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Physical constraints affecting bacterial habitats and activity in unsaturated porous media – a review

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Abstract

The immense diversity of microbial life found in the vadose zone reflects the extremely heterogeneous and highly dynamic aquatic and chemical environments formed within soil pore spaces. The notion of planktonian free swimming microbes is unrealistic under most unsaturated conditions. Experimental and theoretical evidence suggests that surface attachment is the prevailing lifestyle, where bacterial colonies are embedded in biosynthesized extracellular polymeric substances (EPS). This strategy represents a successful adaptation to the variable and unpredictable hydration conditions near the earth surface. The EPS matrix serves as the interface with the environment; it enhances hydration and transport properties in the immediate vicinity of microbial cells, and dampens effects of highly transient fluctuations in water and nutrient fluxes. The primary effect of soil pore geometry and hydration status is on diffusion pathways to and away from stationary microbial colonies. Microbial dependency on diffusion processes occurs at all scales, but is particularly important at the colony scale. We illustrate the critical role of diffusion pathways with their complex spatial and temporal patterns in promoting coexistence and diversity. We review specific features and adaptations of microbial life to the particular conditions of terrestrial soil environments. The physical and related chemical conditions that shape microbial habitats and govern key processes in unsaturated soils are reviewed in a quantitative framework. Key physiological adaptations and biological responses to challenges presented by unsaturated conditions are discussed. Finally, we discuss potential impacts of microbial activity on properties and characteristics of the host porous medium. This review is an attempt to establish an interdisciplinary dialogue between hydrologists and microbiologists towards a quantitative integration of the role of hydrologic conditions on microbial activity and the role of microbiology in controlling macroscopic fluxes within this important compartment of the biosphere.

Keywords: Microbial habitats; Unsaturated soil; Microbial coexistence; Soil pores; Hydration status; Substrate diffusion; Water films; EPS

1. Introduction

The vadose zone probably supports the highest prokaryotic density of all biosphere compartments, despite the relatively harsh conditions prevailing near the earth surface. These include large variations in availability of water and nutrients, temperature fluctuations, constrained spaces, and fragmented aquatic habitats. Soils contain approximately 2.6×10^{29} prokaryotic cells concentrated in a relatively small volume estimated at 10^{15} m^3. In comparison, oceans contain approximately 1.2×10^{29} cells in a total volume of 10^{20} m^3 [176]. Thus, the mean soil prokaryotic density of 2.6×10^{13} m^{-3} in soils vastly exceeds the oceanic value of 1.2×10^{8} m^{-3}. As for the density, the diversity in the soil compartment is also very high. From community

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Nomenclature

A_{svl}	Hamaker constant [J]	$r(\mu)$	radius of curvature of the liquid–gas meniscus [m]
a_m	microbial CO_2 production rate on per-colony forming unit basis at temperature T [kg/m^3 s CFU]	T	temperature [°C]
		x	spatial coordinate
		α	half angle between two mineral surfaces forming a pore corner [°]
C	substrate concentration [kg/m^3]	β	dimensionless scaling factor for film-covered spacing between groove elements
D	substrate diffusion coefficient in the bulk liquid phase [m^2/s]		
$H(\mu)$	effective water film thickness [m]	γ	pit or groove angle [°]
J	diffusive flux of substrate [kg/s m]	μ	chemical or matric water potential [J/kg]
L	depth of a roughness element [m]	ρ	water density [kg/m^3]
l	water film thickness [m]	σ	liquid–gas surface tension [kg/s^2]

DNA reassociation rates, soil genomic diversity estimates exceed those in oceans by 3–4 orders of magnitude [156]. Others have speculated that the diversity found in one ton (roughly 0.7 m^3) of soil exceeds that found in all oceans combined [34].

Prokaryotic density, diversity, and activity arise within one of the most physically and chemically complex zones of the biosphere, one that supports plants and a vast array of other biological activity. The observed prokaryotic density and diversity in soils may be attributed to the extreme heterogeneity of habitats formed within complex pore spaces [30] and to temporal variations in amounts and distributions of water and nutrients. These variations affect the diffusional pathways, which control microbial distribution and activity [83], and sustain a non-uniform and patchy resource distribution, foster dynamic aqueous availability, and trigger transport processes unique to the vadose zone [44,183].

Substrate and metabolite transport at the microbial scale are controlled primarily by diffusion through spatially and temporally variant liquid and gas phases. As a result, the heterogeneous chemical and nutrient distribution greatly impacts microbial activity and distribution in the vadose zone, where microbial mobility is impaired [63]. Indeed, the emerging consensus is that in the unsaturated zone the planktonic form and activity of microbes is very limited (to short episodes of high saturation), and that surface-attached microcolonies and biofilms harbor most of the microbial density in the vadose zone [20,44,183].

Bacteria respond to alterations in soil hydration status with a diverse set of physiological mechanisms. While these responses can be intracellular and individual, the most successful ones are probably those that occur at a communal level [35], including synthesis of extracellular polymeric substances (EPS), which form protective coatings for the embedded microcolonies [70,128]. The EPS layer, in turn, can affect the physical characteristics of the host medium through reduction of available pore spaces for flow [7,11,104] and alteration of water retention and mechanical properties [22,128].

In contrast to a wealth of information on EPS-based biofilms in aquatic systems [103,173], very little is known regarding the spatial structure and properties of biofilms and microbial aggregates under unsaturated conditions [6]. Only a few detailed studies have been made on the biophysical properties of EPS that make it such a useful bacterial matrix under varied environmental conditions [25].

Despite remarkable advances in measurement and modeling of physical and hydrological processes in the vadose zone, the role of microbiological activity in regulating micro- and macro-scale fluxes and its potential impacts on porous media properties remains largely ignored. Gardner [50] stated that "Soil flora and fauna may be inconvenient for the soil physicist, but even we should be taking them seriously and be thinking and writing sensibly about them". Similarly, the microbiological literature is plagued by oversimplified depictions of natural environments and of physical processes affecting microbial activity in unsaturated soils. The need for interdisciplinary approaches to address such complex interactions was identified a decade ago by Potts [115]. Notwithstanding the rapid progress within the separate disciplines, the lack of a coherent conceptual framework has stifled efforts to assemble a more realistic and integral view of the complex interactions between microbes and their physical environment. The objective of this review is to link key physical processes in the vadose zone and their impacts on microbial habitats, activities, and diversity. We focus primarily on bacterial interactions; however, many of the discussions and concepts apply to other microbiota inhabiting the soil. Recent reviews by Ritz and Young [125] on soil-fungi interactions and by Crawford et al. [30] on modeling approaches to soil-microbiota interactions are particularly useful. We begin by examining the characteristics of soil aquatic habitats in relation to hydration status and pore geometry, and highlight the potential impacts on microbial activity through fragmentation, limited mobility, and resource diffusion. A review of basic physiological and morphological characteristics of microbial life in porous media follows, then a discussion on the interactions and feedback mechanisms between physical and biological processes with a focus on the importance of heterogeneity and diffusional limitations on coexistence and diversity, and on microbial modifications of their microenvironment by EPS synthesis to provide shelter and

to cope with temporal variations in key environmental conditions. A review of the major impacts of microbial EPS on soil physical and transport properties concludes the discussion.

2. Specificity of microbial life in terrestrial soil environments

Several features distinguish the terrestrial soil environment from other bacterial habitats. These include the dominant presence of solids which generally account for more than half the volume of soil. The solids are spatially arranged in a very complex fashion, forming tortuous pore spaces. Consequently, soils contain numerous interfaces (solid–liquid, solid–gas and liquid–gas) all of which may contribute to formation of microbially-diverse niches. Microbial activity is vital to a wide range of soil functions. They play critical roles in most biogeochemical cycles on Earth, including those of carbon, nitrogen, phosphorus and sulphur. Among the large array of carbon sources present in soils, very few are specific to terrestrial habitats. Accordingly, although soil bacterial diversity is tremendously high [155], few prokaryotes are unique to soil: the phyla *Verrucomicrobia* and *Acidobacteria* seem to be restricted to soil environments [75,146]. The presence of phyla unique to soils is probably linked to the dominance and high local concentrations of plant-derived materials in this environment. Indeed, plant-derived compounds such as secondary metabolites provide bacteria with vast amounts of (albeit poorly) biodegradable materials [170]. The respiratory metabolisms (aerobic and anaerobic) are the dominant types in soil habitats, although fermentative activity can develop and be maintained in patches where oxygen is absent. Because different types of metabolisms are realized under aerobic, micro-aerobic or anaerobic conditions, the bacterial activity in soil is largely tuned by gaseous exchanges.

Soil is a highly structured environment and some have ascribed it with self-organizing properties [30,183]. Because soil is far from being well-mixed, spatial heterogeneity can arise and persist. The origins of spatial heterogeneity in soil are manifold. First, the input of organic carbon, which constitutes the main source of energy in terrestrial environments, is highly heterogeneous in space and time. Indeed, organic carbon mainly originates from plants, either through exudation from plant roots or through the decay of vegetal tissues. Because the vegetal cover is diverse at several spatial and temporal scales, the nutrient input to soil has similar properties. Additionally, soluble or particulate plant-derived material is transported from the soil surface to deeper layers via the action of soil macro- and micro-biota and, most importantly, via transport with water. The water flow in soil is not homogeneous. Due to preferential flow, solutes may bypass a large part of the soil matrix [17]. The preferential flow paths are often linked to macropores, which consequently are characterized by a higher water-soluble carbon concentration than the adjacent soil matrix [17,167].

The heterogeneous distribution of carbon sources in soil is highlighted by the existence of hot-spots of bacterial activity and abundance (Fig. 1). Indeed, preferential flow paths harbor larger bacterial densities [167] and activities

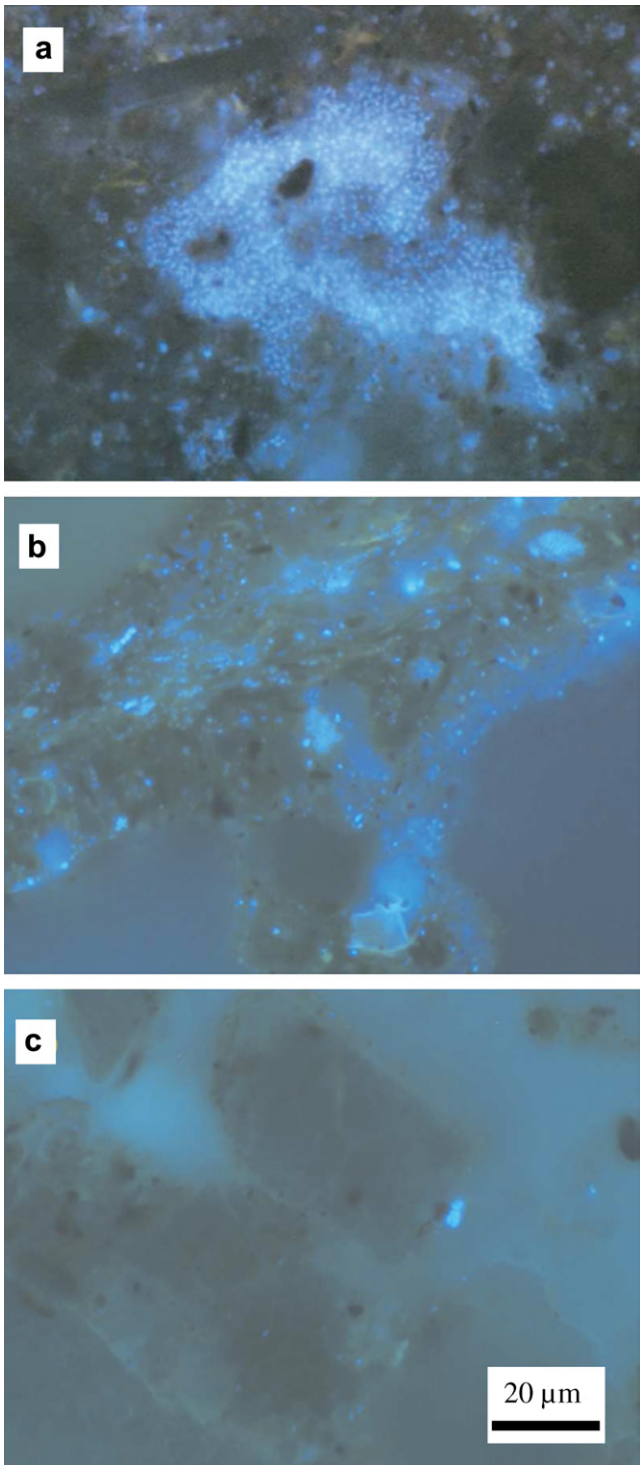


Fig. 1. Examples of bacterial colonization of soil. (a) Substrate amendment (glucose) leads to the development of bacterial hot-spots (here a large biofilm has formed in a pore). Topsoil locally presents relatively high bacterial abundance (b), while subsoil is usually characterized by low bacterial colonization (c). The bar size is 5 μm (from [100]).

