

Rate of Atmospheric Nitrogen Fixation in Temporary Pastures

Carmen Dragomir¹, Nicoleta Dragomir², Ioan Peț¹, Constantina Chiper³

¹Faculty of Animal Sciences and Biotechnologies, Calea Aradului, nr. 119, Timișoara, Romania

²S.C. COMISION DIC SRL, Dumbrăvița, str. Al.Petöfi, nr. 52, Timiș, Romania

³Grass Research-Development Station Timișoara, Calea Urseni, nr. 32, Timișoara, Romania

Abstract

This work presents the influence exerted by the bacterial inoculation of legumes and nitrogen-based fertilization of temporary pastures on the rate of atmospheric nitrogen fixation (BFN). The researches were carried out in an experimental display consisted of a bifactorial experience (A = bacterial inoculation; B = nitrogen doses), during a three-year period, by planting an association of *Dactylis glomerata* (60%) + *Medicago sativa* (40%). The estimated amount of BFN was determined with the method of nitrogen balance with a reference crop. The results achieved showed that, by inoculating the legume within the temporary pasture association, we may obtain an increase of the BFN rate from 2.66 kg/1% alfalfa to 3.24 kg/1% alfalfa, respectively an estimated amount of 0.65 kg N_{fixed}/day/ha, in the inoculated variant.

Keywords: amount of fixed nitrogen remained in soil, mean fixation rate, nitrogen fixation, pasture of *Medicago sativa* + *Dactylis glomerata*

1. Introduction

The importance of forage legumes in the floristic structure of temporary pastures is given by their capacity of providing biologically-fixed nitrogen. The amount of fixed nitrogen is influenced by several factors, among which the legume proportion within the pastures and the length of the vegetation period play a very important role [1-3].

This work presents the estimated amount of nitrogen fixed in the pasture association consisted of *Dactylis glomerata* + *Medicago sativa*, according to alfalfa participation and vegetation duration, and also the amount of fixed nitrogen remained in rhizosphere at the service of the Gramineae plants within the pastures.

2. Materials and methods

The researches were performed at Banat's University of Agricultural Sciences and Veterinary Medicine Timișoara, during 2006 – 2008, on a lick chernozem, moderately gleyed, with pH_(H2O) = 6.21. The planted temporary pasture was consisted of an association of *Dactylis glomerata* + *Medicago sativa*, in a proportion of 60: 40 (%). On this pasture, we placed a bifactorial experience, with the following experimental factors: A – bacterial inoculation of the alfalfa within the association (a₁ = not inoculated, a₂ = inoculated); B – doses of nitrogen-based fertilization (b₁ = N₀, b₂ = N₃₀, b₃ = N₆₀). The alfalfa seeds were inoculated, before planting, with bacteria belonging to the species *Sinorhizobium meliloti*, multiplied on liquid medium with agar. In the preparation of the germinating bed, we applied a general agrifund of fertilization consisted of P₅₀K₇₀. The doses of nitrogen fertilization were applied every year of

* Corresponding author: Carmen Dragomir, 0256/277068, carmendrag9@yahoo.com

vegetation, after each cutting. At the same with experiment displaying, we planted a reference area with *Dactylis glomerata*, as pure crop, with three variants of nitrogen-based fertilization (N_0 , N_{30} , N_{60}), in order to determine the amount of biological nitrogen in the associated crop.

To estimate the amount of biologically-fixed nitrogen (BFN), we used the method of nitrogen difference evaluation, with a reference crop, according to the following formula:

$$1. \text{ BFN (kg/ha)} = N_t(\text{association}) - N_t(\text{reference}),$$

where:

$$2. N_t(\text{association})(\text{kg/ha}) = [(N_{ta} \times S_{ua}) + (N_{tr} \times S_{ur})] / 100;$$

$$3. N_t(\text{reference})(\text{kg/ha}) = [(N_{tar} \times S_{uar}) + (N_{trr} \times S_{urr})] / 100, \text{ where:}$$

- $N_t(\text{association})$ = total nitrogen amount within the biomass of the association *Dactylis glomerata* + *Medicago sativa*;
- N_{ta} = total nitrogen content in the aerial biomass (sprigs + leaves) of the association (%);
- N_{tr} = total nitrogen content in the root biomass of the association (%);
- S_{ua} = aerial DM yield of the association (kg/ha);
- S_{ur} = root DM amount of the association (kg/ha);
- $N_t(\text{reference})$ = total nitrogen amount in the pure reference crop (*Dactylis glomerata*) (kg/ha);
- N_{tar} = total nitrogen content in the reference crop's aerial biomass (sprigs + leaves) (%);
- N_{trr} = total nitrogen content in the reference crop's root biomass (%);
- S_{uar} = aerial DM yield of the reference crop (kg/ha);
- S_{urr} = root DM amount of the reference crop (kg/ha).

