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Tracking C and N flows through microbial biomass with increased soil moisture variability

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ABSTRACT

Changes in soil moisture with cycles of soil wetting and drying are associated with shifts in osmotic potentials that can induce physiological stress for microbial communities. These instances of soil moisture stress can be of sufficient magnitude to alter flows of C and N at an ecosystem scale. In this study we manipulated the duration and severity of soil moisture stress and disturbance in grassland soils from four sites along a precipitation gradient. After subjecting soils to a two-month long incubation under two different wetting-drying regimes, one of high and one of low stress and disturbance, we moistened soils with ^{13}C- and ^{15}N-labeled glycine solution to trace C and N though the soil and its microbial communities as they dried. Contrary to our predictions, we found evidence for preferential use of N-free osmolytes with increased soil moisture stress in soils from the mesic end of the precipitation gradient. Soils from the western, semi-arid end of the gradient were less sensitive to soil moisture stress and did not differ in N demand under high and low stress. Specific respiration rates were higher in all soils under greater soil moisture stress immediately after re-wetting, then returned to levels equal to or below rates in soils under low soil moisture stress regimes. Nitrification outpaced denitrification processes in soils under the highest levels of soil moisture stress. These results suggest increases in both soil CO_2 release and N losses as stress induced by greater soil moisture variability increases in relatively mesic grassland systems, a predicted consequence of climate change in this region.

1. Introduction

Microbial activity in grassland soil systems is primarily limited by moisture and the transitory nature of resource pulses following precipitation, which can create physiologically stressful fluctuations in osmotic potentials (Austin et al., 2004). This variability in soil moisture conditions can be particularly stressful for microorganisms in grasslands given that these systems typically occur where potential evapotranspiration (PET) is equal to or greater than precipitation totals, limiting water availability (Lauenroth et al., 1999). Because climate change in the Great Plains region is expected to promote increases in rainfall variability that will lead to longer, more severe droughts (Easterling et al., 2000), predictions of future functioning of soil microorganisms responsible for biogeochemical processes rely on our understanding of how soil moisture stress controls those microbes' resource demands.

One such resource, soil nitrogen (N), serves multiple roles for soil microorganisms. For example, in addition to its importance as a growth-limiting nutrient, reduced forms of N can be used as electron donors by nitrifiers and oxidized forms as electron acceptors by denitrifiers. Although nitrification and denitrification rates have been linked to soil water potentials, in general little is known about the effects of changes in soil moisture stress, particularly increases in soil moisture variability, on the coupling of N cycling processes such as nitrification and denitrification (Borken and Matzner, 2009). Nitrogen also appears to be an important component of many of the solutes serving as protective osmolytes in systems with fluctuating osmotic potentials. Many microorganisms, especially bacteria, use N-rich protective solutes to guard against cellular water loss in systems where osmotic potential fluctuates due to changes in salinity (Kempf and Bremer, 1998; Schimel et al., 2007). The flux of such solutes into microbial biomass for use as protective osmolytes during one drought period can be equivalent in magnitude to 10–40% of annual net N mineralization in grassland systems (Schimel et al., 2007), but our understanding of the conditions under which microbial communities take up N-rich compounds as protective osmolytes remains limited.

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When microorganisms are relieved of osmotic stress, the fate of the protective osmolytes is unclear. Some researchers suggest that these protective solutes are rapidly and selectively released, intact, to the extracellular environment (Kempf and Bremer, 1998; Halverson et al., 2000). Others suggest that rather than losing this pool of C and N resources to their surroundings, microorganisms may rapidly catabolize the solutes, then release them in mineral form as CO_2 or NH_4^+ (Fierer and Schimel, 2003; Williams and Xia, 2009). In either scenario, as soils dry and osmotic stress returns after a wetting event, a considerable investment must be made by microbes to regain N-rich protective solutes. In a previous study investigating the effects of soil moisture variability on microbial resource use, we found from 360 to 4800% more N mineralized and evidence for a decrease in C use efficiency in grassland soils undergoing wetting-drying cycles, compared to soils kept at a constant soil moisture level (Tiemann and Billings, 2011). This study prompted us to ask if these relatively high rates of N mineralization coupled with low C use efficiency were the result of increases in the acquisition and release of N-rich osmolytes. Because the acquisition and release of protective solutes are apparently of sufficient magnitude to influence ecosystem level N and C fluxes (Schimel et al., 2007; Tiemann and Billings, 2011), understanding the mechanism behind these fluxes is critical for predicting not only microbial community, but ecosystem responses to global climate change.

