Highlights

Integrating microorganisms into community-level ecology remains a critical challenge despite their important influence on macro-organisms.

Insects and their symbionts are a tractable and powerful system to uncover how microbial associations reshape ecological interactions.

Network analysis provides transformative tools to integrate symbionts into ecological frameworks and to test key hypotheses on their community-level roles.

Embedding symbionts into networks can expose hidden drivers and advance mechanistic understanding of community functioning under different scenarios.

Mapping microbial symbiont impacts using ecological networks

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Abstract

Microbial symbionts can dramatically reshape host biology, triggering cascading effects that alter the strength, direction, or even presence of interactions among macro-organisms. However, integrating microbes into community-level understanding remains a major ecological challenge. Using insect–facultative symbiont systems as a model, we show how ecological network analyses can reveal these hidden layers of complexity for a deeper, mechanistic understanding of community functioning. By combining testable hypotheses with tools such as multilayer networks or stochastic co-extinction models and interaction rewiring, we provide a roadmap for uncovering how symbionts drive ecological and evolutionary dynamics, opening new possibilities for empirical studies that link microbial traits to ecosystem-level impacts.

Integrating Microbial Symbionts into Ecological Communities

Ecosystems consist of dynamic networks of interacting species, in which macro-organisms associate with diverse internal and external microorganisms [1]. These microbes can influence their hosts' biology both directly (e.g., facilitating nutrient acquisition, causing disease) [2, 3] and indirectly (e.g., modifying interactions among host organisms or between organisms and their environment) [4]. Relationships with micro-organisms are particularly important for insects, many of which have evolved specialised associations with bacteria, protists, fungi or viruses [5,6]. Disentangling microbial symbionts’ effects on insect community ecology requires incorporating the hidden presence of symbionts within the food web context.

Insect–Symbiont Relationships: Dynamics and Evolution

The extent of interdependence between insects and their symbiotic partners varies greatly. Obligate symbionts are essential for the survival and reproduction of both parties [7]. These associations are evolutionarily ancient, maintained through strict maternal transmission [8], and often have a nutritional function to supplement imbalanced diets [9]. Others mediate antagonistic interactions, as in the symbiotic viruses of ichneumonid and braconid parasitoids that suppress host immune systems [10]. Owing to their extreme functional integration, obligate symbionts and their hosts operate as a single biological unit within ecological communities and can be treated as single entities when analysing species interactions.

Facultative symbionts are not required for host growth and reproduction and are not universally present across natural insect populations [11]. The symbionts rely on hosts for transmission, but the evolutionary interests of host and symbiont are not necessarily aligned, resulting in interactions that span the parasitism–mutualism spectrum. Some facultative symbionts can confer benefits, such as defending their hosts against predators, parasitoids and pathogens [12] or protecting from abiotic challenges such as heat shock [13]. However, another common strategy is manipulating host reproduction to promote their own transmission. Maternally-transmitted facultative symbionts favour a female bias in host offspring sex ratio [14], producing a variety of manipulations—feminisation (see Glossary), parthenogenesis, male killing and cytoplasmic incompatibility [15, 16]. Reproductive manipulation and symbiont-mediated protection are not mutually exclusive [17], and many facultative symbionts function as conditional mutualists: beneficial in certain ecological situations, and costly in others.

Facultative symbionts can influence the outcome of insect pairwise interactions, such as those between prey species and their natural enemies [18,19]. Recent studies have broadened this perspective by examining effects of symbionts within tri-trophic systems, such as plant–herbivore–parasitoid (e.g. [20,21]) and host–parasitoid–hyperparasitoid [22] interactions. Symbiont-mediated traits could propagate through ecosystems, modifying the intensity, direction, or the existence of interactions between macro-organisms [23]. We propose using tools from ecological network theory as a framework for quantifying and analysing impact of facultative symbiotic interactions at the community level.

The Role of Symbionts in Ecological Networks

Application of network analysis in insect–endosymbiont communities

Ecological network analysis quantifies interactions among species to determine structure, function and dynamics of ecological systems [24]. An ecological network is a map of nodes (e.g. species, populations, functional groups) connected by edges (e.g. trophic interactions, mutualism or competition), providing a visual and mathematical representation of a community. The construction and analysis of host–symbiont networks involves the collection of interaction data, typically weighted by the strength or frequency of these interactions [25]; their representation as matrices detailing the links between nodes (Box 1; Box 2); extraction of metrics to interpret the structure [26]; and mathematical modelling to predict responses to change [27,28].

Approaches to Integrating Symbionts in a Network

Incorporating microbial symbionts into ecological network analyses requires careful consideration of the research question and study system. We suggest four approaches to describe different structural representations; outline how hosts, symbionts, and their associations can be treated in a network; and discuss how each approach can be used to explore processes such as coextinction, infection spread, interaction rewiring, or responses to change (Figure 1). Several processes (e.g. responses to change) can be analysed across approaches; what differs is how each framework represents these processes structurally.

