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Nitrogen Use Efficiency, Symbiotic N_2 Fixation and Transfer in Red Clover-Grass Mixtures

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In order to be successful in the mixtures of grass and legume harmonious species should be chosen. This research has been mad eto investigate the nitrogen rate, nitrogen use efficieny, fixation and the transfer of red clover (*Trifolium pratense* L.), smooth brome (*Bromus inermis* Leyss), orchardgrass (*Dactylis glomerata* L.), red fescue (*Festuca rubra* L.) and their binary mixture. According to a two year-average results, the efficiency of pure sown grasses is higher than red clover and mixtures. Nitrogen fixation in the alone sowing of red clover is higher, on the other hand, in mixtures, the fixation amount has been higher in which the legume rate is high. The nitrogen transfer from legume to grass has been occurred most in the mixture of red clover and orchardgrass. As the establishment has aged, the nitrogen transfer to grasses has increased while nitrogen fixation has lessened.

Key Words: Red clover-grass mixtures, N use efficiency, N fixation, N transfer.

INTRODUCTION

In forage cultivation, growing legumes and grasses together is more advantageous than growing their pure stands. The mixtures are effective in preventing some feeding deficiency, wild plant invasion and hazards due to the environmental conditions. The most important characteristic of the mixtures including legumes is making of symbiotic nitrogen fixation and its transfer to the grasses. The mixtures formed from suitable species of legumes and grasses decrease depend on fertilizer nitrogen, thus, it prevents the pollution spread by mineral fertilizers. But for a healthy N fixation and transfer, the mixtures should be formed of suitable species. The amount of nitrogen released from legumes is not exactly known. However utilization of this N by grass was reported under the field conditions differently. In a successful mixture, the amount of nitrogen given out from legumes can rise[1] to 544 kg ha^{-1} and the amount of nitrogen transferred can rise[2] to 45 %.

Red clover is a suitable plant for being grown in mixture. In studies made in Turkey, it has been advised to mix the red clover with red fescue, orchardgrass and smooth brome which are of grasses[3,4]. Serin *et al.*[3] found that while pure sown red clover produced 12,033 kg ha^{-1} hay, this yield rose to 16,703 kg ha^{-1} when mixed with grasses. Thanopuolos & Ledgard[5] determined that the nitrogen amount from the mixture of red clover and perennial ryegrass (*Lolium perenne* L.) were 265 kg N ha^{-1} for the first year and 214 kg N ha^{-1} for the second year.

Studies reveal that the transfer and fixation of nitrogen changes according to species forming the mixtures[6,7]. That is why; determination of mixtures that has high effectiveness of fixation and transfer is important. There are a few researches for mixtures related to nitrogen fixation and transfer in highlands because they have some specific ecological conditions as like short growing season and low temperatures. This study has been planned to investigate the yield, nitrogen rate, botanic composition, symbiotic nitrogen fixation and its transfer in the mixtures of red clover and some grasses in the highland conditions.

EXPERIMENTAL

The study was carried out at Atatürk University, Agriculture Faculty; Research Station in Erzurum which is located on 1853 m altitude, 39º 55' north altitude and 41º 61' east longitude in eastern Anatolia, Turkey. There is a big temperature difference between summer-winter and day-night because of its high altitude and continental climate. While winters are cold and snowy, summers are cool and dry. An important amount of the precipitation is in spring and winter. In the study years, the total rainfall and mean monthly temperature have been almost the same in long-term average. Long term mean rainfall amount and temperature are 424.6 mm and 5.7 ºC, respectively. In the study period (2003 and 2004) annual rainfall were 424.3 and 415.5 mm and temperatures were 5.1 and 5.7 ºC, respectively.

The research area soil is in silt loam structure and its organic matter content is poor (1.02 %) and its pH is neutral (7.6). Phosphorus rate which is useful to plants is insufficient (39 kg P ha^{-1}), whereas potassium is rich (562 kg K ha^{-1}).

