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Received: 25 April 2022 | Revised: 19 July 2022 | Accepted: 10 August 2022

DOI: 10.1111/csp2.12800

CONTRIBUTED PAPER

Conservation Science and Practice Open Access
A Journal of the Society for Conservation Biology

WILEY

Detecting preservation and reintroduction sites for endangered plant species using a two-step modeling and field approach

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Funding information
Botanical Garden of the city of Neuchâtel; Canton de Neuchâtel; Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 31003A_179481; Subvention égalité grant from the University of Neuchâtel; Wüthrich and Mathey-Dupraz Foundation

Abstract

To withstand the surge of species loss worldwide, (re)introduction of endangered plant species has become an increasingly common technique in conservation biology. Successful (re)introduction plans, however, require identifying sites that provide the optimal ecological conditions for the target species to thrive. In this study, we propose a two-step approach to identify appropriate (re)introduction sites. The first step involves modeling the niche and distribution of the species with bioclimatic and topographical predictors, both at continental and at national scales. The second step consists of refining these bioclimatic predictions by analyzing stationary ecological parameters, such as soil conditions, and relating them to population-level fitness values. We demonstrate this methodology using Swiss populations of the lady's slipper orchid (*Cypripedium calceolus* L., Orchidaceae), for which conservation plans have existed for years but have generally been unfruitful. Our workflow identified sites for future (re)introductions based on the species requirements for mid-to-sunny light conditions and specific soil physico-chemical properties, such as basic to neutral pH and low soil organic matter content. Our findings show that by combining wide-scale bioclimatic modeling with fine scale field measurements it is possible to carefully identify the ecological requirements of a target species for successful (re)introductions.

KEYWORDS

ecological niche, endangered plants, ensemble forecasting, population fitness, red list, restoration, soil properties

1 | INTRODUCTION

Earth is experiencing the sixth mass extinction of living species (Barnosky et al., 2011; Ceballos et al., 2017; Cordier et al., 2021; IUCN/SSC, 2008; Primack, 2014). To fight against species disappearance, the field of conservation biology has developed during the last 40 years and has guided the creation of a substantial number of nature reserves and conservation sites, where species can be maintained or reintroduced (Primack, 2014). Nonetheless, many conservation plans are failing and species disappearance is continuing (Gaston & Fuller, 2008; Pimm et al., 2014), suggesting that habitat protection alone is not enough to fully protect all species (Swarts & Dixon, 2009). Indeed, because of habitat loss, fragmentation and change, the restoration of viable populations can rarely be achieved by unaided natural recruitment and dispersal (Seddon, 2010). As a consequence, assisted migration to new sites has been proposed in order to overcome barriers to dispersal and recruitment (Menges, 2008; Seddon, 2010; Swarts & Dixon, 2009).

Conservation translocation is defined as the deliberate movement of living organisms from one area to another and consists of either reinforcement plus reintroduction or introduction plans (IUCN/SSC, 2013). Reinforcement and reintroduction plans entail the translocation of new individuals inside a species indigenous range, while introduction involves moving a species to areas where it is currently not observed (Bottin et al., 2007; IUCN/SSC, 2013). While many translocation attempts have been successful (Bottin et al., 2007; Maschinski et al., 2004; Maschinski & Duquesnel, 2007; Ramsay & Stewart, 1998), they also commonly do not reach the expected results (Bottin et al., 2007; Drayton & Primack, 2012; Heywood & Iriondo, 2003). For instance, according to Godefroid et al. (2011), who analyzed 249 plant species reintroduction plans worldwide, survival, flowering, and fruiting rates are often low following reintroduction, on average 52%, 19%, and 16%, respectively. The reasons that led to these failures include: i) a lack of understanding of primary ecological and biological grounds that caused the decline in population sizes in the first place (Godefroid et al., 2011; Heywood & Iriondo, 2003); ii) a deficit of biological and ecological understanding of the species under scrutiny (Bottin et al., 2007; Guerrant & Kaye, 2007; Menges, 2008); iii) a lack of experimental, scientific and integrated approaches (Seddon et al., 2007); and iv) an insufficient quantitative overview of the parameters that lead to failures (Drayton & Primack, 2012; Godefroid et al., 2011).

Within the framework of plant species translocation efforts, the selection of suitable sites is a particularly complex endeavor. As plants are sessile organisms, survival of seeds, and seedlings can vary on a scale of <1 m^2 (Falk et al., 1996; Guerrant Jr & Kaye, 2007; Maschinski & Haskins, 2012). Accordingly, while in the context of current and future changes in the landscape and climate it is important to select habitats for (re)introduction efforts by modeling current and future bioclimatic envelopes and their geographic projection (Guisan et al., 2006, 2013; Maschinski & Haskins, 2012), the choice of a suitable area for (re)introduction requires a highly sophisticated understanding of the species ecology and biology (Falk et al., 1996; Maschinski & Haskins, 2012). Within this framework, Fiedler and Laven (1996) proposed four classes of site-selection criteria for the (re)introduction of rare plants: 1) physical criteria (including both large-scale geomorphic factors and finer-scale parameters, such as soil types); 2) biological criteria (including autoecological and synecological parameters); 3) logistic criteria (including accessibility, and feasibility); and 4) historical development of the site (Fiedler & Laven, 1996; Guerrant & Kaye, 2007).

The selection of translocation areas has therefore taken two complementary routes. The first route merges physical and biological selection criteria through the use of a spatially implicit habitat suitability modeling approaches (Guisan et al., 2013; Pecchi et al., 2019; Prasad et al., 2016). For instance, Brown et al. (2008) developed a multi-scale analysis for the management of the tree *Syzygium jambos* that included both a landscape- and a population-scale model of the tree distribution. A second route advocates for the use of a finer-scale multidimensional approach for building precise ecological requirements for the target species (Prasad et al., 2016; Richardson et al., 2009). For instance, Vittoz et al. (2006) performed an integrated study of multiple ecological factors, including soil physicochemical properties, hydrology, microtopography, and vegetation community analysis, for identifying the optimal niche of the rare species *Saxifraga hirculus*. Edaphic and environmental factors have also been used to evaluate the ecology of the endangered plant species *Primulina tabacum* (Ren et al., 2010). We here sustain that a more valuable approach would be to combine the two routes and select suitable translocation sites for plants by combining predictions or habitat suitability models with stationary analysis of ecological factors that cannot be retrieved from standard/available raster layers (Seddon et al., 2007). While this has been done to some extent for animals (Borgo, 2003; Cook et al., 2010), to our knowledge this has rarely been attempted for plants.

