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Short communication

Thiosulfate-related microbial communities from four arid soils in the Southwestern United States

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ABSTRACT

Sulfur-deficiency has been observed in arid and semi-arid soils. To explore sulfur cycling in arid soils, four (4) arid soils collected from the Southwestern United States were tested for 12 chemical constituents and moisture content, and soil microbial communities were assayed for the ability to utilize a set of 31 organic substrates using EcoPlates™. Soils were cultured for the presence of sulfur-utilizing bacteria using thiosulfate as the sulfur source. The densities of thiosulfate-utilizing bacterial populations were determined using viable plate counts and differential media. Results showed that the soils contained high amounts of sulfate, calcium and other minerals. Active soil microbial communities utilized 24–31 organic substrates. The density of thiosulfate-utilizing bacterial populations ranged from 10^5 colony forming units per gram (cfu g^{-1}) to greater than 10^6 cfu g^{-1}. Regression analyses showed that there were no statistically significant correlations between the density of thiosulfate-reducing bacteria and other tested variables. However, the high density of sulfur-related bacteria and high organic substrate utilization by the microorganisms suggests that these organisms may be important in sulfur and carbon cycling in these soils.

Factors in soil formation include parent material, climate, topography, biological activity and time (Miller and Spoolman, 2009; Paul, 2007). Microorganisms, including bacteria, fungi and protozoa contribute to soil fertility, and soils usually contain diverse microbial communities. Soil is a good habitat for microorganisms because microbial communities develop on the soil particles and non-arid soils usually contain high amounts of nutrients and moisture. Arid soils lack significant nutrients and moisture. For example, Ensminger (1958) reported that arid soils are often sulfur-deficient, especially if they have been used for agriculture. Therefore, arid soils may not be suitable for large populations of microorganisms and microbial activity may be limited due to variable soil conditions and mineral content.

Four arid soil samples were assessed qualitatively and quantitatively for chemical composition. Most contained a high amount of sulfur in the form of sulfate, possibly due to the weathering of the soils and the presence of gypsum (Paul, 2007). Due to the high concentrations of sulfate, sulfur microbial communities were targeted for further study and thiosulfate-utilizing bacteria were selected as representative sulfur bacteria. Thiosulfate-utilizing bacteria are less fastidious than other sulfur bacteria and able to grow in aerobic and anaerobic environments (Madigan and Martinko, 2006). We asked if samples with higher amounts of sulfate would contain higher densities of sulfur bacterial communities and whether sulfur bacteria activity and density changed relative to soil moisture and nutrient content.

Soil samples were collected in May 2009 from 4 sites in the Southwestern USA: Boulder City, Nevada [35° 58′ 43″N, 114° 49′ 57″W], Lake Mead, Nevada [36° 30′ 01″N, 114° 22′ 34″W], Deming, New Mexico [32° 16′ 07″N, 107° 45′ 31″W], and Kent, Texas [31° 11′ 23″N, 104° 12′ 27″W]. Samples were collected from the top 10 cm of soil using a small shovel and homogenized. The homogenized samples were transferred to sterile, air-tight, glass jars and kept on ice for transport to the laboratory. Samples were stored at 4 °C before culture and chemical analyses. Analyses were within 72 h of collection. Soils were extracted using sodium acetate adjusted to pH 4.8 with acetic acid and a commercial soil test kit was used to test for the presence of 12 chemical constituents according to the manufacturer's instructions (LaMotte Company, Chestertown MD). The chemicals tested were: NO_3^-, K, P, humus (organic C), Mg, Ca, Al, Cl^-, Fe^{3+}, NO_2^-, NH_4, and SO_4^{2-}. Soils were also tested for pH and moisture. Soil moisture content was determined by drying a 5 g

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sample of soil in a drying oven at 105 °C for 12 h and weighing the soil before and after drying (Atlas and Bartha, 1998; Paul, 2007). A 1 g sample of soil from each site was diluted in sterile saline and spread onto solid agar plate media with sodium thiosulfate (0.1 M final concentration). Thiosulfate was chosen because many types of microorganisms have been shown to metabolize this form of sulfur (Dilling and Cypionka, 1990; Dubinina et al., 2011; Magot et al., 1997; Ravot et al., 1995; others) and thiosulfate is considered a microbiologically important form of sulfur found in soil (Paul, 2007). Cultures were incubated anaerobically for 7 d until growth was observed. Density of the microbial community was estimated by multiplying the number of bacterial colonies growing on the plates times the dilution factor. Density was estimated as the average of triplicate culture experiment with standard error. Random bacteria from the thiosulfate plates were replica-plated onto Triple Sugar Iron (TSI) agar to determine if they could metabolize thiosulfate and produce a black phenotype indicative of the production of hydrogen sulfide (Hajna, 1945). Colonies that displayed the black phenotype were designated as TSI-positive (TSI+). Bacteria were gram-stained to determine the cell type and shape.

