Supporting information for

Bioavailability of Soil-Sorbed Tetracycline to *Escherichia coli* under Unsaturated Conditions

Zeyou Chen,[†, ‡], Wei Zhang,[‡] Gang Wang,[§] Yingjie Zhang,[‡] Yanzheng Gao,[†, *] Stephen A. Boyd,[‡] Brian J. Teppen,[‡] James M. Tiedje,[‡] Dongqiang Zhu,[∥] and Hui Li[‡,*]

[†] Institute of Organic Contaminant Control and Soil Remediation, College of Resource and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China

[‡] Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, Michigan 48824, United States

[§]Department of Water and Soil Sciences, China Agricultural University, Beijing 100193, China

[∥] School of Urban and Environmental Sciences, Peking University, Beijing 100871, China

Number of pages: 11

Number of supplemental tables: 3

Number of supplemental figures: 3

Number of supplemental videos: 1

* Corresponding author

Yanzheng Gao

Phone: +86-25-84395019

Fax: +86-25-84395238

Email: gaoyanzheng@njau.edu.cn

Hui Li

Phone: +1-517-353-0151

Fax: +1-517-355-0270

E-mail: lihui@msu.edu

Table S1. Physicochemical properties of the three soils studied.

Table S2. Measured matric potential and osmotic potential of agar media with varying polyethylene glycol (PEG) content and calculated volumetric soil water content.

Table S3. Parameters used to describe bacterial growth and metabolism in the model.

Figure S1. Images of the agar plates for the Webster, Capac and Oshtemo soils with varying sorbed tetracycline concentrations.

Figure S2. Agar diffusion assays of the soils with sorbed tetracycline concentration of 83, 155, 445, 733 mg kg^{-1} by the Webster soil, 78, 143, 417, 698 mg kg^{-1} by the Capac soil, and 86, 160, 460, 757 mg kg^{-1} by the Oshtemo soil. Blue values are the measured diameters of the formed fluorescent rings.

Figure S3. Images of the agar plates for the soils with the lower and higher tetracycline loadings. The lower tetracycline loading in the soils was 14 mg kg^{-1} for the Webster soil, 13 mg kg^{-1} for the Capac soil, and 15 mg kg^{-1} for the Oshtemo soil. The higher tetracycline loading was 148 mg kg^{-1} for the Webster soil, 136 mg kg^{-1} for the Capac soil, and 150 mg kg^{-1} for the Oshtemo soil.

Video 1. The simulated tetracycline diffusion, bacteria growth and fluorescence emission.

Chemicals

Tetracycline hydrate (purity ≥ 99%), ampicillin sodium salt (purity ≥ 95%), methanol (HPLC grade), and 3-(N-morpholino)propanesulfonic acid (MOPS, buffer range 6.5−7.9), histodenz nonionic density gradient medium, polyethylene glycol (molecular weight of 8000 Daltons), and Tween 20 were purchased from Sigma-Aldrich (St. Louis, MO). Sodium chloride, potassium chloride, formic acid, sodium phosphate dibasic, and potassium phosphate monobasic were purchased from J.T. Baker (Philipsburg, NJ). Bacto tryptone, Difco agar noble, and Bacto yeast extracts were purchased from Becton, Dickinson and Company (Sparks, MD). Acetonitrile (HPLC grade) was purchased from EMD Chemicals (Gibbstown, NJ). Sodium pyrophosphate decahydrate (purity ≥ 99%) was purchase from ThermoFisher Scientific (Fremont, CA).

Sorption and Desorption Experiments

Sorption and desorption experiments were conducted to characterize the affinity of tetracycline for the Webster clay loam, the Capac sandy clay loam, and the Oshtemo loamy sand. All glassware used in these experiments was autoclaved. Fifteen mL of 0.22 μm membrane-

filtered tetracycline solution of 0, 0.1, 0.5, 1, 3, 7 and 10 mg L^{-1} was mixed with 1.0 g of each soil in 30-mL Kimble glass centrifuge tubes. The tubes were wrapped with aluminum foil to minimize photolysis. The samples were placed on a rotator at 50 rpm for 24 h at 22 ± 0.5 °C. Preliminary results showed that the sorption equilibrium was achieved within 24 h. The tubes were centrifuged at 1460 *g* for 25 min, and then 1.0 mL of the supernatant was collected for tetracycline analysis using a Shimadzu high-performance liquid chromatography coupled with a Sciex 3200 triple quadrupole mass spectrometer (LC-MS/MS) (Applied Biosystems, Foster City, CA). The amount of tetracycline sorbed by the soils was calculated by the difference between the initial and final tetracycline concentrations in solution phase. After removing the supernatant from the centrifuged tubes, the remaining soils were freeze-dried using a VirTis freeze mobile lyophilizer. The free-dried soils in the first set of the sorption experiments were used in agar diffusion assays later.

