Article

The Contribution of Cultivated and Semi-Natural Patches to the Beta Diversity of Nocturnal Lepidoptera within an Organic Century-Old Olive Grove in a Fragmented Landscape

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Abstract: The simplification of agricultural landscapes that has occurred in recent decades has led to a consequent decline in biodiversity. The aim of this study was to investigate the role of cultivated and semi-natural areas to sustain the beta diversity of nocturnal Lepidoptera. A dataset available for an organic century-old olive farm was analysed. Sampling was carried out from July 2018 to June 2019 within eight sites. The beta diversity was explored using the Bray–Curtis quantitative similarity index. To assess the main process driving community composition, two measures of nestedness were calculated. The analyses showed that most species were more abundant in semi-natural sites, although some species were found to be barycentric or exclusive to the olive groves. The nestedness at farm and site scales confirmed that the main process driving the composition of the moth community was species impoverishment, but this process was not found to be significant when comparisons involved herbaceous semi-natural habitats and cultivated sites. We found out that the contribution of olive groves to the farm beta diversity was small but non-negligible. To improve the sustainable management of an olive grove, it is preferable to promote the presence of tree-covered areas since semi-natural herbaceous cover can be represented within the farm by low-input olive groves.

Keywords: *Olea europaea*; sustainability; biodiversity; pollination; Lepidoptera; Italy

1. Introduction

In the last century, the promotion of monocultures aimed at maximising yields has led to a simplification of agricultural landscapes, with semi-natural habitats reduced to small patches imbedded within an anthropogenic matrix. As a result, local and regional biodiversity decreased and generalist species increased—becoming remaining biodiversity ineffective as a promoter of ecosystem homeostasis [1]. Then, these unbalanced artificial ecosystems became incapable of regulating their own functionality, requiring frequent human intervention [2]. To recover many of the ecosystem services provided by the wild biodiversity, such as pollination, the agricultural policies of many countries invested resources to promote habitat diversification by inserting new semi-natural habitats and preserving those already present in farms [3,4].

Landscape heterogeneity positively affects ecological systems, increasing the abundance and diversity of vascular plants [5], insects [6,7], spiders [8,9], birds [10], and mammals [11]. Grasslands, pastures, woodlands, and hedgerows are foraging and resting areas for beneficial biota, providing nectar sources for pollinators, prey for predators, and hosts for parasitoids, all of which are very scarce within disturbed habitats [12,13]. Furthermore, these semi-natural landscape elements can act as corridors or stepping stones, favouring the permeability of hostile environments [14–17].

Species abundance and/or richness, across a wide range of taxa, tend to be higher in organic than in conventional farms [18]. Organic farming also has an effect on plant

abundance and diversity, moving up the food chain to increase animal abundance at higher trophic levels [19]. However, some studies suggest that the positive effects of organic farming are low compared to the positive effects of high landscape diversity and high proportions of semi-natural areas [20].

The operational unit in agroecosystems is the farm, where management strategies become practical actions. As a consequence, to study the contribution of semi-habitats at the farm scale, it is fundamental to improve and maximise the provision of ecosystem services, promoting the best practices that facilitate the presence of desired biodiversity components [21].

A diversified mosaic of habitats supports a rich beta diversity that contributes to a functional ecosystem. Beta diversity, generally defined as a measure of variability in community composition among sites [22], is used as a key concept to understand how local community assembly (alpha diversity) is linked to the regional species pool (gamma diversity) [23]. It is subdivided into two different antithetical components: turnover and nestedness. Turnover means species replacement, whereas nestedness means species impoverishment. Fragmentation, habitat loss, and degradation increase the importance of nestedness in determining the beta diversity because of the loss of the most vulnerable species in a habitat.

One of the most iconic crops in the Mediterranean basin is the olive, largely diffused from Spain to the Middle East. In southern Italy, olive crops are widespread, and the numbers of organic orchards are increasing due to the favourable climate. In some regions, particular attention is devoted to the cultural and ecological role of century-old olive groves [24], being part of the local history [25,26]. In the past, due to the high adaptability of this plant, in southern Italy, olive groves were primarily planted in hilly areas, whereas natural vegetation was preserved in particularly unfavourable topographic conditions that hamper cultivation. Therefore, in these regions, olive landscapes are usually heterogeneous both at small and large scales, especially in old orchards.

The aim of this paper was to evaluate the relative contribution of cultivated and semi-natural lands to maintain diversity and abundance of a pollinator taxon at farm scale within olive groves, a key agroecosystem in the Mediterranean Basin. The studied pollinator group was the nocturnal Lepidoptera, also called moths, considered one of the major pollinator groups, along with Apoidea, diurnal Lepidoptera, and Diptera Syrphidae [27]. Moths are particularly helpful to investigate beta diversity at a farm scale, as their community changes significantly within a very little space [14], they are useful bioindicators for grasslands and forests [28–30], and automatic operator-unbiased sampling methods are available for rigorous sample comparisons [31,32]. Furthermore, they proved to be good bioindicators in agroecosystems [3,33]. The dataset collected by Sabatino et al. [34] within a century-old olive grove in the hilly territory of the Calabria region, South Italy, is available for this purpose.

