

## The role of shrubs in maintaining butterfly biodiversity in grasslands

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**Summary:** Hedgerows are an essential component of the agricultural and grassland landscapes of many farms and semi-natural grasslands around the world, and their management plays an important role in enhancing habitat biodiversity. In this article, we show how shrub-removal management practices affect butterfly and plant communities in a small region of Transylvania, Romania. We compared across the 15 sites the abundance and diversity of butterfly species in four different shrub managed plots and found for each type of management indicator species, such as *Cupido argiades* in sites where shrubs were cut one year prior to our first year sampling, *Anthocaris cardamines*, *Melitaea phoebe*, *Glaucopteryx alexis* and *Thymelicus sylvestris*, *Thymelicus lineola* where shrubs were removed three years prior to our first year sampling and *Colias alfacariensis* where shrubs were removed more than 3 years prior to our first year sampling.

**Key words:** shrubs, butterflies, grasslands, management, biodiversity, Romania.

### Introduction

Shrubs are important structures in grasslands and agricultural landscapes around the world, providing ecological connectivity, floral diversity that supports insect populations (SARDIÑAS *et al.* 2016, M'GONIGLE *et al.* 2015) improving pollination and crop productivity (MIÑARRO and PRIDA 2013, ALISON *et al.* 2021) and habitat for various species of insects, birds and small mammals (FINCH *et al.* 2020, GARRATT *et al.* 2017, HAENKE *et al.* 2014, KALÁB *et al.* 2020, POLLARD and HOLLAND 2006).

The intensification of agriculture has changed the rural landscape and is one of the most important factors leading to biodiversity decline through excessive cutting of shrubs, leading to a decrease in refuges and feeding spots for Lepidoptera species (KLAUS *et al.* 2015, FROIDEVAUX *et al.* 2019).

Even though it is recommended in the agri-environment work package that farmers should maintain 5-15% of shrubs after hedgerow management to preserve insect diversity, this is still not well understood by some farmers and is not being properly implemented, resulting in unintended negative consequences such as declines in biodiversity, soil erosion, and the degradation of landscape features (CREMENE *et al.* 2005, RÁKOSY 2011, STALEY *et al.* 2012).

In this study, we aim to show to measure the diversity of flowering plants and butterflies and butterfly indicator species where hedgerow conservation measures such as shrub removing have been implemented in 15 sites near Cluj-Napoca, Transylvania.

### Material and methods

We surveyed butterfly communities in early summer and late summer - in May and August in 2022 and 2023 across 15 sites (1 reserve), in the surroundings of Cluj-Napoca, Transylvania (fig. 1). We ensured that daytime sampling is randomized across sites. Transect surveys were conducted using butterfly nets, recording all butterflies observed. Surveys occurred between 10:00 and 17:00 under optimal (>15°C, wind <12 km/h, low cloud cover) conditions, and only on dry days. The sampling design was chosen as four linear 50 m long belt transects of 1.5 m width (7 minutes 30 seconds sampling time per transect) positioned parallel to the field edge at approximately 1 m, 10 m, 20 m and 30 m from the field edge, covering a total area of 300 m<sup>2</sup>. We recorded flowering vascular plant diversity using 10 quadrats of 50x50 cm (0.25 m<sup>2</sup>) per site. Plant diversity was surveyed on the same day as the butterfly transects. To estimate flower cover in shrubby vegetation, hedgerows, we used the same methodology as described above to estimate herbaceous vegetation. We classified hedgerows according to the trimming management type into four groups: 1YC-shrubs that were cut one year prior to first year sampling; 2YC- shrubs cut two years prior to first year sampling; 3YC-shrubs removed three years prior to first year sampling; OYC-shrubs that were removed more than 3 years prior to our first year sampling and control where hedgerows were never trimmed.

