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RESEARCH ARTICLE

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Soil seed-bank germination patterns in natural pastures under different mineral fertilizer treatments

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Abstract

Degraded native grasslands in Mediterranean areas can be improved by encouraging seedling regeneration from soil seed banks using chemical fertilization. The effect of mineral fertilizers on soil seed banks was studied in natural pastures at two locations in southern Italy: Carpino and Rignano Garganico. The aim was to determine if nitrogen (N), phosphorus (P) and combined nitrogen and phosphorus (NP) fertilization can promote increased soil seed density. The seed-bank size and composition were analysed over two growth cycles (2004-2006) at two periods of the year: at the early summer and at the early autumn. The plant species were classified into three functional groups: grasses, legumes and other species (all other dicots). A two-pool model (ephemeral and base pools) derived from the germination patterns was developed to quantify the dynamics of the germinated seed populations. The mean total seed number in the seed bank ranged from 2,915 to 4,782 seed m^{-2} with higher values in early summer than in early autumn. Mineral fertilizer applications increased the seed-bank size (by 27%, 23% and 46%, for N, P and NP, respectively) and modified the composition in both localities. The three plant functional groups showed different potentials for ephemeral and persistent seed-bank production; however, within each plant group, the proportion between the ephemeral and base pool fractions did not change with fertilizer application. These data show that mineral fertilization can have positive effects on the seed-bank size of ungrazed natural pastures, and can be used to improve degraded Mediterranean pastures.

Additional key words: germinable seed population; Mediterranean pastures; fertilizer application; persistent seed bank; transient seed bank.

Introduction

Natural grasslands in the Mediterranean basin occur generally in hilly areas where cropping is not possible because of shallow, stony soils and steep slopes. They consist predominantly of annual species, and their persistence is dependent upon the development and maintenance of a considerable soil seed bank (Sternberg *et al*., 2003). The inland pasture areas of the south of Italy are an important source of feed for livestock. However, although ecologically desirable, extensive use of these areas can causes their degradation, leading to severe nutrient depletion and low crop yields.

In native grasslands, improved productivity is desirable, with the encouraging of seedling regeneration from soil seed banks. This is particularly important in protected areas where the biodiversity of the natural pastures is protected by law, and any recovery is linked to adequate agro-pastoral techniques of management. Many agronomic approaches have been proposed to improve productivity and ecological function of such protected pasturelands, and the most effective is fertilizer application (Dubljević, 2009). Indeed, other practices can be forbidden by law, such as reseeding with exogenous ecotypes, as this promotes genetic contamination of the pasture phytocoenoses.

Numerous studies have shown that nitrogen (N), phosphorous (P) and complex N plus P (NP) mineral fertilizers, without or with potassium (K), can increase soil fertility and promote germination of the indigenous seed bank. This can thus favour growth of the native flora and increase the productivity and nutritive

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Abbreviations used: BP (base pool); EP (ephemeral pool); GSP (germinated seed population); NLIN (non-linear regression).

value in the native Mediterranean pastures for a range of situations (Norman, 1962; Tupper, 1978; Shaw *et al*., 1981; Osman *et al*., 1991; Berg & Sims, 2000; Griffin *et al*., 2002; Martiniello & Berardo, 2005; Vojin *et al*., 2010; Ružić-Muslić *et al*., 2012).

The total seed-bank composition appears to be mainly affected by seed communities in the surface layer (0-10 cm) and a temporal pattern in the number of germinable seeds in the soil has been detected, with a maximum in summer and a minimum in spring, immediately prior to the start of the seed production period (Bartolome, 1979; Young *et al*., 1981; Marañon, 1985; Russi *et al*., 1992). After accumulation of seeds in the soil up to the production peak in late spring to early summer, there is generally a gradual depletion through predation and germination, prior to the period in the autumn after the first effective rains, when most of the germination occurs (Bartolome, 1979; Espigares & Peco, 1993; Ortega *et al*., 1997).

The seed bank analysis can provide a retrospective view of treatment effects (Mall & Singh, 2014). However, the success rate of natural reseeding is affected not only by the seed quantity in the soil, but also by the seed germination patterns and the seed dormancy of the plant species. Indeed, seed banks can be classified as: (i) transient, when the seeds can germinate soon after they are shed from the mother plant; and (ii) persistent, when the seeds can remain in the soil in a state of dormancy and germinate slowly (Thompson & Grime, 1979). Cocks (1992) indicated the importance of the short-term dynamics of these transient and persistent seed banks for the estimation at a preliminary level of the efficiency for pasture improvement.

