Biomass production and N_2-fixation in seven grass-legume mixtures

Jim Rasmussen, Karen Søegaard, and Jørgen Eriksen
Department of Agroecology and Environment, Faculty of Agricultural Sciences, Aarhus University.Post box 50, 8830 Tjele, Denmark.

Abstract: Inclusion of forage legumes in low-input grassland mixtures improves biomass production and soil fertility trough addition of nitrogen (N) from N_2-fixation. The impacts of different mixture of legumes and companion grasses on the N production of the forage mixture have rarely been investigated under comparable soil and climatic conditions. We conducted a field experiment on a sandy soil at two nitrogen levels with seven two-species grassland mixtures: alfalfa (*Medicago sativa*), bird's-foot trefoil (*Lotus corniculatus*), red clover (*Trifolium pratense*), or white clover (*Trifolium repens*) in mixture with perennial ryegrass (*Lolium perenne*), and white clover in mixture with meadow fescue (*Festuca pratensis*), timothy (*Phleum pratense*), or hybrid ryegrass (*Lolium hybridum*). Red clover and alfalfa fixed 400-500 kg N ha^{-1} and bird 's-foot trefoil just above 100 kg N ha^{-1} in aboveground biomass. The white clover N fixation was affected by the companion grass species and ranged from 150 to 175 kg N ha^{-1}. Fertilization had different effects on N_2-fixation among the legumes, but also significant effects on white clover N_2-fixation depending on the companion grass species.

Introduction: The N_2-fixation of forage legumes is an essential input of N in many agricultural systems. Estimation of the amount of N fixed by the legumes is needed in order to know yield potentials, N leaching and gaseous loss risks, and residual N effects of the crop. A great number of estimates of N_2-fixation by white clover, red clover, bird's-foot trefoil, and alfalfa are found in the literature, but the N_2-fixation by these forage legumes have rarely been estimated in the same experiment. Likewise have the influence of the companion grass species in the forage mixture on the N_2-fixation rarely been studied.

The objective of the present study was to compare seven grass-legume mixtures in relation to N_2-fixation as affected by legume species, companion grass species, and N fertilization.

Materials and Methods: The experimental area was located at Foulumgård Experimental Station in Jutland, Denmark. The soil is classified as a Typic Hapludult with the Ap-horizon (0-25 cm) containing 7.7% clay and 1.6% carbon. The mean annual temperature and precipitation of the site are 7.3°C and 627 mm, respectively (1961-1990).

In spring 2006 seven grass-legume mixtures and a pure ryegrass were undersown in spring barley in a randomized block-design with four replications. The seven grass-legume mixtures were: perennial ryegrass in mixture with white clover, red clover, bird's-foot trefoil, or alfalfa, respectively, and white clover in mixture with perennial ryegrass, meadow fescue, timothy, or hybrid ryegrass, respectively. In 2007 all plots received the same treatment i.e. 300 kg total-N ha^{-1} in cattle slurry.

In spring 2008 each plot was divided in two; one receiving no N fertilizer application, and the other receiving 300 kg total-N ha^{-1} applied as cattle slurry at four times during the growth season. In each plot a subplot was irrigated with ^{15}N-$(NH_4)_2SO_4$ to determine legume N_2-fixation (McNeill *et al*., 1994). The forage mixtures were harvested four times during the season: late May, early July, medium August, and early October. At each harvest biomass DM yields and botanical composition were determined alongside measurement of total N and ^{15}N-enrichment of grasses and legumes from the ^{15}N-dilution subplots.

Results and discussion:

Effect of legume species and fertilization on N fixation and N transfer to per. ryegrass

White clover, bird's-foot trefoil, and alfalfa all derived more that 90% of their N from N_2-fixation both unfertilized and fertilized, whereas red clover without fertilization derived 88% from N_2-fixation and 74% when fertilized. However, red clovers large biomass production resulted in by far the largest amount of N fixed (Table 1), almost 500 kg N ha^{-1} without fertilization corresponding to 78% of the total N yield at these plots.

Adding 300 kg total-N ha^{-1} in slurry to monoculture perennial ryegrass increased the N yield from 85 kg N ha^{-1} to 155 kg N ha^{-1}, corresponding to a NUE of 40% of applied ammonium-N in the slurry. In the mixtures red clover and white clover with perennial ryegrass increased the N yield more than monoculture perennial ryegrass when fertilized. For the white clover – perennial ryegrass mixture this was mainly due to increased growth of the grass, whereas for the red clover – perennial ryegrass mixture, the increased yield was caused by an increased growth of red clover. Adding 300 kg N in slurry to the alfalfa and the bird's-foot trefoil mixtures lowered the percentage of legume in the mixture (Table 1), but the increase in perennial ryegrass growth in these two misxtures could not make up for the loss in N_2-fixation of alfalfa and bird's-foot trefoil when fertilized, which was the case for white clover.

Table 1. Sward proportion of legume, N derived from atmosphere (Ndfa), and total N yield with and without fertilization for perennial ryegrass in monoculture or in mixture with either: white clover, red clover, bird's-foot trefoil, or alfalfa.

