Biogeosciences Discuss., 8, 9235–9281, 2011
www.biogeosciences-discuss.net/8/9235/2011/
doi:10.5194/bgd-8-9235-2011

Biogeosciences Discussions

An approach to the investigation of CO_2 uptake by soil microorganisms

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Received: 29 August 2011 – Accepted: 6 September 2011 – Published: 12 September 2011

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Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Sequestration of CO_2 via biological sinks is a matter of great scientific importance due to their potential to lower atmospheric CO_2 levels. In this study a custom built incubation chamber was used to cultivate a soil microbial community, under ideal conditions, to investigate soil chemoautotrophy. The internal atmospheric CO_2 concentrations were monitored and used to estimate the mass of CO_2 uptake. It was found after careful background corrections that 256.4 µg CO_2 kg^{-1} dry soil was removed from the chamber atmosphere over 16 h. Comparisons were made to photosynthetic controls (i.e. grass and soil algae) whereupon it was observed that the chemoautotrophic fraction sequestered 2.6 and 5.4 % of that taken up by grass and soil algae respectively. Using isotopically labelled $^{13}CO_2$ and GCMS-IRMS it was also possible to extract and identify labelled fatty acids after a short incubation time, hence confirming the CO_2 uptake potential of the soil slurry. Provided with favourable conditions, chemoautotrophic soil bacteria have the potential to make a significant impact on inorganic carbon sequestration within the environment. The results of this in vivo study have provided 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

The global soil carbon pool is approximately 3 times the size of the atmospheric pool and 4.5 times that of the biotic pool (Lal, 2004). 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). 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 traditionally thought (Kelleher and Simpson, 2006).

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Furthermore, the concept that extractable SOM is comprised mainly of humic materials has also been challenged. It was shown that the presence of organic material sourced to microbes far exceeds presently accepted values, with large contributions of microbial peptides/proteins found in the HS fraction (Simpson et al., 2007). 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. If we underestimate the contribution of microorganisms to SOM, do we also underestimate their role in carbon sequestration?

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. Cyanobacteria for instance, utilise oxygenic photosynthetic biochemical pathways to fixate atmospheric CO_2 and (but not exclusively) N_2 in soil, freshwater and marine environments (Madigan et al., 2000; Smith, 1983; Staal et al., 2003; Belnap, 2003) thus providing the basis for trophic food webs. Another group of bacteria (and archaea) known to survive autotrophically are the chemoautotrophs (chemolithotrophs). These prokaryotes use inorganic 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 (Alfreider et al., 2009; Waksman and Joffe, 1922; Starkey, 1935; Pedersen, 2000; Amend and Teske, 2005; Sorokin and Kuenen, 2005) as well as some groups being considered fairly ubiquitous across soil landscapes, such as the genus *Thiobacillus* (Chapman, 1990; Smith and Strohl, 1991). Microbial uptake of atmospheric CO_2 via autotrophic processes is a well characterised biological phenomenon, but actual estimates of sequestered CO_2 are rare in the literature (Miltner et al., 2004). Miltner et al. (2004) measured SOM accumulation directly from a non-photosynthetic source and hypothesised that CO_2 uptake came from a combination of

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both autotrophic and heterotrophic growth activities although the authors clearly point out the limitations of autotrophy within their particular sample. Boyd et al. (2009) undertook a study into thermoacidophilic microbial CO_2 uptake and calculated that an average of $13.5 \pm 0.9\,\mu g\,C\ 10^7\ cells^{-1}\ h^{-1}$ was incorporated over a 2 h period. No additional substrates such as electron donors were incorporated in the experimental set up, but the presence of naturally occurring precipitated S^0 was likely the electron donor source.

The possibility of atmospheric carbon sequestration occurring in soil is of great interest and hence chemoautotrophy is the target of this study. The purpose of this study was to develop methodologies to detect and quantify the uptake of CO_2 by soil chemoautotrophs under ideal 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, 2004; Nakanoa et al., 2004) but few studies make attempts at quantifying the volume of CO_2 taken up during incubation. At present we were only able to locate a single study in the literature that measured the robustness of sealed chambers in CO_2 uptake studies (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; Ohasi et al., 2005; Pringault et al., 1996).

Here, we incubate soil in the dark while under the presence of stable isotopic $^{13}CO_2$, make estimations of direct 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. We demonstrate CO_2 uptake by extant soil chemoautotrophic microorganisms that have been provided with a suitable chemical electron donor to observe carbon sequestration. In this environment, carbon fixation was hypothesized to far exceed the CO_2 produced by soil microbial respiration. By utilising isotopically labelled $^{13}CO_2$ and GCMS-IRMS, the fate of this atmospheric carbon within the autotrophic system was monitored. In addition, the incubation of germinated grass under similar environmental conditions was also observed

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to act as a comparative control for CO_2 uptake estimations. The overall aim of the study was to prepare a working method where soil chemoautotrophy can be induced and subsequently, 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 the expansion into various other sample types to determine inorganic carbon sequestration.

2 Experimental

2.1 Site details and pre-treatment

Four soils were used but in the main outlined experiment, the soil designated as "Abbeyside" was an Acid Brown Earths (Fay and Zhang, 2011), retrieved from an open public area adjacent to St. Augustine's Church, Abbeyside, Dungarvan, Co. Waterford, Ireland (52°5′17.36″ N 7°36′38.19″ W). The sampling location was on open grass covered ground within 50 m of the Atlantic coast (Celtic Sea). All samples were collected and transferred aseptically to the laboratory and processed immediately. Roots and large debris were removed manually using aseptic technique. The soil was partially air dried and then sieved using a sterilised stainless steel mechanical sieve with a ≤2 mm aperture size. Sieved soil was stored in an amber jar at 4 °C. Accurately weighed aliquots of soil were dried at 104 °C for 3 days yielding an average moisture content of 22.9 %. A portion of soil was fractionated according to size using a 9 piece aluminium sieve set, range 2000–25 µm (Nickel-Electro, Weston-Super-Mare, United Kingdom) and using the Gradistat soil textural calculator (Blott and Pye, 2001) the soil texture was determined to be a slightly gravelly muddy sand. Using the USDA soil pyramid it was determined that the soil was a loamy sand. A CHN combustion analyser (Exeter Analytical CE440 elemental analyser) was used to determine the soil elemental composition, 4.25 % C, 0.58 % H, 0.15 % N. Phosphorus analysis by wet digestion (April and Kokoasse, 2009) was 0.21 % P. Comparison soils were collected aseptically

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and treated as above. Hampstead Park soil was a Grey Brown Podzolic (Fay and Zhang, 2011), retrieved from an open public area located within Albert College Park (Hampstead Park), Dublin, Ireland (53°22′54.63″ N 6°15′43.72″ W). Soil moisture was 24.5 % and soil texture was determined to be slightly very fine gravelly, very coarse silty medium sand. Using the USDA soil pyramid it was determined that the soil was loamy. The CHN soil composition was, 8.62 % C, 0.97 % H, 0.32 % N. Phosphorus analysis by wet digestion was 0.31 % P. Teagasc soil was a Grey Brown Podzolic (Conry and Ryan, 1967; Fay and Zhang, 2011), retrieved from a continuous barley crop field at the Oak Park Research Centre, Carlow Town, Co. Carlow, Republic of Ireland (52°51′47.24″ N, 6°54′11.34″ W). The soil moisture was 20.2 % at time of sampling and the soil texture was determined to be, a very coarse silty medium sand (muddy sand). The CHN soil composition was, 3.61 % C, 0.33 % H, 0.17 % N. Phosphorus analysis by wet digestion was 0.56 % P. Moscow soil was taken from the Botanic Gardens of Moscow State University, Moscow, Russian Federation (55°42′37″ N, 37°31′87″ E). Soil moisture was 0.45 % at the time of sampling (winter sampling most likely reason for low volume of moisture, March 2009) and the soil texture was determined to be, a very coarse silty medium sand. The CHN soil composition was, 13.08 % C, 1.53 % H, 0.83 % N. Phosphorus analysis by wet digestion was 0.22 % P. 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. Relevant permission from the park authorities, Waterford County Council, Dublin City Council and from the Botanic Gardens of Moscow State University for soil samples was acquired.