Approach 1: Treating symbionts as independent nodes. This approach is suitable for complex ecological systems, in which individual symbiont infection status cannot be monitored (e.g. observational studies, manipulated mixed infected–uninfected populations). Symbionts are considered separate nodes interacting directly and indirectly with other community members, capturing their effects without individual-level data. For example, the bacterium *Hamiltonella defensa* infecting the whitefly *Bemisia tabaci* provides several phenotypic advantages on tomato plants, suppressing plant defence and increasing whitefly reproduction [29, 30]. In *B.tabaci* biotype B, an *H. defensa*-produced protein interacts with the *Tomato yellow leaf curl virus*, facilitating its transmission to the plant [31]. These changes can potentially benefit other herbivores and impact predator–prey dynamics. Representing the symbiont as an independent node linked to the host by infection frequency (Figure 1) would capture its community-level effects, such as altered predatory dynamics, or changes in **apparent mutualism**. A similar approach has been applied in the analysis of parasites as independent nodes [32]. In estuarine ecosystems, parasites can form structural units different from free-living species and significantly affect food web structure and decrease network **robustness** due to their complex interactions [33].

Approach 2: Distinguishing between symbiont-infected and uninfected insect nodes. Infection status (whether a host is infected or symbiont-free) can be treated as a node property. This approach applies when performing experimental manipulation of endosymbiont infection, such as comparing infected and cured communities. Hosts with different infection states can be represented as separate nodes within the same network, or within different networks for infected and symbiont-free populations (Figure 1). This approach could be particularly useful for identifying indirect effects and testing **interaction rewiring** scenarios empirically (see following sections). For example, infection by the symbiont bacterium Wolbachia is often heterogeneous within Drosophila populations. Wolbachia can alter host–parasitoid dynamics by influencing parasitoid developmental success [34]. Separating Drosophila nodes by infection status would account for this specificity when considering interactions such as competition and apparent competition. More broadly, incorporating intraspecific variation can reveal hidden mechanisms: in pollination networks, for instance, accounting for variation in pollen loads and deposition in communities invaded by the alien plant Himalayan balsam (*Impatiens glandulifera*) helped disentangle the ecological processes underlying invasion dynamics [35].

Producing separate ecological networks allows comparison before and after the spread of a symbiont, or in different geographic locations. Comparing networks with uninfected or infected nodes enables quantitative evaluation of network properties such as **modularity** (the extent of sub-structuring within the network), **connectance,** and robustness to species loss, thereby revealing the ecological consequences of symbiont-driven host variation. Similar approaches have been applied to investigate how the pathogen *Anaplasma phagocytophilum* affects the microbiota of *Ixodes scapularis* ticks and their colonisation resistance, finding that *A. phagocytophilum* infection reduces microbial network robustness, making ticks more vulnerable to microbial invasions [36].

Approach 3: k-partite networks. ***k*-partite networks** focus on direct interactions between two discrete groups (bipartite) (e.g., host and symbiont or host and parasitoid) or more (tri-, quadripartite, etc.), with no connections between components of the same trophic level [26]. These networks are, therefore, most appropriate when the focus is on interactions across trophic levels, such as when symbionts perform a protective or nutritional function. For instance, this approach could analyse how environmental stressors (e.g., heat waves, drought, insecticides) affect systems composed of hosts classified by infection status (infected versus uninfected; see Approach 2), interacting with other groups, such as basal resources, parasitoids, predators, or pathogens (Figure 1). In such systems, stressors can act as external modifiers of interaction strength [37]. For example, heat waves disrupt endosymbiont-mediated benefits in parasitoids, weakening their suppression of host immune responses and increasing parasitoid mortality [38]. These disruptions can lead to a decrease in key network properties—such as **interaction frequency**, connectance, or modularity—particularly if parasitoids shift to alternative hosts and appear less specialised [39] or are excluded from parts of the network under stress. *k*-partite networks have been widely applied in plant–pollinator and plant–pest–natural enemy systems to assess how environmental drivers alter interaction structure and network stability [40].

Approach 4: Multilayer networks. These networks integrate different entities, locations, time points and/or interaction types in a single network by distinguishing between types of network edges in different layers [41]. This approach provides a flexible framework for disentangling the context-dependent (e.g. multiple interactions, time, space, etc.) roles of symbionts within ecological systems [42]. Inter-layer edges are key components differentiating multilayer networks from multiple discrete ecological networks (Box 1). These edges link ecological processes (e.g. reduction in parasitism due to host infection status) to interaction networks, allowing species interactions and their drivers to be analysed jointly [43].

Layers could represent distinct interaction types (e.g. symbiosis, parasitism, competition), with interlayer edges connecting the same host across layers (Figure 1). This would enable researchers to analyse (i) how facultative symbionts modify host ecological roles across contexts and (ii) how disturbances (e.g., symbiont loss or acquisition) propagate between layers. As an example, mushroom-feeding *Drosophila* communities often share fungal breeding sites and are commonly infected by the parasitic nematode *Howardula aoronymphium*, which substantially reduces female fertility and increases mortality. Strong interspecific aggregation on mushrooms leads to high cross-species transmission, such that high parasitism in one species can increase infection in others, leading to apparent competition [44]. Within this system, *Drosophila neotestacea* can harbour a defensive *Spiroplasma* strain that protects females from nematode-induced sterility [45], altering infection dynamics at the community level. In a multilayer framework, such effects could be represented through interlayer edges weighted by symbiont effects on nematode infection and linking symbiosis, parasitism, and competition layers (Figure 1).