[113] than the adjacent soil matrix. Similarly, particulate organic carbon (vegetal debris) sustains large microbial populations [49] and high activities, such as methane production [169] and denitrification [111]. If the bacterial distribution is governed by the nutrient distribution, other processes contribute to the complexity of this spatial pattern. Indeed, the presence of a nutrient at a given location in soil does not imply the presence of competent bacteria because, although bacterial abundance is high in soil, at the microscale relatively large stretches of soil surfaces are devoid of bacteria (Fig. 1b and c). The fraction of available surfaces that are effectively colonized by bacteria is estimated to be approximately 0.17% and 0.02%, for organic and sand grain surfaces, respectively [69].

Several processes may contribute to bacterial dispersion and thus colonization of new surfaces. Bacteria may move actively using pili or flagella, be subject to random Brownian motion in water, or be transported convectively either by flowing water or carried by a living organism (plant root, soil microbiota). Some prokaryotes are able to sense gradients of certain compounds, most often nutrient or toxic compounds, and to swim towards or away from the source of the gradient. This property, termed chemotaxis, affects the spatial distribution of bacteria in the rhizosphere [13] and may play a role in soil bioremediation where chemotaxis towards a pollutant at the microscale can be envisioned [10,110]. In saturated porous media, motility may enhance penetration rates [138], but, in soils with small particle (and pore) sizes (less than about 50 μm), the role of chemotaxis probably becomes insignificant at the sample scale of approximately 100 mm [10].

One of the primary factors controlling bacterial movement in soil is the water content. As will be discussed in Section 4, dry conditions strongly limit bacterial motion even at very small scales. Few flagellated cells are reported in soil [62], and the largest fraction of the bacterial populations is comprised of attached bacteria; these constitute responses to limitations in mobility. The dominance of the attached bacterial lifestyle in soils is manifest in the difficulty to release them from soil particles [1]. Attached life differs importantly from the planktonic life modus. Several studies have suggested that attachment influences bacterial metabolism, although the reasons for this remain unresolved [161]. Many molecules relevant to the physiology of bacteria such as C and N substrates, DNA, and enzymes, are found sorbed to soil particles [26]. Therefore, bacteria able to metabolize adsorbed substrates should grow more rapidly in an attached state than in a planktonic state. Contrarily, the diffusion of a dissolved substrate to cells located on crowded surfaces is considerably lower than to planktonic cells [61]. Similarly, attached bacteria may either be more protected [40] or more exposed [142] to predation than their planktonic counterparts because some grazers have a low efficiency against attached bacteria while others show a preference for attached preys [142]. Attachment triggers considerable modifications of bacterial physiology. Some features, such as the excretion of large amount of exopolymeric substances [119,164] and quorum-sensing agents [28] are specifically associated with attached bacteria and will be addressed below.

3. Environmental conditions prevailing in unsaturated soils

3.1. Liquid organization in unsaturated soils

As soils become partially-desaturated, remaining liquid is typically retained in corners and crevices behind liquid–gas interfaces, or adsorbed as thin liquid films on solid surfaces [158]. Sizes of liquid elements and film thicknesses are functions of the prevailing chemical (matric) potential characterizing the energy state of water, and are linked to the degree of dryness and pore space geometry. For example, water film thickness due to van der Waals surface forces is given by

$$l(\mu) = \sqrt[3]{\frac{A_{\mathrm{svl}}}{6\pi\rho\mu}} \tag{1}$$

where l is water film thickness [m], A_{svl} is the Hamaker constant (summarizing interactions between solid surface and gas through a liquid film, $\sim -6\times10^{-20}$ J for water on silicate surfaces), ρ is water density [1000 kg/m^3], and μ the chemical or matric water potential [J/kg] [73,158]. At matric potential values in the range of -10 to -30 J/kg (representing relatively wet conditions or the so-called "field capacity"), the thickness of water film on a smooth mineral surface is about 10 nm, which clearly cannot support complete immersion of typical microbial cells (~1 μm). Furthermore, these conditions restrict microbial mobility between remaining aquatic habitats which are connected primarily by thin liquid films even under relatively wet conditions [63].

Even the larger liquid habitats such as those formed by water retained by capillary forces in crevices and at particle contacts shrink in size with decreasing matric potential and become too small to support full immersion or movement of bacterial cells. Geometric considerations (Fig. 2) show that the maximum size of a spherical or cylindrical bacterium fully immersed in liquid behind a curved liquid–gas meniscus would be constrained by the following relationship:

$$r^* = r(\mu)\frac{1-\sin\alpha}{1+\sin\alpha} \tag{2}$$

where r^* is the maximum radius of the fully immersed bacterium, α is the angle between two mineral surfaces forming a pore corner (Fig. 2c), $r(\mu)$ is the radius of curvature of the liquid–gas meniscus determined by the chemical potential via the Young–Laplace equation: $\mu = \sigma/r\rho$ (σ is the liquid–gas surface tension). The simple relationship suggests that for mildly unsaturated conditions (>-30 J/kg), a typical aqueous element becomes smaller than the average microbial cell size (Fig. 2d). Consequently, the notion of free swimming planktonic microbes in unsaturated soils is

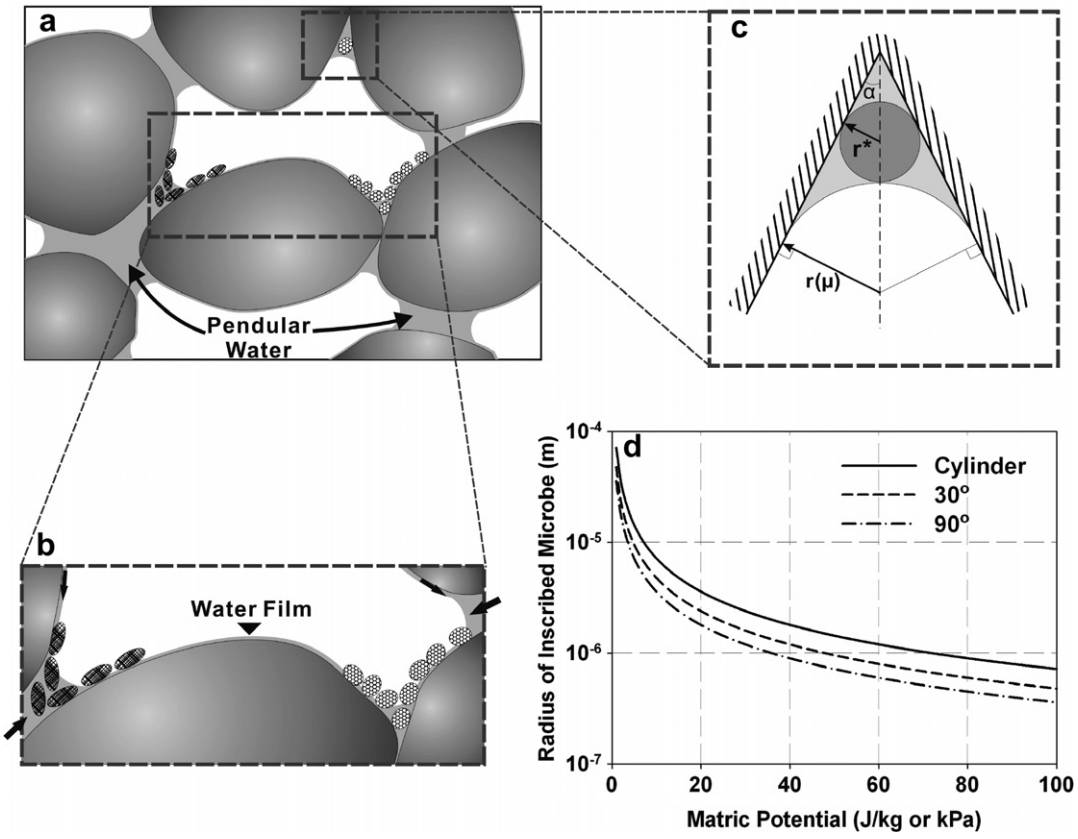


Fig. 2. (a) An illustration of microbes inhabiting soil pore spaces concentrating in corners and crevices where water is comparatively abundant; (b) potential for nutrient flux interception due to diffusion limitation and microbial consumption (arrows indicate nutrient flux); (c) definition sketch for size of aquatic habitat in a corner bounded by liquid vapor interface and a spherical or cylindrical fully-immersed microbe behind the interface; and (d) calculated maximal radius of fully-immersed microbe in a cylindrical capillary (solid line), and in corners with different angles for a range of matric potential values (from [87]).

limited to relatively rare occasions when a soil becomes nearly saturated. Furthermore, because sizes of aquatic habitats in angular pores are defined solely by matric potential and pore shape (not size!), some notions concerning the role of pore size in determining microbial activity during predation [181], and movement of microbes or grazers within certain pore sizes in unsaturated soils may be biased by the traditional "bundle of cylindrical capillaries" representation of soil pores.

Water retained on surface roughness or solid crevices is probably only significant under relatively wet conditions (in the range of 0–20 or −40 J/kg) and may offer an advantage during attachment and initial stages of microbial colony formation. Evidence certainly supports preferential bacterial attachment and habitation of crevices and rough surfaces [90,162] (Fig. 3). The formation of thicker effective liquid films due to rough surfaces can also support larger nutrient diffusion fluxes. This can be illustrated by simple calculations based on idealized surface roughness elements [107]. These express the relationships between ambient matric potential and average liquid cross sectional area available for nutrient diffusion in a unit roughness scaled by its length (see [107]) resulting in an effective film thickness $H(\mu)$:

$$H(\mu) = \frac{l(\mu)\left(\beta L + 2\left[\frac{L}{\cos(\gamma/2)} - \frac{r(\mu)}{\tan(\gamma/2)}\right]\right)}{L[\beta + \tan(\gamma/2)]} \tag{3}$$

where L is the depth of a roughness element, γ is the pit or groove angle, β is a dimensionless scaling factor for film-covered spacing between groove elements (βL) and r is the radius of liquid–vapor interfacial curvature.

