Micro-moth communities mirror environmental stress gradients within a Mediterranean nature reserve

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Abstract

Isolated conservation areas embedded in a hemerobic landscape matrix run the risk of losing their functionality through stress impacts from their surroundings. We studied insects in one of the last remaining coastal forest reserves in northern Italy, viz. the Pineta san Vitale near Ravenna, to assess whether local assemblages are shaped by gradients of external environmental stressors. Specifically, we asked whether proximity to the nearest sources of aerially transported pollutants and extent of soil salinity affect communities. We used nocturnal micro-moths as target group since their usually high degree of host-plant specificity and often small body size lead us to expect these insects to reveal community differentiation at very small spatial scales within the reserve.

We sampled 6085 moths representing 157 species at 16 light-trap sites during the vegetation period in 2013. We then assembled a matrix of 37 traits relating to larval niche, body size, phenology, migration behavior and habitat preferences from literature sources. With these data we established that species composition and overall functional diversity of local micro-moth assemblages was strongly influenced by the distance to pollution sources, and less so by soil salinity. In contrast, species richness and species diversity were not effective in depicting influences of these two environmental gradients. Our results show that micro-moth communities respond sensitively to environmental conditions on small spatial scales, yet not all community attributes are equally suitable to uncover such relationships. Species composition and functional diversity emerged as far more informative than measures of local species diversity.

Zusammenfassung

Isolierte Naturschutzgebiete inmitten einer menschlich überformten Landschaft laufen Gefahr, ihre Funktionalität durch negative Einflüsse von außerhalb zu verlieren. Wir untersuchten Insekten in einem der letzten verbliebenen Küstenwaldreservate in Norditalien, Pineta san Vitale nahe der Stadt Ravenna, um herauszufinden, ob sich lokale Populationen infolge kleinräumiger Umweltgradienten, hervorgerufen durch Störeinflüsse von außen, verändern. Insbesondere prüften wir, ob die Nähe zu Luftverschmutzungsquellen sowie Bodenversalzung die Insekten-Gemeinschaften beeinflussen. Der Fokus lag dabei auf nachtaktiven Kleinschmetterlingen, die oft eng an bestimmte Raupenfraßpflanzen gebunden sind. Daher vermuteten wir, dass diese Insekten besonders geeignet sind, kleinräumige Gradienten widerzuspiegeln.

Während der Vegetationsperiode 2013 erfassten wir 6085 Individuen von 157 verschiedenen Arten an 16 Leuchtstandorten. Wir erstellten eine Matrix mit 37 funktionellen Charakteristika wie larvale Futterpflanzen, Körpergröße, Phänologie,

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Wanderverhalten und Habitatpräferenzen. Wir konnten zeigen, dass Artenzusammensetzung und funktionelle Diversität der lokalen Microlepidopteren-Gemeinschaften stark beeinflusst werden durch die Distanz zur Quelle der Luftverschmutzung sowie –weniger stark –durch die Bodenversalzung. Microlepidopteren-Gemeinschaften reagieren demnach selbst auf kleinräumiger Ebene sensitiv auf Umwelteinflüsse. Aber nicht alle Diversitätsmaße sind gleich gut geeignet, derartige Beziehungen aufzudecken. Funktionelle Diversität und Artenzusammensetzung erwiesen sich als weit informativer als die Betrachtung von Artenreichtum oder Artendiversität.

Introduction

Human impact on biodiversity and ecosystem functioning is omnipresent in the anthropocene, e.g. through habitat loss, fragmentation, pollution or climate change (Zalasiewicz, Williams, Steffen, & Crutzen, 2010). Today small remnant patches of near-natural habitats gain ever more importance as nature reserves (Hanski, 2011). Though aimed at offering refuges to threatened biota, these habitat islands embedded in an anthropogenically transformed matrix do not go unaffected. Habitat quality is fundamental to the persistence of those organismal communities for whom conservation reserves have been designed (Mortelliti, Amori, & Boitani, 2010; Zulka et al., 2014). Therefore, understanding the impact of extrinsic environmental stressors on small-sized nature reserves, and how conservation management can be targeted to protect even small biodiversity islands in a cultivated landscape, is a central point of concern.