2. Materials and Methods

2.1. Study Area

This study was carried out in an olive grove situated in the municipality of Marcellinara, Calabria region, South Italy (38°55′08.4″ N 16°30′05.3″ E), at an altitude of about 200 m (Figure 1).

The climate is typically Mediterranean, with clear differences between seasons. Temperatures generally range from 20 °C to 29 °C in the summer, and from 5 °C to 11 °C in the winter. Yearly mean precipitation ranges from 900 to 1000 mm and precipitation is mainly concentrated in late autumn and winter, with a long dry period from early spring to late summer. Geological substratum is composed by Miocene calcareous sediments including sandstones, clays, and silts, locally with sandy intercalations and gypsum outcrops. Erosion resistance and soil permeability change according to the substratum (Carta Geologica della Calabria, foglio 242 IV-S.O., 1:25,000). Cultivated surfaces have an average SSW inclination of 5 degrees, consisting of some semi-natural areas which are much steeper.
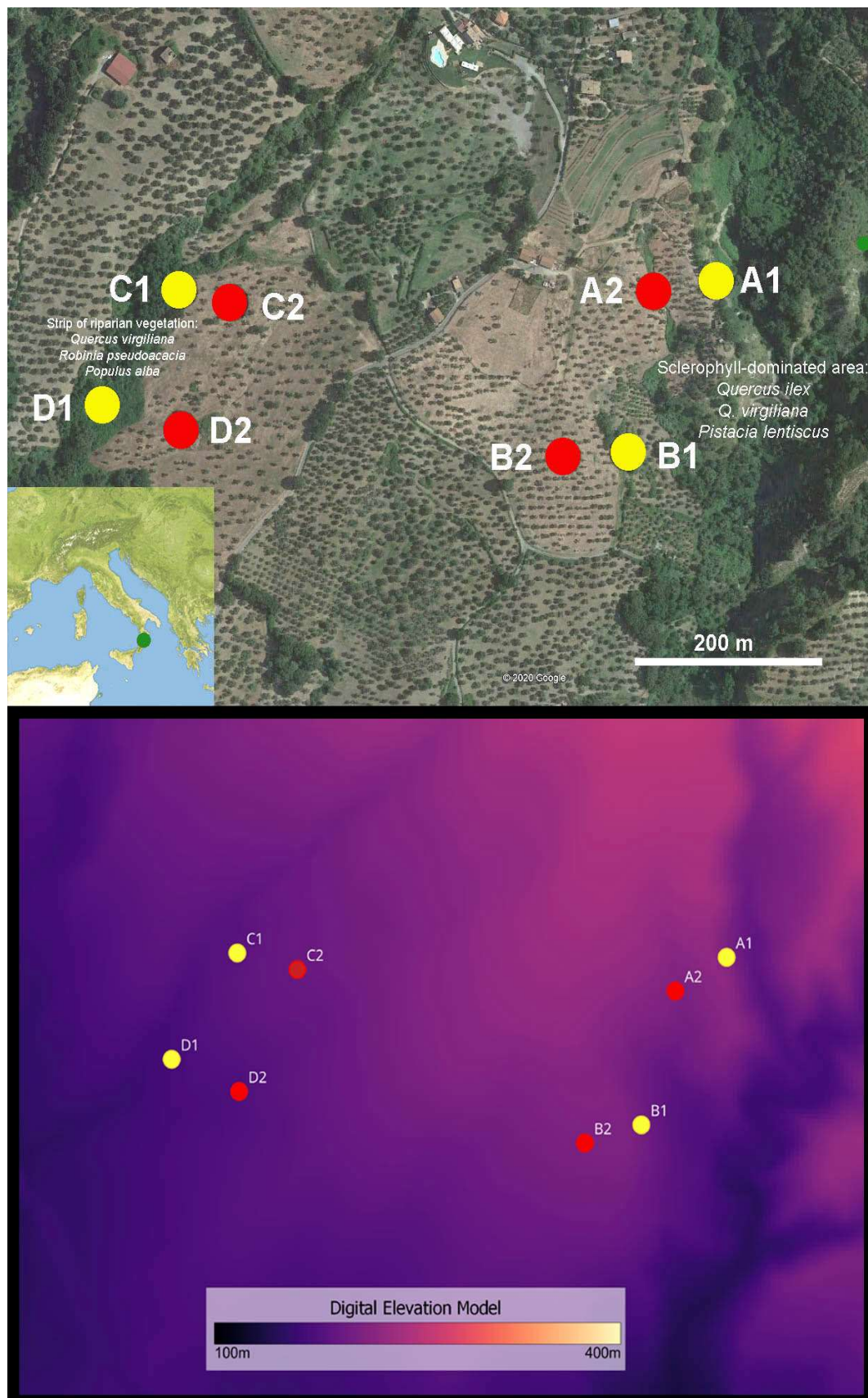
Figure 1. Localisation (green dot) and digital elevation model of study area. Red dots: olive grove light traps; yellow dots: semi-natural light traps (from Sabatino et al., 2021 [34], modified).

