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Performance of White Clover-Grasses Mixtures: Part-II Nitrogen Fixation and Transfer from White Clover to Associates Grasses

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This research was made to investigate fixation and transfer of white clover-grass mixtures. Among legumes, white clover (*Trifolium repens* L.) and grass plants, smooth bromegrass (*Bromus inermis* Leyss.), red fescue (*Festuca rubra* L.) and O. grass (*Dactylis glomerata* L.) were tested in binary mixtures. According to two year-average results, nitrogen fixation in the pure sowing of white clover was higher. On the other hand, in mixtures, the fixation amount has been higher in which the legume rate was high. The nitrogen transfer from legume to grass changed between 37.67 % in white clover-O. grass and 44.04 % in white clover- smooth bromegrass mixtures. Amount of residual N from fertilizer in soil was decreased while grass ratio was increased.

Key Words: White clover-grass mixtures, N Fixation, N Transfer.

INTRODUCTION

Biological nitrogen fixation is an important source of nitrogen in both agricultural and natural ecosystems. Biological nitrogen fixation is between 100 and 290 million tones of year^{-1}, 40-48 million tones year^{-1} of which is fixed by agricultural crops in fields[1]. As biological nitrogen fixation is largely restricted to legumes in agro ecosystems, replacing fertilizer N with symbiotically fixed dinitrogen may require a legume crop to be grown mixed or alone. The amount of dinitrogen fixed and N contribution from leguminous crops are influenced by a number of environmental factors, including soil type, nutritional status of soil, species and varieties, water availability and temperature as well as soil and crop management[2]. Biological nitrogen fixation is very difficult to estimate the amount of N fixed in practice. The annual biological nitrogen fixation in clover grass systems ranges from 13

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to 682 kg N ha^{-1} but most estimates are in the range[3] of 80-300 kg N ha^{-1}. Biological nitrogen fixation, the most important biological process after photosynthesis, is performed by plants using 16 ATPs[4]. There is a need of decreasing the loss in N produced by so large energy. N use of plants sowed together with legumes or following them is desired to be good. Other plants uptakes the N remaining separately from legumes for various reasons. This process is called as N transferring. In this process several point are still not clear. It is known that yield of grass plants is 30 kg/ha or 60 % more when grown in a mixture because of the increased N content[5]. It was stated that in the mixtures, 6 months after sowing N transfer from legumes to grass begins and 79 % of N in grass plants is obtained from this process[6]. Mallarino *et al.*[7] stated that while the amount of transferred N is very little immediately after sowing, in time this amount considerably increases and transferred N amount reaches at a value ranging between 4.5-8.4 kg N ha^{-1}. White clover is a stoloniferous perennial plant capable of symbiotically fixing atmospheric dinitrogen, as fodder legumes commonly grown intermixed with grasses in grazing or cut and carry systems. Elgersma and Hassink[8] reported that the amount of N transferred from white clover to perennial grass is in the range between 13.6 and 28.0 kg N ha^{-1}.

Although N amount in grass plants increases when grown in mixtures, their responses to nitrogenous fertilizers applied decrease. For instance, the fertilizer utilization efficiency in grass plants sowed alone is between 40 and 70 % while in mixtures this percentage is below 25 %, however no loss in yield was reported[9]. Although N amount in grass plants increases when grown in mixtures, their responses to nitrogenous fertilizers applied decrease. For instance, the fertilizer utilization efficiency in grass plants sowed alone is in between 40 and 70 % while in mixtures this percentage is below 25 %, however no loss in yield was reported[9].

The primary objective of this work was to estimate the input of symbiotically fixed N_2 into an organic cropping system *via* a grass-clover mixture and to evaluate its importance for the N supply of crops in the management and to quantify the proportion of these below ground N pools that was derived from the fixation of atmospheric N. At the end of the two production years, the mezotrons were excavated, followed by analysis of the ^{15}N, total N in soil depth 60 cm. To quantify the transfer of atmospherically derived nitrogen from clover to the associated grasses.