Matrix effects on isolated habitat patches are manifold. Especially edge effects or influx of environmental chemicals from the surroundings are expected to prevail in small patches. This can substantially affect habitat quality and consequently species assemblages (Mairota et al., 2015). Essential services for which nature reserves are genuinely preserved and considered important such as providing of genetic resources, biological control, pollination and nutrient cycling (Dobson et al., 2006), might collapse due to human activities in the vicinity.

The status and integrity of conservation areas is usually monitored with the help of selected indicator organisms (Cardoso, Rigal, Fattorini, Terzopoulou, & Borges, 2013). Often the local plant community is assessed, supplemented by selected animal species of special conservation concern (Diaz & Cabido, 2001; Pinho et al., 2012). However, plants do not necessarily provide insight into human influences on higher trophic layers. In fact, climbing the food-web from the plant level, linkages between productivity, stability and biodiversity can be modified, dampened or even reversed (Worm & Duffy, 2003). But if anthropogenic degradation in a habitat were to negatively affect herbivores, this could influence processes along the entire food web by means of trophic cascades.

We here explore nocturnal micro-moths for their potential to reveal anthropogenic threats to habitat quality at small spatial scales. Lepidopterans are well known to respond sensitively to pollution or habitat disturbance through changes in species composition, abundance or diversity. Accordingly, butterflies (Berg, Ahrné, Öckinger, Svensson, & Wissman, 2013) and larger moths (Fiedler, Hilt, Brehm, & Schulze, 2007; Luque, Legal, Winterton, Mariano, & Gers, 2011) have often been surveyed along environmental gradients. Micro-moths, however, have thus far hardly been exploited in that regard, except for a few studies concerning responses of individual species or species groups along pollution gradients (Koricheva & Haukioja, 1992; Rickman & Connor, 2003). This seems surprising, because their large numbers of species and individuals, together with their often high degree of host-plant specificity and habitat fidelity at small spatial scales (Gaston, Reavey, & Valladares, 1992; Menken, Boomsma, & van Nieukerken, 2010), render micro-moths a potentially promising group to reveal ecosystem effects arising from disturbance. Due to their mostly small body size, even isolated conservation areas can be expected to offer a sufficient carrying capacity to sustain micro-moth populations as long as the quality of their habitats is maintained.

In order to empirically test these ideas, we surveyed micro-moth communities along two gradients of environmental stress in an isolated nature reserve embedded in a hemerobic landscape that is otherwise dominated by urbanization and intensive agriculture. Specifically, we investigated if species diversity, functional diversity or species composition of micro-moth communities mirror habitat gradients related to environmental stress that can be observed within a protected area.

Materials and methods

Study site

The coastal forest reserve Pineta san Vitale (PSV hereafter) is part of the "Parco regionale del Delta del Po", located at the Adriatic coast of the Emilia-Romagna (Italy) (Montanari, 2010). Its total extent is about 7 km from north to south and

about 2 km from east to west. Vegetation is dominated by a mix of sub-Mediterranean downy oak and pine forest. PSV is a habitat island, separated from other near-natural forest habitats by kilometer-wide stretches of agricultural land, streets, the city of Ravenna, its harbor and the lagoon "Pialassa Baiona". Only in the north less hemerobic areas are neighboring to the reserve, but contain other vegetation types like hygrophilous forest and open wetlands. South of PSV, the harbor of Ravenna is located which heavily contributes to air pollution (Lucialli, Ugolini, & Pollini, 2007) and the intrusion of wastewaters on the lagoon (Matteucci et al., 2005).