Changing community structure can be captured as shifts in **interlayer connectivity** or modularity, helping quantify the impact of symbiont-mediated indirect effects on overall network structure and stability. Similar approaches have revealed cascading impacts in other systems; for example, invasive ungulates disrupted pollinator-seed dispersal networks by decoupling interaction layers (i.e. pollination and seed dispersal connected via shared plants), reducing robustness and accelerating the spread of disturbance [46].

Multilayer network analyses could model systems with multiple infections within an individual host. The costs of carrying multiple symbionts may combine additively, or there may be negative or positive interference. For example, testing all possible combinations of genotypes of the microbial symbionts *Hamiltonella defensa* and *Fukatsuia symbiotica* in a single pea aphid genotype resulted in a high diversity of phenotypes when exposed to different ecological challenges [47]. In this instance, multilayer networks could generate a more integrative view of direct and indirect effects of symbionts at different levels of the trophic network by analysing the effect of co-infections with different symbiont genotypes on more than one environmental challenge at a time (e.g. host plants and natural enemies). Multilayer networks can be constructed with each layer representing the interactions of symbiont hosts and other partners, such as natural enemies or plants, under a distinct symbiont infection state. Interlayer edges reflect interaction pattern changes across infection states, connecting the same host species or natural enemy across layers. For hosts, these edges can indicate changes in interaction frequency (e.g. attack rate), fitness or interacting partner identity. For natural enemies, interlayer edges can represent changes in attack efficiency across layers. This structure allows for the application of multilayer-specific metrics—such as **versatility** or **interlayer flexibility** to dissect direct and indirect ecological impacts of multiple infections.

Multilayer networks can also capture temporal dynamics (e.g. infection rates by insect symbionts varying seasonally [48]), spatial variation (e.g. symbiont infections correlating with geographic areas [49]) and **horizontal transmission** of symbionts (e.g. symbionts spreading between parasitoid species sharing a single host [50]).

Using ecological network tools to evaluate effects of symbionts at community level

Endosymbiont-mediated host phenotypic changes can lead to emerging indirect effects such as **exploitative competition**, **trophic cascades**, apparent competition or **apparent mutualism** [21,51]. These effects have typically been explored through experimental approaches, where the presence of symbionts is manipulated to observe consequences for third-party species (e.g. [20,21,52]). While effective, such studies only capture a fraction of the indirect interactions present in complex natural communities.

To incorporate the wider community, interaction matrices trace indirect interactions [53] by capturing multi-step relationships (e.g. a symbiont affecting predator populations via changes in host traits). However, identifying underlying mechanisms, such as behavioural or immunological changes in the host, requires integration with other complementary methods that quantify the relative importance of direct and indirect interactions (e.g. **structural equation modelling, path analysis** [54,55]).

Host–symbiont networks have community structures defined as clusters or groups of nodes that are more tightly connected internally than with the rest of the network. In insect–symbiont networks, detecting community patterns could highlight distinct assemblages [56], such as clustering of insect species in well-documented complex networks that rely on similar types of symbionts (e.g. nutritional symbionts in one cluster, defensive symbionts in another). Species that bridge clusters, such as generalist hosts or symbionts with multiple functions, can play a crucial role in facilitating indirect effects. Changes in their interactions can propagate across clusters, influencing species in distant parts of the network [57]. **Community detection methods** provide a wide variety of algorithms (e.g. modularity, **spectral clustering**, or **overlapping community detection**) to explore indirect interactions by identifying and revealing patterns of ecological specialisation and interaction strength [58].

Symbiont Loss, Extinction Cascades, and Network Resilience

Fluctuations in infection frequency in insect-symbiont associations can profoundly reshape community structure and dynamics [59]. By simulating species (node) losses, stochastic co-extinction models offer critical insights into community resilience [60]. In insect–symbiont networks, modelling symbiont extinction could reveal the cascading effects of symbionts on hosts and their broader ecological interactions (Box 3). However, coextinction risks are often mitigated through interaction rewiring [61]: higher-level components consumers switch to alternative resources following the loss of their original partners (e.g., [62]) (Box 3). The introduction of defensive symbionts into an insect community illustrates how biological constraints determine rewiring potential. Defensive endosymbionts can lead to insect hosts becoming unsuitable as resources, thus disrupting interactions with higher-level consumers. Parasitoids can respond by shifting host species, targeting different developmental stages or exploiting hosts on alternative plants [63,64]. Incorporating rewiring into **extinction modelling** would permit exploring both the direct consequences of endosymbiont loss and the conditional capacity of communities to adapt through interaction reconfiguration.

