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## Microbial volatiles: Small molecules with an important role in intra- and inter-kingdom interactions

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1 **Microbial volatiles: Small molecules with an important role in intra- and inter-kingdom**  
2 **interactions**

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18 microbe interactions

19

20

21 **Abstract**

22 During the last decades research on the function of volatile organic compounds focused  
23 primarily on the interactions between plants and insects. However, microorganisms can also  
24 release a plethora of volatiles and it appears that microbial volatile organic compounds  
25 (mVOCs) can play an important role in intra- and inter-kingdom interactions. So far, most  
26 studies are focused on aboveground volatile-mediated interactions and much less information  
27 is available about the function of volatiles belowground. This minireview summarizes the  
28 current knowledge on the biological functions of mVOCs with the focus on mVOCs-mediated  
29 interactions belowground. We pinpointed mVOCs involved in microbe-microbe and microbe-  
30 plant interactions, and highlighted the ecological importance of microbial terpenes as a largely  
31 underexplored group of mVOCs. We indicated challenges in studying belowground mVOCs-  
32 mediated interactions and opportunities for further studies and practical applications.

33

## 1 **Introduction**

2 Many secondary metabolites have been reported to be involved in microbial interactions. One  
3 group of secondary metabolites produced by soil and plant-associated microorganisms, but  
4 largely unexplored to date, are the volatile organic compounds (VOCs). VOCs are typically  
5 small, odorous compounds (< C15) with low molecular mass (< 300 Da), high vapour pressure,  
6 low boiling point, and a lipophilic moiety. These properties facilitate evaporation and diffusion  
7 aboveground and belowground through gas- and water- filled pores in soil and rhizosphere  
8 environments (Vespermann et al., 2007, Insam and Seewald 2010, Effmert et al., 2012).  
9 Microbial VOCs (mVOCs) belong to different chemical classes including alkenes, alcohols,  
10 ketones, benzenoids, pyrazines, sulfides and terpenes (Schulz and Dickschat 2007, Lemfack et  
11 al., 2014, Kanchiswamy et al., 2015, Schmidt et al., 2015, Lemfack et al., 2017). A recent meta-  
12 analysis by Schenkel et al., (2015) provided a comprehensive overview of VOCs derived from  
13 soil-borne microbes.

14 The production of mVOCs in soil is influenced by various factors including the growth stage  
15 of the microbes, nutrient availability, temperature, oxygen availability, pH, and soil moisture  
16 content (Wheatley 2002, Insam and Seewald 2010). Several recent studies reported that the  
17 production of certain mVOCs can be induced or suppressed during inter-specific microbial  
18 interactions (Garbeva et al., 2014a, Schulz-Bohm et al., 2015, Tyc et al., 2015, Piechulla et al.,  
19 2017). mVOCs were often considered to be by-products of primary metabolism, but recent  
20 findings revealed that many mVOCs demonstrate biological activity (Schmidt et al., 2015, Tyc  
21 et al., 2017a). Furthermore, in bacteria, the production of certain mVOCs is dependent on the  
22 GacS/GacA two-component regulatory system (Cheng et al., 2016, Ossowicki et al., 2017).  
23 These findings clearly disagree with the opinion that mVOCs are just waste products.

24 While soluble metabolites are often responsible for short distance interactions, VOCs are  
25 considered to be long-distance messengers (Tyc et al., 2017b, Westhoff et al., 2017). There are  
26 many types of microbial interactions occurring belowground such as bacteria–bacteria, fungi–  
27 fungi, fungi-bacteria, bacteria-protists, fungi–plant, bacteria–plant, and bacteria–fungi–plant  
28 interactions. However, most studies addressing belowground VOCs-mediated interactions are  
29 focused mainly on the root-emitted volatiles (recently reviewed by Delory et al., 2016).

30 The knowledge we have gained from research conducted over the last few years reveals that  
31 mVOCs can have both beneficial and harmful effects on other organisms (Effmert et al., 2012,  
32 Schmidt et al., 2015). mVOCs can provide organisms with rapid and precise ways to recognize  
33 neighbouring organisms (both friends and foe) and to launch proper responses.

1 The aim of this review is to summarize the current knowledge concerning the role of mVOCs  
2 in intra- and inter-kingdom interactions, to pinpoint mVOCs (e.g. terpenes) involved in  
3 microbe-microbe and microbe-plant interactions, to indicate challenges in studying  
4 belowground mVOCs-mediated interactions and opportunities for further studies and practical  
5 applications.