The coastal region of Emilia Romagna also suffers from soil salinization as a result of subsidence processes following gas extraction at offshore platforms (Teatini, Ferronato, Gambolati, Bertoni, & Gonella, 2005). Furthermore, drainage systems enhance the influx of hypersaline lagoon water (Mollema et al., 2013), which has lead to salt concentrations up to 22 g/l in the soil of PSV (Antonellini et al., 2008). Factors concomitant to climate change, such as the increasingly arid summer season with augmented evaporation, further enhance soil salinization (Mollema et al., 2013). As a result, vegetation inside PSV ranges from forest sites that still look healthy to severely degraded forest patches especially at the southern margin (Uhl & Wölfling, 2015).

Study design

Sixteen sites in downy oak and pine forest habitats (at least 100 m away from other vegetation types) were chosen all over the park (Fig. 1). Distances between neighboring sites were about 500–1000 m (depending on accessibility and forest density), to avoid interference between light traps. The mean distance of each trap site to three reference points around the harbor of Ravenna (as a proxy for industrial influence) was measured using GPS data and Google Earth (Fig. 1). Soil salinity was extracted from Antonellini et al. (2008) and Giambastiani (2007). As a proxy of forest structure we calculated the height of the 10 nearest *Quercus robur* trees at each light trap location from scaled digital photographs of every tree, using the software Image J (http://imagej.nih.gov). We also assessed habitat descriptors such as tree crown density, diameter, or basal area of pine and oak. However, none of these were related to moth community traits and accordingly we removed them from in-depth analyses (Uhl, 2014).

Moth sampling and statistical analysis

We used automatic light traps equipped with two 15 W tubes (Truxa & Fiedler, 2012a). These low-power light sources have an attraction range of typically less than 10 m and therefore predominantly sample moths from the immediate surroundings of the trap site (Truxa & Fiedler, 2012a). Sampling occurred four times during 2013 (spring, early summer, summer and autumn), at nights with temperature above

12 °C, no heavy rainfall and no strong wind. Traps were started by photocells at dusk and then operated for 6–8 h depending on battery power.

Micro-moths were identified to species level through wing pattern or genitalia dissection (see Appendix A). Species composition (Bray-Curtis similarity based on square-root transformed abundances) was analyzed in relation to environmental predictors by distance based redundancy analysis (dbRDA; Anderson, Gorley, & Clarke, 2008). We explored relationships of 'ecologically informative' micro-moth species to the environmental gradients under study, defining as 'informative' all those species whose local abundances had a Spearman rank correlation of $r_S > 0.6$ with the canonical axes in reduced ordination space. Species diversity per light-trap site was assessed with the program Spade (Chao & Shen, 2003), using the bias-corrected exponential Shannon index (Beck & Schwanghart, 2010; Fiedler & Truxa, 2012). Species richness was compared by rarefaction of each site's catch to 100 individuals. Functional diversity and its three components functional richness, evenness and divergence (Mouchet, Villéger, Mason, & Mouillot, 2010) were analyzed with a distance-based approach (Laliberté, Legendre, & Shipley, 2015). We collated 37 traits comprising a wide range of descriptors like body size, voltinism, larval food niche and habitat preferences (see Appendices B and C for trait data, trait descriptions, an ordination of moth species in reduced trait space and supplementary references for sources of information). Of these, 29 traits were considered informative if the respective character occurred in at least 5 species. Since some traits were inter-correlated, we subjected them to a PCA (Appendix D) after normalization and based our functional diversity analyses on a Euclidean distance matrix derived from the first 8 PCs (accounting for 62.9% of the variation). Micro-moth community attributes were tested for correlations with the distance of trap sites to industry, or the level of soil salinity.

Results

6085 micro-moth individuals were determined and assigned to 157 species representing 25 families. Functional trait data were available for 147 species (see Appendix B). On average, 380.3 moths (range: 103–1298) were available for analysis per site.

In univariate marginal tests, species composition was significantly structured along the north-to-south gradient and less strongly along the soil salinity gradient or with increasing height of oak trees (see Appendix E). Collectively, these three variables explained 30.6% of variance in species composition (Fig. 2).

