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A Two-Species Test of the Hypothesis That Spatial Isolation Influences Microbial Diversity in Soil

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ABSTRACT

The hypothesis that spatial isolation is a key determinant of microbial community structure in soils was evaluated by examining the competitive dynamics of two species growing on a single resource in a uniform sand matrix under varied moisture content. One species dominated the community under highly connected, saturated treatments, suggesting that these conditions allow competitive interactions to structure the community. As moisture content decreased, however, the less competitive species became established in the community. This effect was most pronounced at a matric water potential of −0.14 MPa where estimates of final population density and species fitness were equal. A second but more closely related species pair exhibited a similar response to decreasing moisture, suggesting that the effects of spatial isolation we observed are not simply a species-pair-specific phenomenon. These findings indicate that spatial isolation, created by low moisture content, plays an important role in structuring soil microbial communities.

Introduction

Although microorganisms are critical to a number of processes that determine soil quality, the key determinants that drive microbial diversity and structure microbial communities in soil are largely undefined. There is now ample evidence suggesting that soils can maintain high levels of microbial diversity [1, 2, 6, 9, 28, 31, 34]. For example, 30 g of forest soil was shown to contain ~4000 completely different bacterial genomes [28], which was estimated to be equivalent to half a million unique microbial species [8].

To better define the structure and controlling forces of existing soil microbial communities, we examined 29 surface, vadose, and saturated soil samples from four geographically distinct locations using a 16S rDNA gene-based cloning approach [35]. In low-carbon saturated soil samples microbial community profiles were dominated by one or a few species, suggesting that competitive dynamics determined the structure of these communities [29, 35]. Competitive communities of this type are also found in aquatic environments [20] and are common to many plant and animal communities. In contrast, low-carbon surface soil samples displayed an unusual diversity profile where

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all species were equally abundant and high microbial diversity was maintained [29, 35]. This type of uniform community structure, also observed in Arctic tundra, tropical, and temperate soils [1, 21, 34], suggests that microbial competition at the soil's surface is not a dominant force.

A key difference between drained surface soils and saturated zone soils is water content. Continuous water films in soil allow nutrient transfer between particles and provide a means by which organisms can move to more favorable locations. As dryer conditions are imposed, a lack of aqueous connectivity between soil particles can reduce the diffusion of substrates [16, 22], and restrict the mobility of microbial populations [12]. Thus, the normally dryer conditions prevalent at the soil surface could act to isolate microbial populations. Once isolated, more competitive species would be unable to displace less fit community members and species diversity in this zone would remain high. While our previous study shows a strong correlation between moisture content and community structure, it does not experimentally test the spatial isolation hypothesis.

The objective of this study was to evaluate the hypothesis that spatial isolation of microbial populations in soil can act to maintain microbial diversity in this habitat. This was achieved by examining the competitive dynamics of two species grown on a single carbon source in a sand matrix under varied moisture content. One species was purposely chosen because it is much more competitive, and thus this species dominates microcosm communities under saturated conditions. In less-connected, dryer treatments we found species abundance and fitness equalized, suggesting that the degree of spatial isolation is indeed an important determinant of microbial community structure in soil.

Methods

Bacterial Strains

Ralstonia eutropha JMP134 (pJP4) [5] and three *Sphingomonas* sp. isolates, strains 9256, 974, and 1443 [14], were chosen because they grow readily on MMO mineral medium [26] supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D). To better distinguish *Sphingomonas* sp. 1443 from strain 974 this isolate was tagged with the *Tn*5-*lac*Z construct pRL1268a [33] by electroporation. The newly constructed strain, 1443Lac, produced blue colonies on LB agar containing 40 μg/ml of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal).

Prior to use in microcosms each isolate was transferred from a frozen 15% glycerol stock to 5% PTYG agar [10] and then a single colony was transferred to 5% PTYG broth and grown overnight at 25°C. Any effect of the spent PTYG broth on the microcosm competition experiments was minimal since each culture was grown into late stationary phase and the cultured cells were highly diluted before being added to microcosms (e.g., 1 μL cultured cells into 30–50 mL MMO).

