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Microbial volatiles as mediators of eco-evolutionary dynamics

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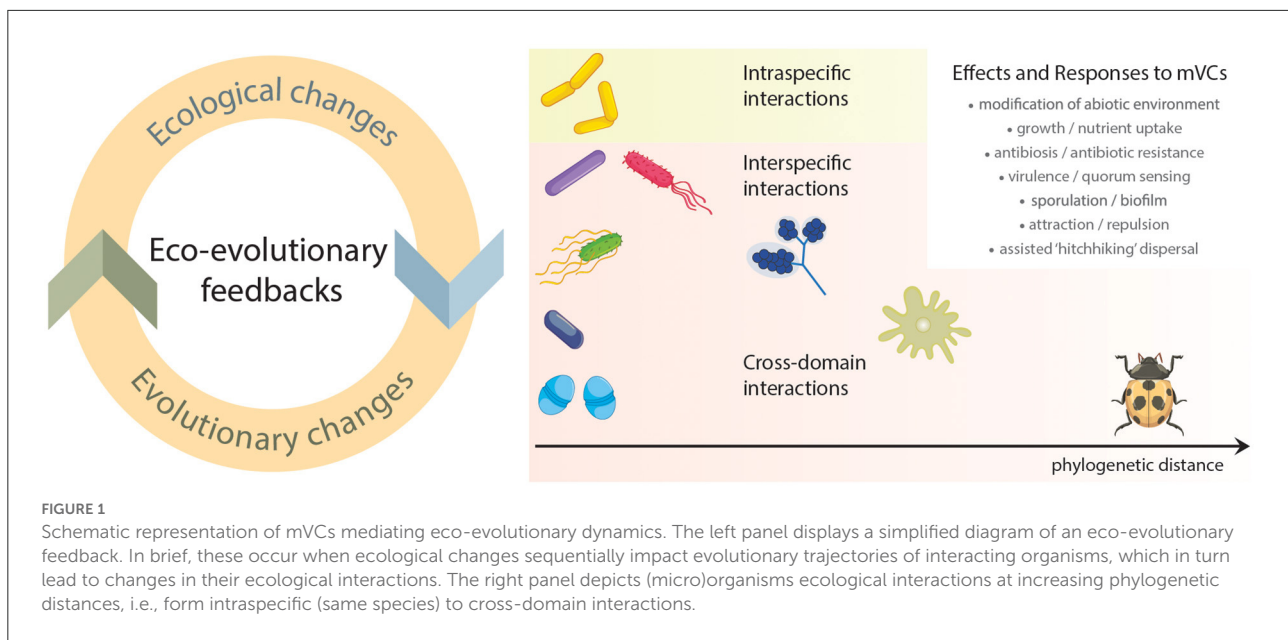
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Introduction

Microbial volatile compounds (mVCs) can provide numerous functional benefits to the producing organism, for instance, by contributing to the invasion of previously unexploited niches and/or inhibiting competitors, by promoting beneficial interactions or regulating abiotic stress tolerance (Tyc et al., 2016; Sharifi and Ryu, 2020). Over time, the benefits provided by mVCs can translate to increased fitness of the volatile-producing microbe, leading to the selection and evolution of specific mVC-associated traits. Simultaneously, interacting (micro)organisms can evolve mechanisms for mVC-perception and tolerance to attain fitness gains. The balancing of fitness gains in producing and perceiving microbial populations can be considered components of eco-evolutionary dynamics, broadly defined as interactions between ecological and evolutionary processes affecting organismal fitness (Hendry, 2017). More specifically, these dynamics occur when ecological changes result in evolutionary changes (e.g., adaptation to new environments) or when evolutionary changes lead to ecological changes (e.g., trait change altering ecosystem function). When these processes reciprocally influence each other, positively or negatively, these dynamics are considered eco-evolutionary feedbacks. To date, however, reliable assessments of the strength of selection acting on mVC-associated traits are still lacking. This occurs primarily because most research focused on mVCs has been dedicated to their ecological functions (e.g., communication, plant growth promotion, ecological competition), relatively with less attention given to their evolution in producing and perceiving microbes. In this opinion, we discuss examples of yet-underexplored evolutionary consequences of mVCs acting as mediators of ecological intra- and interspecific interactions, ranging from microbe-microbe communication to cross-domain interactions. In particular, we discuss whether selection acting on mVC-associated traits can lead to eco-evolutionary dynamics and feedbacks in both producing and perceiving (micro)organisms across varying phylogenetic distances (Figure 1).