	0 N			300 kg N		
Per. ryegrass &	Legume %	Ndfa kg N ha^{-1}	N yield kg N ha^{-1}	Legume %	Ndfa kg N ha^{-1}	N yield kg N ha^{-1}
White clover	44 ± 3	176 ± 18	306 ± 23	34 ± 3	158 ± 19	391 ± 13
Red clover	84 ± 1	498 ± 9	639 ± 16	81 ± 3	456 ± 38	726 ± 17
Bird's-foot trefoil	29 ± 3	115 ± 11	256 ± 6	18 ± 4	77 ± 16	270 ± 8
Alfalfa	79 ± 2	443 ± 13	524 ± 10	70 ± 2	422 ± 11	564 ± 12
P. ryegrass mono			85 ± 8			155 ± 4

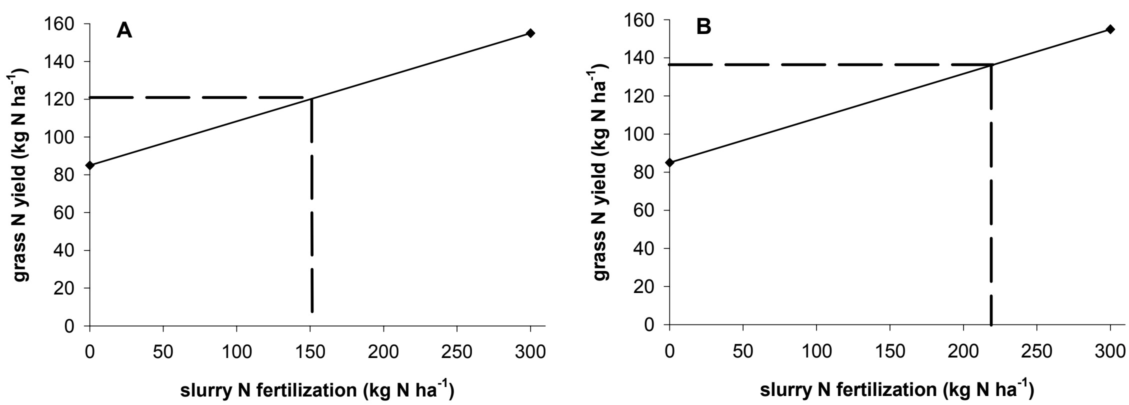

Figure 1. Estimated net N fertilizer effect of N transfer from (A) white clover, and (B) bird's-foot trefoil to companion perennial ryegrass. Estimation based on N yields in monoculture perennial ryegrass receiving 0 and 300 kg total-N ha^{-1} in slurry, and N yields in perennial ryegrass growing in mixture with white clover or bird's-foot trefoil.

Comparing N concentrations in monoculture perennial ryegrass to N concentrations in perennial ryegrass in mixture with one of the four legumes showed that N from the legumes was transferred to the companion grass. Based on N yields in monoculture perennial ryegrass fertilized with 0 and 300 kg total-N ha^{-1} we estimated the N fertilization effect of the N transfer in the unfertilized plots (Figure 1) to correspond to an application of 150 kg total-N ha^{-1} in slurry for white clover and 215 kg total-N ha^{-1} in slurry for bird's-foot trefoil. The cattle slurry added had an ammonium content of 60% of the total-N. It was not possible to make this estimation for alfalfa and red clover as these two species largely outcompeted the companion perennial ryegrass.

Effect of companion grass species and fertilization on white clover N_2-fixation

In mixture with one of four different grasses white clover derived 92-95% from N_2-fixation without fertilization, and 87-91% with fertilization. The total N yields without fertilization did not differ significantly (Table 2), but with fertilization the white clover – hybrid ryegrass mixture had significantly lower N yields than the perennial ryegrass and timothy mixtures.

Table 2. Sward proportion of legume, N derived from atmosphere (Ndfa), and total N yield with and without fertilization for white clover in mixture with either: perennial ryegrass, meadow fescue, timothy, or hybrid ryegrass.

	0 N			300 kg N		
White clover &	Legume %	Ndfa kg N ha^{-1}	N yield kg N ha^{-1}	Legume %	Ndfa kg N ha^{-1}	N yield kg N ha^{-1}
Perennial ryegrass	44 ± 3	176 ± 18	306 ± 23	34 ± 3	158 ± 19	391 ± 13
Meadow fescue	36 ± 2	160 ± 5	327 ± 15	23 ± 1	115 ± 9	366 ± 8
Timothy	33 ± 2	147 ± 13	301 ± 14	29 ± 4	139 ± 22	372 ± 10
Hybrid ryegrass	35 ± 3	155 ± 17	316 ± 21	23 ± 3	110 ± 15	325 ± 16

White clovers N_2-fixation (kg N ha^{-1}) significantly depended on the companion grass species when fertilized. White clover in mixture with perennial ryegrass and with timothy fixed higher amounts of N, than white clover in mixture with meadow fescue and with hybrid ryegrass; most likely due to competitive suppression (Table 2) by meadow fescue and hybrid ryegrass of white clover. This was also reflected in the total N yields of the mixture in response to fertilization, where in particular the white clover – perennial ryegrass mixture, but also the white clover – timothy, responded well to the fertilization, whereas in the other two mixtures the increase in N yields in meadow fescue and hybrid ryegrass could not compensate for the lower white clover N yields. This underlines the highly dynamic interactions found in forage legume-grass mixtures.

References:

McNeill, A.M., Hood, R.C. & Wood, M. (1994). Direct measurement of nitrogen-fixation by *Trifolium repens* L. and *Alnus gultinosa* L. using N-15(2). Journal of Experimental Botany 45, 749-755.