Moisture Characteristics of Quartz Sand

Clean quartz sand with a particle size range of 210–297 μm (Sigma Chemical Co., St. Louis, MO) was used for all microcosm experiments. A ceramic-membrane pressure extractor was used to determine the relationship between gravimetric water content of the sand and matric potential (Soil Moisture Equipment, Santa Barbara, CA). Estimates of pore size diameter were calculated using the equation of capillarity [13]:

$$\psi = 2\gamma(r)^{-1}$$

where ψ is matric potential, r is the pore size radius, and γ is the surface tension of water (0.727 bar μm). A pore size distribution for the quartz sand was calculated as described by Danielson and Sutherland [4].

Microcosms

Microcosms consisted of 20-mL glass vials with plastic screw-cap lids to which a known amount of quartz sand was added. Prior to use, each sand microcosm was sterilized by autoclaving, and then oven-dried at 105°C for at least 8 h. Moisture content was set gravimetrically to a range of matric potentials using MMO mineral medium amended with 2,4-D and a 1:1 mixture of each species. After the addition of amended MMO medium each microcosm was briefly vortexed, capped, sealed with Parafilm, and then incubated at 25°C in an enclosed plastic container.

Moisture loss over the course of a typical experiment (7 days) was negligible, as determined by the lack of change in weight for microcosms set in triplicate at matric potentials of −0.001, −0.008, −0.01, −0.1, and −0.14 MPa by the described method.

Microcosm Sampling

Three microcosms per treatment were sampled destructively immediately after inoculation and then at 24-h intervals by adding 5 mL of MMO medium and briefly vortexing each vial. Concentrations of 2,4-D in the microcosms were determined by high performance liquid chromatography as previously described [15]. Population size of each competing species was determined by plating a dilution series from each microcosm onto either 5% PTYG agar for JMP134 and 9256, or LB agar supplemented with 40 μg/g X-gal for 1443Lac and 974. Under these conditions *R. eutropha* JMP134 and *Sphingomonas* sp. 9256 form

Table 1. Growth characteristics of the two competing species

Isolate	Lag time[a] (h)	Growth rate[a] (h^{-1})	Colony color on 5% PTYG agar	Source
IMP 134 (pJP4)	≈5	0.178	White	Don and Pemberton [5]
9256	≈20	0.161	Yellow	Ka et al. [14]

[a] Determined on duplicate cultures growing at 25°C in MMO medium supplemented with 250 ppm 2,4-D

Table 2. The relationship between matric potential and gravimetric water content and pore neck diameter for the quartz sand matrix

	Matric potential (MPa)					
	−0.01	−0.008	−0.01	−0.05	−0.10	−0.14
Water content (g g^{-1})	0.20	0.08	0.04	0.02	0.012	0.008
Pore neck size[a] (μm)	290	36	30	6	3	2

[a] Refers to the maximum pore neck diameter of the largest water-filled pore

white and yellow colonies, respectively, and *Sphingomonas* sp. 1443Lac and 974 form blue and yellow colonies, respectively.

Fitness Calculations and Statistics

Fitness was calculated as the net rate of increase per day for each species at different moisture levels using the following equation [18]:

$$m_i = (1/t)\ln[N_i(t)/N_i(0)]$$

where t is defined as days, and $N_i(0)$ and $N_i(t)$ are the initial and final population densities. Population density and species fitness differences were compared using Student's t-test; a p value of less than 0.05 was regarded as a significant difference.

Results

Strain Choice and Characteristics

R. eutropha JMP134 and *Sphingomonas* sp. 9256 were chosen for microcosm competition experiments because they readily grow on MMO mineral media supplemented with 2,4-D, but differ in their lag times and specific growth rates (Table 1). Each species also exhibits a unique morphology on PTYG solid medium making the populations distinguishable in mixed culture competition by simple plating methods (Table 1). The higher growth rate and shorter lag time of JMP134 make this strain much more competitive for growth on 2,4-D than strain 9256. Thus, in liquid culture competition, which represents a highly connected environment, JMP134 outcompeted 9256. Although we assume that superior growth kinetics explains the competitive difference between JMP134 and 9256, other mechanisms (e.g., bacteriocin production by JMP134) cannot be ruled out. However, no growth inhibition by JMP134 was observed during competition experiments when cocultures of JMP134 and 9256 were regularly plated onto PTYG medium.

