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EFFECTS OF PHOSPHORUS FERTILIZER AND PHOSPHORUS SOLUBILIZING BACTERIA APPLICATION ON CLOVER DOMINANT MEADOW: I. HAY YIELD AND BOTANICAL COMPOSITION

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ABSTRACT

This study was carried out at Ataturk University farm in Erzurum, Turkey over 4 years (2004-2007) to evaluate the effects of phosphorus fertilization and phosphorus solubilizing bacteria (*Bacillus megaterium var. phosphaticum*) applications on the dry matter production and botanical composition of a natural meadow. Phosphorous fertilizer (0, 11, 22, 33, 44 kg P ha^{-1}) and phosphorus solubilizing bacteria (*Bacillus megaterium var phosphaticum*) were added to study plots. The present study investigated the effects of applying phosphorus and phosphorus solubilizing bacteria on the yield and botanical composition of a clover-dominated meadow in the Eastern Anatolia region of Turkey. Phosphorus **fertilization significantly affected dry matter yield while phosphorus solubilizing bacteria did not show any effect on dry matter yield. While phosphorus fertilization did not affect the botanical composition of grasses, the composition of legumes and other families was affected. There was not any effect of bacteria application like in dry matter yield.**

Keywords: Phosphorus fertilizer, phosphorus solubilizing bacteria, dry matter, botanical composition, meadow

INTRODUCTION

The hay produced from meadows in the highlands of Turkey is the most important winter feed resource for animal husbandry during the winter feedlot period, which may exceed 7 months. Low productivity in highland meadows of Turkey stems from mismanagement and strategies such as early grazing, excessive irrigation and low fertilizer input (Koc and Gokkus 1998). Many studies have been conducted on these issues within the region (Altın 1975; Gokkus 1990; Gokkus and Koc 1995; Comakli et al. 2005; Comakli et al. 2009) and most prominent results were obtained from fertilizer studies (Gokkus 1989; Koc et al. 2005).

The response of meadows to types and quantities of fertilizer varies depending on botanical composition, precipitation and soil properties (Benedycka et al. 1992; Altin et al. 2005). Generally, legume dominant stands show better response to phosphorus application because they obtain their nitrogen requirements via symbiotic pathways (Miller and Reetz 1995). Hence, phosphorus application increases both hay yield and the proportion of legumes within the botanical composition of legume dominant stands (Hatipoglu et al. 2001; Dasci 2008). Hatipoglu et al. (2001) recorded an increase in hay production from 2.95 t ha^{-1} to 5.51 t ha^{-1}, and an increase in legume ratio in the botanical composition from 49.8 % to 80.3 % in legume dominant pasture following 100 kg P_2O_5 ha^{-1} application. Similar results were also reported by Papanastasis and Papachristou (2000) and Dasci (2008).

Chemical fertilizer application is an effective method to increase yields, but is costly and may also lead to environmental problems. In particular, phosphorus fertilizers present a serious risk of cadmium accumulation in soil (Al-Fayiz et al. 2007). Recently, there has been interest in more environmentally sustainable agricultural practices (Orson 1996). A greater number of studies have investigated microorganisms and promising results were obtained from biofertilization studies (Cakmakci et al. 2006; Elkoca et al. 2008). The bacteria used as phosphorus biofertilizers could contribute to increasing the availability of phosphates immobilized in soil and could enhance plant growth by increasing the efficiency of other nutrients (Kucey et al. 1989). Indeed, studies on the application of nitrogen fixing and phosphorus solubilizing bacteria were shown to increase yields in sugar beet (Cakmakci et al. 1999), barley (Salantur et al. 2005), alfalfa (Comakli and Dasci 2009), clover, wheatgrass, perennial ryegrass (Holl et al. 1988) and cicer (Elkoca et al. 2008). *Bacillus megaterium var. phosphaticum*, a phosphorus solubilizing bacteria, release acid into the rhizosphere to enhance nutrient uptake (Lach et al. 1990; Vazquez et al. 2003). This process might reduce pH levels of soils and might alter competition conditions within natural communities.

The aims of this study are to determine the efficiency of phosphorus solubilizing bacteria on hay production and the botanical composition of legume dominant meadows, and; to assess the substitutability of phosphorus solubilizing bacteria for use in commercial phosphorus fertilizer, or to reducing

Table 1. Botanical composition and dry matter yield of the meadow in relation to phosphorus fertilizer and phosphate solubizing bacteria application [(1)].