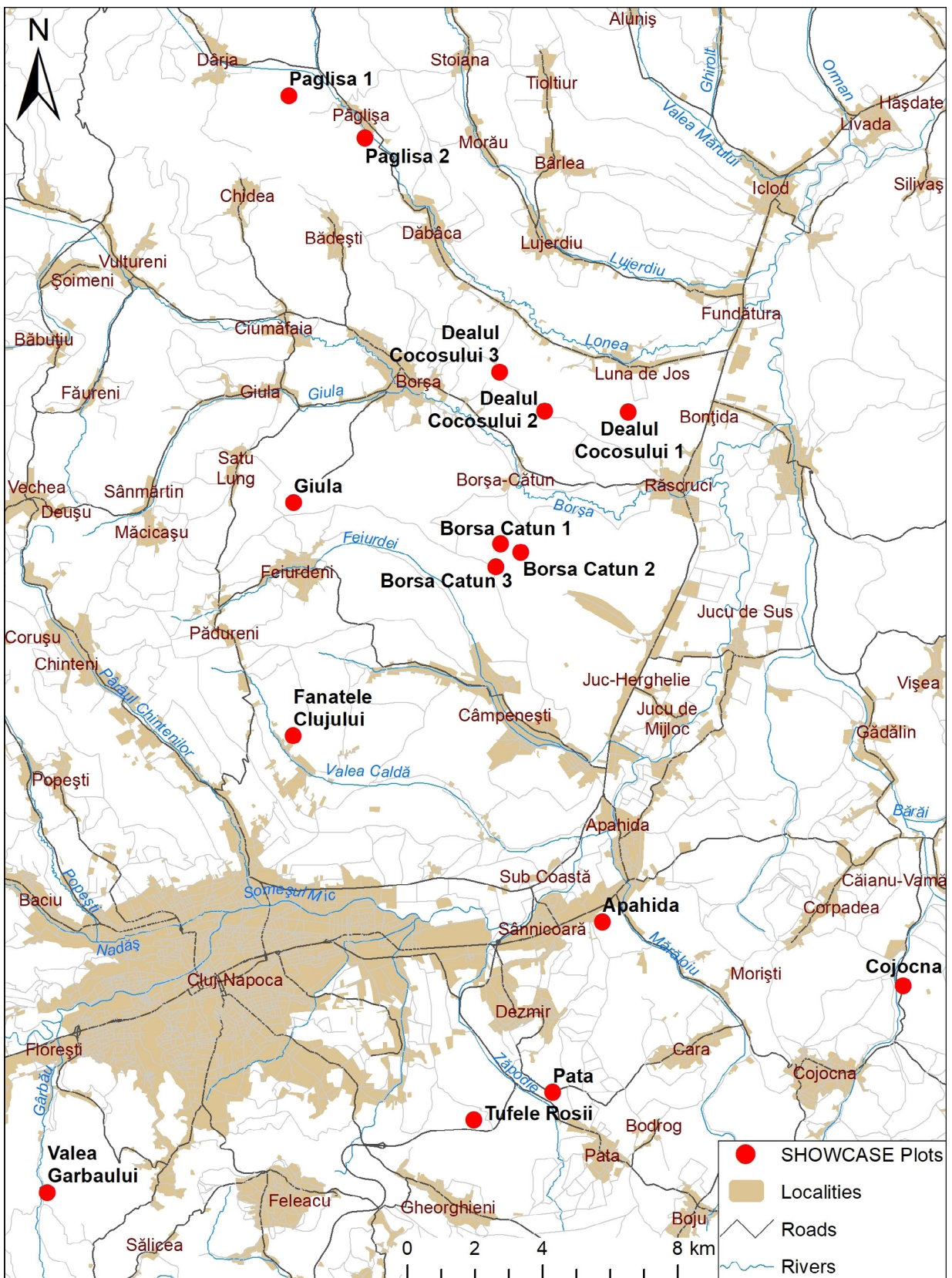


Fig.1. Map of the 16 study sites (red dots) near Cluj-Napoca.

