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

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**A B S T R A C T**

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.

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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 (Kilham 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 fluxes 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...
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 structures 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° 28ʹ N and 088° 47ʹ W) in fall 2009 (Kakumanu et al., 2013). Briefly, the Marietta soil is fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudents 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 Rendolic Eutrudent, 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 17.2 °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 CaCl2 (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 ~5, ~4.5, and ~10 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 preincubation 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 homogeneous 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 potentiometer (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
Sumter soils to achieve the water potentials of −1.5 MPa, −4.5 MPa and −10 MPa, respectively. After reaching the lowest targeted water potential following ~3 days, the soils were left at 22 °C for another 24 h. The following day, samples were taken for CO₂ measurement and soil extracted for microbial metabolites. Samples for PLFA extraction were stored at −80 °C until analysis (Kakumanu et al., 2013).

2.3. Microbial metabolite/osmolyte extraction

Soil samples were extracted for microbial metabolites as per Kakumanu et al. (2013). Briefly, 10 g (dry weight) of soil from each treatment were transferred to separate 160 ml serum bottles and amended with 10 ml chloroform. After 1 min, 40 ml of 0.01 M K₂SO₄ solution was added to each bottle and agitated for 2 h at 250 rpm on an orbital shaker. The samples were centrifuged at 1500 rpm for 10 min to separate the chloroform and aqueous phase. The aqueous supernatant was filtered through a Whatman #1 filter paper and the solution was lyophilized and stored at −80 °C until further analysis.

2.4. Analysis of microbial extracts by phenol-sulfuric acid and ninhydrin methods

The sugars in the extracts were assessed by phenol-sulfuric acid analysis (PSA) (Dubois et al., 1956). Briefly, 15 μl of soil extract was added with 50 μl of 80% phenol solution followed by addition 5 ml of concentrated H₂SO₄ (~18 M). The mixture was incubated for 45 min at room temperature and the absorbance was measured at 480 nm. Glucose was used as calibration standard for the analysis.

Primary and secondary amines (e.g. Amino-N) were determined using the ninhydrin method (Stevenson, 1982). Using 50 μl of soil extract amended with 0.5 ml of citric acid and 2 ml of ninhydrin reagent, samples were incubated at 100 °C for 25 min. The solution was cooled to 22 °C, diluted with 5 ml of 50% ethanol, and the absorbance measured at 570 nm. A standard curve was made ranging from 0.6 to 4.8 μg of L-Leucine-N to estimate the amino acid concentration.

2.5. Quantification and identification of extractable metabolites

The metabolite composition, primarily sugars and amino acids, in the microbial extracts was analyzed using gas chromatography – mass spectroscopy (GC–MS). Prior to the analysis, extracts were derivatized with appropriate reagents and all the derivatization reactions were carried out in silylated (5% dimethylchlorosiloxane) 1 ml reaction vials. For sugar analysis, ~250 μl of soil extracts were dried and converted to trimethylsilyl derivatives by adding N, O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA+1% TMCS) and pyridine in 2:1 ratio and incubating for 3 h at 70 °C (Medeiros et al., 2006). The samples were left overnight at 22 °C and dried under UHP N₂. The residues were redissolved in 110 μl of hexane before GC analysis.

For amino acid analysis, a standard protocol was followed (Fan, 1996). Using 500 μl of soil extract, an equal volume of 1 M HCl was added to lower the solution pH to 2. The solution was then dried and combined with 1 ml of 1:1 mixture of N-Methyl-N-(Tr-butylidimethylsilyl) trifluoroacetamide (MTBSTFA) and acetonitrile and sonicated for 2 h at 60 °C. The solution was left at 22 °C overnight and dried under a gentle stream of UHP N₂. The residues were then reconstituted in 110 μl of hexane for GC analysis. The derivatized soil extracts 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 × 0.25 mm, with film thickness of 0.25 mm) operated using the following conditions: injector temperature, 240 °C, column temperature, 60 °C—280 °C at 8 °C min⁻¹ then held at 280 °C for 5 min; carrier gas, He; injection volume, 1 ml (splitless). The MS mass ranged from 40 to 650 m/z, filament delay of 3 min, target total ion current (TIC) of 20,000, a prescan ionization time of 100 ms, an ion trap temperature of 150 °C, manifold temperature of 60 °C, and a transfer line temperature of 170 °C. Glucose, trehalose, sorbitol, sucrose, proline and glutamine, some of the common microbial osmolytes, were used as standards. The data were compared using the NIST database and available literature. Compounds were quantified using the 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. Phospholipid fatty acid analysis

