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EFFECTS OF PHOSPHORUS FERTILIZER AND PHOSPHORUS SOLUBILIZING BACTERIA APPLICATIONS ON CLOVER DOMINANT MEADOW: II. CHEMICAL 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 chemical composition of forage from a natural meadow area. Study plots were treated with two bacteria levels (B_0 and B_1) and five levels of phosphorus fertilizer (0, 11, 22, 33 and 44 kg ha^{-1}) with four replications. The results of the study indicate that increasing levels of P significantly increased to crude protein content. While phosphorus fertilizer decreased neutral detergent fiber (NDF) content, there were no notable effects on acid detergent fiber (ADF) and total digestible nutrient (TDN) content. The effects of bacteria application included an increase in crude protein content and total digestible nutrient content in forage was increased, and reduced ADF and NDF. Forage P and Ca contents were increased, but K and Mg contents decreased following phosphorus fertilization. While bacteria application increased K and Mg contents of forage, Ca content decreased. There was no notable change in P content of forage following the application of bacteria. The results indicate that P fertilizer and bacteria applications have positive effects on forage quality of meadows. Application of bacteria (alone or combined with P) or 11-22 kg ha^{-1} application of P may increase the forage quality of meadows.

Key words: ADF, crude protein, NDF, meadow, mineral content and TDN

INTRODUCTION

In terms of animal production, the quality of forage is more important than the quantity. Forage quality is determined by the content of different nutrients such as minerals, crude protein and fiber component. The concentration of chemical components in the forage varied depending on many variables such as plant species (Ramirez et al. 2004), harvesting date (Ball et al. 2001), fertilization (Türk et al. 2007), soil properties and other environmental variables (Kulik, 2009).

Nitrogen, an essential component of protein, is crucial for all heterotrophic organisms, and must be acquired by feeding. The critical level of crude protein content is approximately 7 % for ruminants (Meen 2001). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) is the main constituent of plant cell walls. ADF indicates the indigestible portion of the fiber in the forage while NDF indicates the total fiber in the forage (Ball et al. 2001.). There is a strong relationship between ADF content and the total digestible nutrient (TDN) content of forage. The TDN content of forage is calculated using ADF values with a simple equation (Schroeder 1994). The organic constituents, such as ADF, NDF, TDN and crude protein in plant tissue varies due to cultural practices such as fertilization, irrigation, cutting stage etc. (Ball et al. 2001; Türk et al. 2007).

Minerals are important in animal feed, because they have different roles in animal metabolism. Therefore, a balanced nutrient intake, including minerals, is required for optimal animal performance. Mineral deficiency may result in health disorders and reduced animal performance that can only be improved by dietary supplementation (Haenlein and Ramirez 2007). The effect of fertilization on the chemical content of forage was previously reported by several researchers (Bakoğlu et al. 1999; Ramirez et al. 2004; Harrington et al. 2006; Türk et al. 2007).

The objective of the present study was to determine the effect of phosphorus fertilizer and application of phosphorus solubilizing bacteria on the concentration of crude protein, ADF, NDF and mineral components in legume dominant natural meadows.

MATERIALS AND METHODS

This study was conducted on natural meadows of Ataturk University farm in Erzurum, Turkey, over a period of 4 years (2004 to 2007). The experimental area was located 39° 55' N and 41° 61' E at an altitude of 1800 m. Average temperature and total precipitation in the study area are 5.7 °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 °C, respectively. The total precipitation was higher then the long term average in three of the study years (2004, 2005 and 2007) while it was lower than long term average in the third year of experiment. Except during the third year, average temperature was lower than the long term average during the experimental period.

According to laboratory analysis results, the soil of the experimental area was loam, with 3.3% organic matter content, 3.9% lime content, 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 water table was approximately 30-40 cm below the soil surface at the start of the growing season and drops to 1m later are the season, especially toward mid-summer.

The experiment was established on natural meadow which was dominated by alsike clover (*Trifolium hybridum*) with examples of some cool season grasses such as *Alopecurus pratensis, Poa pratensis, Hordeum violaceum* and other forbs, such as *Ranunculus kotschyii, Cerastiun 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 for 4 years on the same ground. Two levels of bacteria (no application and 10^8 cfu ml^{-1}) and five levels of phosphorus (0, 11, 22, 33 and 44 kg P ha^{-1}) were applied, either alone or in combination. *Bacillus megaterium var phosphaticum* culture, isolated from 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 as triple superphosphate, and; bacteria culture were applied at the start of the growing season by sprayed on the plots in order to prevent from infections was changed overshoes every plot. The plots which were treated with bacteria immediately received approximately 10mm water via a sprinkler system. Each plot was 5 by 2 m with a 0.5m buffer inside each plot area and a 2m buffer outside.

