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Methods, Tools, and Technologies

New approach for butterfly conservation through local field-based vegetational and entomological data

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Abstract

In mountain ecosystems, it is crucial to identify conservation strategies to avoid local extinctions mainly due to agropastoral abandonment. For this purpose, identifying appropriate system indicators is required, for example, butterflies, which respond precisely and quickly to environmental changes. *Zerynthia polyxena* is an ecotonal species of butterfly, and thus, through its conservation it could be possible to protect clearing and ecotonal species. To develop conservation measures, we set up a hierarchical investigation that characterizes the ecological preferences of the adult and larval *Z. polyxena*, and host plant—*Aristolochia pallida*—by collecting data on the butterfly abundance (adult and larvae), tree cover, and litter plant features and by phytosociological surveys. Adult preferences change along an altitudinal gradient; the highest butterfly presence is at sites with medium elevation (1100 m above sea level [asl]) with high presence of forest, but even sites at low elevation (975 m asl), a high presence of forest favors butterfly abundance. Larvae prefer partially shaded ecotone plots with abundant host plants and low management intensity (corresponding to abundant litter) and with heterogeneous tree cover. High tree cover (70%) at low altitude and low tree cover (<20%) at high altitude favor larval presence. Larvae prefer plants with large leaves. Host plants were more abundant at low elevation where tree cover was low (<20%). We found that the optimal ecological niches of host plant and larvae have a spatial mismatch. By analyzing vegetation dynamics and butterfly monitoring during one field season, we are able to describe current and past (about 10–50 years ago) management pressures, in order to identify butterfly ecological preferences in relation to local features and therefore to suggest local conservation actions that might support *Z. polyxena*, as well as other butterflies and insects. Specifically, management measures should prevent afforestation (e.g., through irregular mowing) and favor the presence of small clearings and of ecotonal habitats in relation to elevation. Our study suggests that a new multifunctional approach that combines local entomological and vegetational surveys could be applied to define species optimal habitat features and, thus, to address appropriate conservation measures.

KEYWORDS
ecotones, elevation, insects, larvae, litter, management, phytosociological surveys, tree cover, *Zerynthia polyxena*

INTRODUCTION

To reduce biodiversity loss and climate change, and to support water regulation, the European Commission (2020) has proposed—in the EU Forest Strategy—to plant at least 3 billion trees in the EU by 2030 (COM/2020/380). However, a more complex approach than a simplistic planting strategy should be adopted, starting by developing holistic strategies based on available ecological science (e.g., Selva et al., 2020). We should therefore consider that in European Alps, several taxa are threatened by natural reforestation and forest regrowth (Tasser & Tappeiner, 2005), which is related to recent agricultural and pastoral abandonment (Lehikoinen et al., 2019; MacDonald et al., 2020). For this reason, it is crucial to identify some ecological mechanisms to understand where, which species, and how to plant these trees in order to prevent species loss. Alpine forests have developed in concert with human activities (Motta & Nola, 2001), which have driven the coevolution of different taxa, leading to coupled natural–human ecosystems (Palmer et al., 2004). Which human activities and which intensity of these activities have brought forest biodiversity to the current situation? How should forests be managed to support insect biodiversity? What is the role of clearings in the mountains? It is already known that even forest specialists, such as saproxylic beetles dependent on old-growth forest, are favored by the presence of clearings (Horák & Rébl, 2013; Sverdrup-Thygeson & Ims, 2002).

The abundance of insect taxa is experiencing a global decline (Habel et al., 2019; Hallmann et al., 2017). Butterflies are suffering a particular decline; in the United Kingdom, 76% of native and regular migrant butterfly species have declined (Fox et al., 2015) and 8% of species have become extinct (Warren et al., 2021). In the Netherlands, the decline was estimated at about 80% in abundance (Van Strien et al., 2019), while in Belgium, 20 species (29%) became extinct between 1992 and 2007 (Warren et al., 2021). In Italy, several species have already experienced local extinctions (Bonelli et al., 2011). The major threats in lowland plains regard the intensification of human land use, and conversely, in mountains, the abandonment of traditional agropastoral activities and thus afforestation (Fartmann et al., 2013; Helbing et al., 2015; Thomas, 2016). To develop insect conservation plans, we selected butterflies as an indicator system (Rákosy & Schmitt, 2011), because of their quick reaction to environmental changes and of the large knowledge on species (phylogeny, systematics, and ecology). Moreover, for the general interest of a large audience, butterflies are considered a charismatic flagship species. Overall species that are both charismatic and indicators may represent a suitable *umbrella species* group (Fleishman et al., 2000; Fleishman et al., 2001; New, 1997). Indeed, umbrella species should be representative of a habitat, respond to its changes, and be easy to monitor (New, 1997). As a possible butterfly umbrella species, we selected the southern festoon, *Zerynthia polyxena* (Denis & Schiffermüller, 1775), a species that—in Europe—ranges from 400 to 1000 m above sea level (asl), in sparse deciduous woods, orchards, black locust plantation, clearing, and vineyards (Batáry et al., 2008). Considering its ecotonal habitat preferences (Bonelli et al., 2018), its survival is strictly connected to the management of mountain grassland and it could be an appropriate species to conserve other insect species related to clearing and those that have narrow forest or ecotonal requirements (in accordance with Cini et al., 2021). Other insect species may benefit from management that maintains clearings and ecotonal habitats, such as *Parnassius mnemosyne* (Linnaeus, 1758) and *Hamearis Lucina*, which are both oligophagous species that feed on *Corydalis* and *Primula* species in clearings and ecotonal habitats (Anthes et al., 2008; Bergström, 2005). Moreover, some saproxylic beetles, after a larval life linked to deadwood, during their adult stage visit flowers to feed on pollen and nectar (Palm, 1959).

Zerynthia polyxena is included in Annex II of the Bern Convention on the Conservation of European Wildlife since 1979, and in Annex IV of the EU Habitats Directive since 1992 (92/43/EEC). The main threats for the species are agricultural practice changing at low elevations and the proceeding natural succession and reforestation at high elevations (third assessment of conservation status and trends. The State of Nature in the EU (2019)—Article 17 reporting). In Europe, *Z. polyxena* larvae are oligophagous, feeding on *Aristolochia* species; often, they are locally monophagous (Piccini et al., 2021; Slancarova et al., 2015; Vovlas et al., 2014). Indeed, *Z. polyxena* has specialist host plant requirements that are known to make species sensitive to habitat changes (Öckinger et al., 2010).

