Butterfly and grasshopper diversity patterns in humid Mediterranean grasslands: the roles of disturbance and environmental factors

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ORIGINAL PAPER

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Abstract The present paper studies butterfly, grasshopper and vascular plant communities in ten seasonally flooded grasslands with different anthropogenic disturbance regimes (NW Greece). Disturbance intensity was assessed on the basis of disturbance frequency and type (grazing, mowing, trampling, constructions). The distribution patterns of butterflies are regulated by humidity and elevation (Redundancy Analysis). Elevation, flower-heads abundance, low disturbance intensity and plant species richness predict grasshopper species richness well, while the latter together with humidity predict plant species richness (Generalized Linear Models). *Chorthippus lacustris,* a critically endangered endemic grasshopper species, is positively associated with humid microhabitats with high flower-heads abundance. An indicator value procedure reveals four butterfly species as being typical species for habitats with a pronounced character of hedgerows and tree lines. Conservation management of grassland butterflies should focus on the maintenance of the humid character of the humid grasslands as well as on the maintenance of hedgerows and tree lines. The reduction of human-induced disturbance towards occasional grazing and mowing seems to benefit both butterfly and grasshopper communities. Finally, we suggest the use of grasshoppers as surrogates for vascular plants and vice versa, given their congruent species richness patterns.

Keywords Community ecology · Indicators · Intermediate disturbance hypothesis · Lepidoptera · Orthoptera · Vascular plants · Wetlands

Introduction

Grasslands constitute an important habitat type, providing a wide range of ecosystem services including biomass production, nutrient regulation and carbon sequestration (Sala and Paruelo 1997). Seasonally flooded grasslands (humid grasslands), which are inundated during wintertime are dynamic systems that can support rich hygrophilous herbaceous vegetation in spring and summer. Their hydrological regime is the key driver of biotic community composition and ecosystem functioning, which host species dependent on, or tolerating the flooding regime (Gibbs 1995, 2000; Plum 2005). They also support further eco-services such as the regulation of hydrological regime, inundations, drought phenomena and local climate conditions (Mitsch and Gosselink 1993).

In the Mediterranean area, seasonally flooded grasslands are quite rare and their presence is often confined to areas of wetland ecosystems. It is estimated that more than 50% of Mediterranean wetlands have been lost in the last century (Green et al. 2002) and approximately 25% of Europe's remaining wetlands are considered potentially

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endangered (UNEP 2004). The loss of wetland area has reached 63% in Greece, and might even exceed 85% locally (Kati et al. 2006; Psilovikos 1992). The most severe human-induced threat to wetlands, also directly affecting humid grasslands, involves drainage and land conversion mainly to arable, urban or forest land (EEA 2010). Several disturbance types act synergistically to degrade wetland habitat, such as intensive agricultural practices, overgrazing, fragmentation, pollution, alien species and the development of human infrastructure (Battisti et al. 2008). On the other hand, according Connell's (1978) Intermediate Disturbance Hypothesis moderate human-induced disturbance (e.g. grazing, mowing) might have a beneficial rather than a degrading impact on grassland biodiversity. Therefore, by preserving traditional management techniques like low-intensity grazing or mowing once per annum, species rich plant and associated insect communities would normally be expected (Tscharntke and Greiler 1995; Wettstein and Schmid 1999).

The present study attempts to investigate how human-induced disturbance together with other environmental factors affect the insect communities in the ephemeral habitats of seasonally flooded grasslands. We selected butterflies (Rhopalocera, Lepidoptera), as the first target group, being among the best-known and threatened taxonomic groups with a well-developed monitoring scheme across Europe (Thomas et al. 2004). Butterflies are also considered sensitive indicators of environmental change induced by factors such as agricultural intensification, land use change, habitat fragmentation, overgrazing and climate change (e.g. Kruess and Tscharntke 2002; Warren et al. 2001; Wilson et al. 2009). Grasshoppers (Orthoptera) were selected as the second target group, because of their pronounced functional role in food webs, attributed to their ability to recycle ground biomass so as to generate nutrients for other taxa (Samways 1994). They are also good indicators of climatic conditions and vegetation structure at fine scales (Guido and Gianelle 2001; Kati et al. 2004), as well as of human-induced disturbances such as grazing, trampling or mowing (Chambers and Samways 1998; O'Neill et al. 2003). Finally, we considered vascular plant communities, because the above-mentioned target groups are related to their composition and structure.

The goal of our study is to investigate: (a) the diversity patterns of plant, butterfly and grasshopper communities and assess their surrogate value for each other, (b) the main parameters predicting the species richness of these biological groups, (c) the structure of butterfly and grasshopper community, (d) the effect of human-induced disturbance on the groups studied, and finally to interpret our findings into concrete measures for humid grassland management so as to conserve insect communities.

Materials and methods

Study area

The study area includes ten wetland localities, hereafter sites, each of a fixed area of 1 ha. The sites were in the Epirus district in NW Greece (lat. 39°18′–39°55′N, long. 20°11′–20°53′E), ranging from sea level to 480 m (Appendix 1). The climate is Mediterranean with a mean monthly rainfall ranging from 32 to 175 mm and a mean monthly temperature ranging from 4′7 to 24′8°C (HNMS 2000). Habitat types include Mediterranean tall humid herb grasslands of the Molinio-Holoschoenion (habitat code 6420; 8 sites), Mediterranean salt meadow (habitat code 1410; one site) and Hellenic habitat supra-Mediterranean humid meadow (Hellenic habitat code 6450; one site) (Dafis et al. 2001). The first two habitat types are habitats of Community Interest (Annex I, 92/43/EEC). Seven out of the ten sites sampled are included in the European Natura 2000 network (Appendix 1).

Sampling

Butterflies and grasshoppers

To sample butterflies, we established one standard transect of 200 m in each site. We counted all butterfly species occurring within a band of 5 m in each transect, as well as up to 5 m ahead for a fixed time interval of 60 min. We used a hand held net to capture and identify specimens in situ, using appropriate field guides. We sampled three times, with a 25 day interval between samples, in May, June and July of 2008. We also set four standard plots of 10 m^2 (5 m × 2 m), located at every 50 m along the butterfly transect, in order to sample vascular plants, grasshoppers and environmental parameters. We also sampled all grasshopper species occurring within the above plots for a fixed time interval of 10 min. We estimated their relative abundance in situ using a five-grade ordinary scale (1: one individual, 2: 2–5 individuals, 3: 6–10 individuals, 4: 11–50 individuals, 5: >50 individuals). Sampling was conducted once, in July 2008 (last butterfly sampling), at the peak of adult grasshopper activity. Specimens were identified in situ, or using a stereo microscope and the key of Willemse (1985).

