

Review

Exploring interkingdom communication: the case of black soldier fly mass-rearing

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Interkingdom communication through volatile organic compounds influences interactions among organisms at a level often imperceptible to humans. Artificial settings that depend on the biotechnical exploitation of biological processes, such as the rapidly expanding sector of insect farming, are strongly affected by this often-overlooked multiway communication. Here, we aim to portray the significance of interkingdom communication influencing insect behavior. We use the black soldier fly (*Hermetia illucens*) as a model system to introduce the necessary actions to improve our understanding of communication between insects and microbes. Successful exploration of this phenomenon could transform the bioeconomy by improving insect mass-rearing processes and enhancing insect welfare. By interlocking behavioral and molecular ecology, chemistry, microbiology, and bioinformatics, we can uncover the molecular mechanisms underlying these interactions and develop practical applications for improved industrial practices. Future work should focus on pursuing research avenues to untangle the interwoven nature of insect behavior and microbial communities.

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Introduction

Understanding communication is key to comprehending behavior and decision-making processes. While communication is not synonymous with interaction, it refers specifically to the transfer of information from one organism to another, often through variations of cues and signals, thus representing a distinct subtype of biological interactions. Multiway communication between insects and the microbial world, mediated by microbial volatile organic compounds (mVOCs), is pivotal in shaping ecological interactions [1]. As reviewed in Wang et al. [2], microbial symbionts and pathogens can also reprogram their host's chemoreceptive abilities, either by straining its immune response and capacity to sense chemical cues or by increasing the sensitivity. These interactions enable critical processes such as mating, scavenging for resources, pollination, or host–pathogen dynamics. For instance, a specific bouquet of volatiles,

emitted by microbial communities on decaying matter, may guide a female fly (Diptera) to an optimal oviposition site, indirectly indicating resources for its offspring [3,4]. But how do these dynamics translate to industrially farmed insects such as the black soldier fly (BSF)?

The goal of investigating multiway communication is to illuminate these intricate networks and understand their relevance for ecosystems and sustainable agriculture. The ecological impact of volatiles on the microorganisms involved and on higher organisms sensing these compounds is often overlooked. Understanding how microbes in the environment (e.g. rearing facility, feed substrate, air) signal to animals is crucial for industries that farm living organisms beyond traditional livestock, such as insects for food and feed [5,6].

The potential of insect farming

Over the last 15 years, industrial insect farming has become a transformative sphere in the new bioeconomy [7]. Low-value organic matter such as manure [8], agro-industrial by-products [9], and food wastes [10] is bio-converted primarily by the BSF (*Hermetia illucens* Linnaeus 1758, Diptera: Stratiomyidae) — an emerging model system in the field of circular economy. Its larvae transform these materials into high-quality protein and fat for animal feedstock and fertilizer for agriculture [11]. This process reduces greenhouse gas emissions from organic waste treatment by capturing carbon and nitrogen into insect biomass rather than climate-active CO₂, CH₄, and N₂O, while also promoting microbes that oxidize CH₄ and reduce N₂O [12]. Economic and academic interests in this sector have driven the adaptation of regulatory frameworks, further propelling its growth. Despite the euphoria motivating founders to start insect-farming businesses, upscaling production remains a challenge [6,13]. Particularly, the directed oviposition of many fertilized eggs determines the amount of insect biomass produced [14,15]. For this reason, enterprises have specialized in commercializing ‘ready-to-use’ neonate larvae, allowing farmers to outsource fly breeding and reproduction [16,17].

The secretiveness of producers regarding oviposition attractants and the lack of standardization thereof entice the use of diverse organic substrates, from which contamination hazards in breeding environments can result [18]. Understanding and harnessing the instinct of females for optimal oviposition timing and site selection are crucial for production efficiency. This knowledge can improve egg harvesting, prevent biomass loss from misplaced egg clutches, and reduce the manual labor associated with collecting eggs, ultimately creating opportunities for automation. Moreover, directed oviposition may sustain increasingly critical welfare considerations in insect farming [19] by ensuring that

eggs are laid under optimal conditions, thereby enhancing survival and preventing the starvation of neonates because of the economically unfeasible collection of misplaced eggs.