To establish conservation plans for Lepidoptera species, it is crucial to understand species habitat preferences and the current and past management requirements to be able to predict future trends. We focused on the alpine environment at medium altitudes because a large amount of afforestation (70%) occurred in the southern EU countries, especially in the mountain environment due to farm abandonment and rural emigration processes (Mazzoleni et al., 2004). Furthermore, today the mountain environment is crucial for the conservation of species because, due to climate change, the distribution area of the species has undergone a shift at higher altitudes (e.g., Menéndez, 2007). Thus, slope, terrain aspect, latitude, altitude, grassland management, and host plant abundance are key parameters to identify ecological preferences of butterfly species (e.g., Koch et al., 2015; Thomas et al., 1998; Weiss et al., 1988). Considering that *Z. polyxena* is locally monophagous on *Aristolochia pallida* Willd (Piccini et al., 2021), it is important to understand which factors may affect adult and larval presence at different hierarchical scales: site, local microhabitat, and host plant levels. In order to develop optimal-local conservation strategies for the species, first of all the aim of the study was to identify habitat preferences for *Z. polyxena* adults and its host plant *A. pallida* at the site level, and for larvae at microhabitat and host plant levels. To do this, we decided to adopt an innovative hierarchical approach that characterizes the adult and larval ecological niche by collecting data on adults and larvae and through phytosociological surveys of vegetation. In particular, the study focused on:

1. the site, to identify the ecological niche preferences for *Z. polyxena* and *A. pallida*, in relation to forest presence and topographic variables (elevation, slope, and terrain aspect), and also for *Z. polyxena* in relation to host plant abundance;
2. the plot within sites, to identify the microhabitat preference of *Z. polyxena* oviposition and *A. pallida*, in relation to ecological parameters, such as forest variables, herbaceous vegetation, and management intensity, and in relation to host plant abundance for *Z. polyxena*; and
3. the host plant within plots, to identify which host plant features are preferred by *Z. polyxena* larvae.

MATERIALS AND METHODS

Study site

The study area was located in NW Italy (WGS84: 45°07′46.9″ N 6°59′14.7″ E) with an altitude varying between 975 and 1285 m asl. Indeed, in order to conserve the species in the mountain environment and other taxa linked to it, we have focused our study in this range of altitudes that is more suitable to understand forest–clearing dynamics. In addition, we focused on the highest altitudinal range of distribution of the species, as, due to climate change, there is a general upward trend in altitudinal shift (e.g., Menéndez, 2007). The study area was divided into four smaller sites where the presence of *Aristolochia* plants and *Z. polyxena* larvae was previously recorded in 2018. The topographic, forest, herbaceous, and management features of each site are described in Table 1.

Study species

Zerynthia polyxena is a univoltine species belonging to the family Papilionidae. In Italy, the adults fly from March until the beginning of June. The females oviposit on the abaxial surface of *A. pallida* leaves, laying either a single egg or a cluster of eggs (Batáry et al., 2008). Larvae develop from May to June. Once they become pupae, they hibernate and overwinter (Van Swaay et al., 2012).

Aristolochia pallida is a perennial herbaceous species belonging to the Aristolochiaceae family typically occurring in different habitats, such as pastures, meadows, forests, and ecotones from the lowland plain to 1300 m asl (Aeschimann et al., 2004; Pignatti et al., 2017). The plant, a geophyte, is characterized by a globose tuber and develops several annual stems, which can reach a height of 50 cm. The flowering period, in the study area, ranges from May to June.

Sampling design: Hierarchical investigation

We organized the research as a hierarchical investigation, characterizing preferences for *Z. polyxena* and its host plant at the site level and plot level, and the larval stem preferences at the host plant level (Figure 1). The study area covered around 513 ha, sites covered a total area of around 52 ha, and sites were distant from each other by 5.4 ± 3.7 km (min = 0.6 km, max = 9.4 km).

Site level

To count *Z. polyxena* adults, we systematically patrolled areas in each site every other day from 1000 to 1600 from March to June 2019 (57 total days of captures when weather conditions were favorable): capturing, marking individuals, releasing and recapturing butterflies, and recording sex and GPS position (Garmin eTrex 20 with precision ±3 m) at each capture event.

TABLE 1 Area features in relation to topographic, forest, herbaceous variables, and management type

Variable	Unit	A	B	C	D
Mean elevation	m asl	1004	1012	1248	1239
Slope	°	24.7	20.3	18.4	25.9
Aspect	°N	186.8	144.2	124.3	168.1
Total area	ha	10.56	20.17	19.57	1.22
Forest area	ha	6.76 (64%)	6.45 (32%)	9.00 (46%)	0.50 (41%)
Grassland area	ha	3.8 (36%)	13.71 (68%)	10.57 (54%)	0.72 (59%)
Habitat type		Mixture of seminatural grasslands and chestnut orchards	Mixture of terraces and chestnut orchards	Mixture of abandoned terraces and hardwood-dominant neo forest	Abandoned terraces and hardwood-dominant neo forest
Main tree species		*Castanea sativa*, *Fraxinus excelsior*, *Prunus avium*	*Castanea sativa*, *Fraxinus excelsior*, *Prunus avium*	*Fraxinus excelsior*, *Prunus avium*, *Acer pseudoplatanus*, *Corylus avellana*	*Fraxinus excelsior*, *Prunus avium*, *Acer pseudoplatanus*
Main herbaceous species		*Bromus erectus*, *Brachypodium rupestre*, *Festuca ovina* and *Phleum phleoides* [Festuco-brometea] *Arrhenatherum elatius* and *Dactylis glomerata* [Molinio-Arrhenatheretea]	*Bromus erectus*, *Festuca valesiaca*, *Brachypodium rupestre*, *Carex caryophyllea e Helianthemum nummularium* [Festuco-brometea] *Arrhenatherum elatius* and *Trisetum flavescens* [Molinio-Arrhenatheretea]	*Bromus erectus*, *Brachypodium rupestre* [Festuco-brometea] *Arrhenatherum elatius* and *Trisetum flavescens* [Molinio-Arrhenatheretea]	*Bromus erectus*, *Brachypodium rupestre* [Festuco-brometea] *Arrhenatherum elatius* and *Trisetum flavescens* [Molinio-Arrhenatheretea]
Management type		Mowing and grazing (sheep)	Mowing	Mowing and grazing (sheep, wild ungulates)	None (abandoned since some decades)

