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Effect of methodological consideration on soil carbon parameter estimates obtained via the acid hydrolysis-incubation method

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ABSTRACT

Many techniques such as the acid hydrolysis – incubation (AHI) method have been developed with the aim of elucidating the inherent complexity of soil organic carbon (SOC). While the utility of the AHI method has been demonstrated, there is no standardized protocol developed for conducting the long-term incubation component of the method. In the current study we evaluated the effects of chamber venting and mechanical headspace mixing on soil CO_2 flux rates and the resultant size and mean residence time of three operationally defined pools of SOC obtained via the AHI method. Continuous chamber venting resulted in an estimate of the readily mineralized carbon pool that was 2.3 times larger and turned over 2.9 times slower than the same pool estimated using periodically vented chambers. These differences were primarily attributed to the suppression of CO_2 flux in periodically vented chambers as a result of high internal CO_2 concentrations, and a concomitantly reduced diffusivity gradient. Prior to venting the periodically-vented chambers, CO_2 flux rates averaged 2.3 μg C (g soil)$^{-1}$ d^{-1}, while CO_2 flux rates following venting averaged 222.6 μg C (g soil)$^{-1}$ d^{-1}. We did not detect internal stratification of CO_2 suggesting that mechanical headspace mixing is unnecessary in incubation chambers ranging from 1 to 2 L. A standardized protocol is called for that isolates SOC fractions that are useful in hypothesis testing, while simultaneously seeking to minimize laboratory artifacts.

1. Introduction

A wide array of techniques have been employed to fractionate soil organic matter (SOM) into meaningful pools, often with the aim of elucidating ecosystem function and improving biogeochemical modeling efforts (McLauchlan and Hobbie, 2004; Paul et al., 2006; Poirier et al., 2005; von Lutzow et al., 2007). The acid hydrolysis-incubation (AHI) method is one such technique that combines chemical (acid hydrolysis) and biological (long term incubation) fractionation to estimate the size and turnover of three operationally defined SOM pools. In this method, CO_2 respiration rates obtained during soil incubations are used to define the decomposition rates of "active" and slowly mineralized carbon (k_a and k_s, respectively) as well as the size of the active carbon pool (C_a). Acid hydrolysis is used to define a chemically resistant pool (C_r); the decomposition rate of which (k_r) is usually obtained via ^{14}C measurements. The AHI method has demonstrated its utility in reproducibly measuring meaningful pools of SOM (Collins et al., 2000; Fortuna et al., 2003; Haile-Mariam et al., 2000; Paul et al., 2006). Although the method has been extensively reported on, it is not without potential drawbacks and interpretational problems (Bruun and Luxhoi, 2006; Paul et al., 2006). Furthermore, while the issues associated with the use of acid hydrolysis have been discussed at length (Collins et al., 2000; Kogel-Knabner et al., 1994; Schwendenmann and Pendall, 2008), and a rather consistent protocol is in place (Paul et al., 2001b; Sollins et al., 1999), there is no clearly defined and consistent method within the scientific literature regarding the implementation of long term in-vitro soil incubations (as they pertain to the AHI and other methods).

Long term soil incubations are typically conducted in the absence of light to discourage the growth of autotrophic organisms, and at soil moistures that are optimized for microbial growth and respiration (e.g. 60% water filled pore space [WFPS] is common) (Linn and Doran, 1984). The quantity of soil used for incubation studies varies widely from as little as 2.5 g (Risk et al., 2008) to as much as 200 g (Stewart et al., 2009), with 80–150 g most

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commonly reported (Conant et al., 2008a; Plante et al., 2009; Robertson et al., 1999). The size of the incubation chamber varies correspondingly with the quantity of soil used. Discrepancies in soil quantity and chamber size are largely accounted for in calculations of CO_2 efflux per unit mass of soil which have been normalized for headspace volume. As such, these considerations may be of little concern when comparing data between different in-vitro incubation studies. The effects of internal chamber conditions such as CO_2 concentration and headspace mixing however have not been adequately addressed in the incubation literature and may have a marked effect on the outcomes of such studies.

Soil CO_2 flux is strongly governed by the diffusivity gradient generated as a result of concentration differences between the atmosphere and the soil pore space (Healy et al., 1996; Livingston and Hutchinson, 1995). Consequently, if the diffusivity gradient in vitro is drastically different than that encountered in situ, it would be unreasonable to expect realistic soil respiration rates from laboratory incubations. The suppressive effect of non-vented static chambers in field based CO_2 flux measurements has been well documented (Conen and Smith, 2000; Davidson et al., 2002; Kutzbach et al., 2007; Pumpanen et al., 2004). In the field, the reduction in CO_2 flux occurs almost instantaneously and can be attributed in large part to a distortion of the vertical and radial soil gas concentration gradient (Healy et al., 1996). In a controlled laboratory incubation study, Bekku et al. (1997) found that it was necessary to maintain the CO_2 concentration within an incubation chamber at that of the ambient air to determine accurate soil flux rates. In spite of these concerns, headspace CO_2 concentrations between 50,000 and 60,000 mg kg^{-1} (5% and 6%) are often cited as allowable limits (Conant et al., 2008b; Paul et al., 2001b; Steinweg et al., 2008). These limits are usually accepted to provide optimal soil conditions for microbial respiration by maintaining internal humidity to limit soil moisture loss. While such concentrations can occur within soil pore space (Glinski and Stepniewski, 1985; Maier et al., 2010), and are not considered detrimental to microbial growth in the way that low O_2 levels are (Kandeler, 2007), they are not inconsequential to soil CO_2 flux measured above the soil. This is particularly important when high chamber concentrations greatly reduce the diffusivity gradient between the soil pore space and chamber headspace. Santruckova and Simek (1997) clearly demonstrated that in vitro CO_2 concentrations between 2.5 and 5% lead to inhibition of additional soil CO_2 flux.

