Roads affect the spatial structure of butterfly communities in grassland patches (#24703)

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Roads affect the spatial structure of butterfly communities in grassland patches

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Roads may have important negative effect on ~~the~~ animal dispersal rate, mortality and thus functioning of local populations. Road~~s~~ verges may be also surrogate habitat for ~~some species, mostly~~ invertebrates. This creates a conservation dilemma around the impact of roads on invertebrates~~, also because~~ the effect of roads on invertebrates is much less understood than in vertebrates. We studied the structure of butterfly and plant communities in ten grassland patches neighboring roads (~ 50-100 vehicles per hour) and ten control grassland patches located apart from major roads in southern Poland. Five 200-m transects: at a road verge, 25 m apart from the verge inside the patch, inside the patch interior, 25 m from the boundary between grassland and field and at the grassland-arable field boundary ~~were established in every grassland patch~~. Control grasslands bordered ~~with~~ a ~~dirty~~ road (<1 vehicle per day). Within-patch analysis for grasslands bordering ~~with~~ roads indicated that butterfly species richness was similar in different parts of the grassland patch. Abundance of butterflies was higher at road verges and inside the grassland patch than at the boundary with arable fields. In control ~~meadows~~ there were no differences in butterfly species richness nor abundance in different parts of grassland patch. Partial redundancy analysis revealed that roads differentiate butterfly community structure within grassland patches. Road verges, habitat interior and boundary with arable field contributed the most to the species differentiation within grassland patches at roads. These effects were not found in control grassland patches where butterfly communities were more homogenously distributed in a patch. In ~~total,~~ the butterfly but not plant communities in grasslands ~~at~~ roads differed from grasslands located apart. The diversified structure of butterfly community within patches near roads was also visible in total number of species that was higher there than in control grassland patches. ~~Plant community did not differ between two types of grassland.~~ ~~Also,~~ road mortality rate was low and accounted for <5 % of butterflies occurring at the boundary between grassland and a road.

Nevertheless, species composition of roadkilled butterflies was well explained by butterflies community living on road verges rather than total butterfly community structure in grassland patches. These results may be explained by that (1) grasslands located at roads are less isolated from other habitats than control grasslands, (2) road verges could be dispersal corridors for butterflies and (3) that roads create a gradient of local environmental conditions that favour specific species and increase a total species richness in grasslands located at roads. This study is the first one showing that butterfly assemblages are altered by roads. However, no evidence was found for a substantial negative effect of roads on butterfly and plant species richness nor abundance.

Roads affect the spatial structure of butterfly communities in grassland patches

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Abstract

Roads may have important negative effect on the animal dispersal rate, mortality and thus functioning of local populations. Roads verges may be also surrogate habitat for some species, mostly invertebrates. This creates a conservation dilemma around the impact of roads on invertebrates, also because the effect of roads on invertebrates is much less understood than in vertebrates. We studied the structure of butterfly and plant communities in ten grassland patches neighboring roads (~ 50-100 vehicles per hour) and ten control grassland patches located apart from major roads in southern Poland. Five 200-m transects: at a road verge, 25 m apart from the verge inside the patch, inside the patch interior, 25 m from the boundary between grassland and field and at the grassland-arable field boundary were established in every grassland patch. Control grasslands bordered with a field road (<1 vehicle per day).

Within-patch analysis for grasslands bordering with roads indicated that butterfly species richness was similar in different parts of the grassland patch. However, abundance of butterflies was higher at road verges and inside the grassland patch than at the boundary with arable fields. In control meadows there were no differences in butterfly species richness nor abundance in different parts of grassland patch. Partial redundancy analysis revealed that roads differentiate butterfly community structure within grassland patches. Road verges, habitat interior and

boundary with arable field contributed the most to the species differentiation within grassland patches at roads. These effects were not found in control grassland patches where butterfly communities were more homogenously distributed in a patch. In total, the butterfly but not plant communities in grasslands at roads differed from control grasslands located apart. The diversified structure of butterfly community within patches near roads was also visible in total (pooled) number of species that was higher there than in control grassland patches. Plant community did not differ between two types of grassland. Also, road mortality rate was low and accounted for <5 % of butterflies occurring at the boundary between grassland and a road. Nevertheless, species composition of roadkilled butterflies was well explained by butterflies community living on road verges rather than total butterfly community structure in grassland patches. These results may be explained by that (1) grasslands located at roads are less isolated from other habitats than control grasslands, (2) road verges could be dispersal corridors for butterflies and (3) that roads create a gradient of local environmental conditions that favour specific species and increase a total species richness in grasslands located at roads. This study is the first one showing that butterfly assemblages are altered by roads. However, no evidence was found for a negative effect of roads on butterfly and plant species richness nor abundance.

Key words: Road mortality, assemblage, ordination, mitigation, verges, butterfly, plant

Introduction

Roads can exert severe impacts upon ~~the~~ animal populations (Benítez-López, Alkemade & Verweij, 2010; Matos et. al., 2017), either through direct road mortality (collisions with cars), or through habitat fragmentation and barrier effects increasing isolation of populations (Trombulak & Frissell, 2000; Forman et al., 2003; Tanner & Perry, 2007; Schuster, Römer & Germain, 2013). ~~So far~~, most studies focused on the estimates of road mortality since millions of individuals from a wide range of taxonomic groups are being killed every year (Coelho, Kindel & Coelho, 2008; Grilo, Bissonette & Santos-Reis, 2009; Brzeziński, Eliava & Żmihorski, 2012). However, roads also change nearby environment via increasing influx of salt, pollutants and changes in microlimate or water regime (Forman, 2000; Forman et al., 2003; Jackson & Jobàggy, 2005; Green, Machin & Cresser 2006). These changes are especially well exhibited in changes in plant communities near roads compared to more distant habitats (Lee, Davies & Power, 2012; Neher, Asmussen & Lovell, 2013). Many herbivores are dependent on specific plants (Stam et al. 2013). Among insects butterflies are directly dependent on several plant species during different parts of their life. Butterfly larvae usually have specific host-plants species/groups while adults often use specific plant species as the nectar sources (Munguira, Garcia–Barros & Cano, 2009). In a result, there is often observed strong correlation between butterfly and plant species richness (Skórka et al., 2007; Kitahara, Yumoto & Kobayashi, 2009; Chmura, Adamski & Denisiuk 2013). It may thus be expected that all alterations to plant communities by creation or existence of roads should be indirectly visible also in butterfly community structure as well as total species richness and abundance. However, road may also change insect community in a different manner. Road verges, which are linear grassy structures accompanying roads, are regarded as a good surrogate habitat for plants and may act as their dispersal corridors (Tikka et al., 2001; Kalwij, Milton & McGeoch, 2008). This suggests that grassland patches neighboring roads ~~should~~ have higher species richness and abundance of butterflies. This possibly positive effect may be, of course, diminished by road mortality which can be high in butterflies (Skórka et al., 2015; Baxter-Gilbert et al., 2015). Therefore, complexity of direct and indirect possible effects road can exert on butterflies make these insects especially interesting subject to study in order to understand impact of roads on insects at the community level. This is important for effective mitigation measures to minimize impacts of existing and future roads on insect populations – a subject which is rarely studied despite insects are among the most abundant roadkills (Baxter-Gilbert et al., 2015; Munoz, Torres & Gonzalez-Megias, 2015).

