

# The butterflies and burnets (Lepidoptera: Hesperioidea, Lycaenidae, Nymphalidae, Papilionidae, Pieridae, Zygaenidae) of the Nature Reserve Bosco della Fontana (Lombardy, Italy)

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**Abstract** - Insects are declining at an alarming rate in many parts of Europe and this also applies to butterflies, one of the most well-studied groups of insects. They are popular with the public and are considered good biodiversity indicators. Bosco Fontana, an isolated protected area, which contains one of the best preserved lowland forests of northern Italy, is surrounded by a highly modified landscape and is known to host some important butterfly populations. To investigate the butterflies and burnets, a total of 22 standard surveys, lasting 25 minutes each, were carried out in four different habitat types of Bosco Fontana in the years 2020 and 2021. The surveys confirmed the presence of 36 species of butterflies and 3 species of burnets for the reserve. Statistical analysis revealed that the butterfly and burnet assemblages of the grasslands, ecotones and forests were distinct, but similar in the two study years (datasets available as supplementary information). Many nemoral species were confirmed for Bosco Fontana, with relict populations of *Favonius quercus* and *Argynnis paphia* present only in a few residual forests in the Po Plain. Five species typical of grasslands and other open habitats and belonging to the local species pool of the Po Plain in the province of Mantua, were never detected at Bosco Fontana in the study years. The ecological significance of their absence is discussed.

**Key words:** biodiversity, check-list, dark diversity, monitoring, Po Plain.

**Riassunto** - Le farfalle diurne e le zigene (Lepidoptera: Hesperioidea, Lycaenidae, Nymphalidae, Papilionidae, Pieridae, Zygaenidae) della Riserva Naturale Bosco della Fontana (Lombardia, Italia).

Gli insetti stanno diminuendo ad un ritmo allarmante in molte parti d'Europa e questo processo coinvolge quindi anche le farfalle diurne, uno dei gruppi di insetti più studiati, apprezzati dal grande pubblico e considerati buoni indicatori della biodiversità. Bosco Fontana, un'area protetta isolata, che contiene una delle foreste planiziali meglio conservate dell'Italia settentrionale è nota per ospitare alcune importanti popolazioni di farfalle ed è circondata da un paesaggio altamente antropizzato. Negli anni 2020 e 2021, per studiare le farfalle diurne e la famiglia degli Zygaenidae, sono stati effettuati complessivamente 22 rilievi standard, della durata di 25 minuti ciascuno, in quattro diverse tipologie di habitat di Bosco Fontana. Queste indagini hanno confermato la presenza di 36 specie di farfalle diurne e 3 specie di Zygaenidae nel

territorio della riserva. L'analisi statistica ha rivelato che le comunità di farfalle e Zygaenidae delle praterie, degli ecotoni e delle foreste sono ben distinte, mentre le comunità presenti nello stesso tipo di habitat indagate in due anni consecutivi sono molto simili (dati disponibili come informazioni supplementari). Per Bosco Fontana sono state confermate popolazioni di molte specie nemorali e in particolare quelle di *Favonius quercus* e *Argynnis paphia* rappresentano popolazioni relict, che sopravvivono solo in un piccolo numero di lembi forestali della Pianura Padana. Viene discussa l'importanza ecologica dell'assenza di cinque specie di farfalle, tutte tipiche di praterie e altri ambienti aperti e considerate appartenenti al pool di specie della Pianura Padana della provincia di Mantova, ma che non sono mai state segnalate a Bosco Fontana.

**Parole chiave:** biodiversità, check-list, dark diversity, monitoraggio, Pianura Padana.

## INTRODUCTION

Biodiversity loss is one of the most pressing issues facing the planet; in particular, insects are a vital component of biodiversity accounting for more than half of the world's terrestrial species (Warren *et al.*, 2021). The industrialization of agriculture during the second half of the 20<sup>th</sup> century involved farming on a very large scale (monoculture), and the application of increasing amounts of pesticides, both practices destructive to insects and the other components of biodiversity (Raven & Wagner, 2021). For example, grassland butterfly abundance in north-western Europe has declined by 39% since 1990, indicating a dramatic loss of grassland biodiversity (van Swaay *et al.*, 2019). In Flanders (northern Belgium), in the early 2000s, the first Red List of butterflies revealed that 19 out of 64 indigenous species (30%) went extinct (Maes & Van Dyck, 2001). A major driving force behind these losses has been the expansion of intensive agriculture (Raven & Wagner, 2021; Warren *et al.*, 2021). Habitat quality and metapopulation effects explain the disproportionate decline of insects in cultivated landscapes. Degradation in habitat quality as well as habitat fragmentation are responsible for local extinctions for many insect species (Thomas *et al.*, 2001). Not even protected natural areas are immune from decline. In a 27-year study in Germany (Hallmann *et al.*, 2017), 76% of the biomass of flying insects was lost. Similarly, in an 11-year study (Rada *et al.*, 2019), butterfly species richness dropped by 10% in protected areas in Germany. Additionally, impor-

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tant changes in the distribution and abundance of insects, including butterflies, are also caused by climate change (Warren *et al.*, 2021). For example, in Europe the species composition of butterfly communities has shifted approximately 114 km northwards in the period 1990-2008 (Devictor *et al.*, 2012). It is therefore vital to study these important anthropogenic changes in insect and butterfly diversity in the coming decades.

