

ECO-EVOLUTIONARY DYNAMICS OF MICROBIAL COMMUNITY ASSEMBLY: COMPETITION, COOPERATION, HIGHER-ORDER AND KEYSTONE INTERACTION

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Abstract

Microbial communities assemble through reciprocal feedback between ecological interactions and evolutionary change. Classical assembly theory emphasizes selection, dispersal, diversification and ecological drift, but microbial systems can evolve on the same timescales as colonization, competition, facilitation, disturbance and succession. This review develops an explicitly eco-evolutionary framework in which community assembly establishes ecological interactions; those interactions impose selection; genetic and phenotypic adaptation modifies resource use, antagonism, cross-feeding and environmental modification; and the altered interaction network feeds back to subsequent assembly, invasion resistance, stability and ecosystem function. Competition, cooperation and keystone effects are therefore interpreted as dynamic outcomes rather than fixed species properties. Particular emphasis is placed on higher-order interactions, because pairwise measurements can fail when a third species changes metabolite availability, pH, inhibition or spatial access. Recent synthetic-community experiments show that information from three-member combinations can substantially improve bottom-up prediction of multispecies structure. Synthetic microbial communities (SynComs) are highlighted as a central experimental platform for testing interaction mechanisms, keystoneity, spatial organization and evolutionary stability. We also distinguish taxonomic keystones from keystone functions and genes, and critically evaluate co-occurrence networks, centrality, dropout experiments, metabolic modeling, machine learning and longitudinal perturbation as methods for identifying causal community influence. The synthesis argues that predictive microbial ecology will require integration of perturbation experiments, longitudinal multi-omics, spatial measurements, trait evolution and mechanistic modeling. This perspective is relevant to host microbiomes, agriculture, environmental engineering and the rational design of stable microbial consortia.

1. Introduction

Microorganisms rarely exist as isolated populations. In soils, aquatic environments, host-associated microbiomes, biofilms and engineered systems, they form communities linked by competition for resources and space, metabolic exchange, chemical interference, predation, phage pressure and modification of local physicochemical conditions. Community composition is therefore an emergent property of ecological interactions operating within a changing evolutionary landscape [1,2,3].

Community assembly is commonly organized around selection, dispersal, diversification and ecological drift [1]. In microbial systems, however, diversification can occur rapidly enough to feed back on the other assembly processes. Mutation, recombination, horizontal gene transfer, strain replacement and adaptive changes in metabolism or antagonism can alter the strength or even the sign of ecological interactions [4,5,6,7]. The central problem is consequently not only which species arrive and interact, but how those interactions change as community members adapt.

This review is deliberately focused on four linked mechanisms—competition, cooperation, higher-order interactions and keystone effects—within an explicit eco-evolutionary cycle. SynComs and microscale spatial structure are considered only where they provide experimental leverage for these mechanisms. The article is a conceptual synthesis rather than

a meta-analysis, and no hypothetical quantitative outcomes are presented.

2. Review Approach

This article is a structured narrative review designed for mechanistic synthesis rather than statistical pooling. Literature was identified through targeted searches of PubMed/MEDLINE, Google Scholar for discovery, publisher and journal platforms, and DOI/Crossref-style bibliographic verification. The final search and verification update was conducted on 2 September 2026. To avoid an overly broad review, inclusion was restricted to studies that directly clarify eco-evolutionary assembly through competition, cooperation, higher-order interactions, keystone effects, or experimental SynCom/spatial tests of those mechanisms. Recent literature from 2022–2026 was emphasized, while older landmark studies were retained only when they established concepts required for the focused synthesis.

Search concepts were combined using Boolean logic. Studies were included when they directly informed assembly mechanisms, interaction context, evolutionary feedback, invasion, stability, spatial organization, SynCom design or causal keystone identification. Purely descriptive surveys without a clear assembly or interaction component were deprioritized. Evidence was synthesized qualitatively because systems, organisms, experimental designs and outcome measures are heterogeneous [8].

Table 1. Transparent literature-search framework used for this structured narrative review. Search strings are representative rather than an exhaustive database-export protocol

Database/source	Representative search string	Coverage	Inclusion emphasis	Exclusion/deprioritization
PubMed/MEDLINE	("microbial community assembly" OR microbiome assembly) AND (eco-evolution* OR evolution)	Foundational–2 Sep 2026	Peer-reviewed mechanistic or synthesis evidence	Purely descriptive surveys without assembly/interaction relevance
PubMed/MEDLINE	("higher-order interaction*" OR beyond-pairwise) AND microbial	Foundational–2 Sep 2026	Multispecies interaction or evolutionary-context studies	Pairwise-only studies without transferable conceptual relevance

