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

Madhavi L. Kakumanu, Charles L. Cantrell, Mark A. Williams

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 $\sim 3$ 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 $\sim 10$–$25\%$ in phenol sulfuric acid analyzable sugars (PSAs) at intermediate levels ($\sim 4.5$ 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 soil 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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- Compatible solutes
- Matric water potential
- Soil
- EPS
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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 (Tschiccholz 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; Sørensen, 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 contents 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 osmo-lytes, and that the soil with a relatively moist water regime may have communities less adaptable to water stress, have fewer osmo-lytes, 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 Caria 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 Rendolic 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 CaCl₂ (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 potentiometer 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
amino acids (e.g. proline, glutamic acid), sugars (e.g. sucrose, trehalose) and polyols (e.g. sorbitol, mannitol). The physiological and biomass responses of microbial communities were assessed by ninhydrin analysis (amino acids) and phenol sulfuric acid (PSA) analysis for sugars. Metabolite composition was analyzed using GC–MS.

2.3. Extraction of metabolites from soil and associated microorganisms

Microbial metabolites from soil were extracted using a mixture of chloroform and 0.01 M K₂SO₄ (1:4; v/v). Soluble organics were extracted using 0.01 M K₂SO₄ only. The principle behind the extraction of microbial metabolites was based on chloroform lysates of microbial cells and the consequent release and dissolution of intracellular cytosol into the 0.01 M K₂SO₄. We utilized the chloroform slurry method for extraction of microbial metabolites instead of a traditional fumigation–extraction assay for two reasons. First, fumigation of dried soils has been shown to give erratic results (Sparling and West, 1989) that would then make it reasons. First, fumigation of dried soils has been shown to give erratic results (Sparling and West, 1989) that would then make it difficult to compare between drying treatments. Second, the activity of hydrolytic enzymes on proteins and polysaccharides in soils during fumigation (Renella et al., 2002) would result in the contamination of the cytosolic pool of organics and exaggerate concentrations of sugars and amino acids.

For microbial metabolite extraction, 10 ml chloroform was added to 10 g (dry weight) of soil that was transferred to separate 160 ml serum bottle. After one minute, the K₂SO₄ solution was added to bring each bottle up to 40 ml of 0.01 M K₂SO₄ and vigorously agitated for 2 h (250 rpm) on an orbital shaker. Soluble organics were extracted identically, but without chloroform. To separate the chloroform and aqueous phase, the samples were allowed to settle for 15 min and then centrifuged at 1500 rpm for 10 min on IEC bucket centrifuge. The aqueous supernatant was taken and filtered through whatman #1 filter paper and the solution was lyophilized and stored at −80 °C until further analysis.

For measuring microbial and soluble C and N concentrations, soil samples were extracted, filtered, and analyzed on a Shimadzu TOC analyzer. Microbial C and N were determined by subtracting soluble C and N values from chloroform-K₂SO₄ extracts. Unlike the fumigation method (Vance et al., 1987) no correction factor was applied to the values.

2.4. Analysis of soil extracts by colorimetric methods

Reducing sugars in the soil extracts were analyzed by the PSA method (Dubois et al., 1956). Briefly, 50 μl of 80% phenol solution followed by addition 5 ml of concentrated H₂SO₄ (~ 18 M) solution was added to a small volume of soil extract. The mixture was allowed to stand at room temperature for 45 min. The absorbance was measured at 480 nm on UV spectrometer. Absorbance values were used to calculate the concentration of reducing sugars based on an 8-point standard curve of glucose.

Amino acids were determined using the ninhydrin method (Stevenson, 1982). Though ninhydrin also reacts with peptides, proteins, ammonium and other compounds with free α-amino groups it is generally considered sensitive and useful for quantification of amino acids (Jorgenson and Brooks, 1990). The soil extracts along with 0.5 ml of citric acid and 2 ml of ninhydrin reagent were incubated at 100 °C for 25 min. The solution was cooled, diluted with 5 ml of 50% ethanol, and the absorbance was measured at 570 nm on UV spectrometer. A standard curve was made by measuring the absorbance at different concentrations of L-Leucine-N.