In this study, we aimed to further exemplify such a multi-step approach that allows selecting the most appropriate (re)introduction sites (Figure 1). We specifically analyzed naturally-growing and (re)introduced Swiss populations of the orchid species *Cypripedium calceolus* L. (Orchidaceae). In term of conservation status *Cypripedium calceolus* is characterized with different statuses across its distribution; as Least Concern (LC) at global scale, but as Vulnerable (VU) in Switzerland and as Critically Endangered (CR) in the United Kingdom (Bornand et al., 2016; Gargiulo et al., 2018; Bilz, 2011; Stroh et al., 2014). Its complex ecological requirements made it a perfect model species for testing and developing conservation strategies (Gargiulo, Fay, & Kull, 2021; Swarts & Dixon, 2009). While having a Eurasian distribution (Figure S1a), this species is disappearing rapidly in numerous countries, including in Switzerland (Le Lay et al., 2010). For instance, while in the 1960s,

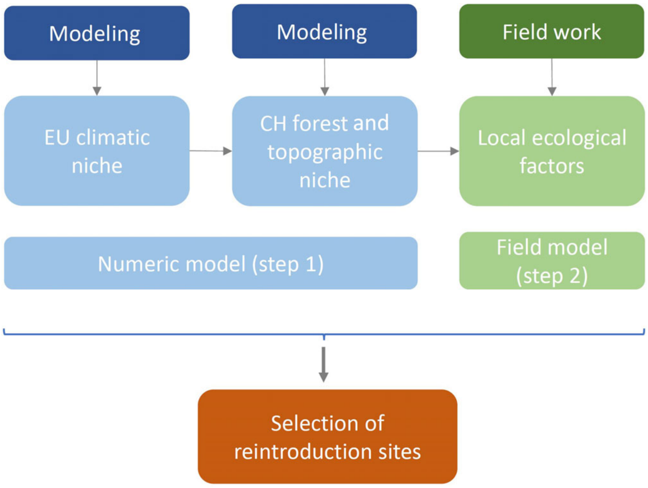


FIGURE 1 Hierarchical workflow for selecting appropriate reintroduction and introduction sites for endangered plant species. The workflow starts by modeling the potential occurrence of target species at the continental scale (numeric model, step 1, EU scale for *Cypripedium calceolus*). Following this, the potential distribution model of the target species can be refined at the national scale using additional layers (e.g., forest edges and topographic factors; numeric model, step 1, national scale). The continental bioclimatic niche is then integrated into the national niche modeling. The modeling step is followed by field observations (field model, step 2), which allows refining the niche of the target species by including additional fine-scale ecological factors that are not available in spatially explicit form, such as soil variables. The merging of step 1 and step 2 should yield a more refined selection of suitable (re) introduction sites for conversation plans

when occurrence data started to be consistently gathered, this species was observed over an area of approximately 400 km^2, in 2019 the area covered by the populations of *C. calceolus* in Switzerland shrunk by almost 50% (*Source*: National Data and Information Center on the Swiss Flora [ww.infoflora.ch], Figure S1b). Because of such a fast decline, this emblematic species has been the target of several conservation plans, with varying degrees of success (e.g., Devillers-Terschuren, 1999; Fay et al., 2018; Ramsay & Stewart, 1998). Its spatial distribution in Switzerland was also modeled based on various environmental maps with the aim to support the detection of new (unsurveyed) populations, but the attempt was unsuccessful (Le Lay et al., 2010). Based on natural history observations, the ecological requirements of *C. calceolus* appear to be centered around the three factors of light, soil moisture and soil bases richness, and practitioners have generally postulated that suitable sites for the growth of this species consist on a limited combination of these parameters range (Devillers-Terschuren, 1999). For instance, *C. calceolus* tends to be mainly found in shady deciduous mixed woodland and sometimes on stone-strewn slopes (Foremnik et al., 2021; Kull, 1999). Regarding edaphic requirements, *C. calceolus* seems to be predominantly associated with basic soils containing calcium carbonates (Kull, 1999) and with moderate soil moisture (Devillers-Terschuren, 1999). Nonetheless, field observations show that the species ecological requirements can be broader. For instance, soil pH requirements for *C. calceolus* have been shown to range from neutral to moderately acidic (Käsermann & Moser, 1999) and, in Switzerland, the species can be found in 14 different habitat types, with a preference for *Cephalanthero-Fagenion* (xerothermophilous beech forest) and *Erico-Pinion* (basophilic subcontinental pine forest) (Delarze et al., 2015; Käsermann & Moser, 1999). Therefore, at first sight, the specific biotic and abiotic requirements for this species tend to be broad and widespread. Accordingly, because field observations highlight strong variation in *C. calceolus* populations size and health condition, we can hypothesize that the performance of the species across habitats vary depending on the local ecological conditions.

To address our aim, we first developed a spatial distribution model that included both current and future climatic scenarios. Second, we performed a field-based sampling approach to correlate stationary edaphic parameters with population fitness variables. Moreover, we compared edaphic properties of natural sites of *C. calceolus* and (re) introduction sites to investigate the potential edaphic mechanisms behind (re)introduction failures. Our approach was based on the hypothesis that successful (re)introductions depend on refining bioclimatic model predictions with in-depth knowledge of which ecological parameters correlate most strongly with the highest population fitness. Our work thus aims to increase the probability of success for conservation plans of endangered plant species that require finding suitable habitats for replenishing or creating novel viable populations.

2 | MATERIALS AND METHODS

2.1 | Step 1: spatial modeling of *C. calceolus* populations at two geographical scales

We developed a hierarchical spatial distribution model based on two scales (Eurasian and Swiss occurrences) to avoid biases related to niche truncation (Chevalier et al., 2021; Mateo et al., 2019).