Microbial cues in ecosystems

Recognizing multiway communication within microbiota, within insects, and between the two in both directions is critical for investigating inter- and intraspecific interactions. While focus typically lies on microorganisms-to-insect signaling and the effects of these cues and signals on insect behavior, insects themselves are also capable of shaping microbial interactions, such as through the excretion of metabolites in their guts that indirectly modulate microbial metabolic activity by altering, for example, the pH or oxygen concentration in the gut environment [20,21]. In mammals, results from a pilot study showed that gut-microbiome-derived mVOCs are excreted through the breath volatilome, allowing inference of gut microbiome composition and health status and emphasizing ‘breathomics’ as a powerful instrument for microbiome assessment [22]. In this context, Cattaneo et al. [23] recently showed how VOCs such as hexadecane, 2,4-dimethyl-1-heptene, and 1-cyclopentyl-ethanone excreted by BSF larvae are candidates for stress indicators. While the direct effects of these larval VOCs on microbiota in the rearing environments are yet to be studied, preliminary evidence suggests interactions: Hexadecane serves as a carbon source for alkene-degrading bacterial genera such as *Pseudomonas*, while 2,4-dimethyl-1-heptene acts as an antimicrobial agent against Gram-positive bacteria [24,25]. Further, larval processing of organic substrates alters microbial community composition, thereby reducing odor-producing taxa such as *Lactobacillus* and *Enterococcus*, potentially through suppression by VOCs [26]. However, whether insect-emitted VOCs function as intentional microbial signals or random by-products with ecological effects remains to be studied.

Profiles of mVOCs are highly diverse and can comprise common, group-specific, or strain-specific compounds [1]. In contrast to quorum sensing, which primarily serves as short-range communication among bacteria and fungi through the transmission and reception of chemical signals (autoinducers) to coordinate collective behaviors such as biofilm formation, recent evidence indicates that such mVOCs can cover greater distances and induce responses in eukaryotes [27]. In fact, quorum-sensing molecules can serve as long-distance mVOCs (i.e. signals or cues) regulating eukaryotic behavior [28]. Ten years ago, Piechulla and Degenhardt [29] asserted that understanding the biological function of mVOCs, particularly in terms of volatile perception, signal transduction, and phenotypical reactions, would be a key mission to understand interkingdom

Table 1**Compounds that induce antennal responses or attract female black soldier flies for oviposition.**

Compound	Chemical class	GC-EAD		Oviposition-active
		Female	Male	
1-Octanol ^a [34]	Alcohol	X		n.t.
(E)-2-Hexen-1-ol ^c [35]	Alcohol	X	X	n.t.
Hexanol ^c [35]	Alcohol	X	X	n.t.
Nonanol ^c [35]	Alcohol		X	n.t.
Phenylethyl alcohol ^a [34]	Alcohol	X		n.t.
Benzaldehyde ^a [34]	Aldehyde	X		n.t.
Decanal ^{a,c} [34,35]	Aldehyde	X	X	X [†]
Nonanal ^a [34]	Aldehyde	X		n.t.
Octanal ^c [35]	Aldehyde	X	X	n.t.
Naphthalene ^a [34]	Aromatic hydrocarbon	X		n.t.
Butyric acid ^c [35]	Carboxylic acid	X		n.t.
Decanoic acid ^b [18]	Carboxylic acid			X
Dodecanoic acid ^b [18]	Carboxylic acid			X
Pentanoic acid ^a [34]	Carboxylic acid	X		X [†]
Propionic acid ^c [35]	Carboxylic acid	X	X	n.t.
Tetradecanoic acid ^b [18]	Carboxylic acid			X
Dodecanoic acid methyl ester ^b [18]	Ester			X
(Z)-3-Hexenyl acetate ^c [35]	Ester	X	X	n.t.
2-Pentyl furan ^c [35]	Furan	X	X	n.t.
2-Heptanone ^b [35]	Ketone	X	X	n.t.
Acetophenone ^a [34]	Ketone	X		X [†]
Sulcatone ^a (6-methyl-5-heptene-2-one) [34]	Ketone	X		X [†]
2-methoxyphenol ^a [34]	Phenol	X		n.t.
p-Cresol ^a [34]	Phenol	X		X [†]
Tetramethyl pyrazine ^a [34]	Pyrazine	X		n.t.
Alpha-pinene ^a [34]	Terpene	X		n.t.
Limonene / 2-Ethyl hexanol ^a [34] [‡]	Terpene / Alcohol	X		n.t.
(-)-Linalool ^c [35]	Terpene alcohol	X	X	n.t.