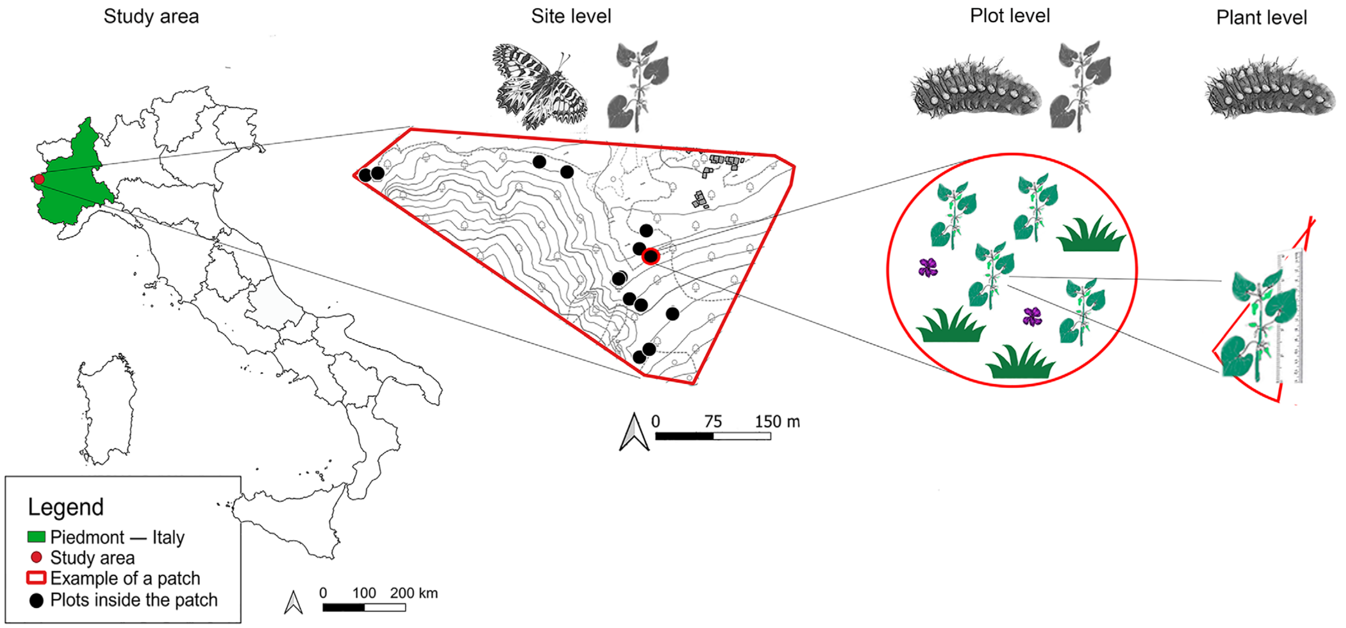

FIGURE 1 Hierarchical investigation: study area (total of 513 ha) in NW Piedmont (Italy); at site level 4, selected sites (total of 52 ha); at plot level, 73 circular plots within sites; and at plant level 8, circular sectors within plots

To analyze the relationship between butterfly abundance and topographical and vegetational parameters, we identified subsites—within sites (see Appendix S1: Figure S1)—most frequently used by butterflies, by performing a cluster analysis using the R packages "*rgdal*" (Keitt et al., 2012) and "*geosphere*" (Hijmans et al., 2017), which divided butterflies into groups according to their capture proximity. To define subsite size, we applied a minimal convex hull to each group (see Appendix S1: Section 1). Subsites have a mean perimeter of 162.36 ± 53.73 (see Appendix S1: Table S1). Once the subsites were defined, we counted the number of adults present in each subsite (hereafter "*Z. polyxena* adults"). In the same subsites, we estimated *A. pallida* stems as the average of total stems between plots (hereafter "*A. pallida* stems"; see Appendix S1: Table S1). Average slope, elevation, and aspect were calculated for each subsite using a digital elevation model (10-m resolution) of the Piedmont Region (Regione Piemonte, 2008. Digital terrain models from CTRN: 10,000—resolution 10 m). The aspect was transformed into southness (southness = 180 − [aspect − 180]) to avoid circular variable issues (Chang et al., 2004).

The proportion of subsites covered by forests (hereafter "Forest") within each study site was defined by visually interpreting aerial photography (Ortofoto Regione Piemonte, 2010) in Quantum GIS environment (QGIS 3.6.0 Development Team, 2016).

Plot level

We set up 73 randomly distributed circular plots (2-m radius) across all sites, in proportional numbers to the area size (Appendix S1: Table S1).

Within each plot, the following activities were carried out:

1. *Zerynthia polyxena* larval count. All eggs, exuviae, larvae, and instar were counted and recorded within each plot (hereafter "*Z. polyxena* larvae") at the beginning of June 2019.
2. *Aristolochia pallida* stem count. All *A. pallida* stems within each plot were counted (hereafter "*A. pallida* stems") at the beginning of June 2019.
3. Environmental data measurement. Within each plot, the elevation, slope, and aspect were measured with a handheld GPS device, a clinometer, and a compass, respectively. The slope was measured in degrees and aspect in north degrees. The latter variable was then converted into southness. Moreover, the distance between each plot and the nearest forest was also calculated in the GIS environment as the distance between the centroid of the plot and the forest layer previously mapped (hereafter "Forest distance").
4. Vegetation surveys. Plant species composition was detected using the phytosociological method (Braun-Blanquet, 1932), according to which all species (herbaceous species, shrubs, and trees) found must be listed, and for each of them, a level of abundance must be specified. Plant species nomenclature followed Aeschimann et al. (2004). The total herbaceous cover (hereafter "Herbaceous cover") and the amount of litter (hereafter "Litter") within each plot were visually estimated in percentage. Then, the quantity of litter was classified into five groups: low (0%–20%), medium (21%–40%), frequent (41%–60%), abundant (61%–80%), and very abundant (81%–100%). Litter can be considered as a reliable proxy of management intensity in the short–medium term in homogeneous agropastoral environments: A higher amount of litter corresponds to lower herbage removal by livestock and wild ungulates or by mowing, corresponding to lower management intensity (Orlandi et al., 2016; Schönbach et al., 2011). To characterize the ecological conditions of each plot, the nitrogen (N) and light (L) ecological indicators proposed by Landolt et al. (2010) were attributed to each plant species, and the mean value was calculated afterward for each vegetation survey by averaging species values weighted on their percentage cover values (hereafter "N Landolt" and "L Landolt").
5. Tree canopy cover quantification. At the center of each understory plot, the canopy cover was measured by taking hemispherical photographs oriented to the zenith at 0.3 m height above the ground with the support of a camera tripod (CANON EOS 350D camera equipped with a LENSBABY Circular Fisheye—5.8 mm f/3.5). Hemispherical photographs were processed with a Gap Light Analyzer (GLA; Frazer et al., 1999) to obtain the tree cover for both the total panorama (hereafter "Tree cover") and each cardinal direction circular sector. To quantify the level of heterogeneity of canopy cover, the coefficient of variation (hereafter "Tree cover CV"), that is, SD divided by the mean, was calculated for each plot by using the cover of the four cardinal direction circular sectors (high values for Tree cover CV indicate high heterogeneity; Appendix S1: Figure S2).