The studied farm is certified as organic from 2018, after a five-year process of conversion during which only copper against fungal diseases and boron to stimulate flowerings were applied. The olive grove is more than 100 years old, and has an irregular planting system with plants set at about 10 m from each other. The cover crop is controlled by mowing in early spring to prevent competition for water and nutrients with olive trees, and in early summer, as a defence against uncontrolled burnings. As a result, the soil of the olive groves is partially bare and mainly covered by thermophilous and xerophilous ruderal plants, including grasses belonging to the genera *Briza*, *Avena*, and *Cynodon*, and other plants such as *Echium* and *Dianthus*. This floristic assemblage changes according to soil moisture.

The farm can be subdivided in two areas, the first at a slightly higher altitude which is warmer and dryer, and the second at a slightly lower altitude, which is cooler and wetter due to thermic inversion. The first area is situated at an altitude of 200 metres and the olive grove is delimited by a large escarpment, with the soil partially covered by a small coppice of *Quercus virgiliana* (Ten.) Ten. and a degraded Mediterranean maquis composed of *Quercus virgiliana*, *Q. ilex* L., *Robinia pseudoacacia* L., *Pistacia lentiscus* L., *Cistus* sp.pl., and sporadic *Spartium junceum* L., *Calicotome spinosa* (L.) Link, *Smilax aspera* L., and *Asparagus albus* L. This area is a part of the 3 km long valley of the Riato stream with large portion of semi-natural habitats. The second area is bordered by a small stream with water present all year, in which hygrophilous vegetation develops. A narrow strip of trees (*Quercus virgiliana*, *Robinia pseudoacacia*, and *Populus alba* L.) and shrubs (*Sambucus nigra* L., *Ficus carica* L., *Rubus* sp., and *Clematis vitalba* L.), surrounded by cultivated areas, partially cover the stream. Where trees and shrubs are absent, the vegetation is dominated by *Pteridium aquilinum* (L.) Kuhn.

Study sites covered the main habitat types present in the farm. A total of eight sampling sites were set up, four within the warmer area (sites A1, A2, B1, B2), and four within the cooler area (sites C1, C2, D1, D2). Four sites were set up within semi-natural habitats (sites 1) and four within olive groves (sites 2). They have been arranged into four pairs (A, B, C, D) consisting of a semi-natural site and the contiguous olive grove at about 50 m.

2.2. Moth Dataset

The dataset used [35] was gathered from Sabatino et al. [34], making only a few taxonomic changes according to Prozorov et al. [36] and Govi et al. [37]. Sampling was carried out by setting up one trap per site using UV LED light traps (emission peak 398 nm light angle per LED 120°; EPISTAR Corporation, Taiwan). They are similar to Heath traps, modified to accommodate a 2.5 m long strip, with a total of 150 LEDs (~15 W), wrapped around and glued to a PVC tube and placed above the collection funnel [32] (Figure 2). Traps were simultaneously activated one night per month, from July 2018 to June 2019, during weather conditions favourable to moth activities and trap efficiency, i.e., with a temperature no lower than the mean of the period, no strong wind, no heavy rainfall, and around the new moon phase. Traps were turned on before sunset and turned off the morning after. For more details see Sabatino et al. [34].

The dataset is composed of 7569 specimens belonging to 332 species. The matrix was filled with abundance data (number of individuals) and submitted for statistical analyses.

Figure 2. Used UV LED light trap positioned within the olive grove.

2.3. Statistical Analysis

Distribution of species richness within the farm was first explored by using the mean of Venn diagrams to show the richness observed in sampled sites, the portion of richness exclusive to semi-natural and cultivated sites paired together, and the abundance of the species therein. Then, to explore the beta diversity of sampled moth communities, we carried out a Cluster Analysis running PAST4.03 [38], using the quantitative Bray–Curtis similarity index which quantifies differences in species composition and abundance between sites [39]. Quantitative indices are often more satisfactory than presence/absence-based indices because they provide more detailed information by considering the individual abundances of species, i.e., the relative dominance of a particular species in a community [40], but they need well-standardised monitoring methods. The index ranges between 1 (samples are identical) and 0 (no shared species). The cophenetic correlation coefficient, a measure of how reliably a dendrogram preserves the pairwise distances between samples [41], was also computed.

As a last step, in order to ascertain if observed differences in species composition is shaped or not shaped by nestedness, a presence–absence matrix was analysed by running the software Nestedness for Dummies (NeD) [42]. In detail, we computed the nestedness measure based on overlap and decreasing fills (NODF) and the matrix temperature (MT) [43,44]. We used the proportional row and column totals as matrix rearrangement rule. In addition to their values, for both statistics we also computed the Z-score, obtained from the value of nestedness for the actual matrix minus the average nestedness of the random replicates (1000) divided by the standard deviation of the replicates [45,46]. Statistics were computed at farm and paired site scales.

3. Results

The moth community of the study area is dominated by the families Noctuidae, Geometridae, and Erebidae representing 96.7% of the entire community (Table 1). Among the species, the most abundant were *Eilema caniola* (N = 455), *Peribatodes rhomboidaria* (N = 452), and *Dysauxes famula* (N = 391), representing 17% of the sampled individuals. To reach the 50% mark of the whole sample, 23 more species are necessary, demonstrating the absence of few very abundant taxa.

Table 1. Number of species and individuals collected for any family of Lepidoptera included in this study.