2.5. Analysis of extractable metabolites by Gas Chromatography–Mass Spectroscopy

The sugars and amino acids in the extracts were characterized using GC–MS. Prior to analysis the samples were derivatized. Derivatization was carried out in 1 ml reaction vials treated with 5% dimethyl dichlorosilane in toluene. For analysis of sugars, the soil extracts were derivatized by N, O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) solution. Approximately 250 μl of the aliquots of soil extract were taken in silylated reaction vials and dried completely under nitrogen. The dried residues were converted to trimethylsilyl derivatives by adding BSTFA containing 1% trimethylchlorosilane (TMCS) and pyridine in 2:1 ratio and incubating them for 3 h at 70 °C (Medeiros et al., 2006). The samples were allowed to stay overnight at room temperature and completely dried under pure nitrogen. The dried residues were redissolved in 110 μl of hexane and sent for GC analysis.

Derivatization of amino acids was done as per the method given by Fan (1996). Approximately 500 μl of soil extracts were taken and the pH was lowered to 2 by adding equal volumes 1 M HCl in reaction vials. The solution was dried completely under pure nitrogen. Dried extracts were sonicated with 1:1 mixture of N-Methyl-N-(Tert-Butyldimethylsilyl) trifluoroacetamide (MTBSTFA) and acetonitrile for 2 h at 60 °C. The solution was left at room temperature overnight and dried under nitrogen. The derivatized extracts were redissolved in 110 μl of hexane before GC analysis.

The samples were analyzed on Varian CP-3800 Gas Chromatograph coupled to a Varian Saturn 2000 MS/MS. The GC was equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm, with film thickness of 0.25 nm) operated using the following conditions: injector temperature, 240 °C; column temperature, 60–280 °C at 8 °C/min then held at 280 °C for 5 min; carrier gas, He; injection volume, 1 mL (splitless). The MS was mass ranged from 40 to 650 m/z, filament delay of 3 min, target TIC of 20,000, a prescan ionization time of 100 μs, an ion trap temperature of 150 °C, manifold temperature of 60 °C, and a transfer line temperature of 170 °C. Individual sugars were identified by comparison of mass spectra with literature and library data, comparison of mass spectra and GC retention times with those authentic standards and/or interpretation of mass spectrometric fragmentation patterns. Standard solutions of glucose and other compounds like trehalose, sorbitol, sucrose, proline, glutamine which are commonly expected as microbial osmolytes, were analyzed (Williams and Xia, 2009). Compounds were quantified using total ion current (TIC) peak area and converted to compound mass using calibration curves of the external standards (glucose for monosaccharides, sorbitol for sugar alcohols and sucrose for disaccharides).

2.6. PLFA analysis

Total lipids were extracted according to the procedure of White and Ringelberg (1998) as modified by Butler et al. (2003). Briefly, 10 g (dry weight) of soil samples from 3 replications of all the treatments were transferred to sterilized 160 ml serum bottles. The soils were extracted overnight using a mixture of 50 mM phosphate buffer (pH 7.1), chloroform and methanol (0.8:1:2). The samples were centrifuged the following day at 1000 rpm for 5 min and filtered using Whatman # 1 filter paper. The filtrate was added with 3 M NaCl solution and a pinch of Na₂SO₄ salt and allowed to stand for ~8 h for phase separation. The chloroform phase was collected into separate glass tubes and dried completely under stream of nitrogen. Dried lipids were redissolved and fractionated into neutral, glyco- and phospholipids using sillicic acid bonded phase extraction columns (Supelco, cat. No. 505048). The neutral and glyco lipids were eluted using chloroform and acetone respectively.
Phospholipids were finally eluted with methanol into separate tubes and completely dried under nitrogen. The dried phospholipid fraction was transformed into fatty acids methyl esters under alkaline conditions and extracted twice in 1:4 chloroform—hexane solution. The chloroform—hexane mixture was completely evaporated under stream of ultra high purity 99.999% N₂ gas and the residue was resuspended in 500 μl of hexane for GC analysis.

The fatty acids were quantified and detected on Agilent 6890 Series gas chromatograph (Santa Clara, CA) equipped with a flame ionization detector, an Ultra-2 column (19091B-102; 0.2 mm by 25 m), and controlled by a computer loaded with ChemStation and Sherlock software. Ultra high purity H₂ was the carrier gas at a column head pressure of 20 kPa, septum purge of 5 ml min⁻¹, a split ratio of 40:1, injection temperature of 300 °C, injection volume of 2 μl. The oven temperature ramps from 170 °C to 288 °C at 28 °C min⁻1 and the analysis time of each sample was 6 min. Peak identification was carried out by the Microbial Identification System (MIDI, Inc.) following calibration with a standard mixture of 17 fatty acid methyl esters (1300A calibration mix).

