Project of Strategic Interest NEXTDATA

WP1.7

D1.7.A – Preliminary version of the mountain faunal biodiversity database and of empirical models for assessing the response of mountain biodiversity to climate change

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This Deliverable describes the assessment of changes in biodiversity and ecosystems in high altitude mountain areas, by statistical analysis and comparisons of the data collected during the project with earlier data, made available in the course of the project.

In this framework, this study aimed to apply and to improve monitoring and data storage methodologies, devoted to explore relationships between animal biodiversity, climate and land use at different spatial scales in alpine protected areas. Long term purpose is to create the baseline against which to assess future changes with a monitoring program that is planned to be repeated every five years.

The study is characterised by two different steps: the conduction of the new monitoring campaign; the identification, collection and analysis of previous data on terrestrial biodiversity in the western Italian Alps. This monitoring project was promoted by Gran Paradiso National Park in 2006 and continued with the cooperation between CNR and other two protected areas in the NW Italian Alps, the Orsiera Rocciavré Natural Park and the Veglia Devero Natural Park.

1. State of the art (2012-2016)

Field research activities have been carried out in fixed plots, already subjected to monitoring during 2007-2008. They represent the implementation and continuation of a previous existing *in situ* project, promoted by Gran Paradiso National Park in 2006 and continued with the cooperation between ISAC-CNR and other two protected areas in NW Italian Alps, Orsiera Rocciavré Natural Park, Veglia Devero Natural Park.

Aim of the research is the development of an historical dataset, on the base of already existing data, and to improve some of the methodologies applied to the monitoring of animal biodiversity.

Monitoring activities during 2012-2013 have been carried out in all the 3 Parks (Gran Paradiso National Park, Orsiera-Rocciavré Natural Park, Veglia Devero Natural Park). Thirteen altitudinal transects were set covering an altitudinal range of 1000 meters, chosen from 500 to 2700 m a.s.l. interesting three vegetation belts (montane, subalpine, alpine).

Sampling units are circular plots (100 m radius), for a total of 75 sampling stations, where monitoring activities have been carried out to provide presence/absence and relative abundance data of species belonging to taxa, selected as bio-indicators.

Selected taxa are: Lepidoptera Rhopalocera (butterflies), Orthoptera (grasshoppers/crickets), birds, surface-active macro-arthropods (Coleoptera Carabidae, Coleoptera Staphylinidae, Araneae, Formicidae).

For each taxonomic group we used semi-quantitative census techniques that are, as much as possible, easy to apply, standardized, cheap and repeatable. Birds were census by means of point counts and each plot was visited twice during the reproductive season. We sampled butterflies and grasshoppers/crickets using walking transects along the diameter of the plot (200 m in length), walked at uniform speed. We collected surface-active arthropods using pitfall traps (plastic cups, diameter of 7 cm, filled with 10 cc of white vinegar). All collected specimens were stored, preserved in alcohol 70%, and identified at the species-level, by expert taxonomists.

Monitoring activities concerned also the collection of:

- micro-climatic data, through the positioning of temperature data-logger (iButton DS1922), one per each sampling station, located in field for all the sampling season;
- macro-environmental (topographic variables) and micro-environmental parameters (percentage of land coverage and estimate of floristic diversity).

During 2014-2016, field activities were carried out in selected plots and focused on birds (30 plots), butterflies (13 plots) and micro-climate (30 plots).

Results of monitoring activities are databases, constituted by lists of species, with data on relative abundance, for each taxon and each sampling plot. Moreover, each plot is accompanied by the characterisation of environment and micro-climate.

These data are now stored in appropriate databases.

Since 2013, three other protected areas, decided to share the objectives of the monitoring activities promoted by PNGP in 2006 and to follow the sampling protocols already adopted by PNGP, PNOR and PNVD. These protected areas are Stelvio National Park (CPNS), Val Grande National Park (PNVG), Dolomiti Bellunesi National Park (PNDB). During spring 2013 (March-April), we formed the personnel of these Parks, in order to obtain comparable data and we create the appropriate databases for common data storage.

Results of the first monitoring campaign (2006-2008), from the first three involved protected areas, have already been analysed, following two approaches: i) the description of α- and β- diversity along the altitudinal gradient; ii) a modelistic approach using Species Distribution Models to forecast biodiversity, considering climate and land-use changes.

As shown in the previous deliverables/reports, results of this first data collection and analysis have been part of Master and PhD Theses and have been presented to different international congresses.

2. *Organisation and storage of the raw data*

The biodiversity data, collected since 2005 by the 3 protected areas partner of the project (Gran Paradiso National Park, PNGP; Orsiera Rocciavrè Natural Park, PNOR; Veglia Devero Natural Park, PNVD), have been stored in a relational database, located at PNGP server, and ready for flowing into the systems of archives and portals of the Next Data project in a summarized way. In the following, and also summarised in Table 1, a brief description of the each database, which are 9 "sampling" databases (one per each of the 8 taxa sampled inside the project plus one related to the faunistic observations), 3 "measure" databases (referred to faunistic contour data), 2 "geographic" databases (describing the geographic position of the sampling sites), 3 "environmental" databases (with floristic, land cover and micro-climatic data).

<u>Sampling databases</u>

- **Coleoptera Carabidae**. Relative abundance and ecological information of the ground beetles (carabids) sampled through pitfall traps during the two sampling periods of the project (PNGP, 2005-2006-2007-2012-2013; PNOR, 2007-2008-2012-2013; PNVD, 2007-2008-2012-2013). The data collected in 2005 are referred to a reduced number of plots. We followed the nomenclature proposed by APAT (2005). The ecological information have been mainly obtained thanks to the expert knowledge (Dr. Gianni Allegro), but also consulting APAT (2005) and Pesarini and Monzini (2010).
- **Coleoptera Staphylinidae**. Relative abundance and ecological information of the rove beetles (staphylinids) sampled through pitfall traps during the two sampling periods of the project (PNGP, 2005-2006-2007-2012-2013; PNOR, 2007-2008-2012-2013; PNVD, 2007-2008-2012-2013). The data collected in 2005 are referred to a reduced number of plots. The specimens belonging to the sub-family Aleocharinae have been determined at the species level only in 2005-2006 in PNGP. We followed the nomenclature proposed by Horion (1963, 1965, 1967). The ecological information have been mainly obtained thanks to the expert knowledge (Dr. Adriano Zanetti), but also consulting Koch (1989) and Horion (1963, 1965, 1967).
- **Araneae**. Relative abundance and ecological information of the spiders sampled through pitfall traps during the first sampling period of the project (PNGP, 2005-2006-2007; PNOR, 2007-2008; PNVD, 2007-2008). The data collected in 2005 are referred to a reduced number of plots. The specimens collected during the second sampling period are still under the control of taxonomist, due to few determinations uncertainties, but soon they will be ready for the storage in the electronic archive. We followed the nomenclature proposed by Platnick (2014). The ecological information have been obtained consulting Isaia et al. (2007) and the web site "Araneae - Spiders of Europe", developed and maintained by the Bern University (www.araneae.unibe.ch).
- **Hymenoptera Formicidae**. Relative abundance and ecological information of the ants sampled through pitfall traps during the second sampling period of the project (PNGP, 2012-2013; PNOR, 2012-2013; PNVD, 2012-2013). We followed the nomenclature proposed by Ruffo and Stoch (2005). The ecological information have been obtained consulting two international web sites (www.antwiki.org, www.antweb.org).
- **Lepidoptera Rhopalocera**. Relative abundance and ecological information of the butterflies sampled through linear transects during the two sampling periods of the project (PNGP, 2006-2007-2012-2013; PNOR, 2007-2008-2012-2013; PNVD, 2007-2008-2012-2013). Butterflies have also been collected every year in 13 selected plots in PNGP (2005-2015). We followed the nomenclature proposed by Balletto et al. (2014). The ecological information have been obtained consulting Balletto et al. (2007) and Balletto et al. (2015).

