Characterization of the water soluble soil organic pool following the rewetting of dry soil in a drought-prone tallgrass prairie

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A B S T R A C T

To better understand the nature of the C flush that follows the rewetting of dry soil, we chemically characterized the water soluble pools following rewetting of soil dried to several different water potentials. To assess the impact that historical soil water status has on the size of the rewetting labile soluble pool, a laboratory water stress gradient was applied to soils that were collected from drought-prone and irrigated tallgrass prairie soils. In the laboratory, soils were either incubated at –33 kPa or dried steadily over a 0.6, 1, 2, or 3 day period to –1.5, –4, –15, and –45 MPa respectively. On the 4th day, samples were wetted back to –33 kPa and immediately assayed for soluble, microbial, or respiratory pools of carbon. After extraction, samples were also assayed using NMR, GC–MS, and LC–MS to assess carbohydrate, amino acid, osmolyte and sugar pools. The greater the degree of drying before rewetting was associated with greater concentrations of microbial, soluble and respiratory pools of carbon, increasing by 50, 400 and 250%, respectively, in the most water stressed compared to continuously moist soil. Compared to drought-prone soils, the amount of soluble C released as a result of rewetting was 30 to 50% greater in soils that were irrigated for 11 years. The pool of organisms was not completely characterized and only small amounts of TBDM and TMS derived compounds accounting for 2–4% of the soluble C pool were detected. In contrast, oligosaccharides constituted approximately 20–25% of the sample C. Our results suggest that the flush of C following wetting of a dry soil is not dominated by common microbial osmolytes (e.g. proline, glycine betaine, ectoine, glycerol, mannitol, trehalose). In light of this finding more research is needed to better understand the adaptations that microbial communities utilize to respond to the rewetting of dried soil.

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1. Introduction

Water dynamics and stress in soil exert a major influence on microbial physiology and function (Harris, 1981; Yancey et al., 1982). The vast majority of ecosystems undergo short- and long-term droughts, which cause soil water contents to drastically decrease. Eventually however, water stress is alleviated either through rainfall or irrigation. While there is widespread agreement that drying and rewetting results in large but temporary pulses of carbon and nutrients (Sorenson, 1974; Bottner, 1985; Appel, 1998), there is still considerable uncertainty about the biotic response which contributes to these pulses. For instance, the flush of soluble and respiratory C following the rewetting of dry soil is considered to be, at least partially, derived from microorganisms. When water potentials decrease, microorganisms have been shown to accumulate a variety of organic solutes to offset cellular water loss (Killham and Firestone, 1984; Kempf and Bremer, 1998; Halverson et al., 2000). When water potentials are then increased again, usually instantaneously, microorganisms typically respond by expelling these accumulated intracellular osmolytes into the extracellular environment. In soils systems, it has been hypothesized that the ejection of intracellular organic solutes into the extracellular soil environment could explain the increase in soluble and respiratory carbon pools that accompany the rewetting of a dry soil (Kieft et al., 1987; Halverson et al., 2000).

While the above mechanism is useful for explaining results from various studies, there have been few definitive tests of this hypothesis. Using a 14C enrichment technique, the enrichment of the respiratory C released immediately after rewetting of dry soil was double the enrichment of the extractable microbial biomass C, suggesting that the CO2 may be of microbial cytoplasmic origin (Fierer and Schimel, 2003). In another study, four soil bacterial isolates were exposed to dilution stress and found to vary in their response, releasing from less than 10 to more than 60% of their intracellular amino acid and low molecular weight sugar pools (Halverson et al., 2000). Similarly, in response to 1 M KCl in the growth medium, the amount of intracellular microbial cytoplasmic...
C and N increased 10-fold in a bacterium (*Pseudomonas* sp.) and a fungus (*Aspergillus flavus*) isolated from soil (Schimel et al., 1989). These lines of evidence for a microbial response to water stress in soil are compelling, but there have been no studies that have attempted to characterize and quantify the chemical nature of the carbon flush and solidify the link between microbial adaptation to dilution stress and the flush of soluble organics that occurs when dry soil is rewetted.