The objectives of the present research were: (i) to investigate the effects of N and P fertilizer addition on the soil seed-bank size for two seasonal periods between the peak of seed input to the soil bank and the period prior to autumn germination; and (ii) to study the germination patterns of the grasses, legumes and other species for two natural grassland locations within the Gargano National Park in southern Italy, with the aim to test this agronomic technique for the improvement of degraded Mediterranean pastures.

Material and methods

Experimental locations

The experiments were conducted over the two years, which are here defined according to the starting year of the 2004-2005 and 2005-2006 growth cycles, at two locations in the Apulia region of southern Italy that lie within the limits of the Gargano National Park (41°48'N, 15°54'E): Carpino and Rignano Garganico (Table 1). These two locations are 30 km apart, and the area is generally steep slopes with hills rising to 1065 m asl. The climate is transitional between a maritime climate and a continental climate, although it varies considerably within the promontory in terms of orientation and altitude. The rainfall and temperature were recorded at meteorological stations about 1 km from each of the experimental locations (Table 2), and their patterns are typical of the meso-Mediterranean zone described by UNESCO-FAO (1963). The aridity indices of De Martonne (1926) for the periods from May to September were 8.0 for Carpino and 9.5 for Rignano Garganico. The experiments were performed in loam soils at Carpino and in clay soils at Rignano Garganico (USDA classification, 1999) (Table 1). The herbages of the pasturelands have been grazed by sheep and goats at Carpino, and by cows at Rignano Garganico.

Table 1. Main geographic and soil characteristics of the study locations

Characteristic	Location	
	Carpino	Rignano Garganico
Geographic		
Altitude (m asl)	147	590
Longitude (E)	15° 52'	15° 35'
Latitude (N)	41° 51'	41° 40'
Soil		
Sand (%)	48	41
Silt (%)	36	17
Clay (%)	16	42
pH (in H_2O)	7.6	7.8
Organic matter[a] (g kg^{-1})	24	18
Total nitrogen (‰)	10.3	3.8
Phosphorus, available[b] (mg kg^{-1})	79.8	51.2
Potassium, exchangeable (mg kg^{-1})	519	462

[a] Walkey-Black method. [b] Olsen method.

Field experiments and management

At each location, the experimental design used in the trials was a randomized complete block with four replicates. Uniform areas of about 900 m^2 were chosen

Table 2. Main climatic characteristics of the study locations, expressed as the means over the two growth years

Month	Location			
	Carpino		Rignano Garganico	
	Rainfall (mm)	Mean temperature (°C)	Rainfall (mm)	Mean temperature (°C)
January	68.6	7.7	83.3	3.6
February	108.6	7.9	126.5	3.5
March	93.9	10.7	134.7	6.0
April	47.6	15.1	66.3	10.0
May	42.6	20.1	20.0	14.9
June	109.4	23.2	111.6	17.9
July	27.6	26.1	36.1	20.4
August	59.6	24.5	48.1	20.6
September	28.2	23.6	52.0	17.0
October	122.3	18.5	141.7	14.5
November	158.7	13.9	156.8	8.3
December	102.3	10.7	143.2	5.3
Annual total	***969.4***		***1120.3***	
Annual mean		***16.8***		***11.8***

the previous autumn and isolated using metal netting and barbed wire, to exclude the grazing animals. The area was divided into four parallel blocks, each of which measured 5 m × 20 m, with 5-m sections between adjacent blocks.

The following fertilizer treatments were used, split within each block: control (no fertilizer applied); N application at 60 kg ha^{-1}, as urea; P application at 80 kg ha^{-1}, as superphosphate; nitrogen and phosphorus (NP) application at 32 kg ha^{-1} and 70 kg ha^{-1}, respectively, as ammonium biphosphate. The plot size within each block was 25 m^2 (5 m × 5 m). For each of the years, the fertilizers were applied to the plots as a single dose in the first 10 days of February. Prior to fertilization, four topsoil cores (30-cm depth, 6-cm diameter) were taken from each plot, for nutrient and soil classification analysis. These samples were air dried, and after root separation, they were used to assess soil texture and pH (in H_2O), organic matter (Walkey-Black method), total N (micro-Kjeldahl), available P (Olsen method) and exchangeable K (NH_4Ac) (Table 1).