Database/source	Representative search string	Coverage	Inclusion emphasis	Exclusion/deprioritization
Publisher/journal platforms	("synthetic microbial communit*" OR SynCom) AND (assembly OR interaction OR stability)	Foundational-2 Sep 2026	Defined-community design, testing, stability, standards	Unverified commentary or non-peer-reviewed claims
PubMed/MEDLINE + publisher verification	(keystone taxa OR keystone species OR keystone function) AND microbiome	Foundational-2 Sep 2026	Causal, modeling or methodological keystone work	Centrality-only claims treated as causal without validation
Google Scholar discovery + DOI verification	(spatial organization OR microscale OR biofilm) AND microbial interaction*	Foundational-2 Sep 2026	Spatially explicit interaction/assembly evidence	Studies lacking microbial-community relevance

3. Eco-Evolutionary Framework for Community Assembly

The organizing principle of this review is a recurrent cycle: community assembly → ecological interactions → selection → genetic/phenotypic adaptation → altered interactions → changed community assembly. This formulation makes evolution a constitutive part of assembly rather than a separate process occurring after ecological structure has formed. Recent modeling of complex communities likewise shows that ecological and evolutionary processes can jointly generate and maintain interaction-network structure [9].

Assembly establishes who encounters whom, at what density and under which abiotic conditions. Those encounters determine access to limiting resources, exposure to antagonists, opportunities for cross-feeding and the spatial neighborhoods in which selection acts.

Selection then changes genotype frequencies or favors phenotypic states that modify resource preferences, secretion, resistance, growth yield, motility or habitat modification. The altered traits reshape the interaction network and therefore change the success of later colonists, the response to disturbance and the stability of the assembled state [6,10,7,11].

This cycle also clarifies why competition, cooperation and keystoneity are context dependent. A lineage can be a strong competitor in one nutrient regime but a facilitator in another; a metabolite producer may be cooperative only when the recipient is sufficiently close; and a low-abundance taxon may become keystone only when it performs a non-redundant function under a particular environmental condition [12,13,14]. This eco-evolutionary feedback structure is summarized in Figure 1.

Eco-evolutionary feedback framework for microbial community assembly

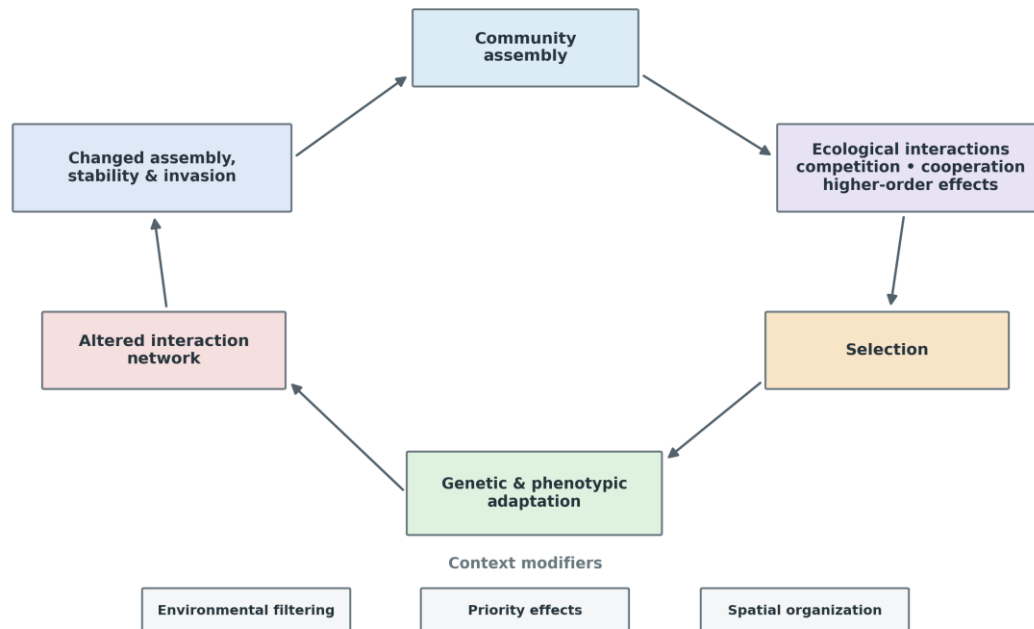


Figure 1. Integrated eco-evolutionary framework for microbial community assembly. Environmental filtering, priority effects and spatial organization modify the cycle at multiple stages; evolutionary change feeds back by altering interaction strengths, invasion resistance, stability and function.

4. Competition as an Eco-Evolutionary Driver

4.1 Resource competition and niche differentiation

Competition occurs whenever organisms share limiting resources, electron acceptors, attachment sites or other essential opportunities. In a 15-member in vitro gut system, resource-use measurements and consumer-resource modeling explained a large fraction of community assembly, while adding cross-feeding and pH-mediated effects improved coexistence predictions [15]. This supports resource competition as a useful baseline, but not a complete description of multispecies assembly.

Competition can produce exclusion, yet it can also generate coexistence when trade-offs, spatial heterogeneity or temporal resource variation prevent a single lineage from dominating. Evolution can reinforce coexistence through resource specialization or character displacement, but can also intensify competition if multiple lineages adapt toward the same high-value substrate. Horizontal gene

transfer further changes the competitive landscape by moving resource-use or resistance traits between lineages [5].

