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Long-term effects of climate and phosphorus fertilisation on serpentine vegetation

Alessandro Chiarucci · Simona Maccherini

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Abstract The long-term effects of phosphorus fertilisation and climate on serpentine plant communities in Tuscany, central Italy have been investigated by using data from a 12 year before-after control-impact (BACI) experiment. Using the point quadrat method, data on plant communities were collected in June of each year from 1994 to 2005 in eight 2 × 2 m plots, four fertilised with phosphorus and four used as controls. Climatic data were obtained from a nearby meteorological station and summarised in 24 variables. Phosphorus addition significantly affected vegetation cover of both vascular and cryptogamic vegetation but did not influence species richness. The effects on species composition were clear but not marked, and consisted in promoting the abundance of some species already present in the community but not leading to the colonisation of other species. Interannual climate differences affected vegetation cover in the fertilised plots but not in the control ones, while climate affected the species richness values of different/various life-forms in both groups of plots, with more evident effects in the fertilised one. The effects of climate on plant community composition were weak once both the variability among individual plots and the successional dynamics were subtracted from the variance in species composition.

Keywords BACI design · Long term effects · P addition · Ultramafic soils · Vegetation dynamics

Introduction

In many parts of the world, ultramafic (serpentine) soils host distinctive vegetation. This is often less productive and stunted with respect to surrounding substrates, but often very rich endemic and rare species (Brooks 1987; Roberts and Proctor 1992; Baker et al. 1992). The peculiar features of serpentine vegetation have been attributed to the chemical and physical properties of the soil. The high concentration of potentially toxic elements, such as chromium, nickel and cobalt, as well as magnesium toxicity, are important limiting factors, but there is evidence that the scarcity of nutrients, calcium deficiency and water stress are also very important in reducing plant growth (Proctor and Nagy 1992).

Several ultramafic outcrops are present along the Alps and the Apennine chain in Italy (Vergnano Gambi 1992). In Tuscany a high number of such small to medium sized outcrops are spread throughout the region, and their most typical

A. Chiarucci (✉) · S. Maccherini
Department of Environmental Science "G. Sarfatti",
University of Siena, Via P.A. Mattioli 4,
53100 Siena, Italy
e-mail: Chiarucci@unisi.it

vegetation cover is garigue (described as *Armerio-Alyssetum bertoloni*). This vegetation type is characterized by reduced plant cover, low species richness and a high proportion of endemic species, including the well-known nickel-hyperaccumulator *Alyssum bertolonii* (Arrigoni et al. 1983; Vergnano Gambi 1992; Chiarucci et al. 1995; Chiarucci 2004). Recent investigations have suggested that the soil metal content is not the most important limiting factor for serpentine vegetation in Tuscany. Pine plantations were found to promote absolute values of species diversity, albeit with a significant shift in species composition and loss in serpentine species (Chiarucci and De Dominicis 1995; Chiarucci 1996). Moreover, in some favourable conditions, vegetation dynamics can lead to mature evergreen forests. Pine plantations and mature vegetation types were both found to have a higher content of exchangeable metals in the soil than garigues and other stunted vegetation types (Chiarucci and De Dominicis 1995; Chiarucci et al. 1998b; c). In a three-year fertilisation experiment, vegetation cover and productivity responded positively to nutrient addition (Chiarucci et al. 1998a, 1999), indicating that nutritional stress represents an important limiting factor for plant productivity. The addition of phosphorus was found to be the most significant in promoting vegetation productivity. An analysis of six years of data on the response of vegetation to fertilisation suggested that the changes induced by the addition of phosphorus remained for years after the cessation of fertilisation and that the interannual climate variability was an important factor in controlling vegetation diversity and productivity (Chiarucci et al. 2004). Significant effects of interannual climate variations on serpentine vegetation have been reported previously on annual serpentine grasslands at the Jasper Ridge Biological Preserve, Northern California (Hobbs and Mooney 1995). In both Tuscany and Northern California, the Mediterranean type of climate determines a summer drought that can last for several weeks. Summer drought is an important factor in Mediterranean ecosystems (Mitrakos 1980; Nahal 1981; Mazzoleni et al. 1992; Manes and Blasi 1995) and can have severe effects on community productivity and composition (e.g. Penuelas et al.2001; Lloret et al. 2004). In some dry years, the effects of summer drought can theoretically be magnified by serpentine soils, since they are subjected to higher drainage.

The aim of the present paper is to test the effects of phosphorus fertilisation and climatic variability on the vegetation cover and diversity of a garigue plant community on serpentine soils in Tuscany. The specific questions asked in our study are: (1) How long do the changes in community structure and diversity induced by fertilisation with phosphorus persist? (2) Does interannual climate variability affect the structure and diversity of the serpentine plant community and how does it interact with the alleviated phosphorus deficiency?

