Supplementary information

A Hydration-Based Biophysical Index for the Onset of Soil Microbial Coexistence

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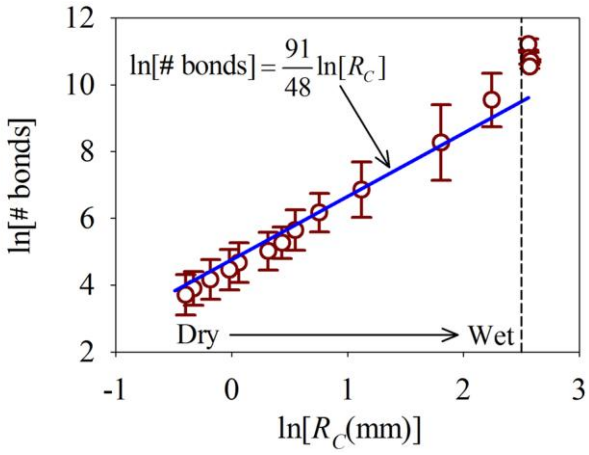
Supplementary Table S1. Physiological parameters for microbial growth, metabolism and nutrient concentrations.

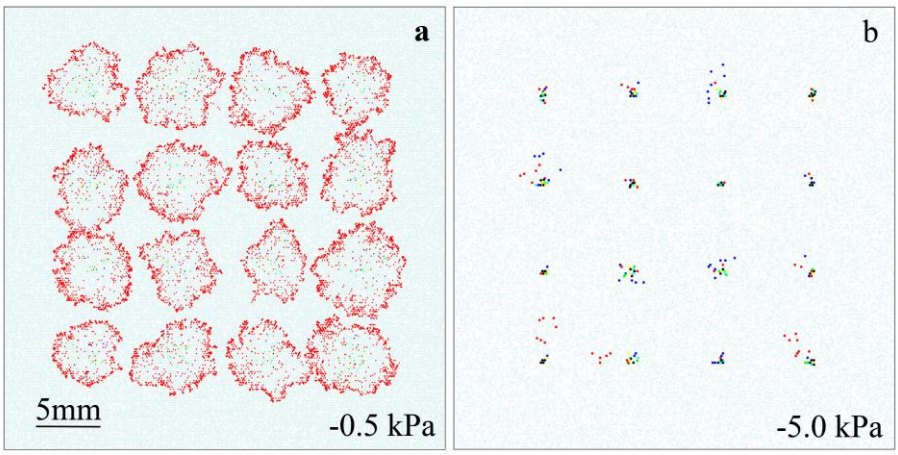
Parameters	Units	Values*
μ_{max}: maximum specific growth rate	hr^{-1}	[0.2~1.2]†
K_S: half-saturation constant	mg l^{-1}	[0.2~1.2]×10^{-3}†
Y_{max}: apparent yield at μ_{max}	g dry mass (g substrate)$^{-1}$	0.44
m: apparent maintenance rate	g substrate (g dry mass)$^{-1}$ hr^{-1}	0.036
$\bar{V}_B$: median cell volume	fl	0.4
$V_{B,d}$: cell volume at division	fl	$2\bar{V}_B$ /1.433
$V_{B,min}$: minimal volume of an active cell	fl	$V_{B,d}$ /5
ρ: cell density (dry mass)	g l^{-1}	290
C: substrate concentration	mg l^{-1}	1

*: For 3D systems with extracted values from Treves *et al*.[1]

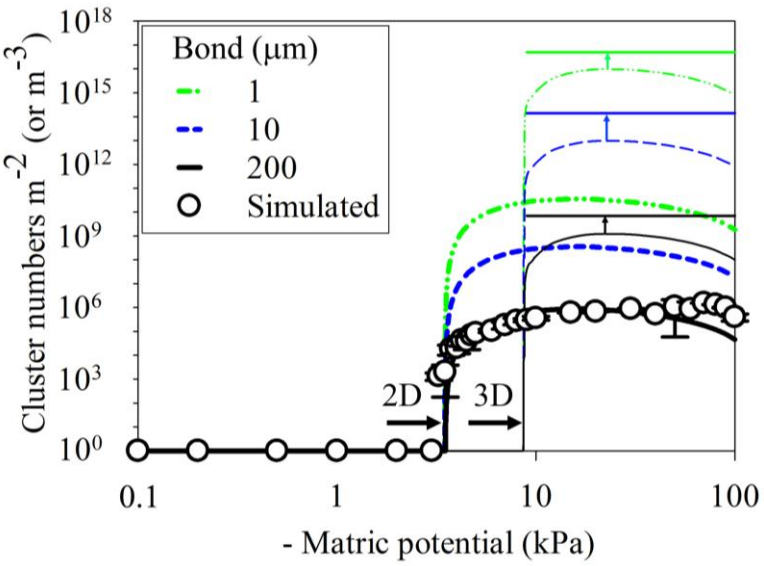
†: Bivariate uniform distribution with correlation coefficient of 0.01.