2.1.1 | Eurasian-scale models

To estimate the current and future bioclimatic niche of *C. calceolus* specifically for Switzerland, we first calibrated Eurasian models with Eurasian occurrences and bioclimatic variables at a 30″ resolution (~1 km^2). We used 2854 occurrence points obtained from the GBIF database (https://gbif.org), and also found in Averyanov (1999) and Gargiulo et al. (2019). We disaggregated occurrences to avoid spatial auto-correlation (grid resolution = 5′, occurrences kept: $n = 1613$) and we selected 100,000 background points in Eurasia (Barbet-Massin et al., 2012; Barve et al., 2011). We used 5 out of 19 bioclimatic (https://chelsa-climate.org/) descriptors to characterize the current climatic niche; (i) bioclim1: mean annual air temperature, (ii) bioclim2: mean diurnal air temperature range (mean of monthly [max. temperature–min. temperature]), (iii) bioclim5: mean daily air temperature of the warmest month, (iv) bioclim12: annual precipitation amount, and (v) bioclim15: precipitation seasonality (Table S1). Those descriptors were the most consistent with species ecology and had a low pair-wise correlation (computation of matrix of Pearson and Spearman rank-correlation <0.7 (Dormann et al., 2013). For future climatic scenarios (2061–2080), we kept the same five Bioclim variables at 1 km^2 resolution, and we selected two Global Circulation Models (GCMs): IPSL-CM5A-LR (Dufresne et al., 2013) and HadGEM2-AO (Collins et al., 2008) together with two Representative Concentration Pathway (RCP): RCP4.5 and RCP8.5 (van Vuuren et al., 2011). We took the conservative decision to use data from GCMs from CMIP5, as studies for comparing CMIP5 and the more recent CMIP6 are still ongoing (e.g., Shu et al., 2020). To model Eurasian niches of *C. calceolus*, we processed three algorithms included in biomod2 (version 3.5.1 Georges & Thuiller, 2013; Thuiller et al., 2016) in R, version 4.1.1 (R Core Development Team, 2021): (i) Generalized Linear Model (GLM), (ii) Boosted Regression Trees (GBM) and (iii) Generalized Additive Model (GAM) (Gallien et al., 2012; Thuiller et al., 2016). For each set of pseudo-absences (1), each modeling technique (3) was run 33 times. To avoid circular reasoning in model evaluations, we used split-sample cross-validation procedure (Gallien et al., 2012). Models were calibrated on 80% of the initial data and evaluated on the 20% left with Probability of detection (POD, Wehling et al., 2011), Receiver operating statistics (ROC, Hanley & McNeil, 1982) and with the maximization of the true skill statistic (hereafter TSS, Allouche et al., 2006; Guisan et al., 2017). We draw response curves for each variable and for each model to show how predicted values change along each variable gradient while keeping all other variable at their mean value (Elith et al., 2005). Finally, we projected Eurasian models on Switzerland using the same bioclimatic variables as the ones used for the calibration at the European scale, but this time from the CHclim25 dataset at a scale of 25 m (Broennimann, 2018). We used Ensemble Forecasting to create final prediction maps for Switzerland. Two maps were produced for each time period; a map showing mean prediction weighted by model quality (TSS), and a map illustrating coefficients of variation between different models. Ensemble Forecasting was also evaluated with TSS. Those maps were used as covariates in the Swiss-scale models described below (Table S1).

2.1.2 | Swiss-scale models

To describe a more detailed climatic and topographic niche of the species at regional scale (Switzerland), we calibrated another model with 1796 Swiss occurrences, which were obtained from the National Data and Information Center on the Swiss Flora (www.infoflora.ch), from the Arbeitsgruppe Einheimische Orchideen Aargau (AGEO, https://ageo.ch/) and from the canton of Fribourg. In this case, we post-processed data in order to suppress records with a precision <25 m, those that were older than 1960 (when occurrence data started to be consistently gathered and scrutinized), and those who were irrelevant (e.g., botanical gardens, urban areas). We also disaggregated data at a grid resolution of 250 m to finally obtain a set of 528 occurrences. As for the European scale, 100,000 background points were selected, but this time within the Swiss territory. We selected six descriptors corresponding to *C. calceolus* ecology to define the Swiss-wide niche at a scale of 25 m^2: (i) the climatic niche obtained at Eurasian scale used as covariate, (ii) the topographic aspect (aspval, corresponding to a sin-transformation of aspect, Aspect Tools of Spatial Analyst, ArcGIS 10.4), (iii) the slope (slp25, Slope Tools of Spatial Analyst, ArcGIS 10.4), (iv) the topographic position (topos, Zimmermann & Roberts, 2001), (v) the maximum height of the canopy (Ginzler & Hobi, 2015), and (vi) the distance between occurrences of *C. calceolus* populations and forest borders calculated with ArcGIS (ArcGIS 10.4). We included those two latter variables because the canopy height gives information about the light reaching the soil, and because digital map observations highlighted that *C. calceolus* populations are frequently located near forest borders (i.e., forest distance smaller than 150 m for 1243 over 1423 occurrences) (Table S1). We next modeled a prediction map for each climatic scenario. To model Swiss niches of *C. calceolus*, we used the same process as at Eurasian scale. For Ensemble Forecasting at Swiss scale, we kept only models with a TSS value higher than 0.5. Models were projected on the same variables used for calibration at a resolution of 25 m. Two maps were produced for each time period; a map showing mean prediction weighted by model quality (TSS), and a map illustrating coefficients of variation between different models. Ensemble Forecasting was also evaluated with TSS. To select potential reintroduction sites on the final current prediction map, we used a threshold of 0.5 as it is the weight given to presence and pseudo-absences when fitting the models (Liu et al., 2013).