Family	Species	Individuals
Noctuidae	135	2727
Geometridae	108	2719
Erebidae	52	1871
Nolidae	12	140
Notodontidae	8	25
Lasiocampidae	5	8
Cossidae	2	2
Sphingidae	1	8
Limacodidae	1	6
Euteliidae	1	5
Brahmaeidae	1	1
Saturnidae	1	1

Richness comprised between 131 and 258 in the natural habitats, and between 80 and 143 in the olive groves. The percentage of species shared by semi-natural habitats and olive groves was higher for the pairs A and B, respectively, 42.7 and 41.3%, than for the pairs C and D, respectively, 29.3 and 34.8%.

In all site-pairs, the number of species shared by semi-natural habitats and olive groves was lower than those exclusively found in the former, and higher than those exclusively found in the latter. However, the absence of tree cover in the semi-natural site D1 strongly reduced such differences, which are very low from a quantitative viewpoint (Figure 3).

The number of individuals belonging to species exclusively found in olive groves was lower in percentage than the number of species within all pairs, except for the pair D where the abundance of species exclusively found in the olive site was higher than those of shared species (Figure 3).

Most species were more abundant within semi-natural sites. However, among those collected species with at least five individuals, five species had more than the 70% of their individuals within the olive grove, namely *Tyta luctuosa*, *Agrotis lata*, *Acontia trabealis*, *Heliothis peltigera* (Noctuidae), and *Apochima flabellaria* (Geometridae). One more species, *Sesamia nonagrioides* (Noctuidae) was exclusively found in the olive grove (Table 2). Fourteen more collected species with less than five individuals were exclusively found within olive sites [35].
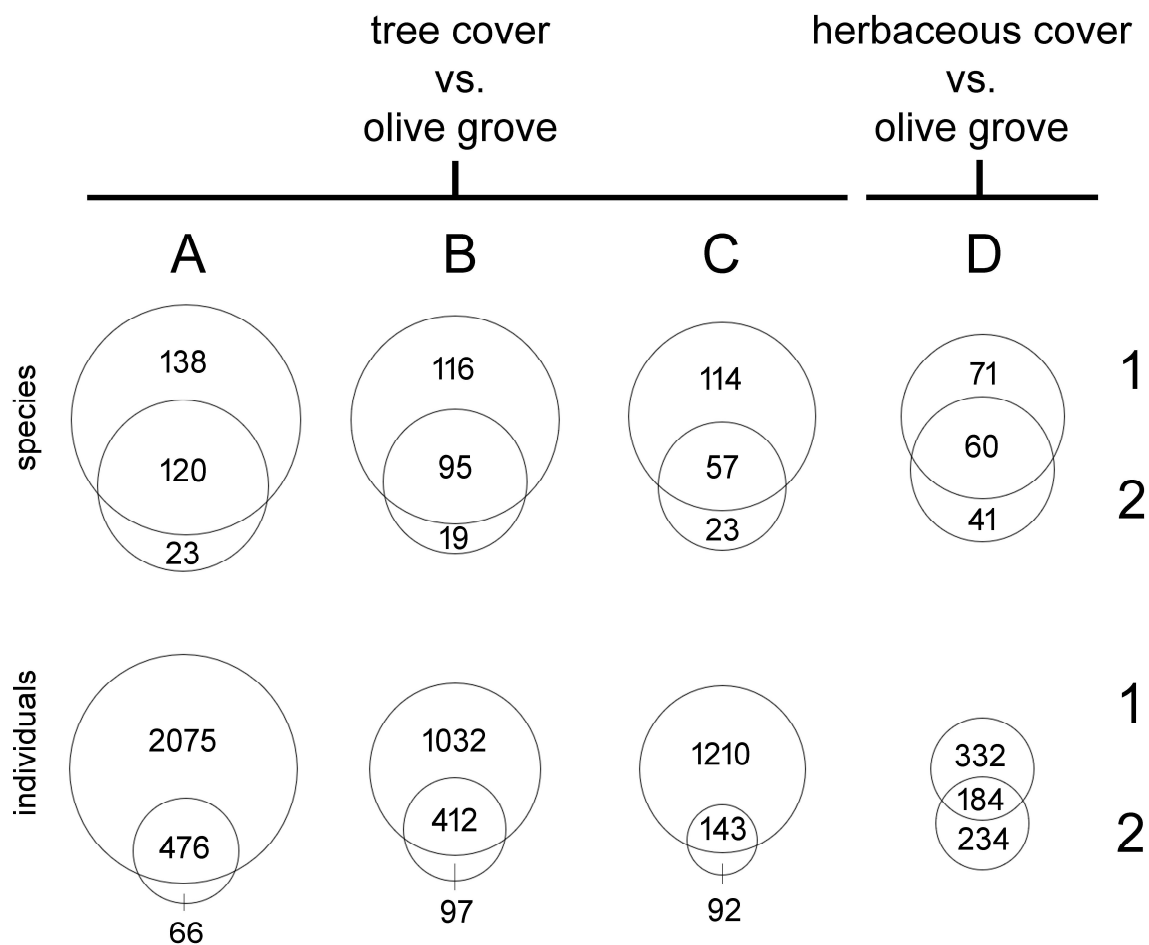
Figure 3. Graphical representation of the relative richness and abundance of moth communities within sampled pairs of semi-natural habitats (number 1) and contiguous olive groves (number 2). Circle areas are proportioned to the number of species and individuals.