Suspensions of soil samples (1 g soil:49 ml of sterile distilled water) were prepared and inoculated into BIOLOG EcoPlates™ (BIOLOG Inc., Hayward CA) to profile the nutrient utilization of the total bacterial community. EcoPlates™ were 96-well plates that contained 31 carbon substrates in triplicate and water as a control. The substrates were: β-Methyl-D-glucoside, D-Galactonic Acid γ-Lactone, L-Arginine, Pyruvic Acid Methyl Ester, D-Xylose, D-Galacturonic Acid, L-Asparagine, Tween 40, i-Erythritol, 2-Hydroxy Benzoic Acid, L-Phenylalanine, Tween 80, D-Mannitol, 4-Hydroxy Benzoic Acid, L-Serine, α-Cyclodextrin, N-Acetyl-D-Glucosamine, γ-Hydroxybutyric Acid, L-Threonine, Glycogen, D-Glucosaminic Acid, Itaconic Acid, L-Glutamic Acid, D-Cellobiose, Glucose-1-Phosphate, α-Ketobutyric Acid, Phenylethylamine, α-D-Lactose, D,L-α-Glycerol Phosphate, D-Malic Acid, and Putrescine. Wells were impregnated with a dye that was activated when the microorganisms metabolized the substrate. The degree of metabolism was proportional to the dye's color intensity and was measured quantitatively using a plate reader (595 nm). EcoPlates™ were used to determine the substrate richness (total number of substrates used) and the total activity of the community. The total activity was the sum of the positive absorbance values taken from the plate reader; total activity was a unit-less measurement (Davelos et al., 2004). Soil samples were viewed with Scanning Electron Microscopy (SEM; Model Evo LS10, Zeiss, Peabody MA) to estimate the soil particle size. Linear regressions were used to compare bacterial density, chemical concentrations, and phenotypes. Regression data were analyzed statically by one-way ANOVA (sum of squares) at a significance of $\alpha = 0.05$ (SAS, 1985).

Based on collection site, particle size and texture, soils were categorized as silty aridisols (United States Department of Agriculture, 2010). The soil pH values ranged from 7.8 to 8 (data not shown) and the moisture content of the soils was ≤1.5% (Table 1). Humus was less than 1 ppm for 3 of the 4 soils; humus can account for 60–70% of organic carbon in soil (Atlas and Bartha, 1998). Our samples were taken from non-vegetated locations which may explain, in part, the low humus content (organic C). The most abundant chemical element, other than sulfur, was calcium (Table 1). Other soil chemical concentrations ranged from <1 ppm (ammonia; Deming, NM) to >400 ppm (potassium; Boulder City, NV and Kent, TX). Boulder City soil contained the highest concentration of sulfate, 2000 ppm (Table 1). It is important to note that the soil chemistry kit used was semi-quantitative. Soil chemical concentrations were within a range of values or the closest estimate of the concentration. That level of accuracy was suitable for our aims. We used the kit to estimate the concentration range of minerals and nutrients in the soils so that we could make comparisons amongst the 4 soil samples and predict the relationship between soil chemistry and the microbial community.

Table 1
Soil physical characteristics and chemical constituents from 4 soils in the Southwestern United States. Measurements were taken from composite samples; therefore, an error term could not be calculated.

Characteristic	Boulder City, NV	Lake Mead, NV	Deming, NM	Kent, TX
Soil Class	Aridisol	Aridisol	Aridisol	Aridisol
Texture	Silty	Silty	Silty	Silty
Average particle size (mm)	0.0021	0.0021	0.0056	0.0056
Moisture (%)	0.1	0.58	1.5	0.08
Sulfate (ppm)	2000	1000	500	50
Nitrate (ppm)	150	150	10	10
Nitrite (ppm)	1	*	*	1
Ammonia (ppm)	10	5	*	5
Potassium (ppm)	400	225	350	400
Phosphorus (ppm)	200	200	200	200
Magnesium (ppm)	25	80	150	25
Calcium (ppm)	2800	2800	1400	2800
Aluminum (ppm)	5	5	10	5
Chloride (ppm)	200	25	200	100
Ferric Iron (ppm)	7.5	7.5	7.5	7.5
Humus (organic C)	1	1	*	1.5

*Below detection.

Density of thiosulfate-utilizing bacteria was highest in the samples from Boulder City, NV and lowest in soils collected from Deming, NM (Table 2). EcoPlate™ assays showed that the soil communities' substrate richness ranged from 24 to 31 (out of 31 tested) organic substrates. Soil from Boulder City displayed high substrate utilization; however, the total activity of the community was the lowest among the 4 soil sites (Table 2). This suggests that Boulder City soils have dense populations of chemoorganotrophic bacteria that can possibly decompose many organic compounds but do not use any particular substrate preferentially or utilize any one substrate to a high degree. By contrast, microbial communities from Deming soils were the least dense and had the lowest number of utilized substrates. However, Deming soil communities displayed a total activity that was second highest among the soils and more than 1.5 times higher than Boulder City communities. Thus, despite using fewer substrates, communities from Deming soil could metabolize substrates better (Table 2). A subset (10–20%) of the thiosulfate-utilizing organisms from each site displayed the black phenotype (TSI+) when grown on triple sugar iron agar (Table 2). TSI+ organisms were typically small (<1 μm), gram-positive, rod-shaped cells with peritrichous flagella (not shown). The presence of flagella demonstrates that these organisms are motile despite the low amount of moisture in the soil. Furthermore, it is possible that the organisms may survive the dry conditions in the soil by forming endospores but we did not examine the organisms for spore formation.

Regression analyses showed that there were no statistically significant correlations between the density of thiosulfate-reducing bacteria and soil moisture (Fig. 1A), sulfate concentration (Fig. 1B), number of organic substrates used (Fig. 1C), total activity (Fig. 1D),

Table 2
Microbial profiles of sulfur-related communities from 4 soils in the Southwestern United States.

Soil site	Thiosulfate microbial density (cfu g^{-1} ± SE)	EcoPlate™ substrates utilized	EcoPlate™ total activity	TSI-positive
Boulder City, NV	$1.92 \times 10^7 \pm 9.60 \times 10^5$	31	11.476	20%
Lake Mead, NV	$7.33 \times 10^6 \pm 3.67 \times 10^5$	28	16.242	10%
Deming, NM	$3.80 \times 10^6 \pm 1.91 \times 10^5$	24	18.912	10%
Kent, TX	$7.73 \times 10^6 \pm 3.89 \times 10^5$	31	27.189	15%