Materials and methods

Study area and vegetation

The fertilisation experiment was performed on an ultramafic outcrop located about 10 km west of Siena (Lat. 43°19′06′′ N, Long. 11°06′33′′ E), in the upper Elsa Valley, near the village of Pievescola. The climate of the site is sub-Mediterranean, with summer drought and a mean annual temperature of 13.5°C . January is the coldest month, with an average temperature of 2.3°C; August is the warmest month with 21.5°C on average. Annual rainfall averages 791 mm. July is the driest month with an average rainfall of 35 mm, and November is the wettest with 119 mm (Barazzuoli 1993).

The site selected for the experiment was a hillside covered by typical serpentine garigue (Chiarucci et al. 1995) with the presence of *Alyssum montanum*, a non-exclusive serpentine plant. Many serpentine endemic and locally preferential *taxa* were also present: *Armeria denticulata*, *Stipa etrusca*, *Thymus acicularis* subsp. *ophioliticus*, *Festuca inops*, *Plantago holosteum*.

Experimental design, treatments and data collection

To rigorously test the effects of fertilisation on the plant community, we conducted a 12 year before-after control-impact (BACI) experiment. The BACI design overcomes the problem of ascribing changes to impact rather than to natural variability (Stewart-Oaten et al. 1986). The eight 2 × 2 m plots used for the study belonged to a group of sixteen plots randomly established in early spring 1994 and submitted to a completely randomized fertilisation experiment, with four treatments and four replicates each. The treatments were: (i) control; (ii) fertilisation with nitrogen; (iii) fertilisation with potassium and (iv) fertilisation with phosphorus. The effects on the vegetation of the nitrogen and potassium fertilised plots disappeared very early (Chiarucci et al. 2004) and these plots were no longer sampled. The eight plots used in the present study were the four control plots (referred to as C) and the four plots that were fertilised with phosphorus (referred to as P). The latter plots were fertilised with 5 g m^{-2} of phosphorus (corresponding to 50 kg ha^{-2}) as NaH_2PO_4* H_2O in autumn 1994 (October 21st) and 1995 (October 21st).

At the end of May 1994, all vascular plants growing in each plot were recorded and their cover accurately estimated using the point quadrat method (Moore and Chapman 1986), with a density of 441 points/plot; this density is very high and allows the estimation of very small differences in vegetation cover. In each subsequent year, from 1995 to 2005, species presence and cover were recorded using the same method as 1994, during the same period (end of May – beginning of June). Vascular plants were all identified at the species or sub-species taxonomic level. Nomenclature of plants was according to Pignatti (1982) for most species and to Chiarucci (2004) for serpentine endemics and locally preferential *taxa*. Cryptogams were described using simple categories (bryophytes, fruticose lichens and foliose lichens). The response variables investigated were the percentage cover for both vascular and cryptogamic vegetation and the species richness data for vascular plants and life-form groups (Raunkiaer 1934; Cain 1950) such as therophytes, chamaephytes and hemicryptophytes.

Climatic data were obtained from the closest climate station (Scorgiano), located at about 10 km from the study site. For the following periods, climatic data were summarized according to the phenology of the vegetation and to the sampling dates, which always were between May 28th and June 6th: *summer* (June-August of the year before the sampling date), *autumn* (September–November), *winter* (December–February), *spring* (March–May). The climatic data were also summarised for the whole *year* before sampling (June of the previous year–May of the year of sampling), for the growing season (September of the previous year–May of the year of sampling), first six months (June–November of the year before sampling) and second six months (December of the previous year–May of the year of sampling). For each of these periods average temperature (in °C), total rainfall (in mm) and the ratio between total rainfall and average temperature were calculated. The climatic data observed during this period were extremely variable, as can be seen by their summary statistics (Table 1).

Table 1 Summary statistics (mean, minimum and maximum) of the climatic data observed in the 12 year study period

Climatic variable	Mean	Minimum	Maximum
T–Sum	21.8	20.2	24.7
P–Sum	106.8	19.0	237.0
P/T–Sum	5.0	0.8	11.1
T–Aut	14.0	12.4	15.5
P–Aut	277.5	96.0	392.5
P/T– Aut	20.0	7.4	30.9
T– Win	5.9	4.3	8.0
P–Win	162.1	85.4	240.0
P/T– Win	29.0	12.5	53.9
T–Spr	12.1	10.5	14.0
P–Spr	164.5	90.0	265.5
P/T–Spr	13.5	8.5	19.2
T–year	13.4	12.2	14.9
P–year	711.0	445.9	980.0
P/T– year	52.9	33.3	74.0
T–Grow	10.6	9.6	12.5
P–Grow	604.2	376.9	834.0
P/T– Grow	56.6	35.6	70.2
T–1–6 m	17.9	16.4	19.2
P–1–6 m	384.3	180.5	629.5
P/T– 1–6 m	21.6	10.8	35.4
T–2–6 m	9.0	7.8	11.0
P–2–6 m	326.7	175.4	504.0
P/T– 2–6 m	36.3	20.2	46.9

T: mean temperature in the period (in °C); P: total rainfall in the period (in mm). The periods are as follows: Sum: summer; Aut: autumn; Win: winter; Spr: spring; Year: whole year; Grow: growing season; 1–6 m: first 6 months; 2–6 m: second 6 months (see text for details).