2.2 | Step 2: field study of *C. calceolus* populations

2.2.1 | Study sites

To conduct field-based surveys of *C. calceolus* populations, we selected 11 naturally occurring sites along the Swiss Jura Mountains (Tables S2 and S3). This region was selected based on the fact that the Jura mountains are predominantly built of limestone, thus likely the optimal substrate for this species, that fit the broader climatic requirements of *C. calceolus*, and that the Jura region has been the subject of multiple (re)introduction attempts since more than 15 years (Figure 2a). In addition, we surveyed five sites selected for reintroduction attempts between 2010 and 2014 (Tables S2 and S3). These reintroduction sites were chosen on the basis of vegetation similarities with historically known forested sites that hosted *C. calceolus* in the past, and the presence of indicator species for mountain spruce forests (*Carex flacca* and *Luzula sylvatica*). Source material for the reintroduced plants came from local seeds to avoid outbreeding depression (e.g., Frankham et al., 2017). Nonetheless, no genetic analyses were performed on these source populations. Plants were cultivated by private plant growers and were reintroduced when they were 4 years old, with a size of about 4 cm, and with two leaves and no flower).

2.2.2 | Edaphic parameters

To assess the edaphic requirements of *C. calceolus* populations, at each site, we dug a soil profile until bedrock, minimum 40 cm away of the studied plant in order to not damage roots. All soil layers were described and soils and humus forms classified according to the international taxonomic keys (Baize & Girard, 2009; IUSS Working Group, 2015; Zanella et al., 2018). Next, in the topsoil layers (OL, OF, OH, and A layers), which corresponded to a depth of 15.5 ± 8.1 cm, and where the rooting of *C. calceolus* takes place, we quantified several physico-chemical parameters, including i) water content, obtained by desiccation at 40°C and weighing, ii) organic carbon relative to total nitrogen (C/N ratio; quantified with an elemental analyzer, Flash 2000, CHN-O Analyzer, Thermo Scientific), iii) total organic matter content (OM), quantified through the ignition loss after heating the samples at 450°C for 2 h, iv) water pH (Metrohm 827 pH-meter, Metrohm AG), v) cationic exchange capacity (CEC), quantified using the "cobalt hexamine trichloride" method (Ciesielski & Sterckeman, 1997), and vi) total carbonates ($CaCO_3$), quantified following the dissolution with HCl 6 M and using The Bernard calcimeter test (Dreimanis, 1962).

2.2.3 | *Cypripedium calceolus* population parameters

In order to understand the relationship between *C. calceolus* populations health status and the edaphic parameters, we measured several plant and population functional traits using non-intrusive methods. *C. calceolus* functional traits (Pérez-Harguindeguy et al., 2013) included plant growth parameters, such as: 1) plant size (measured in centimeters, from the ground to the top of the highest leave), 2) number of leaves per plant, 3) leave area (measured as the median leaf area of the highest stem, calculated using the ellipse area formula), 4) photosynthetic activity (i.e., the median leaf photosynthetic activity, measured with a SPAD chlorophyllometer [Konica Minolta]), and reproduction-related parameters, such as: 5) number of flowers per plant, 6) number of pods per plant, and 7) number of stems per plant (Table S2). Each stem or group of stems spaced with 70 cm from another group of stems were considered as a separated individual (Kull, 1988, 1999). Finally, we assessed 8) a "vitality" parameter (scale 0–3) for scoring an integrative vitality value of each population, where 0 indicates that the population is practically extinct, and 3 indicates that the population is big, plants are healthy, and produce a lot of flowers and fruits. We analyzed traits on 10 randomly chosen plants within 40 m radius, and separated by a minimum of 2 m from each other.

2.3 | Statistical analyses

Based on the SDM analyses, we first assessed the effect of climate warming on the probability of population presence within Switzerland by performing one-way ANOVA on the probability predictions of each population under the four climate warming scenarios and the current predictions.

Next, to assess the correlation between soil quality and *C. calceolus* population health status, we first calculated the horizon depth-weighted, summed, depth-