Phospholipid fatty acids were extracted from the soils as per the procedure of White and Ringelberg (1998) and as modified by Butler et al. (2003). Briefly, 10 g (dry weight) soil was extracted overnight using a mixture of 50 mM phosphate buffer (pH 7.1), chloroform and methanol (0.8:1:2). The following day, the soil solutions were centrifuged for 5 min at 1000 rpm in IEC bucket centrifuge, filtered through whatman #1 filter paper and added with 3 M NaCl solution for phase separation. The chloroform phase was collected and dried completely under UHP N₂. The lipids were then separated into neutral, glyco and phospholipids using sillicic acid bonded phase extraction columns (Supelco, cat. no. 505048). The neutral, glyco- and phospho-lipids were eluted using chloroform, acetone and methanol respectively. The phospholipids were finally transformed into fatty acids methyl esters and suspended 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 program was set to ramp from 170 °C to 288 °C at 28 °C min⁻¹ and then stabilized until the end of the run time of 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.

The PLFA’s i15:0, a15:0, 15:0, i16:0, a16:0, i17:0, a17:0, 17:0 (gram-positive), 16:1o9, 16:1o7t, 18:1o7 and cy19:0 (gram-negative) were considered as bacterial biomarkers, 10:Me 16:0 and 10:Me 18:0 for actinomycetes and 18:1o9 and 18:2o6 as fungal biomarkers (Frostegård and Bååth, 1996; Zhang et al., 2005; Liang et al., 2008; Kakumanu et al., 2013). The ratio of total fungal biomarkers to total bacterial biomarker fatty acids were used to calculate the fungal to bacterial (F/B) ratio (Bosso and Scow, 1998).

2.7. Measurement of soil respiration

Ten grams (dry weight) of soil from each treatment replicate was transferred to 160 ml serum bottles. Three serum bottles with 2 ml of tap water were used to control for background CO₂ production. The bottles were sealed with a rubber septum and crimp caps and incubated at room temperature. The CO₂ samples from the head space of treatment serum bottles were collected every 24 h for 2 days. Bottles were vented for 10 min after each sampling to keep the CO₂ concentrations below 2%. The collected CO₂ samples were analyzed on a gas chromatograph (Varian Model 3600 with a 2 m
3.2.1. Shift in the metabolite composition

3.2. Microbial metabolites in osmotic and matric treatments

Water potentials (matric-induced water deficit, respiration was less affected by osmotic compared to respiration by ~30% more than osmotic de

3. Results

of metabolite and mol% of PLFA data. Scaling (NMS) was used to provide graphical ordination of the mol% of metabolite and mol% of PLFA data.

3.1. Respiratory response to osmotic and matric water potential deficit

Respiration, measured as CO₂ production, declined in both matric and osmotic water potential deficit treatments in both soils (Fig. 1a, & 1b). The largest decline in respiration occurred from the relatively small water potential change between −0.03 and −1.5 MPa. Respiration in the matric and osmotic treatments declined similarly down to −1.5 MPa, but differed by soil, declining −60% and −40% in Marietta and Sumter, respectively. Respiration declined little in the osmotic but declined greatly due to matric deficit, approaching −90% decrease in both soils at −10 MPa compared to those remaining moist (−0.03 MPa). At a given water potential, respiration was less affected by osmotic compared to matric-induced water deficit. For instance, matric deficit reduced respiration by −30% more than osmotic deficit at the two lowest water potentials (−4.5, −10 MPa).