Overview of identified compounds, categorized by their chemical class, along with their GC-EAD activity and their role in attracting females for oviposition (where tested). n.t. = oviposition activity not tested.

Solvents used for the compounds as specified in the references: ^adichloromethane or hexane, ^bethanol, ^cparaffin oil.

[†] These compounds only showed attraction for oviposition when used in combination.

[‡] The authors reported that the respective peaks overlapped in the chromatograms and thus could not be distinguished.

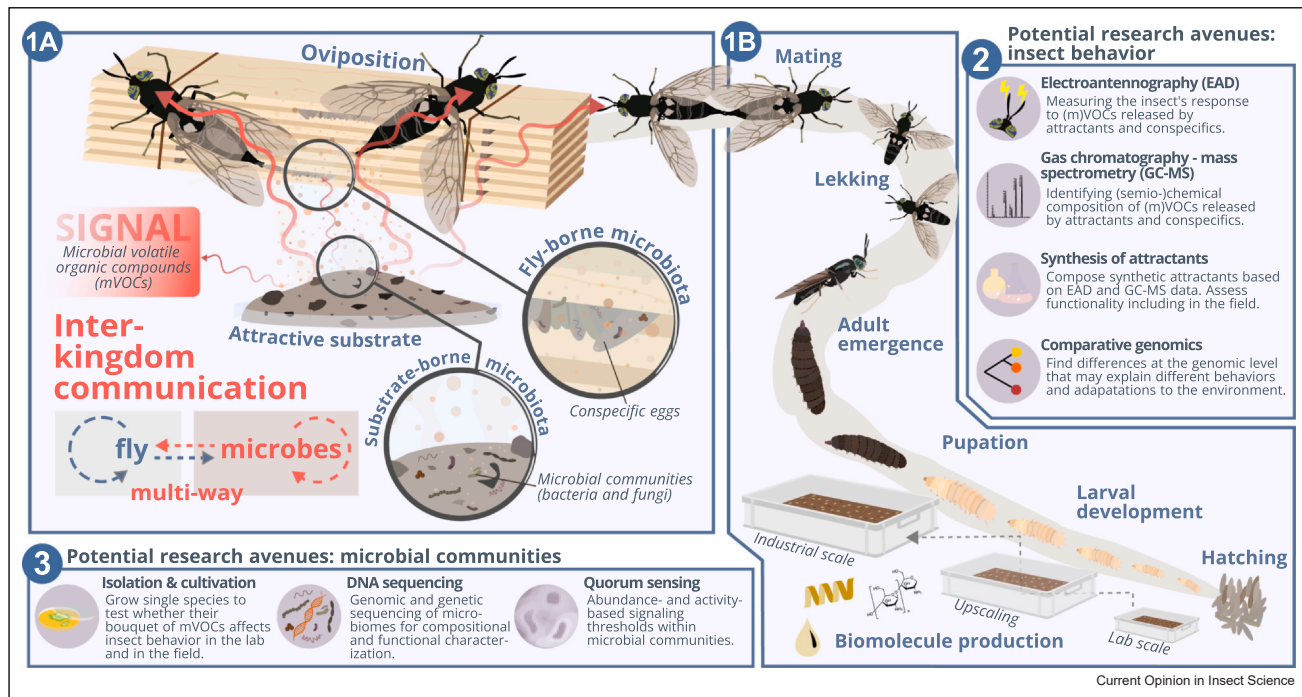
communication. Now, mVOCs are biotechnically applied, among others, in crop protection, food preservation, and microbiome management [30]. A prominent example spanning effects in microorganisms and eukaryotes is the signaling molecule indole, which is produced by many bacteria for quorum sensing [28]. In insects, indole induces behavioral changes: it attracts mosquitoes (Diptera: Culicidae) to humans because of its high concentration in sweat and guides females to suitable oviposition sites, whereas a different indole derivative deters butterflies from laying eggs [31]. So far, > 2000 mVOCs have been identified from > 1000 microbial species and are curated in the mVOC database (<https://bioinformatics.charite.de/mvoc/>) [32]. At a broader scale, the Pherobase database (<https://pherobase.com/>) provides an invaluable resource for pheromones and semiochemicals [33]. Research on signaling molecules that can influence the behavior of mass-reared insect species, such as the BSF, remains scarce [15]. However, understanding which volatile compounds the BSF detects and reacts to is important,

