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A trait-based approach to bacterial biofilms in soil

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SUMMARY

A trait-based approach focuses on attributes of taxa that influence the structure and function of communities. Biofilm production is a common trait among microorganisms in a wide range of environmental, engineered, and host-associated ecosystems. Here, we used *Pseudomonas aeruginosa* to link biofilm production to moisture availability, a common stressor for microorganisms in soil. First, we demonstrate that biofilm production is a response trait that influences the desiccation phenotype by increasing survivorship, 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 no evidence for a trade-off between fitness and biofilm production along a soil moisture gradient. Second, we demonstrated that biofilm production is an effect trait. Specifically, biofilm production increased water retention in soils that were exposed to a series of drying and rewetting cycles. Although this form of niche construction should affect species interactions, we found no evidence that the benefits of biofilm production were extended to another co-occurring soil bacterium. Together, our results support the view that biofilm production is an important trait that may contribute to the distribution, abundance, and functioning of microorganisms in soils.

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 affect 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 (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), 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). In this context, biofilm production is thought to be a "response trait" (Lavorel and Garnier, 2002), which allows microorganisms to contend with environmental conditions that might otherwise reduce survivorship and reproduction.

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.,

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2014). In addition, biofilm production may be an important trait because it has the potential to alter environmental conditions in a way that affects the composition and function of microbial communities. This type of niche construction or ecosystem engineering (Matthews et al., 2014) may have implications for understanding the maintenance of microbial biodiversity and its contribution to ecosystem stability (Fierer and Lennon, 2011). In this context, biofilm production can be viewed as an "effect trait" (Lavorel and Garnier, 2002), which means that a microbial characteristic modifies the environment in ways that create feedbacks, potentially altering the composition and functioning of a microbial community.

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 (Ophir and Gutnick, 1994; Chang et al., 2007). This is important because soil microorganisms are regularly 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.

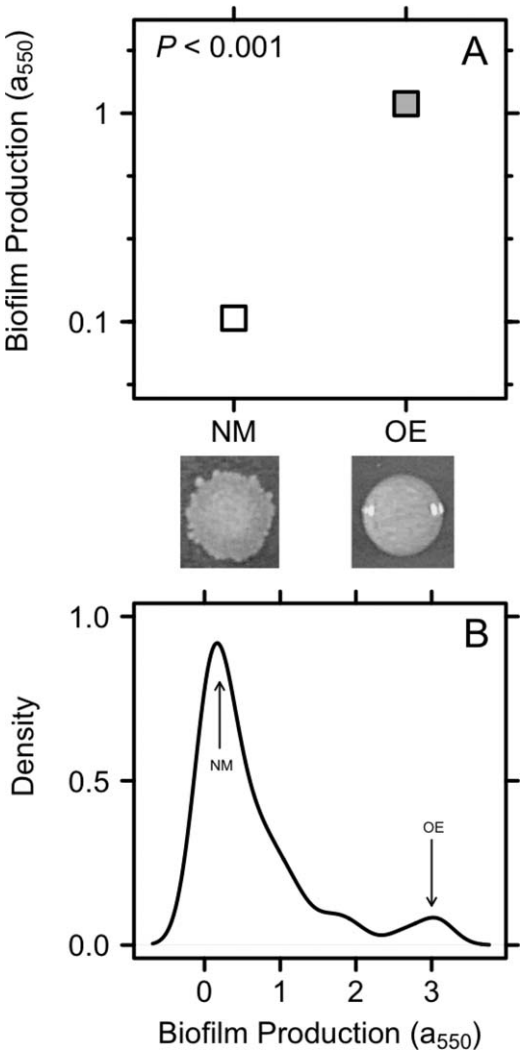

Fig. 1. Biofilm phenotype of *Pseudomonas aeruginosa* was affected by mutations in the regulatory gene *algT* that controls alginate biosynthesis. A. The NM mutant had altered colony morphology and generated 10-fold less biofilm than the wild-type OE strain. B. Kernel density plot showing the distribution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

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 OE compared to the isogenic 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 liquid medium, 25°C, 150 rpm), the two strains have comparable population growth rates (OE = 0.17 ± 0.006 h^{-1} vs. NM = 0.15 ± 0.026 h^{-1} [mean ± SEM], $t_{2.2} = -1.12$, $P = 0.37$) and lag times (OE = 2.9 ± 1.80 h vs. NM = 4.3 ± 0.047 h [mean ± SEM], $t_{2.3} = -0.71$, $P = 0.55$). However, other growth