The estimated amount of BFN remained in rhizosphere (BFN_r)(root system) was determined in relationship with the results obtained by several researchers [4], who consider that at least 22% of the total BFN amount remains in legume roots, at their service, and implicitly at pasture service, according to the following formula:

$$4. \text{ BFN}_r \text{ (kg/ha)} = (22 \times \text{BFN}_t) / 100, \text{ where:}$$

BFN_t = total amount of biologically-fixed nitrogen (kg/ha).

In order to determine the amount of fixed nitrogen returned to soil, under conditions of cattle grazing, we applied the following formula:

$$\text{BFN}_s(\text{kg/ha}) = \text{BFN}_r + \text{BFN}_{dl} + \text{BFN}_{ds} \text{ [5],}$$

where:

BFN_{dl} = estimated amount of fixed nitrogen, excreted by animals through liquid dejections (kg/ha);

BFN_{ds} = estimated amount of fixed nitrogen, excreted by animals through solid dejections (kg/ha).

3. Results and discussion

The results of the researches in the literature show the correlation between the amount of fixed nitrogen and the proportion of legumes in the pasture floristic structure. According to the species and to the ecologic and technological conditions, the fixation rate ranges between large limits, namely 1 – 4 kg BFN/1% legume [1].

The analysis of the mean results presented in Table 1, obtained during our researches, led to the conclusion that successive to the bacterial inoculation of the alfalfa within the association, the mean fixation rate became 3.2 kg/1% alfalfa compared with only 2.66 kg/1% alfalfa, respectively an increase of 21.8 % compared with the not inoculated variant. If we do not take into consideration the nitrogen-based fertilization, then the fixation rate increases from 2.59 kg BFN/1% alfalfa, in the not bacterized variant, to 3.33 kg BFN/1% alfalfa, in the bacterized variant.

There are in the literature several determination methods of the fixed nitrogen amount, according to the legume proportion within the floristic structure, to the duration of the vegetation period or to the dry matter yield. From this viewpoint, Giller [2] considers that the nitrogen fixation rate, for all legume species, is 1 – 2 kg/ha/day. When the fixed nitrogen amount is reported to the DM yield, then the researchers have different conclusions: Moga [6] report an amount of fixed nitrogen of 30 kg/1 t DM in alfalfa; Vinther [7] considers that the association of white clover with Gramineae fixes between 34 – 45 kg/1 t DM; Peoples [8] determines a fixed nitrogen amount of 9 – 16 kg/1 t DM.

During our researches, we observed that the bacterial inoculation of alfalfa seeds has a significant contribution to the increase of the daily nitrogen fixation rate. So, compared to the not inoculated variant, with a mean fixation rate of 0.42 kg N/day/ha, in the inoculated variant we

achieved a daily rate of 0.65 kg N/day/ha (an increase of 54.7%).

The fixed nitrogen transfer, carried out by legumes to the other Gramineae species from the floristic structure of the temporary pasture, has represented the theme of numerous researches, with very various results that range with the species quantifying the amount of transferred nitrogen. So, the researches performed on alfalfa made evident different proportions of transferred nitrogen: 7% (3-27 kg/ha) of the total BFN amount, according to Burity [9], 10% (7-13 kg/ha) according to Ta and Faris [10]; 2% (3-5 kg/ha) according to Hardarson et al. In the case of the red clover, the studies performed by Boller and Nosberger [1] show a transferred BFN amount of 23% (14 – 42 kg/ha), and in white clover, Ledgard [4] observed that the transferred BFN was of 26% (54 – 102 kg/ha).

The analysis of the results regarding the estimation of the BFN amount remained in the rhizosphere of the temporary pasture consisted of *Dactylis glomerata* + *Medicago sativa*; we may observe that this quantified parameter ranges widely, according to the factors studied: bacterial inoculation, nitrogen doses applied and years of vegetation (Table 2).

