

# Paths of nitrogen transfer from *Trifolium repens* to non-legume plants in unfertilised pastures

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## Abstract

Biological N fixation (BNF) is the main N input in unfertilised pastures and can provide transfer N to non-legume plants. This transfer can be coupled spatially or temporally to different degrees. In principle, three mechanisms are conceivable: (i) a direct transfer between living plants, (ii) a local but delayed transfer when non-legumes colonise a patch with decaying legume biomass, and (iii) a spatially diffuse transfer via the excreta of grazing animals. We analysed these mechanisms on 10 pastures grazed by suckler cows and offspring where BNF input was quantified over nine years. Modelled annual BNF yield varied between 1 and 225 kg ha<sup>-1</sup> yr<sup>-1</sup> (mean 30) between paddocks and years. Comparison of <sup>15</sup>N in non-legume plants growing in the immediate vicinity of white clover (*Trifolium repens*) and at large distance indicated negligible direct BNF-N transfer. <sup>15</sup>N analyses of non-legumes growing on legume patches of the previous year also indicated no significant delayed local transfer. Conversely, the <sup>15</sup>N of cattle correlated closely with the BNF input of the previous year. We conclude that BNF-N transfer occurred mainly via the excreta and was independent of the spatial distribution of legumes on the paddock.

Keywords: biological N fixation, excreta, 15-N, isotope, natural abundance, *Trifolium repens*

## Introduction

Nitrogen limits the productivity of most terrestrial ecosystems including non-fertilised grassland. In the latter, biological N fixation (BNF) is the main N input. Transfer of BNF-N to non-legume plants is important for sward productivity and composition and may occur via different mechanisms: (i) direct (spatio-temporally coupled) transfer between legumes and non-legumes, e.g. by mycorrhiza, leaching/interception or trampling, (ii) local but delayed transfer when non-legumes colonise a patch of decaying legumes, (iii) spatially diffuse transfer via grazing/excreta deposition by grazing animals. We studied these mechanisms by taking advantage of the isotopic contrast between BNF-N ( $\delta^{15}\text{N}$ : approx. -2‰) and soil N ( $\delta^{15}\text{N}$  approx. 4‰) and data from 10 pastures analysed over nine years.

## Materials and methods

The study was conducted from 1999 to 2007 on the Grünschwaige Grassland Research Station (near Munich, Germany). The experiment included five old pastures (>45 years old) and five young pastures established between 1998 and 1999 on former cropland. Young pastures were sown with a mixture of grass and white clover (*Trifolium repens*). All pastures were grazed by continuous-stocking with herds of either suckler cows or fattening steers during the entire vegetation period. Further details of the experiment and site are reported elsewhere (Schnyder *et al.*, 2006).

BNF was quantified using the model by Høgh-Jensen *et al.* (2004). The N content of *T. repens* was slightly higher (4.45%) than assumed in the model (4.30%), which is not considered in the following because the difference was smaller than the 95% interval of confidence (0.21%;  $n = 74$ ). Total biomass production was estimated from feed intake by the cattle on each pasture. Sward height was kept constant by adjusting stocking density. No

supplements were fed. Standing biomass of legumes (mainly *Trifolium repens*) was estimated visually by trained persons 2-3 times during the growing period on 40 permanent quadrats (1 m<sup>2</sup>, divided in 10 x 10 cm<sup>2</sup> squares) during nine years.

The direct transfer of BNF-N from legumes to non-legumes was estimated from <sup>15</sup>N analysis of *Lolium perenne* growing in immediate vicinity of *T. repens* and comparison with plants growing at large distance to it on the same pasture. Delayed local transfer of BNF-N from decaying clover residues was assessed by comparing  $\delta^{15}\text{N}$  of *L. perenne* growing on former clover patches (as determined in the previous year) and *L. perenne* on patches with no clover in the previous years. Diffuse transfer was assessed from the relationship between BNF yield (calculated following Høgh-Jensen *et al.*, 2004) and the  $\delta^{15}\text{N}$  of ingested feed-N, as reflected in <sup>15</sup>N of the tail switch hair of the grazing cattle (the  $\delta^{15}\text{N}$  of hair equals that of the diet plus a trophic level shift of 3.2‰; Männel *et al.*, 2007).