Data analysis

To study the effects of fertilisation and time on total species richness, richness of different life-forms and on vascular and cryptogamic vegetation cover, a fixed factor two-way repeated measure analysis of variance was performed, using "year" as the repeated-measure (within-subject) factor in a general linear model (GLM). The data of percentage cover were arcsine-transformed. Mauchly's sphericity test for within-subject factors was also performed (Underwood 1997). When a significant interaction was observed, planned ANOVA comparisons were performed to analyse differences in the mean values of the two treatments.

Departure conditions of the fertilised plots with respect to control plots in terms of community composition were analysed by the principal response curve (PRC) technique (van den Brink and ter Braak 1997, 1998, 1999). This technique, derived from redundancy analysis (RDA), plots the principal components of the effects of treatment, expressed as deviations from the control treatment, against time (van den Brink and ter Braak 1998). PRC consists of the canonical coefficients of a partial redundancy analysis, in which the input data set are the sample-by-species matrix of log-abundance values, the sample-by-year matrix of covariables, and sample-by-treatment in year matrix of explanatory variables. The canonical coefficients of partial RDA (Cdt) quantify the compositional differences between the control and other treatments (d) at each sampling date (t): temporal trends can be visualized by plotting Cdt against t. The species weight b_k indicates the affinity that each individual *taxon* has with the overall community response as displayed in the PRC diagram: species with high positive values follow the overall community response, while in those with negative values the community response is the opposite.

Before performing this multivariate analysis, the abundance of plant species was transformed according to a logarithmic function [$ln(20x + 1)$], to downweight the influence of dominant species (van den Brink et al. 2000). The significance of the PRC diagrams was tested by Monte Carlo permutation tests (999 permutations), permuting whole time series in the partial RDA from which the PRCs were obtained. Statistical analysis was performed using the CANOCO 4.02 software package (ter Braak and Šmilauer 1998).

We used Spearman rank correlations to examine the relationship between total species richness, richness of different life-forms and cover of vascular and cryptogamic vegetation and predictor variables. The effects of climate fluctuations on community composition were tested using the 24 variables in Table 1. Many climate variables were included to reduce the possibility of omitting signals that are weak but biologically important (Taylor et al. 2002). To condense these data and to avoid non-orthogonality, the climatic variables were summarised as the four first axes of a standardized principal component analysis (PCA). A measure for the climate sensitivity of each treatment was derived as the percentage of the total species variation explained by the four composite variables (PCA axes) using partial redundancy analysis (pRDA; Jongman et al. 1995; Legendre and Legendre 1998) performed with CANOCO 4.02 (ter Braak and Šmilauer 1998). The covariables used were: plot identifier (plot ID) and year of sampling. We thus subtracted both the variability among individual plots and the successional changes. The remaining variance is predominantly due to climate. We tested the effects of climate on the control plots and the fertilised plots separately, using 12 years and 11 years respectively (thus excluding the year before treatment in the fertilised plots). Significance of the relationships was tested by Monte Carlo permutation tests (999 permutations).

Results

P fertilisation significantly affected only the cover of vascular and cryptogamic vegetation, while the factor year was significant for all the dependent variables tested (Table 2) except the species richness of chamaephytes, which was the single group of woody plants. This indicated that both the cover and the species richness values considered showed interannual fluctuations. The interaction year * treatment was significant for all the variables with the exclusion of the species richness of

Table 2 Repeated – ANOVA measurements of the effect of fertilisation (control *versus* P addition) on total species richness, richness of chamaephytes, hemicryptophytes and therophytes and percentage cover of vascular and cryptogamic vegetation

Source of variation	Total species richness			Chamaephyte species richness			Hemicryptophyte species richness			Therophyte species richness			Vascular vegetation cover (%)			Cryptogamic vegetation cover (%)		
	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P	DF	MS	P
Between subject																		
Treatment	1	1.76	0.823	1	0.844	0.586	1	1.50	0.615	1	2.344	0.348	1	**1.468**	**<0.001**	1	**0.126**	**<0.05**
Error	6	19.27		6	2.552		6	5.326		6	2.260		6	0.018		6	0.012	
Within subject																		
Year	11	**26.51**	**<0.001**	11	0.238	0.469	11	**9.701**	**<0.001**	11	**11.321**	**<0.001**	11	**0.042**	**<0.001**	11	**0.013**	**<0.001**
Year × Treatment	11	**5.26**	**<0.05**	11	0.139	0.841	11	0.659	0.225	11	**3.185**	**<0.01**	11	**0.030**	**<0.001**	11	**0.017**	**<0.001**
Error	66	2.48		66	0.241		66	0.493		66	1.177		66	0.002		66	0.001	

Sphericity assumption for within-subject factors was met (Mauchly's sphericity test was not significant for all within-subjects factors tested)

chamaephytes and hemicryptophytes, indicating that the effects of the P fertilisation interacted with climate variability (Table 2, Fig. 1). Planned pairwise comparisons showed that significant differences between control and P fertilised plots existed for: total species richness in years 2003 and 2004; therophyte species richness in year 2001 only; vascular vegetation cover from 1995 onward, and for cryptogamic vegetation cover from 2001 onward.