and first compounds have been identified that elicit antennal responses and influence oviposition (Table 1).

Improving insect farming by understanding communication between insects and microbes

Seeing through and optimizing the many factors defining reproductive success is imperative in insect mass-rearing. These include light conditions [36], temperature and humidity [37], sex ratio and fly density [38], fly age [39] and size [40], egg-trap [41] and cage design [42], and ultimately attractants to direct oviposition. Besides established model species such as *Drosophila melanogaster* (Diptera: Drosophilidae), successful advancements in mass rearing were achieved in other dipteran species [43]. For the cattle screwworm (*Cochliomyia hominivorax*, Diptera: Calliphoridae), a parasitic fly attracted to decomposing animal tissues, chemical attractants based on microbially produced VOCs showed great success in establishing eradication programs [44]. The BSF is also attracted to decomposing organic substrates, including

Figure 1



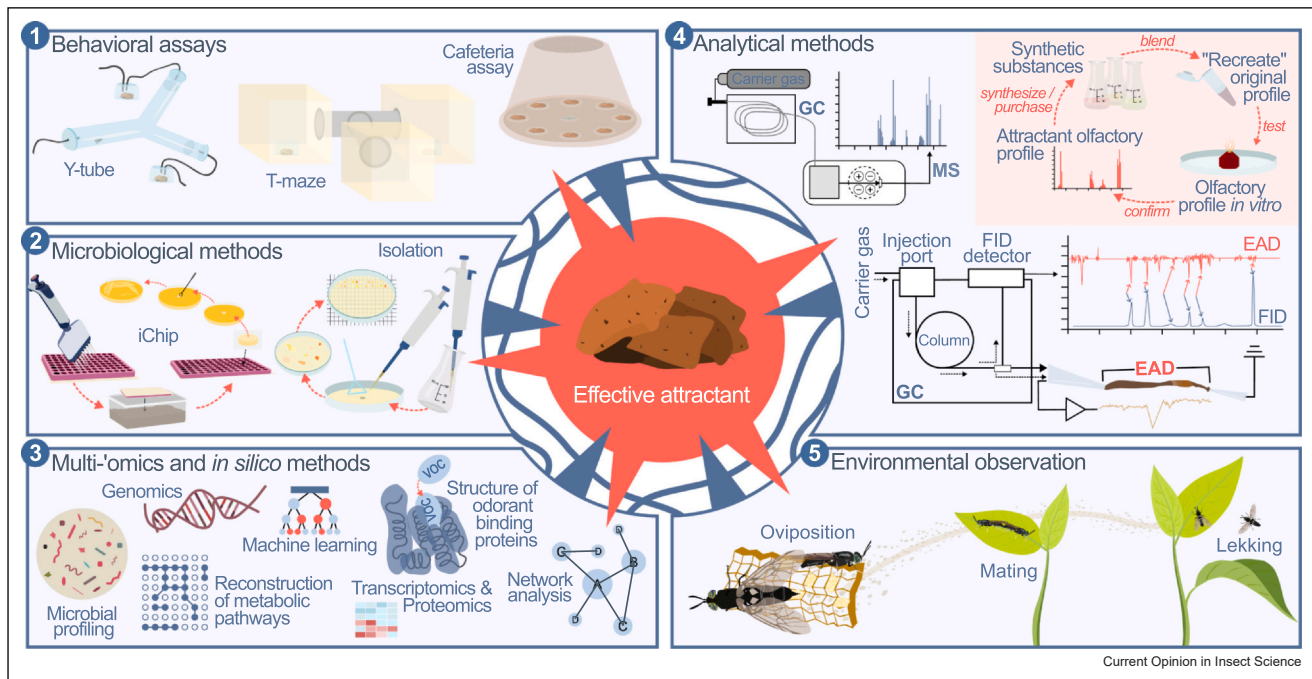
Schematic overview of the black soldier fly rearing process as an emerging model system for studying multiway communication in insect mass-rearing. **(1A)** Multiway communication is central to both laboratory- and industrial-scale processes, with broad implications for rearing efficiency and the extent of manual labor required (e.g. egg harvest). Gravid females, attracted by microbial volatile organic compounds (mVOCs) emitted by decaying organic matter, lay their eggs in small crevices (simulated by egg traps) close to the attractive substrate. Substrate-borne microbes, sometimes complemented with fly-borne microbes on the surface of deposited eggs, generate an attractive profile of mVOCs that signals the presence of ample resources to conspecifics. **(1B)** Reproduction and oviposition are central to the industrial rearing of black soldier flies. A key challenge in upscaling production is obtaining a high number of fertile eggs, which can be addressed through directed oviposition. This approach facilitates egg collection and improves survival by reducing the number of misplaced egg clutches. **(2)** Potential research avenues to study the responses of insects to environmental cues such as mVOCs emitted by attractive substrates. **(3)** Potential research avenues to integrate microbiological aspects in the study of insect behavior, highlighting the interconnection between insect behavior and microbial communities.

