



Bacterial dispersers along preferential flow paths of a clay till depth profile

Krüger, U. S.; Dechesne, Arnaud; Bak, F.; Badawi, N.; Nybroe, O.; Aamand, J.

Published in:
Applied and Environmental Microbiology

Link to article, DOI:
[10.1128/AEM.02658-18](https://doi.org/10.1128/AEM.02658-18)

Publication date:
2019

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Krüger, U. S., Dechesne, A., Bak, F., Badawi, N., Nybroe, O., & Aamand, J. (2019). Bacterial dispersers along preferential flow paths of a clay till depth profile. *Applied and Environmental Microbiology*, 85(6), [e02658-18]. <https://doi.org/10.1128/AEM.02658-18>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 **Bacterial dispersers along preferential flow paths of a clay till depth**
2 **profile**

3 Authors: U. S. Krüger^{1,2}, A. Dechesne³, F. Bak^{1,2}, N. Badawi¹, O. Nybroe², J. Aamand¹

4 ¹Geological Survey of Denmark and Greenland, Copenhagen, Denmark

5 ²University of Copenhagen, Department of Plant and Environmental Sciences, Copenhagen,
6 Denmark

7 ³Technical University of Denmark, Department of Environmental Engineering, Lyngby, Denmark

8 Corresponding authors: Urse Scheel Krüger: usk@geus.dk and Jens Aamand: jeaa@geus.dk

9

10

11

12

13

14

15

16

17

18

19

20 **ABSTRACT**

21 This study assessed the dispersal of five bacterial communities from contrasting compartments
22 along a fractured clay till depth profile comprising plow layer soil, preferential flow paths
23 (biopores and the tectonic fractures below) and matrix sediments, down to 350 cm below the
24 surface. A recently developed expansion of the porous surface model (PSM) was used to capture
25 bacterial communities dispersing under controlled hydration conditions on a soil-like surface. All
26 five communities contained bacteria capable of active dispersal under relatively low hydration
27 conditions (-3.1 kPa). Further testing of the plow layer community revealed active dispersal even
28 at matric potentials of -6.3 to -8.4 kPa, previously thought to be too dry for dispersal on the PSM.
29 Using 16S rRNA gene amplicon sequencing, the dispersing communities were found to be less
30 diverse than their corresponding total communities. The dominant dispersers in most
31 compartments belonged to the genus *Pseudomonas* and, in the plow layer soil, to *Rahnella* too. An
32 exception to this was the dispersing community in the matrix at 350 cm below the surface, which
33 was dominated by *Pantoea*. Hydrologically connected compartments shared proportionally more
34 dispersing than non-dispersing amplicon sequence variants (ASVs), suggesting that active dispersal
35 is important for colonizing these compartments. These results highlight the importance of
36 including soil profile heterogeneity when assessing the role of active dispersal, and contribute to
37 discerning the importance of active dispersal in the soil environment.

38

39

40

41

42 **IMPORTANCE**

43 The ability to disperse is considered essential for soil bacteria colonization and survival, yet very
44 little is known about the dispersal ability of communities from different, heterogeneous soil
45 compartments. An important factor for dispersal is the thickness and connectivity of the liquid film
46 between soil particles. The present results from a fractured clay till depth profile suggest that
47 dispersal ability is common in various soil compartments and that most are dominated by a few
48 dispersing taxa. Importantly, an increase in shared dispersers among the preferential flow paths of
49 the clay till suggests that active dispersal plays a role in the successful colonization of these
50 habitats.

51

52

53 **KEYWORDS**

54 Community motility, liquid film, preferential flow paths, soil, succession

55

56

57

58

59

60

61

62

63 **Introduction**

64 Bacterial dispersal in soil has long been considered an important topic of study for microbiologists
65 in various contexts such as bioremediation, ecology, plant protection and community dynamics
66 (1–5). While these studies provide essential insights, they are mostly based on observations from
67 pure culture studies, leaving much still unknown about dispersal in natural soil communities.

68 Bacteria disperse either passively, *e.g.* by random movement (Brownian motion), transport on
69 plant roots or with water flow, or actively, which requires energy, often using dedicated cellular
70 appendages such as flagella (2, 6–8). In recent studies, there is also an increasing awareness of the
71 potential for cooperative dispersal strategies such as cargo transport of nonmotile bacteria by
72 motile bacterial swarms (3, 9) or interkingdom cooperation with dispersal facilitated by fungi or
73 amoeba (10–13). However, methods for assessing dispersal ability of complex bacterial
74 communities under conditions relevant to soil have only lately become available (14, 15).

75 Bacteria are aquatic organisms by nature and require an aquatic environment for their life
76 functions (16). In soil, water is also crucial to dispersal because bacterial cells generally need to be
77 fully immersed in liquid to move (2, 17). As a consequence, bacterial dispersal in soil is limited to
78 microhabitats that are interconnected by water pathways, such as the liquid films between soil
79 particles (2, 7). This makes soil water a key factor in bacterial dispersal and consequently in
80 bacterial survival and community diversity. Indeed, connectivity, or more accurately the lack of it,
81 is important for maintaining the huge microbial diversity found in the heterogeneous soil
82 environment (2, 18–21). Connectivity in soil can be considered at different scales, from a
83 microscale at which a single bacterium operates to a macroscale, *e.g.* an agricultural field.

84 At the macroscale, the flow of water in well-structured soils is mainly restricted to preferential
85 flow paths, closely connecting some parts of the soil profile while leaving others isolated (7, 22,
86 23). A “text-book” example of connectivity at the macroscale is agricultural clay tills, where most
87 of the water, primarily from rainfall, moves from the plow layer through preferential flow paths
88 towards groundwater reservoirs. These preferential flow paths comprise a complex system of
89 biopores (mainly earthworm burrows and plant root channels) that are connected to tectonic
90 fractures in deeper layers (22, 24, 25).

91 In clay till, preferential flow paths are fairly well characterized from a geological perspective (24–
92 27), particularly as a result of their potential importance in the leaching of pesticides and other
93 contaminants to groundwater (28). However, from a microbial perspective, much is still unclear.
94 Soils separated by a few meters may have very different community structures (2, 6). Indeed,
95 communities separated by as little as few millimeters can vary in composition, activity, and
96 function, *e.g.* the potential for degradation of pesticides (6, 20, 29). This spatial influence on
97 bacterial communities may be pronounced in clay tills, where the soil profile can be viewed as
98 consisting of spatially isolated compartments, and fracture surfaces and matrix sediment for
99 example, which provide bacterial habitats with vastly different physical and chemical compositions
100 (24–27, 30). These varying conditions can select for different bacteria, leading to differences in
101 community composition (31). Dispersal has the potential to redistribute bacteria and spatially
102 homogenize community composition. While preferential flow paths can be a major route for the
103 passive transport of bacteria through soil (7, 16, 32, 33), the contribution of active dispersal to
104 community assembly processes in soil and sediments has not been explored.

105

106 One of the most important factors potentially limiting active dispersal in soil and deeper
107 sediments is fluctuating matric potentials and the subsequent loss of connectivity at the
108 microscale, as has been shown in pure culture studies that have highlighted the negative effect of
109 increasingly thin liquid films on flagella-mediated dispersal (18, 34, 35). However, these limitations
110 might not apply to the same extent to the dispersal of environmental communities. Using the
111 novel and expanded PSM method to study bacterial dispersal under controlled hydration
112 conditions on a soil-like surface, Krüger *et al.* (2018) found that part of environmental
113 communities were able to disperse even under conditions previously thought too dry for dispersal
114 (14, 18, 35). According to their observations, rapid dispersal was possible even at a matric
115 potential of -4.2 kPa, but the community response to increasingly negative matric potentials, and
116 thus decreased liquid film thickness and connectivity, have not been investigated beyond that
117 point.

118 In the present study, the aim was to assess the dispersal potential of bacterial communities from
119 five compartments of a well-defined agricultural soil profile covering the plow layer, deeper
120 preferential flow paths (biopores and tectonic fractures) and adjacent matrix sediments. It was
121 hypothesized that: 1) a sub-community of efficient dispersers is present in each compartment, and
122 2) these bacteria are frequently shared between hydraulically connected compartments.
123 Furthermore, the effect of low matric potential, and thus low liquid film thickness, on dispersal of
124 a plow layer soil bacterial community was studied and it was hypothesized that: 3) only a fraction
125 of the motile community is able to disperse at low hydration conditions.

126

127

128 Results

129 Bacterial communities recovered from the soil profile

130 This study assessed the dispersal of five bacterial communities extracted from five different
131 compartments of a well-defined clay till depth profile (Fig. 1). A newly developed method, the
132 extended porous surface model (PSM), was used in which agar plate imprints are used to reveal
133 the spatial spreading of bacterial communities on a rough hydrated surface resembling soil. This
134 method allows for the recovery and characterization of both the dispersing bacteria and the total
135 community, recovered respectively by pressing a hollowed-out agar plate or a 'full' agar plate onto
136 the PSM surface. The total bacterial communities from the five soil and sediment compartments
137 clearly separated into five clusters on the non-metric multidimensional scaling (NMDS) plot of the
138 community composition from 16S rRNA amplicon sequence data. This was confirmed by
139 PERMANOVA analysis on Bray-Curtis dissimilarities, where 56 % of the variance could be explained
140 by soil compartment ($p < 0.001$) (Fig. 2). Heatmaps and Venn diagrams of the amplicon sequence
141 variants (ASVs) (36) of the total communities also illustrate the different community compositions
142 (Fig. S1- S6). Comparisons between the original soil community, the inoculum (Nycodenz
143 extractions) and the cultivable communities on the full plates and reference plates (inoculum
144 placed directly on agar plate) confirm the expected cultivation bias (Fig. S7 and S8). Yet, in general,
145 the total cultivable communities retained a high level of diversity, with representatives of 109
146 unique genera (belonging to 5 different phyla), across all compartments plus 161 ASVs that were
147 not identifiable at the genus level (Table S1). 27.7 % and 8.7 % of the genera in abundance >0.1 %
148 in the biopores and plow layer soil communities, respectively, were recovered on the full plates
149 pressed onto the PSM incubated for 48h at -3.1 kPa. Similar values were observed for the -0.5 kPa

150 24 h samples (Table S2). Additionally, the ASVs found on the full plates represented 10 % and 1 %
151 of the original community from the biopores and plow layer soil (Table S3). This signifies that the
152 applied method was able to recover a substantial part of the diversity present in the original soil
153 communities.