6

## 7 **VOCs in microbe-microbe interaction**

### 8 *Bacteria-bacteria*

9 Bacterial VOCs can have direct antagonistic effects against other bacteria. For instance, the  
10 sesquiterpene albaflavenone produced by *Streptomyces albidoflavus* revealed activity against  
11 *Bacillus subtilis* (Gürtler et al., 1994) and the emission of dimethyl disulphide by two  
12 rhizospheric bacteria, *Pseudomonas fluorescens* and *Serratia plymuthica*, showed  
13 bacteriostatic effects against two plant bacterial pathogens *Agrobacterium tumefaciens* and  
14 *Agrobacterium vitis* (Dandurishvili et al., 2011). *Pseudomonas fluorescens* WR-1 produces  
15 volatiles such as benzothiazole and 1-methyl naphthalene with bacteriostatic effects against the  
16 tomato pathogen *Ralstonia solanacearum* (Raza et al., 2016a). In fact, many species of  
17 *Pseudomonas* and *Bacillus* that are used as biocontrol agents against plant pathogens, have  
18 been reported to produce VOCs with antibacterial activity (Raza et al., 2016a, Raza et al.,  
19 2016b, Raza et al., 2016c, Xie et al., 2016, Rajer et al., 2017, Tahir et al., 2017a, Tahir et al.,  
20 2017b). For instance, a recent study revealed that VOCs produced by *Bacillus* spp., including  
21 benzaldehyde, 1,2-benzisothiazol-3(2H)-one and 1,3-butadiene, had strong inhibitory activity  
22 against *R. solanacearum*, the causal agent of bacterial wilt disease (Tahir et al., 2017a). The  
23 mVOCs altered the transcriptional expression levels of several genes involved in motility and  
24 pathogenicity (e.g. global virulence regulator *PhcA*, type III secretion system, and extracellular  
25 polysaccharide [EPS] production) and induced systemic resistance by plants, which resulted in  
26 a decrease of wilt disease.

27 Several reports describe the effect of VOCs in bacterial virulence. For instance, 2,3 butanediol  
28 and acetoin are required for full virulence in *Pectobacterium carotovorum*. The same  
29 compounds can increase the production of virulence factors in *Pseudomonas aeruginosa*  
30 (Audrain et al., 2015).

31 In contrast, VOCs produced by some bacteria can also have positive effects on the growth of  
32 other neighbouring bacteria in the rhizosphere. For instance, VOCs from *Collimonas pratensis*  
33 and *S. plymuthica* are able to induce the growth of *P. fluorescens* Pf0-1 (Garbeva et al., 2014a).  
34 These VOCs induced expression of genes involved in motility in *P. fluorescens* Pf0-1 and

1 provoked an increase in the production of secondary metabolites with antibacterial activity  
2 against *Bacillus* (Garbeva et al., 2014a). This suggests that *C. pratensis* and *S. plymuthica* may  
3 be attracting and promoting the growth of *P. fluorescens* in a collaborative attempt to increase  
4 their chances against different bacterial competitors or soil fungal pathogens. Another example  
5 of the growth-promoting effect of VOCs was reported recently by Schulz-Bohm et al. (2015)  
6 which showed that VOCs released by mixtures of root exudate-consuming bacteria stimulated  
7 the activity and growth of distant nutrient-limited bacteria.

8 In addition to exerting antagonistic effects towards other bacteria, VOCs can also modify the  
9 behaviour of other bacteria and modulate their resistance to antibiotics. Bacterial volatiles such  
10 as ammonia, trimethylamine, hydrogen sulfide, nitric oxide, and 2-amino-acetophenone can  
11 alter biofilm formation or dispersal or affect motility of bacteria (Audrain et al., 2015, Raza et  
12 al., 2016a). Bacteria often make use of their motility to move to other areas with more resources  
13 and/or less competitors. In *Streptomyces venezuelae*, a new mode of development, so-called  
14 exploration, has been recently discovered that allows non-motile bacteria to access regions with  
15 more nutrients (Jones et al., 2017). *S. venezuelae* is able to produce hydrophilic fast growing  
16 non-branching vegetative hyphae, triggered by glucose depletion and a rise in pH, to  
17 presumably escape from poor nutrient areas. Interestingly, explorer cells can release signals for  
18 long distance communication with other members of the species to induce their exploratory  
19 growth. One of these signals is trimethylamine, which works not only as a signal to  
20 communicate with distantly located *Streptomyces* and induce exploratory growth but also  
21 displays antibacterial activity against *B. subtilis* and *Micrococcus luteus*, probably by rising the  
22 pH of the medium (Jones et al., 2017).

#### 23 24 *Fungi-bacteria*

25 Fungal VOCs can play an important role in long distance fungal-bacterial interactions and can  
26 lead to different phenotypical responses in the interacting partners. For example, VOCs emitted  
27 by *Trichoderma atroviride* increased the expression of a biocontrol gene (*phlA*) in  
28 *P. fluorescens* that encodes the biosynthesis of 2,4-diacetylphloroglucinol (Lutz et al., 2004).  
29 A few recent studies demonstrated that the growth of some bacterial species can be suppressed  
30 by fungal VOCs (Werner et al., 2016) such as the VOCs that exhibit inhibitory effects on  
31 *Bacillus cereus* and *Bacillus subtilis* produced by the oyster mushroom *Pleurotus ostreatus*  
32 (Pauliuc and Botău 2013).

