*Article*

Macro-moth (*Lepidoptera*) Diversity of a Newly Shaped Ecological Corridor and the Surrounding Forest Area in the Western Italian Alps

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Abstract: In addition to the compilation of biodiversity inventories, checklists, especially if combined with abundance data, are important tools to understand species distribution, habitat use, and community composition over time. Their importance is even higher when ecological indicator taxa are considered, as in the case of moths. In this work, we investigated macro-moth diversity in a forest area (30 ha) in the Western Italian Alps, recently subjected to intense management activities. Indeed, an ecological corridor, which includes 10 clearings, has been shaped thanks to forest compensation related to the construction site of the Turin–Lyon High-Speed Railway. Here, we identified 17 patches (9 clearings and 8 forests), and we conducted moth surveys using UV–LED light traps. A total of 15,614 individuals belonging to 442 species were collected in 2020 and 2021. Two and fifteen species are new records for Piedmont and for Susa Valley, respectively. In addition to the faunistic interest of the data, this study—using a standardized method—provides geo-referenced occurrences, species-richness, and abundance values useful to compile a baseline dataset for future comparisons. Indeed, the replicable and easy shareable method allows us to make comparisons with other research and thus assess the impact of environmental changes.

Keywords: abundance; clearing; diversity indexes; geo-referenced occurrences; *Heterocera*; *Lepidoptera*; species richness; Susa valley

1. Introduction

Datasets of bioindicator taxa are increasingly requested to detect the consequences of land use and climate change [1,2]. Insects are often chosen and used as bioindicators due to their high species richness and diversity. Their biology and ecology are also relatively well-known, especially those of the most charismatic groups. Insects are ubiquitous in terrestrial habitats and their communities quickly respond to environmental variation via changes in species composition or relative abundance [3]. In this context, moths (*Lepidoptera*: *Heterocera*) are good ecological indicators thanks to the high sensitivity of many species to the environmental and bioclimatic conditions occurring in open and forest habitats [4,5]. They are severely affected by small environmental and climate changes causing demographic and/or behavioral modification, easily measurable and observed in the field [6]. Indeed, even little variations in temperature, micro-habitat, management regimes, and pollution might correspond to changes in moth communities [7–11], thus quickly revealing

the type and the direction of the ongoing ecological processes. In particular, since nocturnal *Lepidoptera* depend on plants during their larval stages, moth assemblages often reflect changes in vegetation composition and structure [9,10].

Forests with clearings—i.e., disturbed or fragmented forests—support several micro-habitats characterized by different biotic and abiotic conditions [12], consequently hosting rich invertebrate communities, especially *Lepidoptera* [13,14]. However, those exact habitats are threatened by natural reforestation and forest regrowth [15], which is related to recent agricultural and pastoral abandonment, especially on the Alps [16] Thus, reforestation is major threat not only because it reduces habitat heterogeneity and reduces clearings, but also because it might disrupt connectivity. Ecological corridors play a crucial role in connecting habitats and insects would benefit from this connectivity [14]. For *Lepidoptera*, it is proven that corridors promote mobility [17,18], and increase abundance and species richness [19].

Most of the studies evaluating the effects of environmental and climate changes on species have been focused on distribution only (using opportunistic data), even predicting future elevation shifts and occurrence patterns [20–22]. Abundance is a pivotal variable to understand habitat utilization, to estimate species' declines [23] and to predict future species' distribution, considering density effects [24]. For insects, the large abundance of individuals collected during each survey makes sample management and specimen identification challenging in terms of time and difficulties. Indeed, producing large datasets composed of species-per-site matrices is a laborious task due to difficulties in identifying species, especially for those with high intra-species variability [25]. To tackle these issues, some studies have used proxies for both species' richness and abundance. Biomass has been used as a proxy of abundance [26]. However, biomass does not provide information on species richness and can be problematic if there is a wide range of body sizes within the samples. Other studies used morphospecies as a proxy of species richness [27], but this leads to a strong underestimation of species richness, especially for some insect orders (*Hymenoptera*) [28]. A lack of species-level identification leads to losing ecological insights carried by species and underestimating the risk of extinction [29].

