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# Characterizing ecological interaction networks to support risk assessment in classical biological control of weeds

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A key element in weed biological control is the selection of a biological control agent that minimizes the risks of non-target attack and indirect effects on the recipient community. Network ecology is a promising approach that could help decipher tritrophic interactions in both the native and the invaded ranges, to complement quarantine-based host-specificity tests and gain insights on potential interactions of biological control agents. This review highlights practical questions addressed by networks, including 1) biological control agent selection, based on specialization indices, 2) risk assessment of biological control agent release into a novel environment, *via* particular patterns of association such as apparent competition between agent(s) and native herbivore(s), 3) network comparisons through structural metrics, 4) potential of network modelling and 5) limits of network construction methods.

## Addresses

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## Introduction

Ecological networks (popularized as ‘food-cycles’ by Elton in 1927 [1]) describe flows of matter and energy within a community. For example, trophic networks (food-webs) help to understand antagonistic interactions, e.g. such as predation, parasitism and herbivory [2]. Deciphering such networks is a promising approach to

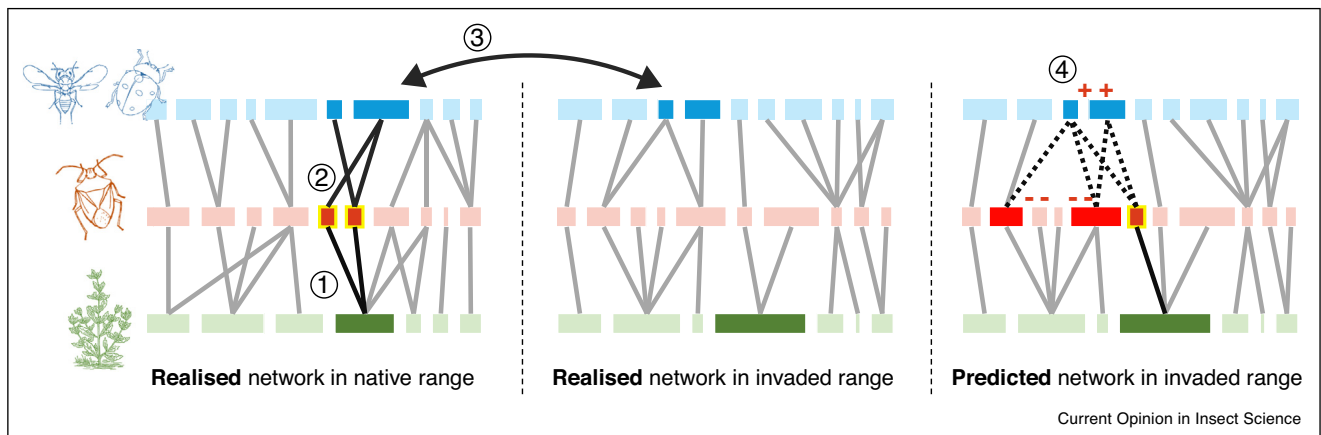
gain insight into niche-based community assembly, reflecting the complexity of species interactions and underlying ecosystem processes [3]. Such analyses can strengthen our understanding of fundamental drivers of community assembly [4,5], co-evolutionary processes [6], ecosystem response to biological invasions and global change [7,8], and ecosystem services management [9,10].

Network ecology could therefore benefit weed biological control, a discipline that aims to re-associate a plant species invading a novel environment with its specialist natural enemies (i.e. biological control agents). Although understanding species interactions has been advocated for more than 20 years [11–13], assessing risks still mostly rely on experimental tests. Network ecology could enhance such research programs through addressing practical questions inherent to weed biological control (Figure 1). This article reviews the approaches and methods that have been used to answer these questions and highlights the potential of ecological network analysis in the context of weed biological control. This review also provides a brief overview of the benefits and pitfalls of main network construction methods.

