

Master of Science in Biogeosciences

Conservation of endangered plants: a roadmap for the selection of translocation sites

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Illustration : Robert Hainard

“On n’est pas riche de ce qu’on prend mais de ce qu’on laisse. »

Robert Hainard

Abstract

The conservation translocations of endangered plant species have become increasingly used techniques for withstanding the increase of species loss worldwide. However, a comprehensive approach that merges natural history with modern modelling techniques to identify suitable restoration sites does not exist today. In this study, I propose a hierarchical and holistic roadmap to identify appropriate reintroduction and introduction sites. The first step of this method includes modelling of bioclimatic and topographical niches of target species. In order to ensure a long-term viability of plants, current and future niches are modelled. The second step consists in analysing ecological parameters such as vegetation and soil of reference populations. In addition, life history traits of the target species are measured in order to establish a connexion between those biological traits and favourable environmental factors. All data are then statistically analysed to extract the most important parameters for the species' ecology. The third step is dedicated to the improvement of prediction maps obtained in step 1 using field data gathered in step 2 and the creation of a field guidebook with crucial parameters to be taken into account when performing translocations. In order to illustrate this methodology, this study focuses on the Lady's slipper Orchid (*Cypripedium calceolus* L., Orchidaceae) growing in Switzerland, for which conservation plans have existed for years, but generally unfruitful. The three steps of this roadmap allowed identifying sites for future reintroduction and introduction corresponding to this species' ecology for studied parameters. In addition, laboratory analysis of soils highlighted several potential causes of weak success of already-performed reintroductions, as well as a better understanding of the ecology of this rare species. This work can be considered as the basis of a broader roadmap that will include more ecological factors, such as biological, historical and demographical ones, in order to lead to more accurate identifications of suitable translocation sites.

Keywords:

Endangered species
Restoration
Reintroduction
Ensemble forecasting
Ecological niche
Soil parameters
Plant vitality

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1. Introduction

According to general agreement, we are currently facing the Earth's sixth mass extinction of living species (Canadell and Noble, 2001; Barnosky et al., 2011; Ceballos et al., 2017). Contrary to previous mass extinctions, current's events are mainly caused by anthropogenic factors (Primack, 2014). Every year, both destruction of natural habitat and over-exploitation of natural resources cause a disheartening loss of the extraordinary diversity of life (IUCN/SSC, 2008; Primack, 2014). In 2017, the IUCN Red List Assessment (<http://www.iucnredlist.org>) reported more than 12,500 species of animals and 11,500 species of plants known to be threatened with extinction (IUCN, 2017). In response to this unprecedented fast loss of biodiversity, the field of Conservation biology has appeared during the last 35 years (Primack, 2014).

Focusing on vegetation, Swarts (2009) argued that conservation through the reserves' system is not enough to comprehensively protect all species. As a matter of fact, because of loss, fragmentation and change of habitats combined with loss of species, restoration of viable populations can rarely be achieved by natural recruitment and dispersal alone (Seddon, 2010). As a consequence, assisted migration of plants to new sites is more and more used to overcome barriers to dispersal (Menges, 2008; Swarts and Dixon, 2009; Seddon, 2010). This kind of actions are even more important in the context of current and future near term changes in landscape and climate (Maschinski and Haskins, 2012). Conservation translocation, as a deliberated movement of living organisms from one area to another, consists in (i) reinforcement and reintroduction, and (ii) introduction procedures (IUCN/SSC, 2013). Reinforcement and reintroduction are the transplantation of new individuals inside its indigenous range, while introduction consists of moving a species to areas where it has not been originally observed (Bottin et al., 2007; IUCN/SSC, 2013).

Plant translocations aim to establish resilient, self-sustaining populations that have sufficient genetic resources to bear adaptive evolutionary changes (Guerrant Jr, 1996; Guerrant Jr and Kaye, 2007). In order to complete reintroduction and introduction task successfully, several steps need to be fulfilled (Kaye, 2008). They consist in (i) planning and identification of objectives, (ii) finding source material, (iii) propagation, (iv) site selection, (v) site preparation, (vi) outplanting, (vii) monitoring, (viii) evaluation and interpretation, (ix) feedback to improve protocols, (x) communication with others, (xi) habitat maintenance and (xii) repeated actions if necessary to meet objectives (Kaye, 2008). In addition, it is crucial to consider genetic and demographic factors before any reintroduction or introduction (Menges, 2008). Each mentioned step is itself divided in different stages.

Even if the theoretical framework of plant translocation has been developed several years ago, numerous failed attempts were recorded (Heywood and Iriondo, 2003; Bottin et al., 2007; Godefroid et al., 2011a; Drayton and Primack, 2012). According to Godefroid et al. (2011a) who analysed 249 plant species reintroductions worldwide, survival, flowering and fruiting rate are often low (on average 52%, 19% and 16% respectively). Multiple reasons that led to failures are listed in literature. The five most often-mentioned ones are reported hereinafter. The first one is the lack of understanding of reasons causing decline in remaining plant populations (Heywood et al., 2003; Godefroid et al., 2011a). The second one is the deficit of biological and ecological understanding of species (Heywood et al., 2003; Bottin et al., 2007; Guerrant Jr et al., 2007; Menges, 2008; Godefroid et al., 2011a). The third one is the insufficient monitoring procedures following reintroduction events (Armstrong and Seddon, 2008; Godefroid et al., 2011a; Drayton et al., 2012). In addition, there is a lack of experimental, scientific and integrated approaches (Heywood et al., 2003; Seddon et al., 2007; Armstrong et al., 2008). Finally, the insufficient quantity of documentation and peer-reviewed articles prevents to have

a real understanding of parameters that may lead to successes, or even more importantly, to failures (Godefroid et al., 2011a; Drayton et al., 2012).

However, it is important to know that some translocation attempts succeeded (Ramsay and Stewart, 1998; Maschinski et al., 2004; Bottin et al., 2007; Maschinski and Duquesnel, 2007; Cogoni et al., 2013). Moreover, failures usually lead scientists to think of better methods and strategies for plant reintroduction and introduction. Some of them are for instance the utilization of ecological niche-based modelling tools (Guisan et al., 2006), the study of biological and ecological factors limiting the establishment of species (Guerrant Jr et al., 2007; Godefroid et al., 2011a; Drayton et al., 2012), a better use of comparison with reference populations as well as a better evaluation of sites prior to reintroduction (Menges, 2008; Drayton et al., 2012).

In the framework of plants translocation, the selection of suitable sites is a particularly complex topic (Falk et al., 1996; Guerrant Jr et al., 2007). Indeed, the choice of a suitable area requires a highly sophisticated understanding of species ecology and biology (Falk et al., 1996; Maschinski et al., 2012). As plants are sessile organisms, survival of seeds and seedlings can vary on a scale of less than 1 m² (Maschinski et al., 2012). Fiedler and Laven (1996) proposed four classes of site-selection criteria. The first class, i.e. physical criteria, involves both large-scale geomorphic factors and finer-scale parameters such as soil types. The second one, i.e. biological criteria, includes autoecological and synecological parameters. The third and the fourth criteria include logistical and historical factors (Fiedler and Laven, 1996; Guerrant Jr et al., 2007).

Studies presenting an explicit methodology for the research and the selection of translocation sites are unusual. Most of the papers that have been reviewed for the present study just mentioned that suitable sites for a particular species are selected, without giving more details. For example, to protect endemic endangered plant species in South Africa, managers and academics decided to translocate them. They selected translocation sites that were edaphically and biologically similar to native ones but did not explain which methods they specifically used to do it (Milton et al., 1999). However, following a part of the above-mentioned selection criteria, authors explained how they analysed species' niches using various tools in order to identify suitable reintroduction and introduction sites. For instance, bioclimatic analysis and modelling of the former and future dispersion range limits of an endangered bird (*Lichenostomus melanops cassidix*) were performed in Southern Australia in order to reintroduce it (Pearce and Lindenmayer, 1998). Another example is the integrated study of soil conditions, hydrology, microtopography, phytosociological analysis and existing literature of an herbaceous plant (*Saxifraga hirculus*) for conservation purpose (Vittoz et al., 2006). Similarly, edaphic and environmental factors were used to evaluate the ecology of an endangered plant species (*Primulina tabacum* Hance) (Ren et al., 2010). In another study, seedling recruitment and survival of endangered endemic limestone plant (*Purshia subintegra*) were tested measuring 16 environmental and edaphic factors in natural and experimental reintroduction sites (Maschinski et al., 2004). Therefore, while several different approaches have been independently proposed, a comprehensive multi-scale approach is still lacking.

I here used a model system (*Cypripedium calceolus* L., Orchidaceae) for the purposes of exemplifying a practical, hierarchical and holistic roadmap for building a species ecological niche. This roadmap was used to select appropriate reintroduction and introduction sites in Swiss Jura, and has the advantage of mixing modelling and field approaches as advised by Seddon et al. (2007). The complexity of their ecological particularities makes orchids perfect model species for testing and developing conservation strategies (Swarts et al., 2009).

I based the elaboration of this roadmap on the hypothesis that a good knowledge of the actual species' ecological and climatic niche allows finding suitable translocation sites. The first step of the roadmap includes modelling of bioclimatic and topographical niche of target species in both current and future scenarios in order to ensure long-term suitability of sites. The second step consists in analysing field parameters and measuring life history traits on native *C. calceolus*. These data were then statistically analysed to understand which parameters were the most important for the species' ecology. The third step consists in the improvement of the map obtained in step 1 using field data as well as the creation of a field guidebook with important parameters to be considered when performing translocations. At the same time, I studied above-mentioned field parameters of sites where reintroductions and introductions had already been carried out with a low success rate.

2. Methods

The hierarchical holistic reintroduction plan presented here is divided into three different steps (Figure 1). After selecting a plant species, determining its geographical distribution and studying its ecology, the first step involves modelling the species' current and future niche at two scales (continent-wide and regional) in order to obtain a prediction map to preselect translocation sites. In the second step, sites with native plants (reference populations) as well as potential sites for reintroduction and introduction are visited. Vegetation, soil and biological traits are studied and analysed in laboratory. In the third and last step, field and laboratory results are used to improve and refine the prediction map obtained in step 1. Finally, a field guidebook could be created as a complement to the map to help professionals to identify reintroduction sites.

The proceedings of the above-mentioned stages using a model species are detailed hereunder.

In this thesis, in step 2, in addition to the methodology mentioned above, I also visited sites with already reintroduced seedlings in order to understand why some reimplantations have not had the expected success.

Each stage of the workflow provided data and results that influence further steps. In the present section, some parts of the crucial results are briefly presented in order for the global process to be clearly explained.

Model species and geographical framework

The present study focuses on Lady's slipper Orchid (*Cypripedium calceolus* L., Orchidaceae, Figure 2) as a model species and Switzerland as geographical framework. The complexity of ecological particularities make orchids perfect model species for testing and developing conservation strategies (Swarts et al., 2009). This study area was selected because reintroductions and introductions of *C. calceolus* were performed in Swiss Jura with relative success rates (Storrer, 2016). Moreover, new reimplantations are planned in the coming years and research of reintroduction and introductions sites is then a high priority.

This orchid is a geophyte with a horizontal rhizome located up to 10 cm underground. Its height ranges from 15 to 50 cm and the plant presents 3 to 5 alternate and oval leaves. Its flowers (1 or 2, exceptionally 3) show a typical yellow clog-shaped labellum (Figure 2b) (Kull, 1999; Lauber et al., 2012).

Model species distribution and vegetation communities

Cypripedium calceolus is a well-known emblematic and patrimonial orchid in Europe. Indeed, this orchid is one of the most famous plants in Europe because of its particular flowers and its exotic look. It has fascinated botanists and non-botanists since the Renaissance (Terschuren, 1999). This Eurasian boreal species is distributed between the United Kingdom and the Pacific Ocean (Figure 3). Its altitudinal range varies from sea level to 2700 m in Switzerland. This represents a large repartition area but actual populations have a scattered distribution (Kull, 1999; Terschuren, 1999; Druart, 2007). Regarding landscape and vegetation, *C. calceolus* is mainly found in shady deciduous and mixed woodland and sometimes in stone-strew slopes (Kull, 1999). At higher altitude, it can grow in full sunlight (Kull, 1999; Terschuren, 1999). That said, in Switzerland, it is identified in 14 different vegetation alliances with an optimum in *Cephalanthero-Fagenion* (xerothermophilous beech forest) and *Erico-Pinion* (basophilic subcontinental pine forest) (Käsermann, 1999; Delarze et al., 2015).

Moreover, Druart (2007) mentions that *C. calceolus* has pioneer characteristics and grows in transition communities which have strong dynamic.

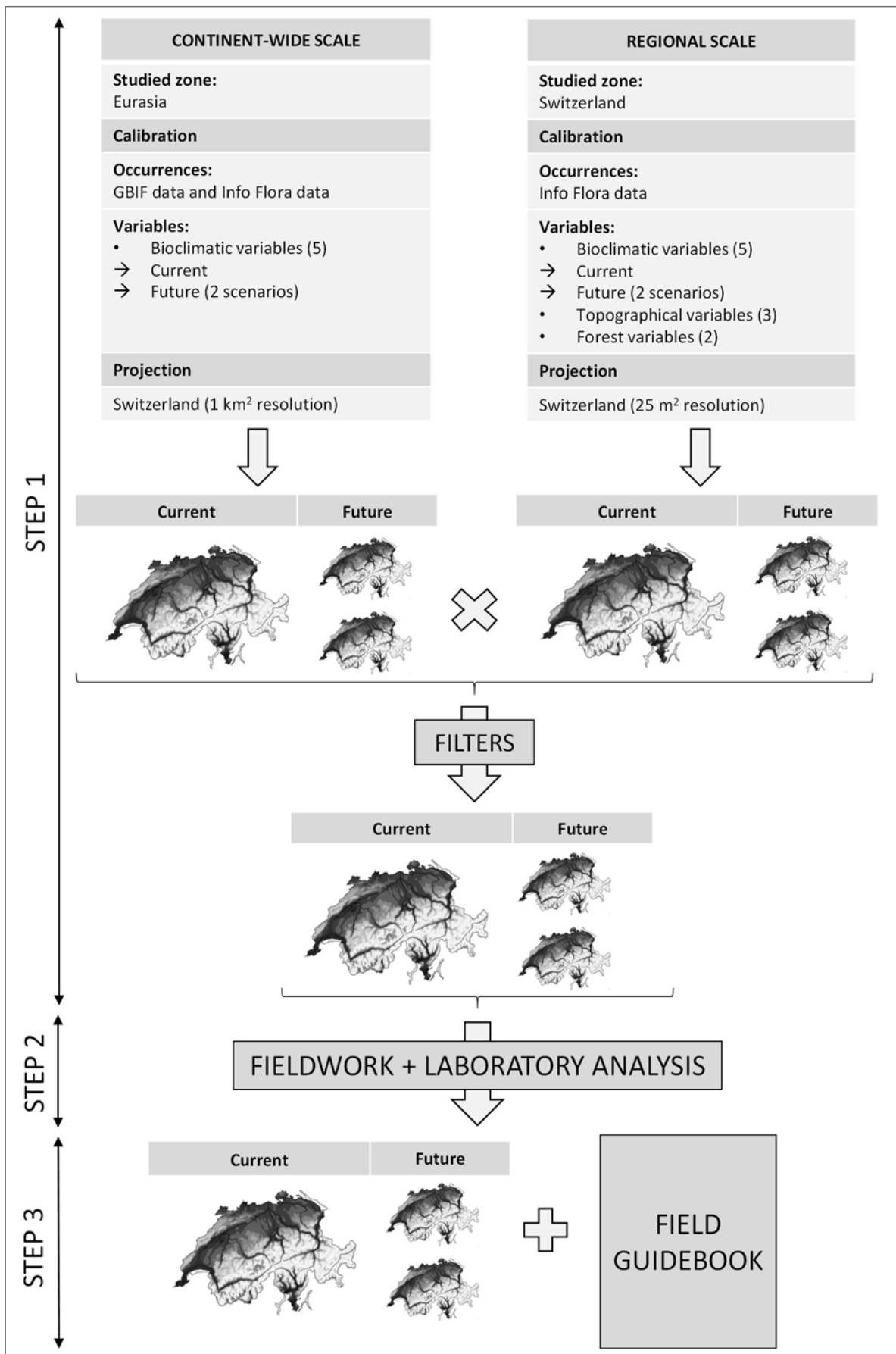


Figure 1 : Hierarchical holistic approach leading to the selection of appropriate reintroduction sites.
For more details, see *Material and Methods* section.

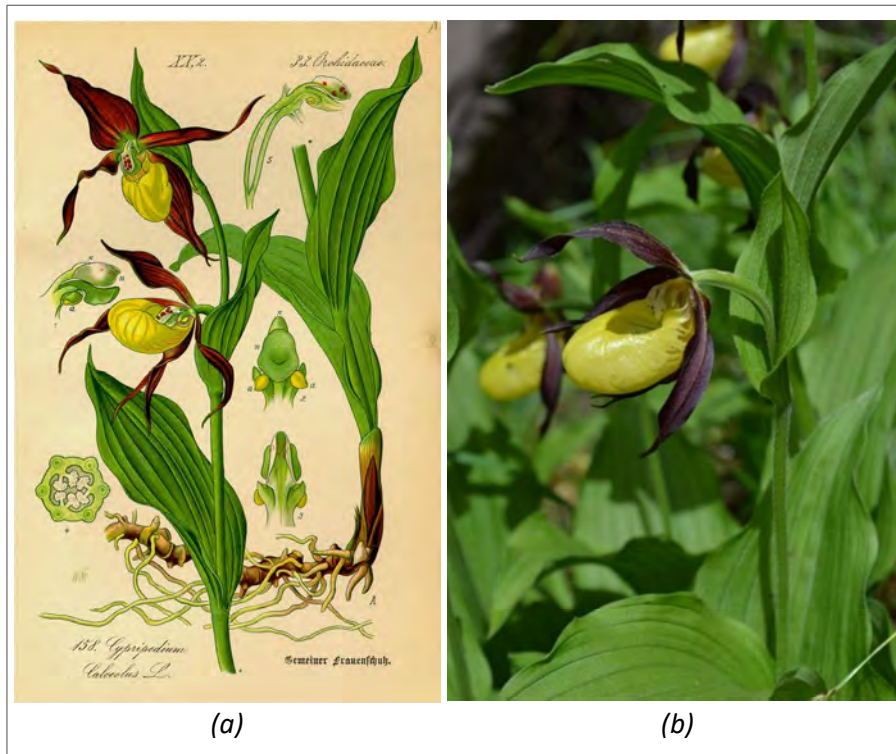


Figure 2 : (a) *C. calceolus* general anatomy (Source: Wilhelm Thomé, 1885). (b) Typical yellow clog-shaped labellum.