Concluding Remarks

Understanding of the ecological processes involving symbionts from a community perspective remains limited. Ecological network analyses can disentangle complex processes, and freely available R-packages, including bipartite [65], igraph [66], NetworkExtinction [67], EMLN [68], and econullnetr [69], now support network construction, extracting metrics and modelling extinctions or resource selection. By explicitly mapping symbiont presence within a network of interactions, researchers could detect how symbionts influence community stability, clustering, and energy flow—patterns that remain hidden when using observational or experimental techniques alone. Incorporating symbionts into network models would therefore not only reveal their ecological importance, but also open the door to a deeper, mechanistic understanding of community functioning under both natural and disturbed conditions. Integrating theoretical models with empirical data from field and laboratory studies, will advance our understanding of host–microbe–environment interactions and help consider microbial symbionts not as peripheral elements, but as active and dynamic components of ecological networks (see Outstanding Questions).

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Declaration of interests

The authors declare no competing interests.

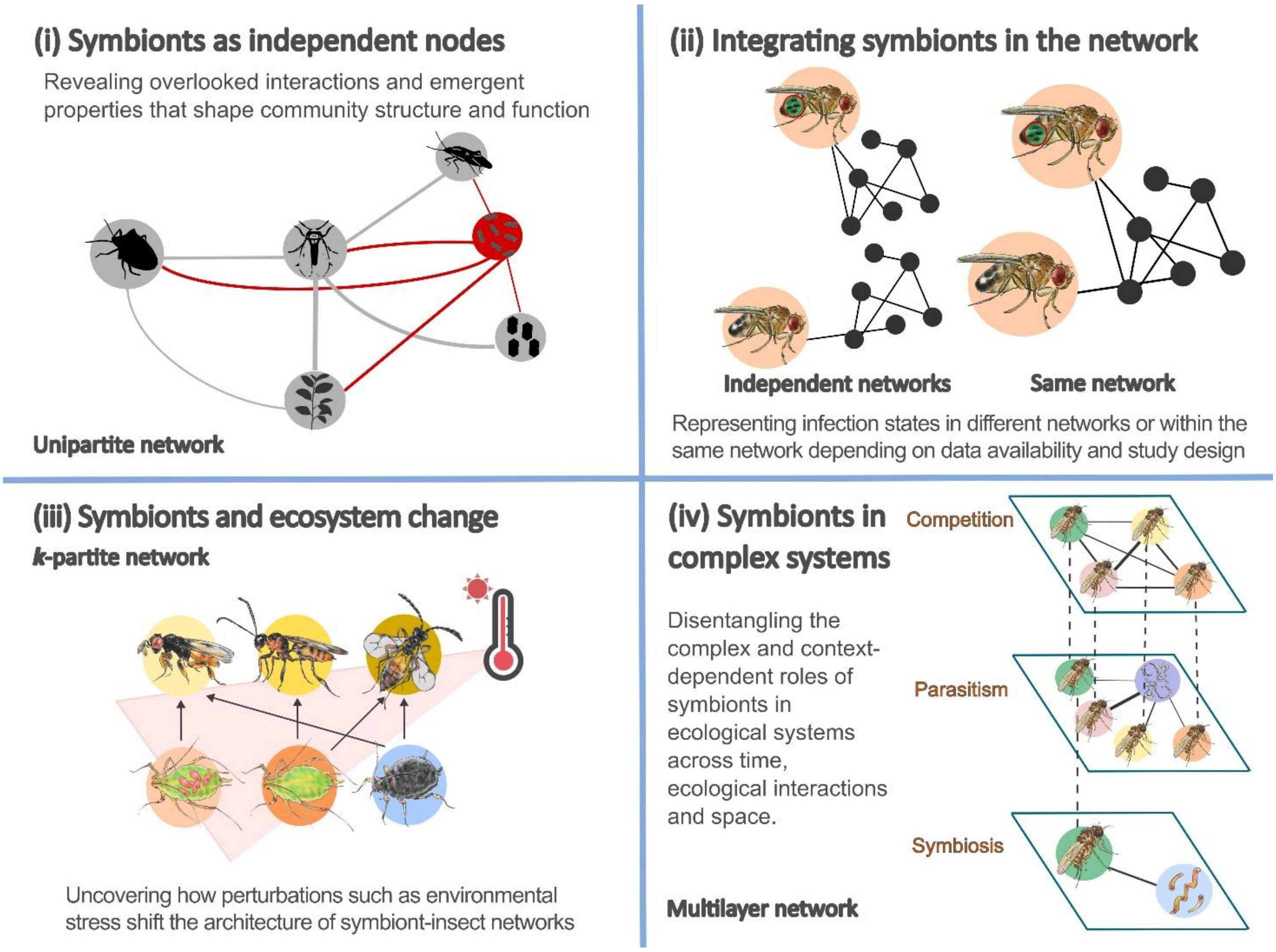

Figure 1. Integrating symbionts into ecological networks. Symbionts can be represented (i) as independent nodes in unipartite networks to reveal hidden interactions; (ii) as a node property to compare infection states in experimental studies; (iii) in k-partite networks to map host–symbiont–partner interactions, across trophic interactions; (iv) in multilayer networks to capture multiple interaction types, temporal changes, or infection states, highlighting context-dependent roles (e.g. the effect of spreading Spiroplasma-infected D. neotestacea on Drosophila communities parasitised by the nematode Howardula aoronymphium. Green node: D. neotestacea, pink node: D. putrida, yellow node: D. falleni, orange node: D. recens). Network icons drawn by Biliana Todorova.