The PLFA’s i15:0, a15:0, i16:0, a16:0, i17:0, a17:0 (gram-positive), 16:1ω9c, 16:1ω7c, 18:1ω7c and cy19:0 (gram-negative) were considered as bacterial biomarkers, 10:Me 16:0 and 10:Me 18:0 for actinomycetes and 18:2ω6c as fungal biomarkers (Frostegård and Bååth, 1996; Zhang et al., 2005; Liang et al., 2008). The ratio of fungal to bacteria biomarker fatty acids were used to indicate change in the fungal to bacterial biomass ratio (Bossio et al., 1998).

2.7. Statistical analysis

Two-way ANOVA was done on all the data to test for effects of soil type, water potential and their interaction. However, irrespective of the interaction effects we looked at each factor separately using one-way ANOVA (Randomized complete block design with factors: soil type, water potential and 3 replications) and the treatments were considered significant at p < 0.05 (Microsoft Excel, 2007; SAS 9.2, Systat software, 2008). Data were transformed when necessary to meet the assumptions of normality. We used PC-ORD version 4 software (MJM Software, Gleneden Beach, OR) for ordination and multivariate analysis of the data as per McCune and Grace (2002).

3. Results

3.1. Quantification of sugars in microbial extracts

Phenol sulfuric acid analyzable sugars (PSA-sugars) in the (chloroform labile) soil extracts indicated that Marietta had significantly greater (~30%) amounts of PSA-sugars than Sumter soil (Fig. 1a). Moreover, PSA concentrations in the two soils responded similarly to water potential deficit. For instance, in Marietta soil PSA-sugars increased significantly (~20–25%) up to ~10 MPa, while Sumter had ~15% greater PSA-sugar in moderately (~4.5 MPa) dry soil compared with the amounts in moist and extremely dry soil.

3.2. PLFAs and fungal to bacterial ratio

The overall abundance of PLFA in Marietta compared to Sumter (Fig. 1b) were in agreement with differences in the size of the PSA-sugar pool, however, the general response to drying tended to contrast with the results of PSA-sugar. Indeed, Marietta tended to show declines and Sumter showed significant increases in PLFA due to drying.

There was no significant effect of water potential deficit on the fungal to bacterial ratio in Marietta; however, there was a significant increase with drying in the Sumter soil (Fig. 2). Sumter had
significantly greater fungal to bacterial ratios than Marietta soil. The increase in fungal to bacterial ratio with soil drying is consistent with higher microbial C:N ratio at dry treatments in Sumter. The relative abundance of PLFA biomarkers indicative of gram-positive bacteria and actinomycetes showed decreases with water deficit whereas fungal biomarkers increased across the water potential gradient of the two soils (Fig. 3).

3.3. Microbial C, N, and soluble C

Microbial C and N were both significantly greater in the Marietta than Sumter. In general, microbial biomass C was not affected by drying (Fig. 4), however, there was an increase at −20 MPa compared to −0.03 MPa in Sumter. Microbial N in Sumter soil decreased when dried to −40 MPa but otherwise was unaffected by drying. In the Marietta soil, in contrast, microbial N tended to decrease during moderate drying compared to moist and extremely dry soil. Sumter had greater levels of soluble C compared to Marietta soil. Soluble C concentrations across the drying gradient changed similarly for both soil types (Fig. 5) increasing significantly at the dryer end of the gradient. Microbial C:N ratios tended to maintain values of ~12, however, when soils dried to the two driest states, ratios increased considerably in the drought-prone Sumter but not the mesic Marietta soil (Fig. 6). Our soil respiration data (not shown) indicated a very typical response to drying and rewetting whereby the respiration declined several fold between moist and...
3.4. Ninhydrin reactive nitrogen (NRN)

Ninhydrin reactive-N (NRN) in chloroform–K₂SO₄ extracts at various intensities of matric stress showed different dynamics in Marietta and Sumter soil (Fig. 7). The initial concentration of NRN in Marietta (7.46 μg g⁻¹ soil) was considerably greater than in Sumter soil (4.62 μg g⁻¹), resembling the relative differences observed for the PSA-sugars. Moreover, the amounts of NRN declined significantly in Marietta but not in the Sumter soil as a result of soil drying.