2.2 Environmental carbon dioxide incubation chamber

The environmental carbon dioxide incubation chamber (ECIC) has the primary function of conducting temperature controlled incubations of environmental samples in the presence of variable concentrations of CO_2. The chamber houses a smaller inner unit (inclusive of internal equipment, the inner chamber has a 40.06 l capacity) which has

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been custom designed to be air tight, yet easily accessible (Fig. 1). 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 CO_2 detector (GMM220, Vaisaila Ltd.) with a detection limit range between 0–2000 ppmv (accuracy, including repeatability, non-linearity and calibration uncertainty ±1.5 % at 25 °C). 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 was detached and inserted into a 100 ml graduated cylinder. The 100 ml graduated cylinder was filled with water and then inverted into a 1000 ml beaker filled with water. Using the chamber computer interface, the CO_2 concentration was set to 1000 ppmv. The peristaltic pump injects CO_2 into the graduated cylinder and a certain volume of water was displaced and the volume recorded manually. From the chamber interface, the pump run time (seconds) was noted. The pump was allowed to inject CO_2 another five times and an average value determined. The following calculation was used to determine the input volume per second (Volume H_2O Displaced (ml)/Pump Run Time (s) = cm^3 s^{-1} CO_2). There is no automated control of the relative humidity (% RH) within the inner chamber but this data is measurable on a real-time basis. Photosynthetic Active Radiation (PAR) lamps are contained within the inner chamber and lamp strength (%) may be adjusted according to requirements. Maximum lamp strength was determined using Maya 2000 Pro spectrometer and SpectraSuite software (Ocean Optics Inc.). Introduction of CO_2 into the inner chamber is carried out using a peristaltic pump fed from a pressurised liquid CO_2 cylinder and a pre-programmed concentration setting in parts per million (ppmv) CO_2. Internal measurements of the CO_2 concentration taken every 30 s are relayed to the onboard computer which compares the required to the actual concentration. If the concentration of CO_2 is lower than the programmed value the peristaltic pump continues in short controlled bursts (measured in seconds) until the required concentration is attained. A data logging system is in place where relevant information can be recorded at 30 s intervals and then transferred to a PC for data

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analysis. The information may be logged according to the operator's requirements and can be readily transferred into user friendly spreadsheets such as Microsoft© Excel. The inner chamber houses the detector used to take measurements of internal CO_2 concentrations and provide a controlled environment where CO_2 may be administered.

Analysis of the chamber performance under blank conditions (i.e. empty and sealed) determined that a very slight but unavoidable leak was present (data set not shown but based upon the observance of a decline of 500 ppmv CO_2 over an average of 52 h; Table 1). The leak rate was proportional to the CO_2 concentration in the chamber, on which basis partial pressure correction rates were determined. These were determined in 100 ppmv fractions between 500–1200 ppmv CO_2. These correction factors have been applied when determining the uptake volume of CO_2 during perceived chemoautotrophic events. Attempts at finding the site of the leakage was unsuccessful using coloured gases (data not shown) and is most likely attributable to diffusion through the door seal. Corrections factors were determined by calculating the average decay rate over defined CO_2 concentration (100 ppmv) ranges and repeated 10× to achieve a sufficient average (Table 1).

2.3 Minimal Salts Medium (MSM) preparation

A modified M9 MSM (0.5 g l^{-1} K_2HPO_4, 0.5 g l^{-1} KH_2PO_4, 0.5 g l^{-1} NH_4Cl, 0.5 g l^{-1} KCl, 0.10 g l^{-1} $MgSO_4$ 7 H_2O, 0.12 g l^{-1} NaCl, 0.05 g l^{-1} CaCl 2 H_2O; Madigan et al., 2009; Shiers et al., 2005) was prepared and 1 ml of a trace metal solution (0.1 g l^{-1} $ZnSO_4$ 7 H_2O, 0.3 g l^{-1} $MnCl_2$ 4 H_2O, 0.3 g l^{-1} H_3BO_3, 0.1 g l^{-1} $CuCl_2$ 2 H_2O, 0.2 g l^{-1} $NiCl_2$ 6 H_2O, 0.3 g l^{-1} $NaMoO_4$ 2 H_2O, 1.0 g l^{-1} $FeSO_4$ 7 H_2O) was added. The MSM was made up to 1000 ml and autoclaved at 121 °C for 15 min.

2.4 $^{12}CO_2$ profile soil incubations

A central hole was inserted into the lid of a 900 ml amber jar, followed by 4 surrounding holes to act as exit vents. The amber jar was then autoclaved at 110 °C for 10 min. A

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known weight of soil (32.06 g) was placed into the sterile amber jar and 300 ml of MSM was added aseptically. Dried silica gel (190 g) was placed onto the bottom shelf of the ECIC to reduce excessive humidity. The sample was placed into the ECIC and the temperature set to 30 ± 0.2 °C. An autoclaved glass pipette was inserted into the central hole in the jar lid. A clean UV sterilised tube with an autoclaved 0.2 µm gas filter was fitted to the glass pipette and to the outlet port of a battery powered air pump (Agile p/n A790). The battery air pump was activated and the chamber doors were sealed. No additional CO_2 was added to the chamber so that CO_2 flux could be monitored via soil respiration and uptake processes (therefore starting atmospheric concentrations were that of the ambient external environment prior to commencement of incubation). Automated data recording took place every 30 s to record internal environmental data (% RH, temp., ppmv CO_2) and the incubation period remained uninterrupted for 8 days. After the 8 day incubation the sample was removed from the ECIC and the silica gel desiccant replaced. The supernatant was removed and replaced with autoclaved MSM. The 300 ml soil slurry was amended with 6.0 ml (0.2 µm sterile filtered) 1000 mM $S_2O_3^{2-}$ stock solution (10.20 g $Na_2S_2O_3$ 50 ml^{-1}) to provide a 20 mM $S_2O_3^{2-}$ electron donor source for soil chemoautotrophic species. The soil was returned to the ECIC and the previous experimental conditions were repeated to observe the manifestation of chemoautotrophic conditions and hence CO_2 uptake.

2.5 Ion chromatography

For each incubation a 10 ml aliquot of supernatant was taken (T_0 and T_{40}) and filtered using a 0.45 µm syringe filter to remove particulate matter. Each sample was diluted 1/1000 and frozen until analysis. Blank incubations consisted of 30.0 g Abbeyside soil suspended in 300 ml MSM medium and incubated for 40 h in the ECIC under the same environmental regime as the experimental soils described below (2.8 Isotopic Labelling Incubation Conditions). For chromatographic separations, a Dionex model DX500 ion chromatograph (Dionex Corporation, Sunnyvale, CA, USA) was employed comprising

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of a GP50 gradient pump at a constant flow rate of 0.30 ml min^{-1}, a CD20 conductivity detector and an EG40 eluent generator with EluGen Potassium Hydroxide cartridge (KOH II EGC) installed. The Rheodyne 7125 injection valve (Rheodyne, Cotati, CA USA) was fitted with a 25 µl sample loop for all work. Separations were carried out using a Dionex IonPac AG16 (2.0 × 50 mm) guard and an AS17 (2.0 × 250 mm) analytical column, with a capacity of approximately 42.5 µ eq./column. All experiments were carried out using suppressed conductivity detection with a Dionex 2 mm bore Atlas suppressor operated at 19 mA in autorecycle mode. For removal of anionic impurities from generated eluents, a Dionex continuously regenerating anion trap column (CR-ATC) was used. For data acquisition, a Dell Optiplex GX-1 personal computer was used with PeakNet 6.60 SP1 data acquisition software (Dionex, Sunnyvale, CA, USA) installed. A five point calibration curve (0–20 mM $S_2O_3^{2-}$) was prepared from a 1000 mM $S_2O_3^{2-}$ stock solution (ReagentPlus, Sigma-Aldrich). Calibration and sample determinations were calculated using peak height ($y = 1624x + 0.0002$; $R^2 = 0.9983$).

2.6 Grass seedling incubation

The basal soil incubation was performed to determine the background respiration rates in order to conduct accurate determinations of CO_2 uptake by germinated grass. A 553 g lot of ≤2 mm manually sieved Abbeyside soil (air dried) was wetted with collected rain water (filter sterilised) and placed into the ECIC for 8 days under 0 % PAR diurnal temperature regime to determine the dark basal respiration. The incubation was repeated with the exception that PAR levels were alternated to resemble diurnal conditions to encourage soil surface algal growth (Table 2). The resultant macrophytes were of an unidentified specie(s) but were likely to be native to the soil. Every effort was taken to ensure no contaminating species were introduced to the soil after sampling by aseptic technique handling and sealed storage/incubations. The dominant observable group formed a thin characteristically green coloured crust across the soil surface after application of 2 % *w/w* glyphosphate herbicide for the control of perennial grass species (41.6 % *w/w* glyphosphate isopropyl amine salt, Monsanto Corp. Surrey, UK).

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Blue Diamond Lea grass seed mixture (29.8 % Cornwall, 12.8 % Soriento, 13.6 % Gilford, 27.7 % Montova, 10.6 % Navan, 5.5 % Huia clover – varieties of *Lolium perenne*, a perennial ryegrass) were stratified for 4 days at 20 °C in 3 ml tap water before planting into the soil sample. The soil was saturated with 200 ml NPK solution (0.02 M KNO_3, 0.02 M K_2PO_4, 0.05 M NH_3NO_3) and incubated for 3 days in direct sunlight while covered with a clear perforated acrylic lid. Germinated grass was placed into the ECIC for 8 days under a 24 h diurnal regime under an atmosphere of 800 ppmv CO_2 (Table 2) with no additional moisture required. Silica gel (190 g) desiccant was placed into the sealed chamber to absorb excess humidity. After the incubation period all roots, stems and leaves were carefully removed and incinerated (440 °C) overnight using a Carbolite muffler furnace to determine residue on ignition (ROI). Figure 2a, b visualise the algal and grass incubations to provide an overview for the reader.

