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Response of Flora and Herbage to Variation of Chemical Elements in Topsoil of Protected European Union Lands of Mediterranean Environments

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ABSTRACT

Pasturelands with bioclimatic mesomediterranean zones in southern Italy amount to 2.5 million ha which represent 44.6% of the national total; 25.8% of pasturelands are covered by National Parks. A further surface area of about 6.02 million ha, named Sites with Community Importance and Zones with Special Protection, has been considered by the European Union as protected areas. The flora and fauna biodiversity of these areas is protected by law. A high request of biomass for animal feeding stressed the sward of pasturelands of these areas. Chemical element-enrichment of topsoil in the protected lands was found to be a feasible method to increase herbage production without changing the ecosystem of native flora. The studies were established on pasturelands of two National Parks. The pastures' surfaces were enriched with nitrogen (N), phosphorous (P) and combined N-P with the aim of assessing the effect of application on biomass yield, herbage quality and flora composition. The environments studied differed in their biomass, qualitative characteristics of the herbage and properties of the topsoil. The effect of chemical compounds on soil and qualitative properties of biomass showed a similar trend of variation between environments. N, P and combined N-P compounds increased biomass production 41%, 56% and 57%, respectively more than the control. At the end of the experiments, combined N-P enrichment treatment lower the content of N_{tot} and P_2O_5 in topsoil and the variation of flora composition of natural pasture control. Combined N-P increased the milk feed unit (MFU) per hectare potential by 2924 compared to 1956 for N and 2769 for P, minimized the variation among flora composition, reduced the content of organic carbon and favoured the content of N and P_2O_5 in topsoil in comparison to the natural pasture control.

Keywords: biomass yield, chemical element variation, flora composition, MFU ha^{-1}, protected pasturelands, topsoil

Abbreviations: DM, Dry matter yield; **E I**, environment I; **E II**, environment II; **EU**, European Union; **ISTAT**, Istituto Nazionale di Statistica Italiano; **MFU**, milk forage unit; **N**, nitrogen element; **N-P**, combined nitrogen-phosphorous elements; **NP**, National Park; $\mathbf{N_{tot}}$, total nitrogen; **OC**, organic carbon; **P**, phosphorous (element); $\mathbf{P_2O_5}$, phosphoric anhydrite; **SCI**, Sites with Community Importance; **ZSP**, Zones with Special Protection

INTRODUCTION

Herbage production of natural pasture was a main agronomic peculiarity for sustaining the economics of animal breeding activity in protected pasturelands of the European Union (EU) of Mediterranean environments. The survey of natural pasturelands in 9 typical Regions of southern Italy, with bioclimatic UNESCO-FAO (1963) mesomediterranean weather condition, amounted to about 2.5 millions ha which represents 44.6% of the national surface (Martiniello and Berardo 2005). 28.5% of these pasturelands was covered by National Parks (NPs) and a further additional surface, defined by the EU as Sites with Community Importance (SCI) by about 4.17 million and Zones with Special Protection (ZSP) and 1.85 million ha are considered protect lands (Boitani *et al*. 2002). The flora and fauna biodiversity existing in all these areas are safeguarded by law (SIC and ZPS by European Directive 92/43CEE 1992; and NP by Italian Legislative Decree 394 1991). Herbage of these areas are used for grazing cows, sheep, goats, buffaloes, horses, donkeys and wild pigs which represent 63% of the national total livestock (ISTAT 1999) and the percentage of these animals was 26, 83, 85, 98, 46, 83 and 1% of the national total, respectively (ISTAT 1998). In the following years the amount of national animals was 58% in 2004 (ISTAT 2004) and 57% in 2006 (ISTAT 2006) and the percentage of cows, buffaloes, sheep horses and donkeys was 3.5, 36, 4.8, 6.3 and 21.9% lower than those of 1998, respectively.

Particularly, in the last decade, the native flora biodiversity of these environments, for neglected utilization of the pastureland swards, was strongly changed. The agro-pastoral farmers, for increasing the biomass feeding demand of the animals, stressed the pasturelands with constrain practices of management causing variation of flora composition and herbage development of the sward (Abdelkefi and Marrakchi 2000; Bounejmate *et al*. 2004; Martiniello and Berardo 2005).

The feeding values (milk feed unit, MFU) in the rangeland of Mediterranean pasture ranged from 1400-1600 MFU ha^{-1} (Crespo 1985; Abdelguerfi and Abdelguerfi-Laouar 2004; Martiniello and Berardo 2005). The recovery of natural herbage production and the ecological function of the protected pasturelands was linked to development of agropastoral management techniques favouring environment sustainability of pasturelands and landscape characteristics of the environments.

In order to increase the productivity of natural pasturelands approaches based on reseeding, irrigation and enrichment of topsoil applications by chemical elements have been proposed to restore natural biodiversity (Decker and Taylor 1985; Follett and Wilkinson 1985; Nicols and Clanton 1985; Darrell *et al*. 1995; Hill *et al*. 2005). Some of

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them, like irrigation, are not realistic while reseeding with allochthonous seed source ecotypes the sward of pasturelands, because the introduction of foreign germplasm promotes a genetic contamination of phytocoenoses, and this practice is forbidden by EU laws (Italian legislative Decree 384, 1991; European Directive 92/43CEE, 1992).

