

## Article

# Assemblage Characteristics of Butterflies and Carabid Beetles as a Function of Soil Characteristics and Plant Diversity in Differently Managed Fields, Forests and Ecotones: A Case Study in Tuczno Forest District, Poland

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**Citation:** Szyszko-Podgórska, K.; Dymitryszyn, I.; Jankiewicz, U.; Kondras, M.; Żyfka-Zagrodzińska, E.; Schwerk, A. Assemblage Characteristics of Butterflies and Carabid Beetles as a Function of Soil Characteristics and Plant Diversity in Differently Managed Fields, Forests and Ecotones: A Case Study in Tuczno Forest District, Poland. *Land* **2022**, *11*, 25. <https://doi.org/10.3390/land11010025>

Academic Editor: Guillermo J. Martinez-Pastur

Received: 15 November 2021

Accepted: 20 December 2021

Published: 24 December 2021

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**Abstract:** A drastic decline in insect fauna on a large scale has been reported. We assume that this is a multifactorial problem involving biotope types and plant diversity, soil characteristics and human activity (management of areas). The aim of our study was to analyze diversity patterns of carabid beetles and butterflies as predatory and phytophagous arthropod groups in response to soil characteristics and plant diversity in different types of ecosystems and ecotones with diverse management situated in a heterogeneous landscape composed of different forests, agricultural and post-agricultural areas of different stages of succession and watercourses and mires in north-western Poland. Three different forests, three fallows, two meadows and two ecotones, differing with respect to the involved ecosystems, were included in the study. Our results showed that the study site types differed with respect to soil characteristics and plant diversity, but ecotones were not characterized by explicitly higher diversity in these parameters. For both carabid beetles and butterflies, characteristic assemblages for individual study sites could be demonstrated. We could also show differences in the most important factors between these two taxonomic groups. We assume that management type is important regarding ecosystem characteristics and biodiversity. Large-scale management strategies are necessary in order to maintain or create landscapes with high natural qualities.

**Keywords:** Carabidae; Lepidoptera; landscape; biological diversity; management

## 1. Introduction

Nowadays, we are confronted with a strong loss in biological diversity. Regarding insects, a drastic decline in insect fauna on a large scale has been reported [1–5]. We assume that this is a multifactorial problem involving biotope types and plant diversity, soil characteristics and human activity (management of areas).

Habitat diversity is an essential factor for species diversity in a given landscape, e.g., [6–9], i.e., the number of species over a large area or region (gamma-diversity) [10,11]. The diversity of ecosystems in the landscape is an important factor for habitat diversity, which depends both on habitat diversity between and within ecosystems, e.g., [12]. In this regard, ecotones deserve special attention because they can be centers of high species richness and can sustain species that are less abundant or do not occur elsewhere [13]. Langhans and Tockner [14] showed the significance of floodplain ecotones for beetle biodiversity. Species richness in ecotones, however, may depend on the degree of anthropization [15].

Individual insect species also show complex interactions with the plant assemblages on the respective sites; this is particularly true for phytophagous species, which depend on the respective host plants [16]. The plant assemblages define the structural and microhabitat features, which are, besides their prey, important for predatory species. However, plants also depend on soil characteristics and human ecosystem management practices, thus acting as mediators between animals and soil and/or human impact. It has been shown that plants mediate multiple interactions of below-ground and above-ground biota [17]. Consequently, soil characteristics have a tremendous impact on biodiversity patterns, not only with respect to the soil fauna, e.g., Ehrnsberger [18], but also regarding above-ground biodiversity [17]. What is more, many insects, and as many carabid beetle species, spend their life cycle partly under the ground (often during the larval stages) and partly above the ground, e.g., Kotze et al. [19].

Management practices have an important influence on arthropod species assemblages too. An overview of the conservation of arthropods in British grasslands including management practices is provided by Morris [20]. The impact on species diversity depends on management intensity. For example, Schwerk and Dymitryszyn [21] showed the importance of the number of cuttings on post-agricultural land for the formation of carabid beetle assemblages. Several studies have shown that some of the cultural landscapes, i.e., landscapes that are “more or less intensively influenced by man” [22] may belong to very species-rich landscapes [23,24].

The aim of our study was to analyze diversity patterns of predatory and phytophagous arthropod groups in response to soil characteristics and plant diversity in different types of ecosystems and ecotones with diverse management. We studied carabid beetles and butterflies.

Carabid beetles are known to react to landscape-level phenomena [25] and to the management practices in grassland habitats [26]. They have also been proven to be suitable indicators of changes in soil conditions such as due to soil contamination or soil preparation techniques [27–29].

Due to their high dispersal ability and sensitivity to changes in environmental conditions, butterflies are used for bioindication by acting as indicators of changes in different types of environments. Butterflies represent a wide variation in geographic distribution and are found in almost all ecosystems. In addition, they are highly diverse in size and food, as well as being studied for their range of tolerance to various factors [30–34]. Butterflies are most often used as indicators of the succession stage, of the presence of certain plants or animals necessary for their growth, and of changes in habitat conditions occurring in the environment [33,35,36]. They are also used as indicators of environmental pollution or radioactive contamination [37–39].

As a study area, a heterogeneous landscape composed of different forests, agricultural and post-agricultural areas at different stages of succession alongside watercourses and mires, located in north-western Poland, was selected [40].

Our hypotheses were:

- (1) The study site types differ in soil characteristics and plants, with ecotones characterized by a higher diversity of environmental characteristics than individual ecosystems, resulting in increased numbers of species, some of them solely found in these areas.
- (2) Carabid beetle and butterfly species assemblages differ between the study site types.
- (3) Carabid beetles and butterflies show differences in response to the studied factors of soil characteristics and plant diversity.

The results are discussed in the context of the impact of management type on site characteristics and biodiversity patterns as an important aspect for developing sustainable strategies of ecosystem and landscape management.

## 2. Materials and Methods

### 2.1. Study Sites

The studies were carried out in the north-western part of Poland (zachodniopomorskie voivodeship) in the Tuczno Forest District. The terrain is situated in the Drawa National Park buffer zone. Three different forests, three fallows and two meadows differing with respect to management type and two ecotones differing with respect to the involved ecosystems were included in our study (Table 1, Figure 1). At each study site, 6 sampling plots were established at a distance of 50 to 100 m from each other. The sampling plots in the forests, fallows and meadows were located at least 30 m apart from the neighboring ecosystems, whereas the sampling plots in the ecotones were located directly in the transition zone of the two involved ecosystems.