## Defining the community of herbivores and their host range to improve prediction of non-target attacks

Classical biological control of weeds uses specialist natural enemies of the target plants to selectively reduce their population dynamics under an acceptable economic threshold. A vital first step in this process is the compilation of inventories of natural enemies associated with the target weed in its native range. The specificity of a candidate biological control agent (BCA) is subsequently explored to reduce adverse effects on non-target plants [14,15]. Such tests are generally designed according to the centrifugal phylogenetic method [16] and performed in standardized environments under choice and no-choice conditions. This conservative approach can lead to false positive interactions as the realized field host range is potentially more restricted than the fundamental host range [17]. Risk evaluation solely under experimental conditions has always been known to be simplistic and increasing emphasis is being placed on field host-range assessments in the native range [15]. This implies characterizing interactions in diversified plant communities and being able to describe the realized field host range of

Figure 1



Illustrative tripartite networks showing interactions among communities of plants (green), herbivores (orange), and natural enemies (blue) (composed of predators and parasitoids). The first occur in native range, the second in invaded range. The third is a putative predictive network in the invaded range. As in conventional representation of tripartite networks, each species is represented by a rectangle, whose width reflects its relative abundance in the community. Analysis of networks is intended to enhance the selection of a biological control agent (BCA) with minimal risk of non-target attacks and indirect effects on the recipient community. The process is divided into several steps. 1: Look for herbivores specific to the target plant (dark green rectangles) in the field and determine potential BCAs (dark orange rectangles). Determining field associations may provide more realistic information about species interactions, than relying solely on tests under controlled conditions. 2: Identify potential natural enemies of these putative BCAs. Natural enemies could *i*) threaten BCA efficiency and, *ii*) be source of indirect effects on recipient community via indirect interactions. 3: Compare realized ecological networks in native versus invaded ranges based on *i*) structural and architectural properties and, *ii*) taxonomy. Networks associated with target species are expected to differ between native and invaded ranges in terms of species richness, trophic guilds and complexity. Moreover, if taxonomically closely related species of natural enemies are found between native and invaded ranges (dark blue rectangles), an introduced BCA is more likely to be attacked by these new natural enemies. 4: Predict possible species associations following the release and establishment of a BCA. The third network presents possible indirect effects (dotted line) of the introduced BCA *via* shared parasitoids with native herbivores (red rectangles). This indirect interaction (apparent competition) is likely to have adverse effects on native herbivores and could cascade across other trophic levels.

arthropods through the construction of bipartite networks (BPNs).

Two recent studies [18,19\*\*] characterized the diet of insects analyzing their gut content and reconstructed BPNs based on metabarcoding (molecular identification of the diet through high throughput amplicon sequencing). Zhu *et al.* [19\*\*] in particular identified host plant species of 239 Lepidoptera species, sampled in subtropical forest in China. By comparing traditional observations of plant–herbivore interactions and morphological identifications versus molecular analyses of gut-content and DNA identifications, this study revealed 46 plant species exclusively detected by molecular methods as well as an overall higher species resolution of ecological interactions than originally thought with traditional observation. On a community-wide scale, environmental DNA from wild flowers also proved useful to discover cryptic and unknown plant–arthropod interactions of diverse ecological groups, for example, pollinators, gall inducers, and herbivore species [20].

In weed biological control, ecological specialization of herbivores is a key requisite in their selection as a BCA. Realized interaction preferences are reflected in network

patterns [21\*] as ecological and evolutionary constraints tend to shape the modular structure of networks (*Modularity*: groups of species, e.g. modules, strongly associated with a particular set of plant species).

The computation of specialization indices is commonly performed for pollination networks [22,23] and capture different aspect of the architecture both for the whole network and individual species. Specialization can be measured by counting the number of resources per species (*Generality*) or by quantifying the dependence of a species upon a given resource (*Interaction strength*), (but see Ref. [22] for more indices and their correlations). Specialization patterns observed may be real, but also due to low sampling completeness, or intrinsic differences in resource attractiveness or abundance. Null models allow correcting for such possible artefacts [24]. Tools like *econullnet* [25\*] have been developed to look for resource preferences of a consumer by comparing observed and expected link strengths for every resource of a given consumer species. Novotny *et al.* [26] investigated the specificity pattern among feeding guilds of herbivorous arthropods by estimating their *effective specialization*, an index defined as the proportion of herbivore species feeding on a particular host plant and being unique to

this plant (detailed in Ref. [27]). A more recent study [28\*] compared the average herbivore specialization between the interior of a tropical forest and edges, that are supposed more disturbed and dominated by generalists. Here, the *specialization index* ( $d'$ ) translates to how the observed interactions of a species differed from randomly sampled interactions with other partners. The recently developed *distance-based specialization index* (DSI) [29], an extension of the *species specificity index* (SSI), accounts for phylogenetic similarity and abundance of hosts plant species (see application of both indices in Ref. [30]). This promising index also accounts for differences in abundance and sampling effort of consumers, which enables robust comparisons among herbivore guilds.