Twenty moth species were particularly informative for the ordination having a Spearman rank correlation of >0.6 with the canonical axes. Among these, the abundance of 14 species increased at sites more distant to industry areas (significant in 12 cases), whereas only four species were significantly
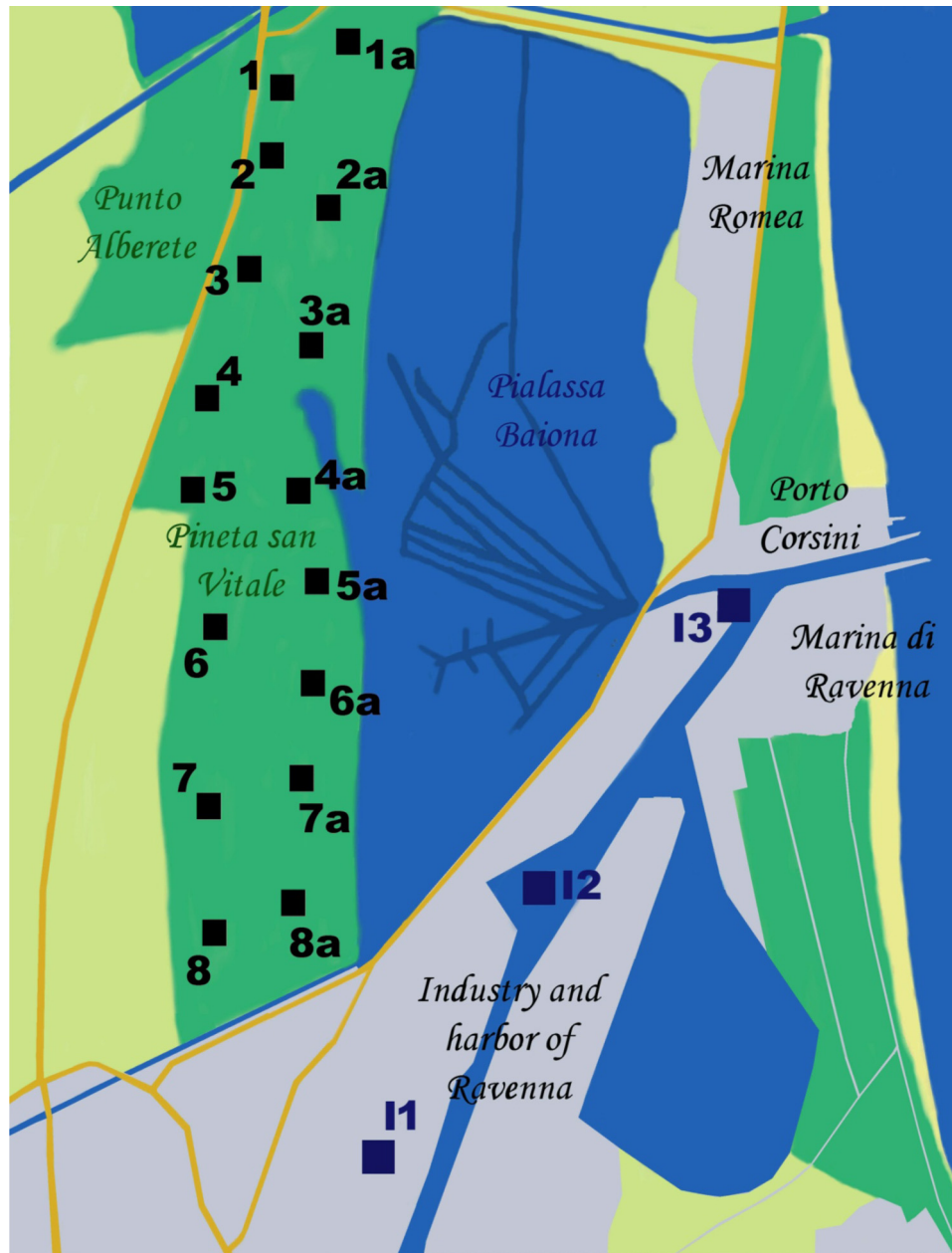
Fig. 1. Map of Pineta san Vitale (PSV) with locations of light traps (1–8 and 1a–8a, see also Uhl & Wölfling, 2015). Reference points to define the distance to industry are marked as I1, I2 and I3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