When dominant plant species were at the flowering stage, representative forage samples were taken by clipping in each plot. The samples were oven dried at 70 ^oC for 48 hours for identification (Jones 1981), and then ground to pass through a 2 mm sieve. Total N content of the samples was determined by the Kjeldahl method and multiplied by 6.25 to give the crude protein content (Jones, 1981). ADF and NDF content were measured using an ANKOM fiber analyzer (ANKOM Technology, Fairport, NY) following the procedure described by Van Soest et al. (1991). TDN of forage was calculated using an equation proposed by Schroeder (1994), which is TDN% = 96.35-(ADF% × 1.15). The minerals, P, K, Ca and Mg, were determined after wet digestion using a nitric (HNO_3) – perchloric ($HClO_4$) acid mixture (4:1 v/v) (AOAC 1990). Phosphorus in the extraction solution was measured spectrophotometrically using the indophenol-blue and ascorbic acid method (Termoelectron Specroscopy LTD, Cambridge, UK). Potassium, Ca and Mg were measured by atomic absorption spectrometry (PerkinElmer 3690) (AOAC 1990).

All data were subjected to analysis of variance (ANOVA) based on general linear models for repeated measurements for factorial arrangement of treatments. Statistical analysis used the SPSS statistical package (SPSS 1999). Means were separated using Duncan's Multiple Range Test.

RESULTS

Both phosphorus fertilizer and phosphorus solubilizing bacteria application significantly affected the crude protein content of forage (P<0.05). Crude protein content showed an increasing trend in line with increasing P doses (Table 1) and the forage harvested from plots treated with bacteria had higher protein content than untreated plots. Crude protein content of forage decreased regularly as the years advanced. In the second year of the experiment, the forage harvested from plots to which bacteria was applied showed higher crude protein content (P<0.05), but there were no differences in the other years. The differences between years in terms of crude protein content depend on bacteria application caused year x bacteria interaction was significant at a level of P<0.05 (Figure 1A).

Table 1. The Effects of P fertilization and bacteria treatments on crude protein, ADF, NDF and TDN content of meadow forage[1].

	Crude Protein (%)	ADF Content (%)	NDF Content (%)	TDN (%)
P_0	9.57 c	41.4	57.0 A	48.7
P_1	9.92 ab	41.3	54.7 BC	48.8
P_2	9.78 b	40.8	53.6 C	49.5
P_3	9.91 ab	41.4	56.0 AB	48.7
P_4	10.43 a	41.3	56.5 AB	48.9
Average	**9.92**	**41.2**	**55.6**	**49.0**
B_0	9.73 b	41.7 A	56.7 A	48.4 B
B_1	10.11 a	40.0 B	54.4 B	49.6 A
Average	**9.92**	**41.2**	**55.6**	**49.0**
2004	13.48 A	38.5 D	52.4 C	52.1 A
2005	11.62 B	42.2 B	52.3 C	47.9 C
2006	7.41 C	40.3 C	57.4 B	50.1 B
2007	7.18 D	44.1 A	60.2 A	45.7 D
Average	**9.92**	**41.2**	**55.6**	**49.0**
Y x B	*	ns	ns	ns
Y x P	ns	ns	**	ns
B x P	ns	ns	**	ns
Y x B x P	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.

