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Novel method reveals a narrow phylogenetic distribution of bacterial dispersers in environmental communities exposed to low hydration conditions

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ABSTRACT

In this study, we developed a method that provides community-level surface dispersal profiles under controlled hydration conditions from environmental samples and enables us to isolate and uncover the diversity of the fastest bacterial dispersers. The method expands on the Porous Surface Model (PSM), previously used to monitor dispersal of individual bacterial strains in liquid films at the surface of a porous ceramic disc. The novel procedure targets complex communities and captures the dispersed bacteria on a solid medium for growth and detection. The method was first validated by distinguishing motile *Pseudomonas putida* and *Flavobacterium johnsoniae* strains from their non-motile mutants. Applying the method to soil and lake water bacterial communities showed that community-scale dispersal declined as conditions became drier. However, for both communities, dispersal was detected even under low hydration conditions (matric potential: -3.1 kPa), previously proven too dry for *P. putida* KT2440 motility. We were then able to specifically recover and characterize the fastest dispersers from the inoculated communities. For both soil and lake samples, 16S rRNA gene amplicon sequencing revealed that the fastest dispersers were substantially less diverse than the total communities. The dispersing fraction of the soil microbial community was dominated by *Pseudomonas* which increased in abundance at low hydration conditions, while the dispersing fraction of the lake community was dominated by *Aeromonas* and, under wet conditions (-0.5 kPa), also by *Exiguobacterium*. The results gained in this study bring us a step closer to assessing the dispersal ability within complex communities under environmentally relevant conditions.

IMPORTANCE

Dispersal is a key process of bacterial community assembly. Yet, very few attempts have been made at assessing bacterial dispersal at the community level as focus has previously been on pure culture studies. A crucial factor for dispersal in habitats where hydration conditions vary, such as soils, is the thickness of the liquid films surrounding solid surfaces, but little is known on how the ability to disperse in such films varies within bacterial communities. Therefore, we developed a method to profile community dispersal and identify fast dispersers on a rough surface resembling soil surfaces. Our results suggest that within the motile fraction of a bacterial community only a minority of the bacterial types are able to disperse in the thinnest liquid films. During dry periods, these efficient dispersers can gain a significant fitness advantage through their ability to colonize new habitats ahead of the rest of the community.

KEYWORDS

Community motility, *Pseudomonas putida* KT2440, liquid film, soil, lake water, succession, porous surface model.

Introduction:

Dispersal is essential in order to ensure fitness in a world of limited and heterogeneously distributed resources and is recognized as a key contributor to community dynamics (1, 2). While dispersal has long been studied as an integral part of the ecology of animals and plants, its contribution to microbial ecology has received less attention (3–5).

Dispersal is traditionally divided into passive (caused e.g. by weather or human activities) and active, also termed motility, which requires metabolic energy (6). Motility is not limited to environments saturated with water, but is also commonly found on or near surfaces in unsaturated environments, such as the thin liquid films between soil particles or on the surface of leaves (7). Bacteria have evolved diverse mechanisms of active dispersal on surfaces including swimming, swarming, twitching, sliding, and gliding, all of which have been mainly described and studied in pure culture settings (8–11) using agar plates or glass slides for capture of motile cells (8, 9, 11, 12). Hence, it remains unclear how well these methods capture dispersal potential in more natural settings, such as in soils, and how the ability to disperse is distributed within the tree of life and within individual communities. There have been a few efforts to uncover the phylogenetic distribution of flagellar motility (13, 14) and gliding motility (12, 15), but a comprehensive view of how the dispersal abilities vary across and within bacterial phyla is still lacking.

This gap in our knowledge partly results from the lack of methods for mass assessment of dispersal potential of bacteria in environmental samples. In a community, not all bacteria have equal potential for dispersal; though this is rarely assessed. To assess the dispersal potential of a community one could, in theory, isolate and test all its members but considering that there can be up to 10^9 bacterial cells in a gram of productive soil (16), this would be practically unfeasible. In addition, by studying strains in isolation, the effects of interaction between strains would be missed. Indeed, most past studies of motility have focused on the motion of single strains (8, 17, 18) largely neglecting the vast possibilities of interactions which have only recently been uncovered (17). Bacterial co-cultures have been observed swarming together, combining their

skills to conquer barriers such as antibiotics (19) or to engage in metabolic cross-feeding (20). Motile bacteria have been demonstrated to carry non-motile bacteria as cargo (21) and inter kingdom cooperation has been described such as bacterial dispersal with the help of fungi and amoeba (22–26). It would seem logical that these complex interactions occur in natural communities but only very few attempts have been made to tackle motile bacteria in environmental samples at the community level (27–31).

A few studies did address community-level motility in aquatic environments. Grossart *et al*. (29), Mitchell *et al.* (31) and Fenchel *et al*. (30), assessed swimming motility in ocean samples using microscopy and revealed a large percentage of motile bacteria, but did not identify these. Dennis *et al*. (27) used a syringe based-assay and 16S rRNA gene amplicon sequencing to uncover the identity of motile lake water bacteria showing a chemotactic response towards inorganic substrates. However, to our knowledge, only one study assessed dispersal and identity of dispersing bacteria in a complex natural community under conditions relevant for partially-water-saturated habitats (e.g. surface or vadose zone soils, phyllosphere) (28). Using sand microcosms, Wolf *et al*. revealed that a subset of a soil community consisting mainly of the family *Enterobacteriaceae* and the genera *Undibacterium*, *Pseudomonas* and *Massilia* were able to expand to a distance of more than 2 cm from the inoculation point within 48 h (28). While this study provided important insights into the identity of dispersers and their expansion rate, they only considered one hydration condition (7.5% moisture w/w, i.e., matric potential in the -20 to -50 kPa range based on the particle size (32)). Yet, previous studies have stated that water is one of the primary factors controlling bacterial motility (33).

In a non-permanently water saturated habitat such as soil, the ability to disperse is primarily dependent on the thickness of the water film surrounding solid surfaces. The hydration status of

soil in the vadose zone is highly variable and can increase or decrease rapidly, e.g. following rainfalls or droughts (7, 18, 33). At low matric potential, the thinning of the aqueous films between soil particles will lead to habitat fragmentation into separate micro habitats (34), with strong effect on the bacterial dispersal ability.

The Porous Surface Model (PSM) is a 2D model system used for studying bacterial motility on the surface of a porous ceramic disc under controlled hydration conditions that mimics unsaturated soil surfaces. Studies of fluorescently tagged pure cultures using the PSM have demonstrated that flagellar motility is restricted to a relatively narrow range of water potential (0 to -2kPa) (18, 34). However, it remains unclear how this knowledge on specific flagellated bacterial isolates can be translated at the scale of complex environmental communities.

Hence, the aims of the current study were to (i) further develop the PSM for its use to assess bacterial dispersal of natural (untagged) bacterial communities and (ii) apply the method to a soil and a lake community to obtain community-level surface motility profiles under controlled hydration conditions and uncover the diversity of the fastest dispersers.

Results

Developing and validating the novel method with pure cultures.

The Porous Surface Model (PSM) has previously been used to monitor bacterial dispersal under controlled hydration conditions (35). The challenge of the current study was to expand the method from being solely usable with fluorescently tagged cultures to evaluate dispersal of a broader range of complex natural communities, tracking the movement from the inoculation point at the center towards the edge of the ceramic disc. To achieve this goal, we devised a procedure

to get an imprint of dispersal on the ceramic disc by pressing agar plates onto the PSM surface (see method overview in the supporting information, Fig. S1). This step resembles the agar lift method used for visualization of the bacterial distribution on soil surfaces described in a previous study (36).