blood, offal, and manure, for oviposition [7]. In the past, methods from open pots filled with these attractants to rearing beds on soil were used for oviposition, and larval-biomass production depended on substrate and environmental conditions [7]. Today, the handling of BSF offspring remains an area where high-tech facilities have yet to achieve the level of process automation required to economically compete with the products they aim to replace, that is, soybeans and fishmeal. Thus, imitating the most attractive oviposition site by leveraging microbes and the VOCs they produce is indispensable in advancing the sector's industrialization (Table 1).

Farmed insects themselves possess intricate microbial communities that influence their digestion [45], immunity [46], and growth [47], contributing to nutrient supplementation and pathogen protection. Characterizing microbiota to improve the biotechnological application of insects has been in focus in the last decade. Tackling communication mechanisms is a logical progression in understanding these microbe–host

interactions, as microbes employ density-dependent quorum sensing to communicate within and across microbial communities, thereby orchestrating their behavior and metabolic activities and emitting VOCs perceptible to higher life such as insects [18,34,35] (Figure 1). Understanding the extent to which inter-kingdom communication influences microbes within and surrounding farmed insects holds potential for advancing disease management, fostering insect health, and optimizing biotechnological applications for bioremediation and waste valorization. Scieuzo et al. [4] characterized 27 odor-binding proteins in adult BSF with differential expression profiles in males and females and tested their binding activity with a palette of VOCs. BSF larvae shape their microbial environment via secreting antimicrobial peptides [48], but it is unclear how this affects the attraction of female BSF in search of an oviposition site [26]. Conspecific offspring, adults, and mVOCs emitted by bacteria isolated from conspecifics may act as attractors for females [3,49]. However, females are also attracted to volatiles like 2-methyl-butanol, produced by