Within the order *Lepidoptera*, which accounts for approximately 180,000 species [30], about 90% are moths, belonging to 129 families [31]. According to [32], 2014 species are present in Italy. Within Italy, one of the most biodiverse sites is the Susa valley (Piedmont, NW, Italy), with several protected and/or endemic or evolutionarily significant unit (ESU) species [33–37]. The moth fauna of this area was largely explored in the past by several authors (e.g., [38], who scored 973 species of moths included in the *Bombicidae*, *Geometridae*, *Sphingidae* and *Noctuidae* families). However, as no record was georeferenced, these data can only be used as a faunal reference and not to assess the effects of land-use changes. Accurate assessment of faunal changes due to habitat modifications can only be carried out if available data are manageable with Geographic Information Systems, i.e., if they have geographic coordinates. The most informative datasets are those composed of presence data coupled with abundance data, in order to facilitate the detection of differences among communities [39].

In this work, we investigated moth diversity in a forest area of the Western Italian Alps, where intense management activities were carried out in recent years in order to improve habitat quality for several open-habitat species, including the protected butterfly *Zerynthia polyxena* (Annex IV, EU Habitats Directive 92/43/CEE), through the realization of an ecological corridor made of stepping-stones clearings [40]. These conservation actions provide the opportunity to study the community of nocturnal *Lepidoptera* in both the previously existing habitat (woodland) and the newly formed one (clearings). The aim is to provide a baseline presence/abundance moth database for the study area, together with basic ecological information concerning the most abundant species.

2. Materials and Methods

2.1. Study Area

We carried out this research in Susa Valley, in the Western Italian Alps (Piedmont). In detail, the study site was located in the Clarea valley (45°08′07.7″ N 6°59′37.8″ E), between 765 m and 983 m a.s.l., in the municipality of Giaglione (TO). The study area is characterized by natural and semi-natural forests dominated by abandoned traditional chestnut (*Castanea sativa* Miller, 1768) orchards, together with a widespread occurrence of oaks (*Quercus* L.) and cherry trees (*Prunus avium* Linnaeus, 1753). In spring 2020, in order to reverse biodiversity loss due to the expansion of an industrial site part of the Turin–Lyon high-speed railway megaproject [40], this continuous forest habitat has been interrupted by the creation of a 500 m ecological corridor. The corridor (overall 10 ha) is made up of a sequence of ten stepping-stones clearings, where all trees were cut down, leaving in place all the woody material as deadwood. After cutting, clearings were left unmanaged, except for the sowing of local herbaceous plant species.

