Plant and Soil (2005) 270: 355–369
DOI 10.1007/s11104-004-1793-z

Vegetation in contrasting soil water sites of upland herbaceous grasslands and N:P ratios as indicators of nutrient limitation

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Received 7 June 2004. Accepted in revised form 4 August 2004

Key words: competition, fertilization experiment, Northern Greece, nutrient use efficiency, resource ratio model

Abstract

Effects of differences in long-term water supply were examined on soil characteristics, primary production and species composition in a wet and a dry site of an upland herbaceous grassland. Also the responses of species to N and P enrichments were examined. N and P concentrations of non-legume species were positively related, indicating that biomass N:P ratios seem to be mainly determined by N:P supply ratios. Forbs had generally higher concentrations than graminoids. Intermittent water inundation of soil in the wet site resulted in greater soil N and P availability. The greater productivity of this site promoted the growth of forbs. A fertilizer experiment showed that biomass was limited by N only in the wet site, but by both nutrients in the dry one. The species with the higher N and P concentrations were favored more after N and P enrichment, respectively; however, species enhancement was not related to N:P ratios of species. This indicates that N and P concentrations of species, rather than N:P ratios of species, are better predictors of species responses to N and P enrichment. N:P ratios of whole communities were 8.73 for the wet and 11.36 for the dry site. These values in comparison with the responses of plant communities to N and P fertilization show that thresholds of N:P ratios indicative of N or P limitation are much lower than those found for European wetlands.

Introduction

The influence of soil resources on primary production, community structure, and diversity has been demonstrated in many plant communities after these communities were enriched with nutrients and water (Elisseou et al., 1995; Goldberg and Miller, 1990; Knapp et al., 2001; Mamolos and Veresoglou, 2000; Mamolos et al., 1995a, b; Wilson and Tilman, 1991) or from observations made along gradients of soil fertility and/or soil moisture (Briggs and Knapp, 1995; Collantes et al., 1999; Lane et al., 2000; Tilman and Wedin, 1991). Effects of soil resources limitation are more pronounced in low-productive soils, where primary production is low and plant community is dominated by species using more efficiently the most limiting recourses (Mamolos et al., 1995b; Tilman, 1982; Tsialtas et al., 2001).

Availability of soil water influences primary productivity and species composition in two ways: (a) directly since water is a resource for plant growth and as such it can expand the growing period during the summer, permitting more species to share the temporal niche dimension, and (b) indirectly by affecting availability of nutrients and modifying soil characteristics like organic matter content of soil (Patric and Mahapatra, 1968). Long-term intermittent saturation of soil with water in dry areas creates anoxic conditions, which promote the accumulation of organic matter and both higher water availability and greater soil organic matter enhance the availability of nutrient and facilitate the penetrability by roots (Rubio et al., 1997). All these processes usually result in increased soil productivity.

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The main growth-limiting nutrients for plants in natural environments are nitrogen and phosphorus (Güsewell and Koerselman, 2002; Koerselman and Meuleman, 1996; Tessier and Reynal, 2003; Vitousek and Howarth, 1991). For this reason studies on mineral nutrition has been focused mainly on these nutrients (Aerts and Chapin, 2000). One way to investigate nutrient-limited plant growth in terrestrial ecosystems is to examine the response of vegetation to nutrient additions. Although often straightforward, these experiments are lengthy and labor-intensive (Bennett and Adams, 2001; Van Duren and Pegtel, 2000). In addition, chemical adsorption and microbial immobilization, in some cases, decrease the availability of supplied nutrients, complicating interpretation of results (Aerts and Chapin, 2000). For these reasons, nutrient ratios have long been used to predict nutrient limitations (Aerts et al., 1992; Ingestad, 1979).

Koerselman and Meuleman (1996) proposed that critical N:P ratios of above-ground biomass (i.e. threshold values) could be used to predict species and community-level N and/or P limitations. Their analysis, based on 40 separate N and P nutrient addition experiments in European wetlands, demonstrated that N:P < 14 was indicative of N limitation and N:P > 16 was indicative of P limitation. The results of Güsewell et al. (2003) were mostly consistent with these critical ratios as regards the distinction between N limitation and P limitation, both at the community and at the species level. Also, Olde Venterink (2000) suggested that an N:P ratio (at the community level) of 14.8 in wetland vegetations discriminates between N- and P limited sites.

Assessments of plant N:P ratios using experimental N and P additions for terrestrial vegetation types other than those for wetlands are fewer and those for grasslands are rare.For upland vegetations, Tessier and Raynal (2003) indicated that the threshold N:P ratios to N- and P-limitation, at the community level, are much lower than those found for European wetlands. Lower thresholds were also found in an African savanna system (Ludwig et al., 2001) and in a semi-arid Australian grassland (Bennett and Adams, 2001), but greater on alpine meadows (Bowman, 1994). These examples indicate that threshold N:P ratios to N- or P-limitation are determined in different N:P ratios from those found in wetlands and may differ in the various grassland types. These examples also show the necessity for further work to be done to determine precisely the thresholds of N:P ratios in the various grassland types.