EXPERIMENTAL

The study was carried out at Atatürk University, Agriculture Faculty; Research Station in Erzurum which is located on 1853 m altitude, 39º55' north altitude and 41º61' 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 was 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. White clover (W clover) (*Trifolium* repens L. var. Tohum Islah), smooth bromegrass (S. brome) (*Bromus inermis* Leyss. var. Tohum Islah.), red fescue (R. fescue) (*Festuca rubra* L.var. Nova Rubra) and O.grass (O. grass) (*Dactylis glomerata* L. var. Tohum Islah) were used as plant materials. The experiment was designed in randomized complete block design with four replicates. In every replication, 4 applications that are formed of plants' pure sowing and their binary mixtures (legume-grass) were placed. Both the mixtures and pure sowing was seeded in a set of 15 cm in distance. The sowing mixtures were made in alternative rows in the rate of 1:1 legume:grass[10]. 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[11] 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[7]. 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 and 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[12]. 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 atom excess (a.e.) %

were determined in the legumes and grasses in the mixtures, the amount of biological nitrogen fixation was estimated using the ^{15}N dilution techniques. The proportion of N derived from the atmosphere (%Ndfa) was calculated using.

$$\%\text{Ndfa} = (1 - \frac{\%\ ^{15}\text{N a.e.}_{(\text{legume})}}{\%\ ^{15}\text{N a.e.}_{(\text{Pure grass})}} \times 100$$

$$\text{Fixed N (kg N/da)} = (\%\ \text{Ndfa}) \times \frac{\text{Total N kg/da}}{100}$$

N transfer was calculated using the formula below:

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

Data were statistically analyzed statistically based on SPSS program every year, separately and in a two year-average. The differences between the averages were explained by LSD multi contrasting test. In the analysis construction year (2002) was excluded.

RESULTS AND DISCUSSION

The Ndfa values in the construction year (2002) ranged between 115.5 and 177.2 kgN ha^{-1}. The lowest values of Ndfa 252.5 kgN ha^{-1} and 61.1 kgN ha^{-1} were measured in 2003 year W clover + S. brome and in 2004 year W clover + R. fescue, respectively. In terms of mean Ndfa, applications showed significant differences considering W clover + O.grass and W clover pure and mixtures in the same group and others in a different group (Table-1).

Biological nitrogen fixation may be affected by various factors, such as plant species grown, amount of legumes on the field, growth period, aim and management of the agricultural foundation, soil type, soil moisture, soil pH, nutrient elements in soil, disease and pests, competition, air and soil temperature, O_2 concentration in air, light intensity and lighting condition, amount and type of treated nitrogenous fertilizers[13,14]. Plant species grown has a very important effect on the amount of nitrogen fixation[13]. Fixed nitrogen amount may show variation depending on the rate of legumes in the composition within especially plants grown in mixtures. The larger the rate of legumes in a mixture, the higher the amount of fixation[14]. The reason why the nitrogen fixation amount is high under the study conditions might have been because in this period O_2 amount was reduced, temperature was at optimum level and lighting amount was high. This condition is confirmed by the meteorological parameters measured in this period, when temperature was between 9 and 26 ºC, which was reported to be the ideal temperature[15].

Fixation amounts showed differences between the mixtures either in the first or the second year. Nitrogen fixation amount may vary depending on the legume rate on the field and competition between the species[14]. It can be seen from the former studies that fixation rates varied between 30 and 640 kg N ha^{-1} depending on plant species. Thus, whether the legume rate is reduced or increased depending on competition and also species features of legumes may cause different fixation rates in the mixtures[14].

The rate of N transferred from legume to grass plants in the construction year was found to be 50.71 %. The highest transfer rate was found in W clover + S brome mixture as 85.23 %. Data obtained in the construction year were excluded from the statistical analyses because plants, especially grass, did not show their real yield. While in the first yield year (2003) mean transfer rate was 35.10 %, it was found to be maximum in the mixture of W clover + S brome with 35.73 % and minimum transfer rate was in W clover + R fescue mixture. In the year 2004, mean transfer rate was 40.86 %. In that year maximum transfer rate was measured in the W clover + S brome mixture. Two-year average transfer rate was 46.62 % (Table-1).