- **Orthoptera**. Relative abundance and ecological information of the grasshoppers and crickets sampled through counts on delimited surface along linear transects during the two sampling periods of the project (PNGP, 2006-2007-2012-2013; PNOR, 2007-2008-2012-2013; PNVD, 2007-2008-2012-2013). Unfortunately, data are not directly comparable between sampling periods, because during the first period have been carried out mainly random samplings, while during the second one a more focused sampling design has been adopted. In particular, during 2006 in PNGP we tested the methodologies and carried out the sampling only in a reduced number of sites. We followed the nomenclature proposed by Massa et al. (2012). The ecological information have been obtained consulting Fontana et al. (2007), Massa et al. (2012) and Hochkirch et al. (2016).
- **Aves**. Relative abundance and ecological information of the birds sampled through point counts during the two sampling periods of the project (PNOR, 2007-2008-2012-2013; PNVD, 2007-2008, 2012-2013) and continuously, every year, in PNGP (2006-2015). We followed the nomenclature proposed by BirdLife International (2015). The ecological information have been obtained consulting Boitani et al. (2002), Hume (2003) and BirdLife International (2015).
- **Odonata**. Relative abundance and ecological information of the damselflies and dragonflies sampled through linear transects around bogs, ponds and wet areas present inside the sampling plots (PNVD, 2007-2008-2013). Suitable plots have been found only inside PNVD. We followed the nomenclature proposed by Riservato et al. (2014). The ecological information have been obtained consulting Utzeri and D'Antonio (2007), Dijkstra and Lewington (2010), Kalkman et al. (2010) and Riservato et al. (2014).
- **Observations**. Faunistic (and also floristic) observations collected in a non-standardised way inside the sampling plots of the project but also along the paths walked by the researchers going from one plot to the other. Such data have been collected during the sampling years of the project (PNGP, from 2006 to 2015; PNOR, 2007-2008-2009-2012-2013; PNVD, 2007-2012-2013). The data collected are not only related to the taxa monitored inside the project, but depend on the competencies and observation abilities/attention of the single researcher. These data are consequently not directly comparable through time and space, and cannot be directly used as semi-quantitative data

Measurements databases

- **Biomass**. Measurements of the volume and the mass occupied by all the arthropods (isolated from the other invertebrates and the few vertebrates), sampled inside the pitfall traps during the second monitoring period (2012-2013, PNGP, PNOR, PNVD).
- **Activity Density**. This represents the number of traps active and emptied during each biweekly sessions of the pitfall traps sampling, in all the years of the project (PNGP, 2006-2007-2012-2013; PNOR, 2007-2008-2012-2013; PNVD, 2007-2008-2012-2013). We distinguished the traps found empty in field from the traps that have been destroyed due to different causes (e.g., animals, particular weather conditions) and consequently didn't provide any sampling unit. Such information is important to obtain a more realistic quantification of the sampling effort for the pitfall trapping and to make fully comparable data coming from different plots and years.
- **Morphometry**. Morphometric measurements done on 5 species of Coleoptera Carabidae (*Carabus depressus*, *Calathus melanocephalus*, *Pterostichus externpunctatus*, *Pterostichus flavofemoratus*, *Pterostichus multipunctatus*), sampled inside the pitfall located in field in the 3 protected areas, during the field season 2012. Eleven morphometric traits have been sampled for each specimen and each measurement have been done independently by three operators. In the attached protocol the morphometric traits have been described and also the tolls used to obtain them.

Geographic databases

- **Traps**. Geographic coordinates of the position of each pitfall traps and of each temperature data-logger positioned in field during the two sampling periods (first period 2006-2007-2008; second period 2012-2013). There could be some small differences in the location of the traps during the first and the second sampling period, due to field error in re-find them or to imprecision in the GPS instrument. Unfortunately, coordinates are not available for all the traps but few of them are missing.

- **Plots**. Coordinates of the plot centre and shapefile of the plots (buffers with a radius of 100 meters around the centre), the sampling units of the project.

Environmental databases

- **Environment**. Information referred to the micro-habitat and the general environmental characteristics of each plot. These data have been collected in 2012-2013.
- **Temperature**. This is a folder of files. Inside this folder, there are different file typologies (hourly measurements of the field located data-logger, validated through a quality control procedure; daily measurements of the ARPA weather stations located inside or near to the bounder of each park; procedure of temperature construction and extrapolation to obtain a temperature map at parks levels). We also stored all the passages which carried to the development of the temperature maps (spatial resolution of 250x250 m), but the detailed procedure is described in a successive paragraph.
- **Vegetation**. This is a folder of files. Inside this folder, there are many different data typologies (floristic lists, semi-quantitative data deriving from linear transects, quantitative description of tree biomass, shapefile deriving from photo-interpretation). Moreover and unfortunately, vegetation/land cover data are not currently homogeneous among the three protected areas. Indeed such data have been stored in separate file for each park. In attach is also present a file with more detailed explanation of the methodologies adopted by each protected area.

Data	**Group**	**Time period**	**Spatial Coverage**
Sampling databases	Coleoptera Carabidae	2005, 2006, 2007, 2012, 2013	PNGP (30 plots)
	Coleoptera Carabidae	2007, 2008, 2012, 2013	PNOR (20 plots), PNVD (24 plots)
	Coleoptera Staphylinidae	2005, 2006, 2007, 2012, 2013	PNGP (30 plots)
	Coleoptera Staphylinidae	2007, 2008, 2012, 2013	PNOR (20 plots), PNVD (24 plots)
	Araneae	2005, 2006, 2007, 2012, 2013	PNGP (30 plots)
	Araneae	2007, 2008, 2012, 2013	PNOR (20 plots), PNVD (24 plots)
	Hymenoptera, Formicidae	2012, 2013	PNGP (30 plots), PNOR (20 plots), PNVD (24 plots)
	Lepidoptera Rhopalocera	2006, 2007, 2012, 2013	PNGP (30 plots)
	Lepidoptera Rhopalocera	2007, 2008, 2012, 2013	PNOR (20 plots), PNVD (24 plots)
	Lepidoptera Rhopalocera	2005-2015 (continuously)	PNGP (selected areas)
	Orthoptera	2006, 2007, 2012, 2013	PNGP (30 plots)
	Orthoptera	2007, 2008,	PNOR (20 plots), PNVD (24 plots)