Initial tests with *P. putida* KT2440 GFP and full agar plates pressed onto the ceramic disc of the PSM showed a clear discrepancy between the bacterial spatial distribution observed on the ceramic disc with epifluorescence microscopy before pressing and that captured on the agar plate. It was clear that pressing the agar plate onto the surface of the ceramic disc disturbed the bacterial distribution, so that the high density of cells at the inoculation point were often spread over a much larger area. To avoid this error, we developed a series of concentric annular hollowed-out agar plates, which were pressed sequentially, starting from the edge of the ceramic disc and leaving the center undisturbed until a full plate was used to capture the total community that had developed on the PSM disc (Fig. 1 and S1).

To test the method, we inoculated a mixture of the motile strain *P. putida* KT2440 GFP and its non-flagellated mutant *P. putida* K2440 dsRed *fliM*$^{-}$ which had previously been used for motility studies on the PSM (18, 35). This pure culture experiment demonstrated the ability of the method to clearly contrast the dispersal potential of these strains (Fig. 1 and Fig. 2). The non-motile strain generally stayed near the inoculation point. For the motile strain, the fastest dispersal was seen at -0.5 kPa with 4 out of 6 replicates reaching the edge of the pressed plate furthest from the inoculation point, i.e. the 25 - 41.3 mm section, after 40 hours incubation (Fig. 2). Dispersal at -1.2 kPa was not significantly different from that at -0.5 kPa, after 24 h ($p= 0.229$) or 40h ($p= 0.857$),

155 and no dispersal was registered at -3.1 kPa. This was consistent with the threshold for flagellar
156 motility of -2.0 kPa previously reported for *P. putida* KT2440 (18).
157
158 To test the ability of the method for capturing other types of motility than flagellum powered
159 swimming we inoculated the gliding bacteria *Flavobacterium johnsoniae* strain CJ1827 (37) and
160 the non-motile mutant *F. johnsoniae* strain 2122 Δ*gldK* (38) on separate PSMs incubated at -0.5
161 kPa. After 48h incubation the non-motile mutant (n=2) stayed near the inoculation point while the
162 gliding bacteria (n=3) were recovered in the 11.5-15 mm section and, in one case, at the edge of
163 the pressed plate, 25-41.3 mm section.
164
165 **Dispersal potential of environmental communities.**
166 Applying the novel method on extracted soil and lake bacterial communities confirmed that
167 dispersal rate declined as conditions became drier (Fig. 3). However, surprisingly, for both
168 community types, dispersal was detected even under the lowest hydration condition tested (-3.1
169 kPa and one sample at -4.2 kPa, not shown), previously proven too dry for *P. putida* KT2440
170 dispersal (Fig. 2). For the soil community, the slowest dispersal was detected at –3.1 kPa with 2
171 out of 3 replicates dispersed to the 11.5-15mm section at 24 h, though all reached the most
172 distant section of the plate (25 - 41.3 mm section) after 48 h (Fig. 3). To record the magnitude of
173 the colonization of the ceramic plate sections a coverage score was introduced for the
174 environmental samples, where the extent of colony coverage of the agar plates was roughly
175 assigned into four categories. This scoring scoring indicated that even though soil bacteria reached
176 the most distant sections of the plates after 48 h, the colonization was less at -3.1 kPa (1-25% to
177 26-50% coverage) than at -1.2 kPa and -0.5 kPa (51-76% to 76-100% coverage).

178 The lake bacterial community study was mainly included for comparison purposes, as we assumed
179 that this community would have experienced a weaker selection for dispersal ability on dry
180 surfaces, and to demonstrate the versatility of the method. It was based on fewer replicates which
181 limits interpretation, but revealed a similar picture with bacteria reaching the edge of the plate
182 after 48 h for both dry (-3.1 kPa) and wet (-0.5 kPa) conditions though the extent of colonization
183 was less for the dry samples (Fig. 3). Direct comparisons of the soil and lake data should be done
184 with caution because the CFU counts suggested that more cultivable cells were inoculated for the
185 lake than for the soil samples (34 x 10^3 vs 2-8 10^3 CFUs per inoculum, respectively). However, for
186 both soil and lake communities it remains clear that we registered much faster dispersal at both -
187 0.5 kPa and -3.1 kPa (Fig. 3) compared to *P. putida* KT2440 (Fig. 1).
188
189 **Diversity of dispersers.**
190 DNA was extracted from the Nycodenz soil extracts and the lake filtrate used for inoculations,
191 from agar plates reflecting the total community present on the ceramic disc (Full Plate), and from
192 the community that developed upon inoculation of the environmental cell extracts onto a
193 'standard' 25% R2A solid medium plate with 20 g agar l^{-1}, which provides conditions that are not
194 conducive to motility (39) (the No Motility Reference Plate, shortened as 'Reference Plate'). All
195 samples were sequenced using Illumina sequencing targeting the V3-V4 regions of the 16S rRNA
196 gene. A total of 3.8 million sequences were kept after filtering for further analysis.
197 Comparisons between the communities in the inoculum (Nycodenz extractions and the Lake
198 filtrate) and the cultivable communities dispersed or not, confirm the expected cultivation bias
199 (Fig S7-8). However, the cultivable community represented on the Full plates and Reference plates
200 retained a high diversity with representatives of 261 unique genera for soil, and 143 for lake. In

addition, 4172 and 665 amplicon sequence variants (ASVs (40)) in soil and lake samples were not identifiable at the genus level. Moreover, the dominating genera in the cultivated soil communities, (*Pseudomonas, Flavobacterium* and *Paenibacillus*) were also among the abundant taxa of the Nycodenz extractions (Fig S7) and lake water filtrate (*Aeromonas Flavobacterium* and *Exiguobacterium*) (Fig S8), respectively.

For each PSM we collected the DNA of the fastest dispersers i.e. that of the colonies of the pressed agar plate the furthest from the point of inoculation that presented growth. As the method did not allow for selective recovery of the cells unable to disperse we compared these 'dispersed' communities to the total community present on the Full Plate and the Reference Plate. Sequencing results from the soil community showed a dominance of *Pseudomonas* in the dispersed communities benefitting from increasingly dry conditions, and achieving almost total dominance at the driest conditions (-3.1 kPa and -4.2 kPa: 99.4 - 98.3% after 48 h) (Fig. 4). Under wet conditions (0.0 kPa and -0.5 kPa) the dispersed bacterial community consisted, besides *Pseudomonas,* mainly of *Paenibacillus, Rahnella, Lysinibacillus* and, after 48 h (-0.5kPa), also of *Flavobacterium* and *Janthinobacterium.* At moderate dryness (-1.2 kPa), *Bacillus* was almost equal in abundance to *Pseudomonas* (47.2% and 50.4% respectively) at 24 h but were reduced over time to 3.7% at 48 h in favor of *Pseudomonas* (50.8%), *Paenibacillus* (19.1%) and to some extent *Janthinobacterium* (11.7%).