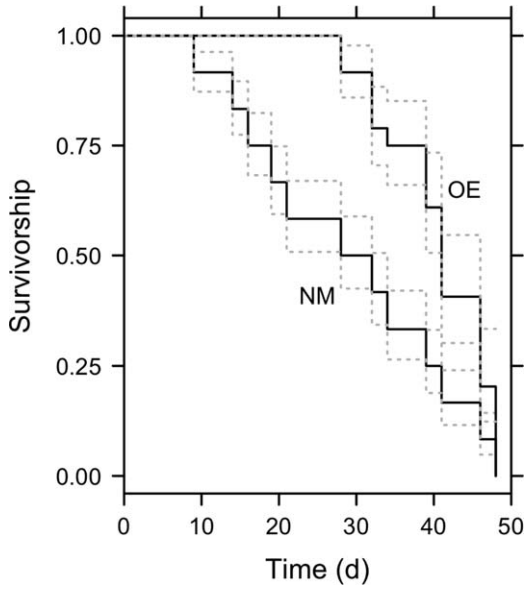

Fig. 2. Survivorship curves for the NM and OE strain of *Pseudomonas aeruginosa* under conditions of desiccation (~35% relative humidity).

characteristics between the strains were different. Specifically, OE had a higher maximum growth rate (μ_{max}) than NM (OE = 0.035 ± 0.0009 h^{-1} vs. NM = 0.025 ± 0.0012 h^{-1} [mean ± SEM], $t_{3.8} = -6.60$, $P = 0.003$), but NM attained higher levels of biomass than OE (OE = 0.96 ± 0.010 vs. NM = 1.44 ± 0.096 [mean ± SEM], $t_{2.0} = 4.97$, $P = 0.036$). The 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). Last, 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).

Survivorship under desiccation

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

Test for biofilm fitness trade-off

Separately, we measured the growth rate of OE and NM in soil microcosms to test for a moisture-dependent cost of biofilm production. After manipulating water content in the microcosms, we expressed moisture availability as water potential in units of megapascals (MPa). This type of measurement most accurately captures the physical forces that determine how moisture affects microbial physiology (Or et al., 2007). As we expected, the growth rate of both bacterial strains decreased as soils became drier (i.e., more negative water potentials). However, a multiple regression model revealed that population growth rates of OE and NM responded differently to soil water potential (Fig. 3, $R^2 = 0.65$, $F_{3,64} = 40.1$, $P < 0.0001$). The intercept of the growth-moisture relationship for OE was significantly higher than that of NM ($P = 0.0004$), but the slopes were the same ($P = 0.94$) suggesting that there was no trade-off between bacterial fitness and a strain's capacity to produce biofilm. Based on these findings, bacterial population growth in soil could be described using two equations:

$$\text{OE growth } \left(d^{-1}\right) = 2.09\ (\pm 0.229) + 2.08\ (\pm 0.337)\Psi \quad (1)$$

$$\text{NM growth } \left(d^{-1}\right) = 0.91\ (\pm 0.315) + 2.04\ (\pm 0.477)\Psi \quad (2)$$

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

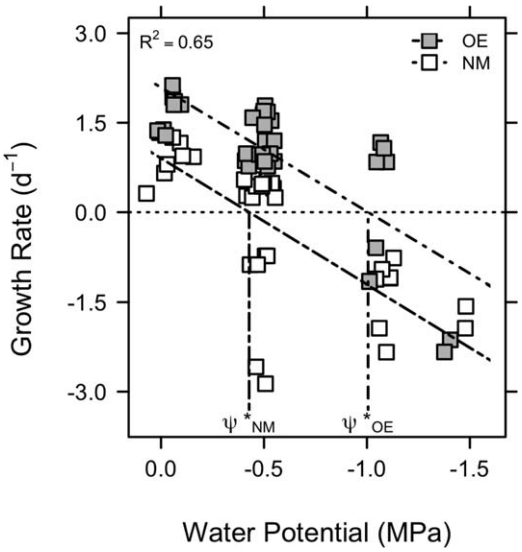

Fig. 3. Fitness (measured as population growth rate) for the NM and OE strain of *Pseudomonas aeruginosa* in soil along a water potential gradient. The minimum water potential needed for positive growth (Ψ^*) is higher (drier) for NM than it is for OE. However, OE has higher fitness than NM at all measured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

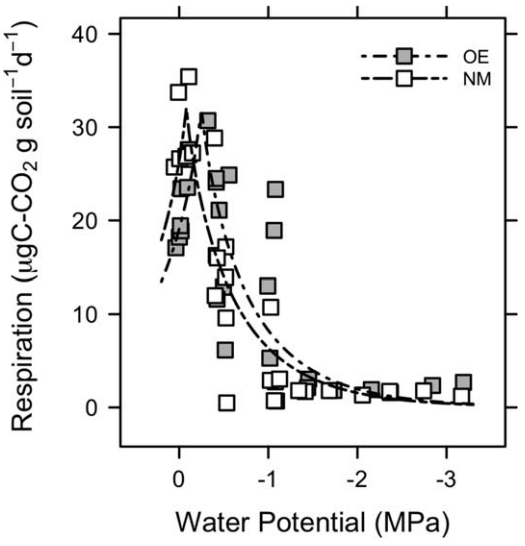

Fig. 4. Moisture niche space using respiration rates as a response variable for NM and OE strain of *Pseudomonas aeruginosa* in soil along a water potential gradient. Maximum likelihood methods indicate that OE had a significantly drier optimal water potential (W_{opt}) than NM. In addition, our data 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.