However, restoring soil fertility by enhancing chemical elements in topsoil favours germination of the indigenous seed born present in the sward, with a consequent development of native flora and productivity of the sward, in many part of the world mainly Syria, Australia, Canada and Italy (Osman *et al*. 1994; Johnston *et al*. 1998; McKenzie *et al*. 1998; Perez *et al*. 1998; Hill *et al*. 2005; Iannucci *et al*. 2005). The enrichment of soil by chemical elements has been a scientific approach widely used around the word (Australia, Italy and US) for increasing sward productivity and nutritive herbage characteristics of native pastures (Norman 1962; Tupper 1978; Oman *et al*. 1994; McKenzie *et al*. 1998; Bounejmate *et al*. 2004). However, the use of soil enrichment by chemical compounds for a limited period has been found to be an approach able to increase qualitative and quantitative biomass production and germination of the indigenous seed born present in the sward favouring development of native flora present in the natural pasture (Perez *et al* 1988; Nie *et al*. 1999; Iannucci *et al*. 2005; Martiniello and Berardo 2007).

The aims of these experiments were to determine the effect of enrichment of topsoil by chemical elements on flora composition, biomass production and chemical composition of the herbage in two National Parks of mesomediterranean natural pasturelands.

MATERIALS AND METHODS

The experiments were established in 2001-2005, in two pasturelands located in the geographical area 15°51′E – 41°50′N, environment I (E I) and 15°35′E – 41°40′N, environment II (E II).

Meteorological characteristics

The mean monthly temperatures and rainfall that occurred in the years of the experiments, in the pasturelands of the two environments, are shown in **Fig. 1**. The volume of rainfall that fell in the period of October to May was more than 74% of the total annual rainfall. In both environments, the amount of rainfall was inversely related to the mean monthly temperature. The period with high temperature and low rainfall was from the end of April to the beginning of October. The pattern of yearly rainfall and temperature that were observed in the period of evaluation were typical to those reported in the UNESCO-FAO (1963)'s mesomediterranean bioclimatic zone and de Martonne (1926)'s aridity index and whose values, which were estimated over the 5 years of the experiments, in the months from May to September, were 4.1 for E I and 6.7 for E II.

Establishment of experiments

The pasture, before and during the experimental evaluation, was grazed for the entire year by sheep in E I and by cows in E II. The amount of nitrogen (N) and phosphorous (P) chemical element needed to increase their contents in the topsoil of natural pasture were established according to information provided by Basso *et al*. (1992) and Osman *et al*. (1994). The amount of chemical element applied was 6.0 g m^{-2} for nitrogen (treatment N), 8.0 g m^{-2} for phosphorous (treatment P) and 3.2 and 7.2 g m^{-2} for combined nitrogen and phosphorous (treatment N-P), respectively. The chemical elements were applied in the period when humidity of soil and weather condition was favourable for the beginning of sward development posterior half of January in 2001 through 2005. The experimental treatments of the study were three different chemical elements and a control without chemical applications. Within experimental entries and control treatments, in November 2000, appropriate areas of pasturelands were protected by manufactured parallel-piped cage (150 cm long, 150 cm wide and 100 cm in height). The lateral sizes and the top of cage, to prevent access to animals, were finished by poultry and rabbit Bekaert welded wire-still fence (**Photo 1**). Sixteen cages were set up on each pastureland.

Four cages for each experimental treatment were allocated at random on the surface of pastureland and represented the replications of the application. The experimental entries of the study were four: three of them for chemical elements and one for control without chemical application.

Soil determinations

The soil characteristics, before the beginning and at end of experi-

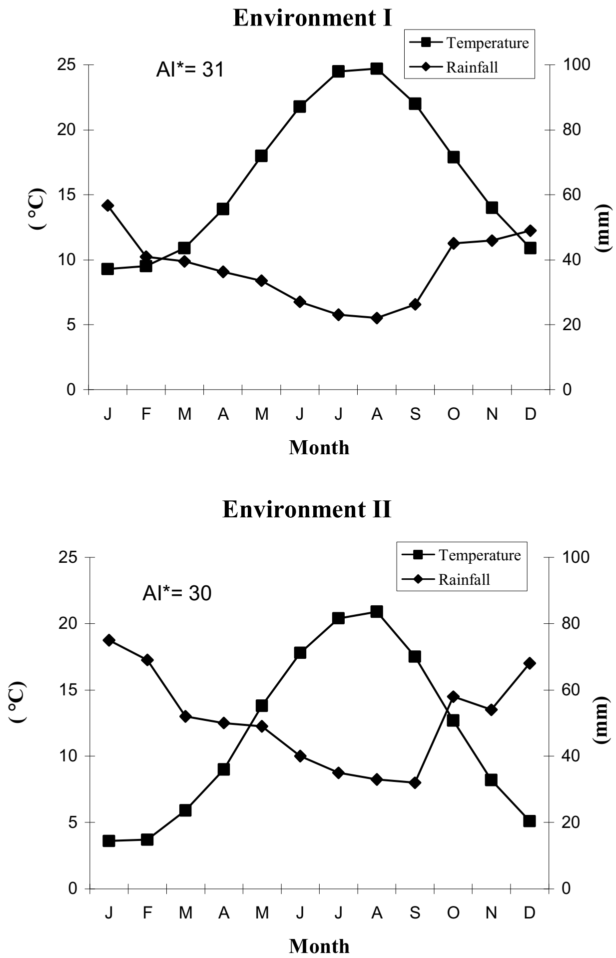


Fig. 1 Mean monthly temperature and rainfall of environments over the period of the study (2001-2005). AI*= Mean of de Martonne's aridity index over the 5 years of experimental period.