Sand Moisture Characteristics

To evaluate the effects of varied degrees of spatial isolation on microbial community structure and competitive dynamics it was necessary to mimic a wide range of moisture conditions typical of the soil environment. We thus determined the relationship between gravimetric water content and pore-neck size at matric potentials ranging from −0.001 to −0.14 MPa in a uniform quartz sand matrix (Table 2). Peak microbial activity generally occurs near field capacity (−0.01 MPa) [19, 23].

Monoculture Microcosms

If the growth rate of either of the test species used in this study is not affected in a proportional way as moisture conditions vary, then further experiments to evaluate the effects of spatial isolation on competitive dynamics would be confounded. To test for this effect, we initiated microcosms with monocultures of each species and tracked their growth over the range of matric potentials used in the mixed culture competition experiments (Fig. 1). Fitness values for both species decreased with decreasing moisture content in a similar fashion (JMP134 slope = 0.10, r^2 = 0.996; strain 9256 slope = 0.07, r^2 = 0.987) suggesting that neither species benefits simply because of the imposed microcosm conditions. The higher fitness values observed for JMP134 compared to 9256 likely reflect the superior growth characteristics of JMP134.

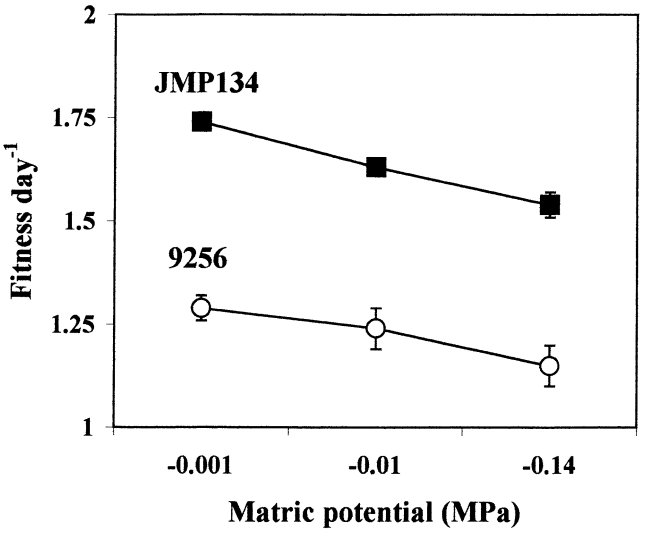

Fig. 1. Effect of varied moisture content on the fitness of JMP134 and 9256 in monoculture sand microcosms. Estimates of species fitness are the mean and SEM from three independent microcosms. Fitness was calculated as the daily rate of increase for each species [18].

Another condition that may result in species coexistence, independent of spatial isolation, arises when one species has an advantage when the resource is common and the other has an advantage when the resource is scarce [30]. However, this effect was ruled out by conducting competition experiments with JMP134 and 9256 in liquid culture with low levels of 2,4-D (25 ppm). Under these conditions JMP134 still dominated the two species community (data not shown).