Seed-bank sampling and measurements

The seed bank was estimated using the seedling emergence method. Soil cores were taken at two periods: early summer (first week in July), when both species with early and late habits had completed their growth cycle, and early autumn (first week in October), following the period of summer soil moisture deficit and prior to the germination 'explosion'.

Soil samples were collected from each plot for all of the treatments by taking 20 cores using a steel probe (10-cm deep, 6-cm diameter). This sampling intensity maintained a total sample area of 562 cm^2 per plot, which was considered to be adequate because 20 randomly selected soil cores with a diameter of 3.8 cm (227 cm^2) have been shown to maintain a stable estimation of the total soil seed bank (Zhang *et al.*, 1998). After collection, each soil sample was placed in a plastic tray (16 × 11 × 4 cm^3) in a greenhouse under natural daily temperature fluctuations (between 5°C and 35°C) and watered regularly, to promote germination. After every 2 weeks, the soil was carefully stirred to facilitate the germination of all of the seeds. The trays were rotated monthly to different areas of the greenhouse, to reduce any effects of their positioning.

The emerged seedlings were removed, counted and identified at weekly intervals over 7 months. The germination test was terminated when no further seedlings had emergence over the previous 20 days. The seedling density was expressed as the number of seedlings that emerged per square metre.

To compare the seedling density from the seed-bank data, the relative contributions were calculated for the different taxonomic groups of the seed bank. For this purpose, the plant species were classified into three functional groups according to agronomic traits and taxonomy: grasses, legumes, and other species (all other dicots), and their relative abundances were totalled to obtain the total germinated seed population (Roberts, 1981; Peco *et al*., 1998).

Data analysis

Combined ANOVA for location, fertilizer treatment, year and seasonal period on the seed bank size (seeds m^{-2}) was performed, considering the year as a random factor (GLM procedure). For each location, three-factor ANOVA was used (*i.e.,* fertilizer treatment, year, seasonal period) to assess the differences in the seed-bank densities. The effects of the principal factors and their interactions were tested with the appropriate error terms (Steel & Torrie, 1980). When F-tests were significant, the means were compared with the LSDs for $p < 0.05$. Prior to ANOVA, all data were tested for normality and homocedasticity. The equations on the relationship between cumulative seedling population and germination time were calculated using the NLIN (non-linear regression) procedure. All of the statistical analyses were performed with the STATISTICA software (StatSoft vers. 7.1; StatSoft, Inc., Tulsa, OK, USA). The relationships between the cumulative germinated seed populations and the germination times for all of the plant functional groups and the treatments can be described by the negative exponential Eq. [1] ($r^2 = 0.89 - 0.96$, $p < 0.001$):

$$y = a\,(1 - be^{-t}) + ct \qquad [1],$$

where y is the cumulative germinated seed population, t is the days of glasshouse incubation, and a, b and c are coefficients.

This allowed further analysis of the germination patterns and the dynamics of the germinable seed populations in the soils. The differentiation Eq. [2] shows a germination pattern in which the germination rate (dy/dt) is determined by the time of incubation (t) and three coefficients (a, b, c):

$$dy\,/\,dt = abe^{-t} + c \qquad [2]$$

Based on Eq. [2], a two-pool model was developed to quantitatively describe the dynamics of the germinable seed reserves in the soil, in terms of the transient seeds or ephemeral pool (EP, seeds m^{-2}) and persistent seeds or base pool (BP, seeds m^{-2}) (Nie *et al*., 1999). According to Eq. [2], BP = cn (where c is the asymptote of the curve and n is the number of the days of incubation) and EP = $Y_{t=n} - cn$ (where $Y_{t=n}$ is the definite integral of the curve from 0 to n). The germinated seed population (GSP, seeds m^{-2}), which represents the soil seed reserve, is the sum of these two pools.