154 The genera *Pseudomonas*, *Flavobacterium* and *Pedobacter* dominated the total communities of
155 the plow layer, biopores, and matrix at 80-120 cmbs (cm below surface) (Fig. S1- S3), while in the
156 fracture community at 300-350 cmbs, *Flavobacterium* was replaced by *Arthrobacter* (Fig. S4). The
157 community from the deep matrix sediment at 300-350 cmbs was dominated by the genus
158 *Pantoea*, followed by *Pseudomonas*, *Chryseobacterium* and *Stenotrophomonas* (Fig. S5). The
159 moisture conditions on the PSM model (-0.5 and -3.1 kPa) had only a minor influence on the total
160 bacterial community composition, contributing just 7 % of the variation in the PERMANOVA
161 analysis of Bray-Curtis dissimilarities ($p < 0.001$) (Fig. 2 and Fig. S1-5). In conclusion, the soil
162 communities recovered from the PSM were distinctly different, although they shared some
163 dominant genera.

164

165 **Community dispersal potential and identity of major dispersers**

166 Rapid dispersal of bacteria was observed for all soil and sediment communities in wet conditions (-
167 0.5 kPa). Except for the plow layer community, there was a clear tendency towards slower
168 dispersal and lower surface coverage scores in dry conditions (-3.1 kPa) compared to wet
169 conditions, indicating dispersal limitation in dry conditions (Fig. 3).

170 Using 16S rRNA gene amplicon sequencing, the dispersing bacteria from the extracted soil and
171 sediment communities were identified. The composition of these dispersing communities was

172 then compared to the total bacterial communities. Both the Shannon diversity and Faith's
173 phylogenetic diversity indices showed that the total communities were more diverse than the
174 dispersed communities, and that the dispersers had a narrow phylogenetic diversity (Fig. S9 and
175 S10). The Shannon diversity index also revealed a lower diversity in dry (-3.1 kPa) compared to wet
176 conditions for all dispersed communities, except for the matrix sediment at 80-120 cmbs, where a
177 high variation between replicates was seen. Dispersing bacteria predominantly belonged to the
178 genus *Pseudomonas* in all but one community at -0.5 kPa. Additionally, under these wet
179 conditions, the plow layer and the biopore dispersers shared a high relative abundance of
180 *Rahnella*, *Paenibacillus*, *Lysinibacillus* and *Kluyvera* (Fig. 3). In dry conditions, *Pseudomonas* almost
181 completely dominated the dispersed communities, except for the matrix soil at 300-350 cmbs.
182 Here *Pantoea* was dominant at -0.5 kPa, while at -3.1 kPa *Pantoea* and *Pseudomonas* were
183 represented equally. In general, the dominant disperser genera were also represented in the total
184 community, but they were greatly enriched in the disperser communities.

185

186 On an NMDS plot (Fig. S11 and S12) the dispersed communities separated from the total
187 communities, as confirmed by PERMANOVA analysis on Bray-Curtis dissimilarities, explaining 9 %
188 and 11 % of the variance for -0.5 and -3.1 kPa (all $p < 0.001$) respectively. However, the strongest
189 effect was still attributed to the compartment type, explaining 47 % and 28 % of the variance in
190 wet and dry conditions. Additionally, there was a significant, but moderate, interaction between
191 dispersed/total community and soil compartment (14 %, $P < 0.001$, for -0.5 kPa, 24 h and 15 %,
192 $P < 0.001$, for -3.1 kPa, 48 h respectively). Significant differences in homogeneity between the
193 dispersed and total communities was found using Betadisperser followed by ANOVA, which tested

194 whether the dispersion of a group from its median was different from the dispersion of other
195 groups ($F=5.9$, $P<0.05$, for -0.5 kPa 24 h and $F=7.1$, $P<0.01$ for -3.1 kPa 48 h). Hence, the
196 dispersed communities had a significantly greater variation than the total communities, indicating
197 a stochastic element in the identity of the bacterial dispersers.

198

199 **Connectivity of dispersing communities from preferential flow paths and matrix**

200 A closer look at the ASVs in the dispersed communities (Fig. 4 A, B and Tables S4-S5) revealed
201 many ASVs shared between the plow layer, biopores and fracture communities. The number of
202 shared ASVs was generally much higher in wet conditions than in dry conditions. The most
203 common genus amongst the shared dispersers between the three communities of the plow layer,
204 biopores and fractures under both hydration conditions was *Pseudomonas* (10 shared ASVs at -0.5
205 kPa and -3.1 kPa), but *Buttiauxella* was also represented (1 shared ASV at -0.5 kPa and -3.1 kPa).
206 The one ASV shared between all compartments under wet conditions belonged to the genus
207 *Pseudomonas*.

208

209 Comparing the percentages of shared ASVs between dispersing and non-dispersing bacteria from
210 the preferential flow paths also revealed a very clear picture of the dispersers being more shared
211 than non-dispersers (Table 1). It should be noted that what are referred to as “non-dispersers” are
212 actually the ASVs in the total communities minus the ASVs observed among the dispersers. This
213 group may therefore also contain some slow dispersers, which were not quick enough to be
214 detected among the dispersers. The proportion of shared dispersers was significantly higher than
215 the proportion of shared non-dispersers in the preferential flow paths for both wet and dry

216 conditions, although in dry conditions this was only significant for the shared communities
217 between the plow layer and fractures. In contrast to the greater sharing of dispersers along the
218 preferential flow paths, there was no significant preferential sharing of dispersers between the
219 preferential flow paths and the adjacent matrix sediments. Indeed, in some cases there was even
220 greater sharing of non-dispersing ASVs between these compartments, as was also the case for
221 vertical sharing between the matrix 80-120 cmbs and matrix 300-350 cmbs sediment
222 communities.

223

224 **Response to increasingly negative matric potentials**

225 To test the effect of increasingly negative matric potentials on the dispersal ability of a soil
226 community, the community extracted from the plow layer soil was exposed to matric potentials
227 from -0.5 to -8.4 kPa. Interestingly, dispersal was seen even in the driest conditions, but the
228 Shannon diversity index of the dispersing community decreased as the conditions became dryer
229 (Fig. 5), with only very few genera present in dry conditions (Fig. 6 and Fig. S13). Furthermore,
230 Faith's diversity index extended the previous results, showing that the narrow phylogenetic
231 distribution of the dispersed communities became narrower in even dryer conditions (Fig. S14).

232 In general, dispersal was increasingly restricted at matrix potentials of -4.1 kPa and below (Fig. 6),
233 with no replicates dispersing beyond the 15-20 mm section for matric potentials of -6.1 and -
234 8.4 kPa. An element of randomness seemed to be involved in the identity of the dispersers at -
235 4.1 to -8.4 kPa, with *Pseudomonas* still being prominent, but in some replicates *Cupriavidus*,
236 *Bacillus*, or *Pseudoduganella* were also dominant dispersers.

237 The element of randomness at decreased matric potentials was also supported by visual
238 observations of the colonization patterns on the surface of the agar plates (Fig. S15). While at -0.5
239 and -3.1 kPa the patterns were characterized by a relatively uniform spread of bacteria from the
240 inoculation point to the edge of the plate at -6.3 and -8.4 kPa, dispersal was limited to a few
241 corridors.

242

243 **Discussion**

244 **Dispersal potential and disperser identity in communities from fractured clay till**

245 While the role of passive transport is well established (2, 32, 33), the importance of active
246 dispersal in soil has been debated for many years (2, 3, 7, 17). Until recently there was no
247 experimental platform to screen for active dispersal at the community level (14, 15). The present
248 study assessed the dispersal of five bacterial communities from matrix sediments and preferential
249 flow paths of a clayey till.

250 The soil and sediment compartments studied here harbored different bacterial communities,
251 reflecting the very heterogeneous nature of clay till profiles and confirming the existence of
252 distinct compartments (30, 31). Dispersing bacteria were found in the plow layer and in all deeper
253 sediments, pointing to the importance of active dispersal in these environments, though these
254 dispersers were not dominant in the total communities (14, 15). *Pseudomonas* was the dominant
255 disperser in the top soil, in agreement with results from the few comparable studies available (14,
256 15). Members of the *Pseudomonadaceae* family have also been found to be early colonizers of
257 plant litter in an agricultural field, also suggesting that *Pseudomonas* is a key disperser in the soil

258 environment (37). In the literature, pseudomonads are known to be efficient dispersers,
259 employing various adaptations such as swimming, swarming and sliding motility (35, 38–40).
260 Additionally, it has been suggested that the ability of *Pseudomonas* to disperse even in dry
261 conditions could be linked to their ability to produce biosurfactants, which can facilitate surface
262 dispersal, especially in fluctuating hydration conditions (14, 41–43). However, the production of
263 surfactants might be linked to specific habitats such as the rhizosphere (41, 44), and the presence
264 of surfactants would therefore have to be proven in soil-like conditions.

265 In general, the dispersing bacterial communities of the soil and sediment compartments had a
266 narrow phylogenetic distribution and many dispersing taxa were shared between the
267 compartments. These dispersers had several genera in common with dispersers from other plow
268 layer soils (14, 15). Besides *Pseudomonas*, these were *Paenibacillus*, *Flavobacterium* and
269 *Janthinobacterium*, as well as *Rahnella* and *Pantoea*, the latter two belonging to the
270 enterobacteria, a group identified as the most abundant disperser in a previous study (15). While
271 *Pseudomonas* flagellar swimming dispersal and *Flavobacterium* gliding dispersal on surfaces have
272 been studied extensively, mainly in pure cultures (18, 35, 45–48), there needs to be a greater
273 focus on the mode of dispersal of other genera, e.g. *Pantoea*, which was found to be dominant in
274 the deep matrix sediment at 300-350 cmbs in the present study.

275 Dispersal rates were severely inhibited in conditions dryer than -3.1 kPa, because smaller liquid
276 film thickness on the ceramic surface prevent active dispersal, as previously demonstrated for
277 both pure bacterial cultures (18, 35) and soil and lake microbial communities (14).