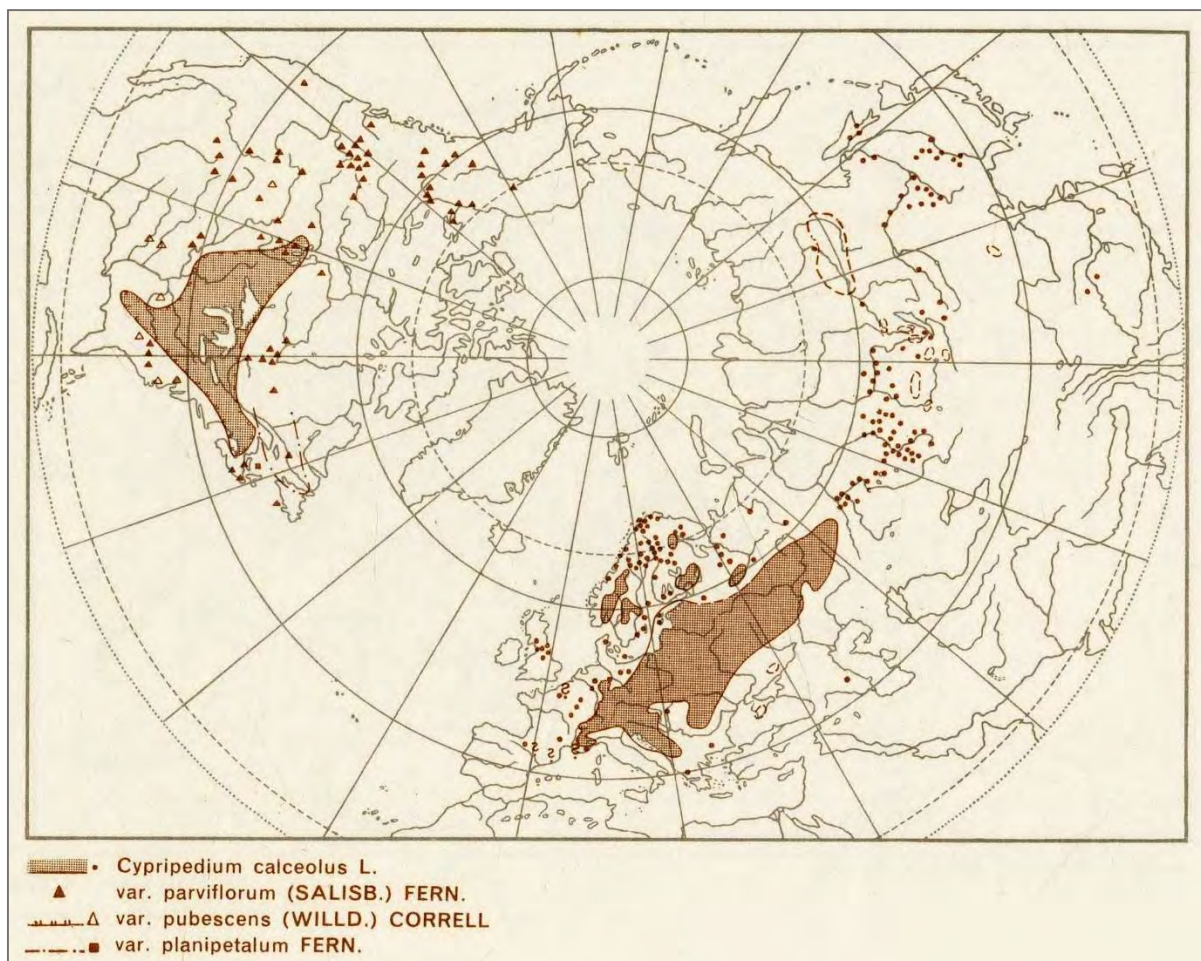


Figure 3 : *C. calceolus* Eurasian distribution (Source: Meusel et al., 1978).

Model species ecological requirements and development

Based on natural history observations, the ecological requirements of *C. calceolus* are principally depending on three factors: (i) light, (ii) soil moisture and (iii) soil bases richness; and practitioners have generally postulated that suitable sites for the growth of this species consists on a restricted combination of these parameters (Terschuren, 1999).

Particularly, due to its high sensitivity to light conditions, this plant suffers from competition with a dense bush layer or with taller plants. On the other hand, studies showed that with more sunlight, plants exhibited shorter leaves and ramets (Kull, 1999). Therefore, *C. calceolus* has been thought to prefer shady areas without direct concurrence for light (Terschuren, 1999).

It has also been argued that *C. calceolus* needs soils containing calcium carbonates, and preferentially should be rich in bases (Kull, 1999). Therefore, parental materials are generally made of limestone or dolomite (Käsermann, 1999; Kull, 1999). According to Käsermann (1999), soil pH requirements range from neutral to moderately acidic, while texture needs to contain fine particles such as clay or silt. It is interesting to notice that this plant will grow on richer substrates when in shade, compared to when growing in sunnier conditions, likely due to the competition effect with other plant species (Käsermann, 1999). Concerning soil moisture, according to Terschuren (1999), *C. calceolus* grows on moderately moist soils that are quiet dry during summer. To conclude with pedological information, this orchid usually grows on Moder humus form.

Model species ecology

C. calceolus has a very slow development and a long life expectancy, potentially living more than 30 years. The first green leaves appear from 1 to 4 years after germination and flowering occurs 6 to 16 years after germination (Fast, 1985; Kull, 1988; Kull and Tuulik, 1994; Rasmussen, 1995).

C. calceolus is either able to reproduce vegetatively or sexually. Vegetative reproduction occurs through the production of tufts of clones from rhizome ramification. A study demonstrates that clones patches are rarely larger than 70 cm in diameter (Kull, 1997; Kull, 1999). Regarding sexual reproduction, *C. calceolus* is most frequently pollinated by solitary bees of genera *Andrena*, *Lasioglossum* and *Halictus*. The floral fragrance emitted by *C. calceolus* has chemical similarities with those pollinator's pheromones (Kull, 1999). Insects enter the front opening of the labellum, fall in it and cannot leave by the same way. They have to cross the labellum, allowing them to touch the stigma and then the anthers before finding the exit (Kull, 1999). This plant does not produce nectar and its pollination system is therefore deceptive. As often, with such pollination systems, pollination success and fructification rate is weak. This rate varies depending on studied populations (Terschuren, 1999). From 1984 to 1995, eight stations were monitored and showed a fructification success rate of 10.5% on average per flower (Kull, 1998). Seeds develop during sixteen to twenty weeks. Then, capsules separate during wet weather and dust-like seeds are dispersed by the wind (Figure 4) (Bockel, 1972; Kull, 1998). No precise data about germination success seems to exist. Kull and Tuulik (1994) and Kull (1998) observed a range from 1 to 10 seedlings out of one seeds emission (5940 to 16700 seeds) (Kull, 1997). During first growing seasons, germination and growth of *C. calceolus* seems to be tightly dependent on orchidaceous mycorrhizal fungi (Kull, 1999), particularly of the genus *Tulasnella* (Shefferson et al., 2007; Oja et al., 2015).



Figure 4 : *C. calceolus* capsule.

Model species conservation

This plant, whose number and size of populations have drastically regressed, is rare and threatened with extinction, especially in Europe, including Switzerland (Figure 5) (Käsermann, 1999; Terschuren, 1999). Its decline is mainly attributed to three factors: (i) loss of habitats, (ii) narrow and specific ecological requirements and (iii) *C. calceolus*' survival and reproduction capacities. Those issues are detailed in Table 1 (Wood et al., 1984; Ramsay et al., 1998; Käsermann, 1999; Terschuren, 1999; Druart, 2007).

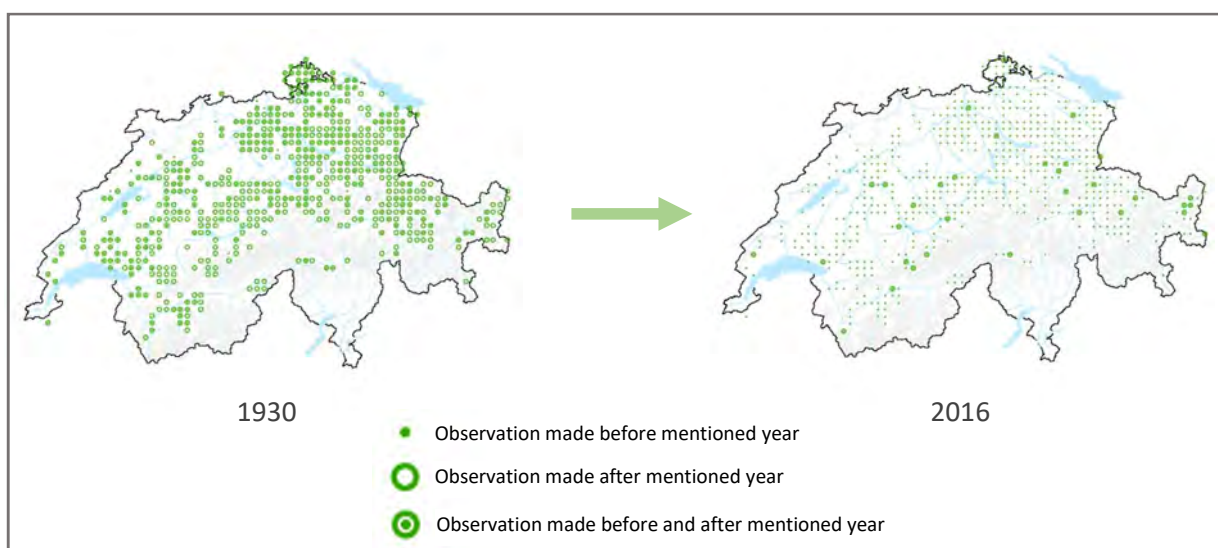


Figure 5 : *C. calceolus* Swiss population evolution between 1930 and 2016. This map doesn't show the actual population of *C. calceolus* for a given year, but the observations that have been, are and will be made for the year in question.
(Source: www.infoflora.ch.)

Table 1 : *C. calceolus* decline causes, grouped in three categories (Wood et al., 1984; Ramsay et al., 1998; Käsermann, 1999; Terschuren, 1999; Druart, 2007).

Decline causes	
Loss of habitats	Forest canopy closure, shadow, bushland expansion
	Forest roads construction
	Pastures in forest
	Intensive forestry
	Change in water regime and lowering of groundwater table
	Trampling
	Eutrophication
Narrow and specific ecological requirements	Lack of knowledge about ecological requirements
	Lack of knowledge about population ecology
<i>C. calceolus</i> survival and reproduction capacities	Picking
	Digging up of rhizomes
	Small and scattered populations
	Grazing
	Lack of pollinator insects
	Weak rate of pollination success
	Weak recruitment rate

Due to the above-mentioned factors, this species is legally protected in European countries and in Russia (Kull, 1999). It has been placed in Appendix I of Bern Convention and in Appendix II of European Union Directive about natural habitats conservation. Moreover, it appears in Appendix II of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Terschuren, 1999).

All over its distribution area, action plans have been set up to try to protect it. In some countries, reintroduction plans have been created (Ramsay et al., 1998; Terschuren, 1999; Druart, 2007). For example, in Britain, a redeployment program has begun years ago. It has involved reinforcement of existing population and reintroductions of new seedlings cultivated in botanical garden from seeds of native plants. This program seems to be really efficient (Ramsay et al., 1998).

In Switzerland, this plant is on the Red List and the International Union for Conservation of Nature (IUCN) considers it as *vulnerable* (Terschuren, 1999; Druart, 2007). In this country, *C. calceolus*' populations have slightly declined in the Alps. However, its decline in Swiss Jura and in Le Plateau is very important, giving to it the UICN status *in danger* in this area (Käsermann, 1999; Druart, 2007). Actions plans exist in different cantons. In addition, reintroductions have been performed in Swiss Jura with a weak rate of success until today. Former selection of reintroduction sites was only based on vegetation community and companion species (*Calamagrostis varia* (Schrab.), *Carex flacca* Schreb. and *Molinia arundinacea* Schrank) (Druart, 2007; Storrer, 2016). As mentioned above, new reintroduction and introductions are planned in this area in the coming years (Storrer, 2017). Selection of ecologically adapted sites is then a priority.

Step 1: Niche modelling

In the modelling step, *C. calceolus*' current and future ecological niches are defined with species distribution models (SDMs) (Guisan and Thuiller, 2005) at two different scales in order to get predictions maps indicating favourable reintroduction sites. First, I calibrated Eurasian models (Figure

1, continent-wide scale: current and future) with Eurasian occurrences and bioclimatic variables. Then, I projected calibration results on Swiss Jura map at 1 km² resolution. I used Ensemble Forecasting to get final predictions maps. Secondly, I calibrated Swiss models (Figure 1, regional scale: current and future) with Swiss occurrences, bioclimatic variables, topographical variables and forest variables. After that, I projected calibration results on Swiss Jura map at 25 m² resolution. As mentioned before, I used Ensemble Forecasting to obtain final predictions maps.

Prediction maps (continent-wide and regional scale) are then multiplied to combine presence probability from European and Swiss calibration. To refine prediction maps, I added geological and environmental filters (Broenimann, 2017b) (Figure 1, Step 1).

All this process is detailed hereunder.

Occurrences

At European scale, I extracted European occurrences from the Global Biodiversity Information Facility (GBIF, www.gbif.org). Then, I post-processed data to remove records that were not accurate enough (more than 1 km precision), without sampling date, collected before 1960, out of distribution area, with unrealistic coordinates or from botanical gardens (Gallien et al., 2012). Following this process, I selected 724 occurrences.

At Swiss scale, occurrences came from Info Flora database (www.infoflora.ch). In this case, I post-processed data in order to suppress records that were not precise enough (more than 25 m) or those that were older than 1960 (Figure 1, Step 1). In this way, 1423 occurrences were picked out.

Then, I disaggregated European and Swiss occurrences to avoid spatial auto-correlation (0.08 decimal degree and 250 m resolution respectively) (Broenimann, 2017b).

Modelling framework

At European scale, I used both GBIF and Info Flora disaggregated occurrences (397) to describe species' global niche. To the same end, I selected 10 sets of pseudo-absences in Eurasia background (Barve et al., 2011; Barbet-Massin et al., 2012). The number of pseudo-absences in each set was the same as the number of presences. Sets of pseudo-absences were selected in 10'000 random pseudo-absences (Barbet-Massin et al., 2012; Broenimann, 2017b).

I chose five descriptors to characterized global niche. For current scenario, those were 5 from 19 Bioclim variables (<http://www.worldclim.org/bioclim>): (i) annual mean temperature, (ii) mean diurnal range (mean of monthly (max. temperature – min. temperature)), (iii) max temperature of the warmest month, (iv) annual precipitation and (v) precipitation seasonality (Table 2). Those descriptors were the most consistent with species ecology and had a low pair-wise correlation (computation of matrix of Person and Spearman rank-correlation < 0.7). Variables resolution was 1 km² (Figure 1, Step 1). For future scenarios (2041-2060), I kept the same five Bioclim variables at 1 km² resolution (Table 2). I 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): RCP2.6 and RCP8.5 (Van Vuuren et al., 2011; Broenimann, 2017b) (Figure 1, Step 1). RCP2.6 assumes aggressive greenhouse gas reduction and sequestration efforts whereas RCP8.5 expects an increase in greenhouse gas emissions until the end of the 21st century (Snober et al., 2013). In order to have only one prediction map by RCP, IPSL-CM5A-LR (RCP2.6) and HadGEM2-AO (RCP2.6) prediction maps were combined. I did the same with IPSL-CM5A-LR (RCP8.5) and HadGEM2-AO (RCP8.5).

To describe a more detailed climatic and topographic niche of the species at regional scale (Switzerland), I used disaggregated Info Flora occurrences (525). As for European scale, 10 sets of pseudo-absences were selected, but this time within the Swiss background. The number of pseudo-absences in each set was the same as the number of presences. Sets of pseudo-absences were selected in 10'000 random pseudo-absences (Broenimann, 2017b). I selected ten descriptors to define national niche. For current scenario, I used the five same Bioclim variables as European scale but with a 25 m² resolution (Table 2). In addition, I selected three topographical variables corresponding to *C. calceolus*' ecology: (i) topographic aspect (aspval, corresponding to a sin-transformation of aspect, Aspect Tools of Spatial Analyst, ArcGIS 10.4), (ii) slope (slp25, Slope Tools of Spatial Analyst, ArcGIS 10.4) and (iii) topographic position (topos, (Zimmermann and Roberts, 2001)). I also added to the study system two variables with regard to: (i) the height of the canopy (Broenimann, 2016) and (ii) distance between occurrences of *C. calceolus* and forest border (Broenimann, 2017a). Those forest variables seemed particularly relevant, first because the canopy height gives information about the light reaching the soil. Secondly, digital map observations indicated that *C. calceolus* plants are frequently located near forest borders (forest distance smaller than 150 m. for 1243 over 1423 occurrences, Figure 6) (Broenimann, 2017a). Topographical and forest variables had 25 m² resolution (Figure 1, Step 1).

Table 2 : Bioclimatic variable used in the modelling process and their signification (<http://www.worldclim.org/bioclim>)

Variable	Signification
Bioclim 1	Annual mean temperature
Bioclim 2	Mean diurnal range (mean of monthly (max. temperature – min. temperature))
Bioclim 5	Max temperature of the warmest month
Bioclim 12	Annual precipitation
Bioclim 15	Precipitation seasonality

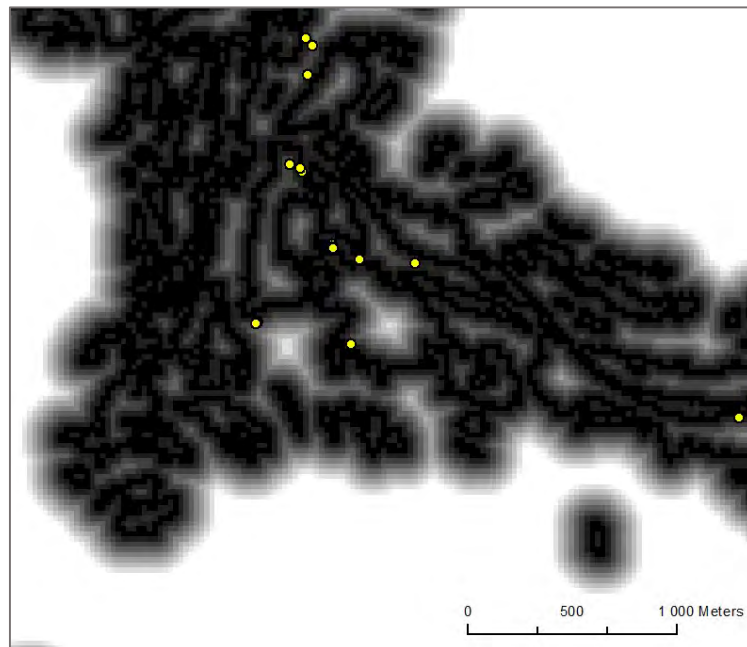


Figure 6 : Example of distance gradient from the forest border (black). *C. calceolus* occurrences are illustrated by yellow dots.

For future scenarios, I kept the same five Bioclim variables at 25 m² resolution (Table 2). However, identical time frame and scenarios as the one used at European scale weren't available. Therefore, I chose period of time and scenarios as similar as possible as European ones. I therefore selected scenario RCP3PD (very low annual total CO₂ emissions) and scenario A2 (high annual total CO₂ emissions) for 2045-2075 (Figure 1, Step 1) (Snoover et al., 2013; Broenimann, 2017b). I kept the same topographical and forest variables as in present scenario.

To model European and Swiss distribution of *C. calceolus*, I processed three algorithms included in biomod2 (Georges and Thuiller, 2013; Thuiller et al., 2016) in R (R Development Core Team, 2014): (i) Generalized Linear Model (GLM), (ii) Generalized Boosting Model, usually called Boosted Regression Trees (GBM) and (iii) Generalized Additive Model (GAM) (Gallien et al., 2012; Thuiller et al., 2016). For each set of pseudo-absences (10), each modelling technique (3) was run three times (Broenimann, 2017b). European and Swiss models were then projected on Switzerland.