3.5. GC–MS characterization of soil microbial extracts

The GC–MS analysis of trimethylsilyl (TMS) derivatives of chloroform labile soil extracts showed the presence of several sugars (monosaccharides and disaccharides) and polyols. The compounds that were confirmed include glucose, galactose, trihydroxy butyric acid, arabinose, glycerol, glutol, xylitol, inositol, myo-inositol, turanose and sucrose. The total sugars detected by GC–MS varied from approximately 20 μg g⁻¹ soil to 120 μg g⁻¹ soil, an amount ~10–50% of the size of the total pool of sugars (Table 1). The changes in the concentration of glucose, polyols and other saccharides at different intensities of matric stress in Marietta and Sumter soil are shown in Fig. 8. Glucose was the most abundant monosaccharide found in all treatments and accounted for 45–60% of total GC–MS metabolites. Compounds like glycerol, galactose, glucose, glutol, myo-inositol and turanose were also found in all treatments. However, certain compounds were not consistently present along the moisture regime. For example, inositol was found only in Marietta soil and not Sumter. Similarly, sugars like arabinose, fructose and polyols like xylitol were found in detectable amounts only in some replications of dried treatments.

The composition and the relative abundance of the saccharides and polyols vary with the soil type (Fig. 8). In this regard, Sumter had significantly greater concentrations of cellular metabolites. In general, drying tended to reduce the metabolite content in Sumter soil and tended to change little in the Marietta soil except for an increase at intermediate levels of drying (~4.5 MPa). Metabolites were thus a greater proportion of total biomass in Sumter compared to Marietta soil.

Amino acids, the other important group of osmolytes were found only in low concentrations in continuously moist treatments but were not detected in dry soils. We identified the amino acids valine, proline, leucine, isoleucine, glutamine, glutamic acids, and some fatty acids in continuously moist treatments. Both NRN and spectroscopic NMR analysis supported the relatively low abundance of amino acids in the extracts (data not shown).

4. Discussion

The primary aim of the research was to test the microbial osmolyte accumulation hypothesis (OAH) in soil. We 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. Sugars, polyols and amino acids have been reported as common osmolytes that accumulate within microbial communities; however, these reports are overwhelmingly based on microbial responses to salt stress when grown in cultures (Galinski and Truper, 1994; Kempf and Bremer, 1998; Poolman and Glaaske, 1998). Generally speaking, the results from this study did not support the OAH hypothesis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Marietta</th>
<th>% Detecteda</th>
<th>Sumter</th>
<th>% Detecteda</th>
</tr>
</thead>
<tbody>
<tr>
<td>−0.03</td>
<td>34.3 (15.9)</td>
<td>14.3 (6.8)</td>
<td>93.5 (13.8)</td>
<td>50.5 (9.6)</td>
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<tr>
<td>−1.5</td>
<td>37.6 (23.1)</td>
<td>15.2 (9.6)</td>
<td>46.2 (10.6)</td>
<td>26.6 (5.4)</td>
</tr>
<tr>
<td>−4.5</td>
<td>69.4 (16.3)</td>
<td>15.0 (5.1)</td>
<td>46.1 (6.9)</td>
<td>21.1 (2.6)</td>
</tr>
<tr>
<td>−10</td>
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<td>12.2 (1.2)</td>
<td>58.0 (18.5)</td>
<td>30.4 (10.2)</td>
</tr>
<tr>
<td>−20</td>
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<td>39.5 (14.8)</td>
<td>23.2 (8.8)</td>
</tr>
<tr>
<td>−40</td>
<td>21.2 (7.5)</td>
<td>7.7 (2.7)</td>
<td>27.0 (3.4)</td>
<td>16.4 (2.0)</td>
</tr>
</tbody>
</table>

Values are means and the figures in the parenthesis indicate the standard error with in the treatments.  

\[
% \text{detected} = \frac{\text{Amount of sugars detected in GC–MS}}{\text{Sugars detected in PSA analysis}} \times 100
\]
not support the OAH as a primary mechanism for microbial adaptation to soil drying. Specific pools of metabolites such as sugars and alcohols did not increase consistently in soil microorganisms due to drying. However, two other insights are important to note. First, the pool of microbial metabolites was relatively large, accounting for a large proportion (20–50%) of the total microbial biomass C (not including the use of $k$-factors to estimate microbial biomass) and suggesting that these molecules are a physiologically important pool of high energy compounds. Second, there was considerable change in the pools of microbial derived PLFA, microbial C, and PSA-sugars that may be important indications of community structure and intracellular re-allocations or C resulting from microbial adaptation to soil drying.