For the lake community, *Aeromonas* was the most abundant genus in all samples and almost completely dominated the dispersed community under dry conditions (91.9% after 24 h and 95.6% after 48 h, -3.1 kPa) (Fig. 5). Under wet conditions *Aeromonas* dispersed and colonized fast (79.1%,

after 24 h) but appeared to experience increased competition from *Exiguobacterium* over time (43.3% and 37.5% respectively after 48 h).

The bacterial diversity, calculated using the Shannon Diversity index (Fig. 6 A, B and S3 A, B), revealed that diversity was significantly affected by the matric potential in the 24 h soil samples ($p < 0.001$, ANOVA based on comparison of the differences between Full Plates and dispersed communities). Both the dry (-3.1 kPa) and moderately dry (-1.2 kPa) conditions were significantly different from the wet (-0.5 kPa) and very wet (0.0 kPa) conditions ($p<0.05$ for all pairwise comparisons). The two dry (-3.1 and -1.2 kPa) and two wet (0.0 and -0.5 kPa) soil communities did not significantly differ from each other (Fig S3 B). At 48 h, the differences between the Shannon Diversity indices at the four matric conditions were not significant ($p=0.121$) in spite of a clear trend for decreasing diversity with drier conditions (Fig. 6 B). The difference in diversity between the dispersed community and that recovered on the Full plate could only be rigorously tested at 48 h, due to the significant effect of matric potential at 24 h, but showed that the dispersed soil community had a significantly lower diversity ($p=0.001$) (Fig. 6 B).

The lake data also indicated a trend for lower diversity in the dispersed community compared to the Full Plate at both 24 h and 48 h (Fig. 6 A and S3 A). As expected, the Shannon diversity values of the Nycodenz extract and the Lake filtrate were much higher than for the samples collected after cultivation on the agar plates (5.57 ± 1.45 S.D (n=4) and 3.82 (n=1), respectively) clearly indicating cultivation bias.

The phylogenetic diversity calculated using Faith's Phylogenetic Diversity index (Fig. 6 C, D and S3 C, D) revealed a consistently narrow phylogenetic diversity of the fastest dispersers at all tested

matric potentials, compared to the total community of the Full Plate and the motility restricted Reference Plate for both the soil and lake community.

A closer look at the phylogenetic distribution of the two dominant genera *Pseudomonas* and *Aeromonas* in soil and lake water, respectively, showed that the dispersed communities at 48 h consisted of multiple and diverse amplicon sequence variants (ASVs) (Fig. S4, Table S1 and Fig. S5, Table S2). Notably, a search of the literature uncovered that all the type strains with the closest sequence similarity to our ASVs possess the ability for active motility mainly by using flagella, except for one for which motility is unknown (table S1 and S2). Neither for the soil community nor for the lake water community was there a clear separation of ASVs between matric conditions visible in the phylogenetic trees (Fig. S4 and S5).

A comparison of the *Pseudomonas* present in the total community of the Full Plate and the dispersed soil community at 48 h shows that only 11 out of 44 ASVs were solely present in the total community, and thus did not disperse from the center of the ceramic disc (Fig S6). This supports the general notion of *Pseudomonads* as efficient dispersers. Furthermore, 9 out of 44 ASVs were solely detected in the dispersed community. This is most likely because they were below detection limit in the total community, as strains present in the dispersed community must also be present in the total community. Other evidence of large enrichments in the dispersed community can be found in the heatmap (Fig. 4), where, in addition to *Pseudomonas, Paenibacillus* (at matric potential below -3.1) and *Bacillus* (at -1.2 kPa) also notably increased their abundance in the dispersed community compared to the total community of the Full Plate and the motility restricted Reference Plate. These results illustrate that there can be a large fitness gain

associated with dispersal for a motile strain i.e. going from being below detection limit to potentially very high relative abundance far from the inoculation point. By moving ahead of the pack, such strains benefit from decreased competition for nutrients and maximize their growth.

Discussion

Performance and limitations of the method

In this study, we developed a method for assessing dispersal of natural bacterial communities under controlled hydration conditions. We achieved this by expanding on the Porous Surface Model already well established for single strain motility studies (18, 35, 39), and using agar plates to get an imprint of the colonization on the surface of the ceramic disc. The method proved effective in separating the dispersal of a motile flagellated *P. putida* strain from a non-motile mutant, which stayed near the inoculation point in the center of the ceramic disc on the agar plate imprints (Fig. 1 and 2). It was also able to capture the effect of lowered matric potential, which resulted in a reduced dispersal rate of the motile strain and a cessation of all movement at -3.1 kPa, in agreement with previous studies (18, 34). In addition, the method was able to detect dispersal of the gliding bacterium *F. johnsoniae*, indicating the potential for detection of other types of motility than swimming.

The possibilities for precise control of hydration conditions are one of the key points that separate this method from the few previous studies on community motility (27–29). Calculations coupled with recent measurements of the liquid film thickness on the surface of the ceramic disc in the PSM model (18, 34, 35) provides us with a unique platform to study the behavior of microbial communities on surfaces as they are affected in their microhabitats by water film thickness. While

292 we only tested the effect of fixed hydration conditions in this study, exploring dynamic conditions
293 such as dry-wet cycles would be straightforward. Indeed, recent studies with a synthetic soil
294 community on the PSM demonstrated a clear effect of such cycles on competition and co-
295 existence (41).
296
297 We recognize that the results from using this method are biased by cultivation and are only valid
298 for the fraction of bacteria able to grow under the selected growth conditions. However, we did
299 find a high diversity of genera among the cultured community and that the most dominant genera
300 e.g. *Pseudomonas* and *Aeromonas* were prominent parts of the original inocula. This indicates
301 that, in spite of the existence of some cultivation bias, our method do provide information of
302 relevance to the original communities.
303
304 In addition, nutrient supplementation is often necessary to detect dispersal (28) and one of the
305 strengths of this setup is that it does allow for easy isolation of strains of interest as we essentially
306 already have them on agar plates. This has led to a culture collection of soil isolates able to
307 disperse at -0.5 and -3.1 kPa for use in future studies (data not shown). A possible venue to
308 decrease cultivation bias is to optimize the medium. We currently use a medium with a relatively
309 low substrate concentration (25% R2A and R2B) to avoid selection of only fast growing bacteria,
310 but this could be further improved by e.g. using a soil extract medium (42). Results are also likely
311 affected by the extraction methods used to obtain microbial inocula from the environment,
312 because extraction, and especially Nycodenz extraction (43, 44), affects the composition of the
313 inoculum. However, this is not a limit to the method itself. The method would also be applicable
314 when using intact environmental samples (for example, soil aggregates) placed in the center of the

ceramic disc. Finally, while there are benefits of using the agar plate sampling method (low detection limit for cultivable bacteria), a possible improvement would be to recover the dispersed community for DNA extraction directly from the surface of the PSM. The recovery rate and detection limit would need to be evaluated carefully.

Dispersal of environmental communities under low hydration conditions

When we applied the method to soil and lake water communities, the results extended previous pure culture studies in confirming that dispersal rates decline as conditions become drier. However, surprisingly, for both communities, relatively rapid dispersal was detected even under the lowest hydration conditions (-3.1 kPa). After 48 h, members of both communities had reached the maximum possible distance of 25-41.3 mm (Fig. 3). It is unlikely that the detection of cells several cm away from the inoculation point could have been caused by simple colonial growth (i.e. cell division and shoving) because colony expansion by growth only is very slow (e.g. diameter expansion rate of 17 µm h^{-1} for a P putida KT2440 at -3.6 kPa) (35). Therefore, this dispersal is likely facilitated by motility. This strong dispersal potential under low hydration conditions was particularly surprising for the lake water community because the selective value of such traits in the original habitat is not obvious.