Considering the simplest form of one-dimensional diffusion, the diffusive flux of substrate J may be expressed as

$$J = -DH(\mu)\frac{\mathrm{d}C}{\mathrm{d}x} \tag{4}$$

where H is the effective film thickness, D is the substrate diffusion coefficient in the bulk liquid phase, C is the substrate concentration, and x is a spatial coordinate. Theoretical calculations of film thickness using data measured by Tokunaga and Wan [154] on rough Tuff rock surface (roughness values: $L = 10^{-3}$ m; $\beta = 4$, and $\gamma = 120°$) are illustrated Figure 4. The results clearly show that rough surfaces support much thicker water films under wet (but unsaturated) conditions (matric potential > -2 J/kg $= -0.2$ m of matric head). Interestingly, a small change in unsaturated conditions erases the differences between effective film thickness on rough vs. smooth surfaces [107]

Fig. 3. Microbial colonies on granular activated carbon three days after incubation. Growth during early stages of colonization occurs primarily in rough areas and within cavities (from [90]).

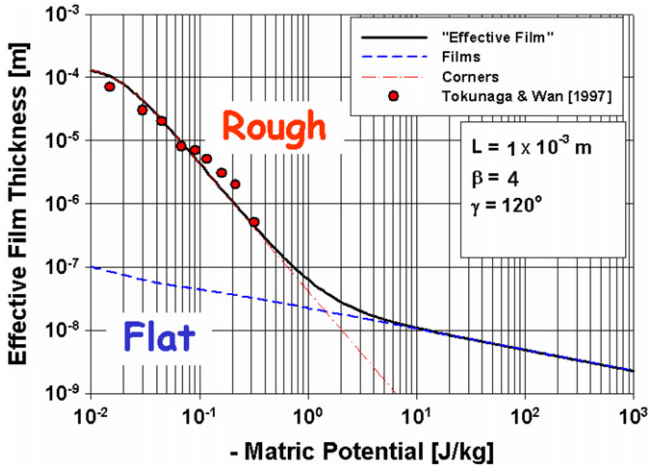


Fig. 4. Comparison of theoretical effective film thickness on smooth and rough surfaces as a function of matric potential [106], based on measurements from Tuff rock surface obtained by Tokunaga and Wan [154].

and the "smooth surface" liquid film $l(\mu)$ (Eq. (1)) would control mass fluxes to microbial colonies as demonstrated by Rivkina et al. [126].

3.2. Scales of processes and microbial interactions in soils

In the discussion of processes affecting microbial habitat diversity, microbial activity, competition and coexistence, we must specify relevant spatial scales. Most diffusional limitations discussed in this review pertain to processes taking place in multiple pores (~10–100 μm) including interacting microbial colonies. At length scales of the order 100–1000 μm diffusion processes govern microbial colony development [32]. At these length scales heterogeneous diffusion pathways and fragmentation of the aqueous phase enhance microbial coexistence and lead to the large microbial diversity observed in soils at small scales [156,184].

At the so-called *sample scale* with lengths in the range of 10–100 mm, macroscopic diffusion coefficients for a porous medium are defined (i.e., diffusional representative elementary volume [REV] scale). Convective transport pathways become prominent in producing nutrient gradients and supporting "hot-spots" [17], or regions with elevated microbial activity. Additionally, this is the scale at which quantitative description of gaseous exchange with atmosphere becomes meaningfully defined [139].

Landscape processes of soil formation and depositional conditions result in differences in soil materials and porous environments that in turn affect diffusion and microbial activity at scales of soil layers (0.1 m) to a soil profile or pedon scale (~10 m). Potential differences in pore spaces between layers would affect long term wetness and aeration conditions, nutrient and gaseous fluxes. Position within the soil profile would impact wetness and access to oxygen and carbon sources resulting in gradients in microbial abundance and composition [44,80]. Nutrient diffusion and microbial migration would not typically exceed the pedon scale, giving rise to non-interacting microbial populations. The primary cross-cutting features at these scales would be convective transport pathways (soil macropores and fractures) and plant roots.

Finally, ecological studies may consider processes taking place at the watershed scale (1 km), where position in the landscape would provide differences in hydration status and dynamics, giving rise to different plant communities, soil types, nutrient fluxes, etc. This leads to climatic spatial scales of 100–1000 km where temperature and precipitation patterns could vary considerably, with associated soils and vegetation types. The extent and hierarchy of the various scales and associated processes are important variables in systematic evaluation of causes and drivers of microbial abundance and diversity in soils [46].

3.3. Water content and spatial organization affecting nutrient and gaseous fluxes

The reduction in conducting liquid pathways as soil dries reduces liquid and nutrient transport and diffusion rates at all scales, particularly at the microscale of individual bacterial communities and within the EPS matrix encasing microbial cells. At the micro-scale (a few pores,

colony) the dominant mechanism of chemical transport is diffusion through virtually stagnant water films and air (with diffusion distance proportional to $t^{1/2}$). At the macro-scale (sample [10–100 mm] to soil profile [0.1–10 m]), the dominant mechanism for supplying dissolved nutrients and oxygen is convection with flowing water and air (convection distance proportional to t). Generally, nutrient and gas concentrations and fluxes are dictated by prevailing boundary conditions at a higher scale in the hierarchy, and by initial concentrations and source-sink distributions at a given scale of interest.

At the macro-scale, the primary effect of changes in water content is on gaseous diffusion and on convective supply of substrates. As water content decreases, soil air content and water–gas interfacial area increase, resulting in enhancement of gaseous diffusion and improved gaseous exchange with atmosphere. Because diffusion coefficients are ~10,000 larger for gas molecules diffusing in air relative to dissolved gases and solutes in aqueous solutions (e.g., oxygen in air 2.0×10^{-5} m^2/s vs. 2.5×10^{-9} m^2/s in water, or glucose in water 7.7×10^{-10} m^2/s), decreasing wetness therefore enhances gas diffusion effectiveness. The interplay between enhanced gaseous diffusion and the concurrent decrease in liquid diffusion pathways with decreasing water content can be formulated as a function of soil porosity and water content. Skopp et al. [139], and more recently Schjonning et al. [134], have analyzed the consequences of such interplay focusing on macroscopic diffusion coefficients for nutrients and gas to identify an "optimal" water content that maximizes microbial activity at soil sample- or profile-scales (Fig. 5). The results in Figure 5b provide limited confirmation to the conceptual framework (Fig. 5a); additional data showing similar trends were obtained by Schjonning et al. [134]. Another important macroscopic impact of water content status in unsaturated porous media is on convective fluxes as determined by the hydraulic conductivity. The hydraulic conductivity and associated convective nutrient fluxes may increase by several orders of magnitude with only a modest increase in the water content of a porous medium, reflecting the strongly nonlinear relationships between hydration status and transport properties of soils.

At the microscale (~100 μm), both structure and position of a bacterial colony within soil pore space are likely to determine the onset of diffusional constraints for different aqueous phase configurations. As indicated above, "dry" conditions are characterized by water films whose thickness and hydraulic connectivity play a primary role in determining substrate diffusion rates [94]. Rivkina et al. [126] show data from Siberian permafrost (Fig. 6) suggestive of diffusional constraints on amounts of ^{14}C-labeled acetate incorporation imposed by thin liquid films, whose thickness is a function of ambient temperature. Note that water film thickness is determined primarily by matric potential and solid surface properties.

Despite numerous complexities associated with a rigorous accounting for the impact of hydration status on

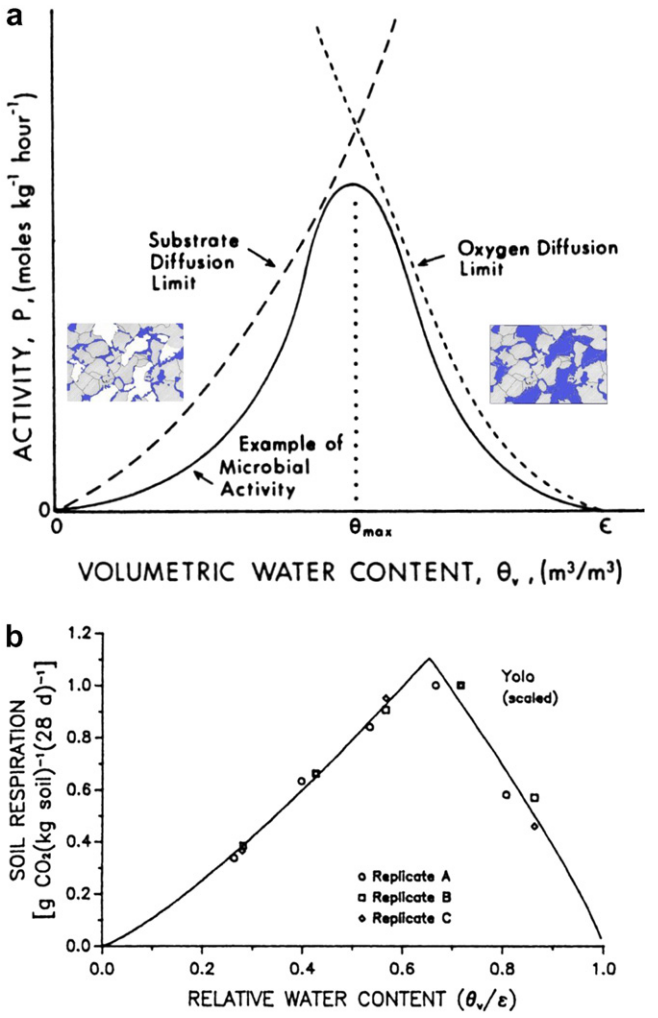


Fig. 5. (a) Conceptual illustration of relationships between macroscopic microbial activity and soil water content. The dotted lines are upper limits imposed by gaseous or substrate diffusion rates; and (b) regression of the conceptual model against respiration measurements from Yolo soil (modified from [139]).

macro- and micro-scale microbial processes, we can make several qualitative observations based on the foregoing analyses. In soils with appreciable surface area or for relatively dry conditions in all soils, most of the water would be associated with solid surfaces as aqueous films whose thickness is proportional to the water content. Under these conditions we would expect linear dependency of microbial activity (controlled by film-limited nutrient diffusion) on water content. With increases in water content, connectivity among pathways dramatically increases (tortuosity decreases at a nonlinear rate), which would lead to rapid (convex) growth in microbial activity with increasing water content. Subsequent increase in microbial activity due to enhanced nutrient availability would be offset by progressively limited gaseous diffusion, resulting in a decrease in microbial activity in the porous medium. Indirect evidence supporting the postulated trends may be seen in the experimental results of Schjonning et al. [134] and Aon et al. [5] (Fig. 7).

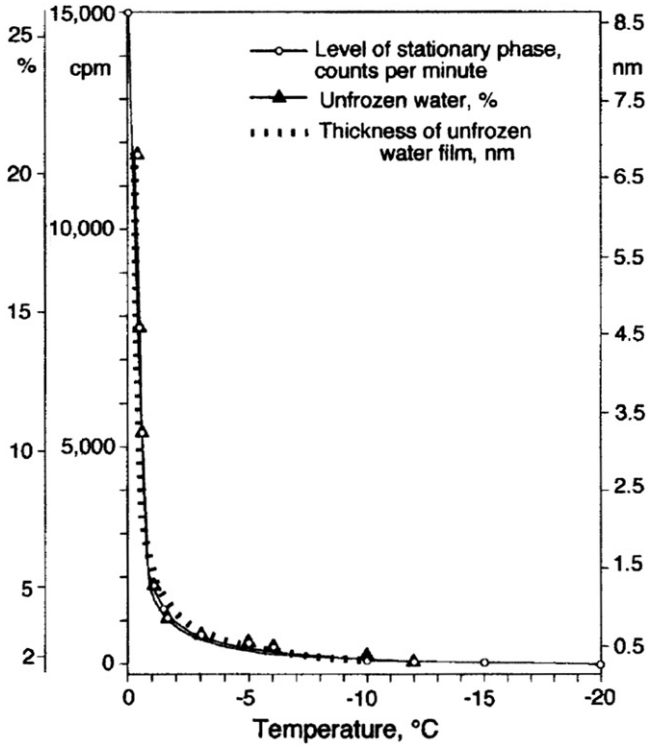


Fig. 6. Levels at which bacterial growth reaches a stationary phase, measured amounts of unfrozen water, and calculated thicknesses of unfrozen water films in permafrost soil versus temperature suggesting diffusion control by unfrozen water film thickness (from [126]).

