Modern isotopic methods to investigate the fate and provenance of C sequestered into soils from livestock derived organic matter

J.A.J. Dungait, R. Bol, I.D. Bull, D. Chadwick, W. Amelung, S. Granger, R.P. Evershed

Rothamsted Research, UK, Email jennifer.dungait@bbsrc.ac.uk

Understanding the fate of dung C in soils is challenging due to the ubiquity of plant-derived organic matter (OM), the source material from which both dung-derived OM and soil organic matter (SOM) predominantly originate. A better understanding of the fate of specific components of this substantial source of OM, and thereby its contribution to C cycling in terrestrial ecosystems, can be achieved through the use of labelled dung treatments. Bulk stable carbon isotope analyses are now used routinely to explore OM matter cycling in soils, and have shown that up to 20% of applied dung C may be incorporated into the surface soil horizons several weeks after application, with up to 8% remaining in the soil profile after one year. However, whole soil $\delta^{13}C$ values represent the average of a wide range of organic components with varying $\delta^{13}C$ values and mean residence times in soils. Several stable ^{13}C isotope ratio mass spectrometric methods have been developed to qualify and quantify different fractions of OM in soils and other complex matrices. Gas chromatography-combustion-IRMS (GC-C-IRMS) analyses have been applied to determine the incorporation and turnover of polymeric plant cell wall materials from C_4 dung into C_3 grassland soils using natural abundance ^{13}C isotope labelling.

The mean residence time of C pools increase with the application of manure. In the Hoosfield Classical Experiment at Rothamsted UK, annual applications of manure at a rate of 35 t ha^{-1} for 140 years resulted in a three-fold increase in soil organic C levels over that in unfertilised plots, and about 50% higher than unfertilised plots 104 years after manure addition had discontinued. This, and other, long-term studies indicate that manure can play a positive role in increasing soil C stocks in soils. However, other studies have concluded that dung application may have no effect or indeed a negative effect on soil C sequestration. Clearly, the dynamics of incorporation of dung C into soil are not well understood, but are highly variable in both space and time.

Natural abundance stable ^{13}C isotope labelling is one of the few proven techniques available for the examination of soil C dynamics in naturally functioning ecosystems. Isotope ratio mass spectrometry is widely used to determine the difference in natural abundance of ^{13}C between C_3 ($\delta^{13}C$ =-32 to -20‰) and C_4 ($\delta^{13}C$ = -9 to -17‰) vegetation which provides the basis for estimating the contribution of ^{13}C-enriched C_4 sources to SOM in ecosystems otherwise dominated by C_3 vegetation.

Naturally, the $\delta^{13}C$ values of cattle dung reflect the stable isotope values of their feed, therefore, cattle fed naturally ^{13}C-enriched C_4 species forage, i.e. *Zea mays*, produce a useful source of natural abundance ^{13}C-labelled dung that can be applied as a treatment to soils in C_3 ecosystems to explore cycling of dung C. Bulk $\delta^{13}C$ values of C_3 and C_4 dung-treated soil can be used in a simple mixing model to estimate dung C incorporation after dung deposition using stable C isotope determinations. A maximum of 20% and 12% dung C was determined in the top 5 cm soil horizon of dung-treated soils after autumn and spring applications. Incorporation of dung C in the spring differed from that in the autumn and was more rapid and fluctuated producing a sigmoid pattern of incorporation in the autumn. In the spring experiment, 8% of the dung derived C remained in the soil after 372 days providing direct evidence for the mechanism for increasing C stocks in soils treated annually with manures.

