The ^{15}N natural abundance and enrichment techniques provide similar estimates of N transfer

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Introduction: Nitrogen (N) transfer from clover to grass could present a significant contribution of symbiotically fixed N to grass nutrition in clover-grass swards. However, depending on the method of estimation, the proportion of transferred N might differ. The aims of this study were (1) to compare the ^{15}N natural abundance (NA) and the ^{15}N enrichment technique to estimate the N transfer from clover to grass and (2) to test the applicability of the NA technique under different fertiliser strategies affecting the ^{15}N soil background value.

Materials and methods: The ^{15}N NA and enrichment (clover leaf labelling) technique were compared in sub plots of a long-term cropping-system field experiment (Hammelehle *et al.*, 2018) using treatments supplied with no fertiliser, animal manure ($\delta^{15}N$ of faeces: 6.7‰ and of slurry: 10‰), or mineral N fertiliser ($\delta^{15}N$: 0.5‰). ^{15}N values and N contents of yields, stubbles, and roots were determined from *Trifolium pratense* – *Lolium perenne* mixed and *L. perenne* pure stands. Estimates of N transfer were determined using different surrogates of plant available soil N and clover and grass plant parts when using NA (Daudin and Sierra, 2008) and using different experimental setups when using ^{15}N enrichment (Giller *et al.*, 1991). The design of the study was a split-split plot. Data were fitted to a mixed effect model.

Results: During two consecutive cultivation years, the proportion of transferred N was considerable and was in the same range for both methods (^{15}N NA: 34%-42% of grass N; ^{15}N enrichment: 27%-46% of grass N). The ^{15}N enrichment technique suffered from a temporally non-uniform ^{15}N labelling of the clover root (results not shown). The NA $\delta^{15}N$ of pure stand grass as surrogate of plant available soil N tended to underestimate the proportion of N transfer, especially in the 1st year of cultivation (Table 1).

Table 1. Development of $\delta^{15}N$ values of clover and grass yields over two years from NA mixed and pure stands (mean of n=15; SEM=0.2).[1]

Species	Stand	$\delta^{15}N$ [‰] yield of harvest		
		2 MC	6 MC	17 MC
Clover	mixed	-0.5 e	-0.5 e	-0.2 e
Grass	mixed	3.9 ab	1.8 d	2.6 cd
Grass	pure	3.8 ab	3.3 bc	4.4 a

[1] Same letter indicates no difference between means at $\alpha<0.05$ (post hoc *t*-test); MC: months of cultivation.

Conclusion: Independent of the ^{15}N method, about 1/3 of grass N derived from N transfer. Adequate NA procedures can be used even in the presence of a range of fertiliser types with differing $\delta^{15}N$ values, if soils have been under these fertilisation strategies for many years and fertilisation remains unchanged during the study.

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Giller K.E., Ormesher J. and Awah F.M. (1991) Nitrogen transfer from *Phaseolus* bean to intercropped maize measured using 15N-enrichment and 15N-isotope dilution methods. *Soil Biology and Biochemistry* 23, 339-346.

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