Soil Biology & Biochemistry 68 (2014) 44–51

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Effects of sample storage on microbial Fe-reduction in tropical rainforest soils

Brian R. Ginn [a], Mussie Y. Habteselassie [b], Christof Meile [c], Aaron Thompson [a,*]

[a] Department of Crop and Soil Sciences, University of Georgia, Athens, GA 30602, USA
[b] Department of Crop and Soil Sciences, University of Georgia, Griffin, GA 30223, USA
[c] Department of Marine Sciences, University of Georgia, Athens, GA 30602, USA

ARTICLE INFO

Article history:
Received 12 December 2012
Received in revised form
4 September 2013
Accepted 7 September 2013
Available online 20 September 2013

Keywords:
Iron reduction
Storage protocol
Storage time
Luquillo Critical Zone Observatory
Soil homogenization
RISA

ABSTRACT

Many biogeochemical investigations of soil processes require temporary and/or long-term sample storage, which may alter soil biogeochemistry and impact the outcome of laboratory experiments. This study examines the influence of storage conditions on soil iron (Fe) reduction. We subjected soil samples from the Luquillo Experimental Forest, Puerto Rico to: (a) 2-mm sieving/homogenization within an anoxic environment, and storage at 4 °C or (b) 22 °C; (c) air-drying at 30 °C followed by 2-mm sieving/homogenization and storage at 22 °C; and (d) storage at 22 °C with no sieving/homogenization (control). We assessed changes in Fe-reduction after one week, six months and 12 months of storage by incubating soils in a 95% N_2:5% H_2 headspace for 7–15 days. After one week of storage, Fe^{II} production was linear with time yielding similar Fe-reduction rates (ave. 2.8 mmol kg^{-1} d^{-1}) in all treatments except the unhomogenized controls, which exhibited only slightly lower rates (2.2 mmol kg^{-1} d^{-1}). Storage for a duration of 12 months at 4 °C decreased Fe^{II} production following 7 days of anoxic incubation, while air-drying soils maintained similar Fe^{II} production levels. Most probable number counts of Fe-reducing microbes were comparable in the air-dried and 4 °C stored soils, but bacterial isolates from the air-dried soils exhibited higher net Fe^{II} production over a one week incubation in pure culture media than strains isolated from soils stored at 4 °C. When microbial activity was not limited by low temperature (e.g., during the 22 °C storage treatment), homogenization slowed Fe^{II} production. The observed Fe^{II} production and cell counts in these tropical soils suggest that storage at cold temperatures, as well as homogenization when microbial activity is not suppressed during storage, reduced the resilience of the resident Fe reducing microbial communities, a finding consistent with studies on other microbial processes in tropical soils. Overall our results suggest air-drying tropical soils maintains their capacity for Fe^{III} reduction during long-term sample storage more effectively than cold-storage.

1. Introduction

Investigations of soil biogeochemistry often require analytical and experimental procedures that cannot be performed *in situ* in the field. For these *ex situ* analyses and experiments it is preferable to use freshly collected soil. But very commonly there is some delay between sample collection and experiment, or analysis, which requires the soils be stored for some period of time.

Cold storage at 4–5 °C is usually recommended for microbial experiments (Brohon et al., 2001; González et al., 2009) because this reduces biomass growth (Anderson, 1987; Bloem et al., 2006) and substrate depletion (Coxson and Parkinson, 1987) relative to storage at higher temperatures. However, cold storage has been shown to permanently decrease soil nitrate reduction potential and microbial enzyme activity (Verchot, 1999; Turner and Romero, 2010). Conversely, samples are often dried to extend storage times, but this too can alter the microbial and chemical composition of the soil (Bartlett and James, 1980; Sheppard and Addison, 2007). Air-dried soils have been shown to exhibit reduced enzyme activity (Turner and Romero, 2010) and altered rates of Cr^{III} oxidation (Bartlett and James, 1980), although the ability to reduce and oxidize sulfur can be retained for many years (Bollen, 1977). One approach to minimize changes in soil metabolism during storage, supported by a literature review by Bloem et al. (2006), is to impose the conditions that slow microbial populations in the native environment. Specifically, Bloem et al. (2006) found that storage at the *in situ* soil temperature best preserved microbial activities. Following USDA temperature classifications (Soil Survey

* Corresponding author. Tel.: +1 706 410 1293.
E-mail address: AaronT@uga.edu (A. Thompson).

0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.09.012

Staff, 2010), the optimal storage method may thus be freezing for *frigid* soils; 4 °C storage for *mesic* soils; and air-drying for *thermic* soils.

Iron cycling is an important mediator of carbon oxidation in many soils (e.g. Dubinsky et al., 2010), and can control the availability of limiting nutrients such as phosphorous (Parfitt, 1989; Guzman et al., 1994; Liptzin and Silver, 2009; Chacon et al., 2006). It has been studied extensively under laboratory conditions following a range of storage treatments including air-drying (e.g. Frenzal et al., 1999; Peretyazhko and Sposito, 2005; Valencia-Cantero et al., 2007; Hansen et al., 2011) or after cold-storage (e.g. Kappler and Brune, 2002; Bennett and Dudas, 2003; Komlos and Jaffé, 2004; Wildung et al., 2004; Weiss et al., 2004; Valencia-Cantero et al., 2007). Qu et al. (2005) and He and Qu (2008) suggest air-drying delays the onset of Fe^{III} reduction but otherwise minimally affects Fe^{III} reduction rates. However, the impact of different storage conditions on soil Fe^{III} reduction has not been comparatively assessed. This is surprising given the large body of work on the effects of storage conditions on metal partitioning and speciation in soils, including iron (e.g., Bartlett and James, 1980; Thomson et al., 1980; Rapin et al., 1986; Pezzolesi et al., 2000).

