Soil Biology & Biochemistry 79 (2014) 14–24

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Osmolyte dynamics and microbial communities vary in response to osmotic more than matric water deficit gradients in two soils

Madhavi L. Kakumanu, Mark A. Williams*

College of Agriculture and Life Sciences, 301 Latham Hall, Virginia Polytechnic and State University, Blacksburg, VA 24060, USA

ARTICLE INFO

Article history:
Received 24 August 2013
Received in revised form
9 August 2014
Accepted 16 August 2014
Available online 28 August 2014

Keywords:
Osmolyte
Matric and osmotic water potential deficit in soil
Alcohols
Sugars
Amino acids
Microbial community PLFA
Respiration
Ninhydrin

ABSTRACT

Sodium chloride and other osmotically active molecules are often applied to microbial cultures or soils to describe microbial responses and adaptation to desiccation in soils. However, salts and other osmolytes may have different effects on microorganisms than matric deficits caused by soil drying. It was thus hypothesized that low matric and osmotic potentials would have different effects on soil microbial communities; and that salt (osmotic) treatments would induce greater mol% change in metabolites than drying (matric). To test this, an experiment was conducted with two soils, a lowland Marietta and an upland Sumter, and exposing them to different levels (−0.03 MPa, −1.5 MPa, −4.5 MPa and −10 MPa) of matric and osmotic potential deficit. The physiological and structural response of soil microbial communities across the water deficit gradient was measured by analyzing the metabolites and phospholipid fatty acids (PLFA), respectively. As hypothesized the matric and osmotic deficits altered the physiology and structure of microbial communities in two soils, however, osmotic induced more change than matric water potential. The mol% of metabolites shifted more in Marietta than the drought-prone Sumter, driven by greater turanose and fructose with degree of osmotic water potential deficit, respectively. Declining matric water potential was associated with inositol and glucitol, respectively, in Marietta and Sumter. The shifts in the metabolite concentration in osmotic treatments resembled osmotic changes often reported in microbial culture. Thus the experiments in soil or in cultures that use osmotic (salt) effects to predict the soil microbial response to matric deficit in soils may not accurately reflect the microbial community response. It is likely that soil microbes use different mechanisms to adapt to salt and matric stresses.

1. Introduction

Drying and rewetting induced soil water potential fluctuations result in numerous physiological challenges on soil microbial communities. It has been hypothesized that microbial communities in soil cope with extreme variations in soil water potential in ways similar to microorganisms in laboratory cultures (Killham and Firestone, 1984b; Kieft, 1987; Van Gestel et al., 1993b; Fierer and Schimel, 2003). Cultured microbes cope with low water potential by accumulating large quantities of compatible solutes, such as glycerol, mannitol, proline, and glutamine (Csonka, 1989; Welsh and Herbert, 1993; Galinski and Trüper, 1994; Halverson et al., 2000; Welsh, 2000). Consistent with osmolyte accumulation during soil drying, microbial biomass often increases in response to water deficit (Halverson et al., 2000; Schimel et al., 2010). Large flushes of soluble organics have also been shown to flood the water-soluble soil pool following re-wetting, also in support of a microbial release of osmolytes during dilution stress (Miller et al., 2005; Williams and Xia, 2009).

Recent studies have called the microbial osmolyte model in soils into question (Boot et al., 2013; Kakumanu et al., 2013). Neither microbial nor soluble pools were observed to contain the osmolytes in quantities that could account for microbial adaptation to water deficit. Indeed, the quantities of specific sugars (e.g. glucose), alcohols (e.g. glycerol), and amino acids (e.g. proline) generally accounted for less than 5% of the water soluble soil organics and less than 1% of the microbial biomass (Kakumanu et al., 2013). Though it cannot be ruled out that a small active microbial biomass in soil utilizes osmolytes to adapt to drying and re-wetting stresses, the broad application of the microbial osmolyte model may not apply to soil microbial communities.

The conditions in cultures do not accurately reflect those in soils and thus may help to explain differences observed between these two habitat types. For instance, the production of osmolytes during

* Corresponding author. Tel.: +1 540 231 2547; fax: +1 540 231 3083.
E-mail address: markwill@vt.edu (M.A. Williams).

http://dx.doi.org/10.1016/j.soilbio.2014.08.015
0038-0717/

low water potential has been shown to be highly regulated by the presence of precursor molecules and nutrients (Sleator and Hill, 2002). As such, soil habitats which are typically low in available C and N may not support the biological expenses and energy needed for osmolyte accumulation; and thus require different mechanisms of microbial adaptation to water deficit.

Water potential deficits in soil are typically imposed by soil (matric) drying, and it is thus important to determine how matric may differ or be similar to those studies that have informed water stress theory but have overwhelmingly emphasized osmotic rather than matric water deficit. Matric and osmotic stress differ in many ways that might impact microbial response. For instance, the presence of very low quantities of water in soils during desiccation restricts the diffusion of molecules and limits microbial mobility toward substrates (Potts, 1994; Chang et al., 2007). The effect of matric deficit could thus lower the availability of nutrient and energy pools compared to osmotic deficits at similar water potentials. The restriction in carbon flow may thus hamper microbial osmolyte accumulation during soil drying.