Box 1. Mathematical Representation of Ecological Networks

Complex communities consist of multiple species—each represented as a node—connected by interactions such as parasitism, mutualism, or predation. These interactions are best captured using networks, where any pair of nodes can interact (unipartite), interactions have a direction (directed), and their strength can vary (weighted). The fundamental mathematical representation of such an interaction network is an adjacency matrix, where rows are resources, columns are consumers, and entry a_{ij} reflects interactions from node j to node i—such as infection frequency or parasitism rate. **Self-interactions** (e.g. cannibalism) appear on the diagonal (Figure IA) [27].

Two species can be connected by more than one interaction type—**multi-edges** (Figure IA) that can be mathematically represented by: (i) separate matrices for each interaction type; (ii) multilayer network models.

To examine structured subsets of complex communities, *k*-partite networks offer a focused representation of interactions restricted to distinct trophic or functional groups. Species are grouped into *k* distinct trophic or functional levels (e.g. plants, herbivores, parasitoids), and interactions occur only between species in different levels.

Multilayer networks extend traditional network representations by incorporating multiple interaction types or contexts (e.g., habitats, temporal dynamics) in separate matrices (layers). Nodes may occur in one or several layers, with inter-layer edges linking them across contexts—e.g. how symbiont-mediated protection alters parasitism (Figure IB) [42]. These networks are represented by a supra-adjacency matrix integrating all layers and their connections. Each layer α has its own $n \times n$ intra-layer adjacency matrix (A^{α})

while inter-layer matrices ($B^{\alpha\beta}$), sized $n_\alpha \times n_\beta$, describe node connections across layers α and β. Just as with edges in a monolayer network, inter-layer edges may take several forms. Inter-layer edges with the same weight (uniform) describe the assumed dependence of layers on each other. In contrast, inter-layer edges with specific weights to each connection (non-uniform) represent the actual or estimated linkage between two nodes and add substantial resolution to the network [43]. For example, in temporal networks, non-uniform inter-layer edges, representing fluctuations in species abundance, detect the species-specific responses to seasonality.

Once a network is constructed, its structure can be analysed using key metrics describing size, interaction density, and connectivity [27]. These metrics underpin models of co-extinction or rewiring, and other analyses of community robustness, dynamics, and evolution.