However, due to the diversity in decomposition dynamics and $\delta^{13}C$ values of individual biochemical components of dung, fluxes in bulk $\delta^{13}C$ values in C_4 dung treated soil may not imply a total loss or gain of dung C. Different components of plant-derived OM, and therefore dung, have a range of $\delta^{13}C$ values due to fractionations against the heavier isotope during biosynthesis. Cow dung is a complex mixture of biochemical components that are likely to decompose at different rates. The contribution of different biochemical fractions to dung estimated using gravimetric procedures showed that the major component is undigested lignocellulosic plant cell wall material. Cow dung derived from *Lolium perenne* and *Z. mays* forages was estimated as 20-30% hemicellulose, 20-30% cellulose, 7% lignin, 12% crude protein and 3-5% fats using the 'Forage Fibre Analysis' procedure. Analyses of dung carbohydrates analysed as alditol acetates

using gas chromatography determined concentrations of up to 80% dry weight as sugars in dung due to the inclusion of the soluble components, which may also derive from microbial debris. Lipids in dung were dominated by the 5β-stanols and C_{26} n-alkanol, with relatively minor contributions from carboxylic acids, wax esters and n-alkanes. Determining accurate concentrations of dung lignin is difficult due to the challenges presented by extraction of monomers with subsequent quantification against an internal standard. Lignin and lipids are depleted compared to bulk plant tissue, whilst cellulose and hemicellulose are 1-2‰ more ^{13}C-enriched. Off-line pyrolysis was used to extract lignin monomers from dung and dung-treated soils for stable ^{13}C isotope analysis using gas chromatography-combustion-IRMS (GC-C-IRMS). The m/z 44 ion current and instantaneous ratio of m/z 45/44 ions recorded for the off-line pyrolysate of lipid-extracted C_4 dung showing base-line resolved pyrolysis products, for which compound-specific $\delta^{13}C$ values could be obtained. Dung lignin-derived moieties in the pyrolysate were up to 7‰ ^{13}C-depleted relative to bulk dung, although syringol and 4-vinylguaiacol were ^{13}C-enriched up to 4‰ and 2‰, respectively. The ^{13}C-depleted values are in agreement with earlier studies, which indicated that plant tissues yield lignin products 2–7‰ depleted in ^{13}C compared with whole tissue. $\delta^{13}C$ values determined for lipids as n-carboxylic acids ($iC_{14:0}$ – $C_{30:0}$) extracted from C_3 and C_4 dung also showed depletion of up to ca. 6‰ compared to bulk dung, with increasing depletion with hydrocarbon chain length in very long chain fatty acids (VLCFA) $>C_{20}$. Carbohydrate $\delta^{13}C$ values determined for arabinose, xylose, galactose and glucose extracted from dung and analysed as their alditol acetates were -10.4 ±0.5‰, -10.4 ±0.4‰, -8.3 ±1.6‰ and -11.5 ±0.6‰ respectively for C_4 dung (bulk $\delta^{13}C$ value -12.6 ±0.3). The variability in concentration and $\delta^{13}C$ values between individual components of dung-derived OM drives the need to develop and apply sensitive tools to determine their contribution to bulk values in order to understand C cycling soils treated with manures as soil improvers or under livestock management.

Using bulk and compound-specific stable IRMS, the spatiotemporal dynamics of whole dung C cycling and that of specific biochemical components of dung in the soil can be determined. Importantly, this work has shown that fluxes of carbon derived from polysaccharides, i.e. as cellulose or monosaccharide components, were more similar to the behaviour of bulk dung C in soil than lignin. However, lignin and its 4-hydroxypropanoid monomers were unexpectedly dynamic in soil. These findings provide further evidence for emerging themes in biogeochemical investigations of soil OM dynamics that challenge perceived concepts of recalcitrance of C pools in soils, which may have profound implications for the assessment of the potential of agricultural soils to influence terrestrial C sinks. Thus, by using bulk and compound-specific stable IRMS, the spatiotemporal dynamics of whole dung C cycling and that of specific biochemical components of dung in the soil can be determined.

Thus, to better understand the contributions made to SOM from different sources, in this case animal wastes, detailed biochemical understanding of the provenance and fate of individual components is required. This paper will provide a review of the state-of-the-art techniques for compound specific analysis of incoming organic materials to soils and quantify the contributions of animal residues to the SOM budget in pastoral agricultural systems.