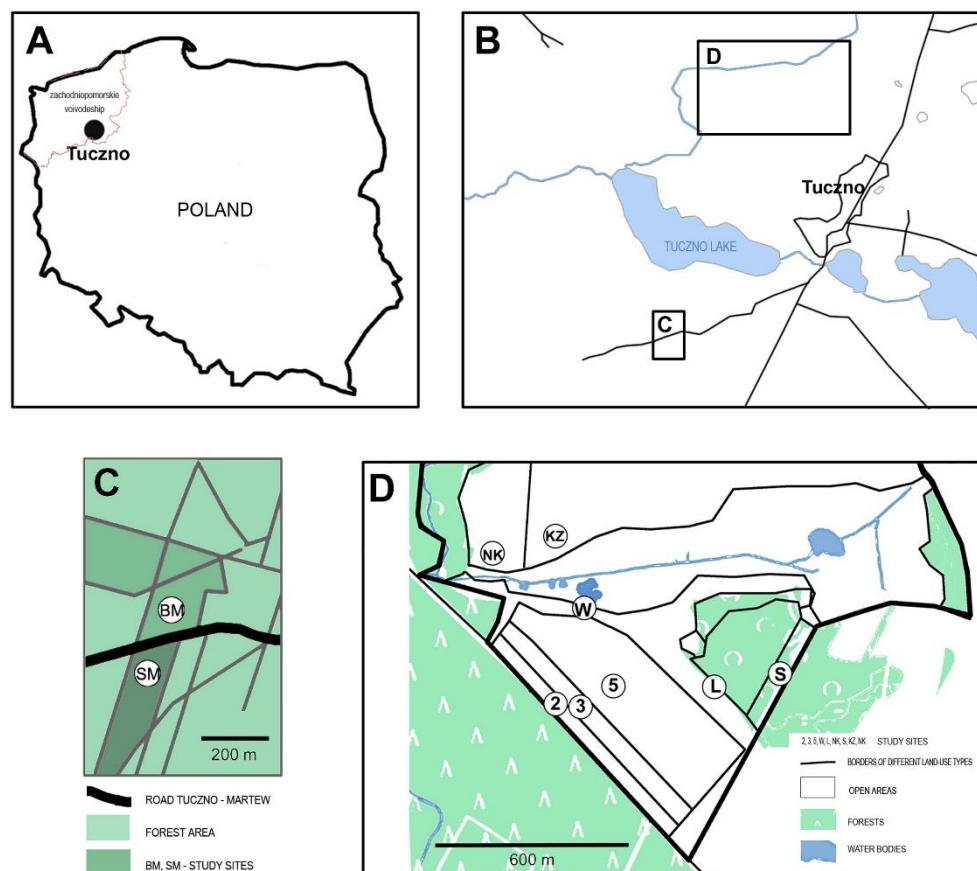
**Table 1.** Type and description of the study sites with information about the most dominant (% share of plant cover) plant species (information on age as of 2018).

Study Site	Type	Description	Dominant Plant Species
2	Fallow	Mown post-agricultural ground without biomass removal.	<i>Anthoxanthum odoratum</i> , <i>Pleurozium schreberi</i> , <i>Holcus lanatus</i> , <i>Deschampsia flexuosa</i>
3	Fallow	Mown post-agricultural ground with biomass removal.	<i>Anthoxanthum odoratum</i> , <i>Hieracium pilosella</i> , <i>Festuca rubra</i> , <i>Armeria elongata</i>
5	Fallow	Non-mown post-agricultural ground.	<i>Anthoxanthum odoratum</i> , <i>Pleurozium schreberi</i> , <i>Deschampsia flexuosa</i> , <i>Phleum pretense</i>
L	Ecotone	Ecotone between forest and fallow ground.	<i>Sarothamnus scoparius</i> , <i>Anthoxanthum odoratum</i> , <i>Pinus silvestris</i> , <i>Agrostis capillaris</i>
W	Ecotone	Ecotone between swamp and fallow ground.	<i>Agrostis capillaris</i> , <i>Arrhenatherum elatius</i> , <i>Festuca rubra</i> , <i>Phalaris arundinacea</i>
NK	Meadow	Non-mown meadow.	<i>Festuca rubra</i> , <i>Pleurozium schreberi</i> , <i>Arrhenatherum elatius</i> , <i>Agrostis capillaris</i>
KZ	Meadow	Mown meadow with biomass removal.	<i>Agrostis capillaris</i> , <i>Arrhenatherum elatius</i> , <i>Anthoxanthum odoratum</i> , <i>Dactylis glomerata</i>
S	Forest	Approximately 19 year old pine forest resulting from natural succession.	<i>Pinus silvestris</i> , <i>Padus serotina</i>
BM	Forest	Approximately 95 year old beech forest.	<i>Fagus sylvatica</i> , <i>Polytrichastrum formosum</i> , <i>Carex pilulifera</i> , <i>Deschampsia flexuosa</i>
SM	Forest	Approximately 46 year old pine forest.	<i>Pinus silvestris</i> , <i>Pleurozium schreberi</i> , <i>Vaccinium myrtillus</i> , <i>Deschampsia flexuosa</i>

### 2.2. Field Methods

#### 2.2.1. Soil Samples and Analyses

The systematic position of the soils was established in accordance with the classification of forest soils in Poland [41] and the FAO–WRB classification [42]. Soil samples were collected at each of the 6 sampling plots at each study site. Soil sampling for physicochemical and physical properties was performed with a sampler from organic and mineral horizons from different depths; in the present study, data from depths of 0–5 cm and 5–10 cm were used. Laboratory analyses for carbon, nitrogen, acidity, sorption capacity and granulometric composition were performed. For the study of biochemical and biological properties, soil samples were taken from the turf and mineral horizons from a depth of 0–20 cm. For analyses of physicochemical and biochemical properties, the bulk sampling method was applied. The samples were taken from 6 randomly selected places within the basic plots and the material was pooled. Sampling was performed during August–September in 2017 and 2018.



**Figure 1.** Location of the study sites in the area of the Tuczno State Forest District. (A) Location of Tuczno in Poland; (B–D) location of the study sites. Designation of study sites as in Table 1.

Soil samples were collected from the distinguished genetic horizons. Particular parameters were determined using the following methods [43–45]: Grain size composition was analyzed using the areometric method by Bouyoucos in the modification of Cassagrande and Prószyński; grain size fractions were determined in line with PTG [46]. An investigation of soil chemical properties included the determination of the following: organic carbon (C<sub>org</sub>) by catalytic combustion to CO<sub>2</sub> at 900 °C on a Shimadzu 5000 A; total nitrogen (N<sub>t</sub>) by modified Kjeldahl method on a Kieltec–Tecator analyzer; pH in 1M KCl—potentiometrically; alkaline cations (Ca, Mg, K, Na); hydrolytic acidity—H<sub>h</sub>. The studies on soil microbiological properties included: the determination of the total number of bacteria on Bunt and Rovira medium using the deep-seeded method, and determination of the total number of microscopic fungi on Martin medium using the deep-seeded method.

Soil dehydrogenase activity was determined by the photometric method described by Casida et al. [47]. TTC (2,3,5-triphenyltetrazolium chloride) was used as a substrate. The colored product (formazan) formed in the reaction after extraction with methanol was determined photometrically at 485 nm against the control samples. We determined soil  $\beta$ -glucosidase activity with the photometric method of Eivazi and Tabatabai [48] using p-nitrophenyl- $\beta$ -D-glucopyranoside as a substrate. Soil protease activity was determined from sodium caseinate as a substrate. The amount of resulting product was determined in compliance with the method of Ladd and Butler [49]. In order to determine urease activity, we used a urea solution. The enzyme activity was determined by the amount of ammonia produced in the reaction. The amount of ammonia was determined by the photometric method by Kandeler and Gerber [50].

### 2.2.2. Inventory of Plants

At each sampling plot, an area of 20 m × 20 m inside the forest ecosystems and 5 m × 5 m in open areas (fallows and meadows) was marked in order to elaborate a phytosociological survey. Altogether, 60 surveys were carried out between 14 and 16 June 2018. The determination of plant species was based on the nomenclature by Mirek et al. [51]. The surveys were elaborated by recording the species and describing their occurrence using the cover-abundance scale of Braun-Blanquet [52]. Total cover for each plant layer of a particular sampling plot was noted in the field. For each phytosociological survey, the values of coverage of the plant species were transformed to a value of mean percentage cover by applying Braun-Blanquet [52]: +—0.1%, 1—5%, 2—17.5%, 3—37.5%, 4—62.5% and 5—87.5%. Later on, mean percentages of species covers for a particular sampling plot (survey) were summed up and subsequently recalculated into 100% to define the value of species dominance (in %) for each study plot.

