Available online at www.sciencedirect.com

ScienceDirect

Geochimica et Cosmochimica Acta 138 (2014) 101–116

Geochimica et Cosmochimica Acta

www.elsevier.com/locate/gca

Microbial activities and phosphorus cycling: An application of oxygen isotope ratios in phosphate

Lisa M. Stout [a], Sunendra R. Joshi [a], Todd M. Kana [b], Deb P. Jaisi [a,*]

[a] Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19716, United States
[b] University of Maryland Center for Environmental Science, Horn Point Laboratory, P.O. Box 775, Cambridge, MD 21613, United States

Received 26 January 2014; accepted in revised form 13 April 2014; Available online 24 April 2014

Abstract

Microorganisms carry out biochemical transformations of nutrients that make up their cells. Therefore, understanding how these nutrients are transformed or cycled in natural environments requires knowledge of microbial activity. Commonly used indicators for microbial activity typically include determining microbial respiration by O_2/CO_2 measurements, cell counts, and measurement of enzyme activities. However, coupled studies on nutrient cycling and microbial activity are not given enough emphasis. Here we apply phosphate oxygen isotope ratios ($\delta^{18}O_p$) as a tool for measurement of microbial activity and compare the rate of isotope exchange with methods of measuring microbial activities that are more commonly applied in environmental studies including respiration, dehydrogenase activity, alkaline phosphatase activity, and cell counts. Our results show that different bacteria may have different strategies for P uptake, storage and release, their respiration and consequently expression of DHA and APase activities, but in general the trend of their enzyme activities are comparable. Phosphate $\delta^{18}O_p$ values correlated well with these other parameters used to measure microbial activity with the strongest linear relationships between $\delta^{18}O_p$ and CO_2 evolution ($r = -0.99$). Even though the rate of isotope exchange for each microorganism used in this study is different, the rate per unit CO_2 respiration showed one general trend, where $\delta^{18}O_p$ values move towards equilibrium while CO_2 is generated. While this suggests that P cycling among microorganisms used in this study can be generalized, further research is needed to determine whether the microorganism-specific isotope exchange trend may occur in natural environments. In summary, phosphate oxygen isotope measurements may offer an alternative for use as a tracer to measure microbial activity in soils, sediments, and many other natural environments.

1. INTRODUCTION

Microorganisms carry out biochemical transformations of various essential elements that make up their cells such as carbon (C), nitrogen (N), phosphorus (P), and sulfur (S). Therefore, understanding how these nutrients are transformed or cycled in natural environments requires measurement of microbial activity through factors including microbial growth and enzyme activity. Various methods have been used in the past to study microbial activity in soils and sediments. One of the most common measurements is soil respiration, often measured as CO_2 produced or O_2 consumed (Nannipieri et al., 1978). Because the respiratory CO_2 production or O_2 consumption by heterotrophic microorganisms is the product of enzyme-catalyzed reactions, careful measurement of CO_2 and O_2 provides information about microbial activity. While it is straightforward to measure respiration in soil, collecting unaltered and representative sample is, however, often more challenging. For example, chambers for gas collection may disturb the ambient air pressure in soil and alter the CO_2

* Corresponding author. Tel.: +1 302 831 1376; fax: +1 302 831 0605.
E-mail address: jaisi@udel.edu (D.P. Jaisi).

http://dx.doi.org/10.1016/j.gca.2014.04.020
0016-7037/

concentration in the soil, among effects of other factors such as rainfall or soil temperature (Tang et al., 2005).

Measurement of microbial enzyme expression is another approach to study environmental microbial activity. While a large number of enzymes are produced by a diverse group of microorganisms, certain enzymes are commonly measured due to their ubiquity in different soil environments (Makoi and Ndakidemi, 2008; Richardson and Simpson, 2011). One of the most frequently measured enzymes is dehydrogenase. This enzyme is found in live cells and is produced as part of microbial oxidative respiration (Skujins, 1973). Dehydrogenase synthesis or repression is the reflection of microorganismal tuning of slow or fast respiration such as during early growth or even in extreme cases such as a dormant or starved state. Dehydrogenase activity (DHA) is also used as a measure of microbial biomass in soil (Mukhopadhyay and Maiti, 2010). Other enzyme activities that are commonly measured are those involved in elemental cycling, including sulfur (such as the arylsulfatase enzymes), carbon (β-glucosidases, cellulases), nitrogen (ureases, proteases), and phosphorus (phosphatases) (Makoi and Ndakidemi, 2008).