4.2 Interference, antagonism and evolutionary response

Bacteriocins, antibiotics, type VI secretion systems and contact-dependent inhibition create direct antagonistic interactions. These mechanisms impose selection for resistance, avoidance, counter-attack and spatial segregation. Because such traits may be carried on mobile elements, adaptation of one population can alter the effective interaction network of the entire community. The ecological consequence of evolution is therefore often distributed across species rather than restricted to the lineage in which the mutation occurs [6,7].

5. Cooperation, Facilitation and Evolutionary Stability**5.1 Cross-feeding and environmental modification**

Positive interactions arise when one organism releases a metabolite used by another, detoxifies a harmful compound, consumes oxygen to create an anaerobic niche, modifies pH, or produces extracellular enzymes that make resources accessible. Cross-feeding can generate trophic networks and metabolic dependencies that increase resource-use efficiency and support coexistence [16,17,18]. Yet the sign of an interaction can change with resource supply: a partner may be beneficial when a metabolite is scarce but become a competitor when both taxa depend on another limiting resource.

Recent synthetic-community work reinforces this context dependence. Nutrient status can shift pairwise interactions toward stronger inhibition under resource limitation and toward greater facilitation when resources are abundant [19]. Community richness and total density also influence measured interaction strength, showing that interaction coefficients are not immutable properties of species pairs [20].

5.2 Extracellular cooperative products, exploitation and evolutionary stability

Extracellular cooperative products—including siderophores, secreted enzymes and extracellular-matrix components—can be costly to synthesize while their benefits remain accessible to neighboring non-producers. This creates selection for exploitative phenotypes that obtain the benefit without contributing to production. Cooperative function can persist when benefits are preferentially returned to producers or reliable partners through limited dispersal, spatial assortment, partner choice, privatization, pleiotropic linkage or ecological feedback [21,22,23].

A key unresolved question is when cooperative function remains stable over evolutionary time. Short-term community performance is not sufficient evidence of long-term stability. Selection can favor reduced contribution, altered partner preference or metabolic streamlining. SynCom engineering should

therefore treat evolutionary stability as a design criterion, not merely an afterthought.

6. Higher-Order Interactions and Emergent Community Structure

Pairwise interaction measurements are experimentally attractive because they reduce combinatorial complexity. However, a pairwise effect can change in the presence of a third species. A third member may remove an inhibitor, change pH, consume a shared resource, supply a metabolite, modify oxygen availability or physically separate competitors. These are higher-order interactions: the effective interaction between two members depends on community context.

A particularly important recent demonstration used a seven-member synthetic bacterial community associated with duckweed. Pairwise cocultures did not fully predict emergent multispecies structure, whereas incorporating information from three-member combinations enabled substantially better bottom-up prediction of community composition and interaction forces [24]. This result is conceptually important because it suggests that exhaustive measurement of all high-dimensional combinations may not always be necessary: triplets can capture a meaningful portion of context dependence.

Other studies similarly show that richness and density attenuate or reconfigure interactions [20], and comparative metabolic modeling of defined communities demonstrates that cooperative and competitive flux relationships can be rewired as species composition changes [25]. Higher-order effects should therefore be understood as mechanistic consequences of altered environmental and metabolic context, not merely statistical correction terms.

The implication for eco-evolutionary dynamics is substantial. Selection acts on traits whose value depends on the surrounding community. A mutation that is beneficial in a pair may be neutral or deleterious in a richer community, while evolution of one species may indirectly alter interactions between two other members. Consequently, evolutionary trajectories and community assembly cannot always be reconstructed from isolated pairwise fitness effects.

Higher-order ecology also implies higher-order selection. The fitness effect of a mutation in species A can depend on species C because C changes the resource, chemical or spatial environment in which A interacts with B. Thus, a mutation favored in monoculture or a pair may be neutral or deleterious in a richer community. Community context can therefore reshape both the direction and magnitude of selection, making evolutionary trajectories

multidimensional rather than a simple sum of pairwise pressures. Recent synthesis of experimental evolution in communities identifies indirect effects and higher-order interactions as central mechanisms by which multispecies context changes selection and evolutionary trajectories [26]. The transition from pairwise effects to context-dependent higher-order selection is illustrated in Figure 2.