Additionally, the common practice of mixing the soil to minimize the impact of spatial variability, often termed homogenization, can also impact the experimental results (e.g., Sheppard and Addison, 2007; Quantin et al., 2002; Thompson et al., 2006; Dubinsky et al., 2010). Homogenization is distinct from making a soil slurry by suspending the soil in a water solution, although most slurried soils have been homogenized previously. Homogenization causes a loss of soil structure that can alter bacterial activity (Teh and Silver, 2006; Teh et al., 2008). As a consequence, some researchers prefer not to alter their samples prior to experimentation, but to perform the experiment directly on the moist field sample (e.g., Liptzin and Silver, 2009).

In this paper, we examine the influence of storage conditions and sample handling on soil iron reduction and microbial composition across one year of storage. We aimed to answer two questions: (1) How do different storage protocol preparations influence the soil's Fe reduction capacity? (2) Which storage condition(s) maintain Fe reduction capacity over 12 months of storage? We do this by applying four common storage protocols (homogenized soil air-dried or stored moist at 4 °C or 22 °C and a no-treatment control stored at 22 °C) to soil from the Luquillo Experimental Forest in Puerto Rico. To answer our first question, we subjected the soils to incubation under anoxic conditions and measured the production of 0.5 M HCl extractable Fe^{II} after one week of storage. To answer our second question we repeated the anoxic incubation after six and 12 months storage.

2. Methods

2.1. Field site

Soils were collected from a single location in Puerto Rico at the Bisley Research Watersheds, Luquillo Experimental Forest (LEF), which is part of the NSF-funded Luquillo Long-term Ecological Research (LTER) site and the NSF-funded Critical Zone Observatory (CZO). Soils in the LEF are classified predominately as Ultisols formed from volcanic parent material with quartz diorite intrusions (Beinroth, 1982; Frizano et al., 2002). The major primary minerals consist of quartz and plagioclase feldspars with lesser amounts of biotite, hornblende, K-feldspar, and accessory magnetite, sphene, apatite, and zircon (White et al., 1998). Prior work by Silver et al. (1999) has identified periodic fluctuations in pO_2 sufficient to generate anoxic conditions within the soil. Peretyazhko and Sposito (2005) identified these soils as Fe-rich, mildly acidic (pH ~5), and nutrient poor. We collected the upper 10 cm of soil from the Bisley watershed valley as an intact soil block at a similar site to that described by Peretyazhko and Sposito (2005). Small portions of a discrete overlying organic horizon were included in the soil block, but their contribution was minimal relative to the total mass of soil collected. Total Fe and P concentrations in the soil were measured by ICP-MS following Li-metaborate fusion (Hossner, 1996) by Acme Labs, Vancouver, BC Canada. Total C was measured by grinding oven-dried samples to less than 250 μm in a ball-mill and combusting them in 5 × 5 mm tin capsules on a Carlo Erba Elemental Analyzer. In addition, we performed a citrate/ascorbate extraction to assess the abundance of short-range-ordered Fe (Reyes and Torrent, 1997). The samples were suspended in a 0.2 M Na-citrate/0.05 M ascorbic acid solution (pH = 6) at 60:1 reagent:sample ratio and shaken for 16 h on a horizontal shaker, and then centrifuged at 11,000 rcf for 30 min. Then the supernatants were analyzed with via atomic absorption (AA) spectroscopy (Perkin Elmer AAnalyst 200).

2.2. Storage protocols

Samples were placed in 4-mil polyethylene ziplock bags and transported without cooling inside an insulated container to the University of Georgia. Within 24 h of sampling all moist soils were processed into four storage treatments (Table 1) by (a) 2-mm sieving/homogenization (to remove large roots and any gravel-sized rocks) under field soil moisture conditions within a 95%:5% (N_2:H_2) glovebox followed by storage at 4 °C or (b) 22 °C; (c) air-drying at 30 °C for 24 h followed by 2-mm dry-sieving/homogenization and storage at 22 °C; and (d) storage at 22 °C with no sieving/homogenization. For the rest of this article, these treatments will be referred to as moist 4 °C, moist 22 °C, air-dried, and controls, respectively. All soils were stored under oxic conditions in the dark.

2.3. Iron reduction experiments

We performed iron reduction experiments on each storage treatment after one week, six months, and 12 months. All solutions and soil samples were stored and prepared under oxic conditions. For the moist 4 °C, moist 22 °C, and air-dried treatments, 4.5 g (dry-weight) of soil was suspended in a 2 mM KCl solution with a 45 g final mass and supplemented with 3 mmol L^{-1} of Na-lactate to eliminate any effects of storage on carbon substrate availability. Triplicate slurries were incubated for 7 d or 14 d in a Coy anaerobic chamber (95% N_2, 5% H_2) on an orbital shaker (Eberbach 6130, Ann Arbor, MI) at 250 rpm. We prepared two different control treatments for our experiments. In the first set of controls, 4.5 g of (dry-weight equivalent) soil was placed into 33 separate centrifuge tubes each at one week, six months and 12 months, sufficient for 11 triplicate time points during each sampling period. An equivalent aliquot of Na-lactate (0.67 mmol g^{-1} soil) was added to eliminate any effects of storage on initial carbon substrate availability, and the tubes were sealed in the anoxic chamber. However, we found substantial drying of the control samples occurred after six months and 12 months of storage under oxic conditions—despite being

Table 1
Storage procedure.

	Temperature	Sieved/homogenized
Moist 4 °C	4 °C	<2 mm
Moist 22 °C	22 °C	<2 mm
Air-dried	22 °C	<2 mm
Non-slurried control	22 °C	None