Specifically, the study was carried out in seventeen small patches: nine clearings belonging to the ecological corridor and eight forest patches (Figure 1). The topography and features of clearing and forest patches are described in Table 1.
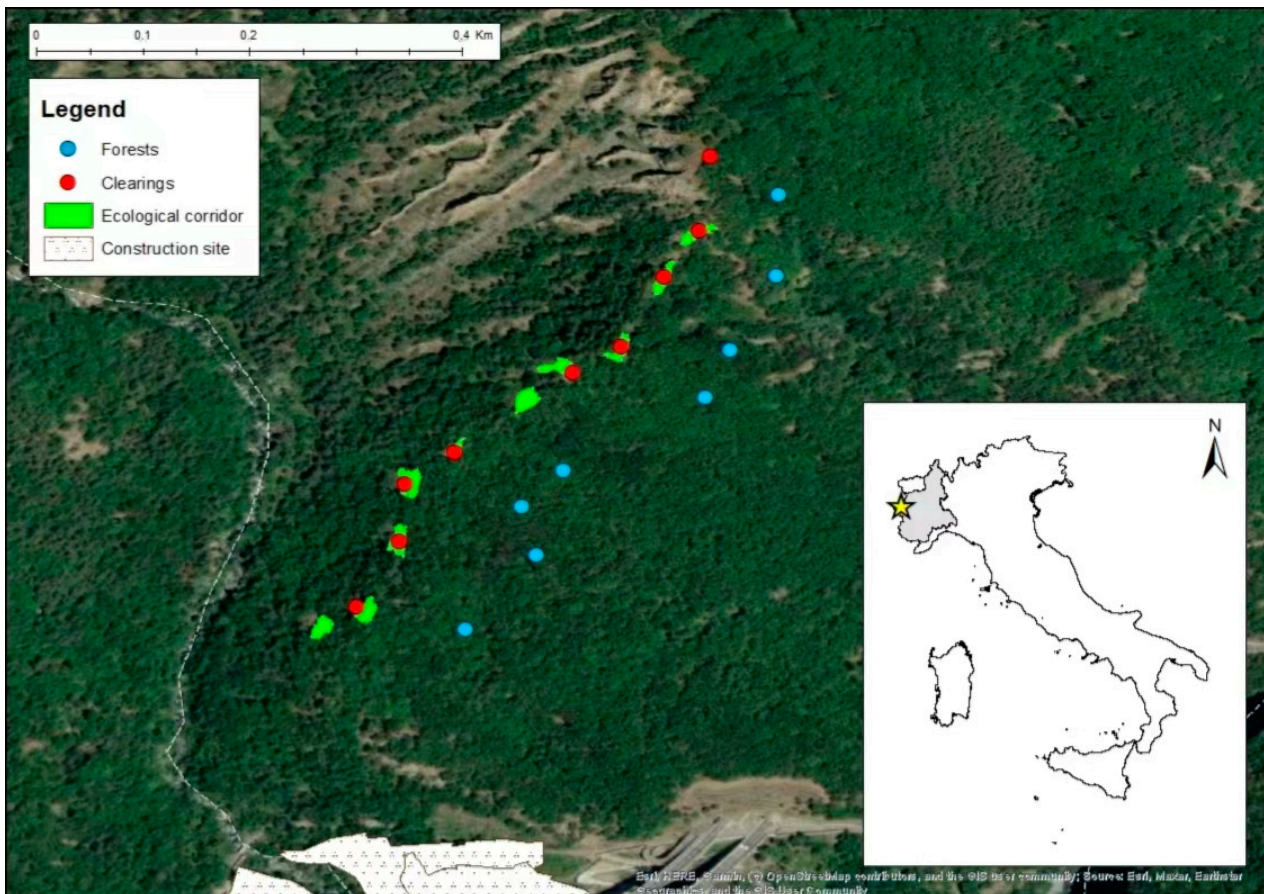

Figure 1. Map of the study area with the sample clearing patches highlighted in pale green. The position of the UV–LED light traps is reported in red for clearings and in blue for the forested patches. In gray, the construction site of the Turin–Lyon High-Speed Railway.

Table 1. Environmental and topographic characterization of the clearings and forest patches where moths were sampled in the study area. In the forests, we did not identify any clearing area.

Patches	Elevation m a.s.l.	Slope (°)	Clearing Area (m^2)	Canopy Cover (%)	Longitude	Latitude
Clearing 2	765.50	32.69	250.81	54.31	6.99101645	45.1326729
Clearing 3	769.30	31.07	268.97	48.85	6.9913708	45.1332286
Clearing 4	770.01	40.52	324.49	53.04	6.99142112	45.1337146
Clearing 5	796.38	33.91	108.05	59.15	6.99184302	45.1339795
Clearing 7	852.24	39.51	240.88	51.68	6.99283393	45.134654
Clearing 8	879.66	25.90	215.31	62.85	6.99324809	45.1348751
Clearing 9	914.83	29.37	235.95	60.96	6.99361194	45.1354649
Clearing 10	944.25	34.92	218.41	44.69	6.99390611	45.1358527
Clearing 11	982.85	12.60	670.03	31.01	6.99400307	45.1364854
Forest 2	764.71	21.66		82.8	6.99193442	45.1324905
Forest 3	782.41	19.32		74.34	6.99253588	45.1331139
Forest 4	794.26	23.67		76.94	6.99241202	45.1335262
Forest 5	816.85	27.52		82.53	6.99276425	45.1338266
Forest 7	850.16	27.57		88.64	6.99396418	45.1344437
Forest 8	873.66	29.16		86.07	6.99416932	45.1348506
Forest 9	909.14	39.27		83.07	6.99455639	45.1354704