33 Recently, Schmidt et al., (2015) screened the phenotypic responses of soil bacterial strains to  
34 volatiles emitted by several fungal and oomycetal soil strains under different nutrient

1 conditions during different growth stages. Out of the phenotypical responses tested such as  
2 growth alteration, antimicrobial activity, biofilm formation or motility, motility of bacteria  
3 (both swimming and swarming) was significantly positively or negatively affected by fungal  
4 and oomycetal VOCs. This finding could, therefore, reflect a potential strategy employed by  
5 the fungus to attract mutualistic bacteria towards itself and to repel competitors by  
6 manipulating their motility through the use of VOCs (Piechulla et al., 2017). Transcriptomics  
7 and proteomics analyses of *Serratia plymuthica* PRI-2C exposed to VOCs emitted by the  
8 fungal pathogen *Fusarium culmorum*, showed that *S. plymuthica* PRI-2C responded to the  
9 fungal VOCs with changes in gene and protein expression related to motility, signal  
10 transduction, energy metabolism, cell envelope biogenesis, and secondary metabolite  
11 production (Schmidt et al., 2017). The metabolomic analyses of *S. plymuthica* PRI-2C exposed  
12 to the fungal VOCs, the gene cluster comparison, and the heterologous co-expression of a  
13 terpene synthase and a methyltransferase revealed the production of the unusual terpene named  
14 sodorifen (Kai et al., 2010, Von Reuß et al., 2010) in response to fungal VOCs. These findings  
15 support the suggested importance of VOCs (and in particular terpenes) as signalling molecules  
16 in fungal–bacterial interactions.

17 Many soil bacteria can produce VOCs with antifungal effects and thus contribute to the  
18 phenomenon known as soil fungistasis where fungal propagules are restricted in their ability to  
19 grow or germinate (Garbeva et al., 2011). Recently, Cordovez et al. (2015) revealed that VOCs  
20 produced by *Streptomyces* spp. exhibit antifungal properties against *Rhizoctonia solani* and  
21 may contribute to plant disease suppressiveness. Ossowicki et al. (2017) showed that VOCs  
22 from the tomato rhizosphere isolate *Pseudomonas donghuensis* P482 have strong antifungal  
23 and anti-oomycete activity which suggests that the antagonistic capabilities of this strain  
24 against plant pathogens are due to their volatile potential (Ossowicki et al., 2017). This effect  
25 of bacterial VOCs against oomycetes is not an isolated case and other *Pseudomonas* strains  
26 have been reported to have anti-oomycete activities (De Vrieze et al., 2015, Hunziker et al.,  
27 2015). In a recent report, VOCs produced by several *Lysobacter* strains growing in a protein-  
28 rich medium showed anti-oomycete activity whereas non-antagonistic VOCs were produced  
29 by these strains when grown on a sugar-rich medium. This indicates that the production of  
30 volatiles is highly dependent on growth conditions and nutrient availability (Lazazzara et al.,  
31 2017).

32

33 *Fungi-fungi*

1 The 1-octen-3-ol, one of the most prominent fungal VOC, known as the mushroom smell, is  
2 produced by a wide range of filamentous fungi and can function as a development signal among  
3 fungi (Miyamoto et al., 2014). The same compound was described to function in *Penibacillum*  
4 *paneum* as a self-inhibitor signal in spore germination (Chitarra et al., 2004). As developmental  
5 signals during population establishment, certain fungal VOCs act in a concentration-dependent  
6 manner to regulate conspecific mycelial growth and spore germination (Nemčovič et al., 2008,  
7 Stoppacher et al., 2010).

8 Fungal VOCs can have inhibitory effects and drive antagonistic interactions among fungi. For  
9 example, the endophytic fungi *Muscodor albus* and *Oxysporus latemarginatus* can strongly  
10 inhibit the growth of several plant pathogenic fungi, including *Botrytis cinerea* and *Rhizoctonia*  
11 *solani* (Strobel et al., 2001). VOCs emitted by *Trichoderma* spp. have a strong effect against  
12 plant pathogenic fungi such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*,  
13 *Sclerotinia sclerotiorum*, and *Alternaria brassicicola* (Amin et al., 2010). Similarly, VOCs  
14 such as 5-hexenoic acid, limonene, octanoic acid and 3,4-2H-dihydropyran produced by the  
15 non-pathogenic fungus *F. oxysporum* CanR-46 could inhibit mycelial growth of 14 fungal  
16 species including the pathogenic *Verticillium dahlia* (Zhang et al., 2015). Recently, a proteomic  
17 study demonstrated that fungal VOCs can interfere with essential metabolic pathways to  
18 prevent fungal growth (Fialho et al., 2016).

19 Some fungal species can detoxify the antifungal compounds produced by their microbial  
20 competitors. For example, *Fusarium graminearum* can detoxify the toxic compound 6-pentyl-  
21 alpha-pyrone, emitted by *Trichoderma harzianum* (Cooney et al., 2001). Fungal VOCs can be  
22 important carbon sources for fungi colonizing carbon-limited environments (Cale et al., 2016).  
23 Conversely, for fungi colonizing a more carbon-rich environment, VOCs may act, in a  
24 concentration-dependent manner, as semio-chemicals to mediate antagonistic and beneficial  
25 interactions between fungi.

