HOW TO QUANTIFY BIOLOGICAL NITROGEN FIXATION IN FORAGE LEGUMES IN THE FIELD

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Reliable measurements of the input of fixed-N from forage legumes are essential in estimating the need for N fertilization in grasslands. Such measurements rely on methods that can be applied in the field for either the entire growing season or parts of it. High precision and reliability should always be aimed at when measuring N_2 fixation. However, depending on the aims and availability of resources (time and equipment), methods with varying precision can be chosen. Methods with high precision are needed if the aim is to reveal factors that affect the N_2-fixation rate, whereas N balances at the field or farm level may be based on N_2 fixation estimates obtained with lower precision. Of the methods listed in Table 1, we only recommend ^{15}N natural abundance (NA) and ^{15}N isotope dilution (ID) for measurements with high precision. In addition, we propose that simple formulas based on legume biomass can be used for rapid estimates with lower precision. Nitrogen difference is not a reliable method for forage legumes because forage grasses usually are more efficient than legumes in taking up soil N (Table 1; Carlsson and Huss-Danell, 2003 and references therein).

In addition, the following is important when comparing NA and ID methods. Although ID gives a measure of N_2 fixation during a defined period, from application of ^{15}N to harvest, NA gives a measure of N_2 fixation integrated over a longer time, up to the whole lifetime of a plant (Huss-Danell and Chaia, 2005). NA is a simpler method than ID because it does not require fertilization with ^{15}N, but NA is sensitive to the B value used (Carlsson et al., 2006; Högberg, 1997). The B value corresponds to the $^{15}N/^{14}N$ ratio of the symbiotic association when grown with N_2 in air as the only N source. Both NA and ID require a reference plant that only uses soil N, and the legume and the reference plant should take up soil N with the same $^{15}N/^{14}N$ ratio (Högberg, 1997).

Grasses may not be ideal reference plants because they have different rooting patterns from forage legumes (Huss-Danell and Chaia, 2005). We therefore recommend using the mean $^{15}N/^{14}N$ ratio of several reference plants, e.g., by using weeds.

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Table 1. Methods to measure N_2 fixation in legume-rhizobia symbioses.

	N content	N difference	$^{15}N_2$ fixation	^{15}N natural abundance	^{15}N isotope dilution	H_2 evolution	C_2H_2 reduction
Substrate for N_2ase	N_2	N_2	N_2	N_2	N_2	H^+	C_2H_2
Study period	Weeks –years	Weeks–years	Hour(s)	Weeks–years	Weeks–years	Minutes–hours	Minutes–hours
Destroys sample?	Yes	Yes	Yes	Yes	Yes	No	No
Analyses needed	N content	N Content	^{15}N content	^{15}N content	^{15}N content	H_2 sensoror GC[a]	GC*
In field	No	Yes (?)	No	Yes	Yes	No	No (yes)
Require-ments, limits	Needs N-free sub-strate, which is rare in the field	Legume and non-legume must take up soil-N with same efficiency	$^{15}N_2$ is expen-sive; gas-tight incub-ation needed	Conc. of ^{15}N in soil and air must differ; B-value in legume should be known	Added ^{15}N must be evenly distri-buted in the soil profile	Uptake H_2ase (Hup) must be inactive; difficult to make gas-tight for H_2	C_2H_2 causes decline in N_2ase activity; conver-sion factor for C_2H_4 to NH_3 needed

[a]GC, gas chromatography

Because forage legumes grown together with grasses consistently derive most of their N from N_2 fixation, rough estimates of N_{fix} (kg N ha^{-1}) can be calculated as: N_{fix} = 0.026 × legume dry matter (DM; kg ha^{-1}) for *Trifolium pratense* L.; N_{fix} = 0.031 × DM for *T. repens* L.; and N_{fix} = 0.021 × DM for *Medicago sativa* L. (Modified from Carlsson and Huss-Danell, 2003.) Such rough but rapid estimates of N_2 fixation may be of great value for establishing on-farm N budgets or N fertilization plans.

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References

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