Bacterial community establishment in native and non-native soils and the effect of fungal colonization

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Abstract

Bacterial communities are essential parts of living soils. However, we understand very little of how soil matrices govern the structure of the local microbiota. Here, we report experiments that address such assembly rules for microbial communities in soil. Thus, indigenous soil bacterial communities were extracted from eight different soils (denoted V, B, G, WD, L, K, S and WG) and used to inoculate four selected sandy soils (V, B, G and WD) in microcosms. Then, these microcosms were subjected to colonization by the soil saprotrophic fungus *Lyophyllum sp.* strain Karsten, or were left uncolonized. Fifteen days following introduction, the microcosms were sampled. Samples were used to enumerate and obtain cultured bacteria, and were also processed for analyses by bacterial quantitative PCR and PCR-DGGE, to assess the abundance and diversity of the bacterial communities.

In all cases and irrespective of the presence of fungal mycelium, bacterial communities of around $10^8$ per g dry soil established. The data further clearly showed the influence of local soil conditions on the establishment of the inoculant bacteria in their native versus non-native soil. Thus, cluster analysis of the PCR-DGGE fingerprints revealed that two soils (V and B) were strong drivers of the established community structures from the eight source communities, whereas the two remaining soils (G and WD) revealed mixed effects. The non-native bacterial communities (as found by PCR-DGGE) obtained from the sandy soils were more similar to the native communities than those from the clayey soils. Overall, the bacterial communities decreased in diversity and richness from non-fungal to fungal-colonized systems (migration and inoculation sites).

In the fungal-affected systems, we found the recipient soil matrix to be of importance at the inoculation site in the V and B soil matrices and at the migration front in the G matrix. Analysis of the culturable bacterial community across different soils revealed that similar types of bacteria, even originating from different sources, were enriched by the colonizing fungus. Particular types, *e.g.* *Burkholderia* and *Pseudomonas* spp. were thus selected by the growing fungal hyphae.
Introduction

Soil is usually characterized as a complex dynamic system in which bacteria and fungi live together (Nazir et al., 2010). These organisms thus occupy the available niche space (Johansson et al., 2004; De Boer et al., 2005) by utilizing the nutrients that are ultimately provided by autotrophic plants or decaying organisms as well as from each other. The microsites in soil where bacteria and fungi meet are called bacterial-fungal interfaces. These are proposed to represent ecological hot spots where the heat of interactions is presumed to occur. A better analysis of such hot spots will enable us to understand the interaction between these two important groups of soil microorganisms. Thus, living together in soil, bacteria and fungi have been hypothesized to exert specific effects on each other (Nazir et al., 2010). Locally-specific conditions drive the selective processes that occur in these soil bacterial/fungal hot spots. For instance, early work by Artursson and colleagues (Artursson, 2005; Artursson et al., 2006) revealed a significant stimulus by the fungus *Glomus mosseae* on some *Paenibacillus* spp. as well as particular *Gammaproteobacteria* in the mycorrhizosphere.

The microbiota of each soil may be unique for that particular soil, as it evolved and matured over the time of existence of the soil. Unfortunately, we currently lack detailed knowledge on how the indigenous microbial communities are shaped in accordance with the local habitat. Moreover, in the light of the uncertainty on the exact nature of the niche spaces in soil, it is unclear to what extent particular bacterial groups are strongly favored or disfavored in soils. On top of this, an important impact by soil fungi can be expected to occur for soil bacterial communities. Singh et al. (2008) examined bacterial and arbuscular mycorrhizal (AM) fungal assemblages in soil and concluded that fungi are indeed major determinants of the local bacterial assemblages. Artursson et al. (2006) reported that specific bacteria were activated in the mycosphere and this was driven by fungal exudates that became available. Mansfeld-Giese et al. (2002) investigated the culturable bacterial communities in the mycorrhizosphere of *Glomus intraradices* and showed that *Paenibacillus* spp. were specifically selected in this habitat.
another investigation, members of the Oxalobacteriaceae were found to be more abundant in mycorrhizal than in nonmycorrhizal root samples (Offre et al., 2007, 2008). In our laboratory, Warmink and van Elsas (2008) showed that the bacterial numbers in the mycosphere of the ectomycorrhizal fungus Laccaria proxima were significantly higher than those in the corresponding bulk soil. Later, Warmink and colleagues (2009) extended the number of fungi examined and found an enhancement of culturable Pseudomonas types in the vicinity of particular fungi, thus reinforcing the concept of selective force exerted by the fungi on the soil bacteria that are locally present.