3.2. Microbial metabolites in osmotic and matric treatments

3.2.2. Quantitative analysis of metabolites

3.2.2.1. PSA-sugars. Concentration of PSA-sugars at different water potentials in matric and osmotic treatments of Marietta and Sumter soils are shown in Fig. 3a and b, respectively. In Marietta, matric and osmotic water deficit resulted in 5–25% increase in PSA-sugars, and was consistent with the idea of osmolyte accumulation under low water potentials. In Sumter, a 2–20% increase in PSA-sugars was observed with lower matric and osmotic potential except at −1.5 MPa, where PSA-sugars decreased relative to the control. Overall, the osmotic had similar amounts of sugars compared to matric treatments; however, Marietta had significantly less PSA-sugar at −10 MPa in osmotic than the respective matric treatment.

3.2.2.2. Ninhydrin-nitrogen (NRN). Overall, the amount of NRN was greater due to osmotic compared to matric water deficit, and in both soils, the osmotic water deficit increased or did not change concentrations with degree of water deficit (Fig. 4a and b). In Marietta, low matric potential decreased NRN by 9–30% across the deficit gradient. In Sumter, the NRN concentration tended to

Porapak Q column) equipped with a thermal conductivity detector and a set oven temperature of 105 °C.

2.8. Statistical analysis

Statistical analysis (3-way ANOVA) was conducted to analyze the effects of soil, stress type, intensity of stress and their interaction on the response variables including PSA-sugars, NRN, metabolites and PLFA concentration. Simultaneously, interaction effects of stress type, intensity were analyzed in each soil independently and the treatments were considered significant at $p < 0.05$ (SAS 9.2, Systat software, 2008). Multivariate analysis of the metabolite data and PLFA data were conducted using PC-ORD version 6 software (MJM Software, Gleneden Beach, OR) as per (McCune and Mefford, 2011). The Multi-response Permutation Procedure (MRPP) was used to test for significant differences between multivariate data sets, such as PLFA and metabolites. Nonmetric multidimensional scaling (NMS) was used to provide graphical ordination of the mol% of metabolite and mol% of PLFA data.

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![Fig. 1. Percent change in the respiration due to matric and osmotic water potential deficits relative to moist control (−0.03 MPa) in (a) Marietta and (b) Sumter soil. Columns represent the average of 3 replicates and the bars represent standard errors. Overall, the amount of respiration was significantly different due to soil, water deficit type, and degree of water deficit. Percent change from moist control (−0.03 MPa) are highlighted and were significantly different from zero (α = 0.05).](image-url)
Fig. 2. Nonmetric multidimensional scaling plot showing the shifts in the relative abundances of metabolites sampled across gradients of matric and osmotic potential deficits in Marietta and Sumter. The bars represent the standard error (n = 3). Percentages on the each axis denote the proportion of variability associated with each axis. Individual Multi-response permutation tests detected significant differences between the main factors of soil type, type of water stress, and degree of water deficit (p < 0.05). An interaction was detected between soil type and water stress type (p < 0.05). However, if grouped chemically, both soil types responded similarly, with sugars and alcohols explaining the shifts due to water potential decline.

Fig. 3. The amount of PSA-sugars (µg g⁻¹ soil) in two soils Marietta (●) and Sumter (○) at different levels of a) matric and b) osmotic potential deficits. Circles represent the mean of 3 replicates and the bars represent the standard error. Treatments not having the same letter are significantly different (α = 0.05) with in the soil. The PSA-sugars are significantly different due to soils and degree of water deficit (p < 0.05) but no significant difference was observed between matric and osmotic stress with in the soil.

Fig. 4. The amount of Ninhydrin-N (µg g⁻¹ soil) in two soils Marietta (●) and Sumter (○) at different levels of a) matric and b) osmotic potential deficits. Circles represent the mean of 3 replicates and the bars represent the standard error. Treatments not having the same letter are significantly different (α = 0.05) with in the soil. The NRN values are significantly affected by soils, stress type and degree of water deficit (p < 0.05).
increase consistently with declining osmotic potential up to \(-4.5\) MPa. There was also an increase in NRN due to matric water potential decline between \(-1.5\) and \(-4.5\) MPa in the Sumter soil, but this was the exception to generally unchanging or lower NRN due to matric potential deficits.