### 2.2.3. Inventory of Carabids and Butterflies

Live traps were set on each sampling plot, i.e., 6 traps per study site (60 traps altogether), to trap epigeic fauna. In 2018, the traps were installed for 24 h at bi-weekly intervals from May to October in order to cover the time of major activity of carabid beetles. The traps consisted of a 2 m plastic fence, which was dug about 10 cm deep into the ground and protruding about 10 cm above the surface of the ground, and 8 plastic cups. Two cups were placed adjacent to each side of the fence, and one cup was placed adjacent to both ends of the fence. Hence, individuals walking towards the fence were directed either into the cups adjacent to the fence sides or the cups adjacent to the ends of the fence. All carabid beetles were picked from the traps and identified at the species level. Identification and nomenclature were performed following the system proposed by Freude et al. [53].

In 2018, butterflies were caught on each study site by using an insect net. Line transects were delineated at each study site in accordance with the methodology adopted in butterfly surveys [54]. A transect was defined as a 5 m wide strip of land, along which the observer moved at a slow pace (about 3 km/h) to catch, record and photograph adult butterflies. The recorded butterflies were assigned to the nearest sampling plot. All individuals assigned to a sampling plot were defined as one sample. Observations were conducted for one day twice per month between May and September 2018. This period was associated with the best weather conditions for butterfly emergence. Butterflies were identified at species level based on the literature [55,56]. Nomenclature was carried out in line with Buszko and Masłowski [55].

## 2.3. Data Analysis

### 2.3.1. Study Site Characterization

For each study site, we calculated the mean value of each soil parameter together with the standard deviation in order to analyze the variability in data of the study sites. With respect to plant diversity, we calculated Bray–Curtis similarity indices between all pairs of the plots at a study site (15 values per study site); we then calculated the respective mean values and standard deviations. The statistical analyses were carried out using PAST v. 4.03 [57].

We applied indirect gradient analysis in order to analyze the variation between the sampling plots with respect to soil parameters and plants using Canoco for Windows v. 4.56 and CanoDraw for Windows v. 4.14 [58,59]. Detrended Correspondence Analyses (DCA) were first applied in order to select the appropriate statistical model based on the longest gradient [60]. Regarding the soil parameters, we applied Principal Components Analysis (PCA) with inter-sample distance scaling and dividing of species scores (i.e., soil data) by standard deviation. The soil data were not transformed. With respect to the plants, a Correspondence Analysis (CA) was carried out with inter-sample distance scaling, specifically Hill's scaling. As the values of species dominance were used, the species data were not transformed. In both the diagram of the PCA and the diagram of the CA, the

ranges of the sampling plots of the respective study sites were visualized by drawing polygons enclosing them.

### 2.3.2. Response of Carabids and Butterflies

With respect to carabid beetles and butterflies, the data collected at the individual study plots were pooled for each study site. For each study site for both taxonomic groups, the dominance values (percentage share of the respective species in a sample) were calculated.

In order to study the distribution of carabid beetles and butterflies on the study sites and their response to the study site characteristics, direct gradient analyses were carried out using Canoco for Windows v. 4.56 and CanoDraw for Windows v. 4.14 [58,59]. The mean values of the studied soil parameters for the study sites and the mean values of Bray–Curtis similarity of plants for the study sites were included as environmental variables. Detrended Canonical Correspondence Analyses (DCCA) were first applied in order to select the appropriate statistical model based on the longest gradient [60]; next, Canonical Correspondence Analyses (CCA) were used. CCA was carried out with inter-sample distance scaling, specifically Hill's scaling. As dominance values were used, the species data were not transformed. The significance of the individual environmental variables included in the CCA was tested using Monte Carlo permutation tests (unrestricted, 1999 permutations), initially for each variable separately and then using automatic forward selection of variables (reduced model) [59].

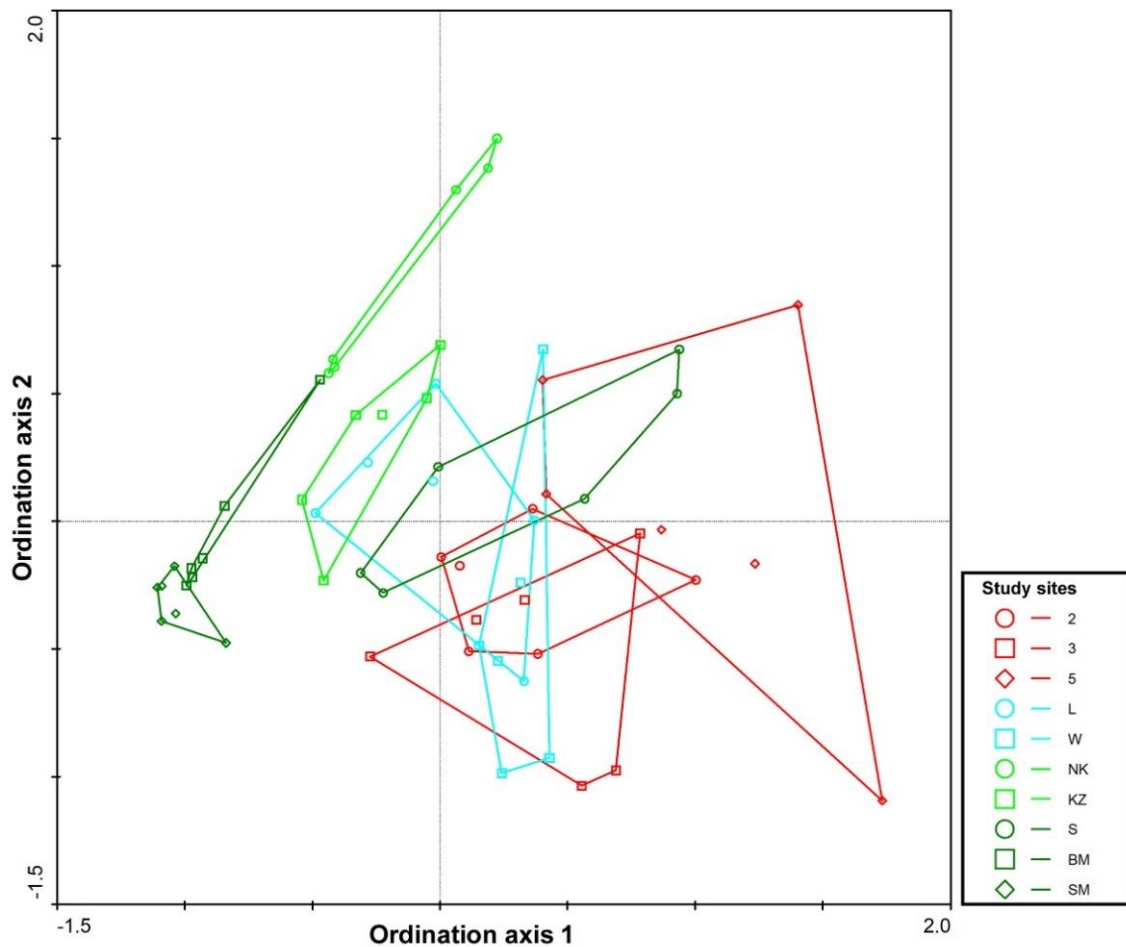
## 3. Results

### 3.1. Study Site Characterization

The sediments, from which the analyzed Brunic Arenosols and Albic Brunic Arenosols were developed, included glacial sands characterized by a loose sand fraction. They were characterized by the largest percentage contribution of three sand fractions (medium, fine and very fine). All studied soils had an acidic reaction in the entire profile, although varying among the genetic horizons and among the subtypes and varieties of subtypes ranging from very strongly acidic (3.15 pH) in the 46 year old pine forest (SM) to slightly acidic (4.67 pH) in the non-mown fallow (5).