2.7 Calculations

High resolution [CO_2] data consisted of measurements taken every 30 s. Average CO_2 decay rate values during the experimental events were derived by selecting the CO_2 data and subtracting the final from the initial recorded value and then dividing by the total time of the CO_2 uptake event (h). These values were corrected against the pre-determined leak rate of the chamber at that particular pCO_2 (ppmv h^{-1}) depending upon the appropriate sub-period. The resulting value (positive values indicate decreased CO_2 (e.g. sequestration) and negative values indicate production of CO_2) could then be converted to mass by multiplying with the time of the event, the chamber volume (0.041 m^3), and the density of CO_2. Standard error values were based on measurements taken during blank atmospheric concentration incubation over 110 h (data not shown).

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2.8 Isotopic labelling incubation conditions

Pre-incubated soil (30.05 g) was placed into an autoclaved 900 ml amber jar (prepared as above) and 300 ml MSM was added. The ECIC was programmed to maintain temperature at 30 ± 0.2 °C and 190 g of dried silica gel was placed onto the bottom shelf to reduce excessive humidity. The ECIC was calibrated using $^{12}CO_2$ (Industrial grade, AirProducts) and $^{13}CO_2$ (99 % atom ^{13}C, Sigma Aldrich). For these calibrations CO_2 was pumped into a filled 100 ml upturned graduated cylinder to measure the volume of water displaced per second of pumping time. The volume (cm^3) of water displaced was divided by the pump running time (s) to determine the $cm^3\ s^{-1}\ CO_2$. The generated figure of 5.166 $cm^3\ s^{-1}\ CO_2$ was used to determine the volume of CO_2 pumped into the chamber during incubation. Twelve seconds pumping time was required to reach 1000 ppmv CO_2 from T_0. The 900 ml amber jar containing the soil sample was placed into the ECIC and an autoclaved glass pipette was inserted into the central hole in the jar lid. A clean UV sterilised 30 cm tube with an autoclaved 0.2 µm gas filter was fitted to the glass pipette and to the outlet port of a battery powered air pump. The 300 ml soil slurry was inoculated with 6.0 ml (0.2 µm sterile filtered) 1000 mM $S_2O_3^{2-}$ stock solution. The battery air pump was activated and the chamber doors were sealed. The chamber was programmed to achieve a CO_2 concentration of 1000 ± 50 ppmv. All incubations took place in the dark and internal lights deactivated. For the $^{12}CO_2$ and the $^{13}CO_2$ experiments, incubations took place over 40 h. Sub-samples of homogenised soil slurry (40.0 ml) were taken at the start (T_0) and the end of both incubations (T_{40}) for fatty acid analysis using GCMS-IRMS.

2.9 Extraction and analysis of soil organic matter

The total lipids were extracted using a modified version of the Bligh & Dyer method developed by Otto and Simpson (2007), carried out in prewashed 40 ml Teflon tubes (Nalgene). This extraction method was selected as it is suitable for low molecular weight (LMW) compounds extracted from incubation mediums and requires relatively

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little sample (Otto and Simpson, 2007). After CO_2 incubation in the chamber, a sample of the soil slurry was centrifuged at 6000 rpm (20 min). The supernatant (medium) was decanted from the soil and biomass and this remaining solid residue was washed twice with a potassium hydrogen phosphate buffer solution. The soil precipitate was freeze dried (0.90 g $^{12}CO_2$ exp, 1.26 g $^{13}CO_2$ exp, dry weight) before extraction with methanol:dichloromethane (ratio 1:0, 1:1 and 0:1) was performed. The total extracts were filtered, concentrated and reconstituted in 1 ml of MeOH:DCM [50:50] for derivatisation and analysis.

The extracts were analysed by gas chromatography coupled to a quadruple Electron Impact Mass Spectrometer (Agilent) and Isotope Ratio Mass Spectrometry (GCMS-IRMS; Isoprime Ltd). The GC column effluent was subsequently split equally between the two detectors. A transmethylation derivatisation was preformed to volatise free fatty acids and phospholipids. Free fatty acids and PLFAs were selected for this experiment to determine the presence of newly formed biomass from a wide as possible array of microbial sources. Comparisons of the lipid profiles at T_0 and T_{40} were intended to show the differences in lipid-mass and ^{13}C incorporation. Although it is well known that PLFAs better represent the living fraction of biomass at the time of sampling, the increase in $\delta^{13}C$ between sampling points should indicate the living biomass fraction regardless. It is for this reason that unenriched fatty acids were not reported, but will be in future work where more specific lipid fractions will be targeted. In the case of the PLFAs, this procedure cleaves the fatty acid side chain from the glycerol backbone/polar phosphorus head group and methylates to form volatile fatty acid methyl esters (FAMEs). An aliquot of the total extract (200 µl) was evaporated to dryness before derivatisation. The method employed was a transesterification reaction involving 50 µl of sodium methoxide (Christie, 1982; Hughes et al., 1986). Determination of monounsaturated fatty acid double-bond position was performed by GCMS analysis of their dimethyl disulphide adducts (Nichols et al., 1986).

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2.10 Analysis by GCMS-IRMS

Samples were analysed using a gas chromatograph (Agilent Model 6890N) mass spectrometer (Agilent Model 5975C Quadropole MS Engine) system equipped with an automatic sampler. This GC was also coupled, via a combustion furnace (GC5) to a continuous flow isotope ratio mass spectrometer (IsoPrime), with a split ratio of approximately 50/50. The column was a fused silica capillary column (30 m × 0.25 mm i.d.) with a film thickness of 0.25 µm (HP-5MS, Agilent). Ultra high purity helium (BIP-X47S grade, Air Products) was used as the carrier gas. The injection port and the GC/MS interface were kept at 250 and 280 °C, respectively. The ion source temperature was 280 °C. The oven temperature of the gas chromatograph was programmed from 100 to 300 °C at a rate of 6 °C min^{-1} after 1.5 min at 100 °C. The column head pressure was 69.4 kPa. An aliquot of each sample (1 µl) was injected into the injection port of the gas chromatograph using the splitless mode of injection, followed by an elution split after column to both mass spectrometry detectors. The GC effluent was diverted via a heart split valve to a ceramic combustion furnace (GC5, 650 × 0.3 mm i.d.) packed with a copper oxide/platinum catalyst heated to 850 °C. Water was removed from the combustion products by passing the effluent through a nafion membrane prior to the CO_2 entering the IRMS (Isoprime Ltd, UK). Reference gas CO_2 of known $\delta^{13}C$ value was introduced from the reference gas injector at the beginning of the run, IRMS system validation was carried out using a stable isotope reference standard (Mixture B2, Indiana University). A standard deviation for the instrument was calculated to be $\pm\delta$ 1.04 over a 10 run sequence of the 15 alkane mixture. Identification of specific lipids was carried out by utilising NIST and Wiley spectral databases, with a spectral accuracy limit of ≥95 %. Evidence of the stability and accuracy of the system can be seen from the $\delta^{13}C$ values of the internal standard, cholestane, which when comparing the delta (δ) values from the $^{12}CO_2$ and $^{13}CO_2$ incubation was within our IRMS standard deviation limits (Table 3). Quantification of fatty acids was performed using a multi-point external standard method. Response factors were calculated using a 0–200 ppm C_{19} FAME

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(nonadecanoic acid methyl ester; Sigma-Aldrich) and a 100 ppm cholestane internal standard.

3 Results

3.1 Carbon dioxide profiles of soil before and after electron donor addition

Incubations of soil were performed on pre-incubated samples (exposed to single inoculation of 20 mM $S_2O_3^{2-}$ for 8 days) to observe the uninterrupted CO_2 flux within the sealed chamber. This was done to provide a visual plot of data without interruption of the automated pump or operator interference (e.g. opening of doors) as would be observed under the $^{13}CO_2$ labelling experiments. The incubation of fresh soil at 30 °C in the MSM under atmospheric concentrations of CO_2 (average indoor atmospheric concentration was determined to be ~450 ppmv CO_2) was performed to map the dynamic flux of CO_2, both under basal (normal background CO_2 flux) and autotrophic induced conditions. These experiments were performed to demonstrate the manifestation of autotrophic conditions in soils. They were not replicated in the ^{13}C-labelling experiment and therefore the $^{12}CO_2$ uptake measurements are only demonstrative to assess the applicability of the technique. The ECIC took regular measurements of CO_2 within the inner unit demonstrating that under favourable conditions (excess nutrients, moisture, constant temperature) the course of CO_2 concentration was, after an initial increasing phase, static against the perceived background leak average of 7.2 ± 1.1 ppmv h^{-1} CO_2 (Fig. 3).

From the raw data it was extrapolated (after leak correction) that the Abbeyside soil had a negative decay rate of -6.4 ± 0.2 ppmv h^{-1} CO_2, indicating that CO_2 production out-competed leaked/sequestered CO_2 (due to the governing effects of partial pressure) over the course of 192 h (8 day) incubation. Thus under the favourable conditions provided in the experiment, the metabolic activity of the resident soil microbiota led to net mineralisation of carbonaceous material naturally abundant in the soil matrix

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(Elberling and Brandt, 2003; Dilustro et al., 2005; Jassal et al., 2005). This is observable in Fig. 3 where the CO_2 gradually increases from 450 ppmv to a maximum concentration of 710 ppmv CO_2, an increase of 260 ppmv over 8 days.