Environmental parameters and plants

For each plot (10 m^2) at each site we measured five environmental parameters, repeating sampling three times, in accordance with butterfly sampling. In each visit, we recorded the soil humidity and air temperature every 5 min for a fixed period of 60 min in the morning (starting at

Table 1 Disturbance regime and species diversity of butterflies, grasshoppers and vascular plants recorded in the study sites

Sites	Disturbance		Butterflies			Grasshoppers			Plants	
	D	D type	S	H	IUCN	S	H	E	S	H
S1	3	G, M	19	2.51		9	1.77	x	17	2.56
S2	0	–	10	1.32		8	1.81	x	27	3.02
S3	2	M	11	1.91		8	1.78	x	11	2.08
S4	6	M, G, T	11	1.95		3	1.00		17	2.49
S5	1	G	23	2.65	NT(2)	6	1.64		39	3.37
S6	3	C	7	1.77		5	1.24	x	19	2.57
S7	3	T, G	10	1.44	NT(1)	3	1.03		31	3.14
S8	4	M	7	1.35		6	1.16	x	16	2.51
S9	7	T, G	3	0.62		3	1.04		15	2.25
S10	6	M, G, T	7	1.65		3	1.10		18	2.53

D Disturbance Index, *D type:* Disturbance type (G: Grazing, T: Trampling, M: Mowing, C: Constructions), *S* Species richness, *H* Shannon index, *IUCN:* threatened status according IUCN criteria *NT* Near Threatened, and the number of occurred species into parenthesis, *E*: presence of the endemic grasshopper *Chorthippus lacustris*

9.00 h), using a Hobo data logger. We then calculated the average values. The cover of vascular plant species within the plots was estimated using the Braun Blanquet method, with species nomenclature following Flora Hellenica and Flora Europaea.We estimated the proportion of herb cover (%) and the maximum height of herbs (cm), as well as the number of flower-heads, using the following ordinal scale (1: <10, 2: 11–50, 3: 51–100, 4: 101–200, 5: 201–400, 6: 401–600, 7: >600). Plant sampling was repeated three times, with the same period between samples as the butterfly sampling. For each site, the cover of shrubs and trees was also recorded at a bandwidth of 20 m at each side of the butterfly transect, using the Braun-Blanquet method, as well as the distance from the nearest tree line or hedgerow from the butterfly transect.

Disturbance regime

We distinguished four types of human-induced disturbances in the sites and we assigned them a weight (w), taking a value of one to three, according to their severity. Livestock grazing and trampling were considered as low-severity disturbances ($w = 1$), grass mowing as a medium-severity disturbance ($w = 2$), and construction works directly converting the habitat as a severe disturbance ($w = 3$). We visited each site four times, recording the type of disturbance encountered, as a measure of disturbance frequency (f). The overall value of site disturbance intensity (D) was assessed with a formula integrating the disturbance severity (w) and the disturbance frequency (f) for the types of disturbances occurring.

$$D = \sum_{i=1}^{4} w_i \times f_i.$$

Given the ephemeral "flooded" character of the study habitats, the human perturbation is considerable only for five months per year. In general, mowing is a mechanic method and occurs once or twice a year, from June to August depending on the grass growth and grazing (usually by cattles) can be observed from May to August, for as long as food source is still available.

Data analysis

The butterfly diversity in each site was estimated in terms of species richness (S) and Shannon–Wiener index (H), considering all the species sampled during the three repetitions and the sum of individuals recorded (Magurran 2004). We used the five-grade ordinal scale of abundance classes of grasshopper and vascular plants in order to estimate the above indices at plot level and we used the average value of the four plots to estimate the above indices at site level. We also estimated the degree of congruence of the species richness patterns of the three biological groups studied, at plot and at site level, using Spearman correlation coefficient.

In order to find the best parameters predicting the species richness of the groups studied, we ran three generalized linear models for butterflies (10 transects), plants (40 plots) and grasshoppers (40 plots). For plants and grasshoppers we first ran a generalized linear mixed model that used plots as a random effect factor. However its optimal variance was zero, so we used the generalized linear model instead. We tested for multicollinearity using the statistic of the Variance Inflation Factor (VIF < 5) and we verified sample independence using the dispersion parameter ($0.5 < \varphi < 1$) as well as residuals normality using the Shapiro–Wilk*o* test. Analyses were performed using the R statistical package (R Development Core Team 2009).

In order to explore the environmental factors shaping the community composition of butterflies and grasshoppers, we conducted a Redundancy Analysis (RDA) using CANOCO software (ter Braak and Smilauer 2002). The environmental parameters inserted in the grasshopper diagram were the average values recorded during the three repeated samples at each plot, whereas the respective parameters inserted in the butterfly diagram were the average values of the four plots sampled in each site. The position of a species in the resulting plot indicates the characteristics of the ecological optima for this species; its abundance or probability of occurrence will decrease with distance from its species point. The diagrams show only species sufficiently influenced by the parameters (fit > 25%) and significant environmental variables ($p < 0.05$) that did not suffer from collinearity (1000 iterations of Monte-Carlo test).

To investigate the ecological structure of the butterfly and grasshopper communities, a hierarchical cluster analysis using Ward's method was performed using the Sorensen index of similarity, considering binary and semi-quantitative data for grasshoppers and butterflies respectively (PCORD v. 4.34 software; McCune and Mefford 1999). Additionally, an indicator value analysis was carried out in order to identify the typical species that characterize each one of the above-defined ecological clusters (Dufrêne and Legendre 1997). The indicator value of each species for a given cluster was calculated as: IndVal = A*B*100, where A = mean number of the individuals across the sites of the cluster/sum of mean number of the individuals over all clusters, and B = number of sites in the cluster where the species is present divided by the total number of sites in that cluster. IndVal is a percentage that ranges between 0 and 100 and takes its maximum value when the species is present only in one cluster and in all sites of this cluster. A species is considered to be a ''symmetrical indicator'' (IndVal > 50%) for one cluster, when it is present in >70% of the sites of the cluster and when >70% of its individuals occurs in the cluster (Options in the IndVal software: 1000 iterations, $p < 0.05$).

Results

The average air temperature and soil humidity recorded in the sites during the three repetitions ranged from 25 to 33°C and from 44 to 65% respectively (Appendix 1). The average maximum herb height ranged between 31 and 99 cm in sites with very high disturbance (S9) to no disturbance (S2) respectively. Grazing was the most frequent disturbance type (60% of sites), followed by mowing (50%) and trampling (40%). Disturbance from construction works was recorded only in one site (10%). The inclusion of a site in the Natura 2000 network of protected areas does not seem to be related with the disturbance regimes encountered and the species richness recorded (Table 1; Appendix 1).

Diversity patterns

We recorded 43 butterfly species (600 individuals), as well as five more butterfly species outside transect sampling (Appendix 2). Three butterfly species, *Thymelicus action, Pseudophilotes vicrama* and *Melitaea trivia* are classified as Near Threatened in the European Union according to van Swaay et al. (2010). We also sampled 19 grasshopper species, as well as four more species outside the plot sampling, including *Saga hellenica,* an endemic species for Greece (Appendix 2). We found only one species of conservation importance, which is *Chorthippus lacustris,* a critically endangered endemic grasshopper species, according to the red data book of Greece (Legakis and Maragou 2009). Finally, we recorded 116 vascular plants, of which no species belong to the known list of alien macrophyte flora of Greece (Zenetos et al. 2009).