Butterflies are one of the most well-studied groups of insects and they are popular with the public. Besides, they are relatively easy to identify, and have been used as model insects for many years (Warren *et al.*, 2021). They are also considered good biodiversity indicators (Blair, 1999; van Swaay *et al.*, 2008; Herrando *et al.*, 2016) particularly for open habitats (van Swaay *et al.*, 2008; Herrando *et al.*, 2016) and their distribution and habitat requirements are generally well known also in Italy (e.g. Balletto & Kudrna, 1985; Bonato *et al.*, 2014; Middleton-Welling *et al.*, 2020). Additionally, butterflies are well represented in the Habitats Directive (Habel *et al.*, 2020). Therefore, this taxonomic group is well suited for studying the effect of anthropogenic changes on insect biodiversity.

The Nature Reserve Bosco della Fontana (hereafter Bosco Fontana) is an isolated protected area in the middle of the Po Plain, with the surrounding landscape highly modified by human activities (agricultural fields, settlements, industry, etc.) both historically and presently. The butterflies of this reserve have been studied in the past (e.g. Triberti, 2002; Poltronieri, 2009; Hardersen & Corezzola, 2014), but a complete checklist and a critical review of the available records is still absent from the literature. Thus, one of the aims of this study was to provide detailed and up-to-date data on butterfly and burnet species composition of Bosco Fontana. A second aim was to investigate the assemblages of adult butterflies and burnets of four different habitat types (lowland hay meadows, dry grasslands, ecotones and forest) and to identify any preference for each of the investigated habitat types. A third aim was to collect a dataset of standardised observations, which will allow for future comparisons. When interpreting the data, the concept of dark diversity (Pärtel *et al.*, 2011; Lewis *et al.*, 2017) was also applied, which considers species absent from an ecosystem but belonging to its species pool, i.e. all species in the region that can potentially live in those specific ecological conditions (Pärtel *et al.*, 2011). Knowing about absent species can help supplement our understanding of the ecology and conservation status of a given area (Lewis *et al.*, 2017).

## MATERIALS AND METHODS

### Study site

The study was carried out in the nature reserve Bosco Fontana (45°12'04"N 10°44'32"E, 25 m a.s.l.), about 5 km NW of Mantua (Lombardy, Italy) (Fig. 1). The reserve covers an area of about 233 ha, of which 215 ha are occupied by mature mixed-deciduous forest. Of these, 189 ha are characterized by Illyrian oak-hornbeam forest (Erythronio-Carpinion) and 26 ha by an alluvial forest with *Alnus glutinosa* (L.) Gaertn. and *Fraxinus angustifolia*

Vahl (Alno-Padion, Alnion incanae, Salicion albae). A total area of 15 ha are meadows (mainly lowland hay meadows (*Arrhenatheretalia elatioris*) and dry grasslands (Festuco-Brometalia)). The forest is one of the best preserved lowland forests of northern Italy as it had continuous woodland cover since at least 1600 (Mason *et al.*, 2002). The reserve is an isolated protected area, as the surrounding landscape is highly modified by human activities, including agricultural fields, rural settlements, industrial buildings (for further details see Mason *et al.*, 2002). The climate is a relatively cool, midlatitude version of the humid subtropical climate. The annual average rainfall is 658 mm, average maximum temperature during the warmest month is 30 °C. The soil types of the reserve are mainly Mollic Gleysols.

### Data collection

Data on the butterflies and burnets of Bosco Fontana were gathered through the critical review of published literature (Triberti, 2002; Huemer, 2004; Corezzola *et al.*, 2012; Hardersen & Corezzola, 2014) and an unpublished dissertation (Poltronieri, 2009), the examination of voucher specimens, the execution of standard surveys of the area (see below) and the collection of opportunistic observations carried out by the author, F. Leandri and G. Sala. Taxonomy and nomenclature follows Wiemers *et al.* (2018) for butterflies, and Naumann *et al.* (1999) for burnets.

To standardize data collection, four different habitat types were selected for monitoring:

L: Lowland hay meadows (*Arrhenatheretalia elatioris* Tüxen 1931)

D: Dry grasslands (Festuco-Brometalia)

F: Forest

E: Ecotones

The distribution of these different habitats in the study area is shown in Fig. 1.