### 4.1. Metabolite composition

The composition of the metabolite pool was consistent with the types of compounds expected to allow for microbial cytoplasmic adjustment to water potential deficits (Brown and Simpson, 1972; Hocking, 1986; Al-Hamdani and Cooke, 1987; Kelly and Budd, 1991; Witteveen and Visser, 1995; Shen et al., 1999). Yet, it is important to also note that these compounds are very common components of cellular metabolism (Fig. 8) and energy cycling. The amounts of metabolites, if utilized for physiological adaptation to water deficit, would have increased and have occurred in near molar concentrations within microbial cells due to desiccation, thus making their detection straightforward (Yancey et al., 1982; Schimel et al., 1989). Overall, the results suggest that microorganisms are not accumulating large concentrations of typical osmolytes during soil drying (Fig. 8), and thus do not support OAH as a primary mechanism of microbial adaptation to soil drying.

It was expected that drought-prone Sumter soils would respond differently to drying than the more mesic Marietta soil. It was therefore interesting that the Sumter soil which contained half the microbial biomass had $2 \times$ the concentration of metabolites relative to the Marietta even when soils were moist (Fig. 8). The microbial metabolite response to drying was also different between the two soils but was inconsistent with the main idea that the drought-prone Sumter would accumulate more osmolytes with drying and that the Marietta soil would show more evidence of microbial death and lysis (Fig. 1a,b; Fig. 4). Indeed, related to the issue of microbial turnover, bulk pools of PLFA and PSA did not change in ways consistent with microbial lysis, and it is thus concluded that microbial lysis in soils did not result during soil drying.

If soil microbial communities are not primarily reliant upon osmolyte accumulation, but are nevertheless resistant and adaptable to drying, then other mechanisms are needed to explain soil microbial responses to desiccation. Rather than having unlimited resources like that typically found during early microbial growth in culture, soil microorganisms under nutrient limited and oligotrophic energy conditions may not be able to physiologically afford the costs of intracellular accumulation of osmolytes. In contrast, more efficient utilization (Tiemann and Billings, 2011) and re-allocation of resources from within microbial cells may be a potentially important mechanism for efficiently coping with water deficit in soils. For example, the production of exopolysaccharides (EPS) has been shown to be an important mechanism for bacterial adaptation to desiccation and other environmental stresses (Roberson and Firestone, 1992).

### 4.2. Microbial pools of PLFA, sugars & NRN

The PSA-sugar concentrations along the drying gradient tended to increase in both soil types during moderate levels of drying (Fig. 1a). The PSA methodology measures both monomeric and oligosaccharide sugars and thus is not a specific determinate of monomer-type sugars that have so often been shown to play a role in osmolyte accumulation (Safarik and Santruckova, 1992; Gallardo and Schlesinger, 1995). It is clear, however, that microbial cells are adapting to soil drying either by increasing microbial biomass or specific pools of cell biomass, a result that is often reported to occur during desiccation in soil (e.g. Lundquist et al., 1999; Williams and Xia, 2009; Schimel et al., 2010). Similarly, it is notable that the droughty Sumter soil had increased levels of NRN at moderate levels of drying and that the Marietta soil showed significant declines in NRN as result of drying. The ninhydrin method, however, much like the PSA method must be interpreted cautiously because it is not specific to known osmolytes, and is also sensitive to peptides, proteins, and ammonium, for example. The concentrations of NRN are also rather low and the changes due to drying were relatively small compared to the size of the water potential deficit (Fig. 7). While the greater PSA and NRN concentrations at intermediate levels of drying could be construed as consistent with a microbial response to desiccation, the small changes observed would explain only a small part of the response needed to confirm a general OAH hypothesis (Killham and Firestone, 1984; Schimel et al., 2007).