26

### 27 *Protists-bacteria*

28 A very diverse and abundant group of soil microorganisms are protists (Protozoa) (Fierer and  
29 Jackson 2006, Geisen et al., 2015). Due to their grazing activities, protists play an important  
30 role in the soil food web and significantly affect carbon allocation and nutrient-cycling in the  
31 soil-plant-interphase (Geisen et al., 2016). Most soil protists are known to be key predators of  
32 bacteria and can shape bacterial communities by selective feeding (Griffiths et al., 1999,  
33 Bonkowski and Brandt 2002, Rosenberg et al., 2009, Glücksman et al., 2010). Reaching  
34 suitable prey is very energy consuming (Jousset 2012). Thus, sensing their prey over long

1 distances in the porous soil matrix would be very beneficial for protists. A recent study by  
2 Schulz-Bohm et al. (2017) revealed that volatile organic compounds can play a key role in  
3 long-distance bacterial-protists interactions. By testing various volatile-mediated interactions  
4 between phylogenetically different soil bacteria and protists and comparing those with direct  
5 trophic interactions, they demonstrated that specific bacterial volatiles can provide early  
6 information about suitable prey. In particular, it was shown that terpenes such as  $\beta$ -linalool,  $\beta$ -  
7 pinene, germacrene D-4-ol or  $\delta$ -cadinene produced by *Collimonas pratensis* Ter91 (Song et  
8 al., 2015b) can stimulate protist activity and motility suggesting that terpenes can be key  
9 components in VOCs-mediated communication between protists and bacteria (Schulz-Bohm  
10 et al., 2017). Interestingly, soil protists such as *Dictyostelium discoideum* (Chen et al., 2016)  
11 produce volatile terpenes. These terpenes might be involved in defence mechanisms, for  
12 example, to repel nematode predators. Similarly, it was shown that soil bacteria can produce  
13 specific volatiles to repel protist predators (Kai et al., 2009, Schulz-Bohm et al., 2017).  
14 Besides bacterivorous protists, obligate and facultative mycophagous (fungus grazing)  
15 protists are common soil inhabitants (Geisen 2016). Mycophagous protists feed mostly on  
16 yeast and fungal spores while some specialists are able to graze directly on the hyphae of  
17 filamentous fungi (Geisen et al., 2016). It is well known that soil fungi such as yeast produce  
18 a wide set of volatile compounds involved in various belowground interactions (Effmert et al.,  
19 2012, Werner et al., 2016). Thus, although not demonstrated yet, it is plausible that fungal  
20 volatiles might play an important role in below-ground communication between soil fungi and  
21 protists, as well.

22

### 23 **VOCs in microbe-plant interactions**

24 In recent years, evidence supporting the idea that plants respond strongly to mVOCs has grown.  
25 Most of the research carried out so far has investigated the impact of microbial VOCs on the  
26 model plant *Arabidopsis thaliana*. This has revealed that, without physical contact,  
27 microorganisms are able to drastically alter plant root system development, plant physiology,  
28 hormonal pathways and biomass production (Ryu et al., 2004, Blom et al., 2011, Wenke et al.,  
29 2012, Bailly et al., 2014, Bitas et al., 2015, Ditengou et al., 2015, Li et al., 2016, Piechulla et  
30 al., 2017). mVOCs can also function as a direct source of nutrients for plants (Meldau et al.,  
31 2013), induce resistance to pathogens in plants (D'Alessandro et al., 2014, Kottb et al., 2015,  
32 Song et al., 2015b, Wintermans et al., 2016), affect plant secondary metabolite production  
33 (Santoro et al., 2011), directly inhibit plant pathogens (Kai et al., 2009, Garbeva et al., 2014b,  
34 De Vrieze et al., 2015, Kottb et al., 2015) and induce soil fungistasis and suppressiveness



1 (Garbeva et al., 2011, Van Agtmaal et al., 2015). Moreover, one single mVOC can show  
2 various functions, such as dimethyl disulfide, which improves plant growth by enhancing the  
3 availability of reduced sulphur (Meldau et al., 2013). It also protects tobacco and corn plants  
4 against *Botrytis cinerea* and *Cochliobolus heterostrophus* by directly inhibiting pathogens and  
5 inducing systemic resistance in plants (Huang et al., 2012a). Likewise, a characteristic  
6 compound of *Trichoderma asperellum*, 6-pentyl-pyrone, can increase plant defence reactions  
7 and at the same time decrease *B. cinerea* and *Alternaria alternata* sporulation (Kottb et al.,  
8 2015).

9 Many independent studies revealed that mVOCs emitted by beneficial soil microorganisms can  
10 affect plant growth but only few studies focused on how VOCs produced by soil-borne plant  
11 pathogens affect plant growth and development. These studies suggest that mVOCs from plant  
12 pathogens may modulate the trade-off between plant growth, development and defence. Bitas  
13 et al., (2015) showed that VOCs emitted by pathogenic *F. oxysporum* promoted the growth of  
14 *A. thaliana* and *Nicotiana tabacum* and affected auxin transport and signalling. VOCs emitted  
15 by the pathogen *Alternaria alternaria* enhanced growth, early flowering and photosynthesis  
16 rates of *Arabidopsis*, maize and pepper by affecting the levels of plastidic cytokinin (Sanchez-  
17 Lopez et al., 2016). A more recent study showed that the soil-borne pathogen *Rhizoctonia*  
18 *solani* produced an array of mVOCs that promote plant growth, accelerate development,  
19 change plant VOCs emission and reduce insect resistance (Cordovez et al., 2017). This must  
20 be a successful strategy for the pathogenic fungi since with increased root biomass and  
21 stimulation of lateral root formation there is a greater surface area for fungal colonization and  
22 infection.