From pairwise interactions to higher-order selection and prediction

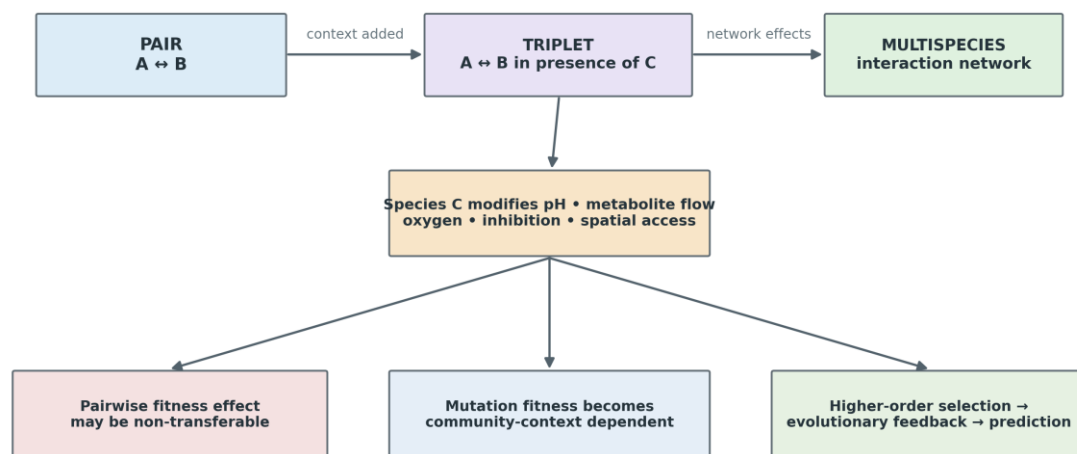


Figure 2. Pair → triplet → multispecies hierarchy. Third-party species can modify pH, metabolite flow, oxygen, inhibition or spatial access, converting a nominally pairwise interaction into a context-dependent fitness landscape. This creates higher-order selection and links ecological complexity directly to evolutionary feedback and prediction.

7. Keystone Taxa, Functions and Genes

7.1 What makes a microbial keystone?

Keystoneness is defined by disproportionate effect rather than abundance. A rare organism can control a limiting transformation, produce a potent inhibitor, modify a critical physicochemical condition or connect otherwise weakly coupled metabolic modules [27,28]. Conversely, a highly abundant organism may be replaceable because its function is redundant.

The modern keystone debate increasingly distinguishes a keystone taxon from a keystone function or gene. This distinction is especially important in microbes because horizontal gene transfer can relocate ecologically influential traits among lineages. In some systems, assigning keystoneness to a metabolic function may therefore be more mechanistically stable than assigning it to a taxonomic label [29,28]. The distinction between an ordinary community member and a low-abundance keystone is illustrated in Figure 3.

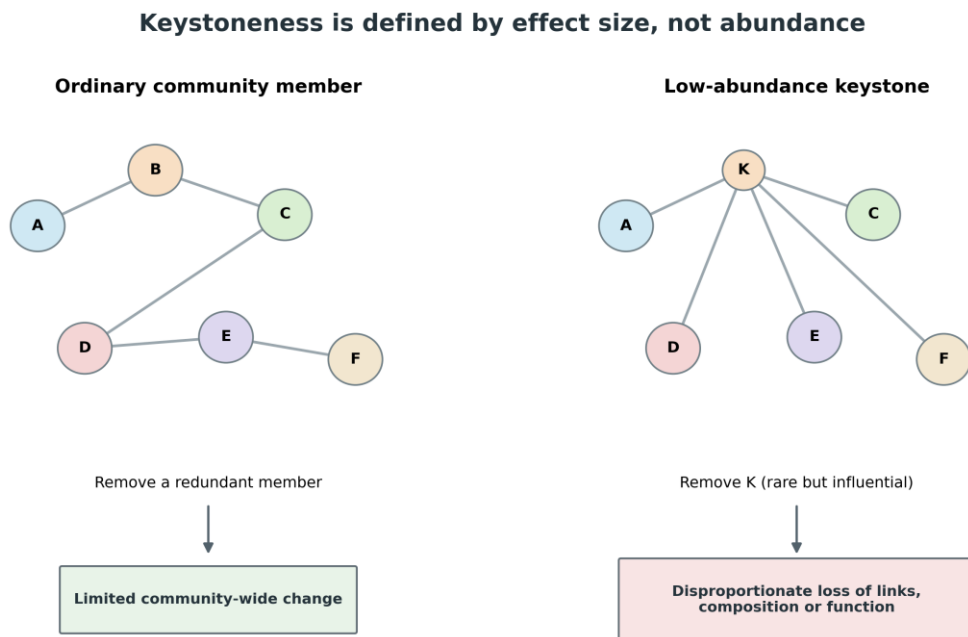


Figure 3. Conceptual contrast between an ordinary community member and a low-abundance keystone. Removal of an ordinary member may have limited effects when interactions or functions are redundant; removal of a keystone produces a disproportionate change in community composition, connectivity or function.

7.2 Methods for identifying keystones

Co-occurrence networks and centrality metrics are useful for generating hypotheses, but correlation does not establish causality and compositional data can generate misleading associations [30,31]. Top-down approaches estimate the total influence associated with a species' presence or absence [32], while deep-learning methods can learn habitat-specific assembly rules and simulate virtual removals [33]. These computational methods expand the search space but remain inferential.

The strongest evidence comes from perturbation. Species-dropout, addition, targeted inhibition and reconstitution experiments directly ask whether changing one member produces a community-wide effect. A recent reassessment of the keystone concept emphasizes taxon-removal experiments as the closest microbial analogue to the causal logic of the original ecological concept [28]. Metabolic modeling can further identify functions that control exchange networks, while longitudinal perturbation can reveal whether influence

persists across time or only appears in a transient state.

