Soil Biology & Biochemistry xxx (2012) 1–10

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

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

CO_2 uptake by a soil microcosm

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ARTICLE INFO

Article history:
Received 1 June 2012
Received in revised form
9 October 2012
Accepted 26 October 2012
Available online xxx

Keywords:
Carbon uptake
Soil microorganisms
Soil organic matter
Lipids
$^{13}CO_2$ enrichment

ABSTRACT

Sequestration of CO_2 via biological sinks is a matter of great scientific importance due to the potential lowering of atmospheric CO_2. In this study, a custom built incubation chamber was used to cultivate a soil microbial community to instigate chemoautotrophy of a temperate soil. Real-time atmospheric CO_2 concentrations were monitored and estimations of total CO_2 uptake were made. After careful background flux corrections, 4.52 ± 0.05 g CO_2 kg^{-1} dry soil was sequestered from the chamber atmosphere over 40 h. Using isotopically labelled $^{13}CO_2$ and GCMS–IRMS, labelled fatty acids were identified after only a short incubation, hence confirming CO_2 sequestration for soil. The results of this *in vivo* study provide the ground work for future studies intending to mimic the *in situ* environment by providing a reliable method for investigating CO_2 uptake by soil microorganisms.

1. Introduction

Soil carbon is reported to be approximately 3 times the size of the atmospheric pool and 4.5 times that of the biotic pool (Lal, 2004; Schmidt et al., 2011) and thus, it is important to develop and verify management procedures that encourage carbon stabilisation in soil. Humic substances (HS) are a large, operationally defined fraction of soil organic matter (SOM). It has traditionally been thought that HS consist of novel categories of cross-linked macromolecular structures that form a distinct class of chemical compounds (Stevenson, 1994). In contrast to traditional thinking however, it was recently concluded that the vast majority of humic material in soils is a very complex mixture of microbial and plant biopolymers and their degradation products, and not a distinct chemical category as is traditionally thought (Kelleher and Simpson, 2006). Furthermore, the concept that extractable SOM is comprised mainly of humic materials has also been challenged and it has been shown that the presence of organic material sourced to microbes (as extant organisms or necromass) far exceeds presently accepted values, with large contributions of microbial peptides/proteins found in the HS fraction (Kögel-Knabner, 2002; Kiem and Kögel-Knabner, 2003; Kindler et al., 2006; Simpson et al., 2007; Potthoff et al., 2008). Based on the amount of fresh cellular material in soil extracts, it is probable that the contributions of microorganisms in the terrestrial environment are seriously underestimated. The activity of soil microorganisms still presents itself as a 'black box' due to the low cultivability of microbes, while being the primary agents of biogeochemical change (Madsen, 2005). Methods that enhance our ability to detect and track the flow of environmentally significant compounds through soil, such as the fate of CO_2, should be developed, so that the scientific community can design experiments usually difficult to monitor *in situ*.

Soil microorganisms are key players in the fixation and mobilisation of carbon and nitrogen, through both heterotrophic and autotrophic metabolic processes (Falkowski and Fenchel, 2008). Certain species of soil bacteria are known to autotrophically fixate mineral forms of gaseous carbon and nitrogen to produce organic cellular matter via various biochemical enzymatic processes. Autotrophic microorganisms (bacteria and archaea) capable of growth in the absence of light are generally described as chemoautotrophs (or chemolithotrophs). These prokaryotes use inorganic

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0038-0717/$ – see front matter © 2012 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.soilbio.2012.10.036

substrates to derive energy for biosynthesis reactions via aerobic or anaerobic CO_2 assimilation (Alfreider et al., 2009). They are unique in their ability to derive energy from sources not related to solar activity and can be found in diverse locations both above and below the Earths crust (Waksman and Joffe, 1922; Starkey, 1935; Pedersen, 2000; Amend and Teske, 2005; Sorokin and Kuenen, 2005; Alfreider et al., 2009). Microbial uptake of atmospheric CO_2 via autotrophic processes is a well characterised biological phenomenon, but actual estimations of sequestration rates are rare in the literature (Miltner et al., 2004). These groups of niche microorganisms are suitable for designing initial experiments as the biomass can be controlled, depending on the supply of key nutrients. Also, the autotrophic nature of these particular species allows for the relatively conducive labelling of biomass in order to determine the flow of CO_2 from the atmosphere directly into the SOM fraction.

The purpose of this study is to develop a methodology to detect and quantify the uptake of CO_2 by soil chemoautotrophs under ideal growth conditions using a custom built environmental incubation chamber. Environmental growth chambers have been utilised for this type of study for various related sample types (Fleisher et al., 2008; Ferguson and Williams, 1974; Nakanoa et al., 2004) but few studies make attempts at quantifying the volume of CO_2 taken up during incubation. The integrity of the data from chamber studies relies heavily on the reliability of measurements (Baker et al., 2004). At present, we were only able to locate a single study in the literature that assessed the accuracy of a sealed chamber when making estimates of CO_2 uptake (Acock and Acock, 1989), with the majority of studies not discussing this experimental aspect despite its relevance to CO_2 uptake determinations (De Morais and Costa, 2007; Ohashi et al., 2005; Pringault et al., 1996). We have therefore developed a mathematical model that takes into account experimental uncertainties such as outgassing and abiotic interactions. The model was used to predict the real-time flux of CO_2 with the aim of estimating CO_2 uptake during the sequestration events of the microbial community.