Site S5 was the most diverse in terms of butterflies and vascular plants, including also two near threatened butterfly species. It was the most humid site, with high herb height and herb cover and low disturbance regime (Table 1, Appendix 1). The most species-rich site for grasshoppers and the second most species-rich site for butterflies was site S1, located within the Natura 2000 network. Situated at a quite high altitude, it had low herb height and included a tree line. The poorest site in terms of butterflies and grasshoppers was site S9, a heavily disturbed salt meadow, with the lowest herb height and cover, low humidity degree, and a pronounced cover of rush shrubs (Appendix 1).

We found a significant relationship between the species richness patterns of grasshopper and of vascular plants ($\rho = 0.462$, $p < 0.01$), using the plot species richness data (N = 40 plots). No significant relationship emerged at the site level among the three studied groups (10 sites).

Species richness drivers

No predictive model was built for butterflies, since the variation inflation factor exceeded the upper threshold (VIF > 5) for the whole set of the explanatory variables. We note however that the butterfly species richness was negatively correlated with disturbance ($\rho = -0.586$, $p < 0.05$) when a simple Spearman correlation coefficient was used.

The conditions of no predictor collinearity (VIF < 5), sample independence and normal residuals distribution were met for grasshoppers and plants. The grasshopper model explained 49% of the variation and was built on the basis of altitude, flower-heads, plant species richness and disturbance (negative influence), while the plant model explained 37% of the variation and was build on the basis of humidity and grasshopper species richness (Table 2).

Community structure

Butterflies

Altitude and humidity appeared in the first two axes of the RDA and explained 52% of the variation in butterfly abundance (Fig. 1a). Elevation (up to 480 m) was mostly associated with *Vanessa cardui* and *G. alexis.* Humidity affected in particular *Pieris rapae* and *Papilio machaon,* as they were placed on the extreme end of the respective axis, as well as two near threatened species: *Pseudophilotes vicrama* and *Thymelicus acteon*.

The hierarchical tree of the butterfly community comprises three levels and four clusters (Fig. 2a). The species *Polyommatus icarus* is a generalist species, located at the top of the hierarchical tree. The first level of the hierarchical clustering distinguishes those grasslands with pronounced field margins at a close distance from the butterfly transect (<80 m) from the others and is characterized by four indicator species. The next level distinguishes the site with the greatest disturbance index from the other group of sites (D < 6). The latter cluster is further divided in two sub-clusters, on the basis of their altitude, so as to distinguish two butterfly species as typical species for higher altitudes (>360 m).

Table 2 General linear models (GLM) for grasshoppers and plants, where coefficients are presented only for significant ($p < 0.05$) and independent predictors (VIF < 5)

	Grasshoppers		Plants	
	Coefficients	VIF	Coefficients	VIF
Intercept	0.0229			
Altitude	0.0009	1.873		1.806
Humidity		2.157	0.0145	1.732
Flower-heads	0.1501	1.727		1.964
Disturbance	−0.1315	3.348		5.211
Grasshoppers			0.0742	2.088
Plants	0.0490	1.941		
Goodness of fit	49%		37%	

Grasshoppers

Five environmental factors, including the number of flower-heads, disturbance intensity, altitude, humidity and the cover of shrubs and trees in the broader plot area affected significantly the distribution patterns of grasshopper community, explaining 32% of the overall variance (Fig. 1b). The endemic grasshopper *Chorthippus lacustris* was strongly associated with the number of flower-heads and humidity, whereas it was negatively associated with disturbance intensity and shrub and tree cover. *Decticus albifrons, Conocephalus discolor* and *Metrioptera roeselii ambitiosa* were also associated with flower-heads abundance, as they were positioned close to the respective axis. Disturbance intensity was mostly associated with *Locusta migratoria* and *Calliptamus italicus,* and the increasing altitude with *Euchorthippus declivus* and *C. dichrous.* Finally no species was strongly associated with the degree of humidity and the cover of shrubs and trees.

The hierarchical tree of grasshopper community comprises five levels and six clusters, but no significant typical grasshopper species was revealed through the Indicator Value Procedure (Fig. 2b). The first cluster includes all sites at sea level and is further divided according to the flower-heads abundance. At the second level all sites above sea level with very low disturbance regime (D < 1) are grouped together, whereas the other cluster is further divided to two more sub-clusters comprising of sites above sea level with medium and high disturbance index. Finally, the latter sub-cluster comprises of one group of sites without any shrub and tree cover and a second group of sites with more pronounced presence of shrub and tree cover.

Discussion

Diversity patterns

Although humid grasslands are a priority habitat for conservation in Europe, we found that they were moderately species-rich in terms of butterflies and grasshoppers, including nevertheless important species of conservation concern. Humid grasslands are considered of moderate ecological value in terms of butterfly and grasshopper species richness when compared with other habitat types, but are ranked as the fifth most important habitat type when the number of threatened butterfly species is concerned (Grotjahn and Handke 2000; Pamperis 2010; van Swaay et al. 2006). We found a strong congruence of the species richness patterns of grasshoppers and vascular plants at the plot level (N = 40), implying the potential use of one