Results

Characteristics of the locations

The total rainfall as the mean over the two years was highest at Rignano Garganico (1120 mm), and from October to April was 26% higher than at Carpino (Table 2). The higher mean annual temperature was seen at Carpino (16.8°C), which was 30% higher than at Rignano Garganico (Table 2). As well as the rainfall and temperatures, the two locations differed in some other characteristics (Table 1). Carpino is at a lower altitude, is nearer to the sea (7 km), and is richer in soil organic matter and mineral elements. In contrast, Rignano Garganico has a stony and clayey soil, with a thin arable layer.

Germinated seed population

The ANOVA calculated to test the effects of all of the principal factors and their interactions showed that the fertilizer treatment, location, and year × seasonal period interaction were statistically significant for all of the plant species and for the three plant functional groups (Table 3).

The pasturelands of the two locations differed for the mean total number of seeds which varied from about 4262 to 3284 seeds m^{-2} for Carpino and Rignano Garganico, respectively (Table 4). The germinable seed bank ranged also between the years and the seasonal periods of the harvest (Table 4). Indeed, the seed-bank size was lower for the second year of evaluation and, combining both locations, the mean number of seeds was higher in summer than in autumn (4217 *vs* 3328 seeds m^{-2}, respectively). Furthermore, the fertilizer treatments influenced the germinable seed bank in this surface (0-10 cm) layer of the soil for both of the loca-

Table 3. Results of the analysis of variance for germinable seed populations of all of the plant species and for the three plant functional groups for the two locations, the two seasonal periods, and the four fertilizer treatments, over the two years of the study

Source of variation	df	Grasses		Legumes		Other dicots		All species	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Location (L)	1	26.8	<0.001	4.8	0.029	47.7	<0.001	24.6	<0.001
Fertilizer treatment (F)	3	13.0	<0.001	13.5	<0.001	5.55	0.001	15.7	<0.001
Year (Y)	1	103.5	<0.001	188.1	<0.001	2.22	NS	31.9	<0.001
Seasonal period (S)	1	14.1	<0.001	39.4	<0.001	3.66	NS	20.3	<0.001
L × F	3	5.2	0.001	1.2	NS	0.4	NS	0.1	NS
L × Y	1	0.1	NS	0.1	NS	0.27	NS	0.2	NS
L × S	1	0.5	NS	0.1	NS	1.47	NS	0.6	NS
F × Y	3	6.0	0.001	4.9	0.002	0.5	NS	0.4	NS
F × S	9	5.2	0.001	1.3	NS	0.2	NS	0.7	NS
Y × S	1	31.9	<0.001	38.4	<0.001	85.8	<0.001	125.0	<0.001
L × F × Y	3	4.5	0.004	1.2	NS	0.3	NS	0.8	NS
L × F × S	3	4.6	0.003	0.6	NS	0.1	NS	0.4	NS
L × Y × S	1	13.3	<0.001	3.7	NS	0.2	NS	3.7	NS
F × Y × S	3	4.6	0.003	2.7	0.046	0.7	NS	2.1	NS
L × F × Y × S	3	6.3	<0.001	0.1	NS	0.7	NS	1.5	NS

NS: not significant.

tions, with the NP application producing the greatest number of seeds that germinated (mean, 4833 seeds m^{-2}). Compared with the control, there were significant increases in the total germinable seed bank for the N, P and NP treatments, of 20%, 16% and 56% at Carpino and of 33%, 29% and 75% at Rignano Garganico, respectively.

Cumulative germination pattern

The pattern of seed germination followed a similar trend for all of the plant functional groups and for both locations, and in general, more than 50% of the seeds germinated in the first 2-3 weeks of incubation (Fig. 1).

The technique used in this study for estimating the population of seeds in plant communities measured the potential germinable seed populations. Based on this condition, the total germinated seed population (GSP) and those of the two pools (EP, BP) according to the different plant functional groups and treatments, were calculated through the mathematical models for these two locations (Tables 5 and 6). The two pasturelands showed different compositions of plant species. The means across the treatments for the GSP of legumes and other dicots were higher at Carpino (660 and 3053 seeds m^{-2}), whereas the density of grass seeds was far greater at Rignano Garganico (839, *vs* 549 seeds m^{-2} for Carpino). Also the transient seed numbers varied among the locations and plant functional groups. In particular, with no fertilizers added (control) at Carpino, the EPs were 273 (60%), 281 (61%) and 1189 (47%) seeds m^{-2} for grass, legumes and other dicots, respectively (Table 5). On the other hand, at Rignano Garganico, the control treatment showed EPs of 384 (72%), 191 (49%) and 1125 (74%) seeds m^{-2} for grass, legumes and other species, respectively (Table 6). As a consequence, the principal components of the persistent seed banks were other dicots and legumes at Carpino (53%) and Rignano Garganico (51%), respectively.

