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Static osmolyte concentrations in microbial biomass during seasonal drought in a California grassland

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ABSTRACT

Microbes cope with environmental changes by physiologically adapting to cues such as moisture and temperature. These responses happen at the molecular scale, but ultimately serve as controls on ecosystem level biogeochemical cycles. One example of this response is the accumulation of osmolytes, cellular compounds used to prevent desiccation under osmotic stress. We explored microbial biomass levels of nitrogenous osmolytes including amino acids glutamate and proline, and the tertiary amine glycine betaine, monthly in a seasonally dry California grassland using high performance liquid chromatography and either fluorescence or mass spectrometry-based detection. We also examined the behavior of amino acids in soil to analyze our ability to accurately measure putative osmolytes and determine contributions of microbial consumption and abiotic adsorption during extraction. We determined there was limited microbial consumption or abiotic adsorption of amino acids during extraction, and found no accumulation of expected nitrogenous osmolytes during the summer dry period with glutamate levels consistently 2–3% of the microbial biomass pool, and below detection limit levels of proline and glycine betaine. The lack of osmolyte use suggests the physiological response expected based on pure culture studies of individual microorganisms, and that of mixed soil consortia may be different due to a lack of sufficient osmotic stress or resource limitation in an environmental system.

1. Introduction

Allocating carbon (C) and nitrogen (N) resources to maintain or suspend metabolic activity in response to environmental stress is a universal biological phenomenon (Kultz, 2005). Soil microorganisms have evolved an array of metabolic modes to survive at a range of pH, temperature, salinity, and moisture conditions. The costs of shifting biochemical products from growth to survival under environmental stress can be extrapolated from microbial intracellular transformations, to ecosystem level balances of C and N (Schimel et al., 2007). Microbial resource allocation in dry ecosystems has potentially important biogeochemical consequences as 30% of the earth is classified as arid (Peel et al., 2007), and models of future climate scenarios predict increases in the length and severity of drought (IPCC, 2007).

As soils dry, the water potential outside of cells decreases relative to that within organisms, creating a gradient that should result in intracellular water loss. This difference in water potential is the result of both matric effects—increasing forces of adhesion and capillarity, and osmotic effects—increasing solute concentration (Papendick and Campbell, 1981). Microorganisms in soil have semipermeable membranes and lack the water impermeable layers common in plant roots (Clarkson, 1993); thus, their internal water potential must equilibrate with the external medium.

Although microbes cannot maintain a cross-membrane water potential gradient, they can adjust their internal solute concentration to reduce the gradient and retain cellular water thus allowing them to maintain physiological activity. Microbial cells accumulate internal solutes by importing or synthesizing compounds to balance the water potential difference when grown in a high salt medium designed to induce osmotic stress (~200–700 mM) (Bonaterra et al., 2005; D'Souza-Ault et al., 1993; Killham and Firestone, 1984b; Sagot et al., 2010). These compounds are termed compatible solutes, or osmolytes, and are typically low molecular weight molecules that do not interfere with cellular processes. Bacteria tend to use nitrogenous osmolytes such as amino acids (glutamate and proline) and tertiary amines (e.g. glycine betaine) while fungi typically use sugar alcohols (e.g mannitol, trehalose) for survival (Csonka, 1989).

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While it is clear that microbes can induce production of compatible solutes to combat water loss and maintain activity in the face of external osmotic stress in culture-based studies, it is not known if microbes can do so in a drying soil. To induce production of osmolytes as soils dry would require substantial resources at a time when diffusion is becoming constrained (Stark and Firestone, 1995), which limits resource availability. Additionally, if microbes manufacture osmolytes in response to stress induced by drought, they must dispose of them quickly when soils rewet; either via respiration, polymerization, or export; otherwise they could burst due to excessive water movement into the cell (Csonka, 1989). Such processes have been argued as the basis for the "Birch effect"—the pulse of respiration when a dry soil is rewet (Kieft et al., 1987; Fierer and Schimel, 2003). Given the amount of osmolyte production in culture-based studies, the ecosystem consequences of production and subsequent loss would be significant, potentially consuming 3–6% of net primary production and accounting for 10–40% of annual net N mineralization in grasslands (Schimel et al., 2007). Alternatively, microbes may balance soil and cellular water potential in dry periods by constitutively producing osmolytes regardless of soil water content (Fig. 1). In either scenario, compatible solutes should account for a substantial proportion of the microbial biomass under dry conditions if they are using an osmotic response. Another of many possible alternatives would be that cells do not produce osmolytes under these conditions, and instead partially dehydrate during drought.

We determined if the microbial community uses osmolytes *in situ* to balance cellular water potential under environmental drought stress, and whether any such accumulation accounts for significant amounts of C and N in terms of ecosystem fluxes of these elements. We quantified osmotic compounds within microbial biomass monthly over two years in a seasonally dry CA grassland. Bacteria are the dominant microbial group in soils from the field site used in the study (Fierer et al., 2003). Therefore, we expected to find a relationship between the accumulation of the nitrogenous osmolytes glutamate, proline and glycine betaine within microbial biomass at low water content in the dry summer months. This is the first *in situ* study to examine the theory of osmolyte production as a microbial defense to water stress in soil ecosystems. Using these data we sought to determine the influence of osmolyte production on ecosystem level C and N cycling in response to seasonal drought.

