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Do microbial osmolytes or extracellular depolymerisation products accumulate as soil dries?

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ABSTRACT

When dry soil is re-wet there is a pulse of C mineralization. It is likely that the pulse of mineralization is fuelled by soluble C that accumulates as soil is drying. When soil is drying soluble C could accumulate as products of exo-enzyme mediated depolymerisation (i.e. protein amino acids and small carbohydrates) in the extracellular fraction of the soil (i.e. adsorbed and in free solution). Alternatively there could be an accumulation of osmolytes within the microbial biomass. To test whether extracellular depolymerisation products and/or microbial osmolytes accumulate as soil dries, soil from a *Themeda triandra* grassland was dehydrated and then depolymerisation products and osmolytes were quantified by capillary electrophoresis-mass spectrometry and gas chromatography–mass spectrometry. A secondary aim of this experiment was to determine if the response of soil to drying is the same when soil is dehydrated rapidly in the laboratory as when an intact soil containing plants is slowly dehydrated by withholding water from large (200 L) mesocosms.

The responses of soil to dehydration differed between lab incubations and mesocosms, despite involving the same soil being dehydrated to the same final water content. When soil was dehydrated slowly in 200-L mesocosms the accumulation of osmolytes was more quantitatively significant than accumulation of depolymerisation products, whereas when soil was dehydrated more rapidly in lab incubations there was negligible accumulation of osmolytes but large accumulation of depolymerisation products. This study has highlighted that when soil dries the accumulation of osmolytes within microbial biomass and depolymerisation products within the extracellular fraction of soil are both quantitatively important and likely underpin the flush of soil CO_2 efflux when dried soil is re-wet.

1. Introduction

Water deficits are a common feature of large areas of the terrestrial biosphere. One globally important consequence of water deficits is their effect on C mineralization (Birch, 1958, 1964; Bottner, 1985; Reichstein et al., 2002; Carbone et al., 2011). In the most general sense, water deficits reduce microbial activity of soils while rewetting increases microbial activity and leads to a pulse of C mineralization (e.g. Birch, 1958, 1964; Bottner, 1985). The pulse of C mineralization upon re-wetting dry soil likely reflects interrelationships among microbial population dynamics, microbial physiology, substrate dynamics and diffusion. One enduring question that is yet to be resolved is the origin of the pulse of C that is mineralized after dry soil is re-wet. It is probable that the pulse of mineralization is fuelled by soluble C that accumulated in the drying soil, but there are two distinct ways in which soluble C could accumulate in drying soil.

Soluble C could accumulate as depolymerisation products in the extracellular fraction of the soil (i.e. adsorbed and in free solution). The reason soluble C is predicted to accumulate is because water stress causes a larger reduction in microbial uptake of exo-enzyme products than in exo-enzyme activity (Stark and Firestone, 1995). The exact reason that microbial uptake is slowed more than exo-enzyme activity remains a matter of conjecture, but it probably relates to a combination of diffusional limitations in drying soil, the spatial arrangement of substrates and microbes, and the direct effects of negative water potentials on microbe physiology. For example, we know that as soil dries there is a progressive loss of hydrologic connectivity that decreases rates of diffusion and access to substrates (Or et al., 2007; Moyano et al., 2013). Moreover, models predict that as soils dry there is an accumulation of soluble C in the extracellular fraction of soil due to reduced microbial uptake (Manzoni et al., 2014) and/or existence of hydraulically-

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disconnected soil patches ("hotspots") (Evans et al., 2016; Manzoni et al., 2016). Irrespective of the underlying physics and physiology, it is predicted that as soil dries exo-enzyme activity continues largely unaffected but there is a large reduction in microbial uptake of exo-enzyme products. The simplifying assumption we make here is that depolymerisation is the predominant function of soil exo-enzymes, and thus we predict that as soil dries there is a net accumulation of the typical products of depolymerisation, *viz*., protein amino acids and mono-saccharides, in free solution and/or adsorbed to the soil stationary phase.

Another way in which soluble C could accumulate in drying soil is as osmolytes within the microbial biomass. The logic behind this hypothesis is that in response to decreasing water potentials soil microbes lower their solute potential by synthesizing osmolytes (Lippert and Galinski, 1992; Kempf and Bremer, 1998b, 1998a; Halverson et al., 2000; Hasegawa et al., 2000; Wood et al., 2001). Osmolytes include 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).