As measures of the seed contributions of each of the plant functional groups to the soil reserve after fertilization, the difference between each of the fertilizer treatments and the control data were calculated (Tables 5 and 6). For both locations, N addition affected the soil seed composition by an increase in the grass species (GSP, 106 and 428 seeds m^{-2}, corresponding to 24% and 81% increases, at Carpino and Rignano Garganico, respectively). In contrast, the leguminous species were favoured by P addition (GSP, 159 and 163 seeds m^{-2}, corresponding to 35% and 42% increases, at Carpino and Rignano Garganico, respectively). The combined

Table 4. Means for the germinable seed bank of all of the plant species for the two locations under the four fertilizer treatments of the two seasonal periods, over the two years of evaluation

Characteristic	Germinable seed bank (seeds m^{-2})	
	Carpino	Rignano Garganico
Fertilizer (F)[1, 2]		
Control	3463.2b	2445.9b
N	4170.7ab (+20%)	3260.0ab (+33%)
P	4029.5ab (+16%)	3146.1ab (+29%)
NP	5384.1a (+56%)	4282.1a (+75%)
Year (Y)		
2004-2005	4863.7	3797.5
2005-2006	3660.1	2769.6
Seasonal period (S)		
Summer	4782.1	3652.3
Autumn	3741.7	2914.8
Mean	*4261.9*	*3283.6*
***F*-test**[3]		
F	**	**
Y	**	**
S	**	*
F × Y	NS	NS
F × S	NS	NS
Y × S	**	**
F × Y × S	NS	*

[1] Control, no fertilizer; N, nitrogen; P, phosphorus; NP, combined N and P. [2] For Fertilizer: values in brackets are increases overcontrol; different small letters indicate significant differences ($p < 0.05$) within each column. [3] * $p < 0.05$; ** $p < 0.01$; NS, not significant.

N and P fertilizer treatments resulted in increased seed bank size of all of the plant functional groups. However, the fertilizer treatments increased the total GSP but had little effect on the relative proportions of both the EP and BP. At Carpino, under all of the fertilizer treatments, there were similar contributions to the total seed bank of grasses (EP, 0.70; BP, 0.30; on average) and legumes (EP, 0.33; BP, 0.67; on average). Under the N application, the other dicots showed a decrease in the BP (0.25) with respect to the other treatments (0.39, on average). Also at Rignano Garganico, there was a similar contribution of the two seed pools to the total seed bank under all of the treatments for the grasses (EP, 0.54; BP, 0.46; on average). Furthermore, at Rignano Garganico, the P application produced an increase in the BP of the other dicots (0.76), whereas NP application resulted in an increase in the EP of the legumes (0.47). Including all of the plant functional groups, the seeds present in the soil under the three fertilizer applications (N, P, NP) that showed characteristics of seed persistence were 2185 (48%) and 1396 (39%) seeds m^{-2} at Carpino and Rignano Garganico, respectively. These represent increased BPs of 27% (Carpino) and 33% (Rignano Garganico) with respect to unfertilized (control) soil.

Discussion

Effects on the germinated seed population

There was a large difference between the two pasturelands for the germinated seed populations, although the seed densities were relatively low compared to other studies in Mediterranean herbaceous communities, such as those of 67,000 seeds m^{-2} (Bartolome, 1979), 6,000-35,000 seeds m^{-2} (Russi *et al.*, 1992), 20,000-40,000 seeds m^{-2} (Levassor *et al.*, 1990) and 110,000 seeds m^{-2} (Ortega *et al.*, 1997). As Sternberg *et al.* (2003) noted, this wide variation in seed-bank densities probably reflects differences in the vegetation characteristics, grazing systems, and climatic conditions. In both locations in the present study, the variations in species number were apparent after the first fertilizer treatment.