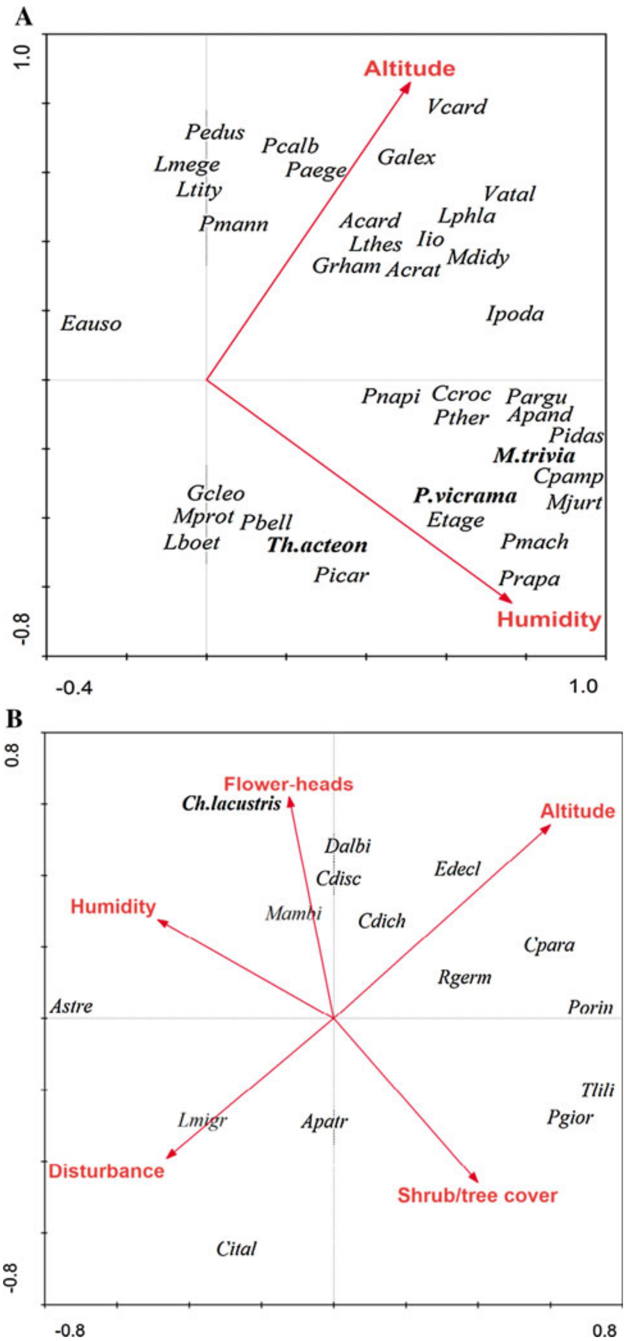

Fig. 1 Redundancy Analysis diagram (RDA) for **a** butterflies and **b** grasshoppers. *Arrows* indicate significant environmental variables. Species abbreviations as referred to Appendix 2. Near threatened butterflies species and the endemic grasshopper are in *bold*

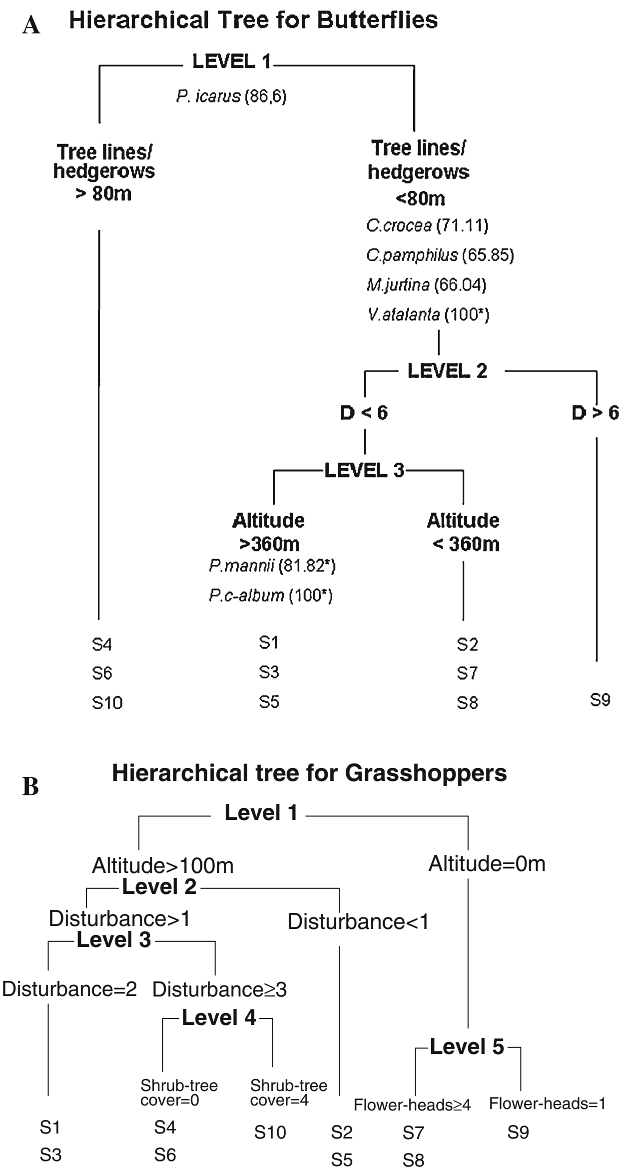

Fig. 2 Hierarchical cluster analysis (Ward's method) for: **a** butterflies and **b** grasshoppers. Indicator species after indicator value analysis (IndVal) ($p < 0.05$), where indicator values are presented in *parenthesis* and maximum indicator values indicated with *asterisk*

group as a surrogate for the other. Other studies have demonstrated a significant species richness patterns covariance between butterflies and grasshoppers, or between butterflies and plants (Grill et al. 2005; Zografou et al. 2009), but such relationships could not be shown due to the small number of samples at the site level (N = 10).

Species richness drivers and community structure

Butterflies

No predictive model was built for butterflies, due to the small number of samples (N = 10). The RDA diagram revealed that humidity and elevation were the main factors regulating butterfly species abundance. Humidity is an important micro-climatic factor affecting butterfly population patterns (Hill 1999), by determining the abundance of larval host-plants and by regulating the nectar concentration in flowers (Corbet et al. 1979). Our results showed the dependence of the three important butterfly species on humid microhabitats. On the other hand, increasing elevation may be positively (Stefanescu et al. 2004; Wettstein and Schmid 1999), or negatively associated with butterfly species richness (Guttiérrez 1997). Finally, another factor that seems to be important for the butterfly habitat structure is the presence of hedgerows and tree lines, as we found four butterfly species to be well-associated with them, according to the indicator value results. These linear structural elements of vegetation are of great importance for several butterfly species, providing wind protection, adequate shade conditions during the warmest part of the day as well as nectar resources (Dover et al. 1997). They can also function as corridors facilitating the movement of butterflies among suitable habitats, especially when the surrounding area has lost its naturalness (Feber and Smith 1995; Pywell et al. 2004).

Grasshoppers

We found that the increasing elevation, flower-heads abundance, plant species richness and the decreasing disturbance regime well predict the overall grasshopper species richness. These parameters together with the cover of trees and shrubs are also shown in the RDA diagram, as significant factors regulating the presence of specific grasshopper species. Flower-heads abundance improves habitat suitability for grasshoppers, since it provides multiple hiding microhabitats from predators (Fielding and Brusven 1995; Gebeyehu et al. 2003) and is a potential feeding resource for some grasshopper juveniles (Wettstein and Schmid 1999). The fact that *Chrthippus lacustris* is strongly associated with the flower-heads axis provides some evidence for a potential feeding preferences of juveniles for flowers rather than grasses, which merits further investigation. Humidity has a regulatory effect on grasshopper hatching and seasonality of their life cycle (Ingrisch 1986), being also important for species of conservation concern such as the critically-endangered *C. lacustris* (Kati et al. 2006). The positive effect of the great number of vascular plant species on grasshopper diversity may be attributed to the greater provision of feeding resources. Finally, the cover of shrubs and trees were not included in the predictive model of grasshopper species richness, but they seem to be an important factor shaping grasshopper habitat, providing shelter, sites for oviposition and feeding resources for several grasshopper species (Guido and Gianelle 2001; Kati et al. 2004; Zografou et al. 2009).

