MINIREVIEW

Gene mobility in microbiomes of the mycosphere and mycorrhizosphere – role of plasmids and bacteriophages

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One sentence summary: This review examines the state-of-the-art in research of gene mobility across bacteria in mycospheres and mycorrhospheres, highlighting the roles of plasmids, phages and genomic islands.

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ABSTRACT

Microbial activity in soil, including horizontal gene transfer (HGT), occurs in soil hot spots and at “hot moments”. Given their capacities to explore soil for nutrients, soil fungi (associated or not with plant roots) can act as (1) selectors of myco(rrhizo)sphere-adapted organisms and (2) accelerators of HGT processes across the cell populations that are locally present. This minireview critically examines our current understanding of the drivers of gene mobility in the myco(rrhizo)sphere. We place a special focus on the role of two major groups of gene mobility agents, i.e. plasmids and bacteriophages. With respect to plasmids, there is mounting evidence that broad-host-range (IncP-1β and PromA group) plasmids are prominent drivers of gene mobility across mycosphere inhabitants. A role of IncP-1β plasmids in Fe uptake processes has been revealed. Moreover, a screening of typical mycosphere-inhabiting Paraburkholderia spp. revealed carriage of integrated plasmids, next to prophages, that presumably confer fitness enhancements. In particular, functions involved in biofilm formation and nutrient uptake were thus identified. The potential of the respective gene mobility agents to promote the movement of such genes is critically examined.

Keywords: mycosphere; horizontal gene transfer; plasmids; bacteriophages; evolution

INTRODUCTION

The living soil is crucial for all life on Earth, and this highlights the relevance of soil microbiomes for key life support (C and N cycling) functions (van Elsas et al. 2019). Next to being spatially heterogeneous, soil microbiomes are also remarkably dynamic in time. Thus, rises and falls, in terms of abundance and activity, of the diverse populations that make up these microbiomes are commonly observed (van Elsas et al. 2019). Varying levels and gradients of, e.g., nutrients, minerals, pH and water in the spatially heterogeneous soil matrix are at the basis of the variability in soil microbiomes (Fierer 2017). In fact, many bacteria in soil commonly occur in a state of nutrient deprivation (leading to dormancy), and they will only incidentally become active. Based on this finding, the concept of “hot moments”
Figure 1. Depiction of the mycosphere and mycorrhizosphere. (A) Heterogeneity of soil creates microhabitats; ecologically relevant processes mainly occur in the small volumes of soil denoted hot spots. These are the most active parts of the soil, in which microbes accelerate metabolic rates and evolutionary processes. Additionally, the enhancement of spatial proximity between the local cell populations in soil hot spots may culminate in the formation of biofilms or similar aggregations. Hot spots include mycospheres (i.e. soil surrounding fungal hyphae), mycorrhizospheres (i.e. soil surrounding intertwined plant roots and fungal hyphae) and rhizospheres (i.e. soil surrounding plant roots). (B) Soil hot spots form as a result of hot moments (i.e. high rates of short-lived microbial activity) of microbial populations, related to soil aggregates, redox potential, nutrient contents and biotic binding agents (e.g. fungal hyphae and roots). Once established (inside hot spots), cells increase metabolic processes and interactions. Consequently, accelerated microbial activities spur genetic exchanges and thus evolution occurs. Upon disintegration and disappearance of the hot spots, formerly enclosed microbiomes mend with the gene pool hosts in the surrounding populations.

(Short time spans characterized by enhanced microbial activities) was recently coined by Kuzyakov and Blagodatskaya (2015). Such hot moments come about as a result of the emergence of local conditions favoring metabolism and growth. The end of such hot moments may be gradual, and the established activity hot spots may still maintain certain levels of bacterial activity (Kuzyakov and Blagodatskaya 2015; see Fig. 1). Microbial life in soil has, thus, been characterized as obeying a "feast and famine" type of existence, with the feast parts being equivalent to the relatively short hot moments and the famine parts often being quite extended.

We here define mycospheres, in a 'loose' sense, as the zones in soil that are influenced by fungal hyphae, whereas mycorrhizospheres can be defined as the soil zones in the vicinity of plant roots that are influenced by mycorrhizal fungi (Sarand et al. 1999; Zhang et al. 2014a). Both habitats are typical examples of microsites where activity hot spots and moments (Kuzyakov and Blagodatskaya 2015) occur, in a highly dynamic manner. How do these come about? Regarding the former habitat, saprotrophic fungi explore the soil for nutrients, establishing nutrient-rich microhabitats. With respect to the latter, it has been estimated that around ~90% of all terrestrial plant species are associated with soil fungi to form mycorrhizas (Selosse et al. 2006), mostly either ectomycorrhizae (EM) or arbuscular mycorrhizae (AM). For bacteria dwelling in soil, carbon thus becomes available from the photosynthates that are transferred from the host plants via the roots to the associated mycorrhiza (Pickles et al. 2017).
Interestingly, Hannula et al. (2012) identified not only mycorrhizal but also saprotrophic fungi, such as Rhizopus oryzae and Rhodocybe mundula, as being involved in the acquisition of recently-formed photosynthetic in the rhizosphere of potato (Solanum tuberosum). Their evidence pointed to the often overlooked fact that such fungi are key early responders to photosynthetic carbon when that becomes available in the rhizosphere. Thus, rhizospheres often contain considerable areas that yield possibilities for activity hot spots/hot moments.

It should be noted that both mycospheres and mycorrhizospheres constitute highly dynamic habitats. The microsites in these habitats are characterized by rather limited lifespans, as fungal tissue first builds up (thus offering dynamic sites for colonization by adapted organisms), increasingly releasing compounds that may attract or feed these, and in a later stage senesces and eventually dies out (Brabcová et al. 2016). The whole lifespan of a local mycosphere may range from days to weeks, and that of a mycorrhizosphere up to several months, depending on the conditions and success of colonization (Warmink, Nazir and van Elsas 2009; see also Fig. 1b). In the light of the variability in space and time, the bacterial dwellers of these two habitats most likely require a suite of intricate metabolic, uptake and stress response systems in order to cope with the local dynamism in terms of nutrient availability and conditions (Table 1). As can be seen from the Table, in both habitats conditions will emerge that favor bacteria endowed with capacities to colonize and acquire local nutrients.

Another key issue with respect to the bacteria living in soil is their frequent inability—due to the barriers posed by the solid soil matrix—to reach other organisms, or a suitable substrate, even if these are present only millimeters away. Thus, bacterial cells are thought to be generally confined to the soil microhabitats they dwell in, except when transport agents are available cells are thought to be generally confined to the soil microhabitats they dwell in, except when transport agents are available (Berthold et al. 2016; Yang, Oliveira da Rocha Calixto and van Elsas 2018). In contrast, most soil fungi—in particular saprotrophic ones—explore the soil for available nutrients, in particular carbohydrate compounds that yield sources of carbon and energy. Their network structures enable them to move around substances (e.g. bound carbon) and so carbon sources that are required for growth can be furnished to local bacteria (Haq et al. 2018). Moreover, the hyphal networks can also translocate bacterial cells through soil (Warmink and van Elsas 2009; Berthold et al. 2016).

Overall, the highly dynamic conditions in mycospheres and mycorrhizospheres pose requirements for strong adaptability of bacteria in order for the latter to establish, colonize the local microhabitat, grow and survive. In other words, ‘survival of the fittest’ is likely to drive the evolution of the fungal-associated bacteria. Which agents affect the evolution of these locally adaptive organisms? Clearly, both agents that enable “optimization” (Torsvik and Thingstad 2007) and “flexibilization” (Nielsen and van Elsas 2019) confer eco-evolutionary advantages, often related to capacities required to acquire locally available carbon and energy sources, as well as other essential nutrients, next to those enabling to withstand local stressors (Thomas and Nielsen 2005).

In this minireview, we briefly examine the selective forces exerted on bacteria in mycospheres and mycorrhizospheres, focusing on the agents of the horizontal gene transfer (HGT) processes in these. We also address relevant evidence regarding the prevalence and/or role of mobile genetic elements (MGEs) in the rhizosphere, in the context of the potential role of fungi in this habitat.