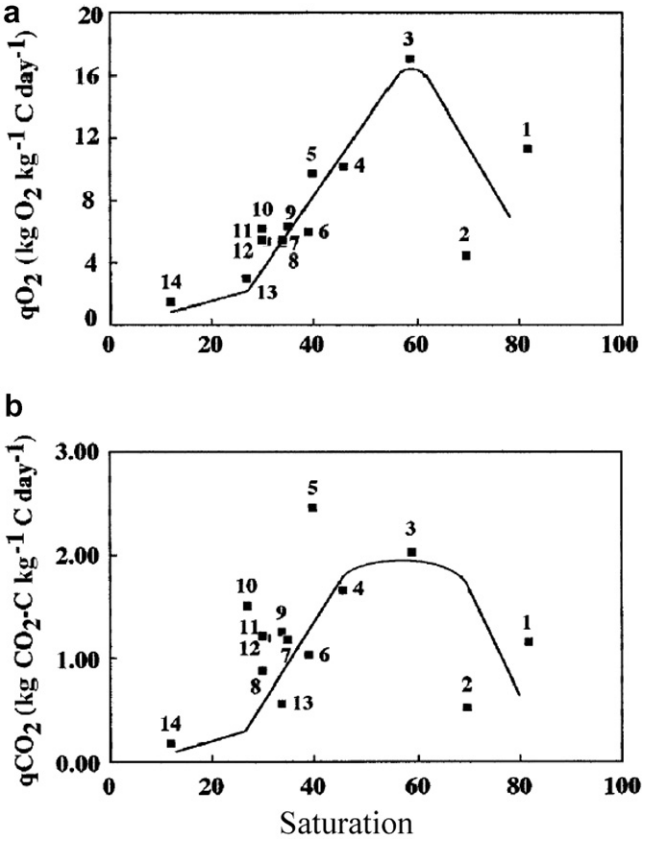


Fig. 7. Microbial respiration rates: (a) oxygen and (b) CO_2 gaseous fluxes as a function of soil water saturation in samples taken from different tillage and fertilizer management treatments (indicated by numbers) (from [5]).

Even at the microbial colony scale, dramatic changes in diffusion rates are experienced as soil dries [25,70]. Attempts to quantify such effects on microbial activity have focused on individual processes, such as the effects of matric potential on diffusion through the EPS matrix [25], effects of matric vs. solute potentials on nitrification [143], and effects of pore space heterogeneity on nutrient acquisition.

3.4. Temporal changes in soil hydration affecting diffusion status

Soil water content is a highly dynamic attribute changing with climatic and hydrological conditions (evaporation, precipitation, drainage, etc.) and with biological activity in soils (plant water uptake). Temporal changes in the amount and energy status of soil water result in dramatic changes in diffusional capacity and the pathways affecting gaseous and nutrient fluxes irrespective of spatial distribution of nutrients. In other words, the nutrient supply network in unsaturated soils is not permanent; microbial colonies must cope with frequent and often dramatic changes in nutrient fluxes, reinforcing the crucial need for developing nutrient storage capacity and various survival strategies in response to nutrient deprivation. Konopka [84] studied the consequences of temporal variations in resource availability on the success of different survival strategies. The study focused on the success of "resting stages" where cells reduce metabolic activity, as contrasted with formation of metabolically inactive endospores in response to an extended period of nutrient deprivation. The theoretical study establishes links between periodicity and duration of the starvation period and the most successful survival strategy. Briefly, resting stages enable rapid transition to capitalize on resource availability, while metabolically inactive endospores survive very long periods of deprivation. For example, Potts [117] studied desiccation tolerance and concluded that "We can be confident that the desiccated cells of some organisms, when stored under appropriate conditions, can remain viable for at least 1000 years". The terrestrial cyanobacterium *Nostoc commune* has a remarkable capacity for desiccation tolerance and can survive storage at −400,000 J/kg (−400 MPa, or ~0% relative humidity) for centuries [115].

3.5. Changes in soil salinity and osmotic environment

Typically, solute concentrations found in soil solutions are not sufficient to produce hypo- or hyper-osmotic stresses in soil microbes. Detrimental salinity effects occur only under extremely high-salinity levels associated with electrical conductivity (EC) of saturation paste extract in excess of 25 dS/m [88], or rapid infiltration of rainwater may

induce hypo-osmotic stress due to an abrupt dilution of soil solution [60]. Nevertheless, solute concentration during dry periods, fertilizer amendments, and conditions at certain spatial locations (near soil surfaces, plant root surfaces) may result in hyper-osmotic stresses limiting microbial growth and activity.

Numerous studies have documented salinity effects on bacterial growth and activity. Recently, Azam and Muller [9] studied effects of sodium chloride (NaCl) on denitrification and exchange of respiratory gases from soils treated with ammonium or nitrate. They demonstrated significant depression of N_2O and CO_2 emissions and O_2 consumption in the presence of NaCl. Chandra et al. [19] showed stimulation of C mineralization at low salt contents followed by a decline at higher concentrations. Tam et al. [147] reported inhibition of growth and biodegradation of phenanthrene of a bacterial isolate at high-salinity levels. Wong et al. [179] tested effects of different application rates of sludge amendments on microbial activity in a sandy soil and concluded that application rates in the range of 50–150 g sludge/kg soil resulted in optimal microbial activity and nutrient transformation, whereas higher application rates suppressed microbial growth and activity due to elevated salinity. In a detailed study, Rietz and Haynes [123] demonstrated effects of soil salinity on various quantifiers of bacterial activity due to irrigation-induced salinity and sodicity in Zimbabwean vertic soils. Their results are characterized by exponential decay with increasing soil salinity, from maximal values of microbial biomass and metabolic or respiratory activities and other activity indicators in non-saline samples to minimal values at salinity levels of 20 dS/m (saturation extract). High salinity levels thus resulted in decreased soil microbial biomass, decreased bacterial growth rates, and extended lag periods after adding a carbon source.

Studies of hypo-osmotic stresses on soil bacteria due to abrupt dilution of soil solution are limited. Halverson et al. [60] observed small amounts of protein and DNA released as water potential increased from -2000 to -1000 J/Kg, with no significant cell lysis. Release of intracellular solutes by Gram-negative bacteria (*Pseudomonas chlororaphis* and *Pseudomonas fluorescens*) was larger than for Gram-positive isolates (*Bacillus pumulis* and *Streptomyces griseus*), suggesting that Gram-positive bacteria are more tolerant than Gram-negative to wetting/dilution shock. The natural response of maintaining constant cell volume and turgor pressure in response to sudden changes in external solution osmolarity implies rapid water efflux out of bacterial cells in hyper-osmotic external solutions and water influx in hypo-osmotic surroundings. In extreme situations, these responses may lead to either loss of cell turgor and plasmolysis or to cell bursting [95]. Gram-negative bacteria are at a higher risk for plasmolysis as compared to Gram-positive, since the binding between their rigid peptidoglycan layer and cytoplasmic membrane is weaker.

Despite numerous investigations, the classical notion concerning additive effects of matric and osmotic potentials on bacterial growth and activity remains problematic [108]. The primary differences between these two forms of stresses are illustrated and explained in detail by Potts [115, p. 764], "There is one distinction between matric and osmotic systems ... The immediate environment of a cell under matric stress is the atmosphere; i.e., the surfaces of their cell walls are exposed to a gas phase, while cells under osmotic stress are bathed in an aqueous solution, albeit one of diminished water activity." Within a typical soil profile, even at scales of water films, the osmotic component of water potential plays a negligible role in modifying convective or diffusive nutrient fluxes to bacterial colonies, in stark contrast with the dominant role of matric potential that determines water content and shapes diffusion pathways.

An additional source of confusion stems from the indiscriminate (and inappropriate) use of water vapor depression by salt solutions in sealed chambers, as surrogate for matric potential effects (e.g., [2]). Potts [115] defined desiccation as removal of substantial amounts of water from bacterial cells by matric stress. The mode of desiccation (and also the rate) plays an important role in the physiological response. Using salt solutions in a closed chamber to depress water vapor in equilibrium with microbial populations may not accurately mimic the range of conditions induced by changes in matric potential within a porous medium. Because of the complex and substantial impacts of matric potential on water organization and related diffusion pathways, it is important to ensure matric stress mediation through the porous medium. When matric potential is reduced through control of the soil liquid phase in the porous medium, cells or colonies remain hydraulically connected and accommodate potential energy differences through mass exchange without localized desiccation of the outer membrane or the surrounding EPS. In contrast, inducing changes directly through vapor pressure modification may involve different time scales for equilibration, and the mechanism of adjustment would most likely be through the vapor phase (evaporation of intracellular water to the drier atmosphere) rather than the liquid. Although the short term result in terms of efflux may be similar, for longer time scales the two water loss (or water gain) mechanisms may follow different pathways and therefore result in differential adaptation by the microbial cells or colony.

In addition to simple osmolarity, salts may have specific chemical and other effects. Hallsworth et al. [58] noted that "chaotropic solutes do not affect turgor pressure, but do reduce water activity, perturb macromolecule–water interactions and thereby destabilize cellular macromolecules, inhibit growth, and are powerful mediators of water stress in a typical soil bacterium, *Pseudomonas putida*". In their study chaotropic solute-induced water stress resulted mostly in enhanced synthesis of proteins involved in stabilization of biological macromolecules and membrane structure. In most circumstances, salts exert both general (osmotic) and specific effects. As Saari et al. [130] concluded, "inhibition of CH_4 oxidation by $(NH_4)_2SO_4$ resulted mainly from a general salt effect (osmotic stress)

though NH_4^+ did have some additional inhibitory properties''. Presence of salts may reduce availability of essential micronutrients (e.g., iron) due to precipitation. Finally, we mention an additional physical effect of increased ionic strength on microbial attachment to solid surfaces (due to reduced electrostatic repulsion; see Elimelech, this issue). High-salinity levels may also limit mobility, as was recently shown [144], causing repression of chemotaxis and motility genes, resulting in severe impairment of the swarming capability of *Bacillus subtilis* cells.

3.6. Diurnal and annual temperature regimes

In the presence of ample nutrients and water, soil temperature plays a key role in microbial growth and activity. In many circumstances and across a wide range of water contents, the dependence on temperature is highly significant as inferred from the strong correlation between microbial activity and seasonal temperature variations [65,68]. Cannavo et al. [18] show high correlation between in situ microbial activity (indicated by elevated CO_2 concentrations in soil atmosphere), and seasonal temperatures at different soil depths. Highest soil CO_2 concentrations occur in the summer, and minimum concentrations in the winter. A gradual decrease in CO_2 concentration with increasing depth is observed during summer (similar to temperature profile), and the concentration increases with depth during the winter [65]. In cases where microbial activity is not correlated with prevailing temperatures, other factors such as water content or nutrient availability must be limiting.