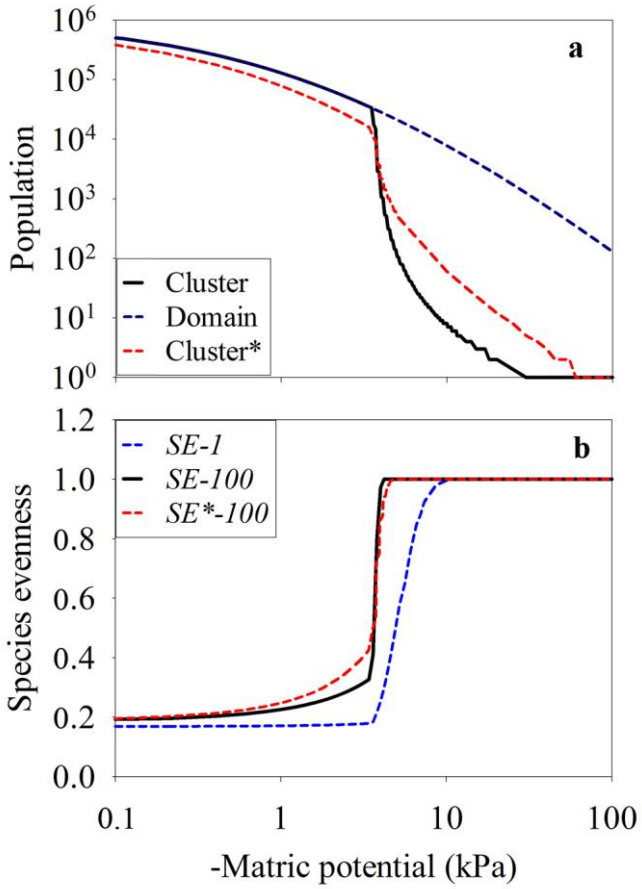
Supplementary Figure S1. Water filled pore cluster characteristics on unsaturated roughness networks. Simulated radius of the maximum water filled pore/channel cluster as a function of numbers of water filled pores/channels (mean±s.d., n=5), in agreement with numerical prediction by percolation theory[2]. Dashed line marks percolation threshold.



Supplementary Figure S2. **Simulated colony growth patterns of multiple competing microbial populations**. Colony growth patterns of a set of competing microbial species on unsaturated roughness network at 60 hr after inoculation under (**a**) -0.5 kPa, and (**b**) -5.0 kPa, respectively. Color spots represent the set of competing microbial species differentiated at intrinsic growth characteristics: maximum specific growth rate of species 1 (SP1) of 1.2 (red), SP2 of 0.6 (blue), SP3 of 0.5 (green), SP4 of 0.4 (cyan), SP5 of 0.3 (yellow) and SP6 of 0.2 hr^{-1} (black), and half saturation constant of SP1 of 1.2, SP2 of 0.6, SP3 of 0.5, SP4 of 0.4, SP5 of 0.3 and SP6 of 0.2 $\mu g\ l^{-1}$, respectively. Populations of 6 mixed species (each consisting 4 cells, totally 24 cells for each inoculation site) were inoculated within 16 sites on a roughness network at the beginning of a simulation.



Supplementary Figure S3. A**nalytical aqueous cluster numbers for 2D surfaces and 3D soil volumes.** Predicted number of aqueous clusters for 2D surfaces (number per m^2) and for 3D soil volumes (number per m^3) with comparisons of numerical simulations (symbols) with measurements[3]. Color bars represent grain numbers per m^3 assuming grain diameters of 1, 10 and 200 μm, these are used as upper bounds for the maximum number of aqueous clusters per soil volume (m^3).



Supplementary Figure S4. A**nalytical nutrient-limited population capacities and species evenness.** (**a**) Analytical predictions of nutrient-limited population capacities per cluster and for the simulation domain, denoting 'Cluster*' for nutrient-limited population capacity per cluster calculated according to equation (S22), and (**b**) analytical prediction of species evenness (*SE*) with initial inoculation size of 1 (*SE-1*) and 100 (*SE-100*) cells of each species, with '*SE*-100*' represents *SE* calculated according to equation (S22) for population capacity per cluster with initial inoculation of 100 cells of each species.