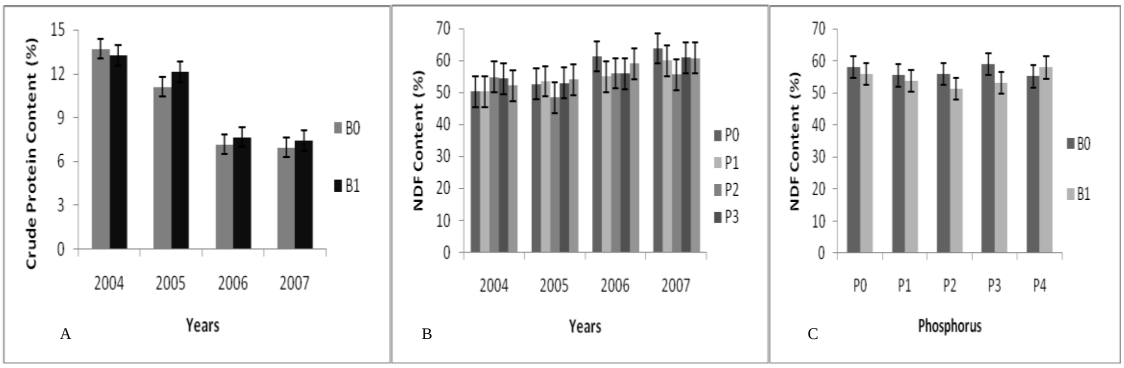

Figure 1. (A) The effect of bacteria application on crude protein content in relation to years. (B) The effect of phosphorus application on NDF content in relation to years. (C) The effect of bacteria and phosphorus application on NDF content.

Phosphorus fertilizer did not significantly affect the ADF content of forage. However, bacteria application caused significant decreases in the ADF content of forage (Table 1). In general, the ADF content of forage increased in the later years of the study, but decreased significantly in the third year of the experiment. There was no dual interaction recorded with regard to the ADF content of forage, but (Year x Bacteria x Phosphorus) interaction was significant at a level of $P<0.01$.

The NDF content of forage was highest at zero P levels and decreased up to application levels of 22 kg P ha^{-1}; thereafter, it showed an increasing trend in line with increasing P levels. Bacteria application caused a significant reduction in the NDF content of forage. The NDF content of forage was lowest during the two years of the experiment but showed a significantly increasing trend in the second two years of the experiment. The NDF content of forage showed different responses to P application during the study years. For example, the lowest NDF value was recorded at zero P levels for the first year of the experiment, and the highest NDF value was recorded at the same P levels in the fourth year of the experiment (Figure 1B). Thus, (Year x Phosphorus) interaction was significant with regard to the NDF content of forage. The NDF content of forage was always higher at the lowest P levels without bacteria application than the first three P applications, but this relationship reversed at the highest P levels and NDF content was higher at the highest P levels, combined with bacteria application. This relationship indicated a significant (Phosphorus x Bacteria) interaction (Figure 1C).

No relationship was found between TDN content of forage and P application, but application of bacteria significantly increased the TDN content of forage (Table 1). Although the TDN content of forage showed a statistically significant increase in the third year of the experiment, in general, it decreased over time. The dual interactions did not significant but the (Year x bacteria x Phosphorus) interaction was significant with regard to the TDN content of forage.

The P content of forage increased in line with increased P doses, but phosphorus solubilizing bacteria application had no effect on the P content of forage (Table 2). P content increased regularly during the first three years of the experiment, but decreased in the fourth year. The P content of forage showed different responses to P doses between the years, thus, the (Year x Phosphorus) interaction was significant (Figure 2A). In the first year, the forage obtained from bacteria-applied plots had higher P content than that of non-applied plots. However, the P content of forage harvested from bacteria applied plots was lower than that of without bacteria applied plots in the third year of the experiment. In the other years, there was no significant difference in P content of forage with regard to bacteria application. Thus, the (Year x Bacteria) interaction was significant in relation to P content (Figure 2A, B). The P content of forage showed different responses to bacteria and phosphorus application combinations by year. This result indicated a significant (Bacteria x Phosphorus) interaction (Figure 2C). It was also found that (Year x Bacteria x Phosphorus) interactions were significant.

Table 2. The Effects of P fertilization and Bacteria treatments on mineral content of meadow forage[1].

	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})
P_0	1859 C	7380 C	12964 E	3481 C
P_1	2178 B	11577 A	15855 A	4281 A
P_2	2186 B	9828 B	14393 C	3433 C
P_3	2316 A	7354 C	14026 D	3810 B
P_4	2248 AB	6283 D	14946 B	3266 D
Average	**2161**	**8484**	**14437**	**3654**
B_0	2177	7107 B	14771 A	3113 B
B_1	2145	9862 A	14102 B	4195 A
Average	**2161**	**8484**	**14437**	**3654**
2004	2189 B	6992 C	14297 C	4479 A
2005	2252 B	9942 B	16191 A	4187 B
2006	2336 A	10652 A	14402 B	3475 C
2007	1867 D	6351 D	12857 D	2475 D
Average	**2161**	**8484**	**14437**	**3654**
Y x B	**	**	**	**
Y x P	**	**	**	**
B x P	**	**	**	**
Y x B x P	**	**	**	**

[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 $p < 0.01$.