Figure 2



Methodological framework to investigate interkingdom communications. **(1)** Behavioral assays examine insect responses to stimuli, such as microbially produced volatile organic compounds (mVOCs), through choice tests and activity monitoring. **(2)** Microbiological methods focus on isolating and characterizing microbial species *ex situ*, identifying mVOC-producing strains and their interactions. **(3)** Multi-omics and *in silico* methods integrate data from microbiomics, genomics, transcriptomics, and proteomics to explore molecular mechanisms and model interactions. **(4)** Analytical methods enable the identification and quantification of VOCs using advanced tools like gas chromatography (GC) coupled with mass spectrometry (MS) and their bioactivity validation using GC-coupled electroantennography (EAD). **(5)** Environmental observation contextualizes findings by examining microbial and insect interactions in natural or applied ecosystems.

Staphylococcus sp. This compound is strongly reduced after the larvae processed the substrate, potentially reducing its olfactory attractiveness [3,4]. Thomas et al. [34] identified decanal, p-cresol, sulcatone, pentanoic acid, and acetophenone as a blend for BSF oviposition (Table 1). When split into two simpler mixtures (Mix 1: decanal, p-cresol; Mix 2: sulcatone, pentanoic acid, acetophenone), oviposition attractiveness decreased, suggesting that at least two compounds are required to trigger oviposition.

Research framework and methodologies

A key mission is to understand the role of microbial sensing in microbe–insect interactions and associated insect responses to their environment (Figure 1). This review addresses the challenge of standardizing these behaviors in artificial environments, given the complex nature of microbe–insect communication. By identifying potential research avenues, knowledge gaps pertaining to the mechanistic understanding of insect VOC perception and processing, as well as the consequences this perception has on insect physiology and behavior, may be addressed. This exploration can be achieved using

interdisciplinary approaches that combine (1) behavioral ecology, (2) microbiology, (3) molecular biology and multi-omics, (4) chemistry, and (5) environmental observation (Figure 2). By integrating methodologies from these disciplines, we outline selected techniques that can together help us understand these complex interkingdom interactions. An implementation of the proposed methods in an industrial setting would involve prioritizing large-scale behavioral screening to quantify responses to attractive substrates (section [Behavioral assays](#)), combined with VOC profiling using GC-EAD-MS or PTR-ToF-MS (section [Analytical techniques](#)). This approach represents the most direct route for generating robust data for attraction formulation and oviposition control, ultimately enabling rapid improvement of rearing procedures. Once the key drivers of oviposition attraction are identified, integrating multi-omics approaches (section [Multi-omics and in silico methods](#)) with traditional microbiological methods (section [Microbiological methods](#)) can further support the validation of VOC-producing microorganisms, which are central to understanding the biological foundation of attraction.

Behavioral assays

Olfactometers have long been used to investigate insect reactions to selected stimuli *ex situ* and under artificial conditions (Figure 2). These systems can work with (e.g. Y-tube olfactometer) or without airflow (e.g. cafeteria experiment), and there are several points to consider for such assays, which have been outlined in the paper by Roberts et al. [50]. Behavioral assays can be used to study the attraction to specific stimuli [18,51] and to verify synthetic compounds in their effectiveness in eliciting specific responses in the organism of study (e.g. oviposition, feeding, deterrence, etc.). Dose-dependent [52] or stage-of-maturity-dependent (i.e. dependent on the age or degradation stage of a no-longer-fresh substrate) [26] effects of substrates can provide information at an even finer resolution. Moreover, these behavioral assays can be enhanced by tracking systems to monitor patterns of insect or antennal movement in response to stimuli [53].

Microbiological methods

After finding attractive substrates, the source of cues can be investigated. While microorganisms produce many detectable VOCs (e.g. in headspace samples), the full microbial diversity remains elusive, with most microorganisms still uncultivable, also due to interdependencies with other species [32,54]. Thus, isolating microorganisms from their natural environment and cultivating them is still a challenge. Moreover, growing microorganisms in artificial media may alter their metabolic activity. This shift can change their metabolic products and obscure insights into their interactions within their native ecosystems, complicating the study of ecological roles and potential functions [55]. Co-culturing, a method to grow two or more microbial isolates together in the same environment, can serve to explore interactions of microorganisms and their combined effects on insect behavior. Novel tools for isolating previously unculturable microorganisms, such as the iChip [54], can be used to investigate the mVOC bouquet *ex situ*. The diffusion chambers on the iChip are sealed off by semi-permeable membranes that only allow in dissolved nutrients, while other microorganisms are kept out, thereby enabling the isolated growth of microbes in their original environment (Figure 2).