In this paper we analyze the effect of road proximity on plant and butterfly communities and we try to disentangle the questions: (1) whether roads change butterfly communities, (2) how plant community and road alone contribute individually to alteration of butterfl~~ies~~ communities ~~at~~ roads, (3) ~~whether butterfly community is correlated with road mortality~~.

Study area and methods

~~The choice of research~~ areas.

The study was conducted in the vicinity of Krakow, Proszowice and Tarnow (southern Poland). We selected 10 ~~meadows~~ adjacent to roads with ~~large~~ traffic (national and provincial roads ~ 50 -

100 vehicles per hour, one lane in each direction), and 10 reference (control) meadows ~~apart (~~at least 200 m~~)~~ from major roads with access via ~~field~~ field road (<1 vehicle per day).

Each meadow was similar in size (7.8-13.5 hectares) and similar type (wet grassland *Molinietialia*) and surrounding landscape. In every grassland patch at a road we established 5 transects 200 long where butterflies and plants were surveyed. ~~Transects were chosen so that ran along a straight line, without shrubs and trees. The first transect was located at the verge (the border between the meadow and the road. The second and third transect was located 25 from the edge of the road and inside the patch interior. The fourth transect ran 25 meters from the border between meadows and arable field. The fifth transect ran on the border between grassland and cropland. Purpose~~ of this design was to create additional control that enabled separation of the sole effect of a road from the impact of a border itself. Changes in habitat conditions (soil, vegetation) apply to most areas to a distance of about 50 meters from the edge of the road (Forman & Alexander, 1998).

In case of control grassland patches the design of transects was identical as grassland at major roads. In all control meadows there were field roads that allowed farmers to reach the area. However, they were grassy and the number of vehicles was less than one per day.

Butterfly and plant surveys

Butterflies were counted on transects during twelve surveys from mid April to mid-September in about 10-14 day intervals in 2013. ~~Each transect was 5 m wide, the observer was moving in the center line of the transect. Transect method,~~ is the standard and most commonly used method to study the population of butterflies (Pollard & Yates, 1993). Observations were carried out during good weather (minimum temperature 17 °C, wind up to 3° ~~in Beaufort~~, cloudy to 25%).

During each visit we also collected roadkilled butterflies at the 200 m part of the road neighbouring with studied meadows (both at major roads and field roads, but we did not find any dead butterfly at the latter). The transect was adjacent to the transect located at road verge where living butterflies were counted.

Plants were surveyed in one 4×10m rectangle plot located in the middle of each transect. Survey was performed at the beginning of July. During the survey, we noted the coverage of each plant species.

Statistical analysis

Within-habitat comparison of butterfly and plant communities

I used generalized linear mixed model (GLMM) with the negative binomial error structure and log-link function to test differences in butterfly and plant species richness, butterfly abundance and ~~cover of plants~~ between transect locations in different parts of the grassland patches. Patch identity was assigned as a random effect. Analyses were conducted separately for grassland patches located at roads and control ones. To find out which levels of the categorical factor were statistically different we used paired contrast analysis.

We used partial canonical correspondence analysis (partial CCA) to test if plant species composition and cover differs between transect locations in different parts of the grassland patches. We assigned patch identity as a supplementary variables that effect was to remove. Plant cover~~s were~~ square-root transformed before analysis. We used 1000 Monte Carlo permutation to test statistical significance of ordination axes and to find out which transect location ~~(categorical variable)~~ contributed significantly to the differentiation of plant communities. The partial CCA was calculated separately for grassland patches located at roads and ~~apart~~.

~~In case of butterfly~~ we used partial redundancy analysis (partial RDA) to test if butterfly species composition and abundance~~s~~ differed between transect locations in different parts of the grassland patches. We used this method instead of partial CCA because the longest ordination axis in detrended canonical correspondence analysis (DCCA) was short (1.4) so linear method was preferred (Jongman, ter Braak & van Tongeren, 1987). In other details the analysis was the same as in plants.

Between habitat comparisons of butterfly and plant communities

We used generalized linear model (GLM) with negative binomial error structure and log-link function to test differences in butterfly and plant species richness, butterfly abundance and plant cover between ~~two grassland types: located at roads and these located apart from road~~. ~~In plants we calculated mean cover for every plant species across five transect locations within a grassland patch.~~ In case of butterflies we calculated sum of the individuals from five transect located within a grassland patch to get proxy of total population sizes (Rosin et al., 2012; Skórka et al., 2013a). In addition, we also used individual-based rarefaction technique (Heck, van Belle & Simberloff, 1975) to test the differences in butterfly species number between grassland types taking sampling effort into the account. This analysis calculate expected number of species based on number of individuals sampled. We did not perform analysis for plants since we do not count individuals as it is difficult to assess what is an individual in plants.

We used partial RDA to test if butterfly species composition and abundances differs between transect locations in different parts of the grassland patches. Grassland type ~~(grassland at road and located apart)~~ was an explanatory variable. We also included covariates that effect was removed: transect location within a patch and plant richness and cover. In addition to partial RDA ~~W~~e performed variance partitioning to test ~~what is~~ relative contribution of (1) grassland type ~~(presence of neighbouring road or its absence)~~, (2) plant ~~data~~ and (3) their joint effect to differentiation of the butterfly community structure. Plant data were represented by the first principal component (PCA1) from number species and total cover of plants.

~~Moreover,~~ we performed partial RDA using butterfly data summed across transects for every grassland patch. However, results were the same. These summed data were used in co-correspondence analysis (Co-CA) (Braak ter & Schaffers, 2004) to reveal if butterfly species composition and abundance can be explained by plant community structure. In this analysis we used butterfly data summed and plant data averaged across the entire grassland patch, respectively, because currently this methods does not allow for effective removal of covariate data (Braak ter & Schaffers, 2004).

In case of plants we used partial canonical correspondence analysis (partial CCA) to test if plant species composition and mean cover differs between grassland located at roads and a~~part~~. We assigned transect location as a supplementary variable~~s~~ that effect was to remove. Plant cover~~s~~ w~~ere~~ square-root transformed before analysis. We used 1000 Monte Carlo permutation to test statistical significance of ordination axes ~~and to find out if two grassland types contribute significantly to differentiation of plant communities~~.