Particularly high standard deviations in many soil parameters were revealed for the meadows, the approximately 95 year old beech forest (BM), the approximately 46 year old pine forest (SM) and the ecotone between swamp and fallow ground (W). The ecotone between forest and fallow ground (L), however, showed comparatively low standard deviations for many of the soil parameters. Regarding plant similarity, the forests showed the highest mean values. The lowest mean value was detected for the ecotone between swamp and fallow ground (W). Low mean values were also revealed for the meadows, whereas the ecotone forest and fallow ground (L) had a mean value similar to the fallow sites (Appendix A, Table A1).

In the PCA of the study sites based on the soil parameters, the first and second ordination axes explained 43.7% and 32.0% of the variance, respectively. In the diagram (Figure 2), the study sites are located along the first ordination axis in the order forests, meadows, ecotones and fallows, from left to right, with the exception of the young pine forest (S), which is located in the center of the diagram. The diagram revealed large polygons for the fallows, especially the non-mown fallow (5), and for both ecotones and the young pine forest (S). The polygon for the mown meadow (KZ) was a bit smaller, and the non-mown meadow (NK), the approximately 95 year old beech forest (BM) and the approximately 46 year old pine forest (SM) had relatively small polygons.



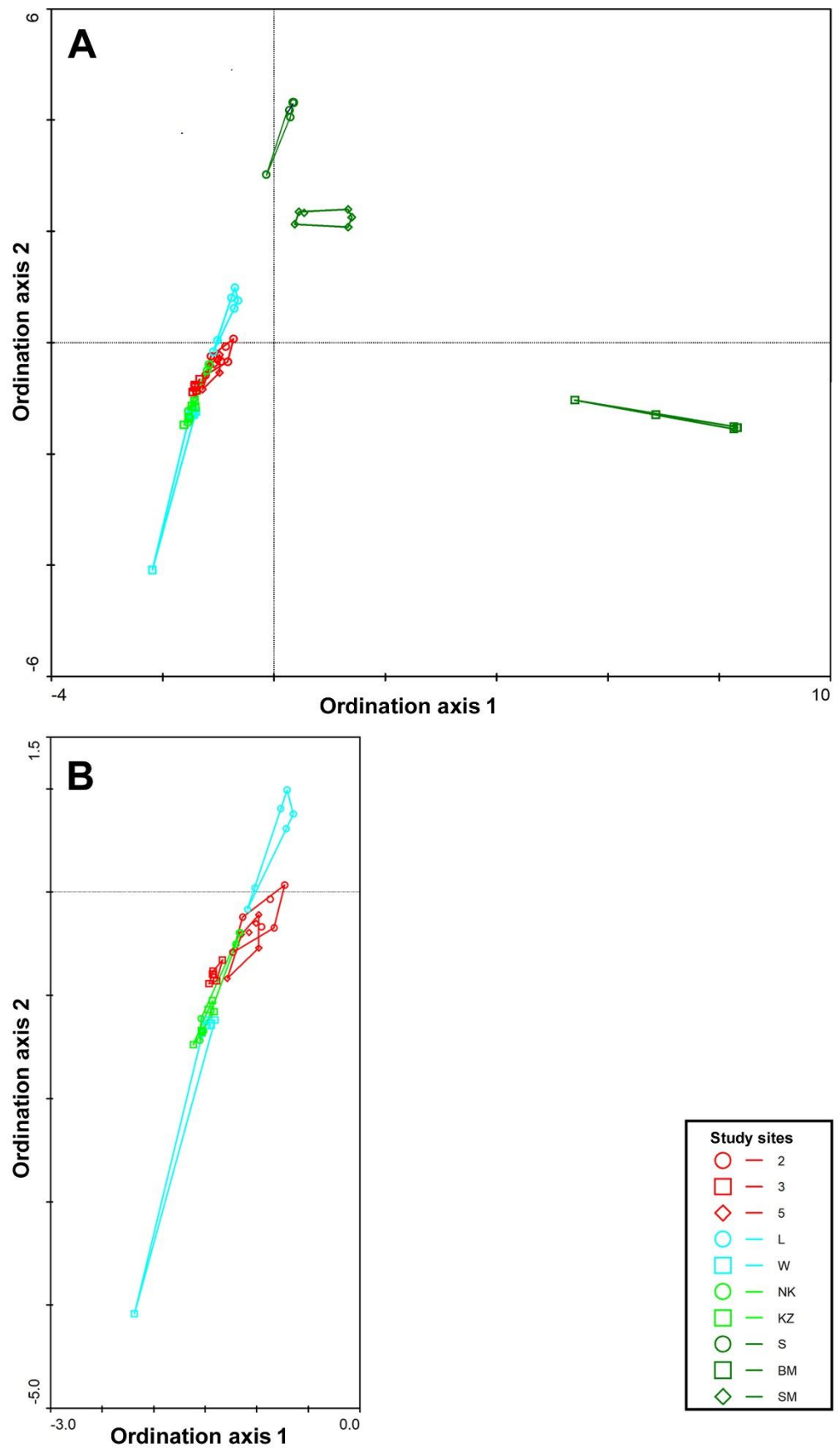
**Figure 2.** Ordination plot based on Principal Components Analysis (PCA) for the sampling plots based on the soil parameters. Numbers of study sites are listed in Table 1.

The first and second ordination axes of the CA with the study sites and the plants explained 12.8% and 11.1% of the variance, respectively. In the diagram (Figure 3), the majority of the study sites are located close to each other, with the exception of the forests. The two pine forests (S, SM) are separated from the other study sites along the second axis and the approximately 95 year old beech forest (BM) is separated along the first axis. In general, small polygons were displayed.

### 3.2. Carabids and Butterflies

Altogether, 2387 individual carabid beetles representing 55 species were collected (Appendix A, Tables A2 and A3). The numbers of species collected in the fallows, ecotones and meadows were similar, but lower numbers were registered in the forests. Particular high numbers of individuals were registered in the fallows; low numbers of individuals were revealed for the ecotone between swamp and fallow ground (W) and the pine forests (S, SM).