To examine the sorption reversibility, the desorption experiments were conducted using a well established decant-and-refill approach.[1] In the second set of the sorption experiments, the remaining supernatant was immediately removed using a disposable glass pipette. The residual solution that could not be removed was determined gravimetrically, and the tetracycline concentration in the residual solution was assumed to be the same as that measured in the bulk supernatant. Then, a certain amount of deionized water was weighed into the centrifuge tubes to reach a final solution volume of 15.0 mL, and the soil pellets were then dispersed. The tubes were shaken for another 24 h, centrifuged at 1460 *g* for 25 min, and the supernatants were collected for the measurement of tetracycline concentrations. The sorption and desorption isotherms were established by plotting the tetracycline concentrations in the soils versus the tetracycline concentrations in the solution phase. The sorption of tetracycline was essentially the same for the two sets of sorption experiments.

Quantification of Tetracycline

Tetracycline concentration was measured using the LC-MS/MS. A C_{18} column (Gemini, 5 μm, 50 × 2.0 mm, Phenomenex Inc., Torrance, CA) was used with a binary mobile phase consisted of phase A 0.3% formic acid solution and phase B acetonitrile/methanol mixture of 1:1 by volume containing 0.3% formic acid at a flow rate of 0.35 mL min^{-1}. The tetracycline

concentration was quantified using the multiple reaction monitoring mode with precursor/product ion pair of m/z 445.4/410.0.

Measurement of Matric Potential and Osmotic Potential

Osmotic potentials (ψ_o) of PEG-free and PEG-infused agar media were measured using a vapor pressure osmometer (Vapro model 5600; Wescor, Logan, UT).[2] At the PEG concentration of 0, 250, 400, 550 and 700 g L^{-1} in the overlying solution, the measured ψ_o of the agar media was −0.83, −1.03, −1.31, −1.88 and −2.46 MPa, respectively. The matric potential (ψ_m) of the agar media was measured using a filter-paper technique modified by Deka *et al.*[3] and Huang *et al.*[4] Briefly, a Whatman No. 42 filter paper (70 mm in diameter) was dried at 105 °C and stored in a desiccator at room temperature before use. The dried filter paper was placed on the surface of PEG-free and PEG-infused agar media in glass Petri dishes. The Petri dishes were then placed in a polyethylene box with an air-tight lid at 25 °C. Water activity in the polyethylene box was controlled in the presence of 100 mL of $BaCl_2$-saturated aqueous solution. The moisture content of the filter paper was found to achieve equilibration after one week. The filter paper was weighed, then dried at 105 °C for 24 h, and reweighed. The matric potential of the agar media was calculated using the equation developed by Deka et al.[3]: $\psi_m = -10^{(2.383-1.309\theta_w)}$, where θ_w is the gravimetric water content of the filter paper (g g^{-1}). Here we approximated the ψ_o and ψ_m of soils deposited on the agar media to the ψ_o and ψ_m of the agar media, because it was impossible to measure those *in situ* with the low amount of soils used in the study. The soils were initially dry, and underwent re-wetting upon deposition on the agar media during which the PEG diffused to soil pore water from the agar media. Also, the ψ_m of the agar media and the soils would become equilibrated over time. Nonetheless, it often takes time to establish the equilibrium of water potential including ψ_o and ψ_m. Thus, it was noted that the actual ψ_m and ψ_o of the soils might deviate slightly from the values measured on the agar media.

Model Simulation

The model simulation was performed on the surface-roughness network consisting of identical roughness elements at a ψ_m of −3.0 kPa. For initial inoculation, 1000 *E. coli* cells were randomly distributed throughout the entire simulation domain. In the simulation domain, 35 mg

L^{-1} of tetracycline (C_0) was initially loaded at four locations similar to that shown in the agar diffusion assay (Figure S1). Tetracycline concentration was assumed to decay according to a pseudo first-order equation of $C_t = C_0 - C_0(\frac{0.082t}{60+0.082t})$, in which C_0 and C_t are the initial tetracycline concentration and its concentration at time t (s), respectively.[5, 6] A modified Monod equation was used to describe the growth kinetics of a single bacterium as:[7, 8]