In total, 22 surveys were carried out in each habitat in the years 2020 (27/04-29/09/2020) and 2021 (10/03-29/09/2021). Although it was planned to start the investigation in March 2020, restrictions imposed by the Covid

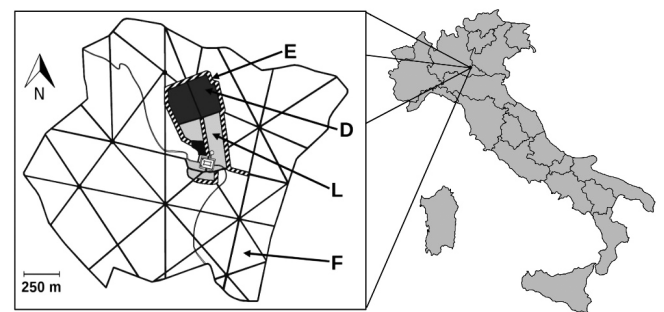


Fig. 1 - Location of the Nature Reserve Bosco della Fontana, with a map of the reserve and indications on the distribution of the four habitat types investigated. L) Lowland hay meadows, D) Dry grasslands, F) Forest, E) Ecotones. / Ubicazione della Riserva Naturale Bosco della Fontana, con una mappa della riserva e indicazioni sulla distribuzione dei quattro tipi di habitat indagati. L) Praterie magre da fieno a bassa altitudine, D) Praterie secche, F) Bosco, E) Ecotoni.

19 pandemic caused the field survey to begin only on 27 April 2020. Conversely, in 2021 the first survey was carried out on 10 March, as planned. All surveys were performed as timed transects lasting 25 minutes each. All four habitat types were investigated on a single day, randomly choosing the order of visit of each habitat during each survey. Only once (14/05/2021) did bad weather prevent all four surveys from being executed, and two were carried out once the weather improved (17/05/2021). A total 36 h 40 min of field work was employed over two years to gather the data, with each habitat type investigated for 9 h 10 min. All surveys were carried out between 10:00 a.m. and 17:00 p.m., under suitable weather conditions (warm, no or light wind, cloud coverage <25%). As one of the study aims was to investigate the assemblages of the butterflies and burnets in the four different habitat types present in the nature reserve, it was chosen not to use fixed transects, as these do not necessarily provide data on the entire habitat present in the reserve. Instead, the “checklist survey” method was followed for the meadows (see Royer *et al.*, 1998), which allows to find a higher number of species (Royer *et al.*, 1998) than the standardized “transect” method (Pollard, 1977), as the observer is free to search out places where butterflies typically would breed or congregate. Meadows which had been mown, were not included in the surveys for the following 10-14 days. In the forest, the network of gravel roads was followed, some of which were chosen at random during each survey. In this habitat, on average, a distance of 1400-1700 m was walked in 25 min. In addition, with regard to the ecotones, during each survey a few sections of this habitat located in the centre of the reserve were randomly chosen and investigated for 25 min.

During each survey, all adult butterflies observed up to a maximum distance of 3 m were identified by the naked eye. If identification from a distance proved difficult, individuals were caught with a sweep net and, if necessary, killed. Voucher specimens of most species were collected and kept in the collection of the Centro Nazionale Carabinieri Biodiversità “Bosco Fontana”, Italy. For some genera (e.g. *Pyrgus*, *Plebejus*), genitalia were prepared to allow for species identification.

For future comparisons, the complete dataset with the quantitative information collected during the standard surveys is provided (Supplementary information: Tab. S1-S4).

## Statistics

In order to investigate how species richness changed during the year, the average number of species recorded was calculated for the first and second half of each month (first half: days 1-15; second half: days 16-30/31). Data for each fortnight included all the records of the four surveys carried out in the different habitat types (L, D, F, E) and the figures are therefore representative for the entire reserve.

Assemblage compositions of the four habitats were compared by two dimensional Nonmetric Multidimensional Scaling (NMDS). In order to compare the butterfly and burnet faunas of the years 2020 and 2021 with the same effort and over the same period, four of the surveys carried out in 2021 were excluded from the analyses. This

resulted in a total of 9 surveys/year, spanning 27 April 2020 - 29 September 2020 and 28 April 2021-29 September 2021. The data of the 9 surveys carried out in one year were pooled and the assemblage compositions of each habitat type was analysed independently for each year, resulting in a total of 8 datasets. NMDS was carried out using the abundance data and employing the function metaMDS, which is incorporated in the statistical package vegan (Oksanen *et al.*, 2020) using R (version 4.2.0 <http://www.r-project.org/>). The zero-adjusted Bray-Curtis coefficient (Clarke *et al.*, 2006) was used as the pairwise distance among samples.

## Dark diversity

Dark diversity (Pärtel *et al.*, 2011) is represented by the species currently absent from the reserve, which nevertheless belong to its potential species pool. The latter consists of all species in the region whose ecological requirements meet the ecological conditions found in the study area. To define the species pool of Bosco Fontana a number of sources were used: bibliographic (Bonato *et al.*, 2014), web-based (iNaturalist: <https://www.inaturalist.org/>) and unpublished data (the author, F. Leandri, M. Ghisolfi).

## RESULTS

A total of 49 species of butterflies and burnets have been recorded in the Nature Reserve Bosco della Fontana since 1977 (Tab. 1). This number includes *Lopinga achine*, which had been caught in the reserve before this study only in 1977 (G. Sala pers. com.).