2.2. Data Collection

Moths were sampled monthly using UV–LED light traps (emission peak 398 nm light angle per LED 120°; EPIS- TAR Corporation, Hsinchu, Taiwan; [41,42], in two consecutive years (2020 and 2021), between May and October. Light traps were designed following [43] and modified to accommodate the UV LEDs, see [41] for further information (see a picture of the trap in Figure S1 in Supplementary Material S2). Ethyl acetate was used as the killing agent [41], and all individuals in the traps were killed. In 2020, we sampled moths in nine clearings and eight forests; the monthly surveys covered four consecutive nights. Every night, the traps were activated in two clearings and two adjacent forests. The average distance between clearings and forests was 78 m, considering the attractiveness of these traps (about 25 m [44] and literature therein); there was no interference between traps. At the fourth night, a trap was also activated in one additional clearing. Differently, in 2021, we sampled moths in nine clearings only; the monthly surveys spanned two consecutive nights, with four and five traps per night, respectively.

Sampling was carried out between 9 p.m. and 6 a.m., activating the traps manually [41] on favorable nights for moth activity (i.e., low wind intensity, no full-moon interference; following [10]), avoiding nights with unstable weather in order to ensure constant meteorological conditions between consecutive nights. We do not have expertise on micro-moths; thus, we identified macro-moths (hereafter "moths") belonging to 12 families *(Hepialidae, Cossidae, Limacodidae, Saturniidae, Sphingidae, Lasiocampidae, Drepanidae, Geometridae, Notodontidae, Noctuidae, Erebidae* and *Nolidae*). Species identification was performed in the laboratory according to the available literature, and additionally by dissecting genitalia for cryptic taxonomic groups. Nomenclature follows [45]. Voucher specimens are preserved in the collections of the Department of Life Sciences and Systems Biology at the University of Turin (Turin, Italy) and of the Italian Research Centre for Forestry and Wood (Rende, Italy).

Topographic and Cover Variable Estimation

In order to provide an environmental description of the sampling patches (clearings and forests), the canopy cover was measured by taking hemispherical photographs oriented to the zenith with an EOS 350D camera (CANON (Tokyo, Japan) equipped with a LENSBABY (Portland, OR, USA) Circular Fisheye (5.8 mm–f/3.5) at the center of each patch. Hemispherical photographs were processed with a Gap Light Analyzer (GLA, Cary Institute of Ecosystem Studies, Millbrook, New York, NY, USA [46]) to obtain the tree cover for the total panorama ("Canopy cover", following [47]). Average slope and elevation values were extracted as topographic parameters for each patch using a digital elevation model (10-m resolution) of the Piedmont Region (Regione Piemonte, 2008. Digital terrain models from CTRN: 10 000). The clearing area per each patch was calculated in the field using a GPS (Garmin® (Olathe, KS, USA) eTrex 20 with a precision of ±3 m).

2.3. Data Analysis

For each sampling patch, species richness (S), abundance (N) and diversity indices (Shannon H', Simpson, Eveness E', Fisher's alpha) were calculated. Diversity indices were obtained using the 'vegan' package in the R software (v.3.2.1).

In addition, the Pearson's correlation coefficient was calculated between moth community parameters (species richness, abundance and diversity indexes, averaged between years for clearings), topographic and canopy variables, using the 'corrplot' package in R. Then, a Kruskal–Wallis non-parametric test was run in R (R v.3.2.1 Development Core Team, 2021) in order to verify whether environmental parameters would explain variations in moth community composition and diversity.

3. Results

Clearings were characterized by an average elevation of 852.78 ± 82.52 m a..s.l, an average slope of 31.16 ± 8.34, and average canopy cover of 51.83% ± 9.74, while forest patches were characterized by an average elevation of 842.76 ± 65.33, an average slope of 27.61 ± 6.37 and average canopy cover of 82.34 ± 4.65.