A possible explanation for the rapid dispersal at conditions previously thought to be too dry could be a difference in cell size between the model strains and the bacteria in the environmental samples. As discussed in Dechesne *et al*. (18) the effective thickness of the liquid-film on the surface of the ceramic disc is the limiting factor for flagellar motility. Pure culture studies on the PSM using the motile strains *Pseudomonas protegens* CHA0 and *Pseudomonas putida* KT2440

report a threshold for swimming and dispersal at -2.0 kPa (18, 34, 35). At -2.0 kPa the predicted effective liquid film thickness on the surface of the ceramic is less than 1.5 µm and decreases to approximately 0.4 µm at -3.6 kPa, close to the shorter dimension of *P. putida* KT2440 rods (measured by others as 0.74 µm (rod shaped) (45) and as 0.6 µm by us under nutrient rich conditions). Hence, motility becomes strongly limited in liquid films thinner than the cell diameter, due to exposing the cell surface to liquid-air interfaces, capillary pressure and pinning forces (18, 35). As many bacteria from soil and aquatic environments are small, with diameters less than 0.4 µm and some even passing through 0.2 µm filters (44, 46–48), it is possible for some of them to be able to actively disperse in the thinnest liquid films tested in this study. It should be noted, however, that as bacterial cell size can vary with the conditions, e.g. Pseudomonads have been known to change both size and shape as a response to starvation or other chemical stressors (45, 49), the size of the bacteria used in this study should be measured under the actual imposed conditions to confirm this theory.

Diversity of efficient dispersers

Our results show that diversity decreased in the dispersed communities compared to the total community in the soil and lake samples. This indicates that, within natural communities, there is a less diverse sub community of bacteria with the potential for dispersal, which will most likely have important consequences for community composition, competition and microbial succession. The study by Wolf *et al.* (28), which is most comparable to ours, identified the most abundant dispersers in their soil community as members of the genera *Enterobacteriaceae*, *Pseudomonas*, *Massilia* and *Undibacterium*, with *Enterobacteriaceae* as the most dominant. Here, we also find both *Pseudomonas* and *Enterobacteriaceae* within the 20 most abundant ASVs in the dispersed

soil community and *Pseudomonas* is the most dominant disperser. We also find *Paenibacillus* and *Cupriavidus*, that Wolf *et al*. (28) detected in low abundance, but along with *Enterobacteriaceae,* they are only present in our study at relatively wet conditions, with matric potentials of -1.2 kPa or lower. *Undibacterium* was not present in either the initial community or in the dispersed, and *Massilia* was only detected in low numbers on one Reference Plate and in one of the Nycodenz extractions. The differences in the abundance and composition of communities between the two studies are most likely caused by a combination of various factors such as different initial communities in the inoculum, medium selection, and variation in hydration conditions. Nonetheless, it remains clear that *Pseudomonads* play a key role in the two soil communities as early colonizers of unoccupied habitats and possibly gaining a further advantage at relatively low hydration conditions where they dominate the community.

Potential modes of dispersal

Under dry conditions *Pseudomonas* and *Aeromonas* dominated the dispersed soil and lake communities (Fig. 4 and 5). Many members of these two genera produce biosurfactants which have been shown to facilitate dispersal on surfaces (50–53) such as leaves, an ability which has been hypothesized to increase fitness for *Pseudomonas* (54, 55). We speculate that biosurfactants also play a role in increasing the connectedness in the liquid film on surfaces. An important factor in our model system is the residual roughness of the ceramic surface, although polished, it can result in the fragmentation of the aqueous habitat as matric potential decreases along with the liquid film thickness, and the topography of the surface, as a result, becomes more apparent. Tecon *et al*. reports that a rapid decrease in connectedness of the aqueous habitat was found at -2.0 to -5.0 kPa which influenced the motility of their tested flagellated bacteria (34). Hence,

biosurfactant production could be a strategy to overcome dispersal limitation under dry conditions for the two genera observed in our study. In addition members of the orders *Exiguobacterales* (*Exiguobacterium*) and *Bacillales* (*Bacillus and Paenibacillus*), which are frequent in the dispersed lake and soil community at wet conditions (Fig. 4 and 5) have also been found to produce biosurfactants giving rise to speculation that the benefit of surfactant production for increased dispersal ability might not only be limited to dry conditions (53). While we did not look for biosurfactant production in this study, it would be straightforward to screen the obtained isolates for biosurfactant production in the future (56).

Alternative modes of surface motility apart from flagella powered swimming might play an increased role as conditions become dryer (7). Therefore, one of the strengths of the PSM for complex community studies is that it is not limited to investigate bacteria with swimming ability, as in the previous work by Grossart *et al.* (29) and Dennis *et al.* (27), but also enables studies of other modes such as sliding, gliding, biosurfactant aided movement, fungal highways (23), or even expansion by filamentous growth (32). The PSM could thus be instrumental to establish which of these modes of motility are relevant on rough unsaturated surfaces.

The pure culture experiment with *F. johnsoniae* CJ1827 confirmed that gliding is possible, and can provide a detectable dispersal advantage, on the rough surface of the PSM. In the soil community experiments, *Flavobacterium* was detected in low abundance in the dispersed communities at -0.5 to -4.2 kPa (Fig. 4). Many members of this genus have been found to possess gliding motility (12, 57, 58), while flagellar motility in the family *Flavobacteriaceae* is almost unheard of (59), and recent isolates of the order *Flavobacteriales* from leaf surfaces have also been reported as biosurfactant producers (53). While the role of chemotaxis was not directly measured, it is

407 possible that chemotactic organisms are enriched at the rim of the ceramic plate where the
408 substrate concentration is highest thanks to the low cell density. As many pseudomonads are
409 known to possess chemosensory systems (60), this might contribute to their prevalence in the
410 dispersed communities.
411
412 In theory, not all the strains we observe in our dispersed community have to possess the ability for
413 active motility themselves, they might be non- motile strains hitching a ride with their flagellated
414 or gliding companions (21). The co-dispersal of multiple species unveils a much more complex
415 picture of interactions that could be addressed by future studies employing the current PSM
416 model system. A possible next step could be to test the isolates obtained in this study to establish
417 which are able to autonomously disperse, versus those that rely on others.
418

419 **Conclusion:**

420 A novel method to study motility at the community level was developed and tested on a soil and a
421 lake microbial community. The results obtained suggest that within the motile fraction of a
422 bacterial community only a minority of the bacteria is able to disperse under relatively low
423 hydration conditions, previously thought too dry for flagellar motility. During dry periods, these
424 highly efficient dispersers will gain a significant advantage with their ability to colonize new
425 habitats ahead of the rest of the community. This highlights the need for increased focus on
426 complex communities, rather than pure culture studies for the prediction of actual dispersal ability
427 on solid surfaces such as soil.
428

429 **Materials and Methods**

Bacterial Strains.

The bacterial strain *Pseudomonas putida* KT2440 GFP, a tagged-derivative of a motile bacterium initially isolated from rhizosphere soil (61) was used as a motile model strain for flagellar motility and a nonflagellated mutant *P. putida* K2440 dsRed *fliM*$^{-}$ previously created (18) was used as a non-motile model strain.