Interestingly, the fungal selective effect appears to affect members of a limited number of bacterial types, which are then strongly selected in the mycosphere. For instance, clear selective effects were found to be exerted by the fruiting bodies of the ectomycorrhizal fungi Laccaria proxima and Russula exalbicans growing in forest soil on members of the Sphingomonadaceae (Boersma et al., 2009). Major Sphingomonas groups from the examined mycospheres did not cluster with Sphingomonadaceae in public databases, which indicates that novel groups of this family were present. Interestingly, similar bacterial community structures were observed for the same fungal species from different sampling sites, whereas the corresponding bulk soil communities differed from each other (Boersma et al., 2009). This indicates a strong selective effect on similar groups of soil bacteria. Furthermore, the two fungal types selected different bacteria of the same family, indicating that different fungi exert different selective forces on soil bacteria. Along with the selective pressure exerted by fungi, the local soil conditions and (a)biotic factors also contribute to the shaping of the local bacterial communities. Nutritional status, local pH and other physico-chemical parameters thus are among the key factors that drive such communities.

There is very little known about how conditions in different soils drive the establishment of bacterial communities upon infestation of a virgin area by diverse communities. Moreover, we normally ignore the effects exerted by local soil fungi. Therefore, the current exploratory study was designed to foster our understanding of the following questions:
(1) Which types of bacterial communities establish when selected sterilized soils are inoculated with communities from different soils? (2) What is the effect of a locally developing soil fungus? (3) Which parameters exert the strongest effect on bacterial community establishment, nativeness, soil, soil type or a colonizing fungus? (4) Are there generalist bacteria that appear under all conditions and how abundant are these?

### Material and Methods

#### Soil characteristics and experimental set-up

Four sandy (Gieterveen - G, Buinen- B, Valthermond- V, Droevendaal - WD) and four clayey (Lelystad- L, Kollumerwaard- K, Steenharst- S, Grebbedijk - WG) were sampled freshly. The characteristics of these soils can be found in Table 1. Bacterial communities were obtained from these soils as soil extracts, as explained below. These were then used as the inoculant communities. These communities were added at 1-2x10^6 CFU per g dry soil. The four sandy (G, B, V, WD) soils were selected to serve as recipient soils for all experiments. They were sterilized by autoclaving twice. Sterility was checked by plating extracts from the soils on R2A plates and incubating the plates at 28 °C; all extracts were found to be sterile, as there was no bacterial or fungal growth on the plates, even after a week.

#### Growth and maintenance of the fungus

The (basidiomycetous) fungus used in this study, i.e. *Lyophyllum* sp. strain Karsten (DSM2979), was grown on oat flake agar (OFA) plates, prepared with 30 g of oat flake (local supermarket) and 15 g of agar (Duchefa, Haarlem, The Netherlands), dissolved in one litre of water, sterilized at 121°C for 21 min. Once every 4 weeks, the fungal strain was transferred to fresh OFA plates for maintenance.
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Soil microcosms
A microcosm system was designed, consisting of three-compartment petri dishes (Greiner Bio-one, Frickenhausen, Germany). Two compartments were filled each with about 10 g of moist, sterilized soil (moisture contents corresponded to 60% of water holding capacity, bulk density \[\text{wt/vol}\] of about 1.3), yielding layers of about 4 mm height. The four sandy soils (G, B, V and WD) were used as matrices that received the inoculant communities. The third compartment was filled with OFA. The physical barriers between the OFA and the two soil compartments prevented compounds from the OFA from reaching the soil compartments. The barriers were overcome by the fungal hyphae, and hence outgrowth of fungal hyphae from the nutrient-rich OFA into the soil was achieved. The system was inoculated with *Lyophyllum sp.* strain Karsten on the OFA and incubated at 28 °C, thereby