The size of the flush released from soil may be related to the moisture regime history of a site. For example, Fierer and Schimel (2003) noted that a drought-prone grassland soil released greater amounts of carbon-dioxide following rewetting of a dry soil than did a more mesic oak soil. Other studies have shown a negative relationship between the number of rewetting events and the amount of C and N that is released following rewetting (Mikha et al., 2005). More direct evidence about how the size of these flushes is related to moisture regime history can help to explain how biotic and abiotic responses to water stress influence ecosystem C dynamics.

We characterized the chemical composition of the soluble organic pool that was extracted from soil following drying and rewetting. We expected that as the degree of drying before rewetting increased, greater concentrations of soluble organics would be produced. We found that they would be characterized by increasing quantities of known microbial osmolytes such as amino acids, sugars, and sugar alcohols. Overlaid on this experiment, we also wanted to determine if the amount of soluble C extractable following the rewetting of dry soil might increase in a historically drought-prone tallgrass prairie that had been irrigated for 11 years.

2. **Materials and methods**

2.1. **Site description**

The soils were collected from a native (annually-burned in spring) upland tallgrass prairie located at the Konza Prairie Biological Station near Manhattan, Kansas. Soils of the upland summit topographic position are dominated by mesic Typic Hapludolls (*Benfield* silty clay loam). Vegetation on the site was a mixture of C3 and C4 species, dominated by big bluestem (*Andropogon gerardii*) and indiangrass (*Sorghastrum nutans* (L.) Nash). Previously, it has been shown that average peak aboveground biomass (1993–2000) was approximately 575 and 455 g m$^{-2}$ in continuously-moist and drought-prone transects, respectively, of which 35–50 g m$^{-2}$ was forbs. Standing root biomass did not differ between treatments and was approximately 785 g m$^{-2}$ in the top 10 cm of soil (Williams, 2001).

Established in 1990 and 1993, the irrigation pipelines traversed a 70 m long by 30 m wide transect in the upland (280 m MSL) topographic position. Penman equations and meteorological data were used to calculate plant water demand, and water was added during the months of May/June through September (Knap et al., 1998). During the other months of the year, precipitation exceeds evapo-transpiration. In the driest year of 1991, an additional 470 mm of water was added in the irrigated treatments, whereas the wettest year of 1993, only 80 mm of water was added. On average, 245 mm of water was added to each growing season, an amount equivalent to 60% of the average total rainfall during the 4-month period. The year of 2003 was a typically dry year with only 500 mm of rainfall recorded during the peak growing season months of May through September, resulting in an additional 245 mm of water added via irrigation (http://climate.konza.kasu.edu). Four replicate irrigation plots (1.0 m$^2$) were interspersed by four un-watered control plots. Details of the irrigation transects in the Konza Prairie LTER and their experimental design have been published previously (O’Lear and Blair, 1999; Williams, 2007). Soils that were irrigated were maintained at water potentials consistently above −1500 kPa during the growing season, whereas soils that receive only natural rainfall inputs often drop well below −5000 kPa for extended periods of time (Williams, 2001).

Twenty 2.2 cm dia by 10 cm deep soil cores were collected and pooled from each plot in late September 2003. After collecting, soils were immediately passed through a 4-mm mesh sieve, meticulously hand-cleaned of roots and organic debris, and stored at 4 °C for less than 2 weeks. Several natural rainfalls occurred during September, and as a result all soils had soil water contents between 32 and 34%. The relationship between soil water content and soil water potential was estimated using standard methods on freshly sieved soils (Klute, 1986; McHnes et al., 1994).