Detailed methods

We considered microbial growth rate represented by the Monod function, with key biological parameters[4] listed in Supplementary Table S1,

$$\mu_{eff} = \frac{\mu_M C}{C+K} - m, \tag{S1}$$

where μ_M and μ_{eff} are the maximum and effective microbial specific growth rates, respectively, K is half-saturation constant marks the value of C at which an active cell achieves half of its maximal growth rate, m is microbial maintenance rate, and C is apparent nutrient concentration exposure to microbial population, which we considered to be proportional to nutrient diffusive flux (expressed as effective nutrient diffusion coefficient, D_{eff}),

$$\langle C \rangle = \frac{D_{eff}}{D_0} C_0, \tag{S2}$$

where D_0 is nutrient diffusion coefficient in bulk water, C_0 is nutrient concentration at boundaries, and D_{eff} is a function of mean volumetric water content ($\langle \theta(\psi) \rangle$) of rough surface[5],

$$D_{eff}(\psi) = D_0 \frac{\langle \theta(\psi) \rangle^2}{\phi^{2/3}}, \tag{S3}$$

where ϕ is the effective "porosity" generated by surface roughness (relative to smooth surface), which is calculated according to,

$$\phi = \frac{2\sqrt{3}}{3\langle H \rangle l} \int_{\Omega_\alpha} \int_{\Omega_H} H^2 \tan(\frac{\alpha}{2}) d\alpha dH, \tag{S4}$$

where $\langle H \rangle$ is the mean height of pores/channels (considering the effective height of the domain equals to the value of 3 times of the mean height of pores/channels), Ω_α and Ω_H are intervals of spanning angle and height of roughness element of the network, respectively, l is the length of a roughness element (pore/channel), α and H are spanning angle and height of a roughness element, respectively[6]. Mean volumetric water content ($\langle \theta(\psi) \rangle$) can be estimated as a function of ambient hydration status (matric potential, ψ) and surface roughness characteristics according to,

$$\langle \theta(\psi) \rangle = \int_{\Omega_\alpha} \int_{\Omega_H} \theta_{\alpha,H}(\psi) f(\alpha, H) d\alpha dH, \tag{S5}$$

with its variance $\sigma^2[\theta_{\alpha,H}(\psi)]$ as,

$$\sigma^2[\theta_{\alpha,H}(\psi)] = \int_{\Omega_\alpha} \int_{\Omega_H} (\theta_{\alpha,H}(\psi) - \langle \theta(\psi) \rangle)^2 f(\alpha, H) d\alpha dH, \tag{S6}$$

with $f(\alpha,H)$ of the bivariate probability density function of α and H, with correlation coefficient of ζ,

$$f(\alpha,H)=\frac{\exp[-\frac{1}{2(1-\zeta^2)}(\frac{(\ln(\pi-\alpha)-\langle\alpha\rangle)^2}{\sigma_\alpha^2}+\frac{(\ln H-\langle H\rangle)^2}{\sigma_H^2}-\frac{2\zeta(\ln(\pi-\alpha)-\langle\alpha\rangle)(\ln H-\langle H\rangle)}{\sigma_\alpha\sigma_H})]}{2\pi(\pi-\alpha)H\sqrt{(1-\zeta^2)\sigma_\alpha^2\sigma_H^2}}, \quad \text{(S7)}$$

where $\theta_{\alpha,H}(\psi)$ is volumetric water content of a roughness element with spanning angle α and height H[5], $\langle\alpha\rangle$ is the mean value of spanning angle for a roughness element. Functional relationships linking mean volumetric water content and matric potential (or relative humidity) are routinely determined in hydrologic studies. The effective growth rate of a specific microbial species (i) is written as,

$$\mu_{eff,i}=\frac{\mu_{M,i}C_0}{C_0+\frac{K_i\phi^{2/3}}{\langle\theta(\psi)\rangle^2}}-m_i. \quad \text{(S8)}$$