Proposed solutions to the problems that can arise from an ever decreasing diffusivity gradient (e.g. inhibition of gaseous flux) have been addressed in the field-based literature by decreasing chamber deployment time (Davidson et al., 2002), using non-linear modeling techniques to account for the non-linear gas flux (Healy et al., 1996; Livingston and Hutchinson, 1995), or including some sort of headspace mixing mechanism such as fans (Christiansen et al., 2011). While the benefit of rigorous headspace mixing as occurs with chamber fans has been questioned by some (Hutchinson and Livingston, 2002; Pumpanen et al., 2004), it is still commonly reported in the CO_2 flux measurement literature as a way to more rapidly obtain an accurate measure of mean chamber CO_2 concentration (Camarda et al., 2009; Nakano et al., 2004; Pihlatie et al., 2007).

Chamber venting has also been discussed at length, but its primary utility has been to equalize pressure between the inside and outside of the chamber and not as a means of maintaining a realistic diffusivity gradient (Livingston and Hutchinson, 1995). While the issues associated with headspace CO_2 accumulation and mixing have been addressed at length as they pertain to in situ gaseous flux measurements, they have not to our knowledge been adequately explored within the in vitro incubation literature. Furthermore, solutions such as decreased chamber deployment time are not practical during long term soil incubations where chambers are necessary to maintain optimal soil moisture levels.

One important advantage of the AHI method is the estimation of SOC parameters that can be used as inputs for models such as CENTURY, EPIC, and ROTHC. The work of Paul et al. (1999, 2006) demonstrated the value and applicability of using such parameters to improve biogeochemical model output. Others have also demonstrated the efficacy of utilizing soil mineralization rates and chemical isolates of stabilized carbon (e.g. C_r) to improve biogeochemical modeling (Juston et al., 2010; Scharnagl et al., 2010). The benefits of this method may be compromised, however, by unknown issues with in vitro chamber based CO_2 flux measurements, or a lack of consistency in the application of long-term incubations. Potential issues include biased CO_2 flux estimates due to high diffusivity gradients within incubation chambers, or artifacts associated with large post venting CO_2 fluxes. Progress toward understanding the impact of such variables, particularly as they pertain to parameter estimation in the AHI method, will further the development of protocols that minimize laboratory artifacts, with the ultimate goal of improving the utility of such techniques for biogeochemical simulation models.

In the present study we evaluated the effects of chamber venting and headspace mixing on internal chamber CO_2 concentrations, and associated CO_2 flux measurements (both pre- and post-venting) in two independent laboratory experiments to determine their impact on carbon parameter estimates obtained via the AHI method. The objectives of this research were to; 1) evaluate how vented versus sealed chamber impacted internal CO_2 concentrations and CO_2 flux measurements within each experiment, 2) evaluate how these flux measurements affected AHI derived SOC parameter estimates, 3) determine whether or not CO_2 stratification was detectable within sealed chambers, and 4) if stratification was found, determine if fans had a beneficial effects in homogenizing headspace CO_2 concentrations. Our initial hypotheses were; 1) that sealed incubation chambers would result in higher internal CO_2 concentration and lower respiration rates relative to continuously vented chambers if the chambers were not opened when estimating CO_2 flux, 2) that this trend would reverse with non-vented chamber generating greater CO_2 fluxes if opened prior to flux measurements, 3) that mechanical headspace mixing would be beneficial in homogenize CO_2 concentrations gradients within the incubation chambers, and that 4) all of these effects would result in significantly different estimate of active and slow carbon pools and their decomposition rates.

2. Materials and methods

2.1. Experimental overview

Two independent laboratory experiments were conducted between June 2009 and March 2012 to evaluate the impact of methodological considerations on soil carbon pool size and carbon pool decomposition estimates as defined by the AHI method (Paul et al., 2006). The first experiment (VENT) was designed to evaluate the effects of continuous chamber venting, versus periodic maintenance venting, on internal chamber CO_2 concentration, CO_2 flux readings, and resultant AHI parameter estimates with the hypothesis that the difference in diffusivity gradients would result in significantly different estimates of SOC pool size and decomposition rate. The second experiment (MIX) was designed to look at the impact of vented versus non-vented chambers on internal chamber CO_2 concentration, CO_2 flux rates, and SOC parameter estimates but under different conditions than those in VENT. In VENT, CO_2 flux rates for sealed chambers were collected while those chambers remained sealed, whereas in MIX, CO_2 flux rates were estimated