	Dry Matter Yield (t ha^{-1})	Botanical Composition (%)		
		Grasses	Legumes	The Other Families
P_0	4.83 C	65.04	16.82 b	18.15 ab
P_{11}	5.64 B	59.83	20.70 ab	19.78 a
P_{22}	5.84 AB	63.70	21.51 a	14.79 b
P_{33}	5.88 AB	62.70	22.83 a	14.47 b
P_{44}	6.36 A	62.98	22.11 a	14.46 b
Average	**5.71**	**62.85**	**20.79**	**16.33**
B_0	5.68	62.62	19.83	17.36
B_1	5.74	63.08	21.76	15.29
Average	**5.71**	**62.85**	**20.79**	**16.33**
2004	3.36 D	18.11 D	63.49 A	18.28 B
2005	7.18 B	66.09 C	19.69 B	14.22 C
2006	4.16 C	77.58 B	0.00	22.42 A
2007	8.13 A	89.62 A	0.00	10.38 D
Average	**5.71**	**62.85**	**20.79**	**16.33**
Y x B	*	Ns	ns	ns
Y x P	**	ns	ns	ns
B x P	ns	ns	ns	ns
Y x B x P	ns	Ns	ns	ns

[1] Values followed by small and capital in a column shows significantly differences at $P< 0.05$ and $P< 0.01$ levels, respectively, using Duncan's multiple range test.

[ns] No statistical difference at $P<0.05$ and $P<0.01$.

[*]Statistical difference at $P< 0.05$.

[**]Statistical difference at $P< 0.01$.

the application ratio required for commercial phosphorus fertilizer.

MATERIALS AND METHODS

This study was conducted on natural meadows of Ataturk University farm in Erzurum, Turkey, for a period of 4 years (2004 to 2007). The experimental area was located 39^o 55^I N and 41^o 61^I E at an altitude of 1800 m. Average temperature and total precipitation at the study site are 5.7 ^{o}C and 425 mm, respectively. During the experimental years, total annual precipitation was 441, 480, 357, 445 mm and average temperatures were 4.4, 5.1, 6.4, 4.5 ^{o}C, respectively. The total precipitation was higher than the long term average in the second year and was lower than the long term average in the third year of the experiment. Except the third year, average temperature during the experimental period was lower than the long term average.

The soil in the experimental area was loam textured, containing 3.3 % organic matter, 3.9 %, lime with EC of 2.9 mhos cm^{-1} and pH of 7.6. The corresponding available K and Olsen P content were 650 and 47 kg ha^{-1}, respectively. The depth of the water table was 30-40 cm from the soil surface at the beginning of the growing season and dropped to 1 m toward mid-summer.

The experiment was established on natural meadow which was dominated by alsike clover (*Trifolium hybridum*) with some cool season grasses such as *Alopecurus pratensis*, *Poa pratensis*, *Hordeum violaceum* and other forbs such as *Ranunculus kotschyi*, and *Cerastium sp*. Alsike clover is a short-lived perennial, but is usually considered as a biennial plant (Townsend 1985).

The experiment used a randomized complete block design with four replications. Two bacteria levels (no application or 10^8 cfu ml^{-1}) and five phosphorus applications (0, 11, 22, 33 and 44 kg P ha^{-1}) were applied alone or in combination. *Bacillus megaterium var phosphaticum* culture, which is isolated within high altitude areas, was obtained from the Biotechnology Laboratory of the Department of Plant Protection, Faculty of Agriculture, Ataturk University, Erzurum. Phosphorus fertilizer was applied as soon as snow melted in the spring. Triple superphosphate and bacteria culture were applied to the plots at the start of the growing by spraying. In order to prevent infections was changed overshoes every plot. A sprinkler system was used to apply approximately 10mm of water to the bacteria-applied plots, immediately after bacteria application. Each plot was 5 x 2 m in size, with a 0.5m buffer inside each edge and a 2m buffer outside.

When the dominant plant species were at the flowering stage, plant samples were taken by clipping four 0.5 by 0.5 m areas within each plot down to the soil surface. The samples were oven dried at 70 ^{o}C for 48 hours prior to identification (Tan and Erkovan 2004). The dried samples cut from a 0.5m by 0.5 m quadrat in each plot were separated by hand and grouped as grasses, legumes and other groups, in order to determine the botanical composition by dried weight.