EFFECTS OF THE MYCOSPHERE AND MYCORRHIZOSPHERE ON SOIL BACTERIA

Characteristics of the mycosphere and mycorrhizosphere as selective habitats in soil

As argued in the foregoing, mycospheres and mycorrhizospheres constitute matrices that enable local bacteria to interact with the fungal host as well as with each other. Table 1 depicts the key conditions in these microhabitats, as well as the resulting effects on the surrounding microbiota. First, the active hyphae of fungi in soil often supply (release) small carbohydrate compounds that support the growth of associated bacteria. Thus, compounds such as: maltose, trehalose, L-rhamnose, D-sorbitol, D-glucuronic acid, p-hydroxy-phenylactic acid, succinamic acid, glucuronamide, L-alanine amine, L-ornithine, glyceral, mannitol, oxalic acid, acetic acid, fumaric acid and CO₂ have all been shown to be released at fungal surfaces (Frey-Klett et al. 2011; Churchland and Grayston 2014; Haq et al. 2018). Moreover, hyphae of particular fungi are known to produce inhibitory (i.e. bactericidal) or signaling compounds (Deveau et al. 2018), such as may have been the case in the study by Sengelav, Kovvalchuk and Sørensen (2000). On another notice, fungal hyphae may locally affect soil structure and/or change (raise) the soil pH (Nazir et al. 2010), the latter effect potentially establishing a hospitable environment for the local bacteria. Finally, fungal hyphae in soil often establish and maintain a liquid film around them, reflecting their capacity of hydraulic redistribution (Guhri et al. 2015). The mycelial networks in soil can thus serve as concentration/transport modules for soil bacteria (Berthold et al. 2016; Yang, Oliveira da Rocha Calixto and van Elsas 2018). Clearly, the extent to which a mycosphere or mycorrhizosphere is conducive to bacterial colonization and activity depends on the characteristics, in terms of the dynamic growth, physiological adaptation and eventual death of the fungal host (Warmink, Nazir and van Elsas 2009). In many cases, the effects of released carbohydrate compounds, next to the pH effect, will tend to create hospitable environments in which bacteria can thrive. However havoc may be incited if antagonistic compounds prevail. As an example, particular saprotrophic basidiomycetes, i.e. agricid fungal species, produce antimicrobial substances that inhibit the growth of Gram-positive bacteria, making their surroundings inhospitable for these bacteria (de Boer et al. 2005). Moreover, wood rot fungi like Hypholoma fasciculare, Phanerochaete velutina, Phallus impudicus, Resinicium bicolor and Phlebia nigra sometimes also strongly inhibit bacteria (El Ariebi et al. 2016). All of these fungi were found to produce sesquiterpene compounds (e.g. cadinene, longifolene, β-patchoulene and caryophyllene oxide), which are known to have antibacterial properties (El Ariebi et al. 2016).

The frequently observed cases in which bacterial abundances and processes in mycospheres/mycorrhizospheres are promoted as compared to those in the surrounding bulk soil (Warmink and van Elsas 2008; Churchland and Grayston 2014; Zhang et al. 2014a; Kuzyakov and Blagodatskaya 2015) are thought to reflect the emergence of local activity hotspots (characterized by raised nutrient levels, a moderate pH and the presence of life-supporting water levels). These factors trigger increases in local cell densities, which may lead to shifted microbiome structures and enhanced activities (Frey-Klett et al. 2011; Deveau et al. 2018).
Table 1. The effects of mycospheres/mycorrhizospheres on (A) local conditions and (B) bacteriomes, in comparison to bulk soil.

| Parameter | Mycosphere | Mycorrhizosphere |
|-----------|------------|------------------|
| **Main effect** | **Specific remarks** | **Main effect** | **Specific remarks** |
| **A. Effect on local conditions** | | | |
| Main compounds | Enhanced level of carbonaceous compounds and mineralization | Compounds found: maltose, trehalose, L-rhamnose, D-sorbitol, D-glucuronic acid, p-hydroxy-phenylacetic acid, succinic acid, glucuronamide, L-alanine amine, L-ornithine, glycerol, mannitol, oxalic acid, acetic acid, formic acid, citric acid, fumaric acid and CO₂ | Enhanced level of carbonaceous compounds and mineralization | Compounds found: oxalic acid, formic acid, citric acid, malonic acid, gluconic acid, succinic acid, protothetric acid, isocitric acid, malate and CO₂ |
| N | Indirect stimulatory influence | Through bacterial N₂ fixation, N assimilation-NH₄⁺ | Through bacterial N₂ fixation, N assimilation-NH₄⁺ |
| P | Enhanced available P levels | Through PO₄³⁻ mineralization, transport, P solubilization and CO₂ release | Enhanced P levels | Through PO₄³⁻ mechanisms, such as uptake and translocation of P |
| Fe | Modulation of Fe levels | Through Fe mobilization | Through Fe mobilization | Root exudates maintain soil Fe concentration |
| Miscellaneous inorganic compounds | Increased level of mineral weathering | Increased Si and K, and decreased Al level | Increased level of mineral weathering | Increase of Si was observed in Norway spruce (Picea abies [L.] Karst) and oak (Quercus sessiliflora Smith) mycorrhizospheres. Increase of K observed in oak. |
| pH | Modulation of mycosphere pH (often a small raise) | Mycosphere in microcosm soil shows pH increase along with fungal growth, i.e., pH from 4.6 to 5.0 | Mycorrhizosphere pH raised towards neutral. |
| Water | Altering of mycosphere water regime | Fungal hyphae alter soil pore structure and water distribution, also impacting nutrient and cell mobilization. Increase in lyphal networks alleviates salt stress. Mechanism: facilitation enhancing nutrient acquisition. | Mycorrhizospheres shape soil hot spots by altering soil structures, water distribution and soil nutrient mobilization. By increasing the colonization by AM fungi. |
| Salinity | Indirect effect on soil salinity | Increase in lyphal networks alleviates salt stress. Mechanism: facilitation enhancing nutrient acquisition. | Mechanism: enhanced nutrient acquisition. |

| **B. Effect on local bacteriomes** | | | |
| Bacteriome density | Enhancement of local density | Raise of up to 10⁵ CFU per gram soil in Lyophyllum sp. strain Karsten mycosphere. Specifically, 10⁷-10⁹ CFU of Variovorax paradoxus and Paraburkholderia terrae. | Increases | Raise of up to 10⁶ CFU per gram soil in the Carex arenaria mycorrhizosphere |
| Bacteriome structure | Modulation of structure due to mycosphere-specific conditions | Different fungi select different bacterial populations. Laccaria proxima increases the abundance of Pseudomonas and Paraburkholderia spp. Typhlis fusus increases Pseudomonas and Ewingella populations | Selection of particular bacteria | Exudates select specific bacterial populations. For example, legume mycorrhizosphere (Gigaspora margarita) increases Paraburkholderia spp. |
| Cell-to-cell interactions and activity⁴ | Enhancement of intensity of interactions | Genetic interactions are promoted | Enhancement | Increase in bacterial interactions and activities due to the increase of available nutrients |

References:
- Warmink, Nazir and van Elsas 2015
- Frey-Klett et al. 2014
- Guhr et al. 2015
- Rashid et al. 2016
- Holz et al. 2016
- Battini et al. 2017
- Calvaruso et al. 2009
- Guhr et al. 2016
- Carrino-Kyker et al. 2016
- Kuzuyakov and Blagodatskaya 2015
- Rillig, Muller and Lehmann 2017
- Liu et al. 2015
- De Boer et al. 2015
- Brabcová et al. 2016
- Kuzuyakov and Blagodatskaya 2015
Table 1. Continued

| Parameter                     | Mycosphere                                      | Mycorrhizosphere                                      |
|-------------------------------|-------------------------------------------------|-------------------------------------------------------|
|                               | Main effect                                     | Main effect                                           |
| Predation                     | Lowering of predation pressure for fungal-associated cells in biofilm. | Lowering of predation pressure                        |
|                               | Bacterial biofilms around hyphae protect against predators, e.g., protozoa | Decrease of protozoan numbers, possibly due to antimicrobials in the mycorrhizosphere; biofilms protect against protozoa. |
|                               | De Boer et al. 2005                            | Frey-Klett et al. 2014                                |
| Antimicrobial activity and toxicity | Increases                                       | Increases                                              |
|                               | Fungal-associated bacteria produce, e.g., phenazines, chitinases and proteases. | Mycorrhizospheres select *Pseudomonas fluorescens* able to produce, e.g., hydrogen cyanide (HCN), that protects against phytopathogens |
|                               |                                                  | Frey-Klett et al. 2014                                |
|                               | Fungi produce antibiotics that inhibit diverse bacteria. |                                                  |

*Refers to bacterial growth, metabolic processes, nutrient acquisition, responses to environmental factors (this regards the presence of fungal and plant roots), interaction towards other bacteria, to the extent of gene transfer.