There was a loss of seeds in the uppermost soil layer between the summer period of the point of peak production, and the autumn germination peak. This appears to be because the seeds of most species of the Mediterranean flora reach maturity after April, and the seeds shed from late May to June, and the decline seen, can be attributed to their burial in the deeper layers (Thompson & Ooi, 2010) or to seed mortality caused by parasitism, desiccation or post-dispersal predation (Hulme, 1998). According to Traba *et al.* (2006), the Mediterranean systems of grasslands that are dominated by annual species depend primarily on the soil seed banks for their regeneration, and a drop in the seed density during the summer season can generate significant effects on the vegetation dynamics. However, in the present study, a loss of 22% (as the mean over both locations) from summer to autumn indicated the small seasonal variation in seed density here.

The macro-elements from fertilization have been shown to be the main factor that influences the permanent grasslands in different environments (Friend *et al.*,

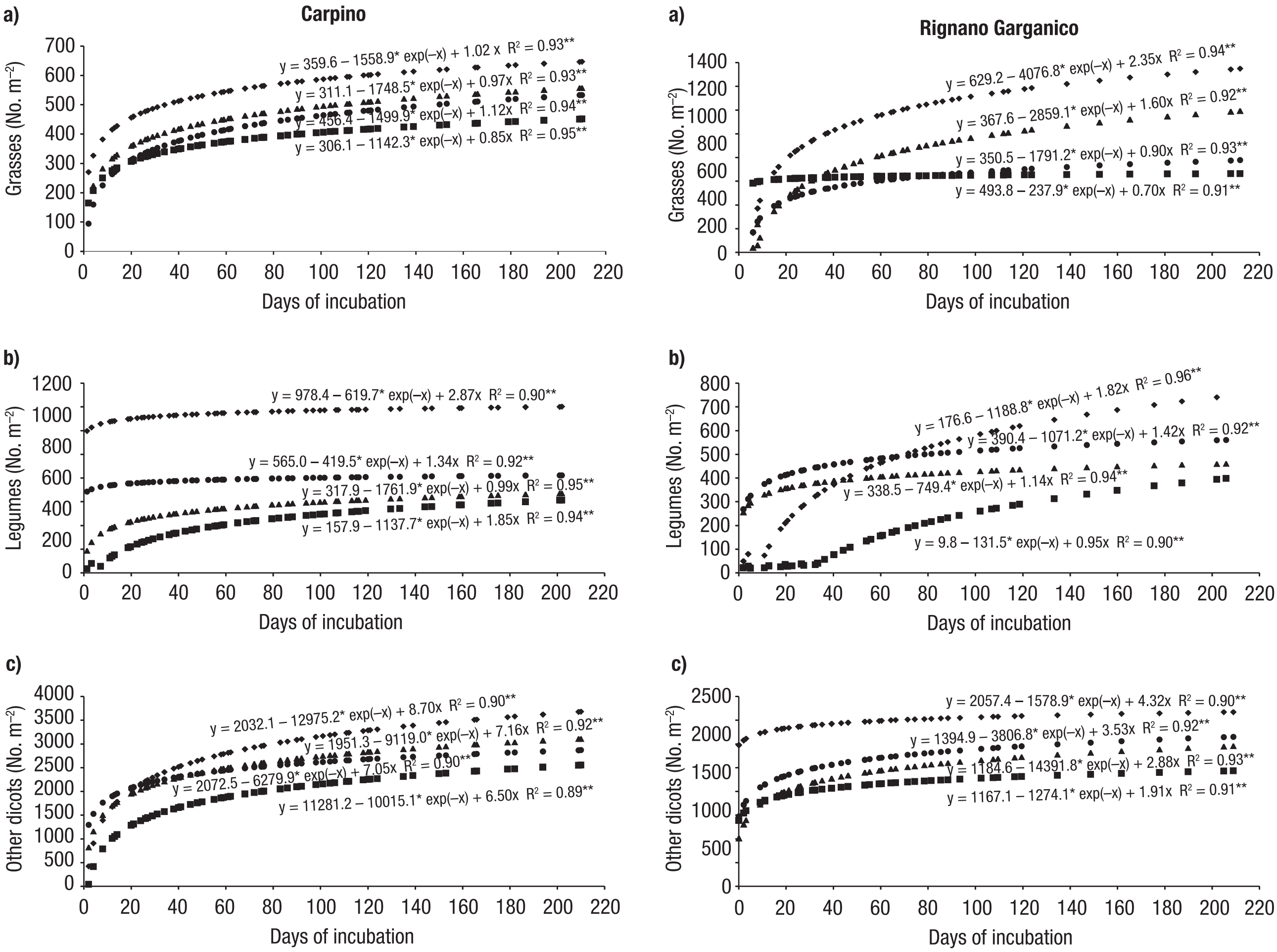

Figure 1. Cumulative grasses (a), legumes (b) and other dicots (c) seedling populations and their curve patterns according to incubation time under the four fertilizer treatments: (■) control (no fertilizer); (▲) nitrogen (N); (●) phosphorus (P); (◆) combined nitrogen and phosphorous (NP).