The results of the Principal Response Curves showed that differences between years explained 48% of the differences in species composition between samples, while differences among replicates explained 35.3%. The remaining 16.7% of the variation in species composition was explained by the differences in treatment (control vs. P-fertilisation). The fertilisation therefore had a lesser effect on species composition than the interannual difference (possibly due to climatic variability). The first PRC diagram was significant ($P = 0.02$) and showed marked differences between treatments (Fig. 2). According to the Monte Carlo permutation test, the second PRC diagram was not significant ($P = 0.52$). The P fertilised plots had a strong similarity with the control plots in 1994, indicating comparable species composition of the communities in the year preceding fertilisation. They became less similar over the subsequent three years of observation, reaching a marked dissimilarity in 1999, which remained almost stable in the following years. *Psilurus incurvus*, *Echium vulgare*, *Sedum album*, *Herniaria glabra*, *Filago germanica*, together with fruticouse and foliose lichens, showed the highest weights (b_k) for the PRC. The cover of these vascular plant species and groups of cryptogams was therefore significantly promoted by P fertilisation (Fig. 2). *Cerastium ligusticum*, *Brachypodium distachyum*, *Sedum rupestre* and bryophytes also showed a relatively higher cover in P fertilised plots with respect to the control plots (Fig. 2). On the other hand, the serpentinophytes *Stipa etrusca* and *Thymus acicularis* ssp. *ophioliticus*, together with *Cistus monspeliensis*, showed a reduction in their cover value in the P fertilised plots.

According to the Spearman rank correlations (Table 3), the climatic variables had different effects on the vegetation. Climate was not found

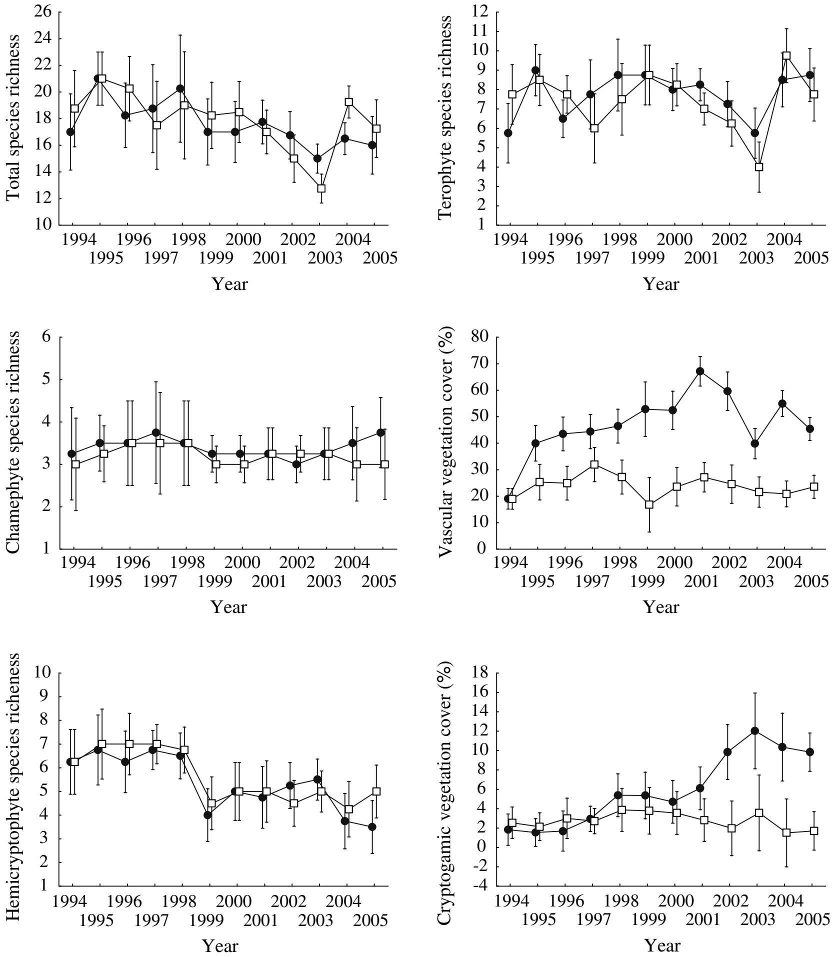

Fig. 1 Yearly patterns of vegetation response variables (mean) in control plots (open squares) and fertilised plots (closed circles) from 1994 to 2005. Vertical bars denote 0.95 confidence intervals

to have significant effects on total cover of vascular and cryptogamic species in control plots, but showed significant effects on the same variables in the fertilised plots. In the P fertilised plots the total cover of vascular vegetation was positively affected by the temperature during the year, especially during the first part of the growing season (*autumn* and *first six months*), and by the amount of rainfall during the spring, i.e. the last period of the growing season. The total cover of the cryptogamic vegetation was positively related to the total amount of rainfall (and P/T ratios) during different periods of the growing season.