more common in the more disturbed fraction of the reserve (Fig. 3, Table 1). Eight of the species that were far more abundant in the north of PSV are oak-feeding specialists like *Carpatolechia aenigma* or *Epagoge grotiana*. Only few open-habitat species like *Ancylis apicella* and *Ematheudes punctella* had their main distribution in more impacted areas very near to industry. Similarly, the abundance of 14 species was negatively related to soil salinity (significant in seven cases), whereas only four species were significantly more common at higher soil salinity (including *Mecyna asinalis*, a coastal species feeding on salt-tolerant herbs). The abundance of *Homaloxestis briantiella* (a detritivore) was highest

at sites with but small oak trees, while *Acrobasis consociella* abundance (an oak herbivore) followed the average height of oaks. Otherwise, the abundance of individual micro-moth species showed little relationship to the height of oak trees (Fig. 3, Table 1).

Neither local species diversity nor species richness was related to the distance of the trap site to the industrial plants (Table 2). However, functional diversity significantly declined along this gradient (Fig. 4A). Regarding the three components of functional diversity – functional richness, evenness and divergence – only functional richness tended to decline at study sites increasingly close to industry

Table 1. Relationships (Spearman rank correlations) between the local abundance of the 20 ecologically most informative micro-moth species and the distance to the industrial plants, soil salinity and oak tree height. Significant correlations (P < 0.05) printed in bold.

	Distance to industry		Soil salinity		Oak tree height	
	r	*P*	*r*	*P*	*r*	*P*
Acrobasis consociella	0.4253	0.1005	**−0.5916**	**0.0158**	**0.5720**	**0.0206**
Ancylis apicella	**−0.7993**	**0.0002**	**0.6420**	**0.0073**	−0.4159	0.1091
Blastobasis glandulella	**0.6336**	**0.0084**	−0.3830	0.1430	0.3695	0.1590
Blastobasis phycidella	**0.6786**	**0.0039**	**−0.5536**	**0.0261**	0.2937	0.2695
Cacoecimorpha pronubana	**−0.6045**	**0.0131**	**0.5383**	**0.0315**	−0.4426	0.0860
Carcina quercana	**0.6529**	**0.0061**	**−0.5478**	**0.0280**	**0.5020**	**0.0475**
Carpatolechia aenigma	**0.8820**	**<0.0001**	**−0.6399**	**0.0076**	0.3183	0.2296
Cydia fagiglandana	**0.6020**	**0.0136**	−0.3927	0.1325	−0.3967	0.1282
Ematheudes punctella	**−0.8069**	**0.0002**	**0.5944**	**0.0152**	−0.1590	0.5564
Endotricha flammealis	**0.5634**	**0.0231**	−0.4544	0.0770	**0.5387**	**0.0313**
Epagoge grotiana	**0.5795**	**0.0186**	−0.4281	0.0980	0.4883	0.0550
Homaloxestis briantiella	−0.3361	0.2031	0.3826	0.1435	**−0.8624**	**<0.0001**
Mecyna asinalis	**−0.7050**	**0.0023**	**0.6082**	**0.0124**	−0.2052	0.4458
Monopis obviella	**0.6758**	**0.0041**	**−0.7418**	**0.0010**	0.4787	0.0607
Paradoxus osyridellus	**0.6735**	**0.0042**	**−0.6226**	**0.0100**	0.2981	0.2622
Parornix anglicella	**0.6918**	**0.0030**	**−0.7095**	**0.0021**	0.2838	0.2869
Pempelia palumbella	**0.6039**	**0.0132**	−0.4874	0.0555	0.3259	0.2180
Piniphila bifasciana	**0.6603**	**0.0054**	−0.3253	0.2190	0.1075	0.6920
Symmoca signatella	0.4889	0.0546	−0.3486	0.1857	**0.5160**	**0.0408**
Yponomeuta plumbella	−0.1848	0.4932	0.1914	0.4776	0.3947	0.1303