Photo 1 Experimental cage. The inside undisturbed biomass was used for determine qualitative and quantitative characteristics and flora composition of herbage.

mental treatments, were assessed on four soil samples taken from 0-30 cm Ap horizon. The four samples were mechanically harvested by a spiral metal core of 60-mm diameter. The samples were air-dried and, after root separation, were mixed, sieved thought a mesh screen with 2 mm Ø, and later used for determination of pH (potentiometrically with a glass electrote using 1:2.5 soil:water ratio), total nitrogen (micro-Kjeldahl), organic matter (Walkey and Black 1934) and available phosphorous (Olsen *et al*. 1954).

Biomass and phytocoenose characteristics

The characteristics of herbage from pasture treatments were determined on the undisturbed biomass developed under cages.

In each year of evaluation, the effect of chemical enrichment treatment was determined on the following traits: biomass production, moisture at harvest, floristic composition and nutritive value of the herbage. Before harvesting biomass, plant height was measured (cm, mean of six random values taken from ground level to apex stem). Harvests generally took place in the last week of May, when the grass herbage was at the flowering phenological stage. In each cage of the experimental treatment, two random contiguous herbage sub-samples of 1 m^2 were manually mowed to the ground level. The neighbouring harvests reduced the variability between samples used for determining quantitative and qualitative characteristics and flora composition of the sward. At the end of harvesting the remnant above-ground biomass was harvested and taken out from the cage.

One sub-sample of herbage was used for assessing dry matter yield (DM), moisture at harvest and flour sample for laboratory analyses. At harvest the biomass of 1 m^2 was weighed and a random sample of about 500 g of fresh biomass was oven-dried (60°C for 72 h) for determination of DM content and then ground in a Cyclotec mill with a mesh screen of 1 mm. Crude protein (CP), crude fibre (CF), neutral-detergent fibre (NDF), acid-detergent fibre (NDF) and acid-detergent lignin (ADL) concentration were chemically determined. The samples were analyzed using a standard procedure for determining CP concentration, according to the Dumas method as modified by Kirsten (1963) while NDF, ADF and ADL concentration were determined as described by Goering and van Soest (1970). The nutritive values of the herbage (MFU), was calculated from the CP and CF concentrations according to the method used by Andrieu and Weiss (1981).

The second sub-sample was used to describe the plant association found in the pasture. The flora species present in the biomass of harvested sample was botanical sieved by the author according to Fiori (1969) and Pignatti (1982) botanical guides. Subsequently, the flora species of herbage were grouped into Gramineae, Leguminosae and Compositae botanical family. Herbarium samples were not created ad deposited. The flora species with less than 3% of the total DM were included in the miscellaneous group belong to the family of Boraginaceae, Ranunculaceae, Scrophulariaceae and Umbelliferae. The botanical family sieved samples were weighed, dried and their DM contribution to herbage determined.

Statistical analysis

Observations of the traits were statistically analysed according to a model with split plots of years, environments and chemical element treatments, where pastures and application treatments were nested within the years. The model considers pasture environments, replicates and chemical elements treatments as fixed effects and year as a random effect (Steel and Torrie 1980). The effect of years, environments and chemical elements treatments and their interactions during the years of evaluation were tested with appropriate error terms (Steel and Torrie 1980). The comparisons among means were performed according to the LSD statistical test at the P=0.05 level of probability.

RESULTS

The natural pasture of E I was 20% more productive in biomass than that of E II (**Table 1**). The biomass production across the environments in the year of evaluation, as an effect of weather conditions during vegetative growth, ranged from 458 to 1342 g m^{-2} in E I and from 370 to 976 g m^{-2} in E II (**Table 1**). The flora of the Gramineae prevailed (over than 50%) over other botanical families in E I while in E II the grass species were less represented (**Table 2**). By contrast, in natural pasture of E II, the Leguminosae, Compositae and miscellaneous group was 40%, 46% and 19%, respectively lower than those of E I (**Table 2**).

The application of chemical elements increased the average of natural pasture biomass production in E I by 50% and in E II by 55% (**Table 1**). Among chemical elements, N application increased the biomass of the control treatment by 35% and 47%, respectively in E I and E II while both P and combined N-P treatments, increased biomass production (average of environments) by 55% in E I and 58% in E II (**Table 1**).

The moisture at harvest of biomass of E I in the control treatment was 6.1% higher than that of E II. The range of moisture content existing in E I (72.8% to 70.3%) and E II (68.4% to 63.3%) was a consequence of the flora composition and development of the biomass in the experiments of the environments (**Fig. 2**). As an effect of weather condition, plant height of the control treatment, across the year of evaluation in both environments, of the first year was higher (54 cm in E I and 35 cm in E II) than the mean of other years (**Fig. 3**). The effect of fertilizer treatments in the years of evaluation influenced plant height differently between environments (control was 15% and 31% lower than mean of fertilizer treatments, respectively in E I and E II) (**Fig. 3**). Plant height, as an effect of the interaction fertilizer applications × year of evaluation, ranged from 45 to 123 cm in E I and from 47 to 98 cm, in E II (**Fig. 3**).