8. Priority Effects, Invasion and Eco-Evolutionary Memory

Priority effects occur when the order or timing of colonization changes subsequent community structure [34]. Early arrivals can occupy space, deplete resources, generate inhibitors or facilitate later taxa. Because early residents can adapt before later colonists arrive, priority effects may acquire an evolutionary component: historical advantage becomes reinforced by local adaptation.

Experimental evidence directly links co-evolution to invasion resistance. In a four-species community inhabiting a toxic medium, resident species initially facilitated some invaders, but after prolonged co-evolution the resident community became more resistant to invasion and less affected by invading taxa [11]. A 2025 synthetic-community study similarly showed that evolution of one dominant species increased protection of a less dominant partner and strengthened resistance to invasion [35].

These results illustrate the full feedback cycle: assembly determines interaction context, selection changes resident traits, and evolved interactions alter future assembly.

Community-level dynamical regimes can also predict invasion success and impact, emphasizing that invasibility is an emergent property of interacting residents rather than simply a characteristic of the invader [36]. This matters for probiotics, microbiome restoration and engineered consortia, where an introduced strain enters an ecosystem with ecological and evolutionary history.

9. Spatial Ecology at the Micrometre Scale

Microbial interactions often occur over micrometre distances. Diffusion, local uptake and physical separation determine whether secreted metabolites reach partners, whether toxins reach competitors and whether cooperative benefits remain localized. Biofilms, soil aggregates, mucosal surfaces and marine particles therefore create interaction neighborhoods that are invisible in bulk sequencing [37].

Recent synthesis of synthetic and engineered communities shows that initial cell density, founder positioning and relative abundance can strongly influence final spatial organization [38]. Low starting density can increase segregation, whereas higher density can increase intermixing; initial producer-to-consumer ratios can determine which spatial pattern emerges; and environmental conditions can alter whether spatial proximity stabilizes facilitation or intensifies interference. Spatial structure thus acts simultaneously as an ecological filter and an evolutionary modifier.

Spatial ecology also complicates the interpretation of cooperation. Close proximity can stabilize cross-feeding by reducing metabolite loss, but it can increase local resource competition. Spatial segregation may protect sensitive strains from antagonists, while mixed neighborhoods can expose them to toxins or, in other systems, allow access to detoxifying extracellular cooperative products. Predictive models therefore need spatially explicit parameters when interaction range is short.

10. Synthetic Microbial Communities as Experimental Ecology

SynComs have moved from being a niche application to a central experimental strategy for microbial ecology. Their defining advantage is controlled complexity: membership is known, strains can be manipulated, and communities can be reconstructed with systematic omissions or additions. This allows direct tests of competition, facilitation, higher-order effects, keystone effects, priority effects and evolutionary stability [13].

A cross-systems primer published in 2024 emphasizes that SynCom design should be aligned with the biological question, host or environmental context, reproducibility and ethical considerations [26]. Parallel guidance in plant-microbiota research calls for reference communities, standardized protocols, benchmark data and shared strain infrastructure [39]. Gut-focused reviews likewise emphasize rational design, defined composition and translational potential [40].

SynComs are especially powerful for causal keystone tests. A candidate can be removed from an otherwise identical community, and the resulting changes in composition, metabolite flow and function can be measured. They are also well suited to higher-order experiments because pairs, triplets and larger combinations can be assembled systematically. In environmental engineering, SynCom design increasingly integrates keystone function, cross-feeding, metabolic redundancy and modeling [41].

The major limitation is external validity. A stable defined consortium in a controlled medium may behave differently when exposed to a host, soil matrix, fluctuating resources or a resident microbiome. Recent work on soil communities illustrates that persistence in the presence of native microbiota remains a major design challenge [42]. SynCom research should therefore connect reductionist mechanism to progressively more realistic ecological contexts.

A useful way to organize next-generation SynCom research is a design-build-test-learn cycle. DESIGN defines the target function and selects candidate members using trait data, metabolic reconstruction and ecological constraints. BUILD assembles strains with

explicit control of initial abundance, spatial arrangement and environmental conditions. TEST measures composition, function, metabolite exchange, robustness, invasion resistance and evolutionary stability. LEARN integrates experimental outcomes with mechanistic models or machine learning to update member selection and community architecture before the next cycle. This framework converts SynCom construction from one-off empirical assembly into iterative predictive engineering [13,39,40].

The newest function-first approaches also combine multi-omics, standardized reporting and AI-assisted design. Rather than choosing strains solely by taxonomy, candidate communities can be prioritized by metabolic capabilities, interaction complementarity and predicted robustness. Such approaches remain hypothesis-generating until experimentally validated, but they provide a practical route for reducing the enormous combinatorial space of possible consortia [43].