The yield of mixtures when nitrogenous fertilizer was not applied or sufficient was so higher than that of pure grass. However, grass plants may dominate legumes increasing their rate in the mixtures. This increase in grass plants is due to the increase in the N transfer from legumes to grass. It was found in the study that transferred N amount in the construction and the first yield years is lower than the second yield year. Because in the construction and first yield years plant growth and *Rhizobium* symbiosis were more gradual and consequently the fixed N amount was lower and the amount of non-living material was little, transfer was also lower. Transfer rate reached at very high levels in the second and third years increasing in the years after sowing[7]. Growth rate of grass and their N contents were higher when grown in the mixtures. This increases the contest of grass plants and consequently their rates in the mixtures. N rate transferred from legumes to grass may reach at 80 % depending on the gap between lines[6]. Transfer amount is higher among grass plants that have higher N intake. In the present study the highest transfer amount was seen in W clover + S brome mixture. This is because of the fact that white clover can leave more nonliving material in addition to high N intake of smooth brome. From two-year mean values this can be seen much more clearly. Mean transfer amount was determined as 40.86 %. In the studies world wide this amount was found to be between 3 and 80 %. Broadbent *et al.*[6] reported that the amount of N transferred from Trifolium repens to Lolium rigidum was 79 %, while Serin and Erkovan[16] 35.03 from red clover to smooth brome, 34.38 from red clover to red fescue and 42.71 from red clover to O. grass.

In the year, when the field was first constituted (2002), nitrogen rate in soil in the depth between 0 and 30 cm was found to be 0.07 %. In 2003, nitrogen rate in soil varied between 0.07 and 0.08 %. In 2004, statistically significant differences between the applications were observed. W clover pure stand was the lowest, whereas W clover + S. brome mixture was found to be the highest. The interaction between year and application was found to be statistically significant ($p < 0.05$; Table-2). Nitrogen amount between 30 and 60 cm soil depths showed variations between 0.04 and 0.08 % in the years 2002, 2003 and 2004. Mean nitrogen rates for the years 2002, 2003 and 2004 were 0.07, 0.07 and 0.05 %, respectively (Table-2).

In general, more than 95 % of total nitrogen is from organic materials and less than 5 % is from inorganic forms, ammonium and nitrate. Organic material amount in soil may be impacted by factors such as climate, vegetation, soil texture and soil processing techniques[17].

For instance, nitrogen rate ranges between 0.07 and 0.12 % in loamy soils and 0.10 and 0.13 % in soils rich in silt when processed while this rate changes between 0.20 and 0.30 %; and 0.25 and 0.35 %, respectively, when grassy plants are grown in these soil types[18].

In the present study, nitrogen rate in soil in 0-30 cm depth was lower in 2003 than that in 2002. This might have been because of the fact that in 2003 inorganic nitrogen amount from organic material was lower. Either slow occurring organic material decomposition or an increase in transferred nitrogen amount might have helped the differences between the applications to take place. Although there are no significant differences between soil depths of 0-30 and 30-60 cm in 2003 for nitrogen rate, in 2004, this rate in 30-60-cm depth was found to be higher in 2004. As can be realized from the soil properties, at soil depth of 0-30 cm, silt rate is lower that at 30-60 cm depth. Thus, this condition may be tied on the fact that nitrogen can be better held by soil layer richer in silt.

Nitrogen rate remained from fertilizer can be seen in Table-3. For this rate, at 0-30 cm soil depth, interactions between years, applications and year *vs.* applications were found to be statistically significant, which is similar with the condition at 30-60-cm soil depth. However, at this depth, interactions between years and year *vs.* application were found to be statistically significant at the significance level of 5 % (Table-3).