Cell-to-cell interactions

That the mycosphere as well as the mycorrhizosphere can establish activity hot spots in soil has been shown by several authors (Nurmiaho-Lassila et al. 1997; Frey-Klett, Garbaye and Tarkka 2007; Warmink and van Elsas 2008; Zhang et al. 2014a).

For instance, in a study performed in our lab, the abundance of *Pseudomonas* populations was found to increase dramatically at the base of the EM fungus *Laccaria proxima* (Warmink and van Elsas 2008), and also particular *Sphingomonas* and *Paraburkholderia* types revealed shifted communities. Figure 1 gives an impression of the stimulatory effects of soil fungi on hypha-associated bacteria. The end result is often an enhanced spatial proximity between the local cell populations, which may culminate in the formation of biofilms or similar aggregations (Berthold et al. 2016; Haq et al. 2016). In theory, such enhanced cell-to-cell proximity, equivalent to raised cell densities (expressed per unit soil volume) will enhance the frequencies of (genetic) interactions between the cells. In more detail, the microcolonies or biofilms at the hyphal surfaces incite, across the constituent cells, varying processes of approximation, colonization, signal exchange and growth. In such processes, the key MGEs that are released, such as phages, plasmids or other cellular DNA, will—due to the mass action effect—have increased chances to be in contact with neighbouring cells. As the fungal surface itself is subjected to phases of hyphal build-up, maturation and senescence, and local conditions may thus continually change, the genetic interactions in the microhabitat are not at all straightforward. Thus, the fate and evolution of locally-adaptive traits is reigned by the system’s conditions, regarding both the gene transfer rates and the selective effects exerted by the system on the recipient cell populations (Zhang et al. 2014a).

Selection of fungal-associated bacteria, in particular *Paraburkholderia* spp.

The notion of soil fungi, including saprotrophs and mycorrhiza, as “selectors” of particular bacterial types is relatively ‘old’ (Warmink, Nazir and van Elsas 2009; Berthold et al. 2016; Pent, Pöldmaa and Bahram 2017). It was recently confirmed by Brabcová et al. (2016), who showed that *Pseudomonas* (next to *Ewingella*) was selectively favored at the EM fungus *Tylopiulus felleus*. Moreover, different fungi may select a suite of similar bacteria (Warmink, Nazir and van Elsas 2009), in addition to diverse ones. As an example, the mycosphere of the EM fungus *L. proxima* was found to primarily select *Paraburkholderia*, *Dyella* and *Pseudomonas* spp., while another EM fungus, *Russula exalbicans*, favored different members of the *Sphingomonadaceae* (Boersma et al. 2009). In other, more recent, work, *Paraburkholderia*, *Bacillus* and *Paenibacillus* spp., next to mycobacteria, were found to be abundant at the EM fungus *Tricholoma matsutake*, while (in addition to *Paraburkholderia*) *Bradyrhizobium* and Acidobacteria spp. were abundant in the mycospheres of *Marasmius* and *Leptotaceae* spp. (Halsey, de Càssia Pereira e Silva and Andreote 2016).

Also, Nguyen and Bruns (2015) recently showed *Paraburkholderia* and *Rhizobium* spp. to be major groups associated with the EM fungus *Pinus murrayica*. *Paraburkholderia*, next to *Dyella*, *Pseudomonas* and *Sphingomonas* spp., were also highly prevalent at *Meliniomyces variabilis*, *Russula* sp. 6 GJ-2013b and *Paenibacillus* involutus (all in *Pinus sylvestris* roots; Marupakula, Mahmood and Finlay 2016). In other recent work, Johnston, Boddy and Weightman (2016) also revealed the prime occurrence of *Paraburkholderia* types as co-migrants with the hyphae of several wood-rot fungi.

From the aforementioned studies, some specific organisms consistently emerged as potentially “universal” fungal-associated bacteria. In other words, these appear to constitute bacterial groups that typically interact with soil fungi. Indeed, in many studies, *Paraburkholderia* spp. (often within the *P. terrae/P. hospita* cluster) have been encountered as prime and consistent colonizers of fungal tissue in soil. In a key laboratory study, Haq et al. (2016) recently provided evidence for the high affinity and chemotactic movement of one such organism, *P. terrae* BS001, toward the hyphae of the soil saprotrophs *Lophyllum* sp. strain Karsten and *Trichoderma asperellum*. Interestingly, an attractive role for several small carbonaceous compounds present at fungal surfaces, e.g. glycerol and oxalic acid, was found (Haq et al. 2016). Here, we will place a focus on mycosphere-derived *Paraburkholderia* as the model/paradigm organism, in order to critically examine the potential of HGT as a mechanism that enhances the adaptability of these organisms to conditions of the mycosphere.
AGENTS OF GENE MOBILITY IN THE MYCOSPHERE AND MYCORRHIZOSPHERE

In a range of studies, the classical HGT mechanisms transformation, transduction and conjugation have been amply shown to be at the basis of the flexibility and adaptability of the genomes of soil bacteria (Nielsen and van Elsas 2019). All three processes require cellular activities, being transformation dependent on particular host genes (including competence factors), transduction on bacteriophage and specific host genes that need to be expressed by the host, and conjugation on systems (often on plasmids, e.g. type-IV secretion systems - T4SS) that allow the construction of cytoplasmic bridges between the donor and recipient cells that interconnect. As the frequencies of all three mechanisms are raised by cell-to-cell proximity as well as cellular activity (Thomas and Nielsen 2005), we hypothesize that they are all potentially promoted in those mycospheres and mycorrhizospheres that confer conditions propitious for cell-to-cell approximations and activities (Zhang et al. 2014a).

A first requirement for host adaptability, irrespective of the types of MGEs, is that particular ephemeral adaptive traits (“accessory” genes) confer fitness advantages under particular ecological conditions, yet are dispensable most of the time) are sufficiently present in the local gene pools. An additional, but not indispensable, asset would be the linkage of such genes to MGEs (Zhang et al. 2014a). Clearly, such linkage facilitates gene transfer, potentially assuring an immediate fitness enhancement of a recipient organism following transfer (if the relevant expression systems are compatible with the recipient organism).

Taking the linkage criterion into our investigations (see Soucy, Huang and Gogarten 2015), we will in the following section examine selected Paraburkholderia strains, in addition to other ones (obtained from mycospheres) for MGEs. We first address the occurrence and role of plasmids as separate genetic entities, and then the respective genomes for inserted MGEs, as the paradigm of adaptive agents in the mycosphere and mycorrhizosphere.

PLASMIDS AS GENE MOBILITY AGENTS IN THE MYCOSPHERE AND MYCORRHIZOSPHERE

Role of plasmids as genetic flexibility agents

Plasmids are here taken as relatively small (< 200 kb) genetic elements that can replicate independently from the chromosomes of their hosts. Most plasmids are either self-transferable, mobilizable and/or retromobilizable and so can move from their host to other bacteria. Regarding the host ranges of plasmids, these vary from narrow (basically confined to a species or genus) to extremely broad (even crossing the Gram boundary). Moreover, some plasmids may occasionally integrate into a recipient chromosome, giving rise to genomic islands. Such integrative events, or the eventual transfer of a transposon from an incoming plasmid to a novel host, are particularly relevant in cases where plasmids have broader transfer than replication ranges (Nielsen and van Elsas 2019). The finding of plasmids (with broad host ranges), such as those of the PromA group, without any discernable accessory genes (termed “cryptic” plasmids; Tauch et al. 2002; Heuer et al. 2004) has given rise to the hypothesis that these may serve the purpose of population-scale gene transfer flexibility. The broad host range character of such plasmids is of prime eco-evolutionary relevance, as it will allow the rapid spread of genetic novelty throughout a major part of a local microbiome. Possibly, bacteria accept and maintain plasmids as tools to, thus, create standing genetic variation within a population, allowing particular constituents of the population to quickly respond to (the adverse) conditions that may occur in the environment. Over time, insertions of relevant genes may thus occur at particular sites. Some scientists will consider this particular plasmid feature to be a token of their “selfishness”. In other words, such plasmids use local (recipient) cell populations to their own benefit, as microbial carrier “donkeys”, and the combination of the plasmid as a gene transfer vehicle, the accessory gene, and the novel host will occasionally constitute an ecologically-viable form under particular conditions.