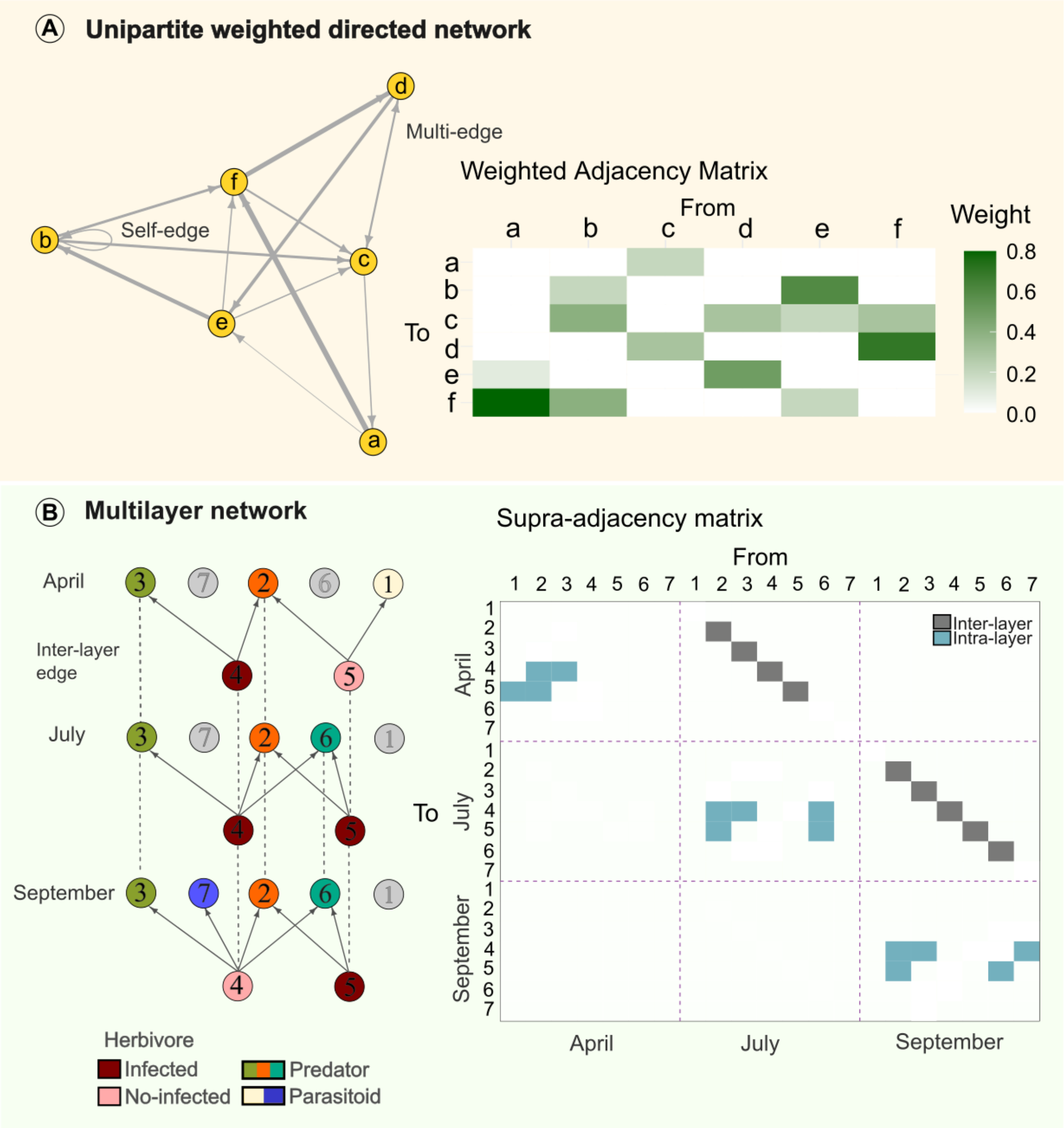

Figure I. Graphical and mathematical representation of (A) a unipartite weighted directed network and (B) host-symbiont network dynamics across seasons (April, July and October) in a multilayer network where every layer represents a bipartite network (in this case herbivore–natural enemy) at different times. The arrow thickness in (A) represents the weight of the link. Dashed lines in (B) indicate interlayer edges and nodes that are in grey-scale are absent in that layer.

Box 2. Identifying Nodes and Links in Insect–Symbiont Ecological Networks

In insect–endosymbiont networks, each species could be represented as a node. Node identification begins with the taxonomic classification of insect hosts. Symbionts are identified primarily through molecular tools such as metabarcoding, single or multiplex PCR, and qPCR. Whether symbionts should be treated as independent nodes or attributes of hosts depends on the ecological question and system (see main text). The choice of symbiont identification technique—quantitative or qualitative—also depends on the study context (e.g., natural communities, experimental systems, or manipulated populations).

Direct interactions between hosts and symbionts can be revealed through molecular detection, while broader ecological interactions—trophic and non-trophic—can be inferred from empirical observations, literature, or expert knowledge [27]. Methods include observational data (e.g. parasitism rates), stable isotope analysis, gut content identification, and controlled experiments (e.g. feeding or parasitism trials). High-resolution molecular tools such as gut content PCR and metabarcoding can further enhance the identification of within-community interactions [70]. However, not all interactions are direct. Indirect effects—often mediated by symbionts—require specialised approaches to infer (see main text).

Understanding the strength (weight) and direction of interactions is essential for ecological interpretation. In host–symbiont networks, infection frequency—quantified via PCR—can serve as a proxy for interaction strength. For symbiont-mediated defensive interactions, weights may be based on parasitism rates or the proportion of prey DNA in predator diets.

Symbiont-mediated effects are highly context-dependent. Their phenotypic outcomes vary based on host genotype, symbiont strain and identity, environmental conditions [71], and co-infection dynamics [72]. For instance, in pea aphids, combining a set of different isolates of *Hamiltonella defensa* and *Fukatsuia symbiotica* in coinfections resulted in diverse symbiont-mediated phenotypes when exposed to different ecological challenges [47]. Therefore, symbiont detection alone is insufficient to assume ecological function. Currently, establishing functional links often requires experimental exposure of hosts with known symbionts to specific challenges. Nevertheless, advances in symbiont genomics are providing promising insights that may in future aid in predicting symbiont-mediated host phenotypes from symbiont genotypes, such as the identification of toxin cassettes on mobile genetic elements. For example, different phage **(APSE)** toxins in *H. defensa* correlate with different levels of protection against parasitism [73].

Box 3. Modelling Symbiont-Driven Network Dynamics

We used data from a field experiment by Rothacher et al. [74], who tested whether the facultative endosymbiont *Hamiltonella defensa* reduces parasitism in the black bean aphid (*Aphis fabae)*. The authors provide a full, species-level record of aphid–primary parasitoid–secondary parasitoid interactions.

To illustrate network analysis in symbiont–insect systems, we constructed a simplified weighted tri-trophic network using the most abundant five primary parasitoids and three secondary parasitoids. Interaction strengths were derived from emergence counts and normalised to represent proportional dependencies among nodes (Fig II A).

We applied a probabilistic co-extinction model [75] to explore cascading consequences of symbiont-mediated host loss. We removed the symbiont-infected H+ aphid phenotype, and, at each step, the algorithm propagates secondary extinctions based on species' proportional dependence on lost resources (Fig. II B). This framework moves beyond deterministic thresholds by incorporating interaction-strength-dependent extinction probabilities (Fig. II B), such that specialists face higher extinction risks than generalists following resource loss.

To increase ecological realism, we extended the model to include rewiring capacity, allowing species to shift resource use after perturbation. Rewiring probabilities integrate: (i) prior use of alternative hosts, (ii) degree of generalism, and (iii) network position, with highly connected species exhibiting greater adaptive potential (Fig. II C). The model tracks cumulative extinctions across cascade stages while recording successful rewiring events, enabling comparisons between rewiring and no-rewiring scenarios. This highlights how behavioural plasticity can buffer symbiont-induced perturbations and influence overall network robustness.