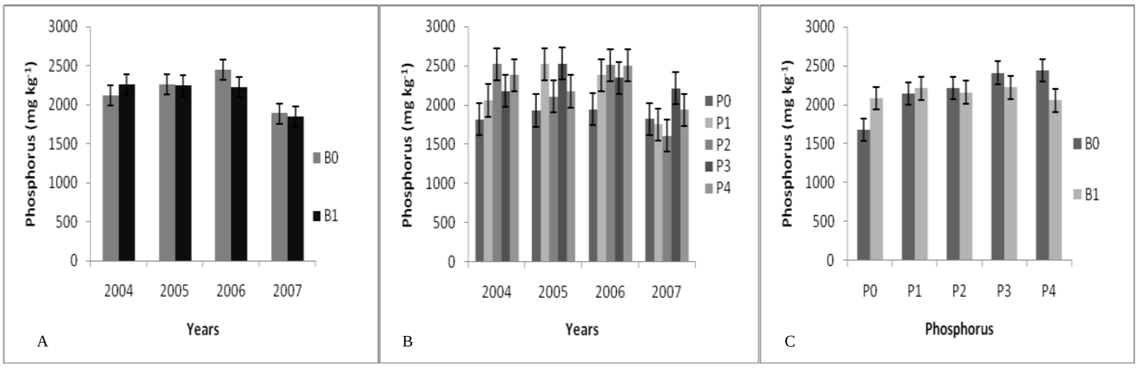

Figure 2. (A) The effect of bacteria application on phosphorus content in relation to years. (B) The effect of phosphorus application on phosphorus content of forage in relation to years. (C) The effect of bacteria and phosphorus application on phosphorus content of forage.

The K content of forage increased in response to increased P doses from 0 to 11 kg P ha^{-1}, but decreased regularly thereafter in line with increased P doses (Table 2).

Bacteria application caused a significant increase in the K content of forage. During the first three years of the experiment, the K content of forage increased regularly each year, but decreased sharply and dropped to the lowest value in the last year of the experiment. The K content of forage showed significant fluctuation according to experimental factors, thus, all interactions related to the K content of forage were significant (Figure 3A, B, C).

The Ca content of forage increased when application of P was increased from 0 to 11 kg ha^{-1}, but decreased thereafter up to an application level of 33 kg P ha^{-1} and increased again at the dose of 44 kg P ha^{-1} (Table 2). Bacteria application caused a significant decrease in the Ca content of forage. The Ca content of forage increased significantly in the second year of the experiment compared to the first year value, thereafter, it decreased regularly each year. There was no significant difference in the Ca content of forage in relation to bacteria application in the second year of the experiment. However, in the other years, Ca content was always higher in the forage harvested from plot without bacteria application. Phosphorus doses caused large fluctuations in the Ca content of forage between years. The Ca content of forage obtained from plots to which bacteria was applied was lower at lower applications of P, but this relationship was reversed at high P level (P_3). Thus, all interactions related to experimental factors for the Ca content of forage were significant (Figure 4A, B, C).

The Mg content of forage increased significantly when P dose increased from 0 to 11 kg ha^{-1} but showed a declining trend in line with increasing P doses after 11 kg ha^{-1} (Table2). Bacteria application caused a large increase in the Mg content of forage. The Mg content of forage decreased over time and the values were significantly different for each year. The forage harvested from bacteria applied plots always had a higher Mg content in each year of the study, but the differences between the values of bacteria applied and non-applied plots showed great variation depending on the year.

As a result of this variation, the (Year x Bacteria) interaction was significant (Figure 5A). The Mg content showed great variation between years with regard to the level of P application. Thus, (Year x Phosphorus) interaction was a significant factor in the Mg content of forage (Figure 5B). Although the forage harvested from plots where bacteria and phosphorus fertilizer were applied together always had high Mg content, the response to P doses showed large fluctuations. Consequently, Bacteria x Phosphorus interaction were significant for Mg content (Figure 5C).