Data	Group	Time period	Spatial Coverage
		2012, 2013	
	Aves	2007, 2008, 2012, 2013	PNOR (20 plots), PNVD (24 plots)
	Aves	2006-2015 (continuously)	PNGP (30 plots)
	Odonata	2007, 2008, 2013	PNVD (5 plots)
	Observations	2006-2015	PNGP, PNOR, PNVD
Measure databases	Activity Density	2006, 2007, 2012, 2013	PNGP (30 plots)
	Activity Density	2007, 2008, 2012, 2013	PNOR (20 plots), PNVD (24 plots)
	Biomass	2012, 2013	PNGP (30 plots), PNOR (20 plots), PNVD (24 plots)
	Morphometry	2012	PNGP (30 plots), PNOR (20 plots), PNVD (24 plots)
Geography databases	Traps	2007, 2012	PNGP (30 plots), PNOR (20 plots), PNVD (24 plots)
	Plots	2007	PNGP (30 plots), PNOR (20 plots), PNVD (24 plots)
Environmental databases	Environment	2012, 2013	PNGP (30 plots), PNOR (20 plots), PNVD (24 plots)
	Temperature	2006, 2007, 2012, 2013	PNGP (30 plots), PNOR (20 plots), PNVD (24 plots)
	Temperature	2006-2014 (continuously)	PNGP (selected areas)
	Vegetation	2007, 2014	PNGP (30 plots)
	Vegetation	2007	PNOR (20 plots), PNVD (24 plots)

Tab. 1. List of the biodiversity data archived and stored. The time period and the spatial coverage of each kind of data are shown.

The metadata referred to this database has been also prepared, in accordance with EnvEurope (LTER-Europe)/ExpeER Metadata Specification for Dataset Level based on EML (Ecological Metadata Language) specification. Our metadata are referred both to the biological databases and the sites of data collection (synthetic information about the three protected areas, PNGP, PNOR, PNVD). In particular, metadata of the biological databases comprehend: i) a title and a abstract describing the main purpose of each database; ii) the extent of the data collection in time and space; iii) a detailed description of the field methodologies adopted to obtain them and of the identification procedures (e.g., the identity of the expert taxonomist who identified the specimens, the followed nomenclature) ; iv) the point contact for the databases (author and owner of the data, both the database and the collected specimens) and its use limitations.

Moreover, the ecological databases are not only related to the presence and abundance of different species in selected plots and time, but also contain some important information: i) a value corresponding to the quality of the data (some specimens can be determined only at the genus or family level, lowering the quality of the determination); ii) the ecological characteristics of each species (derived both from literature and expert opinion, and the related references we used); iii) information, if available, about the single specimens (age classes, sex, behavioural observation).

To obtain a complete collection of data and metadata and to make as precise as possible the future repetition of the sampling procedure (and also any kind of data analysis), we also stored:

- a detailed description of the sampling design and protocol;
- the field sheet use to collect the data;
- a template of the Excel file use to digitally archive all the data.

3. *Data Analysis*

3a) Modelling approach at species level

The aim of these analyses is to develop and test a modellistic approach, to obtain the probability of occurrence of invertebrate target species, using as a starting point the data derived from the Biodiversity Monitoring Project (sampling years 2006-2008; protected areas involved PNGP, PNOR, PNVD).

Modellisation has been carried out at the scale of the whole three protected areas involved in the monitoring and at high spatial resolution (250x250 m cells).

We applied *species distribution models* (SDM) following a multi-scale technique, combining two spatial scales and two different kinds of models (regional and local models).

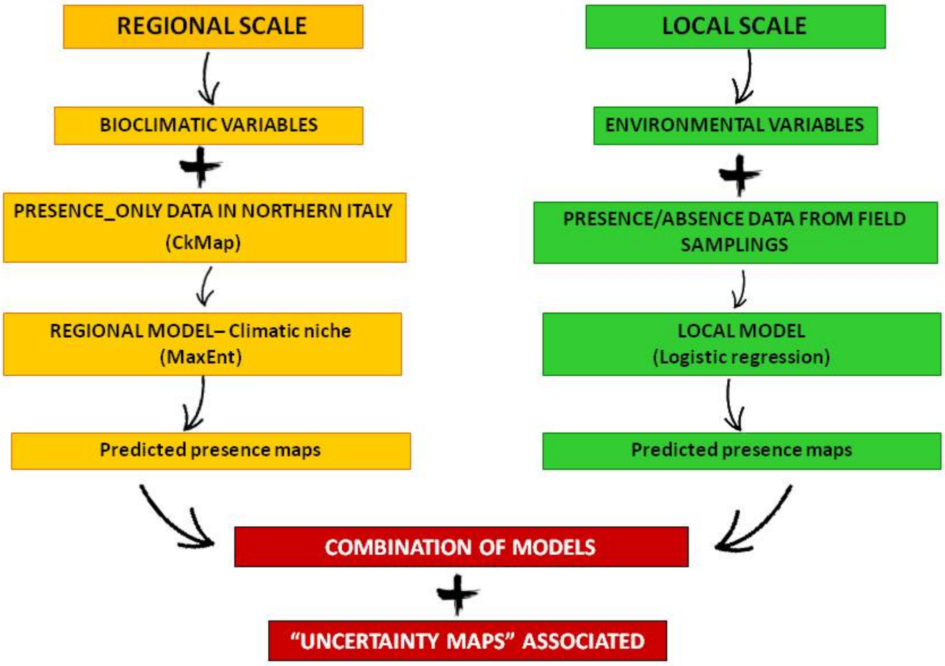
The main steps of this approach were:

- Acquisition and evaluation of predictor variables (temperature and land cover), through the creation of climatic maps of the study areas and the comparison between different sources of land cover data;
- Acquisition of species data and reconstruction of their climatic niche using presence-only data and climatic variables at a regional scale (Northern Italy). The local model instead was made using environmental variables (land cover data) and presence/absence data within Parks boundaries;
- Product of the two models, to obtain potential distribution and uncertainty maps of the chosen species.

During the previous steps of the project, we create temperature maps using in situ data and we selected the "Forestry and other land-use categories map of the Piedmont Region (FPM)" as best available land-cover map.

Our last step was the construction of the species distribution modelling approach, combining two different kind of models with different explanatory variables.

The scheme below briefly summarizes this combined approach.



The selected approach is characterised by the combination of 2 models, developed at different spatial scales, following the suggestion of Vicente et al. (2011). This combined approach differs from traditional approaches (in which all the variables are analysed inside the same modellistic procedure) because it is formed by 2 distinct models, which are based on different predictor variables. Each of the 2 probabilities of presence are then combined to obtain the final model.
These are the steps of our modellistic approach:

- subdivision of response variables in 2 groups (over-regional and local scales);
- modellisation of the selected species, independently for each set of variables;
- projection of each model over the high resolution spatial grids (250x250 m), covering each protected area;
- combination of both projection in the final model, through the product of single probabilities.

The variables used at the over-regional scale are the bioclimatic ones. Indeed, climate can influence species' distribution at a continental scale (Vicente et al. 2011) and to obtain a precise description of the linkage between probability of presence and climate, it is necessary to analyse a large portion of the species range. We indeed considered that our sampling approach (3 protected areas, altitudinal range 800-2600 m) could be not enough to catch the range of climatic tolerance for the selected species.

The variables considered at the local scale are the one related to topography and land cover. Indeed, our sampling stations are distributed over the protected areas territory to cover most of the present environmental variability.

Multi-scale approaches can give information related to variables, which otherwise are difficult to be considered, if working on one exclusive spatial scale (Vicente et al. 2011) and can furnish useful information from the conservationistic point of view, also giving a robust starting point for simulation (Elith and Leathwick 2007).