The chemical enrichment influenced the natural pasture phytocoenoses. N application increased development of

Table 1 Yearly mean of herbage at harvest of the experimental treatments in the two environments evaluated.

Experimental treatment	Fresh biomass					Mean	LSD 0.05
	Year of evaluation						
	I[a]	II[a]	III[a]	IV[a]	V[a]		
	Environment I						
Control	1342	839	1046	458	1073	952	288
Fertilizer:							
Nitrogen (N)	1711	1290	1668	859	1811	1468	345
Phosphorous (P)	2831	1829	2206	1393	2065	2065	463
Combined (N-P)	2286	1900	2802	1429	2470	2177	465
	Environment II						
Control	976	657	559	370	1254	763	308
Fertilizer:							
Nitrogen (N)	1956	1245	1520	737	1747	1441	416
Phosphorous (P)	2594	1572	1739	891	2300	1819	582
Combined (N-P)	1813	1581	2515	1156	2103	1834	451
Mean	1832	1264	1583	789	1851	1464	47
LSD 0.05	163	106	197	80	112	123	

[a] g m^{-2}

Table 2 Botanical family dry matter percentage. Mean of flora species present in the herbage of the experimental treatments in the environments evaluated.

Experimental treatment	Herbage flora composition			
	Graminea[a]	Leguminousae[a]	Compositae[a]	Miscellaneous[a]
	Environment I			
Control	49	9	12	29
Fertilizer:				
Nitrogen (N)	62	3	13	22
Phosphorous (P)	34	32	13	20
Combined (N-P)	51	19	10	19
Mean	49	16	12	23
LSD 0.05				
	Environment II			
Control	34	15	22	30
Fertilizer:				
Nitrogen (N)	63	6	16	16
Phosphorous (P)	36	23	15	27
Combined (N-P)	51	30	5	15
Mean	46	18	14	22
LSD 0.05	5	5	4	5

[a] % over total dry matter of the botanical family present in the biomass.

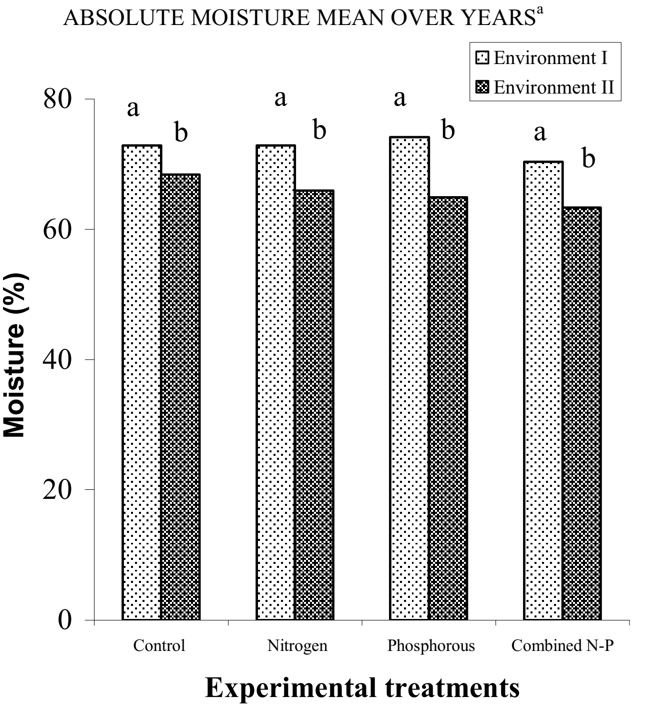


Fig. 2 Mean moisture over the years of biomass at harvest. [a] Treatment means of environments with the same latter are not significantly different at P<0.05 Duncan's Multiple Range Test.

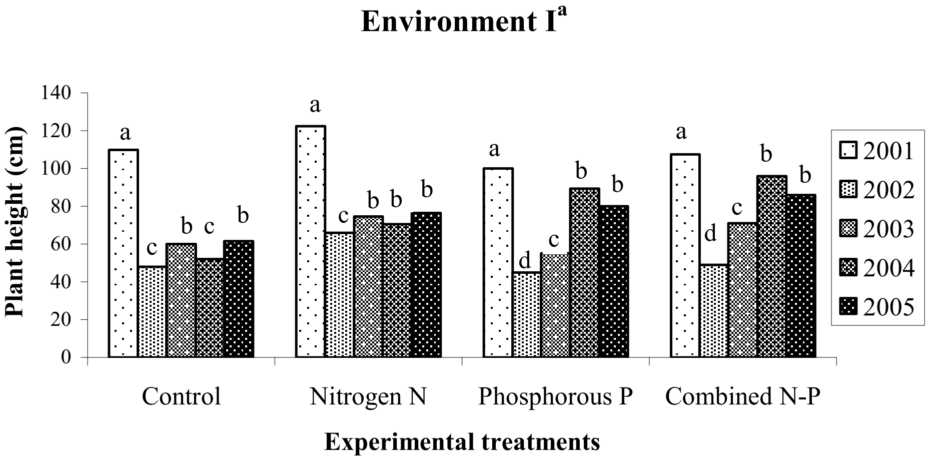
Gramineae (21% in E I and 46% in E II) and reduced the Leguminosae (67% and 39%, respectively in E I and E II) of the natural pasture control treatment (**Table 2**). In contrast, under P application the amount of Gramineae was, in E I, 5.6% lower and Leguminosae 28.1% higher than E II. The effect of both P and combined N-P applications, on flora present in the control treatment, decreased the content of species belonging to the miscellaneous group in E I (29 and 34%, for P and combined N-P respectively) and Compositae E II (31% under P and 50% under N-P) (**Table 2**).