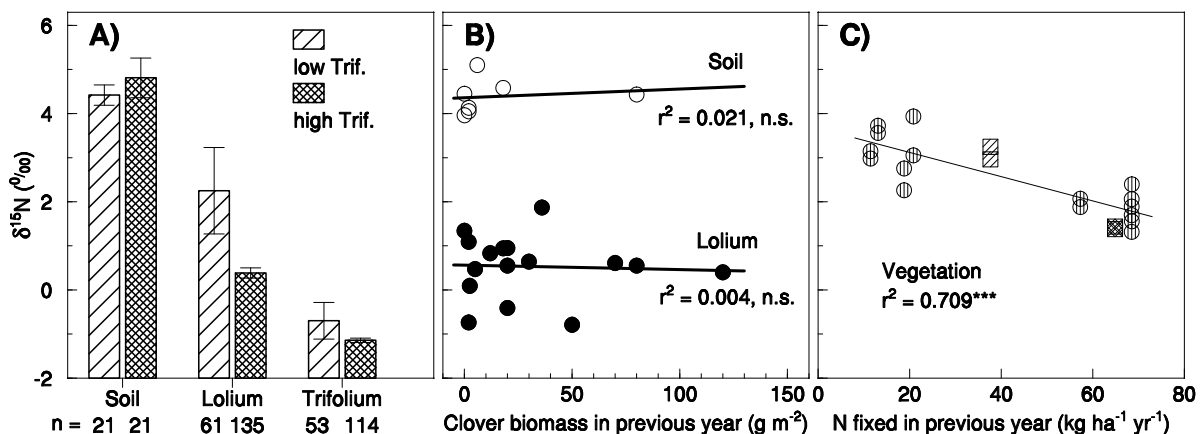


Fig. 1: A) Nitrogen isotopic composition of soil, *Lolium perenne* and *Trifolium repens* in two pastures with low and high *Trifolium repens* content in the previous years (error bars give 95% interval of confidence);

B) Nitrogen isotopic composition in *Lolium perenne* and soil depending on the *Trifolium repens* biomass within the same 10x10 cm<sup>2</sup> square in the previous year;

C) Nitrogen isotopic composition in bulk vegetation of ten pastures derived from cattle hair (two animals each) compared to biological N fixation in the previous year as calculated after Høgh-Jensen *et al.* (2004); square markers denote the two pastures in panel A).

## Results

The BNF varied greatly among pastures and years (total range: 1 – 225 kg ha<sup>-1</sup> yr<sup>-1</sup>). BNF was considerably smaller on old pastures (20 ± CI 5 kg ha<sup>-1</sup> yr<sup>-1</sup>; CI is the 95% interval of confidence) than on young pastures (54 ± CI 15 kg ha<sup>-1</sup> yr<sup>-1</sup>). On young pastures, it was especially high in year 1 after establishment (156 ± CI 49 kg ha<sup>-1</sup> yr<sup>-1</sup>). Thereafter, it decreased linearly ( $r^2 = 0.83$ , n = 30) until year 5 after stand establishment, when it became similar to that of old pastures. The  $\delta^{15}\text{N}$  of *L. perenne* on the high-clover pasture was 1.55‰ lower than on the low-clover pasture although the soils were similar and differed only by 0.35‰ (Fig. 1 A). This shows clearly that the BNF-N contributed significantly to N nutrition of the grass. However, *L. perenne* growing in close vicinity of *T. repens* and *L. perenne* at large distance from *T. repens* did not differ in <sup>15</sup>N in a high-clover pasture. The same was true on a low-clover pasture. The overall  $\delta$ -difference between *L. perenne* growing in close vicinity vs. at large distance to *T. repens* was 0.08‰ ± CI 0.03‰, indicating a negligible direct transfer of BNF-N. Comparing *L. perenne* growing in patches that were occupied by *T. repens* in the year before also did not indicate any transfer of BNF-N from mineralized dead *T. repens* biomass to *L. perenne* or to the soil at the same site (Fig. 1 B). In contrast, BNF-N yield of a

pasture was closely related to  $^{15}\text{N}$  of cattle hair in the following grazing season, indicating that BNF-N was ingested by the grazing animals and redistributed via excreta (Fig. 1 C). The  $^{15}\text{N}$  signal of forage was rapidly incorporated in hair: cattle had near-identical  $^{15}\text{N}$  in new hair growth during wintertime stall feeding when all animals were fed the same diet, but the composition started to change within one week after being put on pastures (data not shown).

## Discussion

The results indicate that direct plant-plant transfer of BNF-N between legumes and non-legumes was negligible and that most of the transfer occurred via the grazing cattle. Thus, N losses from living or mineralising dead *T. repens* plants must have been small in comparison with losses incurred by grazing. There are several mechanisms which underlie this result: (i) N concentration in live leaves of *T. repens* is high, (ii) the harvesting (i.e. grazing) efficiency of live leaf-biomass and -N of *T. repens* is high on pasture, (iii) N allocation to re-growing leaves of defoliated *T. repens* plants has a high priority (Corre *et al.*, 1996), (iv) our data show that losses of N from living *T. repens* other than those associated with grazing losses are small, and (v) N concentration in senesced decaying *T. repens* biomass is low because of mobilisation to other plant parts during senescence. All these mechanisms enhanced the flow of BNF-N from *T. repens* to the grazer and back to the pasture via excreta. These mechanisms explain why *L. perenne* on the high-clover pasture received a high proportion of BNF-derived transfer-N independently of its vicinity to *T. repens*. The mechanisms also explain why patches of decaying (N-poor) *T. repens* biomass provided a relatively unimportant source of transfer-N for *L. perenne* colonising the patch.

## Conclusions

Transfer of BNF-N occurred mainly via the excreta of grazing cattle. Conversely, the direct transfer between living plants or colonisation of decaying legume patches were unimportant paths for BNF-N transfer from *T. repens* to *L. perenne*. Together, these mechanisms enhance the redistribution and cycling of BNF-N in the pasture paddock, but limit the attractiveness of clover patches for colonisation by *L. perenne* and other non-legumes.

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