$$\mu_B = \frac{\mu_{max} C}{K_S(1 + C^*/K_I) + C} - m \,, \qquad (S1)$$

where μ_B and μ_{max} are the specific and the maximum growth rate, respectively, C is the substrate concentration, K_S is the half-saturation constant, C^* is the concentration of inhibitor (i.e. tetracycline in the study), K_I is tetracycline inhibition constant, and m is the cell maintenance rate. The cell growth was evaluated by estimating its volume.[8] When a cell volume (V_B) becomes greater than the critical division volume ($V_{B,d}$), the cell splits into two cells. When V_B is less than the critical minimum volume ($V_{B,min}$), the cell is deemed dead. The $V_{B,d}$ and $V_{B,min}$ are determined based on the Donachie model:[7]

$$V_{B,d} = \frac{2}{1.433}\overline{V}_B \qquad (S2)$$

$$V_{B,min} = \frac{1}{5}V_{B,d} \qquad (S3)$$

where $\overline{V}_B$ (mm^3) is the average volume of an active bacterium. The self-motion of a cell is modeled according to Wang and Or,[9] where both capillary and hydrodynamic limitation to cell motion and bacterial chemotactic motility are considered. The key parameters describing bacteria growth and metabolism are summarized in Table S3.

Table S1. Physicochemical properties of the three soils studied

Soil series	pH	CEC (cmol kg^{-1})	Sand (%)	Silt (%)	Clay (%)	Organic matter (%)	Soil texture
Webster	7.8	23.1	39.9	30.3	29.8	1.8	Clay loam
Capac	7.3	17.2	49.9	27.4	22.7	5.4	Sandy clay loam
Oshtemo	5.3	6.7	84.0	4.1	11.9	0.7	Loamy sand

Table S2. Measured matric potential and osmotic potential of agar media with varying polyethylene glycol (PEG) content and calculated volumetric soil water content

PEG concentration (g L^{-1})[a]	Osmotic potential (MPa)	Matric potential (kPa)	Volumetric soil water content (θv)[b]			Soil water saturation (%)[c]		
			Webster soil	Capac soil	Oshtemo soil	Webster soil	Capac soil	Oshtemo soil
0.0	−0.83	−2.95	0.42	0.34	0.28	89	84	71
250	−1.03	−5.15	0.39	0.3	0.19	82	75	50
400	−1.31	−7.81	0.36	0.27	0.15	76	67	38
550	−1.88	−10.59	0.34	0.25	0.12	72	62	31
700	−2.46	−13.75	0.32	0.23	0.11	69	57	28

[a] The PEG concentrations in the overlying solution.

[b] Volumetric soil water content was calculated according to the van Genuchten equation for the clay loam (the Webster soil), the sandy clay loam (the Capac soil), and the loamy sand (the Oshtemo soil).[10,11]

[c] Soil saturation level was calculated by dividing volumetric soil water content by total soil porosity.

Table S3. Parameters used to describe bacterial growth and metabolism in the model

Parameter	Unit[a]	Value
μ_{max}: maximum specific growth rate	hr^{-1}	1.2
K_S: half-saturation constant	fg fl^{-1}[a]	1.0×10^{-6}
m: apparent maintenance rate	fg glucose (fg mass) $^{-1}$ hr^{-1}	0.036
$\bar{V}_B$: median cell volume	fl	0.4
$V_{B,d}$: cell volume at division	fl	$2\bar{V}_B/1.433$
$V_{B,min}$: minimal cell volume of an active bacterium	fl	$V_{B,d}$ /5
V: maximum cell velocity	μm s^{-1}	0.05
C: glucose concentration	fg fl^{-1}	10×10^{-3}
D: glucose diffusion coefficient	mm^2 hr^{-1}	2.4
K_I: tetracycline inhibition constant	fg fl^{-1}	1.0×10^{-3}

[a] 1 fg = 1×10^{-15} g; 1 fl = 1×10^{-15} L.