| Soils          | Texture           | pHw | OM (%) |
|----------------|-------------------|-----|--------|
| Gieterveen (G) | Sandy loam        | 4.5 | 3.0    |
| Buinen (B)     | Sandy loam        | 4.3 | 3.8    |
| Valthermond (V)| Sandy loam        | 4.7 | 17.8   |
| Droevendaal (*WD)| Sandy clay loam | 5.2 | 3.2    |
| Lelystad (L)   | Clay              | 7.5 | 3.1    |
| Kollumerward (K)| Silty clay loam   | 7.5 | 2.9    |
| Steenharst (S) | Clay              | 5.7 | 5.5    |
| Grebbedijk (*WG)| Clay              | 7.5 | 5.3    |

*Droevendaal and Grebbedijk are sites from Wageningen (W).*
allowing colonization of the OFA plus about 1-2 mm of the sterile soil (prior to introduction of bacterial inocula).

**Preparation of bacterial cell suspension and inoculation**

To prepare soil bacterial cell suspensions, all eight freshly-sampled soils were first homogenized and then added to sterilized milliQ water in a 1:10 proportion (0.5 g of soil in 5 ml of water). The resulting suspensions were homogenized by vigorous shaking on a Vortex mixer (full speed; thrice for 1 min). Following this, soil particles were allowed to settle for 10-15 min on the bench, after which 50 ul of the supernatant was used to establish the aforementioned cell densities in the soil. Briefly, the 50 µl were added by pipetting a 3-mm-wide stripe directly adjacent to the front of the growing hyphae. This inoculation into the four selected soils gave 32 different treatments (Fig. 1). Control treatments without fungi received bacterial inoculation in the same fashion. Afterwards, soil microcosms were incubated at 28 °C. Samples (100 mg) were taken at different time points (mostly at day 15) from different sites within the soil compartments (inoculation and migration front sites, using fungal-affected and unaffected microcosms) by removing 4-mm diameter cores from the soil. These samples were used for further analyses for DNA extraction as well as for cultivation-based techniques.

**CFU counts and isolation of strains**

Selected soil samples were vigorously shaken in 0.85% NaCl buffer using a Vortex (thrice, maximum speed, one min each). Afterwards, the bacterial suspensions were diluted in a tenfold series and subsequently plated on R2A plates. CFU numbers on these plates were enumerated following incubation for 2-5 days at 28 °C. Moreover, three colonies, primarily from the migration front site, were randomly picked for each treatment and streaked to purity. Then, these isolates were subjected to presumptive identification on the basis of their 16S rRNA gene sequence, in accordance with standard techniques (Hirsch et al., 2010).
Fig. 1 Schematic representation of the treatments. The same set of treatments were used for the presence and absence of the fungus, *Lyophyllum* sp. strain Karsten and also for migration front site. Sampling was done after 15-days of bacterial inoculation.

**Extraction of DNA from soil samples**

Total soil DNA was isolated from all samples with the Ultra Clean Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA, catalogue number 12800-100) according to the manufacturer’s protocol. DNA purification was based on the Wizard DNA Clean-Up System kit (Promega, catalogue number 225976), which was used according to the manufacturer’s protocol to remove remaining humic acids. The quality and quantity of the DNA were checked on 1% agarose gels. In all cases, DNA of about 20 kb average fragment size was obtained. The isolated DNA was directly used for PCR-based assessments.

As negative controls, uninoculated sterilized soils were also used in DNA extractions; no detectable DNA could be found with the used procedure.