Testing the relation between communities of living and roadkilled butterflies

We used Co-Ca to test the relation between the butterfly community living in the grassland patch at roads and species composition of roadkilled butterflies. Two Co-Ca analyses were performed.

First we related total abundances of particular species recorded in all locations within grassland patch to see if road mortality is associated with the butterfly community across entire grassland patch. Secondly, we related abundances of species recorded on road verges to species composition and abundances of roadkilled butterflies. Given these two anaylses we could infer if impact of road mortality is grassland-wide or spatially limited to the ~~grassland part close to road~~. In addition to Co-Ca we used correlation analysis to seek for link between total species richness and abundance of living butterflies and species richness and abundance of roadkilled ones. Again, two sets of correlation analysis were performed: (1) for living butterflies summed across entire grassland patch and (2) for butterflies living on road verges only.

All GLMMs, GLM and correlation analyses were performed in SPSS 21 software. All partial CCA, partial RDA and Co-Ca analyses were performed in Canoco 5.0 software.

Results

Within-habitat variation in butterfly communities

In grassland patches located at roads there were no differences in butterfly species richness (GLMM, $F_{4,45} = 1.394$, $P = 0.251$, Figure 1). However, mean abundance of butterflies varied depending on location ~~of~~ within the patch (GLMM, $F_{4,45} = 3.440$, $P = 0.015$, Figure 1). Contrast analysis revealed that abundance was statistically higher inside habitat patch and 25 m from road verges than at the boundary with field boundary (Figure 1).

Partial redundancy ordination showed that location of transect explained 18.4 % variation in butterfly species composition in patches at roads (Figure 2). Ordination axes were statistically significant ($F = 2.0$, $P = 0.0005$). First ordination axis separated butterfly communities near patch boundaries from communities inside the habitat patch (Figure 2). Second ordination axis separated mostly butterfly communities near field boundaries from the communities at road verges (Figure 2). Accordingly, tests indicated that transects located inside habitat patch ($F = 3.0$, $P = 0.0005$), at road verges ($F = 2.3$, $P = 0.01$) and at field boundary ($F = 2.0$, $P = 0.0167$)

contributed significantly to differentiation of butterfly communities within grassland patches at roads (Figure 2).

In ~~grassland patches located apart from roads~~ there were no differences in butterfly species richness (GLMM, $F_{4,45} = 0.142$, $P = 0.966$, Figure 2) nor in mean abundance (GLMM $F_{4,45} = 1.476$, $P = 0.225$, Figure 2) in different parts of the patch.

First two axes of partial redundancy ordination showed that location of transect within the patches explained 11.7 % variation in butterfly species composition in control grasslands. (Figure 2). Ordination axes were statistically significant (test of all axes, $F = 1.5$, $P = 0.0345$). First ordination axis separated butterfly communities transects located 25 m from field boundary and it was statistically significant ($F = 2.6$, $P = 0.04$).

Within-habitat variation in plant communities

In grassland patches located at roads there were no differences in plant species richness (GLMM, $F_{4,45} = 2.359$, $P = 0.068$, Figure S1 in Supplementary information). However, mean plant species cover varied depending on location of within the patch (GLMM, $F_{4,45} = 3.190$, $P = 0.022$, Figure S1 in Supplementary information). Contrast analysis revealed that cover was statistically higher at road verges than in any other parts of the grassland patch but not inside the patch interior (Figure S1 in Supplementary information).

First two axes of partial canonical correspondence ordination explained 10.1 % variation in plant species composition (pseudo $F = 1.4$, $P = 0.0005$) and location of transect within the patch explained 13.7% of this variation (Figure S2 in Supplementary information). First ordination axis separated plant communities ~~located in proximity of a road~~ from plant communities inside the habitat patch (Figure S2 in Supplementary information). Second ordination axis separated plant community at field boundary and 25 m from road verge from plants recorded in other transects within grassland patches (Figure S2 in Supplementary information). Test of variables indicated that only transects located at road verges ($F = 2.6$, $P = 0.0025$) and 25 m from road verges ($F = 1.5$, $P = 0.0187$) contributed significantly to differentiation of plant communities within grassland patches at roads.

In grassland patches ~~located apart from roads~~ there were no differences in plant species richness (GLMM, $F_{4,45} = 0.274$, $P = 0.893$, Figure S1 in Supplementary information) and mean plant species cover between transect locations (GLMM $F_{4,45} = 0.483$, $P = 0.748$, Figure S~~1~~ in Supplementary information).

First two axes of partial canonical correspondence ordination explained 5.9 % variation in plant species composition in control grasslands. Location of transect within the patch explained 9.7% of this variation (Figure S2 in Supplementary information). However, ~~none~~ ordination axis was statistically significant (test of all axes, $F = 1.0$, $P = 0.6217$) and transect location within the grassland patch was non-significant.

~~Between-habitat comparison of butterfly communities~~

In total, there was statistically more butterfly species in grassland patches at roads than in grassland patches located far from roads (GLM $F_{1,18} = 5.545$, $P = 0.034$, Figure 3a) after controlling for plant richness and cover (PCA1plant; $F_{1,17} = 1.327$, $P = 0.265$). There was no differences in mean butterfly abundance (GLM $F_{1,17} = 0.133$, $P = 0.720$, Figure 3b) between two grassland types but abundance was possibly positively correlated with plant species and cover (PCA1plant; GLM $F_{1,17} = 4.397$, $P = 0.051$). Also, rarefaction analysis revealed that estimated number of species was higher in grassland patches located at roads than in control grasslands (Figure 5).

Co-correspondence analysis indicated that plant community (total ~~interia~~ = 7.162) does not explain butterfly species composition (total interia = 0.91): two CoCA axes explained 28 % variation in butterfly community composition, however test of first ordination axis was statistically non-significant (lambda = 0.0093, $P = 0.128$) ~~nor test on both~~ ordination axes (trace = 0.067, $P = 0.238$). Species richness of butterflies correlated with plant species richness ($r = 0.234$, $P = 0.0189$, n = data from all 100 transects) but not plant cover ($r = 0.171$, $P = 0.0898$, $n = 100$). The same was true for butterfly abundance which correlated with plants species richness ($r = 0.347$, $P = 0.0004$, $n = 100$) but not with their cover ($r = 0.075$, $P = 0.456$, $n = 100$).

Partial redundancy analysis showed that butterflies significantly differed between grasslands located at roads and ~~apart~~ ($F = 6.1$, $P = 0.0005$, Figure 4). The two grassland types accounted for 15.4 % in species composition (Figure 4). Moreover, hierarchical partitioning showed that 61 % ($F = 5.8$, $P = 0.0005$) of this variation was explained by grassland type (location), 37 % ($F = 1.6$, $P = 0.001$) by plant data (first principal component calculated from species richness and abundance), and 2 % by joint effect ($F = 2.2$, $P = 0.0005$).