The bacterial strain *Flavobacterium johnsoniae* CJ1827 (37), was used as a model strain for gliding motility, and a non-motile mutant *F. johnsoniae* 2122 Δ*gldK* (38) was used as a non-motile model strain. All strains were routinely maintained on agar plates. *P. putida* strains on R2 agar (R2A, Fluka; Sigma-Aldrich, St. Louis, USA) and *F. johnsoniae* strains on CYE agar (62) medium at 25°C.

Visualizing dispersal of non-fluorescent bacteria from environmental samples on the PSM.

The porous surface model (PSM) has previously been described and used for observing motility and growth of fluorescent strains after their inoculation at the center of a ceramic disc (diameter = 41.3 mm, thickness = 7.1 mm, maximum pore size <1.5 µm, 1 bar bubbling pressure; Soilmoisture, Santa Barbara, USA) simulating a soil surface, under controlled hydration conditions (35). Imposing suction on the disc controls the thickness of the liquid film on the ceramic surface.

In this study, we have expanded the use of the PSM for environmental communities. As non-fluorescent cells are not detectable on the surface of the ceramic disc by standard microscopy, we trapped the bacteria from the PSM by pressing small agar plates on top of the ceramic disc. This allows visualizing the colonization on the ceramic disc by observing the growth on the corresponding agar plates (Fig. S1).

The agar plates were obtained by pouring 6.3 ml 25% R2A with 20 g agar l^{-1} into the lid of a small plastic petri dish (Star^{TM}Dish diameter, 40 mm; height, 12.5 mm; Phoenix Biomedical Products, Mississauga, Canada) filling it to the brim. To further flatten the surface of the agar, the sterile lid of a standard petri dish (diameter 90 mm; height, 14.2 mm; VWR International, Søborg, Denmark) was pressed on top of the small agar plate before it had completely solidified. After drying the small agar plate was transferred into a big petri dish for storage. The PSM reservoirs were filled with 200-250 ml 25% R2B (Alpha Biosciences, Maryland, USA) and autoclaved before use.

Preliminary tests with fluorescent strains revealed that pressing of agar plates on the ceramic discs provided a distorted image of the bacterial spatial pattern because cells are inevitably displaced along the contact plane. Therefore, we detected bacterial colonization in concentric annular sections of the PSM surface. By preparing agar plates with holes of diameters ranging from 11.5 mm, 15 mm, 20 mm and 25 mm (Fig. S1) we could estimate dispersal by sequentially pressing these plates on the PSM starting with that with the biggest hole and finishing with a full plate (Full Plate).

The holes were punched in the agar plates with a custom-made tool consisting of a teflon handle, for safe handling during flame sterilization, fitted to brass tubes of varying diameters (length: 12.5 cm; diameters: 11.5, 15, 20, 25 mm) (Fig. S2). A printed template was placed under the agar plate to help center the holes. All plates were kept for a minimum of 48 h at room temperature before use on the PSM to test for contamination.

Proof of concept with motile and non-motile pure cultures.

We tested the ability of the method for distinguishing the dispersal patterns of *P. Putida* KT2440 GFP and *P. putida* K2440 dsRed *fliM*$^{-}$. The bacteria, cultivated on R2A plates, were suspended in 0.9% NaCl solution and adjusted by optical density measurements at 600 nm to obtain a cell density of ca. 2000 cells μl^{-1}, as confirmed by plate counts. Before inoculation the PSMs were elevated to -4.2 kPa (the length of the hanging water column is 40 cm and equals a suction of -4.2 kPa) for 20 minutes to drain excess fluid from the ceramic surface. The two bacterial suspensions were mixed in equal ratio and 0.5 µl was inoculated in the center of the ceramic disc, where it was rapidly absorbed. The discs were then brought to matric potentials (suction) of -0.5, -1.2 or -3.1 kPa (-5, -12 and -30 cm of water suction) and incubated at room temperature for 14, 24 or 40 hours before sampling by pressing the suite of agar plates onto the surface. Plates were incubated at 25°C for a 48 h growth period before being stored in the fridge at 4°C until observation by microscopy.

To test the applicability of the method for other types of motility, we tested the gliding bacterium *Flavobacterium johnsoniae* strain CJ1827 (37), and a non-motile mutant *F. johnsoniae* 2122 Δ*gldK* (38) on separate PSMs. Bacteria were streaked from CYE agar and grown in overnight cultures at 25°C in motility medium (MM) (63) and adjusted by optical density measurements at 600 nm to obtain a cell density of ca. 63000 cells μl^{-1}, as confirmed by plate counts, before inoculation of 1 µl in the center of the ceramic disc. The PSMs were kept at -0.5 kPa for 48h of incubation at room temperature, using 25% R2B medium in the PSM reservoirs. The 25% R2A pressed plates were kept at 25°C for a 48 h period before growth was recorded.

Microscopy and imaging.

P. putida KT2440 GFP and *P. putida* KT2440 dsRed *fliM*$^-$ spatial patterns on the PSM and on agar plates were determined with a Leica MZ16 FA epifluorescence stereomicroscope equipped for GFP and DsRed detection and fitted with a charge-coupled device (CCD) camera. Each plate was scored for the presence or absence of each strain. For documentation purpose, the entire surface of selected plates was imaged by sequentially capturing several fields of view, using a motorized stage piloted by Image Pro Plus (version 7.1; Media Cybernetics, Silver Spring, MD, USA) and then assembling a tiled image using the same software. The GFP and DsRed images of each plate were captured separately and then combined into one image.

To document the presence of colonies on the plates independently of fluorescence, the plates were subsequently imaged using the camera of a GelDocXR (Bio-Rad), operated in 'epiwhite' mode.

Dispersal potential of environmental communities.

A soil sample was collected from the plow layer (5-15 cm depth) of a Danish agricultural field, included in the Danish Pesticide Risk Assessment Program (PLAP) (64) in March 2016 (Fårdrup, Sjælland). The soil is characterized by clay till and further details can be found at http://pesticidvarsling.dk/. The soil was stored at 4°C. For each experiment 25 g sieved (2 mm) soil was taken by composite sampling, i.e. as small subsamples taken from the original soil sample and then mixed. The soil bacteria were extracted using Nycodenz density gradient centrifugation as in (65), except for the final cell density determination, which was performed directly using a Thoma counting chamber. Cell density was adjusted to $0.5 – 1 \times 10^6$ cells μl^{-1} in 0.9% NaCl solution and 10 µl inoculated as 1 µl drops in the center of the ceramic discs. This inoculum corresponded to ca.

2000 to 8000 CFUs on R2A plates. All plates used for the environmental communities were amended with 100 mg l^{-1} Delvocid to inhibit fungal growth (Natamycin, DSM food specialties, Delft, The Netherlands).

Lake water was sampled from the urban lake Sortedamssøen (Copenhagen), in September 2016. Four litre were collected from the surface water approximately 1.5 m from the shore. The water sample was filtrated first through a 2 µm glass fiber prefilter (Merck Millipore; Tullagreen, Ireland) and then through 0.2 µm polycarbonate filters (GVS Filter Technology; Morecambe, United Kingdom) on a filtration manifold (DHI Lab Products; Hørsholm, Denmark). The filters were transferred into a 15 ml falcon tube with 2.5 ml 0.9% NaCl solution and vortexed for 45 seconds. The filters were removed, and the cell density was adjusted by Thoma count to 2×10^6 cells μl^{-1}. 20 µl of the suspension was inoculated as 1 µl drops, yielding 34125 CFUs per inoculum based on drop plate counts on R2A plates. Both the lake and soil inoculum were kept at 4°C overnight before inoculation on the ceramic discs. After inoculation, the discs were brought to matric potentials of -0.5 and -3.1 kPa and incubated at room temperature for 24 to 48 hours before sampling.

