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Interactive comment on "Compound-specific ^{15}N stable isotope probing of N assimilation by the soil microbial biomass: a new methodological paradigm in soil N cycling" *by* A. F. Charteris et al.

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We also thank Anonymous Referee #2 for their comments. Our responses to their general remarks are given first and we then address their specific comments.

We acknowledge that the experimental protocol for compound-specific measurements of amino acid δ15N values is not new. However, this is not the focus of the paper. This paper highlights an important new way of using the data obtained from 15N tracer experiments (using previously established analytical procedures) in order to quantify the degree of N assimilation by the soil microbial biomass. We have tried to make this clear through the framing of the title, the Abstract, the aims stated at the end of the Introduc-

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tion, the introductory paragraph to the Results and Discussion and the Conclusions. We believe in all these we draw attention to the great value of our newly developed conceptual use of these measurements and their wider potential to investigate the fate of N amendments in soils. As far as we are aware there are only a handful of papers describing the use of 15N-probing of amino acids in soils and no one to our knowledge has ever framed the use of the approach in the way we have here.

In relation to the comment regarding missing information Equations 1-6 provide all information required to calculate the percentage of assimilated N and as discussed below recoveries of added 15N were on average ca. 100%.

Specific comments:

Introduction: Words in quotation marks are intended to imply incomplete understanding, which is well-appreciated by most researchers in this area and stem from the complex nature of the soil system.

Pg 1138, line 28: The term total hydrolysable AA pool has been fairly widely used in related literature and is commonly further abbreviated to THAA pool, which we chose not to do for readability. As we discuss in the paper with reference to Roberts and Jones (2008) the THAA pool effectively equates to soil protein given the low concentrations of free amino acids.

Pg 1139, line 24: 1 month in a -20oC deep freeze following immersion in liquid N2, which is deemed sufficient to instantaneously halt microbial activity, with storage at -20 °C sufficient to prevent it restarting and particularly to an extent that would make a difference to hydrolysable amino acid concentrations and enrichments.

Pg 1139-1140: We have reported the most significant values, notably total 15N enrichments for each treatment are shown in Table 4 - the initial rise in values recorded at t = 3h is sustained throughout the experiment (see mean values also in Table 4). The initial contents/ concentrations of total N and AAs prior to treatment are shown in Tables

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1-3 under the column ‘Time - 0’. The concentrations and enrichments of inorganic N in the soil samples were not measured in the incubation samples, although we agree that these data would be useful to further investigate the wider distribution of N in the system, they are not essential for the calculations we have carried out. Initial inorganic N concentrations were estimated from average values of soil water nitrate and ammonium concentrations and soil mineral nitrate measurements of the whole field from which the soil was taken during the same period in previous years. Particular care was taken to ensure that additions would not overwhelm these native concentrations, with addition concentrations based on typical fertiliser additions of agricultural relevance (see Pg 1144, from line 10).

Pg 1141: Sample amounts for both total N and bulk soil δ15N measurements were based on sample N content to achieve appropriate signals for determination (not too low or overloaded). Typical soil samples were less than 1 mg.

Pg 1143, Equation 6: does assume 100 % conservation of the label based on the incubation set-up which does not allow leaching and minimises volatile losses and the fact that this appears to have been successful as following treatment, bulk soil δ15N values are relatively stable throughout the experiment. Indeed, calculated percentage recoveries were generally close to 100 % in most samples. We agree that it may be worth stating this more clearly for wider use of the method.

Pg 1145: The referee is correct, as shown in Tables 1-3 the total hydrolysable AA pool is consistently larger for the 15N-Glu experiment. The reason for this is simply that the soils used for the 15N-Glu experiment were taken at a different time to those used in the 15N inorganic experiments; importantly all soils were taken from the same plot. The fact that the initial concentrations of amino acids were different between different experiments has no bearing on the outcome of the experiments as the amino acid concentrations remain constant throughout each individual experiment.

Pg 1147: As above, percentage recoveries were generally 100 %.

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Discussion: We provide a context for the state of methods to investigate soil N cycling in the Introduction and have avoided direct comparison of our new method with other methods, (including as suggested the most relevant - 15N measurements of total microbial biomass) because our new method is not intended as a direct improvement or replacement for any of the methods currently available, but rather highlights how currently available experimental techniques may be combined into a powerful novel conceptual method able to provide a wide range of information on soil N cycling that is not currently accessible.

Interactive comment on SOIL Discuss., 2, 1135, 2015.