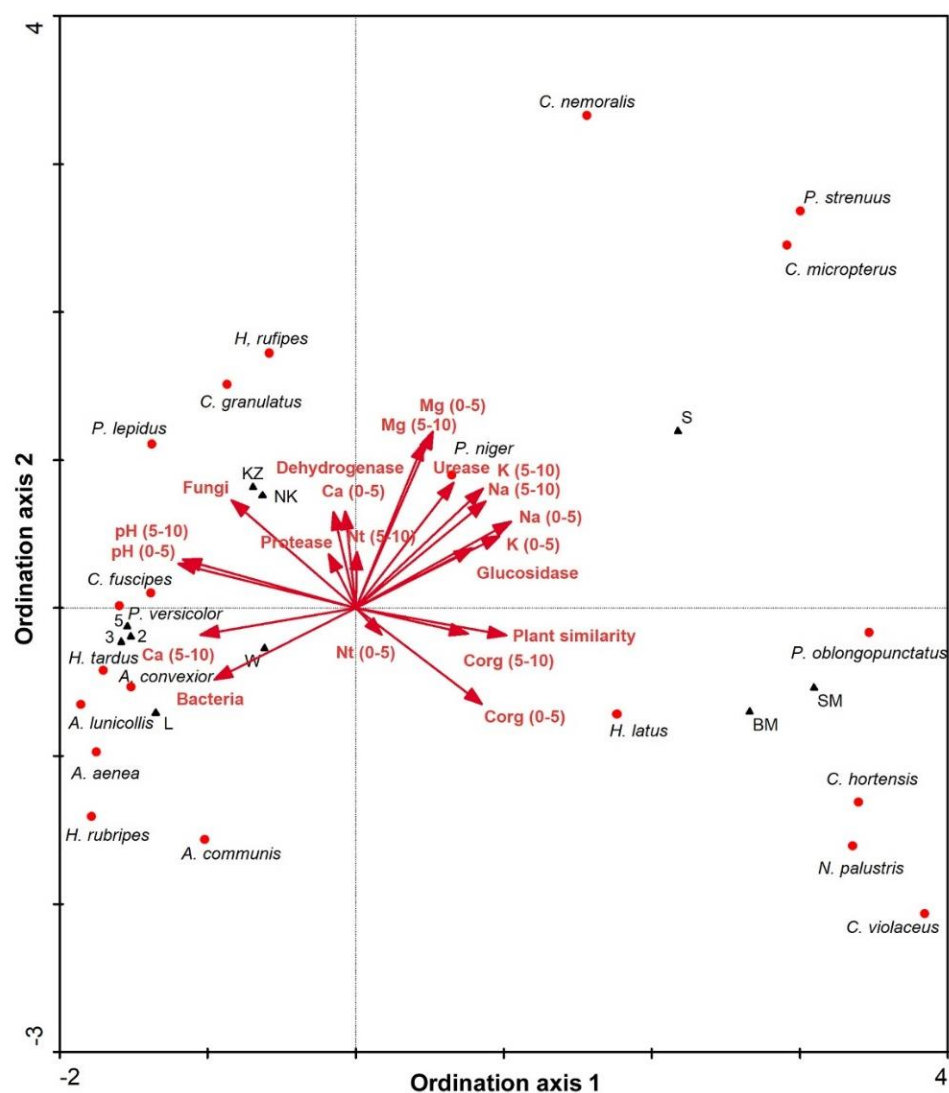
With respect to butterflies, we collected 1373 individuals belonging to 29 species (Appendix A, Tables A2 and A3). As for the carabid beetles, the numbers of species were comparable for fallows, ecotones and meadows, but with a higher variation. The numbers of individuals were very high for the mown fallows (2, 3) and the ecotone between forest and fallow ground (L). Butterflies were only sporadically proven in very low numbers of species and individuals in the forests (S, BM, SM).



**Figure 3.** Ordination plot Correspondence Analysis (CA) for the sampling plots based on plant species ((A): full diagram, (B): detail). Designation of study sites as in Table 1.



In the diagram of the CCA with the carabid beetles (Figure 4), the forests are located on the right side with the young pine forest (S), separated from the older forests (BM, SM) along the second ordination axis. The former is related to high values of NA, K and glucosidase, whereas the latter two are related to high values of Corg and plant diversity. Meadows (NK, KZ) and ecotones (L, W) are located on the left side of the diagram, with the meadows more in the upper part and the ecotones more towards the bottom part. Even more to the left and close to each other, the fallows are located (2, 3, 5). The meadows are particularly related to high values of fungi while ecotones and fallows are related to high values of Ca (5–10 cm) and bacteria. In accordance with the location of the study sites, carabid beetle species characteristics for forest habitats are situated on the right side of the diagram and the species typical of open areas on the left side.

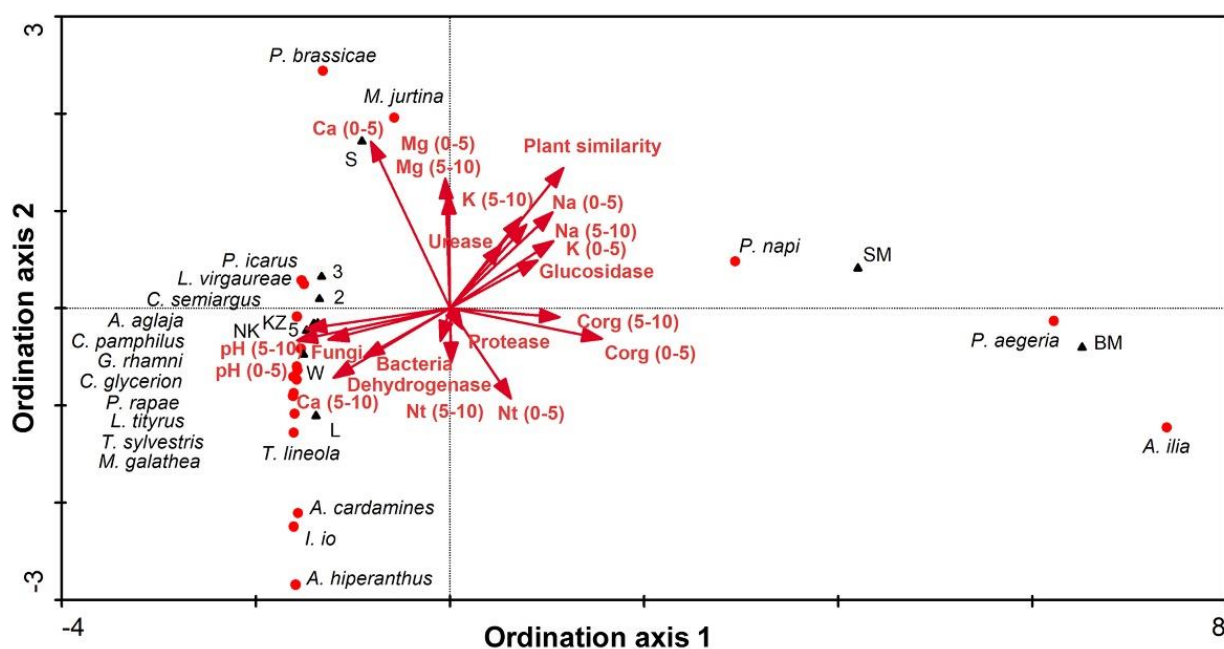


**Figure 4.** Ordination plot Canonical Correspondence Analyses (CCA) for the study sites (black triangles) and 20 carabid beetle species (red dots) that best fit into the ordination space and environmental variables (red arrows). Designation of study sites as in Table 1; environmental variables as in Appendix A, Table A1.

Testing the environmental variables separately revealed significant results for pH (0–5 cm), pH (5–10 cm), Na (0–5 cm), plant similarity, Ca (5–10 cm), K (0–5 cm), bacteria, Na (5–10 cm), Corg (0–5 cm), K (5–10 cm) and glycosidase. However, using forward selection of environmental variables, pH (0–5 cm) ( $\text{LambdaA} = 0.65$ ,  $p < 0.01$ ), Mg (0–5 cm)

( $\text{LambdaA} = 0.28, p < 0.05$ ) and dehydrogenase ( $\text{LambdaA} = 0.24, p < 0.05$ ) were proven to be statistically significant.

In the CCA diagram for the butterflies (Figure 5), the approximately 95 year old beech forest (BM) and the approximately 46 year old pine forest (SM) are located on the right side of the diagram; all other study sites are located on the left side. However, the young pine forest (S) is separated from the open areas (fallow, meadows, ecotones) along the second ordination axis. Corg is pointed at the direction of the older forests (SM, BM), Ca (0–5 cm) and Mg in the direction of the young pine forest (S). The fallows and meadows are particularly related to high pH values, the ecotones to bacteria and fungi. Almost all butterfly species are located on the left side of the diagram, with the exception of *Pieris napi*, *Pararge aegeria* and *Apatura ilia*, which are situated close to the forests. The latter two are species that show preferences for moist forests.



**Figure 5.** Ordination plot Canonical Correspondence Analyses (CCA) for the study sites (black triangles) and 20 butterfly species (red dots) that best fit into the ordination space and environmental variables (red arrows). Designation of study sites as in Table 1; environmental variables as in Appendix A, Table A1.

Separate testing of environmental variables revealed significant results for Corg (0–5 cm), pH (0–5 cm), pH (5–10 cm), plant similarity, fungi, Ca (5–10 cm), Corg (5–10 cm), Na (0–5 cm) and K (0–5 cm). When using forward selection of environmental variables, Corg (0–5 cm) ( $\text{LambdaA} = 0.72, p < 0.05$ ) and plant similarity ( $\text{LambdaA} = 0.33, p < 0.01$ ) were statistically significant.