Between-habitat comparison of plant communities

There were no differences in total plant species richness (GLM $F_{1, 18} = 2.985$, $P = 0.101$, Figure 3c) nor in mean abundance (GLM $F_{1,18} = 2.340$, $P = 0.144$, Figure 3d) in grassland patches located at roads and apart.

~~Despite there was visible slight species separation in ordination plot (Figure 4)~~ the partial canonical correspondence analysis revealed that plant communities did not differ ($F = 0.8$, $P = 0.801$) between grasslands located at roads and apart.

Butterfly community and road mortality

Altogether we recorded 6922 butterflies in grasslands at roads and 154 (2.2%) butterflies had been found dead at road. Co-correspondence analysis showed that total community composition of alive butterflies (total interia = 0.1598) account for 47.4 % species composition of butterflies found road-killed (total interia = 1.0197). However, first ordination axis was statistically non-significant (lambda = 0.0147, $P = 0.0839$) nor was both ordination two axes (trace = 0.0478, $P = 0.305$). We did not find any significant correlation between number of roadkilled butterflies and number of butterflies living in a grassland patches neighbouring with roads ($r = 0.185$, $P = 0.608$, $n = 10$; Figure S3 in Supplementary information).

However, in another co-correspondence analysis we found that 48.3 % of variation species composition of roadkilled butterflies (total interia = 2.737) was explained by species composition of butterflies living on road verges ($n = 1444$ individuals, total interia = 0.676, Figure S4 in Supplementary information). First ordination axis was statistically significant (lambda = 0.0636, $P = 0.0260$) in this analysis. Also, there was statistically significant correlation ($r = 0.685$, $P = 0.029$, $n = 10$) between number of roadkilled butterflies and number of butterflies living on verges of grassland patches neighbouring with roads (Figure 6).

Discussion

Roads had subtle effect on butterfly communities. Contrary to general perception grassland patches neighbouring roads had higher butterfly richness compared to meadows located ~~farer~~ from roads. This pattern was consistent after accounting for sampling effort (number of sampled individuals) as indicated by rarefaction analysis. This important finding~~s~~ may be indication that roads may (1) have no direct negative impact on butterfly populations and (2) road verges that connect different grassland patches located next to roads may be dispersal corridors.

Many studies ~~found roads had negative impact~~ on ~~animals~~ (review in: Munoz, Torres & Gonzalez-Megias, 2015). The influx of pollution may change soil properties and thus conditions for plants that are food resources for adult butterflies and their larva (Munguira, Garcia–Barros & Cano, 2009; Munguira & Thomas, 1992) and road traffic may affect population size (Baxter-Gilbert et al., 2015; Munoz, Torres & Gonzalez-Megias, 2015). ~~Studied grasslands~~ were located at roads that had relatively high traffic. Despite this high traffic level the butterfly communities were not altered negatively. In earlier works it was stated that road verges are often good habitat for butterflies (Ries, Debinski & Wieland, 2001; Wynhoff et al., 2011; Skórka et al., 2013a). Specific conditions at verges may cause that some butterfl~~y~~ of conservation concern may survive in road verges in intensive agricultural landscapes (Wynhoff et al., 2011). In our study verges were integral part of a meadow patch. However, butterfly species composition and abundances were different there compared to that inside habitat patch and at boundary with arable field. Thus, presence of roads probably creates environmental gradient within a grassland patch that may be preferred by different species that ~~in summary~~ enhances species richness in entire grassland patch at roads. This possible gradient may be related to higher plant cover at verges or other factors (soil chemistry, microclimate etc.) that were not investigated in this study. The presence of gradient of conditions may increase available niches and boost species diversity (Amarasekare, 2003; Nord & Forslund, 2015).

Another explanation of the increased number of species in grasslands at roads is that they could be less isolated than control grasslands. Control grasslands were surrounded by arable fields and ~~it was demonstrated that~~ arable land is usually an inhospitable matrix increasing isolation (Luoto et al., 2003; Lenda & Skórka, 2010; Öckinger et al. 2012). As opposed to control grasslands, patches located next to roads were connected with other grasslands via road verges. These ~~marginal~~ habitats may thus improve species turnover among grasslands located at roads or even be a species pool both for butterflies enhancing patch colonization after some disturbances connected with grassland management (Tikka et al., 2001; Moroń et al., 2017).

~~We believe that~~ the positive effect of roads on butterfly diversity would ~~be probably~~ even stronger if road mortality was reduced. However, road mortality was probably negligible in our study. It accounted for less than 5 % of all butterflies recorded in meadow patches at roads. Even if we assume imperfect detection of road killed butterflies (Skórka, 2016) it is still low level. ~~Among roadkilled butterflies dominated species being the most common on a verges and~~ we showed that actually only individuals occurring in grassland part confined to close proximity of a road are really prone to collisions with cars. It would be desirable to test the effect of roads with higher traffic level (e.g. highways) than in this study. This topic should be addressed in further study. However, roads with average and low traffic are the most densely distributed in Polish landscapes and possibly have the most spatially widespread environmental impact (Kotlarek, 2007; Skórka et al., 2015).

Interestingly, both studied boundaries – with road and arable field – had different species composition and were dominated by different butterfly species as indicated by redundancy analysis. This suggest that type of habitat patch boundary has important effect on species composition in the patch (Skórka et al., 2013b). It is noteworthy that in our study, the edge effect was visible in term of species composition rather that total species abundance and richness. Earlier theoretical and empirical works indicated that different species respond in various way (in terms of spatial pattern of abundance) to habitat boundaries. This may be a result of different boundary and matrix types (road and arable field), and also different permeability of these land covers (Skórka et al., 2013b). In reference ~~control~~ meadows, the species composition at boundary with field road was similar to the species composition at the boundary with arable field. Field roads are very different from asphalt roads. They are narrow, mostly covered by grass and often managed in the same way as neighbouring grassland. Thus, it is possible that field roads neighbouring with meadows are perceived by butterflies as ~~integral part of the habitat.~~

Several species showed preferences toward living in that part of habitat patch close to road verges. These are usually low-bodied species such as *Plebeius argyrgnomon* and

Polyommatus coridon and very mobile common species such as *Isoria lathonia* and *Papilio machaon*. Intrestingly, road verges had high abundances of species of conservation concern as *Phengaris nausithous.* This species is especially known for surviving at road verges in intensively managed agricultural landscapes (Wynhoff et al., 2011). This may ~~be~~ also result~~s~~ from the fact that ~~road verges enhance dispersal of ants (DeMers, 1993) and be good habitat for them (Itzhak, 2008; Wynhoff et al., 2011)~~. Ants are hosts for larvae for these small blue butterflies.