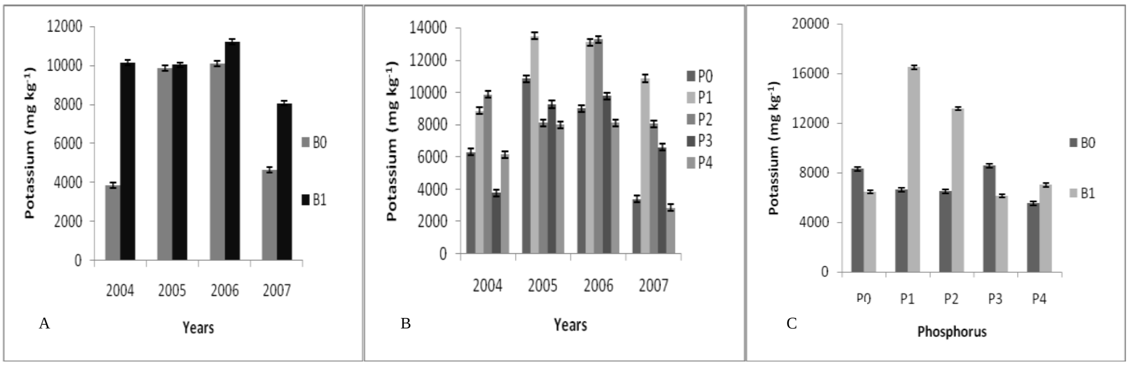

Figure 3. (A) The effect of bacteria application on potassium content in relation to years. (B) The effect of phosphorus application on potassium content in relation to years. (C) The effect of bacteria and phosphorus application on potassium content of forage.

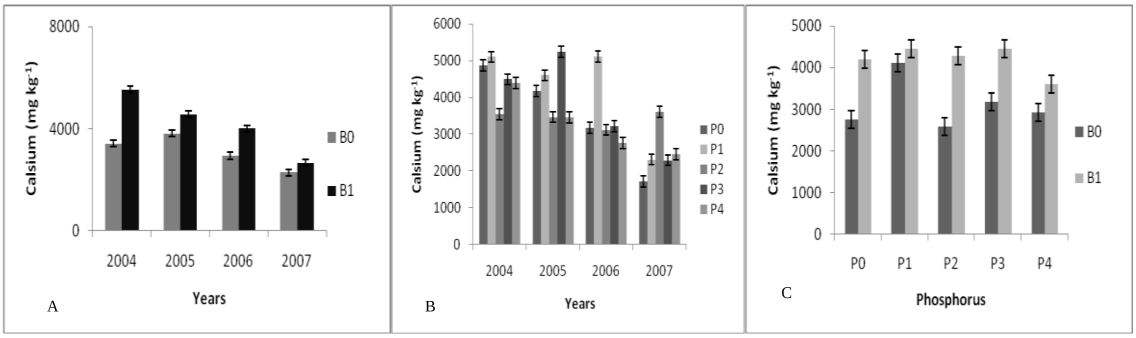

Figure 4. (A) The effect of bacteria application on calcium content in relation to years. (B) The effect of phosphorus application on calcium content in relation to years. (C) The effect of bacteria and phosphorus application on calcium content of forage

DISCUSSION

The most effective and practical method to increase forage production in natural meadow is adequate fertilization. Fertilization enhances not only dry matter production, but also affects the chemical content of the forage (Bell et al. 2001; Türk et al. 2007). In this study, phosphorus fertilizer and phosphorus solubilizing bacteria application significantly enhanced crude protein content of the forage (P < 0.05) (Table 1). P fertilizer enhances nitrogen uptake by plant (Benedycka et al. 1992), and crude protein content may increase in forage harvested from plots where P is applied because nitrogen is the main component of the protein. The reason for the increase of crude protein content in response to the application of phosphorus solubilizing bacteria may be related to nitrogen fixing capability in addition to the phosphorus solubilizing properties of the bacteria (Elkoca et al. 2008) used this experiment. The decreases in crude protein content over time most probably stemmed from a reduction in the proportion of legumes present at the experimental plots over time It is well known that legumes have a higher crude protein content compared to grasses (Gokkus et al. 1999; Erkovan et al. 2008). Although legumes were dominant at the site at the beginning of the study, their proportion of the botanical composition quickly declined over time and there were no legumes identified after second year at the site (detailed in the first part of this article). The harvested hay contained adequate crude protein content for ruminant fodder, irrespective of whether or not fertilizer was used, in the case of harvested true time because crude protein content needs to be around 7% to satisfy the needs of rumen microbes (Meen 2001).