Host plant level

To identify the stem preference for oviposition, we divided each plot into eight circle sectors (1/8 of the circles) within which we recorded the number of larvae (hereafter "Larvae in circular sectors") and their instar stage. It was not possible to disentangle single plant individuals, so different features were measured for each stem with larvae within circular sectors: the average height (hereafter "Stem height"), the average size among the smallest leaves—tender stem parts palatable for larvae (hereafter "Minimum leaf length")—the average number of leaves (hereafter "Leaves"), and the height of the grass (hereafter "Grass height").

Statistical analyses

As the variables were recorded on different scales, they were standardized to their z-scores at each level and were tested to be not correlated with each other. At each hierarchical level, plotting variables related to *Z. polyxena* versus all parameters allowed us to establish which variables were linear and which were nonlinear. All statistical analyses were carried out using R statistical software, v.3.2.1 (R Development Core Team, 2015). Each model was fitted using the "lme4" (Bates et al., 2015) and "mgcv" (Wood, 2004) packages in R. To evaluate the dispersion of models with the Poisson distribution, we used the "Dharma" (Hartig, 2019) package in R.

Site level

To assess which factors influenced *Z. polyxena* abundance at the site level, we modeled adults in a generalised additive mixed modell (GAMM) using slope, *A. pallida* stems, and southness as additive–linear explanatory variables. Considering that at different elevations, site forest presence could have different effects on butterfly abundance, we added the interaction Elevation × Forest as smooth terms (see descriptive statistics in Appendix S1: Table S2). We used a Poisson distribution family, and the model was not over/underdispersed (dispersion test: Obs/Sim $= 1.14$; $p = 0.36$). To take into account that subsites have different sizes, we added the subsite log-transformed perimeter as offset, and site as a random factor in the model.

In order to test whether the same explanatory parameters affected host plant presence, we modeled *A. pallida* stems in a GAMM. We added subsite log-transformed perimeter as offset and site as a random factor. Considering that the response variable (*A. pallida* stems) was an average between plots within areas (see site-level sampling design), we used the Gaussian family and tested that the residuals were normally distributed (Appendix S1: Figure S3).

Plot level

A hierarchical cluster analysis was performed to classify vegetation surveys in vegetation communities with similar ecological and management features (see Appendix S1: Section 1). To assess differences between vegetation communities defined by the hierarchical cluster analysis in terms of number of larvae, a nonparametric Kruskal-Wallis ANOVA was performed ("kruskal.test" function of "stats" R package).

We modeled the *Z. polyxena* larvae in a GAMM using *A. pallida* stems, Litter, Herbaceous cover, N Landolt, and L Landolt as additive–linear explanatory variables while Forest distance, Tree cover CV, and Tree cover by elevation were used as smooth terms (see descriptive statistics in Appendix S1: Table S3). Site was considered as a random factor. We used the Poisson distribution family, and the model was not over/underdispersed (dispersion test: Obs/Sim $= 0.90$, $p = 0.52$).

To evaluate which microhabitat features affect *A. pallida* presence, we modeled *A. pallida* stems in GAMM with the same explanatory variables present in the previous model. Site was considered as a random factor. Considering that the model with the Poisson distribution family was over/underdispersed (dispersion test: Obs/Sim $= 5.32$, $p < 0.001$***), we used the negative binomial distribution family (accordingly with Zuur et al., 2009).

Host plant level

To evaluate which stem features could be preferred by larvae, we modeled Larvae in circular sectors in a GAMM where Stem height, Leaves, and Grass height were used as additive–linear explanatory variables, while Minimum leaf length was used as a smooth term. We added plot and site as random factors and log-transformed instar stage of larvae as offset. We used the Poisson distribution family, and the model was not over/underdispersed (dispersion test: Obs/Sim $= 0.85$, $p = 0.056$).

RESULTS

Site level

Adult butterfly abundance linearly increased when slope decreased (slope: est. $= -0.05$, $Z_{38} = -3.72$, $p < 0.001$***; Figure 2a), and increased at low and medium elevation in subsites with a high proportion of forest presence (Elevation × Forest: edf $= 5.44$, $\chi^2 = 55.59$, $p < 0.001$***;