To avoid circular reasoning in models evaluations, I 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 area under the receiver operating characteristic curve (AUC)(Swets, 1988) and with the true skill statistic (TSS)(Allouche et al., 2006; Gallien et al., 2012; Broenimann, 2017b).

Then, I used Ensemble Forecasting to create prediction maps for Switzerland. Two maps were produced by time scenarios for European and Swiss scale: (i) a map showing mean prediction weighted by models quality (TSS and AUC) and (ii) a map illustrating coefficients of variation between different models. Ensemble Forecasting was also evaluated with TSS and AUC (Broenimann, 2017b).

Final map

In order to combine European and Swiss scale modelling results, probability of presence (prediction maps) by different scenarios (current, future with very low CO₂ emissions, future with high CO₂ emissions) were multiplied to obtain a final probability map for Switzerland (Figure 1, Step 1).

Two filters were also added for a better accuracy of predictions (Figure 1, Step 1). The first one excludes parts of the landscape where *C. calceolus* cannot grow (e.g. lakes, urban areas, snow-covered mountain tops, etc.). Land cover data used for this come from Primary Surfaces layer in VECTOR25, a digital landscape model of Switzerland including natural and artificial objects (www.swisstopo.admin.ch) (Niederöst, 2003). Number of occurrences (Info Flora) per category were counted, and categories with too few occurrences or with improbable presence were removed. The second filter contains bedrock geology of all Switzerland (Carte géotechnique 200, www.map.geo.admin.ch). As for landscape filter, occurrences per category were summed. Once again, those with too few occurrences or unrealistic presences are deleted. For both filters, details about number of occurrences per category can be found in Appendix I. Finally, I post-processed prediction maps with ArcMap (ArcGIS 10.4, 2016).

Step 2: Fieldwork and laboratory analysis

Study area, sites research and selection

For the above-mentioned reasons, the field part of this study was conducted along Jura Mountains in Switzerland (Figure 7). In order to find reintroduction sites and to understand reintroduction inefficiency in this area, I analysed three types of sites: (i) sites containing *C. calceolus* native populations, (ii) sites with reintroduced seedlings and (iii) potential sites for future reintroductions.

Sites localisations were given by the private Swiss foundation Info Flora, by environmental departments of cantons (Genève, Vaud, Neuchâtel, Bern and Aargau), by nature reserves and by professional and amateur botanists.

The translocations studied here took and are going to take place within the historic range of the species but sometimes in sites where this species has never been known to occur. From here and for a question of simplicity, all studied sites are named as “reintroduction sites”.

I selected eleven sites over thirteen localized with native plants. Chosen sites were the less impacted by human action. Five sites with reintroduced seedlings were randomly selected over eight already known. I chose four sites for future reintroduction in areas selected by Neuchâtel environmental department in historical locations within the framework of *C. calceolus* conservation plan (Table 3).

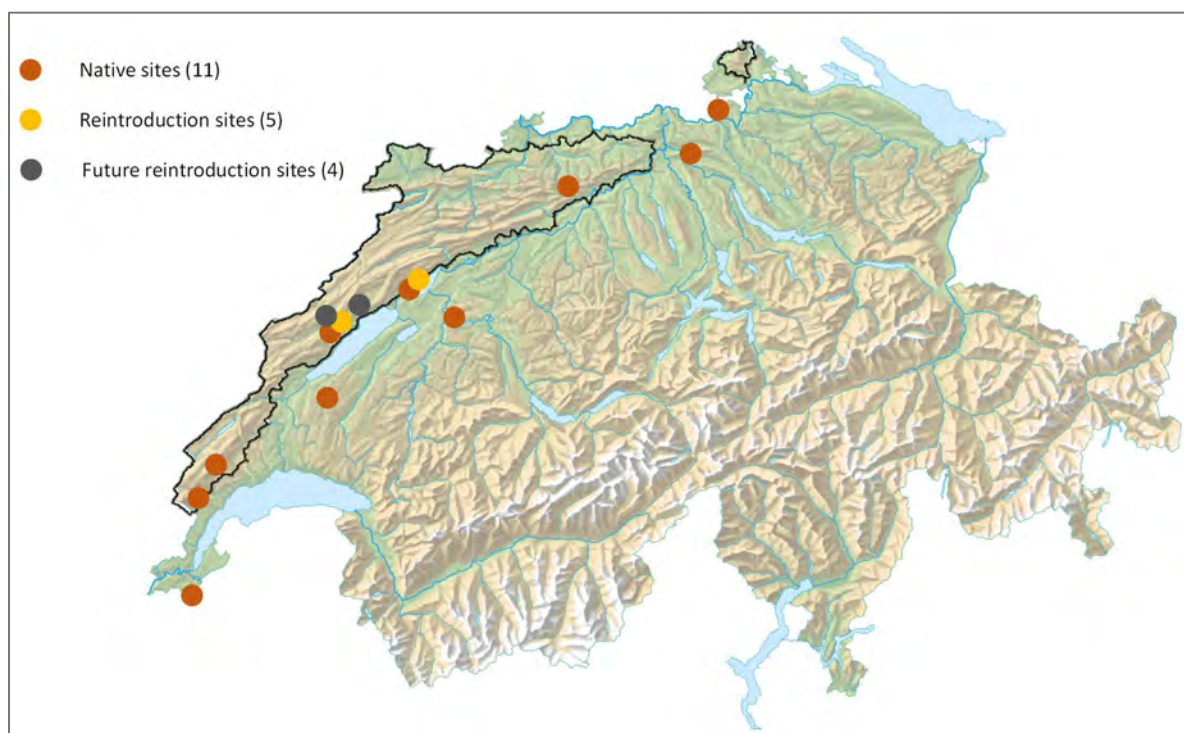


Figure 7 : Locations of study sites: native, reintroduction and future reintroduction. Swiss Jura is highlighted in black.
(Source background image: <http://www.cartograf.fr>.)

Table 3 : List of study sites

Status	Location	Canton/Country	Site name
Native	Blécheins	France	NAT-GE1
Native	Fey	Vaud	NAT-VD1
Native	Chésereux	Vaud	NAT-VD2
Native	Saint-George	Vaud	NAT-VD3
Native	Creux-du-Van	Neuchâtel	NAT-NE1
Native	Creux-du-Van	Neuchâtel	NAT-NE2
Native	Oltigen	Bern	NAT-BE1
Native	La Neuveville	Bern	NAT-BE2
Native	Langenbruck	Basel	NAT-BL1
Native	Freienwil	Aargau	NAT-AG1
Native	Bühl	Germany	NAT-ZH1
Reintroduction	Creux-du-Van	Neuchâtel	REINTRO-NE1

Status	Location	Canton/Country	Site name
Reintroduction	Creux-du-Van	Neuchâtel	REINTRO-NE2
Reintroduction	Creux-du-Van	Neuchâtel	REINTRO-NE3
Reintroduction	Saint-Imier	Bern	REINTRO-BE1
Reintroduction	Neuveville	Bern	REINTRO-BE2
Future reintroduction	Lanvoennes	Neuchâtel	REFUT-NE1
Future reintroduction	Lanvoennes	Neuchâtel	REFUT-NE2
Future reintroduction	Valangin	Neuchâtel	REFUT-NE3
Future reintroduction	Fenin	Neuchâtel	REFUT-NE4

In sites with native and reintroduced subjects, soils sampling localisations were 40 cm away of a randomly selected plant. I visited and studied sites selected for future reintroductions after the other treatments (native and reintroduction sites). I chose sampling points for future reintroduction thanks to their visual similarity with native stations seen previously.

Station parameters

At each site, I initially measured exposition, slope, micro-topography and altitude. In addition, I characterized the number of hours of direct and indirect sunlight reaching *C. calceolus* using a sun-compass based on a 0 to 4 scale where 0 meant full shadow and 4 meant full sunlight (Sonnenkompass, Herzog Forsttechnik AG; Figure 8, Appendix II). Finally, I extracted the same variables (climatic, topographical and forest parameters) I used for the modelling step for each studied site.

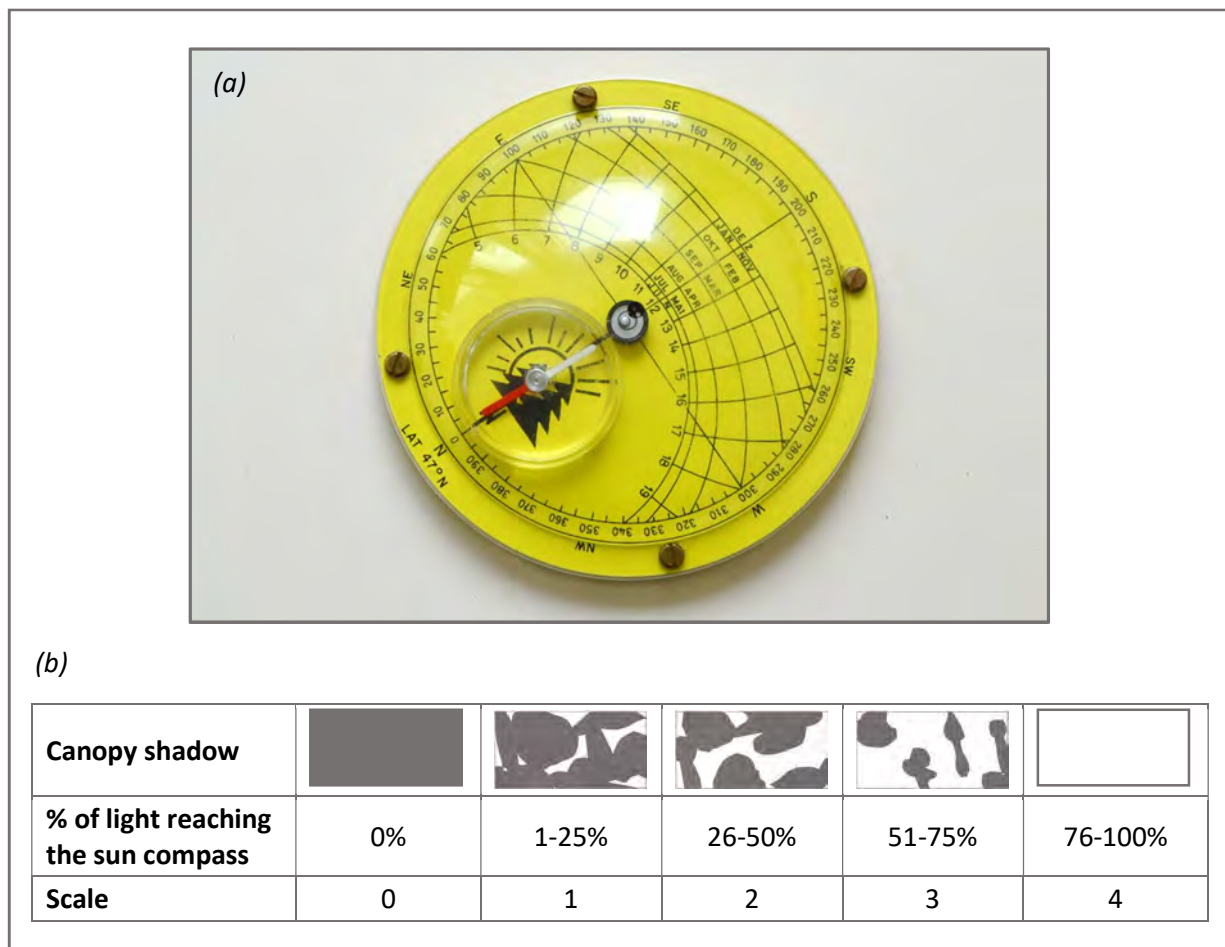


Figure 8 : (a) Sun-compass; (b) Quantification scale of sunlight reaching *C. calceolus* per zone of the solar compass (hours and months): 0 means full shadow, 4 means full sunlight

Vegetation study

In order to describe the vegetation type associated with *C. calceolus* populations, I performed at each site phytosociological surveys using the Braun-Blanquet's method (Braun-Blanquet, 1968). Areas analysed were chosen because of their station representativeness and their homogeneity. Their surface areas were of 100 m². In different vegetation strata (herbaceous, shrubby and woody), I visually evaluated coverage (%) and I realized an exhaustive list of plant species. I performed species identification thanks to reference publications in Switzerland (Aeschimann et al., 2005; Lauber et al., 2012; Eggenberg et al., 2013). For each species, I visually evaluated coverage and aggregation levels thanks to Braun-Blanquet and Pavillard's (Braun-Blanquet and Pavillard, 1928) and Gillet's scales (Gillet, 2000).

In the field, I initially determined vegetation alliance with *Le guide syntaxonomique de la végétation de Suisse* (Gallandat et al., 2012). Afterwards, I realised vegetation tables with plants sorted by different habitats (Delarze et al., 2015) to confirm and sharpen field observations.

Humus forms and edaphic parameters (field work + laboratory)

At each site, I dug a soil profile until bedrock or parental material, minimum 40 cm away of the studied plant, seedling or future reintroduction point in order to not damage roots. I separated soil profiles in organic, organo-mineral and mineral horizons according to each homogenous zone observed. Using *Le Protocole de description des sols* (Gobat et al., 2013), I described and analysed each profile and horizon (Appendix III). In addition, I measured soil temperature in organo-mineral horizon. Then, I identified horizons and solums using *La Clé de Sol* (Gobat et al., 2014) and I named them according to *Le Référentiel pédologique* (Baize and Girard, 2009). Further laboratory analysis allowed confirming first identification. Thanks to Zanella et al. (Zanella et al., 2011), I determined humus forms. I collected samples of each horizon for laboratory analysis.

In order to understand and to describe physicochemical proprieties of soils, I performed laboratory analyses.

In organic horizons (OL, OF, OH), I quantified water content by successive desiccation and weighing (LEF, 2007b). In addition, organic carbon on total nitrogen ratio (C/N) was measured using an elemental analyser (Flash 2000, CHN-O Analyzer, Thermo Scientific) in order to describe biological activity and litter type (LEF, 2015).

In the organo-mineral and mineral horizons, I analysed water content and residual moisture by successive desiccation and weighing (LEF, 2007b).

Regarding organic matter, C/N ratio was also quantified (LEF, 2015). In addition, I performed ignition loss by heating samples at 450°C for two hours (LEF, 2007a).

To evaluate soil acidity, cations and limestone content, I conducted several experiments. First, pH_{H2O} was analysed using an electrode quantifying H⁺ ion in solution (LEF, 2010). Secondly, I measured cationic exchange capacity (CEC) with cobaltihexamine and spectrometer method (LEF, 2014). Thirdly, I evaluated limestone content with the Benard Calcimeter method (LEF, 2012b).

To analyse textural proprieties, mineral grain size was quantified with organic matter destruction, clay dispersion, sedimentation and sieving method (LEF, 2012a). In addition, I estimated percentage of clay per organo-mineral horizon by linking field analysis and textural triangle (Gobat et al., 2010).

To conclude, I determined colour of each horizon using Munsell soil colour chart (Munsell, 2009).

Populations and plants fitness measurements

In order to understand the relationship between *C. calceolus* populations' physiological state and environmental parameters, I measured plant's life history traits. In order not to endanger this extremely fragile species, I selected traits that could be quantified using non-intrusive methods. In sites with native subjects, I analysed traits on ten plants: the studied one and nine others (or less if there is not enough) randomly chosen within a 40 m diameter circle around the initially-selected one. Circle size was the result of a trade-off between having enough plants and keeping an area as homogenous as possible. I considered a subject a clone patch.

Biological traits studied were: vitality, grazing, plant size, number of stems, number of leaves, number of flowers, number of pods, leaves area, photosynthetic activity and presence of seedling (Appendix IV). All those traits are detailed in Table 4.

Table 4 : Measured life history traits of *C. calceolus* per subject.

Biological traits	Description
Vitality	Index from 1 to 3 depending on leaves appearance and colour, number of stems and flowering. 1 means minimum vitality and 3 means maximum vitality.
Size	Plant size, measured in centimetres, from the ground to the top of the highest leaf.
Stems	Number of stems per subject.
Leaves	Number of leaves of the longest stem.
Median leaf area	Median leaf of the highest stem area, calculated using the ellipse area formula.
Photosynthetic activity	Median leaf photosynthetic activity, measured with a chlorophyllometer (SPAD, Minolta)
Flowers	Number of flowers per subject.
Pods	Number of pods per subject.
Seedlings	Presence or absence and number of seedlings (plants smaller than 10 cm) around a subject.
Grazing	Grazing per subject, estimated in percentage.

Statistical analysis

I performed all statistical analysis in R (R Development Core Team, 2014). First, I calculated correlations between measured station parameters and existing data from topographical ESERI calculation layers such as topographic aspect (aspval) and slope (slp25) to validate correspondence between information from databases and measured data.

In addition, I summed sun-compass data per plants per station. Then, I evaluated the correlation between this value and daily average of global potential shortwave radiation per month (srad) (Kumar et al., 1997) using Pearson's regression analysis. For further statistical analysis, summed sun-compass values were included in the category of bioclimatic extracted values.

To understand the relationships between variables, I calculated correlations between them for topographical and bioclimatic extracted data as well as humus and edaphic parameters.

Furthermore, in order to see if differences between statuses and between populations were explained by the studied parameters, I performed multivariate analysis of variance (PERMANOVA, Adonis function, vegan package)(Oksanen et al., 2013) with topographical, bioclimatic, humus and edaphic data. For soils variables, on the one hand, I performed Adonis function on complete soils (all horizons) and on A horizons only and on the other hand, I did PCA only on A horizons.

Populations were then ordinated with principal component analysis (PCA) by environmental parameters to understand effects of variables on population repartition.

Moreover, in order to understand differences and similitudes between statuses (native, reintroduction and future reintroduction stations), I compared variables per status with permutational multivariate analysis of variance (PERMANOVA, `adonis` function, package `lmPerm`) (Wheeler et al., 2016). I chose such a statistical test because data were not distributed normally. In addition, I calculated for each variable the coefficient of variation between statuses. Then, I tested the equality of those coefficients with an Asymptotic test (`asymptotic_test` function, package `cvequality`) (Marwick, 2016).

For soils data, I performed permutational multivariate analysis of variance and coefficient of variation equality only on the organo-mineral horizon (A horizon) of each soil. The choice of A horizon was functional. Indeed, it is in this part of the soil that *C. calceolus* have their roots (Kull, 1999).

Biological traits of native plants were averaged by population. Then, I performed a permutational multivariate analysis of variance (PERMANOVA, `adonis` function, `vegan` package) (Oksanen et al., 2013) on those traits to see if they explained differences between Swiss Jura populations. Afterwards, *C. calceolus* plants were ordinated by biological traits to understand the effect of variables on populations' repartition (PCA).

Finally, I sought to disentangle the influence of environmental variables (topographical, climatic, and edaphic) on the performance of native populations using the function `envfit` (package `vegan`) (Oksanen et al., 2013). This function fits environmental variables onto an ordination (here NMDS). Studied traits (here plants populations) are then projected onto vectors that have maximum correlation with corresponding environmental variables (Oksanen et al., 2013). Moreover, I performed ordination and linear regressions to analyse and to understand the meaning of `envfit` results.

Step 3: Niche modelling improved with field data and field guidebook

The last step of this roadmap consists in improving modelling results with field data. This is only possible if field parameters exist in digitalised version for the study area.

In this case, solar radiation, which is an important factor for this species ecology (see Results section), exists in raster format for Switzerland. Therefore, I added an extra filter with adapted range of values for *C. calceolus* to obtain a final prediction map.

