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A trait-based approach to bacterial biofilms in soil

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ORIGINALITY-SIGNIFICANCE STATEMENT

Biofilm production is a trait found among microbes in a wide range of environmental, engineered, and host-associated ecosystems. In this study, we used *Pseudomonas aeruginosa* as a model system to demonstrate that biofilm production is a "response trait" in that it shapes the desiccation phenotype and moisture niche of bacteria in soil. In addition, we experimentally demonstrate, that biofilm production is an "effect trait" because it alters the environment by increasing water retention in soils, which may have consequences for the diversity and functioning of microbial communities.

SUMMARY

A trait-based approach focuses on attributes of taxa that influence the structure and function of communities. Biofilm production is a trait that is common among microorganisms in a wide range of environmental, engineered, and host-associated ecosystems. In this study, we present experimental evidence linking biofilm production to moisture availability, which is one of the greatest challenges for microorganisms living in the soil environment. Using *Pseudomonas aeruginosa* as a model system, first, we demonstrate that biofilm production is a response trait that affects bacterial performance under dry conditions. In addition to increasing survivorship, biofilm production affected the desiccation phenotype by shifting the niche space and reducing the minimum water potential needed to sustain a net-positive growth rate (Ψ^*). Although the allocation of resources to biofilms is thought to be costly, we found we no evidence to support a hypothesized trade-off between bacterial fitness and biofilm production along a moisture gradient. Second, we demonstrated that biofilm production is an effect trait. Specifically,

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biofilm production increased soil water retention in soils that were exposed to a series of drying and rewetting cycles. Although this form of niche construction via the release of public goods should affect species interactions, we found no evidence that biofilm production modified the performance of another soil bacterium. Together, our results support the view that biofilm production is an important functional trait that may contribute to the distribution, abundance, and functioning of microorganisms in soil environments.

INTRODUCTION

Microbial communities comprise thousands of potentially interacting species that carry out essential ecosystem processes. Insight into the assembly and maintenance of these complex communities may be gained by studying the functional traits of microorganisms (Green et al., 2008; Martiny et al., 2015; Treseder and Lennon, 2015). Functional traits are physiological, morphological, or behavioral characteristics that influence the performance or fitness of organisms under a set of environmental conditions (Lennon et al., 2012). The distribution of traits may reflect adaptations and trade-offs that influence evolutionary and ecological processes that are important for the assembly of communities along environmental gradients (Lebrija-Trejos et al., 2010; Székey and Langenheder 2014; Lennon and Denef 2015). Finally, trait-based approaches provide a framework for predicting how and when microbial taxa should affect ecosystem functioning (Lavorel and Garnier, 2002; Wallenstein and Hall, 2012; Krause et al., 2014).

One trait that can have important consequences for microbial performance is biofilm production. Biofilm production involves the release of exopolymeric substances (EPS),

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including carbohydrates, nucleic acids, and proteins by microorganisms into their surrounding environment. The construction of biofilms with EPS can confer a suite of advantages to microorganisms. For example, once embedded in EPS, individuals attach to surfaces, which prevents them from being washed out of habitats that have short residence times (e.g., guts, waste water treatment facilities, chemostats). In addition, biofilm production creates a multicellular, three-dimensional structure that can confer tolerance to various stressors including antibiotics, grazing, heavy metals, and water limitation (Davey and O'Toole, 2000; Flemming and Wingender, 2010).

Because it modifies microbial interactions, biofilm production is a trait that may have important implications for population- and community-level processes. For example, communication and syntrophic interactions among microorganisms can be facilitated owing to the proximity of individuals living in a biofilm. In addition, when bacteria release EPS, these public goods can be used by other microorganisms (Nadell and Bassler, 2011). This creates opportunities for cheating, which may affect the performance and stability of the assemblage (West et al., 2006; Hillesland and Stahl, 2010). The outcome of such microbial interactions should depend on the cost of biofilm production, since the energy and resources that are allocated to EPS and cell-cell communication cannot be directly invested into growth and reproduction (Penterman et al., 2014). In addition, biofilm production may be an important trait because it has the potential to alter environmental conditions in a way that affect the composition and function of microbial communities. This type of niche construction or ecosystem engineering (Matthews et al., 2014) may have important implications for understanding

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the maintenance of microbial biodiversity and its contribution to ecosystem stability (Fierer and Lennon, 2011).

Biofilm production is a trait that appears to be particularly common among microorganisms living in soil. For example, it has been estimated that EPS may account for up 1.5% of the total soil organic matter (SOM) pool (Chenu, 1995). Despite variation in their chemical composition and structure, biofilms tend to have hydrophobic properties that contribute to water retention in the soil matrix (Chang et al., 2007; Ophir and Gutnick, 1994). This is important because soil microorganisms are commonly challenged by low moisture conditions, which reduces substrate diffusion and restricts motility (Potts, 1994). In addition, biofilm production is beneficial because it reduces desiccation stress in soil environments (Roberson and Firestone, 1992; Ophir and Gutnick, 1994; Li et al., 2010). For example, biofilm production was correlated with the moisture niche in a phylogenetically diverse collection of soil microorganisms (Lennon et al., 2012). Specifically, microorganisms with higher biofilm production had a drier optimum and could tolerate a broader range of soil moisture. However, very little is known about trade-offs and species interactions involving bacterial biofilm production in soils.

In this study, we focus on the ecological implications of biofilm production in soils using *Pseudomonas aueruginosa* as a model system. First, we compare biofilm production, survivorship, growth, and niche space of a non-mucoid (NM) mutant with a wild-type overexpressor (OE) strain. After testing for a trade-off in this functional trait, we evaluated the degree to which biofilm production alters the moisture environment and whether this potential niche construction affects species interactions using head-to-head competition experiments.

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RESULTS

Biofilm phenotype — We used *Pseudomonas aeruginosa* FRDI as the biofilm-producing strain in our study (Ohman and Chakrabarty, 1981). This mucoid variant has a spontaneous mutation in *algT*, which regulates alternate sigma factor (σ^{22}) (Mathee et al., 1997). The mutation results in the constitutive overproduction of alginate, a copolymer of D-mannuronic acid and L-guluronic acid. Alginate is a major component of the EPS that affects biofilm architecture in *Pseudomonas* (Hentzer et al., 2001). Biofilm production was 10-fold higher for the wild type overexpressor (OE) compared to the isogenic non-mucoid ("NM") mutant (*algT*::Tn501; Wozniak and Ohman, 1994) that is deficient in alginate production (Fig. 1A, Welch's t-test, $t_{23.1} = 9.27$, $P < 0.0001$). Under the conditions used for routine maintenance in our laboratory (R2B medium, 150 rpm, 25º C), the two strains have comparable growth rates (OE = 0.17 ± 0.006 h^{-1} vs. NM = 0.15 ± 0.026 h^{-1} [mean ± SEM], $t_{2.2} = -1.12$, $P = 0.37$).

For additional context, we compared biofilm production of OE and NM to biofilm production measured under identical experimental conditions for a collection of soil microorganisms (Lennon et al., 2012). Among the 45 strains examined, OE was in the 87% percentile for biofilm production, while NM was only in the 28% percentile (Fig. 1B). Colony morphology of OE and NM was also distinct. After one week of growth on R2A plates, OE exhibited circular, convex, and glistening colonies with entire margins, while NM formed slightly irregular, dull colonies, with undulate margins (Fig. 1A).