278

279

280 **Bacterial dispersers in preferential flow paths *versus* matrix sediments**

281 The high percentage of shared dispersers between the communities derived from the preferential
282 flow path compartments compared to the matrix sediments indicated that the interchange of
283 dispersing genera is more common along hydrologically connected compartments. This concept is
284 illustrated in Figure 7. As the preferential flow paths are enriched with dissolved nutrients, oxygen
285 and organic carbon transported from the surface by the flow of water (27, 49, 50), they can
286 provide an attractive habitat for soil bacteria. While it has previously been shown that preferential
287 flow paths have the potential to be a major route for the passive transport of bacteria (7, 16, 32,
288 33), the current results support the notion that part of the bacterial communities in the flow
289 paths can also take advantage of active dispersal to spread through and colonize these habitats.
290 This was especially interesting as the benefit of active motility in the presence of water flow was
291 not obvious. Indeed, it might have been expected that the benefit of active motility would be
292 more prominent in the matrix soil where flow is absent.

293

294 The limited number of shared dispersers between the flow paths and the adjacent matrix
295 sediments, especially at 300-350 cmbs (Table 1), may be due to limited connectivity. Given the
296 small particle size of clay particles ($< 2\mu\text{m}$), the pore space in dense clay sediment is generally very
297 small (7), probably impeding bacterial dispersal. Indeed the porosity of the clay till at the current
298 study site decreases with depth (51). Although some bacterial pure cultures are known to be able
299 to swim through apertures as small as $1.1\ \mu\text{m}$ (52), the small pore size and low connectivity of
300 matrix clay tills likely form a barrier to the exchange of bacteria, in particular at 300-350 cmbs. For
301 comparison, deep fractures are reported to have apertures of $100\ \mu\text{m}$ (22) and biopores may have

302 diameters of 8-10 mm, leaving ample space for bacterial dispersal subject to the presence of
303 sufficient liquid films. Furthermore, fractures may be coated with metal oxide precipitates such as
304 iron oxides, which can be almost impermeable to water (27, 53) and therefore probably also a
305 hindrance for bacterial dispersal.

306 It is tempting to speculate that bacterial communities of dense clay matrixes are islands that have
307 little contact with nearby communities. Water percolation through clay till matrix sediments is
308 very limited (22, 23), thereby providing little input of nutrients and organic carbon to the bacteria.
309 The low amount of nutrients in deep sediments (54) may also inhibit active dispersal as nutrient
310 limitation can greatly decrease the fraction of motile bacteria (55). We recognize that the method
311 applied in this study is limited to the fraction of bacteria able to grow under the selected growth
312 conditions excluding contribution of microbes that cannot be active under the conditions of our
313 assay (e.g. strict anaerobes). Nonetheless, while the current study was limited to exploring
314 subgroups of the total diversity of soil bacteria present along the preferential flow paths of a clay
315 till depth profile we believe that we are uncovering important processes relating dispersal
316 potential and connectivity in the heterogeneous soil environment. The observed patterns of
317 intensified cell exchange affected by dispersal potential and soil compartment should apply to
318 other bacteria as well as the principles of soil physics will apply irrespective of bacterial taxonomy.

319

320 **Dispersal at low matric potentials**

321 In unsaturated soil, low matric potentials are known to negatively affect bacterial dispersal (2, 18,
322 35). Here, the matric potential on the PSM was extended to -8.4 kPa, the lowest possible without
323 using a pressurized version of the PSM (56), in order to investigate how low hydration conditions

324 affect active dispersal of soil bacterial communities. The finding of active dispersal even at -8.4
325 kPa, albeit at a decreased rate, was surprising because recent measurements of liquid films on the
326 PSM have shown rapid thinning and disconnection of the liquid films at matric potentials
327 exceeding -2.0 kPa (18), causing severe inhibition of dispersal, as demonstrated for bacterial pure
328 cultures (18, 35, 46). However, due to residual surface roughness on the PSM it is still possible to
329 observe rare thicker liquid films ($\geq 5 \mu\text{m}$) at -2.0 kPa (18). Visual analysis of the agar plates
330 suggested that dispersal at the lowest matrix potentials of -6.3 to -8.4 kPa occurred along a few of
331 such narrow liquid film corridors on the rough surface. At a decreased matric potential (-4.1 to -8.4
332 kPa), *Bacillus* and *Pseudoduganella* rather than *Pseudomonas* were major dispersers in some
333 replicates, indicating a stochastic element with regards to which bacteria disperse when water film
334 thickness becomes limited. Due to the complex heterogeneous nature of soil we speculate that
335 there could also be some open dispersal corridors available in natural soil even under relatively
336 dry conditions. One known option is the use of the thin liquid films surrounding fungal hyphae (i.e.
337 fungal highways) (57, 58). It has been suggested that the abundance of mycelial networks in soil is
338 part of the explanation for the maintenance of the otherwise costly flagella in soil bacteria (11,
339 59).

340

341 It has been claimed that active motility is limited in soil mainly due to dry and unsaturated
342 conditions, which confines active dispersal to transient wet periods, *e.g.* during rain events (2, 7,
343 21). These claims have been supported in part by experimentation done using the porous surface
344 model, showing that bacterial flagellar motility is restricted to a narrow range of high matric water
345 potentials (18, 35). While the relationship between matric potential on and liquid film thickness on

346 the PSM differs from that in soil, it is relevant to ascertain if the range of matric potentials found in
347 soil is compatible with flagella powered swimming. According to data from the Danish Pesticide
348 Leaching Assessment program (PLAP) (60, 61) in fractured clay till, which is a common soil type in
349 Denmark (26), the matric potential of Danish agricultural top soils can fluctuate between -5 and -
350 1500 kPa while deeper clayey matrix sediments (from 60 cmbs and down) remain water-saturated
351 (~ 0 kPa) most of the time (61–63). There should therefore be sufficient liquid films in subsurface
352 clay till to allow active dispersal unless low pore connectivity and fracture coatings create physical
353 barriers that cannot be overcome.

354

355 **Conclusions**

356 This study demonstrated that different compartments of a heterogeneous clay till depth profile
357 harbor bacterial communities that are capable of dispersing in low hydration conditions. The
358 dispersers show narrow phylogenetic diversity and are dominated by pseudomonads and
359 enterobacteria. Active dispersal occurred even within thin and poorly-connected liquid films on the
360 surface of the PSM at matric potentials of -6.3 to -8.4 kPa. These results indicate that active
361 dispersal ability is widespread in soil and sediment communities. An increased proportion of
362 disperser ASVs shared between highly connected compartments (*e.g.* preferential flow paths)
363 points to a role for active dispersal in the spread through, and colonization of, these habitats.
364 Fewer shared disperser ASVs between the preferential flow paths and the matrix sediments
365 illustrated that low porosity of clay tills and metal oxide-coated fracture walls might be barriers to
366 the exchange of bacteria, leaving matrix bacterial communities relatively isolated.

367

368 **Materials and methods**

369 **Soil sampling**

370 Soils were sampled over a three-day period in September 2016 from an agricultural field (Anthric
371 Luvisol) in Lund, Denmark (55°14'49"N, 12°17'24"E) (51). The adjacent field has recently been
372 included in the Danish Pesticide Risk Assessment Program (PLAP)(51, 64). The soil is characterized
373 by clay till and boulder clay, with a very pronounced fracture system down to at least 6 m depth.
374 While the biopores, dominating the top 150 centimeters below the surface (cmbs), mainly consist
375 of earthworm burrows and decayed root channels, the fractures below are mainly of tectonic
376 origin.

377 A multi-bench excavation down to 6 m depth allowed the sampling of sediment from different
378 depths. Soil was sampled from the plow layer (0-20 cmbs), biopores (80-120 cmbs), matrix
379 sediment next to the biopores (80-120 cmbs), oxidized iron-rich red fractures (300-350 cmbs) and
380 matrix sediment next to these fractures (300-350 cmbs) (Fig. 1). Soils were collected as composite
381 samples, *i.e.* as small subsamples combined into one pooled sample for each of the five soil and
382 sediment compartments. One composite sample equaled *ca.* 15-30 subsamples per soil
383 compartment, except for the biopore samples, which consisted of *ca.* 70 subsamples. The
384 subsamples were combined into one composite sample per compartment to ensure sufficient soil
385 from biopores and fractures for further analysis. Samples were secured by carefully removing the
386 outer layer of the soil profile with a knife to avoid cross contamination. Hereafter the freshly
387 exposed soil and sediment were subsampled (carefully scraped off) with a small spoon and stored
388 at 5 °C.

389

390 **Extraction of soil bacteria**

391 The soil and sediment samples from each compartment were homogenized by sieving (2 mm), and
392 mass reduction for laboratory subsampling was performed by bed blending, as described in the
393 “Representative Sampling Horizontal Standard” (65) and by Kardanpour *et al.* (2015) (66). This
394 resulted in 25 g composite soil or sediment sample for each experimental setup.

395 The soil bacterial community from each compartment was extracted using Nycodenz density
396 gradient centrifugation as in (67), except for the final cell density determination, which was
397 performed directly using a Thoma counting chamber. Cell densities of the extracts were adjusted
398 to 0.8×10^6 cells μl^{-1} in 0.9 % NaCl solution. The soil bacterial extracts were kept at 4 °C overnight
399 before inoculation on the ceramic discs of the extended porous surface model system.

400

401 **Dispersal potential of environmental communities using the extended porous surface model**

402 An extended version of the porous surface model (PSM) (14) was used, where the original PSM
403 model (46) had been further developed to encompass the dispersal of non-fluorescent complex
404 communities extracted from environmental samples. The method allows communities to disperse
405 under controlled hydration conditions from the center of a porous ceramic disc (diameter =
406 41.3 mm, thickness = 7.1 mm, maximum pore size <1.5 μm , 1 bar bubbling pressure; Soilmoisture
407 Equipment Corp., Santa Barbara, USA), mimicking a rough soil surface. Imposing suction on the
408 ceramic disc allows for precise control of the liquid film thickness on its surface. The liquid medium
409 used in the PSM was 25 % R2B (Alpha Biosciences, Baltimore, MD). Each experimental setup
410 allowed for the parallel incubation of 9-11 PSMs.

411 Each PSM was inoculated with 10 μl of bacterial extract placed as 1 μl drops at the center of the
412 ceramic disc. Although the inocula for the PSM were adjusted to the same cell densities according
413 to Thoma counts, the cultivable fraction was generally lower in deep sediment samples compared
414 to plow layer and biopore soil. CFU numbers were highest in the plow layer (*ca.* 11,250 CFU) and
415 biopores (130,000 CFU), and decreased in the matrix at 80-120 cmbs (1,000 CFU), red fractures
416 (1,125 CFU) and matrix sediment from 300-350 cmbs (750 CFU). Colonies were enumerated on
417 25 % R2A plates (Fluka R2A; Sigma-Aldrich, St. Louis, MO) after incubation at 25 °C for 48-72 hours.
418 All plates were amended with 100 mg l^{-1} Delvocid to inhibit fungal growth (Natamycin, DSM food
419 specialties, Delft, The Netherlands).