Finally, all ecologically important parameters that had not been digitalised yet were summarized in a field guidebook to help professionals identifying suitable future reintroduction sites.

3. Results

Step 1: Niche modelling

Figure 9 shows different steps of modelling for current scenario in order to highlight the importance of the hierarchical process. Figure 10 and Figure 11 present results of future scenarios.

At continent-wide scale, models showed good performances (TSS > 0.6 and AUC > 0.8 in average for individual projections and for Ensemble Modelling, Table 5). Moreover, Bioclim 1 and Bioclim 5 were the two variables influencing the most the prediction map at this scale (Table 2, Table 6).

At regional scale, models also showed good performances (TSS > 0.6 and AUC > 0.8 in average for individual projections and for Ensemble Modelling (Table 5). Besides, Bioclim 15, canopy height and forest distance were the most determinant variables (Table 2, Table 6).

Final maps

Current scenario

At the continent-wide scale, I obtained a prediction map showing that a large part of Switzerland was favourable for *C. calceolus* according to the used bioclimatic variables (Figure 9a). At regional scale, favourable areas according to the studied parameters were scattered and sparse contrary to wide-scale predictions (Figure 9b).

The multiplication of continent-wide scale with regional prediction map did not exclude many areas, because continent-wide scale prediction map was favourable for nearly all the studied area (Figure 9c). The addition of filters discriminated areas in different parts of Switzerland (Figure 9d). The final map obtained at the end of Step 1 for current scenario (Figure 9d) showed that favourable reintroductions sites stood in the Plateau, the north of the Alps and valleys of western and eastern central Alps. Areas that seemed less conducive for *C. calceolus* are Swiss Jura and the south of the Alps.

Future scenarios

Very low annual total CO₂ emissions (Figure 10)

At continent-wide scale with RCP2.6, both IPSL-CM5A (Figure 10a) and HadGEM2-A0 (Figure 10b) showed mainly unfavourable areas in the north part of Switzerland. HadGEM2-A0 scenario described Switzerland as a less favourable zone for *C. calceolus* than IPSL-CM5A. At regional scale, favourable areas were scattered and rare (Figure 10c). In addition, north part of Switzerland (mainly Swiss Jura) showed less suitable areas than in the current scenario.

The multiplication of continent-wide and regional scales excluded many areas mainly in Swiss Jura and in le Plateau, making favourable areas rarer (Figure 10d). The addition of filters discriminated areas mainly in Ticino. The final map obtained showed very few suitable areas for *C. calceolus*. Those areas were located predominately in the eastern and south part of Switzerland (Figure 10e).

High annual total CO₂ emissions

Prediction maps with high annual CO₂ emissions (RCP8.5) showed the same trends, but with an increased rate of unfavourable areas than those calibrated with RCP2.6 (Figure 11). At continent-wide scale, both IPSL-CM5A (Figure 11a) and HadGEM2-A0 (Figure 11b) showed mainly unsuitable areas in the north part of Switzerland. Once again, HadGEM2-A0 scenario described Switzerland as a less favourable zone for *C. calceolus* than IPSL-CM5A. At regional scale, favourable areas were scattered and rare (Figure 11c). In addition, north part of Switzerland (mainly Swiss Jura) showed less suitable areas than with the current scenario and with RCP2.6 scenario.

The multiplication of continent-wide and regional scales excluded many areas mainly in Swiss Jura and in le Plateau making favourable areas rarer (Figure 11d). The addition of filters discriminated areas mainly in Ticino. The final map obtained showed very few suitable areas for *C. calceolus*. As for RCP2.6, these areas were located predominately in the eastern and south part of Switzerland (Figure 11e).

CURRENT MODELS

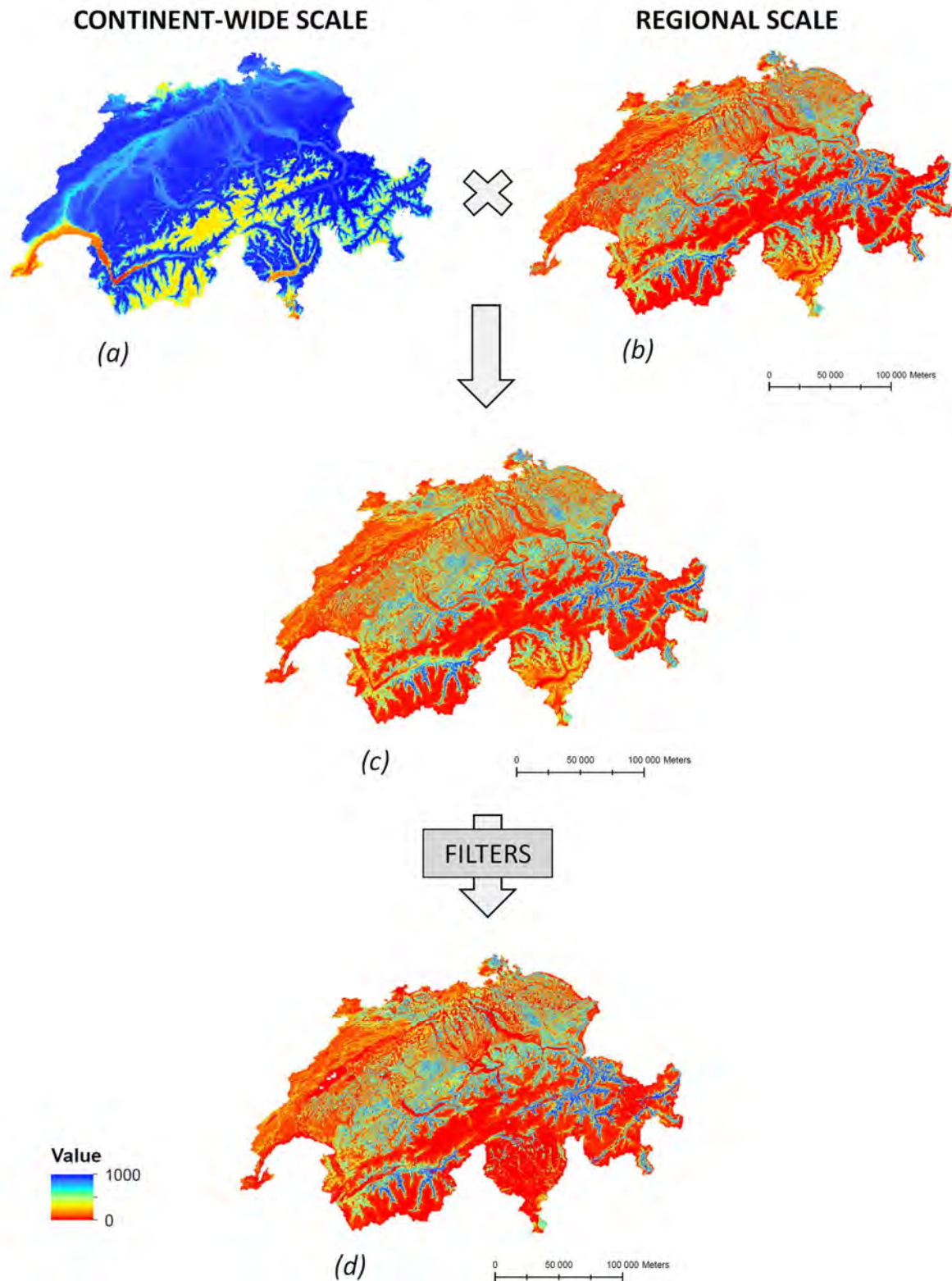


Figure 9 : Results of the modelling of Step 1 for current scenario. (a) Prediction map calibrated with Eurasian occurrences and five bioclimatic variables. (b) Prediction map calibrated with Swiss occurrences and five bioclimatic variables, three topographical variables and two forest variables. (c) Combination of (a) and (b). (d) Prediction map with addition of land cover and geological filters. Legends: Probability of presence according to studied parameters: 0 (red) means area predicted to be not adapted to *C. calceolus*; 1000 (blue) means area predicted to be highly favourable to *C. calceolus*.

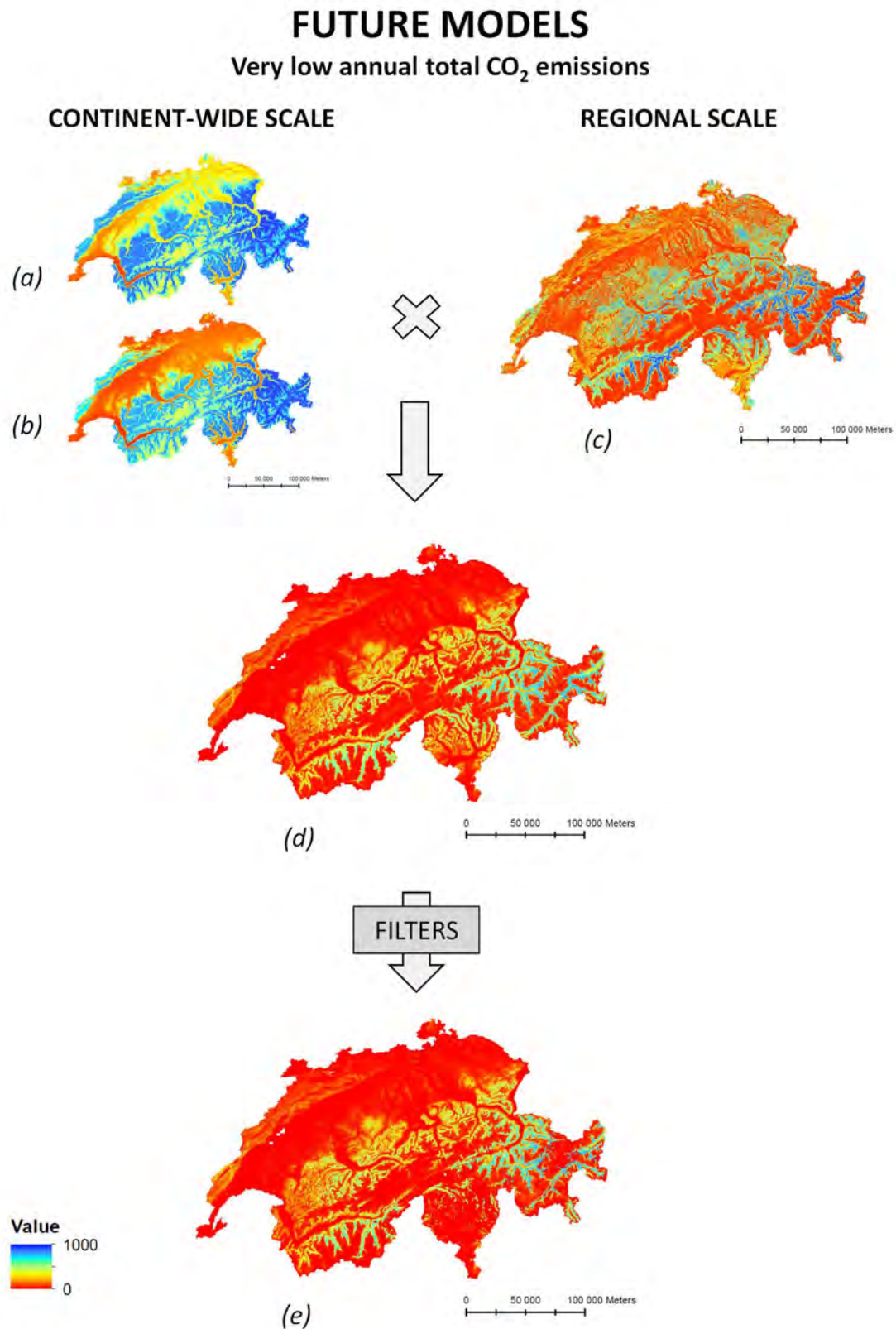


Figure 10 : Results of the modelling of Step 1 for future scenario with very low annual total CO₂ emissions (RCP2.6). (a) Prediction map calibrated with Eurasian occurrences and five bioclimatic variables (IPSL-CM5A-LR). (b) Prediction map calibrated with Eurasian occurrences and five bioclimatic variables (HadGEM-2A0). (c) Prediction map calibrated with Swiss occurrences and five bioclimatic variables (RCP3PD), three topographical variables and two forest variables. (d) Combination of (a), (b) and (c). (e) Prediction map with addition of land cover and geological filters. Legends: Probability of presence according to studied parameters: 0 (red) means area predicted to be not adapted to *C. calceolus*; 1000 (blue) means area predicted to be highly favourable to *C. calceolus*.

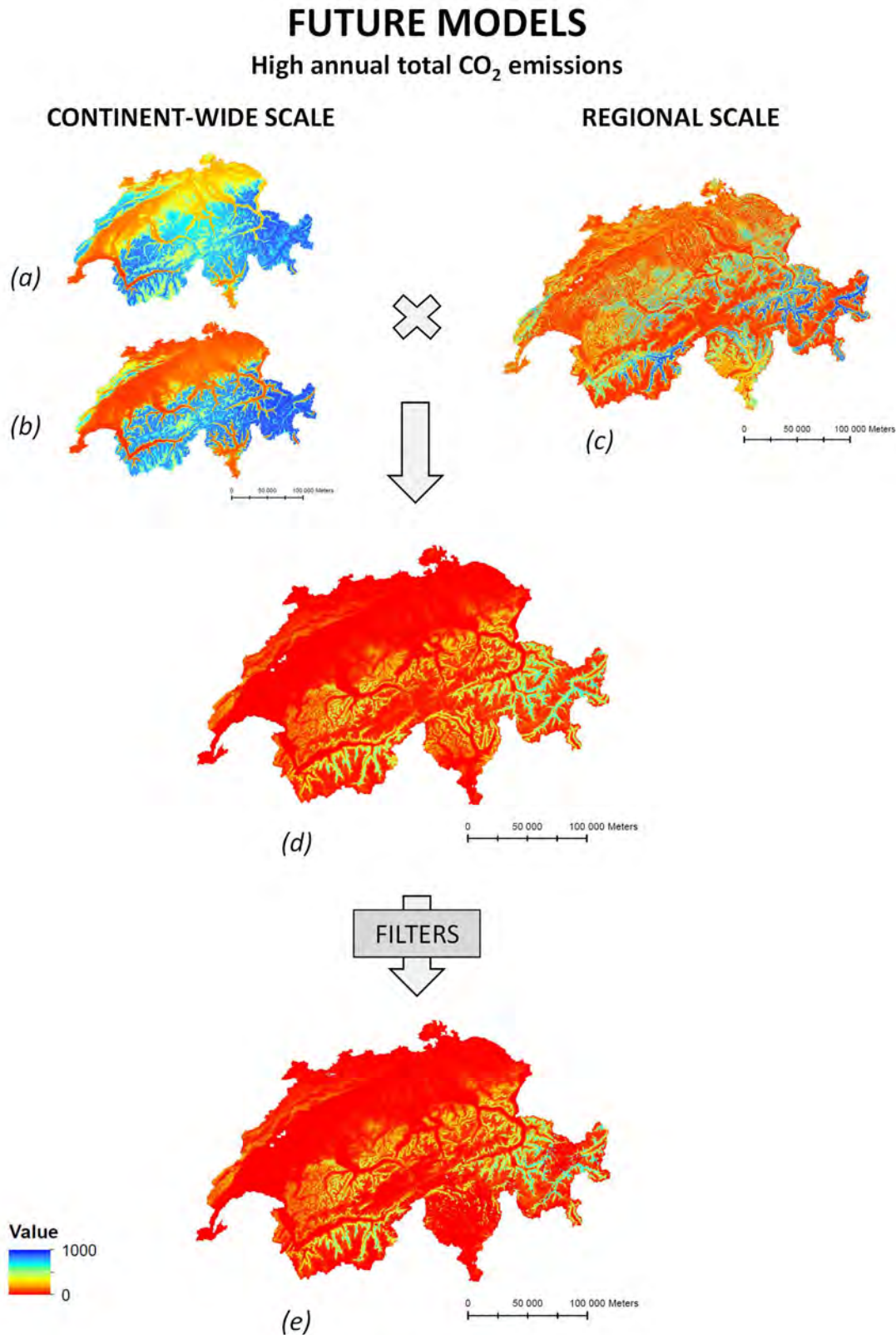


Figure 11 : Results of the modelling of Step 1 for future scenario with high annual total CO₂ emissions (RCP8.5). (a) Prediction map calibrated with Eurasian occurrences and five bioclimatic variables (IPSL-CM5A-LR). (b) Prediction map calibrated with Eurasian occurrences and five bioclimatic variables (HadGEM2-A0). (c) Prediction map calibrated with Swiss occurrences and five bioclimatic variables (A2), three topographical variables and two forest variables. (d) Combination of (a), (b) and (c). (e) Prediction map with addition of land cover and geological filters. Legends: Probability of presence according to studied parameters: 0 (red) means area predicted to be not adapted to *C. calceolus*; 1000 (blue) means area predicted to be highly favourable to *C. calceolus*.

Table 5 : Evaluation values of modelling process. TSS and AUC for the modelling part (M) are mean evaluation values of different algorithms (GLM, GBM and GAM) for all pseudo-absences sets processed. Standard deviations (sd) were calculated as well. Evaluation values of Ensemble Modelling (EM) gave information about prediction maps quality.

	CONTINENT-WIDE SCALE		REGIONAL SCALE	
	TSS	AUC	TSS	AUC
M	0.72 (sd: 0.05)	0.90 (sd: 0.03)	0.62 (sd: 0.05)	0.87 (sd: 0.02)
EM	0.74	0.92	0.65	0.90

Table 6 : Variables importance of current scenarios. At continent-wide scale, Bioclim 1 and Bioclim 5 were the both variables influencing the most the modelling process. At regional scale, Bioclim 15, the canopy height and the forest distance were the most important variables.

	CONTINENT-WIDE SCALE			REGIONAL SCALE		
	GLM	GBM	GAM	GLM	GBM	GAM
Bioclim 1	0.49 (sd: 0.09)	0.20 (sd: 0.04)	0.55 (sd: 0.0)	0.10 (sd: 0.13)	0.01 (sd: 0.01)	0.52 (sd: 0.12)
Bioclim 2	0.04 (sd: 0.03)	0.05 (sd: 0.02)	0.07 (sd: 0.03)	0.01 (sd: 0.02)	0.01 (sd: 0.01)	0.14 (sd: 0.089)
Bioclim 5	0.44 (sd: 0.09)	0.20 (sd: 0.02)	0.46 (sd: 0.08)	0.02 (sd: 0.07)	0.03 (sd: 0.02)	0.23 (sd: 0.10)
Bioclim 12	0.17 (sd: 0.09)	0.53 (sd: 0.05)	0.15 (sd: 0.07)	0.10 (sd: 0.06)	0.06 (sd: 0.02)	0.11 (sd: 0.03)
Bioclim 15	0.01 (sd: 0.02)	0.01 (sd: 0.03)	0.04 (sd: 0.04)	0.13 (sd: 0.06)	0.12 (sd: 0.03)	0.13 (sd: 0.02)
Slope	-	-	-	0.09 (sd: 0.02)	0.02 (sd: 0.01)	0.08 (sd: 0.02)
Aspect	-	-	-	0.01 (sd: 0.01)	0.01 (sd: 0.01)	0.01 (sd: 0.01)
Topos	-	-	-	0.08 (sd: 0.03)	0.05 (sd: 0.02)	0.06 (sd: 0.02)
Canopy height	-	-	-	0.16 (sd: 0.05)	0.35 (sd: 0.05)	0.19 (sd: 0.04)
Forest distance	-	-	-	0.17 (sd: 0.08)	0.06 (sd: 0.02)	0.05 (sd: 0.03)

Step 2: Fieldwork and laboratory analysis

First field impressions

During the fieldwork session, I made several observations that seemed important. They were field feelings and I had not performed any statistical tests at that time.

