Cover Page - Scientific Report

BARD Project Number: US-3377-02

Title: Physical processes affecting microbial habitats and activity in unsaturated agricultural soils

Investigators:

Dani Or

Shmulik Friedman

Jeanette Norton

Affiliated Institutions:

University of Connecticut

Inst. of Soil, Water and Enviro. Sci. (ARO)

Utah State University

Start Date of Project: October 15, 2002

Date of Submission of Report: March 31 2007

First Annual Report: November 30, 2003

Second Annual Report: November 28, 2004

Signature
Principal Investigator (PI)

Carol Welt 04/04/2007

Signature
Institution's Authorizing Official, Principal Institution

Carol Welt, Ph.D.
Executive Director
Office for Sponsored Programs

Cover Page

BARD Project Number: US-3377-02

Date of Submission of the report: March 31 2007

Project Title: Physical processes affecting microbial habitats and activity in unsaturated agricultural soils

Investigators

**Principal Investigator (PI):
Dani Or**

**Co-Principal Investigator (Co-PI):
Shmulik Friedman**

**Collaborating Investigators:
Jeanette Norton**

Institutions

**Univ. of Connecticut, USA
(Presently: EPFL, Switzerland)**

ARO, Israel

Utah State Univ., USA

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Coexistence, Biofilms, Extracellular Polymeric Substances, Growth Models, Nitrification, Genome

Abbreviations commonly used in the report, in alphabetical order:

Budget: IS: \$112,000

US: \$223,000

Total: \$335,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution
Carol Welt, Ph.D.
Executive Director
Office for Sponsored Programs

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1	3		4
Submitted, in review, in preparation			2	2
Invited review papers		1		1
Book chapters				0
Books				0
Master theses			1	1
Ph.D. theses		1		1
Abstracts				2
Not refereed (proceedings, reports, etc.)	1	14	1	16

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	1		2
Longer Visits (Sabbaticals)				

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Abstract

The main objectives of the research project were to develop theoretical tools and experimental methods for quantifying effects of water content and other dynamic environmental factors on bacterial growth in partially-saturated soils. Towards this end we reviewed critically the relevant scientific literature and performed theoretical and experimental studies of bacterial growth and activity in modeled, idealized and real unsaturated soils.

The natural wetting-drying cycles common to agricultural soils affect water content and liquid organization resulting in fragmentation of aquatic habitats and limit hydraulic connections. Consequently, substrate diffusion pathways to soil microbial communities become limiting and reduce nutrient fluxes, microbial growth, and mobility. Key elements that govern the extent and manifestation of such ubiquitous interactions include characteristics of diffusion pathways and pore space, the timing, duration, and extent of environmental perturbations, the nature of microbiological adjustments (short-term and long-term), and spatial distribution and properties of EPS clusters (microcolonies). Of these key elements we have chosen to focus on a manageable subset namely on modeling microbial growth and coexistence on simple rough surfaces, and experiments on bacterial growth in variably saturated sand samples and columns.

Our extensive review paper providing a definitive "snap-shot" of present scientific understanding of microbial behavior in unsaturated soils revealed a lack of modeling tools that are essential for enhanced predictability of microbial processes in soils. We therefore embarked on two pronged approach of development of simple microbial growth models based on diffusion-reaction principles to incorporate key controls for microbial activity in soils such as diffusion coefficients and temporal variations in soil water content (and related substrate diffusion rates), and development of new methodologies in support of experiments on microbial growth in simple and observable porous media under controlled water status conditions. Experimental efforts led to a series of microbial growth experiments in granular media under variable saturation and ambient conditions, and introduction of atomic force microscopy (AFM) and confocal scanning laser microscopy (CSLM) to study cell size, morphology and multi-cell arrangement at a high resolution from growth experiments in various porous media.

The modeling efforts elucidated important links between unsaturated conditions and microbial coexistence which is believed to support the unparallel diversity found in soils. We examined the role of spatial and temporal variation in hydration conditions (such as exist in agricultural soils) on local growth rates and on interactions between two competing microbial species. Interestingly, the complexity of soil spaces and aquatic niches are necessary for supporting a rich microbial diversity and the wide array of microbial functions in unsaturated soils.