420 After inoculation, the discs were brought to matric potentials of -0.5 or -3.1 kPa and incubated at
421 room temperature for 24 or 48 hours before sampling. After incubation, the bacteria were
422 recovered from the surface of the ceramic disc by means of an agar plate lift. This is described in
423 detail in Krüger *et al.* (14) In brief, to visualize the colonization on the ceramic disc, a series of agar
424 plates were used to cover different sections of the ceramic surface. The agar plate series consisted
425 of small flat 25 % R2A plates containing 20 g agar l^{-1} (StarTMDish diameter, 40 mm; height,
426 12.5 mm; Phoenix Biomedical Products, Mississauga, Canada), with holes in four sizes. Sampling
427 was achieved by starting with the plate with the largest hole size, 25 mm, followed by 20, 15,
428 11.5 mm, and ending with the pressing of a full agar plate (full plate). The extent of colonization of
429 the ceramic disc was quantified by evaluating the coverage of bacterial growth on the individual
430 agar plates, after 72 h incubation at 25 °C, and dividing it into four categories: 1-25, 26-50, 51-75
431 and 76-100 % coverage.

432 For each series of five pressed plates, the fastest-dispersing bacteria from the environmental
433 communities were then identified, *i.e.* the colonies of the pressed agar plate the furthest from the
434 point of inoculation (the plate with the largest hole size) that presented growth (referred to as the
435 “dispersers” or “dispersing community”), and the total community present on the full agar plates,
436 by 16S rRNA gene amplicon sequencing. The full plate represented the cultivable community
437 developing on an agar plate covering the entire ceramic plate, *i.e.* both dispersing and non-
438 dispersing bacteria. Additionally, for each separate experiment and soil, a no-motility reference
439 plate, shortened to “reference plate”, was made by drop-plating 10 μ l of each inoculum directly
440 onto the center of a small 25 % R2A plate with 20 g agar l⁻¹, which provided conditions that are not
441 conducive for flagellar motility and are not influenced by the PSM (34). All bacteria were washed
442 off the agar plates using 0.9 % NaCl following the procedure described by Krüger *et al.* (2018) (14).
443 For comparisons with the dispersed communities, total communities present on the full plates
444 were generally preferred, as they captured the double cultivation step (both on the PSM and on
445 the agar plates). However the reference plates were also valuable because they gave an indication
446 of what could be cultivated upon direct inoculation on the agar plates. The cell suspensions from
447 the pressed plates and the reference plates (plate wash) as well as the original Nycodenz extracts
448 were all stored at -80 °C before further processing.

449

450 **Porous surface model with increasingly negative matric potentials**

451 To achieve matric potentials down to -8.4 kPa, the PSM assembly was slightly modified. To limit
452 the amount of air entering the system, the PSM tubing was tightened and partly replaced with
453 stainless steel. To further limit the formation of air bubbles that can form in the medium at

454 lowered matrix potentials, the ceramic plates were degassed for 24 h using a vacuum pump, and
455 the 25 % R2B medium was degassed for 20 minutes in an ultrasound bath. PSMs were assembled
456 submerged in degassed medium.

457

458 **DNA extraction and sequencing**

459 DNA was extracted using the DNeasy Powerlyzer Powersoil kit (Quiagen; Hilden, Germany)
460 following the manufacturer's protocol with a few adjustments, as in Krüger *et al.* (2018). The DNA
461 concentrations were measured on Qubit 2.0 (Life Technologies, Invitrogen; Carlsbad, USA) and
462 samples stored at -80 °C until sequencing. The DNA was PCR-amplified using the primer set 341F
463 (5'-CCTACGGGNGGCWGCAG-3') and 806R (5'-GACTACHVGGGTATCTAATCC-3') (68) targeting the
464 hypervariable V3-V4 regions of bacterial 16S rRNA genes. The purified PCR products (2 x 300-bp
465 reads) were sequenced on the Illumina Miseq platform by Macrogen (Seoul, South Korea).

466 The raw 16S rRNA gene amplicon sequences were processed using the DADA2 pipeline (69) with
467 default parameters. Sequence classification was based on the SILVA prokaryotic reference
468 database version 123 (70). A total of 7.2 million sequences passed the filtering steps, representing
469 an average of 60,500 sequences per sample.

470

471 **Data analysis and statistical methods**

472 Data analysis of sequences and statistics was computed in R (71). The Shannon diversity index was
473 calculated using the "plot_richness" function in the phyloseq package (72). Faith's phylogenetic
474 diversity (PD) was calculated with the "pd.query" function in the PhyloMeasures package (73).

475 Prior to calculating the PD, samples were rarefied to an even depth (mean of 10 iterations) using
476 the “rarefy_even_depth” function in the phyloseq package. Heatmaps were plotted using the
477 “amp_heatmap” function in the ampvis2 package (74). Venn diagrams were plotted using the
478 function “venn” from the gplots package (75). Non-metric multidimensional scaling (NMDS)
479 ordination was undertaken on Bray-Curtis dissimilarities using the “ordinate” function in the
480 phyloseq package. PERMANOVA and analysis of multivariate homogeneity of group dispersions
481 (variances) were computed using the “adonis” and “betadisper” functions in the vegan (2.4-6)
482 package (76), with 999 permutations. Differences in the proportions of shared ASVs between
483 communities were tested using Fisher’s exact test in R (71).

484 Additional statistical analysis was undertaken using Sigmaplot 13 (Systat Software, Inc., San Jose,
485 CA).

486 Differences in Shannon diversity indices between total communities and the fastest dispersers was
487 tested using one-tailed, one sample t-tests (testing for subtracted differences greater than zero).
488 The effects of matric potentials were tested using two-tailed t-tests. P values of < 0.05 were
489 considered significant.

490 **Accession number(s).** All sequencing data have been deposited as an NCBI BioProject under
491 accession number PRJNA483533

492

493 **Acknowledgements**

494 This study was funded by the Villum Kann Rasmussen Foundation through the Center for

495 Environmental and Agricultural Microbiology (CREAM) and by the Independent Research Fund
496 Denmark [5054-00054B]. The authors thank the Pesticide Leaching Assessment Programme for
497 facilitating access to the Lund excavation site.

498

499 **References**

- 500 1. Paul D, Singh R, Jain RK. 2006. Chemotaxis of *Ralstonia* sp. SJ98 towards p-nitrophenol in
501 soil. *Environ Microbiol* 8:1797–1804.
- 502 2. Tecon R, Or D. 2017. Biophysical processes supporting the diversity of microbial life in soil.
503 *FEMS Microbiol Rev* 599–623.
- 504 3. Ben-Jacob E, Finkelshtein A, Ariel G, Ingham C. 2016. Multispecies Swarms of Social
505 Microorganisms as Moving Ecosystems. *Trends Microbiol* 24:257–269.
- 506 4. Schulz-Bohm K, Gerards S, Hundscheid M, Melenhorst J, de Boer W, Garbeva P. 2018.
507 Calling from distance: attraction of soil bacteria by plant root volatiles. *ISME J*.
- 508 5. Deveau A, Bonito G, Uehling J, Paoletti M, Becker M, Bindschedler S, Hacquard S, Hervé V,
509 Labbé J, Lastovetsky OA, Mieszkin S, Millet LJ, Vajna B, Junier P, Bonfante P, Krom BP,
510 Olsson S, Elsas JD van, Wick LY. 2018. Bacterial - Fungal Interactions: ecology, mechanisms
511 and challenges. *FEMS Microbiol Rev* 335–352.
- 512 6. Dechesne A, Badawi N, Aamand J, Smets BF. 2014. Fine scale spatial variability of microbial
513 pesticide degradation in soil: Scales, controlling factors, and implications. *Front Microbiol*
514 5:1–14.

- 515 7. Stotzky G, Bollag J-M. 1992. Soil Biochemistry. Marcel Dekker INC, New York.
- 516 8. Begon M, Harper JL, Townsend CR. 1996. Ecology: Individuals, Populations and
517 Communities, 3rd ed. Blackwell Science.
- 518 9. Finkelshtein A, Roth D, Ben Jacob E, Ingham CJ. 2015. Bacterial Swarms Recruit Cargo
519 Bacteria To Pave the Way in Toxic Environments. MBio 6:1–10.
- 520 10. Warmink JA, Nazir R, Corten B, van Elsas . JD. 2011. Hitchhikers on the fungal highway: The
521 helper effect for bacterial migration via fungal hyphae. Soil Biol Biochem 43:760–765.
- 522 11. Pion M, Bshary R, Bindschedler S, Filippidou S, Wick LY, Job D, Junier P. 2013. Gains of
523 bacterial flagellar motility in a fungal world. Appl Environ Microbiol 79:6862–6867.
- 524 12. Ellegaard-Jensen L, Knudsen BE, Johansen A, Albers CN, Aamand J, Rosendahl S. 2014.
525 Fungal-bacterial consortia increase diuron degradation in water-unsaturated systems. Sci
526 Total Environ 466–467:699–705.
- 527 13. Brock DA, Read S, Bozhchenko A, Queller DC, Strassmann JE. 2013. Social amoeba farmers
528 carry defensive symbionts to protect and privatize their crops. Nat Commun 4:2385.
- 529 14. Krüger US, Bak F, Aamand J, Nybroe O, Badawi N, Smets BF, Dechesne A. 2018. Novel
530 method reveals a narrow phylogenetic distribution of bacterial dispersers in environmental
531 communities exposed to low hydration conditions. Appl Environ Microbiol 84:AEM.02857-
532 17.
- 533 15. Wolf AB, Rudnick M-BB, de Boer W, Kowalchuk GA. 2015. Early colonizers of unoccupied
534 habitats represent a minority of the soil bacterial community. FEMS Microbiol Ecol
535 91:fiv024.

