Soil Biology & Biochemistry 57 (2013) 644–653

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Microbial community response to varying magnitudes of desiccation in soil: A test of the osmolyte accumulation hypothesis

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ARTICLE INFO

Article history:
Received 19 March 2012
Received in revised form
9 August 2012
Accepted 13 August 2012
Available online 10 September 2012

Keywords:
Osmolytes
Compatible solutes
Matric water potential
Soil
EPS
GC–MS

ABSTRACT

Numerous studies have observed the physiological responses of soil microorganisms to water stress caused by soil drying, however, only a few have attempted to assess the microbial response in soil *in situ*. An experiment was conducted to analyze the change in extractable metabolites, particularly sugars and amino acids, in soil and the associated microbial community at various intensities of soil desiccation. Water potential was manipulated in two soils, Marietta and Sumter, representing relatively moist and drought-prone water regimes, respectively. The matric potential of the soils was maintained relatively moist at −0.03 MPa or lowered to −1.5, −4.5, −10, −20 and −40 MPa by air drying over ~3 days. We hypothesized that microbial communities inhabiting the drought-prone Sumter would accumulate more osmolytes, and that the soil with a relatively moist water regime, the Marietta, may have communities less adaptable to water stress, have fewer osmolytes, and show evidence for greater microbial turnover and death. However, there was no evidence that the soils responded to drying by accumulating osmolytes or that there was greater microbial turnover and death related to soil type. Microbial community structure did change with drying, however, with greater fungal-to-bacterial biomass in the Sumter but not in Marietta soil. A significant increase of ~10–25% in phenol sulfuric acid analyzable sugars (PSA-sugars) at intermediate levels (−4.5 MPa) of drying was observed compared to dryer and more moist conditions. However, the GC–MS derived quantities of polyols (glucitol, inositol and xylitol), sugars, and amino acids showed few strong and consistent patterns with level of desiccation. These results provide some of the first evidence that microbial communities in soil *in situ* do not strongly rely on these basic osmolytes to cope with typical soil water deficits. In natural soils, we propose that microbial communities respond differently to water deficits perhaps through re-allocation of C to cell wall mucilage, exopolysaccharides (EPS), and phospholipids, than organisms in culture, perhaps a consequence of low energy and limiting supplies of N.

1. Introduction

The fluctuations in soil water potential caused by episodic dry–rewet events in terrestrial ecosystems exert physiological and energetic challenges to microbial communities. Extremely large fluxes of C, important to the global C cycle, have also been linked to soil drying and re-wetting in ecosystems (Birch, 1958; Fierer and Schimel, 2002). Maintenance of cell turgor, which is vital to microbial cell growth and survival, is strongly regulated by extracellular water dynamics (Bremer and Krämer, 2000; Schimel et al., 2007). Numerous hypotheses have emerged on the adaptation strategies that soil microorganisms utilize to cope with declining and low water potentials. Perhaps the most common hypothesis, supported by observations of soil C flux and microbial biomass dynamics, is that microbial cells accumulate and release intracellular osmolytes to rapidly respond to water dynamics (Brown, 1976; Harris, 1981; Miller and Wood, 1996; Mikha et al., 2005).

Microorganisms exposed to low osmotic potentials in laboratory culture have been shown to accumulate inorganic and/or organic osmolytes in their cytoplasm to maintain cell turgor. The osmolytes include K^+ ions and a group of organic solutes like glutamate, proline, peptides, N-acetylated amino acids (amino acids and their derivatives), sucrose, trehalose (carbohydrates), polyols, glycine betaine, carnitine (quaternary amines) and tetrahydropyramidines like ectoines (Killham and Firestone, 1984; Csonka, 1989; Blomberg and Adler, 1992; Galinski and Truper, 1994; Kempf and Bremer, 1998; Poolman and Glaasker, 1998) rich in C and N. Among these

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http://dx.doi.org/10.1016/j.soilbio.2012.08.014

organic solutes, fungi tend to accumulate polyols whereas bacteria utilize amino acids and sugars to cope with water deficit (Brown, 1976). When exposed to hypo-osmotic conditions, microorganisms expel the accumulated solutes extracellularly to maintain equilibrium (Tschichholz and Trüper, 1990; Halverson et al., 2000).

The sudden flush of C and N mineralization following the rewetting of dry soil has been reported by many researchers and more recently was hypothesized as representing microbial release of intracellular osmolytes (Birch, 1958; Sorensen, 1974; Schimel et al., 1999; Franzluebbers et al., 2000; Chowdhury et al., 2011). Although isotopic studies have revealed that at least part of the C released during the short-term pulse following the rewetting of dry soil is microbial in origin (Kieft et al., 1987; Van Gestel et al., 1992; Magid et al., 1999), it is unclear whether microbial cell lysis, the regulated expulsion of intracellular microbial osmolytes, or other mechanisms are responsible for the pulse.

The response of soil microorganisms to low water potential has been studied by exposing soil isolates to salt induced hyperosmotic stress and through desiccation on a simulated soil matrix (Killham and Firestone, 1984, 1984b; Schimel et al., 1989; Roberson and Firestone, 1992; Halverson et al., 2000). Several of these studies observed increased levels of amino acids, sugars and the accumulation of extracellular polysaccharides in response to declining water potential. Enhanced pools of cytoplasmic C and N content with water potential deficit were also reported. However, to our knowledge no study has attempted to directly measure the pool of soil microbial compatible solutes in response to drying under *in situ* soil conditions. Laboratory cultures are so vastly different from soil conditions that studies under these latter scenarios are greatly needed to understand microbial adaptations to water stress and the controls of soil C and nutrient dynamics in ecosystems.