Vascular plants

We found that humidity and grasshopper species richness predicted the overall species richness of the vascular plants. Humidity is considered a key factor defining vascular plant communities and increasing herb cover (Leuschner and Lendzion 2009).

The role of disturbance

We found that disturbance intensity was important predictor of reducing grasshopper species richness and that it was also negatively correlated with butterfly species richness. The most species-rich sites for butterflies (S5) and grasshoppers (S1) were characterized by weak to medium disturbance intensity, resulted by occasional grazing and mowing. Our results can be explained by the intermediate disturbance hypothesis. It is a well-known mechanism that can explain the coexistence of species in ecological communities at the fine scale, in particular when it is related with patch formation (Roxburgh et al. 2004). Low intensity grazing may enhance habitat heterogeneity at fine scales, generating and maintaining open microhabitats, which are of primordial importance for the life cycle and feeding resources availability of butterflies and grasshoppers (Gebeyehu et al. 2003; Kati et al. 2004; Sergeev 1998; Wettstein and Schmid 1999). More intensive grazing can however have a negative impact on butterfly species, dependent on the complexity of their life cycle (Gibson et al. 1992; Wettstein and Schmid 1999). Extreme disturbance, intensive and perpetual grazing and trampling, as found in the poorest site (S9) resulted in reduced plant diversity, nutrient-rich soils and water retention ability, which in turn cause additional reduction of nectar resources that are crucial for butterfly diversity (Britten and Riley 1994; Dunne et al. 2011). Such intensive disturbance negatively affects grasshopper diversity, due to the perturbations of appropriate microclimates conditions and the loss of appropriate refuge microhabitats that allow hiding from predators (Joern 1979; Kruess and Tscharntke 2002; Sergeev 1998).

Conservation implications

Although seasonally flooded grasslands do not support particularly high butterfly and grasshopper species richness, they support hygrophilous species of conservation concern that are associated with this ephemeral habitat type. Therefore humid grasslands merit special conservation attention for the maintenance of threatened insect species. According to our results we can recommend the following four conservation measures. First we should manage humid grasslands in a way to maintain high humidity, as it is proven to be important for the survival of butterfly and grasshopper species of conservation concern. Second, we should maintain hedgerows and tree lines, as important structural elements that support specific butterfly species. Third, we should manage humid grasslands so as to maintain high vascular plant diversity and abundance of plants in flower, which are found to support grasshopper species richness. We should draw special attention to the conservation of humid grasslands at higher altitudes, as they seem to be important for the insect communities studied. Third, we suggest that only mild disturbance regimes, such as those generated by occasional grazing or mowing can be consistent with butterfly and grasshopper communities conservation. The presence of more intensive human-induced disturbance types such as construction works or trampling, and the co-existence of different disturbance types can have an additive degrading effect on habitats, which might lead to species loss. Finally, we suggest that vascular plants could be used as a surrogate group for grasshoppers and vice versa, although further testing is required before using these surrogates at large scales or in different habitat types.

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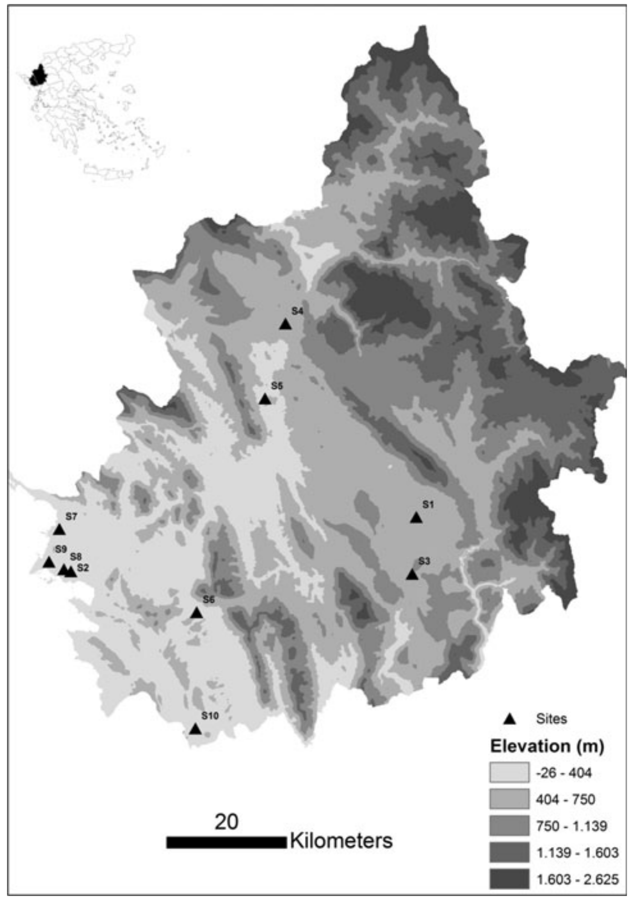
Appendix 1: Study sites



Sites	Coordinates			Environmental parameters							
	Latitude (N)	Longitude (E)	Habitat type	Alt (m)	Temp (°C)	Hum (%)	Herb height (cm)	Herb cover	F	TSh cover (%)	DT (m)
S1	39°38′8.12″	20°53′4.60″	6420*	467	32	53	54	3	3	3	<10
S2	39°32′2.15″	20°13′1.31″	6420*	0	28	57	99	4	4	3	30
S3	39°32′59.57″	20°52′49.80″	6420	480	29	44	69	3	5	0	60
S4	39°55′7.28″	20°36′56.12″	6420	468	30	45	59	3	3	0	>100
S5	39°48′17.50″	20°34′52.57″	6450	378	33	65	94	4	3	3	30
S6	39°28′48.36″	20°27′52.34″	6420*	187	25	47	74	3	3	0	80
S7	39°35′48.66″	20°11′27.92″	6420*	0	30	60	83	4	4	2	45
S8	39°32′11.44″	20°12′11.56″	6420*	0	29	51	64	3	5	1	15
S9	39°32′49.02″	20°10′24.78″	1410*	0	30	45	31	2	1	5	40
S10	39°18′18.79″	20°28′14.52″	6420*	101	31	52	51	3	3	4	>100

* Site of Natura 2000 network, *Alt* altitude, *Temp* mean air temperature, *Hum* mean soil humidity, *Herb height* mean of maximum herb heights at plots, *Herb cover* mean herb cover (1: 1–5%, 2: 6–25%, 3: 25–50%, 4: 51–75%, 5: >75%), *F* average flower-heads (1: <10, 2: 11–50, 3: 50–100, 4: 101–200, 5: 201–400, 6: 401–600, 7: >600), *TSh cover* cover of shrubs and trees at 40 m bandwidth (see herb cover scale), *DT* distance from nearest tree line/hedgerow

Appendix 2: Species inventory

See Tables 3, 4, and 5.

