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Response of osmolytes in soil to drying and rewetting

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ABSTRACT

The accumulation and subsequent release of microbial osmolytes in response to drying and rewetting are thought to be key players in C and N dynamics, yet studies on soils have failed to support this hypothesis. The aim of this experiment was to determine how low-molecular weight compounds, and osmolytes in particular, are affected by drying and rewetting. Water deficits were imposed slowly by withholding water for 21 weeks from large (200 L) mesocosms vegetated with a globally widespread grass *Themeda triandra*. A broad spectrum of small molecules in extracts was identified and quantified by capillary electrophoresis–mass spectrometry and gas chromatography–mass spectrometry. Compared with controls, drought-stressed mesocosms contained >10-fold larger amounts of known microbial osmolytes: ectoine, hydroxyectoine, betaine, proline-betaine, trigonelline, proline, trehalose, arabitol. The pool of osmolytes accounted for 3.6% of $CHCl_3$ labile TOC in control mesocosms and 17% of $CHCl_3$ labile TOC in drought-stressed mesocosms. There was no evidence that rewatering led to a large pulse of osmolytes in free solution. Instead osmolytes decreased to control concentrations within 1–3 h of rewatering – probably indicating rapid uptake by microbes and plants. Results of this study suggest that osmolytes can account for a substantial fraction of microbial C, and are at least one of the ways that soil microbes cope with water deficits.

1. Introduction

Water deficits are a common feature of large tracts of the terrestrial biosphere. Some biomes are regularly exposed to water deficits (e.g. arid, semi-arid and Mediterranean), while prolonged periods of below-average precipitation may lead to drought in biomes that do not normally experience water deficits. For example, recent years have seen prolonged periods of below-average precipitation give rise to several sub-continental scale droughts: central/south-west Asia (1998–2003), western North America (1999–2007), Australia (2002–2003), Europe (2003) and Amazonia (2005) (Cook et al., 2004; Trenberth et al., 2007; Thomas et al., 2009). There is evidence to suggest that the geographic area affected by droughts has increased in the past four decades (Dai et al., 2004) and climate change projections suggest decreased precipitation and increased occurrence and/or severity of drought in most sub-tropical and mid-latitude regions of the globe (Meehl et al., 2007).

One globally important consequence of water deficits is their effect on key soil processes such as C and N mineralization. For example, it has been known for several decades that water deficits reduce microbial activity of soils while rewetting commonly increases microbial activity and leads to a pulse of C and N mineralization (e.g. Birch, 1958, 1964; Bottner, 1985). The mechanisms underpinning the response of soil to drying and rewetting remain controversial (Borken and Matzner, 2009), but almost certainly involve processes altered at the molecular scale (Schimel et al., 2007; Boot et al., 2013).

One mechanism that may at least partially underpin responses to water deficits and rewetting is the synthesis and metabolism of low-molecular weight organic solutes by soil microbes. In response to decreasing water potentials, soil microbes lower their solute potential by synthesizing or importing organic solutes (Lippert and Galinski, 1992; Kempf and Bremer, 1998a,b; Halverson et al., 2000; Hasegawa et al., 2000; Wood et al., 2001). Typically organic solutes accumulate in the cytosol to counter concentrations of inorganic ions in vacuoles (thus contributing to osmotic balance between compartments, and overall turgor). The organic solutes that accumulate are termed compatible solutes, or osmolytes, and tend to be low-molecular weight compounds that do not interfere with metabolism even at very high concentrations. Osmolytes include C- and N-containing compounds such as some sugars and sugar alcohols (e.g. trehalose and arabitol), quaternary ammonium compounds (e.g. betaine) and pyrimidine derivatives (e.g. ectoine) (Csonka, 1989; Lippert and Galinski, 1992; Hasegawa et al., 2000; Wood et al., 2001). At least two mechanisms may underpin how

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the rapid increase in water potential that follows rewetting of dried soils leads to a flush of C- and N-containing substrates and metabolism. First, rewetting of dried soils leads to hydration and lysis of dead microbial cells that accumulated during the drying period. Lysed cells may subsequently serve as substrates for those microbes that survived (Kieft et al., 1987; Wu and Brookes, 2005; Borken and Matzner, 2009). Second, rewetting pose a major stress for those microbes that survived the drying cycle (Schimel et al., 2007). The stress arises because at the end of a drying cycle the surviving soil microbes have a strongly negative solute potential due to accumulated osmolytes, and thus microbes need to dispose of accumulated intracellular osmolytes so as to avoid uncontrolled influx of water (Kieft et al., 1987; Csonka, 1989; Schimel et al., 2007; Borken and Matzner, 2009).

Culture-based and modeling studies suggest accumulation and subsequent release of osmolytes in response to drying and rewetting cycles are key players in C and N dynamics (Schimel et al., 2007), yet there is little empirical support from studies with soil. If microbes use osmolytes as constitutive or inducible defenses against water deficits one would predict that osmolytes would comprise a substantial proportion of microbial biomass in dry soil (Boot et al., 2013) and rewetting would lead to a flush of osmolytes in the extracellular matrix (i.e. soil solution) (Williams and Xia, 2009). At present there is little support for these predictions with recent studies failing to find large constitutive or inducible amounts of osmolytes in a field experiment on seasonally dry grassland soil (Boot et al., 2013) or forest soil (Göransson et al., 2013) or soils exposed to laboratory water stress treatments (Williams and Xia, 2009; Kakumanu et al., 2013). Partial support comes from a study of soil extracts from seasonally dry field sites that reported abundant osmolytes in five out of seven sites (Warren, 2013b). However, limited conclusions could be drawn from the latter experiment because it did not examine seasonal variation in osmolytes or manipulate water availability.