### Statistical Analysis

We used R software (R Core Team) for all statistical analyses. We did the normality and homogeneity tests (Shapiro-Wilk and Levene tests) to assess data normality for butterfly and plant communities. To test differences in butterfly and plant community composition across hedgerows management types

(Control, 1YC, 2YC, 3YC, OYC), we did the Kruskal-Wallis rank sum test as a non-parametric alternative to ANOVA. Where significant differences were detected, Dunn's post hoc test with Bonferroni correction was used to perform pairwise comparisons between treatments. Relationships between butterfly and plant communities were assessed using Spearman's rank correlation coefficient, suitable for non-parametric

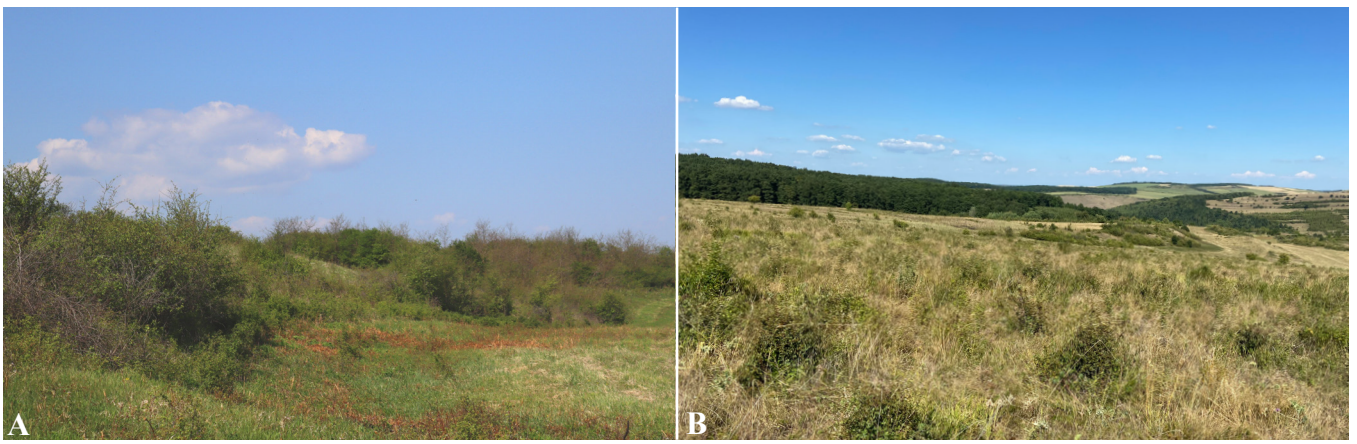


Fig. 2. A) Control plot – shrub belt; B) shrubs in a transect plot.