After sampling by pressing of the agar plate series on the PSMs at appropriate times, plates were incubated for 72 hours at 25°C. In addition to the presence / absence score, used in the pure culture studies, the coverage of bacterial growth on the individual agar plates were roughly estimated by eye using 4 categories; 1-25, 26-50, 51-75 and 76-100% coverage.

After scoring, for each pressed plate series, the plate with the fastest colonizers (bacteria present the furthest from center) and the Full Plate with the total cultivable community were chosen for

amplicon sequencing. In addition to these, for each separate experiment a "Reference Plate" was made, by drop plating 10 µl of the inoculum onto the center of a small 25% R2A plate with 20 g agar l^{-1}. This was meant as a motility-restricted control for the bacteria cultivable on the medium.

The bacteria were then washed from the agar plates by transferring the agar from the small petri dish into a standard size petri dish with a flamed spatula, adding 2 ml 0.9% NaCl solution for 10 minutes and then gently rubbing the surface of the agar with a sterile inoculation loop and collecting the bacterial suspension by pipetting into an Eppendorf tube. The procedure was repeated twice with 1.5 ml 0.9% NaCl and the suspensions collected. The Eppendorf tubes were centrifuged for 5 minutes at 7500 x g before pooling into a single 1 ml sample suspension. The cell suspensions (plate wash) from the pressed plates, the Reference Plates, Nycodenz extracts, lake filtrate and leftover inoculums were all transferred to cryotubes and stored at -80°C.

DNA extraction and sequencing.

DNA was extracted using the Powerlyzer Powersoil kit (MoBio; Carlsbad, USA) following the manufacturer's protocol with a few changes. 500 µl of the thawed plate wash was centrifuged for 5 minutes at 10.000 x g. the supernatant was removed and the pellet dissolved by adding 750 µl bead solution and vortexing. The suspensions were transferred to Glass Bead Tubes, 60 µl C1 solution was added and samples were placed in a Bead Beater for 5 minutes at 2000 RPM. Hereafter the manufacturer's protocol was followed. DNA concentrations were measured on Qubit 2.0 (Life Technologies, Invitrogen; Carlsbad, USA) and stored at -80°C until sequencing.

566 The extracted DNA was PCR-amplified using the universal primer set PRK341F (5'-
567 CCTAYGGGRBGCASCAG-3') and PRK806R (5'-GGACTACNNGGGTATCTAAT-3') that amplify the V3-
568 V4 hypervariable regions of the 16S rRNA genes (66). 2 x 300 bp Purified PCR products were
569 sequenced on the Illumina MiSeq platform at the DTU Multi Assay Core Center (Lyngby, DK). All
570 raw 16S rRNA gene amplicons were processed with the DADA2 pipeline (67) with default
571 parameters. The sequences were classified based on the SILVA prokaryotic reference database
572 version 123 (68). A total of 3.8 million sequences passed the filtering steps, representing an
573 average of $5.3*10^4$ sequences per sample.
574
575 Shannon indices were computed in R software (version 3.3.1; R Core Team (2016)) using the
576 "plot_richness" function in the "phyloseq" package (69). Samples were rarefied to even depth
577 (average of 10 iterations) with the "rarefy_even_depth" function in the "phyloseq" package before
578 calculating Faith's Diversity with the "pd.query" function of the "PhyloMeasures" package (70).
579 Heatmaps were plotted using the "amp_heatmap" function of the "ampvis" package (71), while
580 "ggplot2" (72) and "ggtree" (73) were used for plots and phylogenetic trees, respectively.
581 Type strains were identified using EZBioCloud (www.ezbiocloud.net) (74), and the closest match
582 along with sequences for common *Pseudomonads* and *Aeromonads* were added to the trees for
583 reference. For construction of phylogenetic trees with type strains, sequences were aligned with
584 ClustalW in MEGA7 with the following parameters: Pairwise Alignment: Gap open: 1, extension:
585 6.66, Multiple Alignment: Gap open: 15, extension: 6.66. Sequences were trimmed to even length.
586 Tree was constructed with the "UPGMA" function in package "phangorn" (75).
587

All sequencing data have been deposited as a NCBI BioProject under accession number PRJNA400555.

Statistical analysis.

Mann-Whitney Rank Sum Test in Sigmaplot 13 (Systat Software Inc., San Jose, CA, USA) was used for the dispersal profile data. One-way ANOVA based on comparison of the differences between Full Plates and dispersed communities was used for 24 h Shannon Diversity Indices. Kruskal-Wallis Analysis of Variance on ranks (data was ranked due to unequal variance) and a paired t-test was used for 48 h Shannon Diversity Indices. *P* values < 0.05 were considered significant.

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796 **Figures**
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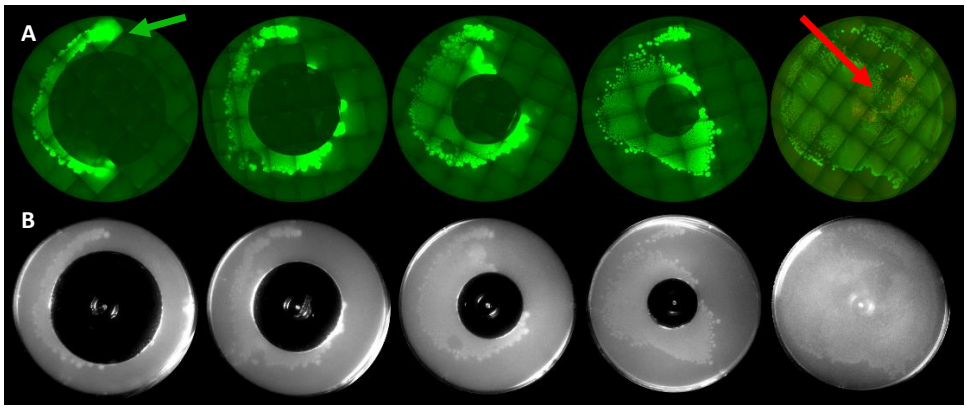


FIG 1 Proof of concept using pure cultures.

A) Separation of the motile strain *P. putida* KT2440 GFP (green) and the non-motile *P. putida* KT2440 dsRed *fliM*$^{-}$(red) on agar plates pressed onto the ceramic disc as pictured with multiple fields of epifluorescence microscopy. The non-motile strain was only detected on the Full Plate press (red arrow), while the motile strain was detected on all of the pressed plates, including the one that captures the zone most distant from the inoculation point (green arrow). B) Dispersal assessed with a camera without fluorescence detection, which is the method used for environmental communities. Contrast has been digitally enhanced. The plates have been pressed after 40 h dispersal at -0.5 kPa.

