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Combined use of empirical data and mathematical modelling to better estimate the microbial turnover of isotopically labelled carbon substrates in soil

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ABSTRACT

The flow of carbon (C) through soil is inherently complex due to the many thousands of different chemical transformations occurring simultaneously within the soil microbial community. The accurate modelling of this C flow therefore represents a major challenge. In response to this, isotopic tracers (e.g. ^{13}C, ^{14}C) are commonly used to experimentally parameterise models describing the fate and residence time of individual C compounds within soil. In this study, we critically evaluated the combined use of experimental ^{14}C labelling and mathematical modelling to estimate C turnover times in soil. We applied ^{14}C-labelled alanine and glucose to an agricultural soil and simultaneously measured their loss from soil solution alongside the rate of microbial C immobilization and mineralization. Our results revealed that chloroform fumigation-extraction (CFE) cannot be used to reliably quantify the amount of isotopically labelled $^{13}C/^{14}C$ immobilised by the microbial biomass. This is due to uncertainty in the extraction efficiency values (k_{ec}) within the CFE methodology which are both substrate and incubation time dependent. Further, the traditional mineralization approach (i.e. measuring $^{14/13}CO_2$ evolution) provided a poor estimate of substrate loss from soil solution and mainly reflected rates of internal microbial C metabolism after substrate uptake from the soil. Therefore, while isotope addition provides a simple mechanism for labelling the microbial biomass it provides limited information on the behaviour of the substrate itself. We used our experimental data to construct a new empirical model to describe the simultaneous flow of substrate-C between key C pools in soil. This model provided a superior estimate of microbial substrate use and microbial respiration flux in comparison to traditional first order kinetic modelling approaches. We also identify a range of fundamental problems associated with the modelling of isotopic-C in soil, including issues with variation in C partitioning within the community, model pool connectivity and variation in isotopic pool dilution, which make interpretation of any C isotopic flux data difficult. We conclude that while convenient, the use of isotopic data (^{13}C, ^{14}C, ^{15}N) has many potential pitfalls necessitating a critical evaluation of both past and future studies.

1. Introduction

Soil carbon (C) turnover is a fundamental process in ecosystem functioning. However, understanding the factors regulating soil C dynamics is difficult due to the many hundreds, or even thousands, of processes occurring simultaneously within soil microbial populations (Hanson et al., 2000; Kuzyakov, 2006). A key challenge in ecosystem science is to understand this inherent complexity.

Below-ground respiration represents the primary flux mediating the passage of terrestrial C back to the atmosphere (Van Hees et al., 2005). This flux is dominated by plant (autotrophic) and soil microbe (heterotrophic) respiration. Soil respiration is controlled by the activity of soil microbial communities, which are typically limited by the availability of labile C (Aldén et al., 2001; Glanville et al., 2012). Root exudation (Jones et al., 2009; Nguyen, 2009) and root and mycorrhizal hyphal turnover (Gill and Jackson, 2000; Wallander, 2006) are the main processes by which labile C enters the soil. Within this, low molecular weight (MW) C compounds are of particular importance because, despite representing <10% of

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total dissolved organic C (DOC), they are postulated to dominate soil CO_2 efflux, due to their rapid rate of production, uptake, assimilation and turnover by the soil microbial community (Jones et al., 2003; Van Hees et al., 2005, 2008). However, it is difficult to gain a detailed mechanistic understanding of how microbial communities mineralize these low MW C substrates as they can undergo a number of fates in soil which are difficult to experimentally separate (e.g. sorption processes and biological transformation).

To accurately model C substrate flow, isotopic tracers (e.g. ^{13}C, ^{14}C) are commonly used to experimentally model the fate and residence time of individual C compounds within soil. In particular, measuring isotopically labelled substrate-derived CO_2 provides an indication as to the speed at which C compounds are specifically biodegraded by the microbial biomass. Measurements of ^{13}C- or ^{14}C-substrate assimilation into the microbial biomass is routinely quantified using the chloroform-fumigation-extraction method of Vance et al. (1987). After extraction of the fumigated sample (e.g. in 0.5 M K_2SO_4), a correction factor (k_{ec}) is applied to the data to account for the fraction of the biomass not soluble in the extractant (e.g. cell walls, insoluble protein; Wu et al., 1990; Joergensen et al., 2011). Correction factors (k_{ec}) applied to microbial biomass data using isotopic tracer studies often vary depending on soil type, soil depth, and rate of substrate addition (Bremer and van Kessel, 1990; Dictor et al., 1998). The k_{ec} value is unlikely to remain constant when using $^{13}C/^{14}C$ pulse-labelled substrates, as the isotopic C becomes progressively transformed within the cells over time (i.e. moves out of the soluble cytosolic pool into insoluble components; Bremer and van Kessel, 1990). In addition, as contrasting C substrates may be partitioned differently within the cell, it is likely that the k_{ec} values also vary significantly between compounds. However, most studies add much higher amounts of labelled substrate than the intrinsic isotopic concentration within the soil solution, therefore stimulating microbial growth and altering partitioning. Here, we directly evaluate the robustness of the k_{ec} approach for tracing isotopically labelled compounds through the soil microbial pool over different chase periods, at native soil solution concentrations not inducing microbial growth.