This project supported collaboration between soil physicists and soil microbiologist that is absolutely essential for making progress in both disciplines. It provided a few basic tools (models, parameterization) for guiding future experiments and for gathering key information necessary for prediction of biological processes in agricultural soils. The project sparked a series of ongoing studies (at DTU and EPFL and in the ARO) into effects of soil hydration dynamics on microbial survival strategy under short term and prolonged desiccation (important for general scientific and agricultural applications)

Achievements

New mechanistic models that realistically consider spatial and temporal variations in hydration and other key environmental factors and detailed experiments yielded new insights on factors affecting bacterial growth and coexistence in partially saturated soils. Such building blocks improve predictive capabilities regarding the role of hydration and certain soil processes in bacterial nutrients cycling, plant pathogenesis, bioremediation of environmental pollutants, and enhance assessment of survival of human pathogens introduced by application of manure, sludge and untreated wastewater. In addition to tools for studying bacterial growth, survival and activity in partially saturated soils, the results of the present project would benefit scientists and practitioners dealing with bacterial growth and activity in other natural and engineered multiphase media such as structured food, plant and animal tissues, and granular bioreactors.

The research area concerning soil-bacteria interactions was new for the PI and co-PI (soil physicists), nevertheless, progress was possible through continuous exchange between modeling effort led by DO, experimental work led by SF, and environmental microbiology colleagues (JN, Sela, Smets and Dechesne). These collaborative efforts are evidenced by co-authorship of two extensive review papers, scientific presentation, and joint student mentoring.

The primary achievements of this project were: (1) establishment of productive collaboration between soil microbiologists and soil physicists; (2) enhancing awareness of the importance of biophysical interactions in unsaturated soils important for both scientific communities; (3) development of new modeling framework for understanding microbial activity and coexistence under dynamic hydration conditions found in irrigated soils; and (4) direct experimental evidence concerning changes in growth and morphology of bacteria in response to changes in hydration, nutrients and oxygen availability, temperature and inoculum size.

To simplify the complex geometry found in soils yet retain the essential elements governing water flow and nutrient diffusion under different water contents, we developed a modeling framework for microbial growth on partially saturated rough surfaces. Two modeling approaches were used to describe changes in water retention and substrate diffusion on rough surfaces. The first is the Roughness Network (RN) model that represents the rough surface as a network of roughness elements (pits and v-shaped channels) with simple geometry, and calculates the details of aqueous phase distribution in the network for any matric potential or relative humidity values (Long and Or, 2007a). The second approach called the Patched Surface (PS) model, represents the rough surface as a lattice of patches each with its own porosity and

water retention properties. The PS is an extension of the approach reported in Long and Or (2005), and offers higher potential for upscaling from mm to dm (sample scale). For either rough surface, once the aqueous phase distribution is determined, a hybrid model based on the Reaction Diffusion Method (RDM) for nutrient (solute) evolution coupled with Individual Based Models (IBM) are employed to simulate microbial growth and expansion under various environmental conditions. Both RN and PS models were used to investigate coexistence of competing species under heterogeneous and diffusion limiting conditions (coexistence being impossible under the wet and homogenous conditions, where the strongest species dominates). The results also highlighted the importance of effective diffusion coefficient of the medium as a primary indicator for microbial growth dynamics in porous media under unsaturated conditions. Despite the many simplifying assumptions involved, this modeling framework provides some of the first steps towards testing of questions relevant to agricultural soils. For example, the impact of availability or limitations of different nutrients on growth, coexistence and activity of different microbial communities occupying the rhizosphere under constant and temporally variable water conditions (induced climatically or by irrigation). Very few models deal with complex conditions that develop in unsaturated soils, and unlike a wealth of modeling tools for microbial growth in aquifers, microbial growth in unsaturated soils was not addressed quantitatively and mechanistically before.