2.2. **Pre-incubation and application of drying and rewetting water stress**

Seventy-five field-moist soils (15 g dry weight) were weighed into 125 ml erlenmeyer flasks and brought up to −33 kPa soil water content and incubated for 3 days (25 °C). Separated into five groups of 12 for each water stress level, soils were either kept moist at −0.033 MPa, or were slowly and linearly dried over a 0.6, 1, 2, or 3 day period to 1.5, 4, 15, and −45 MPa, respectively. On the 4th day, the dried soils were brought back to −0.033 MPa over a ~10 s period with a single addition of deionized water. Three replicate soils from each water stress group were then directly extracted with 75 mL of 0.01 M K$_2$SO$_4$, frozen, and lyophilized. In one set, portions were analyzed by GC–MS, NMR, and LC–MS. The next set was analyzed for several elemental nutrients (Na, Ca, P, K, Mn, Mg, Fe) and inorganic N. The third set of soils were analyzed by immediately fumigating with chloroform for 2 days, and a fourth set was allowed to incubate for 2 days. The fifth set was monitored over 20 h for the production of CO$_2$. The headspace CO$_2$ concentrations were measured using a Varian 3600 gas chromatograph (Varian, Inc., Palo Alto, CA) equipped with a 2-m Porapak Q column and a TCD detector. The amount of CO$_2$ evolved was subtracted from a mason jar containing no soil and 2-mL of water.

2.3. **Determination of microbial biomass and soluble C**

Microbial biomass C was estimated by the chloroform fumigation-extraction method using a $k_c$ of 0.45, (Vance et al., 1987). Samples were extracted and analyzed for soluble C and N according to the method of Bruulsema and Duxbury (1996). One set of samples for estimation of microbial biomass was fumigated for 48 h with chloroform, and another set of non-fumigated controls continued to incubate. After mixing on an orbital shaker (300 rpm) for 1 h, soil extracts (0.01 M K$_2$SO$_4$) were allowed to settle for 20 min, centrifuged at 12,000 × g for 15 min, passed through a 0.22 μm nylon filter, and freeze dried. Subsamples of the solutions were transferred to acetone-rinsed tin squares (Micronalysis, Manchester, UK), dried at 65 °C, and processed for total C and N. A separate measure of soluble C using a Shimadzu TOC-5000 with UV detection corroborated results using the method of Bruulsema and Duxbury (1996).

2.4. **Analysis of extractable metabolites by gas chromatography mass spectrometry and liquid chromatography**

The freeze dried samples were separated into three equal parts. One part was used for GC–MS and NMR analysis, and the other two parts were brought to pH 2 using HCl and lyophilized again. In silanized reaction vials, one freeze dried sample was derivatized with 1:1 acetonitrile: MTRBTFEA (N-methyl-N-tert-butyldimethylsilyl-trifluoroacetamide) while the other was silylated using 22% Trimethylsilylimidazole (TMSI) in pyridine (Regis Technologies, Inc., Morton Grove, IL) as reported by Fan et al. (1997). The tert-butylidimethylsilyl (TBDMS) derivatives were formed after...
sonication for 2 h (60 °C) following an overnight incubation at room temperature (24 °C). Trimethylsilyl (TMS) derivates were formed following sonication for 60 s and heating in a water bath (60 °C) for 25 min. The TMS derivatives were directly analyzed on an ion trap GC/MS (Polaris Q Trace GC, ThermoFinnigan) using electron impact ionization. The TBDMS derivatives were dried to 5 μL and re-dissolved in 100 μL of hexane and also analyzed by GC–MS. An Rtx-5Sil MS (30 m × 0.25 μm × 0.18 mm id) 5%-phenylmethylpolysiloxane capillary column was used (Restek, Bellafonte, PA). One μL sample was injected using splitless mode. The He carrier gas flow rate was maintained at 1.1 mL min⁻¹. The temperatures at the injection port, transfer line, and ion source were 280, 280, and 200 °C, respectively. The initial oven temperature was held at 60 °C for 2 min, ramped to 150 °C at 20 °C min⁻¹, then ramped to a final temperature of 290 °C at 6 °C min⁻¹, and maintained at this temperature for 3 min. The mass spectrum in full scan acquisition mode (47–750 m/z) was collected at 6 min after the start of the GC run. Standards containing mixtures of amino acids, carbohydrates (A6407-1, A9906-1, A6282-5) and pure standards were used to verify and confirm mass spectra (Sigma-Aldrich, St. Louis, MO). Pure standards of proline, trehalose, galactose, mannitol, inositol, sorbitol, xylitol, glutamic acid, glycerol and proline were prepared for derivitization using either MTBSTFA or TMSI and subjected to GC/MS analysis as described above.