A chemical electron donor was added to the slurry in an attempt to stimulate the growth and reproduction of chemoautotrophic microorganisms. The electron donor acts as the sole energy source for these abundantly low numbering chemoautotrophs naturally occurring in the soil profile (Smith and Strohl, 1991). The soil incubation employing the chemical electron donor displayed a different pattern of CO_2 fluctuation over a similar experimental period (see Fig. 4).

The $^{12}CO_2$ data plot shows a short lag phase of approximately 16 h where CO_2 levels remain relatively static. After this initial lag phase, a sharp decline in atmospheric CO_2 for a period of 20 h was observed, eventually reaching a minimum value of 385 ± 13 ppmv. After the minimum value was observed, the atmospheric concentration of CO_2 rapidly increased to levels resembling that of the basal incubation. Extrapolation (taking into account the CO_2 leaked to the laboratory atmosphere due to partial pressure) of the single decay event showed that CO_2 was sequestered to the soil during this period with an average $+3.6\,\text{ppmv}\,\text{h}^{-1}$, equivalent to $256.4\,\mu\text{g}\,CO_2\,\text{kg}^{-1}$ dry soil ($1009.3\,\mu\text{g}\,CO_2\,\text{m}^{-2}$). The decay event can be associated only with the new variable entered into the incubation, the chemical electron donor ($S_2O_3^{2-}$), the presence of which provided an environment conducive for bacterial autotrophy to take place. Interpretation of the CO_2 profile observed in (Fig. 4) must take into account the effects of both partial pressure dependent leak and the production of mineralised CO_2 from the soil matrix. After about 40 h of incubation, the efflux of CO_2 from soil seems to occur at rates exceeding the sum of CO_2 sequestration and leak rate, until a steady state, where production and losses of CO_2 are equalised. It must be stressed that the observed uptake of CO_2 into the soil matrix was not intended to demonstrate permanent sequestration of carbon, but the pattern of CO_2 flow through a complex biological matrix where sufficient environmental conditions were temporarily supplied.

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Isotopic labelling incubations for the Abbeyside soil were carried out in triplicate (^{12}C and $^{13}CO_2$, respectively) with the $^{12}CO_2$ incubations observed in Fig. 5 showing the CO_2 plot maintained at ≥1000 ppmv. The lack of $^{13}CO_2$ data plots is due to the employment of an IR detector calibrated to detect atmospheric CO_2 and employs a wavenumber (cm^{-1}) detection range between 2270–2390 cm^{-1}. The absorbance of $^{13}CO_2$ in the IR spectrum lies between 2250–2290 cm^{-1} (Gosz et al., 1988) and hence the ECIC only reports a small percentage of the true concentration (~20 %) and therefore considered to be unreliable. The $^{12}CO_2$ plots are performed prior to the labelling experiments to act as a control for both CO_2 uptake estimations and GCMS-IRMS investigations. To ensure the consistency of the environments to which the microbiota was exposed, the CO_2 atmosphere was kept at approximately 1000 ppmv (0.1 % *v/v*). This allowed for the eventuality that when concentrations dipped below a control threshold of 950 ppmv CO_2, an automated burst of 99 % CO_2 was introduced into the chamber via a peristaltic pump (hence the zig zag shape of the CO_2 plots). Extrapolation of the CO_2 plot data for each of the decay periods of the individual incubations was estimated to be 149.7, 4359.0 and 8346.7 μg CO_2 kg^{-1} dry soil (589.2, 17161.6 and 32861.1 μg CO_2 m^{-2}) uptake for incubations A, B and C, respectively. The extreme differences in substrate acquisition observed for the three respective incubations require some speculation. The decay periods for incubation A (Abbeyside soil) were less extreme than those observed for incubations B and C. A possible explanation for this discrepancy can be interpreted from the CO_2 flux patterns. To reiterate, the soils were all immersed into a mineral rich medium and incubated at 30 °C for 40 h. Although the addition of a chemical electron donor may induce the growth of chemoautotrophic microorganisms, the growth of heterotrophic species would not be initially retarded. Thus, the production of CO_2 would coincide with autotrophic sequestration. These competing processes would produce a more constrained pattern of CO_2 flux as the two opposing biochemical reactions are carried out. As we are dealing with a biological system of unknown diversity, and an inoculum containing an undefined mix of organic substrates, the level of heterotrophic activity may be more or less random and hence, creates unpredictable CO_2

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plots. Another consideration may be the physical pretreatment the soil was subjected to prior to experimental incubation. Petersen and Klug (1994) conclusively showed that sieving, storage and incubation temperature all caused significant downward shifts of soil extracted PLFAs. The responses of microorganisms reanimated after air drying are likely to be different between sub-samples despite their similar origin. Our current inability to accurately measure rates of respiration is a limitation to the method (although this is a subject of investigation in our laboratory); the sequestration of CO_2 was still obvious for all three experiments.

The sequestration of atmospheric CO_2 was further tested on three other soils to ensure the phenomenon of soil chemoautotrophy was not isolated to the Abbeyside site (Fig. 6). The Abbeyside soil was also exposed to an atmosphere of 1000 ppmv $^{13}CO_2$ to act as complimentary evidence of CO_2 sequestration via isotopic enrichment (Table 3). The blank soil incubation clearly shows the background leak of CO_2 from the chamber according to the partial pressure and the rates of CO_2 decay are measurable. The decay patterns observed for the Hampstead Park and Moscow soils show much more rapid decay plots between CO_2 injection events and are a good visual indicator that CO_2 sequestration is taking place during incubation. Using the partial pressure correction values (determined in 100 ppmv fractions), the approximate mass of CO_2 sequestered between injection events can be easily deduced. It was possible then to calculate the total fixation over 40 h, with Hampstead Park and Moscow soils taking up 1261.7 and 684.8 $\mu g\, CO_2\, kg^{-1}$ dry soil, (4967.2 and 2695.9 $\mu g\, CO_2\, m^2$), respectively. The Teagasc soil showed no significant levels of sequestration. This is an interesting observation requiring further investigation as this soil came from a continuous barley crop field and the sample appears to be a net source of CO_2 and devoid of sulphur based chemoautotrophy under the provided conditions. The site is located at the Teagasc agricultural research facility in County Carlow, Ireland and has undergone continuous barley harvest for approximately 30 yr and therefore represents a possible site of low biological diversity due to a lack of crop rotation. This is an interesting negative result as large tracts of land across the world are subject to monocropping resulting in

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low species diversity (Nsabimana et al., 2004; Dirk van Elas et al., 2002). The data sets in Figs. 5 and 6 show that the experiment was repeatable on the Abbeyside soil and on another two unrelated sites using the same methodologies and hence, uptake was not site specific.

The oxidation of $S_2O_3^{2-}$ was confirmed during the course of the incubation using ion chromatography (IC). The concentration of the $S_2O_3^{2-}$ ion in the soil solution was determined using duplicated samples at both the start and end of the incubations for each soil. Figure 7 clearly demonstrates that for the Abbeyside and Hampstead Park soils, $S_2O_3^{2-}$ was removed from solution over the course of 40 h. However, the concentration of the electron donor remained static for the Teagasc soil (and blanks, respectively). The IC results (Fig. 7) were consistent with the observed CO_2 data (Fig. 6) where consumption of CO_2 was taking place concurrently with $S_2O_3^{2-}$ oxidation and also CO_2 remained largely constant when $S_2O_3^{2-}$ remain unchanged.

3.2 Grass seed incubation

As a comparison, a mixture of agricultural grasses (Diamond Lea mixture) was incubated within the ECIC to determine the CO_2 uptake potential. This experiment was carried out as it is important to determine an experimental control under similar conditions prior to forming conclusions as to the CO_2 uptake capacity of the mixed microbial soil cultures. The grass seed incubation study was performed to compare the capacity of the soil's chemoautotrophic microbiota and that of a typical grass growing on the same soil in the presence of excess energy sources (in this case, photons) allowing for optimum uptake activity. An agricultural grass mixture was used as this is one of the most widespread vegetation types in temperate regions such as Britain and Ireland (Jeffery et al., 1995). Little data exists on the contribution of soil surface algal CO_2 sequestration rates (Betting, 1981) and it had been previously observed (in our laboratory) that using our experimental setup in the presence of PAR, algal growth over the soil surface was significant. We have observed that the algal population tends to

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colonise approximately 5 % of the available surface area for our grass seed incubation containers (data not shown). Therefore algae were grown across the total soil surface, prior to grass seed inoculation (Fig. 8) so that the CO_2 sequestration of soil algae may be estimated and hence a corrective value may be determined when making assumptions of grass CO_2 uptake (based upon the 5 % coverage of algal biomass under normal grass seed incubation conditions). This corrective measure yielded interesting data of its own in regards to rates of CO_2 sequestration by a comparatively smaller biomass community (comprising of a large number of individual algal units). It was observed, that after 8 days incubation under diurnal conditions, the total algal biomass, which colonised approximately 91 % of the soil surface, sequestered 4705.5 µg CO_2 kg^{-1} dry (18525.4 µg CO_2 m^2) soil. It was determined that the 5 % contribution to CO_2 uptake for the total surface area colonized by algae during the subsequent grass incubation was 235.3 µg CO_2 kg^{-1} dry soil (926.3 µg CO_2 m^2), which in itself is a significant contributor to CO_2 fixation, but beyond the scope of this particular study.