3.3. Microbial community response to osmotic and matric water potential deficits

3.3.1. Phospholipid fatty acid (PLFA) abundance

Phospholipid fatty acid abundance increased with water potential deficit in Sumter but tended to decline in Marietta (Fig. 5a, b). The decline in PLFA concentration in Marietta due to osmotic water deficit was much larger and significant \((p < 0.0001)\), declining by 15–85% relative to control. An increase in PLFA-based fungal/bacterial (F/B) ratio was observed under osmotic and matric water deficit in the two soils (Fig. 6c and d), however, the increase was larger and more consistent in the Sumter soil.

The change in the F/B ratio was correlated with shifts in the types of metabolites. For instance, in Sumter the increase in F/B ratio was correlated with greater abundance of polyols, which are typical fungal osmolytes (Fig. 6). In general, osmotic treatments caused significantly greater changes in PLFA abundance and F/B ratios compared to the matric deficit treatment.

3.3.2. PLFA’s across the water deficit gradient

Overall, there was a shift in the relative mol% PLFA due to water potential deficit in both soils (MRPP, \(p < 0.001\); Fig. 7a and b). The shift in PLFAs was much larger and more correlated to the degree of change in osmotic than matric potential, however. Matric deficit treatments tended to cluster together, but independently from the moist \((-0.03\) MPa) soil, indicative of an effect, but that was smaller than the change due to osmotic deficit. In the NMS plot of Marietta, axis 1 and axis 2 explained 81 and 14% of the captured variation, respectively (Fig. 7a). For axis 1, bacterial biomarkers such as \(a15:0\), \(a15:0\), \(16:1\omega7c\) and \(cy17:0\) were negatively correlated \((r > 0.70)\) whereas PLFAs \(18:1\omega6c\), \(11\omega18:0\) and \(cy19:0\) were positively correlated (Fig. 8a). In the NMS plot of Sumter, axis 1 and axis 2 explained 97% and 3% of the variation, respectively (Fig. 7b). The PLFAs \(11\omega4:0\), \(15\omega5:0\), \(11\omega6:0\), \(16:1\omega7c\), \(16:1\omega5c\), \(cy17:0\) were positively correlated with axis 1 and PLFAs \(18:1\omega6c\), \(18:2\omega6c\), \(18:1\omega9c\) and \(cy19:0\) were negatively correlated (Fig. 8b).

3.3.3. Change in microbial groups associated with water stress

Overall, there were alterations in PLFA bacterial biomarkers, which were correlated with changes associated with matric water potential deficit. Longer-chain fatty acids were associated with osmotic water potential deficit in both soils, whereas specific long-chain fatty acids associated with fungal biomarkers were more correlated with osmotic change in the Sumter soil. Similarly, an increase in fungal biomass was observed with both matric and osmotic potential decline, but was much more pronounced and consistent in the Sumter compared to the Marietta soil (Tables 1 and 2). Water potential decline resulted in a decrease in iso- and anteiso-PLFA, which are typically associated with gram-positive bacteria, but are also prone to change based on physiological adaptations to the environment. The ratio of \(cy19:0\) to \(18:1\omega7\) increased with water potential deficit, consistent with its status as a biomarker of physiological stress among bacteria, particularly gram-negative types (Table 3).

4. Discussion

4.1. Microbial pool and metabolite responses to osmotic and matric potential deficit

A consistent body of literature has shown that microbes respond to osmotic salt-based water deficits by accumulating compatible solutes such as osmolytes. Evidence for similar microbial responses in soil have been documented, but only recently have studies attempted to measure osmolyte change in response to drying, and generally have seen little or sometimes small shifts in osmolyte pools (Schimel et al., 2007; Williams and Xia, 2009; Boot et al., 2013; Kakumanu et al., 2013). To understand if salt-induced osmotic water deficit cause similar effects on soil microbial communities, the main objective of this study was to compare the metabolite, physiological, and structural dynamics of soil microbial communities associated with matric and osmotic induced water deficit gradients in two soils. The largest differences in the types of metabolites were related to soil; however, within each soil, change in metabolite fingerprints, community structure, physiology and microbial pool sizes were observable, but changed the most, as hypothesized, in osmotic compared to matric water potential deficit.

