Effects of short-term restoration on plant-pollinator communities in Cantavieja and Ejea de los Caballeros

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Objectives

The objective of this report is to describe the differences between two plant-pollinator communities located in Cantavieja and Ejea de los Caballeros before and after restoration practices. In this report, we compare the two plant-pollinator communities using: 1) rarefaction analysis of the number of species and unique interactions; and 2) computation and evaluation of general aspects of network structure.

All data manipulation and analyses were conducted in R version 4.1.2 (R Core Team 2021).

Sampling method

Two distinct locations were monitored with different historical land-use and management in the region of Aragón (Spain): Cantavieja (40° 30′ 44″, N 0° 22′ 59″ W) at 1450m above sea level with a total of 30 plots and Ejea de los Caballeros (42° 01′ 06″ N, 1° 08′ 53″ W) at 350m. In each location, a total of 21 plots were monitored in 2021 and 2024. Plot size is 2m by 2m and they are randomly distributed within each location (see **Figure 1**). Importantly, since the start of the project vegetation has remained undisturbed without grazing or clearing.

Each plot was surveyed three times at the begining of the season, at the peak of flowering and at the end of the season. In each occasion, all plant-pollinator interactions observed were documented. Pollinator specimens not identified in the field were captured and identified in the lab.



Figure 1. Cantavieja (left) and Ejea de los Caballeros (right) locations, with the different sampling plots indicated by an orange square along with the plot identifier. The positions of the bee hives are shown with a white cross in both locations.

Comparative analysis

Species Richness and Interactions

In this section, we first described the species accumulation curves per location and year. For this, we used rarefaction analysis to compare the number of observed species at equal number of sampling rounds. Thus, several species accumulation curves are created by randomly selecting each sampling round, and then calculating the mean of all these curves. Cantavieja and Ejea de los Caballeros showed a higher number of pollinator species in 2024. (**Figure 3**). However, the difference between years was only particularly pronounced for Ejea de los Caballeros.

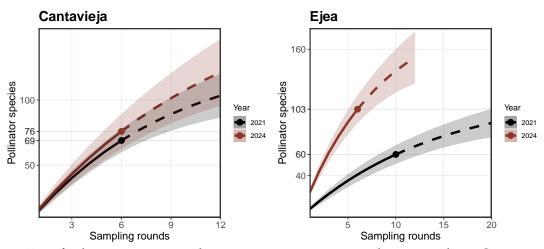


Figure 2. Rarefied species accumulation curves across sampling rounds in Cantavieja and Ejea de los Caballeros. The solid line indicates the rarefied number of species per sampling round, the dot represents the observed number of species, and the dashed line shows the extrapolated number of species if additional rounds were conducted.

Second, we compared the number of observed pollinator and plant species per location and year. As showed in **Figure 3**, both Cantavieja and Ejea de los Caballeros showed more pollinator species in the year 2024 (**Figure 4**). However, the number of observed plant species was higher in 2021 for both locations.

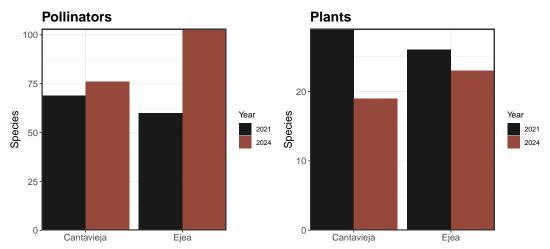


Figure 3. Pollinator and plant richness in Cantavieja and Ejea de los Caballeros for the sampling periods 2021 and 2024.

Third, the rarefied accumulation curve of unique interactions was calculated for each location and year. Note that *Apis mellifera* was excluded, as their individuals do not directly respond to the applied restoration practices. An example of unique interaction is the visit of *Amegilla quadrifasciata* on *Eryngium campestre*, which was observed in both locations. Here, we observed similar patterns to the ones observed for the accumulation curve of pollinator species, where Cantavieja shows little differences between years but Ejea de los Caballeros shows notably more unique interactions for the year 2024.

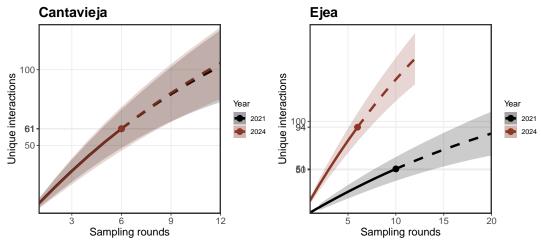


Figure 4. Comparison of the rarefied number of unique interactions across years in Cantavieja and Ejea de los Caballeros. Note that *Apis mellifera* were excluded from this analysis.

Fourth, we compared the degree distributions between the pollinator and plant communities of Cantavieja and Ejea de los Caballeros across different years. Pollinators exhibited a classical decaying distribution for ecological networks (Jordano, Bascompte, and Olesen 2003), while plants had a more gradual decaying distribution. Pollinators did not show statistical differences between their distributions between years for both locations (Kruskal-Wallis P > 0.05). However, plants had statistically different distributions for both locations (Kruskal-Wallis P < 0.05).