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Survivorship under desiccation — To test whether biofilm production affected bacterial persistence, we quantified survivorship under desiccation. Survivorship over the 48 d experiment was significantly affected by bacterial biofilm production ($\chi_1^2 = 41.3$, $P < 0.0001$). The median survival time for air-dried OE was 41 days (41 - 46 days = 95% CI), while the median survival time for NM under the same conditions was 30 days (28 - 34 days = 95% CI).

Test for biofilm fitness trade-off — Separately, we measured the growth rate of OE and NM along a moisture gradient in soil microcosms to test for a moisture-dependent cost of biofilm production. A multiple regression model revealed that population growth rates of OE and NM responded differently to soil water potential (Fig. 3, $R^2 = 0.65$, $F_{3,64} = 40.1$, $P < 0.0001$), but there was no evidence for a trade-off between bacterial fitness and biofilm production. The intercept of the growth-moisture relationship for OE was significantly higher than that of NM ($P < 0.0001$), but the slopes were the same ($P = 0.94$). Based on these findings, bacterial population growth could be described using two equations:

$$\text{OE growth } (d^{-1}) = 2.09\ (\pm 0.229) + 2.08\ (\pm 0.337)\ \Psi \qquad \text{(eq. 1)}$$

$$\text{NM growth } (d^{-1}) = 0.91\ (\pm 0.315) + 2.04\ (\pm 0.477)\ \Psi \qquad \text{(eq. 2)}$$

where values represent parameter estimates (± SEM) and Ψ is water potential measured in megapascals (MPa). From this analysis, we also determined that the minimum water

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potential to sustain positive population growth for the NM strain (Ψ^*_{NM} = -0.43) was 57% higher (i.e., wetter) than that of the OE strain (Ψ^*_{OE} = -1.01) (Fig. 3).

Moisture niche space — We measured the peformance (i.e., respiration) of OE and NM along a soil moisture gradient to test whether biofilm production is a trait that can alter microbial niche space. The respiration rates of the two strains responded differently to changes in water potential (Fig 4). Based on AIC, the best models included separate moisture niche parameters (eq. 3) for OE and NM (Table 1). Of the 17 models that were evaluated, we determined that the top six models performed equally well as indicated by ΔAIC values that ranged from 0 to 2.06. The optimum water potential (W_{opt}) was the only parameter that was consistently identified among all of the top models, so we largely restrict our interpretation to this niche characteristic. The W_{opt} for NM (-0.08 ± 0.023 MPa) was 68% lower than the W_{opt} for OE (-0.25 ± 0.063 MPa).

Niche construction by bacterial biofilms — We incubated NM and OE separately into replicate soil columns for three months to test whether biofilm production is a trait that can modify soil moisture. Soil moisture dynamics were significantly affected by a strain's ability to produce biofilm (RM-ANOVA, time x strain, $F_{38,190} = 4.43$, $P < 0.0001$). During the initial establishment period, soil moisture in the experimental columns remained relatively constant and moist with an average water potential of approximately -0.0001 MPa. Water potential became more negative (i.e., drier) during each of the four dry-down periods, but to a much lesser degree in columns that had been inoculated with OE (Fig. 5). Based on the marginal means (or least square means [LSM]

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± SEM) generated from the RM-ANOVA, the water potential of soils inoculated with OE (-0.0017 ± 0.00023 MPa) was two-fold wetter than soils inoculated with NM (-0.0034 ± 0.00020 MPa). In gravimetric units, this difference in moisture content amounted to 0.0875 g $H_2O \cdot g\ soil^{-1}$. At the end of the experiment, there was no difference in the abundance (mean ± SEM) of OE ($4.6 \times 10^7 \pm 1.82 \times 10^7$ CFU g^{-1} soil) and NM ($5.4 \times 10^7 \pm 1.68 \times 10^7$ CFU g^{-1} soil) in the soil columns (Welch's *t*-test, $t_{5.96} = -0.29$, $P = 0.78$).

Biofilm production and species interactions — We found no evidence to support the hypothesis that biofilm production by one strain of bacteria can alleviate the desiccation stress of an unrelated bacterial strain in a soil matrix. For this comparison, we used *Pedobacter* KBS0701 as a desiccation-sensitive, low biofilm-producing "tester" strain. Our multiple regression model was effective at explaining *Pedobacter* growth rates ($R^2 = 0.74$, $F_{5,28} = 16.5$, $P < 0.0001$). As expected, *Pedobacter* fitness declined with decreasing water potential. However, the parameters describing *Pedobacter* growth (i.e., slope and intercept) along the moisture gradient were not modified by the presence of OE or NM ($P > 0.22$), which resulted in a Ψ^* for KBS0701 of -0.51 MPa.

DISCUSSION

Biofilm production is a trait that affects the performance of microorganisms in environmental, engineered, and host-associated ecosystems. Using *Pseudomonas aeruginosa* as a model system, we demonstrate that biofilm production increases desiccation tolerance, an important phenotype that may influence soil microbial structure and function. The wild-type overexpressor strain (OE) survived longer and had a drier moisture optimum (W_{opt}) than the mutant non-mucoid strain (NM) that lacked the ability

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to produce alginate. Despite not being able to detect a fitness cost of biofilm production, we demonstrate that this trait has the ability to alter the soil moisture dynamics in a way that is consistent with niche construction. However, the release of public goods in the form of exopolymeric substances (EPS) did not alleviate desiccation stress for an unrelated bacterium. Thus, our results suggest that the benefits of biofilms in soils may not always be extended to co-occurring non-producer populations.

Biofilms increase survivorship — Desiccation tolerance is a complex phenotype that can be influenced by various traits, including the upregulation of compatible solutes, dormancy, variation in cell wall structure, and biofilm production (Potts, 1994; Lennon et al., 2012). Many of these traits are adaptive because desiccation can oxidatively damage nucleic acids and proteins that are essential for bacterial survival (Dose et al., 1992; Fredrickson et al., 2008). Our results revealed that biofilm production increased survivorship under conditions of desiccation. On average, the OE variant of *Pseudomonas aeruginosa* with high biofilm production survived 37% longer than the NM variant that was deficient in alginate biosynthesis. Our findings are consistent with other studies that have generated mutants to explore the effects of biofilm production on desiccation tolerance. For example, mutations in *cps*, which controls capsule biosynthesis led to a six-fold reduction in survivorship for *Escherichia coli*, *Erwinia stewartii*, and *Acinetobacter calcoaceticus* (Ophir and Gutnick, 1994). Similarly, mutations in *algD*, an important structural gene involved in alginate biosynthesis significantly reduced survivorship in three different species of *Pseudomonas* (Chang et al., 2007). Alginate

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biosynthesis is required for constructing thicker biofilms, which have been shown to be effective at reducing evaporative water loss (Chang et al., 2007).

Our results demonstrate that the disruption of alginate biosynthesis via mutation of the *algT* regulatory gene led to a reduction in median survivorship by 11 days under conditions of desiccation. The magnitude of this biofilm-mediated effect may be critical for the persistence of some bacterial populations in the soil environment. Soils commonly experience desiccation for varying lengths of time depending on geography, climate, and soil properties. For example, in mesic habitats, rain events may occur every few days (or less) and alleviate desiccation stress (Aanderud et al., 2011). However, in more arid ecosystems, biofilm production may help bacteria survive dry conditions for extended periods of time.