The improvement of molecular techniques coupled with contemporary analyses of BPNs offers multiple opportunities for acquiring insights on interactions occurring in natural environments, and may thus help to characterize field host range of biological control candidates, and complement host-specificity tests.

### Looking for predators and parasitoids of herbivores to improve predictions of indirect effects

Adding parasitoids and predators to convert bipartite networks (BPNs) into tripartite networks (TPNs) can also assist weed biological control. Describing such networks in native and invaded ranges could help analyze the influence of the third trophic level on BCA efficiency and detect the likelihood of indirect effects on the community dynamics.

Knowledge about parasitoids of BCAs is usually obtained as part of rearing BCAs identified in native range surveys. Characterizing predators of herbivores is more challenging as direct observation is required. Metabarcoding enables the detection of prey in arthropod gut-content but also early stage parasitoids in their hosts. In insect biological control, the use of advanced molecular technologies for constructing ecological networks has been recently developed [31\*\*,32] and could be directly transferable to weed biological control. The exploitation of newly introduced organisms by parasitoids of the recipient community is a novel association that has been repeatedly found in the context of biological invasions [33,34\*]. Likewise, predation of the BCA by native natural enemies is a pattern that has also been observed [11,35,36]. These discoveries confirm the ability of introduced organisms to modify food web structure. In a more recent study [37], a post-release food web was constructed involving the two BCAs of the weed *Melaleuca quinquenervia*. The results showed that generalists predators impact the population dynamics of the two released BCAs, although not significantly to diminish biological control efficacy. The community-wide effects of BCAs introduced to Hawaii were also explored *via* the

construction of a TPN and non-target effects have been identified on native communities [38]. Although suggestions have been made to use network analyses in assessing post-release safety of BCAs [11,13], they could also have value in pre-release assessments of indirect effects.

To our knowledge, in classical biological control of weeds, the only study attempting to quantify risk of indirect impacts before the release of the BCA has been in Portugal on *Trichilogaster acaciaelongifoliae*, a gall insect on *Acacia longifolia* [39\*\*]. The authors focused on apparent competition between the BCA and native herbivores due to a shared natural enemy, which in the worst case can lead to the extinction of the native species [40]. From a plant-gall insect-parasitoid TPN, they calculated the proportion of shared parasitoids between the BCA and a native galler. They estimated the potential for the galling BCA to affect the community according to two scenarios: 1) the BCA interacts only with similar species as those currently known to be in interaction with in its area of origin; 2) the galler interacts with all species belonging to the same family as the parasitoids currently known to attack it in its area of origin. In doing so, they predicted the potential for the BCA to interact with native parasitoids and resulting in highly significant indirect effects on the native gall insect.

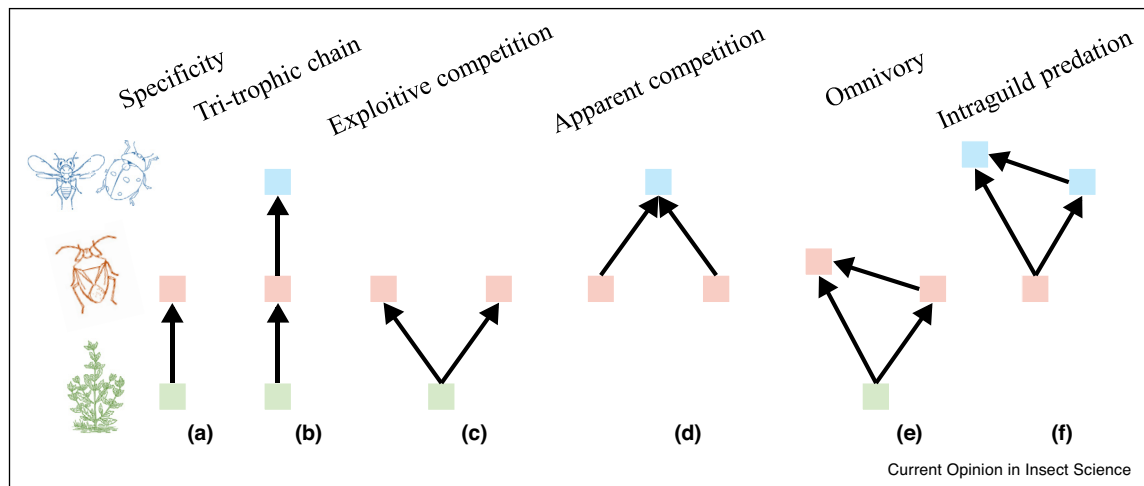
In addition to apparent competition, other indirect effects could be monitored through network analyses (Figure 2). Network motifs capture the meso-scale structure of a particular species assemblage. Tools like *bmotif* [41] can help count motifs, and species occurrence within motifs, of a BPN and could be employed to look for particular motifs involving BCAs. Despite their potential value, there has been limited use of ecological networks analyses in pre-release assessment in weed biological control programs.