Clearly, examinations of extant mycosphere / mycorrhizosphere plasmids, along with their accessory gene loads, will yield a legacy of past events of gene gain (and loss) as well as selection. Unfortunately, extensive overviews of this kind have not been made so far.

That transfer of plasmids from a current host to fitter host organisms (yielding novel gene/host combinations) is at the basis of their persistence in bacterial populations was already suggested by Bergstrom, Lipsitch and Levin (2000). According to their theory, plasmids are indeed driven by events of periodic selection of novel combinations of plasmid, accessory gene and host. In the next section, we address to what extent mycospheres and mycorrhizospheres enable plasmids to persist and evolve across the fungal-associated bacterial populations.

Plasmids in the mycosphere and mycorrhizosphere: occurrence and putative roles

Initial studies on the potential role of plasmids in the mycosphere were performed by Sarand et al. (1998, 1999). The roles of the biodegradative TOL plasmid pWW0 (117 kb) (Sarand et al. 1998), and of a similar plasmid (approximately 150 kb) (Sarand et al. 1999) were tested in a Pseudomonas fluorescens host at the EM fungus Suillus bovinus associated with Pinus sylvestris roots. Both plasmids had capacities to degrade m-toluate and m-xylene (next to benzene, ethylbenzene and o-, m- and p-xylene), and so they might enhance host fitness in systems containing m-toluate. The data revealed that, in such systems containing the plasmid-endowed P. fluorescens with S. bovinus, the m-toluate levels decreased by 29–50% (day 1) to 58–79% (day 4), as compared to the control (fungus alone) (Sarand et al. 1999). Thus, maintaining the plasmid was beneficial to the bacterial host and fungus alike. In a recent study in agar microcosms set up so as to measure the effect of bacterial motility (high, medium and low) on plasmid pWW0 transfer between Pseudomonas putida strains, pronounced emergence of transconjugants in microcosms with (Pythium ultimum) mycelium was found (Berthold et al. 2016). This suggested the existence of preferential dispersal pathways and enhancement of HGT in the presence of fungal mycelium.

Importance of broad-host-range character of plasmids

Broad-host-range plasmids constitute key connectors of genomes across suites of separate bacterial lineages (Tauch et al. 2002; Sen et al. 2011; Shintani et al. 2014; Klümper et al. 2015). That a scenario of rapid plasmid spread is actually likely to occur under transfer-conducive conditions, was demonstrated in a study by Klümper et al. (2015). In their study, the authors showed that with P. putida KT2440, Escherichia coli MG1655 and Kluyvera sp. as donor strains, the transfer of IncP-1 and PromA-type plasmids to diverse hosts from agricultural soils present in soil microorganisms, when the latter are exposed to
“crowded” and nutrient-rich conditions, is a realistic scenario. In their experiment on agar plates, transfers were found to a large suite of diverse bacterial phyla, both Gram-negative and -positive, i.e., (α–) Proteobacteria, Acidobacteria. Actinobacteria, Bacteroidetes, Firmicutes, Fusobacter, Gemmatimonadetes, Planctomycetes, spirochaetes, candidate division TM7 and Verrucomicrobia. Arguably, these plasmids can also interconnect bacterial with Eukaryote genomes (Zhang et al. 2014a). Given the propensity of mycospheres and mycorrhizospheres to enhance local cell densities of diverse bacterial types, we posit that broad-host-range plasmids are key gene mobility agents in these natural mycosphere bacteriomes. Several recent studies have indeed implicated the highly promiscuous IncP-1β as well as PromA group plasmids as potentially important agents of HGT in mycospheres. In the following, we place emphasis on each of these two plasmid groups.

**IncP-1β plasmids**

Recent evidence has identified plasmids of the IncP-1β group as major MGEs in rhizosphere bacteriomes (Bziuk et al. 2016). Thus, using molecular quantification of the IncP-1β marker gene korB, enrichments of such plasmids were found in the rhizospheres of lettuce and tomato. Furthermore, triparental matings, using an IncQ plasmid as the tracer, captured high levels of IncP-1β plasmids from these habitats (Nour et al. 2017). Whereas this finding can be taken as evidence for an IncP-1β selective effect of plant roots, a possible role for fungi of the rhizosphere (and so mycospheres and/or mycorrhizospheres) cannot be excluded. Indeed, as described in Zhang et al. (2015), IncP-1β plasmids were typically present in several Variorax paradoxus isolates obtained from the mycospheres of L. proxima. In other work, they were also encountered in the microbiomes of various other mycospheres, such as those of Russula, Inocybe and Ampulloclitocybe spp. (Zhang et al. 2014b). Selected plasmids, i.e. pHB44 and PBS64, were found to stimulate the production of biofilms in their V. paradoxus BS64 hosts as compared to plasmid-less variants (Zhang et al. 2015). Moreover, a presumed iron uptake system present on the IncP-1β plasmid pHB44 was found to assist the fitness of the host organism, P. terrae strain BS001, in the mycosphere of Lyophyllum sp. strain Karsten, but only under low-Fe conditions (Zhang, Yang and van Elsas 2016). In contrast, in soil with sufficient Fe, plasmid carriage resulted in a lowering of host fitness, revealing a predicted effect of an enhanced energy or other burden on the host cell (Fig. 2). Finally, the in-mycosphere transfer of plasmid pHB44 from P. terrae BS001 to the co-inhabitant V. paradoxus BS64 was also strongly enhanced under the low-Fe conditions as compared to higher Fe levels. Overall, these data yield evidence for the tenet that, indeed, plasmids of the IncP-1β type constitute agents of genetic flexibility, across taxonomic borders that enable mycosphere-dwelling bacteria to quickly take profit of key plasmid-borne functions that are instantaneously required to survive.

**PromA type plasmids**

Whereas the first (cryptic) PromA group plasmid, pIP02, was obtained from a rhizosphere soil (van Elsas et al. 1998; Tauch et al. 2002), another highly homologous PromA group plasmid, pTer331, was found in a mycosphere-isolated Collimonas fungivorans strain (Mela et al. 2008). Furthermore, the early work on the prototype PromA plasmid pIP02 (Tauch et al. 2002) pointed to a prevalence of PromA target sequences in rhizospheres of diverse plant species. As argued in the foregoing, a role for fungal drivers in these rhizospheres cannot be excluded. On the basis of the repA gene, Zhang et al. (2014b) found PromA sequences to be highly prevalent at the EM fungi Russula, Inocybe and Ampulloclitocybe spp., as well as directly in the forest floor soil. Moreover, PromA type plasmids were also found to transfer frequently into hosts used in triparental exogenous isolation experiments (Zhang et al. 2014b). However, these mycosphere plasmids have been given the epithet cryptic, as finding clear functional genes on them has been difficult. Interestingly, the para locus on PromA plasmids was identified as being conducive to insertions of functional genes (Schneiker et al. 2001; Dias et al. 2018), and so a polymerase chain reaction-based detection system of such insertions was designed (Dias et al. 2018). Indeed, in a recent study three typical insertions into the para locus of PromA group plasmids were encountered in DNA derived from the mycospheres of different fungi. All of these insertions were different from those typically found in two bulk soils (which included genes for an esterase and an endo-1,4-beta-xylanase), one resembling a gene for a putative dehydrogenase (from an uncultured bacterium) (Dias et al. 2018). In future work, it will be a challenge to determine the extent to which PromA type plasmids are indeed important carriers, as well as genetic flexibility agents, of relevant mycosphere-adaptive traits across members of mycosphere microbiomes.