Biofilms alter moisture niche — Traits like biofilm production may affect the distribution and abundance of microorganisms along environmental gradients. If there is too much overlap in niche space, one species may drive another species locally extinct via competitive exclusion. In contrast, divergence in trait values should allow for coexistence of species over a broader range of environmental conditions (Chase and Leibold, 2003). Our results demonstrate that a knockout of alginate biosynthesis regulatory gene (*algT*) led to dramatic changes in the biofilm phenotype, which corresponded with a shift in the moisture niche of *Pseudomonas aeruginosa*. In addition to altering colony morphology, the non-mucoid (NM) mutant produced 10-fold less biofilm than the alginate overexpressing (OE) wild-type strain (Fig. 1A). For additional context, we compared biofilm production from the two *Pseudomonas* strains (OE and

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NM) to biofilm production measured on a phylogenetically diverse collection of soil microorganisms (Lennon et al., 2012). The mutation in alginate biosynthesis resulted in *Pseudomonas aeruginosa* dropping from the 87th percentile to the 28th percentile in biofilm production (Fig. 1B).

Previously, we demonstrated that natural variation in biofilm production was correlated with parameters that describe the moisture niche of soil microorganisms (Lennon et al., 2012). Specifically, higher biofilm production corresponded with a drier optimum water potential (W_{opt}) and a wider niche breadth (b). In addition, soil microorganisms that produced more biofilm had longer lag times (Fig. 1S), suggesting that there could be a cost associated with quorum sensing and/or the production of exopolymeric substances (EPS). In the current study, we followed up on the comparative analysis by experimentally demonstrating that biofilm production altered the moisture niche of *Pseudomonas aeruginosa*. Specifically, W_{opt} of the non-mucoid (NM) variant was 68% lower than the wild-type (OE) strain (Fig. 4). In addition, our maximum likelihood analysis supported the interpretation that alginate-based biofilm production may increase the niche breadth *(b*) and maximum respiration rate (R_{max}) of *Pseudomonas aeruginosa* (Table 1). If these findings hold for other taxa, then biofilm-producing bacteria may be classified as habitat (i.e., moisture) generalists that can alter ecosystem processes (e.g., CO_2 flux).

Is there a fitness cost of biofilm production? — Biofilm production is viewed as a costly trait. First, because biofilm production is controlled by quorum sensing, energy is required to regulate many genes that are involved in cell-cell communication (Keller and

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Surette, 2006). Second, during biofilm production, energy- and nutrient-rich molecules (polysaccharides, proteins, and nucleic acids) are diverted from growth and reproduction towards exopolymeric substances (EPS). For these reasons, we hypothesized that there would be a fitness cost associated with biofilm production in *Pseudomonas aeruginosa*, but that this cost would depend on soil moisture content. Specifically, we predicted that OE would perform better than NM under dry soil conditions owing to the benefits of biofilm production. In contrast, we predicted that NM would outperform OE under moist soil conditions because it would not pay the cost of biofilm production in an environment where it would be less advantageous.

We found no evidence to support a trade-off involving biofilm production and moisture availability. Instead, population growth of NM and OE declined linearly and at the same rate with decreasing water potential (Fig. 3). However, the intercepts describing the relationship between growth rate and water potential were significantly different for OE and NM. As a result, the minimum water potential required for positive population growth (Ψ^*) was 57% lower for OE than it was for NM. This finding lends further support to the view that biofilm production mitigates desiccation stress. Owing to the superior overall performance of OE relative to NM, our results raise the question of whether biofilm production may reflect a syndrome of correlated traits that improves microbial performance in the soil environment.

Our findings are at odds with the view that bacteria must pay a cost to build a biofilm. For example, the wrinkly-spreader (WS) phenotype of *Pseudomonas fluorescens* experiences a 20% reduction in fitness compared to the ancestral smooth (SM) population owing to the costs associated with overexpressing the cellulose component of the biofilm

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that is required for occupying the air-water interface (Rainey and Rainey, 2003). Other studies assume an even larger cost of biofilm production (Mitri et al. 2011).

There are a number of potential explanations for why biofilm production was not accompanied by a fitness cost in our study. First, costs can be challenging to measure. From an eco-evolutionary perspective, a 1% reduction in relative fitness is sufficient to rapidly drive a population extinct. However, it is often difficult to achieve the statistical power needed to reliably detect a 1% fitness cost (see Lennon et al., 2007). Second, the magnitude of a fitness cost is affected by the environment and is expected to be highest under competitive or stressful conditions (Agrawal et al., 2010). For example, the cost of biofilm production for *Pseudomonas fluorescens* was >50% under low resource conditions, but < 10% under high resource conditions (Brockhurst et al., 2008). Third, while our study was designed to test for a trade-off involving a single-trait (i.e., moisture), trade-offs are often constrained by other traits (Agrawal et al., 2010). For example, biofilm production is intimately linked to dispersal capacity (Nadell and Bassler, 2011). Finally, our study examined the biofilm phenotype that resulted from the modification of a single regulatory gene (*algT*). However, biofilm production in *Pseudomonas* is complex trait that involves multiple genes encoding for other polysaccharides, extracellular DNA, and proteins including type IV pili, flagella, and fimbrae (Wei and Ma, 2013). Perhaps disruption of other biofilm pathways would lead to different phenotypes that would be accompanied by different fitness costs.

Biofilm and niche construction — We have demonstrated that biofilm production is a "response trait" (*sensu* Lavorel and Garnier, 2002) that alleviates desiccation stress. Our

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results also suggest that biofilm production may be an "effect trait" that alters the soil environment (Lavorel and Garnier, 2002). Over the course of three months, we measured the moisture content of soil columns that were inoculated with either the non-mucoid (NM) mutant or the alginate overexpressing (OE) wild-type strain of *Pseudomonas aeruginosa*. Soil inoculated with OE retained significantly more moisture than the soils inoculated with NM (Fig. 5). We attribute these differences in water retention to the per capita effect of biofilm production since the densities of *Pseudomonas* in the two treatments were comparable at the end of the experiment.

Growing evidence suggests that microbial communities are sensitive to changes in soil moisture availability (e.g., Aanderud et al., 2015; Maestre et al., 2015). Moisture-mediated changes in microbial composition may be due to filtering, a process whereby environmental conditions select for taxa with certain traits. However, the distribution and abundance of species can also be affected by species interactions such as facilitation, which can arise via niche construction when species modify their environment (Kylafis and Loreau, 2011). Because the OE strain increased water retention in soils (Fig. 5), we hypothesized that biofilm production might affect the performance of desiccation-sensitive soil bacteria. Using *Pedobacter* as a "tester" strain, microcosm experiments revealed that fitness declined with decreasing water potential. Contrary to our expectation, the slope of this relationship did not become shallower in presence of OE, which would be consistent with the niche construction hypothesis. Nor did the slope of *Pedobacter* growth vs. water potential relationship become steeper in the presence of *Pseudomonas*, which might occur due to increased competition (Oliveira et al., 2015) or exposure to toxins produced by *Pseudomonas aeruginosa* (e.g., pyocyanin). It is also

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possible that the extent of biofilm development during the relatively short experiment was not sufficient to modify the soil matrix in ways that would affect performance of the tester strain.