Several underivatized samples from each treatment were also analyzed using an HPLC system (Waters2695, Alliance, Waters) equipped with an anHrla ms NH2 HPLC Column (5 μm particle size, 15 cm × 4.6 mm, Supelco) and a light scattering detector (ELSD800, Alltech). The isocratic mobile phase containing acetonitrile/water (80/20, v/v) was used. Proline, erythritol, betaine, ectoine, mannitol, and glycerol were used as standards for the HPLC analysis.

2.5. Elemental nutrients and inorganic N analysis

Equal portions were used from the extraction of one set of replicate soils for analysis of inorganic N and elemental nutrients (Na, Ca, P, K, Mn, Mg, Fe). The NH₄¹-N and NO₃⁻N concentrations were measured colorimetrically on an Alpkem Autoanalyzer (Alpkem Corp., Clackamas, OR). Ammonium-N was determined by the Griess–Ilosvay technique (Keeney and Nelson, 1982). The treatment effects of field irrigation and laboratory drying-rewetting. LSD tests were used to determine where significant differences occurred.

3. Results

3.1. Pools of microbial, soluble, and respiratory C following the rewetting of dried soil

The amounts of microbial and soluble pools of C following the rewetting of dried soil are shown in Fig. 1a,b respectively. In the case of microbial C, the most noticeable effect was related to the lower amount of microbial C extractable in relatively non-water stressed soil (−0.9, and −1.5) compared to soils dried to lower water potentials (−4, −15 MPa) before rewetting. The effect of water potential on the amount of microbial extractable C was somewhat reduced in the driest soil (−45 MPa) compared to the soils at intermediate levels of drying (−4, −15 MPa). Moreover, there was an effect of field irrigation on the amount of microbial extractable C at the intermediate level of drying (−4 KPa). These trends were consistent using several different methods of calculating the microbial biomass. For instance, whether or not we subtracted the soluble C derived from the extraction of the control soil or the immediately rewetted soil, the same general trends were apparent.

The trend for soluble C was more consistent across the water stress gradient than that of microbial C. Compared to those with lower water stress, soils that were dried to lower water potentials before rewetting tended to have greater amounts of extractable soluble C (Fig. 1b). A 6-fold increase in the amount of soluble C was
extracted from the most water stressed soil compared to the continuously moist soil in the irrigated treatment. The effect of field irrigation was also very strong, with greater amounts of soluble C made extractable with degree of water stress when compared to soils that were naturally drought-prone. The two most extreme water stress treatments had 30–60% more extractable soluble C in the field irrigated compared to the non-irrigated-drought-prone soil.

At all levels of water stress, the respiratory pools of CO₂–C over the 20 h incubation were very similar in size to those of extractable soluble C (Fig. 2). The overall trend across the water stress gradient was also similar, showing greater release of C upon rewetting as the soils were dried to lower soil water potentials. Drying and rewetting of field irrigated soils showed a greater respiratory response than drought-prone soils, with the greatest effect of irrigation on both soluble and respiratory pools of CO₂–C at the two most extreme water stress treatments (Figs. 1 and 2).

Differences in inorganic N were not found along the water stress gradient, averaging 0.84 µg N g⁻¹ soil. Related to this there was no affect of irrigation nor was there an irrigation by laboratory water stress interaction. The C:N ratio of the soluble pool averaged 4.5, 2.7, 3.9, 4.3, and 7.3 in the soils dried −0.033, −1.5, −4.0, −15, −45 MPa, respectively, before rewetting. The soil dried to the lowest water potential (−45 MPa) and rewetted had a significantly greater C:N ratio than the other water stress treatments. Overall, the low C:N ratio of the soluble pool indicates that N, perhaps in the form of proteins or high N containing humic materials, comprise an important part of the remaining undescribed pool of organics.