Compared to the relatively small changes in metabolite pools due to matric deficit, increasing alcohols and sugars in response to osmotic deficit treatments closely resembled osmolyte accumulation patterns observed in microbial cultures growing in salt enriched media (Adler et al., 1982; Anderson and Witter, 1982; Brown, 1990; Madkour et al., 1990; Kelly and Budd, 1991; Shen et al., 1999; Empadinhas and da Costa, 2008). Hence, mechanisms used by microbes in soil to adapt to salt-induced osmotic water deficit appear to rely more on osmolyte accumulation than...
adaptations to matric deficit. Culture based responses of microbes to salt thus may not reflect how microbes in soil respond to soil drying (Williams and Xia, 2009; Kakumanu et al., 2013).

Energy and nutrient availability may limit the capacity of microbes to respond to soil desiccation (Schimel et al., 2007; Boot et al., 2013; Kakumanu et al., 2013). Soils that are dry may also hamper microbial adaptation because of inaccessibility to watersoluble pools of energy and nutrients and their resupply through transport, mass flow, and diffusion. A new model focused more on re-allocation of resources within microbes and that consider energetic and nutrient dynamics may shed light on microbial processes important to the biogeochemistry of drought.

Though the overall pattern of metabolite, structural and physiological changes were greatest due to osmotic compared to matric deficit, there were also some unique responses from each soil. Most notable was the greater increase in fungal to bacterial ratios in Sumter relative to Marietta with water deficit. The mol% of metabolites, particularly polyols and fructose, were positively correlated with increasing F/B ratios, and consistent with the role that these molecules can play as osmolytes during salt stress.

Water regime legacy has been observed as a possible factor determining microbial responses to drying and ultimately re-wetting (Van Gestel et al., 1993a; Fierer et al., 2003; Williams, 2007; Jin et al., 2011; Evans and Wallenstein, 2012). However,
there are also many differences in soil properties that could account for the differences between soils with different water regime legacies. Except for the overall large drop in PLFA abundance in Marietta, and largely associated with osmotic water deficit, there were numerous similar PLFA responses to drying (Williams and Xia, 2009; Boot et al., 2013; Kakumanu et al., 2013) that also indicate the potential for a core set of metabolic responses to water deficit and soil community adaptions to drying.

Broadening the pool size of potential microbial metabolites to include PSA-sugars and NRN reactive molecules provided less detailed but useful descriptions of metabolite change Ninhydrin-N is often used as a proxy for amino acid-N, and since many amino acids can be used as intracellular osmolytes, this pool can be useful for providing insight into the soil microbial response to water deficit (Killham and Firestone, 1984a; Csonka, 1989). Overall, the change in NRN tend to support the idea that osmotic induced water potential deficits increase the abundance of the microbial osmolyte pool, but the impacts of matric deficit were less clear. In the Marietta soil there was a decline in NRN (~20%) due to matric water deficit. In the Sumter soil there was an increase followed by a decrease at the lowest matric water potential. These changes in nitrogen may be ecologically relevant; however its small pool size

% change indicates the percent increase (positive values) or decrease (negative values) in the relative proportion of the indicative group at respective treatment compared to control (Treatment/control)/control)

Fig. 8. Two-dimensional correlation between microbial PLFA’s and their correlation with the multivariate spread due to type of water deficit and degree of water deficit in (a) Marietta and (b) Sumter soils.

| Table 1 | Relative proportion (mol %) of PLFA biomarkers indicative of various microbial groups across gradient of low matric and osmotic potentials in Marietta soil. |
|-----------------|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|
| Treatments      | Gram positive % change | Gram negative % change | Actinomycetes % change | Fungi % change |
| Control         | 19.54 (0.01)   | 28.25 (0.03)   | 13.50 (0.03)   | 7.25 (0.03)   |
| Matric          |                |                |                |                |
| –1.5            | 19.37 (0.07)   | 28.06 (0.02)   | 13.81 (0.04)   | 7.48 (0.05)   |
| –4.5            | 19.59 (0.04)   | 28.16 (0.06)   | 13.70 (0.07)   | 7.39 (0.03)   |
| –10             | 19.34 (0.02)   | 28.26 (0.09)   | 13.51 (0.04)   | 7.41 (0.05)   |
| Osmotic         |                |                |                |                |
| –1.5            | 18.84 (0.06)   | 28.81 (0.08)   | 13.61 (0.23)   | 7.42 (0.03)   |
| –4.5            | 18.60 (0.04)   | 28.91 (0.06)   | 13.56 (0.05)   | 7.48 (0.01)   |
| –10             | 19.34 (0.02)   | 28.12 (0.09)   | 13.18 (0.04)   | 7.3 (0.001)   |

Values are means and the numbers in the parenthesis indicate the standard error with in the treatment. Values within a column designated with same smaller case letters are not significantly different (p ≤ 0.05).