First, the stations I visited were different and diversified both at soil and vegetation levels (Appendix V and Appendix VI). In reintroduction sites, I realized that even if vegetation was similar and companion species above-mentioned were present, soils could be very different from the ones in native stations. Indeed, I noted that soil pH measured on the field was more acidic in some reintroductions sites than in native sites. In addition, soils of native sites presented more calcium carbonates than those with reintroduced plants.

I also observed that the majority of natives sites were rather cool and often north exposed, that was not always the case in reintroduction sites.

In addition, I observed that native stations had a very sparse bush layer and an important woody layer. Moreover, in a native station of *C. calceolus* I visited in canton of Aargau (not selected in this study) where all the surrounding trees had been cut a few years ago, plants showed a weak fitness. Indeed, subjects were a lot smaller than average and presented a greenish-yellowish colour. Those two observations both confirmed the importance of the light availability for plant fitness (later measured with the sun-compass).

Station parameters

Results of each studied variable per station are presented in Appendix VII.

Correlations between field variables and existing data from topographical ESERI calculation layers gave expected results. Slope measured on the field and slp25 were highly correlated ($n=20$, $r^2=0.69$, $p<0.001$) as well as topographical aspect quantified on the field and aspval ($n=20$, $r^2=0.74$, $p<0.001$). Moreover, sun-compass summed values and srاد were also correlated ($n=20$, $r^2=0.16$, $p=0.041$). For subsequent statistical analysis, I used slp25, aspval and srاد values instead of field values.

To characterize station parameters, I measured and extracted values of topographical variables (topos, topographic aspect, slope, canopy height and forest distance) and bioclimatic variables (sun, Bioclim 1, Bioclim 2, Bioclim 5, Bioclim 12 and Bioclim 15) (Table 2). Statistically significant correlations between station parameters are presented in Figure 12 (a) and (b). For topographical variables, I measured significant negative correlation for topographical aspect and forest distance ($n=20$, $r^2=0.19$, $p=0.029$). Concerning bioclimatic variables, I found significant positive correlation for Bioclim 1 and Bioclim 5 ($n=20$, $r^2=0.96$, $p<0.001$), Bioclim 1 and Bioclim 15 ($n=20$, $r^2=0.43$, $p<0.001$), Bioclim 5 and Bioclim 15 ($n=20$, $r^2=0.38$, $p=0.002$). Bioclim 1 and Bioclim 12 ($n=20$, $r^2=0.78$, $p<0.001$), Bioclim 5 and Bioclim 12 ($n=20$, $r^2=0.78$, $p<0.001$), Bioclim 12 and Bioclim 15 ($n=20$, $r^2=0.31$, $p=0.006$) were significantly negatively correlated (Figure 12 (a) and (b)).

Taken together, topographical and bioclimatic variables did not explain differences between studied stations neither between populations nor between statuses (PCA plots, Figure 13a and Figure 13b, respectively; Adonis per population for topographical variables: $F_{x,y} = 0$, $p = 1$, and for bioclimatic variables: $F_{x,y} = 0$, $p = 1$; Adonis per statuses for topographical variables: $F_{x,y} = 1.653$, $p = 0.161$, and for bioclimatic variables: $F_{x,y} = 0.902$, $p = 0.414$).

Concerning topographical variables (Figure 13a), the aspect and the forest distance were the variables influencing the most the distribution of sites. In addition, parameters values from reintroduction and future reintroduction had bigger amplitudes than those from native sites. However, the three niches drawn by topographical values coincided well (Figure 13a). Concerning bioclimatic variables (Figure 13b), Bioclim 1 and Bioclim 5 are the variables influencing the most sites distribution. Future reintroduction sites had the smallest amplitude of variables values. In addition, those values are nearly totally included in the range of native sites. On the other hand, reintroduction sites had a great amplitude and did not coincide well with native sites (Figure 13b).

I measured no significant difference between statuses compared with PERMANOVA except for precipitation seasonality bioclimatic variable (Bioclimatic 15, $p=0.005$) (Figure 14 and Figure 15). Using Asymptotic test, I found significantly different coefficients of variation between statuses for the canopy height (native stations had a variability 24 times bigger than future reintroduction sites), for Bioclim 5 (native stations had a variability 5 times higher than future reintroduction sites) and for Bioclim 15 (native stations had a variability 5 times higher than future reintroduction sites and reintroductions sites) (Figure 14 and Figure 15, different letters above plots indicate significantly different coefficients of variation between statuses).

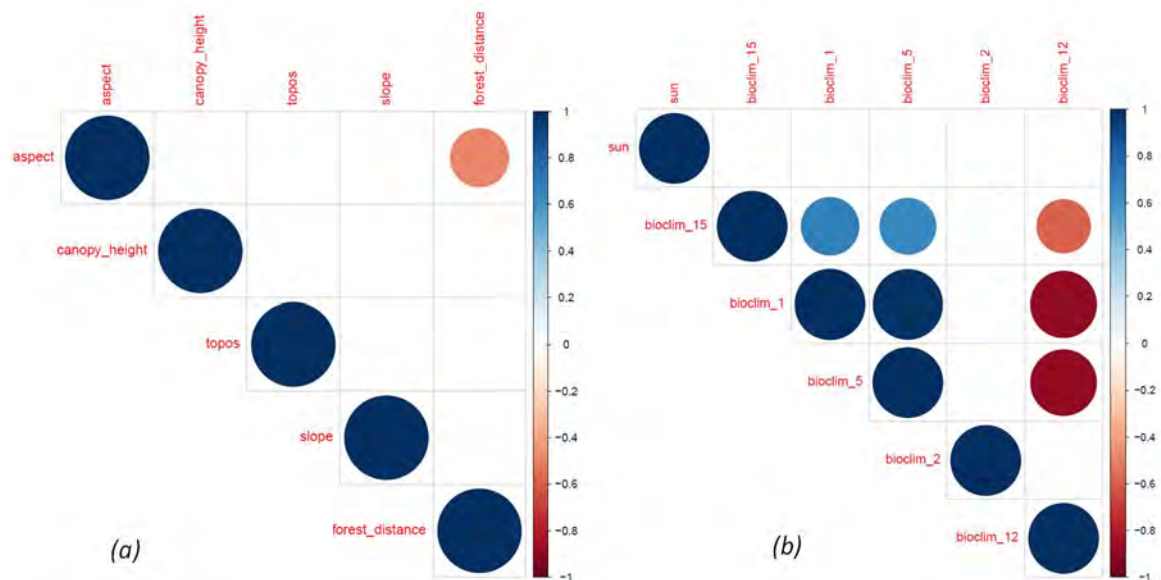


Figure 12 : (a) Significant correlations ($p < 0.05$) are indicated with red (negative correlation) or with blue (positive correlation) dots. (b) The meaning of blue and reds dots is the same as in the (a) part of the figure.

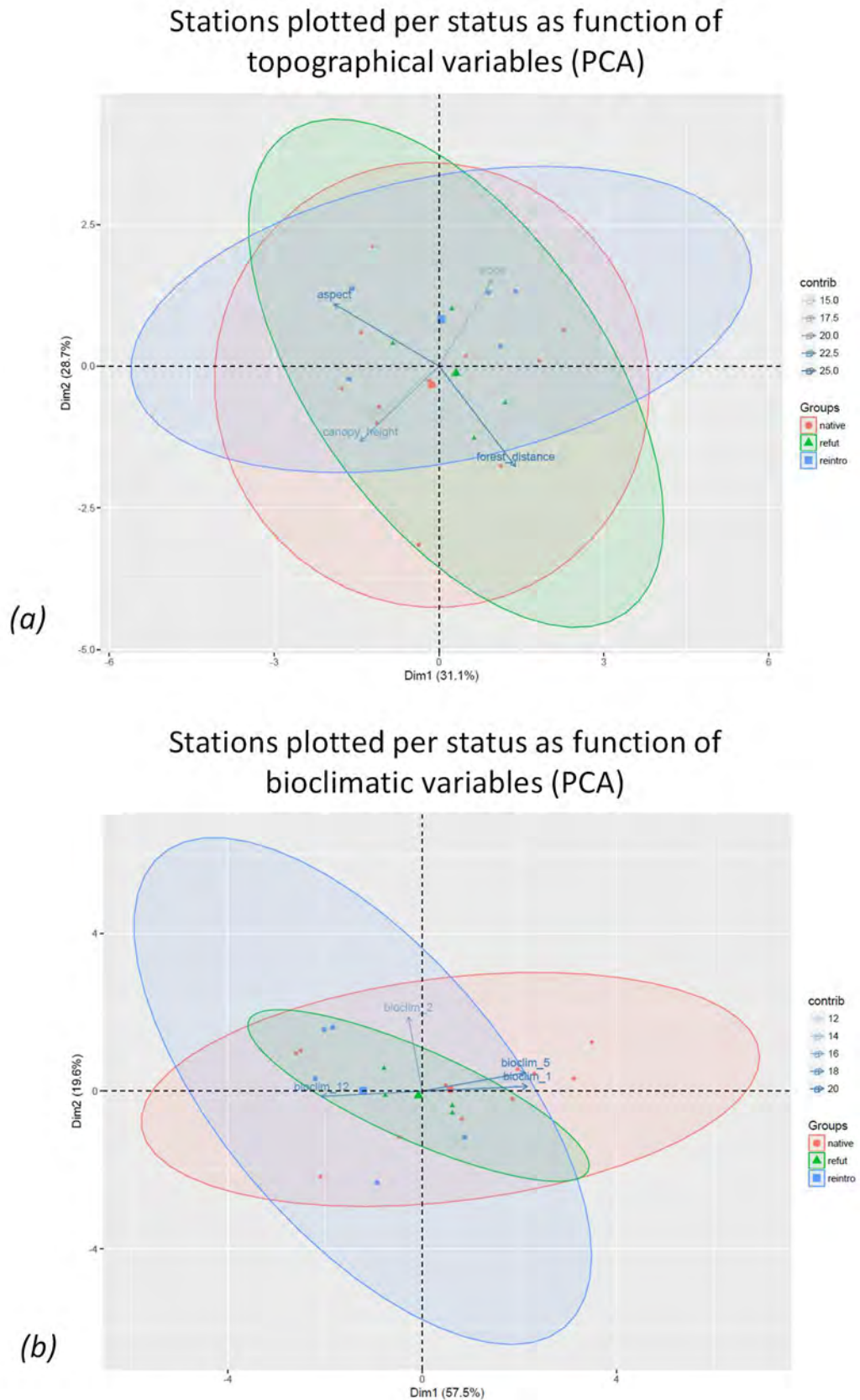


Figure 13: PCA illustrating stations plotted per status as function of topographical variables (a) or of bioclimatic variables (b). Relative contributions of each variables are illustrated from light blue (small importance) to dark blue (great importance). Legends for statuses: native: native sites; refut: future reintroduction sites; reintro: reintroduction sites.

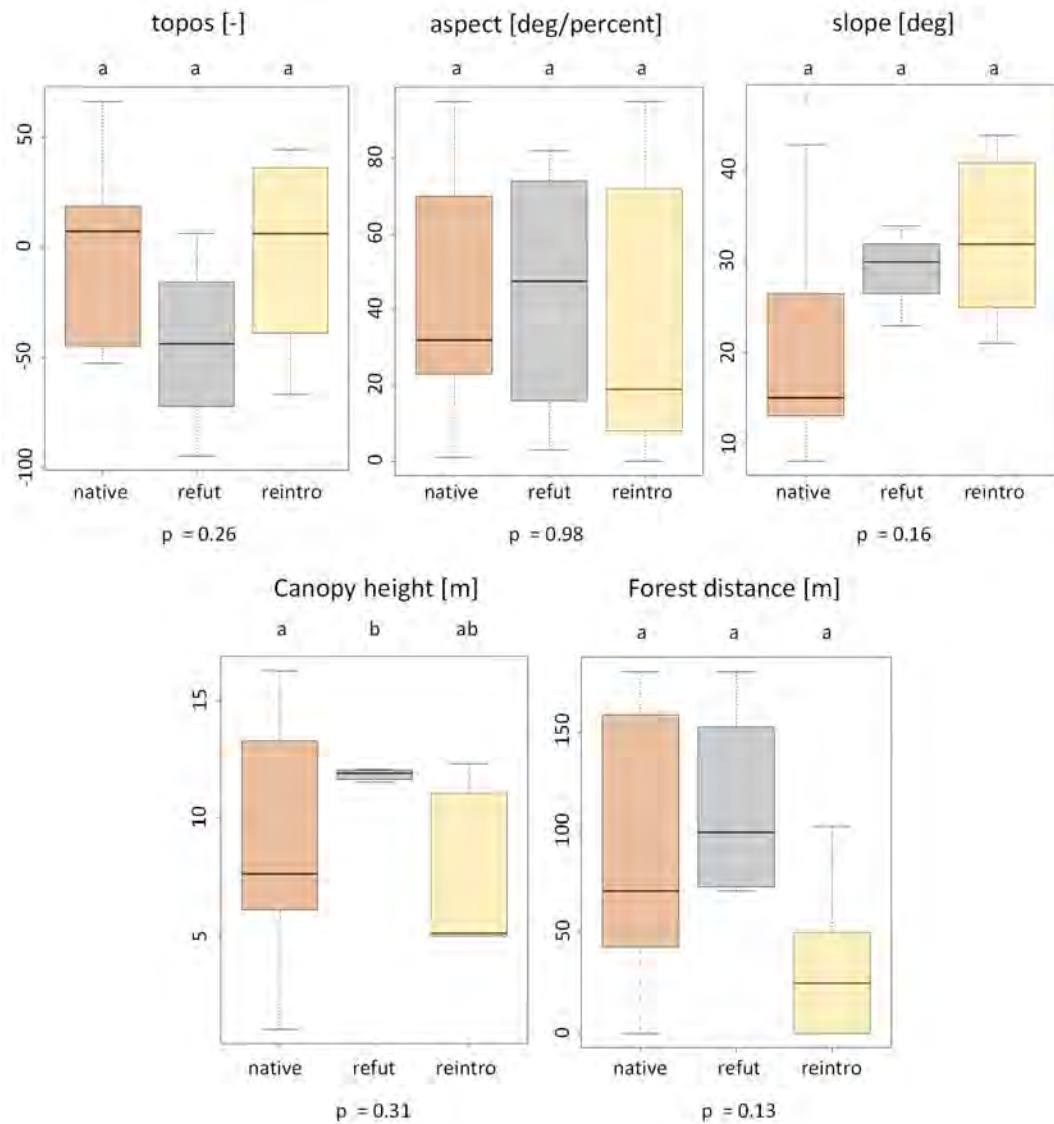


Figure 14 : Range of topographical variables are indicated per status. In each boxplot, central line represents the median. The two boxplot edges are the first (25% of values greater than the median) and the third quartiles (25% of values smaller than the median). The two whiskers mean the maximum and the minimum values (25% of the values on each side out of the plot, except outliers). Little circles hereafter whiskers are the outliers. P-values of PERMANOVA are indicated under each plot. Different letters above boxplots mean significant difference measured with coefficient of variation. Legends for statuses: native: native sites; refut: future reintroduction sites; reintro: reintroduction sites.

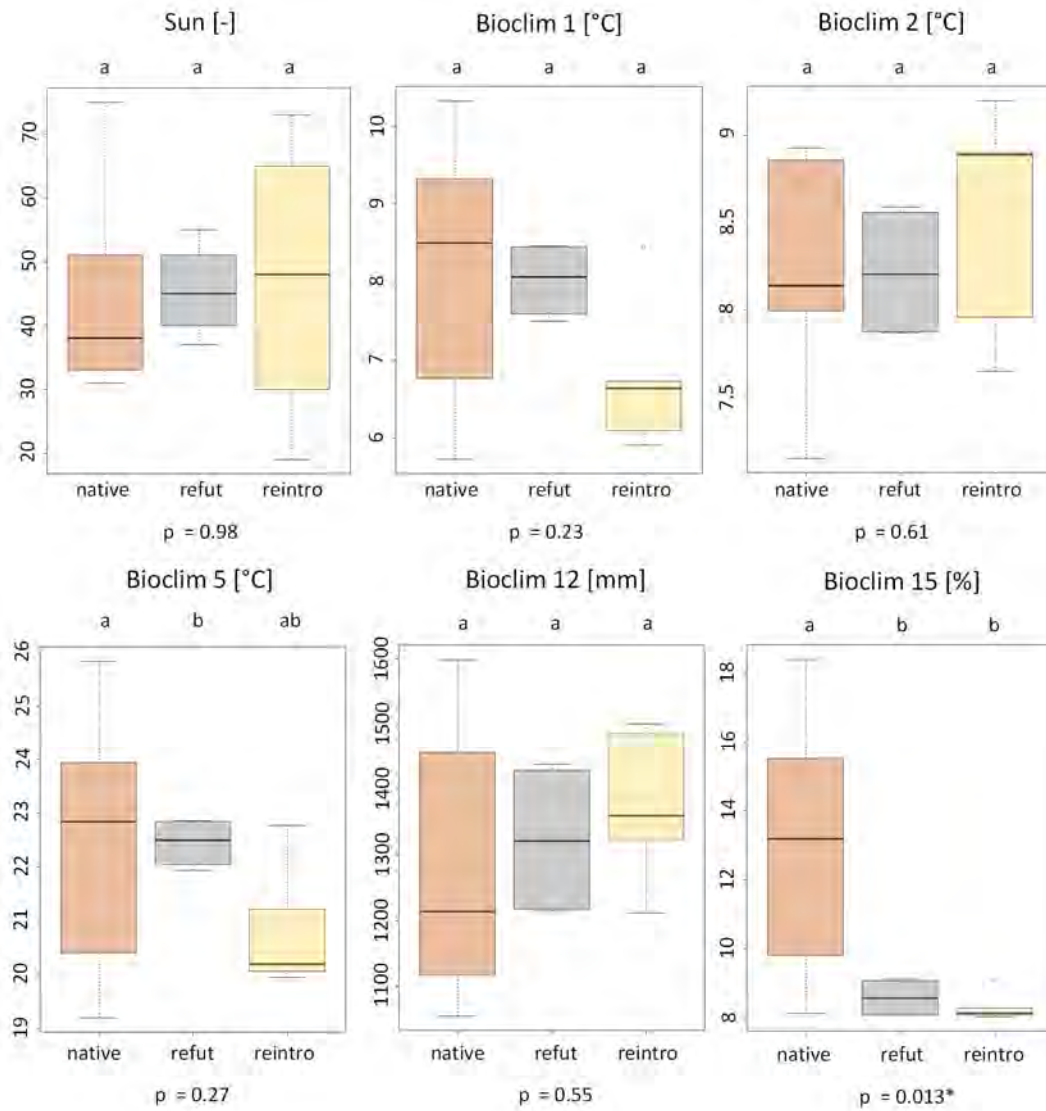


Figure 15 : Range of bioclimatic variables are indicated per status. In each boxplot, central line represents the median. The two boxplot edges are the first (25% of values greater than the median) and the third quartiles (25% of values smaller than the median). The two whiskers mean the maximum and the minimum values (25% of the values on each side out of the plot, except outliers). Little circles hereafter whiskers are the outliers. P-values of PERMANOVA are indicated under each plot. Different letters above boxplots mean significant difference measured with coefficient of variation. Legends for statuses: native: native sites; refut: future reintroduction sites; reintro: reintroduction sites.