The 22 surveys carried out during 2020-2021 in each of the four habitat types confirmed the presence of 36 species of butterflies and 3 species of burnets (Tab. 2). Of these, three are reported for Bosco Fontana for the first time: *Lycaena tityrus*, *Leptotes pirithous*, and *Cacyreus marshalli*. Additionally, three species of butterflies (*Apatura ilia*, *Lycaena dispar* and *Satyrium w-album*) were confirmed outside the standard surveys in the years 2020-2021. A total of 7 species were not confirmed during the biennial research. These species are listed hereafter followed by the last year in which they were recorded in the nature reserve: *Anthocharis cardamines* (2022), *Colias hyale* (2004), *C. alfacariensis* (2009), *L. achine* (1977), *Melitaea diamina* (2019), *Aglais urticae* (2022) and *Nymphalis polychloros* (2004).

Some species showed a clear preference for one of the habitat types investigated and these are here briefly listed. The following species were observed with the highest abundances in the lowland hay meadows (L): *Pyrgus armoricanus*, *Lycaena phlaeas*, *Polyommatus icarus*, *Melitaea phoebe*, *M. celadussa*, *Zygaena filipendulae*. Whereas the following species were encountered with the highest abundance in the dry grassland (D): *Iphiclides podalirius*, *Pontia edusa*, *Lysandra bellargus*, *Zygaena loti*. The species *Celastrina argiolus* was the only one that was most common in the ecotones (E), whereas the following species were observed with the highest abundance in the forest (F): *Pieris napi*, *Favonius quercus*, *Argynnis paphia*, *Vanessa atalanta*, *Pararge aegeria*.

Tab. 1 - Checklist of the butterfly and burnet species recorded in the Nature Reserve between 1977 and 2021. X: the species was listed as present in the relative publication; (X): the species was listed as present in the relative publication but under a different species name. \*: recorded only by G. Sala in 1977. / Checklist delle specie di farfalle e Zygaenidae segnalate nella Riserva Naturale dal 1977 al 2021. X: la specie è stata indicata come presente nella relativa pubblicazione; (X): la specie era elencata come presente nella relativa pubblicazione, ma con un nome diverso. \*: segnalata solo da G. Sala nel 1977.

Reference	Triberti, 2002	Huemer, 2004	Poltronieri, 2009	Hardersen & Corezzola, 2014	Hardersen (present study)
Study period	1998-2000	2004	2009	2010-2011	2020-2021
<i>Iphiclides podalirius</i> (Linnaeus 1758)	X	X	X	X	X
<i>Papilio machaon</i> Linnaeus 1758	X	X	X	X	X
<i>Ochlodes sylvanus</i> (Esper 1777)	X	X	X	X	X
<i>Carcharodus alceae</i> (Esper 1780)		X	X	X	X
<i>Pyrgus malvoides</i> (Elwes & Edwards 1897)	X	X			X
<i>Pyrgus armoricanus</i> (Oberthür 1910)			(X)	(X)	X
<i>Gonepteryx rhamni</i> (Linnaeus 1758)		X			X
<i>Colias alfacariensis</i> Ribbe 1905	X		X		
<i>Colias hyale</i> (Linnaeus 1758)		X			
<i>Colias crocea</i> (Geoffroy 1785)	X	X	X	X	X
<i>Pontia edusa</i> (Fabricius 1777)			X	X	X
<i>Pieris brassicae</i> (Linnaeus 1758)			X		X
<i>Pieris rapae</i> (Linnaeus 1758)		X	X	X	X
<i>Pieris napi</i> (Linnaeus 1758)	X	X	X	X	X
<i>Anthocharis cardamines</i> (Linnaeus 1758)	X	X	X		
<i>Lycaena dispar</i> ([Haworth] 1802)	X	X			X
<i>Lycaena phlaeas</i> (Linnaeus [1760])		X	X	X	X
<i>Lycaena tityrus</i> (Poda 1761)					X
<i>Favonius quercus</i> (Linnaeus 1758)	X	X	X		X
<i>Leptotes pirithous</i> (Linnaeus 1767)					X
<i>Satyrrium w-album</i> (Knoch 1782)			X		X
<i>Lampides boeticus</i> (Linnaeus 1767)			X		X
<i>Cacyreus marshalli</i> Butler 1898					X
<i>Celastrina argiolus</i> (Linnaeus 1758)	X	X	X		X
<i>Cupido argiades</i> (Pallas 1771)		X	X	X	X
<i>Plebejus argyrognomon</i> (Bergsträsser 1779)	(X)	X	X	(X)	X
<i>Aricia agestis</i> ([Denis & Schiffermüller] 1775)		X	X	X	X
<i>Lysandra bellargus</i> (Rottensburg 1775)			X	X	X
<i>Polyommatus icarus</i> (Rottensburg 1775)		X	X	X	X
<i>Argynnis paphia</i> (Linnaeus 1758)	X	X	X	X	X
<i>Issoria lathonia</i> (Linnaeus 1758)			X		X
<i>Apatura ilia</i> ([Denis & Schiffermüller] 1775)	X	X			X
<i>Vanessa cardui</i> (Linnaeus 1758)	X	X	X	X	X
<i>Vanessa atalanta</i> (Linnaeus 1758)	X	X	X	X	X
<i>Aglais io</i> (Linnaeus 1758)	X	X	X	X	X
<i>Aglais urticae</i> (Linnaeus 1758)		X			
<i>Polygonia c-album</i> (Linnaeus 1758)	X	X	X		X
<i>Nymphalis polychloros</i> (Linnaeus 1758)		X			
<i>Melitaea didyma</i> (Esper 1778)	X	X	X	X	X
<i>Melitaea phoebe</i> ([Denis & Schiffermüller] 1775)	X	X	X	X	X
<i>Melitaea diamina</i> (Lang 1789)				X	
<i>Melitaea celadussa</i> Fruhstorfer 1910	X	X	X	X	X
<i>Coenonympha pamphilus</i> (Linnaeus 1758)	X	X	X	X	X
<i>Lopinga achine</i> (Scopoli 1763)*					
<i>Pararge aegeria</i> (Linnaeus 1758)	X	X	X		X
<i>Lasiommata megera</i> (Linnaeus 1767)	X	X	X		X
<i>Zygaena loti</i> ([Denis & Schiffermüller] 1775)	X	X			X
<i>Zygaena filipendulae</i> (Linnaeus 1758)		X			X
<i>Zygaena ephialtes</i> (Linnaeus 1767)		X			X