We tested our approach on 3 taxa (Coleoptera Carabidae, Orthoptera, Lepidoptera Rhopalocera), selected due to the presence of bibliographic references about their climatic tolerance. We choose 2 species per taxon, choosing species with different altitudinal specialisation.

Over-regional scale

We estimated at the over-regional scale the climatic niche of the selected species, using presence data furnished by CKMap (Ruffo and Stoch 2005). CkMap is an atlas of the Italian fauna, with spatial information collected over low resolution spatial grids (10x10 km), deriving from unstandardised data (only presence information). In our case, we focused on -the grids over North Italy (1397 cells). Each cell is characterised by the value of "1" in case of presence and "NA" in case of unknown information. Temperature data have been obtained by the maps of Metz et al. (2014).

We obtained the climatic niche using MaxEnt (Philips et al. 2006).

The analysis have been done with the software R, using the package *biomod2* (Thuiller et al. 2014). We used 80% of the initial dataset as a *training set*, to calibrate each models, and the remaining 20% as a *test set*, to validate the models (100 iterations). For each iteration, we evaluate model goodness of fit through AUC (Receiver Operating Characteristica/Area Under the Curve) and TSS (True Skill Statistic) (Thuiller et al. 2014). We used the iteration with a TSS value > 0.7 to execute an *ensemble forecasting*, a global model representing the climatic niche of each species.

Local scale

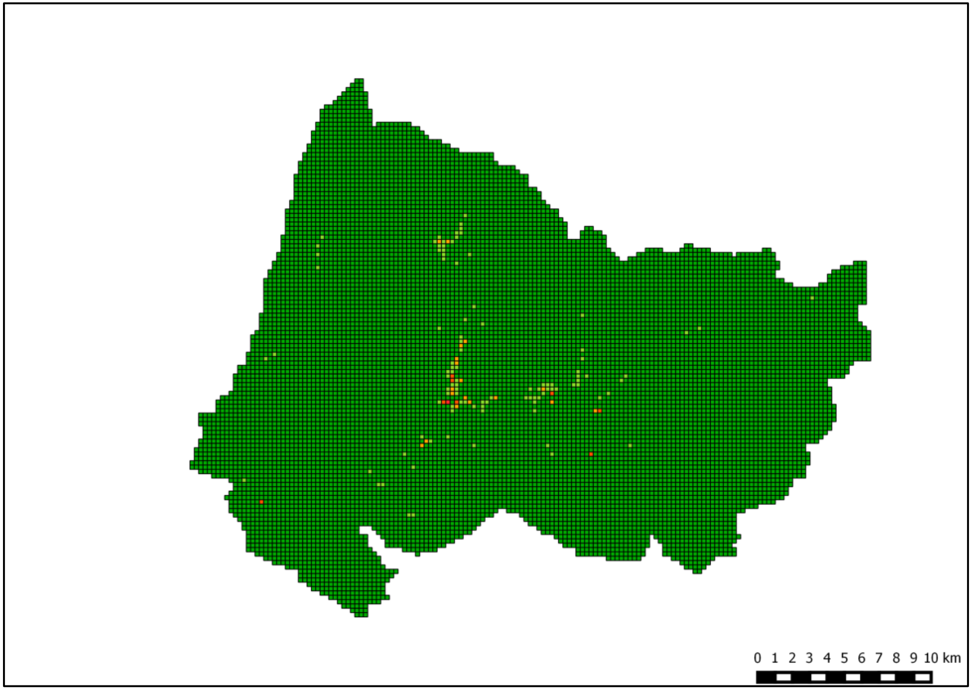
The local scale analysis, used as a starting point, field data collected inside the Biodiversity Monitoring Project during the previous years (since 2006), to determine through logistic regression the relationships between presence/absence data and topographic and land cover data.

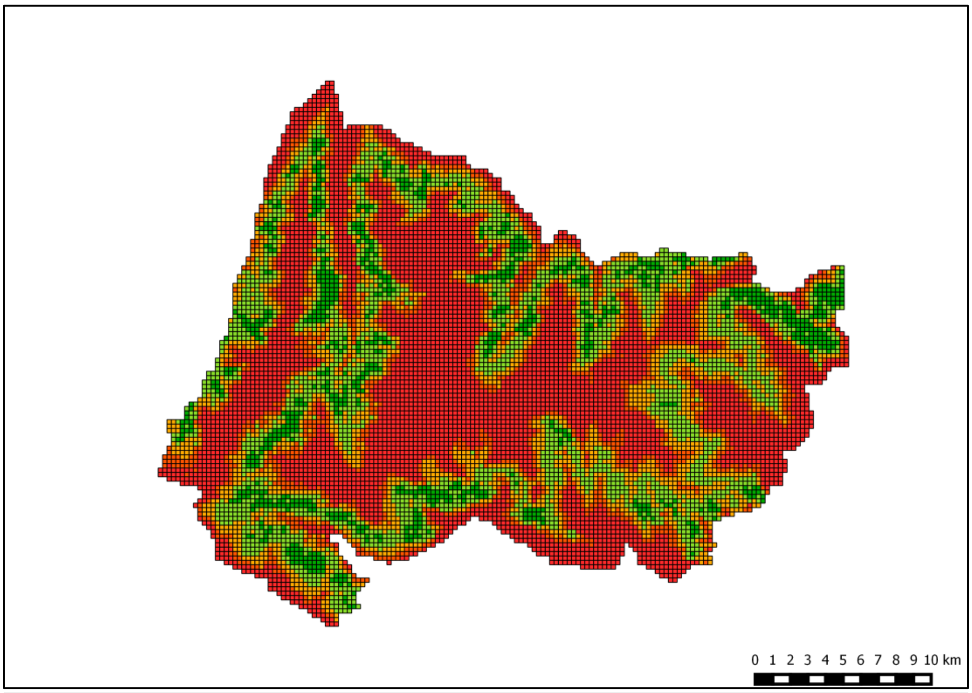
Explicative variables are:

- altitude (100 m bands);
- percentage of tree cover (boradleaves and coniferous – 20% increase);
- percentage of herbaceous layer (20% increase);
- rock presence (yes/no);
- slope (low/high);
- aspect (cosin component);
- index of structural diversity (Shannon index of different structural layer).

Also for this modelisation, we used the R package *biomod2* (Thuiller et al. 2014). But in this case, we used as a training set the 60% of the original data (40% as a test set; 100 iterations). Model evaluation and ensemble forecasting have been done in the same way as for the over-regional scale. To obtain the final representation of probability of presence for each cell, we made the product of each single probability (over-regional and local scale), assuming as independent the values derived from the 2 models.

As an example, the maps obtained for *Aeropus sibiricus* (Orthoptera, Acrididae) in the Gran Paradiso National Park are shown (Fig. 1).