**Real-time quantitative PCR (qPCR)**

A primer pair (hrcR-F GCGATCATCGTCTCGGTGTA and hrcR-R CGAA CGCAGGAAGAACTGTC) was designed (see chapter 7) specifically to target the *Burkholderia terrae* type hrcR gene as a proxy for its type three secretion system.
DNA templates (1 ng per reaction) with SYBR Green qPCR master mix (Maxima, Applied Biosystem) was used to quantify the 16S rRNA as well as hrcR gene copy abundance. One µl of each DNA sample was used as the template in a 25-µl qPCR reaction containing 10 nM of each primer and 1 × Maxima qPCR SYBR green master mix (11.5 µl). Real-time PCR was performed using an AB-7300 thermal cycler (Applied Biosystems) with the following conditions: initial cycle 95°C for 15 min and 40 cycles of: 95°C for 20 s; 57°C for 20 s and 72°C for 30 s. The threshold cycle (Ct) was determined by SYBR Green incorporation into double-stranded DNA. These Ct values identified the PCR cycle number at which the amplicon numbers exceeded the detection limit. The relative standard curve method was applied to estimate the gene copy numbers.

**Analysis of total bacterial community structure with PCR-DGGE**

All soil DNA samples were subjected to PCR with bacterial primers F968-GC and 1378R (Nubel et al., 1996; Heuer and Smalla, 1997) using the GeneAmp PCR System9700 (Applied Biosystems). The 50 ul PCR mix consisted of 5 µl 10x Stoffel buffer (Roche), 200 mM of each deoxynucleoside triphosphate, 400 nM of each primer, 1% formamide, 0.05U Stoffel Taq DNA polymerase (Roche), 31 µl H2O and 1 ul (about 20 ng) of soil DNA. The following touchdown PCR program was used: 94 °C for 3 min; 94 °C for 1 min, 65 °C (decrease per cycle 1 °C) for 1 min, 72 °C for 3 min (10 cycles); 94 °C for 1 min, 55 °C for 1 min, 72 °C for 3 min (35 cycles); 72 °C for30 min. PCR products were checked for quantity and quality by electrophoresis in 1% agarose gels. PCR products were then used in denaturing gradient gel electrophoresis (DGGE) analyses. DGGE was performed on a PhorU2 system (Ingeny International, Goes, The Netherlands) according to the manufacturer’s protocol, using a gradient (Muyzer et al., 1993) of 40% to 60% denaturants (ureum/formamide) (100% denaturants is 8M urea plus 40% formamide), a buffer temperature of 60 °C and 110V for 18 h. Following electrophoresis, the polyacrylamide gels were stained using SYBR green.
Analysis of community profiles and statistics

The patterns generated via PCR-DGGE were evaluated with the program GelComparII (AppliedMaths, Sint-Martens-Latem, Belgium). As a proxy for bacterial richness, we used band (species) richness $S$; Species richness $S$ was thus defined as the number of DGGE detected bands. Evenness was estimated on the basis of the relative band intensities. Together, this yielded the Shannon-Wiener index of bacterial diversity, $H$, which characterizes the diversity of the dominant species in soil microbial community (> 0.1% according to Gelsomino et al. (Gelsomino et al., 1999). These data were used to determine the influence of L. sp. strain Karsten on the diversity of the dominant members of the soil bacterial community. The Shannon-Wiener index was calculated as $H = -\sum P_i \log P_i$ based on the relative band intensities ($P_i$) as formulated by Eichner (Eichner et al., 1999). Means and standard deviations of $S$ and $H$ were calculated for every treatment. Clustering was done using the unweighted pair group method with mathematical averages (UPGMA; Sneath and Sokal, 1973).

Results

Lyophyllum sp. strain Karsten colonization and effect of bacterial inocula

Growing from the OFA compartment, L. sp. strain Karsten started to colonize the soil matrices as from day zero. The fungal growth rates varied in the different soil matrices, from a maximum (4.4 mm/day) in the WD soil and a minimum (1.7 mm/day) in the V soil, while it was 2.6 and 3.6 mm/day in the B and G soils, respectively.

Bacterial inoculation, on the other hand, caused decreases in the fungal growth rate. Grossly, these rates were constant, but had dropped to between 1.5 and 1.0 mm/day for all four (WD, V, B and G) sandy soil matrices. We could not discern any statistical differences in such rates, although different bacterial inocula may have had different visual effects on fungal colonization. Importantly, fungal growth was never fully inhibited by the presence of any bacterial inoculum. Thus, a hyphal
migration front always moved towards the edge of the petri dish within experimental time for all the treatments in the four sandy soil matrices.

