, Shin Y.J, Visseren-Hamakers I.J, Willis K.J, Zayas C.N. 2019.** IPBES (2019): Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. *IPBES secretariat*.
- Diekmann M. 2002.** Species indicator values as an important tool in applied plant ecology - a review. *Basic and Applied Ecology*. 4.493-506.
- Durak T, Jan Holeksa J. 2015.** Biotic homogenisation and differentiation along a habitat gradient resulting from the ageing of managed beech stands. *Forest Ecology and Management*. 351. 47–56.
- Hautier Y, Niklaus P.A, Hector A. 2009.** The effects of light and soil conditions on the species richness of the ground vegetation of deciduous forests in northern Germany (Schleswig-Holstein). *SCIENCE*. 324, 636-639.
- Härdtle W, Von Oheimb G, Westphal C. 2003.** Competition for Light Causes Plant Biodiversity Loss After Eutrophication. *Forest Ecology and Management*. 182. 327–338.

- Heino J, Melo A.S, Bini L.M. 2015.** Reconceptualising the beta diversity-environmental heterogeneity relationship in running water systems. *Freshwater Biology*. 60. 223–235.
- Hemrová L, Milan Kotlínek M, Marie Konečná M, Radim Paulič R, ana Jersáková J, Tamara Těšitelová T, Jana Knappová J, Zuzana Münzbergová Z. 2019.** Identification of drivers of landscape distribution of forest orchids using germination experiment and species distribution models. *Oecologia*. 190. 411–423.
- Hens H, Pakanen V-M, Jäkäläniemi A, Tuomi J, Kvist L. 2017.** Low population viability in small endangered orchid populations: Genetic variation, seedling recruitment and stochasticity. *Biological Conservation*. 210. 174–183.
- Humbert J-Y, Dwyer J.M, Andrey A, Arlettaz R. 2016.** Impacts of nitrogen addition on plant biodiversity in mountain grasslands depend on dose, application duration and climate: a systematic review. *Global Change Biology*. 22. 110-120.
- Jimenez-Alfaro B, Gavilan R.G, Escudero A, Iriondo JM, Fernandez-Gonzalez F. 2014.** Decline of dry grassland specialists in Mediterranean high-mountain communities influenced by recent climate warming. *Journal of Vegetation Science*. 25. 1394–1404.
- Josefsson T, Hörnberg G, Östlund L. 2009.** Long-Term Human Impact and Vegetation Changes in a Boreal Forest Reserve: Implications for the Use of Protected Areas as Ecological References. *Ecosystems*. 12. 1017–1036.
- Keppel G, Gillespie T.W, Ormerod P, Fricker G.A. 2016.** Habitat diversity predicts orchid diversity in the tropical south-west Pacific. *Journal of Biogeography*. 43. 2332–2342.
- Klanderud K. 2005.** Climate change effects on species interactions in an alpine plant community. *Journal of Ecology*. 93. 127–137.
- Lauber K, Wagner G. 2014.** Flora Helvetica. *Haupt Verlag*. Bern. 5. Auflage.
- Landolt E, Bäumler B. 2010.** Flora Indicativa. Ökologische Zeigerwerte und biologische Kennzeichen zur Flora der Schweiz und der Alpen. 2., völlig neu bearbeitete und erweiterte Auflage der Ökologischen Zeigerwerte zur Flora der Schweiz, 1977. *Haupt Verlag*. Bern.
- Lennartson T. 2002.** Extinction threshold and disrupted plant-pollinator interactions in fragmented plant populations. *Ecology*. 11. 3060–3072.
- Lundholm J.T. 2009.** Plant species diversity and environmental heterogeneity: spatial scale and competing hypotheses. *Journal of Vegetation Science*. 20. 377–391.
- Malavasi M, Vojta Bartak V, Carranza M.L, Petra Simova P, Acosta A.T.R. 2018.** Landscape pattern and plant biodiversity in Mediterranean coastal dune ecosystems: Do habitat loss and fragmentation really matter? *Journal of Biogeography*. 45. 1367–1377.
- Moeslund J.E, Arge L, Bøcher R.K, Dalgaard T, Svenning J-C. 2013.** Topography as a driver of local terrestrial vascular plant diversity patterns. *Nordic Journal of Botany*. 31. 129–144.
- Mujica M.I, Saez N, Mauricio Cisternas M, Manzano M, Armesto J.J, Perez F. 2016.** Relationship between soil nutrients and mycorrhizal associations of two *Bipinnula* species (Orchidaceae) from central Chile. *Annals of Botany*. 118. 149–158.