The influence of these factors may be observed in the analysis of the very large limits of the BFN remained in rhizosphere, during the vegetation periods: between 18 – 39 kg/ha, in the first year, 13 – 48 kg/ha, in the second, and between 2 – 42 kg/ha, in the third year of vegetation. On the average, in the association studied, a BFN amount of 11 – 43 kg/ha/year remains in the rhizosphere

after aerial vegetative biomass removal by cutting. If we consider the entire period of utilization, the BFN amount accumulated in soil during the years of vegetation ranges between 33 – 129 kg/ha. These fixed nitrogen amounts represent, according to Ledgard [4] about 2% of the total amounts of legume-fixed nitrogen, at the level of N₆₀ kg/ha.

Among the factors studied, the bacterial inoculation enhances mostly the BFN amount remained in the rhizosphere. In these terms, the contribution of alfalfa bacterization is 55%-fold bigger than the one of the control variant – not bacterized.

The amount of biologically-fixed nitrogen remained in soil (returned to soil) is mainly influenced by the method of pasture utilization. So, the researches performed by Cuttle [5] proved an amount returned to soil of 64 kg/ha/year, of a total BFN amount of 100 kg/ha, in the case of a temporary pasture consisted of white clover and perennial Gramineae. Our researches led to the conclusion that, in the temporary pastures used for cutting, the amount of fixed nitrogen remained in soil is 30 kg/ha/year (25%), and in the case of grazing, the amount returned to soil is 73 kg/ha/year (61%), of which 43 kg/ha/year (60%) gets to soil through animal dejections (Figure 1).

From the amount of fixed nitrogen remained in soil, a big part will be used in the next year of vegetation by the plants within the floristic structure of the vegetal cover, and if the pasture will be broken up, the agricultural crop planted here will benefit by this biological nitrogen. With this, we may save significant quantities of nitrogen-based fertilizers.

Table 1. Mean rate of atmospheric nitrogen fixation (BFN) in the temporary pasture consisted of *Dactylis glomerata* + *Medicago sativa*

Treatments		BFN amount reported to 1% alfalfa participation in the association		BFN amount reported to the duration of the vegetation period	
Alfalfa cultivation method in the association	Nitrogen doses (applied after each cutting)	Kg/1%	%	Kg/ha/day	%
Not inoculated	N ₀	2,59	100	0,63	100
	N ₃₀	2,71	104,6	0,38	60,3
	N ₆₀	2,68	103,5	0,25	39,7
Inoculated	N ₀	3,33	128,6	1,00	158,7
	N ₃₀	3,37	130,1	0,57	90,4
	N ₆₀	3,03	116,9	0,37	58,7
Mean of results	Not inoculated	2,66	100	0,42	100
	Inoculated	3,24	121,8	0,65	154,7

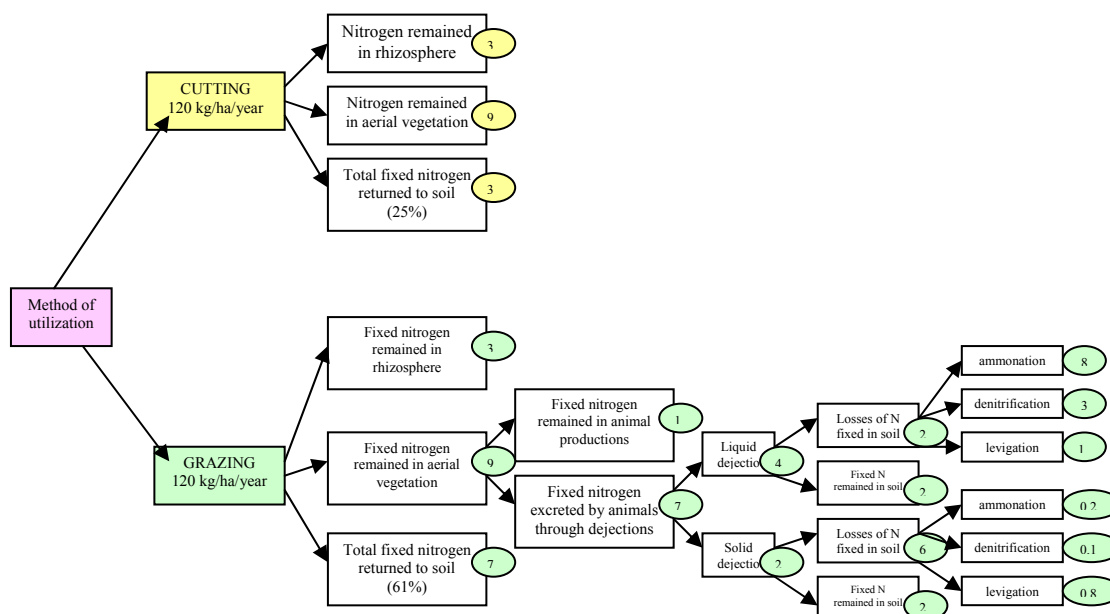


Figure 1. Estimate balance of biologically fixed nitrogen quantity (NFB/kg/ha/an), in temporary pastures used for cutting or grazing