The experimental phase comprised two types of systems: incubation experiments in spatially homogenous and closed-systems, and growth experiments in short columns under steady-state water flow conditions. *Pseudomonas aeruginosa* (biofilm-forming aerobic soil bacteria) was grown in acid-washed quartz sand. The two primary factors determining maximum (stationary phase) number of heterotrophic bacteria in closed-system were the amount of carbon source and the amount of oxygen and its supply rate. Inoculum size and temperature did not affect the maximum bacterial concentration. Bacterial growth rate was determined primarily by temperature, increasing temperatures shortened duration of the lag phase. The specific growth rate in partially-saturated sand was higher than in free solution, as well as the apparent activation energy (dependence of growth rate on temperature). Increasing nutrient concentrations beyond a certain value extended the duration of the lag phase and impeded bacterial growth rate. The maximum specific growth rate dependence on saturation degree was of optimum type with minimal growth rates at low and high water saturations and a maximum growth rate at some intermediate saturation degree of about 0.7 in our experiments (similar to results reported in literature). The specific growth rate increases moderately with inoculum size.

The growth of aerobic heterotrophic bacteria under steady-state water flow conditions was controlled by nutrient concentration and oxygen supply rate. Bacterial growth was maximal near the surface and decreased with soil depth, namely it decreased with increasing water contents. In contrast, under no-flow conditions local nutrient diffusion rates became limiting and maximum bacterial growth migrated to a certain depth below soil surface. Bacterial growth increased with nutrient concentration in a Monod saturation pattern such that larger water fluxes were unable to compensate for lower nutrient concentrations. We measured reduction in bacterial growth in the upper part of the column at higher water fluxes in both low and high nutrient concentrations indicating potential impeding effects of dilution of a stimulating autoinducer.. CSLM micrographs show bacteria attached to sand grains as discontinuous microcolonies.

The two types of closed, stagnant, and open, flow-thorough conditions allowed characterization of different effects of environmental factors relative to real conditions in partially saturated agricultural soils. For example, it was found that at a level of nutrient concentration (0.4% citrate + M63) for which bacterial growth is still increasing with increasing nutrient concentration in flow-through conditions, it is decreasing with increasing nutrient concentration in closed-system, batch conditions due to deficient oxygen supply and accumulation of poisonous materials.

A summary of scales observed by different methods (numbers refer to reviewed publications)

List of reviewed publications

- (1) Or, D., B. F. Smets, J.M. Wraith, A. Dechesne, and Friedman, S. P. (2007). Physical Constraints Affecting Microbial Habitats and Activity in Unsaturated Porous Media – A Review. *Advances in Water Resources, In Press, Available online 11 September 2006.*
- (2) Long, T., Or, D. (2005). Aquatic habitats and diffusion constraints affecting microbial coexistence in unsaturated porous media. *Water Resources Research* 41(8): Art. No. W08408 AUG 12 2005.
- (3) Long, T., Or, D. (2007). Microbial growth on partially saturated rough surfaces: Simulations in idealized roughness networks. *Water Resources Research* 43(2): Art. No. W02409 FEB 10 2007.
- (4) Or, D., Phutane, S. and A. Dechesne (2007). Extracellular polymeric substances (EPS) affecting pore-scale hydrologic conditions for bacterial activity in unsaturated soils. *Vadose Zone J. (In press)*
- (5) Long, T., and D. Or, 2007, Dynamics of microbial growth and coexistence on variably hydrated rough surfaces, *J. Theor. Biol., (in review)*
- (6) Rol, J., Sela, S., Pinto, R. and Friedman, S.P. (In preparation). Incubation experiments for characterizing the effects of environmental conditions on growth of *Pseudomonas aeruginosa* in partially-saturated sands. To be submitted to *Vadose Zone J.*
- (7) Rol, J., Sela, S., Naftalejev, B. and Friedman, S.P. (In preparation). Flow-through experiments for exploring the effects of water and nutrient fluxes on vertical distribution of *Pseudomonas aeruginosa* in partially-saturated sands. To be submitted to *Vadose Zone J.*
- (8) Norton, J. M. D. J. Arp, P. J. Bottomley, P. S.G. Chain, L. J. Hauser, M. G. Klotz, M. L. Land, F. W. Larimer, M.W. Shin, S. R. Starkenburg, L. Y. Stein. (In preparation) Complete genome sequence of *Nitrospira multiformis*, an ammonia-oxidizing bacterium from the soil environment. To be submitted to *Appl. Environ. Microbiol.*
- (9) Koper, T. E., JM Stark, MY Habteselassie, JM Norton. (in preparation). Nitrification exhibits Haldane kinetics in an agricultural soil treated with ammonium and dairy waste. To be submitted to *Soil Biol. Biochem.*