Microbial volatiles mediating intraspecific interactions

The ability of mVCs to steer evolutionary outcomes within species is linked to their capacity to change the chemical properties of the niche (e.g., medium alkalization), to interfere with chemical communication between taxa (e.g., disruption of quorum sensing), and regulate virulence (e.g., toxin production). For example, a recent study revealed that the bacterium *Streptomyces venezuelae* synthesizes trimethylamine during its exploratory behavior. This volatile compound acts by increasing the local pH, which in turn reduces local iron availability (Jones et al., 2019) in the system – as similarly shown by the production of the alkaline mVC ammonia. In doing so, the producing strain gains a competitive advantage by reducing the fitness (i.e., the efficiency of iron acquisition) of competing species. It is tempting to speculate, as it remains to be tested, whether this trait in *S. venezuelae* might have co-evolved with siderophore biosynthesis and uptake, thus providing an additional fitness gain to the mVC-producing bacterium (Jones et al., 2019). Besides, there are also examples of intraspecific modulation of chemical communication and physiology by mVCs. For example, the blend of mVCs produced by *Pseudomonas chlororaphis* – composed of 1-undecene, methyl thiolacetate and dimethyl disulfide – was shown to quench quorum-sensing signals required for phenazine biosynthesis, and suppress the transcription of the biosynthetic genes for *N*-acyl-homoserine lactones (Chernin et al., 2011). In another example, the volatile compound leudiazene was shown to regulate the production of mangotoxin in *Pseudomonas syringae* pv. *syringae* (Sieber et al., 2021). This volatile – produced by the

non-ribosomal peptide synthetase *MgoA* – triggers its own mangotoxin production that not only causes disease symptoms in plants but also acts as an antimicrobial molecule, facilitating colonization of the phyllosphere by *P. syringae* pv. *syringae*.

Microbial volatiles mediating interspecific interactions

The broad diversity of mVCs mediates complex and yet-underexplored interactions between different microbial taxa. These occur via diverse mechanisms and can be associated with eco-evolutionary dynamics, and in some cases, with the establishment of eco-evolutionary feedbacks. First, mVCs can reduce the metabolic cost associated with the biosynthesis of other active metabolites. For example, some *Streptomyces* strains produce ammonia (a “low cost” molecule) at high enough concentrations to kill other bacteria at a distance and to potentiate the activity of soluble antibiotics (e.g., polyketides, non-ribosomal peptides, β -lactams – “high cost” molecules) produced locally by other *Streptomyces* species (Avalos et al., 2020). In another example, a yet-undefined mVOC produced by *Xanthomonas perforans* (a non-motile organism) was shown to trigger surface motility in *Paenibacillus vortex* (Hagai et al., 2014). The same study showed that *X. perforans* was able to “hitchhike” with the swarming colony of *P. vortex* to reach new sites for infection on tomato leaves. As such, it is plausible the plant pathogenic Xanthomonads are benefiting from the volatile-mediated interspecific interaction by reducing the cost of an energetically costly trait, in this case, motility. Whether this type of ecological interaction can be characterized as mutualism or commensalism is still unclear. For different bacterial taxa,

however, the “hitchhiking” bacteria provide functional benefits to their interactive motile partners. These benefits can occur via local degradation of antibiotics (Finkelshtein et al., 2015) or by breaking down complex carbon sources leading to co-metabolism (Wang et al., 2021). Hence, given the mutual fitness benefits to both interacting partners described in these studies, it is more likely that these interactions are in most cases mutualistic.

Perhaps one of the most known examples of how microbial volatiles mediate eco-evolutionary feedbacks is illustrated by the interaction between the taxonomically distant organisms *Streptomyces* and the soil arthropod *Folsomia candida*. This example originated from studies addressing the longstanding question about the possible biological function(s) of the ubiquitous soil volatile geosmin (Becher et al., 2020). Several studies have shown that the production of geosmin and 2-methylisoborneol by *Streptomyces* attracts *F. candida*. This arthropod assists in the dispersal of bacterial spores through feeding and their attachment to the arthropod’s cuticle, whereas the arthropod benefits from this attraction by feeding on the bacteria, directly increasing its reproductive success (i.e., higher arthropod molting and egg laying). Interestingly, the biosynthesis of these mVCs by *Streptomyces* is restricted to the sporulating life stage of the bacterium, which is tightly controlled by the transcription factors *BldM* and *WhiH*. Moreover, the hydrophobic cuticle of this arthropod favors the attachment of hydrophobic *Streptomyces* spores. Taken together, the functional interplay between these traits possesses signatures of an eco-evolutionary feedback between distantly related organisms across domains of life. Another example is provided by the interaction between the bacteria *Listeria monocytogenes* and the protozoan *Euglena gracilis* in soil. *Listeria monocytogenes* can produce an array of mVCs (including decanal, 2-ethylhexyl acetate, 3,5-dimethylbenzaldehyde, and ethyl acetate) that attract *E. gracilis* (Gaines et al., 2019). This protozoan feeds on specific bacterial taxa, imposing a strong selective pressure by favoring the persistence and evolution of adaptive traits to resist predation (e.g., secondary metabolites, motility, cell surface, and multicellular behaviors, such as biofilm formation) (Matz and Kjelleberg, 2005).