Table 2. Relationships between community attributes of 16 local micro-moth assemblages and the distance of sampling sites to industry and soil salinity. Given are Pearson correlation coefficients r and their *P*-values. Significant results (P < 0.05) printed in bold.

Community trait	Distance to Industry		Soil salinity	
	r	*P*	*r*	*P*
Catch size	0.4551	0.0765	−0.4225	0.1030
Rarefied species richness (100 individuals)	−0.0339	0.9009	0.2625	0.3259
Species diversity	−0.0039	0.9884	0.2049	0.4464
Functional diversity	**0.7005**	**0.0025**	−0.3316	0.2096
Functional richness	**0.5752**	**0.0197**	−0.3483	0.1861
Functional evenness	−0.2217	0.4092	0.2940	0.2690
Functional divergence	−0.3583	0.1730	0.4024	0.1223

(Table 1). Species diversity and richness were not measurably affected by soil salinity (Table 1). Functional diversity very weakly (though not significantly) declined at higher salinity (Fig. 4B), and also for functional richness, evenness and divergence no significant correlations with soil salinity were observed (Table 1).

Discussion

We established significant correlations between micro-moth communities and two environmental stress gradients acting from the landscape matrix within a small Mediterranean coastal forest reserve. Species richness and diversity were not significantly correlated to any of our investigated gradients but we observed clear shifts in community composition that could be traced down to abundance patterns of individual species. Even at this small spatial scale, forest habitat specialists – especially oak feeders – were more scarce in the southern part of the area, whereas a small number of micro-moth species characteristic for open habitats became increasingly abundant near industrial plants. Clearly, beneficiaries of habitat degradation were less numerous than species that concentrated towards the less impacted part of the reserve. These community changes at the level of herbivores and few detritivores reflect alterations in vegetation, with more dense oak forest sites persisting in the north of the reserve, whereas badly harmed, grassy sites with many dead trees prevail in the south (Uhl & Wölfling, 2015). From the perspective of the larval stages of micro-moths, which are predominantly quite host specific, habitat quality is essentially determined by vegetation (Summerville & Crist, 2003;
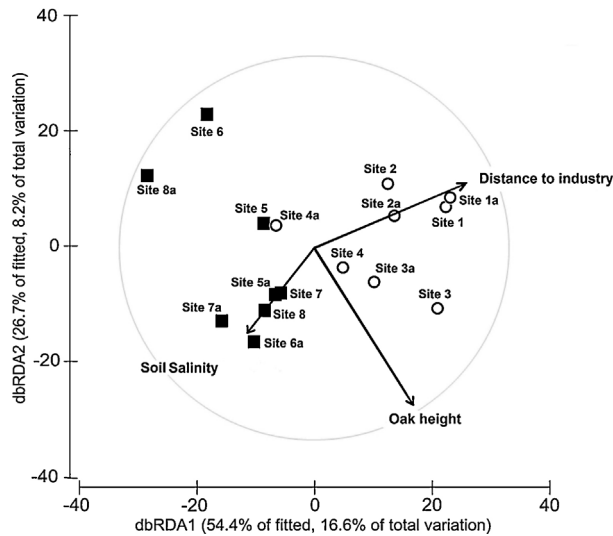
Fig. 2. Constrained ordination diagram (distance-based redundancy analysis, dbRDA) showing species composition of micro-moth assemblages at the 16 study sites. Black squares: sites in the southern half of PSV (close to industrial plants); empty circles: sites in the north of the park. Vector lengths indicate partial correlation coefficients of the predictors with the canonical axes. The three predictors collectively explain 30.6% of variance in species composition.
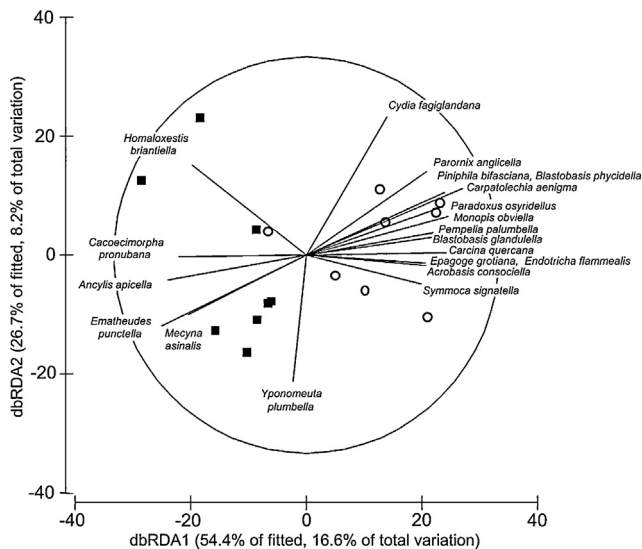