Tab. 2 - Butterfly and burnet species and the number of individuals recorded in 2020-2021 during 22 surveys carried out in four habitat types (L: Lowland hay meadows, D: Dry grasslands, F: Forest, E: Ecotones). / Specie di farfalle e Zyganidae e numero di individui registrati nel 2020-2021 durante 22 indagini effettuati in quattro tipi di habitat (L: Praterie magre da fieno a bassa altitudine, D: Praterie secche, F: Bosco, E: Ecotoni).

Species	Habitat type			
	L	D	E	F
<i>Iphiclides podalirius</i> (Linnaeus 1758)	5	14	2	-
<i>Papilio machaon</i> Linnaeus 1758	-	1	1	-
<i>Ochlodes sylvanus</i> (Esper 1777)	27	10	37	2
<i>Carcharodus alceae</i> (Esper 1780)	2	2	3	-
<i>Pyrgus malvoides</i> (Elwes & Edwards 1897)	10	8	2	-
<i>Pyrgus armoricanus</i> (Oberthür 1910)	9	2	-	-
<i>Gonepteryx rhamni</i> (Linnaeus 1758)	-	-	2	2
<i>Colias crocea</i> (Geoffroy 1785)	49	87	6	-
<i>Pontia edusa</i> (Fabricius 1777)	5	17	-	-
<i>Pieris brassicae</i> (Linnaeus 1758)	-	-		3
<i>Pieris rapae</i> (Linnaeus 1758)	24	22	32	25
<i>Pieris napi</i> (Linnaeus 1758)	21	25	101	169
<i>Lycaena phlaeas</i> (Linnaeus 1761)	35	8	12	-
<i>Lycaena tityrus</i> (Poda 1761)	6	7	4	-
<i>Favonius quercus</i> (Linnaeus 1758)	-	-	-	1
<i>Leptotes pirithous</i> (Linnaeus 1767)	-	1	1	-
<i>Cacyreus marshalli</i> Butler 1898	-	-	1	-
<i>Lampides boeticus</i> (Linnaeus 1767)	1	-	1	-
<i>Celastrina argiolus</i> (Linnaeus 1758)	-	-	34	15
<i>Cupido argiades</i> (Pallas 1771)	49	41	20	-
<i>Plebejus argyrognomon</i> (Bergsträsser 1779)	45	43	27	-
<i>Aricia agestis</i> ([Denis & Schiffermüller] 1775)	1	1	1	1
<i>Lysandra bellargus</i> (Rottemburg 1775)	23	160	12	-
<i>Polyommatus icarus</i> (Rottemburg 1775)	91	55	17	-
<i>Issoria lathonia</i> (Linnaeus 1758)	2	3	-	-
<i>Argynnis paphia</i> (Linnaeus 1758)	-	1	15	29
<i>Vanessa cardui</i> (Linnaeus 1758)	1	-	5	-
<i>Vanessa atalanta</i> (Linnaeus 1758)	1	3	9	19
<i>Aglais io</i> (Linnaeus 1758)	5	3	6	3
<i>Polygonia c-album</i> (Linnaeus 1758)	2	-	7	8
<i>Melitaea didyma</i> (Esper 1779)	294	234	51	-
<i>Melitaea phoebe</i> (Goeze 1779)	132	58	25	-
<i>Melitaea celadussa</i> (Rottemburg 1775)	613	179	95	-
<i>Coenonympha pamphilus</i> (Linnaeus 1758)	160	178	26	-
<i>Pararge aegeria</i> (Linnaeus 1758)	-	-	2	83
<i>Lasiommata megera</i> (Linnaeus 1767)	15	10	11	-
<i>Zygaena loti</i> ([Denis & Schiffermüller] 1775)	12	117	2	-
<i>Zygaena ephialtes</i> (Linnaeus 1767)	1	1	-	-
<i>Zygaena filipendulae</i> (Linnaeus 1758)	39	21	2	-

Species richness of adult butterflies and burnets during the year is presented in in Fig. 2. The average number of species recorded was lowest in the first half of march (5 species) and increased almost continuously up to August, when 22.5 species were observed on average during the surveys. Thereafter the average number of species declined.