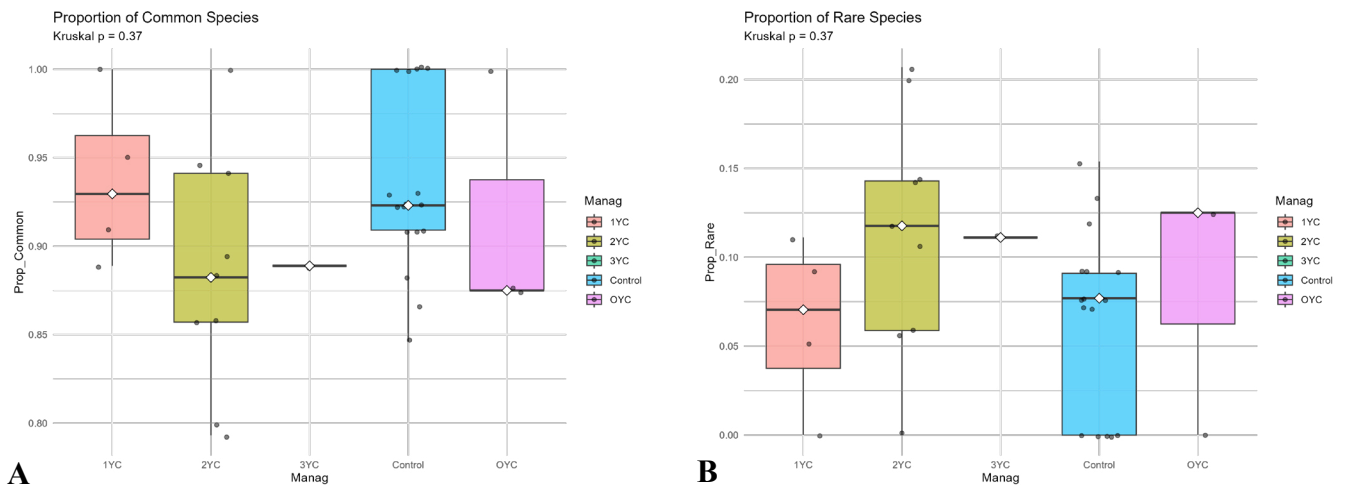


Fig. 3. Proportion of common species A) and rare species B) in different shrub managed plots using the Kruskal-Wallis test,  $p=0.37$

data. A multilevel pattern analysis using the IndVal.g function was employed to identify species significantly associated with specific grassland management regimes. A significance level of  $\alpha = 0.05$  was applied. To observe the differences in community composition among the various sites under different management types, we used Beta diversity analysis. We applied the Kruskal-Wallis test to assess significant differences in species frequency and ecological category abundance across the four management treatments.

### Results

The analysis of frequency categories revealed no statistically significant differences (Kruskal-Wallis test,  $p = 0.37$ ) in the occurrence of common butterfly species across different grassland management regimes. However, a higher frequency of common species was observed in the control plots, followed by 1YC and 2YC plots (Fig. 3A).

A similar pattern was identified for rare species (Fig 3B). Although the highest proportions of rare species were recorded in 2YC and OYC sites, followed by 1YC and control plots, the differences

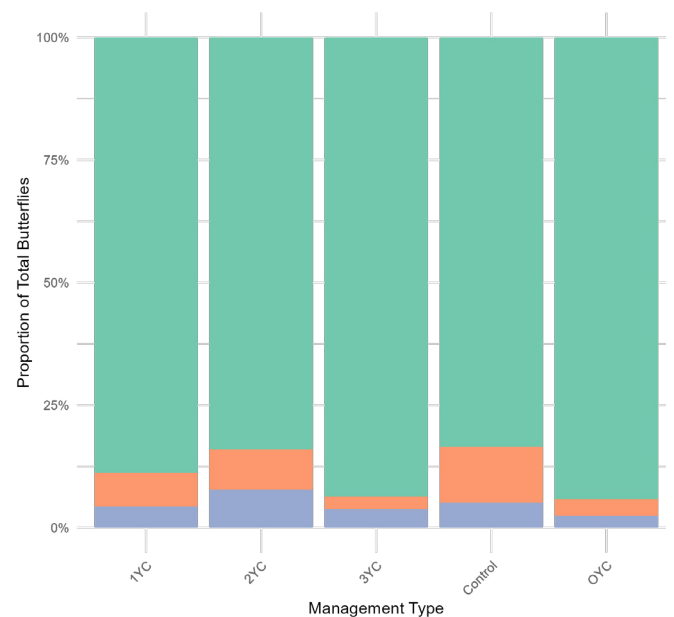


Fig. 4. Proportion of butterfly species traits abundance by management type (generalist – green bars, generalist/specialist – orange bars, specialist – grey bars)