2001; Susan *et al.*, 2004; Radkowski, 2006). In the present study, the application of mineral fertilizers can result in significant increases in the sizes of the germinated seed populations of all of the plant species considered, with the effect being greater with the NP treatment. Furthermore, the increase in the germinated seed population appears to arise because the reseeding processes were due to the prevalence of annual species in the pasturelands, and they are dependent on the environmental conditions and the soil characteristics of the location (Martiniello, 1998; Martiniello *et al.*, 2000).

Effects on the seed germination pattern

The fertilizer treatments affected the proportion of each of the plant functional groups in the germinable seed bank, with the degree of this effect dependent on the mineral fertilizer applied. In particular, N addition increased the grass species, probably through its more efficient use by these species and through a reduction in the nitrogen-fixating abilities of the leguminous species, which leads to a decrease in their competitive abilities (Ružić-Muslić *et al.*, 2012). In contrast, P addition favoured leguminous species for both of the locations. Increased vegetal cover especially due to annual legumes has been found after P fertilizer application, supplied through different methods and at different times of application (Jeangros *et al.*, 1999; Viguera *et al.*, 2000; Arévalo & Chinea, 2009). Indeed, Tisdale *et al.* (1985) reported that P is crucial to root development in legumes, as it promotes the development nitrogen-fixing bacterial nodules. The combined fertilizer treatment (NP) used in the present study improved the

Table 5. Germinated seed populations calculated for 210 days of incubation, and the sizes of the ephemeral and base pools for the grasses, legumes and other dicots under the four fertilizer treatments at Carpino

Plant functional group	Fertilizer[1]	GSP[2] (seeds m^{-2})	EP[3] (seeds m^{-2})	BP[4] (seeds m^{-2})
Grasses	Control	451.2	272.7	178.5
	N	557.6	344.0	213.6
	P	541.1	337.9	203.2
	NP	646.9	412.5	234.4
	Mean	*549.2*	*341.8*	*207.4*
	N-Control[5]	106.4 (1.00)	71.3 (0.67)	35.1 (0.33)
	P-Control[5]	90.0 (1.00)	65.3 (0.73)	24.7 (0.27)
	NP-Control[5]	195.7 (1.00)	139.9 (0.71)	55.9 (0.29)
Legumes	Control	458.9	280.5	178.3
	N	503.6	295.3	208.3
	P	618.2	337.3	280.9
	NP	1057.1	454.6	602.5
	Mean	*659.5*	*341.9*	*317.5*
	N-Control[5]	44.7 (1.00)	14.8 (0.33)	29.9 (0.67)
	P-Control[5]	159.3 (1.00)	56.8 (0.36)	102.6 (0.64)
	NP-Control[5]	598.3 (1.00)	174.1 (0.29)	424.2 (0.71)
Other dicots	Control	2553.2	1188.7	1364.5
	N	3109.5	1605.1	1504.4
	P	2870.1	1389.2	1480.9
	NP	3680.1	1853.5	1826.7
	Mean	*3053.2*	*1509.1*	*1544.1*
	N-Control[5]	556.3 (1.00)	416.4 (0.75)	139.9 (0.25)
	P-Control[5]	316.9 (1.00)	200.5 (0.63)	116.4 (0.37)
	NP-Control[5]	1126.9 (1.00)	664.8 (0.59)	462.2 (0.41)
All species	***Mean***	***4261.9***	***2192.8***	***2069.1***

[1] Control, no fertilizer; N, nitrogen; P, phosphorus; NP, combined N and P. [2] GSP, germinated seed populations. [3] EP, ephemeral pool. [4] BP, base pool. [5] Fertilizer treatment minus control treatment. Values in brackets are the proportion in relation to the GSP.

seed-bank size and reduced the effects of competition among the botanical families of these natural pasturelands (Martiniello, 1998; Dubljević, 2009).