Considering the assemblage composition of all butterflies, the NMDS had a calculated Stress of 0.042, showing that the configuration is excellent and allows for a detailed inspection (Zuur *et al.*, 2007) (Fig. 3). The assemblages of the grasslands, ecotones and forests were well separated and the assemblages of the ecotones were placed half-way between those of the forest and of the grasslands.

**DISCUSSION**

The surveys carried out in the nature reserve Bosco Fontana confirmed the presence of 36 species of butterflies and 3 species of burnets. At least four additional species (*A. cardamines*, *A. ilia*, *L. dispar* and *S. w-album*) were not detected, even though they were certainly present as they were confirmed in the years 2020-2021 outside the standard surveys. This confirms that even a relatively large effort cannot detect all diurnal lepidoptera present in an area, as has already been documented in various studies (Wikström *et al.*, 2009; Jonason *et al.*, 2010; Hardersen & Corezzola, 2014). As a consequence, this checklist compiled from a limited number of surveys, is unlikely to be complete. A case in point is the fact that some of the rarest species have been observed in only one of the two years of the study, e.g. *F. quercus* (observed in 2020) and *Aricia agestis* (observed in 2021). Conversely, *A. cardamines* was not found during the present study, but it was confirmed in 2022 during research focusing on this species. At Bosco Fontana this species is present at low density for a brief period of time (M. Ghisolfi, pers. com.).

The large number of studies carried out over the past four decades has resulted in a rather consistent data set on

the butterflies and burnets present in Bosco Fontana. Since 1977, a total of 46 species of butterflies and 3 species of burnets have been observed in the reserve (Tab. 1). This list includes both sister-species *Colias alfacariensis* and *C. hyale*, which can only be reliably discriminated by examining the larvae (Villa *et al.*, 2009). Table 1 does not include some species reported for Bosco Fontana, which are believed to have been wrongly classified: *Pyrgus alveus*, *P. cirsii*, *Plebejus argus*, *Plebejus idas*, *Melitaea cinxia* and *Hipparchia fagi*. *Pyrgus alveus* had been reported by Poltronieri (2009) and Hardersen & Corezzola (2014), but examination of the genitalia revealed that they belong to *P. armoricanus*. *P. cirsii*, reported by Huemer (2004) for Bosco Fontana, is known in Italy only from Liguria (Villa *et al.*, 2009). *H. fagi* and *P. idas* reported by Triberti (2002) were excluded. The first species is unknown for the Po Valley, it has never been reported again for Bosco Fontana and is not present in the entomological collection of the Centro Nazionale Carabinieri Biodiversità “Bosco Fontana”. The genitalia of specimens assigned to *P. idas* by Triberti (2002) were examined revealing that they belong to *P. argyrognomon*. *Plebejus argus* had been reported by Hardersen & Corezzola (2014); however, the genitalia revealed that they too belong to *P. argyrognomon*. *Melitaea cinxia*, reported by Huemer (2004), is not known from the central Po Plain and is not present in the entomological collection of the Centro Nazionale Carabinieri Biodiversità “Bosco Fontana”.

Some of the unconfirmed taxa (e.g. *Colias alfacariensis*, *A. urticae*) occur only sporadically in the Po Valley (Bonato *et al.*, 2014) and it was therefore no surprise that they were not found. The only important loss registered concerns *L. achine* (a species listed in appendix IV of the Habitats Directive), which presumably became extinct sometime after 1977, probably as a consequence of changes

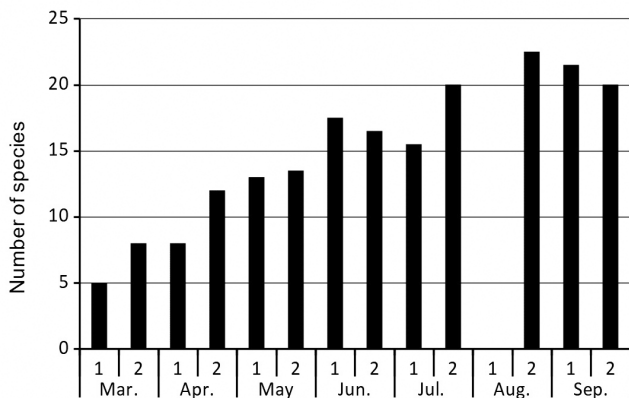


Fig. 2 - The average number of species of adult butterflies and burnets recorded during the year. Data are presented for the first and second half of each of the months (first half: days 1-15; second half: days 16-30/31). No data were available for the first half of August. / Il numero medio di specie di farfalle e Zygaenidae adulte registrate durante l'anno. I dati sono presentati per la prima e la seconda metà di ogni mese (prima metà: giorni 1-15; seconda metà: giorni 16-30/31). Per la prima metà di agosto non sono disponibili dati.