Table 1. Comparison of models used to compare the moisture niche of bacterial strains with contrasting biofilm production.

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

We measured respiration rates for NM and 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.

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 Akaike information criteria (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 OE (-0.25 ± 0.063 MPa) was 68% lower (i.e., drier) than the W_{opt} for NM (-0.08 ± 0.023 MPa).

Niche construction by bacterial biofilms

We incubated NM and OE separately in replicate soil columns for three months to test whether biofilm production is an effect trait (*sensu* Lavorel and Garnier, 2002) that can modify physical properties of its environment (i.e., soil moisture). Soil moisture dynamics were significantly affected by the genetic background of the bacterial strain (OE vs. NM) that was introduced to the columns (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

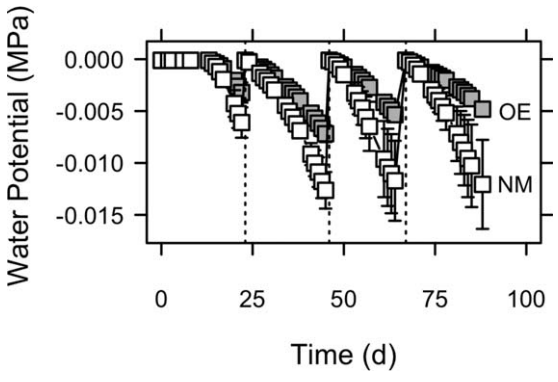

Fig. 5. Water potential measured in soil columns that were inoculated with NM and OE strains of *Pseudomonas aeruginosa*. Moisture content was maintained for an initial establishment phase, which was followed by a series of dry-down and rewetting cycles (vertical dashed lines). Water potential was significantly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

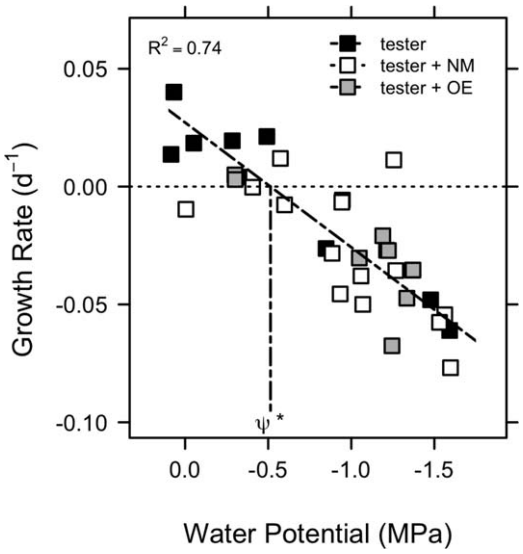

Fig. 6. Fitness (measured as population growth rate) for a desiccation-sensitive "tester" strain *Pedobacter* sp. KBS0701, which belongs to the Sphingobacteriaceae (Bacteroidetes).
The tester strain was inoculated into soil microorganisms alone (black), with NM (white), or with OE (grey). We found no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

square means [LSM] $\pm$ 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 $\pm$ 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 the 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 as soils became drier (i.e., more negative water potentials). 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 (Fig. 6).