Mixed Culture Microcosms

To evaluate the effects of varied degrees of spatial isolation on microbial community structure we carried out mixed culture competitions in sand microcosms at matric potentials that ranged from −0.001 to −0.14 MPa. With nearly complete saturation of the sand matrix (−0.001 MPa), JMP134 outcompeted 9256 over the course of 7 days, during which time the 2,4-D initially added to the microcosm diminished to undetectable levels (Fig. 2). JMP134 also outcompeted 9256 at matric potentials of −0.001, −0.0035, −0.005, and −0.009 MPa (data not shown) and at −0.008 MPa (Fig. 2). In the two most saturated treatments (−0.001 and −0.008 MPa) 9256 reached population densities that were below the level of detection in our plating regime (Fig. 2). Values for 9256 were set at zero for these sample points (e.g., after day 4 for −0.001 MPa treatment and after day 5 for −0.008 MPa treatment). It is likely that 9256 maintained a low population density in these treatments although an additional inhibitory mechanism by JMP134 cannot be completely ruled out. However, no evidence of such inhibition or killing was observed in liquid culture competition experiments between JMP134 and 9256. In contrast to the more saturated treatments, mixed species competitions in sand with matric potentials maintained between −0.01 and −0.14 MPa revealed a progressive increase in the population density of 9256 with decreasing matric potential (Fig. 2). This effect was most dramatic at −0.14 MPa where growth of 9256 was nearly identical to that of JMP134, suggesting that 9256 is no longer experiencing the strong competitive exclusion by JMP134 that was observed in the wetter treatments (Fig. 2). The more rapid 2,4-D degradation rates observed in the less saturated treatments (−0.01 MPa and below) likely indicate that more optimal conditions for microbial growth are present [19].

Final Population Densities

To begin examining the effects of spatial isolation on the competitive dynamics of the two species, the final population density of each species was compared after the substrate had been removed to undetectable levels (Fig. 3A). In wetter treatments, JMP134 dominated the microcosm community by either completely outcompeting 9256 (e.g., at −0.001 and −0.008 MPa), or by becoming established at levels that were nearly two orders of magnitude greater than 9256 (e.g., −0.01 MPa), all of which were highly significant differences ($p < 0.001$). At matric potentials below −0.01 MPa, differences between final population densities of the two species diminished progressively (Fig. 3A), and at −0.14 MPa final population density estimates of each species were statistically indistinguishable ($p > 0.05$).

Species Fitness

The simple design of the microcosm communities allowed for precise tracking of the dynamics of each species. Thus in addition to assessing community diversity by examining final population densities (e.g., Fig. 3A), the overall fitness of each species vs matric potential was also examined (Fig. 3B). These measures are useful because they take into account the initial and final population sizes of the competitors for each day of the experiment, and therefore