Interestingly plant species richness and composition were not affected by roads. However, plant cover was very similar to the pattern found in a~~bunance~~ of butterflies. Butterflies are herbivores and many of them depend on specific plants during different life stages (Kitahara, Yumoto & Kobayashi, 2008). Our co-correspondence analysis also confirmed strong dependency of butterfly species composition on plants. However, the effect of roads on butterfly community remained after controlling the effects of plants and this indicates that roads modify insect herbivore community composition also in different manner. As it was mentioned above this may be a direct effect of road mortality, alteration in species behaviour near roads and changes in microclimate conditions at roads (Jackson & Jobàggy 2005; Green, Machin & Cresser; Skórka et al. 2013b).

Conclusions

Results suggest that proximity of a road has specific spatial effect on butterfly communities but little on plants. Butterfly communities were more spatially diversified within a patch and species rich in grasslands located next to roads. Moreover, butterfly diversity was higher in grasslands neighboring with roads than in grassland far from roads. This suggests that grasslands located at roads with moderate traffic may be at least as good habitat for butterflies as these meadows located apart. The road mortality was not very high and possibly affected predominantly individuals living on grassland edges at roads indicating that not entire patch is equally affected by a road. It is thus crucial to appropriately manage these parts of grassland patches located next to roads. It would enhance road verge as dispersal corridor and habitat and would reduce road mortality. We propose to mown grassland patches at road verges partially because it was shown in former study (Skórka et al., 2013a) that this measure increases suitability of a verge as the habitat for butterflies and reduce collisions with cars.

Data Availability

The following information was supplied regarding data availability: The raw data is provided as a Supplemental File 2.

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Figure captions

Figure 1. The impact of transect location on mean number of species (a,b) and individuals (c,d) within grassland patches bordering with road (a,c) and apart from roads (b, d). Whiskers are 95 % confidence intervals. The only statistically significant difference were found for abundance of butterflies at roads (c): levels not connected by the same capital letter denote statistically significant differences.

Figure 2. Ordination of butterfly species in partial redundancy analysis in different parts of the grassland patches bordering with roads (a, b) and apart from a road (c, d). Explanation: road verge – transect on a road verge, 25m from road verge - transect located inside meadow patch 25 m from a road verge, inside – transect located inside the grassland patch, 25m from field boundary – transect located inside meadow patch 25 m from a border between the patch and arable field, field boundary – transect located at the border between the habitat patch and arable field. In case of control grasslands (grassland located apart from a road) "road verge" was a transect located along a field road used by farmers. Species abbreviations are first letters of the genus and species names.

Figure 3. The impact of grassland location on the mean number of butterfly species (a) and individuals (c), and plant number of species (c) and cover (d) within grassland patches bordering with road and apart from roads. Whiskers are 95 % confidence intervals. Explanation: * - statistically significant difference at $P < 0.05$.

Figure 4. Ordination of butterfly (a, b) and plant (c, d) species in grassland patches bordering with roads and apart from roads. Redundancy (butterflies) and partial canonical correspondence (plants)analyses were used for ordination of species after removing the effects of transect location within a patch (see Figure 2). Explanations: Road – grassland patches bordering with roads, Control – grassland patches located apart from roads. Species abbreviations are first letters of the genus and species names.

Figure 5. Rarefaction depicting the estimated number of species as the function of number of sampled individuals. Whiskers are 95% confidence intervals.

Figure 6. Correlation between number of butterflies living on road verges and number of roadkilled butterflies.

Figure 1

The impact of transect location within a grassland patch on the number of butterfly species and individuals

The impact of transect location on mean number ofbutterfly species (a,b) and individuals (c,d) within grassland patches bordering with road (a,c) and apart from roads (b, d). Whiskers are 95 % confidence intervals. The only statistically significant difference were found for abundance of butterflies at roads (c): levels not connected by the same capital letter denote statistically significant differences.

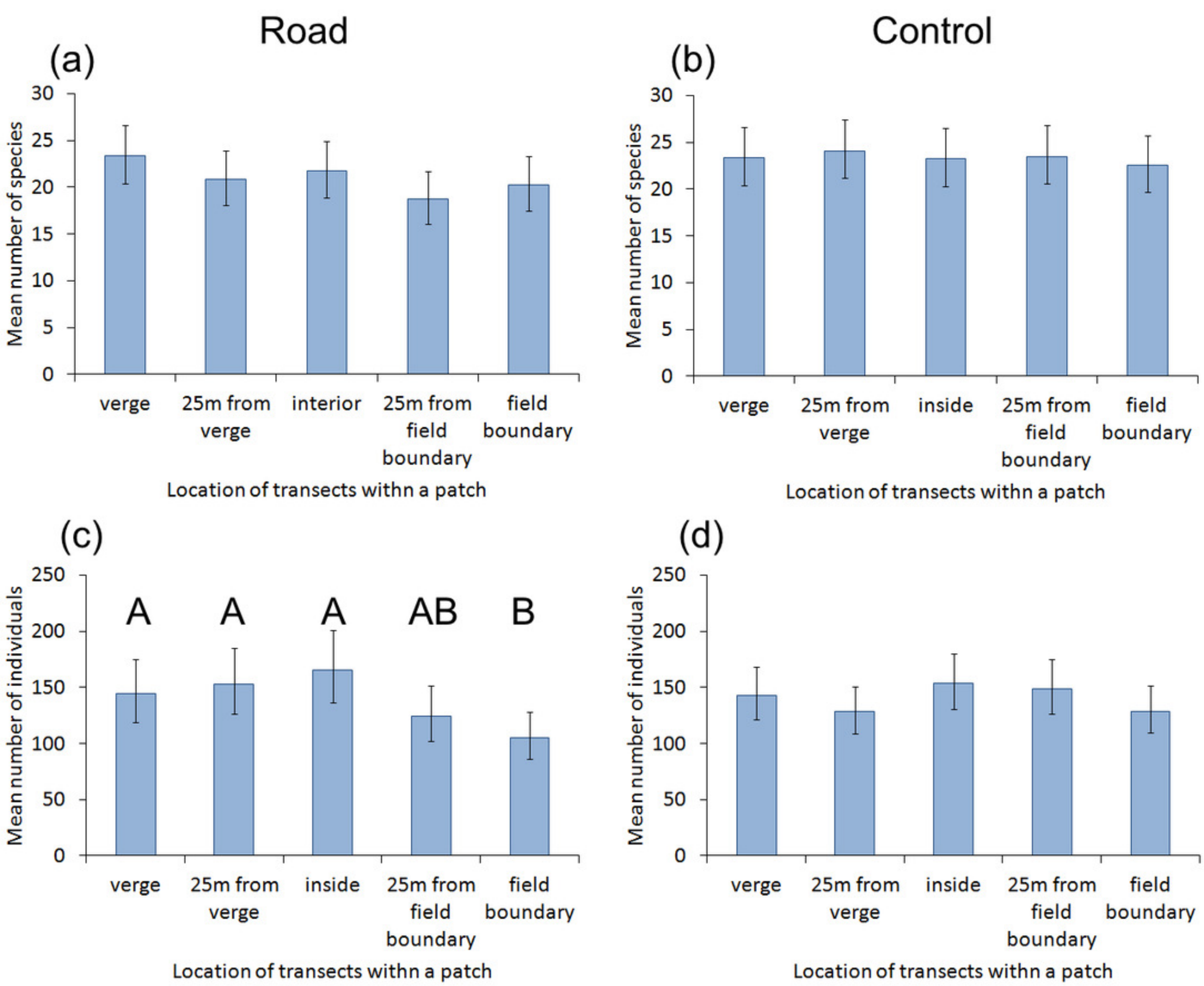


Figure 2

Differentiation of butterfly species in different parts of the grassland patches bordering with roads and apart from a road

