P396-TH

ISOTOPIC (^{13}C AND ^{15}N) LABELLING OF ORGANIC COMPOUNDS IN COW DUNG FOR USE IN ECOLOGICAL INVESTIGATIONS

Luke DICKSON[1], Richard EVERSHED[1] and Richard WALL[2]

1. Organic Geochemistry Unit, University of Bristol, School of Chemistry, Cantock's Close, Bristol, BS8 1TS
2. School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG

The return of organic matter from cow pats to soil is important for global food production and will be critical for pastoral carbon sequestration. Even so, dung organic matter processing in the environment (especially that mediated by invertebrate ecosystem engineers) is poorly understood. Natural abundance isotope tracer techniques have been used successfully to quantify dung carbon movement (e.g. Dungait *et al.*, 2005), but were found to be insufficiently sensitive where the contribution of dung carbon was low (Bol *et al.*, 2000). High enrichment of the stable isotopes ^{13}C and ^{15}N in contextually realistic molecular forms, coupled with compound-specific isotope ratio measurements, is enabling the study of microbial and invertebrate functional relationships within the dung ecosystem. Further, molecular detail may be added to the developing quantitative model of dung degradation and, using the enhanced sensitivity of organic matter tracing afforded by the high isotopic enrichment, the model may be extended to deeper soil layers.

Laboratory dung incubations using ^{13}C-glucose and $^{15}NH_4{}^{15}NO_3$ and have shown that the microbial community in cow dung used the labelled glucose as a respiratory substrate; $\delta^{13}C(CO_2)$ values peaking at 7000 ‰ after 14 h. Despite this supplementation with glucose, whose C was respired in preference to endogenous dung-C, the microbial respiration rate was similar to that in control dung, indicating that the microbial community was not greatly perturbed. Early data suggest that some amino acid abundances were slightly affected by these additions in the first few days; an indication that nitrogen-immobilising microorganisms found in cow dung by Yokoyama, *et al*. (1991) may have exploited added $^{15}NH_4{}^{15}NO_3$ and generated a ^{15}N label in the organic nitrogen pool. Immanent compound-specific isotope ratio analyses of dung amino acids will be used to confirm this incorporation.

Dung microorganisms also used the ^{13}C-glucose in biosynthesis of their phospholipid fatty acids (PLFA), again, in preference to other available carbon sources. This was demonstrated by δ^{13}C(PLFA) values that reached and maintained enrichments of thousands of per mil within 14 h. Phospholipids are rapidly hydrolysed on cell death, in this case introducing ^{13}C-labelled fatty acids into the much larger total fatty acid pool. Despite this feed of labelled components, the total fatty acid pool maintained large differences in their δ^{13}C

values compared with corresponding PLFA (>1000 ‰ below PLFA in some cases). This means that microorganisms are isotopically distinct from other dung organic matter, enabling diagnosis of the selective grazing of microorganisms by coprophagous larvae as has been suggested for *Musca domestica* (Levinson, 1960). After 21 d incubation, the total fatty acid fraction, although remaining much less enriched in ^{13}C than the PLFA, was sufficiently enriched (see fig.1) for high sensitivity dung tracer experiments and trophic investigations of the dung invertebrate community. The 16:0 fatty acid was only modestly enriched (178 ‰) but the large quantity present makes it an important part of the dung label. Further, it is not a uniquely microbial fatty acid but is of general utility to organisms in the dung-soil ecosystem. For example, the dipteran coprophage *Neomyia cornicina*, which assimilates much fatty acid without modification, will assimilate 16:0 and use it unmodified. Coprophagous insect larvae can be fed 16:0 labelled with ^{13}C and will become similarly labelled, an important prerequisite for investigating this cryptic and probably much preyed-upon (Kirk, 1999) trophic group.

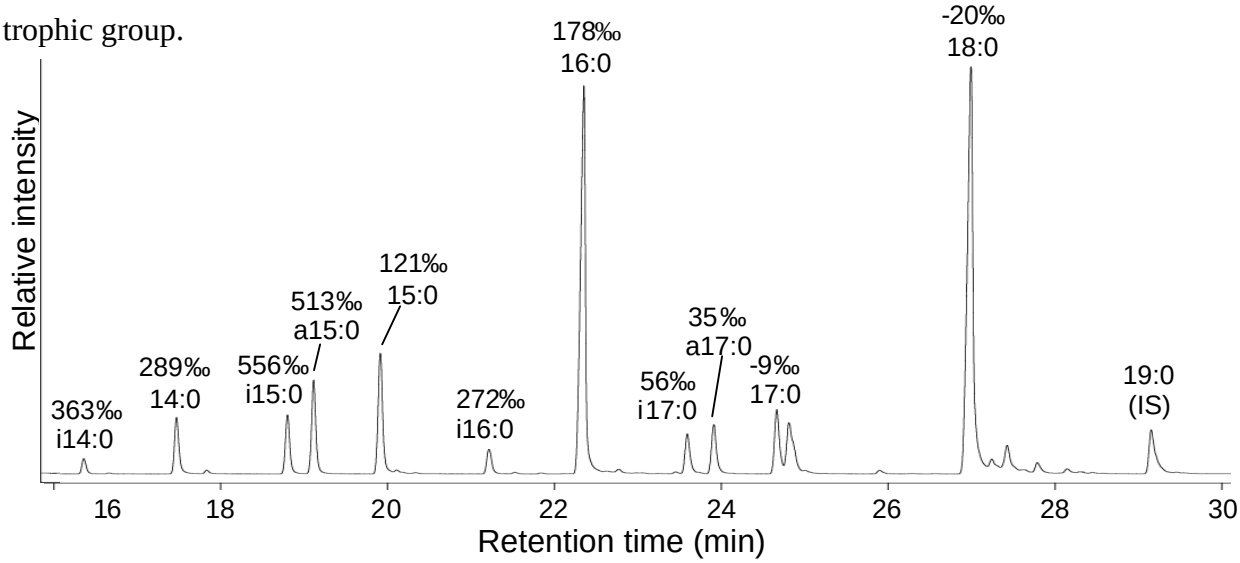

Figure 1. Partial gas chromatogram of the total fatty acid fraction of cow dung after 21d incubation with ^{13}C-glucose and $^{15}NH_4{}^{15}NO_3$. Peak labels detail $\delta^{13}C$ value and peak identity.

REFERENCES

Dungait, J.A.J., Bol, R. and Evershed, R.P. (2005) Quantification of dung C incorporation in a temperate grassland soil following spring application using bulk stable carbon isotope determinations. *Isotopes in Environmental and Health Studies*, **41**:1, 3-11.

Bol R., Amelung W., Friedrich C. and Ostle N.J. (2000) Tracing dung-derived carbon in temperate grassland using 13C natural abundance measurements. *Soil Biol. Biochem.*, **32**, 1337.

Levinson, ZH (1960) Food of housefly larvae. *Nature*, **188**, 427-428.

Kirk, A.A. (1999) The effect of dung pad fauna on the emergence of *Musca tempestiva* (*Dipt, Muscidae*) from dung pads in southern France. *Entomophaga,* **37**:4, 507-514.

Yokoyama, K., Kai H., Koga T. and Aibe T., Nitrogen mineralization and microbial populations in cow dung, dung balls and underlying soil affected by paracoprid dung beetles (1991) *Soil Biol. Biochem.*, **23**:7, 649-653.