The removal of H^+ aphids generated substantial secondary losses in the network, with extinction risks closely tracking species' proportional dependence on the H^+ phenotype. Under the probabilistic co-extinction model, two primary parasitoids were lost: *L. confusus*, which relied almost entirely on H^+ aphids, and *B. angelicae*, which, despite also attacking H^- aphids, was strongly affected by the disappearance of its preferred host. Secondary parasitoids, however, largely persisted because many primary hosts remained available. Incorporating rewiring reduced these losses: specialists were able to shift to alternative hosts, allowing *B. angelicae* to survive via increased use of H^- aphids. Overall, rewiring increased final community richness and boosted system robustness, illustrating how adaptive flexibility can buffer symbiont-driven perturbations.

This framework is intended as a conceptual demonstration. Applying it to real systems would require empirical data on probabilistic dependencies, temporal changes in resource availability, and context-dependent attack rates to parameterise extinction and rewiring processes accurately.

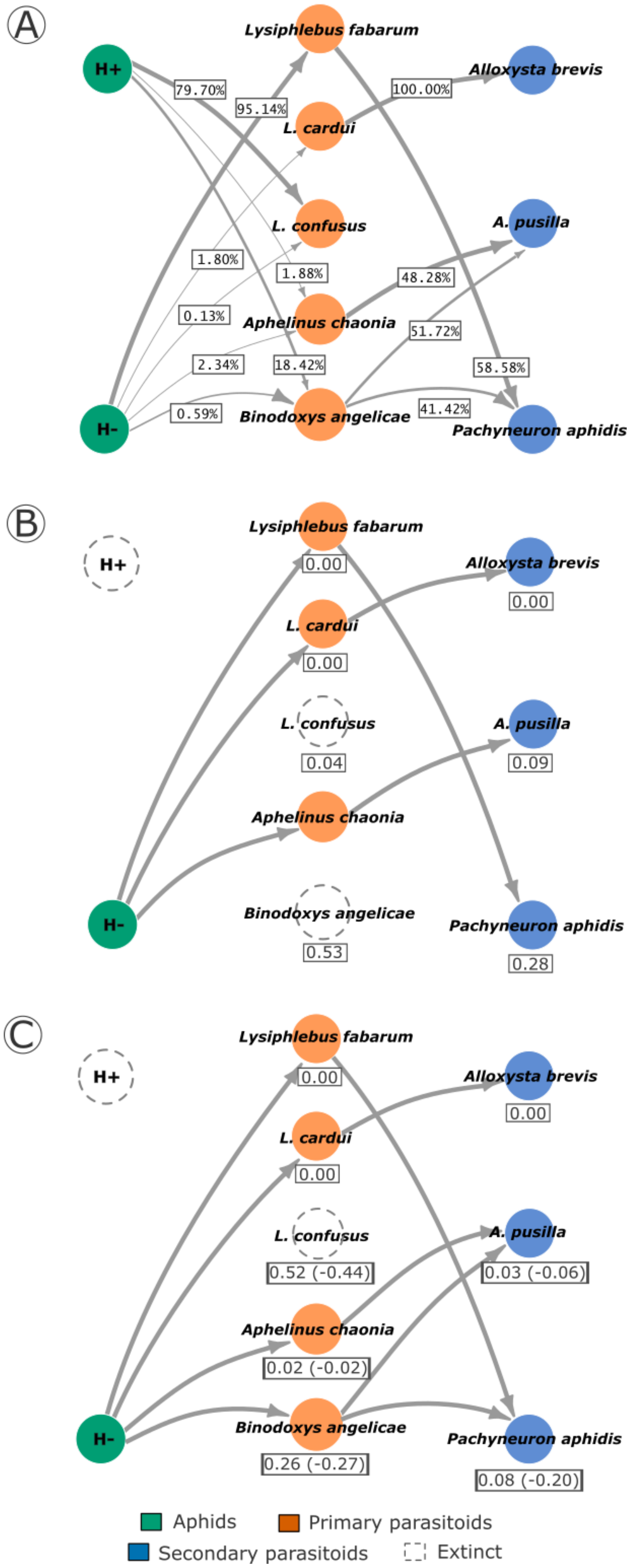
A
H+
H-
Lysiphlebus fabarum
L. cardui
L. confusus
Aphelinus chaonia
Binodoxys angelicae
Alloxysta brevis
A. pusilla
Pachyneuron aphidis
79.70%
95.14%
100.00%
1.80%
1.88%
48.28%
0.13%
51.72%
2.34%
18.42%
58.58%
0.59%
41.42%
B
H+
Lysiphlebus fabarum
0.00
Alloxysta brevis
0.00
L. cardui
0.00
L. confusus
0.04
A. pusilla
0.09
Aphelinus chaonia
H-
Binodoxys angelicae
0.53
Pachyneuron aphidis
0.28
C
H+
Lysiphlebus fabarum
0.00
Alloxysta brevis
0.00
L. cardui
0.00
L. confusus
0.52 (-0.44)
A. pusilla
0.03 (-0.06)
Aphelinus chaonia
0.02 (-0.02)
Binodoxys angelicae
0.26 (-0.27)
H-
Pachyneuron aphidis
0.08 (-0.20)
Aphids
Primary parasitoids
Secondary parasitoids
Extinct

Figure II: Symbiont-driven tri-trophic "toy" network dynamics based on Rothacher et al. [74]. (A) Weighted aphid–primary parasitoid–secondary parasitoid network with proportional dependencies shown in squares. (B) Network after removal of H^+ aphids under a probabilistic co-extinction model; squares indicate extinction probabilities. (C) Network including rewiring; squares show extinction probabilities with changes relative to no rewiring in parentheses.