Many field investigations, laboratory studies under controlled conditions, and computer simulations demonstrate temperature effects on bacterial abundance [42], growth rate [51], respiration or CO_2 production rates [21,65,180] and pesticide or hydrocarbon degradation [41,43,74,145,166,177]. In ambient and laboratory temperature ranges the response is usually positive, of enhanced growth and activity at higher temperatures, described mathematically by an exponential, Arrhenius-type relationship [166,180]:

$$a_m = a_{m0} e^{k(T-T_0)} \tag{5}$$

where a_m is the microbial activity rate (e.g., CO_2 production rate) for a given bacterial population at temperature T, a_{m0} is the activity rate at a reference temperature T_0 and k is a constant. Figure 8 [180] is one such example demonstrating a fourfold increase in microbial CO_2 production upon temperature increase from 5 to 20 °C.

The primary effect of temperature is physiological through internal cell functions, similar to other organisms. These effects are not universal and vary between different microbial communities. For most bacteria activity and growth increase with temperature up to an optimal range of 30–40 °C, followed by a decrease at higher temperatures. Certain bacteria behave differently under extreme conditions of low (e.g., freezing) and high temperatures. For example, microbes that thrive at high temperature (extremophiles) have been studied in geothermal environments that also exhibit severe chemical conditions of very low or high pH and high concentrations of metabolic toxins such as arsenic (e.g., [45,89,98]). Potts [116] reports on growth of a *coccoid cyanobacterium* (dominant in rock-dwelling communities of hot and cold deserts) on roof shingles where during summer days the dried, dark-pigmented, spherical colonies of this organism are baked at temperatures in excess of 85 °C. Such temperature-adapted organisms do not do well in the ambient environments favored by normo-thermic organisms.

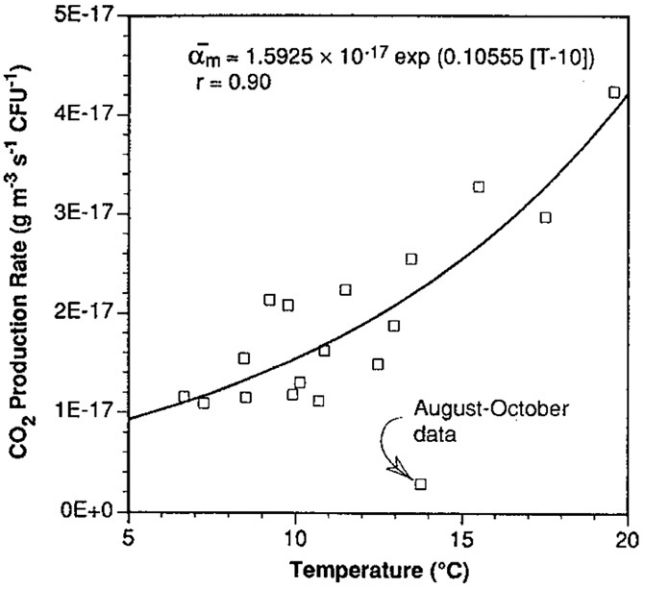


Fig. 8. Soil CO_2 production rate on per-colony forming unit (CFU) basis as a function of subsurface temperature. Deviation during the August–October period may represent a nutrient limitation (from [180]).

Temperature has commonly been shown to affect the growth rates and activity of microorganisms, but in some circumstances it has been demonstrated that temperature affected primarily the lag period, which was shorter at higher temperatures [43]. Possible minor temperature effects, especially under conditions of low nutrient availability, are through the hydraulic conductivity and molecular diffusion coefficients increasing at a rate of about 2%/°C as a result of decreasing water viscosity with temperature.

In contrast to natural aquatic environments (oceans, lakes, and rivers), soil temperature fluctuates daily and annually due to changes in radiation intensity, wetness and air temperature. The components of soil surface energy balance determine the amplitude of the diurnal fluctuation (highest near the surface and damped with depth). In deserts the amplitude of the annual fluctuation can reach 50 °C and greater, but in more moderate climates the fluctuations are smaller and penetrate to depths of a few meters, allowing microbial activity at different, temperature-dependent rates during the year. The amplitude of the daily temperature fluctuations is smaller and penetrates to only a few tens of centimeters.

In addition to its role in damping the impacts of desiccation and potentially harmful rapid re-hydration, EPS might offer an advantage in mitigating diurnal and other rapid temperature changes. The enhanced water retention of EPS results in high heat capacity, thereby buffering temperature changes, and may have lower thermal conductivity (relative to free water) especially in the presence of channels and voids. The authors are not aware of studies concerning temperature regulation of soil biofilms; this advantageous regulating effect was demonstrated by e.g., Perrot et al. [112], who reported enhanced survival of gel-immobilized *Escherichia coli* (an artificial biofilm) as compared to planktonic bacteria after 4 weeks exposure to spring water at sub- and supra-optimal temperatures of 4 and 18 °C. The number of planktonic bacteria recovered on non-selective medium decreased by 3 logarithmic units during exposure at 4 °C and was reduced to an undetectable level at 18 °C. However, the gel-coated microbial population did not decrease by more than one log unit over the exposure period at either temperature.

3.7. Radiation sheltering

An important aspect of microbial life in the opaque soil environment is light and radiation sheltering that protects microbes from potentially harmful UV radiation. Penetration depth of UV radiation in soils is relatively small; a 1-mm soil cover prevents lethal radiation damage at simulated high levels of UV and visible light fluxes resembling conditions on the Mars surface [29]. This advantage becomes more significant when considering the time span of spores and other resistant life forms that must endure cumulative radiation damage over centuries on the one hand, and the relatively small number of strand breaks critical for survival (e.g., 50 breaks for *E. coli* that carry one copy of its genome [116]). Considering background depurination rates under unsaturated and desiccation conditions [116], and cumulative damage to microbial DNA due to exposure to UV and solar radiation [140], soil radiation sheltering offers a clear advantage, especially to microbial species that do not have protective pigments and advanced DNA repair mechanisms [116]. Newer studies show potential impact of enhanced solar and UV radiation on microbial communities affected by the thinning atmospheric ozone layer [140].

3.8. Mechanical forces – interfacial snap-off and shrinkage stresses

Drainage processes in soils are associated with rapid and violent liquid–gas interface reorganization and snapping as the gaseous phase invades the receding water-filled pores. Such processes present a potential for significant mechanical damage to cell membranes and cellular structures [118], especially considering the preferential accumulation of microbial cells near liquid gas interfaces. Thompson and Yates [151] have demonstrated in dynamic batch experiments enhancement of bacteriophage deactivation in the presence of solid–water–air interfaces. In addition to potential damage due to the passage of interfaces, the shrinking matrix and pore spaces in clayey soils may exert stresses in excess of ten thousands of J/kg (tens of MPa), well beyond the resistance of most microbial cells. The role of EPS in providing mechanical protection against interfacial snap-off and soil shrinkage is not well documented.

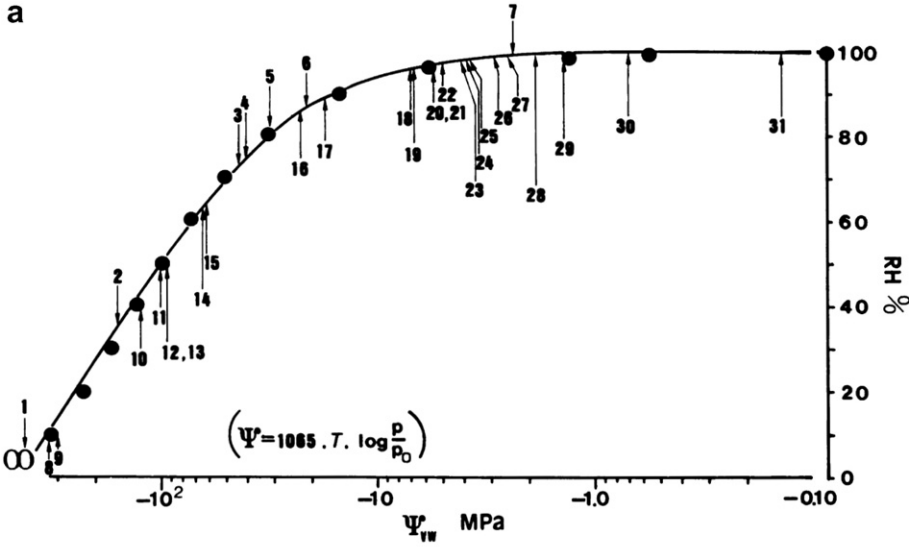
4. Bacterial adaptations and responses to fluctuating soil water content and soil structure

The vadose zone is strikingly different from the saturated zone, as explored above, and is characterized by spatially fragmented and limited water availability. The hydration status in soils is highly dynamic, resulting from wetting then subsequent drying, and characterized by lower water contents and rapidly decreasing water potentials (primarily due to matric rather than solute potential). It seems inevitable that survivability and viability of microbial life in such environments requires considerable physiological and other adjustments. It is informative to examine what would happen to a microbial cell under decreasing water availability if it were not able to respond.

Reduced extracellular water availability would result in a decrease in external water activity, water would flow out of the cells, intracellular turgor pressure would decrease, and cells would plasmolyse if the stress were not addressed. Indeed, reduction in extracellular water potential would result in the removal of a substantial fraction of the bulk water from cells, termed desiccation [115]. One straightforward response would be to adjust the intracellular water availability congruently with changes in extracellular water availability, and indeed one common biological (including microbial) response is through the accumulation of compatible solutes [115]. Under conditions of cell dehydration, it is likely that many cellular macromolecules would lose the 3D structure required to remain biologically active (e.g., by denaturing of outer membrane and cytosolic proteins, nucleic acids, and by fluidity loss of membrane phospholipids). Obvious responses to this effect would be processes that stabilize proteins (e.g., synthesis of chaperone molecules, or cis-to-trans isomerization of fatty acids in the phospholipids of the cell envelope [59]).

Clearly, this brief scenario indicates that microorganisms *must* respond to dehydration in order to remain viable. What then are the mechanisms of response? It is fair to say that to date our knowledge of the mechanisms, or regulation thereof, of physiological responses to dehydration stress (absolute matric potential decrease) is very limited, but recent insights have been gained, and an overall hierarchy of responses can be sketched. The outstanding, albeit slightly dated, review on this topic by Potts [115] deserves explicit mention. It is important to realize the extremely narrow range of water potentials (and related relative humidity values) supporting growth and activity of microbial life in soils and other systems. Figure 9

a



b

No.	Pressure (MPa)	Comment
1	0	Atmosphere over fuming P_2O_5
8	−400	*Crinalium epipsammum* and *Tychonema* spp. survive
9	−300	*Enterobacter cloacae* and *Alcaligenes eutrophus* become nonculturable
2	−168	Saturated solution of $CaCl_2$
10	−129	Limit for survival of *Rhizobium meliloti* in alginate beads
11	−100	Typical exposure of *Nostoc* colonies in situ
12	−99.5	Nitrogenase activity lost in 30 min in *Nostoc* strain UTEX 584
13	−99.5	Polysomes of *Nostoc* strain UTEX 584 intact after 2 h
14	−66	Mean lowest value in Antarctic rocks
15	−62.5	Ambient values above marine *Scytonema* mats
3	−44	Saturated solution of urea
4	−41	Saturated solution of NaCl
5	−31	Nucleic acids and proteins fully hydrated
16	−26	Cortex and core of *Bacillus* spores
6	−22	Saturated solution of sucrose
17	−17	Lower limit for growth of *Arthrobacter* spp.
18	−7	Lower limit for growth of *Bacillus subtilis*
19	−6.9	Minimum required for photosynthesis by *Chroococcidiopsis* in hot-desert rocks
20	−5.6	Nitrogenase active for at least 3 h
21	−5.6	Minimum for growth of *Flavobacterium*, *Pseudomonas*, and *Rhizobium* spp.
22	−5	Bacterial respiration ceases
23	−4.6	Growth of *E. coli* ceases
24	−4.4	Competitive bacterial growth ceases
25	−4.2	Nitrification and sulfur oxidation cease
26	−2.8	Inhibition of photosynthesis in desert crusts of *Microcoleus* spp.
27	−2.7	35‰ seawater at 34°C
28	−1.8	Inhibition of growth of *Microcoleus* spp.
29	−1.5	*E. coli* MM294(pEMR1) nonviable
30	−0.7	Mean of minimum value that supports bacterial growth
31	−0.14	Motility of bacteria ceases

Fig. 9. (a) Relationships between water potential and relative humidity; numbers above curve indicate physical constraints and those below the curve indicate water potential values that limit various physiological processes, and (b) comments on key values ordered from most to least extreme water deficit (from [115]).

adapted from Potts [115] provides a general overview of relationships between key physiological processes and water potential or relative humidity. The data illustrate that at relative humidity values not far from 99%, microbial growth becomes limited, and at water potential of −5000 J/kg (−5 MPa or ~96% RH) bacterial respiration ceases.