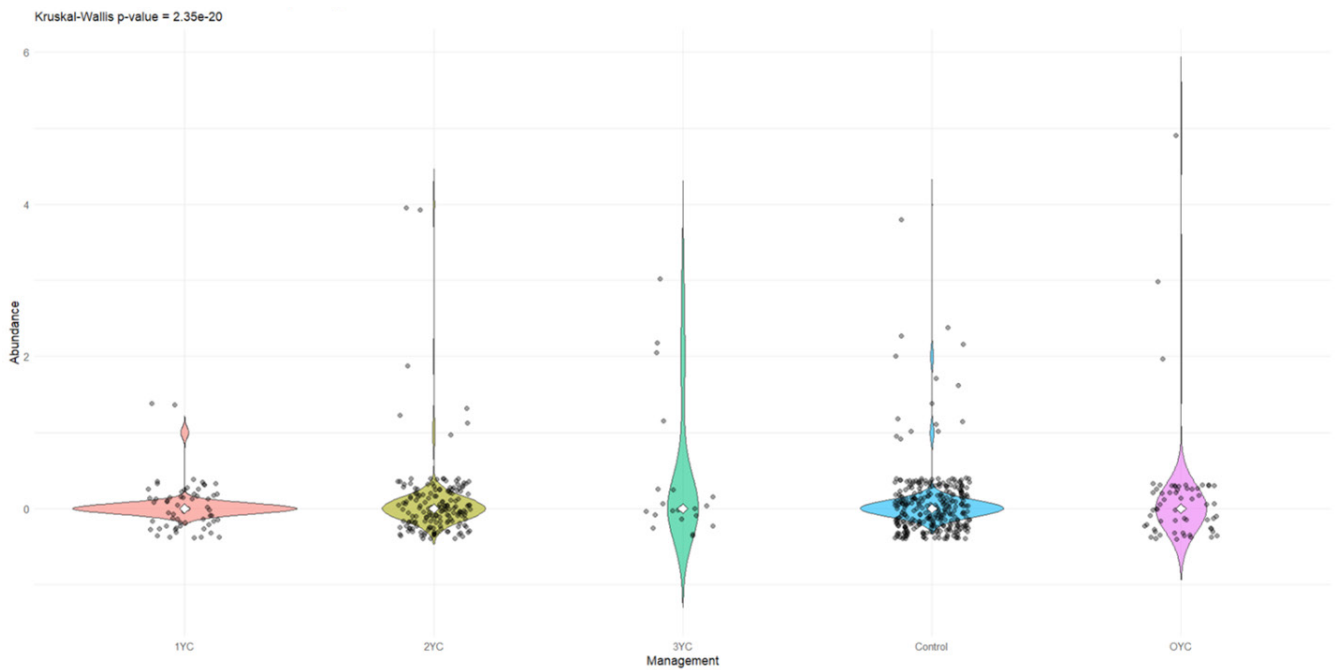


Fig. 5. Distribution of generalist butterfly species abundance by shrub removal management type