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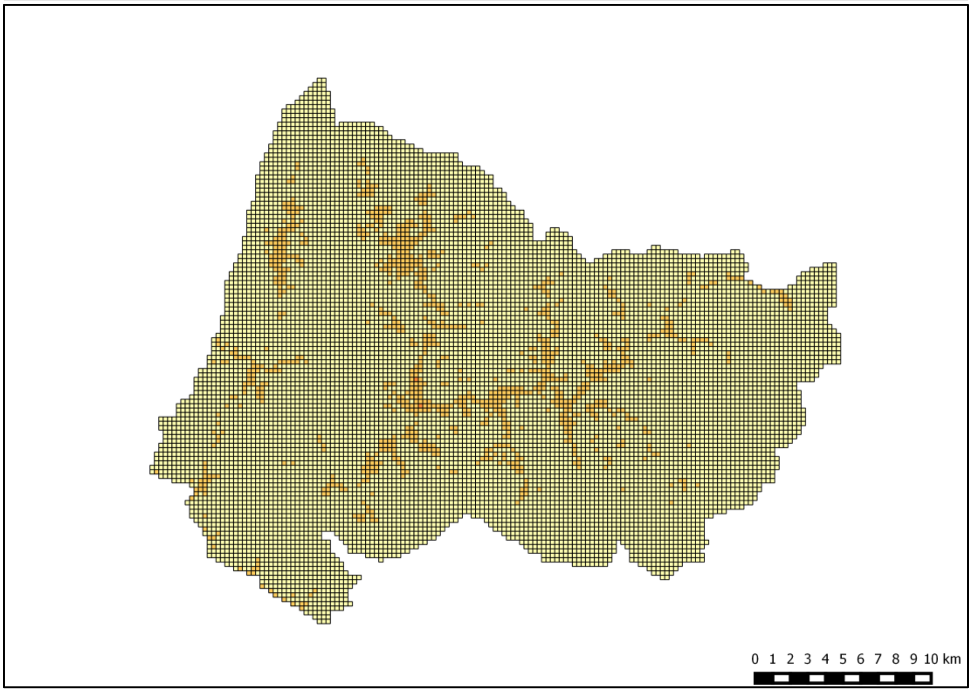
0 1 2 3 4 5 6 7 8 9 10 km

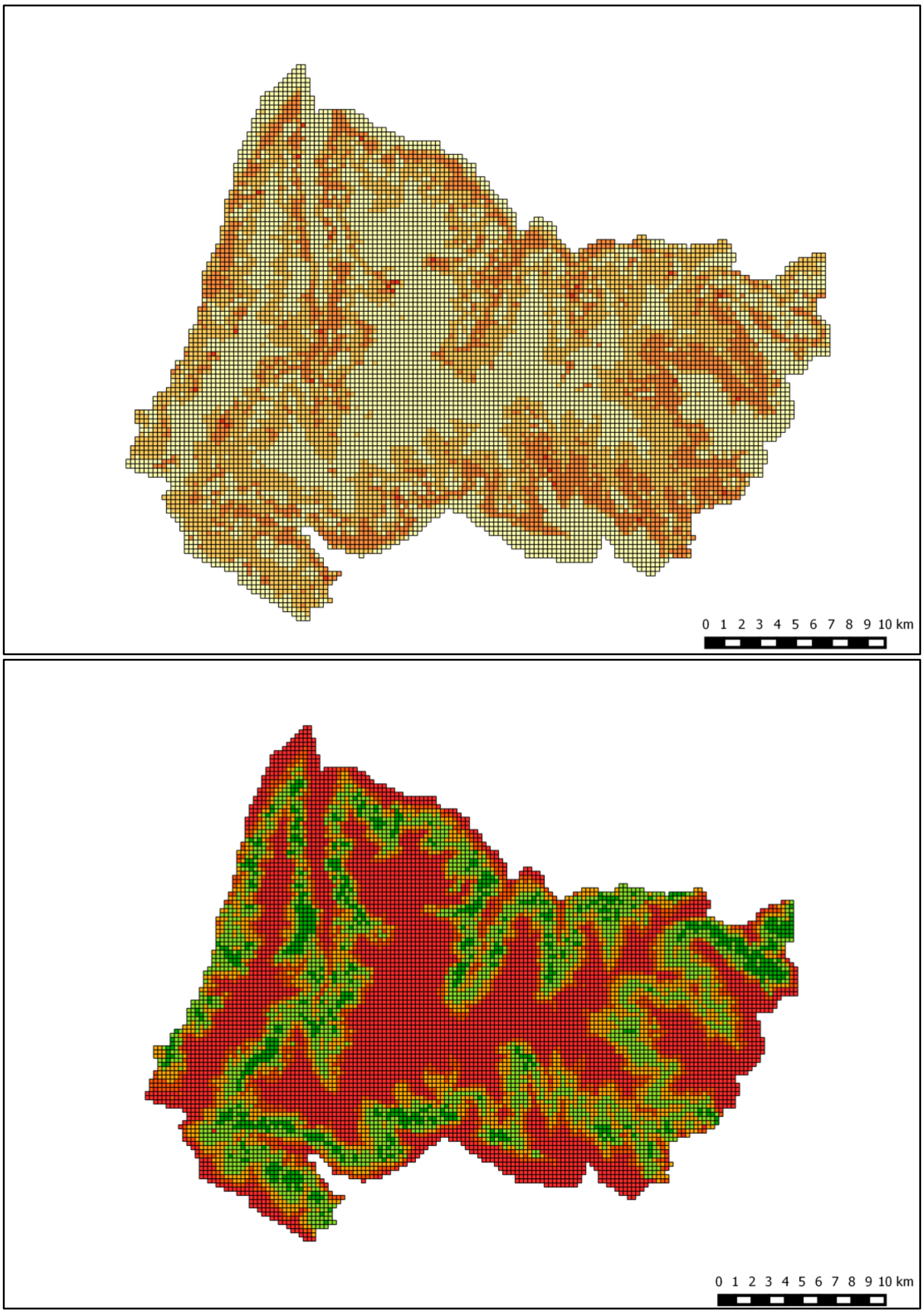

Fig. 1. Maps representing the probability of presence for Aeropus sibiricus in the Gran Paradiso National park, following the model at the over-regional scale (climatic niche; A) and its confidence interval (C) and the model at the local scale (B) and its confidence interval (D). In the maps E, the final probability, given by the products of both models is represented.

As a general conclusion, we can summarise that:

- our modellistic approach represent a good compromise to obtain probability of presence at high resolution spatial scale, also integrating data from the whole climatic niche of the species. Such a modelistic approach can be used for conservation purposes and as a starting point for the application of climate and environmental change scenarios;
- some drawbacks and weak points have been identified. In particular, the data at the over-regional scale are at a too high spatial resolution (10x10 km), while the low precision of land cover maps at the local scale can strongly influence the quality of the results.

Moreover, we should work on a better way to combine the two models (over-regional and local scale) in the final one, both in term of probability of occurrence and in term of uncertainty maps.

3b) Butterfly communities along altitudinal gradients: 5 years data from the NW Italian Alps

The main threats to biodiversity are climate warming and land use changes. Other drivers may also interact with climate change and land use changes to impact biodiversity however, substantial changes in terrestrial species' populations and distributions have already been detected world-wide mainly in response to both of these impacts (Mantyka-Pringle et al. 2012). Exploring temporal patterns of biodiversity is of great relevance because future warming and related environmental changes are expected to cause substantial changes not only in spatial species distribution but also in species turnover in time. Long-term monitoring programs are for sure fundamental tools in order to assess temporal changes of biodiversity on a long time scale. However, temporal data series need time to reach a long time extent. Therefore, it could be intriguing meanwhile to focus on short-time scales, for investigating short-term biodiversity responses and understanding if there is a beginning of biodiversity temporal patterns or just a temporal fluctuations.

In this framework, we analysed butterfly data deriving from the two sampling periods (1st, 2006-2008; 2nd, 2012-2013) of the Biodiversity Monitoring Project and we compared them both at species and at community level.