The concentrations of elemental nutrients were generally very low (<0.1 µg/g soil) and did not differ between treatments

3.2. Characterization of the soluble and microbial organic pools by NMR, LC–MS, and GC–MS

Characterization of silyated amino acids and short chain carbohydrates using gas chromatography was sensitive enough to detect ~1 ng µl⁻¹ quantities. In the case of the sample extracted with 0.01 M K₂SO₄ directly after rewetting of the dried soils, each 1 µl of derivatized sample injected on the GC–MS would have been derived from a sample containing between 150 and 500 µg extractable soluble organic carbon per 100 µl. This translates into a capacity to detect a derivatized carbohydrate or amino acid if it comprised 0.1% (w/w) of the sample. We confirmed the paucity of extractable soluble sugars and amino acids by repeating the most extreme drying (−45 MPa) and rewetting experiment by combining the extracts from a 120-g (dry weight) soil sample. Detection limits using HPLC were two to four times greater than that for GC–MS. Using these multiple methods, however, there were no detectable levels of amino acids in our samples (Fig. 3a). Mass spectra that were indicative of glycerol, pyranoses, urea, and several fatty acids were detectable. However, lacking appropriate standards, these compounds could not be absolutely identified or quantified. When we assumed a similar response of these peaks to that of glucose, we collectively could account for approximately 2–4% of the soluble C pool.

In contrast, we were able to detect copious amounts of saccharides, organic acids, and amino acids from extracts of the fumigated samples (amino acids are shown in Fig. 3b). Yet, their collective mass could account for only 10–18% of the C in the fumigated sample extract. Most of the soluble sugars and amino acids in these extracts probably derive from microbial cytoplasm. However, if enzyme activity remains intact, the released intracellular enzymes may breakdown proteins and oligosaccharides into their constituent monomers. Protease activity, for example, remains functional directly after chloroform fumigation of soil (Zelles et al., 1997). For the purposes of this paper, the primary emphasis of reporting the results of the fumigated samples was to serve as a positive control indicating that our methodology for derivitization and detection of the metabolites was viable.

Nuclear magnetic resonance analysis of the soluble organic pools from the −0.033, −4.0, and −45 MPa provided further insight into the chemical characteristics of the extracts (Figs. 4a–c), and reflect the difference in the amount of extractable organics as shown in Fig 1b. The ¹H spectra suggests a mixture of compounds such as carbohydrates (¹H chemical shift: 3.0–4.5 ppm), alcohols, organic acids, and amino acids, collectively resonating mainly in the ¹H chemical shift region of 0.7–3.0 ppm. Several large peaks in the downfield region, especially the peak at 8.42 ppm, are likely derived from amino groups in compounds such as glycine, alanine, valine, leucine, serine, thereonene, asparagine, glutamine, glutamate, and lysine.

Overall, the chemical composition of the soluble organic pool indicates some degree of similarity between the water deficit treatments. For example, the large peaks at 0.12, 1.30, 1.78, and 3.92 among others, are found to be dominant members in all of the extracts. However, there are large differences in the relative size of these peaks across the samples, suggesting that the chemical composition of the extracts differ from each other. Perhaps the most striking difference is the shift in peak dominance in the upfield and aliphatic region of the spectrum as the level of water stress is increased. In Fig. 4c for example, the two peaks at 2.02 and 1.87 ppm increased in intensity relative to Fig. 4a,b. In a complex mixture these peaks could be represented by many possibilities. However, peaks with similar retention times have been previously identified as belonging to N-acetyl aspartine/gamma-butyrobetaine/acetate and aminobutyric acid (GABA)/methyl acetate, respectively, in extracts of plant tissues (Fan, 1996).

Given the paucity of detectable monomeric amino acid and sugar derivatives, it seems likely that the peaks in the NMR spectra are probably derived from amino acid and sugar residues that are components of longer chain carbohydrate and peptide molecules. This finding is further corroborated by the presence of crosspeaks between the O-alkyl and the aliphatic region in the TOCSY (Total Correlation Spectroscopy)-NMR spectrum that are indicative of amino acid residues (data not shown).