In the control plots, total species richness was negatively affected by the total amount of rainfall during both the winter and the whole year preceding sampling, and also by spring temperature. In fertilised plots, total species richness was affected positively by winter temperature and negatively by the winter P/T ratio. Climate had significant effects on the species richness of different life–forms, which were quite similar between control and fertilised plots, but more pronounced in the former (Table 3). In particular, the species richness of geophytes was negatively affected by the total amount of rainfall and the P/T ratio during different periods of the growing season. The species richness of hemicryptophytes was negatively affected by the temperature during different periods of the growing season. On the other hand, species richness of therophytes was negatively affected by the total amount of rainfall during the first part or the whole length of the growing season.

The first four axes of the PCA performed on the climatic variables explained about 90% of the total variance in the original climatic data (see Table 4). The coefficients of the principal

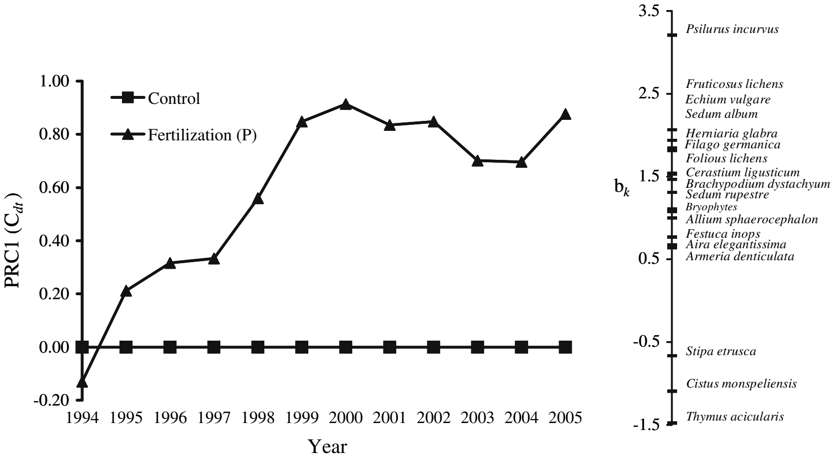

Fig. 2 First Principal Response Curves diagram, showing the effects of fertilization on serpentine vegetation over time. Species weight on the right (b_k) can be read as the affinity of every species with the diagram shown (PRC 1). The *taxa* with a variance in the abundance values of less than 5%, as explained by PRC1, are not displayed (but not omitted from the analyses). The reasons for low values are several: they showed no differences between treatments, showed a very different response than indicated by PRC1, or occurred in very low numbers

Table 3 Spearman rank correlation between climatic factors and vegetation response variables: total species richness (TSR); species richness of chamaephytes (ChR), hemicryptophytes (HR), geophytes (GR), therophytes (TR); vascular vegetation cover (VVC) and cryptogamic vegetation cover (CVC)

Climatic variable	Control Plots							Fertilised Plots						
	VVC	CVC	TSR	ChR	HR	GR	TR	VVC	CVC	TSR	ChR	HR	GR	TR
T–Sum	−0.53	−0.39	−0.09	−0.45	**−0.81**	0.08	0.56	0.56	0.32	−0.43	**−0.74**	**−0.77**	0.08	0.31
P–Sum	0.49	0.26	−0.30	0.46	0.35	**−0.58**	**−0.60**	−0.13	0.26	0.00	0.51	0.28	**−0.67**	−0.18
P/T–Sum	0.55	0.27	−0.26	0.49	0.46	−0.53	**−0.63**	−0.20	0.17	0.09	**0.61**	0.38	**−0.60**	−0.19
T–Aut	0.10	−0.15	−0.43	−0.43	−0.41	−0.03	−0.11	**0.59**	0.42	−0.23	−0.17	−0.36	−0.12	0.15
P–Aut	−0.01	0.00	−0.48	−0.21	−0.26	**−0.65**	−0.48	−0.06	0.50	−0.30	0.01	−0.06	−0.52	−0.20
P/T– Aut	−0.10	0.10	−0.37	−0.14	−0.15	−0.57	−0.37	−0.21	0.30	−0.19	−0.08	0.01	−0.44	−0.18
T– Win	0.55	0.14	0.34	−0.08	0.32	0.38	0.03	0.01	−0.36	**0.67**	0.16	0.29	0.40	0.47
P–Win	−0.05	0.11	**−0.58**	0.16	−0.37	**−0.85**	−0.31	0.36	**0.70**	−0.49	0.01	−0.49	**−0.79**	−0.08
P/T– Win	−0.33	−0.06	−0.55	0.19	−0.47	**−0.78**	−0.13	0.35	**0.63**	**−0.65**	−0.13	−0.56	**−0.74**	−0.20
T–Spr	−0.13	0.45	**−0.68**	**−0.63**	−0.50	−0.22	−0.24	0.45	0.44	−0.29	−0.29	−0.48	−0.40	0.04
P–Spr	0.24	0.25	−0.01	−0.13	−0.28	0.03	−0.01	**0.60**	0.32	0.03	0.20	−0.43	0.04	0.18
P/T–Spr	0.28	0.08	0.10	0.00	−0.15	0.13	0.03	0.57	0.16	0.09	0.25	−0.34	0.17	0.07
T–year	−0.05	0.03	−0.24	−0.57	**−0.65**	0.05	0.18	**0.67**	0.43	−0.17	−0.38	**−0.59**	−0.04	0.45
P–year	0.24	0.20	**−0.62**	0.06	−0.22	−0.82	**−0.58**	0.27	**0.68**	−0.30	0.21	−0.19	**−0.82**	−0.09
P/T– year	0.18	0.26	−0.54	0.16	−0.14	**−0.82**	−0.51	0.13	0.57	−0.26	0.16	−0.14	**−0.82**	−0.08
T–Grow	0.19	0.35	−0.32	**−0.60**	−0.22	0.12	−0.17	0.31	0.22	0.08	−0.08	−0.13	−0.03	0.29
P–Grow	0.08	0.25	−0.52	−0.13	−0.41	**−0.70**	−0.36	0.34	**0.66**	−0.29	0.03	−0.40	**−0.66**	0.08
P/T– Grow	−0.03	0.10	−0.51	0.07	−0.39	**−0.88**	−0.38	0.20	**0.73**	−0.41	0.06	−0.31	**−0.76**	−0.04
T–1–6 m	−0.33	−0.36	−0.17	−0.48	**−0.79**	0.00	0.39	**0.67**	0.48	−0.41	**−0.59**	**−0.76**	0.04	0.33
P–1–6 m	0.22	0.22	−0.46	−0.02	−0.06	**−0.66**	**−0.60**	−0.07	0.49	−0.17	0.18	0.08	**−0.64**	−0.16
P/T– 1–6 m	0.21	0.24	−0.48	−0.06	−0.01	**−0.63**	**−0.63**	−0.13	0.43	−0.14	0.18	0.13	**−0.60**	−0.21
T–2–6−m	0.17	0.43	−0.30	**−0.64**	−0.19	0.13	−0.23	0.24	0.13	0.17	−0.08	−0.07	0.04	0.20
P–2–6–m	−0.03	0.10	−0.29	0.19	−0.40	−0.57	−0.03	0.56	**0.62**	−0.35	−0.02	**−0.62**	−0.45	0.13
P/T– 2–6 m	−0.01	−0.08	−0.27	0.31	−0.29	**−0.58**	−0.10	0.50	0.56	−0.38	0.12	−0.54	−0.41	−0.08