These analyses are part of a Master Thesis of Massimo Brunetti and PHd Thesis of Emanuel Rocchia, and a first draft has been presented as an oral communication at the congress "Future of Butterflies", organised by the Butterfly Conservation Europe at the Wageningen University (April 2016).

Species Distribution and Altitudinal Range

To understand if and how species' distribution changes over time, we analysed changes in occupancy between time periods. We defined occupancy as the number of plots occupied by each species in each sampling session (1st vs 2nd) and compared it by using a t-test for paired samples (significance level assessed after 999 randomizations, following Legendre and Legendre 2012). To identify which group of species changes the most through time, we analysed if the occupancy equally increase/decrease between functional groups. We compared the changes in the number of plot per species (delta plot, 2^{nd} sampling session *minus* 1^{st} sampling session), among the ecological groups of conservation interest by using non-parametric test (Kruskal-Wallis or Mann-Whitney tests, depending on the number of ecological categories). We followed the classification proposed by Balletto et al. (2015), concerning: feeding specialization (from polyphagous to monophagous); altitudinal range (generalist, medium altitude, specialised); alpine species; light ("shade loving", "sun loving"), temperature and water preferences; dispersal capacity; habitat preferences (woodland, ecotone, open areas, screes); length of flight period; voltinism (monovoltine, multivoltine); hibernation strategy (egg, larva, pupa, adult).

We also described the altitudinal range of each species with the following parameters:

- altitudinal optimum (mean and median value);
- higher limit (absolute maximum, 90th percentile);
- lower limit (absolute minimum, 10th percentile).

To quantify the amount of change, we compared these parameters between sampling periods with t-test for paired samples (significance level assessed after 999 randomizations, following Legendre and Legendre 2012). As in the case of occupancy rates, we also compared the changes in altitudinal range among the ecological groups.

As a general result, we observed an increase in mean occupancy levels (n = 150, t = -8.15, p = 0.001; plot/species 1st = 8.85 ± 0.74, 2nd = 12.50 ± 0.96, change = 3.65 ± 0.45).

The occupancy did not change equally among ecological groups. Concerning feeding specialisation, specialised (monophagous) species differed from the other feeding groups (KW test, $\chi2$ = 9.82, df = 3, p = 0.020), even showing a slight decrease in the number of plot per species (polyphagous = 3.26 ± 0.99, one family = 4.10 ± 0.59, one genus = 3.68 ± 1.05, monophagous = -1.17 ± 0.98). We also recorded significant differences regarding the relationship with altitude. Altitudinal specialists increased less than the generalists (KW test, $\chi2$ = 13.13, df = 2, p = 0.001; generalists = 6.12 ± 1.03, medium = 3.04 ± 0.48, specialists = 2.32 ± 1.55) and also high altitude species showed a significant less marked increase (MW test, W = 1070, p = 0.013, high altitude = 1.76 ± 1.32, others = 4.02 ± 0.46). "Shade loving" species showed on the opposite the highest increase in mean occupancy levels (MW test, W = 2269, p = 0.041, "shade loving" = 4.53 ± 0.67, "sun loving" = 2.71 ± 0.57).
Concerning altitudinal range, we observed significant changes both at the minimum and at the maximum boundary of species' altitudinal range. At the lower altitudinal limit, we observed a significant decrease in the absolute minimum value (t-test, n = 133, t = 3.03, p = 0.004, change = -96.62 ± 31.85). At the higher one, we observed an increase in both the absolute maximum (t-test, n = 133, t = -3.08, p = 0.006, change = -75.19 ± 24.01) and in the 90th percentile (t-test, n = 133, t = -2.63, p = 0.014, change = 55.15 ± 20.97).
We also observed significant differences in the changes in the altitudinal range between ecological groups. In particular "high altitude" species, compared to the others, showed a significant increase in the mean (MW test, W = 1657.5, p = 0.041; high altitude = 59.21 ± 21.24, others = -11.07 ± 18.09), median (MW test, W = 1657, p = 0.040; high altitude = 59.37 ± 19.90, others = -8.72 ± 17.58) and 10th percentile values (MW test, W = 1737, p = 0.012; high altitude = 68.12 ± 50.24, others = -72.11 ± 28.05). "Shade loving" species, compared to the "sun loving" one, lowered their minimum (MW test, W = 2738.5, p = 0.014; shade = -164.18 ± 45.34, sun = -28.03 ± 43.47) and 10th percentile values (MW test, W = 2702, p = 0.027; shade = -74.10 ± 36.66, sun = -19.09 ± 34.14). Strongly vagile species increased their minimum boundary, while the others lowered it (KW test, $\chi2$ = 8.34, df = 2, p = 0.015; high = 139.29 ± 110.22, medium = -103.49 ± 37.93, low = -178.79 ± 62.12).

In our analysis, butterflies showed a significant increase in mean occupancy levels and this result is consistent with most of the patterns observed on these taxonomic groups over longer periods. Ecological and life-history traits may be good predictors of species distributional changes and shifts in their upper-elevation boundaries. However, few studies to date have examined their explanatory value, and results thus far are equivocal (Angert et al. 2011).
Monophagous, altitudinal specialists and high-altitude species appeared to be more limited than the others were. Such categories comprehend species with high level of specialisation, consequently less prone to colonise new environments, even if climatic or environmental constraints will be relaxed. In particular, monophagous species are strictly limited by the presence and the quality of their single larval host plant and are already observed and also predicted to be highly vulnerable to climatic/environmental changes (Romo et al. 2014). Our results concerning butterfly specialisation are quite interesting and mirror what has been observed in central Europe concerning habitat specialisation, where a decrease of specialised and low vagile species has been observed along with an increase of generalist and good disperser (Habel et al. 2016).
High-altitude species are already limited in their distribution. Their presence is, in many cases, limited by minimum temperatures (Pellisier et al. 2013) and, consequently, they cannot lower their altitudinal range, neither in many cases, raise it, due to drastic changes in vegetation cover (a high occurrence in rock cover and a strong reduction of the availability of herbs and grasses). Moreover, the permafrost reduction, which is a relatively new and rapid phenomenon, make instable high altitude rock and screes, preventing the colonisation by plant species (Cannone et al. 2007). Indeed, high altitude species also showed a significantly higher increase in their mean, median and 10th percentile altitudinal parameters, corresponding to a reduction in their lower altitudinal boundary and in the surface available. However, we should also consider a limit of our sampling design, as

we do not consider plots above 2700 m a.s.l., consequently reducing our possibility to observe an expansion toward higher altitude and a colonisation of new plots by high altitude species.

A significant higher increase in plot occupancy by "shade-loving" species compared to the others can be associated to a tendency towards a higher coverage of shrubs in the open areas at low and medium altitude. In the European Alps, the effect of climate change is regionally confused by human activities. Cattle grazing in the alpine pastures has been decreasing throughout the last century, allowing a fast recolonization by trees and shrubs, where the treeline had been artificially lowered (e.g., Vittoz et al. 2008; EEA 2010).