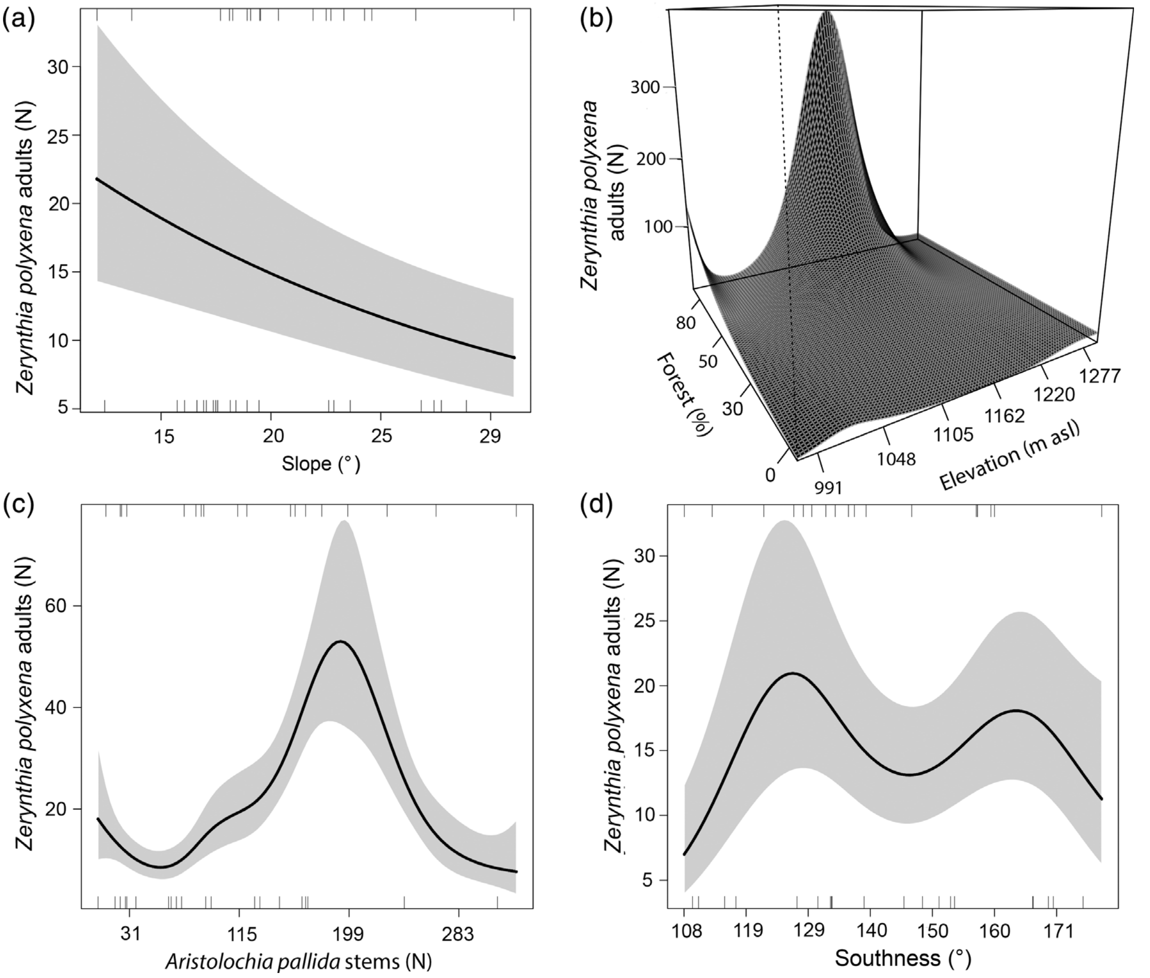

FIGURE 2 Linear relationship at site level between *Zerynthia polyxena* butterfly adults counted in subsites and (a) slope, and smooth relationship between adults and (b) the interaction between forest and elevation, (c) *Aristolochia pallida* stems and (d) southness. Estimated values (black lines) and CIs for the estimated values (gray band)

Figure 2b). Moreover, butterfly abundance increased with the increase in food plant abundance up to about 200 *A. pallida* stems per plot, then it decreased (*A. pallida* stems: edf = 6.80, $\chi^2 = 121.23$, $p < 0.001$***; Figure 2c). Butterfly abundance shows two peaks at 125° and 165°N—corresponding to areas facing southwest and south (southness: edf = 3.94, $\chi^2 = 31.63$, $p < 0.001$***; Figure 2d and Appendix S1: Table S4).

Aristolochia pallida stem abundance decreased when elevation increased (edf = 1.00, $\chi^2 = 5.05$, $p = 0.032$*; Appendix S1: Figure S4 and Table S5).

Plot level

The hierarchical cluster analysis on vegetation surveys identified eight clusters belonging to three main vegetation communities: (1) woodland and fringe, (2) ruderal, and (3) seminatural dry grassland (Appendix S1: Section S2.2 and Figure S5). *Zerynthia polyxena* larvae occurred in 52 (71%) of the 73 plots considered for the analysis, for a total of 160 larvae recorded. The number of larvae tended to be higher in the plots classified as dry grasslands compared with ruderal or woodland and fringe communities ($\chi^2 = 4.9201$, df = 2, $p = 0.085$; Appendix S1: Figure S5).

Larvae decreased with increasing light index (L Landolt: est. = −0.80, $Z_{63} = -2.60$, $p = 0.009$**), while they increased with increasing *A. pallida* presence (*A. pallida* stems: est. = 0.70, $Z_{63} = 5.76$, $p < 0.001$***; Figure 3a) and with increasing amount of litter (Litter: est. = 0.48, $Z_{63} = 3.29$, $p < 0.001$***; Figure 3b; Appendix S1: Table S6). The number of larvae changed nonlinearly in relation to elevation and tree cover (Tree cover × Elevation: $\chi^2 = 12.1$, edf = 4.61, $p = 0.040$*; Figure 3c), and larvae preferred higher cover heterogeneity (Tree cover CV: $\chi^2 = 24.79$, edf = 8.58, $p = 0.003$**; Figure 3d; Appendix S1: Table S6).