Correlations are calculated separately for control and fertilised plots. Significant values ($P < 0.05$) are in bold. Climatic variables are abbreviated as in Table 1

Table 4 The eigenvector coefficients (loadings) of standardized principal component analysis of original climatic variables for the period 1994–2005 (see Table 1 for abbreviations of variables)

Climatic variable	Axis 1	Axis 2	Axis 3	Axis 4
T–Sum	0.0597	**−0.535**	−0.0824	**−0.7688**
P–Sum	**0.6649**	**0.5231**	0.1439	0.3253
P/T–Sum	**0.6288**	**0.5522**	0.1439	0.3735
T–Aut	0.3046	**−0.7256**	−0.0594	−0.0506
P–Aut	**0.7164**	0.2201	**−0.5769**	−0.2329
P/T– Aut	**0.6306**	0.375	**−0.5613**	−0.2162
T– Win	0.2193	−0.444	**−0.4635**	**0.5868**
P–Win	**0.8231**	0.0439	0.4323	−0.2306
P/T– Win	**0.53**	0.2668	0.5671	−0.4422
T–Spr	**0.5768**	**−0.5211**	−0.1607	−0.0215
P–Spr	0.3285	**−0.6879**	0.4131	0.3787
P/T–Spr	0.1566	**−0.5545**	**0.5507**	0.3865
T–year	0.428	**−0.8252**	−0.3087	−0.1441
P–year	**0.9912**	0.107	0.0271	0.0479
P/T– year	**0.9336**	0.3361	0.0793	0.0692
T–Grow	**0.4769**	**−0.7367**	−0.3248	0.2467
P–Grow	**0.9641**	−0.1175	−0.0353	−0.0974
P/T– Grow	**0.8941**	0.179	0.0593	−0.2331
T–1–6 m	0.1699	**−0.7353**	−0.0995	**−0.5879**
P–1–6 m	**0.8387**	0.4201	−0.3293	0.0033
P/T– 1–6 m	**0.7795**	**0.5258**	−0.323	0.0847
T–2–6–m	**0.504**	**−0.6376**	−0.4092	0.3608
P–2–6–m	**0.7288**	−0.4011	**0.5328**	0.0895
P/T– 2–6 m	**0.5657**	−0.1573	**0.7953**	−0.0802

The eigenvalues of the first four axes are: axis 1 = 0.406, axis 2 = 0.245, axis 3 = 0.143, axis 4 0.103 (sum = 89.7% of the total variance explained). In bold are the loadings >0.45, to highlight the meanings of the respective axes. Climatic variables are abbreviated as in Table 1

components (loadings) for all standardized original climatic variables indicated that the first PCA axis primarily accounted for the rainfall and P/T ratio, while the second PCA axis mainly accounted for the temperature and spring rainfall. Axis 3 accounted for the effects of winter temperature and P/T ratio during the second part of the growing year, while axis four mainly accounted for summer and winter temperatures. These composite variables fluctuated over time (Fig. 3) and showed: (i) a slight increase for axes one and three; (ii) a slight decrease for axes two and four. Neither the control nor the fertilisation treatments were significantly associated with climate: variance explained by pure climate effect was 24.4% ($P = 1$) for control plots and 24% ($P = 0.196$) for plots fertilised with P.