Both transient and persistent types of seed banks were represented at each of the two locations in the present study indicating the presence of the complementary mechanisms to vegetation regeneration. However, the sizes of the EP and BP, as calculated by the mathematical model based on the germination curve, are influenced by the amount of seed produced by the resident pasture plants and the seed characteristics of the various plant species. Indeed, the high transient seed fraction (*i.e.,* the EP) that was recorded at Rignano Garganico suggests a lower potential for persistence of the seed bank, due principally to the grass and other dicots functional groups. However, at Carpino, the BP size was represented principally by the seeds of the other dicots. The different potential for producing persistent seed banks might indicate a different floristic composition within each plant functional group. Furthermore, according to Pugnaire & Làzaro (2000), in terms of the contribution to the persistent seed bank, the results confirm the highly conservative role of the native species in buffering against changes in the pasture plant communities.

As reported above, the germinated seed population increased after the mineral fertilization applications, and as suggested by Egley (1986) and Arévalo & Chinea (2009), this appears to be due to the chemical modification of the soil that stimulates the seeds to germinate,

Table 6. Germinated seed population calculated for 210 days of incubation, and the sizes of the ephemeral and base pools for the grasses, legumes and other dicots under the four fertilizer treatments at Rignano Garganico

Plant functional group	Fertilizer[1]	GSP[2] (seeds m^{-2})	EP[3] (seeds m^{-2})	BP[4] (seeds m^{-2})
Grasses	Control	529.8	383.5	146.3
	N	958.1	621.7	336.4
	P	621.9	433.5	188.4
	NP	1246.8	753.9	493.0
	Mean	*839.2*	*548.2*	*291.0*
	N-Control[5]	428.2 (1.00)	238.1 (0.56)	190.1 (0.44)
	P-Control[5]	92.1 (1.00)	50.0 (0.54)	42.1 (0.46)
	NP-Control[5]	717.0 (1.00)	370.3 (0.52)	346.7 (0.48)
Legumes	Control	389.3	190.8	198.4
	N	452.0	213.3	238.8
	P	552.5	254.4	298.1
	NP	738.7	356.7	382.0
	Mean	*533.1*	*253.8*	*279.3*
	N-Control[5]	62.8 (1.00)	22.4 (0.36)	40.3 (0.64)
	P-Control[5]	163.2 (1.00)	63.6 (0.39)	99.7 (0.61)
	NP-Control[5]	349.4 (1.00)	165.8 (0.47)	183.6 (0.53)
Other dicots	Control	1526.8	1125.0	401.8
	N	1850.0	1245.7	604.3
	P	1971.7	1231.2	740.5
	NP	2296.6	1389.4	907.2
	Mean	*1911.3*	*1247.8*	*663.5*
	N-Control[5]	323.1 (1.00)	120.7 (0.37)	202.5 (0.63)
	P-Control[5]	444.9 (1.00)	106.2 (0.24)	338.7 (0.76)
	NP-Control[5]	769.8 (1.00)	264.4 (0.34)	505.4 (0.66)
All species	***Mean***	***3283.6***	***2049.8***	***1233.8***

[1] Control, no fertilizer; N, nitrogen; P, phosphorus; NP, combined N and P. [2] GSP, germinated seed populations. [3] EP, ephemeral pool. [4] BP, base pool. [5] Fertilizer treatment minus control treatment. Values in brackets are the proportion in relation to the GSP.

which will also cause a decrease in the persistent seed bank. Nevertheless, as shown here, the fertilization also increases the BP fraction, and thus it appears that the germinable seed bank increases after these fertilizer treatments mainly as a result of a greater seed production by the vegetation.

In conclusion, fertilizer treatments can be considered as useful agronomic practices for the improvement of the seed banks of native pastures in the Mediterranean environment, and especially in areas protected by landscape ties. At least in the absence of grazing, the most balanced result appears to be achieved by the combined N and P treatment, which increased the seed density of all of the plant functional groups. However, to maintain the native species permanently incorporated in the soil seed bank after the fertilizer treatments, the further management should ensure some seed production each year; *e.g.*, by suspending grazing of the pasture during the flowering and seeding periods.

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