Conceptually, responses to dehydration stress can be global or specific. Phenomena like substrate deprivation (starvation) or extremes in temperature and osmolarity all elicit responses and the known overlap in regulatory networks for stresses may make some of these also present under water stress. Some of these responses may be controlled by specific sigma factors (elements of RNA polymerases that to a large extent control transcription of specific genes): sigma S, σ^S, (or RpoS) for stationary phase and sigma factors of the extracytoplasmic function subfamily, σ^E, which respond to external stress [66]. For example, σ^E is a critical determinant in the adaptation of *P. fluorescens* to dry conditions and hyperosmolarity [135].

Based on the 'mode of action', we divide specific dehydration stress responses into three categories. First are those that function outside of the cell (e.g., attempting to modify external water availability), second are those that function inside the cytosol, and third are those that function at the border between the cytosol and the external environment by modifying the envelope barrier.

The maintenance of envelope integrity is essential for the survival of bacteria. During dehydration or rehydration, the phospholipids composing the bacterial membrane

may undergo a phase transition from the liquid crystalline to the gel phase or vice versa, provoking leakage of cellular solutes and thus greatly impairing bacterial survival [115]. The nonreducing disaccharides sucrose and trehalose efficiently protect bacterial membranes as described by the "water replacement hypothesis" [31] because they interact with the polar head groups of the phospholipids. As a result the normal liquid-crystal state of the membrane is maintained and deleterious phase transitions to the gel phase is prevented [175]. In addition, a range of carbohydrates may protect the membrane through their free radical scavenging activity [86].

Cytosolic changes are mediated by the uptake and/or de novo synthesis of different osmolytes that act as compatible solutes, including proline, betaine, trehalose, ectoine, hydroxyectoine, and mannitol. The role of compatible solutes is, however, much more complex than balancing transmembrane osmotic pressures, as suggested above. Current understanding is that compatible solutes stabilize native protein structures via preferential exclusion of harmful solutes from the protein's surface [174].

EPS synthesis likely affects water availability because of its high water holding capacity, and desiccation tolerance is indeed adversely affected by mutations in EPS biosynthesis genes, as has been observed in detailed mutant studies with *E. coli* (mucoidy from colonic acid regulated by the *cps* genes [105]) and *P. fluorescens* (EPS synthesis regulated by the *alg* gene cluster [135]). Studies have shown that at the onset of drying conditions microbial colonies respond by enhanced production of EPS [3]. In *P. putida* and *P. fluorescens* this expression is controlled by AlgT (also known as AlgU, one of the ECF sigma factors in *P. putida*), *algU* controls desiccation and osmotic stress tolerance [135] and *algA* (controlled by the former) is overexpressed in *P. putida* under matric stress [160]. The benefits of EPS synthesis are manifold (protection, hydration, anchoring, etc.) and evident at the microcolony – rather than the individual cell – scale. Morphological changes in the EPS 3-D structure upon dessication are illustrated in Figure 10 [127]. In contrast to the fibrous and open structure under wet condition (Fig. 10a), the EPS becomes dense and amorphous when dried (Fig. 10b). It was hypothesized that such a change reduces rates of water loss (reduces diffusion rates through the denser coating) and possibly traps nutrients within the dense protective coating thereby assisting bacteria to survive desiccation [22]. Dehydration of non-submerged biofilms, similarly, causes collapse of the open EPS structure and likely affects other transport properties in the biofilm [70].

The discussion above clearly reflects a fragmented understanding of the process, but progress is being made on elucidating more of the genetic cascades involved in desiccation tolerance. An interesting mutant screening approach was introduced by van de Mortel and Halverson [160] to detect genes whose expression is differentially affected by desiccation. They discovered approximately 30 genes, one third of which were also upregulated during stationary phase (and thus likely RpoS controlled). However, the remaining genes covered a wide spectrum of physiological functions spanning protein fate, solute or nutrient acquisition, energy generation, motility, alginate biosynthesis, and cell envelope structure, emphasizing both the redundancy and the multiplicity of responses to dehydration.

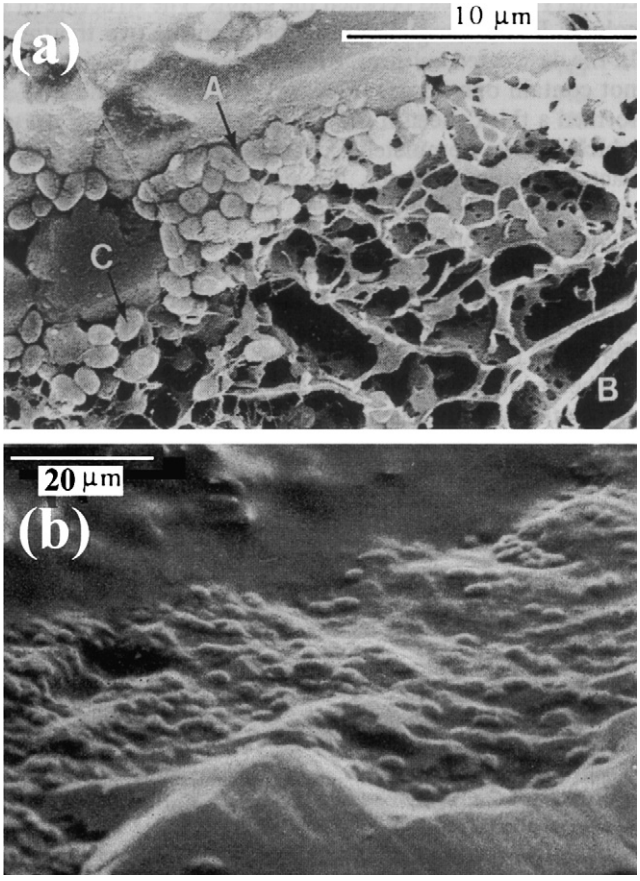


Fig. 10. Cryoscanning electron micrographs of (a) wet [−25 J/kg or −0.025 MPa], and (b) desiccated [−1000 J/kg or −1.0 MPa] cultures of *Pseudomonas* embedded in EPS in sand matrix. Note the change in morphology from open structure under wet conditions to dense EPS structure in response to desiccation (modified from [127]).

The *responses* described above beg the question as to how the decrease in external water availability is first *sensed* by a bacterium. It seems that the primary response to desiccation (resulting in osmotic upshifts) would involve the activation of transporters to allow rapid accumulation of osmoprotectants, and of sensor kinases to increase the transport or biosynthesis of such solutes [114]. It appears that intracellular ionic solutes (or ionic strength) serve as the signal for activation of these membrane-bound proteins, because of accompanying changes in the physicochemical properties of the cytoplasmic membrane (e.g., lateral membrane tension) which would alter the protein/lipid interactions and trigger protein activation [114]. The *pro*U operon has been associated with uptake of various compatible solutes, a typical mechanism of osmoadaptation in *P. putida* and other strains, although *de novo* synthe-

sis of compatible solutes also occurs [8,77,78]. A biosensor construct consisting of the promoter of the *pro*U operon (P_{proU}) transcriptionally fused to *gfp* has been used convincingly to examine response to water availability (−100 J/kg and below) [8]. However, it is more likely that proU is somewhere downward on the regulatory cascade and does not really measure water availability.

While bacteria can successfully respond to unsaturated conditions at an individual level and thus can survive and/or maintain activity during drying events, drying conditions have nevertheless a large impact on microbial processes. Indeed, the fragmentation of aqueous habitats, lack of connectivity, and ever thinner liquid films [107] limit bacterial mobility and affect the nature of interactions within confined communities such as predation by nematodes [131], quorum-sensing and gene transfers between bacteria [97], nutrient deprivation, and accumulation of potentially harmful metabolic by-products. The reduction in overall nutrient diffusive capacity and changes in the spatial distribution of hydrated pathways may limit competition and facilitate coexistence at the small scale, hence promoting microbial diversity [157]. Next we review several key interactions and adjustments at the microbial community level in response to changes in soil hydration status.

4.1. Limited mobility and convective transport under unsaturated conditions

Microbial mobility and expansion rates under unsaturated conditions are significantly reduced relative to those found in liquid media or on wet agar surfaces [64]. In a saturated porous medium with ample nutrient supply, penetration rates of bacteria may amount to as much as 2.8 mm/h [122]. Decreasing soil water content results in a drastic reduction of penetration rates; for example, *Pseudomonas aeruginosa* penetrated more than 20 mm in 24 h when water content was 39%, dropping to only 5 mm in 48 h as water content was reduced to 33%. At water contents below 28% no movement was detected within 48 h [52]. Similar results were obtained with *Rhizobium meliloti* NR203 [141].

As swimming by flagellar motility becomes limited with decreasing water content, other mechanisms for microbial movement may become important on the liquid film-coated surfaces, including swarming, gliding, twitching, and sliding [64]. The extent and manifestation of these surface translocation mechanisms in soils are not yet known. However, in other systems (e.g., on agar surfaces) it has been shown that such mechanisms support surface translocation and expansion of microbial communities to colonize new surfaces and adapt to changes in nutrient diffusion flux patterns [12]. This surface-associated motility is realized through several bacterial appendages (e.g., type IV pilus for twitching motility [76]) and in the case of *Serratia liquefaciens* MG1 through the differentiation into cells adapted to motility [38]. Bacteria can also excrete compounds to facilitate their surface motility. For example, EPS secretion assists surface gliding of certain filamentous cyanobacteria [92] and *Serratia marcescens* produces wetting agents to overcome the direct influence of attractive intermolecular forces due to proximity to solid surface and the strong surface tension of the water–gas interface [91]. An interesting mechanism of *B. subtilis* movement on film-coated surface involves dragging of multicellular aggregates and other structures using supercoiled macrofibers that reduce their length [93]. The resistive forces for motion in water films (in the order of 10 nN) are higher than when microbes are fully immersed in liquid (approx. 1 nN), the difference being due to capillary pinning forces under partial immersion [93]. By analogy with limitations to motion on agar surfaces due to changes in hydration described by [64], we expect increased drag and pinning forces limiting mobility across thin liquid films (thickness on the order of nm).