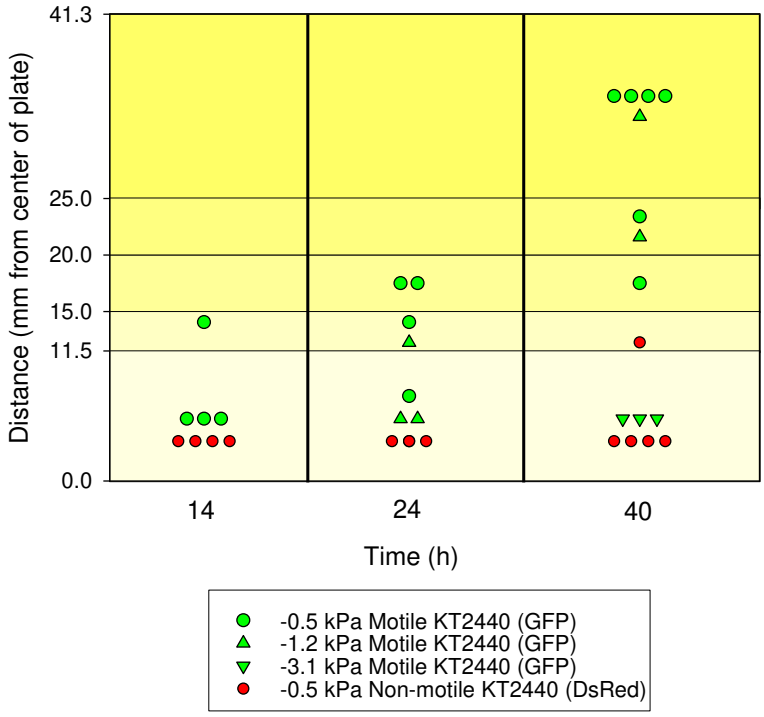


FIG 2 Dispersal dynamics of motile and non-motile strains as affected by the matric potential. The progressive dispersal of the motile strain *P. putida* KT2440 GFP was captured by our method, as well as the inability of the non-motile *P. putida* KT2440 *fliM*⁻ DsRed to disperse away from center of the ceramic disc. Both motile (green) and non-motile (red) strains were tested at three matric potentials (kPa). For the non-motile, only -0.5 kPa is depicted as the other values were similar, with bacteria solely present at the center. The distances shown are ranges, e.g. colonies have been observed on the agar ring at a distance between 11.5 to 15 mm from center. Numbers of replicate dispersal experiments vary from 2 to 5.

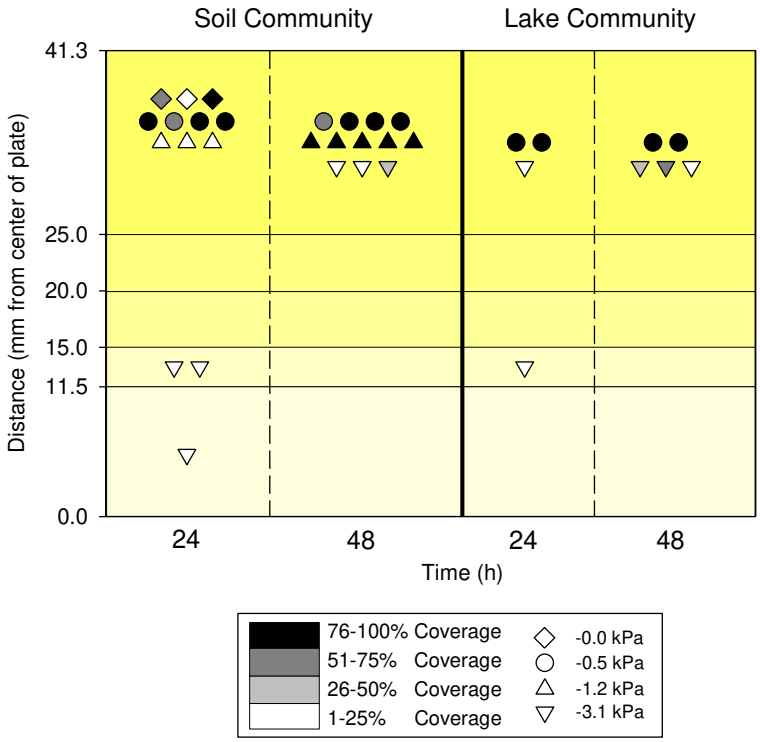
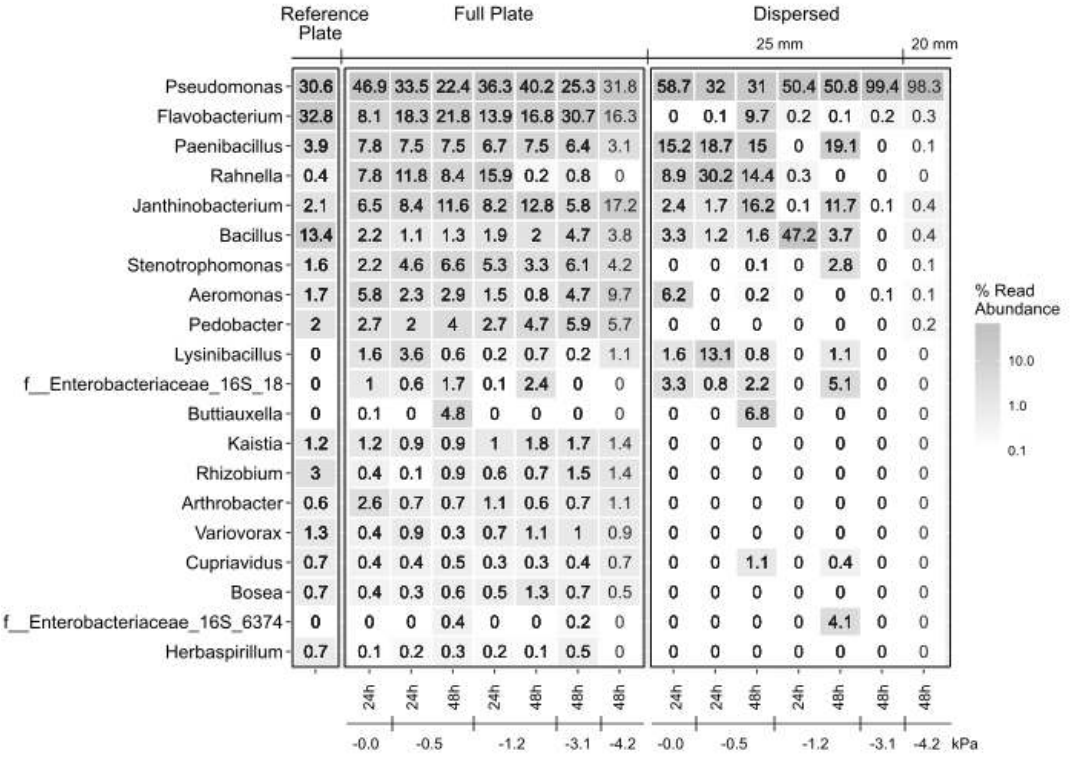


FIG 3 Dispersal of a soil and a lake community over time at different matric potentials. Symbol shading depicts bacterial coverage of the pressed agar plate, giving an indication of the extent of colonization. The lake community was tested at two matric potentials vs four for the soil one; the number of replication varied from two to five.