4. Discussion

The primary objective of this experiment was to characterize the molecular composition of the organic pool made soluble following
In our study, soils that were dried to the two lowest (−15, and −45 MPa) but not the greater (−0.33, −1.5, −4 MPa) soil water potentials, rewetting resulted in the production of very low concentrations of identifiable organics. Collectively these organics accounted for ~2–4% of the total soluble C pool across all treatments. In contrast to our hypothesis we thus found little evidence that the extractable organic pool and the flush of organics, regardless of degree of drying before rewetting, was composed of significant concentrations of microbial amino acids, simple sugars, or other metabolites such as glycine betaine, ectoine, and mannitol. We cannot rule out the possibility that much of the soluble material released following rewetting is derived from the physical forces that cause slaking or the breakdown of aggregates. Our results are in agreement with the common finding that soils are typically comprised of de pauparate concentrations of water soluble low molecular weight organics (Van Hees et al., 2005; Jones and Willet, 2006; Fischer et al., 2007).

Contradictory to the de pauparate concentrations of amino acids and sugars in our samples, the release of intracellular protein, amino acids, and sugars that were measured following the exposure of four soil bacterial isolates to dilution stress (Halverson et al., 2000) were large, releasing of up to 20% of the intracellular amino acid pool when water potential was increased from −2 MPa to −0.33 MPa. However, it is possible that the amino acid pool in that study may also contain polypeptides and/or proteins. Sizable pools of soluble sugars were also detected following dilution stress. In closer agreement with our work, one of the four isolates (Bacillus pumilis) studied by Halverson et al. (2000) did not release any measurable amount of amino acid or sugar following dilution stress. This latter finding indicates that not all soil microorganisms respond to dilution stress by releasing intracellular amino acids and sugars into the extracellular soil environment.

Our inability to measure detectable concentrations of sugars, amino acids, alcohols and specific osmolytes show some agreement with the study by Fierer and Schimel (2003). Following the rewetting of dry soil they found a sizeable respiratory CO2 release of previously microbial metabolized 14C. Much less of this labeled material, interpreted as coming from the cytoplasm, contributed to the flush of carbon made soluble following the rewetting of dry soil. Perhaps soil microbial communities utilize a different mechanism for coping with drying and dilution stresses than the expulsion of low molecular weight organic osmolytes that are reported in studies of microbial cultures (Tschicholz and Truper, 1990; Schleyer et al., 1993). The costs of osmolyte production for a single drought event have been calculated to consume up to 5% of net annual productivity in certain grassland systems (Schimel et al., 2007). From that perspective, it seems ecologically and energetically sensible that microbial communities in soils may have adapted alternative coping mechanisms for dealing with drying and dilution stresses other than dumping precious high energy compounds into the extracellular soil matrix.

Several questions regarding the soil and microbial response to drying and dilution stress in soil remain unresolved but the results of this study bring to light further questions. Because there have been no other studies characterizing the chemical nature of the pool of soluble organics as a result of drying and dilution stress in soil, we can not make direct comparisons to other in situ soil communities. Several research papers have studied how microbial cultures derived from soil isolates respond to water stress, however, and we can thus begin to speculate on some of the reasons for
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microbial communities are composed of extraordinary phylogenetic diversity. If metabolic

diversity follows a similar trend to phylogenetic diversity then it would not be surprising if the pool of organics made extractable

was composed of a highly diverse array of microbial organic osmolytes. If, for example, several hundred different types of

osmolytes constituted the sample, then they may not have been at detectable concentrations. Indeed, several recent works show that

a diversity of osmolyte types exist, with new and novel microbial osmolytes having been recently discovered (Martin et al., 1999; Roebler and Muller, 2001).

In conjunction with our $^1$H NMR results, it is clear that there is a diverse array of peaks that could represent a rather large diversity

type of compound. Dominance of peaks in the alkyl and especially the O-alkyl region are certainly reminiscent of the compounds

containing oxygen and nitrogen groups with aliphatic side chains, which could easily match the description of many soluble organic

osmolytes and carbohydrates. Regardless of the degree of water

stress, ~25% of the soluble C reacted using the phenol sulfuric acid

assay (Dubois et al., 1956), suggesting that oligosaccharides make up

a substantial proportion of the rewetting labile pool (data not shown). It is interesting furthermore that our C:N ratios were

similar to estimates for cytoplasm C:N in a Psudomonas sp. but 5–

6× lower than that for the fungus Aspergillus flavus (Schimel et al.,

1999). Suffice it to say, the $^1$H spectra are consistent with a broad

range of compounds. We suspect that further chemical character-

ization of the unknown 70% in these extracts will yield insights

about how soil and microbial communities contribute to rewetting

pulses in dry soils.