The results presented here contrast with the 11–60% increase in N and amino acids by cultivated soil microorganisms (Killham and Firestone, 1984b; Schimel et al., 1989) in response to matric deficit.
However, unlike that of most culture conditions, C and N are limiting nutrients for microbial biomass production in most non-agricultural soils and may help to explain the small pool of amino-type N found in our study. Moreover, under dry conditions the thinning of water films and the discontinuity between films can result in substantial reductions in substrate diffusion (Schimel et al., 1989) and nutrient availability to microorganisms. Under such conditions the limitations in nutrient (e.g. nitrogen) and energy availability could lower the capacity for an organism to produce/accumulate appropriate concentrations of osmolytes to counterbalance cellular water loss (Stark and Firestone, 1995). Could microorganisms in C and nutrient limited soil environments have evolved different mechanisms to cope with soil drying (and re-wetting) than mechanisms observed to occur by microbes in nutrient-rich laboratory culture (Tschichholz and Trüper, 1990; Schleyer et al., 1993)?

Microbial respiration rates in this study (data not shown) declined in a typical manner with degree of drying and following the re-wetting of soil (e.g. Birch, 1958; Williams and Xia, 2009). If other microbial responses are similarly tied to desiccation level, then it might be expected that intracellular osmolyte molecule concentrations might show similar patterns of change to desiccation intensity. Indeed, the flush of soluble C (Fig. 5) released following re-wetting of soil has been widely hypothesized to represent the intracellular release of microbial osmolytes and also tends to correlate well with the degree of drying (Schimel et al., 1999; Williams and Xia, 2009; Chowdhury et al., 2011), however, the chemical composition of this pool has not been observed to contain high concentrations of putative osmolytes (Williams and Xia, 2009).

The increase in PSA-sugars and NRN in the moderately dry compared to both dry and moist soils may be interpreted to arise from completely different microbial adaptations. Under extremely dry conditions (−20 and −40 MPa) the costs of cell maintenance rise and the availability of substrate to sustain microbial acclimation would tend to decline, perhaps favoring lower cost solutions such as re-allocation of resources or transition into inactive and dormant states. Cell maintenance activities under low water potential, for example, are used to maintain cell wall fluidity, metabolite regulation, and reallocation of resources (Potts et al., 2005). In this regard, the dynamics of sugar and nitrogen provide clues to understanding how soil communities respond to desiccation and that may not be necessarily tied to osmolyte accumulation. The shifting of resources, such as PLFA, microbial C, and N within a desiccation resistant microbial community suggests that microbes could be re-allocating resources to cope with soil drying (Singh et al., 2002).

It has been hypothesized that the increases in microbial C that often occur during soil drying may be the result of re-distribution of cellular C from structural to cytoplasmic cellular material (Schimel et al., 1989; Williams and Xia, 2009). However, the re-allocation in the opposite direction, from cytoplasmic to structural cell wall material may also occur (Kieft et al., 1994; Singh et al., 2002). In this regard, it is interesting that the abundance of PLFA showed opposing trends to PSA-sugar abundance, for example, increasing the PLFA concentration in the drought-prone Sumter and declining in the more mesic Marietta soil in response to drought (Fig. 1b). The different response of the cellular sugar (carbohydrate) and PLFA pool sizes further confirm the different community responses, but are also consistent with a cellular re-allocation hypothesis in response to water deficit. The reallocation of resources between high energy carbohydrates and wall membranes may be an important means of cellular adaptation to desiccation for microbes in soils (Roberson and Firestone, 1992; Potts, 2001; Gustavs et al., 2009; Hocking et al., 2012).

4.3. Effect of water deficit on microbial communities and fungal/bacterial (F/B) ratio

The response of the microbial community to desiccation may simultaneously result in physiological re-allocation between cellular pools and compositional community change. Shifts in community composition were much larger for the Sumter than the Marietta (Fig. 3) soil, indicating that the soils contain communities with different desiccation coping mechanisms. In particular, the greater community dynamics (greater F/B ratio) in the drought-prone Sumter may suggest the presence of adaptations that allow greater levels of activity and functioning during desiccation, an observation consistent with the idea that fungi are more likely to remain active at very low water potentials than bacteria (Harris, 1981; Shipton and Burggraaf, 1982). The more mesic and less drought-prone Marietta soil communities may adapt to drying, in contrast, by reducing activity and overall community change. This interpretation is consistent with the 50–75% reduction in microbial respiration in Marietta relative to the Sumter soil (data not shown).