852
853 **FIG 4**. Heatmap of the relative abundance of the 20 most dominant genera across communities
854 derived from a soil extract and differing in their dispersal after being incubated at prescribed
855 matric potential for 24h or 48h. For 24 h, two additional matric potentials of 0.0 kPa and -4.2 kPa
856 (only one sample recovered at 20 mm) were added. Columns present the average of triplicate
857 communities, except for the motility restricted control (Reference Plate; n=4), the total
858 community on the Full Plate at -4.2 kPa (n= 1) and the fastest dispersed community at -1.2 for 24 h
859 (n=2) and at -3.1 kPa (n=2), and -4.2 kPa (n=1) for 48 h.

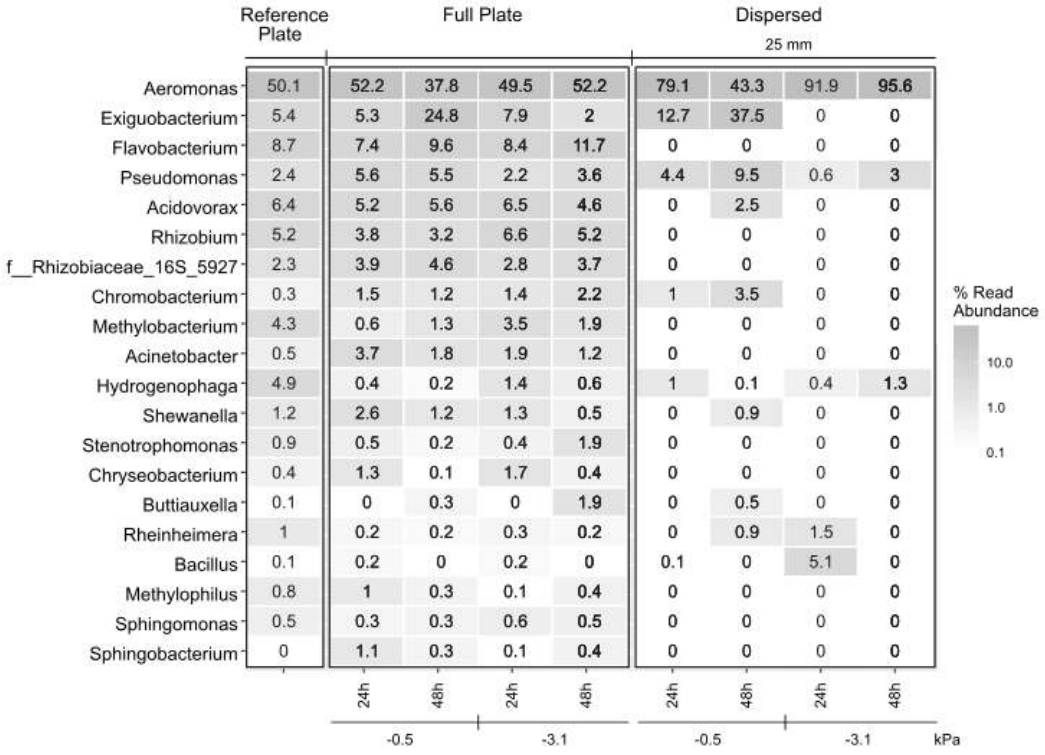
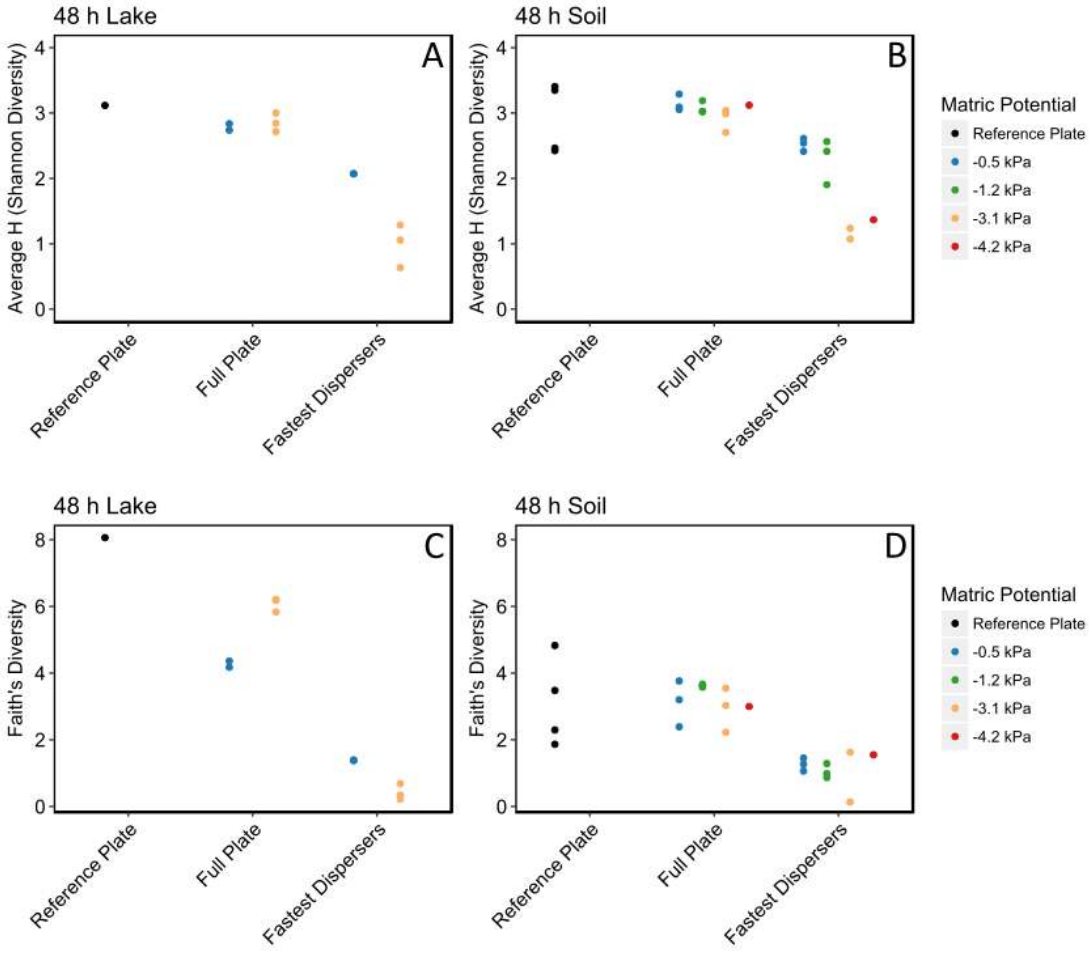


FIG 5 Heatmap of the relative abundance of the 20 most dominant genera across communities derived from a lake filtrate and differing in their dispersal after being incubated at prescribed matric potential for 24 h or 48 h. Columns present the average of duplicate communities, except for the motility restricted control (Reference Plate, n=1), the total community on the Full Plate at -3.1 kPa (n= 3) for 48 h and the fastest dispersed community at -3.1 kPa for 24 h (n=1).



868

869 **FIG 6** Estimates of alpha-diversity (Shannon Diversity Index and Faith´s Phylogenetic Diversity
870 index) for communities derived from soil or from a lake, after 48 hours incubation at prescribed
871 matric potentials. For each matric potential, the total community recovered from the full agar
872 plate (Full Plate) and the fastest dispersed community is presented. A motility restricted control
873 (Reference Plate) is also included. Replicates are depicted as separate dots. The Faith´s
874 Phylogenetic Diversity indices reported are the average of values obtained for 10 random
875 rarefactions.

876