Combining detailed experimental data with mathematical modelling approaches, can help provide insight into the mechanisms involved in complex processes such as soil C cycling (Roose and Schnepf, 2008). Applying kinetic equations to experimental data makes it possible to parameterise mathematical models, which can then be used to estimate future changes in soil C storage and other key ecosystem services.

First-order reaction kinetics have been used to build a variety of soil organic matter (SOM) decomposition models for over fifty years, mathematically modelling the turnover of multiple C pools within soils and sediments (Salter and Green, 1933; Berner, 1964; Middleburg, 1989; Ostle et al., 2009). First-order reaction kinetics are commonly used for modelling enzyme kinetics and are frequently used within multi-component models describing not only SOM turnover, but also large-scale soil nutrient dynamics (e.g. CENTURY, RothC, ECOSYS; Paul, 2007). Within, this study, we are interested in applying first-order kinetic reaction kinetics to explicitly consider C flows through the soil microbial biomass, focusing specifically on respiration of substrate-derived C from the soluble biomass pool. Commonly, research mainly considers substrate C decomposition by soil microorganisms, rather than specifically including C dynamics within the microbial biomass (Middleburg, 1989; Bosatta and Ågren, 1995; Manzoni et al., 2012). Some other models consider microbial growth and activity when investigating microbial biomass dynamics and apply second order Michaelis-Menton type kinetics. However, these studies often add enough substrate to change the concentration of the intrinsic soil solution pool and stimulate microbial growth (Whitmore, 1996; Nguyen and Guckert, 2001). Here, we directly label the soil solution so as to not induce a microbial growth response beyond that which is naturally occurring, to investigate how C is processed within the microbial biomass.

Following introduction of isotopically labelled low MW compounds into soil, a biphasic pattern of CO_2 evolution is often observed which is suggestive that C can be partitioned into two major compartments/pools (Saggar et al., 1996; Chotte et al., 1998; Boddy et al., 2007). To describe the microbial-driven substrate mineralization kinetics of these two pools, a double first-order exponential decay model is often applied to the experimental data (Boddy et al., 2007). Mineralization experiments using isotopically labelled substrates are conducted over varying time frames; from minutes (Hill et al., 2008; Fujii et al., 2010), days (Coody et al., 1986; Glanville et al., 2012), weeks and years (Simfukwe et al., 2011; Farrar et al., 2012).

However, a two pool model may be too simplistic to describe how some substrates are compartmentalized during their metabolism and a three pool model may describe data more accurately (Hill et al., 2011; Farrar et al., 2012). The first pool of this model is attributed to a rapidly cycled microbial labile C pool (half-lives for this pool reported in minutes/hours; Hill et al., 2008; Farrar et al., 2012; Glanville et al., 2012). The second pool represents microbial structural or stored C (pool half-lives in days/weeks; Boddy et al., 2007; Farrar et al., 2012; Glanville et al., 2012) while the third, slowest turning over pool represents recalcitrant extracellular SOM (pool half-lives in weeks/months; Farrar et al., 2012). In addition, the duration of incubations may influence how pools are attributed to biological function. In particular, for long incubations (months/years), the slowest pool may represent turnover of quasi-stable soil C rather than being representative of microbial C. Further, biological attribution of modelled pools is not only time and substrate specific, but very likely also depends on a range of soil chemical, physical and biological factors (Van Hees et al., 2005).

Even if attribution of biological function to the different pools is achievable, a further major caveat associated with using exponential decay models is the assumed lack of interaction between the discrete pools and the lack of experimental techniques to validate them. To address this issue, we have developed a new empirical model based on our measured pools and applied a set of new, independent equations which allow for interactions between pools.

The first aim of this study was to monitor the temporal dynamics of two contrasting ^{14}C-labelled C substrates through the microbial biomass within an agricultural soil. Secondly, we used this experiment to evaluate whether a single k_{ec} value can be used reliably to assess the amount of substrate-^{14}C contained within the microbial biomass. Thirdly, we used the experimental data to test how the duration of the isotopic tracer experiment influences the choice of modelling approach. Fourthly, we attempted to validate components of the model. Lastly, we used our experimental data to develop a new integrated empirical model (based on measured C pools) which describes the different interactions of C between key pools in soil.

2. Materials and methods

2.1. Field site

Soil was obtained from a hyper-oceanic, freely draining, temperate agricultural grassland located in Abergwyngregyn, Gwynedd, North Wales (53°14′N, 4°1′W). The mean annual rainfall is 1250 mm and the mean annual soil temperature (at 10 cm depth) is 11 °C. The soil is classified as a Dystric Eutrudepts (US Soil Taxonomy). The vegetation at the site consists of perennial ryegrass