Conclusions and Implications — Our results indicate that biofilm production is a response trait that allows bacteria to tolerate desiccation by shifting critical life-history parameters along with the moisture niche. Somewhat surprisingly, we documented no cost associated with biofilm production. Perhaps this is due to the fact that biofilms perform other functions beyond desiccation tolerance, which constrain trade-offs. As such, biofilm production may reflect a syndrome of correlated traits that are advantageous in the soil environment. The lack of cost associated with the production and release of public goods, however, has interesting eco-evolutionary implications for social interactions and the degree of "cheating" among microbes in soils (West et al., 2006). Our results also indicate that biofilm production is an effect trait that modifies its environment. In our study, this niche construction did not influence species interactions as expected, but this does not preclude biofilm production from having important trait-mediated effects in nature. Although findings from our study are useful for testing mechanisms underlying species interactions, future studies taking advantage of environmental genomics could examine the distribution of biofilm genes and pathways along gradients, which would help advance trait-based microbial ecology.

MATERIALS AND METHODS

Biofilm production — We estimated biofilm production for the OE and NM isolates using the crystal violet assay (O'Toole et al., 1999). Briefly, we added 100 µL of R2B

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356 medium to a 96-well plate. We inoculated replicate wells (n = 12) with 1 μL of an exponentially growing OE or NM culture and incubated for 48 hrs at 25º C. We then
358 added 25 μL of 1% crystal violet to each well for 20 min. After rinsing the plates, we measured biofilm production as the absorbance of the wells at 550 nm using a Molecular
360 Devices SpectraMax5 spectrophotometer. We tested for differences in biofilm production between OE and NM using a Welch's *t*-test.

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Survivorship — We measured survivorship for the OE and NM isolates using a
364 desiccation assay. We added 10 μL of an exponentially growing culture (~10^6 cells) to replicate wells of a 96-well plate. We allowed these cell suspensions to evaporate under
366 ambient laboratory conditions (~35 % relative humidity), which took approximately 15 min. Over a period of 48 d, we periodically added 200 μL of R2B medium back to eight
368 of the dried wells and visually monitored them for five days. If the wells became turbid after resupplying medium, we scored the population as "alive"; if the wells remained
370 clear, we scored the population as "dead". We analyzed the resulting binary data using the 'survival' package (version 2.37.7) in R (R Core Development Team, 2009). We
372 tested for differences in survivorship between OE and NM using the *survdiff* function. We used this function to generate Kaplan-Meier estimates of survival with G-rho family
374 of weighting on death of bacteria in a well at a given time point implementing the log-rank test (i.e., rho = 0, Harrington and Fleming, 1982). With the resulting data, we
376 created survival curves using the *survfit* function in R.

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Biofilm trade-off — We used experimental microcosms to test for a tradeoff between biofilm production and fitness in the soil enviroment. Specifically, we hypothesized that biofilm production reduces desiccation stress in dry soils, but comes at cost in wet soils. The microcosms consisted of 25 mL glass scintillation vials containing 10 g of a sterilized soil matrix (70% quartz sand, 20% kaolinite clay, and 10% bentonite clay) amended with 300 mg of R2B medium as the sole resource. We established a moisture gradient by adding different amounts of water to the microcosms to create of water potentials (-0.01 to -1.41 megapascals [MPa]) that had previously been shown to affect the growth and physiology of soil microorganisms (Lennon et al., 2012). We quantified the water potential of each microcosm by constructing a water-retention curve that equated volumetric water content (cm^3 H_2O/cm^3 soil) with water potential of the soil matrix. We measured soil water potential with a WP4-T Dewpoint Meter and a T5 Mini Tensiometer attached to an Infield 7 Datalogger (Decagon Devices, Pullman, Washington).

We initiated the experiment by inoculating a microcosm with a suspension of OE or NM (100 µL of log phase culture). The microcosms were immediately sealed with a septum cap and incubated at 25 °C for 72 hrs. As a measure of fitness, we quantified population growth rates as ln (N_f/N_0)/t where N_0 is the cell concentration at the beginning of the experiment, N_f is the cell concentration at the end of the experiment, and t is the duration of the experiment in hrs. We estimated cell concentrations from the appearance of colony forming units (CFU) on R2A plates after extracting 1 g of soil in 10 mL of 1% sodium pyrosphosphate. We used indicator variables multiple regression to test whether or not the two strains performed differently along the moisture gradient, where water

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potential was treated as a continuous variable and strain type (OE vs. NM) was treated as a categorical variable (Lennon and Pfaff, 2005). In addition, we measured Ψ^*, which we define as the minimum water potential at which population growth rate equaled zero. Mathematically, we quantified Ψ^* as the x-intercept in a population growth-water potential regression model.

Niche space — We used the same soil microcosm system described in the previous section to test whether biofilm production modifies the bacterial moisture niche. We did this by measuring the respiration rates of OE and NM along a soil moisture gradient, which is described in greater detail elsewhere (Lennon et al., 2012). Briefly, we quantified respiration as the amount of CO_2 that evolved in the microcosm headspace during the incubation using a LI-COR LI-820 infrared gas analyzer. We then modeled the respiration response of the two strains using the following equation:

$$R = R_{max}\left(exp\left[-\left|\frac{W-W_{opt}}{\sigma}\right|^{\tau}\right]\right) \qquad \text{eq. 3}$$

where R is the respiration rate, R_{max} is the maximum respiration rate, W is soil water potential, W_{opt} is the soil water potential corresponding to R_{max} (i.e., the optimum), σ describes the rate that respiration declines as a strain moves away from W_{opt}, and τ is the kernel that defines the general shape of the response curve. We then estimated niche breadth (b) using the following equation:

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$$b = \sigma(-\log_{10} x)^{1/\tau} \quad \text{eq. 4}$$

where x defines the range of water potential based on a proportion of R_{max}. We assigned a value of 0.5 to x, which means that niche breadth defines the range of water potential where R is ≥ 50% of R_{max} (Lennon et al., 2012). We used the maximum likelihood package 'bblme' (version 1.0.17) in R to estimate model parameters. We used Akaike Information Criteria (AIC) to determine whether or not the best model included distinct parameters from eq. 3 and eq. 4 for the OE and NM isolates.

Niche construction — We conducted an experiment to test whether biofilm production is a trait that modifies soil moisture dynamics in way that is consistent with niche construction. Eight glass columns (30 cm long, 2.8 cm inner diameter) were filled with 150 g of a sterilized soil matrix (95% sand, 5% vermiculite). The columns were vertically supported in a fume hood with extension clamps mounted to ring stands. The top and bottom of each tube were fit with sterilized rubber stoppers to prevent contamination and soil loss. To control the soil drying rate, we inserted two pieces of glass tubing (4 mm inner diameter) through the top rubber stopper of each column. One of the glass tubes delivered 0.1 μm-filtered air at low pressure to the column, while the second glass tube allowed for degassing and the maintenance of ambient pressure. We initiated the experiment by adding ~10^9 cells from a log phase culture of either OE or NM to a column along with 45 mL of R2B medium. To prevent contamination, we supplemented the R2B medium with 25 μg mL^{-1} spectinomycin and 50 μg mL^{-1} cycloheximide (OE and NM are resistant to these two inhibitors at these concentrations.) During the first 13 d of the

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446 study, we added enough water to each column to offset evaporation. This initial phase of the experiment was intended to create optimal soil moisture conditions to allow for the 448 establishment of the bacteria in the soil matrix of the columns.