All data were subjected to analysis of variance based on general linear models in order to determine factorial

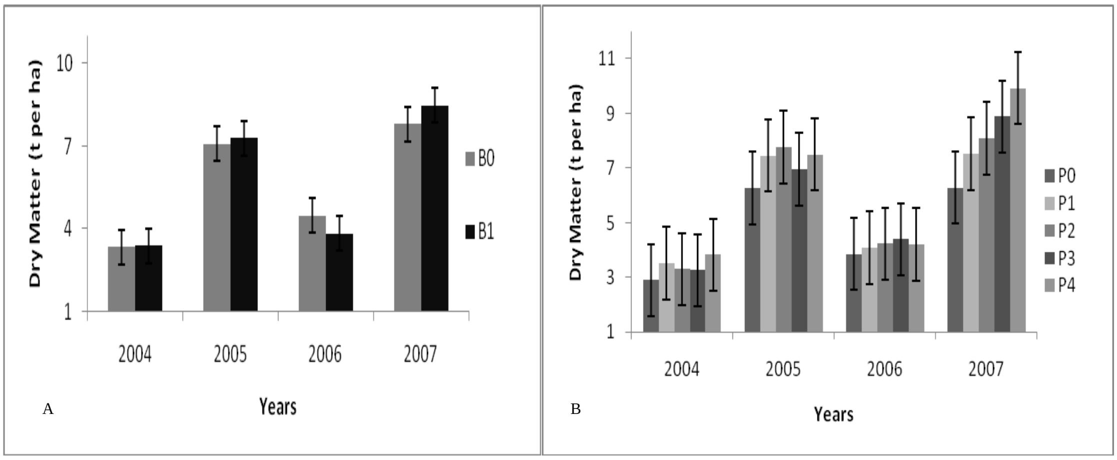

Figure 1. (A) The effect of bacteria application on dry matter production in relation to years. (B) The effect of phosphorus doses on dry matter production in relation to years.

arrangement of treatments and repeated to determine the factorial arrangement of treatments using the SPSS statistical package (SPSS 1999). Means were separated using Duncan's Multiple Range Test.

RESULTS

Phosphorus fertilizer application significantly affected both botanical composition and dry matter production, while applications of phosphorus solubilizing bacteria had no effect on either botanical composition or dry matter production in the alsike clover dominant natural meadow. ANOVA results indicated that there was no significant interaction effect for botanical composition values, while for dry matter production (year x bacteria) and (year x phosphorus) interactions were significant at a level of $P<0.05$ and $P<0.01$, respectively (Table 1). Dry matter production increased in response to increasing phosphorus fertilizer. While the lowest dry matter production was recorded for P_0 application, the highest dry matter yield was recorded for P_{44}, the highest application used. However, the increases recorded above the P_{22} application level were not statistically significant. Phosphate solubilizing bacteria application had no effect on dry matter production. Dry matter production varied significantly between years. The lowest dry matter production was recorded in the first year of the experiment, the highest yield in the fourth year of the experiment. Dry matter production during the third year was lower than the second and the fourth year values but was higher than the first year value. In the first three years, there were no significant differences in dry matter production between applications of phosphate solubilizing bacteria, but application of phosphate solubilizing bacteria caused a significant increase in dry matter production in the fourth year (Figure 1A). This may be due to (year x bacteria) application interaction effects ($P<0.05$).

In the first and third years, there were no significant responses to phosphorus fertilizer application in terms of dry matter production, while significantly different responses were recorded in relation to phosphorus fertilizer application in the second and fourth years (Figure 1B). Particularly in the fourth year, the response to phosphorus fertilizer were more pronounced than in other years, in which linear responses were recorded in line with increasing P doses. This may explain (year x phosphorus) interaction effects ($P<0.01$).

The proportion of grasses in the herbage varied between years, but did not show any variation according to phosphorus and bacteria application (Table1). The percentage of grasses in the herbage increased with each year of the study, and the ratio of grasses in the herbage showed a statistically significant variation between each year. In the first year, grass comprised 18.11% of the herbage, increasing each year to 89.62% in the fourth years of the experiment. No statistically significant interaction was found between the different applications and the grass ratio.

The percentage of legumes in the herbage was not affected by bacteria application but was affected by phosphorus application and by year. The percentage of legumes increased in line with phosphorus application but the increases were not statistically significant above 11 kg P ha^{-1} application. The percentage of legumes decreased over time and there were no legumes recorded in the herbage after the third year. No significant interaction effects were found between treatments and the percentage of legumes in the herbage.

Over the 4-year duration of the study, the average percentage of other plant families in the herbage decreased in line with increased P application, but this decrease was not notable and was not statistically significant at application levels above 11 kg P ha^{-1}. The application of bacteria did not affect the proportion of other plant families in the herbage. However, the percentage of other families varied according to year, with the highest percentage recorded in the third year and the lowest percentage in the fourth year of the study. As with the grass and legume groups, no statistically significant interaction was found between treatments and the percentage of "other plant families" in the herbage.