Fig. 3. Ordination diagram (as in Fig. 2) with species positions in reduced ordination space illustrated as vectors. Selected species had a Spearman correlation of >0.6 with one of the investigated environmental parameters.

Fig. 4. Functional diversity of 16 micro-moth assemblages in the reserve PSV relative to the distance of light-trap sites from the industrial plants (A) and soil salinity (B). Lines are ordinary least squares regressions. For statistical data see Table 2.

Pellissier et al., 2013;). Accordingly, changes in vegetation may be inferred as major drivers behind the observed insect community shifts, whereas it is less likely that many of these insects respond directly to air pollution.

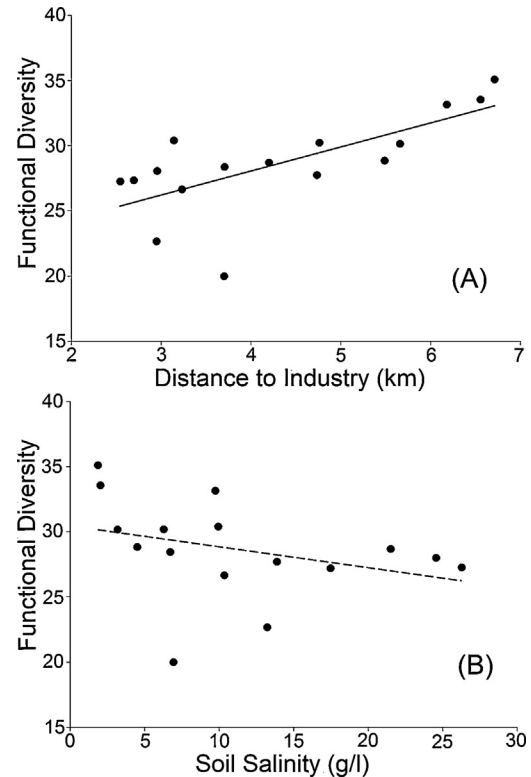Soil salinity effects on the micro-moth community were less pronounced, but also detectable on the species composition level. As with the proximity to potential sources of pollution, the abundance of most micro-moth species was negatively affected by soil salinity. Only four open habitat species, appearing mostly in the south of PSV, were positively associated with high soil salinity. One of them is *M. asinalis*, the larvae of which feed on *Crucianella maritima* and therefore are affiliated to coastal habitats with high salinity. For the other three species reasons for their positive associations with soil salinity remain unclear. Two of them are polyphagous and rather ubiquitous species in cultivated landscapes (*C. pronubana*, *A. apicella*), whereas the life history of *E. punctella* remains unknown.