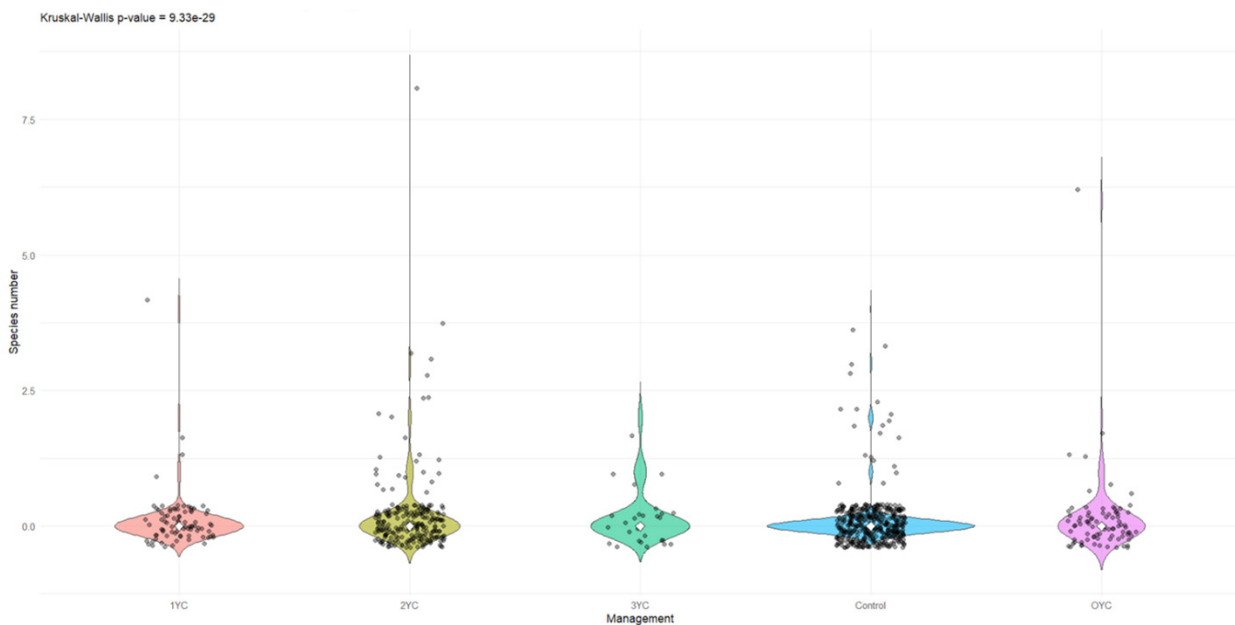


Fig. 6. Distribution of specialist butterfly species number by shrub removal management type

across management types were not statistically significant (Kruskal-Wallis test,  $p = 0.37$ ).

When examining the distribution of ecological categories, generalist species were the most prevalent, followed by specialist species and last were those with mixed traits (generalist/specialist).

However, when these categories were analysed separately, distinct patterns emerged. For generalist butterfly species, statistically significant differences were found across management regimes (Kruskal-Wallis test,  $p < 0.05$ ), with higher abundances recorded in both 2YC and control plots. For generalist/specialist species, the highest abundance was found in 3YC sites. While the most specialist species were

found in 2YC sites.

The Dunn post-hoc test indicated significant differences between 2YC and control sites ( $p = 0.05$ ), suggesting that both types of management support high abundances of generalist butterfly species (Fig. 5).

The Dunn post-hoc test showed significant differences between 2YC and control plots, corresponding to the areas with the highest species abundances (Fig. 6). Thus, the 2YC management regime supports not only generalist but also specialist butterfly species in relatively high numbers.

Both butterfly and plant communities have more species in the sites where shrubs were removed prior to our study than the control plots (Fig. 7 A, B).