23 When analysing mVOCs effects on plant growth, it is important to take into account, that  
24 microorganisms can produce high amounts of CO<sub>2</sub> that can promote plant growth (Kai and  
25 Piechulla 2009, Piechulla 2017). Hence, a good experimental setup with appropriate controls  
26 are required to avoid artefacts in the results (Kai et al., 2016, Piechulla 2017).

27 Alternatively, plants are able to mediate the belowground plant-microbe interactions via root-  
28 emitted VOCs (Wenke et al., 2010). Root-derived VOCs may serve multiple roles such as  
29 carbon sources, defence metabolites and chemo-attractants (Van Dam et al., 2016).  
30 Rhizobacteria such as *Pseudomonas fluorescens* and *Alcaligenes xylosoxidans* have been  
31 shown to metabolize  $\alpha$ - pinene as their sole carbon source (Kleinheinz et al., 1999). Del Giudice  
32 et al. (2008) also reported that bacteria associated with the roots of vetiver grass (*Vetiveria*  
33 *zizanioides*) use sesquiterpenes as a carbon source. Undoubtedly, plants and soil  
34 microorganisms are engaged via VOCs in long-distance interactions (Van Dam et al., 2016).

1 However, so far, limited knowledge exists concerning the role of plant VOCs in attracting  
2 beneficial organisms and how plant-associated microorganisms affect the quantity and quality  
3 of plant volatile emission. Only recently, using a glass olfactometer system, the attraction of  
4 distant soil bacteria by VOCs emitted by plant roots was revealed (Schulz-Bohm et al.,  
5 accepted). Olfactometer systems have been used successfully to study aboveground plant-  
6 herbivores interactions (Ballhorn and Kautz 2013) or belowground plant-nematode interactions  
7 (Rasmann et al., 2005). However, this is the first case to apply an olfactometer to study plant-  
8 microbe interactions. Moreover, the same study revealed that upon fungal infection, the blend  
9 of root VOCs changed and specific bacteria with antifungal properties were attracted (Schulz-  
10 Bohm et al., accepted).

11

### 12 **mVOCs-mediated dialogue**

13 Several reports describe the chemical dialogue between microbes, plants and other organisms  
14 by the exchange of soluble compounds (Badri et al., 2009, Lira et al., 2015, Song et al., 2015a,  
15 Liu et al., 2016). Most of the studies reporting mVOCs-mediated communication belowground  
16 focus on the uni-directional responses and only a few studies reported on bi-directional  
17 mVOCs-mediated interactions. For instance, the importance of mVOCs in the dialogue  
18 between the fungal plant pathogen *Verticillium longisporum* and its bacterial antagonist  
19 *Paenibacillus polymyxa* was recently revealed in both *in vitro* and *in planta* experiments  
20 (Rybakova et al., 2017). Both microorganisms responded to one another's VOCs and this  
21 specific mVOCs-mediated interaction resulted in the inhibition of cellular metabolism and  
22 growth reduction of the fungal pathogen.

23 A VOCs-mediated dialogue between bacteria and fungi was also reported by Spraker et al.  
24 (2014) where VOCs of the fungal plant pathogen *Aspergillus flavus* reduced the production of  
25 the major virulence factor EPS of the bacterial plant pathogen *Ralstonia solanacearum*. In  
26 parallel, *A. flavus* responded to VOCs of *R. solanacearum* by reducing conidia production and  
27 increasing aflatoxin production.

28

### 29 **Conclusions and outlook**

30 Over the last decade, our understanding of the chemical complexity of mVOCs produced by  
31 many different soil microorganisms has grown. It is clear that these small and odorous  
32 molecules can modify the behaviour and promote or inhibit growth of neighbouring organisms  
33 (Figure 1).

1 Most existing studies on mVOCs are focused on describing the uni-directional effect of  
2 mVOCs produced by a single organism and the responses of the organisms perceiving them  
3 without considering mVOCs-mediated dialogue and the bi-directional responses to one  
4 another. Furthermore, microbial interactions taking place belowground are far more complex  
5 than single one-to-one interactions and involve more organisms, which can significantly affect  
6 mVOCs emission. For example, fungal-associated bacteria have been shown to affect the  
7 production of VOCs in fungi (Schulz-Bohm et al., 2015, Splivallo et al., 2015) and in addition,  
8 they can affect the fungal plant-pathogenicity and repress the expression of fungal virulence  
9 genes (Minerdi et al., 2009). Therefore, a holistic approach considering the effect of mVOCs  
10 on belowground soil community is needed. For instance, using a metagenomics approach Yuan  
11 et al., (2017) revealed that mVOCs could alter the composition of soil bacterial and fungal  
12 communities and significantly increased the relative abundance of *Proteobacteria*,  
13 *Bacteroidetes*, *Firmicutes* and *Ascomycota*. Furthermore, mVOCs influenced genes involved  
14 in important soil functions such as N-fixation (*nifH*), nitrification (*amoA*), denitrification (*nirS*)  
15 and antibiotic production (NRPS) (Yuan et al., 2017).