It is important to consider whether the concentrations of organic osmolytes in soil microbial cells could theoretically yield
detectable concentrations using our analytical methods. Under osmotic stress, microorganisms have been shown to accumulate

molar concentrations of organic osmolytes in the cytoplasm (Yancy et al., 1982). An estimate of cytosolic volume (four times the dry

weight; 50% C) of 8 mL g$^{-1}$ soil could thus contain 8 mmol of compatible solutes. For simplicity, if proline was the sole compat-

ible solute accumulated in a microorganism during soil drying, then up to 800 µg of proline might be anticipated to accumulate in

microbial cells in each gram of soil. At this concentration the amount of carbon contributed by proline would be substantially

larger than the entire soluble carbon pool made extractable from the soil dried to the lowest water potential of ~45 MPa (~400 vs

60 µg C soil; Fig. 1). These calculations serve to illustrate the copious amounts of osmolytes that could be released into the extracellular

matrix upon osmotic upshock that ensues during the wetting of dried soils. The cytoplasmic volume of microbial cells is sensitive to

many factors such as cell size and physiological state, so lower concentrations of osmolytes are likely. Moreover, the proportion of

the microbial community in a state of dormancy in soil is also large. However, the amounts suggested by the theoretical calculation

above would need to be at least three to four orders of magnitude lower before they drop below our limit of detection.

The observation that microbial biomass C tended to increase with degree of water stress is not unprecedented (Lundquist et al.,

1999), but has been observed at a lower frequency than unchanged or decreasing microbial biomass in response to water stress (e.g.

van Gestel et al., 1993; Wu and Brookes, 2005; Gordon et al., 2008).

In our experiment where we measured microbial biomass by

differences between our results and those of cultured microor-

organisms. For one, studies that have tested for the microbial

production and extracellular release of osmolytes in response to

water stress have typically utilized some form of salt rather than

the removal of water through drying to induce a decrease in water

potential. Other differences in these studies are also evident.

Microorganisms in culture studies are typically grown under water

stress conditions in the presence of copious carbon and nutrients, whereas the microbial communities in soil in situ, are predomi-
nantly in resting states that exhibit much lower rate of growth. To

understand how soil microbial communities respond to drying and rewetting stress, it may be necessary to focus on in situ responses and further mimic the true matrix effects that are associated with soil drying and rewetting.

Several alternative hypotheses may also serve to explain the absence of detectable concentrations of soluble organic osmolytes in our samples. For one, the sorption of charged low molecular weight compounds to clays and soil organic matter may reduce the concentrations of these compounds in the soil solution (Kalbitz et al., 2000). In that case, we may not be able to detect the microbial response to dilution stress. Microbial recapture and uptake of released osmolytes following dilution stress have also been noted to occur (Tschicholz and Trüper, 1990). It is possible that before

extraction of the metabolites that many of them were taken back up by the cells and/or metabolized. As well, during the 30 min

extraction of the soil on the reciprocal shaker, it is possible that the microbial cells had the opportunity to re-assimilate many of the

metabolites that were released following dilution stress. Microor-

ganisms in soil may also utilize an undiscovered mechanism for

contending with dilution stress that does not rely on the expected osmolyte stress response. And lastly, soil microbial communities

are composed of extraordinary phylogenetic diversity. If metabolic

diversity follows a similar trend to phylogenetic diversity then it would not be surprising if the pool of organics made extractable

was composed of a highly diverse array of microbial organic osmolytes. If, for example, several hundred different types of

osmolytes constituted the sample, then they may not have been at detectable concentrations. Indeed, several recent works show that

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larger than the entire soluble carbon pool made extractable from the soil dried to the lowest water potential of ~45 MPa (~400 vs

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the microbial community in a state of dormancy in soil is also large. However, the amounts suggested by the theoretical calculation

above would need to be at least three to four orders of magnitude lower before they drop below our limit of detection.