To date no studies have directly tested both hypotheses in the same experimental system, while studies testing only the osmotic adjustment hypothesis have yielded contradictory results. Culture-based and modelling studies suggest accumulation and subsequent release of osmolytes in response to drying and rewetting cycles could be key players in C and N dynamics (Schimel et al., 2007), but there has been mixed support from experiments with soil. One recent study observed >10-fold larger amounts of known osmolytes (ectoine, hydroxyectoine, betaine, proline—betaine, trigonelline, proline, trehalose, arabitol) in soils that had been water stressed (Warren, 2014b), consistent with microbes adopting a strategy of osmotic adjustment. In contrast, other studies have not observed osmolyte accumulation in drying soils (Williams and Xia, 2009; Boot et al., 2013; Göransson et al., 2013; Kakumanu et al., 2013; Warren, 2014a), perhaps indicating that in some soils the most favourable strategy for coping with water stress is not osmotic adjustment but instead accumulation of inorganic ions and/or dormancy (Manzoni et al., 2014).

The mixed evidence for osmotic adjustment may reflect true biological differences among soils, but a proportion could be due to the experimental system and procedures used to impose water stress. Some studies have used an experimental system involving intact soil with plants, and allowed soil to dry slowly over weeks to months as part of a field experiment (Boot et al., 2013) or large mesocosms (Warren, 2014b). Other studies have used lab incubations in which soil is dehydrated under strictly controlled conditions, often for a as little as a few days (Williams and Xia, 2009; Kakumanu et al., 2013) but sometimes for as long as a year or more (Meisner et al., 2013, 2015). Lab incubations are simpler and easier to control than field or mesocosm experiments, but we do not yet know if lab incubations are a reasonable proxy for what occurs in an intact soil exposed to a realistic drying cycle. There are at least three reasons that responses of soil to drying lab incubations could differ from what is observed in an intact soil. First, lab incubations do not include on-going inputs of C from plants and thus soil microbes may be more C limited than would be the case for an intact soil. Consequently the synthesis of osmolytes in lab incubations could be constrained by C limitations. Second, lab incubations often involve short-duration dehydration treatments (e.g. 4 days: Williams and Xia, 2009) that may be too rapid for prompting significant osmolyte accumulation (Turner, 1986) and may not encompass changes that occur only after prolonged drought (Meisner et al., 2013, 2015). Third, lab incubations typically involve disruption of soil structure, which can artefactually change dynamics of small organic compounds (Inselsbacher, 2014), microbe-substrate distances and responses to water stress (Manzoni et al., 2016).

The primary aim of this experiment was to directly test whether drying of soil leads to accumulation of extracellular depolymerisation products (e.g. protein amino acids, mono-saccharides) and/or microbial osmolytes. A secondary aim of this experiment was to determine if the response of soil to drying is the same when soil is dehydrated in the laboratory as when an intact soil containing plants is dehydrated by withholding water from large (200 L) mesocosms for 132 days. The experimental system used for these experiments were mesocosms were filled with soil and seedlings from *Themeda triandra* Forssk. Grassland that have been described previously (Warren, 2014b). The lab dehydration treatment collected soil from well-watered mesocosms, and then dehydrated it over a period of seven days. Osmolytes and depolymerisation products were localised to the extracellular fraction (adsorbed + free) by extraction of soil with 0.5 M K_2SO_4 and localised to the microbial biomass by extraction with 0.5 M K_2SO_4 + chloroform (chloroform direct extraction: Warren, 2015a). A broad spectrum of C- and N-containing osmolytes and depolymerisation products were quantified by gas chromatography—mass spectrometry (Roessner et al., 2000; Warren et al., 2012) and capillary electrophoresis-mass spectrometry (Warren, 2013a).

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 sulphate (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

This experiment used soil mesocosms that have been described previously (Warren, 2014b). In June 2009 16 replicate mesocosms (painted steel drums, 572 mm diameter, 851 mm high, 200 L volume) 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). Soil water retention characteristics were estimated from van Genuchten parameters for soil with the same texture (Van Genuchten, 1980; Leij et al., 1996). After collecting in the field, soil was sieved to 4 mm, mixed, and then each mesocosm was filled with 200 L of soil at approximately the bulk density of field soil. In November 2009 mesocosms were planted with six-month-old seedlings of two perennial native grasses *T. triandra* and *Microlaena stipoides*.