Using motifs in ecological networks to identify the role of plants in crop margins for multiple agriculture functions.

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Abstract

Advances in network ecology offer new tools for the sustainable management of agroecosystems. Plants in field margins may be involved in different processes of agronomic importance - indirectly affecting crops by supporting shared pollinators, hosting natural enemies of pests or acting as pest reservoirs**.** In this work, we explored the use of motifs (patterns of interaction between a subset of species) in agricultural ecological networks incorporating multiple types of trophic interactions to identify plant species with a key role in field margins. We searched for plant species benefiting crops via (1) shared pollinators and (2) supporting parasitoids of crop pests; or (3) negatively affecting crops through attracting pest herbivore species. We focused on determining whether species' showed consistent affiliation with these roles across the landscape and evaluated the potential of plant species to influence crops indirectly via shared insect partners. To showcase our framework, we used a unique data set of antagonistic and mutualistic interactions recorded in soybean crops and wild plant species in field margins, that despite its limitations (e.g., spanning one growing season) allowed us to test our approach using multiple types of interactions simultaneously. Here, we support the use of motifs in multi-trophic interaction networks in agroecosystems to reveal the role of key plant species in sustaining ecological functions involving crops and other plant species, enhancing the evidence base for management recommendations in the future.

Keywords: Indirect interactions; Multi-trophic networks; Pollination; Pest control; Herbivory.

1. Introduction

Non-crop plants in field margins (e.g., hedgerows or flower strips) are essential for biodiversity conservation, sustainability and functioning in agro-ecosystems (Albrecht et al., 2020; see definition in Supplementary Material 1). A significant mechanism by which they affect crops is through spillover effects (i.e., cross-boundary movement of individuals; Rand et al., 2006; Supplementary Material 1). Via these reciprocal transfers plants may indirectly benefit the surrounding crops via bottom-up effects on their shared partners, e.g., increases in flower-visitor or parasitoid abundance leading to increased pollination and biological control. However, the same plants might negatively affect crops by attracting agricultural pests (Bianchi et al., 2006; González et al., 2016).

Plants are embedded in complex networks of interactions (Pocock et al., 2012) through which they can affect other organisms via a number of direct and indirect pathways (Bohan et al., 2013). A useful approach to capture this complexity is merging different types of interactions into a single multi-trophic network (Fontaine et al., 2011; Pocock et al., 2012; Supplementary Material 1). This is particularly relevant in agroecosystems considering plant multi-functionality and the potential trade-offs between positive and negative effects of maintaining surrounding natural vegetation on field margins (Windsor et al., 2021). By constructing these networks, it is possible to investigate the roles of species' involved in multiple different ecological processes.

Motifs, are the building blocks of larger networks formed by at least two species (Simmons et al., 2019), and can be used to track the role of species in networks and measure their prevalence across ecological communities (e.g., McLeod et al., 2020; Baker et al., 2015; Supplementary Material 1). Additionally, motifs allow for the detection of certain species that have high fidelity to their ecological role by consistently occupying the same position in networks (e.g., hosts and parasitoids interacting in the same way across time and space; Baker et al., 2015). Moreover, motifs are particularly useful for understanding indirect interactions occurring in ecological communities (Simmons et al., 2019; Supplementary Material 1), such as those that occur between plants on field margins and their adjacent crops. Knowing how network structure and species roles' influence ecosystem functioning is fundamental for advancing crop management in a biodiverse landscape (Bohan et al., 2013).

We studied a soybean crop-margin system in central Argentina using multi-trophic networks, composed of different types of antagonistic and mutualistic interactions (pollination, herbivory and parasitism interactions), to identify plant species in field margins with key roles relevant to agronomic management. We applied a network motif approach to assess the potential trade-offs between different roles of species and whether the species roles remained constant across different fields, potentially offering ecosystem services or disservices at the landscape scale. We specifically addressed three questions: i) Can motifs be used to detect plant species in field margins involved in key agronomical processes? ii) Do species show a consistent affiliation with certain roles across the landscape? iii) What is the potential of key species to influence crops indirectly?