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 demonstrated that biofilm production increases desiccation tolerance, an important phenotype that may influence soil microbial structure and function. The wild-type OE strain survived longer and had a drier moisture optimum (W_{opt}) than the mutant NM strain that lacked the ability to produce alginate. Despite not being able to detect a fitness cost of biofilm production in soil, we demonstrated that this trait has the ability to alter moisture dynamics in a way that is consistent with niche construction. However, the release of public goods in the form of EPS did not alleviate desiccation stress for an unrelated soil 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*, which has the capacity for 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 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 the knockout of an 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 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 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 (Supporting Information Fig. 1S), suggesting that there could be a cost associated with quorum sensing and/or the production of 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 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 commonly 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 Surette, 2006). Second, during biofilm production, energy- and nutrient-rich molecules (polysaccharides, proteins, and nucleic acids) are diverted from growth and reproduction towards 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. In contrast to our previous findings (Lennon et al., 2012), lag time of *Pseudomonas aeruginosa* in liquid culture was unaffected by a strain's capacity to produce biofilm. However, we did observe other growth characteristics that were suggestive of a biofilm-related trade-off. Namely, OE had a statistically higher maximum growth rate (μ_{max}) than NM, but this was accompanied by a reduction in the maximum biomass that was attained. This finding is consistent with expectations of a trade-off between rapid growth rate, which is advantageous for individuals and maximizing yield, which is advantageous for the population (Bachmann et al. 2013). In the soil environment, however, we did not find evidence for a trade-off involving the capacity for biofilm production and tolerance to desiccation stress. Specifically, there was no statistical interaction in our multiple regression model between strain identity (NM vs. OE) and water potential. Instead, the population growth rates of NM and OE declined at the same rate as soils became drier (i.e., more negative water potentials) (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 net-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 somewhat 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 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). The discrepancy between our finding and what is reported in other studies has important implications for understanding the social aspects of biofilm production and microbial interactions in the soil matrix. When a strain of bacteria allocates energy and resources towards a product that is released into the environment, there is the potential for cheater strains to evolve. These variants enjoy the benefits of the public good without an investment. However, diversity is maintained by the fact that the cheater population cannot outcompete the producer strain if the public good is required for persistence. In the soil environment, if biofilm production is advantageous but comes at no cost, then one would not expect the evolution of cheaters or social complexity that has been reported in other spatially structured habitats (West et al., 2006; Hillesland and Stahl, 2010).

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 helps bacteria contend with an important environmental variable; moisture availability. Our 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 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 (OE vs. NM) were comparable at the end of the experiment. Although our results suggest that OE should outperform NM at all moisture levels (Fig. 3), equal densities of *Pseudomonas* in the column experiments could arise due to the lack of head-to-head competition or the ability of NM to persist in the soil matrix in a quiescent or dormant state (Lennon and Jones, 2011).

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 as soils became drier (i.e., more negative water potentials). Contrary to our expectation, the slope of this relationship did not become shallower in the 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 one might expect if the tester strain was negatively affected by resource competition or interference from exposure to toxins produced by *Pseudomonas aeruginosa* (Oliveira et al., 2015). It is also 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 in the soil environment. 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 a 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

Growth characteristics

We characterized the growth characteristics two isolates derived from *Pseudomonas aeruginosa* FRDI: the wild-type OE and the isogenic NM mutant (*algT*::Tn501; Wozniak and Ohman, 1994). In triplicte, we conducted growth curve assays by taking optincal desnity readings at 600 nM (OD600) with a Molecular Devices SpectraMax5 spectrophotometer. We grew the cultures in R2B medium at 25°C on a shaker table (150 rpm). We estimated population growth rates using the Malthusian equation: $[\ln(OD_{t_f})/\ln(OD_{t_0})]/t$, where t is the duration of the growth curve assay in hrs, and $\ln(OD_{t_0})$ and $\ln(OD_{t_f})$ are the natural logarithms of OD600 readings at the beginning and end of the experiment, respectively. With the growth curve data, we also estimated lag time (L), maximum growth rate (μ_{max}), and the maximum biomass (or yield) for OE and NM using the modified Gompertz equation (Lennon et al., 2007). We compared the growth characteristics of the two *Pseudomonas* strains using Welch's *t*-tests.

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 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 25°C. We then 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 Devices SpectraMax5 spectrophotometer. We tested for differences in biofilm production between OE and NM using a Welch's *t*-test.

Survivorship

We measured survivorship for the OE and NM isolates using a desiccation assay. We added 10 μL of an exponentially growing culture ($\sim 10^6$ cells) to replicate wells of a 96-well plate. We allowed these cell suspensions to evaporate under 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 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 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 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 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 created survival curves using the *survfit* function in R.

Biofilm trade-off

We used experimental microcosms to test for a trade-off 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 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 h. 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 hours. 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 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 vs. 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:

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

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:

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

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 $\geq 50\%$ 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 Eqs. (3) and (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 $\sim 10^9$ 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 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 establishment of the bacteria in the soil matrix of the columns.

After the establishment phase, we created a series of dry-down and rewetting 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 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 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 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 bacterial strain (OE vs. NM) on soil water potential using repeated measures (RM) ANOVA (SAS PROC MIXED) with covariance structures selected using the Bayesian 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 pyrosphosphate solution. We plated these extracts onto R2A medium (without inhibitors) to check for contamination and to compare densities of OE and NM across treatments.

Species interactions experiments

Biofilm production is a trait that could modify the direction and magnitude of interaction between microbial species. We tested this hypothesis by measuring the population growth rates of a desiccation-sensitive isolate along a soil moisture gradient in the presence or absence of OE and NM using the 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 ($W_{\text{opt}} = -0.0007$ 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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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

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 rho correlations.