The observation that perceiving microorganisms can develop resistance mechanisms to mVCs, i.e., becoming “deaf” to mVCs as chemical signals, may affect eco-evolutionary dynamics and feedbacks. Examples of such mechanisms include the reduced transcription of the *ompR* porin master regulator in *Escherichia coli*, preventing the influx of ammonia into the cells, thereby enabling this bacterium to grow at high ammonia concentrations (Avalos et al., 2020). However, when *E. coli* was exposed to sub-inhibitory concentrations of ammonia, this volatile served as an intracellular signal enhancing the production of polyamines. This, in turn, resulted in a reduction of cell membrane permeability and enhanced resistance to antibiotics (Bernier et al., 2011). When mVCs

exert specific regulatory roles inside cells, it is likely that developing such resistance mechanisms will have consequences on the perception and functioning of these compounds in the perceiving organisms. This is also exemplified by the toxic inorganic volatile hydrogen cyanide (HCN). In this case, perceiving microorganisms have evolved strategies to resist its toxicity via cyanide-resistant respiratory pathways or via the breakdown of HCN into the less toxic formamide (Duffy et al., 2003). Volatiles can also have broader impacts on the evolution of interactive microbial species by specifically targeting signaling cascades. This was demonstrated in the case of the mVCs 2,3-butanedione and glyoxylic acid produced by *Bacillus subtilis*. In brief, these molecules were shown to negatively regulate the expression of the gene *ypdB* in *E. coli* and the respective downstream transcription factors (*soxS*, *rpoS* and *yhjU*) (Kim et al., 2013) involved in cell motility and antibiotic resistance.

The evolutionary role of mVCs can be further inferred from the emergence of congruent patterns when assessing microbial phylogeny from metabolomics (Elmassry et al., 2020), or “chemotyping.” For example, a phylogenetic signal for the volatile profile was observed in *Actinobacteria* (Choudoir et al., 2019), with metabolomics leading to more refined phylogenies in a collection of *Streptomyces* species as compared to classical taxonomic markers (i.e., 16S rRNA, *atpD* and *recA* genes) (Cordovez et al., 2015). On the other hand, when analyzing biosynthetic genes of the 10 most abundant terpene classes in *Streptomyces*, the overall phylogenomic and phylogenetic analyses were incongruent (Martín-Sánchez et al., 2019). This points to the fact that the evolution of mVCs genes may differ in terms of their vertical and horizontal gene transfer, where the latter may lead to a more rapid and phylogenomic-disconnected evolution of specific mVCs, in this case, terpenes. However, some terpene synthases were shown to be restricted to one phylogenomic (sub)group, such as epi-isozizaene, cyclooctat-9-en-7-ol, and isoafrikanol synthases, suggesting functional specialization and highlighting the ecological relevance of these terpenoid compounds for specific phylogenetically related *Streptomyces* species.

Perspectives and concluding remarks

Here, we postulate that mVCs offer several opportunities for the creation, mediation, and manipulation of intra- and inter-specific eco-evolutionary feedbacks. Nevertheless, challenges remain in quantifying and determining how specific mVCs contribute to the fitness of particular microbial species (both, producer and perceiver). This is primarily due to the complexity of properly identifying and quantifying eco-evolutionary dynamics across multiple potential biological interactions. Besides, this challenge can be exacerbated by

the chemical nature of volatile molecules, which due to their high diffusibility, can serve as “common goods” in the system (Netzker et al., 2020). This means that locally produced mVCs can be accessed by a diverse set of (micro)organisms in a non-specific and concentration-dependent manner, further confounding the identification of their role as signals modulating eco-evolutionary dynamics. The local microbial community composition can also affect the production and functioning of mVCs, adding another layer of complexity. This can occur, for example, when bacteria are interacting at a distance and changes in interacting partners elicit changes in emitted mVCs (Tyc et al., 2015), or by non-enzymatic reactions between compounds produced by two distinct bacterial populations leading to the formation of novel compounds (Kai et al., 2015). The environmental conditions and nutrient availability also play key roles in defining the chemical diversity and concentration of mVCs produced by microbial populations (Choudoir et al., 2019). Since most of the current data derives from *in vitro* studies, significant constraints in our interpretation of the true eco-evolutionary consequences of mVCs in natural settings still exist. Nevertheless, we consider that *in vitro* studies offer optimal conditions, currently not fully leveraged in literature, to test whether specific mVCs are the key molecules driving eco-evolutionary dynamics and feedbacks. This can be based on the design of prospective experimental evolution assays aiming at unraveling the mechanisms of species interactions mediated by mVCs, thus discerning between correlation (based on indirect experimental evidence) and causation (biological mechanistic evidence). Last, embracing the complexity of mVC-mediated communication from an eco-evolutionary perspective is a necessary step toward advancing our understanding of the mechanisms driving the emergence and evolution of interactions between producing and perceiving microbial populations across ecosystems.

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Author contributions

RM and FD-A discussed and wrote the first draft. The concepts were refined and further discussed by all authors who also provided comments on and edited all versions of the manuscripts. All authors contributed and approved the submitted version.

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