Table 2. Estimated amount of fixed nitrogen (BFN) remained in rhizosphere, in the temporary pasture consisted of *Dactylis glomerata* + *Medicago sativa* (under conditions of cutting)

Treatments		Year I		Year II		Year III		Total		Mean	
Alfalfa cultivation method	Nitrogen doses (applied after each cutting)	Kg/ha	%	Kg/ha	%	Kg/ha	%	Kg/ha	%	Kg/ha	%
Not inoculated	N ₀	25	100	26	100	30	100	81	100	27	100
	N ₃₀	27	108	16	61	6	20	49	60	15	59
	N ₆₀	18	72	13	50	2	7	33	41	11	41
Inoculated	N ₀	39	156	48	185	42	140	129	159	43	159
	N ₃₀	29	116	28	108	17	57	74	91	24	89
	N ₆₀	20	80	23	88	5	17	48	59	16	59
Mean of results	Not inoculated	23	100	18	100	13	100	54	100	18	100
	Inoculated	29	126	33	183	21	161	84	155	28	155

4. Conclusions

During our researches, we observed that successive to the bacterial inoculation of the legumes within the temporary pasture's floristic structure, the rate of the atmospheric nitrogen biological fixation increases from 2.66 kg/1% alfalfa to 3.24 kg/1% alfalfa, or from 0.42 kgN/day/ha, in the not inoculated variant, to 0.65 kgN/day/ha, in the inoculated variant.

The BFN amount remained in rhizosphere (in roots), after aerial vegetative biomass removal by

cutting, ranges between 11 – 43 kg/ha/year, according to the factors studied.

According to pasture utilization, the estimated BFN amount remained in soil is, on the average, 30 kg/ha/year, under cutting, and 73 kg/ha/year, under animal grazing.

References

1. Boller B C and Nosberger J - Symbiotically fixed nitrogen from field-grown white and red clover mixed with ryegrasses at low levels of ^{15}N -fertilization. *Plant and Soil* 104, 1987, 219-226.
2. Giller K. E.- Nitrogen fixation in tropical cropping systems. CABI Publishing, 2001, Wallingford, UK
3. Kristensen E. S. et al.- A simple model for estimation of atmospherically – derived nitrogen in grass – clover systems. *Biological Agriculture and Horticulture*, 12, 1995, 263-276
4. Ledgard, S. F. – Transfer of fixed nitrogen from white clover to associated grasses in swards grazed by dairy cows, estimated using ^{15}N methods. *Plant Soil* 131, 1991, pp. 215-223.
5. Cuttle S. et al.- A review of leguminous fertility – building crops, with particular refence to nitrogen fixation and utilisation. Project OF0316, Institute of Grassland and Environmentl Research, Plas Goferrdan, Aberystwyth SY 23 – 3 EB, 2003.
6. Moga I., Schitea Maria, Mateiaş M., - Plante furajere, Ed. Ceres, 1996, Bucureşti.
7. Vinther F. P. et al. – Estimating legume N_2 fixation in a grass – clover mixtures of grazed organic cropping systems using two ^{15}N methods. *Agriculture, Ecosystems & Environment*, 78, 2000,139-147
8. Peoples, M. B., Bowman, A. M., Gault, R. R., Herridge, D. F., Mc Callum, M. H., McCormick, K. M., Norton, R. M., Rochester, I. J., Scammell, G. J. & Schwenke, G. D.- Factors regulating the the contributions of fixed nitrogen by pasture and crop legumes to different farming systems of eastern Australia. *Plant and Soil*, 228, 2001, pp 29-41
9. Burity H A, Ta T C, Faris M A and Coulman B E- Estimation of nitrogen fixation and transfer from alfalfa to associated grasses in mixed swards under field conditions. *Plant and Soil* 114, 1989, 249-255.
10. Ta, T. C., and M. A Faris- Effects of alfalfa proportions and clipping frequencies on timothy-alfalfa mixtures: II. Nitrogen fixation and transfer. *Agron. J.* 79:820-824, 1987.