The observation that microbial biomass C tended to increase with degree of water stress is not unprecedented (Lundquist et al.,

1999), but has been observed at a lower frequency than unchanged or decreasing microbial biomass in response to water stress (e.g.

van Gestel et al., 1993; Wu and Brookes, 2005; Gordon et al., 2008).

In our experiment where we measured microbial biomass by

Fig. 4. $^1$H NMR spectra of soluble carbon extracted from continuously moist soils (a) and following the rewetting of soil previously dried to –4 (b) and –45 MPa (c).
extracted directly following the rewetting of the dry soil, it seems obvious that significant microbial growth would not have had time to occur as a result of rewetting. Perhaps then it is a function of native tallgrass prairie soils containing very large reserves of organic matter and microbial biomass. High structural stability of the soil and its aggregates may have the tendency to protect microorganisms from extraction using the traditional chloroform fumigation method. While the exact role that rewetting of a dry soil has on aggregate stability and turnover is still uncertain (Degens and Sparling, 1995; Denef et al., 2001; Mikha et al., 2005; Cosentino et al., 2006), the breakdown of aggregate fractions which protect microorganisms from environmental stresses may work in association with the upshock stress to weaken microbial cells and make them more susceptible to chloroform fumigation. Redistribution of total microbial carbon to the cytoplasm during water stress may also make the proportion of carbon in soil microbial cells more amenable to extraction using the fumigation-extraction assay (Schimel et al., 1989). Likewise, the mode of microbial adaptation to water stress, perhaps through the release of intracellular cytoplasmic solutes in response to dilution stress may serve to explain why some studies show reductions in extractable microbial biomass when dry soils are rewetted.

As part of our second objective we found a consistently higher flux of soluble carbon in the continuously moist compared to the drought-prone soils. Perhaps this result is a consequence of changing microbial sensitivity to drying after 11 years without water stress. If the microbial community composition or structure was altered in such a way to allow water stress intolerant microorganisms to grow in the irrigated soils then certainly some of the extra pulse due to the continuous moisture may be derived from morbidity or osmolyte release by the microbial community (Kieft et al., 1987; Halverson et al., 2000). Previous research has suggested that microbial communities are altered in tallgrass prairie soil due to continuously greater water availability in irrigated compared to drought-prone soil (Williams and Rice, 2007). However, the degree to which the change in the community is due to differences in water availability or to indirect impacts that result from changing soil water dynamics are not known. Another possible explanation for the greater flux of soluble carbon in continuously moist soil may come from the accumulation of organics in aggregates and on colloidal surfaces that are normally labile to the effects of water stress, but tend to accumulate in its absence. To our knowledge, no other studies have looked at the effects of long-term water availability on the susceptibility of organic matter to drying and rewetting. Because we detected such low concentrations of microbial derived metabolites, we are not able to discern whether some of the differences between the continuously moist and drought-prone soils was a consequence of abiotic or biotic forces. However, it has been shown that instantaneous drying and rewetting pulses of C and N can be diminished by an amount that is proportional to the number of preceding drying and rewetting events (Mikha et al., 2005). Hence, the build up of labile carbon susceptible to drying and rewetting might be expected in soil following 11 years of irrigation. It has been shown that soil communities can be highly resilient to drying and rewetting stresses (Lundquist et al., 1999; Fierer et al., 2002). Indeed previous research on these same soils came to the conclusion that the structure of microbial communities in native tallgrass prairie soils are resistant to the effects of water stress (Williams, 2007). Measurements on the whole and actively growing community using PLFA and 13C-PLFA stable isotope probing showed only modest changes in the community profiles exposed to dilution stress. Physiological adaptation to water stress by microorganisms appears to be a widespread trait in soil.

In this paper we wanted to determine if the flux of soluble carbon that occurs following the wetting of dry soil could be accounted for by the microbial mechanism of organic osmolyte expulsion. While we were not able to conclusively determine the extent to which microorganisms are responsible for the flush, we have shed light on the chemical characteristics of the soluble pool made extractable following the wetting of a dry soil. The flush response was not dominated by compounds that would suggest a strong microbial release of amino acids, sugars and osmolytes. Further studies on the chemical composition of this pool may shed further light on the microbial and non-microbial sources of the rewetting flush in dry soil.

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