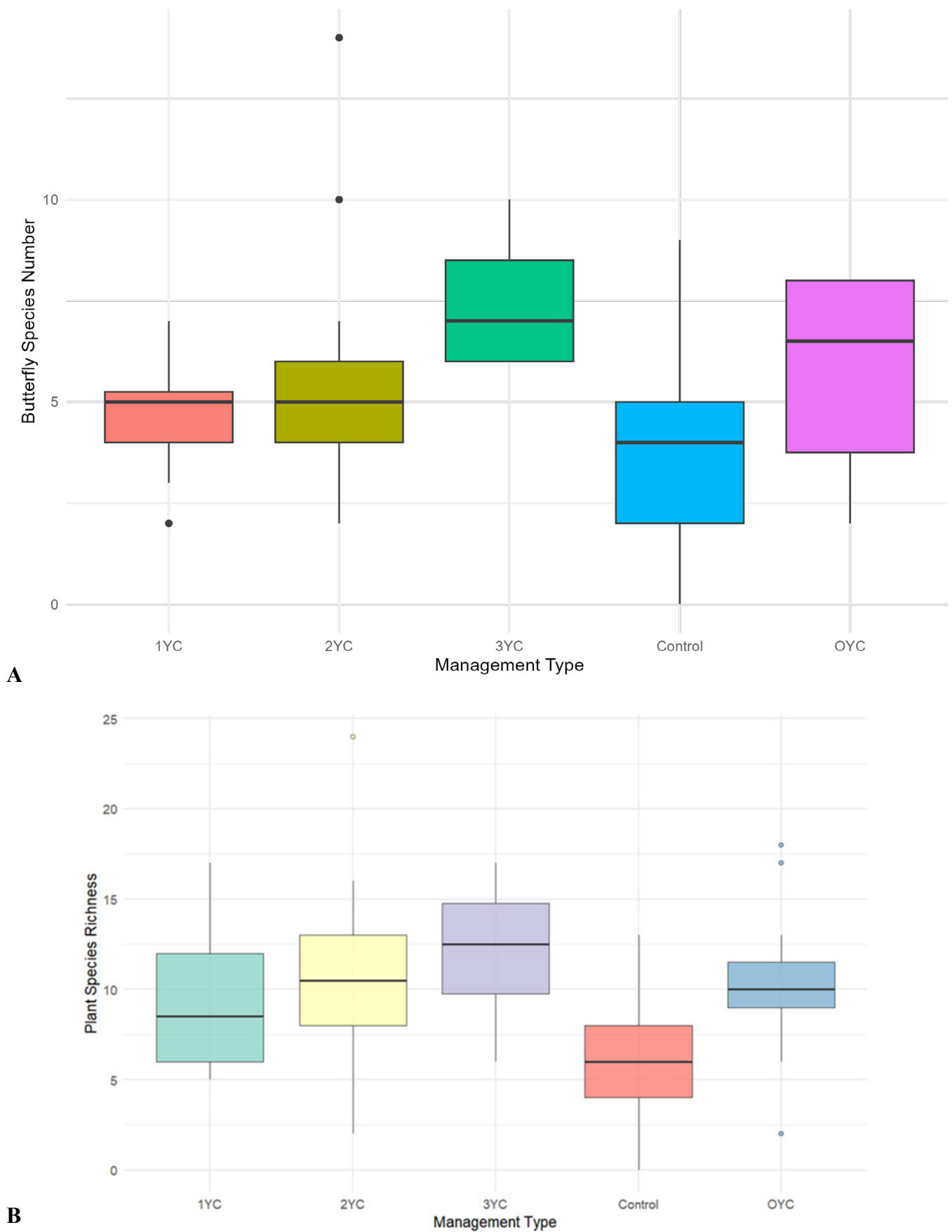


Fig. 7 A) Butterfly species richness and B) plant species richness by management type

The indicator species analysis detected 15 species associated to specific shrub treatments, such as *Cupido argiades* for 1YC plots, *Anthocaris*

*cardamines*, *Melitaea phoebe*, *Glaucopsyche alexis* and *Thymelicus sylvestris*, *Thymelicus lineola* for 3YC plots and *Colias alfacariensis* for OYC plots.

## Discussions

Hedgerows support insect diversity by offering important microhabitats (VOLPATO *et al.* 2019) and increase pollinator populations by ensuring consistent nectar and pollen availability throughout the season (MORANDIN and KREMEN 2013). They are ecological corridors that connect fragmented habitats, supporting insect movement and gene flow (DAINESE *et al.* 2016) in intensively farmed areas (RIVERS-MOORE *et al.* 2020).

In our study, the interventions primarily involved shrub removal, which was conducted either by hand or using small machinery with a maximum weight of 450 kg, including double-cutting equipment and mulchers. These interventions were carried out one to three years prior to the present study. The studied sites were owned by farmers and in proximity of their crop fields or wildflower fields. The hedgerows increase insect biodiversity by creating ecotonal zones that support a variety of species due to their structural and floral diversity (COULTHARD *et al.* 2016), important for species that rely on both hedgerow and field for different life cycle stages (PETER *et al.* 2025).

The studied sites, each managed under different regimes, host distinct butterfly and plant communities. These communities are not merely richer or poorer versions of a single species pool but rather consist of entirely different species assemblages. Consequently, rather than experiencing a gradual gain or loss of species across sites, there is a complete turnover in species composition. This suggests that the observed differences are not solely due to relative abundance but reflect fundamentally different combinations of species shaped by specific environmental and management-related factors.

With no statistical difference, species frequency patterns of common species were more in Control and 1YC plots than 2YC and OYC plots and rare species were slightly high in 2YC and OYC plots, as FROIDEVAUX *et al.* (2019) reported that less frequent hedgerow trimming increases populations of disturbance-sensitive insects. Ecological category analysis showed that specialists, generalist and generalist/specialists all significantly varied among treatments, with the highest abundances found in 2YC and Control, which were particularly higher than 3YC, highlighting the ecological filtering effect of the different removal management treatments (KIMBERLEY *et al.* 2020). Thus, the agri-environmental schemes aiming at preserving butterfly diversity should promote landscape and shrubs management heterogeneity and offer incentives to conserve grasslands and agricultural lands with shrubs.

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