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Response of organic N monomers in a sub-alpine soil to a dry–wet cycle

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ABSTRACT

Cycles of soil drying followed by rewetting occur in most terrestrial ecosystems, but there is conflicting evidence as to the role of osmolytes in dry–wet cycles. The broad aim of this experiment was to determine how N-containing osmolytes and other organic N monomers are affected by rewetting of a moderately dry soil. In a sub-alpine grassland, experimental plots were irrigated with 50 mm of water near the conclusion of a typical late-summer drying cycle. Twelve putative osmolytes (proline, 8 quaternary ammonium compounds, trimethylamine N-oxide, ectoine, hydroxyectoine) and 60 other organic N monomers were identified and quantified by capillary electrophoresis-mass spectrometry of the free/exchangeable pool of soil water (0.5 M K_2SO_4 extracts) and microbial biomass (via chloroform fumigation extraction). The total concentration of organic N monomers was 25-times greater in fumigated than unfumigated extracts. Differences in relative abundance of compound classes and compounds between fumigated and unfumigated extracts suggested some compounds were localized to the free/exchangeable pool; others were predominantly microbial, whereas many were shared between pools. A striking feature of the free/exchangeable pool was that on an N-basis alkylamines were the most abundant compound class and accounted for 34% of the pool of organic N monomers. There was no evidence that osmolytes were the primary means soil microbes coped with dry–wet cycles. Instead, the pool of osmolytes was an invariant 4% of the pool of CE-MS detected monomers in K_2SO_4 extracts and 7% of the pool of CE-MS detected monomers in the chloroform-labile (microbial) fraction. The absence of substantial amounts of osmolytes may be because water stress was too mild or brief, or because osmolyte synthesis was limited by availability of energy, N or C and some alternative strategy was used to cope with water deficits.

1. Introduction

Cycles of soil drying followed by rewetting occur in most terrestrial ecosystems, and can have a profound effect on soil function. One globally important consequence of dry–wet cycles is their effect on ecosystem cycles of C and N. For example, 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; Birch, 1964; Bottner, 1985). These pulses of N mineralization and soil CO_2 efflux are globally important because they can account for a substantial fraction of annual fluxes (Reichstein et al., 2002; Carbone et al., 2011).

What remains unclear is the balance of physical versus biological mechanisms that underpin responses of soil to dry–wet cycles (Fierer and Schimel, 2003; Schimel et al., 2011; Göransson et al., 2013). Physical limitations arise because as soil dries there is a decrease in hydrologic connectivity between soil pores and this decreases rates of diffusion and microbial access to substrates (Or et al., 2007; Moyano et al., 2013). Exoenzyme activity continues, but there is limited movement of exoenzyme products from substrate to microbe, and thus products accumulate in soil. In the case of physical limitations, rewetting leads to a pulse of N and C mineralization because it permits transport of the accumulated products of exoenzyme activity to microbes, and physically disrupts soil thereby exposing previously protected substrates to microbial attack (Schimel et al., 2011). The biological mechanisms that can drive responses to dry–wet cycles are manifestations of how soil microbes cope with, and are affected by, water deficits. Soil microbes can employ different strategies to cope with water deficits (Manzoni et al., 2014). One way that soil microbes can cope with dry–wet cycles is via active osmoregulation. Osmoregulation with organic osmolytes involves soil microbes lowering their solute potential (thus maintaining the turgor pressure necessary to

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remain active) by synthesizing or importing organic osmolytes such as sugars and sugar alcohols, quaternary ammonium compounds and pyrimidine derivatives (Csonka, 1989; Lippert and Galinski, 1992; Hasegawa et al., 2000; Wood et al., 2001). Rewetting leads to lysis of microbes and excretion of osmolytes, with released osmolytes serving as substrates for those microbes that survived water stress – and thereby underpinning the flush of N and C mineralization (Fierer and Schimel, 2003).

There is conflicting evidence for accumulation of osmolytes in soil under water stress. For example, accumulation of large amounts of osmolytes was found in mesocosms of grassland soil exposed to a drying cycle (Warren, 2014), whereas large amounts of osmolytes were not observed in a field experiment with a grassland (Boot et al., 2013) or temperate forest soil (Göransson et al., 2013) or soils exposed to laboratory water stress treatments (Williams and Xia, 2009; Kakumanu et al., 2013). These differences among studies may be partly due to differences among ecosystems in how microbes respond to water deficits. Model predictions suggest that active osmoregulation is a favourable strategy in soils that experience moderate water deficits, whereas in soils that become very dry the optimal strategy is instead dormancy (Manzoni et al., 2014). Hence, the mix of microbial strategies used to cope with water deficits may differ among ecosystems according to the severity of water deficits that the ecosystem normally experiences.