Ordination of butterfly species in partial redundancy analysis in different parts of the grassland patches bordering with roads (a, b) and apart from a road (c, d). Explanation: road verge – transect on a road verge, 25m from road verge - transect located inside meadow patch 25 m from a road verge, inside – transect located inside the grassland patch, 25m from field boundary – transect located inside meadow patch 25 m from a border between the patch and arable field, field boundary – transect located at the border between the habitat patch and arable field. In case of control grasslands (grassland located apart from a road) “road verge” was a transect located along a field road used by farmers. Species abbreviations are first letters of the genus and species names.

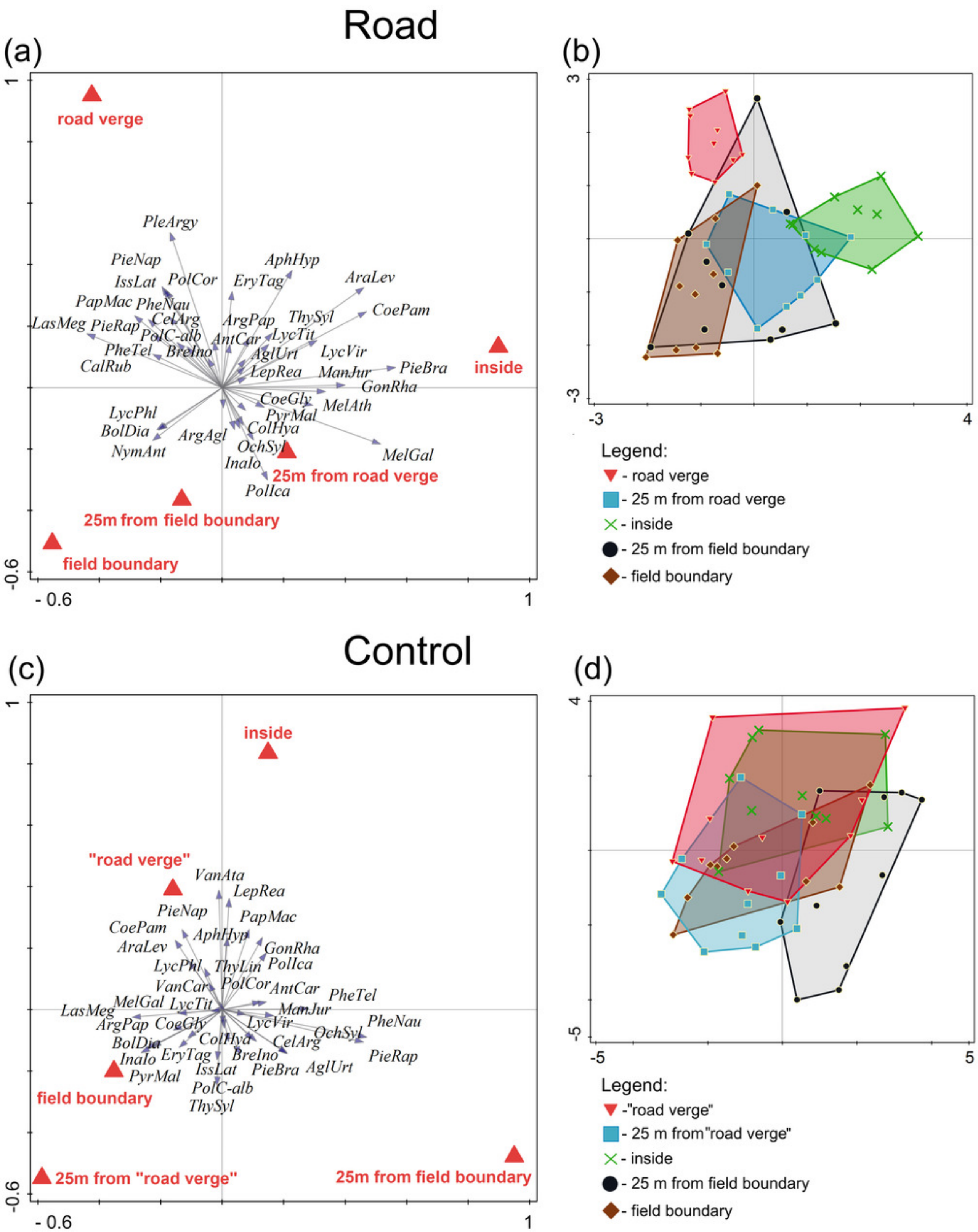

Road
(a)
road verge
inside
25m from road verge
25m from field boundary
field boundary
- 0.6
1
-0.6
1
(b)
3
-3
-3
4
Legend:
- road verge
- 25 m from road verge
- inside
- 25 m from field boundary
- field boundary
Control
(c)
inside
"road verge"
field boundary
25m from "road verge"
25m from field boundary
- 0.6
1
-0.6
1
(d)
4
-5
-5
5
Legend:
-"road verge"
- 25 m from"road verge"
- inside
- 25 m from field boundary
- field boundary

Figure 3

The impact of grassland location within a grassland patch on the number of butterfly species and individuals

The impact of grassland location on the mean number of butterfly species (a) and individuals (c), and plant number of species (c) and cover (d) within grassland patches bordering with road and apart from roads. Whiskers are 95 % confidence intervals. Explanation: * - statistically significant difference at $P < 0.05$.