2. Materials and methods

2.1 Applying motifs in multi-trophic networks from a Soybean-margin system

We studied a soybean-margin system from the Pampas, the main agricultural region in Argentina. We used a species interaction dataset from a field experiment carried out in the vicinity of “San Claudio" ranch in Buenos Aires (36° 00′ S, 61°5′ W) during the soybean flowering season in January 2018. We use data from 20 soybean plots to construct 20 multi-trophic networks involving mutualistic and antagonistic species interactions linking together soybean crops (*Glycine max* L.), plant communities in their field margins, pollinators (Diptera, Hymenoptera and Lepidoptera), herbivores (Lepidoptera caterpillars), and their parasitoids (Hymenoptera and Diptera) (the study system and data collection is described in more detail in Supplementary Material 2). Although this dataset had limitations (e.g. short period of time surveyed in one year; see Discussion), it provides a unique opportunity to test the utility of motifs as it consists of multiple types of interactions.

2.2. Key species in motifs

We used three motifs of agronomic interest, revolving around pollination, herbivory and parasitism, to identify key plant species roles on each multi-trophic network at each plot studied (Fig. 1). Pollination and herbivory motifs consisted of three taxa: a crop plant, a plant species from the field margin and a shared insect (pollinator or herbivore, depending on the type of motif). Parasitism motifs involved five taxa: a crop plant, a pest herbivore of the crop plant, a plant species from the field margin, a non-pest herbivore interacting with the non-crop plant and a shared parasitoid interacting with both the crop herbivore and the non-pest herbivore (Fig. 1). We searched the networks for the selected motifs and extracted the plant species identity and the number of times the plant occurred in each of the motifs across the different field sites (R code is available at github.com/Royal-Society-Agricultural-Networks/multitrophic-networks-motifs).

2.3. Fidelity of species to their role

In each plot, the roles of each plant species were defined as a vector, in which each element is the number of times the species is recorded in a given motif. For example, the role of species “a” in plot “5” is $\boldsymbol{f}_{a,5} = \{3,1,0\}$, corresponding to their incidences in pollination (3), herbivory (1), and parasitism (0) motifs, respectively. As a way to quantify fidelity of species to their roles, we evaluated if plant species were significantly associated with the different motifs studied across plots performing a non-parametric permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001). For the PERMANOVA, we used a Bray-Curtis dissimilarity matrix of species’ roles in plots as the response variable and the species identity as the grouping factor (4999 permuted values for the null distribution). We used the “adonis” function from the “vegan” package (Oksanen et al., 2020). See Supplementary Material 3 for a detailed version of the methods.

2.4. Potential for indirect effects

We evaluated the indirect interactions between a plant from the margins and the crop via shared partners, using the species composition of the different studied motifs. To do so, we estimated the potential for indirect interactions (PII, *sensu* Tack et al., 2011; Müller and Godfray, 1999). The index considers that if two species share consumers (e.g., pollinators), the host species (acting plant) that has more shared individuals (consumers) would have higher indirect impact on the other species (target plant). In this study, for each plot we obtained values of PII for each plant species from the margin participating as both an acting plant and a target plant when interacting with the crop. We estimated PII using the "PAC" function available in the "bipartite" package (Dormann et al., 2008). We compared the obtained values as target and acting plant between species to assess for the balance of interactions in motifs between the margin and the crop. To do so we implemented Linear Mixed Effect Models using the "nlme" package (Pinheiro et al., 2021). All analyses were performed using R 3.6.1 (R Core Team 2019). An extended version of the methods is available in Supplementary Material 4.

3. Results

In 15 of the 20 multi-trophic networks at least one motif was detected. The pollination motif was the most represented in the studied plots (N = 14), followed by the herbivory motif (N = 5; Supplementary Material 5). The parasitism motif was not registered at any plot, i.e., no parasitoids were shared between a pest host in the crop and a host in the margins. Moreover, species identities were significantly related to the different key roles studied, with the subset of plant species related to pollination motifs being different from those composing herbivory motifs (PERMANOVA: $F_{7,25} = 9.942$, $p < 0.001$).

Regarding indirect effects, measured as PII, when we compared the values of individual species as target and acting roles at each plot, we found varied responses in pollination motifs. *Eryngium* sp. showed a tendency to higher values as an acting than as a target plant, i.e., a source of pollinators to the crop rather than a sink ($F_{1,2} = 9.950$, $p = 0.088$). Conversely, *Hirschfeldia incana* acted primarily as a target species, receiving pollinators from the crop ($F_{1,4} = 7.578$, $p = 0.051$). Other species, such as *Melilotus albus* and *Carduus acanthoides* did not have differences in their role as target or acting plants ($F_{1,8} = 1.568$, $p = 0.246$ and $F_{1,6} = 0.260$, $p = 0.629$, respectively). In herbivory motifs, *Conyza* sp., the only plant species assessed showed higher values playing a role as target plant, receiving herbivores from the crop ($F_{1,2} = 19.639$, $p = 0.047$).