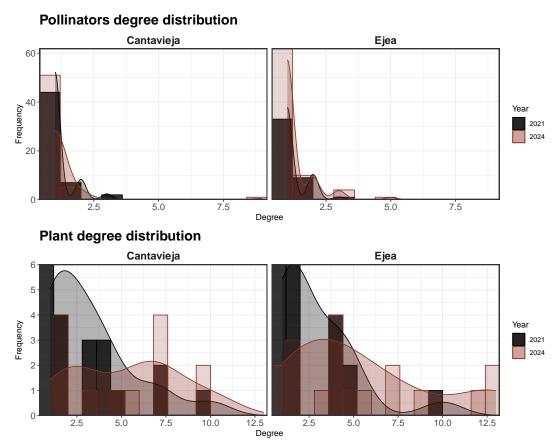


Figure 5. Comparison of the pollinator and plant degree distributions for Cantavieja and Ejea de los Caballeros between years.

Network structure

The structure of plant-pollinator networks per location and year was compared using the following network descriptors:

- (i) Network-level specialization (H2'), which indicates how selective the species are at the network level.
- (ii) Connectance, which refers to the number of links compared to all possible links.
- (iii) Robustness, which evaluates how resilient the network is to species loss.
- (iv) Nestedness, which describes how well the interactions of specialist species are subsets of those of generalist species.

Network-level specialization, connectance, and robustness were calculated using the *bipartite* package (Dormann, Gruber, and Fründ 2008). Note that for robustness, we evaluated the impact of excluding plant species, pollinator species, and excluding both. To compare nestedness across networks, we used a normalized metric (NODFc) that is independent of network size (Song, Rohr, and Saavedra 2017). This was implemented using the *Maxnodf* package (Hoeppke and Simmons 2021).

Cantavieja

Cantavieja had 65 pollinators and 20 plant species in 2021 and 71 pollinators and 13 plants in 2024. The total number of recorded interactions was 130 in 2021 and 247 in 2024. The level of network specialization was slightly higher in 2021 (H2' = 0.56 than in 2024 (H2' = 0.54), while connectance was notably higher in 2024 (C = 0.07) than in 2021 (C = 0.05). Robustness to plant loss was 0.51 in 2021 and 0.48 in 2024, robustness to pollinator loss was 0.68 in 2021 and 0.78 in 2024, and robustness at the network level was 0.37 in 2021 and 0.32 in 2024. Nomalized nestedness showed the opposite trend to network robustness, with 0.08 in 2021 and 0.14 in 2024.

Ejea de los Caballeros

Ejea de los Caballeros had 53 pollinators and 20 plant species in 2021 and 18 pollinators and 18 plants in 2024. The total number of recorded interactions was 121 in 2021 and 187 in 2024. The level of network specialization was slightly higher in 2021 (H2' = 0.58 than in 2024 (H2' = 0.52), while connectance was also slightly higher in 2024 (C = 0.06) than in 2021 (C = 0.05). Robustness to plant loss was 0.52 in 2021 and 0.51 in 2024, robustness to pollinator loss was 0.67 in 2021 and 0.79 in 2024, and robustness at the network level was 0.38 in 2021 and 0.32 in 2024. Nomalized nestedness showed the opposite trend to network robustness, with 0.09 in 2021 and 0.11 in 2024.

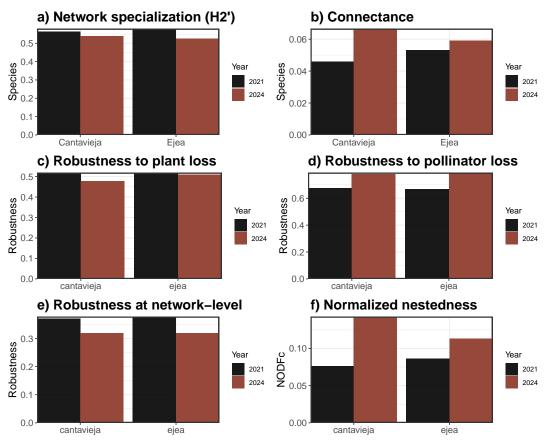


Figure 6. Comparison of network specialization (a), connectance (b), robustness to plant loss (c), robustness to pollinator loss (d), robustness at network level (e), and normalized nestedness (f) for Cantavieja and Ejea de los Caballeros across sampling years.

Concluding remarks

Overall, the total number of pollinators was higher after restoration practices along with key network descriptors such as connectance or normalized nestedness for both locations. However, it is worth noting that the accumulation curves of species richness and interactions does not saturate, likely indicating that further sampling effort to characterize the full plant-pollinator communities may be needed. In addition, in line with these results, the robustness to pollinator loss was higher in 2024. However, the networks showed lower number of floral resources, which have important implications for the robustness of the overall networks, as the loss of few species can lead more rapidly to the loss of pollinators. The fact that networks are more connected and slightly less specialized in 2024 could be due to a lower plant:pollinator ratio, resulting in a higher number of partners per plant species, more connected networks and lower chances of being selective for pollinators. Thus, while restoration practices may have increased pollinator diversity, the lower number of floral resources could threaten these ongoing conservation efforts.

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