Table 2. Total abundance of olive grove barycentric species collected with more than five individuals.

	Individuals	Semi-Natural	Olive Grove
	(N)	**(%)**	**(%)**
Acontia trabealis (Scopoli, 1763)	180	17.2	82.8
Tyta luctuosa (Denis & Schiffermüller, 1775)	91	26.4	73.6
Apochima flabellaria (Heeger, 1838)	27	29.6	70.4
Agrotis lata (Treitschke, 1835)	13	15.4	84.6
Heliothis peltigera (Denis & Schiffermüller, 1775)	13	15.4	84.6
Sesamia nonagrioides (Lefèbvre, 1827)	5	0	100

Cluster Analysis (cophenetic correlation coefficient: 0.8416) showed that moth communities of olive groves clearly differentiated from those of semi-natural habitats, being the node of these two clusters at very low similarity value (Figure 4).

The site pairs located in the dryer area (A and B sites) were more similar than those located in the wetter area (site C and D), and this occurred for both olive groves and semi-natural habitats with Bray–Curtis similarity values of around 60%. The branch of the site D1, covered by herbs, originated at the base of the cluster composed of semi-natural habitats, separating D1 from the other sites covered by trees.
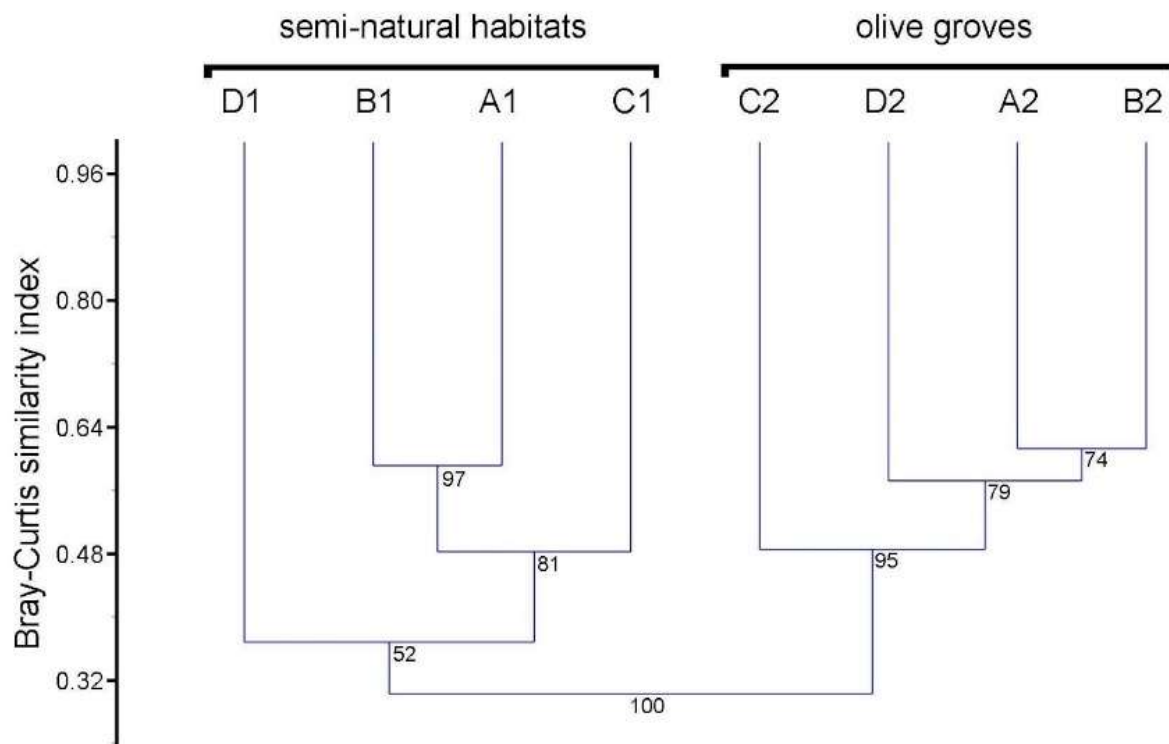
Figure 4. Results of the Cluster Analysis (algorithm: paired groups; similarity index: Bray–Curtis; 1000 bootstraps) carried out on the moth samples collected in the eight study sites. The percentage of node robustness is reported.

The computation of nestedness at farm and paired-site scales confirmed that the main process involved in shaping the moth community of olive groves was the loss of most of the species found in semi-natural habitats (Table 3). Both of the tests showed the moth community of the farm as nested due to the species impoverishment in olive groves (Figure 5). Nestedness was confirmed for three out of four site pairs with strong significance, but in the pair D, in which the semi-natural habitat has a herbaceous cover, it was only confirmed with a low significance by the MT (Table 3).

Table 3. Results of nestedness analyses based on overlap and decreasing fills (NODF) and matrix temperature (MT). We reported the values of statistics, their Z-scores, and associated *p*-values. N.S. means Not Significant.