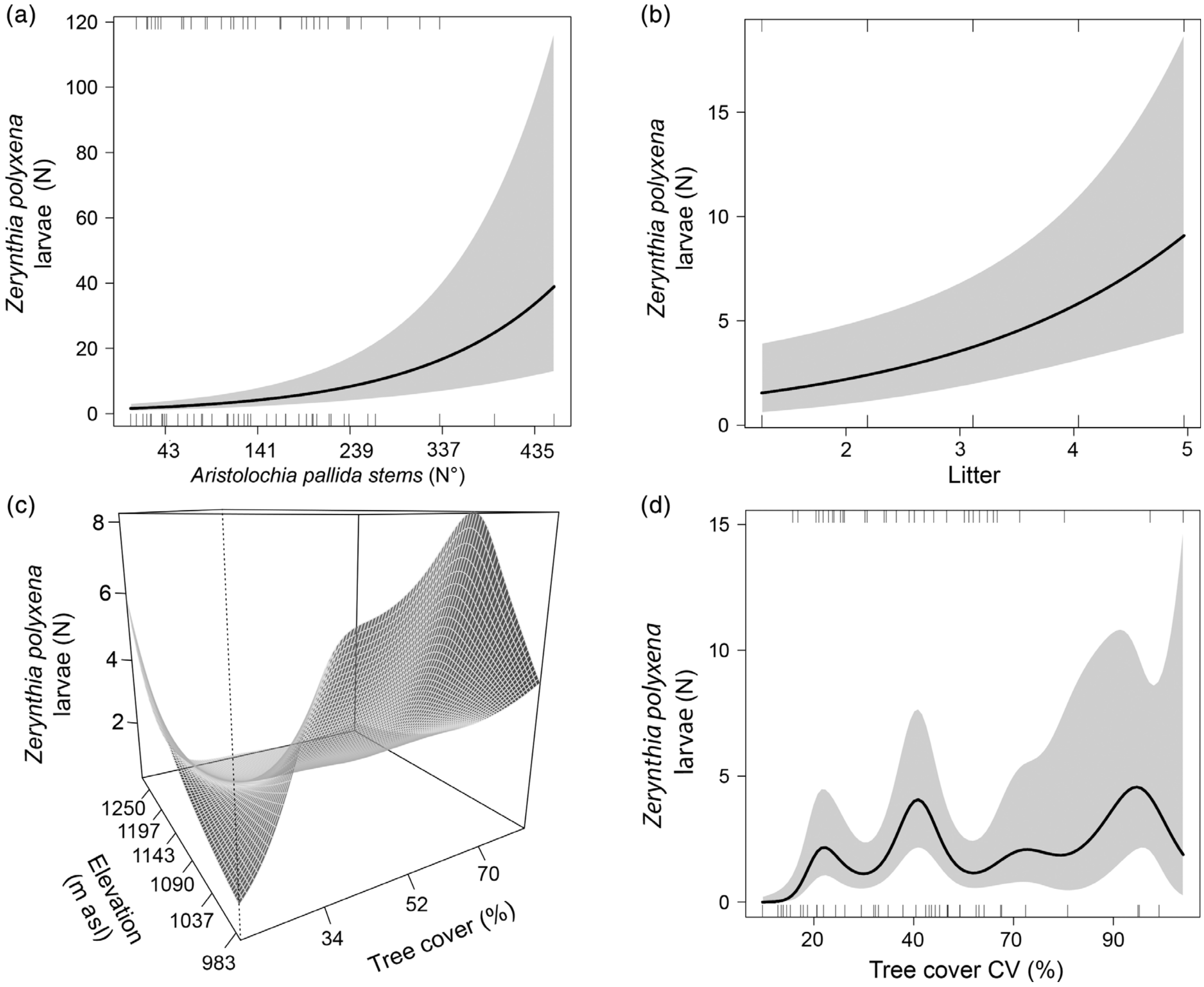

FIGURE 3 Linear relationship at plot level between *Zerynthia polyxena* larvae and (a) *Aristolochia pallida* stems and (b) litter and the smooth relationship between larvae and (c) interaction between Tree cover and elevation and (d) Tree cover CV (coefficient of variation). Estimated values (black lines) and CIs for the estimated values (gray band)

Herbaceous cover negatively and linearly affected *A. pallida presence* (est. = −0.19, $Z_{63} = -1.97$, $p = 0.05$*; Figure 4a), while tree cover nonlinearly affected *A. pallida* presence in relation to elevation ($\chi^2 = 24.72$, edf = 2.90, $p < 0.001$***; Figure 4b; Appendix S1: Table S7). Host plants were more abundant at low elevations where tree cover was low (<20%). Medium–high abundance of host plants was found at both low and high elevations with high tree cover (70%).

Host plant level

Zerynthia polyxena larvae preferred stems with larger-sized smaller leaves (young or apical leaves; Minimum leaf length: edf = 3.81, $\chi^2 = 8.83$, $p = 0.006$**; Appendix S1: Table S8 and Figure S7).

DISCUSSION

Ecotonal habitat preferences of *Z. polyxena*

Ecotones are often species-rich and display specific resources and environmental conditions that favor invertebrates' presence (e.g., Wielgolaski et al., 2017). High diversity in the transition areas could be related to overlapping of the distribution of species belonging to the surrounding habitats, and to a wide range of temperature and humidity that form a diversity-rich microhabitat (Körner, 2003; Nagy & Grabherr, 2009). For butterflies in Europe, it is known that open areas support a higher diversity richness, but the surrounding habitats—especially forest (Bergman et al., 2018)—could also be important, especially for specialist species that have limited requirements (Krämer et al., 2012). Transition zones

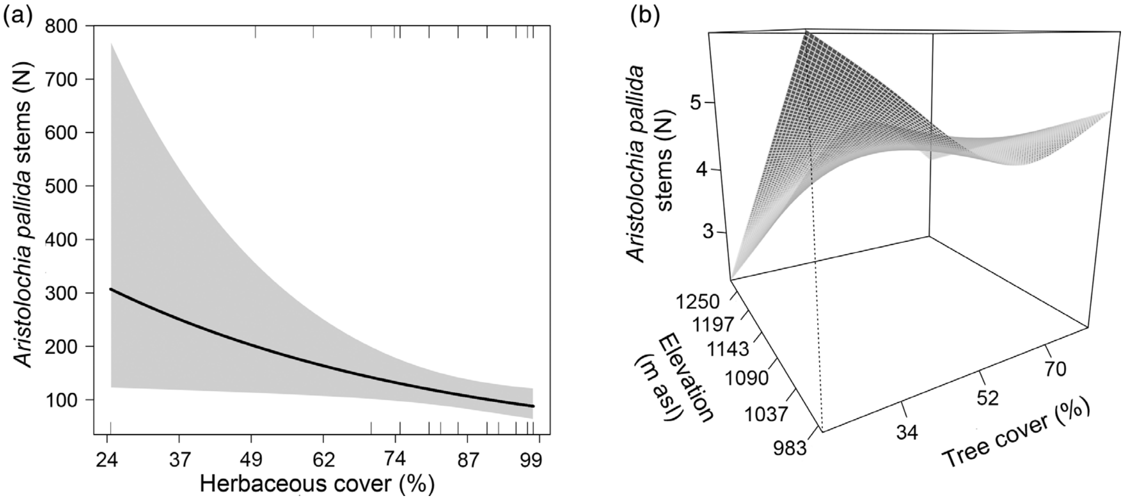

FIGURE 4 Linear relationship at the plot level between *Aristolochia pallida* stems and (a) herbaceous cover and smooth relationship between stems and (b) the interaction between tree cover and elevation. Estimated values (black line) and CIs for the estimated values (gray band)

could be suitable habitats for some specialist species, such as *H. lucina*, which prefers calcareous grasslands surrounded by old woodland (Anthes et al., 2008; Fartmann, 2006). In accordance with Bonelli et al. (2018) and Čelik (2012), we confirm that *Z. polyxena* has ecotonal preferences, and protection of this species could also conserve other clearings and ecotonal insect species.

Zerynthia polyxena adults inhabit a wide range of biotopes in Europe (van Sway et al., 2006), including meadows (Vovlas et al., 2014), black locust plantations, and hummocks (Batáry et al., 2008; Örvössy et al., 2014), as well as open woodland and close forest (Slancarova et al., 2015). Conversely to what Hayes et al. (2018) found for *H. lucina*, we found that adults preferred gentler slopes facing southwest or south (see also Weiss et al., 1988) with a modest presence of forest area. The preference for gentler slopes may be associated with the fact that these terrain conditions are less susceptible to leaching and the soil nutrient availability is higher than in steep slopes, resulting in high plant vigor (Corona et al., 1998; Whitehead, 2000). Moreover, areas facing south and southwest have all-day sunlight, which is favorable for larval development (Vovlas et al., 2014), and could be the reason why adults preferred south-facing areas (to link adult choices to larval development; see Thompson, 1988). This could be related to habits of subnemoral butterfly species (forest-edge butterflies), which generally feed in open areas and may use forest edge and ecotones as an oviposition site (Balletto & Kudrna, 1985). The host plant abundance was already known to be a pivotal factor determining the spatial distribution of *Z. polyxena* adults (Čelik, 2012). Moreover, we found that there is a threshold limit of 200 mean stems, most of which do not determine higher butterfly abundance. Considering that areas with higher abundance of host plants are rarer, the cost for butterflies in finding these areas could outweigh the benefits.