In the current study, we manipulated the severity of soil moisture stress that microorganisms must overcome by altering the magnitude and frequency of soil wetting events. We conducted these manipulations using grassland soils collected from four different native precipitation regimes, along part of the Great Plains precipitation gradient in Kansas, USA. For two months we treated these soils with either a high frequency, low water addition treatment that induced relatively low levels of osmotic and water availability stress, or a low frequency, high magnitude water addition treatment that induced relatively high osmotic and water availability stresses. At the end of two months, after soil microorganisms had presumably acclimated to the different wetting-drying regimes, we applied a final water amendment to the soils that included ^{13}C- and ^{15}N-labeled glycine. To our knowledge this is the first use of a dual labeling approach to track microbial resource use during soil wetting-drying cycles. We selected glycine as a tracer compound because it represents a simple organic C and N source that can be directly incorporated into proteins, easily deaminated for use as an energy source or for biosynthesis, and it can be methylated to form one of the most preferred bacterial osmolytes, glycine betaine (Kempf and Bremer, 1998; RoeBler and Muller, 2001; Kim and Gadd, 2008; Kimura et al., 2010).

This dual isotopic labeling approach allowed us to track the fate of added C and N as *potential* metabolites or protective solutes as the soils dried and osmotic stress increased to address the following questions: 1) Does microbial N demand reflect the severity of soil moisture stress, and is this relationship variable across a precipitation gradient? 2) Is an increase in N demand associated with an increase in soil C losses through higher microbial respiration rates? 3) Are protective solutes returned, largely intact, to the extracellular environment during osmotic downshock, or are these substrates rapidly catabolized to support cell maintenance and activity, then released in mineral form as CO_2 or NH_4^+? 4) Do N cycling processes such as nitrification and denitrification differ in their responses to moisture variability and thus become uncoupled as soil moisture stress increases?

2. Methods

2.1. Study sites

Soils used in this study were collected from four sites across a precipitation gradient in Kansas, USA, which contain mesic tallgrass prairie in the east and semi-arid mixed grass and shortgrass steppe in the west. The eastern most site, part of the Kansas University Field Station lands (KUFS), (W 95°14′35″ N 38°10′21″) receives an average of 1003 mm of precipitation annually. The soils at this location are gravelly silt loams (smectitic, thermic, Typic paleudolls). Moving west, the second site, located at the Konza Prairie LTER (KNZ, W 96°33′18″ N 39°5′2″), averages 835 mm precipitation annually and soils are a mix of silty loams (smectitic mesic Typic natrusolls) and silty clay loams (fine mixed superactive mesic Pachic argiustolls). Our third site, part of Kansas State University's Western Kansas Agricultural Research Center, (HYS, W 99°17′46″ N 38°50′13″) receives an average of 578 mm precipitation annually and the soils are silt loams (fine-silty, mixed, superactive, mesic, Cumulic haplustolls). The final and western most site, The Nature Conservancy's Smokey Valley Ranch, (SVR, W 100°58′55″ N 38°51′50″) receives on average 485 mm of precipitation annually. The soils are silt loams (fine-silty, mixed, superactive, mesic, Aridic haplustolls). All sites are part of actively grazed rangeland and are burned annually, with the exception of SVR, which is not burned.

2.2. Soil collection

We collected soils from each of the four study sites on May 28 and May 29, 2009. We used PVC cores (10 cm diameter, 10 cm long) to collect three soil cores, approximately 10 m apart, from established 130 m^2 plots at each location. On the day of collection, soils were returned to the lab at the University of Kansas where roots greater than 2 mm in diameter were removed and soil from each core was homogenized. Immediately after this processing, we weighed one sub-sample from each soil core collected and dried them at 60 °C for >48 h to determine gravimetric soil water content (SWC).

2.3. Soil wetting and drying incubation

We weighed the equivalent of 100 g dry soil from each soil sample into two separate, pre-weighed, 5 cm diameter by 5 cm long PVC collars fitted with two sheets of coarse filter paper on the bottom. The soils inside the PVC collars were then placed into 1 L jars on top of a wire mesh support. For each of the three soil cores, sampled from each location, we created two incubation vessels, such that $n = 6$ for each soil origin and wetting-drying treatment. Half of these were used for glycine additions ($n = 3$) at the end of the incubation (see below), and half for water-only controls ($n = 3$). The first treatment, a large water pulse followed by a long drying interval (LI), was achieved by applying enough water to bring the soils to 100% water holding capacity (WHC) and allowing the soils to dry for two weeks. For the second treatment, we applied a water pulse 25% the size of the LI treatment at a short time interval (SI), every 3–4 days, so that these soils received the same total amount of water over a two-week period as the soils undergoing the LI treatment. Because of the longer and more severe drought interval, followed by a more extreme wetting event, the LI treatment created an environment of generally higher osmotic stress compared to SI soils. The incubation was conducted for a total of five, two-week long wetting-drying cycles, or 72 days. Based on results from our previous study, this was enough time for microbial communities to acclimate to the different soil moisture regimes (Tiemann and Billings, 2011). We applied the water using a needle and syringe to assure even coverage. All soils, regardless of treatment, were gently mixed twice a week during the dry down periods to achieve homogeneous soil moisture.

During the incubation, we measured soil respiration and N_2O production by capping the jars with lids fitted with septa and