An experiment was designed to test the physiological and structural response of soil microbial communities to drying across a water deficit gradient using two soils that developed within contrasting water regimes. The first and primary objective was to determine if microorganisms adapt to soil drying by accumulating compatible solutes. It was hypothesized that as the soils were dried, greater amounts of organic osmolytes would be detected. We expected an increase in fungal polyols, and bacterial derived sugars and amino acids in response to water deficit. The second objective was to assess whether microbial communities in two different soil types with contrasting water regime histories would respond differently to drying and whether changes in fungal to bacterial ratios would coincide with the composition of fungal and bacterial osmolyte pools. It was hypothesized that microbial communities inhabiting the drought-prone soil would accumulate more osmolytes, and that the soil with a relatively moist water regime may have communities less adaptable to water stress, have fewer osmolytes, and show evidence for greater microbial turnover and death.

2. Materials and methods

2.1. Site description

The experiment was conducted on two soils, the wetter lowland Marietta and dryer upland Sumter series located near Mississippi State University, Mississippi, USA (33° 28′ N and 088° 47′ W) in Fall 2009. These soils have been previously sampled across a 5 county area in northern Mississippi and were shown to have similar pH, texture, color, and hue as predicted by the soil type. For the purposes of the work in this paper, a 100-m transect across a 2–5 Ha forest was used within each of the respective soil types to collect soil to a 10-cm depth. Five-kg of soil was collected every 20-m from within a central location of each forest. At sampling, the soils were relatively moist with water content of 34–36% (~−150 kPa). The soils were sieved to 4 mm and thoroughly cleaned of obvious plant litter and rocks, and refrigerated at 4 °C until use.

Rainfall across the area, for both soil types, averages 51 inches and the mean annual temperature is 17.7 °C. The Marietta soils are (fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts) derived from deep alluvial deposits near streams in the blackland prairie region of Mississippi. These soils drain areas of the mixed uplands of the Southern Coastal Plain. The soils are subject to frequent flooding. Mottles and stains starting at the depth of 10-cm and shallow water table are indicators of the generally moist water status of these soils. The site was forested with >50-y old deciduous vegetation dominated by *Carya illinoinensis*. The C:N content of the Marietta soils was 2.35% and 0.17% respectively with pH of 6.2. The Sumter soil is silty clay, with medium granular structure, moderately deep, well drained, with rapid runoff. These upland soils were formed in marly clays and chalk of the blackland prairies (fine-silty, carbonatic, thermic Rendollic Eutrudepts) and drain to lowlands (e.g. Marietta). The water table is deep and the permeability of the soil is slow. The soil has a pH of 6.3 with C and N content of 2.56 and 0.15% respectively. Total soil organic C and N contents were measured on a Vario MAX CNS macro elemental analyzer (Elementar Americas, Inc., Mt. Laurel, NJ). Soil pH was measured after shaking the soil with 0.01 M $CaCl_2$ (1:1, mass: volume) suspension for 30 min.

2.2. Experimental setup

A laboratory experiment was conducted to study the physiological response of soil microbial communities to water potential deficits (matric) caused by air drying. Keeping in view of the textural differences in the soil types and their water holding capacities, we used water potential to measure the drying effect on the soil microbial communities. Each treatment (soil, $n = 2$; water potential, $n = 6$) was replicated three times, thus resulting in a total of 36 separate samples. Each sample consisted of 10 g (dry weight) of well homogenized soil weighed into 150 ml volume specimen cups. This experimental setup was repeated 5× to have enough material for all the analysis (e.g. Phospholipid fatty acids (PLFA), osmolytes, respiration, microbial C, N and soluble C). Keeping the soil mass low within each specimen cup also allowed for control and homogeneity of the soil drying process. The water content of all the samples was adjusted to their respective field capacities (−0.03 MPa) by adding sterile distilled water. Soils were pre-incubated at room temperature (22 °C) for five days to reduce disturbance effects related to sampling, sieving, and storage.

The pre-incubated soils were either maintained moist (−0.03 MPa) or slowly air dried to five different water potentials of −1.5, −4.5, −10, −20, and −40 MPa at 22 °C. For three consecutive days, soils were dried for 6–12 h each day until reaching target water potential. The soils took approximately 16, 22, 29, 33 and 36 drying hours to reach the water potentials of −1.5 MPa, −4.5 MPa, −10 MPa, −20 MPa, −40 MPa, respectively. The relation between the soil water content and water potential were analyzed prior to the experiment by filter paper method (McInnes et al., 1994). As well, the water potentials of the soils were cross-validated and monitored using a WP4 dewpoint potentiameter by (Decagon, Inc., Pullman, WA).

The soils remained at respective water potentials for 24 h before any further analysis. PLFA analysis was done to understand the structural and physiological changes in the community with drying. The respective soil samples were stored at −80 °C until analysis. All the other extractions except for the PLFAs were done the following day. Microbial osmolytes are known to comprise