## 4. Discussion

### 4.1. Limitation of the Study Design

The design of the study was not free from limitations. For example, the species assemblage of a given study site might be influenced by the surroundings [61,62]. We tried to reduce this impact by locating the sampling plots in the forests, fallows and meadows at least 30 m apart from the neighboring ecosystems.

Due to the high costs of biochemical analyses, soil sampling was performed only during August–September in 2017 and 2018. However, temperature and rainfall did not significantly affect the physicochemical properties of the soils under study, even if they could have an influence on the biochemical properties. Hence, our samples generally illustrate the properties of the soils under study.

We limited the period for the inventory of carabid beetles and butterflies. With respect to butterflies, we sampled during the period of best weather conditions for their emergence in 2018. Regarding carabid beetles, the sampling design, including the use of live traps, was used in order to reduce interference with the study sites and to avoid the death of other animals as far as possible. However, trapping was carried out during the major activity period for carabid beetles and the low amount of collecting days was compensated by using very effective traps with fences [63].

#### 4.2. Study Site Characterization

Our results showed that the study site types differed with respect to soil characteristics and plant diversity, but ecotones were not characterized by explicitly higher diversity in these parameters (hypothesis (1)). Differences between ecosystem types in mean values were basically as expected, such as the comparatively high carbon values in the soil of the two old forests, as also shown from former studies [40]. However, of special interest to our study were the standard deviations in soil characteristics because they express variability between the plots within one study site. The high variability (i.e., high standard variations) with respect to various parameters in the meadows may be due to the fact that these areas were located comparatively close to swamps. Some flooding events had taken place in the decades before the study, which may have caused the high variability. Research on meadows in Germany treated by flood pulse irrigation [64] showed high standard variations in soil parameters. This may also explain the more pronounced high standard deviations in the ecotone between swamp and fallow ground compared to the ecotone between forest and fallow ground. When looking at the full set of studied parameters together, high variability is indicated by large polygons in the PCA diagram for the fallows, especially the non-mown fallow (5), both ecotones and the young pine forest (S).

The small polygons in the ordination diagram based on the plants indicate that, generally, rather high similarities between the plots of individual study sites were obtained. The higher plant similarities between plots within forests were expected because a homogeneous regime of forest management influences, among other things, plant biodiversity [65]. Relatively small, but nonetheless present, differences in similarity within each of the forest study sites can be explained by the diversity of environmental conditions [66], such as the diversity of some soil parameters. While the plant communities of the young pine forest (S) and the 46 year old pine forest (SM) were relatively similar, due to the dominance of Scots pine in the stand, the beech-dominated study site (BM) was distinctly different from the previous ones. Alongside the management regime, the history of land use may also be of importance [67]. Ecotones at least partly fulfill the expectations of comparatively low plant similarity values, especially the ecotone between swamp and fallow ground, which is characterized by a steep moisture gradient; soil moisture is known to be an important environmental factor affecting plant species composition and diversity, e.g., Hettenbergerová et al. [68]. As with the soil parameters, flooding events may have had an influence too. On the other hand, diverse plant communities might be more tolerant to environmental stress caused by flooding as studies on the effect of a flood event on a plant diversity experiment in Germany indicated that more diverse communities grew more immediately following the flood [69]. Noticeably, the meadows are characterized by low plant similarities.

### 4.3. Carabids and Butterflies

For both carabid beetles and butterflies, characteristic assemblages for individual study sites could be demonstrated and expressed by the location of the study sites along the ordination axes in the CCA diagram (hypothesis (2)). We could also show differences in the most important factors between the groups (hypothesis (3)). The formation of characteristic assemblages in these two taxonomic groups for the study sites in the research area has been shown in former studies, e.g., Szyszko-Podgórska [33]; Schwerk et al. [70]. The sensitive reaction of carabid beetles to soil pH has also been previously reported, e.g., Koivula [25]; Zumstein et al. [71]; Nietupski et al. [72]. It can also be assumed that many other soil parameters correlate with soil pH [73,74]. Beside pH and the correlated parameters, Mg (0–5) and dehydrogenase were of impact, as shown by forward selection of variables. In our study, differences in moisture conditions, also caused by flooding events, also seemed to be of importance. The carabid species with preferences for moist conditions (see Appendix A, Table A3) were found in the ecotone between swamp and fallow ground (W) and also in low numbers in the meadows (MK, KZ). With respect to the butterflies, organic carbon in the soil was the most significant parameter. Hyvönen et al. [75], who compared biodiversity responses to seed mixtures and mowing in a long-term set-aside experiment, detected significant differences in butterfly species richness between study sites, which also differed significantly in soil carbon. Plausibly, forward selection of variables indicated plant similarity as an important factor. A comprehensive review of reports on insect decline revealed habitat loss by conversion to intensive agriculture, agro-chemical pollutants, invasive species and climate change as main drivers of these declines [76]. Differences in the most important factors between the two taxonomic groups can be explained by differences with respect to their ecological characteristics. Being very important in this context, food preferences can be assessed. Many carabid beetles are mostly carnivorous or omnivorous [19], whereas butterflies are herbivorous insects that depend on nectar-producing flowers and host plants for the caterpillars [56]. For example, *Apatura ilia*, whose caterpillars feed on deciduous tree species, was exclusively found in the approximately 95 year old beech forest (BM). Accordingly, the factor plant similarity occurred to be of higher importance for the butterfly assemblages. Differences in responses to environmental factors may lead to poor analogies in patterns between different taxonomic groups. For example, Koivula [25] stated that sets of carabid beetles are often poorly correlated with those of other taxonomic groups, e.g., spiders. Furthermore, studies on the effects of fen management in butterfly, grasshopper, and carabid beetle assemblages revealed taxon-specific responses [77]. In the farmland edges in the Czech Republic, carabids, butterflies, birds and small mammals showed only weak between-taxon correlations and often taxon-specific responses to geography, vegetation, adjoining site management and surrounding habitat diversity and edge density [78]. In our study, differences in moisture conditions, also due to flooding events, seemed to be an additionally important factor.

An essential question regarding the practical aspects of nature conservation asks to what degree the management type of ecosystems has an impact on their characteristics and biodiversity. Management practices in agricultural and forest ecosystems have been proven to have an impact on the formation of carabid beetle assemblages, e.g., Koivula and Niemelä [79], Kosewska [80], Skłodowski [28]. The carabid species *Calathus fuscipes* has been proven to benefit from mowing measures [21] and was very common in the mown fallows (2, 3) in our study. Moreover, agricultural management, plant species richness and landscape diversification had a significantly positive effect on butterfly species richness in a grassland–forest mosaic in the Italian Alps [81]. Based on studies in tallgrass prairie and pine barrens, Swengel [82] concluded that for the protection of specialist butterfly species, consistency of management within a site alongside differentiation among sites is desirable. For grasslands, Morris [20] emphasized the importance of integrating the theoretical and experimental aspects of grassland ecology with the practical expertise of reserve managers and conservation site officers. As individual taxonomic groups react differently, different strategies are demanded that match the respective taxonomic groups.

In this context, the spatial scale is also of importance. A study of the effects of agri-environmental measures on butterflies in Switzerland using a multiscale approach indicated that the effectiveness of ecological compensation areas depended both on local site conditions and the amount of ecological compensation areas and semi-natural elements in the surrounding landscape [83]. Scheper et al. [84], in their meta-analysis, showed that agri-environmental schemes can create a contrast in floral resources that impact the response of pollinators as butterflies, and this response is moderated by landscape context and farmland type. Moreover, some species demand different succession stages or ecosystems in a wider landscape for the establishment of their populations [85]. In order to protect such species, large-scale management strategies are necessary to maintain or create landscapes of high natural qualities.