**ANALYSIS OF THE GENOMES OF MYCOSPHERE-COMPETENT Paraburkholderia spp. FOR GENE MOBILITY AGENTS**

RGP – genomic islands with features of integrated MGEs

Regions of genomic plasticity (RGPs) are here defined as genomic regions that are acquired through HGT, often containing genes that encode behaviour/fitness-modulating functions, including pathogenicity and symbiosis (Dobrindt et al. 2004). Bacteria that have access to the horizontal gene pool often contain large numbers of RGPs, as well as other mobile regions (Dobrindt et al. 2004). In an initial screening, Haq et al. (2014) reported a total of 16% of the P. terrae BS001 genome to be inside RGPs (detected by the Microscope platform, about 79 regions). In this organism, RG76 (70.4 kb), RG24 (31.45 kb), RG61 (51.15 kb) and RG62 (53.22 kb) were reported to carry typical plasmid-related genes, suggesting their plasmid-derived ancestry. Most conspicuously, the 70.4-kb RG76 contained an entire T4SS with a full set of archetypical functional genes, thus revealing clear features of a plasmid with full transfer potential. RG76 further contained genes for a DNA replication protein (repB), for stable maintenance and partitioning (korC, kkLA, para, parB, parD, parE and parF) and for mobilization/transfer (mobB and mobC), next to multiple genes for transposases (e.g. tnpA, tnpB and insH) and integrases. Further evidence for the contention that the RG76 region was plasmid-derived was provided by the finding that its T4SS was highly syntenous to that of the (functional) megaplasmid pIPHY02 of Paraburkholderia phymatum STM815. Six additional RGPs found in the strain BS001 genome (RG47 - 46.2 kb; RG20 - 48.1 kb; RG25 - 46.4 kb; RG38 - 53.8 kb, RG49 - 96.2 kb and RG53 - 102.9 kb) carried genes for transposases and integrases, also suggesting past events of gene mobility. Moreover, RG53 carried genes for a siderophore-binding protein, an invading plasmid antigen J (ipaJ) and a type-III effector protein (hopJ); this points to functions relevant for host fitness in the soil/mycosphere.
Figure 2. Roles of two groups of broad-host-range plasmids in the mycosphere. (A) The IncP-1β plasmid pHB44 enhances *Paraburkholderia terrae* BS001 fitness under low-iron conditions in the mycosphere and also transfers to *Variovorax paradoxus* HB44 under these conditions (Zhang et al. 2014a, 2014b, 2015). (B) The broad-host-range PromA group plasmids act as agents of highly promiscuous and frequent HGT, including insertions of accessory genes into the parA locus (van Elsas et al. 1998; Zhang et al. 2015; Dias et al. 2018).

Additional analyses of the genomes of the mycospheric *P. terrae* strains BS007, BS110 and BS437 (Pratama et al. 2017), along with the *Paraburkholderia* type strains *P. terrae* DSM 17804T, *P. hospita* DSM 17164T and *P. caribensis* DSM 13236T, showed the presence of T4SSs in all genomes (incomplete in BS007) but that of *P. caribensis* DSM 13236T. In *P. terrae* DSM 17804T and *P. hospita* DSM 17164T, the T4SSs were located inside RGP97 (157 kb) and RGP99 (520 kb), respectively. In all cases, complete sets of T4SS genes, i.e. virB1 to virB11, next to VirD4, were found to be flanked by typical (integrative/conjugative) plasmid-related genes (see Table 2). Furthermore, other conserved plasmidic regions (i.e. plasmid-related genes, such as those encoding the antitoxins ParE1 and ParB) were also found, next to diverse insertions (i.e. accessory genes) and deletions (Fig. 3). Some of these accessory genes (Table 2) potentially encode traits that may ephemeraly enhance fitness of the host in soil. For example, genes for the biosynthesis of glutamate, tyrosine, threonine and methionine, of antibiotics (phenazine), for degradative functions (of ethylbenzene and chlorocatechol), for chitinase, next to genes for additional metabolic steps (of butanoate, amino acids, nitrogen and methane) were encountered. Furthermore, genes for antibiotic resistance, stress responses and an antitoxin protein were also found (see Table 2). These analyses showed that: (i) RGPs are very common across the examined *Paraburkholderia* genomes, which is consistent with the versatile and plastic lifestyles of these organisms, and (ii) presumed gene mobility through the T4SSs was conserved among these *Paraburkholderia* spp., with the notion that the identified regions were variable across the strains (see Table 2). Thus, the presumed integrated plasmids were unique per strain/species, as also found by Mannaa, Park and Seo (2019). A recent study of the genomes of *P. phymatum*, *P. caribensis* and *P. phenoliruptrix*, for which a relationship with plants was cogitated, showed the presence of RGPs endowed with genes for plant-symbiotic and -growth-promoting roles (e.g. nod, nif, ACC deaminase) (Mannaa, Park and Seo 2019). Together, these data indicate that, along evolutionary time, the genomes of the mycosphere-dwelling *Paraburkholderia* species that were examined, in addition to those of the type strains, have been amply subjected to diverse invasions by plasmid-like elements of diverse sizes, i.e. 60-kb up to 500-kb. It is likely that
| Strain [Acc. No] | Source | RGP | Size (bp) | # genes | Typical genes [predicted functions] | MGE | Reference |
|-----------------|--------|-----|-----------|---------|-----------------------------------|-----|-----------|
| P. terrae BS001 [AKAU00000000] | Mycosphere of 1lyophyllum sp. strain Karsten | RGP76 | 70 422 | 89 | Presumed relevant accessory genes: | 6 transposases, 9 integrases, ParA, ParB, ParD, ParE, korC, repB, MobB, MobC, KicA. | Haq et al. 2014 |
| P. terrae BS110 [NFVD00000000] | Mycosphere of Laccaria proxima | RGP45 | 116 299 | 159 | Presumed relevant accessory genes: | 6 transposases, 8 integrases (2 phage integrases), toxin ParE1, antitoxin ParD, ParB, Rep8 | Pratama et al. 2017 |
| P. terrae BS437 [NFVC00000000] | Mycosphere of 1lyophyllum sp. strain Karsten | RGP25 | 151 786 | 184 | Presumed relevant accessory genes: | 8 transposases, plasmid pBRH01 gene of unknown function | Pratama et al. 2017 |
| | | | | | Chitin deacetylase (Hydrolase), | | |
| | | | | Xylanase (xylan degradation), | | |
| | | | | Aminoglycoside phosphotransferase (antibiotic resistance), | | |
| | | | | Nitroreductase | | |
| | | | | Oxygen-insensitive NADPH nitroreductase | | |
| | | | | Other genes: | | |
| | | | | 3-methyl-2-oxobutanoate hydroxymethyltransferase (pantothenate and CoA biosynthesis), | | |
| | | | | Phosphate acetyltransferase (taurine and hypotaurine metabolism), pyruvate metabolism and propanoate metabolism), | | |
| | | | | Stress protein UspA (universal stress protein), | | |
| | | | | Acetyl-CoA synthetase, | | |
| | | | | Alcohol dehydrogenase, | | |
| | | | | Putative phosphoketolase (carbohydrate metabolism), | | |
| | | | | Amidohydrolase 2 (hydralase), | | |
| | | | | 4-hydroxythreonine-4-phosphate dehydrogenase (vitamin B6 metabolism) | | |
Table 2. Continued

| Strain [Acc. No]a | Source | RGPb | Size (bp) | # genes | Typical genes [predicted functions] | MGEc genes | Reference |
|------------------|--------|------|-----------|---------|------------------------------------|------------|----------|
| P. terrae DSM 17804T [CP026111-CP026114] | Forest soil | RGP97 | 157,478 | 174 | Presumed relevant accessory genes:  
  - Phthalate 4,5-dioxygenase oxygenase subunit (Xenobiotic metabolism),  
  - Rhodocoxin reductase (degradation of the thiocarbamate herbicide),  
  - (5)-1-Phenylethanol dehydrogenase (aromatic degradation),  
  - Ferredoxin-NAD(P)(H) reductase fdr (aromatic carbazole metabolism),  
  - Ferredoxin Cark (carbazole metabolism) | 3 transposases, Integrase family protein, ParB family protein | Yang et al. 2006 |
| P. hospita DSM 1764T [CP026105-CP026110] | Soil | RGP99 | 419,946 | 689 | Presumed relevant accessory genes:  
  - Protein YdeP (possible acid resistance),  
  - Manganese catalase (antioxidant enzyme),  
  - S-(hydroxymethyl)glutathione dehydrogenase (methane metabolism),  
  - Zinc-dependent sulfurtransferase sufUSCBD glyoxalase/bleomycin resistance protein/dioxygenase,  
  - Phasin family protein (carbon and energy reservation),  
  - Carboxylic anhydride (interconvert carbon dioxide and bicarbonate),  
  - Amino acid (glutamine synthetase, glutamate biosynthesis, cysteine metabolism),  
  - Exonuclease,  
  - Oxidase,  
  - D-erythronate dehydrogenase (carbohydrate metabolic oxireductase activity),  | ParB, 5 integrases, 20 transposases, 2 IS1 element protein InsF, 3 IS2 insertion element transposase InsAB, Intron glide, one plasmid stabilization system, two KpLE2 phage-like element, toxin ParE1, two antitoxin ParD | Goris et al. 2002 |

aNCBI accession number; bThe genomes are hosted at MicroScope platform (https://www.genoscope.cns.fr/agc/microscope/home/) (last accessed 2019), where RGPs analyses were performed; cMGE: mobile genetic element. Data fully published in Pratama et al. (in prep).

different events of gene loss have also occurred. The resulting inserts were found to be mosaic, and diverse, in structure. It will be a challenge to determine what types of selective pressures have been involved in favoring the emergence and persistence of such elements in the respective bacterial genomes.