Species richness

To analyse how species richness per plot changed through time, we compared it between sampling periods with t-test for paired samples (significance level assessed after 999 randomizations, following Legendre and Legendre 2012). To understand if changes in species richness were mainly related to specific plots' characteristics, we analysed it as a function of the following variables: altitude, temperature, geographic location, dominant vegetation cover (habitat type), dominant land use. We considered as dependent variable the rate-of-change (hereinafter ROC), defined as the differences in species richness between sampling sessions, divided per the species richness of the first sampling session. We analysed ROC through linear regression and we compared variables in a multi-model context, according to two criteria: (i) avoiding the simultaneous use in the same model of highly correlated predictors (Spearman's rs >0.5); (ii) choosing predictors to represent biologically meaningful combinations of predictive variables and consequently avoiding data dredging. All models were compared with the null model (intercept only) and all continuous variables were standardised, to permit comparisons among variables. The multivariate model selection was performed using Akaike information criterion, in its form corrected for small samples (AICc). As measures of goodness of fit, we calculated the adjusted R2. These analyses were performed by R package MuMIn 1.7.2 (Barton 2012).

Species richness significantly increased from the first to the second sampling season (t-test, n = 62, t = -9.76, p = 0.001, change = 8.82 ± 0.90).

The analysis of the ROC showed a significant effect of both land cover and land use: wooded habitats and managed plots increased the most (Table 2).

	alt	**alt^2**	**park**	**rme**	**rmi**	**vegetation**		**use**		**Tmin**	**adj r^2**
butterflies						ecotone	-0.415 ± 0.137 ***	yes	0.284 ± 0.100 **	0.109 ± 0.124 *	24.64
						meadows	-0.192 ± 0.124 **				
						rock	-0.179 ± 0.199				

Tab. 2. Best linear regression model for each taxon. In the cells are indicated the coefficients (± standard error) of the selected variables. Adjusted r squared is indicated as a measure of goodness of fit. alt = altitude; alt^2 = altitude (second order); park = geographic location; rme = change in mean temperature; rmi = change in minimum temperature; vegetation = dominant cover type (land cover); use = land use; Tmin = mean seasonal minimum temperature during the first season. adj r^2 = adjusted R^2. *** $p < 0.001$; ** $p = 0.01$; * $p = 0.05$.

Consequently, we can assess that butterflies showed significant changes in species richness per plot inside the analysed period. Butterfly communities are known to quickly change their arrangement because of environmental changes (Thomas 2005), and previous studies indicated that butterflies might be responding even faster than other taxa (Devictor et al. 2012).

We observed a clear and significant increase in species richness within our temporal frame, mainly related to land cover and land management. The highest rate of change was clearly observed in the wooded areas, while ecotonal places (transitional areas, dominated by shrubs and mainly located inside the subalpine belts) showed the lowest rate. Also other authors observed similar results, although mainly related to individual species abundances. For instance, they observed a higher increase in species abundances simultaneously to an increase of temperature within forest areas respect to elsewhere (Sgardeli et al. 2016). Indeed, in days with high temperature and solar radiation, wooded areas can exert a tampon effects, protecting the individuals from extreme temperature and reducing temperature leaps, on the opposite of what happen in the open areas where they are exacerbated (Oliver and Morecrof 2014).

In the grazed areas, we observed an increase in species richness twice as much as in the unmanaged one. This can be probably explained with the low intensity, sustainable grazing we had there. Indeed, it has been already observed that grazing can increase the presence of plant species belonging to Poaceae and Fabaceae (Fischer and Wipf 2002), which represent the most used plant families as larval host plant by many butterfly species. Moreover, grazing maintains woodland clearings and open herbaceous areas below the tree line, which without management activities would be fast colonised by shrubs and trees (Nagy and Grabherr 2009).

Community composition

We analysed community compositions by testing both for changes in location (significant changes in community composition per site over time) and dispersion over the years (significant changes in observed differences in community composition among sites, over time). Changes in location were tested by applying non-parametric MANOVA to Bray-Curtis distance matrixes, to test if the multivariate centroids of species composition were, or were not, similar in the two groups (Anderson 2001). Non-parametric MANOVA is an analysis of variance using distance matrixes and was performed by the function *adonis* of the *vegan* package (Oksanen et al. 2016). The significance of the test was assessed by using F-tests based on sequential sums of squares obtained from permutations of the raw data (999 permutations). Since we had to keep in mind the temporal structure and the spatial dependencies of our sampling design (62 sites at 2 points in time), we applied a restricted randomisation, which did not allow for permutations across samples.

Changes in dispersion were tested by the *betadisper* function of the package *vegan*, a multivariate analogous of the Levene's test for comparing group variances (Anderson 2001). Non-Euclidean distances between objects and group centroids were handled by reducing the original distances to principal coordinates. To test for significance, we applied a similar randomisation approach, as previously explained.

We observed that all the variables significantly influence butterfly community composition, but focusing on the R2, geographic location (park) and altitude showed the most important role (Table 3). The interaction of season and each variables is significant but, in all cases, of low importance.

Variable		F-value	R2	p	p (dispersion)
season		5.87	1.91	0.001	0.001
park	**first**	5.31	21.54	0.001	0.006
	second	7.1	26.85	0.001	
	***season**	1.93	1.88	0.001	
altitude	**first**	8.14	29.63	0.001	0.015

Variable		F-value	R2	p	p (dispersion)
	second	10.16	34.44	0.001	
	***season**	1.57	1.56	0.002	
vegetation	**first**	4.59	19.19	0.001	0.206
	second	4.82	19.96	0.001	
	*season	1.18	1.19	0.076	
use	**first**	2.11	3.39	0.029	0.001
	second	1.99	3.21	0.045	
	***season**	1.69	0.57	0.006	

Tab. 3. Results of the non-parametric MANOVA. We showed the relative role (expressed as R squared) of sampling season and of categorical variables in determining community composition. For each categorical variable, we showed its effects during the first and the second sampling season, and its interaction through time. To represent the importance of each variable, we indicated the F-value, the p-values (after 999 randomisations) and the R squared of the variable. For each variable, we also indicate if the multivariate dispersion was significantly different among categories. Significant variables ($p < 0.005$) are indicated in bold.

Interestingly, we observe a significant changes in dispersion between season, with a lower dispersion around the median during the second sampling session (Fig. 2, indicating a tendency towards homogenisation).

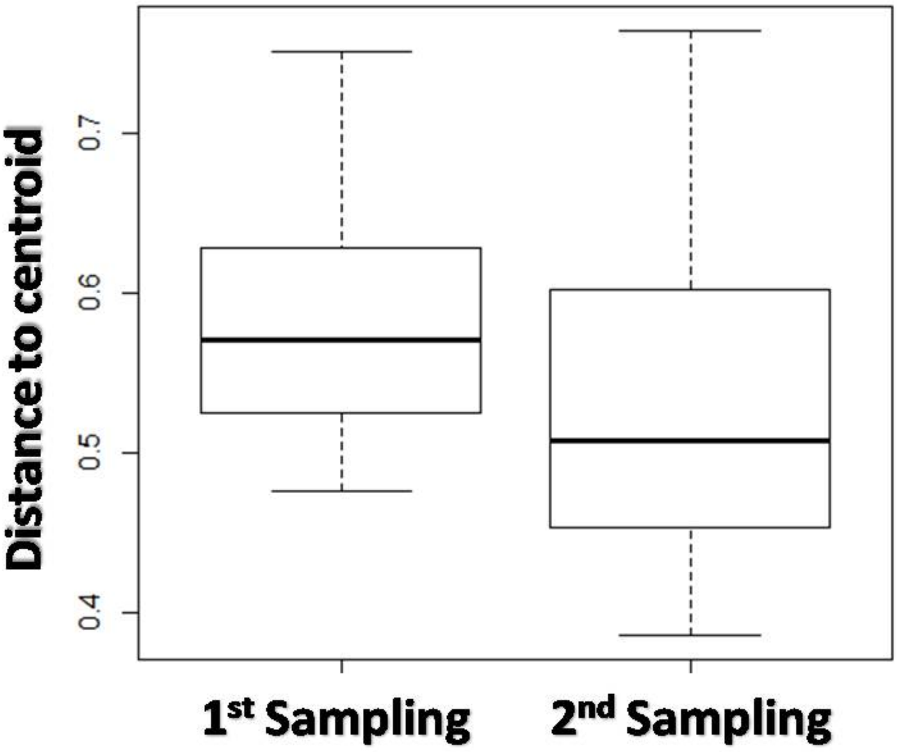

Fig. 2. Box-plot of the distances to the centroid of community composition, during each of the sampling period. A reduction in species heterogeneity at community level from the first to the second period can be clearly seen.

