Fate of ¹³C-labelled microbial carbon and nitrogen in soil

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Introduction

• Most soil N is organic

- To understand more about soil N we need to consider microbial structural nitrogen – one of the least well understood components
- •Microorganisms contain a diverse range of N-containing structural compounds
- •To understand more about the mineralization of this N we can look at the mineralization of C from these compounds
- •Previous work has shown differences in mineralization between microbial and nonmicrobial amino acids
- •By using ¹³C-labelled compounds we can "follow" the mineralization process

Compounds examined

L-alanineD-alanineD-amino acids → bacteria



Figure 1. Structure of Dand L- forms of amino acids. These are stereoisomers – i.e. nonsuperimposable mirrorimages of each other.

Hypotheses

- Different mechanism for mineralization of D- and L-alanine – different compounds identified by NMR analysis
- •C in CO₂ coming directly from added substrates

Method

•Soil was sampled from sites under arable and grass from each of two soils with contrasting textures (Gleneagles series and Stirling series soils)

•Isotopically labelled D- or L-alanine was added to each soil (2.5 mg g⁻¹ soil)

•Samples were incubated at 22°C in sealed jars

•Alkali traps were included to capture respired CO₂

•Soil samples were frozen at 0, 24, 72 & 150 hours

•Soil was centrifuged in two-part centrifuge tubes to extract soluble fractions for liquid-state NMR spectroscopy:

-Water was added and soil centrifuged to extract the water soluble C fraction

 $-K_2SO_4$ was added and soil centrifuged to extract the adsorbed C fraction

Results: Liquid-state NMR



Figure 2. Relative signal intensities of NMR spectra of water-soluble and adsorbed fractions of soils with ¹³C-labelled D-alanine



Figure 3. Relative signal intensities of NMR spectra of water-soluble and adsorbed fractions of soils with ¹³C-labelled L-alanine •The only signals produced in the NMR analysis were those of alanine

•No intermediate or other compounds were seen

- •The signal intensities were normalised relative to each other and these values are plotted against time here
- •For both D- and Lalanine, more signal was seen in the water soluble fraction than in that which was adsorbed to the clay particles in the soil
- •In most cases, the signal had disappeared by 150 hours
- •The decrease in signal intensity was generally more rapid with L-alanine

Results: Mass spectroscopy & respiration



Figure 4. ¹³C recovered as CO₂ from soil with ¹³C Dor L-alanine



Figure 5. Substrate-induced respiration with un-labelled D- and L-alanine in each of the soils

•Respired ¹³C is shown as a percentage of the ¹³C added as D-or Lalanine

•¹³C-labelled CO₂ was present in all cases

•The ¹³C respired increased more rapidly with L- than D-alanine

•In all cases, respiration was initially faster with Lalanine than with Dalanine

•By approximately 150 hours, the rate of respiration with Dalanine had caught up with that of L-alanine

Conclusions

•No intermediate compounds identified in liquid-state NMR analysis for D- or L-alanine - suggested no major differences between mechanisms of mineralization of these compounds.

•Solid-state NMR analysis of soil is yet to be completed – may identify any differences not seen in water-soluble and adsorbed fractions.

•NMR signal disappearing by 150 hrs suggested complete mineralization

•More rapid decrease in NMR signal intensity with L-alanine corresponded with faster initial respiration rate

•As anticipated, mass spectroscopy showed that the C respired as CO_2 came directly from the substrates added

•The initially more rapid increase in ${}^{13}C$ in CO₂ with L-alanine corresponded with the initially more rapid respiration rate shown

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