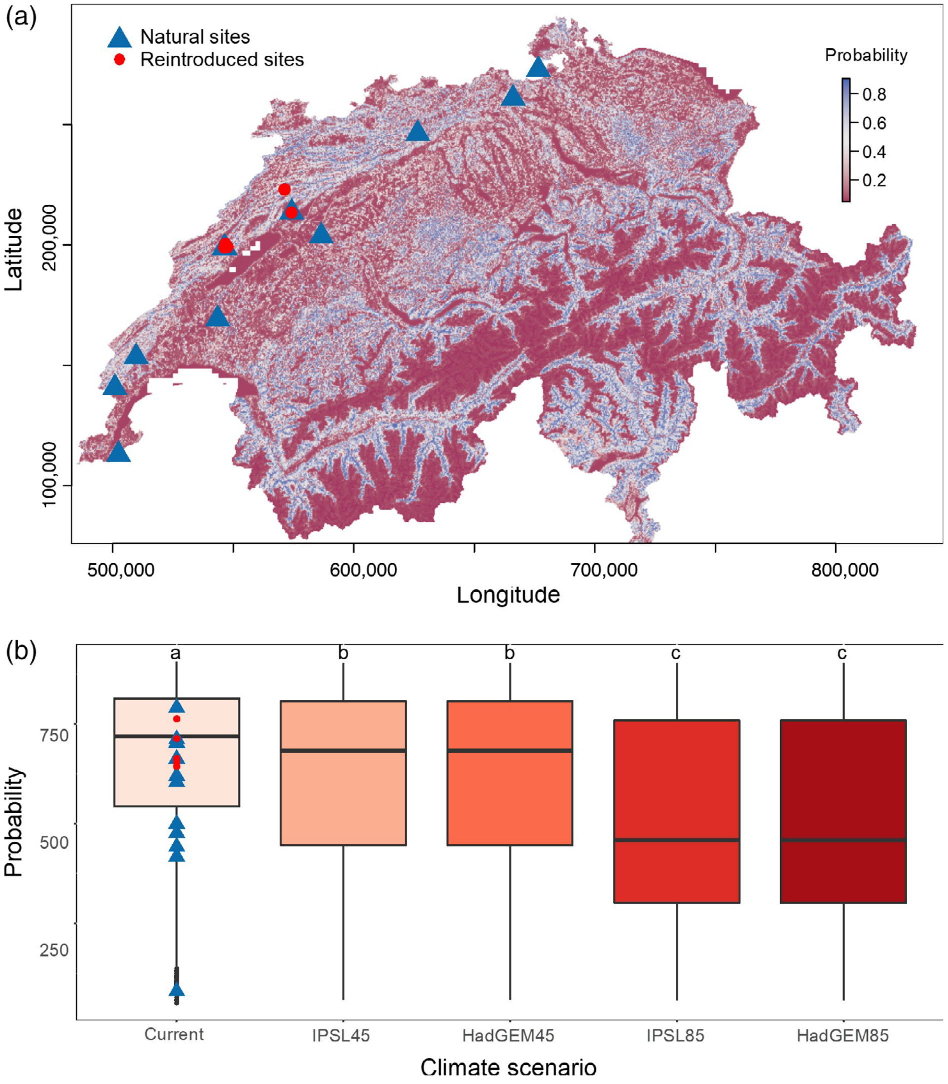


FIGURE 2 *Cypripedium calceolus* populations and probability of occurrence in Switzerland. (a) The map is colored based on the spatial modeling integrating Swiss and Eurasian populations, whereby red indicates low probability of occurrence and blue indicates high probability of occurrence based on the climatic and topographic variables at 25 m resolution scale. Also shown are the 11 natural populations (blue triangles) and five reintroduction sites (red dots) chosen for field work. (b) Boxplots showing medians and quantiles of probability of occurrence values for current climate scenarios (a) and the two GCMs: IPSL-CM5A-LR (IPSL45 and IPSL85) and HadGEM2-AO (HadGEM45 and HadGEM85) together with two RCP scenarios: RCP4.5 and RCP8.5 (Figure S3). Letters above boxplots indicate significant differences among climatic scenarios (Tuckey HSD, $p < .05$). GCM, global circulation model; RCP, representative concentration pathway

segment-aligned dissimilarity across the 11 soil pedological profiles based on pH, CEC, $CaCO_3$, OM, and CN using the *profile_compare* function in the package *aqp* (version 1.40, Beaudette et al., 2013). Second, we calculated the Euclidean distance across *C. calceolus* populations along the two first axes of a principal component analysis based on the growth and reproductive traits measured. Third, we measured the correlation between the two matrices (dissimilarity and distance matrices, respectively) using a mantel test (function *mantel.test* in the package *vegan*, version 2.5.7). Finally, because the mantel test revealed a significant correlation (Section 3), we performed a multivariate correlation between the measured average values of the soil parameters (pH, CEC, $CaCO_3$, OM, and CN) found in the first 20 cm of each pedological profile, and the *C. calceolus* growth and reproduction traits using the *envfit* function in *vegan*. We performed this analysis in order to highlight the soil parameters with the best fitting correlation with the measured *C. calceolus* functional traits.

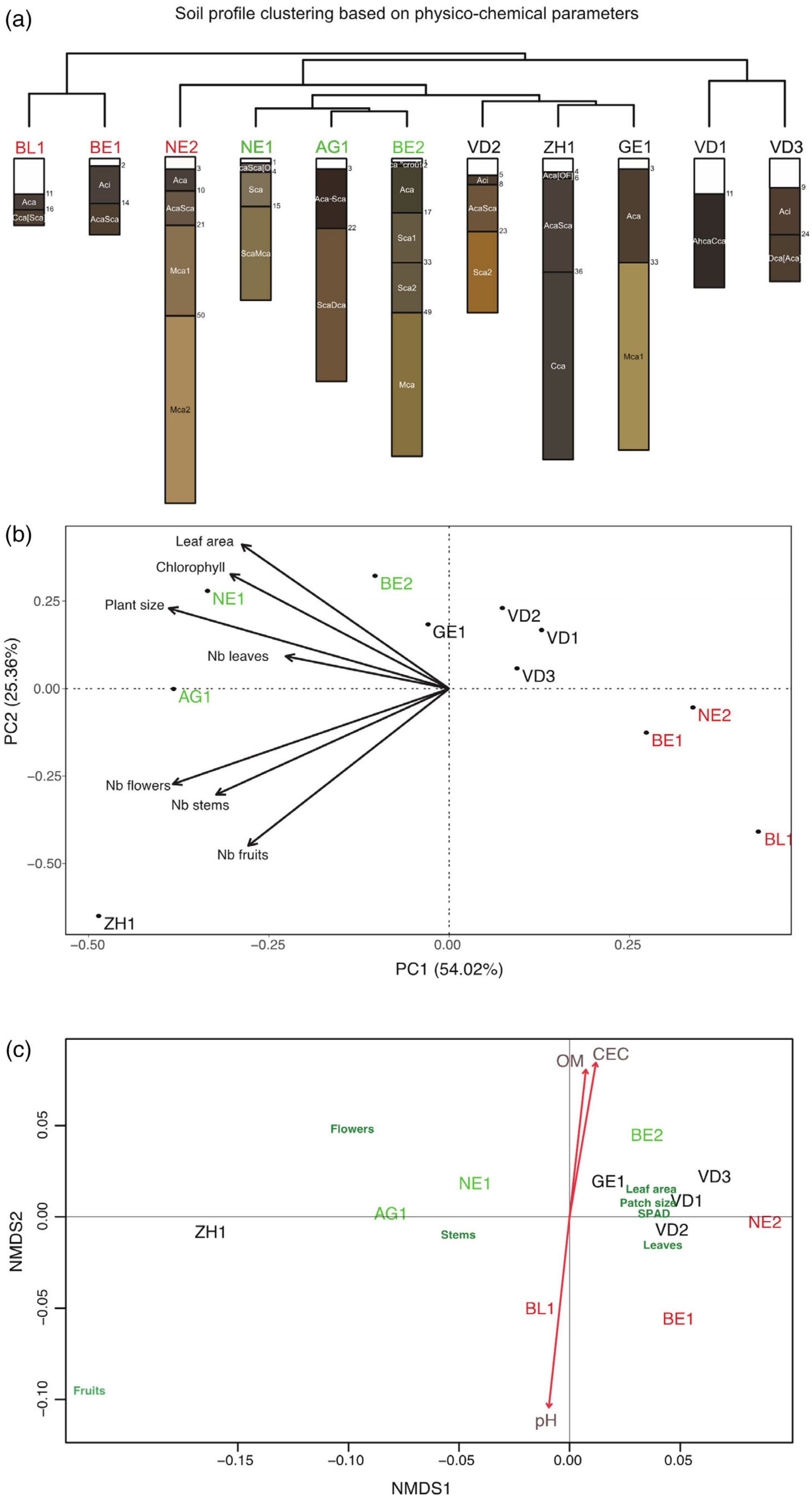
Finally, we compared the soil physico-chemical parameters (pH, CEC, $CaCO_3$, OM, and CN) of the 11 natural population sites with the 5 reintroduction sites across aggregated variables within horizon depth (*slab* function in the *aqp* package (Beaudette et al., 2013).