Table 3 Inventory of the butterfly species and their conservation status for the 27 member states of the EU after van Swaay et al. (2010)

Abbreviations	PAPILIONIDAE	Abbreviations	NYMPHALIDAE
Ipoda	*Iphiclides podalirius* (8)	Apand	*Argynnis pandora* (1)
Pmach	*Papilio machaon* (3)	Apaph	*Argynnis paphia* (1)
	PIERIDAE	Iio	*Inachis io* (1)
Acard	*Anthochariscardamines* (2)	Mardu	*Melitaea arduinna* (1)
Acrat	*Aporia crataegi* (10)	Mdidy	*Melitaea didyma* (18)
Ccroc	*Colias crocea* (75)	Mphoe	*Melitaea phoebe* (4)
Eauso	*Euchloe ausonia* (1)	Mtriv	***Melitaea trivia*** (1)
Gcleo	*Gonepteryx cleopatra* (1)	Pcalb	*Polygonia c-album* (2)
Grham	*Gonepteryx rhamni* (1)	Vatal	*Vanessa atalanta* (4)
Pbrass	*Pieris brassicae* (1)	Vcard	*Vanessa cardui* (4)
Pmann	*Pieris mannii* (4)		*Danaus chrysippus*
Pnapi	*Pieris napi* (5)		*Nymphalis antiopa*
Prapa	*Pieris rapae* (8)		**LIBYTHEIDAE**
Pedus	*Pontia edusa* (19)		*Libythea celtis*
	LYCAENIDAE		**SATYRIDAE**
Galex	*Glaucopsyche alexis* (1)	Cpamp	*Coenonympha pamphilus* (98)
Lboet	*Lampides boeticus* (1)	Lmege	*Lasiommata megera* (1)
Lphla	*Lycaena phlaeas* (1)	Mjurt	*Maniola jurtina* (71)
Lther	*Lycaena thersamon* (2)	Paege	*Pararge aegeria* (4)
Ltity	*Lycaena tityrus* (1)	Ptith	*Pyronia tithonus* (10)
Pargu	*Plebejus argus* (18)		**HESPERIIDAE**
Pidas	*Plebejus idas* (10)	Calce	*Carcharodus alceae* (3)
Pbell	*Polyommatus bellargus* (1)	Etage	*Erynnis tages* (1)
Picar	*Polyommatus icarus* (193)	Mprot	*Muschampia proto* (4)
Pther	*Polyommatus thersites* (2)	Tacte	***Thymelicus acteon*** (2)
Pvicr	***Pseudophilotes vicrama*** (1)		*Gegenes nostrodamus*
	Leptotes pirithous		

Numbers in parenthesis indicate the sum of individuals recorded during transect sampling. All species are least concerned apart from species in bold that are near threatened. No abbreviations are given for species sampled out of plots

Table 4 Inventory of grasshopper species sampled

Abbreviations	ACRIDIDAE	Abbreviations	TETTIGONIIDAE
Aunga	*Acrida ungarica* (1)	Cdisc	*Conocephalus discolor* (5)
Apatr	*Acrotylus patruelis* (1)	Dalbi	*Decticus albifrons* (2)
Astre	*Aiolopus strepens* (16)	Mambi	*Metrioptera roeselii ambitiosa* (3)
Cital	*Calliptamus italicus* (8)	Porin	*Platycleis(Tessellana) orina* (6)
Cdich	*Chorthippus dichrous* (12)	Rgerm	*Rhacocleis germanica* (3)
Cpara	*Chorthippus parallelus tenuis* (5)	Rniti	*Ruspolia nitidula* (2)
Clacu	*Chorthippus lacustris* (13)	Tlili	*Tylopsis lilifolia* (4)
Edecl	*Euchorthippus declivus* (8)		*Saga hellenica*
Lmigr	*Locusta migratoria* (3)		*Tettigonia viridissima*

Table 4 continued

Abbreviations	ACRIDIDAE	Abbreviations	TETTIGONIIDAE
Pgior	*Pezotettix giornai* (4)		**TETRIGIDAE**
	Anacridium aegyptium	Pmeri	*Paratettix meridionalis* (1)
	Paracinema tricolor		
	GRYLLIDAE		
Gcam	*Gryllus campestris* (1)		

Numbers in parenthesis indicate the number of plots that the species were encountered. No abbreviations are given for species sampled out of plots

Table 5 Inventory of the vascular plant species sampled

ALISMATACEAE	**LINACEAE**	*Galium debile* (7)
Alisma lanceolatum (4)	*Linum bienne* (1)	*Galium lucidum* (1)
ORCHIDACEAE	**FABACEAE**	**LAMIACEAE**
Orchis laxiflora (1)	*Galega officinalis* (3)	*Mentha aquatica* (1)
XANTORRHOEACEAE	*Lathyrus aphaca* (1)	*Mentha pulegium* (14)
Asphodelus albus (1)	*Lotus corniculatus* (15)	*Teucrium sp.* (1)
CYPERACEAE	*Medicago arabica* (3)	*Vitex agnus-castus* (1)
Carex distans (4)	*Medicago intertexta* (1)	**PLANTAGINACEAE**
Carex hirta (4)	*Medicago minima* (1)	*Plantago coronopus* (6)
Carex muricata (9)	*Medicago orbicularis* (1)	*Plantago lanceolata* (12)
Cyperus longus (3)	*Medicago sativa* (1)	**SCROPHULARIACEAE**
Eleocharis palustris (4)	*Melilotus infestus* (7)	*Bellardia trixago* (1)
Schoenoplectus lacustris (3)	*Ononis spinosa* (4)	*Parentucellia viscosa* (8)
Scirpoides holoschoenus (2)	*Trifolium alexandrinum* (7)	*Verbascum blattaria* (2)
JUNCACEAE	*Trifolium campestre* (1)	**VERBENACEA**
Juncus acutus (2)	*Trifolium fragiferum* (10)	*Verbena officinalis* (3)
Juncus sp. (1)	*Trifolium nigrescens* (8)	**CONVOLVULACEAE**
POACEAE	*Trifolium pallidum* (3)	*Convolvulus arvensis* (17)
Aegilops neglecta (1)	*Trifolium patens* (8)	*Cuscuta campestris* (2)
Agrostis capillaris (5)	*Trifolium repens* (7)	**ASTERACEAE**
Agrostis stolonifera (3)	*Trifolium resupinatum* (23)	*Anthemis cotula* (1)
Aira elegantissima (2)	*Trifolium sp.* (1)	*Carduus acicularis* (4)
Alopecurus rendlei (14)	*Vicia peregrina* (1)	*Carthamus lanatus* (1)
Brachypodium sylvaticum (1)	**ROSACEAE**	*Centaurea calcitrapa* (2)
Bromus racemosus (2)	*Potentilla reptans* (2)	*Chamaemelum mixtum* (1)
Bromus secalinus (2)	*Prunus spinosa* (1)	*Chrysanthemum segetum* (1)
Bromus sterilis (4)	**GERANIACEAE**	*Cichorium intybus* (13)
Cynodon dactylon (30)	*Geranium dissectum* (3)	*Cichorium pumilum* (7)
Dactylis glomerata (2)	*Geranium lucidum* (1)	*Cirsium arvense* (3)
Digitaria sanguinalis (5)	**BRASSICACEAE**	*Cirsium italicum* (1)
Hordeum marinum (1)	*Rorippa sylvestris* (1)	*Crepis setosa* (1)
Hordeum vulgare (4)	*Sinapis arvensis* (6)	*Helmintoheca echioides* (3)
Lolium multiflorum (2)	**MALVACEAE**	*Helmintoheca prolifera* (1)
Lolium rigidum (2)	*Althaea officinalis* (1)	*Hypochaeris cretensis* (1)
Phleum pratense (1)	*Malva sylvestris* (3)	*Inula britannica* (1)
Phragmites australis (3)	**CARYOPHYLLACEAE**	*Lactuca saligna* (1)
Poa trivialis subsp. sylvicola (17)	*Moenchia mantica* (2)	*Pulicaria dysenterica* (2)
Secale strictum (5)	*Petrorhagia prolifera* (1)	*Silybum marianum* (1)