In our analyses, differentiation diversity (sensu Jurasinski, Retzer, & Beierkuhnlein, 2009) turned out to sensitively reflect community changes along environmental gradients. With roughly 30% of variance explained in reduced ordination space, our modeling success was in the same range as frequently observed with arthropod community data (Blanchet, Legendre, Bergeron, & He, 2014). Indeed, we were able to trace compositional changes along small-scaled gradients down to the level of individual species and their life-history traits. Earlier studies on moth communities likewise established clear relationships with local environmental conditions (Summerville, Steichen, & Lewis, 2005; Babin-Fenske & Anand, 2011; Truxa & Fiedler, 2012b), even though moths as rather mobile organisms may be thought of being

less suitable to mirror small-scale gradients. More generally, species composition often reveals far more distinct relationships to putative drivers of biodiversity than measures of inventory diversity (Fiedler et al., 2007; Reiss, Bridle, Montoya, & Woodward, 2009). In line with such findings, we observed that neither species diversity nor species richness at the level of individual trap sites were associated with the two stress gradients under study.

In recent years, trait-based (rather than species-based) functional analyses have become promising tools to detect the impact of disturbance factors on ecosystems (Cadotte, Carscadden, & Mirotchnick, 2011; Mouillot, Graham, Villéger, Mason, & Bellwood, 2013). Until very recently (Gagic et al., 2015), most studies on functional diversity focused on plant communities (Diaz & Cabido, 2001; Valencia, Maestre, Bagousse-Pinguet, Le Quero, & Tamme, 2015), because far more trait information is available for vascular plants (Kleyer et al., 2008) than for most invertebrate animals. However, trait-based approaches also become increasingly important to analyze higher trophic layers or the linkages between plants, animals and ecosystem processes (Cadotte, Arnillas, Livingstone, & Yasui, 2015). Our results on micro-moth communities confirm the usefulness and sensitivity of trait-based approaches, even though the quality and amount of trait information for micro-moths is still moderate as compared to organisms with a longer history of autecological research. Most recently, Thorn et al. (2015) likewise observed that micro-moths may respond particularly sensitively to habitat conditions. As a cautionary note, our results were derived from surveys in just one study area. Tests across additional sites will be needed to assess the generality of our findings.

Trait selection is extremely important for the outcome of an analysis (Petchey & Gaston, 2006; Calba, Maris, & Devictor, 2014), yet it depends on the availability of reliable information and on the functional significance of traits. We were able to characterize many aspects of the functional niches of micro-moths by assembling a trait matrix that was mainly devoted to the larval fraction of the moths' life cycles. This is for sure useful considering the role of these insects in food webs. Moreover, we took care to avoid inclusion of traits with too sparse data and we accounted for inter-correlations between life-history dimensions. However, traits like lifespan, fecundity or range of active flight could not be used as they are unknown for most species. Future inclusion of such traits would obviously be important for a better understanding of the population and metapopulation dynamics of these moths and accordingly for modeling their long-term persistence in an isolated nature reserve (Slade et al., 2013).

Conclusions

Even though an analysis of Mediterranean micro-moth communities met challenges in terms of species identifications and the assembly of a functional trait matrix, our study revealed that these organisms are highly suitable to unravel environmentally induced changes in species composition and functional diversity. We attribute this to the high host plant specificity and strong habitat fidelity shown by many species of micro-moths. For the conservation status of PSV we conclude that active management of habitat quality should be adopted immediately to counter-act adverse effects imposed by environmental pollutants from outside the nature reserve. Such effects are already visible in terms of vegetation structure (Uhl & Wölfling 2015) and, as shown above, have repercussions on organisms at higher trophic levels. For example, local measures to reduce soil salinization or assisted regeneration of degraded vegetation units would likely contribute to restoring herbivore insect communities (Summerville et al., 2005) whose biodiversity is currently eroding under the negative influences that are swamping the reserve PSV.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.baae.2015.10.002.

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