- 536 16. Pepper IL, Gerba CP, Gentry T, Maier RM. 2008. Environmental Microbiology 2nd Edition.
537 Academic Press.
- 538 17. Or D, Smets BF, Wraith JM, Dechesne A, Friedman SP. 2007. Physical constraints affecting
539 bacterial habitats and activity in unsaturated porous media - a review. *Adv Water Resour*
540 30:1505–1527.
- 541 18. Tecon R, Or D. 2016. Bacterial flagellar motility on hydrated rough surfaces controlled by
542 aqueous film thickness and connectedness. *Sci Rep* 6:19409.
- 543 19. Carson JK, Gonzalez-Quiñones V, Murphy D V., Hinz C, Shaw JA, Gleeson DB. 2010. Low pore
544 connectivity increases bacterial diversity in soil. *Appl Environ Microbiol* 76:3936–3942.
- 545 20. Fierer N. 2017. Embracing the unknown: disentangling the complexities of the soil
546 microbiome. *Nat Rev Microbiol* 15:579–590.
- 547 21. Holden PA. 2011. How do the Microhabitats Framed by Soil Structure Impact Soil Bacteria
548 and the Processes that they Regulate?, p. 118–148. *In* Ritz, K, Young, I (eds.), *The*
549 *Architecture and Biology of Soils: Life in Inner Space*. CAB International.
- 550 22. Rosenbom AE, Therrien R, Refsgaard JC, Jensen KH, Ernstsens V, Klint KES. 2009. Numerical
551 analysis of water and solute transport in variably-saturated fractured clayey till. *J Contam*
552 *Hydrol* 104:137–152.
- 553 23. Jørgensen PR, Hoffmann M, Kistrup JP, Bryde C, Bossi R, Villholth KG. 2002. Preferential flow
554 and pesticide transport in a clay-rich till: Field, laboratory, and modeling analysis. *Water*
555 *Resour Res* 38:28-1-28–15.
- 556 24. Allaire SE, Roulier S, Cessna AJ. 2009. Quantifying preferential flow in soils: A review of

- 557 different techniques. *J Hydrol* 378:179–204.
- 558 25. Klint KES, Abildtrup CH, Gravesen P, Jacobsen PR, Vosgerau H. 2001. Sprækkers oprindelse
559 og udbredelse i moræneler. *Vand og Jord* 8:111–119.
- 560 26. Klint KES, Gravesen P. 1999. Fractures and biopores in Weichselian clayey till aquitards at
561 Flakkebjerg, Denmark. *Nord Hydrol* 30:267–284.
- 562 27. Rosenbom AE, Ernstsens V, Flühler H, Jensen KH, Refsgaard JC, Wydler H. 2008. Fluorescence
563 Imaging Applied to Tracer Distributions in Variably Saturated Fractured Clayey Till. *J Environ*
564 *Qual* 37:448.
- 565 28. Jørgensen PR, Klint KES, Kistrup JP. 2003. Monitoring Well Interception with Fractures in
566 Clayey Till. *Ground Water* 41:772–779.
- 567 29. Grundmann GL. 2004. Spatial scales of soil bacterial diversity - The size of a clone. *FEMS*
568 *Microbiol Ecol* 48:119–127.
- 569 30. Bundt M, Widmer F, Pesaro M, Zeyer J, Blaser P. 2001. Preferential flow paths: Biological
570 “hot spots” in soils. *Soil Biol Biochem* 33:729–738.
- 571 31. Bak F. 2017. Microbial communities of subsurface biopores and fractures in clay tills mirror
572 the communities of the overlying plough layer soil. The international Society for Subsurface
573 Microbiology (ISSM). Conference abstract.
- 574 32. Unc A, Goss MJ. 2004. Transport of bacteria from manure and protection of water
575 resources. *Appl Soil Ecol* 25:1–8.
- 576 33. Dibbern D, Schmalwasser A, Lueders T, Totsche KU. 2014. Selective transport of plant root-

- 577 associated bacterial populations in agricultural soils upon snowmelt. *Soil Biol Biochem*
578 69:187–196.
- 579 34. Dechesne A, Smets BF. 2012. Pseudomonad Swarming Motility Is Restricted to a Narrow
580 Range of High Matric Water Potentials. *Appl Environ Microbiol* 78:2936–2940.
- 581 35. Dechesne A, Wang G, Gulez G, Or D, Smets BF. 2010. Hydration-controlled bacterial motility
582 and dispersal on surfaces. *Soil Biol Biochem* 107:14369–14372.
- 583 36. Callahan BJ, McMurdie PJ, Holmes SP. 2017. Exact sequence variants should replace
584 operational taxonomic units in marker-gene data analysis. *ISME J* 11:2639–2643.
- 585 37. Albright MBN, Martiny JBH. 2017. Dispersal alters bacterial diversity and composition in a
586 natural community. *ISME J* 12:296–299.
- 587 38. Kearns DB. 2010. A field guide to bacterial swarming motility. *Nat Rev Microbiol* 8:634–44.
- 588 39. Hölscher T, Kovács ÁT. 2017. Sliding on the surface: Bacterial spreading without an active
589 motor. *Environ Microbiol* 00:2537–2545.
- 590 40. Thormann KM, Paulick A. 2010. Tuning the flagellar motor. *Microbiology* 156:1275–1283.
- 591 41. Raaijmakers JM, De Bruijn I, Nybroe O, Ongena M. 2010. Natural functions of lipopeptides
592 from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS Microbiol Rev*
593 34:1037–1062.
- 594 42. Lindow SE, Brandl MT. 2003. Microbiology of the Phyllosphere MINIREVIEW Microbiology of
595 the Phyllosphere. *Appl Environ Microbiol* 69:1875–1883.
- 596 43. Burch AY, Zeisler V, Yokota K, Schreiber L, Lindow SE. 2014. The hygroscopic biosurfactant

- 597 syringafactin produced by *Pseudomonas syringae* enhances fitness on leaf surfaces during
598 fluctuating humidity. *Environ Microbiol* 16:2086–98.
- 599 44. Nielsen TH, Sørensen J, Nielsen TH, Sørensen J. 2003. Production of Cyclic Lipopeptides by
600 *Pseudomonas fluorescens* Strains in Bulk Soil and in the Sugar Beet Rhizosphere Production
601 of Cyclic Lipopeptides by *Pseudomonas fluorescens* Strains in Bulk Soil and in the Sugar Beet
602 Rhizosphere 69:861–868.
- 603 45. Haefele DM, Lindow SE. 1987. Flagellar Motility Confers Epiphytic Fitness Advantages upon
604 *Pseudomonas syringae*. *Appl Environ Microbiol* 53:2528–2533.
- 605 46. Dechesne A, Or D, Gulez G, Smets BF. 2008. The porous surface model, a novel
606 experimental system for online quantitative observation of microbial processes under
607 unsaturated conditions. *Appl Environ Microbiol* 74:5195–5200.
- 608 47. McBride MJ, Nakane D. 2015. *Flavobacterium* gliding motility and the type IX secretion
609 system. *Curr Opin Microbiol* 28:72–77.
- 610 48. Shrivastava A, Lele PP, Berg HC. 2015. A rotary motor drives *Flavobacterium* gliding. *Curr*
611 *Biol* 25:338–341.
- 612 49. Arnaud E, Best A, Parker BL, Aravena R, Dunfield K. 2015. Transport of *Escherichia coli*
613 through a Thick Vadose Zone. *J Environ Qual* 44:1424.
- 614 50. Nielsen MH, Styczen M, Ernstsén V, Petersen CT, Hansen S. 2010. Field Study of Preferential
615 Flow Pathways in and between Drain Trenches. *Vadose Zo J* 9:1073–1079.
- 616 51. Bojsen EH, Albers C, Olsen P, Jacobsen PR, Iversen B, Rosenbom AE. 2018. The Danish
617 Pesticide Leaching Assessment Programme: Site Characterization and Monitoring Design of

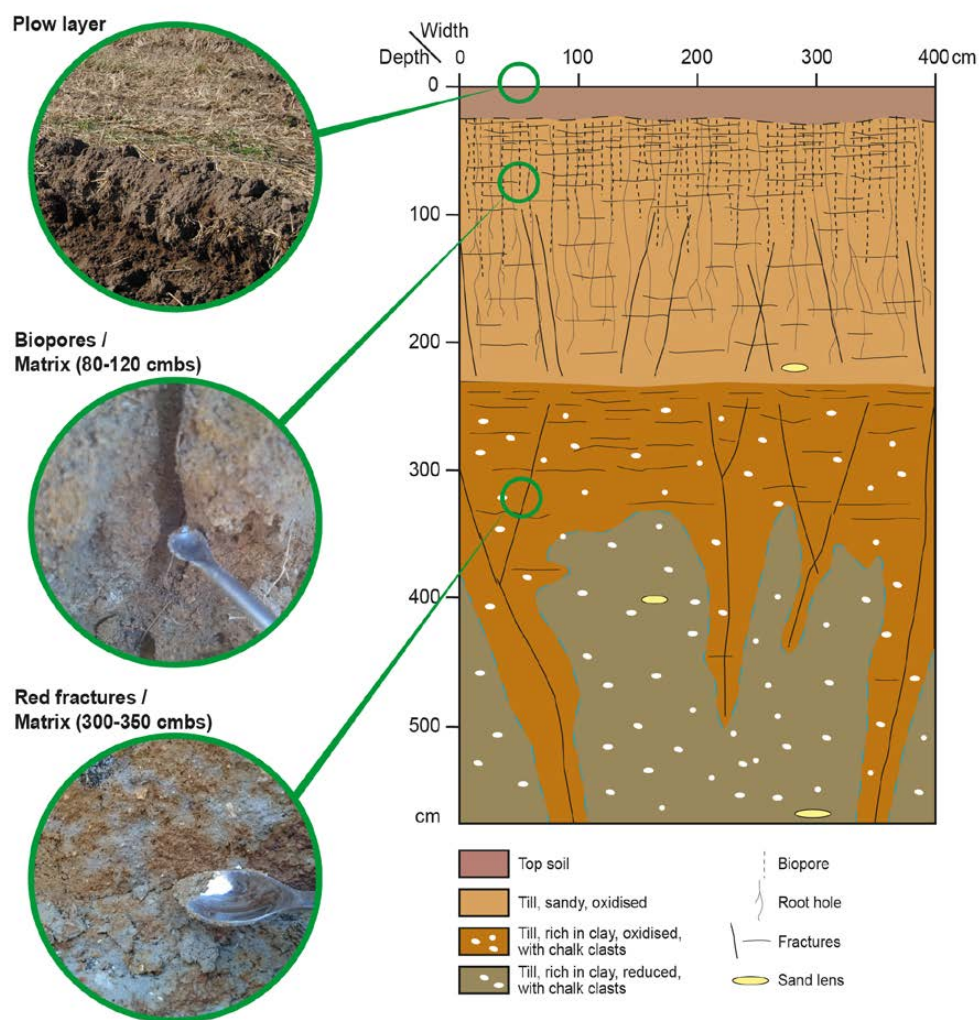
- 618 the Lund field, Geological Survey of Denmark and Greenland.
- 619 52. Mannik J, Driessen R, Galajda P, Keymer JE, Dekker C. 2009. Bacterial growth and motility in
620 sub-micron constrictions. *Proc Natl Acad Sci* 106:14861–14866.
- 621 53. Rosenbom AE, Hansen M, Klint KES. 2001. Image and SEM-analysis of fractures in clay till.
622 TRACe-Fracture toward an improved risk assessment of the contaminant spreading in
623 fractured underground reservoirs progress report. Rapport 2001/37.
- 624 54. Fierer N, Schimel JP, Holden PA. 2003. Variations in microbial community composition
625 through two soil depth profiles. *Soil Biol Biochem* 35:167–176.
- 626 55. Mitchell JG, Pearson L, Bonazinga A, Dillon S, Khouri H, Paxinos R. 1995. Long lag times and
627 high velocities in the motility of natural assemblages of marine bacteria. *Appl Environ*
628 *Microbiol* 61:877–882.
- 629 56. Gülez G, Dechesne A, Smets BF. 2010. The Pressurized Porous Surface Model: An improved
630 tool to study bacterial behavior under a wide range of environmentally relevant matrix
631 potentials. *J Microbiol Methods* 82:324–326.
- 632 57. Kohlmeier S, Smits THM, Ford RM, Keel C, Harms H, Wick LY. 2005. Taking the fungal
633 highway: Mobilization of pollutant-degrading bacteria by fungi. *Environ Sci Technol*
634 39:4640–4646.
- 635 58. Nazir R, Warmink JA, Boersma H, Van Elsas JD. 2010. Mechanisms that promote bacterial
636 fitness in fungal-affected soil microhabitats. *FEMS Microbiol Ecol* 71:169–185.
- 637 59. Worrlich A, König S, Miltner A, Banitz T, Centler F, Frank K, Thullner M, Harms H. 2016.
638 Mycelium-Like Networks Increase Bacterial Dispersal, Growth, and Biodegradation in a