Equation (S8) highlights dependency of microbial growth rate on environmental characteristics and physiological traits, and provides a means to estimate microbial mean generation time (binary fission or doubling time), $T_{G,i}$,

$$T_{G,i}=1/\mu_{eff,i}. \quad \text{(S9)}$$

The mean effective aqueous film thickness of a roughness network, $\langle d(\psi)\rangle$, is calculated as,

$$\langle d(\psi)\rangle=\int_{\Omega_\alpha}\int_{\Omega_H} d_{\alpha,H}(\psi)f(\alpha,H)d\alpha dH, \quad \text{(S10)}$$

where $d_{\alpha,H}(\psi)$ is effective aqueous film thickness of a roughness element of specified geometry (with spanning angle α and height H) that can be easily calculated[6]. It enables estimation of microbial cell velocity expressed as a function of mean aqueous film thickness according to, $V_{\alpha,H}(\psi)=V_0\frac{F_M-F_C-F_\lambda}{F_M}$, with V_0 of mean cell velocity in bulk water, F_M, F_C and F_λ are the viscous drag force opposing motion in bulk water (equal to the maximum flagellar propulsive force), the viscous force associated with cell-surface hydrodynamic interactions, and the capillary pinning force, respectively[5]. Microbial mean cell velocity on a roughness network is calculated as,

$$\langle V(\psi)\rangle=\int_{\Omega_\alpha}\int_{\Omega_H} V_0\frac{F_M-F_C-F_\lambda}{F_M}f^*(\alpha,H)d\alpha dH, \quad \text{(S11)}$$

where $f^*(\alpha,H)$ is bivariate probability density function of α and H considering six neighboring roughness elements,

$$f^*(\alpha,H)=\frac{\exp[-\frac{6}{2(1-\zeta^2)}(\frac{(\ln(\pi-\alpha)-\langle\alpha\rangle)^2}{\sigma_\alpha^2}+\frac{(\ln H-\langle H\rangle)^2}{\sigma_H^2}-\frac{2\zeta(\ln(\pi-\alpha)-\langle\alpha\rangle)(\ln H-\langle H\rangle)}{\sigma_\alpha\sigma_H})]}{\frac{\pi(\pi-\alpha)H}{3}\sqrt{(1-\zeta^2)\sigma_\alpha^2\sigma_H^2}}. \quad \text{(S12)}$$

This in turn enables determination of microbial mobility defined as, $M_C=\langle V(\psi)\rangle^2\tau/2$, with τ the mean interval of microbial motile duration[7]. The mean square displacement of a cell within one generation time is estimated as[7],

$$\langle R^2\rangle=4M_CT_G, \quad \text{(S13)}$$

which enables estimation of microbial mean generation (doubling) length as,

$$\langle R_G\rangle=\sqrt{2\langle V(\psi)\rangle^2\tau/\mu_{eff,i}}. \quad \text{(S14)}$$

The application of percolation theory to porous media represented by network models has yielded insight into physical behaviors at scales representing pores to larger/natural scales in porous media[2]. The dynamics of a spanning aqueous cluster via percolation transition into aqueous fragmentation is well described by percolation theory[2],

$$R_C(\psi)=R_0(\frac{N_C}{N_0})^{1/\chi}, \quad \text{(S15)}$$

and

$$N_C(p)=\begin{cases}p_{C,a}(p_C-p)^{-\gamma}, & (p\le p_{C,a})\\ N_0p, & (p>p_{C,a})\end{cases}, \quad \text{(S16)}$$

where N_C and N_0 are numbers of pores/channels of the maximum cluster and number of total pores/channels of a system, respectively, R_C and R_0 are effective radii of the maximum cluster and system size (for a finite domain), respectively, γ is an exponent having value of 43/18 for 2D and 1.80 for 3D systems, χ is statistical fractal with value of 91/48 for 2D systems and 2.52 for 3D systems, p_C is percolation threshold with value of 0.35 for 2D and 0.18 for 3D infinite systems, respectively, $p_{C,a}$ ($p_{C,a}=p_C-N_0^{-1/\gamma}$) is effective percolation threshold of finite systems, and p is probability of a pore/channel that has significant water retention that supports typical cell motility, which can be expressed as a function of hydration status (matric potential or relative humidity),

$$p(\psi)=\int_{\Omega_{\alpha*}}\int_{\Omega_{H*}}f(\alpha,H)d\alpha dH, \quad \text{(S17)}$$

where Ω_{α^*} and Ω_{H^*} are ranges of spanning angle and height of roughness element, respectively, within which effective aqueous film thickness ($d_{\alpha,H}(\psi)$) of a roughness element is sufficient for typical cell motion – flagellar motility[6]. The number of water filled pore/channel clusters (N, minimum cluster number considering the maximum cluster size) can be expressed according to,

$$N(\psi) = \frac{N_0 p}{N_C} . \tag{S18}$$

The biophysical variables described above enable predictive and quantitative assessment of coexistence potential of multiple microbial species on a rough surface as a function of hydration status. We propose a single coexistence index (CI) defined as the ratio of microbial mean generation length to connected aqueous cluster size,

$$CI(\psi) = \langle R_G(\psi) \rangle / R_C(\psi) . \tag{S19}$$

This ratio compares the size of connected aqueous clusters (islands) hosting multiple species with cell displacement distances during one binary fission (generation), where the displacement distance encapsulate both net motion and nutrient interception required for cell growth and division.