10.1 Multi-omics and spatial multi-omics

Multi-omics can connect community membership to mechanism at several biological levels. Metagenomics identifies genetic potential and strain variation; metatranscriptomics indicates which pathways are actively expressed; metaproteomics captures translated functional machinery; and metabolomics measures exchanged or transformed molecules. Integrated longitudinal sampling can therefore distinguish the presence of a pathway from its actual activity and connect evolutionary change to altered community function.

Spatial information is the missing dimension in many bulk omics datasets. Imaging, spatially resolved metabolite measurements, microfluidics and emerging spatial-omics approaches can identify which cells are sufficiently close to exchange metabolites or inhibitors and how gradients in oxygen, pH and nutrients structure local selection. Recent synthesis of engineered communities highlights initial density, relative abundance, cellular traits, interaction range and environmental conditions as determinants of spatial organization [38].

11. Environmental Filtering, Disturbance and Stability

Temperature, pH, salinity, oxygen, nutrient supply, host immunity and antimicrobials alter both direct growth and the interaction network. Environmental stress can shift the balance between deterministic selection and other assembly processes; groundwater studies, for example, demonstrate strong effects of environmental stress on microbial assembly [44].

Resistance describes the degree of change during disturbance, whereas resilience describes recovery afterward [45]. Both can depend on functional redundancy, spatial refuges, standing genetic variation and the presence of taxa or functions that restore metabolic connectivity. Narrow-spectrum resource users may contribute disproportionately to synthetic-community stability by strengthening complementary metabolic interactions [46]. Yet concentration of an essential function in one lineage can also create fragility, reinforcing the distinction between beneficial keystone and systemic dependence.

12. Methods for Causal and Predictive Microbial Ecology

No single method can resolve community causality. Longitudinal metagenomics detects strain replacement and within-lineage evolution; isolate genomics links genetic change to traits; metabolomics identifies exchanged compounds; spatial imaging reveals neighborhood structure; lineage tracking measures selection; and SynCom perturbations test causal membership effects. Network inference and machine learning can prioritize hypotheses, but their predictions should be validated experimentally [32,33,31].

Mechanistic consumer-resource and metabolic models are particularly useful when parameters have interpretable biological meaning. Generalized Lotka-Volterra models remain useful summaries but may hide context dependence inside fitted coefficients. Higher-order models, genome-scale metabolic models and spatially explicit approaches can represent mechanisms that pairwise phenomenological coefficients miss [24,25]. The strongest future

designs will combine prediction and perturbation iteratively.

12.1 Predictive Framework: From Profiling to Validated Prediction

A predictive eco-evolutionary workflow can be organized as a hierarchy: descriptive community profiling → pairwise inference → targeted higher-order interaction testing → mechanistic metabolic or consumer-resource modeling → evolutionary validation → causal perturbation → out-of-sample prediction. Each stage resolves a different source of uncertainty. Profiling identifies candidate members and functions; pairwise assays establish a tractable baseline; triplet or targeted multispecies tests expose context dependence; mechanistic models generate interpretable hypotheses; evolution

experiments test whether interaction rules persist under selection; perturbations establish causality; and prediction is evaluated in new environmental or community contexts.

The framework is deliberately iterative rather than linear. Failed predictions should determine which additional interaction, spatial or evolutionary measurements are needed. In this sense, prediction is not the endpoint of a static model but the output of repeated cycles of model confrontation with perturbation data. Recent rational-design studies using epistatic interaction information illustrate how explicit non-additivity can be incorporated into SynCom construction rather than treated only as unexplained error [47].

13. Synthesis Tables

Table 1 summarizes representative recent studies that materially extend the eco-evolutionary interpretation of microbial community assembly.

Study	System	Interaction mechanism	Approach	Principal finding
Good & Rosenfeld, 2023 [6]	Human gut microbiome	Within-host evolution / community feedback	Longitudinal genomic analysis	Evolutionary change is linked to broader ecological shifts.
Weiss et al., 2023 [12]	Synthetic gut community	Context-dependent keystone	Species dropout across environments	Keystone effects depend on nutrition and host context.
Ho et al., 2024 [15]	15-member gut SynCom	Resource competition + cross-feeding	Metabolomics + consumer-resource model	Resource competition predicts much assembly; pH/cross-feeding improve coexistence predictions.
Ishizawa et al., 2024 [24]	Duckweed-associated 7-member SynCom	Higher-order interactions	Pairs, triplets and multispecies combinations	Triplet information improves bottom-up prediction beyond pairwise data.
McEnany & Good, 2024 [10]	Randomly assembled communities	Early evolutionary steps	Theory / eco-evolutionary modeling	Mutant fate depends strongly on community ecological context.
Rios Garza et al.,	Cross-system	Keystone causality	Conceptual +	Taxon removal

Study	System	Interaction mechanism /	Approach	Principal finding
2026 [28]	synthesis		methodological review	and mechanistic validation are central to causal keystone claims.
de Scally & McDonald, 2025 [35]	E. coli-yeast SynCom	Evolution and invasion resistance	Long-term coevolution + invasion assays	Evolution of one member strengthens community resistance to invasion.
Wang et al., 2025 [46]	Synthetic communities	Stability through resource complementarity	Defined communities + metabolic analysis	Narrow-spectrum resource use can stabilize communities through enhanced metabolic interactions.
Araujo & Lurgi, 2026 [9]	Complex model communities	Eco-evolutionary assembly	Network eco-evolutionary model	Ecology and evolution jointly generate interaction-network structure.

