Short communication

Stabilisation of soil organic matter in invertebrate faecal pellets through leaf litter grazing

Andrew J. Rawlinsa, Ian D. Bulla, Philip Inesonb, Richard P. Eversheda,*

a Organic Geochemistry Unit, Bristol Biogeochemistry Research Centre, School of Chemistry, University of Bristol, Cantock’s Close, Bristol BS8 1TS, UK
b Department of Biology, University of York, P.O. Box 373, York YO10 5YW, UK

Received 24 April 2006; received in revised form 13 October 2006; accepted 18 October 2006
Available online 30 November 2006

Abstract

Soils were amended with either leaf litter or faeces from pill millipedes fed on the leaf litter, then incubated at 20 °C for 130 days whilst monitoring the respiration rates. Significantly more CO2 was respired from soil containing leaf litter than that amended with an equivalent weight of faecal matter, whilst the unamended soil exhibited a respiration rate similar to soil amended with faecal material. Consideration of these findings with recently observed differences in biochemical compositions of litter and faeces suggests that processing of plant litter by detritivores leads to more stabilised forms of organic matter by removal of biochemical components essential to the nutrient requirements of the invertebrate and the soil microbial biomass.

Keywords: Decomposition; Leaf litter; Millipede faecal pellets; Respiration; Stabilization

Macroinvertebrates are important in the decomposition of plant litter and the formation of stable forms of soil organic matter (SOM) in the terrestrial environment; humic material being primarily composed of animal faeces (Kubiena, 1953). Pill millipedes are important detritivores in some terrestrial biomes and can be found in densities of up to 24 m−2 (Thiele, 1956), ingesting leaf litter, with 89–95% of the litter reportedly passing through the millipede unused (Bocock, 1967). However, recent work has shown that the processing of leaf litter by pill millipedes produces faecal material with a markedly differing chemical composition to that of the leaf litter diet (Rawlins et al., 2006). The changes in chemical composition suggest that a degree of stabilisation is affected by pill millipedes. This suggests that faecal matter will act as a more stabilised form of organic matter and decompose at a lower rate than that of the leaf litter from which it is derived. This study was carried out to test this hypothesis using faeces from the pill millipede (Glomeris marginata) fed on a monotonous diet of oak (Quercus robur) leaf litter.

Soil was collected from Cheddar Gorge, UK (Grid reference 348250, 154750) and dry sieved to 500 μm. Leaf litter was cut and pill millipede faeces sieved to 200 μm. The soil (2 g, 48% moisture) was amended with 40 mg of leaf litter or millipede faeces, intimately mixed and incubated at 20 °C along with unamended soil only controls (n = 3). Analysis of CO2 concentrations was carried out on a Carlo Erba 5300 gas chromatograph fitted with a packed column (Porapak Q, 3 m, He carrier gas) with a 500 μl sample loop. After separation on the GC column the effluent passed through a methaniser, containing a Ni/Pt catalyst held at 325 °C to convert CO2 to CH4, then to a flame ionisation detector (FID) maintained at 200 °C.

Fig. 1 depicts the respiration rates for the soil amended with leaf litter. Respiration increases from day 2 to 4 where it reaches a maximum, decreasing to day 30, where a steady ‘background’ level of respiration is reached that is maintained throughout the remainder of the experiment to day 130.

Maximum respiration rates are normally observed during the early stages of leaf litter decomposition in soil resulting from the accelerated mineralisation of carbon present in the soil microbial biomass (Ekblad and Högberg, 2000). The stabilisation of organic matter by
digestion has been reported previously for mixtures of soil and sheep faeces; ascertained by the $\delta^{13}$C analysis of respired CO$_2$ (Kristiansen et al., 2004). Moreover, this effect has also been observed by Maraun and Scheu (1996) for leaf litter and the associated faecal pellets from Glomeris marginata using a combination of microbial biomass and respiration measurements although the latter study was concerned only with the isolated substrates rather than their behaviour in situ within the soil environment.

Respiration rates of unamended soil are also shown in Fig. 1, revealing very similar rates to those of soil mixed with pill millipede faeces; maximising early in the experiment and proceeding to decrease until day 30, after which a steady rate of respiration continues through to day 130. Respiration from these soils follows a similar trend to that of soil amended with leaf litter but with a far lower rate of respiration. This can be clearly seen in Fig. 2 where respiration is rapid during the initial period of decomposition but quickly begins to plateau from day 12 onwards with little further divergence. The differences in respiration suggest that leaf litter, as a substrate, is more readily utilisable by microorganisms or fulfils an essential metabolic requirement, compared with faecal matter or soil, thereby supporting higher microbial respirative activity over the course of the experiment.