- 639 Model Ecosystem at Various Water Potentials 82:2902–2908.
- 640 60. Rosenbom AE, Brüsich W, Badawi N, Olsen P. 2016. Monitoring of pesticide leaching from
641 cultivated fields in Denmark Monitoring of pesticide leaching from cultivated fields in
642 Denmark. Geol Surv Denmark Greenl Bull 35:17–22.
- 643 61. Rosenbom AE, Bojsen EH, Badawi N, Gudmundsson L, Platten-Hallermund F von, Hansen
644 CH, Nielsen CB, Plauborg F, Olsen P. 2017. The Danish Pesticide Leaching Programme –
645 Monitoring results. May 1999- June 2016.
- 646 62. Lindhardt B, Abildtrup C, Vosgerau H, Olsen P, Torp S, Iversen B, Jørgensen JO, Plauborg F,
647 Rasmussen P, Gravesen P. 2001. The Danish Pesticide Leaching Assesment programme –
648 Site Characterization and Monitoring design.
- 649 63. Nagy D, Rosenbom AE, Iversen B V., Jabloun M, Plauborg F. 2018. Estimating the degree of
650 macropore flow to drainage at an agricultural clay till field for a 10-years period. Prepared
651 for submission in review.
- 652 64. Danish Pesticide Leaching Assessment Programme (PLAP). <http://pesticidvarsling.dk>.
- 653 65. DS3077. 2013. Representative Sampling- Horizontal Standard. Danish Stand Auth 44:1–38.
- 654 66. Kardanpour Z, Jacobsen OS, Esbensen KH. 2015. Counteracting soil heterogeneity sampling
655 for environmental studies (pesticide residues, contaminant transformation) – TOS is critical
656 1–5.
- 657 67. Klümper U, Dechesne A, Smets B. 2014. Protocol for Evaluating the Permissiveness of
658 Bacterial Communities Toward Conjugal Plasmids by Quantification and Isolation of
659 Transconjugants. Hydrocarb Lipid Microbiol Protoc Springer Protoc Handb 1–14.

- 660 68. Muyzer G, Waal ECDE, Uitterlinden AG. 1993. Profiling of Complex Microbial Populations by
661 Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-Amplified
662 Genes Coding for 16S rRNA 59:695–700.
- 663 69. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-
664 resolution sample inference from Illumina amplicon data. *Nat Methods* 13:581–583.
- 665 70. Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glöckner FO. 2007. SILVA: A
666 comprehensive online resource for quality checked and aligned ribosomal RNA sequence
667 data compatible with ARB. *Nucleic Acids Res* 35:7188–7196.
- 668 71. R Core Team. 2016. R: A language and environment for statistical computing. R Foundation
669 for Statistical Computing,. 3.3.1. Vienna, Austria.
- 670 72. McMurdie PJ, Holmes S. 2013. Phyloseq: An R Package for Reproducible Interactive Analysis
671 and Graphics of Microbiome Census Data. *PLoS One* 8.
- 672 73. Tsirogianis C, Sandel B. 2016. PhyloMeasures: a package for computing phylogenetic
673 biodiversity measures and their statistical moments. *Ecography (Cop)* 39:709–714.
- 674 74. Albertsen M, Karst SM, Ziegler AS, Kirkegaard RH, Nielsen PH, Stokholm-Bjerregaard M.
675 2015. Back to Basics – The Influence of DNA Extraction and Primer Choice on Phylogenetic
676 Analysis of Activated Sludge Communities. *PLoS One* 10:e0132783.
- 677 75. Warnes GR, Bolker B, Bonebakker L, Gentleman R, Liaw WHA, Lumley T, Maechler M,
678 Magnusson A, Moeller S, Schwartz M, Venables B. 2016. gplots: Various R Programming
679 Tools for Plotting Data. R package version 3.0.1. [https://CRAN.R-](https://CRAN.R-project.org/package=gplots)
680 [project.org/package=gplots](https://CRAN.R-project.org/package=gplots).

- 681 76. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, Mcglinn D, Minchin PR, O'hara RB,
 682 Simpson GL, Solymos P, Henry M, Stevens H, Szoecs E, Wagner H, Oksanen MJ. 2018. vegan:
 683 Community Ecology Package.

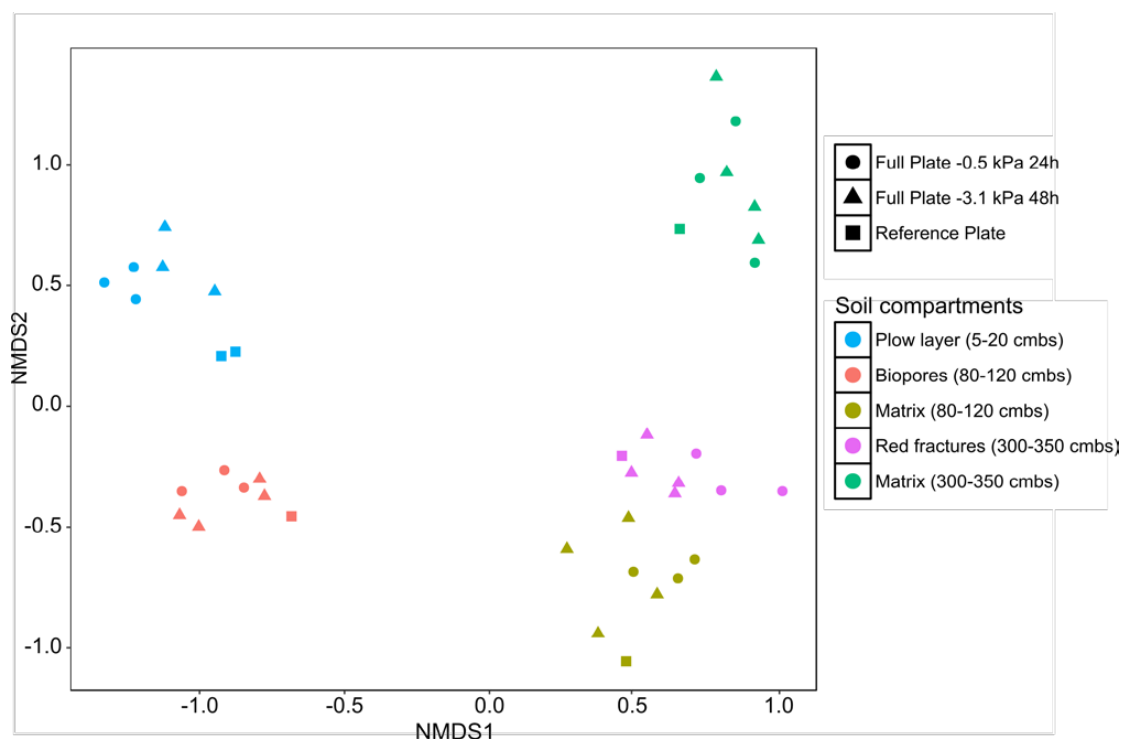
684 **Figures**



685

686 **FIG 1 Schematic illustration of the soil profile with highlighted sampling points.** Plow layer
 687 samples were obtained from 20 cmbs (cm below surface). Biopores and matrix sediment samples
 688 were from 80-120 cmbs, and red fractures and matrix sediment were sampled from 300-350
 689 cmbs. The illustration is adapted with permission from a report from the Danish Pesticide Leaching
 690 Risk Assessment Program (PLAP) (64).