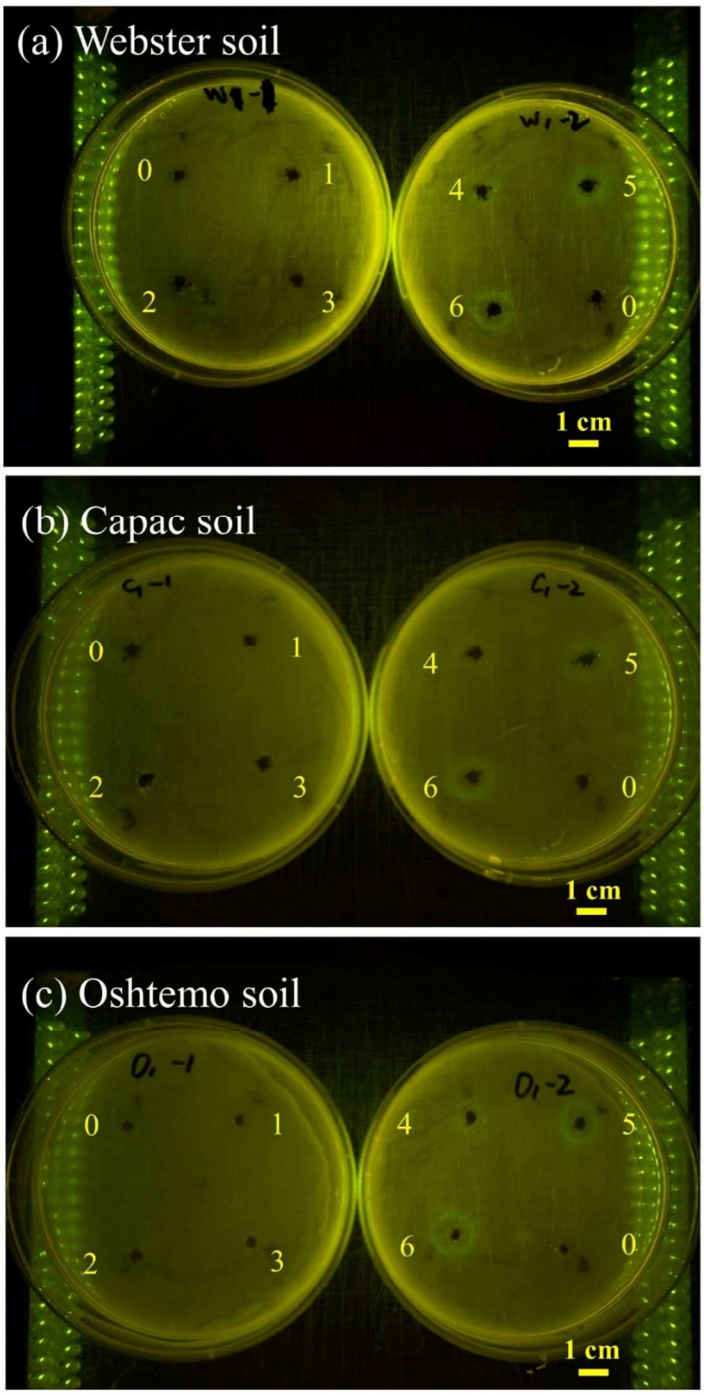

Figure S1. Images of the agar plates for the Webster, Capac and Oshtemo soils with varying sorbed tetracycline concentrations. Numbers 0, 1, 2, 3, 4, 5 and 6 represent (a) the Webster soil with sorbed tetracycline concentration of 0, 0.8, 6.9, 14, 44, 104, and 148 mg kg^{-1}, (b) 0, 0.8, 6.4, 13, 41, 96 and 136 mg kg^{-1} for the Capac soil, and (c) 0, 1.0, 7, 15, 45, 105 and 150 mg kg^{-1} for the Oshtemo soil. Soils are shown as the dark spots on the agar medium surface.

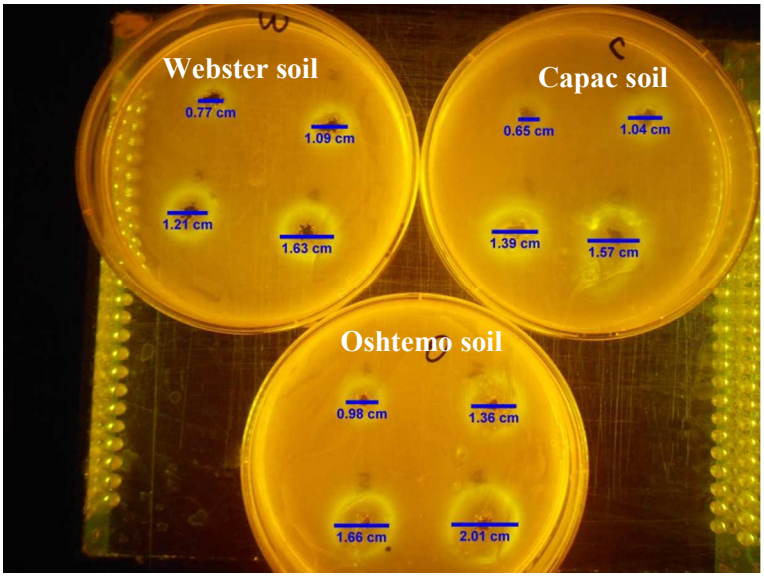

Figure S2: Agar diffusion assays of the soils with sorbed tetracycline concentration of 83, 155, 445, 733 mg kg^{-1} by the Webster soil, 78, 143, 417, 698 mg kg^{-1} by the Capac soil, and 86, 160, 460, 757 mg kg^{-1} by the Oshtemo soil. Blue values are the diameters of the formed fluorescent rings.