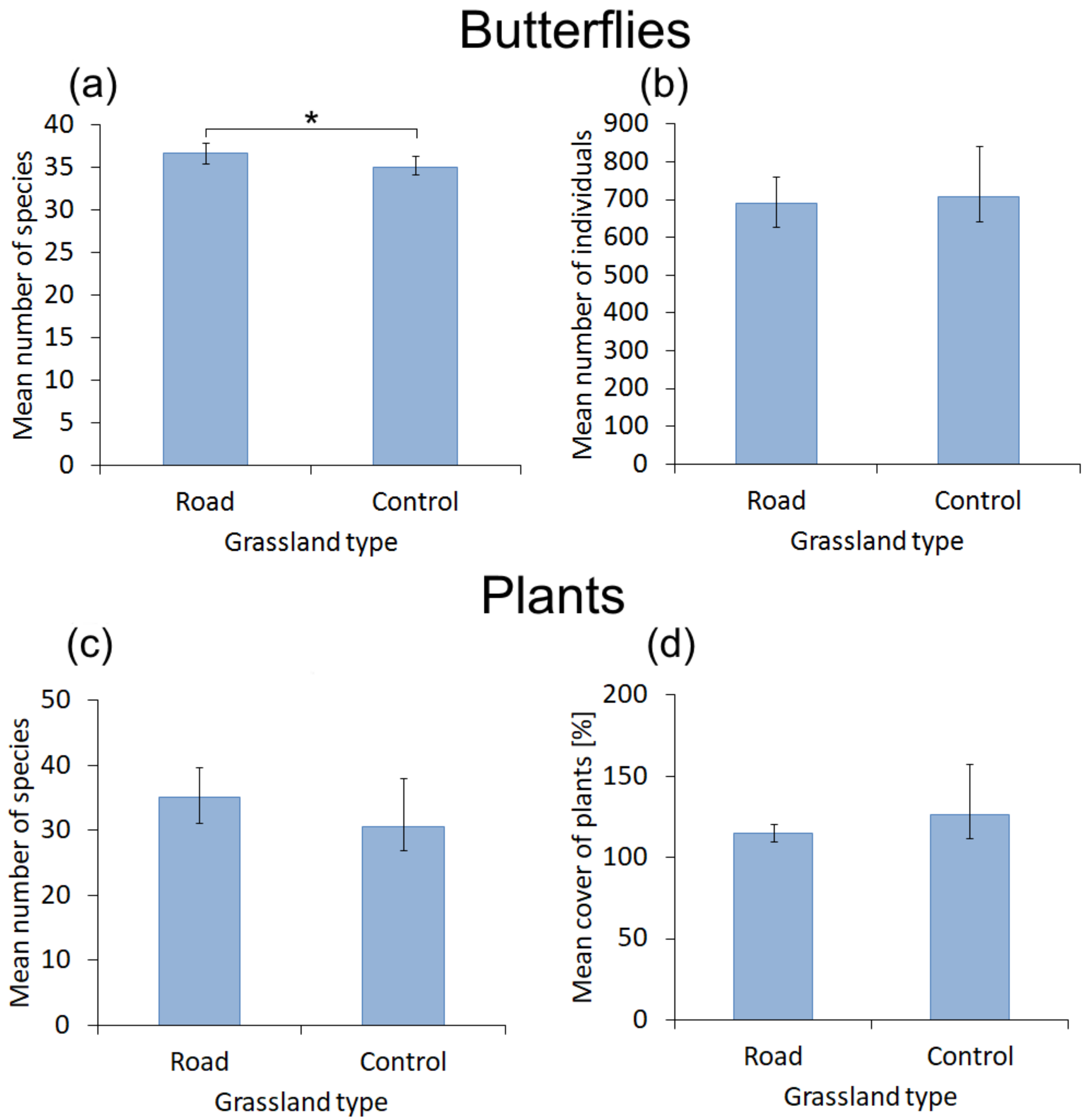
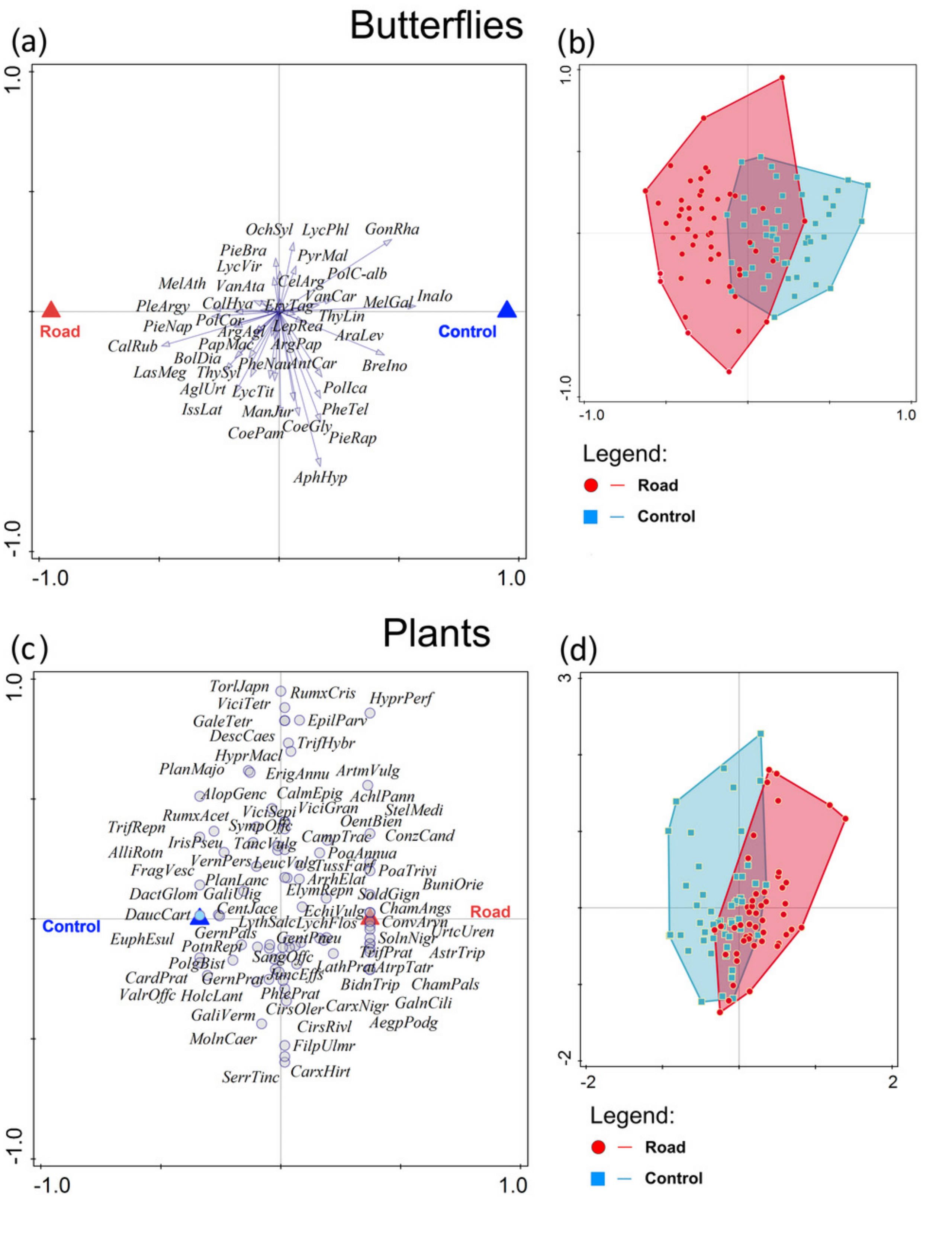

Butterflies
(a)
Mean number of species
40
35
30
25
20
15
10
5
0
*
Road
Control
Grassland type
(b)
Mean number of individuals
900
800
700
600
500
400
300
200
100
0
Road
Control
Grassland type
Plants
(c)
Mean number of species
50
40
30
20
10
0
Road
Control
Grassland type
(d)
Mean cover of plants [%]
200
150
100
50
0
Road
Control
Grassland type

Figure 4

Differentiation of butterfly and plant species composition in grassland patches bordering with roads and apart from roads

Ordination of butterfly (a, b) and plant (c, d) species in grassland patches bordering with roads and apart from roads. Redundancy (butterflies) and partial canonical correspondence (plants)analyses were used for ordination of species after removing the effects of transect location within a patch (see Figure 2). Explanations: Road – grassland patches bordering with roads, Control – grassland patches located apart from roads. Species abbreviations are first letters of the genus and species names.