Subjects of considerable debate are related to whether N:P ratios of individual species appear to depend on the particular ecological context in which species have grown or by species-specific physiological requirements for N and P, and whether N:P ratios of individual species could be used as predictors of N or P limitation. In his "resource ratio model", Tilman (1997) postulates that plants tend to take up nutrients in the proportions needed for growth, independently of the supply ratio. On the other hand, Güsewell et al. (2003) found that N:P ratio in plant biomass reflect the relative amounts of N and P available to plants, as was assumed by Koerselman and Mauleman (1996), resulting in significant correlation between variation in N:P ratios of individual species and variation in N:P ratios of the whole vegetation (Braakhekke and Hooftmann, 1999; Roem and Berendse, 2000).

Koerselman and Mauleman (1996) hypothesized that N:P ratios of individual plant species might indicate their responses to fertilizer in a similar way as for the whole vegetation: species with high N:P ratios would be enhanced by P enrichment, while species with low N:P ratios would be enhanced by N enrichment. On the other hand, the ‘resource ratio model’ (Tilman, 1982) predicts that if N and P are potentially limiting for plant growth in a community, fertilization with N will cause P to be relatively scarcer and promote species that are strong competitors for P. In the long term, the latter may outcompete species that were the strongest competitors as long as N was scarce. Fertilization with P would induce the opposite effect. Furthermore, the model proposes that the concentration of a nutrient in plant biomass indicates the competitive ability of a plant species for this nutrient: strong competitors are assumed to have a low concentration and weak competitors a high concentration (Tilman, 1990). According to this model, the species with the lowest tissue concentrations for the most limiting nutrient would dominate in a plant community, as Mamolos et al. (1995b) found, and fertilization with this nutrient should favor proportionally more the growth of species which have the highest concentrations of this nutrient (Mamolos and Veresoglou, 2000; Mamolos et al., 1995b; Tilman et al., 1999).

Herbaceous grasslands in northern Greece, which are found in altitudes more than 1000-m, consist almost of perennial C_3 species, are dominated usually by grasses and limited by soil water, N and P (Elisseou et al., 1995; Mamolos et al., 1995a; Tsialtas et al., 2001). Low winter temperatures and a summer drought shorten the plant growth from mid April to late August (Papanastasis, 1981). Soil physicochemical characteristics and relief influence primary productivity, which is usually low, and the species composition**.** In some grasslands the surface soil is inundated in patches by the upward movement of water from greater depths. It enhances variation in physicochemical characteristics of soil and consequently variation in productivity and species composition of plant communities.

By working in two sites, differing in water regime, of upland grassland, the first aim of this work was to examine the long-term effect of soil saturation with water on the physicochemical characteristics of soil, especially on availability of N and P as well as on primary productivity and plant species composition. The second aim was to examine whether total vegetation and individual species could respond to fertilization with N and P. We addressed the following questions: (1) could responses of vegetation and of individual species, after N and P enrichment, be predicted by N:P ratios? If so, (2) are the threshold N:P ratios of N- or P-limitation for plant growth similar to those found for wetlands? (3) Could N:P ratios of individual species be determined by the relative availability of N and P or by species-specific physiological requirements for N and P? (4) Are the N:P ratios or the absolute N and P concentrations of individual species better indicators of species responses to N or P fertilization?

Materials and methods

Study site

The study site is an upland grasslands 160 km west of Thessaloniki in northern Greece (40°48′ N, 21°23′ E; 1340 m alt.) with soil classified as Typic Xerothrents (Soil Survey Staff, 1975) and annual precipitation 805 ± 37 mm (mean ± 1 SD) for the period from 1980 to 1994 (Elisseou et al., 1995). The most common herbaceous species are shown in Table 1. Vegetation is quite heterogeneous in species composition as a result of high variations in relief and soil depth and soil water content as well as nutrients availability. In an about 5 ha flattened area, two sites were selected, which have similar soil depths (no more than 35 cm) and consist of the same plant species, but differ substantially in both water availability and species composition. Within each site the above-ground biomass and species composition was visually homogeneous. Some of the physicochemical characteristics of soil in the two sites are shown in Table 2. In the wet site, soil is inundated by the upward movement of soil water; soil is saturated with water for a long time continuously during winter and spring. Since the two sites are about 100-m apart, and soil in both sites must have been developed from the same parental rock, we could hypothesize that differences between sites in primary productivity and plant species composition are reflections of long term differences in water availability.