The CO_2 flux data of the grass seed incubation (Fig. 9) represents the diurnal regime of photosynthesis (25–100 % PAR) and night time respiration (0 % PAR) over the course of the study and from the intake and output plots it is possible to determine the overall fixation of atmospheric carbon to organic matter. After 8 days it was determined that 9801.2 µg CO_2 kg^{-1} dry soil (38587.6 µg CO_2 m^2) was sequestered into plant material once algal photosynthetic uptake, nocturnal respiration and soil CO_2 respiration (constant) were taken into account. Based upon the 5 % soil coverage of photosynthesising algae during the grass seed experiment, an approximate corrective value of ~235.3 µg CO_2 kg^{-1} (926.3 µg CO_2 m^2) has been attributed to these organisms and was incorporated into the grass CO_2 uptake calculation. The residue on ignition (ROI) test revealed that 60.08 % of the dry grass solids contained volatile organic solids.

The control experiment was performed to act as a comparative figure to refer to when discussing microbial uptake of inorganic carbon within soil matrices, as research on the available literature failed to provide adequate data. Our data suggests that provided with adequate conditions, several forms of CO_2 sequestration may be taking place

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on the terrestrial surface especially in areas heavily fertilised with inorganic fertilisers containing electron donor sources.

3.3 Isotopic labelling incubations

The incubations described so far show the net result of CO_2 uptake and efflux mechanisms. It has previously been demonstrated that CO_2 fixation and subsequent production of organic matter occurs within soil matrices with or without the addition of carbonaceous growth substrates (Miltner et al., 2004, 2005; Santruckova et al., 2005) and that heterotrophic CO_2 sequestration is possibly a significant factor in these incubation experiments. Also, the presence of organisms such as *Ralstonia eutropha* which exist as facultative heterotrophs (Pohlmann et al., 2007) must be considered as potential sources of carbon fixation through means other than $S_2O_3^{2-}$ oxidation e.g. H_2 chemoautotrophy. In the studies carried out by Miltner et al. (2004, 2005) and Santruckova et al. (2005), considerable time was required for isotopic incorporation and before quantifiable results could be reported. To investigate the occurrence and effects of heterotrophic CO_2 sequestration during the basal incubation described above, an experiment designed to measure CO_2 sequestration without the presence of an electron donor was performed with 99 % isotopically labelled $^{13}CO_2$. The soil was subjected to the same extraction and derivitisation procedure (vide supra) as employed for the GCMS-IRMS analysis. Table 3 shows the $\delta^{13}C$ values (i.e. isotopic ratio) of the $^{13}CO_2$ blank incubation NaOMe derivitised extract. The lack of enrichment in the IRMS spectrum indicates that $^{13}CO_2$ was not significantly sequestered in the absence of an electron donor over the 8 day incubation.

3.4 Identification of ^{13}C enriched lipids from soil organic matter

For all experiments involving potential enrichment of SOM, a control experiment using $^{12}CO_2$ was performed. This served two functions; firstly it demonstrated that the soil organic matter detected in the enriched samples had come about through CO_2

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uptake, but secondly and more importantly it provided unenriched spectra for identification and comparison purposes. Analysed lipids were selected based on an increased $\delta^{13}C$ value (≥+50 ‰), compared to the corresponding value from the $^{12}CO_2$ incubation. Please note that the large standard deviations observed between the CO_2 uptake measurements and the $\delta^{13}C$ values are because they were both based upon different incubation types. The ×3 replicate $^{12}CO_2$ incubations were used to generate the CO_2 uptake estimates whereas the $\delta^{13}C$ values were taken from the samples ran in triplicate producing more conserved values. The δ values for fatty acids were corrected after IRMS analysis. This was done because after derivitisation to the corresponding FAME, 94 % of the carbon isotope measured is from the fatty acid itself and the remaining 6 % as a result of the methoxy carbon (Docherty et al., 2001). This results in a very minor alteration in the δ value, especially when dealing with largely enriched lipids as reported here.

A total of 11 normal, branched and monounsaturated fatty acids were all significantly enriched in $\delta^{13}C$. Saturated fatty acids ranged from C_{14} to C_{18} and displayed very strong even to odd predominance. Odd-chained fatty acids consisted of C_{15} only whereas long-chain ($>C_{20}$) fatty acids were absent. Monounsaturated fatty acids ranged from C_{16} to C_{18}, with a maximum at 18:1ω11. The double bonds were localised to position ω9 and ω11 for both fatty acids. A single "anteiso" branched fatty acid was detected (*a*16:0) and the unusual 14Me15:0 branched fatty acid. A single cyclopropane was observed in the "iso" branched position. Enrichment of $\delta^{13}C$ was spread unevenly throughout the FAMEs with monounsaturated species 16:1ω9 and 18:1ω11 containing seven times more enrichment than the average of the 9 remaining fatty acids. The alkanes, 14:0 and 16:0 also display strong isotopic enrichment. Table 3 displays the results of the IRMS analysis for the sodium methoxide derivitised extract while Fig. 10 identifies the labelled lipids in the GC chromatogram.

The identification of specific fatty acids displaying strong ^{13}C enrichment should be investigated further to determine the possible relevance to carbon sequestering microorganisms. FAMES have been used as taxonomic markers for the quantification and

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classification of microorganisms for a long time (Tunlid and White, 1992; Frostegård and Bååth, 1996; Zelles, 1997, 1999). Enriched FAMEs can be used as biomarkers for Gram-positive (odd-chained and iso/anteiso fatty acids) and Gram-negative bacteria (monounsaturated and cyclic fatty acids), fungi (octadecadienoic acid) and general membrane lipids (C_{16} and C_{18}). For the extracted samples, a strong predominance for even-numbered, straight chain fatty acids and a single cyclopropane indicates the dominant presence of Gram-negative bacteria (Kandeler, 2007). The presence of iso/anteiso methyl-branched fatty acids indicates the presence of Gram-positive bacteria (Zelles, 1997, 1999) although, a wider spread of branched fatty acids would be expected for greater biodiversity. The branched fatty acid 12Me15:0 is uncommon but is found in rhizosphere strains (Yang et al., 2011) and marine strains such as *Vibrio alginolyticus* (Carballeira et al., 1997). The dominance of short straight-chains ($<C_{20}$) and the cyclopropane, *cy*16:0, indicates that Gram-negative bacteria were the dominant genera's. Also, the significant $\delta^{13}C$ measurements made for 18:1ω11 further compounds the evidence for Gram-negative activity as this is a known pre-cursor molecule for the production of cyclopropanes (Grogan and Cronan, 1997; Zechmeister-Bolternstern et al., 2011) which were notably absent for the $^{13}CO_2$ blank incubation. Biomarker PLFAs reported for *Thiobacillus*-like species such as *i*17:1ω5, 10Me18:1ω6 and 11Me18:1ω6 (Kerger et al., 1986; Piotrowska-Seget and Mrozik, 2003) were not observed in this experiment but the chemoautotrophic genera from environmental samples are not very well represented in the literature and therefore the significance of these particular biomarkers is tentative at present.

Employing biomarker methods for soil samples is difficult as the lipid composition of terrestial species is less known to that of marine species (Kattner et al., 2003; Stübing et al., 2003; Stevens et al., 2004a, b). Many of the lipids observed such as straight chain fatty acids (e.g. 16:0, and 18:0) are common across taxa from diverse locations (Ruess and Chamberlain, 2010). This similarity is caused by the uniform biosynthesis of fatty acid production within the animal, plant and microbial cell. For instance, acetyl-CoA is used as the primer and the carbon chain is elongated by the condensation of

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malonyl-CoA to the primer. This process yeilds palmitic acid (16:0) as a major lipid product (Weete, 1980; Ratledge and Wilkinson, 1988) which was also a dominant lipid observed in this experiment. The high recovery of 16:0 PLFA for both isotopic incubations (Table 4) along with considerable $\delta^{13}C$ enrichment, back up this statement and indicate that chemoautotrophic bacteria were producing this PLFA during the active growth phase. The quantification of FAMEs from the three respective incubations have shown a general decrease in lipid mass (but not exclusively) with only 18:1ω11 and 18:0 showing consistent increases. A decrease in general lipid mass could be due to sudden environmental changes to the original inoculum leading to rapid changes in the microbial population, be it from the introduction of unusual chemical electron donors or simply the disruption caused by air drying and sieving of the soil during sample preparation (Petersen and Klug, 1994).