After the establishment phase, we created a series of dry-down and rewetting 450 events, which are common in natural soils (Schimel et al., 2007). There were four dry-down periods lasting 10, 23, 21, and 21 days. At the end of the first three dry-down 452 periods, we rewetted the soil by resupplying enough R2B medium to achieve the soil moisture content of the initial establishment phase. During the rewetting events, we 454 removed the bottom rubber stopper to allow for water drainage from the column. For three months, we estimated gravimetric water content of the soil every 2-3 days based on 456 changes in the mass of each column. We then converted gravimetric water content into water potential (MPa) using the procedures described above. We tested for the effect of 458 bacterial strain (OE vs. NM) on soil water potential using repeated measures (RM) ANOVA (SAS PROC MIXED) with covariance structures selected using the Bayesian 460 Information Criterion (Wolfinger and Chang, 1999). Finally, we extracted bacteria from a soil sample collected from each column on the last day of the experiment in a 1% sodium 462 pyrosphosphate solution. We plated these extracts onto R2 agar (without inhibitors) to check for contamination and to compare densities of OE and NM across treatments.

464

Species interactions experiments. Biofilm production is a trait that could modify the 466 direction and magnitude of interaction between microbial species. We tested this hypothesis by measuring the population growth rates of a desiccation-sensitive isolate 468 along a soil moisture gradient in the presence or absence of OE and NM using the

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microcosms method described above. We used *Pedobacter* sp. KBS0701, which belongs to the Sphingobacteriaceae (Bacteroidetes), as the desiccation-sensitive "tester" strain. From a collection of 45 well-characterized soil isolates, we found that KBS0701 had the second lowest (i.e., wettest) moisture optimum (W_{opt} = -0.0007 MPa) and the third narrowest niche breadth (b = 0.0007 MPa) (Lennon et al., 2012). Like some other *Pedobacter* isolates, KBS0701 produces a pink pigment (Krieg et al., 2010). This phenotype allowed us to differentiate strains and thus estimate the population growth rates of *Pedobacter* and *Pseudomonas* strains (OE or NM) when mixed cultures were plated on R2A plates.

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Table 1. Comparison of models used to compare the moisture niche of bacterial strains with contrasting biofilm production. We measured respiration rates for non-mucoid (NM) and overexpressor (OE) strains of *Pseudomonas aeruginosa* along a water potential gradient. We described the responses using maximum likelihood and the niche models (eqs. 3 and 4). W_{opt} = optimum water potential, R_{max} = maximum respiration rate, b = niche breadth, τ = shape kernel describing respiration response, "no difference" corresponds to a model where OE and NM have identical niche parameters, and "null" means that respiration was fit to the global mean respiration rate. Better models have lower ΔAIC and higher weights.

niche parameters	ΔAIC	df	weights
W_{opt}, b	0.00	7	0.2481
W_{opt}	0.06	6	0.2408
W_{opt}, R_{max}	1.23	7	0.1341
W_{opt}, b, τ	2.00	8	0.0913
W_{opt}, R_{max}, b	2.00	8	0.0913
W_{opt}, τ	2.06	7	0.0886
W_{opt}, R_{max}, τ	3.25	8	0.0489
W_{opt}, b, R_{max}, τ	4.01	9	0.0334
b	6.43	6	0.0100
R_{max}, b	8.43	7	0.0037
b, τ	8.43	7	0.0037
no difference	9.15	5	0.0026
R_{max}, b, τ	10.4	8	0.0013
τ	11.15	6	0.0009
R_{max}	11.2	6	0.0009
R_{max}, τ	13.2	7	0.0003
null	77.0	2	<0.0001

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FIGURE CAPTIONS

Fig. 1. Biofilm phenotype of *Pseudomonas aeruginosa* was affected by mutations in the regulatory gene *algT* that controls alginate biosynthesis. **A.** The non-mucoid (NM) mutant had altered colony morphology and generated 10-fold less biofilm than the wild-type overexpressor (OE) strain. **B.** Kernel density plot showing the distribution of biofilm production for NM and OE in reference to a diverse collection (n = 45) of soil microorganisms (see Lennon et al., 2012).

Fig. 2. Survivorship curves for the non-mucoid (NM) and overexpressor (OE) strain of *Pseudomonas aeruginosa* under conditions of desiccation (~35 % relative humidity).

Fig. 3. Fitness (measured as population growth rate) for the non-mucoid (NM) and overexpressor (OE) strain of *Pseudomonas aeruginosa* in soil along a water potential gradient. The minimum water potential needed for positive growth (Ψ^*) is higher (drier) for NM than it is for OE. However, OE has higher fitness than NM at all measured water potentials.

Fig. 4. Moisture niche space using respiration rates as a response variable for non-mucoid (NM) and overexpressor (OE) strain of *Pseudomonas aeruginosa* in soil along a water potential gradient. Maximum likelihood methods indicate that OE had a significantly drier optimal water potential (W_{opt}) than NM. In addition, our data support models where OE had a wider moisture niche breadth (b) and higher maximum respiration rate (R_{max}) than NM. Note: drier soils have more negative water potentials.

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654

Fig. 5. Water potential measured in soil columns that were inoculated with non-mucoid
656 (NM) and overexpressor (OE) strains of *Pseudomonas aeruginosa*. Moisture content was maintained for an initial establishment phase, which was followed by a series of dry-
658 down and rewetting cycles (vertical dashed lines). Water potential was significantly higher (wetter) in soil columns inoculated with OE than in columns inoculated with NM.

660

Fig. 6. Fitness (measured as population growth rate) for a desiccation-sensitive "tester"
662 strain *Pedobacter* sp. KBS0701, which belongs to the Sphingobacteriaceae (Bacteroidetes). The tester strain was inoculated into soil microorganisms alone (black),
664 with NM (white), or with OE (grey). We found no evidence to support the hypothesis that biofilm production could alter the performance of unrelated bacteria via facilitation. Ψ^* is
666 the minimum water potential required for positive growth rate in the tester strain. Note: drier soils have more negative water potentials.