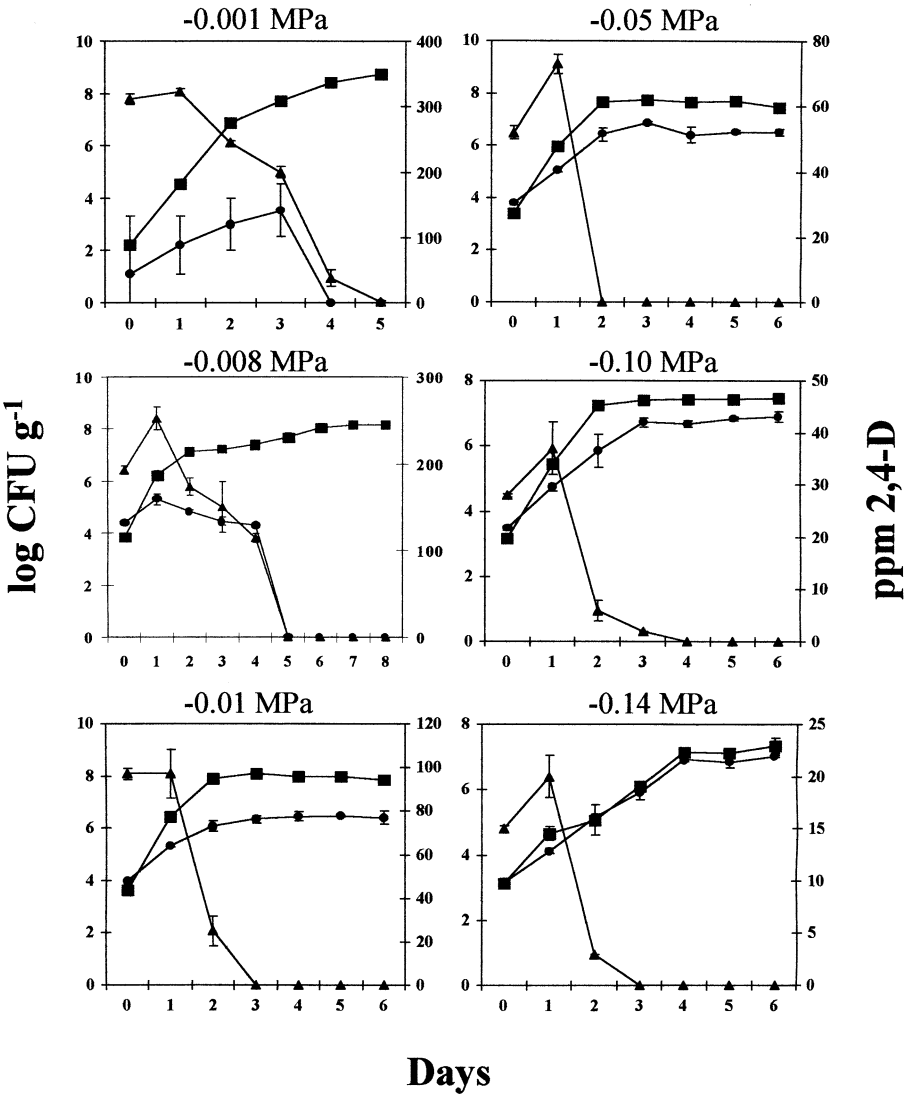

Fig. 2. Mixed culture competition experiments between JMP134 (■) and 9256 (●) for 2,4-D (▲) in sand microcosms maintained at matric potentials ranging from −0.001 to −0.14 Mpa (corresponding matric potentials are indicated above each panel). Estimates of population density and ppm 2,4-D were taken at daily intervals and are the mean and SEM from three independent microcosms.

encompass the entire growth cycle of each species rather than just a final time point [18]. This type of estimate has been used frequently to evaluate the competitive ability of evolved and ancestral microbial species [7, 18, 25, 32] and has been recommended to assess the persistence of microbial populations after introduction into a target environment [17]. Species fitness estimates were done only on the growth phase of the two species, and therefore avoided the long stationary phases observed in some treatments after 2,4-D removal (e.g., in the −0.01 MPa treatment days 2–6 were not used to calculate fitness; Fig. 2). Since the populations of 9256 dipped below the level of detection at matric potentials of −0.001 and −0.008 MPa it was assumed that 9256 maintained at least its starting inoculum, which set the fitness estimates for 9256 to zero in these treatments (Fig. 3B).

Overall, species fitness responded to matric potential (Fig. 3B) in the same manner as was observed for the final population density estimates. At matric potentials ranging from −0.001 to −0.10 MPa JMP134 fitness was significantly higher than that of 9256 ($p < 0.001$), suggesting that at these moistures the communities were structured by competitive dynamics. However, when moisture content was decreased to a matric potential of −0.14 MPa the fitness values for the two species became statistically indistinguishable ($p > 0.05$) (Fig. 3B), suggesting that competition was reduced in this treatment.

Do the Effects of Spatial Isolation Apply Generally?

To ensure that the evaluation of the spatial isolation hypothesis is a more general phenomenon and not strain-pair dependent, the dynamics of a second species pair competing for 2,4-D under varied moisture conditions was also examined. This second pair consisted of two closely related *Sphingomonas* sp. isolated from the same location

