Title: In field N transfer, build-up, and leaching in ryegrass-clover mixtures

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Abstract:
Two field experiments investigating dynamics in grass-clover mixtures were conducted, using ^{15}N- and ^{14}C-labelling to trace carbon (C) and nitrogen (N) from grass (*Lolium perenne* L.) and clover (*Trifolium repens* L. and *Trifolium pratense* L.). The leaching of dissolved inorganic nitrogen (DIN), as measured in pore water sampled by suction cups, increased during the autumn and winter, whereas the leaching of dissolved organic nitrogen (DON) was fairly constant during this period. Leaching of ^{15}N from the sward indicated that ryegrass was the direct source of less than 1-2 percent of the total N leaching measured, whereas N dynamics pointed to clover as an important contributor to N leaching. Sampling of roots indicates that the dynamics in smaller roots were responsible for N and C build-up in the sward, and that N became available for transfer among species and leaching from the root zone. The bi-directional transfer of N between ryegrass and clover could however not be explained only by root turnover. Other processes like direct uptake of organic N compounds, may have contributed.

Introduction
Efficient utilisation of nutrients in agricultural systems is essential in order to meet a sustainable production of food and reduce environmental harmful losses. Grass-clover mixtures are a key crop at e.g. dairy farms supplying high quality fodder and at the same time building up soil fertility. The main input of N in such systems is derived from N_2-fixation of clover, which means that understanding the below ground dynamics is essential to reduce N loss. In recent years there has been a growing awareness that investigations of N losses have to include organically bound N as this can contribute significantly to the total N loss.

The aim of the present studies was to investigate the N dynamics in perennial ryegrass-clover mixtures that lead to transfer among species, C and N build-up, and dissolved organic C (DOC), dissolved inorganic N (DIN), and dissolved organic N (DON) leaching.

Materials and methods

In the two field experiments PVC cylinders (ø 30 cm) were inserted into existing grass-clover swards to confine the system under investigation. Beneath the cylinders Teflon suction cups were installed in order to collect pore water percolating the cylinders. N dynamics was studied by ^{15}N and ^{14}C labelling, added in one experiment directly in the field through leaf-labelling of either grass or clover (Rasmussen et al., 2007), and in the other experiment via ^{15}N- and ^{14}C-labelled residues, which was incubated in the cylinders (Rasmussen et al. *submitted*). The leaf-labelling experiment lasted for three months during the summer, whereas the residue incubation experiments lasted for 16 months, covering two growth seasons and one autumn-winter period with high pore water leaching. In both experiments N concentration and ^{15}N- and ^{14}C-enrichment in leaf material, roots, soil and percolating pore water was determined. The latter measured in form of both DIN and total dissolved N (TDN), which allow for the calculation of DON by simple subtraction.

Results and discussion

The two experiments gave many interesting findings of which two concerning the ^{15}N-data will be discussed here.