Table 1. Vascular plant species present in the two studied sites: The 16 (sufficiently abundant species in both sites) species with abbreviations in parentheses were included in the statistical analysis shown in Table 3. Nomenclature follows Tutin et al. (1964–80)

Graminae	**Other families**
Anthoxanthum odoratum (Ao)	*Achillea ageratifolia*
Agrostis capillaris (Ac)	*Achillea millefolium*
Dactylis glomerata	*Centaurea affinis* (Ca)
Cynosurus crystatus (Cc)	*Cerastium semidecandrum* (Cs)
Phleum pratense (Pp)	*Cichorium intybus*
Poa bulbosa (Pb)	*Euphorbia cyparissias*
	Euphrasia pectinala
Cyperaceae	*Galium lucidum* (Gl)
Carex nigra (Cn)	*Linum trigynum*
Carex caryophyllea (Cca)	*Origanum vulgare*
	Ornithogalum sp.
Leguminosae	*Plantago lanceolata* (Pl)
Dorynchium herbaceum	*Potentilla argentea*
Lotus corniculatus (Lc)	*Prunella vulgaris* (Pv)
Lotus angustisimus	*Ranunculus psilostachys* (Rp)
Trifolium heldreichianum (Th)	*Rumex acetosella*
Trifolium medium	*Sanquisorba minor*
Trifolium repens (Tr)	*Taraxacum officinale*
Vicia vilosa	*Teucrium chamaedrys*
	Thymus sibthorpii

Fertilization experiment

In each site, a 2 × 2 factorial combination of N (0 and 15 g m^{-2}, as urea) and P (0 and 10 g m^{-2}, as superphosphate) was applied in October of 1996 to the plots, which were fully randomized within each of the four blocks. The dimensions of plots were 6.0 × 2.0-m in the control plots and 2.0 × 2.0-m in the other plots.

The above-ground biomass of every species was assessed on 25 June in 1997 by cutting the above-ground vegetation at ground level from two 50 × 50-cm quadrats randomly selected in the central 1.5 × 1.5-m square of each of the enriched with nutrients plots and of the centre of the one third (i.e. 2.0 × 2.0-m) of the control plots. The above-ground biomass was separated and sorted into the component species, dried at 75 °C for 48 h and weighed.

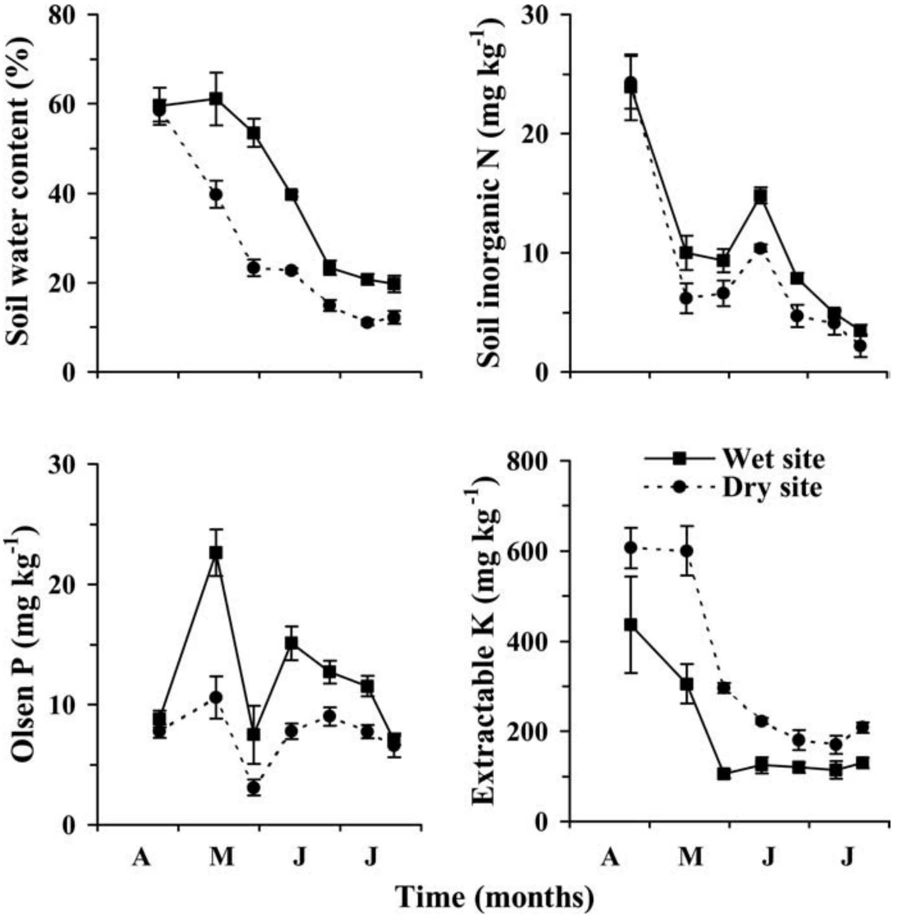

Figure 1. Changes in water content, inorganic N, Olsen extractable P and extractable K of soil during the growing season in 1997. Error bars show ± 1 SE; $n = 4$.