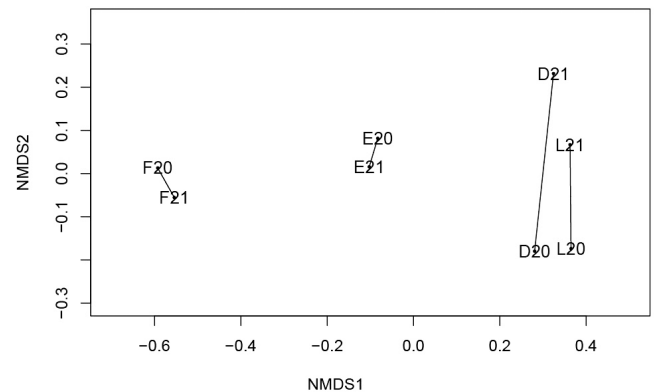


Fig. 3 - Nonmetric Multidimensional Scaling (NMDS) of the abundance data collected during 9 surveys in each year (2020-2021), using the zero-adjusted Bray-Curtis coefficient (Clarke *et al.*, 2006) to calculate pair wise distances. Groups are connected by a line using the function “ordispider” (statistical package Vegan). L: Lowland hay meadows, D: Dry grasslands, F: Forest, E: Ecotones; 20: 2020, 21: 2021). / Multidimensional scaling non metrico (NMDS) dei dati di abbondanza raccolti durante 9 indagini negli anni 2020 e 2021, utilizzando il zero-adjusted Bray-Curtis coefficient (Clarke *et al.*, 2006) per calcolare le distanze tra coppie. I gruppi sono collegati da una linea utilizzando la funzione “ordispider” (pacchetto statistico Vegan). (L: Praterie magre da fieno a bassa altitudine, D: Praterie secche, F: Bosco, E: Ecotoni).

in the management of the forest and the decrease in the number of trees felled. Timber harvesting in Bosco Fontana has been practised for centuries, but has declined since 1983 and stopped altogether in 1994 (Mason, 2002); this has certainly led to a higher canopy density, making the forest less suitable for *L. achine*, as this species requires open canopies (Streitberger *et al.*, 2012). The populations of *F. quercus* and *Argynnis paphia* represent relict populations present in a small number in remains of lowland forests in northern Italy (Bonato *et al.*, 2014; Ghisolfi *et al.*, 2021). The distribution of *F. quercus* seems particularly fragmented as it is extremely localized in the Po Plain of Lombardy where recent records are known only from a handful of sites (Gatti, 2021). Bosco Fontana thus appears to host one of the few relict populations. Also in Veneto region, the species is not known from the Po Valley (Bonato *et al.*, 2014). However, in all likelihood, this species is more widespread than has been documented, as it rarely descends to ground level; moreover, being small in size, it is difficult to observe (Gatti, 2021).

It seems that the assemblage of butterflies and burnets present in this protected area was relatively stable over the last 20 years (Tab. 1). However, as various studies about Bosco Fontana (Triberti, 2002; Huemer, 2004; Poltronieri, 2009; Hardersen & Corezzola 2014) did not follow the same standard monitoring protocol, minor trends in assemblage composition could not have been detected. It is therefore advocated to apply the method used during this study for any future work on the butterflies and burnets of Bosco Fontana to allow for detailed comparisons. This is particularly important considering that the assemblage composition might vary in the future due to climate change. For example, Devictor *et al.* (2012) showed that the composition of the butterfly community has shifted northwards by 114 km in 18 years, similar to Warren *et al.* (2021) who reported the massive northward spread of certain thermophilic species in Europe.

The four habitat types investigated hosted different assemblages of species, which were not significantly different for each habitat type in the two consecutive years of the study. However, the assemblage of the grasslands varied somewhat more between years than those of the ecotones and the forest, with the most pronounced difference observed in the dry grassland habitat. This is probably attributable to the fact that some species were not recorded there in one year. For example, *Lasiommata megera* and *Melitaea phoebe*, most common in 2020, were not recorded in the dry grassland in 2021. The general stability of the assemblages recorded over two years also indicates that the 9 standard surveys made it possible to collect valid and comparable data on the butterfly and burnet assemblages of the various habitat types, a result in line with Hardersen & Corezzola (2014). Consequently, the data collected in this study will allow for a meaningful comparison of the butterfly and burnet assemblages of Bosco Fontana for the years to come.

The species composition of butterflies in the two grassland habitats was comparable, while the ecotone assemblages were predictably found between grassland and forest. The large differences in assemblage compositions are explained by the fact that each butterfly species occupies

preferential habitat types (e.g. van Swaay *et al.* 2006, Wikström *et al.* 2009). Recently, van Swaay *et al.* (2006) classified the most important biotopes for European butterflies and these classifications largely correspond to the demonstrated preferences for one of the habitat types analysed here. However, some differences emerged in this study; for example, *Pyrgus armoricanus* was mostly found in lowland hay meadows. In contrast, van Swaay *et al.* (2006) and Gatti (2021) reported that this species is most common in dry grasslands. In Bosco Fontana, *Iphiclides podalirius* and *Pontia edusa* were most frequent in the dry grassland, contrary to van Swaay *et al.* (2006) and Gatti (2021) that observed the species in a much wider variety of biotopes. During this study, *Pieris napi* was most frequently observed in the forest, as already recorded in woods of the Po Plain where the species is commonly the dominant species in the forest interior, probably because of thermal characteristics of this lowland habitat (Balletto *et al.*, 1982). Also, Gatti (2021) described this species as subnemoral and sciaphilous. In contrast, van Swaay *et al.* (2006) reported that, in Europe as a whole, *P. napi* uses a wider variety of biotopes.