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Figure 1

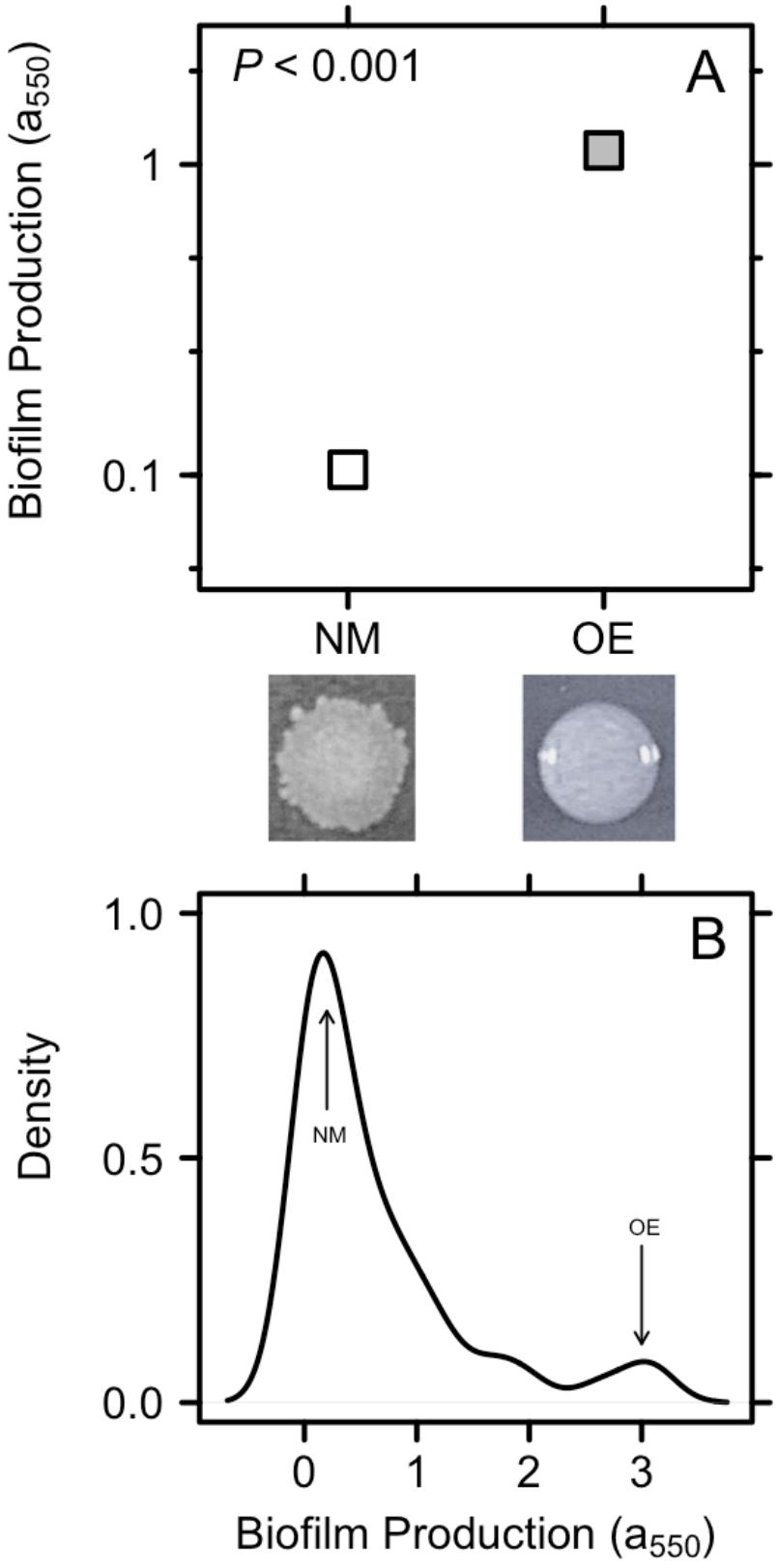

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Figure 2

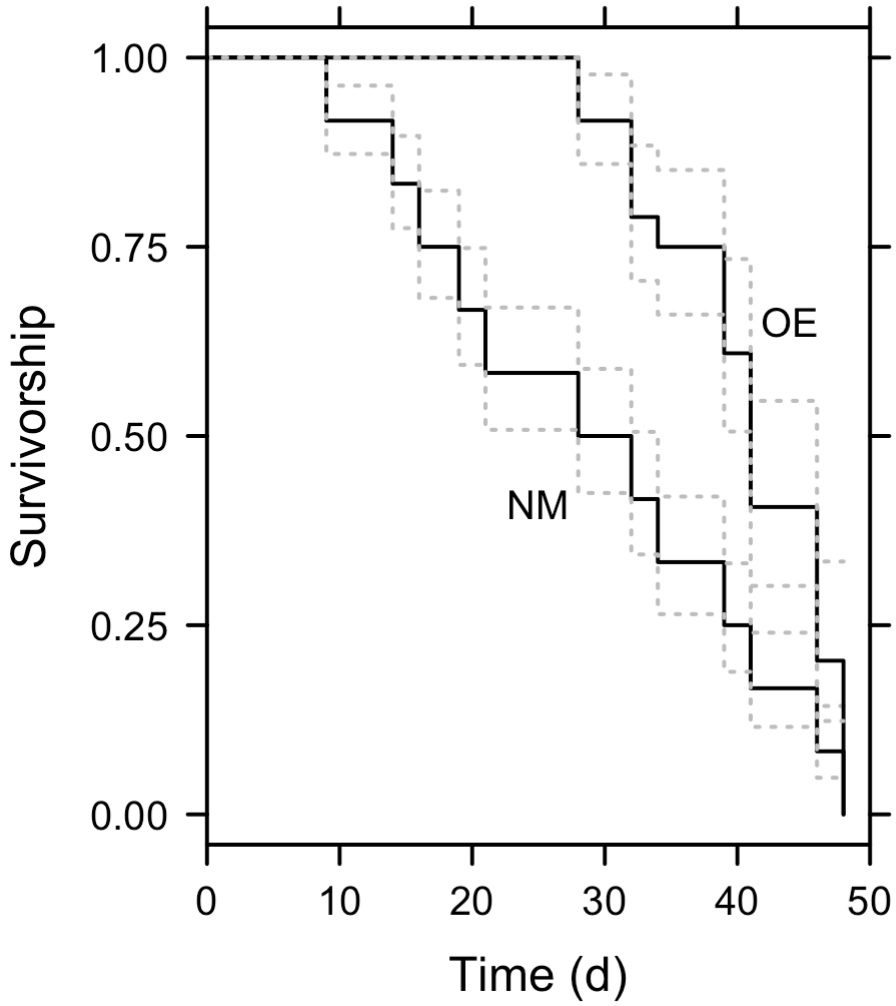

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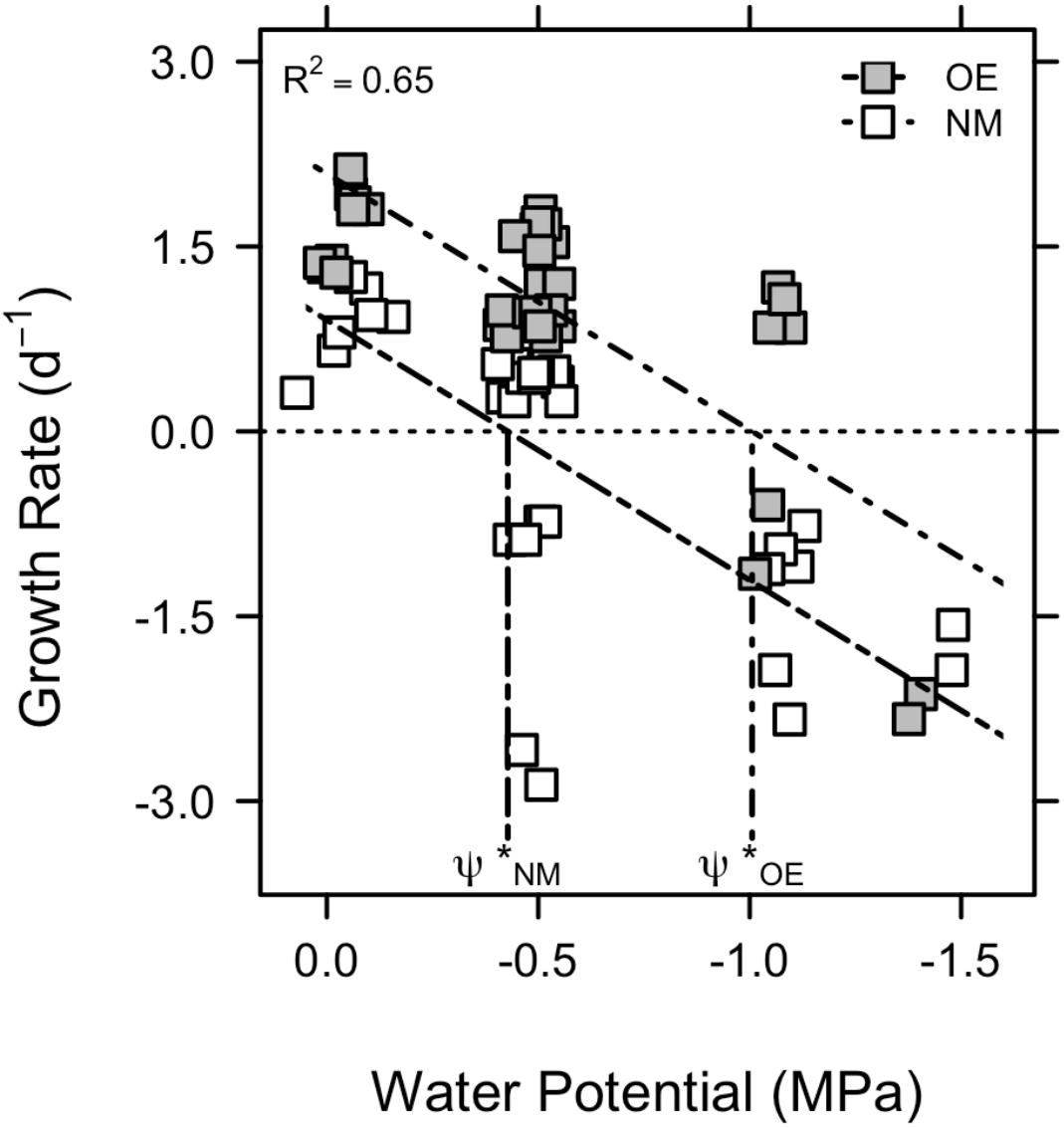
Figure 3 672