691



692

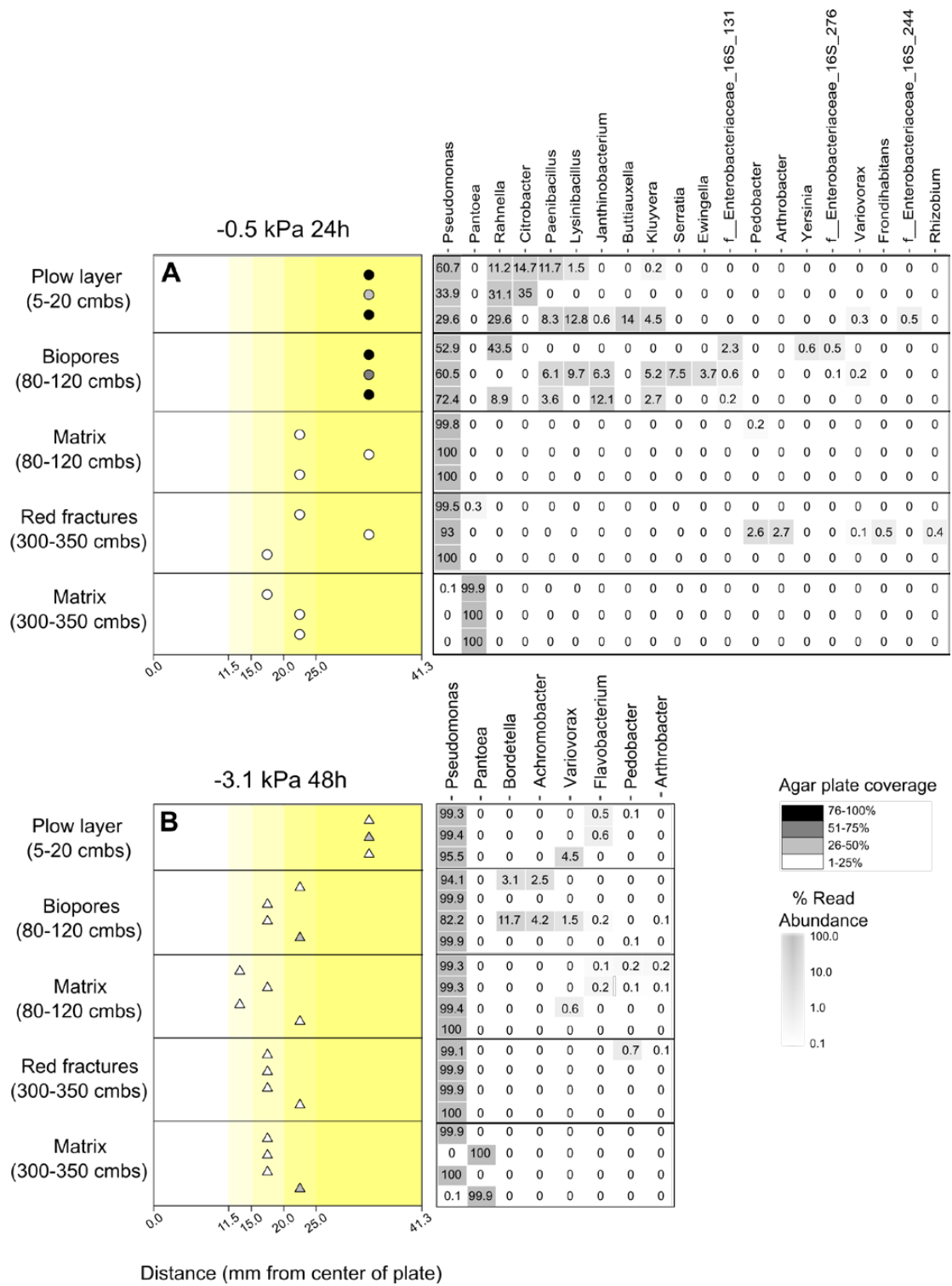
693 **FIG2** NMDS plot of the composition of the total communities derived from five compartments of a
 694 well-defined soil profile. Stress = 0.13. Bray-Curtis dissimilarities calculated from 16S rRNA genes.
 695 The total communities were tested at two matric potentials in the PSM experiments, -0.5 kPa
 696 (circles) and -3.1 kPa (triangles), and recovered on full agar plates (full plate). The motility-

697 restricted controls (reference plate) are marked with squares. Replicates are depicted as separate

698 dots.

699

700

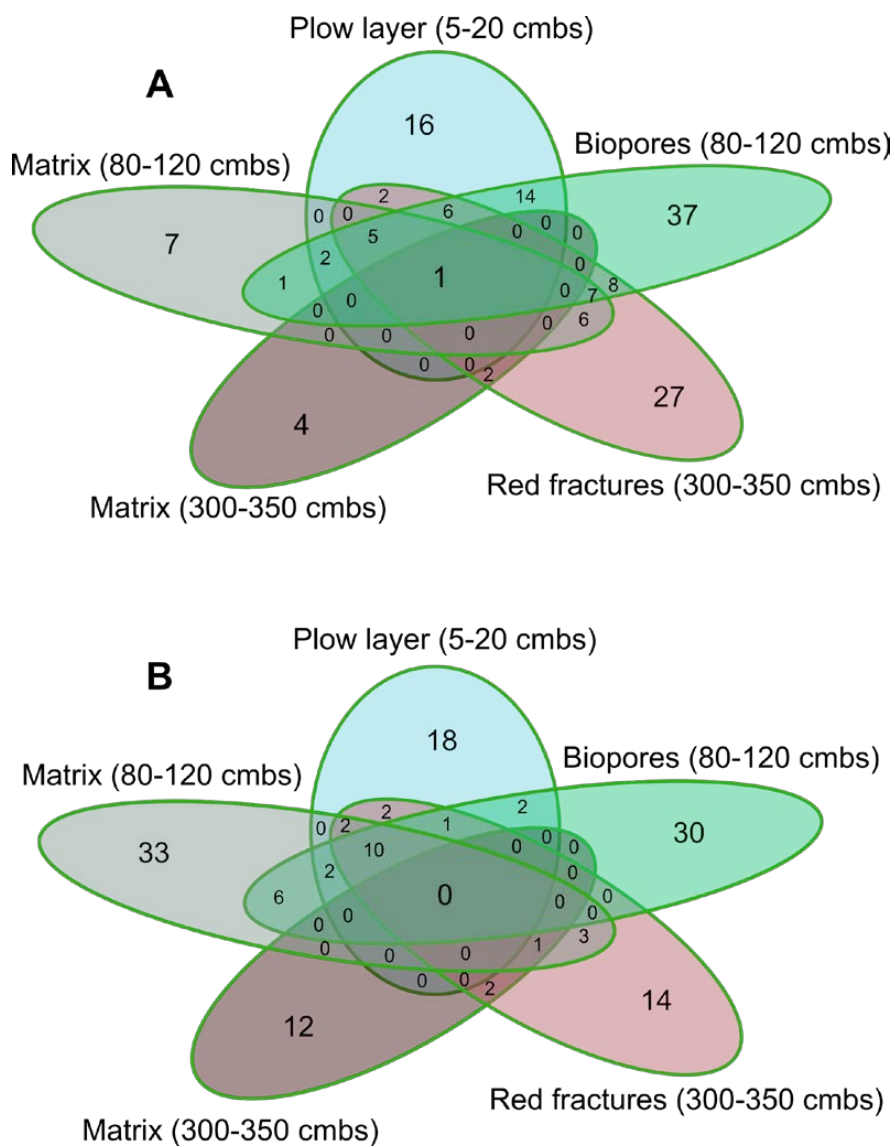


701

702 **FIG 3** Dispersal and composition of communities derived from five compartments of a well-defined
703 soil profile and incubated at matric potential -0.5 kPa for 24 h (**A**) and -3.1 kPa for 48 h (**B**). Left:
704 Symbol shading depicts bacterial coverage of the pressed agar plate, giving an indication of the
705 extent of colonization. The distances shown are ranges, *e.g.* colonies were observed on the agar
706 ring at a distance of between 11.5 and 15 mm from the inoculation point at the center. Right:
707 Heatmap of the relative abundance of the most dominant genera among the dispersing bacteria
708 across five soil communities. The replicates are depicted as separate dots and replication numbers
709 varied from three to four.

710

711



712

713

714 **FIG 4** Venn diagrams depicting the shared and unique ASVs between the dispersed communities

715 from five compartments of a well-defined soil profile. A) Communities exposed to

716 -0.5 kPa for 24 h, with a total of 145 unique ASVs. B) Communities exposed to -3.1 kPa for 48 h

717 with a total of 138 unique ASVs.