- Munteanua C, Kuemmerleb T, Keulerd N.S, Müllerb D, Balázsi P, Doboszg M, Griffiths P, Haladaf L, Kaimg D, Királyh G, Konkoly-Gyurói E, Kozakg J, Lieskovskyf J, Ostafing K, Ostapowicza K, Shandraj O, Radeloffa V.C. 2015.** Legacies of 19th century land use shape contemporary forest cover. *Global Environmental Change*. 34. 83-94.
- Nicolé F, Brzosko E, Till-Bottraud I. 2005.** Population viability analysis of *Cypripedium calceolus* in a protected area: longevity, stability and persistence. *Journal of Ecology*. 93. 716–726.
- Niu S. and Wan S. 2008.** Warming changes plant competitive hierarchy in a temperate steppe in northern China. *Journal of Plant Ecology*. Vol.1. Nr.2. 103-110.
- Parmesan C. 2006.** Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics*. 37. 637–669
- Partzsch M. 2019.** Warming differently affects the inter- and intraspecific interactions among semi-dry grassland species. *Perspectives in Plant Ecology, Evolution and Systematics*. Vol.40.
- Pearson S, Eggenberg S, 2010.** Liste der National Prioritären Arten. *Bundesamt für Umwelt*.
- Pellegrino G, Bellusci F. 2014.** Effects of human disturbance on reproductive success and population viability of *Serapias cordigera* (Orchidaceae). *Botanical Journal of the Linnean Society*. 176. 408–420.
- Pellegrino G, Gargano D, Noce M.E, Musacchio A. 2005.** Reproductive biology and pollinator limitation in a deceptive orchid, *Serapias vomeracea* (Orchidaceae). *Plant Species Biology*. 20. 33-39.
- Rodríguez V.M, Soengas P, Alonso-Villaverde V, Sotelo T, Cartea M.E, Velasco P, 2015.** Effect of temperature stress on the early vegetative development of *Brassica oleracea* L. *BMC Plant Biology*. 15:145.
- Ross L.C, Woodin S.J, Hester A.J, Thompson D.B.A, Birks H.J.B. 2012.** Biotic homogenization of upland vegetation: patterns and drivers at multiple spatial scales over five decades. *Journal of Vegetation Science*. 23. 755–770.
- Scanga S.E. 2014.** Population dynamics in canopy gaps: nonlinear response to variable light regimes by an understory plant. *Plant Ecology*. 215. 927–935.
- Stevens C.J, Dise N.B, Mountford J.O, David J. Gowing. 2004.** Impact of Nitrogen Deposition on the Species Richness of Grasslands. *Science*. Vol.303. 1876-1879.
- Travis J.M.J. 2003.** Climate change and habitat destruction: a deadly anthropogenic cocktail. *Proc. R. Soc. Lond. B*. 270. 467–473.
- Tsai H-C, Chiang J-M, McEwan R.W, Lin T-C. 2018.** Decadal effects of thinning on understory light environments and plant community structure in a subtropical forest. *Ecosphere*. Vol.9.
- Vitousek P.M, Aber J.D, Howarth R.W, Likens G.E, Matson P.A, Schindler D.W, Schlesinger W.H, Tilman D.G. 1997.** Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*. Vol. 7. 737-750.
- Walther G-R. 2003.** Plants in a warmer world. *Perspectives in Plant Ecology, Evolution and Systematics*. Vol. 6(3). 169–185.

**Walther G-R, Beissner S, Burga C.A. 2005** Trends in the upward shift of alpine plants. *Journal of Vegetation Science*. Vol. 16. 541-548.