**Bacterial community establishment in the soil matrices**

**Bacterial abundance in the soil matrices**

Samples taken after 15 d from the inoculation and migration front sites, both in fungal-less and fungal-containing microcosms, were subjected to DNA extraction and analyzed. Quantitative PCR of these DNA extracts revealed that bacterial communities in the range corresponding with 16S rRNA gene copy numbers of up to $10^8$ per gram of soil had established in all soils, except for the extracts obtained from the virtual migration site in the fungal-less systems (Table 2). These observations confirmed the occurrence of migration of a migrator community only in the presence of *L. sp.* strain Karsten. Compared to the applied inoculum level of about $10^6$ per g of soil, it also indicated in all cases fair to good colonization of the soil matrices. In all soil matrices, there was a tendency for the bacterial densities to increase in accordance with the presence of *L. sp* strain Karsten (Table 2).

**Bacterial diversity in the soil matrices**

We compared the established bacterial communities (after 15 days) in their native as well as their non-native soils. All native communities re-established in their own soils, at richness values of about 32 to 37 bands, as evidenced by the PCR-DGGE profiles (data not shown). The soil matrix that allowed the highest discernible native diversity to establish was soil B. In this soil, the native community yielded PCR-DGGE community fingerprints consisting of 37 bands (Fig. 2). Furthermore, the non-native communities were established in all four matrices at between 22 and 40 bands (WGwd and Gv, respectively). We then analyzed what richness, on average, would be established per recipient soil matrix. The analysis revealed the communities of the B and V soil matrices to sustain a higher richness (33 bands) than those of the G and WD soil matrices (29 bands). Furthermore, we noticed that the non-native sandy soil source communities (G, B, V and WD) established at
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Table 2 Bacterial community establishment in different soil matrices

| Soil matrices | Without colonization of L. sp. strain Karsten | With the colonization of L. sp. strain Karsten |
|---------------|-----------------------------------------------|-----------------------------------------------|
|               | Inoculation site | Migration front | Inoculation site | Migration front |
| G             | 7.85 (0.31)     | BD              | 8.14 (0.35)     | 8.27 (0.37)    |
| B             | 8.24 (0.27)     | BD              | 8.10 (0.31)     | 8.34 (0.30)    |
| V             | 7.84 (0.45)     | BD              | 8.14 (0.38)     | 8.17 (0.53)    |
| WD            | 7.94 (0.17)     | BD              | 8.04 (0.28)     | 8.27 (0.31)    |
| G             | 29 (3.2)        | N/A             | 23 (4.7)        | 19 (4.2)       |
| B             | 32 (4.1)        | N/A             | 25 (5.0)        | 17 (5.2)       |
| V             | 33 (5.0)        | N/A             | 20 (6.0)        | 14 (5.0)       |
| WD            | 29 (4.5)        | N/A             | 19 (8.2)        | 12 (8.7)       |
| G             | 3.29 (0.13)     | N/A             | 2.97 (0.29)     | 2.76 (0.33)    |
| B             | 3.37 (0.15)     | N/A             | 3.15 (0.23)     | 2.71 (0.33)    |
| V             | 3.40 (0.16)     | N/A             | 2.84 (0.77)     | 2.08 (0.93)    |
| WD            | 3.26 (0.20)     | N/A             | 2.77 (0.69)     | 2.21 (0.87)    |

BD: below detection (10^2 gene copies per g dry soil). N/A: not applicable; Abundance: log 16S rRNA gene copies per g dry soil. Richness: number of bands; Diversity: indicated by the Shannon index (H').

higher richness (ranging from 29 to 40 bands) in the test matrices compared to the non-native communities (ranging from 22 to 36 bands) originating from clayey soils (L, K, S, WG).