3 | RESULTS

3.1 | Step 1: spatial modeling of *C. calceolus* populations at two geographical scales

3.1.1 | Bioclimatic spatial modeling

We built species distribution models for *C. calceolus* and generated maps of probability of occurrences for this species (Figures 2a and S2). At both Eurasian and Swiss scales, model performance was based on TSS (Eurasian scale [EU]: mean: 0.75, SD: 0.04; Swiss scale [CH]: mean:



Soil profile clustering based on physico-chemical parameters

FIGURE 3 Effect of local edaphic factors on *Cypripedium calceolus* population growth- and reproduction-related parameters. (a) Variation in soil color profiles of the 11 natural field populations, aggregated based on soil physicochemical properties. Soil horizon colors are based on the Munsell code (Munsell, 2015). (b) Principal Component Analysis (PCA) of population fitness parameters, based on the following traits: Number of fruits, number of stems, number of flowers, number of leaves, plant size, chlorophyll, and leaf area in multidimensional space. (c) Results of the *envfit* analysis for correlating population fitness traits with soil properties in the first organo-mineral horizon (a horizon). Red arrows indicate the three soil factors (total OM, CEC, and pH = soil pH) that correlated most strongly with the population trait matrix, $p < .05$ for pH and CEC, and $p < .1$ for OM). Populations are color-coded based on population trait similarity (panel b). Letter codes for *C. calceolus* populations are described in supplementary data D1. CEC, cation exchange capacity; OM, organic matter

0.56, SD: 0.04), POD (EU: mean: 0.99, SD: 0.001; CH: mean: 0.99, SD: 0.007), and ROC (EU: mean: 0.92, SD: 0.03; CH: mean: 0.83, SD: 0.03). At the Eurasian scale, variable importance analysis highlighted mean annual air temperature (bioclim1), mean diurnal air temperature range (bioclim2) and mean daily air temperature of the warmest month (bioclim5) as the main drivers of this species distribution. At the Swiss scale, distance of *C. calceolus* populations to a forest border and canopy height were the two variables that mostly influenced model accuracy (Figure S3). Accordingly, in Switzerland, this species is preferentially found growing up to 40 m from the forest border and under a canopy measuring between 20 and 50 m. Finally, we combined best performing models to create final maps displaying the probability of occurrence for each climatic scenario (evaluation with TSS:EU: 0.774, CH: 0.61). Under a current climatic scenario, the areas predicted to be most favorable for *C. calceolus* are the Jura, the lower alpine valleys and the southern Swiss Alps (Figure 2a). When compared to current climate, future global circulation models RCP4.5 (IPSL45 HadGEM45) and RCP8.5 (IPSL85 HadGEM85) decrease the overall average probability of population occurrence by 6% and by 22%, respectively (Figure 2b; $F_{4,6505} = 105.5$, $p < .001$). Such a decline was the strongest for the RCP8.5 model, particularly in the low altitude areas and the southern Swiss Alps (Figure S4).

3.2 | Step 2: field study of *C. calceolus* populations

3.2.1 | Edaphic parameters

We found variation across *C. calceolus* populations in terms of soil physico-chemical properties (Figures 3a and S5). Nonetheless, the studied natural populations were only present on calcareous soils: 11 CALCOSOLS (Calcaric Cambisols) and 3 RENDOSOLS (Calcaric Leptosols). We characterized humus forms as Oligomull (2), Dysmull (5), Hemimoder (2), and Dysmoder (2). In reintroduction sites, we identified 3 CALCOSOLS (Calcaric Cambisols), 1 RENDOSOL (Calcaric Leptosol), and 1 BRUNISOL (Cambisol). Humus forms were Dysmull (4) and Oligomull (1) (see Supplementary data D1 for soil profile descriptions). Analyses of the first organo-mineral horizons showed that in natural populations, soil pH ranged from 7.4 to 7.83, CEC from 23.9 to 70.8 cmolc/kg, $CaCO_3$ from 4.43% to 63.4%, organic matter from 7.4% to 45.6% and C/N ratio from 10.4 to 29.6. In reintroduction sites, pH ranged from 5.77 to 8.18, CEC from 11.4 to 70.5 cmolc/kg, $CaCO_3$ from 0% to 67.7%, OM from 13.5% to 65.8%, and C/N ratio from 2.3 to 33.6 (Figure 3 and Table S3).