Related to the EPS hypothesis, it was observed that desiccation increased the F/B ratio of Sumter but not the Marietta soil. Bacteria are well known for EPS production and fungi are likely to modify cell wall composition during stress periods (Csonka and Hanson, 1991; Blomberg and Adler, 1992; Kieft et al., 1994; Sajbidor, 1997; Vargas, 2005). In this regard, the greater F/B ratio and increasing pool size of membrane PLFA is consistent with fungal cell membrane based responses to water potential deficit in the drought-prone Sumter. Though the relatively high activity of fungi may increase cellular turnover and perhaps result in the loss of some modular biomass and hyphae when dry soil is re-wetted (Williams, 2007), it also provides the opportunity for foraging by hyphae and access to resources with less competition from relatively localized and sessile taxa, as bacteria.

Change in microbial C and N pool sizes to desiccation can also be explained by community changes. Because fungi generally require less N than bacteria to build cellular biomass, the increasing PLFA-based fungal to bacterial biomass ratio (Fig. 2) is consistent with the greater microbial C:N ratio with drying in the Sumter soil (Williams and Xia, 2009). Decreasing the water potential, furthermore, may also increase cell wall growth relative to cytoplasmic growth, thus increasing the C:N ratio of fungal organisms (Paustian and Schnürer, 1987) and provide an explanation for the increasing microbial C:N ratios with drying. This mechanism assumes limiting resources are re-directed to hyphal growth to enhance resource exploitation. The difference in microbial N demand in fungi compared to bacteria could thus effect microbial physiological responses and explain some of the different community responses to drying between soils.

Among the bacterial groups, gram-positive bacteria, and to some extent actinomycetes showed declines in their relative abundance, an observation that goes against the idea that these bacteria, because of their enhanced peptidoglycan layer should be more resistant to drying (Potts, 1994). Indeed, recent studies have shown opposite trends, that gram-negative are more well adapted than gram-positive bacteria to water stress (Williams, 2007; Williams and Rice, 2007; Aanderud and Lennon, 2011, Fig. 3.). It is similarly possible that changes in methyl-branched PLFA, for example, are indicative of physiological change in cell membranes used to cope with drying (Nordström, 1993).

Though fungi benefited relative to bacterial biomass as a result of desiccation in the drought-prone Sumter soil, there was again no indication that specific fungal osmolytes, such as alcohols, were produced to adapt to drying soil. In contrast, fungi may alter the allocation of energy and C to cell wall production, and especially change fatty acid synthesis during desiccation (Fig. 2; Blomberg...
and Adler, 1992). Bacteria, in contrast, may respond to desiccation by increasing the allocation of C to EPS on the outer surface of the cell wall (Roberson and Firestone, 1992), an interpretation consistent with increases in PSA-sugars in the Marietta soil (Fig. 1a).

5. Conclusion

The study was focused on testing the osmolyte accumulation hypothesis (OAH) in soil by characterizing the concentrations of common microbial osmolytes across a gradient of desiccation stress under in situ soil conditions. Eleven putative osmolytes (sugars and alcohols) were characterized but showed no consistent increases with water potential deficit that would link their production to microbial adaptation to water deficit. In contrast, the dynamics in microbial PLFA, microbial C, and PSA-sugars indicate that microorganisms are responding to water deficit. It is hypothesized that microbes are adapting to desiccation, perhaps through the re-allocation of molecular resources to the cell wall (fungi) and extracellular cell wall EPS (bacteria). Some of the variability in microbial responses to soil drying may also be explained by differences in microbial community composition, structure, and activity. The utilization of EPS production by bacteria to adapt to desiccation has been described previously, however, in a native soil context it has not been explicitly tested as a means of microbial adaptation. Compared to OAH, these hypothesized mechanisms of microbial response may be more consistent with the oligotrophic energy and nutrient conditions typically found in soils. The research suggests that current conceptual models of microbial adaptation to water deficit need re-evaluation.

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Appendix A. Supplementary data

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References