Presentations:

- (1) Long, T. and D. Or (2006), Modeling microbial growth on unsaturated rough surfaces – effects of roughness heterogeneity and matric potential dynamics, Gordon Research Conference: Flow and Transport in Permeable Media, 2006.
- (2) Norton, J.M., M.Y. Habteselassie, T.E. Koper, J.M. Stark and B.E. Miller. 2006. Connecting Community Structure, Kinetics and Process: Nitrification in Dairy Waste Treated Soils. ASA-CSSA-SSSA Abstracts. Indianapolis, IN
- (3) Long, T. and D. Or (2005), Microbial growth on partially saturated rough surfaces – simulations in idealized roughness networks, AGU Fall Meeting, San Francisco
- (4) Or D., and S. R. Phutane (2005), The role of EPS in microhydrology and transport processes affecting microbial activity in unsaturated porous media, Eos Trans. AGU, 86(52), Fall Meet. Suppl., Abstract H41H-01
- (5) Dechesne A., Or D., Smets B.F. (2005) Substrate diffusion heterogeneity controls bacterial competition and coexistence. *American Geophysical Union Fall Meeting*. San Francisco. December 5-9.
- (6) Long, T. and D. Or (2005), Hydration and roughness effects on microbial growth on rough surfaces, AGU Joint Assembly, New Orleans
- (7) Long, T. and D. Or (2004), A theoretical framework for microbial diversity and coexistence in unsaturated soils, Kirkham conference, Utah State Univ.
- (8) Phutane, S.R., and D. Or (2005). Role of extra-polymeric substances (EPS) on the microbial activity in unsaturated porous media, Eos Trans. AGU, 86(18), Abs. B33C-02
- (9) Or, D., (2005). The role of exopolymeric substances (EPS) in the micro-hydrology of soil microbial communities. Agronomy Abstracts, ASA, Madison, WI.
- (10) Long, T. and D. Or (2004), Effects of diffusion fragmentation and spatial constraints on soil microbial diversity, AGU Joint Assembly, Montréal, Canada
- (11) Norton J.M., M.G. Klotz, L.Y. Stein, P.J. Bottomley and D.J. Arp. 2005. Nitrifier genomics: links between habitats and functional gene diversity. ASA-CSSA-SSSA Abstracts. SLC, UT.
- (12) Koper, T. E., JM Stark, MY Habteselassie, JM Norton. 2005. Nitrification exhibits Haldane kinetics in an agricultural soil treated with ammonium and dairy waste. ASA-CSSA-SSSA Abstracts. Salt Lake City, UT.
- (13) Habteselassie, M.Y, J.M. Stark , B.E. Miller S. Thacker, J.M. Norton. 2005. Use of real-time and conventional PCR to quantify and profile ammonia-oxidizing

bacteria in dairy waste treated agricultural soil. ASA-CSSA-SSSA Abstracts. Salt Lake City, UT.

- (14) Norton J.M, L.Y. Stein M.G. Klotz, P. S. G. Chain, F.W. Larimer, M. Land and D.J. Arp. 2005 The genome of *Nitrosospira multiformis*: functional and evolutionary insights from a comparison with *Nitrosomonas europaea*. ASM Abstracts. Atlanta, GA.
- (15) Rol, D., Sela, S., Pinto, R., and Friedman, S. P. 2005. The effect of water content on microbial growth in unsaturated sand. An International Workshop on Microorganisms-Water and aquifers, Sde-Boker, Israel, September 20-21.
- (16) Or, D., B. F. Smets, J.M. Wraith, A. Dechesne, and S. Friedman. 2005. Physical constraints affecting mMicrobial habitats and activity in unsaturated porous media – A few points for discussion. An International Workshop on Microorganisms-Water and aquifers, Sde-Boker, Israel, September 20-21.