The enrichment of fatty acids with ^{13}C during the course of the experiment was intended to further demonstrate the hypothesis that chemoautotrophy is stimulated after addition of $Na_2S_2O_3$. Fatty acid profiles provided by GCMS-IRMS have clearly demonstrated the enrichment of the stable isotope into biomass over the course of the experiment. The significance that certain PLFAs became much more enriched in ^{13}C over others is difficult to put into context as all the straight chain alkanes observed are between C_{14}–C_{18} and, the most commonly found in environmental samples (Finean and Mitchell, 1981; Morgan and Winstanley, 1997). The presence of only short chained PLFAs ($\leq C_{20}$) indicates microbial input to SOM (Ruess and Chamberlain, 2010; Hart et al., 2011) rather than fresh inputs by higher plant material. A lack of PLFAs with enriched fatty acid chains $>C_{20}$ or polyunsaturated species, indicates that no higher plant (including algae) or fungal activity was directly related to carbon capture (Ruess and Chamberlain, 2010). Several monounsaturated PLFAs were observed that are common across taxa, but 18:1ω9 is common to fungi and Gram-positive bacteria (Bååth, 2003; Vestal and White, 1989). Further, the lack of general fungal biomarkers such as 16:1ω5 (Olsson et al., 1995, 2003), 18:1ω7 (Olsson, 1999) and 18:2ω6,9 (Frostegård and Bååth, 1996; Zelles, 1999) indicates that etomycorrhizal and

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arbuscular mycorrhizal fungi were not actively consuming biomass and/or exudates of the $S_2O_3^{2-}$ activated consortium. The incorporation of the isotopic label into lipids and specifically PLFAs has shown that CO_2 was the primary carbon source for chemoautotrophic microorganisms (as a lack of plant biomarkers indicated no photosynthetic activity).

4 Conclusions

The biofixation of CO_2 from a mechanism other than photoautotrophy is of considerable interest. Although the soil sample was provided with conditions conducive for chemoautotrophy and hence no direct comparisons can be made to current in situ activity, it is known, that due to land fertilisation practices biofixation occurs through this chemical pathway, e.g. the additions of sulphur-based fertilisers to agricultural lands (Garcia de la Fuente et al., 2007; Yang et al., 2010). Therefore it is conceivable that CO_2 sequestration may be occurring within the upper zones of the soil matrix, on a slow but continuous basis in conjunction with surface level photosynthesis and hence, the significance of this initial study. The methods applied above could be easily applied to variations of inoculum, substrate, nutrients, electron donors (inc. photons) and environmental conditions in order to elucidate the impact on carbon sequestration. The most obvious expansion of this method would be to perform a long-term incubation following a 24 h $^{13}CO_2$ pulse event to track the fate of sequestered carbon into microbial biomass.

It is apparent that further investigations are required into understanding the complex relationships between soil biotic and abiotic components. For instance, further intergration of geomorphological and geological investigations are required to assertain the presence of electron donor bearing minerals, such as pyrite or monazite in the test soils. Additional, PCR techniques should be used to investigate the contribution to soil S^0 oxidation by heterotrophic bacteria and eukaryotic organisms (fungi) as their contribution has also been documented in the literature (Vidyalakshmi et al., 2009; Yang et

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al., 2010). Further work using molecular biology techniques should include CsCl gradient ultracentrifugation, as this would greatly enhance our undertanding using cutting edge protocols and instrumentation. To our knowledge, no measurements or estimations of CO_2 sequestration resulting from agricultural S^0 oxidation have been carried out in either in vivo or in vitro studies. This implies that a significant gap in the knowledge of CO_2 transfer between the atmosphere and the pedosphere exists. This unique opportunity to provide applicable data to soil carbon dynamics and atmospheric flux ratios was the primary driving force behind this experiment.

The overall aim of this study was to prepare a working method in which multiple techniques may be eventually employed to study carbon uptake by a single soil sample, subjected to various conditional changes. The unavoidable leak in the chamber was reproducible and its linear nature led to the calculation of partial pressure dependent correction rates, providing accurate CO_2 flux data. The addition of the chemical electron donor to the soil sample has been shown to be the main energy source and hence essential for the biofixation of CO_2 within the system. The CO_2 fluxes of the incubation samples have been tracked and quantified using high resolution data sets, leading to estimations of inorganic carbon uptake. Specifically, the 8 day soil autotroph enrichment study yielded an uptake value of 256.4 $\mu g\, CO_2\, kg^{-1}$ dry soil. The significance of such sequestration may seem minimal when compared to macroorganisms such as grass, but the global contribution may be more tenable. The isotopic enrichment of lipids provided further evidence of incorporation of inorganic atmospheric CO_2 into the soil matrix. The next goal is to apply this methodology to study more natural environments to assess carbon uptake under different conditions. Identification of microbial species involved in CO_2 uptake, profiling of chemoautotrophic species in different soil samples, and analysis of the fate of organic carbon are only some of further applications that can be envisaged for the current methodology.

Acknowledgements. The authors thank the Irish Environmental Protection Agency (STRIVE program), Science Foundation of Ireland (GEOF509) and the Geological Survey of Ireland for funding. We would also like to thank Tomasz Piasecki and Mirek Macka, School of Chemical

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Sciences and National Centre for Sensor Research, DCU for the use and assistance taking radiometer measurements and Michael Williams, Trinity College Dublin, School of Natural Sciences for his kind assistance and technical advice. Thank you to Michael McKeown, Silverbridge, Co. Armagh for the kind donation of agricultural grass seed, to the Teagasc agricultural and food development authority for providing soil samples from the Oak Park research facility and to Tim Stevens of TAS Ltd for his patience in helping to design the environmental chamber.

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Table 1. Average decay rates extrapolated from 10× CO_2 decay incubations under blank conditions.

Decay Period (ppmv CO_2)	Decay Rate (ppmv h^{-1} CO_2)	Standard Error (ppmv h^{-1} CO_2)
1200–1100	16.529	4.361
1100–1000	15.009	4.860
1000–900	13.054	0.764
900–800	9.703	1.625
800–700	6.804	1.423
700–600	5.354	1.055
600–500	2.651	0.493

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Table 2. Automated daily ECIC programme cycle for 7 day diurnal regime.

Time (min)	Temperature (°C)	PAR (%)*
0	20	100
360	18	50
180	12	25
180	10	0
240	10	0
120	12	25
120	16	50
240	20	100

* PAR (%): 100 % = Maximum Photosynthetic Photon Flux Density (PPFD) 90 µmol m^{-2} s^{-1}

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Table 3. Enrichment results of NaOMe derivatisations showing delta values (δ) and standard deviations (δ^{13}C). Lipid nomenclature *a* = anteiso, *i* = iso, *cy* = cyclopropane, *ω* = location of double bond, ND = Non-detected.

	$^{13}CO_2$ Blank		$^{12}CO_2$ incubation		$^{13}CO_2$ incubation	
Lipid	δ^{13}C (‰)	st. dev. (± ‰)	δ^{13}C (‰)	st. dev. (± ‰)	δ^{13}C (‰)	st. dev. (± ‰)
14:0	−28.9	0.5	−30.9	1.6	1766	22
12Me15:0	−27.1	0.3	−33.2	0.8	200	16
15:0	−22.1	0.4	−34.4	0.5	103	35
*a*16:0	−21.5	0.3	−16.0	2.7	404	49
16:1*ω*9	−25.9	0.2	−28.3	2.6	5228	19
16:1*ω*11	−33.3	0.1	−32.5	2.3	342	41
16:0	−28.0	0.2	−26.4	2.1	3877	328
*cy*16:0	ND	ND	−31.4	2.1	472	18
18:1*ω*9	−21.5	2.4	−25.1	2.4	389	21
18:1*ω*11	−28.9	0.3	−33.7	0.9	6732	397
18:0	−34.8	0.1	−31.8	1.5	458	5
Cholestane (IS)	−27.9	0.3	−22.4	2.3	−22	2

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Table 4. Quantification of FAMEs extracted per 1.0 g of Abbeyside soil (freeze dried) before and after 40 h incubation exposed to the inorganic electron donor, $S_2O_3^{2-}$.

	^{12}C-T_0		^{12}C-T_{40}		^{13}C-T_0		^{13}C-T_{40}	
Lipid	$\mu g\,g^{-1}$	st. dev.	$\mu g\,g^{-1}$	st. dev.	$\mu g\,g^{-1}$	st. dev.	$\mu g\,g^{-1}$	st. dev.
14:0	147.2	3.5	172.8	6.1	119.0	4.5	104.1	3.7
12Me15:0	159.0	3.4	148.5	5.6	125.9	3.3	114.2	2.9
15:0	182.4	12.7	119.4	3.3	108.2	3.0	219.4	6.1
*a*16:0	130.5	3.1	113.8	4.2	100.5	1.0	96.7	2.1
16:1ω7	310.4	14.6	526.0	66.8	191.8	4.7	163.7	9.0
16:1ω9	209.5	7.0	201.2	10.8	141.7	4.3	117.3	3.2
16:0	435.1	11.6	755.7	34.3	331.4	26.1	312.5	21.5
*cy*16:0	135.2	1.9	172.5	4.5	104.0	2.0	97.6	3.0
18:1ω9	263.8	2.3	241.8	16.9	247.9	32.7	162.3	6.3
18:1ω11	304.5	15.0	746.3	27.7	209.7	24.3	217.2	7.5
18:0	195.3	3.5	221.8	1.9	0.0	0.0	223.9	6.6