### Comparing ecological networks between native and invaded ranges to describe novel interactions

The introduction of organisms into established communities raises the prospect of novel associations created in the recipient community. Assessing the extent of modification caused by either invasive alien plants (IAPs) or BCAs to recipient communities requires comparison with a reference, that is, communities from the native range, for network structure and species composition. Trophic networks associated with IAP species are hypothesized to 1) be composed of more generalist species and 2) be less diversified (at the herbivore and higher trophic levels) than the native community structure [42,43].

Memmott *et al.* [42] compared, between native and invaded habitats, the arthropod fauna on the IAP Scotch broom, *Cystisus scoparius*, before the BCA release. They

Figure 2



Common motifs studied in ecological networks to explore community assembly. (a) 2-node motif that can be encountered in bipartite and tripartite networks (a) specific relationship between a plant and a herbivore species). Motifs (b)–(d) are 3-node motifs present in bipartite and tripartite structures. (b) Tri-trophic chain (a) plant consumed by a herbivore, which is then preyed upon by its natural enemy). (c) Exploitive competition (a) similar resource shared by two consumers). (d) Apparent competition (a) shared consumer between two resource species). Motifs (e) and (f) cannot be represented in bipartite or tripartite structure, since they represent species interacting within the same community. However, these kinds of interactions occur frequently in natural ecosystems and can be visualized and studied in more complex graphs displaying intermediate trophic levels. (e) Omnivory (a) consumer feeding on diversified food sources, including plants and arthropods, for example, carabid beetles feeding of crop pests and weed seeds). (f) Intraguild predation (predation among a group of natural enemies also sharing a same resource, for example, among natural enemies of aphids, mirids can feed on syrphid eggs).

confirmed that the generalist species were more abundant in the exotic range, while specialist species were dominant in the native range. By analyzing TPNs, authors observed higher herbivore richness in the native range, divided into seven feeding guilds, whereas some guilds (seed and flower feeders) were absent in the invaded range. The increased biomass and abundance of herbivores in the native range coincided with higher natural enemy biomass and abundance. Similar observations have been made by comparing the structure of parasitoid complexes associated with herbivores in their native and invaded range [43] that also pointed out a correlation between the abundance of parasitoids attacking a host in its native versus invaded ranges. In a rare study that investigated realized interactions through network comparison after release of a BCA [44], food webs constructed from the two galling BCAs of the IAP *A. longifolia*, revealed similar taxonomic patterns at the family and super family levels and guild compositions. This study indicates the predictive power of food webs.

When comparing taxa compositions, the *Bray–Curtis similarity index* is the most commonly used. It allows assessing the difference in species composition between two samples considering abundance data [28,45,46]. Structural comparisons of trophic networks rely on the use of networks descriptors to extract information on species properties (e.g. *Ratio of prey to consumers*, *Proportion of species per*

*trophic level*), link properties (e.g. *Link density*, *Connectance*), and consumer-prey asymmetries (e.g. *Generality*, *Vulnerability*) [47]. These metrics, that can be elucidated using various analytical packages (e.g. *bipartite* [48], *cheddar* [49], *foodweb* [50], and *enaR* [51]), can enable a richer understanding of potential ecological interactions of candidate BCAs in the native versus invaded ranges.

### Predicting interactions to assess risks also means modelling

Predictive models of food webs are an additional important and helpful tool in biological control of weeds [52]. By combining the description of a static food web structure with dynamic population models, dynamic food webs could further our understanding and ability to predict changes due to species introductions [52,53]. In a recent study [54], a network model was proposed, based on phosphorus flows, to assess the direct and indirect effects of different biological control methods on the dynamics of algal blooms. Key nodes were identified in the network as particularly efficient to control algal blooms, and strong indirect influences were observed between functional groups. This methodology could be adapted to classical biological control. Sophisticated development of models in closely related research fields of invasion biology [55] and ecosystem management [56,57] would also be transferable to weed biological control.