Samples	Statistics	Values	Z-Scores	Nested	*p*
Farm	NODF	67.55	19.28	yes	$p < 0.001$
	MT	20.25	-14.35	yes	$p < 0.001$
pair A	NODF	49.11	8.61	yes	$p < 0.001$
	MT	10.10	-8.64	yes	$p < 0.001$
pair B	NODF	48.91	17.45	yes	$p < 0.001$
	MT	8.46	-8.31	yes	$p < 0.001$
pair C	NODF	42.26	11.08	yes	$p < 0.001$
	MT	15.57	-7.08	yes	$p < 0.001$
pair D	NODF	46.17	0.32	no	N.S.
	MT	26.21	-1.75	yes	$p < 0.05$

Grouping samples according to their land use, i.e., semi-natural patches and olive groves, confirmed a significant effect of nestedness in shaping moth communities (Table 4), with the site D covered by only herbaceous plants, ranking as the worst. However, the ranking of cultivated patches was not consistent with semi-natural ones, showing a worse ranking of the site C, located among olive groves (Figure 6). Grouping samples according to

their abiotic characteristics, i.e., dry versus wet farm portions, we again observed the major role of nestedness (Table 4) and found a similar scaling of sites ranking. In fact, whilst in the dry farm, olive groves ranked similarly to the their neighbouring semi-natural patches, in the dry farm, this did not occur again, because of the worse rank of the cultivated site C (Figure 6).
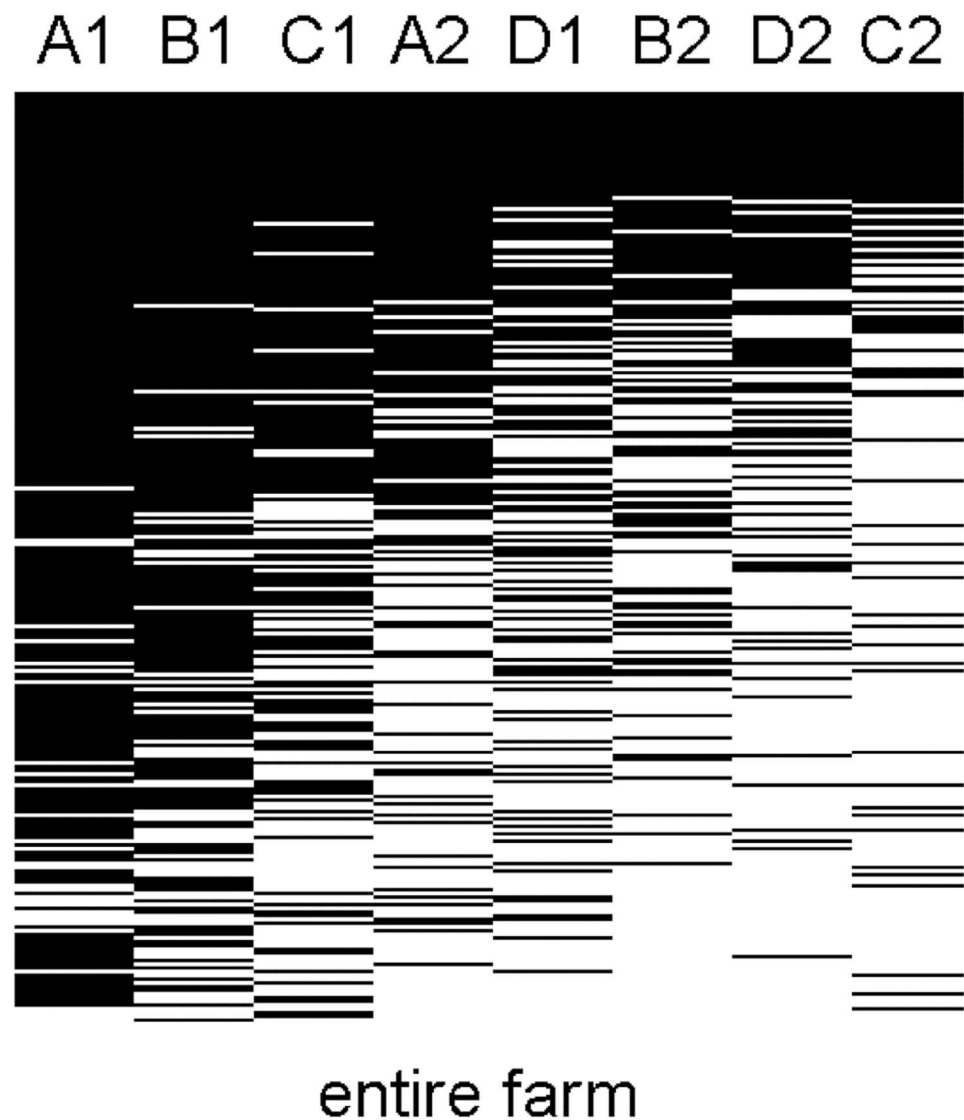

Figure 5. Packed matrix graph of sampled sites obtained by running the software Nestedness for Dummies (NeD).

Table 4. Results of nestedness analyses based on overlap and decreasing fills (NODF) and matrix temperature (MT). We reported the values of statistics, their Z-scores, and associated *p*-values.