% change indicates the percent increase (positive values) or decrease (negative values) in the relative proportion of the indicative group at respective treatment compared to control (Treatment – control)/control × 100.

| Table 2 | Relative proportion (mol %) of PLFA biomarkers indicative of various microbial groups across gradient of low matric and osmotic potentials in Sumter soil. |
|-----------------|-----------------|-----------------|----------------|-----------------|-----------------|
| Treatments      | Gram positive % change | Gram negative % change | Actinomycetes % change | Fungi % change |
| Control         | 20.87 (0.19)   | 28.79 (0.19)   | 10.03 (0.11)   | 11.34 (0.09)   |
| Matric          |                |                |                |                |
| –1.5            | 20.99 (0.09)   | 28.37 (0.13)   | 9.59 (0.02)    | 11.89 (0.11)   |
| –4.5            | 20.41 (0.32)   | 28.08 (0.17)   | 9.67 (0.03)    | 12.90 (0.36)   |
| –10             | 20.39 (0.01)   | 28.21 (0.05)   | 9.57 (0.07)    | 12.70 (0.07)   |
| Osmotic         |                |                |                |                |
| –1.5            | 20.05 (0.11)   | 29.56 (0.11)   | 10.03 (0.02)   | 12.39 (0.18)   |
| –4.5            | 19.79 (0.12)   | 29.28 (0.08)   | 9.76 (0.08)    | 13.47 (0.36)   |
| –10             | 18.99 (0.27)   | 29.73 (0.30)   | 9.62 (0.09)    | 14.43 (0.07)   |

Values are means and the numbers in the parenthesis indicate the standard error with in the treatment. Values within a column designated with same smaller case letters are not significantly different (p ≤ 0.05).

% change indicates the percent increase (positive values) or decrease (negative values) in the relative proportion of the indicative group at respective treatment compared to control.
induced microbial death. These trends in biomass accumulation and the generally weak or sporadic findings of osmolyte upregulation due to water deficit (Kakumanu et al., 2013; Warren, 2014) in soil microbes point to multiple mechanisms of adaptation that may be common in soils.

Differences in community composition and activity might also help to explain some of the microbial responses to water potential deficits. In particular, the Sumter soil has almost twice the fungal PLFA than Marietta. Though both soils showed evidence for increasing fungal dominance and greater % of fungal polyols as water potential declined (Reichardt et al., 2001; DeGrood et al., 2005; Wichern et al., 2006), the effects were greatest in the drought-prone Sumter regardless of water deficit type. These results thus highlight important differences between microbial communities that can help to explain different responses to water deficit in soil (Griffin, 1981; Luard and Griffin, 1981; Freckman, 1986; Deacon, 2009). Greater respiration, degree of fungal biomarker change, and the 10–40% increase in PLFA abundance in Sumter relative to Marietta soil highlight different strategies used by microbes to adapt to water potential decline (Schimel et al., 2007; Aanderud and Lennon, 2011).

Though fungal biomarkers changed much more in Sumter than Marietta in response to water deficit, there were patterns of mol% PLFA change that also indicated similar membrane-based adaptations to osmotic and matric deficits.

Levels of PSA-sugar tended to increase with water potential deficit, and highlighted a consistent response between the two water deficit types in both soils. The PSA pool is likely composed of many different types of sugar molecules, and given its size, which is close to half of the microbial biomass (100–150 μg g\(^{-1}\) soil), it may also be partially indicative of water deficit induced changes in microbial biomass size. The interpretation fits with other studies indicating that drying, or drying and rewetting tend to result in greater pools of microbial biomass (Franzluebbers et al., 1996; Lundquist et al., 1999; Fierer et al., 2003; Xiang et al., 2008). This has been interpreted in several ways, including the microbial accumulation of sugars, carbohydrates, and exopolysaccharides to soil drying. These results thus support the occurrence of a global response by soil microbes to water deficit, but it remains unclear if this pool is a direct adaptation to water loss or might contain yet to be measured types of osmolytes.