**Wartmann B.A. 2006.** Die Orchideen der Schweiz - Ein Feldführer. *Sternenvogel*. 29-33.

**Weaver K, Morales V, Dunn S.L, Godde K and Weaver P.F. 2018.** An Introduction to Statistical Analysis in Research:With Applications in the Biological and Life Sciences. *John Wiley and Sons*. First Edition. 435-471.

**Wilson M.V and Shmida A. 1984.**Measuring beta-diversity with presence-absence data. *Journal of Ecology*. 72. 1055-1064.

**Wraith J, Pickering C. 2019.**A continental scale analysis of threats to orchids. *Biological Conservation*. 234. 7-17.

**Vogt-Schilb H, Munoz F, Richard F, Schatz B. 2015.** Recent declines and range changes of orchids in Western Europe (France, Belgium and Luxembourg). *Biological Conservation* 190. 133–141.

## 8 Supplementary information

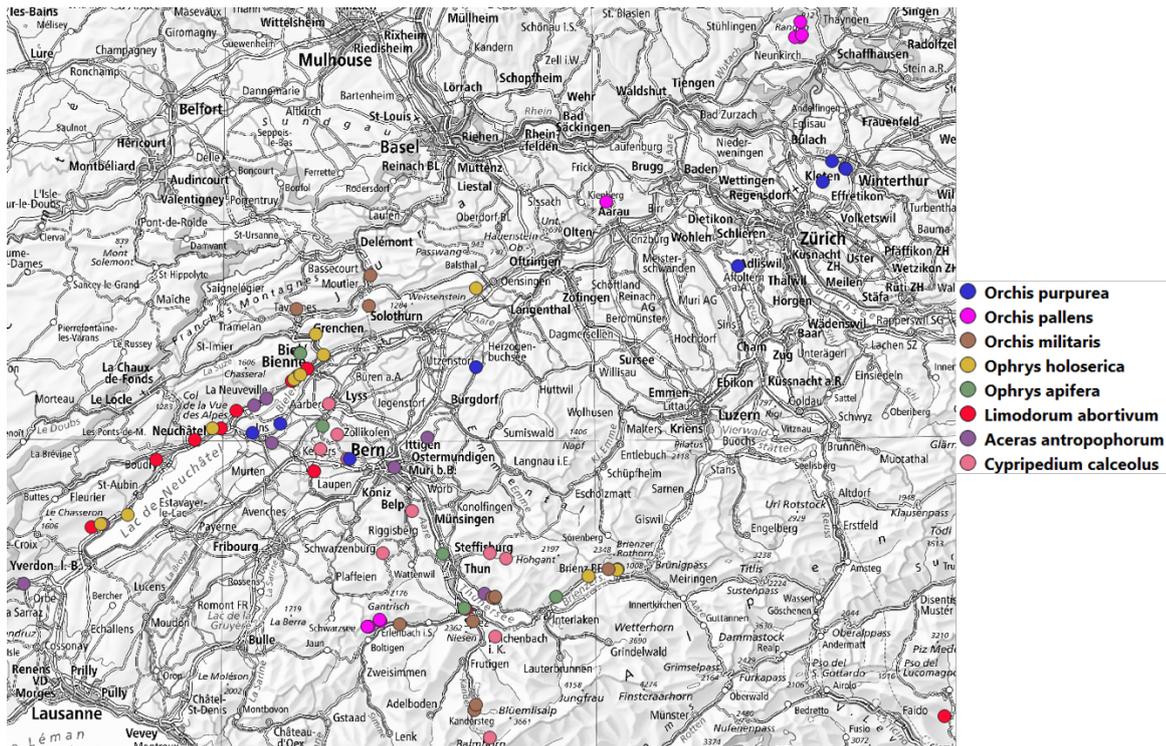


Figure S1: Map of Switzerland showing the location of 76 studied populations. The different colours indicate the eight different orchid species.