Vegetation study

In order to characterise vegetation, I performed phytosociological surveys in each station. Vegetation alliance were then determined (Table 7 and Appendix V). Alliance identifications were complex because of *C. calceolus* ecology. Indeed, this species lives often in transition habitats. For this reason and in order to give a precise overview of field reality, alliances were determined in herbaceous and in woody strata (Table 7). The identified alliances were predominantly forest ones. Beech wood (*Fagenion*) and pine forest (*Pinion*) were the two most commonly encountered forest types (Table 7). In almost all surveys, typical species of meadows and forest borders were also identified (Appendix V).

Table 7 : Vegetation alliances characterised using phytosociological surveys and Brown-Blanquet method in native sites (NAT), sites with already performed reintroduction (REINTRO) and future reintroduction sites (REFUT).

Station	Dominant vegetation alliance in the woody strata	Dominant vegetation alliance in herbaceous strata	Special characteristics
NAT-GE1	<i>Molinio-Pinion</i>	<i>Molinio-Pinion</i>	Presence of species of <i>Quercion pubescenti-petraeae</i>
NAT-VD1	<i>Galio-Fagenion</i>	<i>Galio-Fagenion</i>	Former plantation of <i>Picea abies</i>
NAT-VD2	<i>Molinio-Pinion</i>	<i>Molinio-Pinion</i>	-
NAT-VD3	<i>Abieti-Fagenion</i>	<i>Abieti-Fagenion</i>	Presence of species of <i>Cephalanthero-Fagenion</i> in herbaceous strata
NAT-NE1	<i>Lonicero-Fagenion</i>	<i>Lonicero-Fagenion</i>	Presence of species of <i>Sesslerion</i> in herbaceous strata and of <i>Picea abies</i> in woody strata
NAT-NE2	<i>Abieti-Fagenion</i>	<i>Abieti-Fagenion</i>	Presence of species of <i>Cephalanthero-Fagenion</i> in herbaceous strata
NAT-BE1	<i>Cephalanthero-Fagenion</i>	<i>Cephalanthero-Fagenion</i>	-
NAT-BE2	<i>Lonicero-Fagenion</i>	<i>Lonicero-Fagenion</i>	-
NAT-BL1	<i>Abieti-Fagenion</i>	<i>Abieti-Fagenion</i>	Presence of species of <i>Cephalanthero-Fagenion</i> in herbaceous strata
NAT-AG1	<i>Lonicero-Fagenion</i>	<i>Lonicero-Fagenion</i>	-
NAT-ZH1	<i>Abieti-Fagenion</i>	<i>Atropion</i>	Transition between <i>Atropion</i> and <i>Abieti-Fagenion</i> with the presence of numerous forest species
REINTRO-NE1	<i>Abieti-Fagenion</i>	<i>Abieti-Fagenion</i>	Presence of species of <i>Cephalanthero-Fagenion</i> in herbaceous strata
REINTRO-NE2	<i>Abieti-Fagenion</i>	<i>Cephalanthero-Fagenion</i>	-
REINTRO-NE3	<i>Abieti-Fagenion</i>	<i>Abieti-Fagenion</i>	Presence of species of <i>Cephalanthero-Fagenion</i> in herbaceous strata
REINTRO-BE1	<i>Abieti-Fagenion</i>	<i>Cephalanthero-Fagenion</i>	-
REINTRO-BE2	<i>Cephalanthero-Fagenion</i>	<i>Cephalanthero-Fagenion</i>	-
REFUT-NE1	<i>Molinio-Pinion</i>	<i>Molinio-Pinion</i>	-
REFUT-NE2	<i>Molinio-Pinion</i>	<i>Molinio-Pinion</i>	-
REFUT-NE3	<i>Cephalanthero-Fagenion</i>	<i>Cephalanthero-Fagenion</i>	Presence of numerous species from other forest alliances
REFUT-NE4	<i>Abieti-Fagenion</i>	<i>Cephalanthero-Fagenion</i>	-

Humus forms, edaphic parameters and laboratory analysis

Humus forms and soil type were first described and determined using field measurements (Table 8 and Appendix VI). To confirm those results and to have a better understanding of *C. calceolus* edaphic niche, I also performed laboratory analysis. Fieldwork measurements and laboratory analysis results of each humus and edaphic variable per station are presented in Appendix VIII and Appendix IX.

In native stations, all soil types were calcareous ones (CALCOSOL or RENDOSOL, Table 8). Humus forms were either Oligomull, Dysmull, Hémimoder or Dysmoder. In reintroduction sites, soils were also calcareous except for one (REINTRO-BE2, Table 8). I characterised humus forms as Oligomull or Dysmull. In future reintroduction stations, soils were all CALCOSOL with Dysmull humus forms (Table 8).

Table 8 : Soil types and humus forms described using le Référentiel pédologique (Baize et al., 2009) and Zanella (2011) in native sites (NAT), sites with already performed reintroduction (REINTRO) and future reintroduction sites (REFUT).

Station	Sol type according to le Référentiel pédologique (Baize et al., 2009)	Humus form according to Zanella (2011)
NAT-GE1	CALCOSOL	Oligomull
NAT-VD1	RENDOSOL pachique issu de molasse	Hémimoder
NAT-VD2	CALCOSOL	Dysmull
NAT-VD3	CALCOSOL décarbonaté en surface	Dysmull
NAT-NE1	CALCOSOL colluvial, leptique issu d'éboulis calcaires	Dysmoder
NAT-NE2	RENDOSOL colluvial issu d'éboulis calcaires	Dysmoder
NAT-BE1	CALCOSOL issu de molasse	Oligomull
NAT-BE2	CALCOSOL issu de calcaires du Portlandien	Dysmull
NAT-BL1	RENDOSOL colluvial issu d'éboulis calcaires	Hémimoder
NAT-AG1	CALCOSOL issu de molasse	Dysmull
NAT-ZH1	CALCOSOL issu de molasse	Dysmull
REINTRO-NE1	CALCOSOL leptique issu d'éboulis calcaires	Dysmull
REINTRO-NE2	CALCOSOL présentant des cutanes issu d'éboulis calcaires	Dysmull
REINTRO-NE3	CALCOSOL présentant des cutanes issu d'éboulis calcaires	Dysmull
REINTRO-BE1	RENDOSOL décarbonaté en surface issu de dalle calcaire	Oligomull
REINTRO-BE2	BRUNISOL issu de lœss	Dysmull
REFUT-NE1	CALCOSOL issu d'éboulis calcaires	Dysmull
REFUT-NE2	CALCOSOL issu de substrat calcaire meuble	Dysmull
REFUT-NE3	CALCOSOL	Dysmull
REFUT-NE4	CALCOSOL	Dysmull

During laboratory analysis for characterising the humus forms, I measured the water content and the C/N ratio in humic horizons of studied soils. Both measured variables did not show significant correlation ($n=15$, $r^2=0.09$, $p=0.142$). Taken together, water content and C/N values did not explain differences between the humus forms neither across statuses (Adonis, $F_{x,y}=2.262$, $p=0.129$) nor population (Adonis, $F_{x,y}=0$, $p=1$). Along the same lines, I indeed found no status differences for water content (PERMANOVA, $p=0.123$) as well as for C/N (PERMANOVA, $p=0.194$). Similarly, I did not detect significant difference between coefficients of variation calculated between statuses (Asymptotic test, Figure 16, different letters above plots indicate significantly different coefficients of variation between statuses).

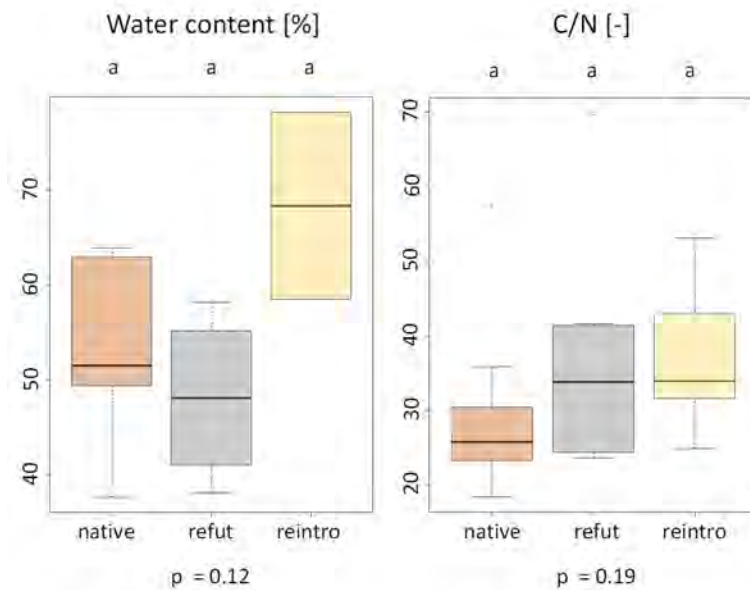


Figure 16 : Range of humus variables are indicated per status. In each boxplot, central line represents the median. The two boxplot edges are the first (25% of values greater than the median) and the third quartiles (25% of values smaller than the median). The two whiskers mean the maximum and the minimum values (25% of the values on each side out of the plot, except outliers). Little circles hereafter whiskers are the outliers. P-values of PERMANOVA are indicated under each plot. Different letters above boxplots mean significant difference measured with coefficient of variation. Legends for statuses: native: native sites; refut: future reintroduction sites; reintro: reintroduction sites.

In order to complete field descriptions of soils, I studied soil temperature, water content, residual moisture, ignition loss, cationic exchange capacity, $\text{pH}_{\text{H}_2\text{O}}$, total limestone, C/N ratio and clay content. In addition, I performed mineral grain size analysis, which did not give any significant results because of the high amount of organic matter or clay particles contained in samples. These results were therefore not considered. Statistically significant correlations between soil variables in A horizons are shown in Figure 17. I measured strong negative correlations between $\text{pH}_{\text{H}_2\text{O}}$ and C/N ratio ($n=20$, $r^2=0.59$, $p<0.001$) as well as between soil temperature and water content ($n=20$, $r^2=0.36$, $p=0.003$). On the other hand, I found strong positive correlations between water content and CEC ($n=20$, $r^2=0.54$, $p=0.001$), loss ignition and CEC ($n=20$, $r^2=0.68$, $p<0.001$), loss ignition and residual moisture ($n=20$, $r^2=0.77$, $p<0.001$), residual moisture and CEC ($n=20$, $r^2=0.83$, $p<0.001$).

Taken together, soil variables studied in all horizons explained differences between populations (Adonis, $F_{x,y}=6.35$, $p<0.001$). However, differences between statuses were not explained (Adonis, $F_{x,y}=0.81$, $p=0.531$). Moreover, soil variables taken only in A horizons did not explain differences neither between populations (Adonis, $F_{x,y}=0$, $p=1$) nor between statuses (PCA plot, Figure 18; Adonis, $F_{x,y}=0.66$, $p=0.707$).

Ignition loss, representing the amount of organic matter, and cationic exchange capacity were the parameters influencing the most the distribution of sites (Figure 18). Variable values of future reintroduction sites had the smallest amplitudes and were totally included in native sites range of measured values. On the other hand, future reintroduction sites range of values is far much larger than for the two other statuses (Figure 18).

I only found statistically significant difference between statuses compared with PERMANOVA for $\text{pH}_{\text{H}_2\text{O}}$ ($p=0.032$) (Figure 19). I measured significantly different coefficients of variation between statuses for soil temperature (reintroduction station had a variability eight times higher than future reintroduction sites), $\text{pH}_{\text{H}_2\text{O}}$ (reintroduction sites had a variability nine times higher on average than native and future reintroduction sites) and C/N ratio (reintroduction sites had a variability three times

higher on average than native and future reintroduction sites) (Figure 19, different letters above plots indicate significantly different coefficients of variation between statuses).

Even if the other PERMANOVA and coefficient of variation results are not significant, it is very interesting to notice that future reintroduction range of values are always included in native sites ranges. On the other hand, reintroduction sites range of values did not coincide with native sites ranges.

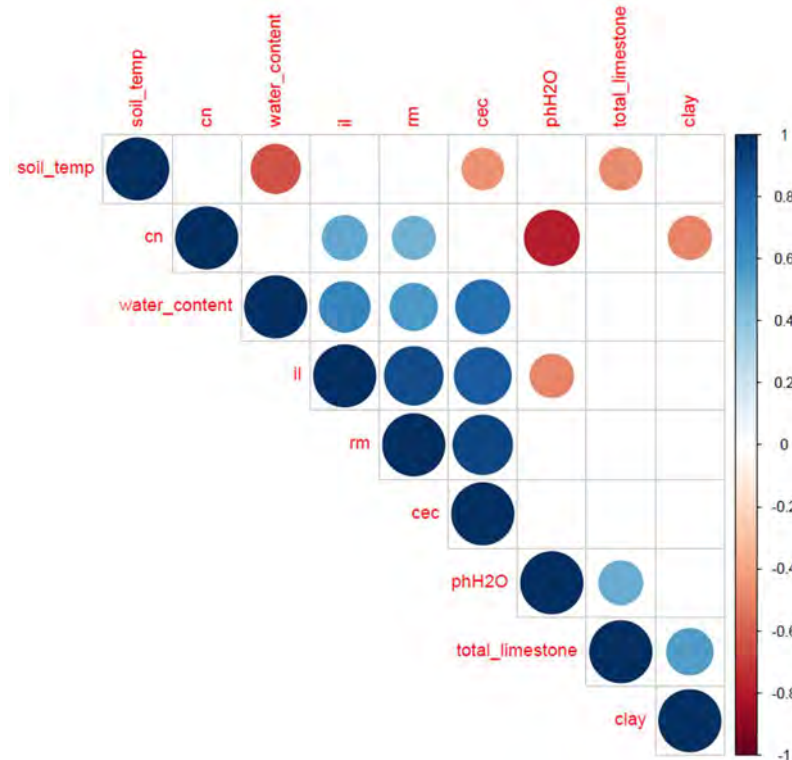


Figure 17 : Correlation between soil variables in A horizons. Significant correlations ($p < 0.05$) are indicated with red (negative correlation) or with blue (positive correlation) dots. Legends: soil_temp: soil temperature; cn: C/N ratio; water_content: water content; il: ignition loss; rm: residual moisture; cec: cationic exchange capacity; pH2O: pH₂O; total_limestone: total limestone; clay: percentage of clay.

Populations and plants fitness measurements

Results of each fitness measurements per subject per station are presented in Appendix X.

Along Swiss Jura, *C. calceolus* populations were significantly different according to biological traits (Adonis, $F_{x,y} = 4.41$, $p = 0.001$). Results of populations ordinated in function of biological traits (PCA) are presented in Figure 20. Populations are mainly distributed according to two principal axes: (i) vitality (biological traits: size, vitality, number of leaves, leaf area, photosynthetic activity (SPAD)) and (ii) reproduction (biological traits: number of flowers, number of stems, number of pods, seedlings). Vitality variables were anti-correlated with the grazing variable.

Envfit function

I found that the two environmental variable that influenced the most *C. calceolus* biological traits are soil temperature ($n = 20$, $r^2 = 0.68$, $p = 0.017$) and the aspect (marginally significant, $n = 20$, $r^2 = 0.44$, $p = 0.075$) (Figure 21). After processing ordination and linear regression on envfit results, I concluded that the more plants were north exposed; the better vitality they had. Moreover, lower soil temperatures allowed a better vitality in studied population. To conclude, I found that populations

that lived at fresher soil temperature allocated more resources on vitality and those that were at warmer soil temperature allocated more resources on reproduction.

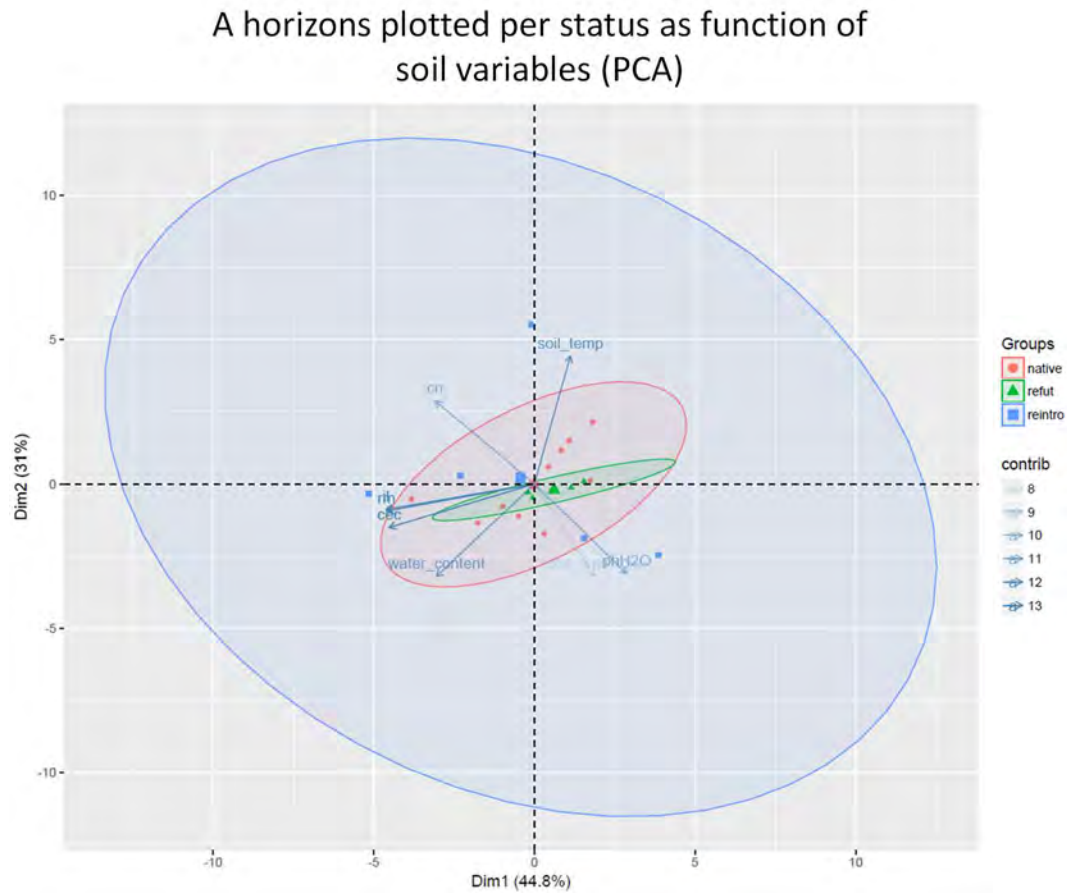


Figure 18 : PCA illustrating stations plotted per status as function of soil variables. Relative contributions of each variables are illustrated from light blue (small importance) to dark blue (great importance). Legends for statuses: native: native sites; refut: future reintroduction sites; reintro: reintroduction sites.