Prophages and phages

Our current knowledge with respect to prophage prevalence in mycosphere and soil dwellers is still scarce. Not only do we largely ignore the extent of phage prevalence, but also do we understand little about their roles and functions. To what extent are prophages anchored in host genomes able to exert tasks like population control or HGT? With respect to soil bacteria, a classical study established a role of phages as transfer vehicles of small tetracycline resistance plasmids across soil-derived Bacillus strains (van Elsas and Pereira 1987). Moreover, other studies on such phages (van Elsas and Penido 1982; Quesada, Soriano and Espinosa-Urgel 2012) have offered snapshots of potentially vast soil phage communities.

With respect to the model mycosphere bacterium Paraburkholderia, recent compelling evidence has identified a potential role of their (pro)phages. The initial finding of a typical phage gene, i.e. an integrase, in the genome of P. terrae BS001 (Haq et al. 2014) led to the discovery of ‘remnant’ prophage regions, most of which had been subjected to deletion bias (i.e. the loss of viral reproduction and structural genes). More recent work showed that prophages indeed occur across the genomes of diverse mycosphere-derived P. terrae and P. hospita strains (Pratama and van Elsas 2017; Pratama,
Figure 3. Analysis of (30–40) genes flanking the T4SS (type-IV secretion system) across the genomes of three mycosphere-associated Paraburkholderia strains, next to two “type strains”. The commonalities of large regions versus the mosaic structures of other regions are shown. The percentages of similarity (100–27%) are shown in the grey-shared bars.

Chaib De Mares and van Elsas (2018). In detail, such prophages were omnipresent and occupied up to 4% of genome space, specifically, in the mycosphere (Trichoderma harzianum)-derived Paraburkholderia sp. MF2-27. Most regions identified as prophage, however, did not contain all genes necessary for completion of a lytic cycle (Pratama, Chaib De Mares and van Elsas 2018). Therefore, notwithstanding technical constraints, we here posit that, following the events of prophage insertion, multiple gene losses occurred in these organisms, in the context of the highly-dynamic soil and mycosphere sites inhabited by them.

A number of questions arises here. What is the cost of carrying these prophages? To what extent do they offer immediate benefit to the host? What are those benefits? What is the evolutionary consequence of carrying these prophages? What can we say, for instance, about the Paraburkholderia sp. MF2-27 prophages found in multiple copies in the genome (Pratama, Chaib De Mares and van Elsas 2018), with respect to its habitat (the Trichoderma harzianum mycosphere), in comparison to other mycospheres? Theoretically, lysogenic phages may benefit their host through: (i) “engineering” their host genome, (ii) changing host physiology by new genes, (iii) facilitation of gene mobilization through transduction and (iv) controlling competitor bacteria following release of phage (Howard-Varona et al. 2017). Additionally, prophages promote diversification in bacterial hosts, via integration of prophage encoded-genes (Ohnishi, Kurokawa and Hayashi 2001). Undoubtedly, carrying prophages imposes benefit-harm trade-offs to the bacterial hosts. Concerning the latter, energy costs due to transcription of additional genes and lowering of smoothness of biochemical processes due to prophage integration may occur (Casjens 2003; Obeng, Pratama and van Elsas 2016). With respect to the former, carrying prophages may allow hosts to cope with adverse environments (Wang et al. 2010), such as the pressure posed by similar lytic phages from the outside. Here, the multiple prophages infesting Paraburkholderia sp. MF2-27 are interesting, raising questions about the true benefits of carrying such prophages versus their potential harm.

In mycosphere and soil habitats, the phage-bacterium interaction dynamics, and hence phage activity and lysogenic lifestyle, may be related to phage:bacterium ratio’s (PBRs; in other studies denoted as VBRs [virus:bacterium ratio’s]). These PBRs, defined as the relative abundance of phage particles compared to that of bacterial cells, have been proposed as proxies of viral significance in a given ecosystem (Williamson, Radosevich and Wommack 2005). Although no data are as yet available for mycospheres/mycorrhizospheres, rhizospheres have revealed values (e.g. 0.27) below those of bulk soils (e.g. 4.68 [Swanson et al. 2009] and 8.20 in hyper-arid desert soils [Zablocki, Adriaenssens and Cowan 2015]). Possibly, the phage lysis-lysogeny decision is connected to local host cell densities (see Silpe and Bassler 2019), and hence PBR values might be reduced in mycospheres / mycorrhizospheres. Interestingly, in mycosphere-inhabiting bacteria other than Paraburkholderia, considerable prophage regions, up to 13% of the genomes were encountered (Pratama and van Elsas 2017). Hence, we posit that lysogeny is common across mycosphere and soil dwellers; furthermore, the extent to which the phages affect their hosts in the mycosphere depends on local events of activation of phage cycle and other genes, as discussed in the following.

Prophages of P. terrae and their presumed roles: Φ437 as the paradigm

To the best of our knowledge, prophage Φ437 represents the first phage that was detected in a mycosphere-inhabiting Paraburkholderia species (Pratama and van Elsas 2017). The entire 54-kb prophage (containing the minimal set of phage genes) found in the P. terrae strain BS437 genome, denoted Φ437, was released upon mitomycin C induction (Pratama and van Elsas 2017). It was later found to be also spontaneously released by
the host cells, and hence questions were raised as to the occurrence of this phenomenon in the mycosphere. Here, we propose two scenarios by which phage /Phi1437 may affect its host in the mycosphere (see Fig. 4). First, prophage-carried “moron” (more-on DNA) gene encoded proteins may confer fitness advantages to host cells in the form of, for instance, auxiliary metabolic genes (Breitbart et al. 2018). Interestingly, one such moron gene was found to be present in /Phi1437; it was predicted to encode a gene similar to the amrZ gene (PSI-BLAST-P 55% homology, 79% coverage; Phyre2 analyses 55% homology, 80% coverage, 100% confidence value). This gene is linked to a transcription factor (Pratama and van Elsas 2017) in Pseudomonas aeruginosa, where it regulates transcription/repression, primarily of algD2, the first gene of the alginate biosynthesis operon (involved in bacterial biofilm formation). Thus, AmrZ is thought to be involved in enhanced biofilm formation. Moreover, it also controls genes involved in type IV pili (T4P) and twitching motility, and it represses flgE, an activator of flagellar expression (Tart, Blanks and Wozniak 2006). Interestingly, the alginate biosynthetic system was also found to be present in the BS437 genome (Pratama et al. 2017). It is worth to note that, among the Paraburkholderia genomes tested, phage /Phi1437 was only found in P. terrae BS437, suggesting that it offers a unique accessory mechanism that enhances host biofilm formation. In the second scenario, host biofilm formation may be modulated by spontaneously released phage /Phi1437, which may cause local holes (and so channels) in the biofilm (Pratama and van Elsas 2017). This is in line with other studies that have pinpointed prophage-mediated lysis as the cause of the release of crucial biofilm-promoting factors, i.e. extrachromosomal DNA and exopolysaccharides (Gödeke et al. 2011; Seoret al. 2015). We hypothesize that phage /Phi1437 may have such a stimulatory role in P. terrae BS437 as well.