Butterflies
(a)
(b)
Road
Control
OchSyl LycPhl GonRha
PieBra PyrMal
LycVir CelArg PolC-alb
MelAth VanAta VanCar
PleArgy ColHya EryTag MelGal InaIo
PieNap PolCor ThyLin
ArgAgl LepRea AraLev
CalRub PapMac ArgPap
BolDia PheNau AntCar BreIno
LasMeg ThySyl
AglUrt LycTit PolIca
IssLat ManJur PheTel
CoePam CoeGly PieRap
AphHyp
1.0
-1.0
Legend:
Road
Control
Plants
(c)
(d)
Control
Road
TorlJapn RumxCris HyprPerf
ViciTetr
GaleTetr EpilParv
DescCaes TrifHybr
HyprMacl
PlanMajo ErigAnnu ArtmVulg
AlopGenc CalmEpig AchlPann
RumxAcet ViciSepi ViciGran StelMedi
TrifRepn SympOffc OentBien
IrisPseu TancVulg CampTrac ConzCand
AlliRotn PoaAnnua
VernPers LeucVulg TussFarf PoaTrivi
FragVesc PlanLanc ArrhElat BuniOrie
DactGlom GaliUlig ElymRepn SoldGign
DaucCart CentJace EchiVulg ChamAngs
LythSalc LychFlos ConvArvn
EuphEsul GernPals GentPneu SolnNigr UrtcUren
PotnRept TrifPrat AstrTrip
PolgBist SangOffc LathPrat AtrpTatr
CardPrat GernPrat JuncEffs BidnTrip ChamPals
ValrOffc HolcLant PhlePrat GalnCili
GaliVerm CirsOler CarxNigr AegpPodg
CirsRivl
MolnCaer FilpUlmr
SerrTinc CarxHirt
3
-2
2

Figure 5

Rarefaction depicting the estimated number of species as the function of number of sampled individuals. Whiskers are 95% confidence intervals.

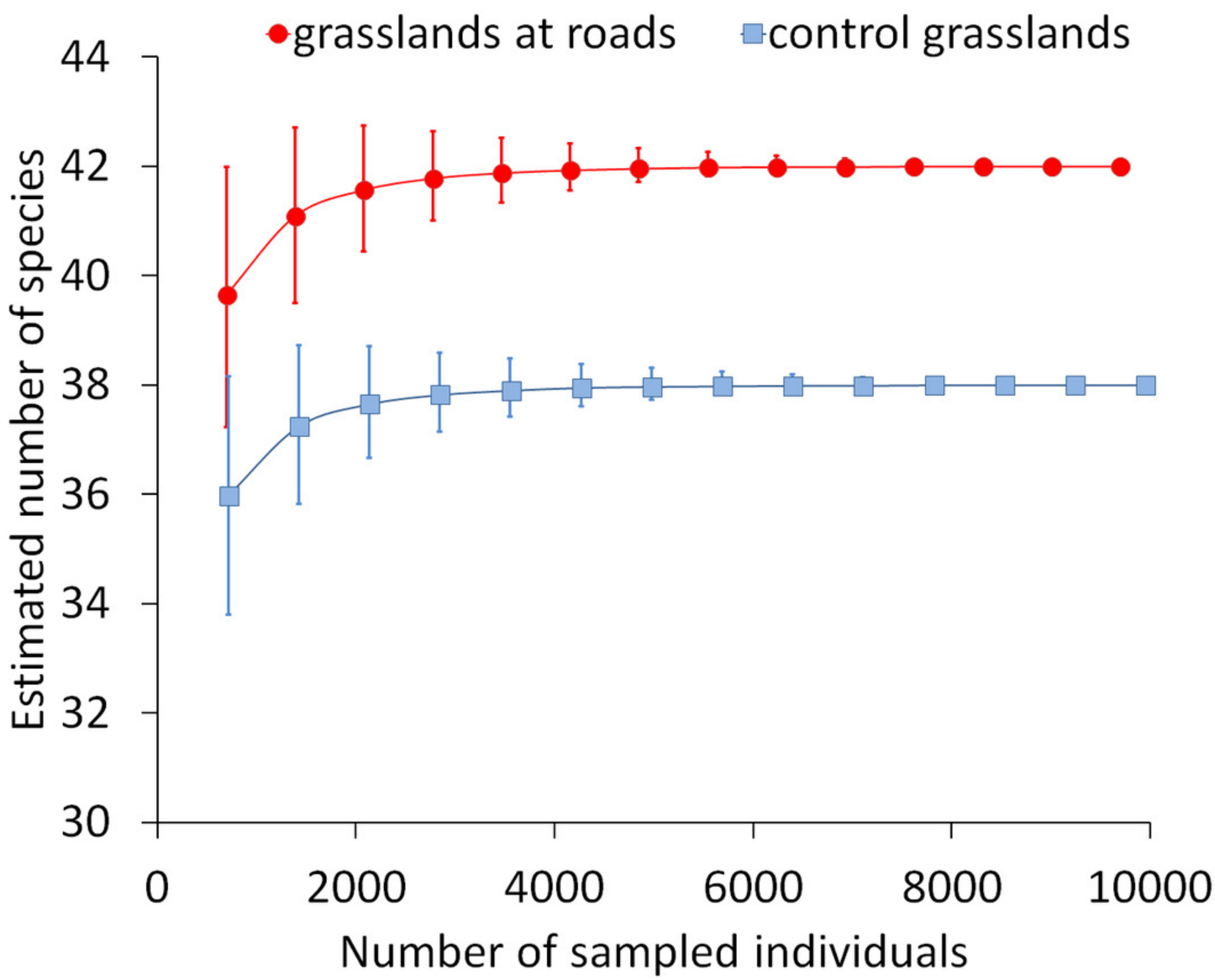


Figure 6

Correlation between number of butterflies living on road verges and number of roadkilled butterflies