The sowings were done in 2002 and data were obtained in 2003 and 2004. Red clover (*Trifolium pratense* L. var. Tohum Islah), smooth brome (*Bromus inermis* Leyss. var. Tohum Islah.), orchardgrass (*Dactylis glomerata* L. var. Tohum Islah) and red fescue (*Festuca rubra* L.var. Nova Rubra) were used as plant materials. The experiment was designed in randomized complete block design with four replicates. In every replication, 7 applications that are formed of plants' pure sowings and their binary mixtures (legume-grass) were placed. Both the mixtures and pure sowings

were seeded in a set of 15 cm in distance. The sowing mixture were made[8] in alternative rows in the rate of 1:1 legume:grass. Each plot had a 1.2 m width and a 2 m length (2.4 m^2).

By forming micro-plots (0.6 m × 1 m) in the main plots[2] labelled ammonium sulfate [$(^{15}NH_4)_2SO_4$)] including 5 % atom excess (a.e.) in every year beginning of spring was applied. Signed fertilizer was sprayed to ground surface as 2.5 kg ha^{-1}, adding 5 L of water to it[9]. In the samples taken from these micro parcels, symbiotic nitrogen fixation and transfer with the help of labelled nitrogen were examined. In the areas that are out of micro plots, dry matter yield and botanic composition were determined by applying 120 kg P_2O_5 ha^{-1} and nitrogen fertilizer equal to labelled fertilizer amount (50 kg N ha^{-1}). After the end of the spring rainfalls, the plots were irrigated at least twice in every cutting. When the plants started blooming in the plots, harvest was made. In each year, pure sown grasses were cut once and three times in red clover and mixtures. During the harvest, micro parcels and other parts were cut separately; mixtures were separated into species and weighed. Samples' dry matter yields were determined by being dried out first and then, being dried for 48 h in an oven with a 78 ºC heat. Each sample was grinded and its nitrogen concentrations were determined by Kjeldahl method in Nuclear Research and Training Centre, Turkish Atomic Energy Authority in Ankara[10]. From these samples, specimens of 5 mL were taken and the amount of nitrogen signed with % was determined in emission spectrophotometer. After the values of ^{15}N a.e.% were determined in the legumes and grasses in the mixtures, N transfer was calculated using the formula below[10,11].

$$\text{Transferred N \%} = \frac{[\%\,^{15}\text{N a.e.}_{(\text{Mixture grass})} - \%^{15}\text{N a.e.}_{(\text{Pure grass})}]}{[\%\,^{15}\text{N a.e.}_{(\text{Mixture legumes})} - \%^{15}\text{N a.e.}_{(\text{Pure grass})}]} \times 100$$

Data were statistically analyzed statistically based on MSTAT-C program every year, separately and in a two year-average[12]. The differences between the averages were explained by LSD multi contrasting test.

RESULTS AND DISCUSSION

In the first year of the research, nitrogen use efficiency has been higher than (46.65 %) in the second year (34.35 %, Table-1). In the second year, the reaction to the fertilizer nitrogen has become less, probably the plants have become enough for meeting needs from symbiotic fixation more. In both years, N use efficiency of pure sown grasses is more because grasses use from fertilizer nitrogen easier[1]. According to the two-year average, nitrogen use efficiency is high in orchardgrass and smooth brome (56.94 and 56.75 %). As in our research, nitrogen use efficiency changes according to plant species. In red clover-smooth brome and red clover-orchardgrass mixtures, nitrogen use efficiency has been lower (29.75 and 28.44 %).

TABLE-1
N USE EFFICIENCY OF RED CLOVER, SOME GRASSES AND THEIR BINARY MIXTURES (%)

Treatments/years	2003	2004	Average
Red clover	43.05 C	26.42 D	34.74 C
Smooth brome	67.12 A	46.37 B	56.75 A
Red fescue	44.05 C	40.22 C	42.14 B
Orchardgrass	53.65 B	60.24 A	56.94 A
Red clover-smooth brome	40.59 C	18.90 E	29.75 D
Red clover-red fescue	43.76 C	25.79 D	34.77 C
Red clover-orchardgrass	34.34 D	22.53 DE	28.44 D
Average	46.65 A	34.35 B	40.50

LSD (Year × mixture): 4.03
Number with the same letters in a column are not statistically different according to LSD multiple range test ($p < 0.05$).