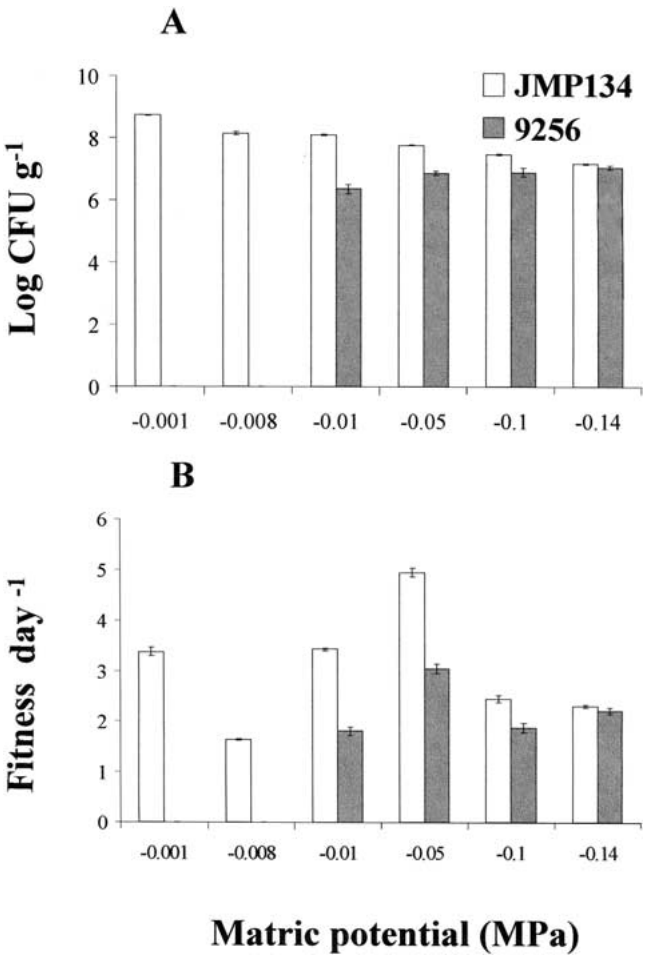

Fig. 3. Final population densities (A) and fitness estimates (B) for JMP134 and 9256 maintained at matric potentials ranging from −0.001 to −0.14 MPa in quartz sand. Values are the mean and SEM from three independent microcosms. Fitness was calculated as the daily rate of increase for each species [18].

of an agroecosystem study site [14]. One member of this pair, *Sphingomonas* sp. 1443Lac, was tagged with *Tn*5-*lac*Z to distinguish it from *Sphingomonas* sp. 974.

To test the stability of the 1443Lac construct, this strain was inoculated into a sand microcosm maintained at −0.01 MPa with 2,4-D as the sole carbon source, and its growth was followed for 7 days (at which time the 2,4-D was reduced to undetectable levels). Daily dilutions from the microcosms yielded only blue-colored colonies, suggesting that the *lac*Z-tagged *Sphingomonas* sp. did not revert to its former non-*lac*Z phenotype.

Because the microcosm experiments with the first species pair showed that competitive interactions apparently dominated the community at matric potentials above −0.008 MPa, the examination of competitive interactions in the second species pair was conducted at and below this point. In mixed culture competitions with matric potentials ranging from −0.008 to −0.05 MPa the final population densities of 1443Lac were significantly greater than those of *Sphingomonas* sp. 974 ($p < 0.05$) (Fig. 4A). In contrast, when matric potential was maintained at −0.14 MPa estimates for the final population sizes of the two species were not significantly different ($p > 0.05$). Fitness over the course of the competition experiments was also significantly greater for *Sphingomonas* sp. 1443Lac in the −0.008 to −0.05 MPa range (Fig. 4B; $p < 0.05$), but not when matric potentials were maintained at −0.14 MPa (Fig. 4B; $p > 0.05$). Although the differences in final population densities and species fitness for the second species pair were significant in the more saturated treatments (e.g., −0.008 to −0.05 MPa) they are not nearly as dramatic as the differences observed for the first species pair (Figs. 3A and 3B). One explanation for this difference may lie in the relatedness of the second species pair. Both *Sphingomonas* spp. were isolated from the same agroecosystem study site and have more similar growth kinetics on 2,4-D, whereas the difference in growth response to 2,4-D for the first species pair was much more dramatic (see Table 1).

Discussion

Using a 16S rDNA gene-based cloning approach we previously found that microbial communities were structured differently at the surface and saturated zones of low-carbon soils [35]. The saturated zone soils showed a competitive diversity pattern where one or a few species dominated the community. In contrast, surface soils displayed a more unusual pattern where each species was equally rare, suggesting that reduced competitive interactions existed in these environments. Mathematical modeling of the relationship between microbial biomass and nutrient exchange indicated that spatial isolation could explain the noncompetitive diversity pattern we observed in numerous surface soil samples [35].