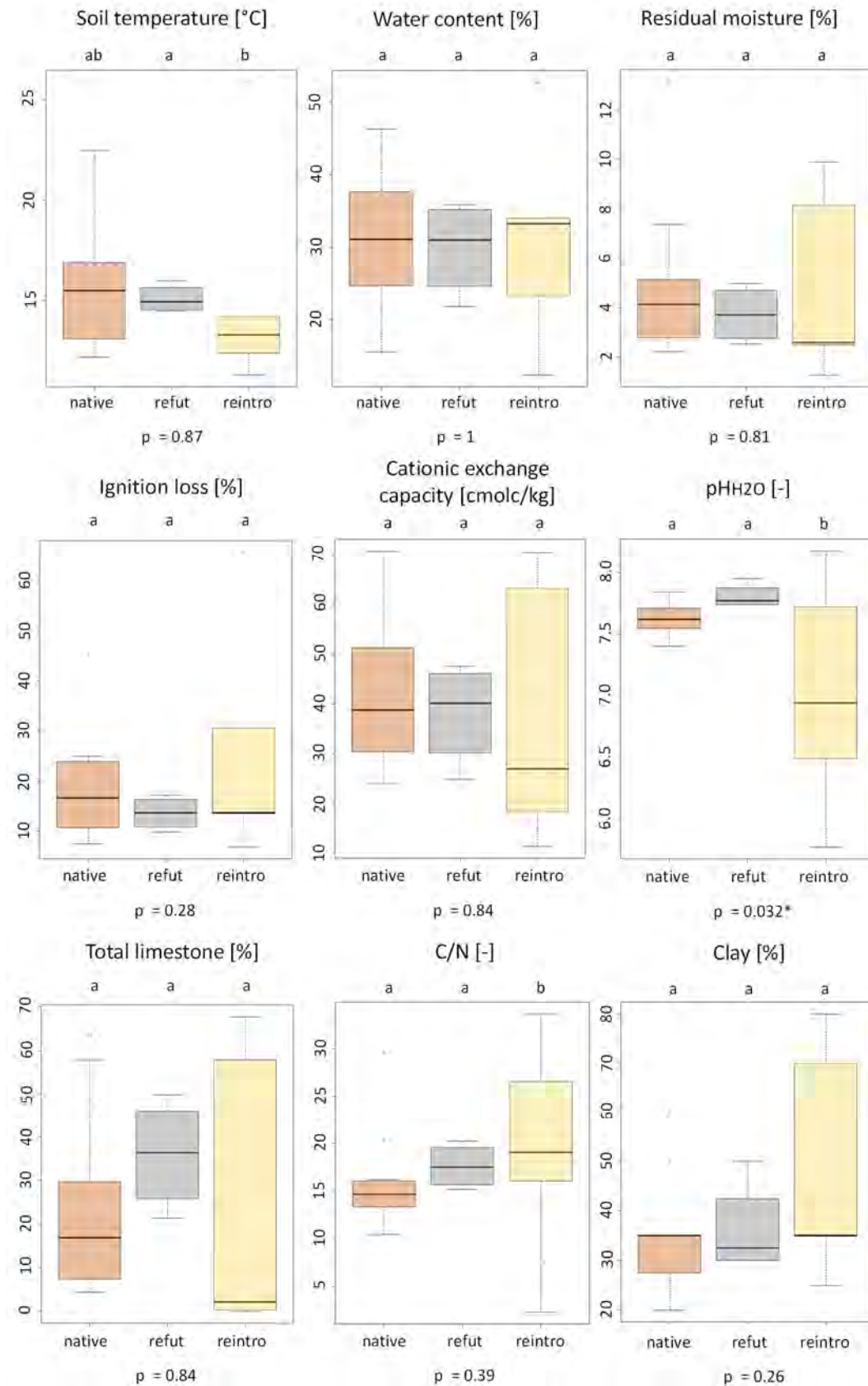


Figure 19 : Range of soil variables are indicated per status. In each boxplot, central line represents the median. The two boxplot edges are the first (25% of values greater than the median) and the third quartiles (25% of values smaller than the median). The two whiskers mean the maximum and the minimum values (25% of the values on each side out of the plot, except outliers). Little circles hereafter whiskers are the outliers. P-values of PERMANOVA are indicated under each plot. Different letters above boxplots mean significant difference measured with coefficient of variation. Legends for statuses: native: native sites; refut: future reintroduction sites; reintro: reintroduction sites.

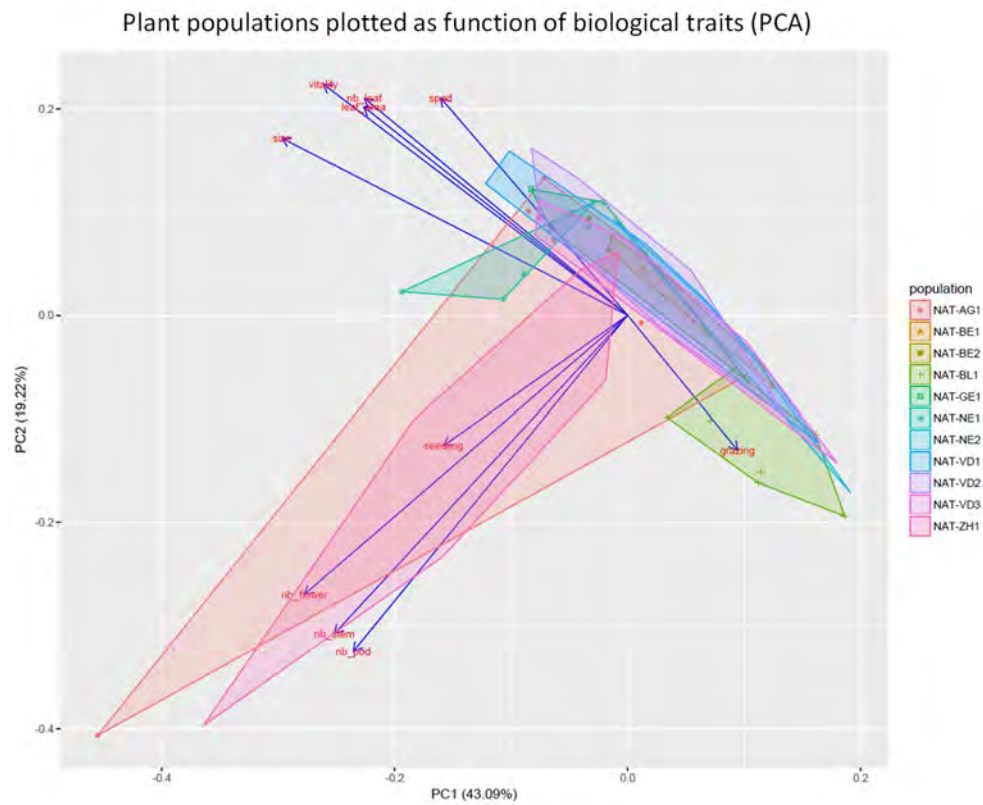


Figure 20 : PCA illustrating plants plotted per populations as function of biological traits (vitality, size, stems (nb_stem), leaves (nb_leave), median leaf area (leaf-area), photosynthetic activity (spad), flowers (nb_flower), pods (nb_pod), seedlings (seedling) and grazing).

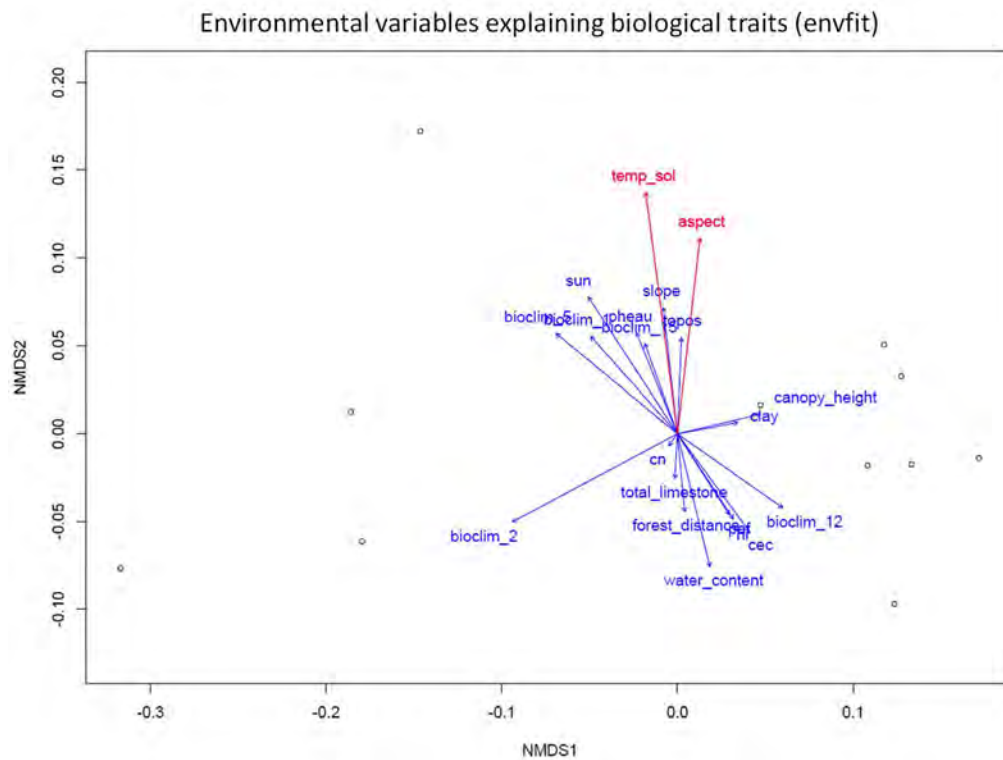


Figure 21 : Results of envfit function highlighting the influence of environmental variables on the performance of native populations. Legends: blue arrows: environmental variables that did not influence significantly plants populations ($p > 0.1$); reds arrows: environmental variables that did influence significantly or marginally significantly plants populations ($p < 0.1$); dots: plants populations.

Step 3: Niche modelling improved with field data

As mentioned above, sun-compass results were correlated with daily average of global potential shortwave radiation per month (srad) (Kumar et al., 1997). After fieldwork, light reaching the plants proved to be an important factor for *C. calceolus* fitness. Because of that, I added adapted ranges of values (Figure 22) on the previously obtained map (Step 1) in order to get final prediction maps (Figure 23, Figure 24 and Figure 25).

As the map obtained at the end of Step 1, the current final map obtained here (Figure 23) showed that favourable reintroductions sites stood in the Plateau, the north of the Alps and valleys of western and eastern central Alps. Areas that seemed less conducive for *C. calceolus* are Swiss Jura and the south of the Alps.

Concerning very low annual total CO₂ emissions scenario (Figure 24), the final prediction map looked like the one obtained at the end of Step 1. Suitable areas for *C. calceolus* were scattered and mainly located in valley bottom in eastern (Grisons) and south part (Wallis) of Switzerland.

The final map of high annual total CO₂ emissions scenario (Figure 25) showed the same trends than very low annual total CO₂ emissions scenario. However, favourable areas were even more rare and scattered.

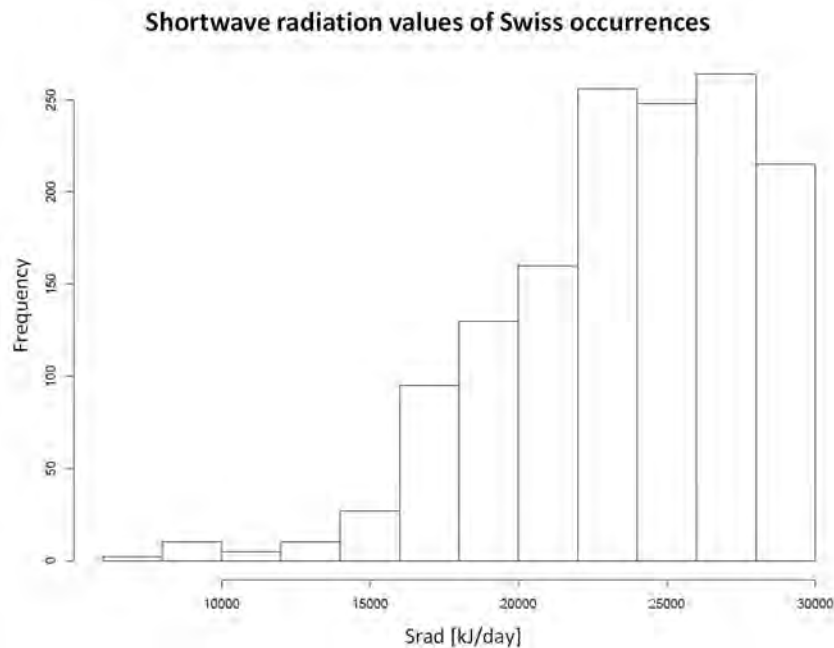


Figure 22 : Range of shortwave radiation values (srad) of Swiss occurrences (Info Flora data).

In addition, I listed important values for *C. calceolus* fitness that were not available in raster layers such as soil pH, soil amount of calcium carbonates or soil C/N ratio in a field guidebook (Appendix XI). In order to make the use of this guidebook as practical and useful as possible, I detailed field measurements protocols and their costs if they need to be done in a laboratory.

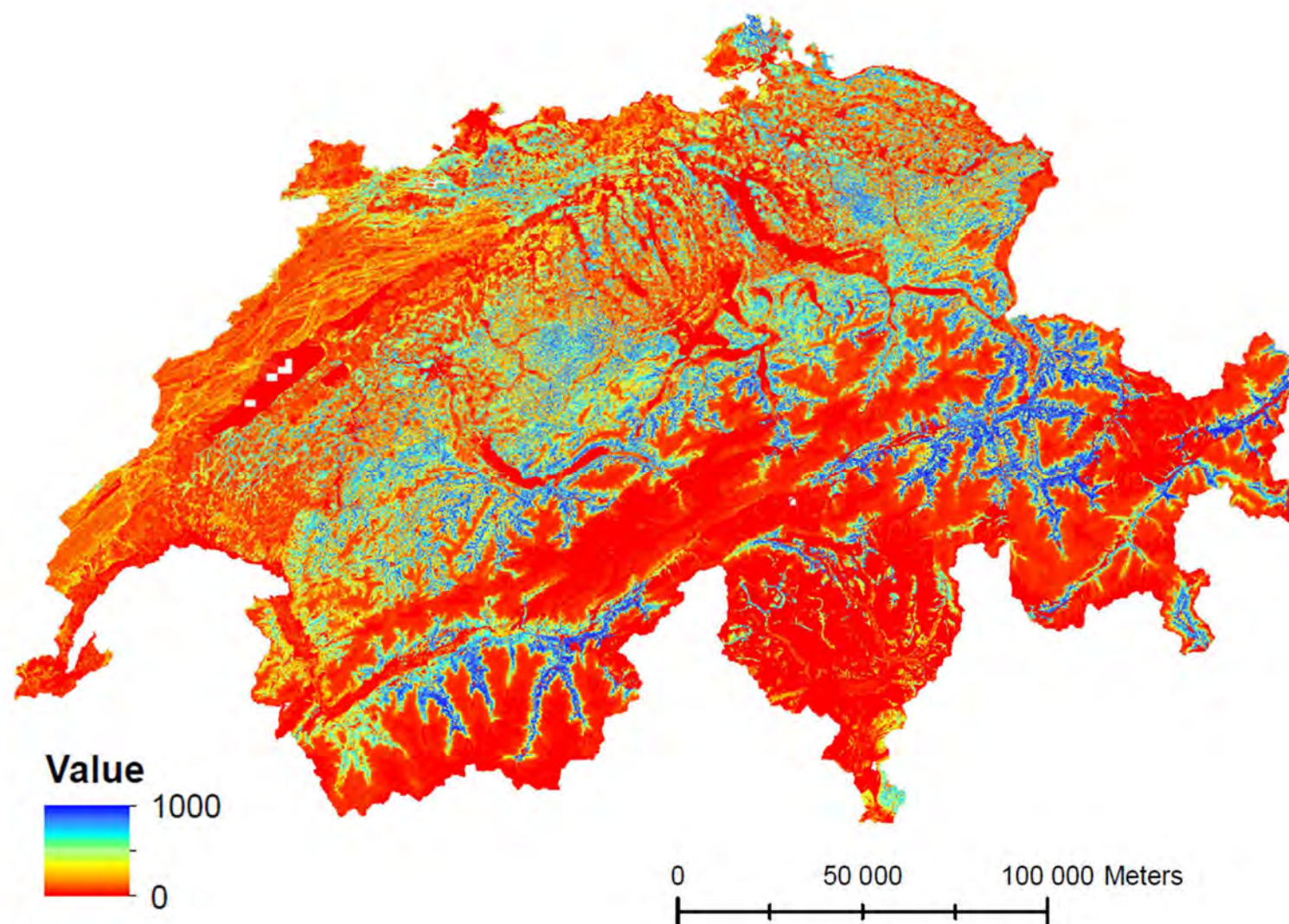


Figure 23 : Final prediction map obtained after the addition of fieldwork results (Step 3) for current scenario. Legends: Probability of presence according to studied parameters: 0 (red) means area predicted to be not adapted to *C. calceolus*; 1000 (blue) means area predicted to be highly favourable to *C. calceolus*. White squares are areas with lacking data.

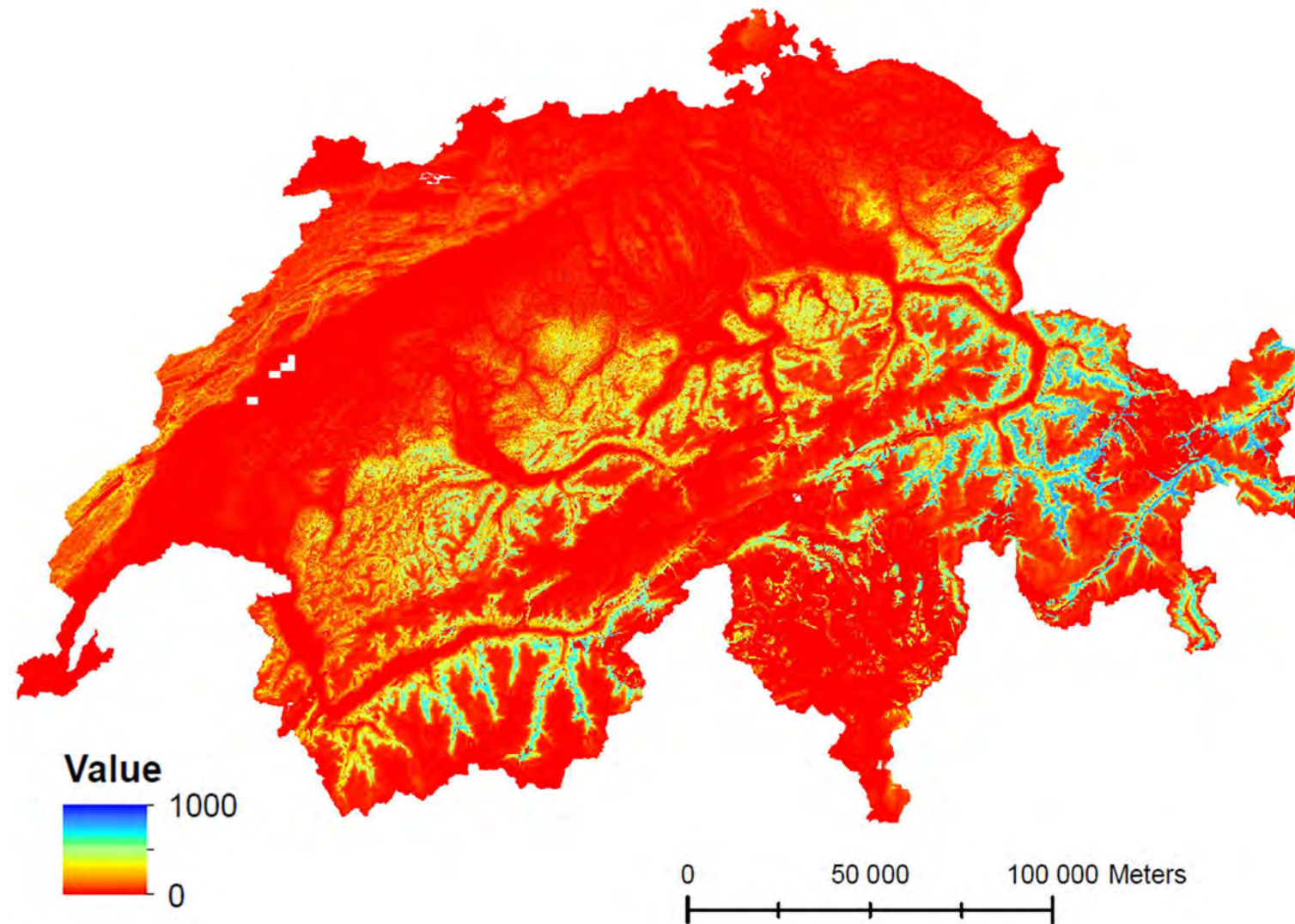


Figure 24 : Final prediction map obtained after the addition of fieldwork results (Step 3) for very low annual total CO₂ emissions scenario. Legends: Probability of presence according to studied parameters: 0 (red) means area predicted to be not adapted to *C. calceolus*; 1000 (blue) means area predicted to be highly favourable to *C. calceolus*. White squares are areas with lacking data.