4. Discussion

Studying motifs in multi-trophic networks is a useful approach to assess key plant species in field margins and their potential to be involved in different agronomical processes. To demonstrate our perspective, we explored multi-trophic networks from soybean crop-margin systems and detected different plant species distinctly involved either in pollination or in herbivory motifs. However, we did not detect parasitism motifs, with no plant species in margins observed supporting parasitoids of crop pests. The commonness of pollination motifs could be explained by the presence of super generalist species (such as *Apis mellifera* L. and *Palpada* sp. Macquart) associated with numerous plant species leading to an increase in the number of interactions and consequently the number of pollination motifs (see Supplementary Material Table S5.2). Conversely, the absence or a low number of parasitism and herbivory motifs could be related to species rarity and the high interaction intimacy with their hosts, which could reduce the occurrence of interactions (Valladares et al., 2012, Tylianakis and Morris 2017). Moreover, more complex motifs with high number of species and interactions are in general less frequent than more simple ones, such as three species motifs (Simmons et al., 2019).

Motifs allowed us to identify that plant species in the field margins exhibited differential fidelity to the investigated roles. This highlights the importance of considering the use of a pool of plant species to design multifunctional margins, allowing to supply multiple ecosystem services while minimizing the disservices (Power 2010; Windsor et al. 2021). Moreover, we observed many plant species playing the same role (i.e., occupying the same position in motifs), mainly in pollination motifs, across the studied plots. This is relevant since the diversity (i.e., redundancy) in the resources offered to consumers (e.g., pollinators) could help maintain ecosystem functionality in these highly simplified systems.

Studying motifs enabled us to assess indirect interactions, capturing the potential of these plants to influence crops via shared partners. We observed, especially for pollination motifs, that plants from margins show different trends in their potential to indirectly impact the crop. This suggest that the different plant species could differentially benefit the adjacent crops acting as a source of resources and thus potentially promoting enhanced pollinator visitation in proximal crops. However, it is important to note that complementary research is needed to determine changes on tendencies across the time (i.e., phenological changes in the role of the plant in the network), or which are the processes (facilitation or competition) driving these indirect interactions (see Carvalheiro et al., 2014).

Constructing multi-trophic interactions networks is a challenge, requiring substantial effort to observe a sizable proportion of the interactions present within an ecosystem. Regarding the use of our dataset, we recognize several limitations; for example, we measured interactions over a single year only. Consequently, we may have missed changes in species composition and relative abundances, as well as changes in interactions between years as a result of variable biotic and abiotic conditions. Also, surveys were completed over a restricted time span

(January), centered on the soybean flowering season. This could restrict the detection of antagonistic interactions by not sampling other growth stages of plant species that might be more attractive to herbivores and, consequently, to their parasitoids. Moreover, all these limitations could result in a low number of recorded motifs and, therefore, a low number of replicates, reducing the robustness of statistical analyses and comparisons performed with these data. Despite these limitations, our dataset allowed us to trial the motif-based approach, which is applicable to different types of interaction or study design, including bipartite networks (see definitions in Supplementary Material 1). By now, we were able of identify potential candidates that could improve ecosystem services provided by biodiversity to soybean plantation. We hope that future studies can develop approaches to optimize the plant sets that would best benefit plant crops (e.g., Windsor et al. 2021).

Bridging the gap between fundamental and applied ecology, network motifs appear to be a useful tool to aid our understanding of the role of (semi)natural vegetation on agroecosystems with a view to making management recommendations in the future. To our knowledge, this is one of the first studies to use multi-trophic interaction networks to study the role of plant species with potential to influence the provision of different ecosystem services and disservices simultaneously (but also see Pocock et al., 2012; Windsor et al., 2021). Applying our approach to different crops and interactions of interest could reveal important ecological data informing the design of integrative farm-level field margin management strategies. We encourage other researchers to continue in this way.

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on field and identified specimens, all authors contributed to discussion and writing of the manuscript.