Table 5 continued

Setaria viridis (2)	*Spergularia salina* (1)	*Tragopogon pratensis* (4)
Sorghum halepense (1)	**POLYGONACEAE**	*Xanthium strumarium* (8)
Trisetum aureum (2)	*Rumex conglomeratus* (9)	**CAPRIFOLIACEAE**
RANUNCULACEAE	*Rumex crispus* (13)	*Dipsacus laciniatus* (1)
Ranunculus sardous (22)	**BORAGINACEAE**	**APIACEAE**
CLUSIACEAE	*Echium plantagineum* (1)	*Daucus carota* (3)
Hypericum spruneri (1)	*Myosotis sicula* (1)	*Eryngium amethystinum* (5)
EUPHORBIACEA	**RUBIACEAE**	*Eryngium campestre* (1)
Chrozophora tinctoria (1)	*Cruciata laevipes* (1)	*Oenanthe pimpinelloides* (27)
Euphorbia platyphyllos (3)	*Galium aparine* (1)	

Numbers in parenthesis indicate the number of plots that species were encountered (40 plots sampled)

References

Battisti C, Luiselli L, Pantano D et al (2008) On threats analysis approach applied to a Mediterranean remnant wetland: is the assessment of human-induced threats related into different level of expertise of respondents? Biodivers Conserv 16:1529–1542. doi:10.1007/s10531-008-9360-1

Britten HB, Riley L (1994) Nectar source diversity as an indicator of habitat suitability for the endangered Uncompahgre fritillary, *Boloria acrocnema* (Nymphalidae). J Lep Soc 48:173–179

Chambers BQ, Samways MJ (1998) Grasshopper response to a 40-year experimental burning and mowing regime, with recommendations for invertebrate conservation management. Biodivers Conserv 7:985–1012. doi:10.1023/A:1008803513740

Connell HJ (1978) Diversity in tropical rain forests and coral reefs. Science 199:1302–1310

Corbet SA, Unwin DM, Prûs-Jones OE (1979) Humidity, nectar and insect visits to flowers, with special reference to *Crataegus, Tilia* and *Echium*. Ecol Entomol 4:9–22. doi:10.1111/j.1365-2311.1979.tb00557

Dafis S, Papastergiadou E, Lazaridou E, Tsiafouli M (2001) Technical manual of identification, description and mapping of Greek habitat types. Greek Biotope–Wetland Center, (EKBY), Thessaloniki (in Greek)

Dover JW, Sparks TH, Greatorex-Davies JN (1997) The importance of shelter for butterflies in open landscapes. J Insect Conserv 1:89–97. doi:10.1023/A:1018487127174

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol Monogr 67:345–366. doi:10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2

Dunne T, Western D, Dietrich WE (2011) Effect of cattle trampling on vegetation, infiltration, and erosion in a tropical rangeland. J Arid Environ 75:58–69. doi:10.1016/j.jaridenv.2010.09.001

EEA (2010) EU 2010 Biodiversity Baseline. EEA Technical report No 12/2010. European Environment Agency, Copenhagen

Feber RE, Smith H (1995) Butterfly conservation on arable farmland. In: Pullin AS (ed) Ecology and conservation of butterflies. Chapman & Hall, London, pp 84–97

Fielding DG, Brusven MA (1995) Ecological correlates between rangeland grasshoppers (Orthoptera: Acrididae) and plant communities of southern Idaho. Environ Entomol 24:1432–1441

Gebeyehu S, Michael J, Samways MJ (2003) Responses of grasshopper assemblages to long-term grazing management in a semi-arid African savanna. Agric Ecosyst Environ 95:613–622. doi:10.1016/S0167-8809(02)00178-0

Gibbs JP (1995) Hydrologic needs of wetland animals. In: Nierenberg WA (ed) Encyclopedia of environmental biology, vol 2. Academic Press, New York, pp 267–276

Gibbs JP (2000) Wetland loss and biodiversity conservation. Conserv Biol 14:314–317. doi:10.1046/j.1523-1739.2000.98608.x

Gibson CWD, Brown VK, Losito L, McGavin GC (1992) The response of invertebrate assemblies to grazing. Ecography 15:166–176. doi:10.1111/j.1600-0587.1992.tb00020.x

Green AJ, Hamzaoui MEL, Agbani MAEL, Franchimont J (2002) The conservation status of Moroccan wetlands with particular reference to waterbirds and to changes since 1978. Biol Conserv 104:71–82. doi:10.1016/S0006-3207(01)00155-0

Grill A, Barbara K, Cleary DFR, Kati V (2005) Butterfly, spider, and plant communities in different land-use types in Sardinia, Italy. Biodivers Conserv 14:1281–1300. doi:10.1007/s10531-004-1661-4

Grotjahn F, Handke K (2000) Changes in the grasshopper fauna of the river marshes in Bremen from 1986 to 1997-increase of numbers of species and individuals in grassland areas. Naturschutz und Landschaftsplanung 32:97–105

Guido M, Gianelle D (2001) Distribution patterns of four Orthoptera species in relation to microhabitat heterogeneity in an ecotonal area. Acta Oecol 22:175–185. doi:10.1016/S1146-609X(01)01109-2

Guttiérrez D (1997) Importance of historical factors on species richness and composition of butterfly assemblages (Lepidoptera: Rhopalocera) in a northern Iberian mountain range. J Biogeogr 24:77–88. doi:10.1111/j.1365-2699.1997.tb00052.x