2. Materials and methods

2.1. Soil collection and analyses

The study site is located at the University of California Sedgwick Reserve (34°41.60′ N; 119°43.57′ W) in the lower part of the Figueroa Creek drainage. It has a Mediterranean climate with hot, dry summers, and cool, wet winters. Mean annual precipitation is 38 cm, although it is highly variable, falling primarily between October and April. Vegetation is characterized by Mediterranean annual grasses including *Bromus diandrus*, *Bromus hordaceous*, and *Avena fatua*. Soils from our collection site are Pachic Argixerolls in the Salinas series (Gessler et al., 2000), texture is a sandy clay loam (51% sand, 27% silt, 22% clay), saturation percentage (θ_s) is 46%, pH is 6.45 (saturated paste), electrical conductivity (ECe) is 0.28 dS m^{-1} (saturated paste) determined at UC Davis soil analysis laboratory. Calculated bulk density based on soil texture is 1.4 g cm^{-1} (Saxton et al., 1986) and was used to calculate volumetric water content (θ).

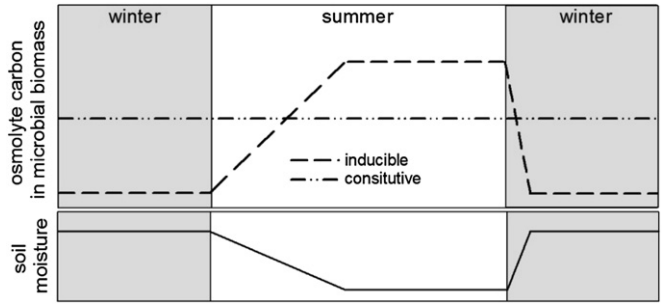

Fig. 1. Conceptual diagram illustrating the theoretical relationship between soil moisture and osmolyte production.

Soils were sampled (top 10 cm, 4 replicates) using a 5 cm diameter corer approximately monthly from June 2007 to August 2009. Cores were transferred to the laboratory and either processed immediately or stored at 10 °C and processed within 48 h. Cores were broken up by hand; rocks, plant material and fine roots were removed, and soil was passed through a 4 mm sieve.

Cytoplasmic constituents of soil microbes were extracted by lysing cells with 1% chloroform soil slurry (fumigated), and background soil organic materials were extracted in parallel with water (unfumigated) (Fierer et al., 2003). The chloroform slurry method rather than gas fumigation was employed so that adjusting the soil water content was not required to reach equal fumigation efficiency in seasonal samples with variable water content. All extractions were performed using 10 g soil (wet weight) and 40 mL extractant in 125 mL glass Erlenmeyer flasks that were acid-washed and ashed prior to use. Unfumigated extracts were mixed on a rotary shaker for 2 h and fumigated extracts were mixed for 4 h. Following shaking, soil suspensions were separated by vacuum filtration (glass fiber filters, 1 μm nominal pore size), collected, and stored at −20 °C until analysis. Subsamples were used for soil moisture; gravimetric water content was calculated by oven drying to constant weight at 80 °C.

2.2. Chemical characterization of osmolytes

The extractable organic carbon (EOC) from the unfumigated extract, and the combined EOC and cytoplasmic constituents released in fumigated extracts were measured using persulfate digestion (Doyle et al., 2004). The microbial biomass carbon (MBC) flush equates to the difference between the fumigated and unfumigated extracts and is not corrected for fumigation efficiency. Amino acids in both the EOC and MBC pools were quantified with the Waters AccQ-Tag system. The AccQ-Fluor reagent adds a fluorescent tag to primary and secondary nitrogens, facilitating detection following chromatographic separation. The AccQ-Fluor reagent was used to derivatize 40 μL of the fumigated and unfumigated H_2O extracts; amino acids were then separated using an HPLC system equipped with a fluorescence detector. Amino acids were quantified using a standard amino acid mixture derivatized in the same fashion. A subset of the samples at the lowest summer and peak winter soil water content were analyzed for glycine betaine (GB); they were freeze-dried and suspended in 50:50 H_2O:acetonitrile. Samples were centrifuged at 10,000*g* for 10 min, supernatant was analyzed on an HPLC equipped with a 3 μm silica HILIC column (4.6 × 100 mm) using isocratic separation (75:25 acetonitrile:H_2O with 10 mM ammonium formate) and time of flight mass spectrometry (TOF-MS) detection. Glycine betaine standards were run with analytical samples for quantification. Fluorescence detection could not be used for glycine betaine due to the tertiary N, precluding the use of the AccQ-Fluor reagent.

2.3. Addition experiment

In addition to the characterization of the osmolyte pool we wanted to test the effectiveness of our method for quantifying