The effect of chemical application on the herbage composition depended on the type of element (**Table 3**). N, in both environments, reduced the content of CP and increased that of CF, NDF and ADF of the control treatment (**Table 3**). An opposite effect was observed under P and combined N-P applications. The content of CP in the biomass increased (8.3% in E I and 16.1% in E II) and that of CF decreased under both P and combined N-P applications (6.0% and 3.7%, respectively in E I and E II) (**Table 3**). Variation observed among fibre components (NDF, ADF and ADL) in the two environments was ascribed to the interaction among chemical element applications and the weather condition that occurred in the year of evaluation on flora composition of the sward (**Table 3**). Thus, the effect of P application in E I and E II (measured as difference between the percentage of the qualitative value determined on dry matter of P and natural pasture control treatments) increased the CP content of the control treatment (2.6 and 1.5 percentage point, respectively) and reduced the amount of CF (1.2 under E I and 1.5 under E II percentage point) (**Table 3**). Variation of NDF, ADF and ADL reflect the effect of phosphorous application on floral composition of pastures. Particularly, the lower value of fibre components (CF, NDF, ADF and ADL) observed under P and combined N-P treatments of E II was a consequence of reduced plant height of biomass caused by the elements (plant height in E I was 7.4% and 12.5% under P and combined N-P higher than E II, respectively) (**Table 3** and **Fig. 3**).

The effect of chemical treatment on MFU was related to the influence of weather condition of the environments (MFU of E I was 2.3% higher than E II) (**Table 4**). N application in E I, favouring the increase of plant height (5% higher than mean of P and N-P treatments) and botanical family biomass of Gramineae (31.5% higher than mean P and N-P treatments), reduced the content of MFU of the herbage of natural pasture control by 3.8 and 1.9% in E I and E II, respectively (**Table 2** and **Fig. 3**). By contrast, the effect of P application increased the content of MFU of the natural pasture (1.8% and 3.6% in E I and E II, respectively) and those of N application (5.4% in both environments) (**Table 4**). Furthermore, because combined N-P application in both environments increased the percentage of Leguminosae and Gramineae in E I and E II over the natural pasture control, with a different and opposite trend (Gramineae was higher 3.9% in E I and 33% in E II than the control while the increase of Leguminosae was about 50% in both environments) (**Table 2**), the content of MFU was 3.6% lower in E I and 3.4% higher in E II than the control. Furthermore, the higher percentage of Leguminosae in biomass in E II rather than E I, under N-P treatment increased by 5.4% the content of MFU of E II over E I (**Table 4**).

The MFU ha^{-1} herbage of natural pasture of E I was 18.6% higher than E II (**Table 4**). The effect of chemical treatment increased the MFU ha^{-1} of the natural pasture by 27, 48 and 53.9%, respectively under N, P and combined N-P in E I and 48.5% for N, 63.9% for P and 63.1% for N-P in E II (**Table 4**).

The organic carbon (OC) and phosphoric anhydrite (P_2O_5) contents in topsoil of natural pasture in E I was



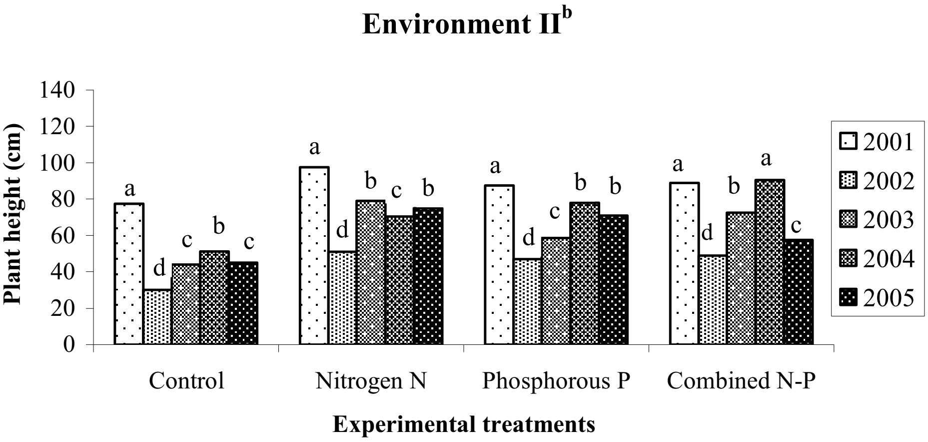


Fig. 3 Yearly mean of plant height (cm) of herbage across the experimental treatment entries in the two environments. [a] means in environment I and [b] means in environment II, with the same letter in the treatment, are not significantly different at P<0.05 according to Duncan's Multiple Range Test.

Table 3 Mean over the years of herbage qualitative characteristics in the fertilizer treatments in the environments evaluated.