Discussion

The addition of a limited amount of phosphorus for two consecutive years resulted in a marked increase in vegetation cover that was maintained for many years. These results confirm those provided by the analysis of the first six years of this experiment (Chiarucci et al. 2004) and those of a three year experiment on the serpentine vegetation of another site in Tuscany (Chiarucci et al. 1998a, 1999). Other papers have reported comparable results for the serpentine sites of California (Koide et al. 1988; Huenneke et al. 1990) and the Shetland Islands (Spence and Millar 1963; Carter et al. 1988; Slingsby 1991). The long-lasting increase in vegetation cover observed in the present study is noteworthy since the plant communities of the plots studied were mainly composed of annual species (therophytes) which, by their nature, are not able to accumulate biomass in their vegetative structure.

The effects of phosphorus fertilisation on species richness were not as significant and contrasted with the positive effects of phosphorus fertilisation observed in a shorter (three year) experiment performed on another serpentine site in Tuscany (Chiarucci et al. 1998a). Similar fertilisation experiments on a serpentine soil on the Isle of Rum, Scotland, showed an increase in total ground cover and species richness, together with changes in species composition (Ferreira and Wormell 1971; Looney and Proctor 1989). These changes were also maintained for several years after the cessation of fertilisation (Slingsby 1991; Proctor and Nagy 1992). On the other hand, Huenneke et al. (1990) reported a decrease in species richness and an invasion of alien species after soil enrichment with nitrogen and phosphorus in Californian serpentine grasslands. Different results regarding species richness were reported after the addition of phosphorus to other soil types. For example, P fertilisation on a calcareous grassland was found to not affect overall species richness species but only the species richness of annual legumes (Sternberg et al. 1999). Grime's model (1979) states that the addition of nutrients such as phosphorus should increase productivity by promoting the abundance of dominant species (Wilson et al. 1996); accordingly, the increase of

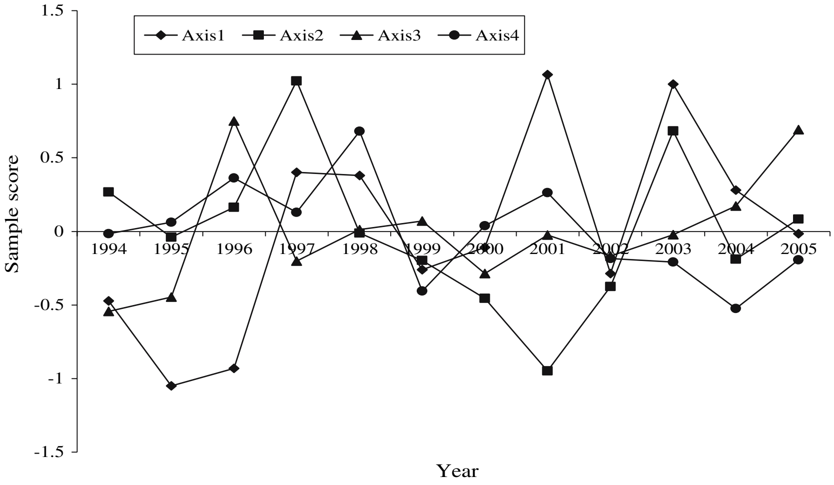

Fig. 3 Fluctuations of four PCA axes (composite variables = sample scores) over the total study period, representing the variance of 24 original climatic variables

dominant species in a resource limited habitat such as serpentine could determine a reduction in the richness of local species. In this experiment, a marked and long-lasting effect on vegetation productivity (quantified by the proxy of the plant cover), although it had limited effects on plant species richness.

The P fertilisation determined significant changes in the abundance of some species, but important shifts in species composition were not observed. Some species were positively affected by the addition of phosphorus: in particular *Psilurus incurvus*, *Echium vulgare*, *Sedum album*, *Herniaria glabra*, *Filago germanica*, together with fruticouse and foliose lichens. All these species are very common but not exclusive to the natural vegetation of this and other serpentine sites in Tuscany (Chiarucci et al. 1995). On the other hand, the endemic serpentine *taxa Stipa etrusca* and *Thymus acicularis* subsp. *ophioliticus*, together with *Cistus monspeliensis*, showed their highest cover values in the control treatment but were not excluded from the fertilised plots. A marked shift in species composition and a decrease in the typical serpentine species was reported for P fertilisation experiments in other serpentine sites. In an experiment performed on a serpentine site in California, P fertilisation permitted the invasion and dominance of non-native annual grasses in patches originally dominated by native annual forbs (Huenneke et al. 1990). In the fertilisation experiments performed on the Isle of Rum, Scotland, the addition of nutrients also determined a shift in vegetation composition from barren debris with small patches of species rich *Calluna vulgaris* heath to species-rich *Agrostis-Festuca* grassland (Looney and Proctor 1989). The experiments performed in California and Scotland suggested that the colonisation of oligotrophic serpentine plant communities by non-serpentine plants is a consequence of changes in soil nutrient availability. This hypothesis was not confirmed by the present study and it is likely that factors other than nutrient availability prevented the invasion of serpentine substrates by other species on this site.