16 From the current scientific literature, it is clear that the most studied belowground mVOCs-  
17 mediated interactions are the interactions between bacteria, fungi and plants (Figure 1). There  
18 is a lack of knowledge relating to the emission of VOCs by protists, archaea or other  
19 rhizosphere organisms, such as nematodes or earthworms, indicating that these groups are  
20 currently understudied with regards to this aspect.

21 Several VOCs are commonly produced and emitted by both plant roots, fungi, bacteria and  
22 protists and it is possible that these compounds act as a 'lingua franca' for intra- and inter-  
23 kingdom communication between these organisms. Let us take as an example only one  
24 chemical class, the terpenes. Terpenes are the largest and most diverse class of metabolites  
25 known to date. They are best known to humans as plants metabolites. However, recent studies  
26 revealed that terpenes can be produced by all kingdoms of life including prokaryotes  
27 (Takamatsu et al., 2011, Yamada et al., 2012, Song et al., 2015b, Yamada et al., 2015, Chen et  
28 al., 2016). Recently, Yamada et al., (2015) described a powerful bioinformatics method based  
29 on the use of Hidden Markov Models (HMMs) and Protein Families Database (PFAM) search  
30 that has allowed the discovery of terpene synthases of bacterial origin and showed that  
31 phylogenetically different bacteria can be a rich source of terpenes. Both the number, the wide  
32 distribution, and the structural diversity of terpenes provide enormous potential for mediating  
33 significant chemical interactions and communication belowground. Examples of terpene-  
34 mediated microbial interactions are presented in Figure 2 and Table 1, indicating the ecological

1 importance of terpenes in interactions between soil micro- and macro-organisms, including  
2 plant roots.

3 Despite the rapid increasing numbers of studies showing the importance of mVOCs in the long-  
4 distance belowground chemical interactions, we still do not know exactly how VOCs are  
5 recognized and perceived. VOCs receptors or other perception mechanisms have not been  
6 identified in any of the described cases. The big challenge is to determine whether VOCs are  
7 internalized and transduced by receptor-mediated processes, whether they interact with the cell  
8 membrane to initiate signal transduction cascades or whether they are simply taken up by the  
9 cell and metabolized (Widhalm et al., 2015, Adebessin et al., 2017, Tissier et al., 2017). For  
10 plants, the current view is that due to their lipophilic nature, VOCs such as mono- and  
11 sesquiterpenes may interfere with membrane structures, thereby causing depolarization of the  
12 membranes and triggering  $\text{Ca}^{2+}$ -signalling in plants (Maffei et al., 2001, Heil and Land 2014).  
13 For further deciphering of mVOC-mediated microbe-microbe interactions, the mVOCs  
14 microbial perception mechanism needs to be elucidated. The application of methods for  
15 screening of mutant strains may be useful for that purpose, to identify microbial genes and  
16 proteins that are required for VOCs perception.

17 Another big challenge is to determine what concentrations of mVOCs are produced in soil and  
18 at what distances these mVOCs are eliciting a biological response in other organisms. There is  
19 the possibility that, similar to the roles of antibiotics in nature (Davies et al., 2006, Yim et al.,  
20 2006, Romero et al., 2011), mVOCs could have concentration-dependent function either as  
21 weapons in intercellular chemical warfare or as signalling compounds when they are present  
22 in low concentrations.

23 Concerning the implementations of mVOCs, our knowledge on the potential use of those  
24 compounds in large-scale agriculture and horticulture is still limited. In agriculture systems,  
25 mVOCs have to be applied under open-field conditions, which are very different from the *in*  
26 *vitro* conditions currently used in most studies. There are very few studies assessing the effects  
27 of mVOCs application under open conditions and they have been summarized in a recent  
28 review from Chung et al., (2016). Since it was discovered that the 2,3-butanediol elicited plant  
29 growth and induced systemic resistance (Ryu et al., 2003, Ryu et al., 2004), several studies  
30 have applied this compound or the producing strains to the soil of open fields to test its effects  
31 under agricultural conditions and have revealed promising results (Velivelli et al., 2015).  
32 Dimethyl disulfide, frequently emitted by many bacteria, is another compound used in recent  
33 years in the novel soil fumigant PALADIN® that targets nematodes and soil-borne pathogens.  
34 However, the research concerning the application of other mVOCs in agriculture is still in its

1 infancy. We now live in a time in which the old methods of using chemicals to protect crops  
2 need to be replaced with and, in some cases, complemented by green solutions. The traditional  
3 harmful synthetic fungicides currently used could be replaced with the so far under-explored  
4 and unique mVOCs for which significant proof of plant growth promoting effects and plant  
5 protection ability already exists. In spite of the obvious potential of mVOCs in agriculture, the  
6 field suffers from the common 'translational gap' because of a lack of studies evaluating other  
7 unexpected effects of those bioactive molecules on non-target beneficial soil organisms.