Experimental treatment	Qualitative characteristics of herbage				
	CP[a]	CF[a]	NDF[a]	ADF[a]	ADL[a]
	Environment I				
Control	11.4	37.4	60.2	40.1	8.2
Fertilizer:					
Nitrogen (N)	10.9	38.2	66.1	43.8	6.1
Phosphorous (P)	14.0	36.7	62.1	39.2	8.9
Combined (N-P)	13.9	33.6	62.4	41.0	8.1
Mean	12.6	36.5	62.7	41.0	7.8
LSD 0.05	0.5	0.7	2.4	0.9	0.2
	Environment II				
Control	10.7	33.6	60.2	35.9	10.2
Fertilizer:					
Nitrogen (N)	8.9	37.6	62.7	38.7	6.3
Phosphorous (P)	12.2	34.1	61.5	36.5	7.9
Combined (N-P)	13.3	34.5	61.4	37.5	8.8
Mean	11.3	35.0	61.5	37.2	8.3
LSD 0.05	0.5	0.5	0.5	0.7	0.2

[a] % of chemical compound on dry matter

Table 4 Effect of experimental treatments on qualitative characteristics of herbage (mink forage unit, MFU) and MFU produced per hectare in the pastures of two environments.

Experimental treatment	Nutritive value of the herbage		
	Environment I[a]	Environment II[a]	LSD 0.05
	MFU[a]		
Control	0.55	0.54	0.01
Fertilizer:			
Nitrogen (N)	0.53	0.53	NS
Phosphorous (P)	0.56	0.56	NS
Combined (N-P)	0.53	0.56	0.02
Mean	0.54	0.55	0.01
LSD 0.05	0.01	0.01	
	MFU per ha[b]		
Control	1326	1079	127
Fertilizer:			
Nitrogen (N)	1817	1468	145
Phosphorous (P)	2551	2065	124
Combined (N-P)	2876	2177	93
Mean	2143	2289	47
LSD 0.05	112	123	

[a] kg DM^{-1}
[b] numero MFU ha^{-1}
NS= not statistically significant at P<0.05

17.6% and 30.9% higher, respectively than those of E II while total nitrogen (N_{tot}) content of E I was 6.1% lower than that of E II (**Table 5**). Topsoil chemical determination between environments under N treatment presented a similar trend of variation. The soil content of parameters N_{tot}, OC and P_2O_5 under N-enrichment treatments at the end of experiments were 13.6%, 25.5% and 24.8%, respectively lower than the values recorded at the beginning of trials (**Table 5**). By contrast, P enhancement increased 11.4% and 23.2% at the end of the experiment in E I and E II, respectively for N_{tot}, 27.% and 9.6% for OC, and 25.0% and 48.1% for P_2O_5 (**Table 5**). The effect of combined N-P enrichment in topsoil increased, at the end of experiments, the content (mean over environments) of chemical elements of the control natural pasture by 9.5%, and 21.3%, respectively for N_{tot} and P_2O_5 and reduced the OC by 1.3% (**Table 5**). Furthermore, the effect of combined N-P in comparison to P treatment lowered the content significantly (mean over environments) of chemical content in the soil by 0.2 mg kg^{-1} for N_{tot}, 0.2 g kg^{-1} for OC and 8.5 ppm for P_2O_5. However, the amount of soil parameters (mean over two environments) at end of experiments of combined N-P application was 14.9% higher for N_{tot} and 14.5% for P_2O_5 and 2.1% lower for OC than the same parameters of the natural pasture (**Table 5**).

Table 5 Effect of fertilizers on the content of soil characteristics: total nitrogen (N_{tot}), organic carbon (OC) and phosphoric anhydride (P_2O_5) in the Ap horizon in the years at starting (2001) and at end (2005) of experimental treatments.

Experimental treatment	Topsoil characteristics								
	N_{tot} [a]			OC [b]			P_2O_5 [c]		
	2001	2005	LSD 0.05	2001	2005	LSD 0.05	2001	2005	LSD 0.05
					Environment I				
Control	3.1	3.1	NS	12.9	12.1	NS	26.6	27.0	NS
Fertilizer:									
Nitrogen (N)	3.1	2.9	0.2	13.9	10.9	2.2	27.6	24.0	1.9
Phosphorous (P)	3.1	3.5	0.3	12.9	12.9	NS	27.0	36.0	1.1
Combined (N-P)	3.1	3.3	0.2	12.8	12.8	NS	27.1	28.0	1.1
Mean	3.1	3.2		13.1	12.2		27.1	28.8	
LSD 0.05	NS	0.2		NS	0.6		NS	2.8	
					Environment II				
Control	3.4	3.2	NS	10.4	10.2	NS	17.0	20.0	NS
Fertilizer:									
Nitrogen (N)	3.1	2.8	0.2	11.6	9.4	0.6	17.6	17.0	1.9
Phosphorous (P)	3.3	4.3	0.2	10.4	11.5	0.6	18.7	36.0	8.9
Combined (N-P)	3.6	4.1	0.3	10.3	11.2	1.0	16.2	27.0	5.6
Mean	3.4	3.6		10.7	10.6		17.4	25.0	
LSD 0.05	NS	0.3		NS	0.5		NS	25.0	

[a] mg kg^{-1}
[b] g kg^{-1}
[c] ppm
NS = not statistically significant at P<0.05

DISCUSSION

The botanical composition of pastures used in this study is in agreement with that in studies by Argenti *et al.* (1999), Bernués *et al.* (2004), Abdelguerfi and Abdelguerfi-Laouar (2004), Casasús *et al.* (2004) and Martiniello and Berardo (2005), which are characterized by species of typical botanical families and soil parameters of pastures belonging to UNESCO-FAO (1963)'s mesomediterranean bioclimatic zone.