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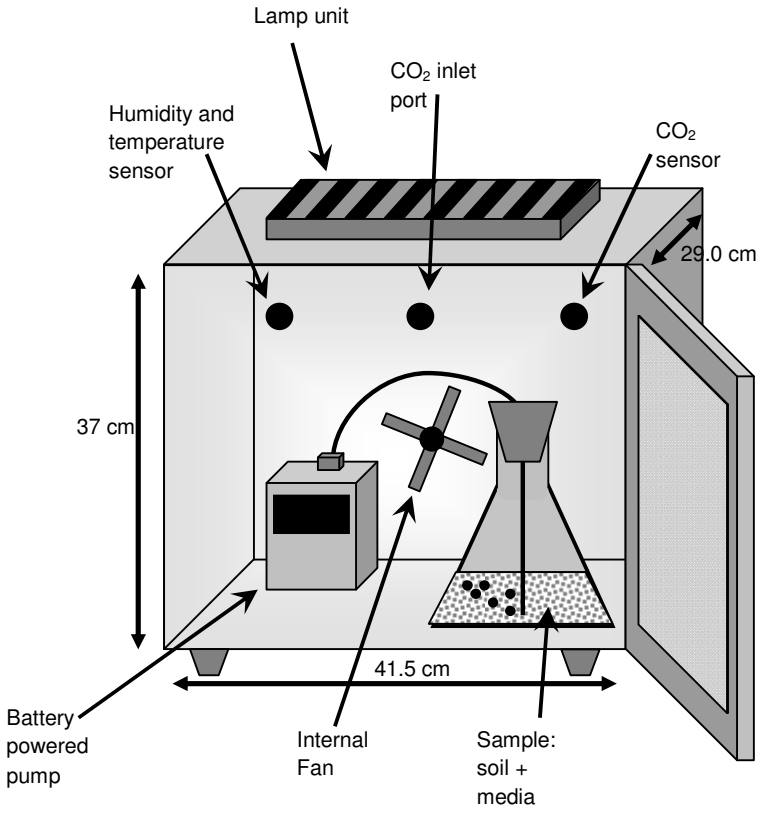

Fig. 1. Diagram of the inner chamber component (unsealed) of the ECIC and the incubation method. Outer chamber unit houses the temperature control equipment and the onboard CPU/interface.

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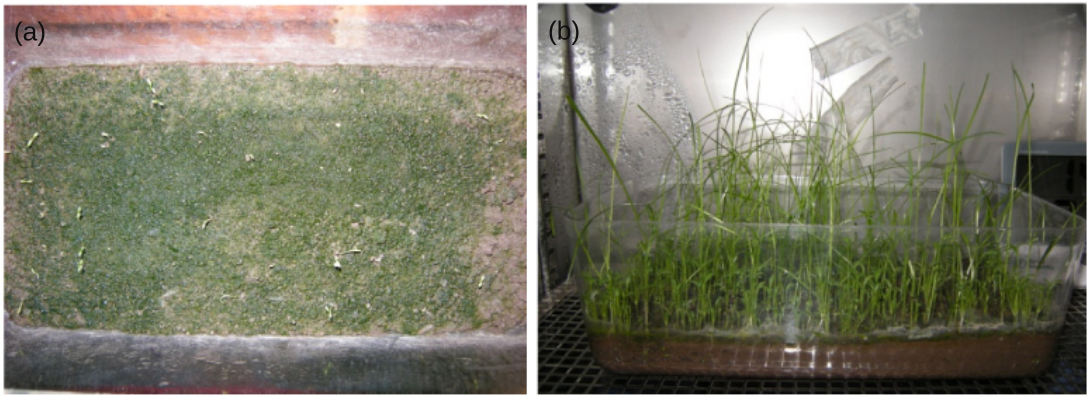

Fig. 2. **(a)** Soil algae incubation; bird's eye view showing surface coverage of the unidentified macrophyte species after 8 days diurnal conditions **(a)**. **(b)** Grass incubation; side on view after 8 days diurnal conditions within the ECIC.

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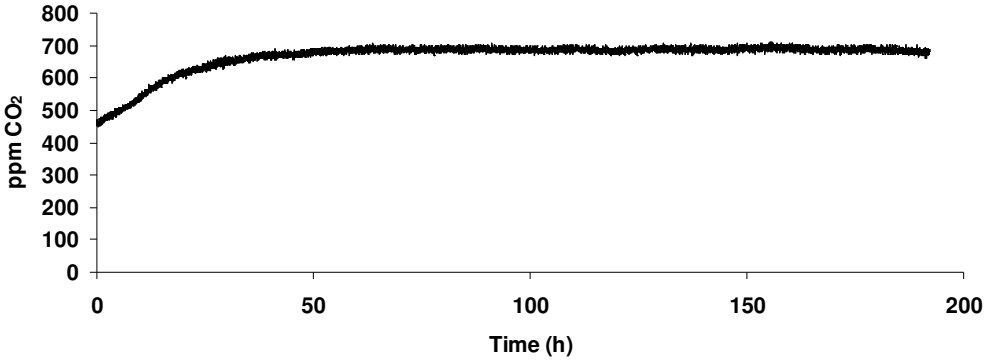

Fig. 3. Basal incubation taking place under $^{12}CO_2$ atmosphere over a 192 h period at 30 °C. Real-time data points are uncorrected for the perceived leak but still considered to be representative of CO_2 flux. All calculations derived from this data had correction factors applied prior to reporting.

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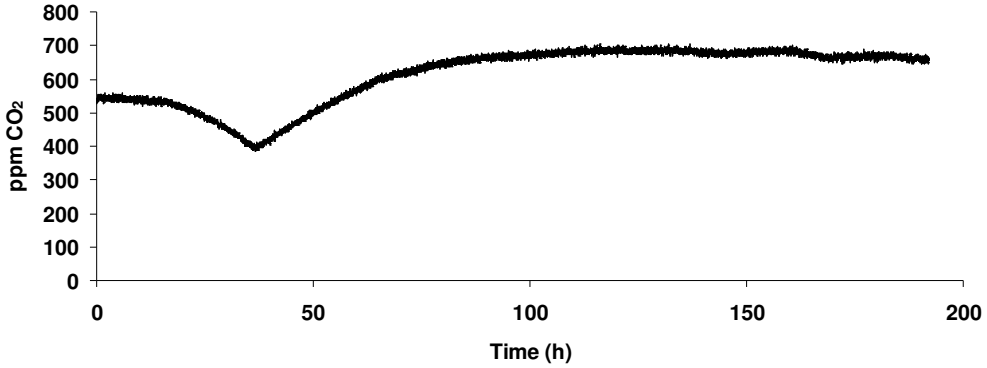

Fig. 4. Real-time data plot of a 192 h soil and electron donor incubation $^{12}CO_2$ profile at 30 °C. Real-time data points are uncorrected for the perceived leak but still considered to be representative of CO_2 flux. All calculations derived from this data had correction factors applied prior to reporting.

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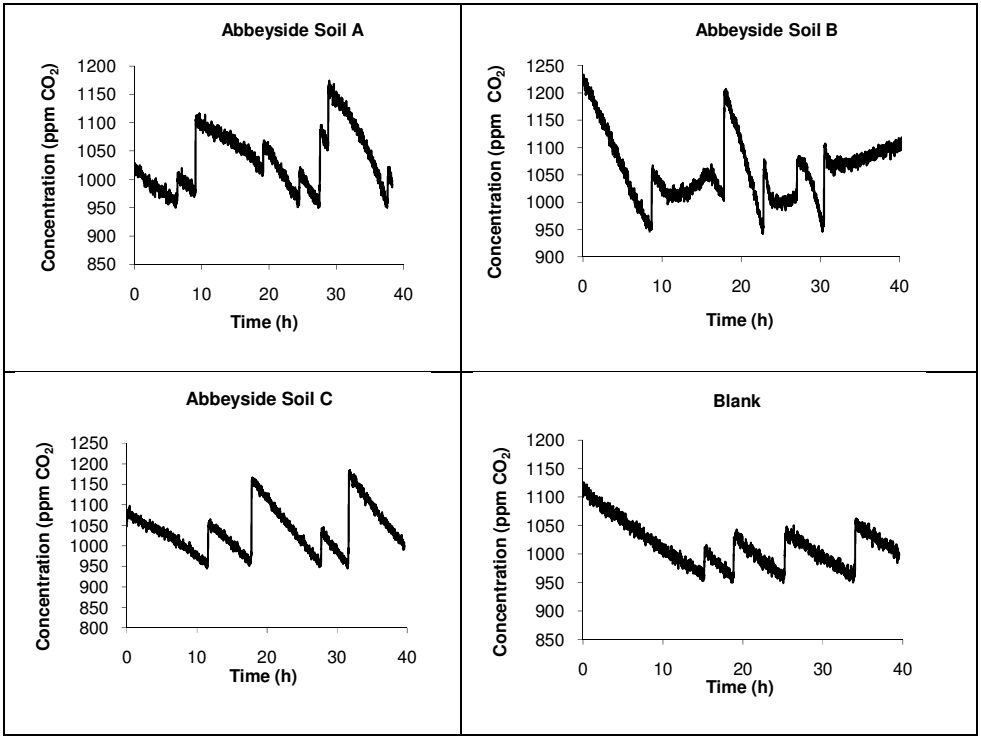

Fig. 5. CO_2 plots of Abbeyside soil incubations performed in triplicate prior to isotopic labelling with 99 % $^{13}CO_2$. Real-time data points are uncorrected for the perceived leak but still considered to be representative of CO_2 flux. All calculations derived from this data had correction factors applied prior to reporting.