Phosphatases are a large group of enzymes that include both phosphomonoesterases and phosphodiesterases, and those that are active at either acid or alkaline pH values. In general, acid phosphatases are more common in acidic environments and alkaline phosphatases are more common in alkaline environments, and the prevalence of either in an environment can change seasonally. These enzymes are produced by plants and microorganisms, and can be intracellular or extracellular (Nannipieri et al., 2011). Extracellular phosphatase activity tends to increase in low phosphate concentrations, although the nature of this response seems to be organism-dependent (Kuhn et al., 2002; Allison and Vitousek, 2005). However, activity of any enzyme in soil may not only be controlled by active organisms. This is because a substantial amount of extracellular enzymes, naturally present or released, such as exoenzymes released from living cells or endoenzymes released from disintegrated cells, may be involved in cycling of a particular element (Dilly and Nannipieri, 1998). For example, alkaline phosphatase activities are often expressed extracellularly by many microorganisms. Measurement of extracellular enzyme activity has advanced since the introduction of fluorescent artificial substrates incorporating methylumbelliferone (MUF), which have the advantage of short incubation times and low detection limits, ideal for application to natural samples (Hoppe, 1983; Boschker and Cappenberg, 1994).

Biogeochemical cycling of P is carried out almost entirely by biota and the role of extracellular and intracellular phosphatase enzymes in P cycling is reflected in oxygen isotope ratios ($\delta^{18}O_p$) (Blake et al., 2005). Among different phosphatase enzymes, inorganic pyrophosphatase (PPase) catalyzes the wholesale exchange of O (i.e., exchange of all four oxygen atoms in phosphate with oxygen in ambient water) is required to achieve O isotopic equilibrium between phosphate and water (Blake et al., 1998, 2005). Details of this group of enzyme are discussed in Jaisi et al. (2014). Alkaline phosphatase (APase), on the other hand, is a non-specific enzyme that hydrolyzes a variety of phosphomonoester compounds as well as pyrophosphate and phosphite (Metcalf and Wolfe, 1998). One major distinction is that the APase reaction is unidirectional and thus does not promote wholesale phosphate–water isotope exchange like PPase. Therefore it imparts a large kinetic isotope fractionation ($-30 \pm 8‰$) during conversion of organic P to inorganic P (Liang and Blake, 2006). A recent study found a 5–7.5‰ difference in $\delta^{18}O_p$ values between alkaline and acid phosphatase-catalyzed degradation of two monoesters (adenosine 5′ monophosphate and glycerol phosphate) (von Sperber et al., 2014). This difference was attributed to the difference in the sources of oxygen incorporated, OH vs. H_2O, which have isotope fractionations of $40 \pm 3‰$ at 25 °C (Green and Taube, 1963) into the newly formed inorganic P. Intact microbial cells include a series of phosphoenzymes, including APase and PPase, acting together to promote rapid enzyme catalyzed O-isotope exchange between phosphate and water that may attain equilibrium isotope composition (Longinelli and Nuti, 1973; Blake et al., 2005).

Fundamental understanding on O-isotope systematics (Blake et al., 1997, 2005; Paytan et al., 2002; Colman et al., 2005; Liang and Blake, 2006, 2009) including isotope signatures associated with specific P pools in soil and sediments (Jaisi and Blake, 2010; Zohar et al., 2010; Goldhammer et al., 2011; Jaisi et al., 2011; Tamburini et al., 2012; Jaisi, 2013) and most recent advances in methods of sample purification and isotopic analysis, particularly a significant decrease in sample mass requirement have led to rapid expansion of the application of oxygen isotopic composition of phosphate to a wide range of environments from soils (Tamburini et al., 2013), sediments and natural waters to biomass (see Jaisi and Blake, 2014 for detailed review). Similarly, enrichment and natural abundance studies have been used to identify microbial P cycling in soil (Larsen et al., 1989; Johansen et al., 1991; Melby et al., 2011; Angert et al., 2012; Tamburini et al., 2012), and tracking of atmospheric, riverine, fertilizer, and marine P sources (McLaughlin et al., 2006; Elsbury et al., 2009; Paytan and McLaughlin, 2011; Gross et al., 2013).

Isotopic techniques such as $\delta^{13}C$ and $\delta^{15}N$ have been used to obtain direct and quantitative links to plant and microbial driven soil respiration (Hanson et al., 2000; Trumbore, 2006; Sims et al., 2007; Bowling et al., 2008; Paterson et al., 2009). In this regard, isotope methods have been thought as most reliable methods for quantifying denitrification in soils (Butterbach-Bahl et al., 2002) as well as identifying ecosystem pool and CO_2 fluxes (e.g., Bowling et al., 2008). Analogous studies on phosphate oxygen isotopes are rare (Larsen et al., 1989; Johansen et al., 1991). As illustrated and exemplified above, phosphate isotopic composition has increasingly been applied to better understand the physicochemical and biological pathways of P cycling and its fate in agricultural and non-agricultural soils. However, there is a gap in knowledge on how this cycling compares to existing methods of measuring microbial activities in soils and sediments. Because nutrient cycling is caused by microbial activity and the presence of nutrients supports microbial activities in