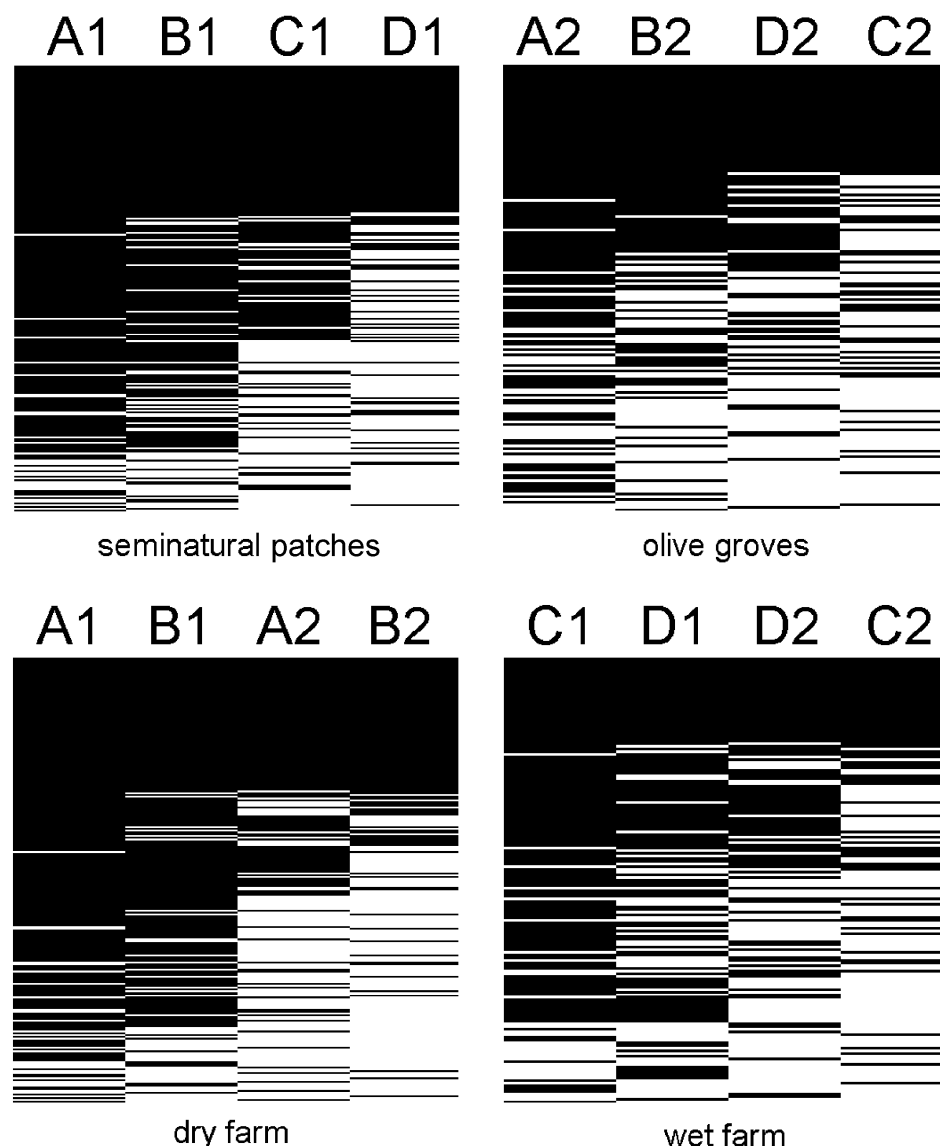| Samples	Statistics	Value	Z-Score	Nested	*p*
semi-natural patches	NODF	66.81	9.77	yes	$p < 0.001$
	MT	17.02	−11.24	yes	$p < 0.001$
olive groves	NODF	59.95	6.46	yes	$p < 0.001$
	MT	26.26	−7.98	yes	$p < 0.001$
dry farm	NODF	67.69	11.60	yes	$p < 0.001$
	MT	14.31	−11.17	yes	$p < 0.001$
wet farm	NODF	58.54	9.82	yes	$p < 0.001$
	MT	25.44	−9.285	yes	$p < 0.001$

Figure 6. Packed matrix graph of sampled sites grouped according to land use and abiotic characteristics obtained by running the software Nestedness for Dummies (NeD).

4. Discussion

In this study, we found that moth communities sampled in olive groves are nested within those of contiguous semi-natural habitats. However, the discriminant for this beta diversity pattern seems to be the type of vegetation cover, being the presence of trees determinant.

The observed community composition showed characteristics very similar to those observed in other similar habitats of South Italy. In fact, *Eilema caniola* (Hübner, 1808), *Dysauxes famula* (Freyer, 1836) (Family Erebidae) and *Peribatodes rhomboidaria* (Denis & Schiffermüller, 1775) (family Geometridae) were among the most abundant species in other cultivated Mediterranean landscapes, such as another organic olive grove on the Ionian Coast of Calabria [14], but also within a suburban area in the same region [47], demonstrating that these moth populations are not sensitive to human habitat alterations. However, these species represent a relatively small fraction of the total abundance that was more homogeneously distributed between species. Indeed, whilst in this study 26 species were necessary to reach 50% of the sample, in Scalercio et al. [14], 19 species were sufficient. Moth communities observed within fragmented landscapes with a matrix of semi-natural patches are frequently dominated by the oak feeder *Eupithecia dodoneata* Guenée, 1857 [48]—

of which only few individuals were found in the study area— and *E. caniola*, *P. rhomboidaria*, and *D. famula,* which were still common or abundant, but with a lower ranking.