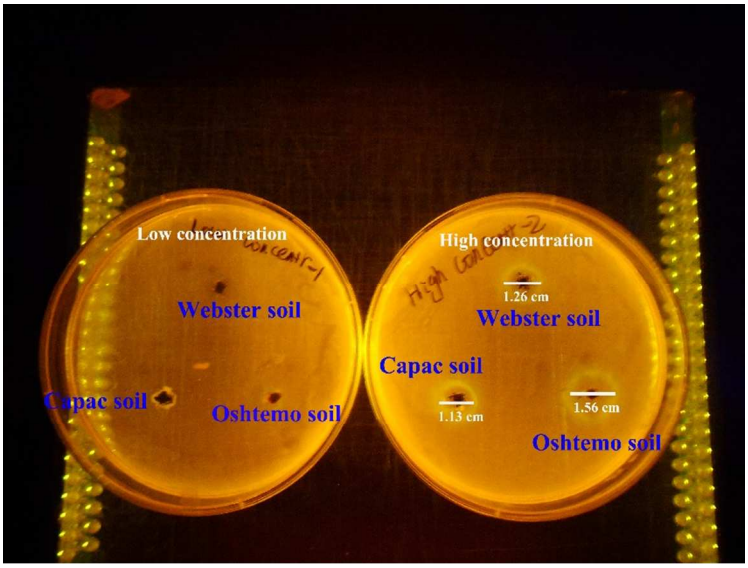

Figure S3. Images of the agar plates for the soils with the lower and higher tetracycline loadings. The lower tetracycline loading in the soils was 14 mg kg^{-1} for the Webster soil, 13 mg kg^{-1} for the Capac soil, and 15 mg kg^{-1} for the Oshtemo soil. The higher tetracycline loading was 148 mg kg^{-1} for the Webster soil, 136 mg kg^{-1} for the Capac soil, and 150 mg kg^{-1} for the Oshtemo soil.

.

References

1. Li, H.; Sheng, G.; Teppen, B. J.; Johnston, C. T.; Boyd, S. A. Sorption and desorption of pesticides by clay minerals and humic acid-clay complexes. *Soil Sci. Soc. Am. J.* **2003,** *67* (1), 122-131.
2. Money, N. P. Osmotic pressure of aqueous polyethylene glycols relationship between molecular weight and vapor pressure deficit. *Plant Physiol.* **1989,** *91*(2), 766-769.
3. Deka, R.; Wairiu, M.; Mtakwa, P.; Mullins, C.; Veenendaal, E.; Townend, J. Use and accuracy of the filter-paper technique for measurement of soil matric potential. *Eur. J. Soil Sci.* **1995,** *46* (2), 233-238.
4. Huang, Y.; Chapman, B.; Wilson, M.; Hocking, A. D. Effect of agar concentration on the matric potential of glycerol agar media and the germination and growth of xerophilic and non-xerophilic fungi. *Int. J. Food Microbiol.* **2009,** *133*(1), 179-185.
5. Aharoni, C.; Sparks, D. L. Kinetics of soil chemical reactions—a theoretical treatment. *Rates of soil chemical processes* **1991**, 1-18.
6. Fernández-Calviño, D.; Bermúdez-Couso, A.; Arias-Estévez, M.; Nóvoa-Muñoz, J. C.; Fernández-Sanjurjo, M. J.; Álvarez-Rodríguez, E.; Núñez-Delgado, A. Kinetics of tetracycline, oxytetracycline, and chlortetracycline adsorption and desorption on two acid soils. *Environ. Sci. Pollut. Res.* **2015,** *22*(1), 425-433.
7. Kreft, J.-U.; Booth, G.; Wimpenny, J. W., BacSim, a simulator for individual-based modelling of bacterial colony growth. *Microbiology* **1998,** *144*(12), 3275-3287.
8. Long, T.; Or, D. Microbial growth on partially saturated rough surfaces: Simulations in idealized roughness networks. *Water Resour. Res.* **2007,** *43*(2), w02409.
9. Wang, G.; Or, D. Trophic interactions induce spatial self-organization of microbial consortia on rough surfaces. *Sci. Rep.* **2014,** *4*, 6757.
10. Van Genuchten, M. T. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. *Soil Sci. Soc. Am. J.* **1980,** *44* (5), 892-898.
11. Tuller, M.; Or, D. Retention of water in soil and the soil water characteristic curve. *Encyclopedia of Soils in the Environment* **2004,** *4*, 278-289.