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PREFERENCES FOR DIFFERENT NITROGEN FORMS BY COEXISTING PLANT SPECIES AND SOIL MICROBES: COMMENT

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Harrison et al. (2007) reported on an interesting 15N labeling study. Under field conditions, they assessed whether coexisting plant species of temperate grasslands show preferences for different chemical forms of nitrogen (N), including ammonium nitrate (inorganic N) and three amino acids of varying complexity (organic N). The authors found that all plant species were able to take up the full range of amino acids offered to them, as shown by 15N and 13C enrichment in plant tissues. However, plants all preferred inorganic over organic N, indicated by higher 15N enrichments after ammonium nitrate compared to organic N labeling. We do not object to the general interpretation of the results and the authors’ main conclusions. Yet, we would like to comment on the plant uptake of intact amino acids. When testing for significant relationships between excess 13C and 15N of plants to infer direct uptake of amino acids (Na¨sholm et al. 1998), Harrison et al. (2007) should have accounted for the different C:N ratios of the amino acids used. The amino acid tracers were U-13C2-15N-glycine, U-13C3-15N-serine, and U-13C9-15N-phenylalanine (all 15N 98% and 13C 98%), and their ratios of C:N atoms are 2:1, 3:1, and 9:1 respectively. While the authors point out that these differences in available C may affect the preferences of plants and microbes, they omitted to consider the methodological consequences. One common problem (see e.g., Jones et al. 2005) when using dual-labeled amino acids to study organic N uptake by plants is detecting the 13C label in plants. Due to the high C:N ratio of plants and the high abundance of 13C (~1.08 atom % in C3 plants), the dilution of C is usually 60–150 times higher than that of 15N (Nåsholm and Persson 2001). Finding a significant relationship between excess 13C and 15N requires separating the shift in 13C resulting from direct amino acid uptake from natural variation and analytical error. However, this is often not possible, due to rather low concentrations of tracer 13C. As a solution, Nåsholm and Persson (2001) suggested to concentrate the labeled fraction of the plant material studied, by extracting the soluble fraction containing the label. For assessing the uptake of intact amino acids using the dual-labeling approach, the critical step is to assure that there is a theoretical possibility of detecting this uptake. From the measured values of δ15N (after labeling with 15N) the theoretical shift in δ13C corresponding to 100% intact uptake can be calculated (Nåsholm and Persson 2001). Thereby it can be determined whether this shift is distinguishable from “noise.”

Given the high amount of C in phenylalanine, it is not surprising that Harrison et al. (2007) found a significant relationship between excess 13C and 15N across all species for this amino acid, but not for glycine and serine. In their paper, Fig. 2A shows that shoot 15N enrichment over all plant species was highest for glycine and lowest for phenylalanine (among organic N forms), while shoot 13C enrichment was similar for all amino acids (Fig. 2C). This almost opposite pattern for 13C and 15N enrichment also applies for single species (Fig. 1), roots (Fig. 3), and microbes (Fig. 4). In the latter, 13C enrichment was actually highest when labeled with phenylalanine, and lowest in the case of glycine. We think that these results are due to the different C:N ratios of the three amino acids rather than indicating higher uptake of phenylalanine compared to glycine and serine, which is particularly unlikely given that phenylalanine is the largest and most complex amino acid tested. However, without significant relationships between excess 13C and 15N in plant tissues, the proportion of amino acids taken up as intact molecule cannot be estimated for glycine and serine. Moreover, although no data on amino acid concentration in the soil solution are shown, it is likely that phenylalanine is the least abundant of the three amino acids, and glycine the most abundant. Thus, the dilution of the added 15N tracer (equal for all N forms) by the natural abundance pool was probably least for phenylalanine and strongest for glycine, again leading to an overestimation of phenylalanine uptake when assessed by 15N labeling.