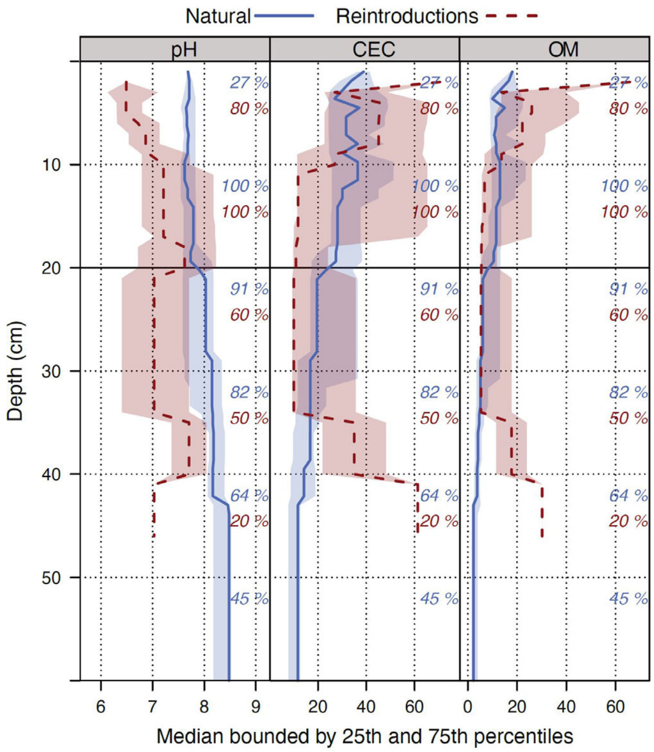


FIGURE 4 Soil profile comparisons across *Cypripedium calceolus* populations. The profiles show average changes in soil parameters (pH, CEC, and OM content) with soil depth for natural sites (blue lines; $n = 11$) and reintroduction sites (red lines; $n = 5$) of *C. calceolus* populations. Shaded areas around the median lines represent 75% confidence intervals. CEC, cation exchange capacity; OM, organic matter

3.2.2 | *Cypripedium calceolus* population parameters and correlation with soil parameters

We measured plant traits only in natural populations because no reintroduced plants survived through the years. The number of individuals per population varied from 1 to 50. Per individual, the number of stems varied from 1 to 60, the number of flowers from 1 to 38, the number of pods from 1 to 15, and the median leaf area from 29 to 140 cm^3 (Table S2). We found that across *C. calceolus* populations soil physico-chemical parameters within pedological profiles (Figure 3a) correlated with growth- and reproduction-related plant traits (Figure 3b; mantel test based on 1000 permutations, $p = .03$). We also found that the soil properties that mostly discriminated *C. calceolus* populations were pH ($r = 0.84$, $p = .002$), CEC ($r = 0.56$, $p = .04$), and OM ($r = 0.72$, $p = .07$) (Table S4, and Figure 3c). Generally, we found that fitter and bigger populations occur on soils that with moderate pH, OM, and CEC values (Figure 3c; pH: 7–8, CEC: 30–40 cmoloc/kg, and total organic matter: 15%–20%). Finally, we observed that the average horizon depth-weighted pH values for the natural population soils was 7.9, while the soil pH for the reintroduction site was 7.2 (i.e., 10% lower; Figure 4). We also found higher variability in $CaCO_3$ values for the reintroduction sites than for the natural population sites, and that reintroduction sites had 8% higher soil CN than the natural populations (Figure 4).

4 | DISCUSSION

We developed a two-step approach for identifying suitable sites for the (re)introduction of *C. calceolus* populations. First, we found that climatic predictions alone were rather broad in precision, but nevertheless could highlight a likely decline of the species range during the next 50 years of climate warming. Next, when incorporating topographical layers for the modeling approach at the regional scale, we found that the species was more likely to occur near forest edges. Finally, by analyzing in situ soil parameters, we found that soil organic matter, cation-exchange capacity and pH correlated most strongly with *C. calceolus* population fitness variables as described by multivariate function trait space. We thus advocate for the combination of modeling tools with fine-scale ecological surveys to identify suitable reintroduction sites for this, and potentially other, endangered plant species.

4.1 | Spatial niche modeling for conservation efforts

When modeling a current climate scenario, the continent-wide model parameterized with only bioclimatic variables did not give sufficient information, in that it did not exclude many areas of the Swiss map. Indeed, according to GBIF occurrences and the literature (e.g., Braunschmid et al., 2017; Käsermann & Moser, 1999; Kull, 1999), *C. calceolus* does appear to be adapted to a wide range of climatic conditions, from England to Japan and from Finland to Spain and Bulgaria (Devillers-Terschuren, 1999). Therefore, it is perhaps not surprising that the climatic niche modeling alone shows little predictive power in relation to the population health status of this species (Kull, 1999). By contrast, the regional scale model, which included bioclimatic as well as topographical and forest edge and forest height variables, was much more informative, highlighting that the highest probability of finding this species is tightly linked to how far the population is from forest edges and depends on canopy height (Foremnik et al., 2021; Hurskainen et al., 2017). A previous modeling study of the species in part of Switzerland, which included topographic, climatic and soil variables and a measure of vegetation productivity but no distance to forest edge, could successfully predict the species distribution but failed to support the identification of any new population in the field (Le Lay et al., 2010). Indeed, several authors have suggested that the requirements of this species in terms of light conditions correspond to open forest gaps (Fay & Taylor, 2015; Hurskainen et al., 2017; Käsermann & Moser, 1999; Kull, 1999; Ramsay & Stewart, 1998). Therefore, from a general point of view, our results corroborate previous theory suggesting that rare species geographic range distributions are less linked to macroclimatic patterns but more to microclimatic conditions, such as those that are created by the forest-open space ecotone, or in forest gaps (Wamelink et al., 2014).

Nonetheless, when modeling future climate scenarios, which considered both medium (RCP4.5) and high (RCP8.5) annual total greenhouse gas emission predictions, our modeling predicted declines in suitable future areas of *C. calceolus* population growth. This result suggests that while this species has broad climatic requirements, when growing on the warmer boundary of its climate envelope (e.g., the Mediterranean regions, or bottom of valleys), populations may already be growing at their maximum temperature tolerance. In this case, an average increase of 2°C–4°C is likely to be sufficient to bring multiple populations to the brink of collapse (Blinova & Chmielewski, 2008; Kolanowska & Jakubska-Busse, 2020). This, coupled with the long life expectancy and slow life cycle of *C. calceolus*, makes the inclusion of future prediction maps when selecting reintroduction and introduction sites particularly relevant in the context of climate change (Guisan et al., 2013; Maschinski & Haskins, 2012; Primack, 2014). However, it is important to underline that the association of old occurrences (from 1960) and more recent climatic data could underestimate the response to the species to climate change.