## 5. Conclusions

In the paper, our aim was to show the differences in soil characteristics and plants between a number of selected ecosystems and ecotones, as well as the differences in assemblages of carabid beetles and butterflies. We also focused on analyzing to what degree the two taxonomic groups of animals differed in their response to these characteristics.

The variation in characteristics between the studied ecosystems and ecotones was due to the type of area, the land use history and the management; flooding events were assumed to be an additionally important factor. Carabid beetles and butterflies showed distinct responses to the different characteristics of the individual study sites, which can be explained by differences with respect to their ecological traits, such as their food preferences. We conclude that management practices in agricultural and forest ecosystems, such as those studied by us, have an important impact on the formation of carabid beetle and butterfly assemblages. In order to effectively protect species diversity, large-scale management strategies are necessary.

**Author Contributions:** Conceptualization: K.S.-P. and A.S.; methodology: K.S.-P., I.D., U.J., M.K., E.Ż.-Z. and A.S.; validation: K.S.-P., I.D., U.J., M.K., E.Ż.-Z. and A.S.; investigation: K.S.-P., I.D., U.J., M.K., E.Ż.-Z. and A.S.; resources: K.S.-P.; data curation: K.S.-P. and A.S.; writing—original draft preparation: K.S.-P., I.D., M.K. and A.S.; writing—review and editing: K.S.-P., I.D., U.J., M.K., E.Ż.-Z. and A.S.; visualization: A.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Data Availability Statement:** The data belong to the Institute of Environmental Protection-National Research Institute, Warsaw. Data may be accessed by contacting the first author of the publication.

**Acknowledgments:** This paper is communication no. 540 in the tradition of the Laboratory of Evaluation and Assessment of Natural Resources, Warsaw University of Life Sciences–SGGW.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A

**Table A1.** Characterization of the study sites by type, soil parameters and plant similarities (Bray–Curtis index). For soil parameters and plant similarities, mean values and standard deviations are shown.

Characteristic	Study Site									
	2	3	5	L	W	NK	KZ	S	BM	SM
Type	Fallow	Fallow	Fallow	Ecotone	Ecotone	Meadow	Meadow	Forest	Forest	Forest
Corg (0–5 cm) %	1.890 ± 0.178	2.183 ± 0.427	2.249 ± 0.473	1.618 ± 0.844	2.384 ± 0.697	2.204 ± 0.238	2.431 ± 0.467	1.458 ± 0.261	3.772 ± 1.515	4.023 ± 1.790
Corg (5–10 cm) %	1.248 ± 0.124	1.231 ± 0.155	1.234 ± 0.311	1.046 ± 0.236	1.536 ± 0.553	1.613 ± 0.454	2.288 ± 0.546	1.048 ± 0.091	2.006 ± 0.273	2.877 ± 2.580
Nt (0–5 cm) %	0.126 ± 0.011	0.152 ± 0.025	0.168 ± 0.051	0.119 ± 0.056	0.182 ± 0.053	0.180 ± 0.029	0.195 ± 0.033	0.102 ± 0.020	0.181 ± 0.069	0.189 ± 0.079
Nt (5–10 cm) %	0.091 ± 0.010	0.093 ± 0.013	0.079 ± 0.021	0.080 ± 0.017	0.124 ± 0.047	0.140 ± 0.037	0.192 ± 0.054	0.066 ± 0.004	0.095 ± 0.012	0.136 ± 0.122
Ca (0–5 cm) mg·100 g <sup>-1</sup>	43.804 ± 4.595	53.519 ± 13.777	69.658 ± 21.384	32.544 ± 18.074	54.508 ± 28.990	7.382 ± 4.624	22.167 ± 9.881	134.876 ± 40.245	4.283 ± 2.298	4.752 ± 2.000
Ca (5–10 cm) mg·100 g <sup>-1</sup>	27.734 ± 6.597	25.868 ± 7.870	31.298 ± 11.955	21.803 ± 8.323	33.900 ± 16.500	4.078 ± 3.120	23.921 ± 14.851	9.447 ± 3.781	2.473 ± 0.471	3.199 ± 1.445
Mg (0–5 cm) mg·100 g <sup>-1</sup>	0.070 ± 0.018	0.084 ± 0.045	0.276 ± 0.237	0.088 ± 0.099	0.130 ± 0.084	12.751 ± 7.955	8.333 ± 4.537	12.851 ± 6.642	3.347 ± 1.542	4.110 ± 2.241
Mg (5–10 cm) mg·100 g <sup>-1</sup>	0.022 ± 0.007	0.014 ± 0.006	0.034 ± 0.022	0.023 ± 0.014	0.033 ± 0.012	4.532 ± 2.431	11.995 ± 7.132	9.389 ± 6.525	3.016 ± 1.491	2.657 ± 1.407
K (0–5 cm) mg·100 g <sup>-1</sup>	0.027 ± 0.007	0.039 ± 0.017	0.056 ± 0.021	0.020 ± 0.011	0.029 ± 0.010	1.866 ± 0.982	0.979 ± 0.889	1.329 ± 0.489	1.912 ± 0.619	1.328 ± 0.697
K (5–10 cm) mg·100 g <sup>-1</sup>	0.014 ± 0.003	0.010 ± 0.003	0.023 ± 0.015	0.010 ± 0.004	0.013 ± 0.002	0.811 ± 0.521	0.748 ± 0.542	0.769 ± 0.339	0.748 ± 0.235	0.519 ± 0.496
Na (0–5 cm) mg·100 g <sup>-1</sup>	0.013 ± 0.002	0.016 ± 0.005	0.020 ± 0.007	0.009 ± 0.004	0.012 ± 0.002	0.249 ± 0.001	0.271 ± 0.051	0.291 ± 0.064	0.333 ± 0.064	0.270 ± 0.051
Na (5–10 cm) mg·100 g <sup>-1</sup>	0.006 ± 0.002	0.006 ± 0.003	0.011 ± 0.005	0.006 ± 0.002	0.007 ± 0.001	0.270 ± 0.050	0.333 ± 0.064	0.249 ± 0.001	0.249 ± 0.001	0.270 ± 0.051
pH (0–5 cm)	4.655 ± 0.132	4.582 ± 0.226	4.673 ± 0.405	4.313 ± 0.072	4.453 ± 0.548	4.000 ± 0.110	4.480 ± 0.351	4.058 ± 0.122	3.317 ± 0.059	3.157 ± 0.076
pH (5–10 cm)	4.650 ± 0.156	4.613 ± 0.518	4.292 ± 0.237	4.235 ± 0.144	4.232 ± 0.307	4.030 ± 0.065	4.445 ± 0.353	4.060 ± 0.204	3.557 ± 0.067	3.557 ± 0.067
Dehydrogenase (0–20 cm) µg TFP 24 h 10 g <sup>-1</sup>	0.153 ± 0.052	0.174 ± 0.052	0.197 ± 0.065	0.119 ± 0.029	0.123 ± 0.030	0.322 ± 0.133	0.315 ± 0.108	0.122 ± 0.049	0.182 ± 0.086	0.187 ± 0.068
Protease (0–20 cm) mg tyrosine kg <sup>-1</sup> h <sup>-1</sup>	0.263 ± 0.041	0.272 ± 0.075	0.348 ± 0.153	0.206 ± 0.070	0.246 ± 0.122	0.304 ± 0.118	0.334 ± 0.029	0.244 ± 0.108	0.320 ± 0.337	0.235 ± 0.085
Glukosidase(0–20 cm) mM pN P·kg <sup>-1</sup> h <sup>-1</sup>	0.446 ± 0.161	0.491 ± 0.086	0.617 ± 0.248	0.404 ± 0.146	0.316 ± 0.095	1.242 ± 0.507	1.412 ± 0.516	0.799 ± 0.272	1.072 ± 0.358	1.585 ± 1.032
Urease (0–20 cm) mg NH <sub>3</sub> g <sup>-1</sup> 24 h <sup>-1</sup>	0.555 ± 0.238	0.668 ± 0.138	0.704 ± 0.183	0.572 ± 0.176	0.548 ± 0.221	3.730 ± 0.791	3.563 ± 1.246	2.595 ± 0.805	2.620 ± 0.631	2.380 ± 0.937
Bacteria (0–20 cm) CFU/g <sup>-1</sup>	82.500 ± 29.912	97.000 ± 37.342	103.000 ± 57.838	47.500 ± 37.023	81.667 ± 20.801	7.317 ± 3.975	21.300 ± 8.080	12.567 ± 4.668	9.683 ± 1.869	12.100 ± 11.606
Fungi (0–20 cm) CFU/g <sup>-1</sup>	79.667 ± 25.216	62.333 ± 28.069	128.333 ± 37.425	82.333 ± 21.658	65.500 ± 43.514	138.833 ± 45.305	86.000 ± 30.509	81.833 ± 44.853	37.833 ± 29.728	11.000 ± 5.288
Plant similarity	0.503 ± 0.100	0.599 ± 0.088	0.511 ± 0.094	0.550 ± 0.101	0.366 ± 0.157	0.400 ± 0.188	0.474 ± 0.099	0.745 ± 0.236	0.701 ± 0.207	0.759 ± 0.083