Significant correlations were found between the climate variables and vegetation, even though the vegetation response was different in control and P fertilised plots. Other studies have reported significant effects of the climate on serpentine vegetation. For example, climatic effects were found to control the diversity of the flora at the outcrop scale in the serpentines of California (Harrison et al. 2000). Plot scale investigations on serpentine soils of climate-vegetation relationships have only been performed by Hobbs and Mooney (1991, 1995), whose main aim was to analyse the relation to gopher disturbance. In the present study, the climatic variables showed different effects on plant community cover and species richness. Interestingly, the effects of climate on total species richness differed between the control and fertilised plots, suggesting that, in this nutrient poor habitat, the effects of climate may change according to the nutritional status of the soil. In control plots, total species richness was negatively affected by the total amount of rainfall during both the winter and the whole year preceding sampling, as well as by the spring temperature. Thus, a wet climate reduced the species richness within a plot, as did a warm period in the last vegetative period. Total species richness in the fertilised plots was negatively related to the winter P/T ratio and positively affected by winter temperature. In the same fertilised plots the winter rainfall promoted the total vegetation cover. Thus, when soil is amended, the winter rainfall could favour the dominant species of the community at the expenses of less abundant species, resulting in a lower value of species richness. This model is more likely to fit Grime's model (1979) and it is likely that when soil resources are less limited the serpentine vegetation resembles more "normal" vegetation. In grasslands growing on other soil types, positive relationships between precipitation and richness are common (Cornwell and Grubb 2003; Adler et al. 2005). However, as observed by Adler and Levine (2007), the effects of climate on species richness at the plot scale are often difficult to demonstrate, even when the relationship between these two variables is clear at a larger, regional scale: this is probably due to the community's lagged response to the climate.

The species richness of the different life-forms responded differently to the climate. The species richness of geophytes was negatively influenced by rainfall during most of the periods considered. This result apparently contrasts the findings of Avinoam and Orshan (1990), who found a positive correlation between the number of geophytes and the amount of rainfall. However, throughout the twelve year study period the number of geophytes per plot in this study was very low (from 0 to 2), thus limiting the value of the correlation; Avinoam and Orshan (1990) found a higher number of geophytes also at very low rainfall levels. The very different spatial scale of these studies is likely to represent the key to this marked difference. The negative relationship between temperature during different time periods and the species richness of hemicryptophytes confirmed that this group, which is dominant in temperate ecosystems, is only marginal in xeric habitats under a marked Mediterranean climate and can be significantly reduced in richness by limited fluctuations in xericity (Raunkiaer 1934; Cain 1950; Orshan 1986). On the contrary, the species richness of therophytes was negatively related to the total amount of rainfall during the first part or the whole length of the growing season, emphasising their strong relationship with the xeric conditions of Mediterranean habitats (Raunkiaer 1934; Cain 1950; Orshan 1986; Kutiel et al. 2000) that are particularly marked on serpentine soils (Proctor and Nagy 1992; Vergnano Gambi 1992; Chiarucci 2004). As observed by Lloret et al (2004), experimental evidence on the effects of climate change on the richness of plant species under field conditions is still lacking and long-term studies in xeric habitats, such as on serpentine soils, could provide insights into earlier trends of species diversity in response to climatic changes.

According to our analyses, there was no significant effect of climate on community composition. This was an unexpected finding, since Mediterranean vegetation is strongly dependent on climatic parameters, with special regard to very xeric habitats (Raunkiaer 1934; Avinoam and Orshan 1990; Blasi et al. 1990; Federici and Pignatti 1991; Mazzoleni et al. 1992; Lloret et al. 2004). This observation, combined with the finding that the most important climatic effects on species richness and vegetation cover were found in the fertilised plots but not in the control plots, suggests that these serpentine plant communities are subjected to a combination of climatic and nutritional stress that prevents their dynamic succession. Only when nutritional stress is removed, or at least reduced, by P fertilisation does the climatic effects become more evident, albeit still weakly. It can be hypothesized that reducing soil nutritional stress by P fertilisation makes these serpentine plant communities more similar to other vegetation types under a Mediterranean climate that are subjected to well defined climatic determinants (Raunkiaer 1934; Mitrakos 1980; Avinoam and Orshan 1990; Lloret et al. 2004). The observations that emerged from this long-term investigation lead us to conclude that the plant communities on serpentine soils in Tuscany represent a steady-state community type, with marked adaptations to the xeric and nutrient poor conditions of this habitat and minimally prone to changes in species composition, except than when extensively subjected to pine plantations as demonstrated by Chiarucci and De Dominicis (1995) and Chiarucci (1996).

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