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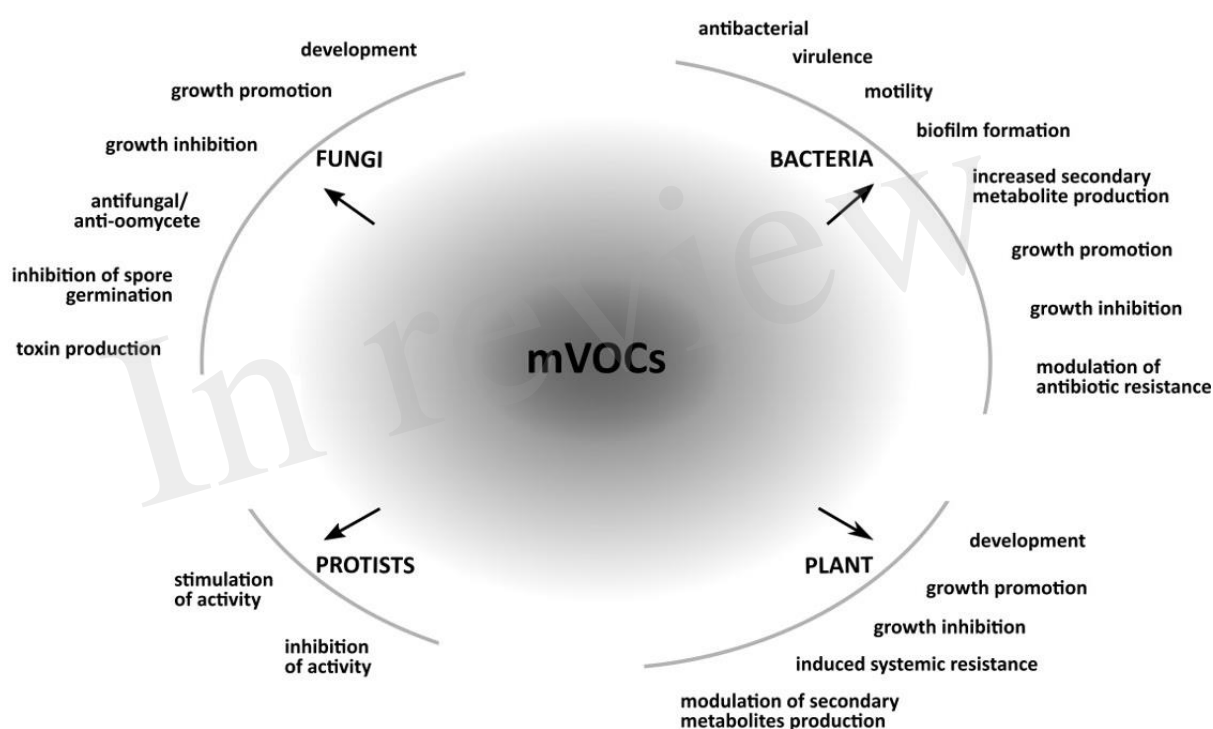
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### 15 **Figure 1. Responses in bacteria, fungi, protists and plants caused by mVOCs**

16 The figure shows examples of responses caused by mVOCs in bacteria, fungi, protists and  
 17 plants.

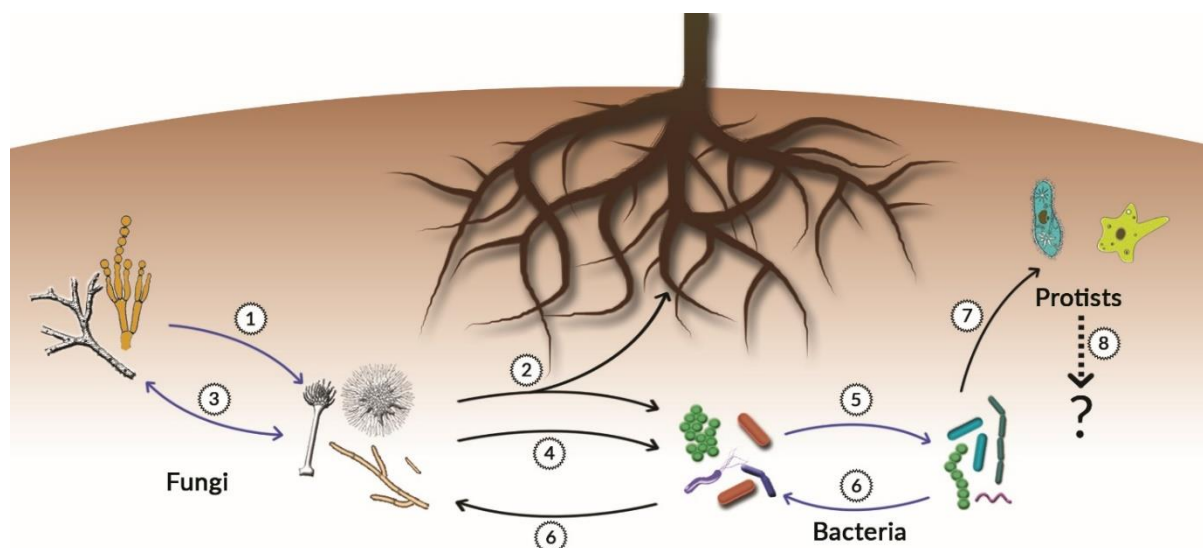
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**Figure 2. Terpenes-mediated belowground interactions**

The figure shows examples of interactions between different organisms in the rhizosphere that are mediated by microbial terpenes. Blue arrows indicate intra-kingdom interactions while black arrows indicate inter-kingdom interactions. The numbers in the figure correspond with the numbers in Table 1.