The time course of the experiment was analysed statistically using a repeated measures analysis of variance (ANOVA). This shows that the null hypothesis is rejected ($P > 0.05$) and that respiration from the microcosms does differ significantly with time between the soils amended with faecal pellets and the leaf litter. Single-factor ANOVA on the cumulative respiration from the microcosms shows that, between treatments, the samples do differ significantly at the end of the experiment ($P > 0.05$). However, variance shows that the soil amended with faeces and soil controls do not differ significantly ($P < 0.05$), thereby supporting the proposition that pill millipede faeces are a highly stabilised form of organic carbon in the terrestrial environment with the gut acting as a selective vector in the stabilisation process.

Fig. 3 depicts respiration rates of amended soils normalised to that of the soil only controls. It reveals that the flux of carbon from the soil after the addition of leaf litter decreases with time. In contrast, the addition of pill millipede faeces results in a smaller more variable flux. Between days 50 and 130, respiration from soil amended with leaf litter and pill millipede faeces follows identical trends, suggesting that by this stage the substrates possess a chemical composition of similar stability. Both substrates suppressed respiration from the microcosms over a period of 20 days, suggesting a temporary inhibitory effect on the microbial community.

The differences observed in the microbial response to the addition of organic matter to soil can be explained to a large extent by the changes in the biochemical composition that occur when pill millipedes consume leaf litter. We recently showed (Rawlins et al., 2006) that the biochemical composition of oak leaf litter was significantly different to pill millipede faeces. A clear loss of at least 70% was observed for carbohydrates and lipids rising to 85% for hydrolysable protein. Much of these losses may be accounted for by microbial degradation occurring in the gut of the millipede, e.g. $\beta$-oxidation of fatty acids (Voet and Voet, 1995 and references therein). However, it is the preferential, large loss of short chain fatty acids, triglycerides, hydrolysable carbohydrates and proteins that is key to understanding the different rates of respiration observed.
for the leaf litter and millipede faeces. These components all possess a high metabolic value for the millipede and as such are readily assimilated by the organism. Previous work has already demonstrated the short residence time of such compounds in the soil environment most likely as a result of microflora-mediated decomposition (e.g. Hita et al., 1996). Moreover, this selective assimilation must be a relatively facile process given the relatively brief increase in the rate of respiration observed when the soils amended with leaf litter are incubated. Rawlins and others (2006) also demonstrated the preferential loss of phytosterols, presumably to meet the invertebrates’ physiological requirement for cholesterol (Svoboda and Thompson, 1985), although such losses are unlikely to have any significant effect on respiration rates resulting from the action of soil microflora that have no such physiological requirement.

A comparison of the elemental composition of the leaf litter and the millipede faeces quite clearly shows that whilst the more labile, metabolically useful carbon results in a loss of organic matter it is only a minor loss (~4%). The majority of organic matter (non-hydrolysable macromolecules, lignin, etc.) remains in a more intractable, condensed state following passage through the millipede gut (Rawlins et al., 2006). In addition, leaf litter organic matter will be further stabilised, post ingestion within the faecal pellets, due to the physical protection afforded during pellet formation. This is comparable to the stabilisation of organic matter within earthworm casts observed by Golchin and others (1994) and

![Fig. 2. Cumulative respiration of soil incubated with leaf litter, pill millipede faeces and soil without organic amendment. Bars indicate standard deviation (n = 3).](image1)

![Fig. 3. The effect of the addition of organic matter added to soil on respiration normalised to the soil only controls.](image2)
Guggenberger and others (1996). This results in a physically protected organic carbon pool, which may be physically released with the disintegration of the faecal pellets but will remain chemically resistant to all but the more specialised degrading saprophytes such as white rot fungi and ascomycetes. In summary, the addition of leaf litter to soil results in increased respiration from the microcosms, most likely due to an increase in microbial activity resulting in accelerated organic matter mineralisation. The addition of invertebrate faecal matter to soil results in no significant increase in the mineralisation of organic matter. This strongly supports the conclusion that such faecal material constitutes a highly stabilised and recalcitrant form of organic matter in the terrestrial environment with the changes in biochemical composition brought about by gut passage being a significant factor in stabilisation.

Acknowledgements

This work was undertaken within the Organic Geochemistry Unit (OGU), a subdivision of the Bristol Biogeochemistry Research Centre at the University of Bristol. The authors would like to thank Dr. Ed Hornibrook and Dr. Rob Berstan for extensive assistance and the NERC for funding the Bristol node of the NERC Life Sciences Mass Spectrometry Facility (http://www.lsmsf.co.uk).

References

Maraun, M., Scheu, S., 1996. Changes in microbial biomass, respiration and nutrient status of beech (Fagus sylvatica) leaf litter processed by millipedes (Glomeris marginata). Oecologia 107, 131–140.