In our study, dry grassland ecotonal habitats, partially shaded and rich in sciaphilous plants (e.g., species belonging to woodland and fringe communities), were the best oviposition sites (in accordance with Batáry et al., 2008; Örvössy et al., 2014) supporting large amounts of larvae. This larval preference could be due to the higher host plant quality in ecotones compared with host plants under a closed canopy (in accordance with Konvička & Kuras, 1999). Conversely, in the Mediterranean area of Italy, larvae of a congeneric species, *Z. cassandra*, were found in clearings in full sunlight, far from the ecotonal area rich in host plants *A. rotunda* (Vovlas et al., 2014). Furthermore, the best oviposition sites were characterized by low management intensity—evaluated by high litter presence (in line with Ernst et al., 2017; Zingg et al., 2018). At low elevations, a higher tree cover (70%) supports a higher number of larvae, while at high elevation, there was a lower tree cover (<20%). In addition, heterogeneity of tree cover was preferred by larvae, and higher preference was found at 90%, followed by 40% and 20%. In these cover conditions, the overall moist and temperate (with less excursion between day and night) microclimate conditions may favor larval development, avoiding desiccation in partially shaded conditions between clearings and forests at low elevation. Conversely, at high elevation in early spring the temperatures are still too low even during the day; thus, larval development could be better in open areas (see Anthes et al., 2008).

We found significantly more abundance of larvae on host plants with large leaves (i.e., larger smallest leaves). Indeed, host plant features may be an important factor in larval survival and development (Örvössy et al., 2014).

In this study, we did not investigate which management and environmental parameters favor leaf width of the nurse plant; however, based on ecological preferences found with the GAMM on *A. pallida* habitat preferences (see next section), extensive management could favor more vigorous individuals, but more detailed research on this topic is needed. However, the suggested low management intensity, which does not favor fast-growing species linked to frequent mowing and intense livestock grazing, should reduce competition and favor slow-growing plants (Kemp & King, 2001), such as *A. pallida*.

Habitat preferences of *A. pallida*

Aristolochia pallida is a plant species with a very wide ecological niche, and its presence is not strictly linked to a specific vegetation community. In Italy, *A. pallida* is a frequent or abundant species in the sub-alliance *Ulmenion minoris* (wooded floodplains dominated by *Ulmus minor*; Biondi & Blasi, 2015). In the Alpine chain, the phytosociological optimum is indicated in the alliance *Carpinion orientalis* (xerothermic woods dominated by *Quercus pubescens*; Aeschimann et al., 2004). Indeed, the species is common in several habitats, such as screes, meso-thermophilous shrub communities, mesophilous hardwood forests, dry grasslands, and meso-thermophilous fringe communities (Aeschimann et al., 2004; Pignatti, 1982), demonstrating its wide ecological plasticity. However, even though its presence is recorded up to 1300 m asl (Pignatti et al., 2017), in this study *A. pallida* was significantly less abundant with increasing elevation.

However, the GAMM at the plot level showed that *A. pallida* abundance was associated with spots with a low herbaceous cover. As a semishade species (Landolt et al., 2010), *A. pallida* prefers sparse vegetation, whose structure allows the sunlight to penetrate down to the bottom of the vegetation layer. In addition, plant abundance was affected by elevation and tree cover interaction. Along the altitudinal gradient explored in this research, *A. pallida* was more abundant at low elevations and less abundant at high elevations, as its ecological optimum is up to the hill belt (Aeschimann et al., 2004). Nevertheless, tree cover played an important role in expanding or reducing the ecological niche of *A. pallida* along the altitudinal gradient, as it can significantly affect the microclimate in the understory. At low elevations, *A. pallida* abundance decreased, with higher tree cover values, as the species does not tolerate too-shaded habitats. Conversely, at higher elevations, the number of *A. pallida* individuals increased when the tree canopy cover was relatively high. This could be explained by the fact that higher elevation sites are characterized by low temperatures and the presence of a not-too-dense tree cover could guarantee thermal comfort between night and day by reducing the temperature excursion, allowing the vital cycle of *A. pallida*.

Mismatch on host plant and larval microhabitat ecological optimum

Nowadays, it is common to find a temporal mismatch between herbivore insects and host plants due to climate change (e.g., Gilman et al., 2010), which has led to important negative consequences for pollination and other ecosystem services (e.g., Hegland et al., 2009). We recorded *Z. polyxena* populations at higher altitudes (>1200 m asl) than those already present in the literature (Bonelli et al., 2018). This could be an effect of temperature increases that cause an altitudinal shift of insects (e.g., Menéndez, 2007). Moreover, we found that the optimal ecological niche of host plant and butterfly species has a spatial mismatch. At this point, the species still have an overlapping area in ecotonal habitats, but climate change could eventually disrupt trophic interactions, as predicted for *Boloria titania* and its larval host plant *Polygonum bistorta* (Schweiger et al., 2008).

It is known that host plant ecological preferences are often less narrow than those of oviposition made by butterflies (e.g., Ghidotti et al., 2018). In this study, we found that at low elevation, most host plants were found where tree cover was lower, but the few plants found at the border of the clearings with high tree cover (70%) were preferred for oviposition. The situation was even more complicated at high elevation (>1200 m asl), where generally host plants were less abundant. At those elevations, the host plants were found preferably where tree cover was medium–high (from 52% to 70%), conditions that may be unfavorable for larval development.

Even though the optimal habitat conditions for larvae and host plants did not totally converge, they currently still have an overlapping area where favorable conditions for both species are present. From a conservation point of view, it is crucial to maintain overlapping areas between host plant and butterfly species, to favor ecotonal environments, by an active but not intensive management of grassland either by mowing or by grazing.

New approach for butterfly conservation

Insects are globally facing decline, even if some temperate species have increased in abundance and range. Conversely, insects have already experienced the loss of rare taxa and decline in formerly abundant species, which could have negative consequences on ecosystem functioning (Wagner et al., 2021). In order to prevent insect and butterfly decline, it is crucial to identify conservation plans specifically designed for local environmental conditions. Indeed, for example, in the Alpine environment reforestation, linked to land abandonment (Chemini & Rizzoli, 2003), is one of the major threats for insect taxa (Tasser et al., 2007; Tocco et al., 2013), including butterflies (third assessment of conservation status and trends. The State of Nature in the EU—Article 17 reporting).