Phosphorus fertilizer had no significant effect on the ADF and TDN content of forage, but led to a reduction in the NDF content of forage (P<0.01). Bacteria application decreased ADF and NDF content but increased TDN content. The results of previous studies on the effect of fertilization on ADF, NDF and TDN content are not consistent. While some studies reported a positive effect (Messman et al. 1991; Ball et al. 2001; Rayburn 2004; Daşcı 2008), others reported negative or no effect (Pieper et al. 1974; Daşcı 2008;). Despite the statistical significance of the bacteria application and P fertilization effects, the extent of the changes observed is not enough to alter the forage quality. It is suggested that the changes in ADF, NDF and TDN content over time stemmed from the variation in botanical composition observed between study years; stemmed from the variation in botanical composition observed between study years, such as the increase in the proportion of grass and the decline of legume species observed over time (detailed in the first part of this article). It is well known that grasses have a higher ADF and NDF but a lower TDN content than that of legumes (Ball et al. 2001; Rayburn 2004).

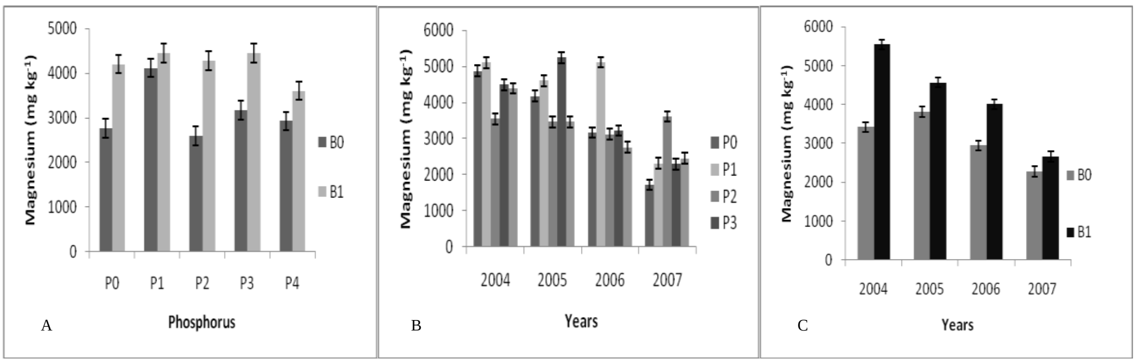

Figure 5. (A) The effect of bacteria application on magnesium content in relation to years. (B) The effect of phosphorus application on magnesium content in relation to years. (C) The effect of bacteria and phosphorus application on magnesium content of forage.

The phosphorus content of forage increased as P doses increased (Table 2). This increase may derive from the increasing availability of P in the soil as a consequence of applying P fertilizer, because nutrient sorption by plants increases when the soil nutrients are abundant (Kulik 2009). The phosphorus content of the forage decreased over time (Table 2), which may be related to changes in botanical composition because the legume proportion in the botanical composition decreased over time. Legumes have a higher P content than grasses (Ramirez-Orduna et al. 2005). The phosphorus content of forage exceeded the critical level for maintenance of ruminants (2100 mg kg^{-1}) upon the application of P at the experimental site (NRC 2000).

The other minerals which were observed in this study showed significant fluctuations in response to the various treatments (Table 2). The potassium, Ca and Mg contents of forage were generally higher at low P doses. This may be due to nutrient availability affected by P fertilization, bacteria application and also changes in the root environment. The Ca and Mg content of forage exceed the critical level for maintenance of ruminants (2100 mg kg^{-1}) upon the application of P when compared to non-application of P at the experimental site, but K content remained below the necessary value at all treatments (NRC 2000).

CONCLUSION

The results indicate that phosphorus fertilizer is important for improving hay quality in addition to increasing hay yield in legume dominant natural meadows. Although there was no clear effect of phosphorus solubilizing bacteria on hay yield and botanical composition (Part I of this paper), hay quality was positively affected by the application of bacteria. Hence, phosphorus solubilizing bacteria may be applied alone or in combination with P fertilizer to improve forage quality. Under similar ecological conditions, the application of 11 or 22 kg P ha^{-1} and P solubilizing bacteria should be suggested in order to improve hay quality in addition to increasing hay production in legume dominant meadow.

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