We fully agree with Harrison et al. (2007), that a rigorous test to detect organic N uptake by plants requires compound specific isotope analysis (a combination of gas chromatography with isotope ratio mass spectrometry; see e.g., Persson and Nåsholm 2001). But clearly, the results of Harrison et al. (2007) demonstrate that the use of the Nåsholm et al. (1998) method to infer
The comment of von Felten et al. (2008) raises a number of interesting and valid points concerning the measurement of direct uptake of amino acids by plants. But, as they highlight, none invalidate our main finding that all plant species tested were able to take up the full range of amino acids presented to them, but that all preferred inorganic over organic N forms (Harrison et al. 2007). Hence, our findings do not support the idea that all plant species tested were able to take up the full range of amino acids when in the presence of organic N forms.

The second point raised by von Felten et al. concerns the apparent opposite patterns of uptake of 15N and 13C in plant shoots, roots and microbes (Harrison et al. 2007: Figs. 1–4). Looking at the data however, we feel this is not the case for all fractions. For example, in Fig. 1, while there is greater uptake of 15N from glycine than phenylalanine for all species tested, there is not a significant difference in uptake of 13C from each of the three N forms presented. Similarly, looking at Fig. 2c, we found no significant difference in uptake of 13C across all of the N forms tested; we also found the same to be true for uptake of 13C into plant roots (data not presented). As highlighted by von Felten et al., microbial enrichment by 13C (Fig. 4) did indicate greater uptake of phenylalanine compared to glycine and serine. However, the potential overestimation of phenylalanine uptake into the microbial fraction was noted in our discussion: although we found significantly greater 13C from

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**Literature cited**


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phenylalanine than glycine and serine in the microbial biomass, we highlight that this was most likely due to differences in the number of carbon atoms present in a molecule of each N form, rather than an indication of preferential use of phenylalanine by microbes. (Glycine and phenylalanine have the same number of N atoms per molecule, but the number of C atoms differs dramatically, with glycine having two and phenylalanine having nine. Therefore, a greater amount of $^{13}$C from phenylalanine than glycine will be detected per unit uptake of N by microbes.)

The final point raised by von Felten et al. concerns the potential for differential dilution of $^{15}$N tracer due to variable concentrations of individual amino acids in soil solution. We fully agree with this comment and for this reason we were cautious in drawing conclusions on differential uptake of amino acids, especially since we did not measure concentrations of individual amino acids in soil solution; it is notoriously difficult to obtain realistic measures of soil solution amino acid concentrations because the turnover of amino acids is so rapid (Boddy et al. 2007). We were careful to highlight this issue in our discussion, i.e., that soil concentrations of phenylalanine and serine are likely relative to other amino acids tested, especially glycine, and hence the uptake of phenylalanine and serine might have been overestimated relative to its importance. While we did not take this dilution effect into account in our study, we believe that it doesn’t invalidate our main finding that plants preferred glycine over phenylalanine because shoot $^{15}$N concentration was significantly lower for phenylalanine than for all other N forms tested. If anything, the likely overestimation of phenylalanine uptake in our study serves to strengthen our conclusion that this amino acid is less preferred by plants than are the simpler N forms, such as glycine.

In sum, we argue that the points raised by von Felten et al. do not alter our main conclusions, which concern preferential use of, and competition for, different chemical forms of N by plants and microbes. Also, while they do raise valid points concerning the need for caution in using dual labeled ($^{15}$N, $^{13}$C) stable isotopes for detecting and quantifying direct uptake of amino acids by plants, as has as been called for by other authors (e.g., Jones et al. 2005), we do not believe our findings are misleading; rather, we draw cautious conclusions from our data which take into account the uncertainty about direct uptake that they raise. One key issue that the comment that Felten et al. does raise, however, is the need to develop suitable analytical techniques which enable improved measurement of the pool size and flux of different amino acids in soil, thereby providing more realistic measures of the availability of these N forms to plants, and assessment of the direct uptake of different N forms by plants.

**Literature cited**