4.2 | Ecological study and field parameters analyses

We based our study on the hypothesis that examining local ecological factors to identify suitable reintroduction and introduction sites increases the probability of plant establishment success (Maschinski & Haskins, 2012). Indeed, it has been argued that failures in reintroductions and introductions are often the consequence of a lack of ecological data (Heywood & Iriondo, 2003). In our case, soil variables showed a narrower range of values in native sites (e.g., pH, soil organic matter, CN ratio) than reintroduced population sites and were also correlated to changes in population fitness traits. These results highlight the importance of considering soil variables to make selection of translocation sites more accurate. Accordingly, the studied reintroduction sites for this species have historically been chosen based solely on vegetation and on plant companion species observations (Storrer, Y., personal communication). Indeed, of the two indicator species used for the selection on reintroduction sites, *C. flacca* generally grows on calcareous soils, while *L. sylvatica* typically grows on acidic soils (Landolt et al., 2010). Therefore, one of the factors most likely to explain reintroduction failures is the difference in soil composition between natural and reintroduced sites. Moreover, by introducing small (4 cm tall) juvenile (4 years old) *C. calceolus* plants has probably had a negative impact on the success of these reintroductions (Gargiulo, Adamo, et al., 2021). Indeed, Fay and Taylor (2015) advocate for reintroducing *C. calceolus* plants of an already sufficient large size to reduce risks of premature death of the seedlings. That said, our results do not fully correspond to what was previously argued in the classical literature. For instance, Käsermann and Moser (1999) suggested that the optimal pH requirements for this species range from neutral to moderately acidic, whereas our measurements of the A soil layer in native stations were neutral to moderately alkaline (pH 7.6). Secondly, Devillers-Terschuren (1999) described *C. calceolus* sites as being dry during summer months, while native sites studies here were often north-exposed and rather cool. Thirdly, as mentioned above, *C. calceolus* is thought to need full sun conditions for developing well (Devillers-Terschuren, 1999). On the contrary, we observed that for best population performance, solar radiation reaching the plants should be indirect (i.e., close to forest edges, but not in the forest per se). We therefore could propose that the recruitment and establishment phases of this species might require full sun conditions, whereas further growth and flowering conditions is more appropriate under indirect light conditions. If true, *C. calceolus* would thus need pioneer conditions, such as after natural disturbance by tree felling, during early stages of development, and then a more mature ecosystem without a dense bushy understory for the rest of its development (Nicole et al., 2005). If this hypothesis were to be experimentally confirmed, such dynamic ecological requirements would further complicate the selection process of sites for conservation, reintroduction and introduction. Despite these findings, other ecological parameters found in the literature correspond to those we observed. Notably, soils of native populations always contained calcium carbonates (Käsermann & Moser, 1999; Kļaviņa & Osvalde, 2017; Kull, 1999). In summary, we observe that *C. calceolus* needs soils with a neutral to basic pH containing calcium carbonates, and requires a delicate balance of light, moisture, and bases richness (Devillers-Terschuren, 1999). Such ecological requirements must therefore be taken into account in future reintroduction and introduction plans.

4.3 | Merging spatial niche modeling with ecological surveys

This study empirically confirms what Maschinski & Hankins (2012) previously hypothesized: that it is essential to consider both broad- and fine-scale environmental qualities when choosing reintroduction and introduction sites. Indeed, heterogeneity in environmental conditions can influence reintroduction and introduction success on scales <1 m^2 (Maschinski & Haskins, 2012). Ideally, one would thus integrate field data with the broad-scale climate-based prediction maps to make identification of reintroduction and introduction sites more accurate (i.e., the first modeling approach step should already reveal high- and low-priority sites, whose suitability should then then be assessed in later field-based data collection campaigns) (Chevalier et al., 2021; Mateo et al., 2019). Such a modeling approach is moreover currently limited by the fact that few variables exist in raster layer format, particularly those describing edaphic properties for large areas (Cianfrani et al., 2018); and see Descombes et al. (2020) and Hengl et al. (2017) for recent efforts to interpolate soil properties in geographic space. Moreover, considering future bioclimatic scenarios is likely to improve the success rate of reintroduction and introduction in the context of a rapidly changing climate (Marini et al., 2009). While promising, this methodological approach must now be confirmed by testing it across a large range of different species and by implementing an experimental design allowing long-term comparisons between reference sites and those selected by such a method (Seddon et al., 2007). Finally, all conservation attempts, regardless of whether they are successful or not, should be published in open-source databases, which would allow comparing and evaluating the relevance and success of different reintroduction and introduction attempts worldwide (Gargiulo, Adamo, et al., 2021; Godefroid et al., 2011; Godefroid & Vanderborght, 2011; Heywood & Iriondo, 2003; Seddon et al., 2007).

AUTHOR CONTRIBUTIONS

Olivia Rusconi and Sergio Rasmann developed the ideas and the sampling design. Olivia Rusconi, Yannick Storrer, and Renée-Claire Le Bayon collected the data. Olivier Broennimann and Antoine Guisan provided the data and know-how for spatial modeling. Olivia Rusconi, Olivier Broennimann, and Sergio Rasmann analyzed the data. Olivia Rusconi and Sergio Rasmann wrote the first draft of the manuscript, and all co-authors contributed to editing.

ACKNOWLEDGMENTS

We thank Amandine Pillonel, and Molly Baur for helping with soil analyses, Dominique Piazza for helping with field sampling and Tom Walker for editing and commenting of previous versions of the manuscript. This project was financed by the Botanical Garden of the city of Neuchâtel, the Canton of Neuchâtel, the Wüthrich and Mathey-Dupraz Foundation, the Subvention égalité grant from the University of Neuchâtel, and a Swiss Science Foundation Grant 31003A_179481 to Sergio Rasmann.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data for plant traits and soil properties is available at https://datadryad.org/stash/share/bBC-NV1T-y57vOYSJJjfsf-aBV6Ll8Gb1usYmBecn54 (doi: 10.5061/dryad.n8pk0p2zk).

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How to cite this article: Rusconi, O., Broennimann, O., Storrer, Y., Le Bayon, R.-C., Guisan, A., & Rasmann, S. (2022). Detecting preservation and reintroduction sites for endangered plant species using a two-step modeling and field approach. *Conservation Science and Practice*, e12800. https://doi.org/10.1111/csp2.12800