Here, we incubated soils in the dark, while under elevated $^{12}CO_2$ and $^{13}CO_2$ respectively, make estimations of direct $^{12}CO_2$ uptake and employ compound specific gas chromatography mass spectrometry–isotope ratio mass spectrometry (GCMS–IRMS) to provide evidence of the uptake of CO_2 by soil microorganisms via the production of fatty acids (reported as fatty acid methyl esters [FAMEs]). We demonstrate CO_2 uptake by extant soil chemoautotrophic microorganisms that have been provided with a suitable chemical electron donor to observe carbon sequestration. The overall aim of the study was to prepare a working method where soil chemoautotrophy can be induced and a single soil sample may be subjected to a suite of techniques to assist in the elucidation of soil carbon dynamics. It is hoped that the techniques developed herein will allow for investigation into CO_2 sequestering microcosms that attempt to mimic *in situ* conditions.

2. Experimental

2.1. Site details and pre-treatment

Four separate soils were collected for CO_2 uptake measurements and each have been designated an identification name (see Table 1). Only one of the soils (AS) was exposed to $^{13}CO_2$ in order to demonstrate the sequestration of ^{13}C into fatty acids including the blank-control ^{13}C incubation. Table 1 provides relevant characteristic information including sampling location and edaphic properties. Surface epipedon (A horizon) samples were collected and transferred aseptically to the laboratory and processed immediately. Roots and large debris were removed manually using aseptic technique. A CHN combustion analyser (Exeter Analytical CE440 elemental analyser) was used to determine the elemental composition and phosphorus (P) analysis by wet digestion according to April and Kokoasse (2009). All chemicals and solvents were purchased from Sigma Aldrich. The chemicals were of the highest purity grade available and all solvents used were of PESTANAL® quality. Permission from the relevant authorities at, Waterford County Council, Dublin City Council and the Botanic Gardens of Moscow State University was acquired.

2.2. Environmental carbon dioxide incubation chamber

The environmental carbon dioxide incubation chamber (ECIC) conducts temperature-controlled incubations of environmental samples in the presence of varying concentrations of CO_2. The ECIC consists of two units (Fig. 1). The outer unit controls temperature and houses the onboard CPU. The smaller inner unit has a 40.06 l capacity (inclusive of internal equipment and reaction vessel) and the outer door is sealed using a screw clamp, silicone foam strip and a thin layer of high vacuum grease to create an airtight seal. The ECIC is primarily used to measure and maintain the internal atmospheric concentration of CO_2 over short to long-term incubations while under constant temperature and atmospheric pressure. The inner chamber employs an infra-red (IR) CO_2 detector (GMM220, Vaisaila Ltd.) with a detection limit range between 0 and 2000 ppmv (accuracy, including repeatability, non-linearity and calibration uncertainty ±1.5% at 25 °C). The IR detector has been calibrated to detect atmospheric CO_2 and employs a wavenumber (cm^{-1}) detection range between 2270 and 2390 cm^{-1}. The absorbance of $^{13}CO_2$ in the IR spectrum lies between 2250 and 2290 cm^{-1} (Gosz et al., 1988) and hence, the ECIC only reports a small percentage of the true concentration of $^{13}CO_2$ (~20%), and therefore plots of this data have not been used to make quantitative measurements.

A calibration procedure for determining the pumping rate of CO_2 s^{-1} was performed manually each time a new incubation experiment was performed. Briefly, the liquid CO_2 inlet tube leading into the inner chamber (from a pressurised gas cylinder) was detached and inserted into a 100 ml graduated cylinder. The

Table 1
Soil identifications, sampling locations and some edaphic properties.

Name	Location and sampling date	Soil designation	Coordinates	Moisture (%)	pH	C (%)	H (%)	N (%)	P (%)
AS	Abbeyside, Dungarven, Co. Waterford, Ireland (04/2009)	Acid brown earths	52° 5′ 17.36″ N, 7° 36′ 38.19″ W	22.9	7.3	4.25	0.58	0.15	0.21
HS	Alberts College Park (Hampstead Park), Glasnevin, Dublin, Ireland (04/2009)	Grey brown podzolic	53° 22′ 54.63″ N, 6° 15′ 43.72″ W	24.5	7.6	8.62	0.97	0.32	0.31
MS	Botanic Gardens of Moscow State University, Moscow, Russian Federation (03/2009)	Histosols cryic	55° 42′ 37″ N, 37° 31′ 87″ E	0.45[a]	6.8	13.08	1.53	0.83	0.22
TS	Oak Park Research Centre, Carlow Town, Co. Carlow, Ireland (09/2008)	Grey brown podzolic	52° 51′ 47.24″ N, 6° 54′ 11.34″ W	20.2	6.5	3.61	0.33	0.17	0.56

[a] Winter sampling most likely reason for low volume of moisture due to freezing conditions at location, March 2009.

Please cite this article in press as: Hart, K.M., et al., CO_2 uptake by a soil microcosm, Soil Biology & Biochemistry (2012), http://dx.doi.org/10.1016/j.soilbio.2012.10.036