In a screening for phage /Phi1437 sequences in microbiomes derived from the mycospheres of Russula ochroleuca and R. emetica, next to corresponding bulk soil, such sequences were consistently found only in bacteriaeomes derived from the two mycospheres (Pratama and van Elsas 2018). This finding suggested the presence of susceptible P. terrae cells and a key role of /Phi1437 in the respective mycospheres, but not in the bulk soil. Interestingly, a viral contig representing another phage, denoted VC14, from populations derived from non-mycosphere soil and predicted to infect Paraburkholderia spp., was found to contain a gene encoding a phasin-like protein. PSI-BLASTP analysis of the (translated) gene showed 94% homology and 94% coverage to a phasin element of Burkholderia sp. GAS332 as well as P. xenovorans (59% homology and 92% coverage). In Pseudomonas oleovorans GP01, phasin (phaC1) was predicted to advance host growth by encoding medium-chain-length polyhydroxyalkanoate synthase under excess carbon and limiting nitrogen conditions (Priet et al. 1999). These conditions may be equivalent to those typically found in region in forest floors. This led to the hypothesis that – indeed – genes for such accessory functions might be prevalent in the (phage) horizontal gene pool. In addition, other novel auxiliary metabolic genes/morons typically found in the phage contigs from the mycosphere included genes for glyoxalase/dioxygenase superfamily proteins (involved in catabolism of aromatics) (Pratama and van Elsas 2018). Moreover, novel auxiliary metabolic genes/morons related to soil carbon cycling were also found, e.g. genes for glycoside hydrolases, next to those for chitinase, peptidoglycan lyase and polygalacturonidase (Pratama and van Elsas 2018). Glycoside hydrolases are involved in degradation of polymers like cellulose, hemicelulose, chitin and/or amylase. Chitin, for example, is a component of the fungal cell wall, and so the presence of chitinase genes indicated the involvement of phages as carriers of genes for mycosphere degradation processes. These initial insights into mycosphere viromes open up a ‘pandora box’ of unexplored ‘flexible functions’ that play roles in these soil hotspots.

OUTLOOK

As argued in a previous review (Zhang et al. 2014a), the mycosphere (and mycorrhizosphere) established in soil by, respectively, saprotrophic and mycorrhizal fungi, act to provide both hot spots and hot moments for the activity of soil bacteria. The occurrence of such sites and time spans of enhanced cell density and activity at soil fungi is clearly propitious to HGT processes across local bacteria, much like was previously demonstrated for the rhizosphere (van Elsas et al. 1986; Deveau et al. 2018). However, a note of caution should be placed here, as mycospheres and mycorrhospheres do differ in their degree of hospitality for soil bacteria. This may range from being extremely hospitable by, for instance, the provision of colonizable surfaces, nutrients and a hospitable pH (allowing large cell densities to build up), to extremely inhospitable, for instance by secretion of antibiotic compounds or lowering the pH to inhospitable levels, e.g. below 5.0. Here, we selected Paraburkholderia of the terrae/hospita species cluster as the model or reference mycosphere organisms for analysis of the presence and relevance of MGEs as well as RGPs. Clearly, although there is overwhelming evidence that these organisms are mycosphere-competent, we basically ignore other parts of their lifestyle and so the data discussed herein need to be regarded with a note of caution. Moreover, other mycosphere-competent organisms may have evolved by parallel routes, and so the analyses may be organismal type-confined.

A hospitable mycosphere or mycorrhizosphere should be viewed as an arena in which cells are in continuous search for available substrate. Competition for this or other substrate is likely to be fierce, and cells that are able to grow and divide will do so in close connection or proximity to other cells, which promotes occasional cell-to-cell interactions. Such active and spatially proximal cells are likely to become involved in HGT processes. Hence, in this context, one may envision a role for plasmids and phages in this competitive life modus. The effects of such HGT processes are exacerbated as the products of HGT constitute potentially strongly-selected evolutionary/adaptive assets that enable mycosphere inhabitants to be ecologically successful. In this scenario, even if transfer rates are local and low-frequency, any adaptive trait (which may be of only ephemeral value), can be quickly shared across local hosts. However, as soon as these elements have turned obsolete, for instance due to shifted conditions, they may be deleted, e.g. by the processes underlying deletion bias or plasmid loss. This fast removal of transiently-required genetic information may thus reduce any metabolic or other burden posed on the host cell populations.

As argued in the foregoing, plasmids and phages may also play roles as ‘physical’ agents that modulate biofilm quality and so may promote biofilm health and strength. Given the relevance of bacterial biofilms in the mycosphere for bacterial survival, we postulate this physical effect to be a second important ‘population-level’ driver of fitness in the mycosphere. As a paucity of knowledge exists on this interesting topic, key research into it is encouraged.

Finally, in the age of massive gathering of microbiome data by soil -omics approaches, the mycosphere has been
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Figure 4. Proposed role of bacteriophages in the mycosphere and/or the mycorrhizosphere. (A) Mycosphere-inhabiting bacteria, e.g., *Paraburkholderia terrae* BS437 carrying prophage φ437, potentially modulate biofilm formation through the prophage-born transcriptional regulator encoded by the *amrZ*-like gene, which regulates biofilm formation via the alginate system and the type four pilus. (B) Spontaneous release of φ437 particles will lyse local host cells in the biofilm. In such local spots, exo-polysaccharides and eDNA (extra-chromosomal DNA) may contribute to the structure of the biofilm.

greatly ignored. It is exactly at the level of the mycosphere/mycorrhizosphere soil hot spots, and at hot moments in these, that the greatest value of these technologies can be found. Future work on mycosphere omics (“mycospheromics”) requires an a priori consideration of research aims and objectives, which ideally lie in fostering our understanding of the local interactions such as those addressed in this review.

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REFERENCES

Battini F, Grønlund M, Agnolucci M et al. Facilitation of phosphorus uptake in maize plants by mycorrhizosphere bacteria. *Sci Rep* 2017;7:1–11.

Bergstrom CT, Lipsitch M, Levin BR. Natural selection, infectious transfer and the existence conditions for bacterial plasmids. *Genetics* 2000;155:1505–19.

Berthold T, Centler F, Hübbschmann T et al. Mycelia as a focal point for horizontal gene transfer among soil bacteria. *Sci Rep* 2016;6:1–8.

Boersma FGH, Otten R, Warmink JA et al. Selection of *Variovorax paradoxus*-like bacteria in the mycosphere and the role of fungal-released compounds. *Soil Biol Biochem* 2010;42:2137–45.

Boersma FGH, Warmink JA, Andreote FA et al. Selection of *Sphingomonadaceae* at the base of *Laccaria proxima* and *Russula exalbicans* fruiting bodies. *Appl Environ Microbiol* 2009;75:1797–89.

Brabcová V, Nováková M, Davidová A et al. Dead fungal mycelium in forest soil represents a decomposition hotspot and a habitat for a specific microbial community. *New Phytol* 2016;210:1369–81.

Breitbart M, Bonnain C, Malki K et al. Phage puppet masters of the marine microbial realm. *Nat Microbiol* 2018;3:754–766.

Bziuk N, Fornefeld E, Nour E et al. Enrichment of IncP-1 plasmid carrying bacte- ria in the rhizosphere of lettuce and tomato – is there a fitness advantage? 9th Young Scientist Meeting. 2016. 9th-11th November in Quedlinburg - Abstracts - (Berichte aus dem Julius Kühn-Institut 186), Quedlinburg, 43.
Calvaruso C, Mareschal L, Turpault M-P et al. Rapid clay weathering in the rhizosphere of Norway spruce and oak in an acid forest ecosystem. Soil Sci Soc Am J 2009;73:331.

Carrino-Kyker SR, Kluber LA, Petersen SM et al. Mycorrhizal fungal communities respond to experimental elevation of soil pH and P availability in temperate hardwood forests. FEMS Microbiol Ecol 2016;92:1–19.

Cajens S. Prophages and bacterial genomics: what have we learned so far? Mol Microbiol 2003;49:277–300.

Churchland C, Grayston SJ. Specificity of plant-microbe interactions: ecology, mechanisms and challenges. FEMS Microbiol Rev 2018;42:335–52.

Dias ACF, Cotta SR, Andreote FD et al. Antifungal rhizosphere bacteria can increase as response to the presence of saprotrophic fungi. PLoS One 2015;10:1–15.

De Boer W, Holman LB, Summerbell RC et al. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev 2005;29:795–811.

De Boer W, Hundscheid MPJ, Gunnewiek PJAK et al. Studying the response of bacterial communities to elevated soil pH: a plethora of outstanding interactive capabilities unveiled. Genome Biol Ecol 2014;6:1652–68.

Haq IU, Rocha Calixto RO da, Yang P et al. Chemotaxis and adherence to fungal surfaces are key components of the behavioral response of Burkholderia terrae BS001 to two selected soil fungi. FEMS Microbiol Ecol 2016;92:1–14.

Haq IU, Zwahlen RD, Yang P et al. The response of Paraburkholderia terrae strains to two soil fungi and the potential role of oxalate. Front Microbiol 2018;9:989.
Ohnishi M, Kurokawa K, Hayashi T. Diversification of Escherichia coli genomes: are bacteriophages the major contributors? Trends Microbiol 2001;9:481–5.

Pent M, Pöldmaa K, Bahram M. Bacterial communities in boreal forest mushrooms are shaped both by soil parameters and host identity. Front Microbiol 2017;8:1–13.