The modeled rough surface network under study is of physical size of 34.4 × 34.4 mm with 200 × 173 sites on hexagonal lattice[6]. Systematic testing of the proposed analytical CI was based primarily on a mechanistic hybrid individual-based model[6,8], considering explicitly autonomous cell motility, nutrient interception and cell growth, leading to trophic interactions among populations within hydration-controlled diffusion fields and aqueous films. We performed simulations on unsaturated model roughness networks[6], considering 6 motile microbial species differentiated by their intrinsic-growth characteristics, inoculated in 16 sites each consisting of 6 species (see Supplementary Fig. S2). The mean CI values extracted from a series of numerical simulations considered displacement distances by individual cells within one generation and the sizes of connected aqueous clusters on a simulated surface. Mean species evenness and mean relative abundance values extracted from numerical simulations were based on populations of 16 mixed inoculated colonies.

As a reference state we have considered population size of microbial species at a time where nutrient consumption has reached supply limit or capacity for an aqueous cluster. The population size at the nutrient-limiting state was used for estimating survivability within a prescribed domain, with the average elapsed time calculated according to:

$$W^* = \sum_i (W_{i0} 2^{\mu_{eff,i} T^*}) , \tag{S20}$$

where W_{i0} is initial population of the microbial species *i* inoculated at the center of the aqueous cluster, $\mu_{eff,i}$ is effective microbial specific growth rate of species *i*, and *W** is nutrient-limited population size for an aqueous cluster, which can be estimated from microbial physiological maintenance (assuming similar maintenance for all species) and the potential maximum nutrient flux arriving at boundary of the simulation domain and the number of aqueous clusters according to:

$$W^* = \begin{cases} \dfrac{12 L_0 \langle H \rangle D_{eff}(\psi) \dfrac{C_0}{l}}{N_{2D}(\psi)} / \Delta m, & \text{(for 2D system)} \\ \dfrac{6 L_0^2 D_{eff}(\psi) \dfrac{C_0}{l}}{N_{3D}(\psi)} / \Delta m, & \text{(for 3D system)} \end{cases}, \tag{S21}$$

where L_0 is the side length of the domain under consideration, C_0 is constant nutrient concentration at the boundary of the domain, Δm is the mean nutrient mass flux required for physiological maintenance of individual microbial cell, and $N(\psi)$ is the number of aqueous clusters. Alternatively, we may calculate the nutrient-limited population size of an aqueous cluster considering maximum nutrient flux arriving at boundary of a single (typical) cluster according to:

$$W^{*\prime} = \begin{cases} 6\pi R_C(\psi) \langle H \rangle D_{eff}(\psi) \dfrac{C_0}{l} / \Delta m, & \text{(for 2D system)} \\ 4\pi R_C^2(\psi) D_{eff}(\psi) \dfrac{C_0}{l} / \Delta m, & \text{(for 3D system)} \end{cases}. \tag{S22}$$

Comparisons of analytical predictions of the largest population size supported by diffusion for an aqueous cluster (equations (S21) and (S22)) and for the entire domain show consistent agreement for pre-percolation of the systems which diverge after percolation threshold due to aqueous phase fragmentation (Fig. S4a). Additionally, variation in initial inoculation size has minor influence in species evenness (Fig. S4b), reflecting the generality of the switch manner across a narrow range of hydration conditions.

The resolved species population sizes ($W_i = W_{i0} 2^{\mu_{eff,i} T^*}$) at T^* are subsequently used to estimate species evenness, abundance and fitness parameters. For the example of two species, the relative fitness (*RF*) of the inferior species (SP-I) relative to that of the superior species (SP-S) was calculated as[9],

$$RF = \left(\frac{W_I}{W_{I0}}\right) / \left(\frac{W_S}{W_{S0}}\right), \tag{S23}$$

where W_{I0} and W_I are initial and final populations of the inferior species, and W_{S0} and W_S are those of the superior species. Simpson species evenness (*SE*) was calculated as[10],

$$SE_{Simpson} = \frac{1}{Z \times \sum_i (w_i^2)}, \quad \text{(S24)}$$

where Z is number of total microbial species under consideration, w_i is relative abundance of species i, with $w_i = \frac{W_i}{\sum_i (W_i)}$, and W_i is the population of species i.

References

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