Table 2. Representative recent studies linking interaction mechanisms, evolution and microbial community assembly.

Method	Strength	Main limitation	Best use	Key refs
Co-occurrence network centrality /	Scalable; uses observational data	Association is not causation; compositional bias	Hypothesis generation only	30,27,31
Top-down influence inference	Estimates total species influence without all pairwise links	Dependent on model assumptions and data quality	Prioritize perturbation targets	32
Deep learning / virtual removal	Captures nonlinear habitat-specific patterns	Interpretability and transportability limitations	Screen candidate keystones	33
Species-dropout / addition	Direct causal test; clear counterfactual	Combinatorial cost; laboratory context	Strongest taxon-level evidence	12,28
Metabolic modeling	Mechanistic link to exchange and resource control	Requires accurate reconstructions / environment	Identify keystone functions	25,28
Longitudinal perturbation	Tests persistence and recovery over time	Resource-intensive; confounding possible	Validate dynamic influence	45,11
Targeted inhibition / phage	Can manipulate members in	Off-target and ecological side	In situ causal testing	28

Method	Strength	Main limitation	Best use	Key refs
/ CRISPR perturbation	complex communities	effects		

Table 3. Comparison of approaches for identifying microbial keystones.

Approach	What it resolves	Main limitation	Best eco-evolutionary question
Pairwise co-culture	Direct sign/strength under defined conditions	Scales as N^2 ; may not transfer to rich communities	Baseline interaction mapping
Three-member combinations	Captures smallest higher-order context	Still combinatorial for large N	Efficient bridge from pairs to multispecies prediction
Full SynCom perturbation	High causal relevance within defined system	Large experimental burden	Keystone and stability testing
Consumer-resource modeling	Mechanistic resource interpretation	Requires resource-use data	Competition, coexistence and niche overlap
Genome-scale metabolic modeling	Predicts exchange and metabolic dependency	Reconstruction and flux assumptions	Cross-feeding and keystone functions
Spatial imaging / microfluidics	Resolves interaction neighborhoods	Limited scale and technical complexity	Microscale cooperation, competition, founder effects
Longitudinal evolution experiment	Measures adaptive feedback directly	Time and replication intensive	Evolutionary stability and invasion resistance

Table 4. Experimental and modeling strategies for moving from pairwise descriptions to eco-evolutionary prediction.

14. Critical Discussion and Unresolved Questions

Controversy	Interpretation A	Interpretation B / challenge	Best resolution strategy
What is the keystone?	Taxon as causal unit	Function/gene may be more stable when traits move or are redundant	Use perturbation plus functional validation
Can pairs predict communities?	Pairwise coefficients provide a useful baseline	Richness, density and third species can alter interaction strength/sign	Test transferability with triplets and targeted multispecies assays
What governs assembly?	Environmental selection can create repeatable outcomes	Priority effects, founder structure, drift and evolution create contingency	Partition deterministic filters from historical starting conditions
Is cooperation stable?	Spatial assortment and privatization can stabilize benefits	Cheaters and changing environments can erode contribution	Evaluate function across evolutionary time
How should keystones be identified?	Networks/centrality efficiently nominate candidates	Association can be non-causal or redundant	Require removal/addition or targeted perturbation
What does stability mean?	Ecological resistance/resilience can	Evolution may change traits and destabilize	Report ecological and evolutionary stability

Controversy	Interpretation A	Interpretation B / challenge	Best resolution strategy
	be measured over short time	function later	separately
How realistic are SynComs?	Defined membership enables mechanism and causality	Reduced systems may fail in hosts, soils or resident microbiomes	Validate across increasing ecological realism

Table 5. Major controversies in eco-evolutionary microbial community ecology and experimental strategies for resolving them.

Is the keystone property best assigned to a taxon, function or gene? Taxon-level language is experimentally convenient, but mobile genes and functional redundancy can decouple ecological role from taxonomic identity. A stronger framework would specify the causal mechanism and then ask whether that mechanism is uniquely carried by a taxon or distributed across the community [29,28].

Can pairwise interactions predict complex communities? Sometimes they can approximate important features, particularly when resource competition dominates and context is similar [15]. But higher-order effects, richness-dependent attenuation and environmental modification can make pairwise coefficients non-transferable [20,24]. The relevant question is not whether pairwise models are universally valid, but under what ecological conditions their approximation error is acceptably small.

When is cooperation evolutionarily stable? Mechanisms such as spatial assortment and privatization can stabilize contribution, yet changing resource regimes or mutation can erode cooperation. Long-term evolution experiments should therefore accompany SynCom performance assays. A consortium that performs well for a few transfers may be evolutionarily fragile.