Species richness increased during the summer and peaked in August. Bonato *et al.* (2009) found a similar trend for riparian habitats of the Venetian Prealps and observed maximum species richness in mid-August. Similarly, Borghesio (2009) found the highest species richness in a lowland heath of northern Italy in the same month. It seems likely that this late maximum is influenced by the relatively large number of multivoltine species present at these low altitudes (Bonato *et al.*, 2009; Hardersen & Corezzola, 2014).

Species diversity of butterflies is correlated with habitat area (Baz & Garcia-Boyer, 1995; Kraus *et al.*, 2003), as are rates of immigration, extinction, and turnover (Kraus *et al.*, 2003). Additionally, habitat quality and site isolation are both important determinants of where populations persist in modern landscapes (Thomas *et al.*, 2001) and habitat fragmentation is now a serious concern for many butterflies (Warren *et al.*, 2021). Thus, it is expected that a relatively small isolated nature reserve, such as Bosco Fontana, will not host all the species which could potentially be present (dark diversity: Pärtel *et al.*, 2011). The following species, which have never been reported from Bosco Fontana, and which belong to the local species pool, are here considered to be the dark diversity of butterflies of Bosco Fontana: *Thymelicus lineola* (Ochsenheimer 1808), *Erynnis tages* (Linnaeus 1758), *Callophrys rubi* (Linnaeus 1758), *Melanargia galathea* (Linnaeus 1758), *Maniola jurtina* (Linnaeus 1758). Remarkably, most of these species are typical of grasslands and open habitats (van Swaay, 2006; Bonato *et al.*, 2014), with the only exception of *C. rubi*, which has a broader ecological amplitude and typically lives in shrubland, forest margins and grasslands (Bonato *et al.*, 2014; Gatti, 2021). The meadows of Bosco Fontana occupy approximately 15 ha only (6.5% of the reserve area) and host a limited number of plant species: for this reason, they can be defined as poor forms of *Arrhenatheretalia* and *Festuco-Brometalia* (Rossato, 2015). Thus, this restricted area of grassland is evidently too small and

does not show the necessary diversity of host plants to support the entire local butterfly species pool. In contrast, the 189 ha area of forest seems to contain the complete set of forest species typical of the central Po Plain and even to host relict populations of typical forest species, e.g. *F. quercus* and *A. paphia* (Bonato *et al.*, 2014). The sole exception is represented by *Lopinga achine*, which became extinct in Bosco Fontana sometime after 1977 (Campanaro *et al.*, 2014). However, it is doubtful if the current forest habitat is still suitable for this species, as the changed forest management resulted in a more closed canopy. Additionally, *L. achine* seems currently absent from the Mantua Po Plain and for this further reason is not to be considered as belonging to the local species pool.

## CONCLUSION

At least, 40 species of butterflies and 3 species of burnets are currently present in the nature reserve Bosco Fontana and some of these (e.g. *F. quercus*, *A. paphia*) represent relict populations of typical forest species, now absent from many areas of the Po Plain. Also for birds (Grattini *et al.*, 2016), plants (Prosser, 2014) and beetles (e.g. Vigna Taglianti, 2004), the forest of Bosco Fontana hosts some of the last populations of typical forest species of the central Po Plain. These relict populations underline the importance of the forest of this reserve for nature conservation, also as potential source populations for new forests that have recently been planted in the Lombardy Po Plain, for example as part of the project “10,000 hectares of new forests and multifunctional green systems”. In contrast, the meadows of Bosco Fontana are probably too small in extent and poor of host plant species to support the complete local butterfly species pool of grassland species. This applies even though the nature reserve is surrounded by meadows. However, these grasslands are intensively farmed and it is known that agricultural intensification causes the vanishing of a major proportion of butterfly species (Habel *et al.*, 2019). It is hoped that, in the future, functional connections with the surrounding Natura 2000 sites will be established to improve the ecological coherence. This can be achieved by developing features of the landscape of major importance for fauna and flora, as set out in Article 3.3 of the Habitats Directive, and that this will reduce habitat fragmentation for butterflies and other fragile animal populations.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online for this article.

Tab. S1-S4 - Butterfly and burnet species recorded in 2020-2021 in the Nature Reserve Bosco della Fontana - complete dataset with quantitative information standardly collected during each survey in the four different habitat types investigated. / Farfalle diurne e zigene registrate nella Riserva Naturale Bosco della Fontana 2020-2021 - set di dati completo con informazioni quantitative raccolte in modo standard durante ogni indagine nei quattro diversi tipi di habitat indagati.