Biomass characteristics

The adopted chemical soil enrichments influenced the pastures' herbage of the NP protected areas favouring an increase of herbage biomass and nutritive value of pasturelands of the southern Mediterranean environments of the EU.

In both environments, as a consequences of P and N-P soil enrichment at the end of the experiments, in comparison to the control treatment, the contents of N_{tot} and available P_2O_5 in the soil (respectively, 22.1% and 34.7% under P, and 17.2% and 14.5% under N-P) were enhanced (**Table 5**). Thus, the increase of available N_{tot} and P_2O_5 in the topsoil is in agreement with the results obtained in other environments in Australia, Italy and the USA by Sneva *et al.* (1958), Norman (1962), Hubbart and Manson (1967), Tupper (1978), Berg and Sims (2000), Martiniello and Paoletti (2002) and Martiniello and Berardo (2007), favoured higher DM yield and plant height than the natural pasture (mean over environments, 56.5% and 23.2%, respectively higher than the control) (**Fig. 3** and **Table 1**).

Sward phytocoenoses composition

The lower content of N_{tot} topsoil at end of the experiment under N application was a consequence of the reduced Leguminosae and Gamineae ratio in the sward of pasture (mean over environments) than that of the control treatment (Leguminosae and Gramineae ratio, 0.29 and 0.07, respectively in the control and N application) (**Table 2**). Furthermore, under nitrogen treatment at the end of the experiments, the statistical significant content of the top soil parameters was 0.3 mg kg^{-1}, 1.8 g kg^{-1} and 3.1 ppm lower than those of natural pasture control, respectively for N_{tot}, OC and P_2O_5 (**Table 5**).

In agreement with Selling and Zasoski (1993), Sanchez-Maran *et al.* (2002), Rutigliano *et al.* (2005) and Li *et al.* (2007), the reduced variation of soil OC at the end of the experiment between control and N treatment may evidence a soil microbial community which decompose organic matter determining the release of mineral nutrient in soil and, consequently, influencing primary productivity and nutrients cycling for sustaining the increase of biomass production (**Tables 2, 5**). The larger amount of $N_{tot,}$ OC and P_2O_5 parameters in the soil under P than N and N-P treatments changed the proportion of botanical composition of the natural pasture control (mean over environments) favouring an increase of Leguminosae (56.4%) and a decrease of Gramineae (15.7%), Compositae (17.6%) and the miscellaneous group (20.3%) (**Table 2**). According to Osman and Bahhady (2000) and Hill *et al.* (2005), the effect of P and N-P combined application treatments, in Mediterranean environments (Syria) and southern Australia, benefits the increase of P_2O_5 in soil which favoured the development of Leguminouse on floral composition and consequently influenced the quality of the herbage composition. In addition, on the basis of literature, soil enriched with chemical elements after a short period (four-five years) was possible to evidence a residual effect of chemical elements on sward of pastureland. The residual effect, left in the soil after withhold chemical application are evidenced all over the world influenced favourably the quality and production of biomass and the flora species in the sward. The highlighted results obtained on the topic of residual effect are briefly reported next:

a) Hubbard and Mason (1967) evaluated the residual effect of ammonium nitrate and phosphate on some native species in two sites of British Columbia (Canada). The response of residual effect was determined up to a period of 5 years of continuous applications and gave favourable advantages economically feasible for a period of six years of evaluation. The residual effect mainly increased dry matter of native flora species by 76% and 44% in Summerlands and Kamloops sites, respectively.

b) In south-eastern Australia, experiments carried out by Tupper (1978) on the evaluation of residual effect of nitrogen, phosphorous and gypsum. The residual effect of nitrogen element increased the dry matter production, of *Danthonia ceaspitosa* Gaudich. and *Stipa variabilis* Huges, only after the first year withhold while phosphorous residual effect favoured leguminous production for four years after the last application;

c) In western Oklahoma USA, Berg and Sims (2000) evaluated the effect of residual nitrogen following five years of continuous fertilization on *Bothriochloa ischae-*

mum L. The authors evidenced a substantial effect of residual on biomass weight gain for three years following the five years of N fertilization;

d) In Argentina, Berardo and Marino (2001) determined residual effect of phosphorous application on cultivated lucerne (*Medicago sativa* L.) crop. The authors fertilized for four continuously years the crop with phosphorous. The effect of phosphorous application increased dry matter but the gain in biomass production still continued for other two years benefiting the residual effect of previous phosphorous application;

e) In Italy, Martiniello and Berardo (2007) conducted an experiment in southern part of the Country using nitrogen, phosphorous and combined nitrogen-phosphorous elements at different rate of applications. Pasturelands were fertilized continuously for six years. After a 2 years of withholding chemical elements applications, was assessed the effect of residual fertilizers on quality and quantity of biomass of pastures. Results evidenced that the residual effect differently influenced the dry matter production, quality of herbage and flora composition of the sward. Among chemical elements, the residual effect was mainly evident with phosphorous and combined nitrogen-phosphorous elements.