Pickles BJ, Wilhelm R, Asay AK et al. Transfer of $^{13}$C between paired Douglas-fir seedlings reveals plant kinship effects and uptake of exudates by ectomycorrhizas. New Phytol 2017;214:400–11.

Pratama AA, Chaib De Mares M, van Elsas JD. Evolutionary history of bacteriophages in the genus Paraburkholderia. Front Microbiol 2018;9:853.

Pratama AA, Haq IU, Nazir R et al. Draft genome sequences of three fungal-interactive Paraburkholderia terrae strains, BS007, BS110 and BS437. Stand Genomic Sci 2017;12:81.

Pratama AA, van Elsas JD. A novel inducible prophage from the mycosphere inhabitant Paraburkholderia terrae BS437. Sci Rep 2017;7:9156.

Pratama AA, van Elsas JD. The ecology and evolution of bacteriophages of mycosphere-inhabiting Paraburkholderia spp. (doctoral dissertation). 2018. http://hdl.handle.net/11370/65283 (15 March 2019, date last accessed).

Priet JA, Bühler B, Jung K et al. PhaF, a polyhydroxyalkanoate-granule-associated protein of Pseudomonas oleovorans GPa1 involved in the regulatory expression system for pha genes. J Bacteriol 1999;181:858–68.

Quesada JM, Soriano MI, Espinosa-Urgell M. Stability of a Pseudomonas putida KT2440 bacteriophage-carrying genomic island and its impact on rhizosphere fitness. Appl Environ Microbiol 2012;78:6963–74.

Rashid MI, Mujawar LH, Shahzad T et al. Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. Microbiol Res 2016;183:26–41.

Rillig MC, Muller LA, Lehmann A. Soil aggregates as massively concurrent evolutionary incubators. ISME J 2017;11(9):1943–48.

Rand I, Timonen S, Koivula T et al. Tolerance and biodegradation of m-toluolate by Scots pine, a mycorrhizal fungus and fluorescent pseudomonads individually and under associative conditions. J Appl Microbiol 1999;86:817–26.

Rand I, Timonen S, Nurmiho-Lassila EL et al. Microbial biofilms and catabolic plasmid harbouring degradative fluorescent pseudomonads in Scots pine mycorrhizospheres developed on petroleum contaminated soil. FEMS Microbiol Ecol 1998;27:115–26.

Schneider S, Keller M, Dröge M et al. The genetic organization and evolution of the broad host range mercury resistance plasmid pSB102 isolated from a microbial population residing in the rhizosphere of alfalfa. Nucleic Acids Res 2001;29:5169–81.

Secor PR, Sweere JM, Michaels LA et al. Filamentous bacteriophage promote biofilm assembly and function. Cell Host Microbe 2015;18:549–59.

Selosse MA, Richard F, He X et al. Mycorrhizal networks: des liaisons dangereuses? Trends Ecol Evol 2006;21:621–8.

Sen D, Van der Auwera GA, Rogers LM et al. Broad-host-range plasmids from agricultural soils have IncP-1 backbones with diverse accessory genes. Appl Environ Microbiol 2011;77:7975–83.

Sengeløv G, Kovalchuk GA, Sørensen SJ. Influence of fungal-bacterial interactions on bacterial conjugation in the residuesphere. FEMS Microbiol Ecol 2000;31:39–45.

Shintani M, Matsui K, Inoue J et al. Single-cell analyses revealed transfer ranges of incP-1, incP-7, and incP-9 plasmids in a soil bacterial community. Appl Environ Microbiol 2014;80:138–45.

Silpe JE, Bassler BL. A host-produced quorum-sensing autoinducer controls a phase lysis-lysojeny decision. Cell 2019;176(1-2):268–80 e13.

Soucy SM, Huang J, Gogarten JP. Horizontal gene transfer: building the web of life. Nat Rev Genet 2015;16:472–82.

Swanson MM, Fraser G, Daniell TJ et al. Viruses in soils: morphological diversity and abundance in the rhizosphere. Ann Appl Biol 2009;155:51–60.

Tart AH, Blanks MJ, Wozniak DJ. The AlgT-dependent transcriptional regulator AmrZ (AlgZ) inhibits flagellum biosynthesis in mucoid, nonmotile Pseudomonas aeruginosa cystic fibrosis isolates. J Bacteriol 2006;188:6483–9.

Tauch A, Schneiker S, Selbitzschka W et al. The complete nucleotide sequence and environmental distribution of the cryptic, conjugative, broad-host-range plasmid pPFO2 isolated from bacteria of the wheat rhizosphere. Microbiology 2002;148:1637–53.

Thomas CM, Nielsen KM. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol 2005;3:711–21.

Torsvik V, Thingstad TF. Prokaryotic diversity-magnitude, dynamics, and controlling factors. Science 2007;296:1064–6.

Van Elsas JD, Dijkstra AF, Govaert JM et al. Survival of Pseudomonas fluorescens and Bacillus subtilis introduced into two soils of different texture in field microplots. FEMS Microbiol Lett 1986;38:151–60.

Van Elsas JD, McSpadden Gardener BB, Wolters AC et al. Isolation, characterization, and transfer of cryptic gene-mobilizing plasmids in the wheat rhizosphere. Appl Environ Microbiol 1998;64:880–9.

Van Elsas JD, Penido EGC. Characterization of a new Bacillus megaterium bacteriophage, MJ-1, from tropical soil. Antonie Van Leeuwenhoek 1982;48:365–71.

Van Elsas JD, Pereira MTPR. Characteristics of a soil-isolated Bacillus subtilis phage, GS1 and GS1-mediated plasmid transduction. Centrol Mikrobiol 1987;142:63–70.

Van Elsas Trevors JT, Rosado AS et al. Modern Soil Microbiology III. New York: Taylor and Francis, 2019. In press.

Wamberg C, Christensen S, Jakobsen I et al. The mycorrhizal fungus (Glomus intraradices) affects microbial activity in the rhizosphere of pea plants (Pismum sativum). Soil Biol Biochem 2003;35:1349–57.

Wang X, Kim Y, Ma Q et al. Cryptic prophages help bacteria cope with adverse environments. Nat Commun 2010;1:147.

Warmink JA, Nazir R, van Elsas JD. Universal and species-specific bacterial “fungiphiles” in the mycospheres of different basidiomycetes fungi. Environ Microbiol 2009;11:300–12.

Warmink JA, van Elsas JD. Migratory response of soil bacteria to Lophyllum sp. strain Karsten in soil micromosa. Appl Environ Microbiol 2009;75:2820–30.

Warmink JA, van Elsas JD. Selection of bacterial populations in the mycosphere of Laccaria proxima: is type III secretion involved? ISME J 2008;2:887–900.

Williamson KE, Radoschew M, Woomack KE. Abundance and diversity of virus in six Delaware soils. Appl Environ Microbiol 2005;71:3119–25.

Yang HC, Im WT, Kim KK et al. Burkholderia terrae sp. nov., isolated from a forest soil. Int J Syst Evol Microbiol 2006;56:453–7.

Yang F, Oliveira da Rocha Calixto R, van Elsas JD. Migration of Paraburkholderia terrae BS001 along old fungal hyphae in soil at various pH levels. Microb Ecol 2018;76:443–52.
Zablocki O, Adriaenssens EM, Cowan D. Diversity and ecology of viruses in hyperarid desert soils. *Appl Environ Microbiol* 2015;82:770–7.

Zhang M, Pereira e Silva M de C, Chaib De Mares M et al. The mycosphere constitutes an arena for horizontal gene transfer with strong evolutionary implications for bacterial-fungal interactions. *FEMS Microbiol Ecol* 2014a;89:516–26.

Zhang M, Visser S, Pereira e Silva MC et al. IncP-1 and PromA group plasmids are major providers of horizontal gene transfer capacities across bacteria in the mycosphere of different soil fungi. *Microb Ecol* 2014b;69:169–79.

Zhang M, Warmink J, Pereira e Silva MC et al. IncP-1β plasmids are important carriers of fitness traits for *Variovorax* species in the mycosphere—two novel plasmids, pH844 and pBS64, with differential effects unveiled. *Microb Ecol* 2015;70:141–53.

Zhang M, Yang P, van Elsas JD. Effect of the IncP-1β plasmid pH844 on the population dynamics of *Burkholderia terrae* BS001 in the *Lyophyllum* sp. strain Karsten mycosphere under different iron conditions. *FEMS Microbiol Ecol* 2016;92:1–10.