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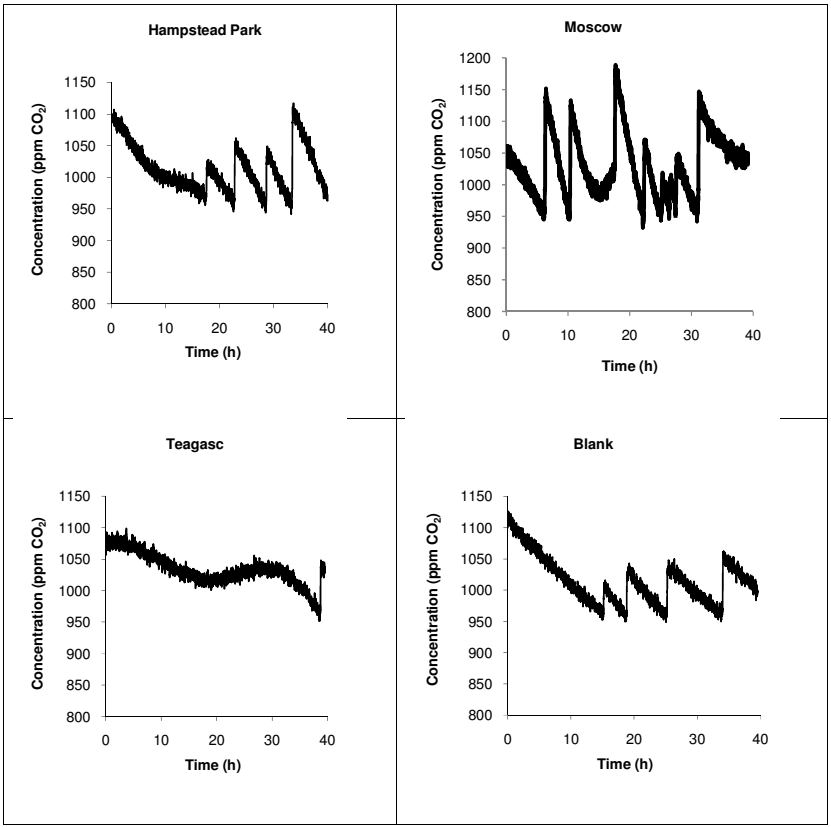

Fig. 6. CO_2 data plots for incubated soil slurries exposed to the 20 mM sodium thiosulphate from three sample sites and blank. Real-time data points are uncorrected for the perceived leak but still considered to be representative of CO_2 flux. All calculations derived from this data had correction factors applied prior to reporting.

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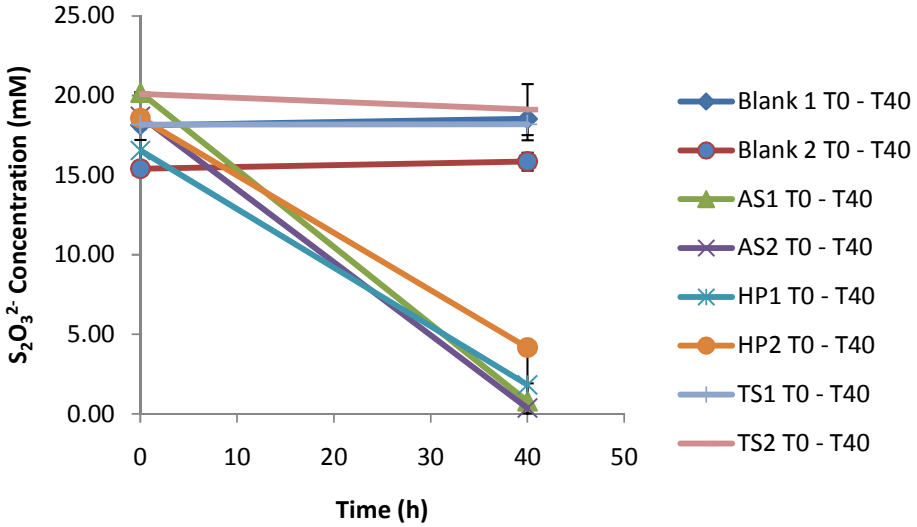

Fig. 7. Concentration of $S_2O_3^{2-}$ in soil slurry suspension. Determined using IC with each soil incubated in duplicate and sampling points taken in duplicate. Only two sampling time points were available due to the incubation chamber being sealed during the experimental period. Key: AS = Abbeyside Soil, HP = Hampstead Park, TS = Teagasc Soil.

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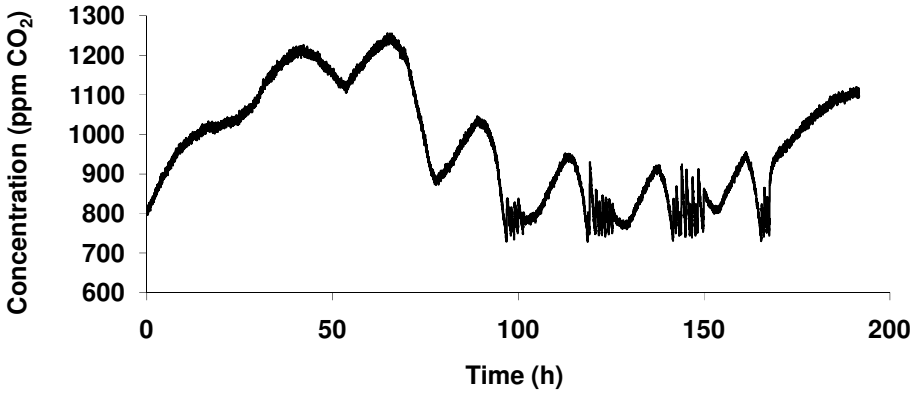

Fig. 8. CO_2 real-time plot for soil algae diurnal incubation for 8 days under a diurnal regime. Please note that the extreme fluctuations at the peak troughs between 95–175 h are caused by the automated re-introduction of substrate CO_2 to maintain ~800 ppmv CO_2. Real-time data points are uncorrected for the perceived leak but still considered to be representative of CO_2 flux. All calculations derived from this data had correction factors applied prior to reporting.

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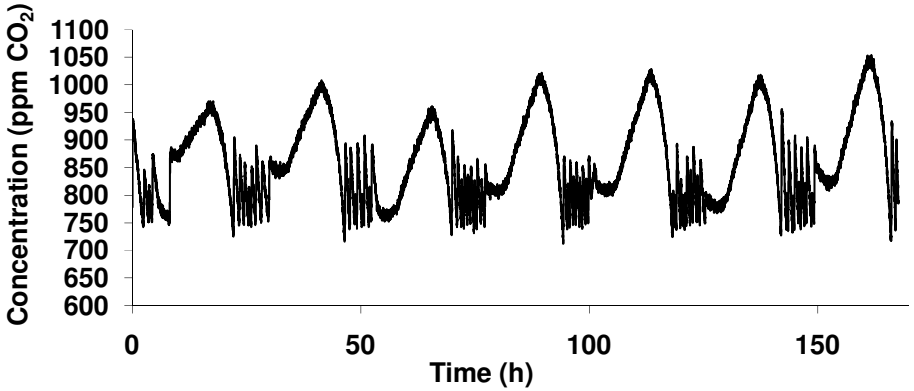

Fig. 9. CO_2 real-time plot for grass incubated at 800 ppmv CO_2, for 8 days under a diurnal regime. Real-time data points are uncorrected for the perceived leak but still considered to be representative of CO_2 flux. All calculations derived from this data had correction factors applied prior to reporting.

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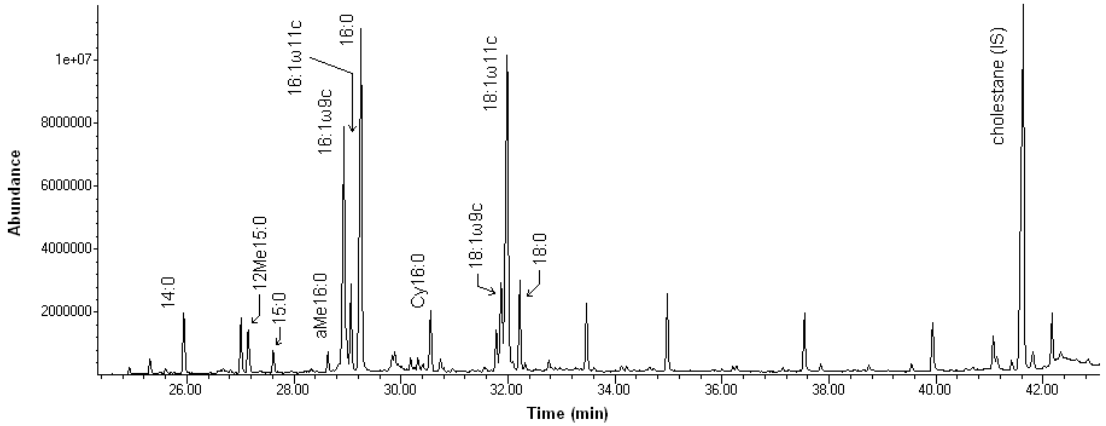

Fig. 10. GC chromatogram of NaOMe derivatised extract (Abbeyside soil) identifying enriched FAMEs in order of elution (time in minutes).

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