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674 **Figure 4**

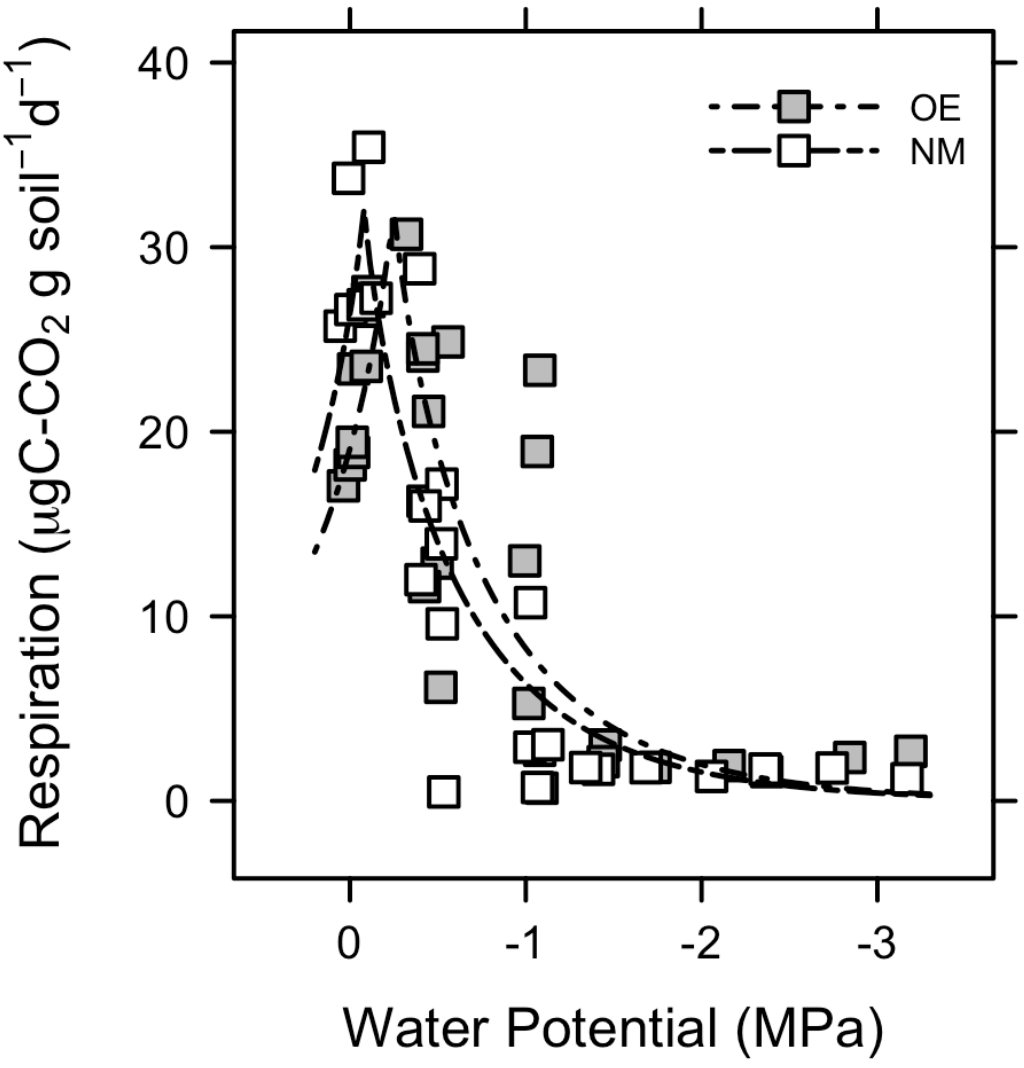

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Figure 5

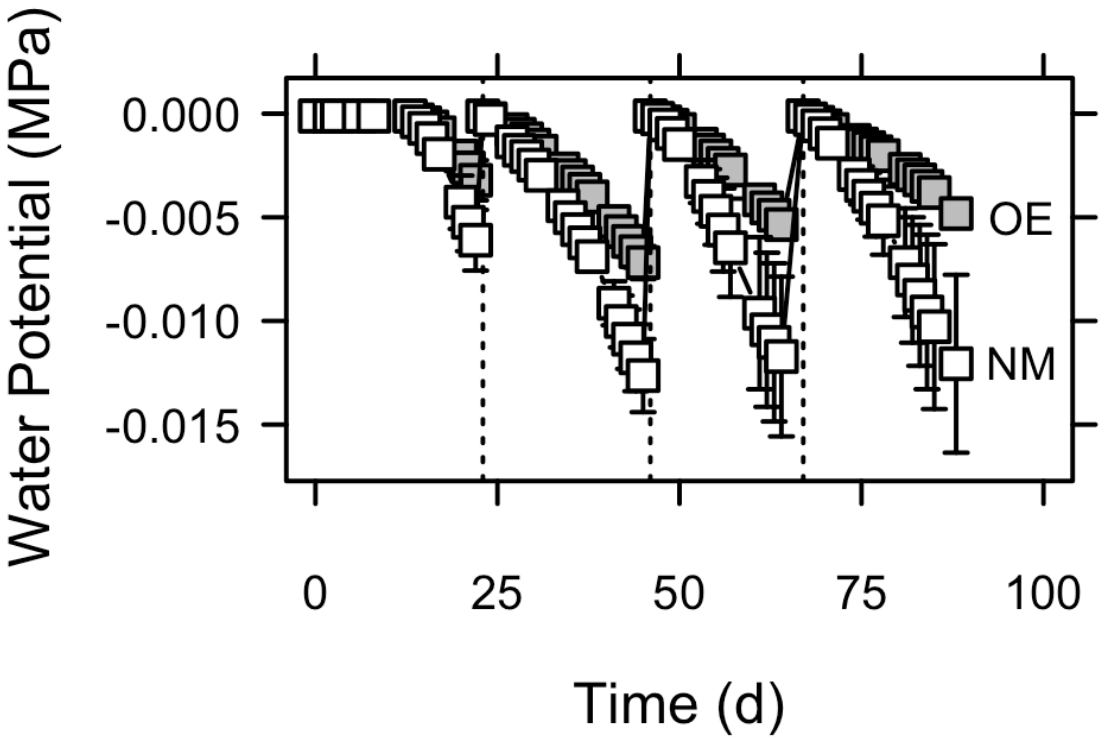

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Figure 6

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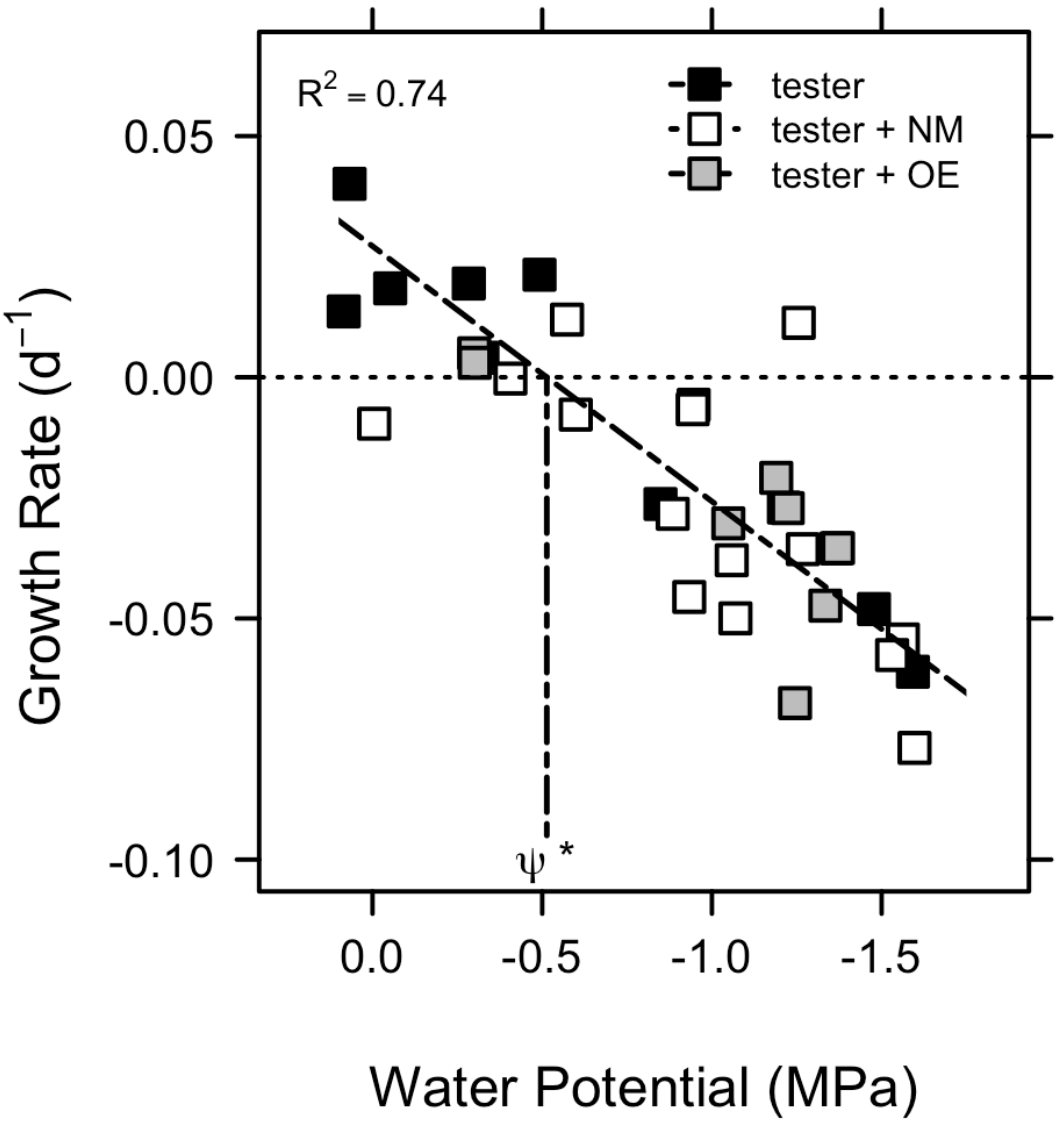