In the first year, the amount of N from symbiotic way has been 323.2 kg ha^{-1}, in the second year has decreased to 291.4 kg ha^{-1} (Table-2). Decreasing might have occurred because red clover that has short longevity and perennial has started to become sparse from the second year. There has been no difference between the applications in 2003. But in every two year and average, N fixation of pure sown red clover has been higher. The mixture of red fescue that has a higher legume ratio follows the pure sowing of red clover (Fig. 1). Ta and Faris[13] have declared that the amount of N rises as the amount of legume rises in the mixture. In two years average, the N fixations of pure sown red clover, red clover-smooth brome, red clover-orchardgrass and red clover-smooth brome treatments have been found as, in the same order, 321.0, 309.1, 306.6 and 292.3 kg ha^{-1} (Table-2).

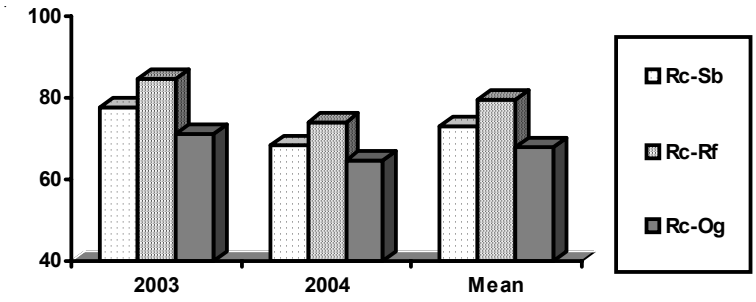

Fig. 1. Legume rates in red clover and grass mixtures (%)

TABLE-2
N FIXATION OF RED CLOVER AND RED CLOVER-GRASS MIXTURES (kg ha^{-1})

Treatment/year	2003	2004	Average
Red clover	331.7	310.4 A	321.0 A
Red clover-smooth brome	309.5	275.1 B	292.3 B
Red clover-red fescue	319.5	298.7 AB	309.1 AB
Red clover-orchardgrass	331.9	281.2 B	306.6 AB
Average	323.2 A	291.4 B	307.3

LSD (Year × mixture): ns
Number with the same letters in a column are not statistically different according to LSD multiple range test ($p < 0.05$).

The transfer of nitrogen from red clover to grasses in mixture has been 25.02 % for the first year but 49.73 % for the second year (Table-3). The nitrogen transfer is the taking of the loss from legumes in different ways by grasses. In order to be taken nitrogen by grasses should be release to the soil by legumes. The most effective way of this is the passing of the nitrogen through the dying of legumes' root and nodules. And this happens through the aging of the plants. Because of this, the transfer has remained low for the first year and the transfer has increased to 98.8 % the following year. Mallarino *et al.*[9] informs that the transfer has increased in the years after sowing and has reached to the highest level in the second and third years. The most of transfer in 2003 and in the average of a two year time has occurred in red clover-orchardgrass mixture. The nitrogen transfer in red clover that puts a lot of dead material and orchardgrass that gets a lot of nitrogen is in high amounts.

TABLE-3
N TRANSFER FROM RED CLOVER TO GRASSES IN BINARY MIXTURES (%)

Treatment/year	2003	2004	Average
Red clover-smooth brome	18.16 B	51.90 A	35.03 B
Red clover-red fescue	15.80 B	52.97 A	34.38 B
Red clover-orchardgrass	41.09 A	44.32 B	42.71 A
Average	25.02 B	49.73 A	37.37

LSD (Year × mixture): 16.31
Number with the same letters in a column are not statistically different according to LSD multiple range test ($p < 0.05$).

Depending on the characteristic of species which forms the mixtures, the dynamic of mixture shows changes. In order to get required advantage from the mixture, the mixture species should be harmonious. In this research,

according to the characteristics of grasses included in mixture distinctions have been found in mixtures. Mixtures have been more productive than both red clover and grasses pure sowing. The mixture of smooth brome and orchardgrass is more productive than the mixture of red fescue because red fescue competes less with red clover. Its rate in mixture and its contribution to yield has remained low.

The rate of N in each three mixtures has been close to each other but has been lower than red clover and higher than pure sowing of grasses. The nitrogen use efficiency of pure sown grasses has been determined quite high. On the other hand, in mixtures, the N efficiency has been lower because they were not depending on fertilizer nitrogen.

Due to the species which form the mixtures, the symbiotic nitrogen fixation and transfer also have been different. The most of nitrogen fixation has been found in pure sown red clover and the most of transfer in red clover-orchardgrass mixture. The nitrogen fixation has decreased as the establishment aged but nitrogen transfer from legumes to grasses has increased.

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