The presence of a diversified landscape composition is known to increase the beta diversity of several animal taxa [49–51] and this is of great importance to maintain a high diversity rate within agroecosystems that assume a key role for the provision of ecosystem services [52]. In olive groves, it has been observed that small patches of semi-natural habitats can provide resting places and can act as stepping stones, favouring dispersal of species [14,53,54], and that the presence of semi-natural patches affects the traits of species found in olive groves [55]. In our study, we found that olive groves inhabit impoverished versions of the moth communities found in semi-natural areas, being nested within them. This finding is quite expected, as a similar pattern has been observed for several taxa in other agroecosystems [56,57]. This can be attributed to the fact that agricultural practices strongly modify the vegetation cover by mechanical and chemical means, and have a detrimental effect on biodiversity.

According to the source-sink theory [58], the diversity of cultivated sites (sinks) should mostly depend on those of neighbouring semi-natural patches (sources). As a consequence, we expected that a sink benefitting from a rich source should be richer than a sink benefitting from a poor source. Contrary to our expectations, we observed that this did not occur in the wetter part of the farm for the pair C, where the moth community of the cultivated C2 site (sink) was much poorer than the cultivated D2 site. So, a sink near a source (D1) shows a lower diversity than the C1 site. This anomalous pattern can be explained thanks to the ecology of several species, found mostly within the C1 site (semi-natural, tree covered, and with running water) that should act as a source for the C2 site (cultivated). The C1 moth community is characterised by hygrophilous and sciaphilous species such as *Herminia grisealis* (Denis & Schiffermüller, 1775), *H. tarsipennalis* Treitschke, 1835 (family Erebidae), and *Tephronia teophilaria* Hausmann, 2019 (family Geometridae), which composed more than 50% of the site's individuals. For a community composed of such species, we can hypothesise that the ecological border between the riparian woodlot and the cultivated patch is very strong, reducing the emigration rates between these patches [59].

Despite being nested, the moth community found in the studied olive grove is barycentric for a small but not negligible portion of the beta diversity, contributing to the total farm diversity. This portion is composed of species sharing some autoecological traits. The most representative taxa of this group (*Tyta luctuosa, Agrotis lata, Acontia trabealis, Heliothis peltigera, Apochima flabellaria*, and *Sesamia nonagrioides*) have larvae that feed on herbaceous plants, such as heliophilous, xerophilous and thermophilous plants. They prefer open habitats, that in the study area, are represented by the low-input century-old olive groves with plants set at about ten metres from each other.

In the absence of cultivation, the study area should be covered by an alternance of thermophilous and hygrophilous forests according to the edaphic conditions, as observed in the sampled semi-natural areas—otherwise, it lacks a habitat for the above-mentioned species. Definitively, the presence of olive groves offers ecologically suitable conditions to an ecologically well-defined group of species and, consequently, increases the diversity at the local scale.

The lack of pesticide spraying and mowing to which the studied olive farm was subjected could further explain the barycentric presence of some species in the cultivated portion of the study area. In fact, pollinator insects are known to be sensitive to chemicals with direct negative effects of pesticides on their survival rates [60], and indirect negative effects of herbicides on floristic diversity [61], reducing feeding sources for both larvae and adults. Soil management strategies, such as ploughing and milling, are also known to be detrimental to pollinator diversity and abundance. Plant diversity is reduced [62] and this can cause high mortality rates among immature moths living in the soil due to exposure to bird predation, as observed for the pest Lepidoptera [63]. Preferred soil management strategies should be grassing and mowing [64], which provide more nectar sources to adults and are harmless to moths that have not reached maturity.

The ecological management of cultivated areas, which represents the largest portion of the territory, was likely positive for semi-natural barycentric species. In fact, the studied farm processes that are detrimental to biodiversity, such as pesticide drift [65], are prevented beyond the farm, and good ecological conditions of cultivated land favour the permeability of forest species into the open habitat [53], increasing their survival rate within small habitat patches. However, as demonstrated in this paper, the fidelity of moth communities to their habitats is still very strong, as evidenced by the low similarity values of species assemblages found at distances of tens of meters. This was especially true when abiotic factors established stronger ecological barriers to moth movement. In our study, the highest species richness observed in the drier and warmer semi-natural area is at least partly due to the larger extension of the Riato valley, being 3 km long and with a maximum width of 1 km, compared with the wetter and colder semi-natural vegetation strips, which are only 600 m long and 100 m at their maximum width. Moth diversity increases with the increase in habitat quantity [66]. However, habitat quality also plays an important role [28], as demonstrated by the small differences in richness and abundance between cultivated sites and the corresponding semi-natural sites without tree cover.

5. Conclusions

Century-old olive groves have a lower profitability than modern plantations, increasable with appropriate management [67], but they can have a great ecological importance. We demonstrated that their presence increases the beta diversity of a pollinator insect group at the farm scale, hosting species with particular ecological needs. Furthermore, they can be assimilable to semi-natural open habitats when agronomic inputs such as chemical applications and soil management are reduced to a minimum. Promoting the presence of tree-covered semi-natural patches, particularly if they are ecologically well differentiated and alternated to low-input olive groves instead of semi-natural grasslands, could represent an interesting solution to increase the diversity and ecological functioning of cultivated landscapes, maintaining economic profitability on larger surfaces in the Mediterranean Basin.

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