Outstanding questions

1. What role do symbionts play in shaping the structure and dynamics of ecological networks?

Despite their well-known effects on host traits and their direct interactions, symbionts are never included as explicit nodes in ecological network studies. As a result, their potential to modulate structure, stability, and resilience at the community level remains largely unexplored. Integrating symbionts into complex systems research could reveal hidden interaction pathways, uncover overlooked drivers of network dynamics, and fundamentally change our understanding of how ecological communities function.

2. How do symbiont–host coevolutionary dynamics shape long-term patterns of specialisation, diversification, and interaction reallocation in ecological networks?

Symbionts can influence host range, defence traits, and resource use, potentially accelerating or constraining evolutionary trajectories. Incorporating symbionts into network analyses could help reveal how coevolution—between hosts, symbionts, and their natural enemies—drives patterns of modularity, nestedness, and reallocation through time, improving predictions of community reorganisation under changing ecological and evolutionary pressures.

3. Do symbiont-mediated phenotypic shifts driven by global change scale up from individual hosts to alter food-web structure and stability under climatic stress?

Facultative symbionts can modify key host traits—from thermal tolerance to defence against natural enemies and resource use—that often vary with environmental conditions. As warming, altered seasonality, and landscape change reshape ecological contexts, shifts in symbiont composition, density, and genotype may influence host performance and population dynamics in complex, context-dependent ways. Whether these trait-level responses propagate to reshape species interactions is crucial for predicting community resilience under global change.

4. Can symbiont-mediated traits in invasive insect hosts reshape native ecological networks?

Invasive insects may carry symbionts that confer novel traits—such as resistance to enemies, thermal tolerance, or altered behaviour—which can disrupt native species interactions. These shifts may trigger indirect effects on uninfected native species, ultimately altering the structure and dynamics of local ecological networks.

Glossary

Apparent competition: Indirect negative interaction between species mediated through shared natural enemies

Apparent mutualism: Indirect positive interaction between species mediated through reduction in shared natural enemies

APSE: A phage known from the insect bacterial symbiont *Hamiltonella defensa* (*Acyrthosiphon pisum* Secondary Endosymbiont phage)

Community detection methods: Procedures for identifying groups of highly connected nodes in a network based upon network structural properties

Connectance: Measure of community complexity based on the proportion of realised interactions from the pool of all possible interactions between the species of a network

Cytoplasmic incompatibility: Post-mating incompatibility causing F1 inviability when females are infected with different symbiont strains to males (or males are uninfected)

Exploitative competition: Species compete for the same limited resource

Extinction modelling: Use of network science to evaluate how the extinction of one species can trigger the extinction of other species

Feminisation: Genotypic males develop as functional phenotypic females

Horizontal transmission: Transfer of genetic material within or between host species that does not occur via inheritance from parents to offspring

Interaction frequency: Rate at which different species interact with each other

Interaction rewiring: Process by which higher-level components of the food web re-allocate to alternative resources following the loss of their original partners

Interlayer connectivity: Structural property of multilayer networks, defined by links connecting species across layers, used to quantify how processes in one layer relate to others

Interlayer flexibility: The ability of a node to be active across different layers of the multilayer network

***k*-partite network:** A network composed of a defined number, *k, of* non-overlapping node sets, with edges allowed only between (and not within) those sets.

Male killing: Symbiont infection of a female results in the production of female-biased broods because male progeny dies post-fertilization

Modularity: Metric quantifying how strongly a network is divided into groups (modules), comparing interactions within modules to those between modules.

Multi-edges: The same two nodes are linked by more than one type of edge

Overlapping community detection: Set of algorithms to identify the multiple community memberships of a node

Parthenogenesis: Form of asexual reproduction where an embryo develops from an unfertilized egg

Path analysis: Statistical method to evaluate the direct and indirect effects of variables on a specified outcome and the relative strength of each causal pathway

Robustness: Tolerance of the network to species extinctions, quantified as the fraction of species that had to be removed to result in a total loss of ≥ 50% of the species

Self-interaction: A node is linked to itself; for example, autophagy or cannibalism

Spectral clustering: Set of algorithms that divide a graph into clusters using the eigenvectors of the input data matrix

Structural equation modelling: Multivariate statistical framework used to model complex relationships between directly and indirectly observed variables

Trophic cascade: Indirect species interactions that originate with the removal/addition of components in one trophic level and propagate down a food web (sometimes also used for similar upward consequences)

Versatility: Node role in the cohesion of the multilayer network structure, building bridges between different types of relationships