The importance of local bacterial motion notwithstanding, the primary mechanism for bacterial transport in unsaturated soils across macro-scale distances (>10 mm) remains convective, as discussed in several papers in this special issue. Typically, bacterial convective transport decreases with decreasing water contents and water flow velocities [48]. The preferential entrapment of hydrophobic bacteria at water–air interfaces [171] may retard transport if the interfaces are stagnant [132,172], or enhanced transport relative to water-saturated conditions if interfaces advance.

4.2. Heterogeneity and fragmentation – microbial coexistence and diversity

The bacterial diversity in soils is strikingly high even when considered at small spatial scales (e.g., [14,54,102]). Heterogeneity of soil environmental conditions that comprise microbial habitats is generally considered to be the main driver of this unparalleled diversity [71]. Studies have demonstrated that the availability of multiple niches resulted in rapid emergence of microbial diversity from an initial monoclonal bacterial population [120]. The environmental conditions in soil, including solid surface characteristics, oxidative/reducing status, nutrient concentrations, etc., are considered as very heterogeneous within small spatial and moderate time scales, thereby providing a diversity of niches available for exploitation. It has been experimentally [85] and theoretically [36,37] documented that existence of spatial structure, as opposed to well-mixed conditions, promotes diversity even in otherwise homogenous environments. Papke and Ward [109] established the role of spatial isolation in bacterial diversification in natural populations, as well. Recent studies [14,27,53,102] have focused on endemism (i.e., the condition of being restricted to a particular area) of soil bacteria, which is closely related to spatial isolation. These and other studies [14,27,168] indicated that while the endemism of free-living soil bacteria is strict at regional scales, at scales ranging from millimetres to centimetres bacterial endemism appears to

be less exact because clone-mates can be identified in separated microhabitats. Nevertheless, the interactions between bacteria located relatively close to each other but occupying different soil microhabitats, are likely to be limited. Indeed, the bulk of microbial interactions are mediated by the amount, configuration, and energy status of soil water. Whenever the soil water potential is low, these interactions are impaired. Treves et al. [157] showed that competitive interactions between two bacterial species were decreased in a dried sand matrix relative to the same matrix under wetter conditions, leading to species coexistence in the driest environment. Spatial isolation due to insufficient pore scale water connectivity has also been proposed [184] as explanation for the relatively large bacterial diversity found in unsaturated compared to saturated soils.

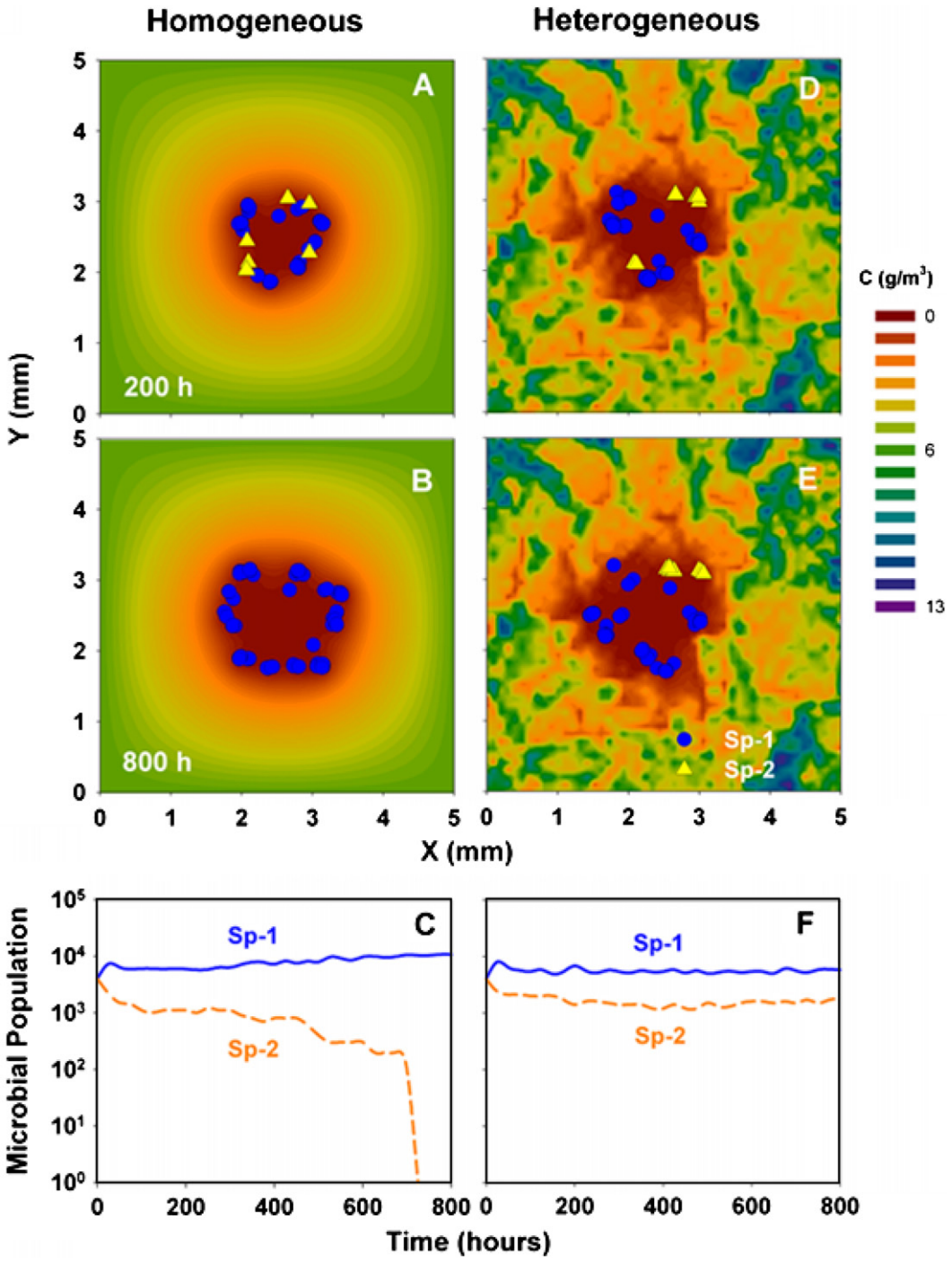


Fig. 11. Spatial distributions of clusters of two microbial species (Sp1 – strong competitor; Sp2 – weaker species) in homogeneous (A) and (B) and heterogeneous (D) and (E) domains, 200 h (A) and (D) and 800 h (B) and (E) after initial inoculation. Panels (C) and (F) describe temporal changes in population size of each of the two species in domains I and II (each cluster represents ~100 microbial cells). From [87].

Even when effectively connected by water films, low diffusion rates characteristic of unsaturated soils may support simultaneous occupancy by bacterial species that otherwise would not coexist. Indeed, low diffusion rates can theoretically result in a sustained heterogeneous pattern of nutrient accessibility, promoting the coexistence of microbial species that compete for the same nutrients (Fig. 11) [87]. Results of Thomas et al. [150] support this contention, as they reported that low diffusion rates decreased inhibitive interactions leading to coexistence of bacterial species within a porous medium.

Spatial isolation also arises as a result of complex geometric arrangement of the soil pore network. Microcolonies separated even by very small distances will not interact if their respective locations within soil pores are not, or are only poorly, connected. Further, minimal pore neck diameters in excess of nominal bacterial dimension are required for direct cell-to-cell contact. The commonly-observed phenomenon of bacteria confined within pores [47], as well as the soil porosity component having narrow pores such that they remain inaccessible to bacteria (estimates range from 15% to 54% of total pore space, depending on soil type) [26] clearly illustrate the important role of pore geometry on bacterial isolation.

In addition to the implications of habitat fragmentation in terms of bacterial isolation, the sheer wealth and often patchy distribution of environmental conditions in soils also contributes to the generation and maintenance of high bacterial diversity. This multiplicity of niches arises from chemical and physical properties of soils, including the diversity of nutrient sources [184] and varied nature of solid surfaces [72] which will not be reviewed here. The magnitude of bacterial niches in soil is extended even further if we consider the temporal variations of environmental conditions and the role of perturbations on the maintenance of diversity [16].

5. The effect of biological activity on host soil properties

5.1. Soil structure

Formation of microbial colonies on solid surfaces impacts soil structural properties, primarily through the formation of polymer bridges that bind soil particles [22,24]. The microbial enmeshing of soil particles shown in Figure 12 has a dual role in forming microaggregates [101]. The spatial arrangement of microbial activity (and associated microbial products such as EPS) must play an important role in the structural efficiency of such stabilizing agents (e.g., accumulation at grain contacts). Moreover, we expect that soil strength and structural stability acquired by accumulation of microbial remnants should be strongly correlated to the mechanical properties of EPS forming the bacterial colonies. An important characteristic is the response of EPS to changes in its hydration status. It has been observed with similar biopolymers [153] that the tensile strength and the Young's modulus increase by several orders of magnitude as the relative humidity (or water potential) decreases below saturation. Moreover, the biopolymer changes from soft and ductile at high humidity to stiff and brittle at low humidity. If such mechanical changes also occur in bacterial EPS, they could provide mechanical and diffusional protection for bacterial biofilms during desiccation. In addition, the mechanical properties of EPS would result in a marked increase in soil strength with decreasing soil water content.

As clearly illustrated in Figure 12, the presence of EPS helps maintain an open structure among clay particles and at an aggregate bed scale. Such an open structure is favorable for soil transport properties. Hadas et al. [55] attributed the increase in soil aggregate size and strength one week after plant residue addition to reinforcement by fungal hyphae, whereas changes appearing after the sixth

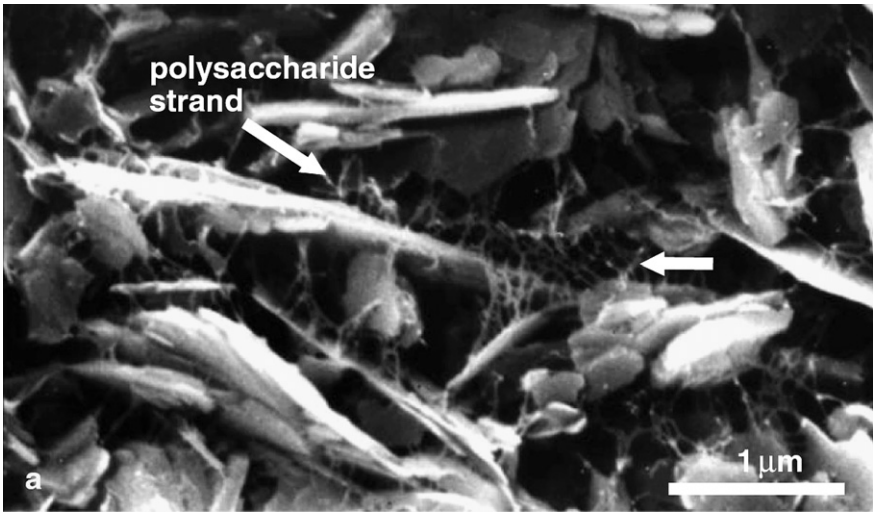


Fig. 12. Bridging of kaolinite particles by microbial scleroglucan (EPS) strands (from [22]).

week were attributed to bacterial secretions. Following intense colonization of the wheat rhizosphere by EPS-producing bacteria, Amellal et al. [4] observed significant increase in soil aggregation and concluded that *Pantoea agglomerans* plays an important role in soil water regulation by improving aggregation.

5.2. Water retention and wettability

Seasonal changes of soil water retention properties in response to organic matter amendments can be highly correlated with microbial activity [121,133]. Microbial activity, especially the secretion of EPS, can affect the soil water retention characteristic through several different mechanisms. These include the additional water retention capacity of the EPS material itself, the partial EPS coating of soil minerals which alters their wettability, soil structural changes mediated by EPS that alter the pore size distribution, and alteration of the water–air surface tension [129].