### Protocol for germination of orchids (Roland Schafflützel, Christoph Zwahlen and Giotto Roberti)

#### Nutrient Media

Add the nutrient medium (MURASHIGE AND SKOOG, Duchefa Biochemie) to a standard Agar (7.5 g/L) solution and use it to prepare Petri-dishes

#### Seeds preparation

Seeds are put in tubes filled with soap-water and left there for 2-3 days, until they sink

Pour the seeds in a frit and add 1ml of H<sub>2</sub>O<sub>2</sub>

After one minute, wash the seeds with abundant tap water

Pour 4 ml of 1.5% Javelwasser (Bleach) in the frit

Put the frit for 15 min. in the Ultrasound bath at 25°C. The seeds should turn white

#### **Important: The next steps need to be done on a sterile workbench**

Wash the seeds with sterile deionized Water, until the pH is neutral

Transfer the seeds on the prepared Petri-dished and close them

Store the dishes at 20°C in the dark

Figure S2: Protocol for the in-vitro germination of orchid's seeds

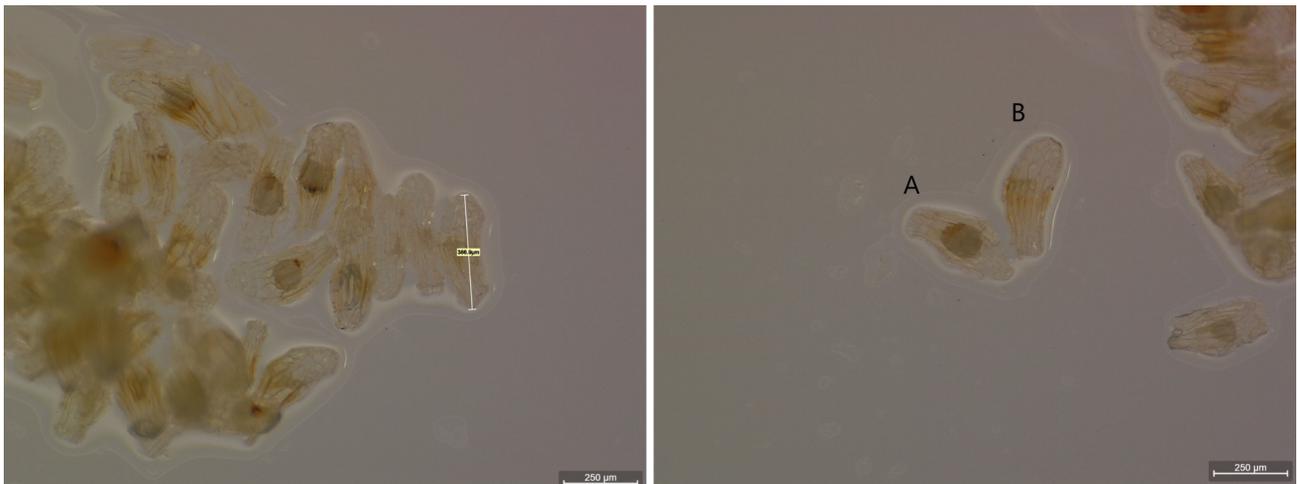


Figure S3: Picture of the seeds taken under the binocular. The seed indicated with A is germinating whereas the seed indicated with B is not.

