



Compound-specific ^{15}N analysis of amino acids in ^{15}N tracer experiments provide an estimate of newly synthesised soil protein from inorganic and organic substrates

Alice Charteris (1), Katerina Michaelides (2), and Richard Evershed (1)

(1) Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol, BS8 1TS, UK, (2) School of Geographical Sciences, University of Bristol, University Road, Bristol, BS8 1SS, UK

Organic N concentrations far exceed those of inorganic N in most soils and despite much investigation, the composition and cycling of this complex pool of SOM remains poorly understood. A particular problem has been separating more recalcitrant soil organic N from that actively cycling through the soil system; an important consideration in N cycling studies and for the soil's nutrient supplying capacity. The use of ^{15}N -labelled substrates as stable isotope tracers has contributed much to our understanding of the soil system, but the complexity and heterogeneity of soil organic N prevents thorough compound-specific ^{15}N analyses of organic N compounds and makes it difficult to examine any ^{15}N -labelled organic products in any detail. As a result, a significant proportion of previous work has either simply assumed that since the majority of soil N is organic, all of the ^{15}N retained in the soil is organic N (e.g. Sebilo et al., 2013) or subtracted ^{15}N -labelled inorganic compounds from bulk values (e.g. Pilbeam et al., 1997). While the latter approach is more accurate, these methods only provide an estimate of the bulk ^{15}N value of an extremely complex and non-uniformly labelled organic pool. A more detailed approach has been to use microbial biomass extraction (Brookes et al., 1985) and subsequent N isotopic analysis to determine the ^{15}N value of biomass-N, representing the fraction of ^{15}N assimilated by microbes or the ^{15}N cycling through the 'living' or 'active' portion of soil organic N. However, this extraction method can only generate estimates and some lack of confidence in its validity and reliability remains. Here, we present an alternative technique to obtain a measure of the assimilation of an applied ^{15}N substrate by the soil microbial biomass and an estimate of the newly synthesized soil protein, which is representative of the magnitude of the active soil microbial biomass. The technique uses a stable isotope tracer and compound-specific ^{15}N analysis, but unlike previous works analyses for amino acids (representing organic products) rather than ammonium (NH_4^+) and nitrate (NO_3^-). Amino acids are commonly referred to as 'the building blocks of life' as they form the proteins which regulate life's essential biochemical reactions. Proteinaceous matter generally comprises 20-40% of total soil N and is ubiquitous in living organisms, so is a likely 'organic product' of microbial activity/assimilation. Hence, we consider it likely that amino acids represent the major organic nitrogenous products and a reasonable 'proxy' for/measure of the assimilation of an applied ^{15}N substrate by the soil microbial biomass and an estimate of the newly synthesized soil protein.

Brookes, P. C. et al. *Soil Biol Biochem.* 1985, 17, 837-842.

Jenkinson, D. S. et al. *Soil Biol Biochem.* 2004, 36, 5-7.

Nannipieri, P. et al. *Plant Soil.* 1999, 208, 43-56.

Pilbeam, C. J. et al. *J Agr Sci.* 1997, 128, 415-424.

Sebilo, M. et al. *PNAS.* 2013, 110, 18185-18189.