The primary objective of the research was to assess whether the osmotic and matric induced water deficits cause comparable metabolite and structural dynamics in soil microbial communities. It was hypothesized that compared to matric, osmotic potential deficit would induce greater change in the mol % of osmolytes, increase osmolyte concentration, and alter microbial community structure and physiology. This hypothesis is supported by observations that in soil, osmolyte pools tend to be stable and microbial community structure is often resilient to large change in response to soil drying (Boot et al., 2013; Kakumanu et al., 2013; Warren, 2014). In contrast, osmolytes accumulate and change in composition when microbes are exposed to salts in culture. Simultaneously, we also determined the effect of soil type and intensity of the water potential deficit on microbial community physiology and structure. It was hypothesized that metabolite pools and community structure would change and that two soils would have different metabolite dynamics in response to the two types of water potential change.

2. Materials and methods

2.1. Site description and soil collection

The experiment was conducted on two soils, the Marietta and Sumter located near Mississippi State University, Mississippi, USA (33° 28′ N and 088° 47′ W) in fall 2009 (Kakumanu et al., 2013). Briefly, the Marietta soil is fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts derived from deep alluvial deposits near streams in the black land prairie region of Mississippi. Marietta soils are located in the drainage areas of the mixed uplands of the Southern Coastal Plain and subjected to frequent flooding. Mottles and stains starting at the depth of 10 cm are one primary indicator of the generally moist water status of this soil. The C, N content of the Marietta soil is 2.4% and 0.17% respectively with pH of 6.2.

The Sumter soil is a carbonatic, thermic Rendollic Eutrudept, silty clayformed in marly clays and chalk of the black land prairies. It is moderately deep, well drained, upland with medium granular structure and rapid runoff. The water table is deep and the permeability of the soil is slow. The Sumter soil has pH of 6.3 with C and N content of 2.6% and 0.15% respectively. The rainfall across the area averages 1300 mm and the mean annual temperature is 17.7 °C.

Top soil from ~10 cm depth was collected at 0, 50, and 100-m from three locations along a 100-m transect at each of the 2–5 Ha forested soil types. At sampling, the soils were relatively moist (34–36% w/w; ~−150 KPa). The soils were sieved through 4 mm mesh, thoroughly cleaned of obvious plant litter and rocks, and stored at 4 °C. 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 and structural response of soil microbial communities to matric (air drying) and osmotic (salt addition) potential deficits. Both osmotic and matric water potentials of the soils were adjusted, incrementally, over a period of 3 days. This was done to simulate the natural change in water potential during soil drying, and to aide comparison between osmotic and matric deficit. The experiment was a 3-way factorial design, with two soils, Marietta and Sumter, two forms of water deficit, matric and osmotic, and three water potential deficits of −1.5, −4.5 and −10 MPa and one moist (no water deficit) control (−0.03 MPa). Each treatment combination was conducted on three independently collected field replicates across a 100-m transect of the representative soil series (Total = 42 samples).

Approximately 10 g (dry weight) of homogenized soil was weighed into individual 150 ml volume (measured to the nearest ml) specimen cups. These conditions resulted in a relatively thin 1 cm layer of soil spread evenly across the bottom of the cup to provide control and reduce the heterogeneity of the drying process. This resulted in the need to have 3 repetitions of each replicate treatment (42 × 3) to provide sample for analyses.

The water content of all samples were first adjusted to their respective field capacities (−0.03 MPa) using sterile distilled water and incubated at room temperature (22 °C) for 5 days. The pre-incubation following the small adjustment of water in soil served the purpose of reducing disturbance effects related to sampling, sieving, and storage.

After 5 days of pre-incubation, the soil samples were randomly assigned to treatments. The soils under matric treatments were slowly air dried at 22 °C for 6–12 h each day. When the targeted water potential (−1.5, −4.5 or −10 MPa) for a treatment was reached, those samples were covered to stop water loss (Kakumanu et al., 2013). Remaining soils continued to dry until reaching their target water potential, with the lowest water potential achieved after ~3 days.

For the soil samples amended with the salt (osmotic) treatment, preliminary experiments were conducted to find the best method for lowering the soil osmotic potential in a stepwise and homogenous way. Incremental additions of salt solution allowed the soil and biological communities to adjust slowly to changing solution concentrations that mimicked changes in water potential during soil drying. The water potential of the NaCl solutions were confirmed and consisted of 3–4 separate daily additions of NaCl solution until the soils reached their target osmotic water potential. In practice, step-wise additions of 25 μl were added to the soils until 200 μl of 1 M solution had been homogeneously amended. The water additions resulted in an increase in soil water content of ~5%, after which time the soil was allowed to equilibrate and slowly lose the 0.2 ml of added water over 2–3 h. On the second and third days, 3 M solutions were used to decrease soil osmotic potential from −4.5 to −10 MPa. After equilibration of each salt solution and before the addition of the subsequent solutions, separate samples were taken to confirm the target water potential using a WP4 dewpoint potentiameter (Decagon, Inc., Pullman, WA). Overall, 58.5 mg, 117 mg and 234 mg of NaCl was added to 10 g (dry weight) of Marietta soils and 67.2 mg, 131.6 mg and 257.2 mg NaCl to 10 g of