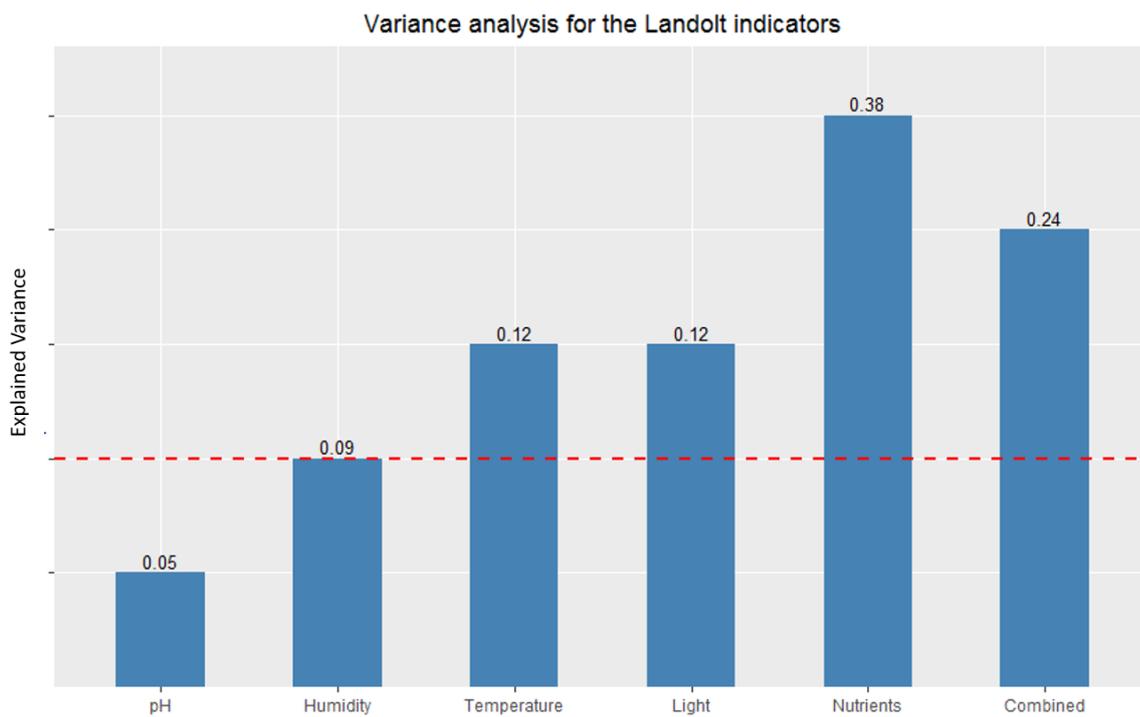


Figure S4: Barplots of the variance explained by each of the Landolt indicator values resulting from the individual linear models. The red, dashed line indicates the cutoff threshold if the respective Landolt indicator value was further considered in the analysis.

NUTRIENTS					
	delta_nut	df	F-value	p-value	adjusted R <sup>2</sup>
<i>Aceras antropophorum</i>	1.375	8	0.532	0.486	0
<i>Ophrys apifera</i>	-0.335	6	0.056	0.819	0
<i>Ophrys holoserica</i>	2.401	8	9.706	<b>0.014</b>	0.491
<i>Orchis militaris</i>	5.627	8	4.716	0.061	0.292
<i>Cypripedium calceolus</i>	4.01	8	7.227	<b>0.027</b>	0.408
<i>Limodorum abortivum</i>	1.763	8	2.6	0.145	0.15
<i>Orchis pallens</i>	-1.843	7	0.84	0.389	0
<i>Orchis purpurea</i>	0.497	7	0.123	0.736	0
	delta_nut <sup>2</sup>				
LME (delta_nut + delta_nut <sup>2</sup> )	-2.366	66	7.538	<b>0.007</b>	
	delta_nut				
LME (grassland)	2.395	33	16.311	<b>0.0003</b>	
	delta_nut <sup>2</sup>				
LME (forest)	-2.857	32	9.141	<b>0.004</b>	

Veg_Height					
	Veg_Height	df	F-value	p-value	adjusted R <sup>2</sup>
<i>Aceras antropophorum</i>	0.027	8	0.221	0.65	0
<i>Ophrys apifera</i>	-0.016	6	0.05	0.83	0
<i>Ophrys holoserica</i>	-0.079	8	5.973	<b>0.04</b>	0.355
<i>Orchis militaris</i>	-0.124	8	2.814	0.131	0.167
	Veg_height				
LME	-0.056	33	4.286	<b>0.046</b>	

HABITAT X NUTRIENTS				
	Value	df	F-value	p-value
delta_nut (Forest)	-1.283	64	2.316	0.133
delta_nut <sup>2</sup> (Forest)	-3.077	64	10.191	<b>0.002</b>
delta_nut:Grassland	5.649	64	5.658	<b>0.020</b>
delta_nut <sup>2</sup> :Grassland	5.558	64	4.751	<b>0.033</b>

Figure S5: Output of the models for the separate analysis of the nutrients in grassland and forest (A), for the model with vegetation height as explanatory variable and population size as response variable (B) and for the interaction between nutrients and habitat type (C).