Overall, 15,614 individuals belonging to 442 species were collected. In more detail, we collected 11,066 specimens belonging to 399 species in 2020, and 4548 individuals belonging to 300 species in 2021 (Table S1; for the complete checklist see Supplementary Material S2). The most common species in the whole study area in 2020 were *Agrotis trux* (*Noctuidae*), with 833 individuals (about 8% of the total abundance in 2020), *Paracolax tristalis* (*Erebidae*), with 625 individuals (about 6%), and *Lithosia quadra* (*Erebidae*), with 440 individuals (about 4%). In 2021, *Agrotis trux* (*Noctuidae*), with 322 individuals (about 7% of total abundance in 2021), *Thaumetopoea pityocampa* (*Notodontidae*) with 323 individuals (about 5%), and *Aplocera plagiata* (*Geometridae*), with 208 individuals (about 6%), were the most abundant taxa in the clearing patches (Figure 2).
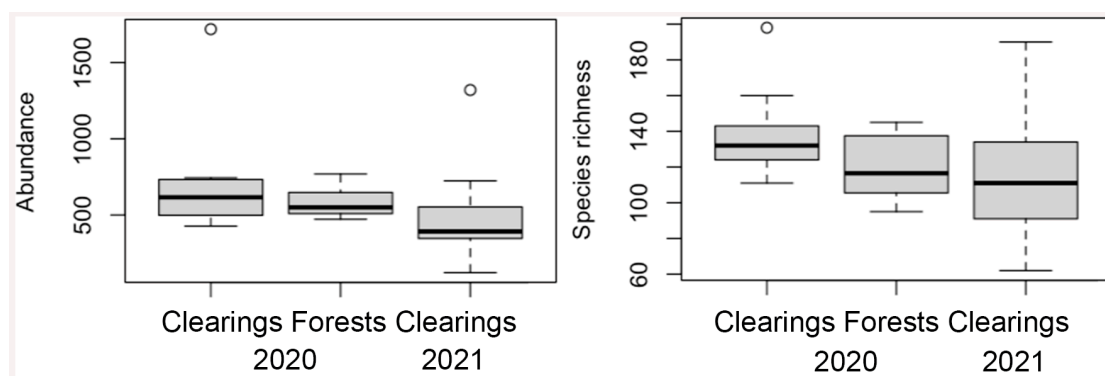

Figure 2. Moth species richness and abundance observed in clearings and forests in 2020 and 2021.

In comparison, the moth community in clearings was composed of 335 species (6462 individuals) in 2020, while 301 species (4.655 individuals) were found in the forest patches in the same year (Table S1 in Supplementary Material S2). In 2020, the most abundant species in clearings were *Agrotis trux* (*Noctuidae*), with 555 individuals, *Lithosia quadra* (*Erebidae*), with 339 individuals, and *Thaumetopoea pityocampa* (*Notodontidae*), with 325 individuals. Conversely, in the same year, the most abundant species in forest patches were *Paracolax tristalis* (*Erebidae*), with 459 individuals, *Agrotis trux* (*Noctuidae*), with 278 individuals, and *Eilema lurideola* (*Erebidae*), with 219 individuals (Figure 2).

No significant correlations between moth community parameters (species richness, abundance and diversity indexes) and environmental variables (topography and canopy cover) were found (Figure S2 in Supplementary Material S2). Similarly, the Wilcoxon test did not detect significant relationships between moth community parameters and environmental variables (Table S2 in Supplementary Material S2).

4. Discussion

To date, the available knowledge on nocturnal *Lepidoptera* in the investigated site of the Western Italian Alps is mainly provided by the work of [38], who report 973 species of macro-moths occurring in the Susa Valley. Despite the focus on nocturnal sampling only, the small area surveyed, the relative homogeneity of habitats, and the short duration of the sampling period (only two years, excluding November to April), the 442 species observed in this study correspond to the 45.4% of the species richness reported by [38], disclosing an unexpected diversity in the study area for this insect group.

Particularly important is the collection of some species never observed before in Piedmont and/or new to the Susa Valley, together with the confirmation of the occurrence of other taxa unreported since 1852:

- *Eupithecia gueneata* (Millière, 1862) and *Eupithecia spissilineata* (Metzner, 1846) are new for the moth fauna of Piedmont. *E. gueneata* (Millière, 1862) is a Western-Palaearctic species, inhabiting warm and dry habitats where its caterpillar feeds on *Pimpinella saxifrage* L. [48], known in Southern and Eastern Europe, with only isolated data reported for Italy. In Piedmont, recent unpublished observations report its occurrence not far from the study area, in the Alpi Cozie Natural Park (Massimo Rosso pers. comm.). Conversely, *E. spissilineata* is an Eastern-Mediterranean species, inhabiting rocky habitats with an unknown larval biology [47], with the westernmost populations being known in Southeastern France. Since, in Italy, this species has only been observed in Latium, Abruzzo, and doubtfully in Tuscany [32], the data reported in this paper significantly extends its Italian range northwards.
- *Caradrina (Platyperigea) aspersa* (Rambur, 1834) is confirmed in Piedmont after [49], as reported in [50].
- *Synopsia sociaria* (Hübner, 1799), *Colostygia pectinataria* (Knoch, 1781), *Epirrita christyi* (Allen, 1906), *Hydriomena impluviata* (Denis and Schiffermüller, 1775), *Perizoma blandiata* (Denis and Schiffermüller, 1775), *Charissa (Costignophos) pullata* (Denis and Schiffermüller, 1775), *Scopula (Scopula) submutata* (Treitschke, 1828), *Cucullia (Shargacucullia) lychnitis* (Rambur, 1833), *Cucullia (Shargacucullia) prenanthis* (Boisduval, 1840), *Apamea aquila* (Donzel, 1837), *Hoplodrina alsinides* (Constantini, 1922), *Mythimna (Anapoma) riparia* (Rambur, 1829), *Noctua interposita* (Hübner, 1790), *Grammodes stolida* (Fabricius, 1775), and *Nola confusalis* (Herrich-Schäffer, 1847) are new to the moth fauna of Susa Valley, according to [38];

  Cleoceris scoriacea (Esper, 1789) is confirmed in the Susa Valley after Ghiliani (1852).

We also collected 16 *Euplagia quadripunctaria* individuals, a protected species listed in the Habitats Directive (92/43/CEE). This species is widespread and common in Italy.

There were not significant differences between abundance, species richness and indexes between clearings and forests (Table S2 in Supplementary Materials S2). However, irrespective of their limited extent, clearings proved to host a higher moth diversity than the surrounding forest patches, in terms of species richness, abundance, and diversity in-

dices. Furthermore, the communities between clearings and forests were different, hosting different species. Indeed, *Agrotis trux*, known to prefer open areas, was the most abundant species in clearings. Similarly, *Thaumetopoea pityocampa*, the third most abundant species in clearings, is a heliophile species; indeed, its nests are found on forest edges or on isolated pines (*Pinus* L.) [51]. The second most abundant species *Lithosia quadra* is a vagile species, more frequently found in open areas (Scalercio pers. obs.). Conversely, the most abundant species found in forests were *Paracolax tristalis* and *Eilema lurideola*, linked to litter and lichens, respectively. Thus, a mosaic within a forest dominated by chestnut trees supports a diverse community of moths. Chestnut stands are recognized as an important and protected habitat (habitat 9260; listed in Annex I in the Habitats Directive 92/43/CEE). However, few faunal studies have been focused on chestnut forests [52,53]. Some studies have focused on moths in chestnut forests and surroundings [10,54,55]. From those results, it is proven that a mosaic-like landscape composed of mature and early managed chestnut forests—as is our study area—supports a diversified moth community. Indeed, our study area is composed of chestnut coppices abandoned for 40 years and newly formed clearings.

The topographic and canopy variables were similar among clearing and forest patches. Indeed, we did not find any significant correlation between those variables. This is in line with the fact that the investigated area was small (30 ha), considering the potential mobility capabilities of moths and the close proximity between patches (max linear distance around 500 m). However, vegetational variables can explain even small differences between clearings and forests (Piccini pers. obs.).

5. Conclusions

In conclusion, most of the available checklists present only opportunistic data (e.g., [56]); conversely, we also included georeferenced and abundance data. Specifically, abundance is relevant for understanding possible future decline [23], the utilization of habitats and estimating ecosystem functioning [57]. This study provides a useful baseline dataset on moth diversity concerning a forest area where deep management interventions were recently carried out. Moreover, the availability of geo-referenced occurrences, species richness, and abundance data, collected with a standardized sampling method, provides the opportunity for future comparisons in the same area. In addition, the checklist provided here is also of particular interest from a faunistic point of view due to the high number of new records for the territory under investigation, confirming the need for more faunistic research to deeply investigate and preserve local biodiversity (particularly true for micro-moths).

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