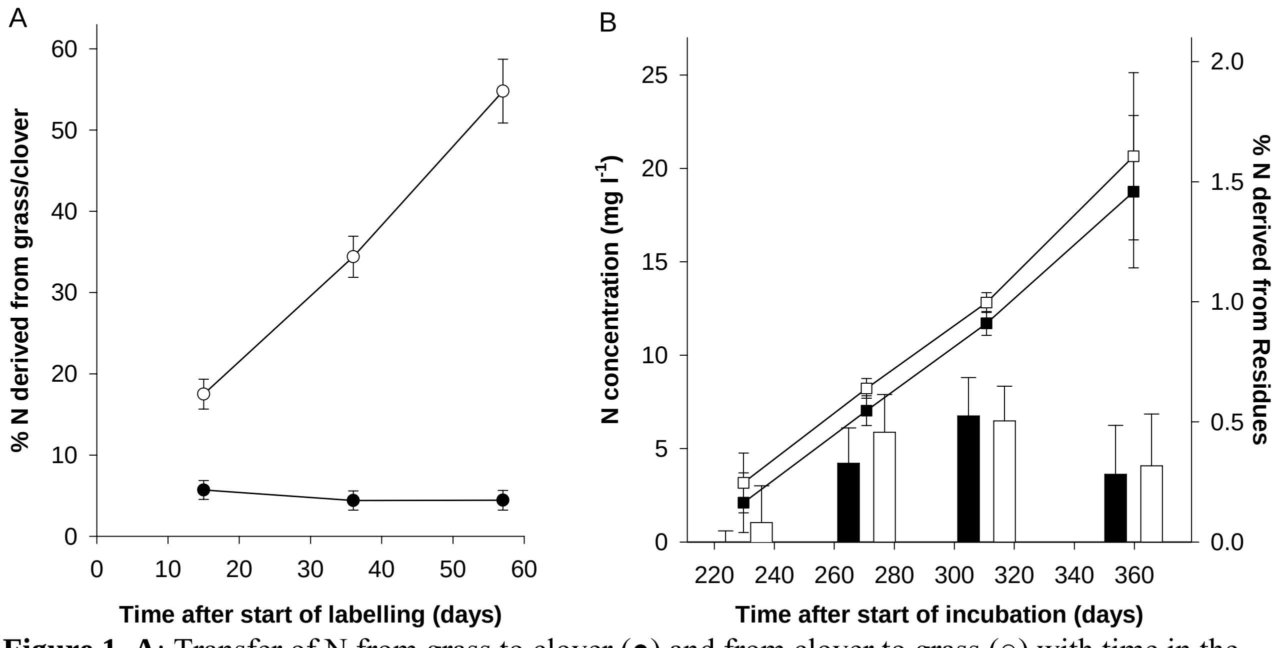

Figure 1. A: Transfer of N from grass to clover (●) and from clover to grass (○) with time in the leaf-labelling experiment. Error bars shows standard error (S.E.) for nine replicates. **B**: Curves show leaching of DIN (■) and TDN (□), and bars show ^{15}N-enrichment as percent N derived from Residues of DIN (black bar) and TDN (white bar) in the residue incubation experiment. The plot show as an example the data for the incubation of ^{15}N-labelled clover roots. Error bars shows standard error (S.E.) for four replicates.

Of major agronomic importance is the transfer of N between the two species. The experiment with direct leaf-labelling of either ryegrass or clover confirmed that N transfer in such mixtures are bi-directional (Høgh-Jensen and Schjoerring, 2000) with a net-transfer from clover to ryegrass. We found that 40 percent of the N in ryegrass originated from clover, whereas 5 percent of N in clover came from the companion ryegrass. The mode of N transfer has been discussed in the literature (e.g. Goodman, 1991; Lipson and Nasholm, 2001; Moyer-Henry et al., 2006). Our results (Figure 1A) indicate that different modes of N transfer are in action when comparing the bi-directional transfer. Transfer of ^{15}N from ryegrass to companion clover was highest initially after leaf-labelling and thereafter decreasing, which point to fast processes like clover uptake of inorganic or organic root depositions from grass or direct root-root interconnections via mychorrhiza. Transfer of ^{15}N from clover to ryegrass was fairly high immediately after leaf-labelling, meaning that the same process above was in play, but as the ^{15}N transfer increased markedly with time, it is likely that clover root turnover is of major importance for the total N transfer.

The environmental concern regarding DIN and DON leaching was studied in the experiment with dual-labelled residues (white clover root, ryegrass root, white clover leaves, ryegrass leaves). The leaching of DON and DIN did not seem to come directly from the labelled residues. Instead we found a positive correlation between the uptake of ^{15}N by ryegrass in the growth season prior to the period of leaching in autumn and winter and the ^{15}N-enrichment of DON and DIN. The results point to turnover of ryegrass roots to be a main source of the ^{15}N-enriched DON and DIN percolating from the cylinders; this not exceeding 1.5 percent though. The total N loss during autumn and winter was in the studied system 10 kg N ha^{-1} of which 15 percent was found as DON. During autumn and winter there was an increasing leaching of DIN, whereas the leaching of DON (TDN – DIN) was fairly constant (Figure 1B). Interestingly there was also a shift in the ^{15}N-enrichment of N lost from DON in autumn to DIN by the end of winter.

Conclusions

The fairly slow process of clover root turnover was found to be the most important regarding N transfer to companion grass, although faster processes was also in play. Transfer in the direction from grass to clover was fast and therefore expected to be in form of either clover uptake of root deposits or direct root-root interconnections.

Turnover of ryegrass roots was directly related to the leaching of ^{15}N-enriched DON and DIN, although this only constituted a minor proportion of the total N leaching. The N leaching increased during autumn and winter due to an increase in DIN loss. Also the ^{15}N-enrichment of lost N shifted from DON to DIN in the same period.

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