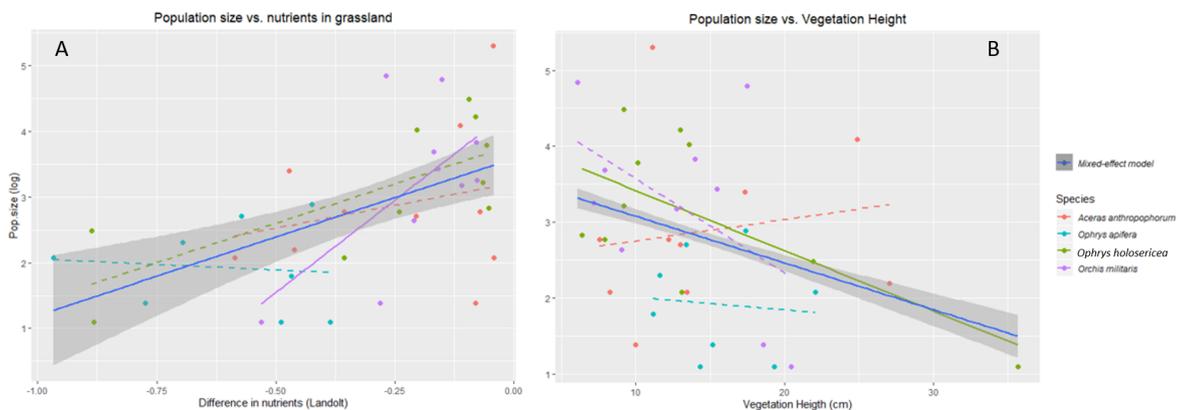


Figure S6: Results of the separate models for grassland with difference in nutrients (A) and vegetation height (B) as explanatory variables. Dashed lines indicate that the relationship is not significant whereas full lines indicate a significant relationship ( $p < 0.05$ ), different colors indicate the different species, see legend.

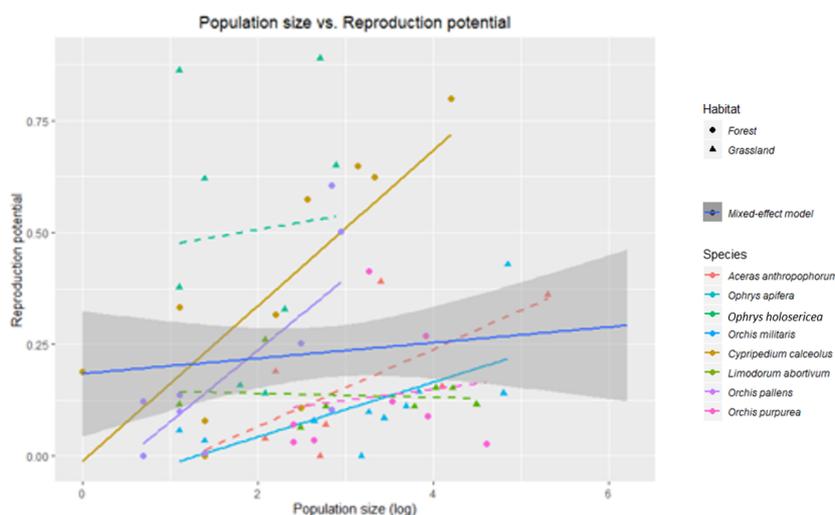


Figure S7: Results of the model with reproduction potential (% of Fruits \* Germination rate) as response variable and population size as explanatory variable. Dashed lines indicate that the relationship is not significant whereas full lines indicate a significant relationship ( $p < 0.05$ ), different colors indicate the different species, see legend.

## Declaration of consent

on the basis of Article 30 of the RSL Phil.-nat. 18

Name/First Name:

Registration Number:

Study program:

Bachelor       Master       Dissertation

Title of the thesis:

Supervisor:

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