Clearly, recipient soil matrix was the major driver of the community structures established after 15 days, as evidenced by PCR-DGGE. Figure 3 shows the data. It shows that the V as well as B soils were strong determinants of the structures. All eight source communities clustered together in one cluster, denoted the V soil cluster, whereas 6/8 clustered in a B soil cluster. Furthermore, there was one mixed (WD / G soil) cluster. Interestingly in the B soil group, five out of seven communities had a sandy soil origin. On the other hand, five out of nine in the V soil group were from clayey soils, and four from sandy ones.
**Bacterial community establishment and the effect of fungal colonization**

The presence of the fungus *L. sp.* strain Karsten tended to decrease the diversities of the established bacterial communities (Table 2). Thus, *L. sp.* strain Karsten exerted specific selective force on the bacterial communities that caused decreases in bacterial diversity (Shannon index H’ as a proxy) in the fungal-colonized soil compared to the one without fungus. Along with the H’ values, bacterial richness also tended to decrease in the fungal-affected treatments compared to the ones without fungus (Table 2). In the fungal treatments, there was a clear difference at the inoculation and migration front sites. The richness was significantly lower at the migration front site than that at the inoculation site (P<0.05). We then analyzed the PCR-DGGE patterns from all samples and treatments by principle components analysis (PCA), as shown in Fig. 4. The ordination revealed complex yet differentiating patterns. A major discernible driver of community structure was the absence or presence of the migrating fungus *L. sp.* strain Karsten, as evidenced from the clear separation of the two groups of data points along the x axis (Fig. 4). Interestingly, the communities with the fungus at the inoculation site were intermediate between the two groups. The fungal-colonized cluster could further be differentiated into two subclusters, i.e. a lower-left cluster having samples at inoculation spot while the upper left group contains the samples from the migration front. UPGMA-based cluster analysis of the communities in the fungal-affected microcosms from migration front site, revealed two distinct clusters (Fig. 5).
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The largest cluster encompassed 13 communities in mixed soil matrices, 9 of which had received sandy soil source communities (1, 5, 5 and 2 communities, respectively in four G, B, V and WD matrices). Thus, the clustering was mainly driven by the source communities rather than by the recipient soil matrices. The other large cluster contained 9 communities across different soil matrices, 6 originating from sandy and 3 from clayey source communities. Two smaller clusters were also found, encompassing clayey soil source communities. The rest of the samples (4) was mixed and cannot be put together either on the basis of source community or of the soil matrix.
**Fig. 3** Bacterial community establishment in soil. Bold letters aside brackets and vertical lines represent the host matrices where bacterial communities were established. SC: source community originating from sandy soils; CC: source community originating from clayey soils.

**Enrichment of the type three secretion system (T3SS) as evidenced by hrcR**

The *B. terrae* type hrcR gene was used as a proxy for the T3SS to evaluate its enrichment in the experimental set ups. We analyzed the abundance of this gene across all soils and treatments. Overall, the abundance of hrcR was between below
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Fig. 4 PCA biplot for all the samples considering their DGGE profiles. Treatments are similar as in figure 1 while ‘f’ and ‘m’ respectively represent the samples with the fungus at inoculation spot (red squares) and at migrating front site (green diamonds). While blue rounds represent the sample without fungal colonization.

detection (about $5 \times 10^2$ per g) and about $10^3$ gene copies per g soil in the absence of the fungus. Interestingly, fungal colonization enhanced the abundance of *B. terrae* BS001 specific *hrcR* up to $10^6$ copies per g soil, respectively, at the inoculation site and migration front. Compared to the treatments without fungal colonization, there was a significant increase in the abundance of this gene in the presence of *L. sp.* strain Karsten (P<0.05). The *hrcR* (*B. terrae* type) enrichment by fungal colonization was particularly evident in the B soil matrix, though from several non-native source communities.
Culturable bacterial communities

Bacterial counts taken from the inoculation and migration front sites revealed the presence of, roughly, $2 \times 10^8$ CFU per g soil in all the experimental treatments (soils). A suite of different morphological types was present in each sample.
Therefore, around 200 random isolates were picked from the bacterial growth plates (R2A; at least three colonies per treatment) prepared from the migration front and a selection of these was identified. Following streaking to purity, the partial 16S rRNA gene was amplified and sequenced to assess strain identity.