DISCUSSION

The data from the present 4-year study indicated that hay production in a clover-dominated natural meadow increased using phosphorus application but that the application of phosphorus solubilizing bacteria had no significant effect on hay production. Increase in hay production recorded only in the fourth year, due to (year x bacteria application) interaction, but bacteria treatment had no statistically significant effect (as determined using ANOVA) on dry matter production or botanical composition component. Previous trials with *Basillus* species showed considerable yield increases in many crops (de Freitas et al. 1997; Cakmakci et al. 2005; Cakmakci et al. 2006; Elkoca et al. 2008). However, the present study did not find similar evidence of increased hay production following the application of *Bacillus megaterium var phosphaticum* in a clover-dominated natural meadow

The meadow vegetation showed increased hay production in response to P fertilizer. Higher or sustainable hay production was achieved from the application of 22 kg P ha^{-1} in the first 3 years of the experiment, but required higher phosphorus doses in the fourth year. The increases in hay production in the first three years of the study might be explained by legumes response to P application, as it is well known that legumes show better response to P application (Miles 1957; Miller and Reetz 1995). The increase in hay production in terms of P application in the advanced years of the experiment might be explained by nitrogen and phosphorus interaction in the root zone of the soil, because availability of P increase N uptake of plants (Benedycka et al. 1992). Indeed, nitrogen availability in the root zone must be increased due to the release of N from symbiotic nodules and dead roots of legumes (Walley et al. 1996) in the later years of the experiment, because there was no legume component in the hay in those years (Table 1). Hay production increased over time, except during the third year of the experiment. General increases in hay production over time might be due to increased soil nutrient levels as a result of the residual effect of P and N released due to the decomposition of roots of die down legumes. Dry and warmer climatic condition prevailing in the third year of the experiment may be responsible for the sharp reduction in hay production, because dry climatic conditions reduce the photosynthetic period and trigger earlier maturity in plants (Ball et al. 2001). As a result, photosynthate accumulation decreases; hence, this may be the explanation for reduced hay production in the third year of the experiment.

While the application of bacteria had no effect, phosphorus application had a significant effect on the botanical composition. The proportion of grasses in the forage showed no response to P application, but the proportion of legumes increased with the increasing P availability. However, the increase at applications above 11 kg P ha^{-1} was not statistically significant. Hence, as reported by other researchers (Papanastasis and Papachristou 2000; Hatipoglu et al. 2001; Dasci, 2008) the better response of legumes to P fertilizer might be responsible for these increases in legumes proportion. The percentage of species belonging to other families within the forage showed a slight decrease in line with increased P doses. However, this decrease was not statistically significant at applications greater than 22 kg P ha^{-1}. As the ratio of legumes increased, the proportion of species of other families decreased in line with increased P dose. This result might be a result of the better response of legumes to P application than other plant families.

The ratio of grasses increased regularly during later years, while the ratio of legumes ratio decreased over time; the ratio of other plant families fluctuated between years. The causes of the decrease in legumes are unclear, because neither P nor bacteria application had any negative effect on its ratio in the botanical composition. Nevertheless, legumes disappeared at the experimental site and from the neighboring area (personal observation) after the second year of the experiment. This disappearance may be related to the nature of alsike clover, as this species is a short lived perennial (2-3 years) and is sensitive to shade and temperature (Davies 2001). Prevailing cool and humid climatic condition in the first 2 years of the experiment may favor cool season grasses, which show better response to cool, humid conditions than legumes (Davidson and Robson 1986). Alternatively, nitrogen released from nodules and decomposed root may also favor cool season grasses, which dominated the botanical composition of the experimental site in the latter years of the experiment (Table 1). As a consequence of these circumstances, grasses become dominant at the experimental sites over time.

CONCLUSION

The results of the present study indicate that phosphorus fertilizer is an important factor affecting hay yield in legume dominant natural meadows. No clear effect was observed related to the application of phosphorus solubilizing bacteria in terms of hay yield and botanical composition. The method used for application of bacteria involved spraying the test-plots at the start of the growing season. Bacteria was subsequently introduced in irrigation water via a sprinkler system. It could be concluded that the spraying method was ineffective and, therefore, different application methods would be necessary to permit definitive conclusions regarding the application of phosphorus solubilizing bacteria in the legume-dominated meadow. Under similar ecological conditions, an application of 22 kg P ha^{-1} is suggested in order to increase hay production in legume dominant meadow.

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