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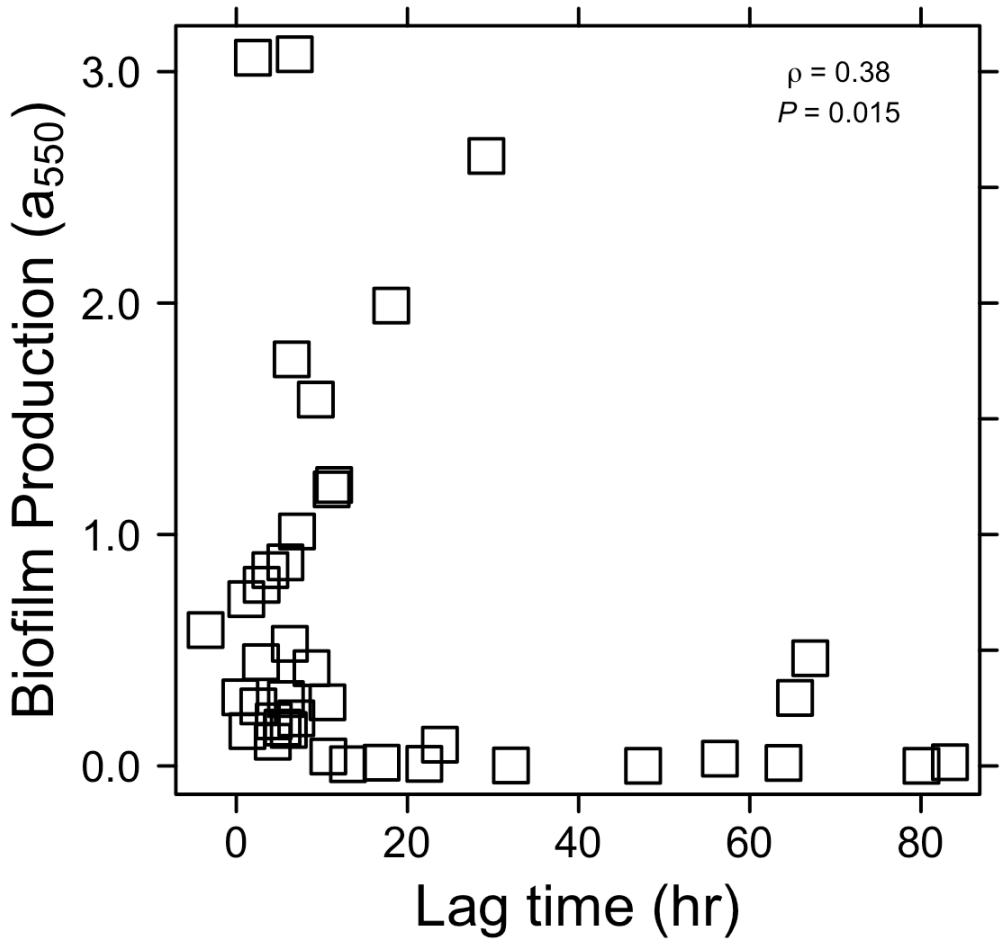
SUPPLEMENTARY FIGURE

678 **Fig. S1.** Relationship between biofilm production and lag time measured on 45 strains of soil microorganisms. Data from (Lennon et al., 2012). Statistics correspond to Spearman
680 rho correlations.

682



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