Analytical techniques

Well-established methods, such as gas chromatography (GC) coupled with electroantennographic detection (EAD) and mass spectrometry (MS), to characterize volatile patterns and simultaneously assess insect antennal reactions, are fundamental to pinpoint behaviorally active mVOCs in substrate samples. The recent advancement of combining GC, EAD, and MS into a single system can improve efficiency and reduce errors for cross-platform comparisons [56]. Proton-transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS)

provides real-time, high-throughput analysis of VOCs, and its high resolution could help functionally characterize genes involved in VOC biosynthesis [57]. Cultivable microbial strains can be used in headspace analysis combined with these methods to link specific microbial emissions to insect attraction [1]. Collectively, these techniques enhance the resolution of dose- and substrate-dependent effects of VOCs on insect behavior.

Multi-'omics and *in silico* methods

Integrating multi-'omics data (e.g. genomics, transcriptomics, proteomics) can systematically combine molecular pathways involved in VOC biosynthesis and their effect on insect chemosensation. RNA-Seq paired with proteomic analysis can reveal how genes involved in mVOC biosynthesis align with odorant-binding-protein expression in insect antennae and can validate receptors for synthetic attractants [4,58]. *In silico* simulation of docking mechanisms can predict interactions between VOCs and chemosensory proteins, while metagenomics of insect- and substrate-associated microbiomes can identify VOC producers [3,4,35].

Collectively, attraction assays, analytical methods, multi-omics data analysis along with validation in natural environments can resolve forces of attraction and determine the compatibility of specific semiochemicals with an insect's array of olfactory receptors [4,6].

Future challenges

Determining the biologically relevant concentrations of bioactive mVOCs that induce specific reactions and assessing whether these compounds are effective alone or in blends remains a key challenge in uncovering volatile-mediated interkingdom communication [29] (Table 1). Another difficulty is characterizing the mVOC patterns of isolated microorganisms outside their natural habitat, as laboratory conditions will alter their metabolism and thus the production and emission of volatiles (Figure 1). However, 'omics methods have provided promising results to eliminate this obstacle *in silico* before testing compounds in *in vitro* experiments (Figure 2). Once attractive substances have been identified, their general validity will be open for scrutiny also in field studies, thus allowing proper ground truthing by assessing the ecological realism of these findings (also see Figure 2). The phenomenon of natal habitat-preference induction, which occurs in fruit and house flies and suggests that larval experiences shape adult preferences, may pose an obstacle given the diverse rearing substrates employed by insect farmers [59]. Decoupling production and breeding lines may resolve this issue.

Conclusion

This review aims to provide inspiration and a framework for conducting foundational research within applied ecosystems. By deepening our understanding of multiway

interkingdom communication, we can contribute to more efficient and sustainable agricultural practices such as advanced conversion of agro-industrial by-products into insect biomass and frass fertilizer, enhancing food and feed security and promoting protein self-sufficiency by improving the scalability of rearing operations and supporting localized protein production. The complexity of advancing insect farming for food and feed through interkingdom communication — an area with broad implications for waste valorization and greenhouse gas mitigation — requires a multidisciplinary approach. This must integrate behavioral and molecular ecology, chemistry, microbiology, bioinformatics, and economics. Addressing this complexity through case studies is crucial, as it not only fills knowledge gaps but also drives innovation in ecological understanding and the industrial application of insect mass rearing, paving the way for more resilient and sustainable agricultural systems. Zooming now into the BSF system will then allow us to zoom out by testing the robustness of the findings obtained across kingdoms, both at the basic and the applied level.

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Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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