Hill JK (1999) Butterfly spatial distribution and habitat requirements in a tropical forest: impacts of selective logging. J Appl Ecol 36:564–572. doi:10.1046/j.1365-2664.1999.00424.x

HNMS (2000) Hellenic National Meteorological Society Web. http://www.hnms.gr/hnms/greek/index_html

Ingrisch S (1986) The plurennial life cycles of the European Tettigoniidae (Insecta: Orthoptera), 3. The effect of drought and the variable duration of the initial diapauses. Oecologia 70:624–630. doi:10.1007/BF00379915

Joern A (1979) Feeding patterns in grasshoppers (Orthoptera: Acrididae): factors influencing diet specialization. Oecologia 38:325–347. doi:10.1007/BF00345192

Kati V, Dufrêne M, Legakis A et al (2004) Conservation management for the Orthoptera in the Dadia reserve, Greece. Biol Conserv 115:33–44. doi:10.1016/S0006-3207(03)00091-0

Kati V, Mani P, von Helversen O et al (2006) Human land use threatens endemic wetland species: the case of *Chorthippus lacustris* (La Greca and Messina 1975) (Orthoptera: Acrididae) in Epirus, Greece. J Insect Conserv 10:65–74. doi:10.1007/s10841-005-2642-y

Kruess A, Tscharntke T (2002) Contrasting responses of plant and insect diversity to variation in grazing intensity. Biol Conserv 106:293–302. doi:10.1016/S0006-3207(01)00255-5

Legakis A, Maragou P (2009) The red data book of threatened vertebrates of Greece. Hellenic Zoological Society, Athens

Leuschner C, Lendzion J (2009) Air humidity, soil moisture and soil chemistry as determinants of the herb layer composition in European beech forests. J Veg Sci 20:288–298. doi:10.1111/j.1654-1103.2009.05641.x

Magurran AE (2004) Measuring biological diversity. Blackwell, Oxford

McCune B, Mefford MJ (1999) PC–ORD. Multivariate analysis of ecological data. Version 4. MjM Software Design, Gleneden Beach

Mitsch WJ, Gosselink JG (1993) Wetlands, 2nd edn. Wiley (formerly Van Nostrand Reinhold), New York

O'Neill KM, Olson BE, Rolston MG, Wallander R, Larson DP, Seibert CE (2003) Effects of livestock grazing on rangeland grasshopper (Orthoptera: Acrididae) abundance. Agr Ecosyst Environ 97:51–64. doi:10.1016/S0167-8809(03)00136-1

Pamperis LN (2010) The butterflies of Greece, 2nd edn. Koan, Athens

Plum N (2005) Terrestrial invertebrates in flooded grassland: a literature review. Wetlands 25:721–737. doi:10.1672/0277-5212(2005)025[0721:TIIFGA]2.0.CO;2

Psilovikos A (1992) Prospects for wetlands and waterfowl in Greece. In: Finlayson CM, Hollis T, Davis T (eds) Managing Mediterranean wetlands and their birds. Proceedings of an IWRB international symposium, Grado, Italy. IWRB Special Publication no. 20, pp 53–55

Pywell RF, Warman EA, Sparks TH, Greatorex-Davies JN, Walker KJ, Meek WR, Carvell C, Petit S, Firbank LG (2004) Assessing habitat quality for butterflies on intensively managed arable farmland. Biol Conserv 118:313–325. doi:10.1016/j.biocon.2003.09.011

Roxburgh SH, Shea K, Wilson JB (2004) The intermediate disturbance hypothesis: patch dynamics and mechanisms of species coexistence. Ecology 85:359–371

R Development Core Team (2009) R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available at: http://www.R-project.org

Sala OE, Paruelo JM (1997) Ecosystem services in grasslands. In: Daily GC (ed) Nature's services: societal dependence on natural ecosystems. Island Press, Washington, pp 237–252

Samways MJ (1994) Insect conservation biology. Chapman & Hall, London

Sergeev MG (1998) Conservation of orthopteran biological diversity relative to landscape change in temperate Eurasia. J Insect Conserv 2:247–252. doi:10.1023/A:1009620519058

Stefanescu C, Herrando S, Páramo F (2004) Butterfly species richness in the north–west Mediterranean Basin: the role of natural and human-induced factors. J Biogeogr 31:905–915. doi:10.1111/j.1365-2699.2004.01088.x

ter Braak CJF, Smilauer P (2002) CANOCO Reference manual and canoco draw for windows user's guide: software for canonical community ordination (version 4.5) microcomputer power. Ithaca, New York

Thomas JA, Telfer MG, Roy DB, Preston CD, Greenwood JJD, Asher J, Fox R, Clarke RT, Lawton JH (2004) Comparative losses of British butterflies, birds, and plants and the global extinction crisis. Science 303:1879–1881. doi:10.1126/science.1095046

Tscharntke T, Greiler HJ (1995) Insect communities, grasses, and grasslands. Annu Rev Entomol 40:535–558. doi:10.1146/annurev.en.40.010195.002535

UNEP (2004) Freshwater in Europe—Facts, Figures and Maps. United Nations Environment Programme-Division of Early Warming and Assessment-Europe, Chatelaine, Switzerland

Van Swaay C, Warren M, Loïs G (2006) Biotope use and trends of European butterflies. J Insect Conserv 10:189–209. doi:10.1007/s10841-006-6293-4

Van Swaay C, Cuttelod A, Collins S, Maes D, López Munguira M, Šašić M, Settele J, Verovnik R, Verstrael T, Warren M, Wiemers M, Wynhof I (2010) European red list of butterflies. Publications Office of the European Union, Luxembourg

Warren MS, Hill JK, Thomas JA et al (2001) Rapid responses of British butterflies to opposing forces of climate and habitat change. Nature 414:65–69. doi:10.1038/35102054

Wettstein W, Schmid B (1999) Conservation of arthropod diversity in montane wetlands: effect of altitude, habitat quality and habitat fragmentation on butterflies and grasshoppers. J Appl Ecol 36:363–373. doi:10.1046/j.1365-2664.1999.00404.x

Willemse F (1985) A key to the orthoptera species of Greece. Hellenic Zoological Society, Athens

Wilson RJ, Davies ZG, Thomas CD (2009) Modelling the effect of habitat fragmentation on range expansion in a butterfly. Proc R Soc Lond B 276:1421–1427. doi:10.1098/rspb.2008.0724

Zenetos A, Pancucci-Papadopoulou MA, Zogaris S et al (2009) Aquatic alien species in Greece (2009): tracking sources, patterns and effects on the ecosystem. J Biol Res-Thessalon 12:135–172. Available at: http://www.jbr.gr/papers20092/03-Zenetos%20et%20al.pdf

Zografou K, Sfenthourakis S, Pullin A, Kati V (2009) On the surrogate value of red-listed butterflies for butterflies and grasshoppers: a case study in Grammos site of Natura 2000, Greece. J Insect Conserv 13:505–514. doi:10.1007/s10841-008-9198-6