The data affirmed that fungal colonization selected certain bacteria in the mycosphere. Bacteria of the genus *Burkholderia*, *Pseudomonas* and *Achromobacter* were prevalent at the migration front compared to the inoculation site. Detailed analysis of culturable bacteria at the migration front revealed, next to the three big clusters encompassing strains from the genera *Burkholderia*, *Pseudomonas* and *Achromobacter*, four smaller groups consisting of *Stenotrophomonas*,
Sphingobacterium, Dyella and Chryseobacterium (Fig. 6). Pandorea and Staphylococcus were found as well. Interestingly, all three Dyella strains were found to originate from G soil, although they were enriched by the fungus in the G and B soil matrices. Likewise, four out of five Stenotrophomonas strains originating from G soil were enriched in the G and B soil matrices while the 5th was from the Vv sample. Sphingobacterium, on the other hand, was enriched in the same matrix, i.e. the B soil, while originating from G and B source communities. Six out of twelve Achromobacter strains were selected at the migration front in the B soil matrix, and four and two, respectively, in the G and V soil matrices. All these originated from different sources. The WD soil matrix was not conducive to these Achromobacter strains to migrate along growing fungal hyphae (Fig. 6). Pseudomonas and Burkholderia, on the other hand, were enriched by fungal migration in all four matrices and they originated from different sources as well.

Discussion

To foster our understanding of how soil microbial communities function, it is very important to understand the assembly rules that govern the patterns of bacterial community establishment. An excellent strategy to assess these rules is provided by soil inoculation experiments, in which inocula from one soil are added to selected presterilized soils and the establishment of particular community structures is compared. This study provides data that support an analysis of the foundations of the bacterial community establishment.

A first strong conclusion from our data was that local soil conditions are very important for invading bacterial communities to establish. These local conditions caused significant differences in the established communities as a whole. Previous studies have shown the importance of soil factors, in particular soil pH and organic matter (carbon and nitrogen) content (Nazir et al., 2010b; Pereira e Silva et al., 2011), in shaping the local microbial communities. Bacteria living in these varying environmental conditions may have adapted over evolutionary time to the local soil parameters. In our data, there was a clear trend in clustering of the communities per soil matrix even when originating from different soil sources.
However, and in particular under the influence of the soil saprotrophic fungus *L. sp.* strain Karsten, we could distinguish grouping of the bacterial communities by their “broad” origin, *i.e.* originating from clayey versus sandy soil sources (Fig. 3). Thus, the presence of a soil-colonizing fungus represents a key factor that governs bacterial community established in a new environment. In our study and similar to previous studies (Warmink and van Elsas, 2009), the presence of *L. sp.* strain Karsten was found to restrict the bacterial diversity. This was evidenced by the fungal-infested treatment being less diverse than the non-fungal one. Finally, the presence of particular bacteria uniquely at the fungal migration front identified these as community migrators, indicating their capability to benefit from the available mycelial network for shelter and nutrition. It was striking that this capacity appeared to be present in the bacterial communities across all eight soils and through all four selected sandy soil matrices. Hence, bacterial movement with growing hyphae is a ubiquitous phenomenon.

In terms of culturable bacteria, a suite of different types was found to be capable of migrating along with the moving hyphae of *L. sp.* strain Karsten. In particular, we found that members of the *Burkholderiales* increased at the migration front, which indicates the migration capacity of these bacteria with the saprotrophic fungus *L. sp.* strain Karsten (Warmink and van Elsas 2009; Nazir et al., 2012). The presence of other bacteria at the migration front could either be the result of their own migration capability or of the *Burkholderia* helper effect for co-migration along growing fungal hyphae, as was recently reported (Warmink et al., 2011). For instance, *Dyella japonica* BS003 was found to be able to comigrate with the help of *B. terrae* BS001. The three *Dyella* strains found at the migration front together with *Burkholderia* types may reinforce this finding.

Furthermore, the enrichment of the *B. terrae* BS001 like hrcR gene at the migration front also affirms the selection of *B. terrae* like bacteria by the fungal migration front. Interestingly, TTSS was hypothesized to be required for bacterial migration along growing fungal hyphae (Warmink and van Elsas 2009). Our data support this contention, as both *Burkholderia* types and the TTSS were significantly
enriched by the migration front of *L. sp.* strain Karsten moving through different soil matrices.

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