**Table A2.** Numbers of species and individuals of carabid beetles (Carabidae) and butterflies (Lepidoptera) collected at the study sites.

Parameter	Study Site									
	2	3	5	L	W	NK	KZ	S	BM	SM
Type	Fallow	Fallow	Fallow	Ecotone	Ecotone	Meadow	Meadow	Forest	Forest	Forest
Carabidae (species)	20	18	18	23	20	22	23	13	16	9
Carabidae (individuals)	445	627	380	197	68	130	211	69	188	72
Lepidoptera (species)	21	17	15	20	13	17	16	3	2	3
Lepidoptera (individuals)	398	233	114	256	68	164	126	5	4	5

**Table A3.** List of collected carabid beetle species (in alphabetical order) and collected butterfly species (in alphabetical order) with information about preferred habitats. Habitat preferences were elaborated based on the literature [53,55,56,86–89].

Species	Habitat Preference
<b>Carabid Beetles (Carabidae)</b>	
<i>Agonum fuliginosum</i>	Forest habitats
<i>Amara aenea</i>	Open habitats
<i>Amara communis</i>	Eurytopic species
<i>Amara consularis</i>	Open habitats
<i>Amara convexior</i>	Open habitats
<i>Amara equestris</i>	Open habitats
<i>Amara familiaris</i>	Eurytopic species
<i>Amara lunicollis</i>	Eurytopic species
<i>Amara ovata</i>	Open habitats
<i>Amara plebeja</i>	Open habitats
<i>Amara similata</i>	Open habitats
<i>Anisodactylus nemorivagus</i>	Open habitats
<i>Badister bullatus</i>	Open habitats
<i>Badister lacertosus</i>	Forest habitats
<i>Bembidion lampros</i>	Open habitats
<i>Calathus erratus</i>	Eurytopic species
<i>Calathus fuscipes</i>	Open habitats
<i>Calathus melanocephalus</i>	Eurytopic species
<i>Calathus micropterus</i>	Forest habitats
<i>Carabus granulatus</i>	Eurytopic species/moist
<i>Carabus hortensis</i>	Forest habitats
<i>Carabus nemoralis</i>	Forest habitats
<i>Carabus violaceus</i>	Forest habitats
<i>Clivina fossor</i>	Open habitats

Table A3. Cont.

Species	Habitat Preference
<i>Cychnus caraboides</i>	Forest habitats
<i>Elaphrus riparius</i>	Open habitats/moist
<i>Harpalus griseus</i>	Open habitats
<i>Harpalus latus</i>	Eurytopic species
<i>Harpalus luteicornis</i>	Eurytopic species
<i>Harpalus pumilus</i>	Open habitats
<i>Harpalus rubripes</i>	Open habitats
<i>Harpalus rufipalpis</i>	Eurytopic species
<i>Harpalus rufipes</i>	Open habitats
<i>Harpalus tardus</i>	Eurytopic species
<i>Harpalus xanthopus</i>	Forest habitats
<i>Lebia chlorocephala</i>	Eurytopic species
<i>Leistus terminatus</i>	Forest habitats
<i>Notiophilus palustris</i>	Forest habitats
<i>Oodes helopioides</i>	Open habitats/moist
<i>Oxypselaphus obscurus</i>	Forest habitats
<i>Poecilus cupreus</i>	Open habitats
<i>Poecilus lepidus</i>	Open habitats
<i>Poecilus versicolor</i>	Open habitats
<i>Pterostichus diligens</i>	Forest habitats/moist
<i>Pterostichus melanarius</i>	Eurytopic species
<i>Pterostichus niger</i>	Forest habitats
<i>Pterostichus nigrita</i>	Eurytopic species/moist
<i>Pterostichus oblongopunctatus</i>	Forest habitats
<i>Pterostichus rhaeticus</i>	Eurytopic species/moist
<i>Pterostichus strenuus</i>	Forest habitats
<i>Pterostichus vernalis</i>	Eurytopic species
<i>Syntomus foveatus</i>	Open habitats
<i>Syntomus truncatellus</i>	Open habitats
<i>Synuchus vivalis</i>	Eurytopic species
<i>Zabrus tenebrioides</i>	Open habitats
<b>Butterflies (Lepidoptera)</b>	
<i>Anthocharis cardamines</i>	Open areas, forest edges
<i>Apatura ilia</i>	Open areas, forest edges, forests/moist
<i>Aphantopus hyperanthus</i>	Open areas
<i>Araschnia levana</i>	Open areas, forest edges, forests
<i>Argynnis aglaja</i>	Open areas, forest edges
<i>Argynnis paphia</i>	Open areas, forest edges
<i>Coenonympha glycerion</i>	Open areas, forest edges



Table A3. Cont.

Species	Habitat Preference
<i>Coenonympha pamphilus</i>	Open areas, forest edges
<i>Colias hyale</i>	Open areas
<i>Cyaniris semiargus</i>	Open areas, forest edges
<i>Gonepteryx rhamni</i>	Open areas, forest edges, forests
<i>Inachis io</i>	Open areas, forest edges
<i>Issoria lathonia</i>	Open areas
<i>Lycaena dispar</i>	Open areas
<i>Lycaena tityrus</i>	Open areas, forest edges
<i>Lycaena virgaureae</i>	Open areas, forest edges
<i>Maniola jurtina</i>	Open areas, forest edges
<i>Melanargia galathea</i>	Open areas, forest edges
<i>Nymphalis antiopa</i>	Open areas, forest edges, forests
<i>Papilio machaon</i>	Open areas
<i>Pararge aegeria</i>	Forests/moist
<i>Pieris brassicae</i>	Open areas, forest edges
<i>Pieris daplidice</i>	Open areas
<i>Pieris napi</i>	Open areas, forest edges
<i>Pieris rapae</i>	Open areas, forest edges
<i>Polyommatus icarus</i>	Open areas, forest edges
<i>Thymelicus sylvestris</i>	Open areas, forest edges
<i>Thymelicus lineola</i>	Open areas, forest edges
<i>Vanessa atalanta</i>	Open areas, forest edges

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