Here we tested the hypothesis that spatial isolation is a key determinant of microbial community structure in soils by allowing two species to compete for a single resource in a uniform sand matrix under varied moisture content. As expected, the more competitive of the two species dominated the community under saturated conditions. However, as we increased the degree of spatial isolation, by decreasing the moisture content of the microcosms, the less competitive species also became established in the

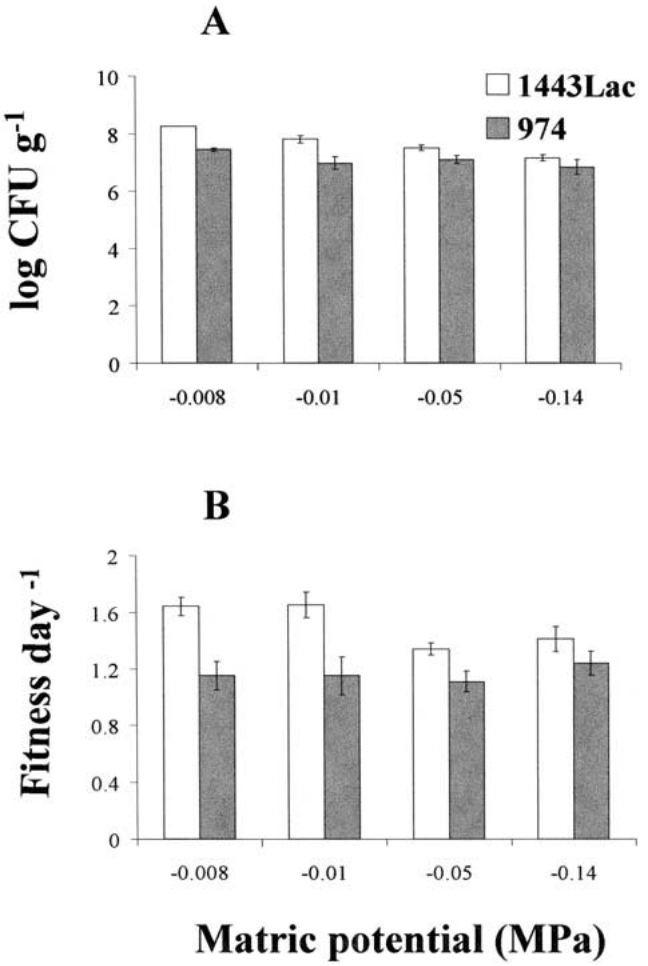

Fig. 4. Final population densities (A) and fitness values (B) for *Sphingomonas* sp. 1443Lac and 974 maintained at matric potentials ranging from −0.008 to −0.14 MPa in quartz sand. Values are the mean and SEM from three independent microcosms. Fitness was calculated as the daily rate of increase for each species [18].

community. This effect was most pronounced when the matric potential was maintained at −0.14 MPa. Under these conditions the final population density of each competing species and their respective fitness values were not significantly different from one another. We observed this same trend with a second pair of closely related isolates, suggesting that the effects of spatial isolation are likely to be a general phenomenon rather than simply species-pair dependent.

Our results suggest that as spatial isolation increases, a community that displays a competitive diversity pattern, where one or a few species dominate, can be shifted to exhibit a community profile that appears to lack competitive dynamics. We thus conclude that spatial isolation is an important driving force that shapes the structure of soil microbial communities. The competition experiments presented here are similar to Gause's batch culture studies with *P. caudatum* and *P. bursaria* [11]. However, whereas Gause observed displacement of one species because of the uniformity of the culture flask environment, this study shows that spatial separation in a simulated soil environment results in species coexistence.

Certainly, the two species sand microcosm communities described in this work are highly simplified compared to many natural soil microbial communities. However, it is reasonable to assume that the effects of spatial isolation would affect microbial community structure in a similar fashion whether the community contains few or many species. The advantage of using such a simple test system is that we could construct our communities with well-characterized competitors, precisely track the dynamics of each population at a number of time points, easily replicate experimental conditions, and eliminate the confounding effects of additional carbon resources. The current methods to track changes in microbial communities and the complexity of these communities in soil would make our study of the effects of spatial isolation in an extant soil community much more laborious and possibly prohibitive.