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Figure legends

Fig. 1. Ecological definition of motifs of interest of this study (Pollination, Parasitism and Herbivory) and their agronomic importance. Nodes are species with colors indicating interacting groups (aquamarine = soybean, green = plants from margin, red = herbivores, yellow = pollinators, violet = parasitoids), and lines represent links or connections between them. In circles are detailed the different studied motifs. In addition, we present a glossary with terms related to the topic of study. Authorship of the taxa images: Thomas Hegna (pollinators) and Kamil S. Jaron (parasitoid wasp) from http://phylopic.org/.

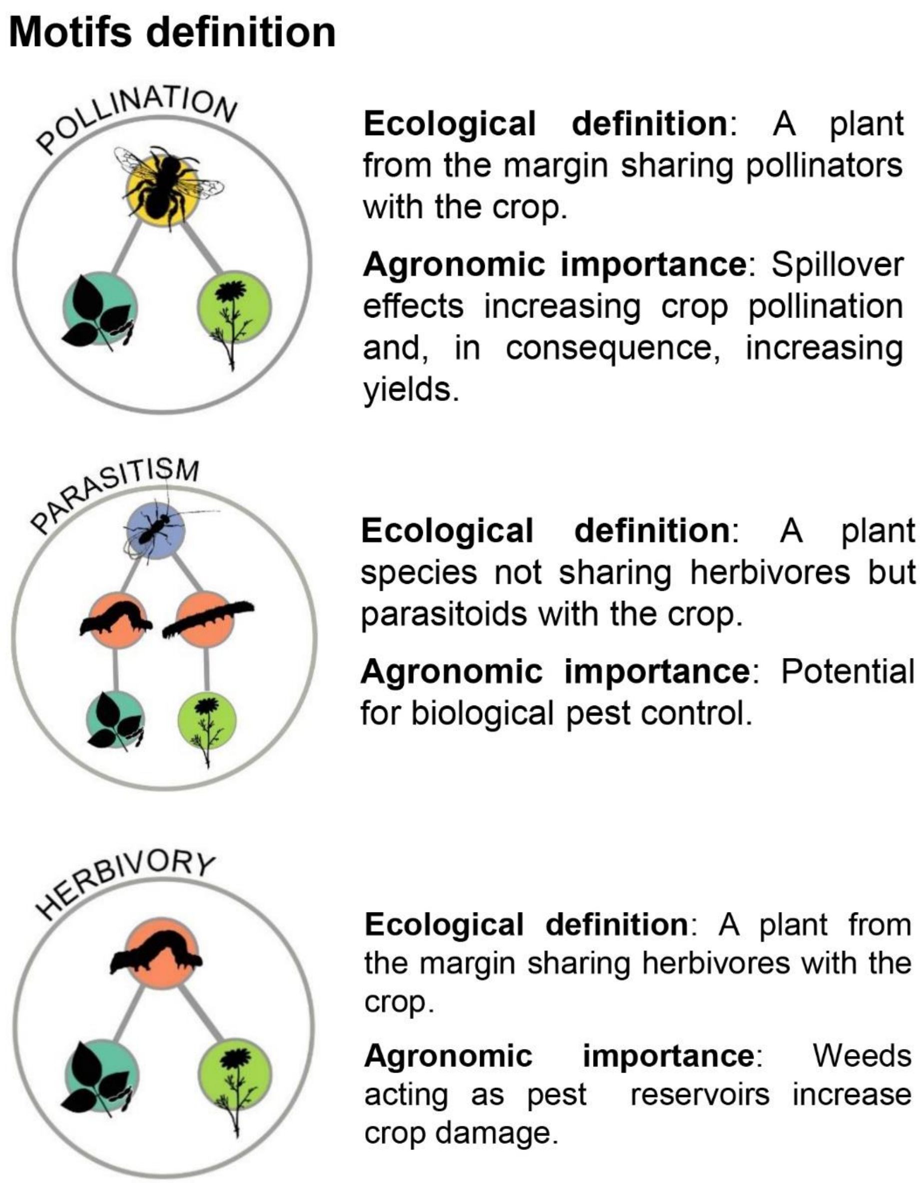
Motifs definition
POLLINATION
Ecological definition: A plant from the margin sharing pollinators with the crop.
Agronomic importance: Spillover effects increasing crop pollination and, in consequence, increasing yields.
PARASITISM
Ecological definition: A plant species not sharing herbivores but parasitoids with the crop.
Agronomic importance: Potential for biological pest control.
HERBIVORY
Ecological definition: A plant from the margin sharing herbivores with the crop.
Agronomic importance: Weeds acting as pest reservoirs increase crop damage.

Figure 1