Seed-borne properties

The soil enrichment by chemical compounds has been found to be an approach able to increase qualitative and quantitative biomass production and germination of the indigenous seed born present in the sward favouring development of native flora present in the natural pasture. A study carried out by Iannucci *et al*. (2005) on samples of soils of the treatments of the E I and E II after three years evaluation evidenced that the merged seedling of the enriched soil in both environments were higher than the natural control. The total number of seedlings geminated in both environments was similar (over 5500 seedlings m^{-2}). The natural pasture of E I showed more seedlings belonging to the Leguminosae family and miscellaneous group and fewer Gramineae than E II. The seedlings that emerged from soil enriched by N element were, when all environments were averaged, 29% less than those that emerged in a natural pasture. By contrast, mean of seedlings of soil of two environments, enriched with P and combined N-P elements was 40.5% and 16.3% higher than those sprout from soil of natural pasture and those treated with N, respectively. However, the seedlings belonging to the Gramineae family under both P and combined N-P treatments, was 48% and 52% lower than those of Leguminous and miscellaneous group, respectively. The results on the seed-bank reported by Iannucci *et al*. (2005) evidenced wide variability of dormant seed present in the soil of natural pasture able to recover seed germination for restoring the native ecological flora of natural pasture. However, since the seeding classification was made on germinated sprouts, the only botanical attribution possible to determine was the botanical family. So, considering the large number of seedlings m^{-2}, according Tracy and Sandersen (2000), it is possible to claim that the seed-bank species composition of the soil may represent little similarity (44%) to the existing vegetation. A further study carried out on a seed-bank indicated that the biological mechanisms which lift dormancy of seeds were influenced: in New Zealand by modality of grazing management of the sward (Nie *et al*. 1999), in northeastern United States by intensive grazing and hay production (Tray and Sanderson 2000) and in Nebraska a sand-hill prairie by depth of the topsoil of pasture, environmental factors and grazing management practices (Perez *et al*. 1998). However, the study reported by Perez *et al*. (1998), Nie *et al*. (1999), Tracy and Sunders (2000) and Ianucci *et al*. (2005) evidenced a wide presence of dormant seed-bank in the soil of pasturelands which represent an ecological potential resource for renewing the property and function of native pasturelands.

Qualitative components of the herbage

In line with Streeter and Barta (1988), the lower P_2O_5 content observed in the topsoil of treatment receiving N application than the pasture control, was ascribed to the higher P uptake of the grasses for sustaining higher biomass production in the N application treatment. Because P and combined N-P applications favoured the development of Leguminosae rather than others families, the CP content (mean over the environments) in the herbage was 17.2% higher than the control treatment while N application favoured development of the Gramineae and a reduction of CP in the biomass (10.0% lower than the value assessed in the herbage of natural pasture control) (**Table 3**).

According to Mckenzie *et al*. (1998), Johnston *et al*. (1998) and Jouven *et al*. (2006), the higher concentration of CF, NDF and ADF observed in the herbage under chemical element enrichments than those of the natural pasture control (**Table 3**) was due to a greater morphological structures as consequences of greater plant height under chemical-enriched treatments (plant height under chemical treatments was 16.4% and 38.8% higher than the comtrol in E I and E II, respectively; **Fig. 3**). However, according to Mckenzie *et al*. (1998), the lower 3.0 percentage point, (difference between mean of E I and E II of control and N application) content in ADL observed under N application than the control treatment, was due to the reduced (mean over environments) plant height (26% short) and Gramineae (34% lower) of natural pasture biomass which influence the quality of structural fibre in the herbage (**Fig. 3** and **Table 2**).

Since chemical elements induced variation in biomass yield and affected – to differing degrees – the qualitative characteristics of herbage (CP, CF, NDF, ADF and ADL), they favoured a higher production of MFU ha^{-1} than the natural pasture. The gain in MFU ha^{-1} over the control treatment was 755, 1566, and 1696 units for N, P and combined N-P applications, respectively. So, considering the effect of the combined N-P enrichment on herbage and biodiversity of the flora in the sward, this application may be considered as a useful approach to increase the biomass and qualitative characteristics of the herbage for preserving biodiversity in the protected pasturelands of the EU. The chemical elements used in the study may achieve a possible residual effect that can left in topsoil residual chemical enrichment which benefits biomass yield, nutritive value of the herbage and flora composition without genetic pollution of the phytocoenes present in the sward of protected EU pasturelands.

CONCLUSIONS

The effect of combined N-P fertilizers, in comparison to those of N, enhanced the P_2O_5 content and N_{tot} in the soil and favoured the increase of biomass development and MFU ha^{-1} of the natural pasture. In addition, the effects of the combined N-P treatment increased biomass yield and nutritive value of the herbage, and altered the floristic composition of the pastures beneficially compared with the control treatment. Thus, fertilizer application for a period of time considered in this study based on combined N-P elements in pastures of mesomediteranean environments may be useful to better exploit the development of herbage favouring increases in forage production and nutritive value and preserving biodiversity in protected natural pasturelands (NP, SCI and ZSP) of EU.

IMPLICATIONS

The effect of combined N-P fertilizers in pasturelands favoured the development of herbage, preserved the floral biodiversity and the chemical inorganic characteristics of natural pasture topsoils. The low variation of soil organic carbon between control and N-P fertilizers was evidence that the treatment promoted soil microbial activity which plays a fundamental role in the ecosystem functioning and which influenced the process of primary nutrient cycling and exploitation of native dormant seed-bank in the topsoil. The combined N-P rather than separate N and P applications allows a more favourable condition in the topsoil able to favour the development of herbage and small variation of floral species present in the sward of natural pastures.

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