Nitrogen amount remained in soil from marked fertilizer applied on the field in the constitution year is generally higher than that in maintenance years due to the facts that in this first year vegetation period is relatively shorter and real crop-yield can not be obtained from forage plants. This condition can be confirmed when considered dry material yield and nitrogen utility efficiency[14]. In the maintenance years, nitrogen amount remained at 0-30 cm soil depth was very little. For nitrogen amount remained in soil,

TABLE-1
N FIXATION AND TRANSFER FROM WHITE CLOVER TO GRASSES IN BINARY MIXTURES

Mixtures	Ndfa (kg N ha^{-1})				Nitrogen transfer (%)			
	2002	2003	2004	Mean	2002	2003	2004	Mean
W clover(Wc)	177.2	327.9	83.5 A	205.7	–	–	–	–
Wc + S brome	139.8	252.5	73.2 AB	162.9	85.23	35.73	52.36	44.04
Wc + R fescue	115.5	296.1	61.1 B	178.6	32.13	34.70	47.03	40.87
Wc + O. grass	124.4	256.7	89.7 A	173.2	34.78	34.88	40.46	37.67
Mean	**139.2**	**283.3 A**	**769.0 B**	180.1	**50.71**	**35.10**	**46.62**	40.86
	LSD (Year × treatments) = ns				LSD (Year × treatments) = ns			

TABLE-2
NITROGEN IN SOIL OF WHITE CLOVER-GRASS MIXTURES FIELDS

Mixtures	0–30 cm N (%)				30–60 cm N (%)			
	2002	2003	2004	Mean	2002	2003	2004	Mean
W clover (Wc)	0.05	0.08	0.03 B	0.05	0.05	0.08	0.06 A	0.06 a
Wc + S brome	0.07	0.07	0.06 A	0.06	0.07	0.07	0.06 A	0.06 a
Wc + R fescue	0.06	0.07	0.05 AB	0.06	0.06	0.07	0.06 A	0.06 a
Wc + O. grass	0.08	0.08	0.05 AB	0.06	0.08	0.08	0.04 B	0.05 b
Mean	**0.07**	**0.07 A**	**0.05 B**	0.06	**0.07**	**0.07 A**	**0.05 B**	0.06
	LSD (Year × treatments) = 0.002				LSD (Year × treatments) = ns			

TABLE-3
NITROGEN[15] IN SOIL OF WHITE CLOVER-GRASS MIXTURES FIELDS

Mixtures	0–30 cm N^{15} (%)				30–60 cm N^{15} (%)			
	2002	2003	2004	Mean	2002	2003	2004	Mean
W clover (Wc)	2.53	0.62 A	0.37 B	0.50 A	3.18	0.74 A	0.61 A	0.68 A
Wc + S brome	2.60	0.45 B	0.19 C	0.32 B	3.10	0.37 B	0.21 C	0.29 C
Wc + R fescue	2.40	0.52 B	0.46 A	0.49 A	2.90	0.41 B	0.34 B	0.38 B
Wc + O. grass	2.40	0.28 C	0.35 B	0.32 B	2.15	0.19 C	0.29 BC	0.24 C
Mean	**2.48**	**0.47 A**	**0.34 B**	0.40	**2.83**	**0.43 a**	**0.36 b**	0.39
	LSD (Year × treatments) = 0.108				LSD (Year × treatments) = 0.113			

Tables 1-3 = Means with capital letters and small letters are not significantly different $p < 0.01$ and 0.05, respectively.

year to year and application to application differences may be tied on the rate of grassy species in the composition.

Nitrogen amount remained in soil in 2003 was more than in 2004. Crop yields of applications in 2003 were higher than that obtained in 2004. The reason for this condition may be because in 2004, the rate of legumes in the composition reduced and that of grassy species increased. Since the rate of grassy plants which can better use soil nitrogen compared to legumes, increased in the composition, nitrogen amount remained in soil decreased depending on this increase. A slight increase in the nitrogen amount remained in soil from marked fertilizer applied on the field was observed in the parallel of increase in soil depth. As a mobile element, nitrogen can penetrate into underlying soil layers as the result of washing off process depending on the types of soil[12]. Increase in the nitrogen rate, which plants cannot utilize and removal of this nitrogen via washing off process from soil may vary according to species. In 2003, nitrogen amount remained in 30-60 cm soil layer was higher than in 2004, which may be tied on lower grassy plant rate in the first year.

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