The severity and duration of dry–wet cycles vary enormously among ecosystems. Soils from Mediterranean and (semi-)arid ecosystems may experience one prolonged and severe dry–wet cycle per year, whereas dry–wet cycles are less severe and of shorter duration in ecosystems that are less seasonal and have weaker water deficits. For example, in many temperate ecosystems rain-free periods of days to weeks cause soils to dry and become moderately water stressed before being rapidly re-wet when it rains. A suite of studies have examined responses of osmolytes to rewetting of soils exposed to long-term (months) water deficits (Boot et al., 2013; Göransson et al., 2013; Warren, 2014a), but much less is known about the role of osmolytes in the response of soil to a short-term, moderate dry–wet cycle. Responses of osmolytes to short-term water deficit and rewetting were examined in a laboratory study (Williams and Xia, 2009); however, data may not be applicable to field soils because water stress was imposed very rapidly under laboratory conditions and might have been too rapid for prompting significant osmolyte accumulation (Turner, 1986). Additional studies under realistic field (or field-like) conditions are required to ascertain the role of osmolytes in the response of soil to a short-term, moderate dry–wet cycle.

The broad aim of this experiment was to examine how the pool of N-containing osmolytes and organic N monomers was affected by (artificial) rewetting in a moderately dry soil. Experiments took place during late summer in a sub-alpine grassland in south-eastern Australia. To measure the largest responses likely in this ecosystem, the experiment was carried out when the soil had already undergone several natural dry–wet cycles and was at its driest point in the season. To determine how organic N monomers were affected by rewetting, experimental plots were irrigated with 50 mm of water near the conclusion of a typical late-summer drying cycle. Samples were collected immediately before rewetting, then 1, 7, 21 and 70 days after rewetting. A broad spectrum of N-containing osmolytes were quantified by capillary electrophoresis-mass spectrometry (Warren, 2013a) of the free/exchangeable pool of soil water (0.5 M K_2SO_4 extracts) and microbial biomass via chloroform fumigation extraction (Brookes et al., 1985). Data permitted testing of the hypotheses a) in dry soil the pool of osmolytes in the microbial biomass is large, b) rewetting leads to a large decrease in concentrations of osmolytes in the microbial biomass, and c) rewetting leads to a large pulse of osmolytes in free solution.

2. Materials and methods

2.1. Study site

The study site was a sub-alpine grassland in the Snowy Mountains of south-eastern Australia (36° 06′S; 148° 32′E; 1500–1600 m above sea level) that has been described previously (Warren and Taranto, 2010, 2011). The soil was a humic umbrosol (World Reference Base) derived from granodiorite. The soil is widespread across most of the alpine and sub-alpine regions of south-eastern Australia, albeit with variations in depth of the profile, presence of coarse fragments, and amount of organic material (Costin et al., 1952, 1964; McKenzie et al., 2004). From 0 to 30 cm the soil was a well-drained sandy loam without coarse fragments >2 mm. Below 30 cm there were abundant coarse fragments of granodiorite. In the upper 30 cm, pH (H_2O) was 4.5, organic C (Walkley and Black) was 12–17%, and total N was 0.2–0.3%. Soil water retention characteristics have not been measured at the site, nevertheless the volumetric water content at soil water potential (Ψ) of −1.5 MPa was estimated to be between 11 and 14%. These estimates are based on previous studies on similar soils in sub-alpine and alpine south-eastern Australia that reported the measured volumetric water content corresponding to −1.5 MPa was 13.8% (Griffin and Hoffmann, 2011) or 11–14% (Costin et al., 1964), while 11% was estimated from van Genuchten parameters for soil with the same texture (Van Genuchten, 1980; Leij et al., 1996).

A weather station was established at the field site in May 2007. Volumetric soil water content was measured at depths of 30, 10, and 5 cm with a standing wave probe (MP 406, ICT International, Armidale, Australia). Data were stored as half-hourly averages on a data logger (Smart Logger, ICT International). Based on long-term measurements interpolated from nearby permanent weather stations, the annual maximum temperature is 13 °C, while the mean annual minimum is 0.5 °C. Annual precipitation is in the order of 1200 mm. The mean duration of snow cover is 2–3 months, though this varies among years.

2.2. Experimental design

In 2008 12 experimental plots were established over an area of approximately 1 km^2. Plots were 2 m × 2 m. In January 2009 six plots were randomly allocated to a control treatment while six were allocated to an irrigation treatment. The aim of the irrigation treatment was to mimic the re-watering events that naturally occur during late summer and autumn. Data from the weather station indicated that natural re-watering events associated with thunderstorms or passage of major cold fronts commonly involved 25–50 mm of rainfall, and these tended to be more common in the later afternoon. To mimic these natural events it was decided to irrigate plots with 50 mm of water during the late afternoon. On 27 February 2009 between 1600 and 1800 h 50 mm of deionized water was applied to the irrigation treatment plots. Water was applied slowly with watering cans to permit infiltration, and at no time was any run-off observed. On each of the six control and six irrigated plots destructive harvests and measurements of gas exchange were made immediately before irrigation, then 1, 7, 21 and 70 days after irrigation.

2.3. Destructive harvests and soil collection

Aboveground biomass and soil samples were collected immediately before irrigation, 1, 7, 21 and 70 days after irrigation. On each