Even if we observed a quite huge amount of differences between our sampling seasons, we did not observe substantial differences in community composition. These results were expected, as a consequence of the short time frame of analysis.

Works that demonstrated variation in butterfly community composition take into account clearly longer time frames (e.g., Habel et al. 2016) and, at our knowledge, no works observe significant changes in community composition in such a short time frame. Anyway, we interestingly noticed a tendency towards biotic homogenization in butterfly community composition. With the term biotic homogenization, we refer to the increase in biological similarity among communities, a replacement process leading to a decrease in distinctiveness in community composition over time, as a result of the replacement of some specialist species with other generalists, which become more uniformly distributed across previously different assemblages (Olden and Rooney 2006). Indeed, species respond individually to the changing environmental conditions, depending mainly on their physiological characteristics and habitat requirements. This determines new species assemblages, which can be appreciated only by the examination of the entire communities throughout time (e.g., Wilson et al. al 2007).

For example, a similar change in community composition over time, accompanied by an increase in community similarity, has been observed in the analysis of data from the UK Butterfly Monitoring Scheme through a period of 20 years (Gonzalez-Megias et al. 2008). This tendency towards biotic homogenisation has been observed during the last decades in different taxa, following land cover and climatic changes (e.g., Buhler and Roth 2011). Such phenomena often determine an increase in the generalist and highly vagile species, to the detriment of the others (e.g., Bonelli et al. 2011).

Community Temperature Index

With the term "Species Temperature Index" (STI), we referred to a quantitative description of the realised climatic niche of a species (Tayleur et al. 2016).

To obtain such quantification over the Italian territory, we used presence data given by the database CkMap (Ruffo and Stoch 2005). CkMap is an atlas of the Italian fauna, promoted by the Italian Ministry of the Environment, which summarised data related to the distribution of the Italian fauna on a gridded map (10x10 km). In our case, because we wanted STI referred to the Alpine populations, we only focused on North Italy, considering 1396 cells. Every cell had a value of "1" (if occupied by the species) or "0" (if the species presence wasn't certain). Temperature data were obtained by the maps of Metz et al. (2014), already described in the previous paragraphs of this report. In this way, we calculated mean temperatura values for each species (realised niche optimum).

We used STI to calculate the "Community Temperature Index" (CTI), quantified as the mean STI of all the species present in a given community. We calculated CTI for each community (plot) and sampling period and we analysed the changes in CTI through time.

We compared CTI between sampling period by using a t-test for paired samples. As in the case of ROC, we analysed the temporal change in CTI (Δ CTI) through linear regression in a multi-model context regression, as a function of the same environmental variables and models.

We observed that CTI significantly increased from the first to the second sampling period (t=-3.59; p=0.001), indicating a common trend toward termophily in all the sampled areas. Moreover, interestingly, we observed that change in CTI through time was mainly dependent on the geographic position of the sampling plots, with a significantly higher increase in the plots located in the colder areas (Fig. 3; R^2=14.17, p=0.007; geographic location, p=0.007).

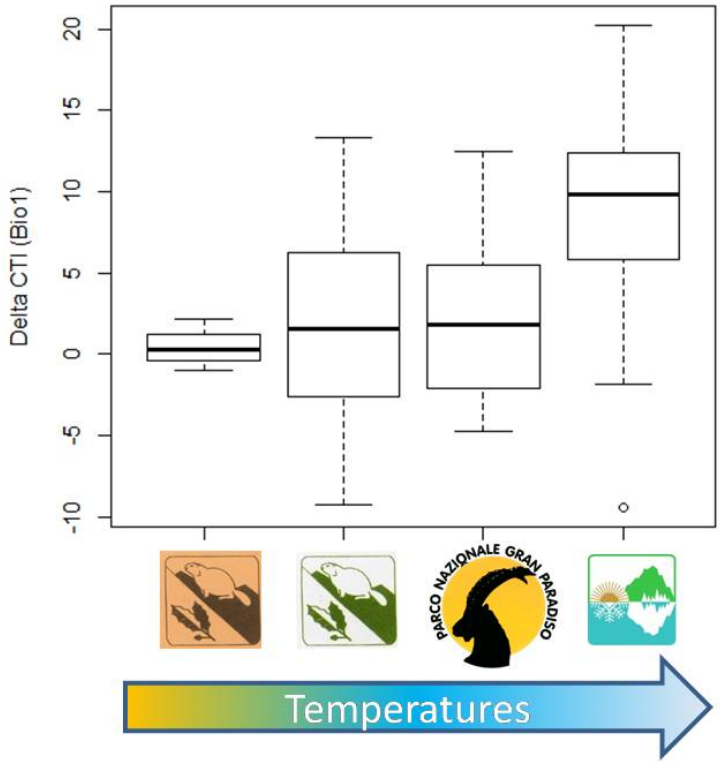

Fig. 3. We observed significant differences in ΔCTI between geographic locations (protected areas). The park located in the colder areas clearly show the highest increase in CTI (calculated considering mean temperature values, expressed as the Bioclimatic Bio1).

Such an increase in CTI derived from the increase in mean occupancy levels recorded by many generalist, more termophilous species from the first to the second sampling period. This phenomenon was particularly marked in the coldest areas, representing a clear threat for the altitudinal specialist and microthermic species of the alpine belt. Our results are consistent with an increase in CTI already observed in other geographic areas (e.g., in Greece by Zografou et al. 2014 over 13 years; in the Swiss Alps by Roth et al. 2014 over 8 years). In any case, our trend has been observed over a shorter time frame, and if confirmed during the next monitoring sessions could represent a warning signal for the alpine butterfly fauna.

Conclusions

Interestingly, and partly alarming, our results suggest that, even if the time-frame under analysis is relatively short, we already observed a huge amount of changes, in particular considering that we are working in protected areas, where habitat alteration by direct human effects is strongly reduced.
To summarise, only in 5 years, we observed:

- a *general increase* in *mean occupancy* level and in *species richness*;
- *no significant changes* in *mean altitudinal optimum*, but *significant changes* at *both altitudinal limits*.

Moreover, the observed changes differed across species, determining:

- an increase in shared species (tendency to *homogenisation*) among communities, even if the *overall community composition* didn't change;
- a significant increase in *Community Temperature Index (CTI)*.

Considering these results, it is now even more important to continue our monitoring to understand in the next future if our observed patterns represent only transient changes or are the first signals of an imminent trend.

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