718

719

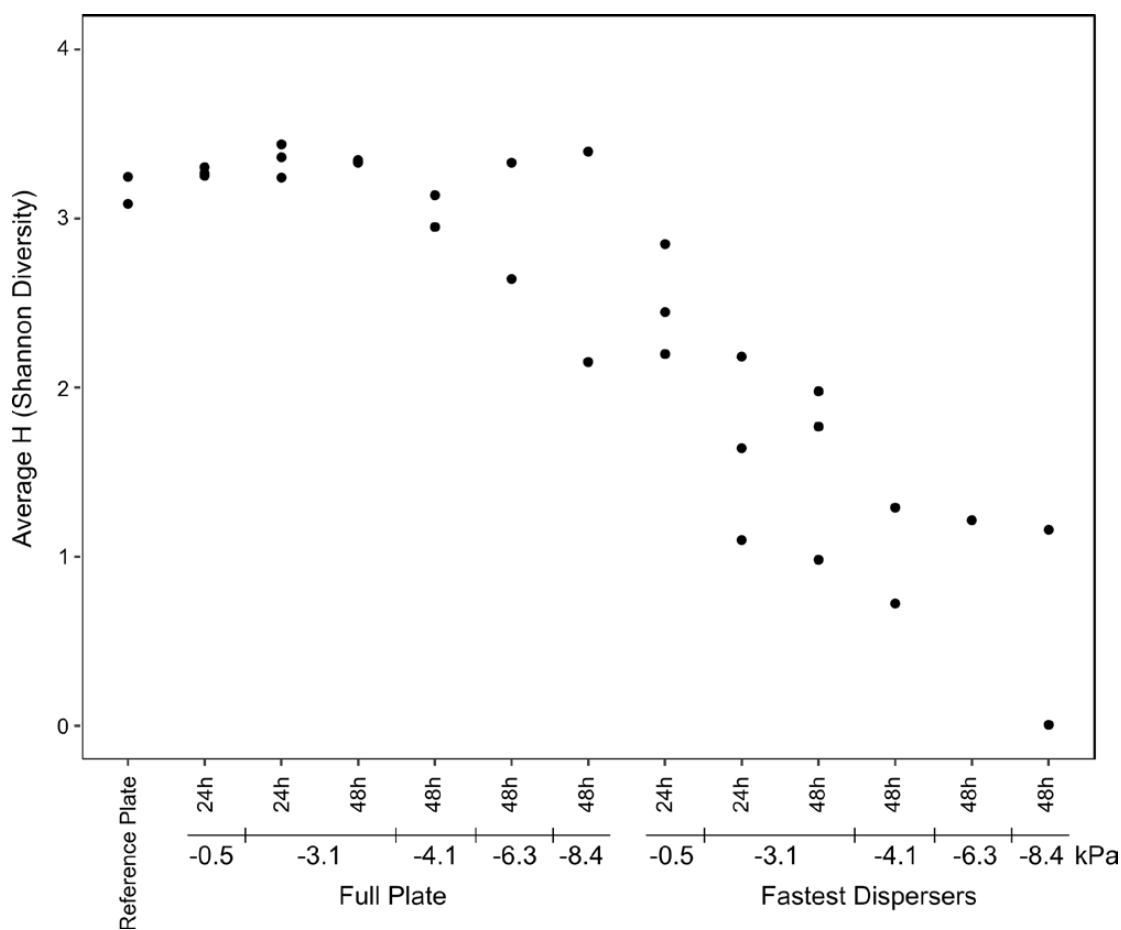
Table 1. Shared dispersing and non-dispersing ASVs between communities derived from five compartments of a well-defined soil profile				
		Percentage of shared dispersers ^a	Percentage of shared non-dispersers ^b	P value Fisher's exact test
Preferential flow paths				
Plow layer vs. biopores	-0.5 kPa	28.9 %	12.2 %	P< 0.001
	-3.1 kPa	20.0 %	15.0 %	0.2892
Biopores vs. fractures	-0.5 kPa	22.9 %	5.6 %	P< 0.001
	-3.1 kPa	14.7 %	7.6 %	0.1042
Plow layer vs. fractures	-0.5 kPa	14.6 %	3.8 %	P<0.01
	-3.1 kPa	26.3 %	6.9 %	P<0.001
Preferential flow path vs. matrix				
Biopores vs. matrix at 80-120 cmbs	-0.5 kPa	17.0 %	13.6 %	0.5636
	-3.1 kPa	20.0 %	12.6 %	0.1093
Fractures vs. matrix at 300-350 cmbs	-0.5 kPa	4.4 %	13.3 %	0.0826
	-3.1 kPa	6.4 %	17.8 %	0.07794
Matrix vs. matrix				
Matrix 80-120 vs. matrix 300- 350 cmbs	-0.5 kPa	2.9 %	10.1 %	0.2712
	-3.1 kPa	1.4 %	11.9 %	P< 0.05

720

721 ^a Dispersed bacteria recovered the furthest from the inoculation point (at least 11.5 mm).722 ^b Non-dispersing bacteria were calculated by subtracting the unique ASVs observed in the

723 dispersed community from the ASVs observed in the total community.

724



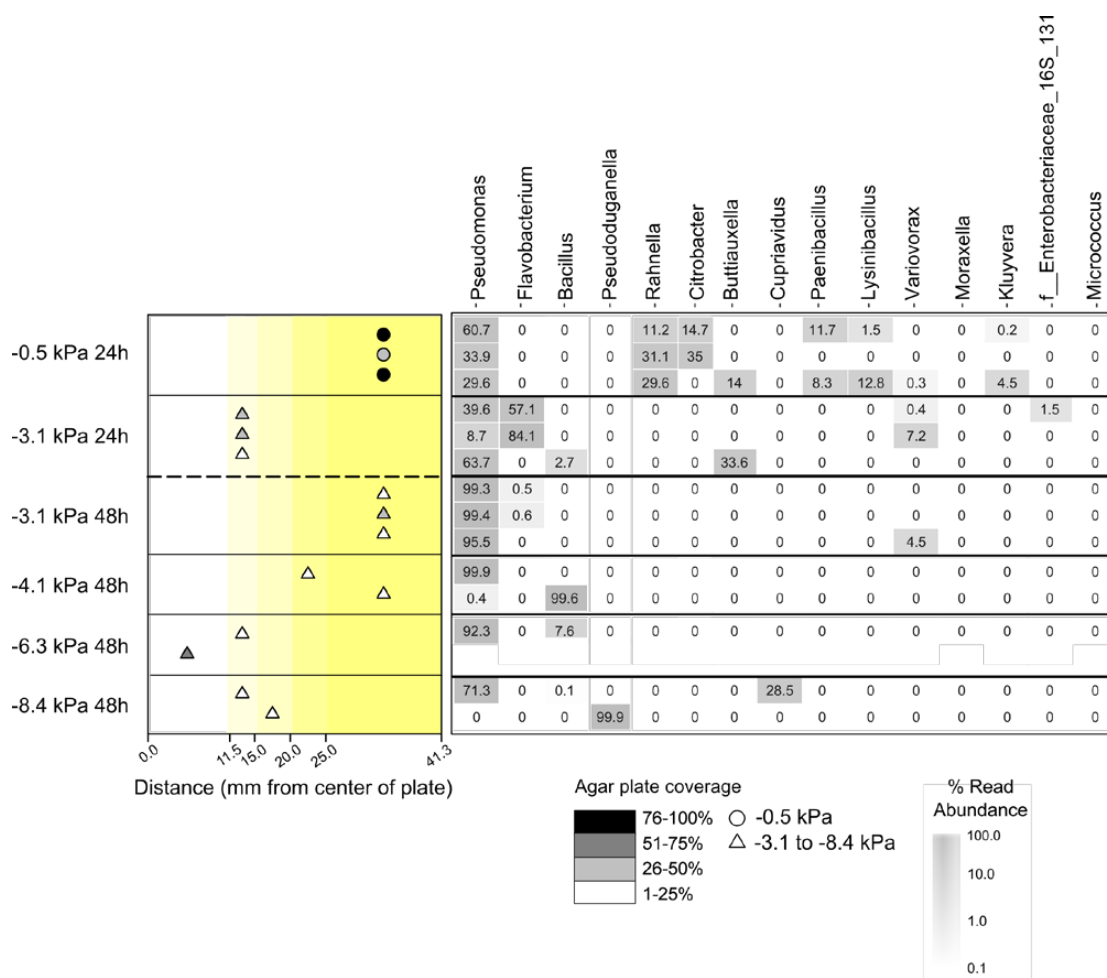
725

726 **FIG 5** Estimates of alpha-diversity (Shannon diversity index) for communities derived from plow
 727 layer soil samples and incubated at a range of negative matric potentials (-0.5 to -8.4 kPa) for 24 h
 728 or 48 h. For each replicate PSM, the total community recovered from the full agar plate (full plate)
 729 and the dispersed community is presented. A motility-restricted control (reference plate) is also
 730 included. Replicates are depicted as separate dots.

731

732

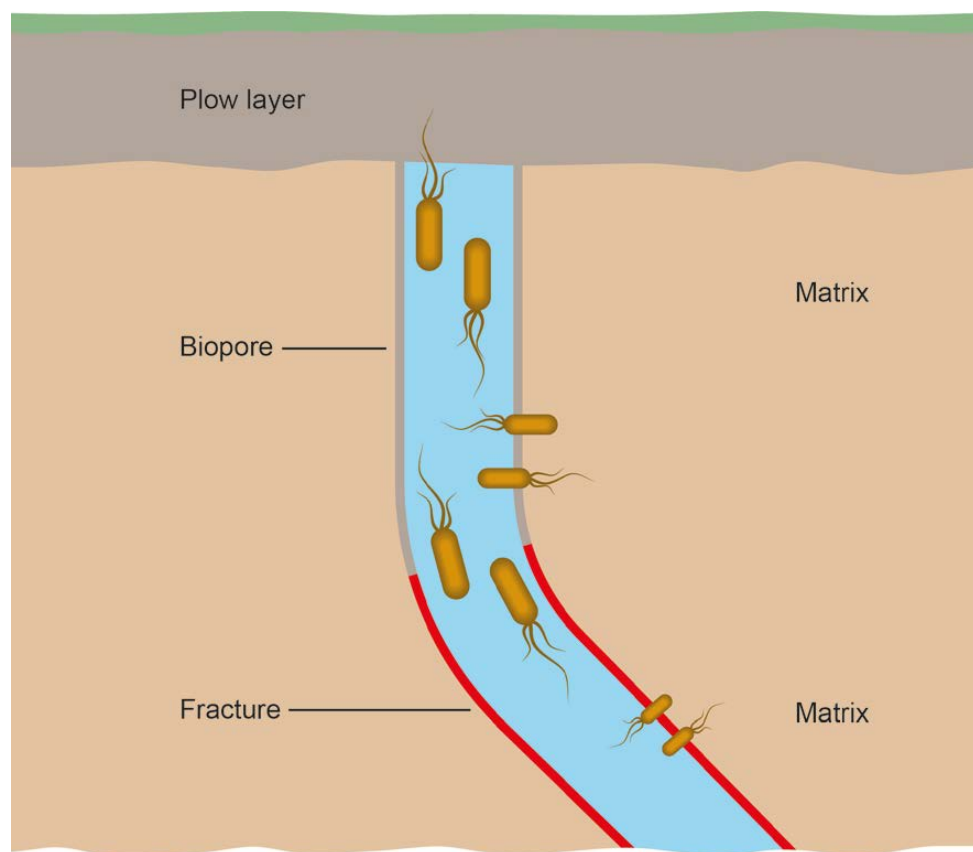
733



734

735 **FIG 6** Dispersal and composition of a community extracted from plow layer soil and incubated at
 736 matric potentials from -0.5 kPa to -8.4 kPa for 24 h or 48 h. Left: Symbol shading depicts bacterial
 737 coverage of the pressed agar plate, giving an indication of the extent of colonization. The distances
 738 shown are ranges, *e.g.* colonies were observed on the agar ring at a distance of between 11.5 to 15
 739 mm from the inoculation point at the center. Right: Heatmap of the relative abundance of the
 740 most dominant genera among the dispersers. Replication number varied from two to three.

741



742

743 **FIG 7** Conceptual model of preferential flow paths as facilitators of connectivity between
744 communities and “hotspots” for the exchange of motile bacteria. The highest number of shared
745 dispersers were observed along the preferential flow path (plow layer vs. biopores and biopores
746 vs. fracture), fewer shared dispersers between the biopores and matrix, and almost none shared
747 between the fracture and deep matrix. The size of the depicted bacteria represents the intensity
748 of shared dispersers between compartments.

749

750

751