How deterministic is assembly? Environmental filtering and resource competition can create repeatable structure, but priority effects, founder positioning, drift and evolutionary contingency can generate alternative states [34,38,44]. Determinism and historical contingency are not opposites: deterministic selection can act on historically different starting states and thereby produce different but reproducible trajectories.

Can network centrality identify keystones without perturbation? Centrality may identify

influential candidates, but a highly connected node can be redundant, and an apparently peripheral low-abundance member can control a critical transformation. Perturbation remains necessary for strong causal claims [28].

Finally, can SynComs become predictive rather than merely illustrative? The answer depends on whether principles discovered in reduced systems survive increasing ecological realism. Standardized reference communities, explicit environmental metadata, spatial measurements and validation against natural communities will be crucial [26,39].

15. Applications

15.1 Human and animal microbiomes

Probiotics, live biotherapeutics, fecal microbiota interventions and dietary strategies introduce or favor organisms within communities that already possess ecological and evolutionary histories. Establishment depends on niche availability, resident competition, cross-feeding partners, priority effects and invasion resistance. Defined gut SynComs offer a tractable bridge between mechanism and translation [40].

15.2 Agriculture and plant-associated microbiomes

Plant microbiome engineering seeks stable functions such as nutrient acquisition, pathogen suppression and stress tolerance. Recent work shows that seed-borne bacteria can shape rhizosphere assembly through niche partitioning and facilitation [48]. SynCom standards and field validation are therefore essential for translating laboratory interaction mechanisms into robust agricultural inoculants [39].

15.3 Biotechnology and environmental engineering

Waste treatment, fermentation, bioremediation and biomanufacturing depend on communities that must maintain function under fluctuating conditions. SynCom design can distribute metabolic burden, create division of labor and improve pathway efficiency, but evolution can destabilize costly contributions. Engineering should therefore couple desired community function to individual fitness where possible and incorporate redundancy, spatial organization and invasion resistance [41,49].

16. Future Research Priorities

- Integrate evolution into assembly experiments by tracking strain-level genetic and phenotypic change during colonization, disturbance and recovery.
- Use triplet and targeted higher-order experiments to identify when pairwise models fail, rather than assuming either universal pairwise sufficiency or universal high-order complexity.
- Define keystone causally through removal, addition or targeted perturbation and distinguish taxonomic from functional keystones.
- Measure microscale spatial structure explicitly, including founder density, relative abundance, interaction range and metabolite gradients.
- Evaluate SynComs over evolutionary timescales and in progressively more realistic environments containing resident microbiota.
- Develop models that combine consumer-resource mechanisms, metabolic exchange, spatial structure and trait evolution, with experimental perturbations used for validation.
- Adopt transparent reporting standards for narrative and structured reviews, including databases, search dates, search concepts and study-selection principles.

17. Limitations of This Review

This article is a structured narrative review rather than a formal systematic review or meta-analysis. Its purpose is mechanistic integration across microbial systems, and the heterogeneity

of habitats, organisms, interaction definitions, experimental durations and outcome measures precludes meaningful quantitative pooling. The literature search was made transparent, but it was not designed to provide exhaustive PRISMA-style screening counts. The review also spans studies that define interaction strength, stability and keystone differently, which can limit direct comparison across systems.

A second limitation is that much causal evidence comes from simplified laboratory communities. SynComs and model systems provide experimental control but may not capture the taxonomic diversity, spatial heterogeneity, immigration, phage dynamics and environmental fluctuation of natural microbiomes. Conversely, observational multi-omics in natural systems provides realism but can weaken causal inference. The conclusions of this review therefore place greatest weight on convergence between mechanistic models, controlled perturbation and longitudinal or natural-system validation.

18. Conclusion

Microbial community assembly is best understood as an eco-evolutionary feedback process rather than a sequence in which ecology occurs first and evolution later. Assembly creates interaction context; competition, cooperation and environmental modification impose selection; adaptation changes traits; and those changes reshape interaction networks, invasion resistance, stability and subsequent assembly. Higher-order interactions demonstrate why the effect of one species on another cannot always be treated as fixed, while spatial ecology shows that the relevant interaction environment often exists at micrometre scales.

Keystone effects further illustrate the limits of abundance-based reasoning. The most influential element may be a rare taxon, a metabolic function or even a transferable gene, and causal perturbation is essential for distinguishing true keystones from correlated network hubs. SynComs provide a particularly powerful platform for these tests because they allow systematic control of membership, order of arrival, spatial structure and evolutionary history.

The next stage of microbial community ecology should therefore connect controlled perturbation with longitudinal evolution, multi-omics, spatial measurement and mechanistic modeling. Such integration offers the strongest path toward predicting how communities assemble, why they resist or admit invaders, how stable functions emerge and how microbial consortia can be rationally managed in medicine, agriculture, biotechnology and environmental systems.

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