## Selecting the best methods for reconstructing reliable ecological networks

The relevance of ecological networks for weed biological control depends on the reliability of the data and the methods used to build them. According to the method, ecological networks summarize different kind of species interactions [45\*\*]. Field collection provides networks representing realized interactions, but may be subject to false negative inference due to insufficient sampling effort [21\*,58]. Networks based on literature or database surveys [59,60] for supplementing field observations lead to likely interactions. In addition, models and machine-learning algorithms may be used on data such as presence-absence [61], body size [62,63] or species traits [64,65], to generate predicted interaction networks.

Constructing reliable ecological networks requires knowledge about the benefits and limits inherent to each method in order to choose the methodology suited to the studied system and the research questions addressed. Revealing realized trophic links traditionally rely on labor-intensive techniques based on direct field observations, rearing, or microscopic dissections of gut content and faeces [66]. While providing meaningful behavioral information, these approaches present major limitations when working on below-ground or nocturnal species and prevent the dietary study of sap feeders insects [67]. Approaches relying on plant alkane fingerprints, protein electrophoresis of gut content, stable isotope analysis, detection of prey proteins based on polyclonal and monoclonal antibodies (ELISA) and DNA-based methods can help overcome barriers of visual identification [12,68]. However, the performances of these techniques are context-dependent [67]. Plant alkane fingerprints and protein electrophoresis are not suited to reflect diet breadth of generalist species (providing uninterpretable overlapping banding patterns) [69]. Isotopic enrichment studies have the advantage of providing information over longer temporal scales, integrating past energy flows rather than just the most recent meal. However, isotopic signatures are subject to variations among species that can lead to inconsistent and unclear trophic links [70]. After DNA-based techniques, the monoclonal antibody approach is the second-most used method for the evaluation of food webs in agriculture [71]. Preys antigens offer the benefit of being detectable for a longer period following their consumption [72], compared with rapid degradation of prey DNA in consumer gut content. Although antigens are good markers for screening the consumption of a specific prey by a range of predators, they are not suitable for complex food web analyses as their development would be expensive and time-intensive [73].

DNA-based methods are increasingly used in contemporary food web elucidation in agriculture [66,71,74]. Most commonly, DNA metabarcoding [75] associated with Next Generation Sequencing (NGS) technologies [76]

offers us the possibility to efficiently process large number of samples in the context of biological control. For example, from a field-collected sample of arthropod gut contents, food-range can be tracked and difficult-to-observe interactions, such as host-parasitoid interactions, can be revealed, regardless of insect life stage [77,78]. However, these methods are also prone to potential sources of errors. Sampling device and storage can infer false positive interactions (through external contaminations, secondary predation or scavenging) [31\*\*,79,80]. Insects would be best collected individually, using an aspirator or by hand directly with sterile forceps [80]. This time-intensive method can be adapted by limiting collection time to standard periods at each collection site, normalizing the sampling effort for between site comparisons. Besides sampling incompleteness [21\*], DNA stability and detectability are also a source of false negative interactions. Sensitivity tests may be used to assess how long after ingesting a prey or plant DNA can be detected in consumer gut [80]. Multiple primer set combinations are also recommended to amplify DNA with a large taxonomic coverage [81]. While the mitochondrial gene COI is generally recognized for its properties in arthropod species delineation [82], multiple plant markers are needed for determining plant species [19\*\*]. Lastly, the accurate identification of DNA fragments will fully depend on the quality and completeness of the reference database queried [83,84].

Since sampling incompleteness and the general ability to accurately reveal species interactions may introduce bias to a majority of network descriptors [21\*], the analyses and comparisons of resulting networks require practitioners to be fully aware of the pitfalls and potential that a chosen method offers.

## Conclusion

Characterizing and analyzing ecological interaction networks structure in both, native and invaded ranges generates insights on the processes underpinning effective biological control. It also enables projections of the direct and indirect effects that a biological control agent would have and assist choosing a species that would: 1) be specific to the plant based on natural interactions recorded, 2) possess few natural enemies or natural enemies that would belong to different taxonomic groups as those encountered in the range of introduction. Network analyses, supplemented by advanced molecular methods, could enhance the development of safe biological control strategies and also improve the confidence in biological control among regulators and the general public

## Conflict of interest statement

Nothing declared.

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