Vegetational structure and composition have already been used to predict butterfly ecological preferences (Marini et al., 2009). Local phytosociological surveys have also been shown to influence insect communities (Burgio et al., 2015; Ewing et al., 2020). Here, we used vegetational surveys to characterize vegetation communities, where it was possible to understand the current and past (around 10–50 years ago) management of grasslands (Tasser & Tappeiner, 2005). Information gathered from vegetation communities can be useful, within a relative homogeneous study area (e.g., with little variation in climate and precipitation), to provide management implications for maintaining local butterfly populations. Indeed, by analyzing phytosociological consociations, we were able to determine that the entire area was shaped by past agropastoral activities, which have sharply declined in recent decades in the entire study area. Indeed, plots were mainly composed of seminatural dry grassland communities (80% of vegetation surveys), belonging to the class *Festuco valesiacae–Brometea erecti* Br.-Bl. & Tüxen ex Br.-Bl. 1949 (Biondi & Blasi, 2015), which are related to past agropastoral activities (i.e., mowing and livestock grazing). Furthermore, the dominance of *Brachypodium rupestre*, a light-shade-tolerant species, denotes an involution of managed grasslands to the early stages of a shrub- and tree-encroachment process (as demonstrated by a non-negligible abundance of *Fraxinus excelsior* L. renewal). The occurrence of *Arrhenatherum elatius* and *Trisetum flavescens* could indicate a past presence of mown meadows. In ecotonal areas, we found light-demanding woody species and shade-tolerant herbaceous species typical of fringe communities (i.e., *Hypericum perforatum* L., *Origanum vulgare* L., and other species belonging to the class *Trifolio medii-Geranietea sanguinei* Müller 1962). These species characterize a secondary succession of grasslands that have not been managed for around 10 years, resulting in an accumulation of litter (Schmidt, 2005). The recent effect of grazing and mowing activities, which have shaped the vegetational and butterfly communities, is reflected by litter abundance that could be used as a proxy of grazing intensity and mowing frequency (Orlandi et al., 2016). Herbage removal by ruminants and mowing has a strong effect on vegetation structure and composition (Kohler et al., 2004), and it was assumed that a high presence of litter is an indicator of low recent grazing intensity or mowing frequency. Litter abundance has already been seen as a possible factor influencing butterfly oviposition (Ewing et al., 2020; Stuhldreher & Fartmann, 2015), highlighting its microclimate function that reduces extreme temperature fluctuations. Here, we used litter for its additional indicator of management intensity, suggesting that butterfly presence was favored by a low management intensity (corresponding to a high presence of litter; Orlandi et al., 2016), probably related to occasional mowing or low-intensity grazing.

By means of the current local vegetation and entomological surveys, we were able to understand the recent and current management intensity, and the butterfly ecological preferences at different elevations, and we were therefore able to design specific conservation measures to maintain butterfly populations and communities. For *Z. polyxena*, we found that at low elevation (975 m asl), maintenance of small clearings (<200 m^2) in a not-dense forest (with heterogeneous tree cover of about 70%) and at high elevation (>1200 m asl), medium-sized clearings (around 1 ha with low heterogeneous tree cover <20%) would support the species and likely a cascade of other butterflies. To maintain these populations, according to the general suggested management actions for butterfly species belonging to Habitats Directive (Van Swaay et al., 2012), we highlight the importance of maintaining not-dense forests, ecotonal habitats, and clearing (different sizes in relation to elevation) through low management intensity linked to occasional mowing and/or planned livestock grazing. Similarly, Cini et al. (2021) have proved that low management intensity (one late mowing every 2 years) would favor larval presence of a congeneric species, *Z. cassandra*. Adopting a rotational grazing system for domestic livestock, which considers periods of grazing and rest among paddocks and defines the stocking rate considering the vegetation composition (Probo et al., 2014), would be a valuable tool. Indeed, it allows the adjustment of livestock grazing pressure and distribution with the aim of keeping grasslands open but without threatening the butterfly's habitat conditions. For instance, late passages of animals into different portions of the grassland over the years could be ensured so that litter can be slightly accumulated and allow the butterflies to complete their reproductive cycle in different areas and in different years. Conversely, the spatial distribution of wild animals, and therefore their stocking rate, cannot be managed as well as for domestic livestock. The EU's Common Agricultural Policy, launched in 1962, includes the possibility for farmers to be refunded for the low livestock grazing in those areas identified as priorities for biodiversity conservation, especially in the Alps (measures 10.1.9 "Eco-sustainable management of pastures" included in the Rural Development Program of Piedmont Region). A proactive management that favors the maintenance of a mosaic of different habitats, such as forests, ecotones, and clearings, would support high diversity (Vimal et al., 2017). This identified low-intense management may benefit other species linked to clearings and forest margins, such as the congeneric species *Z. cassandra* (Cini et al., 2021), *P. mnemosyne* (Cini et al., 2020), *H. lucina*, and some saproxylic beetles. Moreover, we were able to identify local ecological preferences of the species and to understand the past management that led to the current situation, and thus design possible management measures. This approach may be exported to other species in other environments that could lead to different management measures.

Accordingly with Gómez-González et al. (2020), instead of a massive tree plantation program—which may threaten biodiversity linked to open areas (e.g., Bond et al., 2019)—maintaining a diverse mosaic composed of several habitats (including areas devoted to production and natural areas) would maximize biodiversity, ecosystem resilience, and related services. Over one season, local data, and a new combined vegetational and entomological approach, we were able to identify management actions to support and maintain local butterfly populations. Activities that favor a mosaic of diverse habitats are optimal for the target species and crucial for biodiversity in mountain areas. The same field-based approach, including a multidisciplinary way (e.g., vegetational and entomological surveys) and some crucial local factors (e.g., elevation, litter, and tree cover), can be scaled up to plan conservation strategies for different organisms in different habitats.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Irene Piccini, Viviana Di Pietro, Marco Pittarello, Michele Lonati, and Simona Bonelli conceived the ideas and designed the methodology; Irene Piccini, Viviana Di Pietro, Michele Lonati, and Marco Pittarello collected the data; Irene Piccini, Viviana Di Pietro, Michele Lonati, and Marco Pittarello analyzed the data; and Irene Piccini led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data (Piccini et al., 2022) are available from Figshare: https://doi.org/10.6084/m9.figshare.19248371.v1.

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SUPPORTING INFORMATION

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