The soil water-holding capacity increases in the presence of appreciable amounts of EPS, as demonstrated with purified EPS [128] and with different model polysaccharides [22] adsorbed to sand, kaolinite and Ca-montmorillonite. The increased water retention is attributed primarily to the direct addition of EPS with its very high water holding capacity e.g., up to 70 g/g for xanthan at high water potentials, and also to creation of a more open pore space and separation between solid particles to which a fibrous network is attached [22,23]. The largest relative increases in water holding capacity at high and low water potentials were for sand with xanthan addition. The increase with dextran amendment was negligible, reinforcing the importance of the water-holding capacity of EPS itself [22].

The addition of EPS to clay minerals induces a secondary mechanism for water retention, namely the formation of a more open inter-particle space. Enhanced water uptake after air drying was documented with scleroglucan-amended montmorillonite. However, upon rewetting of kaolinite–EPS complexes, smaller amounts of water were absorbed by the EPS-amended kaolinite as compared to clean kaolinite [22], perhaps due to irreversible structural changes in EPS during drying, or EPS coating of kaolinite particles preventing their slaking thereby lowering their water absorption rates. The persistence of montmorillonite–EPS water holding capacity upon rehydration implies that large polymer molecules cannot enter inter-sheet mineral spaces, and the main role of EPS was creating an open and accessible spatial arrangement of montomorillonite tactoids.

Typically, an increase in EPS content enhances soil water holding capacity. However, in some cases soil wettability may be reduced. Hallett and Young [57] reported cases of water-repellency induced by EPS. Kidron et al. [79] attributed runoff over microbiotic crusts to water absorption and swelling of EPS causing pore clogging. These conflicting results reflect the complexity of the interactions and composition of EPS. Differences in wettability properties of organic "spots" on mineral surfaces (remnants of bacterial colonies) could affect water retention behavior, for example, by mechanisms of fractional wettability reported in Bradford and Leij [15] and Ustohal et al. [159].

Hallett and Young [57] defined a water-repellency parameter related to the sorptivity ratio of 95% ethanol and water, and demonstrated increased water repellency after C (glucose) and N (ammonium nitrate) amendments to soil aggregates followed by increased respiration rates. Subsequently, Hallett et al. [56] evaluated the spatial variability of water repellency on an intact 0.9 m wide, 1.3 m long and 0.25 m deep block of grassland soil and discovered high mm-scale variability with little evidence of spatial autocorrelation. Although demonstrated only for a single 1 m^2 land area, it is likely that many soils exhibit subcritical water repellency, where despite the soil appearing to take up water readily, partially-hydrophobic soil particle surfaces impede the rate of infiltration [56]. This corresponds well with the postulated microbial origin of water repellency, and with the sub-millimeter scales and temporal variability of organic matter, organisms, and microbial environments in soils [99].

5.3. Transport properties for water, solutes and gas

The reduction of hydraulic conductivity in saturated systems due to accumulation of biological mass and activity, usually termed bioclogging, has been observed at many length scales in media including glass micromodels [82,165], single fractures [67], soil columns [33,96,104,136,148,162], Hele–Shaw cells [81,152] and aquifers in the vicinity of pumping and recharge wells [11]. Possible mechanisms for microbially-induced reduction of the hydraulic conductivity of water-saturated porous media include decreased effective pore sizes and reduced porosity due to biomass accumulation, and reduced effective pore space due to microbially-induced mineral precipitation and also from biogenic air bubbles. Increased viscosity and density of soil water due to planktonic bacteria [129] has a negligible impact.

Microscopic observations in porous medium or glass micromodel systems indicate that in some cases a continuous biofilm covering the grains may develop [33], in other cases discrete microcolonies form [162], or both phenomena may occur [82,165]. Estimates of biofilm thickness in the range of 15 μm (for 0.12 mm sand grains) to 150 μm (for 0.7 mm sand grains) could explain the significant observed porosity and permeability reductions [33]. Starvation conditions can result in partial biofilm sloughing and commensurate permeability recovery [82,165]. Rittmann [124] claimed that the most important factor determining the extent of bacterial deposition is the substrate supply rate, and proposed a normalized surface loading criterion, which is defined to be the actual substrate flux (i.e., rate of removal per unit surface area) divided by the minimum flux capable of supporting a deep biofilm. When this number is

larger than 1, a continuous biofilm develops, and when less than 0.25 patchy biofilms develop, mostly in pore throats. Additional geometrical (pore size), and hydrodynamic factors also affect the spatial arrangement of bacterial populations.

Different approaches to describe the saturated hydraulic conductivity of the clean porous media have been used to model bioclogging effects on permeability reduction: a single cylindrical capillary model [104], cut-and-rejoin bundle of capillaries models [148,163] and the Kozeny–Carmen equation [148,163]. In all the above models a uniform narrowing of the pore space by the developing biofilm is assumed, which is highly unlikely. A more realistic assumption is that biofilms develop preferentially in larger pores with larger water flow (favoring substrate supply and toxic compound removal), whereas discrete colonies probably develop at pore constrictions and near grain contacts. Such combination of continuous microscopic biofilm development and single bacterial microcolony deposition patterns are characterized by different macroscopic permeability–porosity relationships along the bioclogging continuum. Experiments have illustrated the relatively moderate hydraulic conductivity reduction due to a continuous biofilm growth model relative to more abrupt reduction expected for pore throat blocking (up to 2 or 3 orders of magnitude difference) [129].

Certain bacteria are involved in pedogenic and anthropogenic mineralization processes, and depending on the type of bacteria and available energy and nutrient sources, such microbial activity can result in either dissolution or precipitation of rock and soil minerals (for details see a recent review by [39]). For example, reduction of soil solution pH due to bacterial activity may dissolve carbonate and other minerals, resulting in an increase in saturated hydraulic conductivity. The opposite effect is also encountered, for example the precipitation of sulfides caused by sulfate-reducing bacteria, which can decrease the saturated hydraulic conductivity [178]. In contrast to temporal effects on hydraulic conductivity due to bubble formation and dissolution, the reversal of mineral precipitation and consequent hydraulic conductivity recovery would be much slower processes.

In contrast to the wealth of experimental data and modeling of bioclogging in water-saturated porous media, systematic experimental studies in unsaturated soils are relatively nonexistent. In the few reported studies [96,136] the flow conditions were not controlled and determination of biomass and/or EPS accumulation was incomplete for proper quantification of observed phenomena. The factors mentioned above including reduced wettability are expected to reduce unsaturated hydraulic conductivity. However, for the unsaturated hydraulic conductivity, reduced pore sizes can increase water holding capacity at low matric potentials and the hydraulic conductivity of EPS-amended soils at such potentials can be higher than that of unmodified soils.

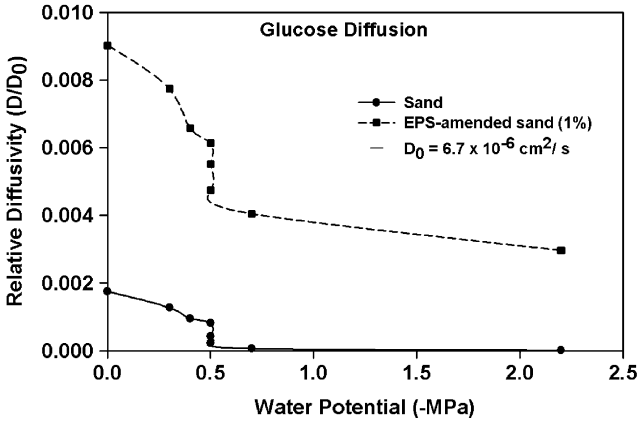


Fig. 13. Relative diffusion coefficient of glucose in sand and xanthan-amended sand as a function of water potential (modified from [25]).

The effect of bacterial activity on solute transport properties of soils has been studied primarily in saturated systems. Taylor et al. [149] demonstrated experimentally and theoretically order of magnitude increases in the dispersivity of a water-saturated sand column as a result of significant biofilm growth. In a recent study Seymour et al. [137] demonstrated a transition from normal to anomalous hydrodynamic dispersion in water-saturated porous media due to biofilm growth, and modeled the transport with a fractional advection-diffusion equation. Similar findings were reported by Hill and Sleep [67] studying flow and solute transport through a sandblasted glass parallel plate fracture. Analyses of pre-biofilm tracer tests revealed that both Taylor (normal) dispersion and macrodispersion were influencing transport. After biofilm growth, only macrodispersion was dominant, with the macrodispersion coefficient increasing logarithmically with hydraulic conductivity reduction. Only a few studies address the effects of microbial activity on hydraulic dispersivity of unsaturated porous materials [182].

Indirect evidence suggests that microbial activity enhances macroscopic diffusion properties of porous media. A remarkable increase in glucose diffusion coefficient through unsaturated sand was obtained with addition of only 1% EPS as demonstrated in Figure 13 [25]. This large increase is attributed to the overall increase in water holding capacity of EPS that controls the substrate diffusion coefficient.

Other transport properties of soils and rocks such as the thermal and electrical conductivities are also expected to be affected by accumulation of EPS but to a lesser extent as compared to the reduction in hydraulic conductivity, because of weaker sensitivity of these properties to the medium's pore size distribution.

6. Summary and conclusions

The soil environment presents a myriad of physical complexities and constraints that make for a unique setting in

which microbes must seek to thrive. The pore-scale water configuration in variably-unsaturated soils is likely the greatest single controller of microbial habitats and activities. Cells are fully immersed only under specific circumstances and mobility is greatly constrained under most unsaturated conditions. Diffusion rates for substrate and products are governed by the geometry and properties of the gas and liquid phases, which are continually rearranged in response to external conditions and physico-chemical environments in the soil. Soil transport properties change nonlinearly with changes in wetness and temperature.

Perhaps the most significant adaptation to fluctuating hydration conditions in the vadose zone is the formation of colonies embedded in a matrix of extracellular polymeric substances (EPS). These biopolymers modify the microenvironment of the mostly stationary microbes by enhancing hydration conditions, dampening rapid fluctuations, and providing an effective interface with the surroundings as soils intermittently or progressively desaturate. EPS in turn serves to modify certain physical characteristics of the soil matrix, often to the advantage of the embedded microbial colonies. Unlike the extensive biofilms observed under saturated or near-saturated conditions, limitations induced by unsaturated conditions result in much more localized EPS production around small colonies or cell aggregates.

The spatial isolation imposed by the soil aquatic landscape of small 'pools' connected by extensive thin liquid films engenders much greater bacterial diversity than is possible under saturated conditions where mobility and diffusion processes are not greatly constrained. In other words, diffusion and resource heterogeneity provide niches that shelter weaker species and promote coexistence of species that otherwise would have been eliminated. This unique feature, governed by soil physical properties and processes, is likely responsible for the immense microbial diversity in soils relative to the oceanic or free aquatic habitats, which might initially appear as more favorable environments to support microbial diversity.

Though fraught with potential complication, full consideration of the interactions of soil microorganisms with their physical and chemical environments will substantially advance our understanding of microbial ecology. Investigations concerning the preponderance of bacteria and archaea that are considered as 'unculturable' will be facilitated by provision of realistic created habitats that are based on a fidelity to their true natural surroundings. An integrative bio-physical synthesis will also provide enhanced understanding of pore-scale physics and transport phenomena, as the soil microbiota, their activities and their residues are a ubiquitous and critical feature of soils and engineered porous media.

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