### Table 3

Concentration of PLFA biomarkers (nm g\(^{-1}\) soil) indicative of stress (Cy19:0/18:1ω7 and monounsaturated fatty acids/saturated fatty acids) across the gradients of matric and osmotic potentials in Marietta and Sumter soils.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Marietta</th>
<th>Sumter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cy19/18:1ω7c</td>
<td>Mono/sat</td>
</tr>
<tr>
<td>Control</td>
<td>0.682 (0.001)(^a)</td>
<td>1.941 (0.004)(^b)</td>
</tr>
<tr>
<td>Matric</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1.5D</td>
<td>0.730 (0.006)(^bc)</td>
<td>1.986 (0.003)(^bc)</td>
</tr>
<tr>
<td>−4.5D</td>
<td>0.710 (0.004)(^bc)</td>
<td>2.053 (0.015)(^bc)</td>
</tr>
<tr>
<td>−10D</td>
<td>0.719 (0.002)(^bc)</td>
<td>2.031 (0.021)(^bc)</td>
</tr>
<tr>
<td>Osmotic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1.5SS</td>
<td>0.713 (0.013)(^a)</td>
<td>2.078 (0.026)(^a)</td>
</tr>
<tr>
<td>−4.5SS</td>
<td>0.769 (0.005)(^a)</td>
<td>2.033 (0.009)(^ab)</td>
</tr>
<tr>
<td>−10SS</td>
<td>0.746 (0.004)(^a)</td>
<td>1.961 (0.011)(^a)</td>
</tr>
</tbody>
</table>

Values are means and the numbers in the parenthesis indicate the standard error within the treatment. Values within a column designated with same smaller case letters are not significantly different (\(p \leq 0.05\)).

\(\text{−}4.5\text{–}5\text{ μg g}^{-1}\text{ soil}\) suggests it may not play a primary role in microbial adaptation to water potential deficit. Or, there may be a small pool of microbes that are responding to matric water deficit using osmolytes. Deeper molecular characterization of the microbial molecular pools and their turnover should provide further insight into the mechanisms of microbial adaptation to osmotic and matric deficits.

4.2. Microbial community structure, physiology, and activity change in response to osmotic and matric deficit

The size of the microbial PLFA pool, often used as a proxy for microbial biomass (Pankhurst et al., 2001; Wilkinson et al., 2002; Sardinha et al., 2003; Williams and Rice, 2007), was affected more by osmotic than matric deficit, and was most obvious in Marietta soil where the largest decreases were associated with deficits down to −4.5 and −10 MPa. The significant decline in the PLFA pool in Marietta reveals the presence of microbes that are physiologically sensitive to salt induced water deficit, resulting in considerable cell shrinkage (loss of PLFA) or perhaps reallocation from PLFA to other cellular pools. In this way, changes in cellular pools are linked to cellular physiology, but could also be coupled with changes in community composition. Cell death has been a common interpretation for microbial biomass or PLFA decline in response to water deficit stress (West et al., 1988; Van Gestel et al., 1993b; Wu and Brookes, 2005; Gordon et al., 2008), however, the stable or increasing abundance of PSA-sugars in response to both types of water potential deficits, and the fact that the PSA pool is 5 times larger than PLFA, does not support the idea of water stress

5. Conclusion

Overall, the results in this study suggest that osmotic deficit had a considerably larger effect on soil metabolite profiles and microbial community biomass pools than matric deficit. The change in metabolite pools in response to osmotic but not matric water deficit also more closely mimicked osmolyte changes observed to occur in
culture-based microbial studies. The mol% change in metabolites associated with matrix stress did indicate a small relative increase in alcohol pools and perhaps suggesting that some part of the microbial community utilizes these molecules as osmolytes to adapt to water deficit. However, soil microbes thus appear to respond to osmotic stress like those of cultured microbes. Soil drying causes a smaller osmolyte change than osmotic deficit but appears to induce other microbial aclimations and adaptations that require further study.

References