The effect of spatial isolation on microbial competitive dynamics we observed is likely to be related to a number of interacting factors. The lowered water activity of dry soil limits substrate diffusion to microbial cells [22] and can inhibit microbial activity [3]. Indeed, the combined effects of diffusional limitation and cell dehydration explained the decline in activity of nitrifying bacteria in a dry silt loam [27]. Dry soils can also limit microbial movement. For example, severe limitations to microbial movement occur when water-filled pore neck diameters are maintained below 2–3 μm [12], which is consistent with the dramatic effects of spatial isolation we observed when matric potential was maintained at −0.14 MPa (which is equivalent to water-filled pore neck diameters of ≤ 2 μm; see Table 2). Interestingly, the enhanced survival of bacteria introduced into drier soils [24], which has been interpreted to mean that the bacteria are escaping predation by entering smaller pores [16], may now also be explained in terms of the effects of spatial isolation on competitive interactions.

The quartz sand used in our microcosms represents a large particle matrix. A pore-size distribution of this matrix shows that only 4% of the total pore space was occupied by pores with a neck diameter of ≤ 2μm (Fig. 5),

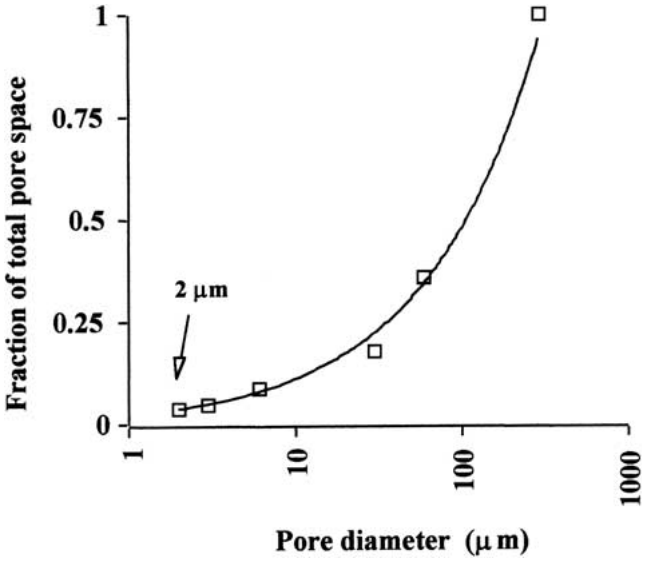

Fig. 5. Pore-size distribution for the quartz sand matrix showing the fraction of total pore space at different pore sizes. The relationship fits the equation $y = 0.026x^{0.631}$ ($r^2 = 0.99$).

and this likely explains why relatively dry microcosm conditions were necessary to significantly reduce competitive interactions among the two species pairs. However, it is important to note that the weak competitor of each two-species pair became established in the microcosms at more saturated levels (e.g., −0.01 MPa for the first species pair). Thus the influence of spatial isolation on microbial diversity may be important even at moisture levels that represent more natural field conditions. With more information on the competitive dynamics of microorganisms in soils of different textures, it should be possible to construct a predictive model of microbial community structure based on site data such as soil particle size and moisture content.

Although the microcosm studies presented here indicate that spatial isolation alone is an important factor for microbial community structure, resource heterogeneity in soils may also lead to a community profile that lacks competitive interactions. The appeal of this proposal is that the amount [23] and probably also the varieties of carbon decrease with increasing soil depth. Thus the uniform diversity pattern observed in surface soil samples could be the result of coexistence of many species on a variety of resources. It would therefore be informative to model the combined effects of spatial isolation and resource heterogeneity on microbial community structure across a range of moistures. In our previous study high carbon content in saturated subsurface soils produced a community with high microbial diversity and a clear lack of dominant species [35].

If the conclusion that spatial isolation is a driving force for soil microbial community structure is true, these findings have broad implications for soil microbial ecology. Understanding the key factors that lead to growth and dominance in a soil matrix is critical information for improved agricultural productivity, for the development of more efficient bioremediation practices, and for understanding the maintenance of soil microbial diversity.

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