



Organic N cycling revealed through ^{15}N , ^{13}C -stable isotope probing of the soil amino acid metabolome

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The availability of biologically accessible forms of N influences the diversity, dynamics and functioning of many ecosystems including soils. Although the cycling and interconversions between inorganic forms of N in soils are relatively well constrained, much less is currently known about the fate of organic N-containing compounds. Organic N represents the vast majority of natural N inputs to soils and of these, proteins, peptides and amino acids are the most significant. Determining the rates and pathways of N and C flux from such sources is vital to gain a detailed understanding of natural (and perturbed system) N cycling. Herein we adopted a compound-specific dual-stable isotope probing approach to trace N and C within the soil amino acid metabolome.

A grassland soil was incubated with dual ^{15}N , ^{13}C -labelled substrates (glutamate, glycine and cow dung) in separate incubations, enabling the fate of their N and C to be followed over a time course (between 3 h and 32 d). A molecular level approach was adopted throughout, whereby chemically and isotopically defined substrates were added to soil before isotopic analyses of individual soil amino acids, respired CO_2 and bulk soil. Compound-specific N and C isotope analyses of soil amino acids, following addition of individual amino acid substrates, revealed highly conservative biochemical processing by the soil microbial community, such that the biochemical behaviour of a whole soil reflects that of an individual microorganism; highlighting the conservative nature of primary cellular metabolism even at a systems level. The mass balance approach adopted throughout and the application of linear and non linear regressions enabled rate constants and fluxes of ^{15}N and ^{13}C label into *de novo* biosynthesised amino acids to be determined in addition to measures of the 'biochemical proximity' of substrates and products. Such information is vital to the creation of models of soil N dynamics whose structure is based in fundamental cellular biochemistry, which would potentially demonstrate a wider applicability to a large range of systems. Significant decoupling of the soil N and C cycles was demonstrated at the amino acid level, whereby the disparate fates of C and N from substrates in which they were covalently linked are clearly apparent.

This approach was extended to investigations into the physical transport and biochemical transformations of dung-derived N and C shortly after its addition to soil. These experiments revealed valuable information regarding the transport processes involved in dung N and C incorporation into soil, and the chemical forms in which it is transported. The application of these techniques to studies of metabolic processes is not limited to soils and sediments; valuable information regarding C and N metabolism in other ecosystems and, indeed, individual species of micro- and macro- organisms could be derived in this way.