The inconsistency of results may reflect true biological differences, but a proportion can probably be explained by varying durations of water stress (Borken and Matzner, 2009), and the comprehensiveness with which osmolytes were profiled (Warren, 2013a). For example, studies that impose water stress for a short periods (e.g. 4 days: Williams and Xia, 2009) may be too rapid for prompting significant osmolyte accumulation (Turner, 1986) and would not encompass changes in the microbial response to rewatering that occur only after prolonged drought (Meisner et al., 2013). A diversity of different C- and N-containing molecules can be accumulated as osmolytes (Csonka, 1989; Lippert and Galinski, 1992; Hasegawa et al., 2000; Wood et al., 2001), and thus studies that quantify only N-containing osmolytes (Boot et al., 2013; Warren, 2013b) may fail to see quantitatively significant changes in C-based osmolytes (e.g. sugars and sugar alcohols) while data on compound classes (e.g. monosaccharides and amino acids, Göransson et al., 2013) are difficult to interpret in terms of osmolyte accumulation because the assays do not distinguish osmolytes from the large background of non-osmolytes.

The aim of this experiment was to determine how low-molecular weight osmolytes are affected by water deficit and rewetting. Mesocosms were filled with soil and seedlings from *Themeda triandra* Forssk. grassland. The response of *T. triandra* grassland to drying and rewetting may be globally significant because it is one of the most widespread grasses in grassland ecosystems of Africa, Asia and Australia and is regularly exposed to water deficits (Dell'Acqua et al., 2013). Moreover, climate change projections suggest the future will see increased frequency and severity of water deficits across much of the species range (Meehl et al., 2007). To assess the significance of osmolyte accumulation in responses to water deficits and rewetting, I tested a) if osmolytes were a larger fraction of microbial C in mesocosms from which water had been withheld than control mesocosms kept well watered, and b) if rewetting led to a large pulse of osmolytes in free solution. Water deficits were imposed slowly by withholding water from large (200 L) mesocosms so as to mimic the situation in nature and allow sufficient time for osmolytes to accumulate. A broad spectrum of C- and N-containing osmolytes was quantified by gas chromatography–mass spectrometry (Roessner et al., 2000; Warren et al., 2012) and capillary electrophoresis–mass spectrometry (Warren, 2013a), while measurements of CO_2 efflux from the soil surface was used as an index of microbial activity and to place the putative flush of osmolytes in the context of the large flush of CO_2 efflux induced by rewetting of dried soils (Birch, 1958, 1964; Jarvis et al., 2007).

2. Materials and methods

2.1. Chemicals

Methanol, acetonitrile and formic acid were LC/MS (Optima) grade from Fisher Chemical (Scoresby, Vic, Australia). Ammonium formate (Acros Organics, Geel, Belgium), ammonium hydroxide (28–30% NH_3, Sigma, Sydney, Australia), potassium sulfate (Sigma), methoxyamine hydrochloride (Sigma) and iodomethane (Sigma) were analytical grade, while pyridine, chloroform, and *N*-Methyl-*N*-trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) were derivatization or GC grade.

All electrolytes, rinsing solutions, standards and samples were prepared with 18.2 MΩ cm resistivity ultra-pure water (Arium, Sartorius, Goettingen, Germany). Approximately 140 standards (comprising organic N monomers, small carbohydrates, and organic acids) were prepared from their free acids or salts purchased from Sigma. α-*N*-methyl-histidine, α-*N*,*N*-dimethyl-histidine and *N*-methyl-proline were from Chem-Impex (Chem-Impex International, Wood Dale, IL, USA). All standards of chiral amino acids were L enantiomers, while carbohydrates were D enantiomers. Hercynine (*Nα*,*Nα*,*Nα*-trimethyl-L-histidine) was synthesized according to Reinhold et al. (1968), as described recently (Warren, 2013b).

2.2. Soil mesocosms

In June 2009 eight replicate mesocosms (painted steel drums, 572 mm diameter, 851 mm high) were filled with loam soil collected from A1 and A2 horizons of *T. triandra* grassland in western Sydney (34.0 S, 150.6 E, 75 m above sea-level). The intact soil was an abruptic lixisol and chemical properties have been described recently (Warren, 2013b). After collecting in the field, soil was sieved to 4 mm, mixed, and then mesocosms were filled with 200 L of soil at approximately the bulk density of field soil. Calibrated soil moisture probes (Hobo EC-5 Soil Moisture Smart Sensor, Onset Corp, Pocasset, MA, USA) were installed horizontally at a depth of 15 cm in four mesocosms (two control and two drought treatment). Volumetric water content was recorded every 30 min and stored on a datalogger (Hobo). In November 2009 mesocosms were planted with six-month-old seedlings of two perennial native grasses *T. triandra* and *Microlaena stipoides*. Mesocosms were held within a sunlit polythene-covered greenhouse that transmitted around 70% of sunlight. A ventilation system ensured that air within the greenhouse was well mixed and exchanged with external air, while a thermostated cooling system maintained maximum temperatures 5 °C above ambient from May to October and at ambient temperature from October to May (Fig. 1). Mesocosms were watered every 5–15 days so as to avoid development of water stress. When treatments commenced in September 2012 the above-ground dry mass of plants varied between 1000 and