**Table 1. Examples of terpenes involved in belowground microbial interactions**

Origin	Nr	Compound	Biological activity	Ref.
Fungal	1	$\alpha$ -Humulene	Antimicrobial (antifungal)	(Minerdi et al., 2009)
	2	$\beta$ -Caryophyllene	Antimicrobial (antibacterial) Plant growth promotion	(Huang et al., 2012b) (Minerdi et al., 2011)
	3	Farnesol	Infochemical	(Hornby et al., 2001, Martins et al., 2007)
	4	$\beta$ -Phellandrene	Affects motility	(Schmidt et al., 2017)
Bacterial	5	Albaflavenone	Antimicrobial (antibacterial)	(Gürtler et al., 1994)
	6	$\beta$ -Pinene	Antimicrobial (antifungal, antibacterial)	(Garbeva et al., 2014b, Song et al., 2015b)
	7	Volatile terpenes from <i>Collimonas</i>	Stimulation of protists activity	(Schulz-Bohm et al., 2017)
Protist	8	(E,E)- $\alpha$ -farnesene $\beta$ -barbatene	Unknown	(Chen et al., 2016)

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Figure 1.JPEG

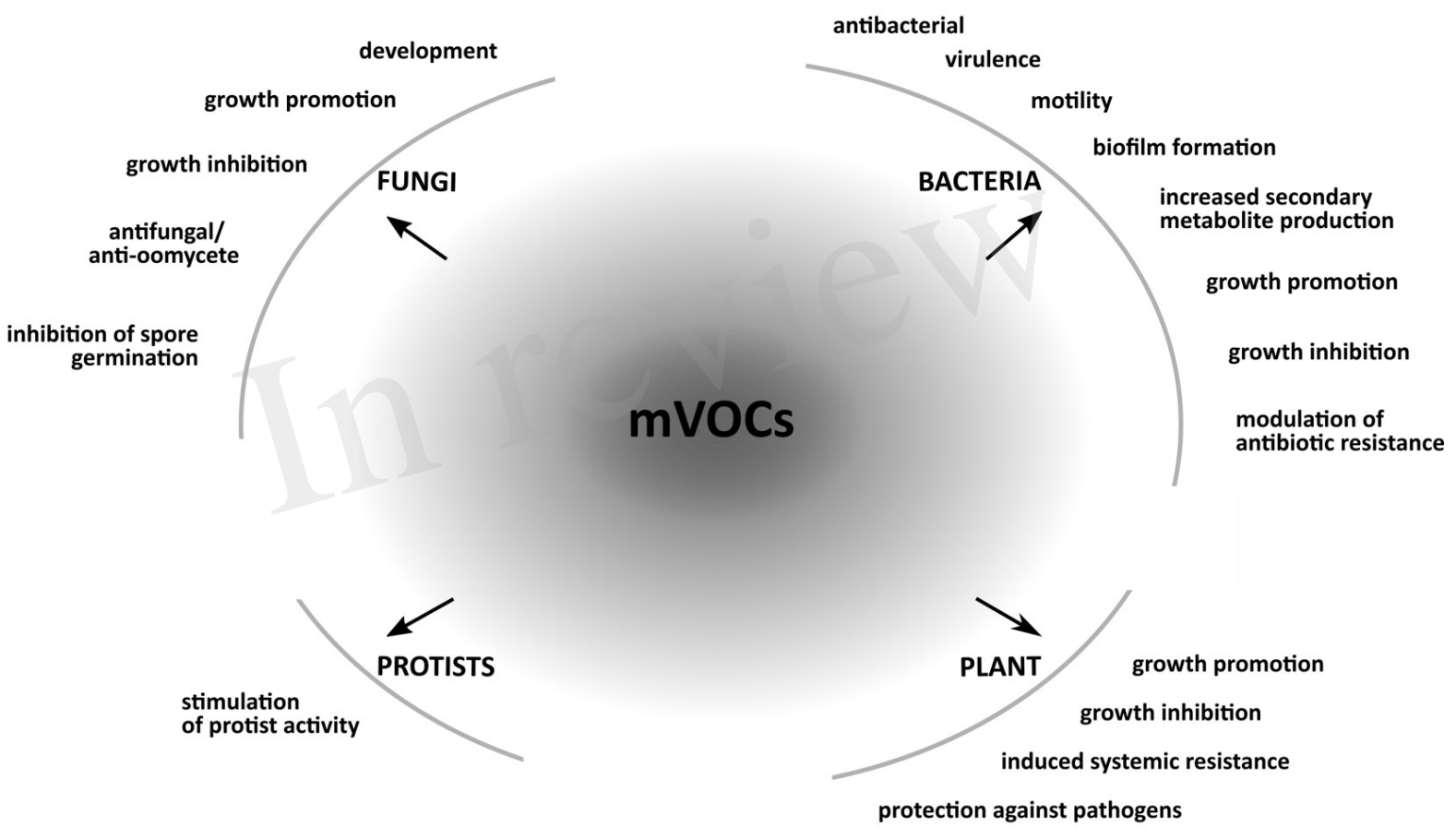


Figure 2.JPEG