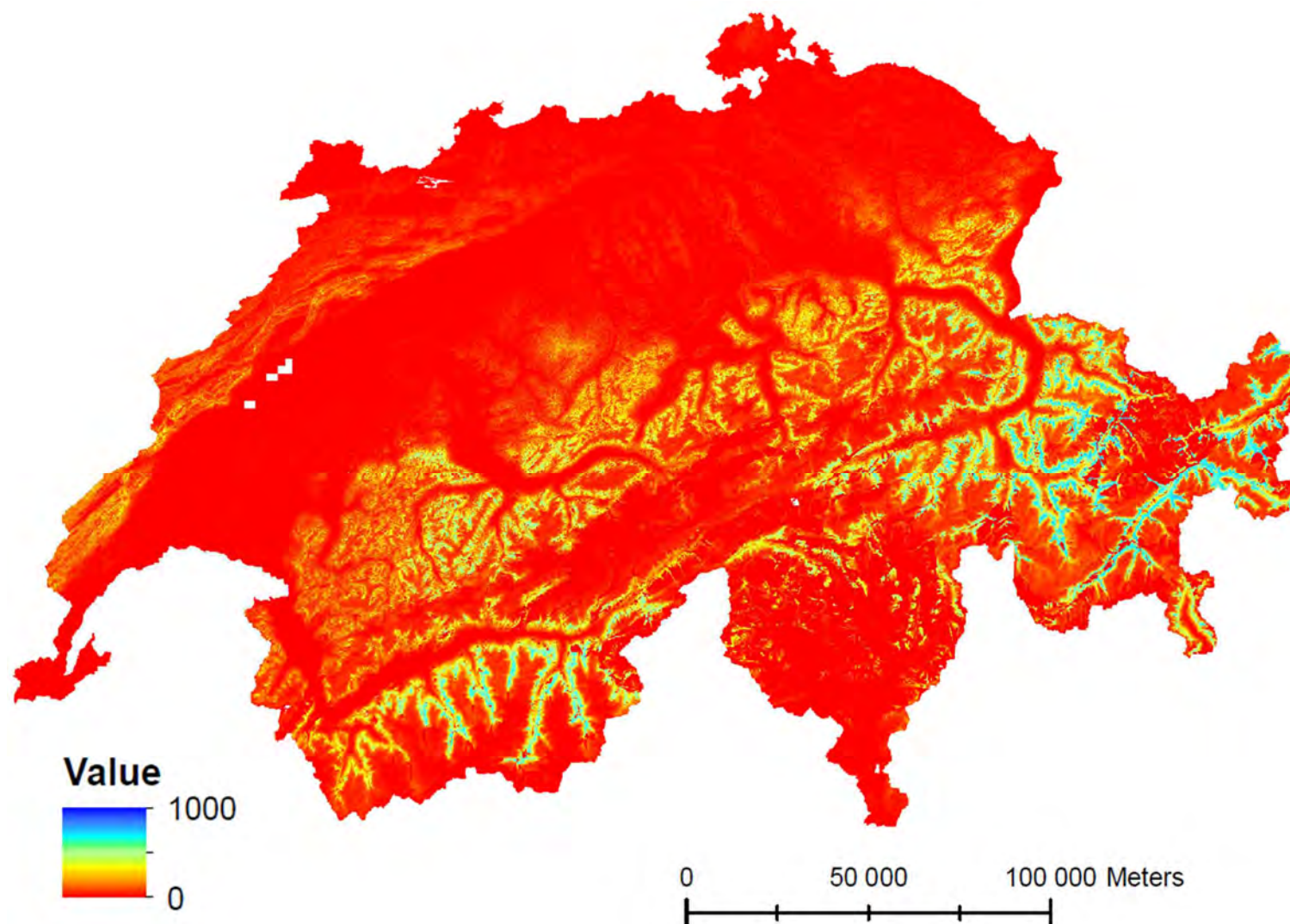


Figure 25 : Final prediction map obtained after the addition of fieldwork results (Step 3) for very low annual total CO₂ emissions scenario. Legends: Probability of presence according to studied parameters: 0 (red) means area predicted to be not adapted to *C. calceolus*; 1000 (blue) means area predicted to be highly favourable to *C. calceolus*. White squares are areas with lacking data.

4. Discussion

The three steps of the roadmap were successively and successfully achieved. Modelling step gave precise current and future prediction maps for preselection of translocation sites. In the second step, analysis of ecological and edaphic parameters of native stations allowed identifying the most important parameters that must be taken into account in the field when restoration sites are selected using prediction maps. The addition of field parameter that can be modelled on prediction maps allowed making them more accurate and closer to reality. Moreover, the comparison of ecological parameters of native stations and stations selected for previous reintroductions helped explaining the low success of those reintroductions.

Modelling of realized current and future, bioclimatic and climatic niche

The obtained prediction maps gave precise and detailed locations for reintroduction and introduction sites. A 25 m² accuracy seemed ecologically relevant because heterogeneity in environmental conditions can influence translocation success on a scale smaller than 1 m² (Maschinski et al., 2012). On the other hand, modelling with a precision of 25 m² using 10 variables is a long process requiring high computing resources. The modelling process performed with the settings exposed in the Methods section for current scenario took more than 4 days using a 3.20 GHz processor and 16 Go RAM, and produced a volume of files equivalent to 314 GB. Consequently, such a modelling process was possible in the studied area (Switzerland, 41,000 km²), but could become more complicated and tedious for larger regions. In such a case, a coarser scale must be selected, for example 100 m², with a loss of ecological accuracy that could be problematic in such a site selection (Maschinski et al., 2012). Then, in a second phase, an area that seems favourable at 100 m² scale could be used to perform a more precise modelling, for instance at a 25 m² scale. Another solution would be reducing the number of algorithms and runs performed, however with a consequential loss of robustness in the calibration and evaluation phases of modelling.

In the current scenario, the continent-wide scale model calibrated only with bioclimatic variables did not give much information and did not exclude many areas. Indeed, referring to GBIF occurrences, the *C. calceolus* seems to be adapted to nearly all investigated area at a bioclimatic level. On the other hand, the regional scale model, which includes bioclimatic, topographical and forest variables was much more informative and highlighted suitable areas. It is interesting to note that canopy height and precipitation seasonality were the two variable influencing the model at regional scale. The multiplication of continent-wide scale and regional models gave a prediction map identical to regional scale model for the above-mentioned reasons. The addition of filters allowed excluding areas that were not suitable to the species at land use and geological levels, therefore relevant to include.

Concerning future scenarios, considering both very low and high annual total CO₂ emissions, continent-wide scale models (IPSL-CM5A-LR and HadGEM2-A0) calibrated only with bioclimatic variables discriminated large parts of the studied zone. Again, the regional scale modelling process calibrated with bioclimatic, topographical and forest variables eliminated numerous regions of the studied zone and underlined suitable restoration sites. The multiplication of continent-wide and regional maps highlighted unsuitable areas from prediction maps of both scales. Besides, the addition of filters excluded also parts of the studied area. In summary, each step of future scenarios modelling discriminated areas of prediction maps, making each step relevant.

At continent-wide scale, more occurrences than the ones provided by GBIF could improve prediction maps. For instance, Balkan and Baltic areas were under-represented compared to the real distribution area of the species. At regional-scale, variables such as soil pH could have improved prediction maps but have not been available yet in a compatible format with modelling tools.

In the specific case of *C. calceolus*, it is very interesting to note that although great efforts of conservation, reintroduction and introduction in Swiss Jura, this region does not seem, according to the modelled parameters, very suitable for this species neither currently nor in the future. According to the prediction maps obtained, this area might be an edge of *C. calceolus*' realized niche.

The inclusion of future prediction maps in the process of translocation sites selection is particularly relevant in the context of climate changes (Primack, 2014). In this particular case, in view of the long life expectancy and the slow life cycle of *C. calceolus*, correct identification of long-term suitable restoration sites is even more important.

Ecological study and field parameters analyses

It has been shown that the use of ecological quantitative assessments to identify suitable reintroduction and introduction sites increases the rate of plant establishment success (Maschinski et al., 2012), making the second step of the roadmap crucial. Indeed, failures in reintroductions and introductions are often the consequence of a lack of biological data (Heywood et al., 2003).

Statistical analysis performed on extracted bioclimatic and topographical data as well as on field data allowed to confirm field observations. In addition, the envfit function (Oksanen et al., 2013) was particularly adapted to such a study. Indeed, thanks to it, it is possible to understand which environmental factors influence the most the biological traits of the studied species.

In the particular case of *C. calceolus*, the station, vegetation and edaphic parameters selected after a study of species ecology were relevant and gave useful information for confirming sites selected in Step 1. Indeed, some easily measurable parameters (on the field or in laboratory) showed a very narrow range of values in native stations (e.g. pH, C/N ratio) and were therefore crucial to consider for future site selections. In addition, the results of envfit function showed that the topographical aspect and soil temperature should absolutely be taken into account during sites selection.

Concerning *C. calceolus*' ecology, some results obtained in this study did not correspond to those found in literature. First, Käsermann (1999) argued for an optimal soil pH ranging from neutral to moderately acidic, whereas my measurements of the A horizons in native stations were all around 7.6 (i.e. neutral to moderately alkaline). Secondly, Terschuren (1999) described *C. calceolus* sites as dry during summer while the native stations I visited were often moist. Thirdly, humus forms were not only Moder as mentioned by Käsermann (1999). As a matter of fact, I also identified Oligomull and Dysmull. In addition, concerning sites hosting reintroduced seedlings and measures taken to preserve native populations, I noted confusion between the notions of sun and light. As mentioned before, *C. calceolus* is said to need bright conditions for developing well (Terschuren, 1999). Indeed, this is true but the light reaching the plant should be indirect. Direct sun burns adult plants and makes them weak and small. Moreover, based on my field observations and phytosociological surveys, I hypothesised that *C. calceolus* does not need the same amount of light according to its development stage. As a matter of fact, I assume that recruitment and establishment conditions have to be well sun-exposed whereas further growth and flowering conditions have to be bright but not directly sunny. This would mean that *C. calceolus* needs pioneer conditions at early stage of development, and then a more mature

ecosystem without a dense bushy understory layer for the rest of its development. In stations hosting mature plants, the mix of forest and meadows plant species confirms a stable but bright ecosystem. Those assumptions contradict the proposed ecological requirements exposed by Druart (2007), describing *C. calceolus* as a species that needs ecosystems with a strong dynamics.

On the other hand, other ecological traits found in literature correspond to those I observed. For instance, soils of native populations always contained calcium carbonates and were rather permeable. In addition, the failure of mineral grain size analysis because of the high content of organic matter or clay confirmed soil base richness (Terschuren, 1999). Moreover, vegetation alliances identified corresponded to those mentioned by Druart (2007).

To summarize the most important field observations and laboratory analysis results, *C. calceolus* needs soils with a neutral to basic pH with presence of calcium carbonates. In addition, a delicate balance of light, moisture and bases richness influences the presence of this plant as described by Terschuren (1999). Those ecological requirements have to be taken into account for further reintroductions and introductions.

Talking about *C. calceolus* life history traits, results showed that populations invest their resources either in vital functions or in reproduction functions (see e.g. negative correlation between plant size and fruit number). This observation can be useful for further conservation actions, reintroductions and introductions. Indeed, if all optimal conditions for plant vitality are fulfilled, they can invest more energy in reproduction, which is an absolute necessity for seeds production and seedling recruitment as stated Seddon (1999).

Incomplete (or incorrect) reports can cause serious problems when plants are reintroduced or introduced. In addition, as mentioned before, a general lack of biological and ecological data is the cause of a lot conservation failures (Heywood et al., 2003). It is therefore crucial to review carefully existing literature and evaluate ecological parameters, especially in the case of species having a complex ecology, such as orchids (Swarts et al., 2009). In the case of *C. calceolus* reintroductions in Swiss Jura, results obtained in this study allow explaining low reintroduction success. As mentioned above, the first problem was the gaps in ecological knowledge of this species preventing the selection of suitable reintroduction sites. In addition, those sites were chosen on a phytosociological evaluation, excluding other parameters such as the edaphic ones. Soils parameters in those stations did therefore unfortunately not correspond to the edaphic native range of *C. calceolus*. Comparisons with reference populations' parameters, as advised by Menges (2008), were not performed before site selection. Sites chosen for future reintroductions in the framework of this study, after a close visual analysis of native stations, were much more similar to native conditions than those already hosting reintroductions. These results confirm once again the necessity of comparison with reference population.

Moreover, it is interesting to note that edaphic and bioclimatic parameters of native populations match very well with those of future reintroductions site whereas topographic parameters between those two treatments are less alike. The difference in topographic parameters can be explained by the topographic singularity of the area where two of the native sites and the future reintroduction sites were located. Indeed, they were much sloppier than the rest of native range. This highlights the importance of studying the broadest possible range of reference populations in order not to underestimate species realized niche.

My results highlight that choosing sites in historical range of a species is not enough to ensure reintroduction success. As mentioned before, because of the heterogeneity of the environment, reintroduction success can vary even at very low spatial scales (< of 1 m²) (Maschinski et al., 2012). In addition, historical areas may have changed and not correspond anymore to ecological requirements

of the target species (Heywood et al., 2003). As Maschinski (2013) stated, in restoration science, local is not always the best choice.

Above-mentioned problems encountered during reintroductions of *C. calceolus* in Swiss Jura, such as lack in species biology or reintroduction in not suitable area for a species, is regrettably not an isolated case and supports data found in literature (Heywood et al., 2003; Seddon et al., 2007; Drayton et al., 2012). The roadmap presented in this study gives adequate solutions for avoiding such problems.

Merging ecological surveys with spatial modelling

The third step allowed the integration of field data on the previously obtained prediction maps. This made the final map for reintroduction and introduction sites more accurate. This approach is however limited by the few variables existing in raster version for large areas such as Switzerland. In the future, if the number of modelisable ecological variables increases, quality of predictions maps will be improve.

In the particular case of *C. calceolus*, the addition of solar radiation did not exclude many areas. Regions with inadequate solar radiation had already been removed in Step 1. As mentioned just above, the addition of ecological filters such as soil pH would have drastically improve the final prediction maps but such raster layers were not available at the time of this study.

In addition, the final current prediction map elaborated in this study needs to be validated on the field. If important field variables available in raster format were shown not having been taken into account, they could be added at any time.

The combination of modelling tools, fieldwork and naturalist approach gives a complete and interesting strategy to identify suitable reintroduction sites. In addition, as mentioned above, the improvement of prediction maps obtained through modelling with field data allows highlighting more accurately suitable sites. Moreover, the consideration of future bioclimatic scenarios optimizes the success rate of translocation in a context of fast changing climate (Marini et al., 2009).

The hierarchical and holistic approach presented here contains specific steps that are reproducible for other plants species. As mentioned in the introduction, studies explicitly describing a methodology to research and select restoration are sites rare. This study combined different already existing methods and allowed filling a part of the current lack of reintroduction and introduction sites selection theory.

Future challenges for the presented conservation roadmap and *C. calceolus*

For now, the quality and the efficiency of the proposed roadmap is difficult to assess because of the lack of similar approaches found in conservation biology literature. In order to validate and evaluate this method, it has to be tested with different species in different kinds of ecosystems. In addition, those attempts need to follow an experimental approach in order to allow comparative evaluation of reference sites and those selected with the proposed method (Seddon et al., 2007). After that, reintroduction and introduction sites need to be carefully and long-term monitored (Seddon et al., 2007). Then, attempts (regardless of whether they are successful or not) should be published in peer-reviewed literature in order to create a database allowing to compare and to evaluate relevance of this method and success of translocation attempts (Godefroid and Vanderborght, 2011b). Even if reintroduction and introduction are long processes yielding no immediate results, the effort of publishing the lessons learnt is a high priority to avoid repeating the same mistakes (Heywood et al., 2003; Seddon et al., 2007; Godefroid et al., 2011a).

As a first trial, sites chosen thanks to this roadmap will host reintroduced and introduced seedlings of *C. calceolus* in Swiss Jura in 2018. As mentioned above, this will be an excellent first test for this method even if the long life cycle of this species makes it difficult to draw rapid conclusions (Heywood et al., 2003). In addition, this will confirm or infirm the initial hypothesis that good knowledge of the actual species' niche allows finding suitable reintroduction and introduction sites. As advised above, results of this first attempt have then to be published in peer-reviewed literature in order to make lessons learnt through success and failure accessible and useful for further translocation.

5. Conclusion and future prospects

The holistic and hierarchical approach presented in this study is a good and promising first step in the direction of repeatable strategy to identify suitable translocation sites and it begins to fill a consequent gap in biological conservation and in restoration science.

As a matter of fact, the combination of modelling tools, field analysis and ecology study allowed a more efficient and relevant selection of restoration sites. Besides, the combination of broad- and fine-scale made the choice of those sites more accurate.

In addition, as already done in subsequent studies, the results of the modelling step of this roadmap can be used for other conservation purposes than those presented above. For example, rare species prospection and selection of sites for natural reserves are two potential uses.

This roadmap included vegetation, edaphic, topographical and bioclimatic parameters but on the other hand, did not considerate numerous primordial factors such as genetic, demographic, logistical, historical and especially biological ones. As a matter of fact, genetic, demographic and metapopulation studies are essential to increase the success rate of restoration. For successful reintroductions and further introduced population recruitment, plants need to be correctly inserted in the web of existing populations. In addition, some logistical parameters such as sites access can be a limiting factor and have to be considered. Historical factors and former distribution give great information about population dynamics and decline causes. Moreover, biological interactions such as herbivory, parasitism or mutualism are crucial in reintroduction and introduction success. That is especially true in the case of this study. Indeed, as an orchid, *C. calceolus* has a symbiotic relation with mycorrhizal fungi that is essential for seeds germination. This fungus is then a crucial parameter to take in account for reintroduction and introduction sites selection. In addition, the very specific pollinators of this species must be present in restoration sites to make population recruitment possible. A close study of insect species occupying selected sites is therefore necessary too.

The presented roadmap has to be considered as the foundations of a more accurate and multidisciplinary roadmap, which would include the above-mentioned parameters lacking in this case. The more multifactorial the next roadmap will be, the higher success rate it will yield.

I conclude this study with a thought of Godefroid et al. (2011a) saying that translocation of species is the point of nature destruction to which we should never arrive.

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