Soil and vegetation samplings during the growing period

Seven vegetation samplings were conducted (23 April, 14 and 28 May, 12 and 25 June, and 8 and 17 July) on the 4.0 × 2.0-m area of the both sites control plots during the growing period of 1997. On each occasion, three 25 × 25-cm quadrats were selected randomly within each plot and above-ground vegetation was cut at ground level, separated and sorted into the component species, dried at 75 °C for 48 h, weighed and ground. The ground material of the three samples of each species was mixed for every control plot and a sub-sample was analyzed for N and P determination. Nitrogen was determined by the Kjeldahl method. For P, the ground material was digested with triple acid reagent HNO_3: H_2SO_4: $HClO_4$; 10:1:1 (Allen, 1989) and total P was determined spectophotometrically at 882 nm, using Murphy and Riley's (1962) reagent, with Perkin-Elmer Lambda 5, Norwalk, Connecticut, USA.

From the centre of every 25 × 25-cm quadrat a soil sample was taken by the 0–15 cm soil layer to assess temporal variation of soil characteristics. Before chemical analysis the air-dried soils of the three samples of each control plot were mixed. Soil moisture content was determined by the soil weight difference before and after drying at 105 °C for 48 h. Inorganic N was extracted by air-dried soil with $2M$ KCl (Keeney and Nelson, 1982) and was determined by micro-Kjeldahl method (Allen, 1989). Olsen extracted P was determined as for plant material. Potassium was extracted with neutral $1M$ NH_4COOCH_3 and measured by flame photometry with Jenway Pep 7, Dunmow, Essex, UK.

Table 2. Physicochemical characteristics of the surface soil layer (0–15 cm) of the experiment sites

Site	Soil type^a	Sand (%)^a	Silt (%)^a	Clay (%)^a	pH^b matter (%)^c	Organic (g kg^{-1})	Total N (cmol$^{(+)}$ kg^{-1})^d	CEC
Wet	SL	68	19	13	6.40	28.52	13.74	31.20
Dry	SL	74	18	8	5.80	20.35	10.78	26.40

[a] Hydrometer method.
[b] In 0.01 *M* $CaCl_2$ 1:2·5 (w/v).
[c] Wet oxidation.
[d] Soils were extracted with 0.5 *M* $BaCl_2$-TEA, pH 8.2.

Calculations

Because concentrations in plants vary during the growing period, we used, as more appropriate, the weighted above-ground N and P concentrations for the whole growing period (Mamolos et al., 1995b). They were calculated for every of the sufficiently abundant species in the control plots by the ratio of species above-ground N (or P) content duration to species above-ground biomass duration (Hunt, 1978; Mamolos et al., 1995b). Above-ground biomass (or N or P content) duration was calculated by the stepwise calculation, using the trapezoidal rule [surface area $= 0.5 \cdot (B_1 + B_2) \cdot (t_2 - t_1)$, where B_1 and $B_2$the above-ground biomass (or N or P content) at the times t_1 and t_2] and assuming that above-ground biomass (or N or P content) varies linearly between two successive harvests.

Statistical analyses

Data of above-ground biomass of total community, total grasses, total forbs and total legumes as well as the sufficiently abundant individual species were subjected to two way ANOVA analysis, separately for each site, using N and P additions as main factors. Furthermore, combined ANOVA analyses of the two sites data were performed to evaluate effects of site.

Combined ANOVA analysis was performed on data of weighted N or P concentrations of the 16 sufficiently abundant species and common in the two sites using site and species as main factors. This analysis was completed with orthogonal comparisons between species groups, i.e. grasses, forbs (i.e. non-legume dicots), legumes and sedges, within each site and between sites within each group of species.

Linear correlation analyses were performed between N and P concentrations of species to find out whether these concentrations are related. Respective analyses were also performed between changes in abundance of individual species after N (or P) fertilization and either N (or P) weighted concentrations or N:P contents ratio in biomass of individual species to find out whether responses of individual species after N or P fertilization were related to nutrient concentrations and to N:P ratios.

Results

Temporal variation in soil characteristics

All the four soil characteristics measured during the growing period of 1997 showed similar trends in the two sites. The wet site had always higher soil water content, inorganic N and Olsen extractable P, values, but lower extractable K (Figure 1). Soil water content continuously declined during the growing period. There was a similar pattern for extractable K, which tended to be flattened after May. A continuous decline was also evident for inorganic N, except for a rise occurring between the third and the forth sampling. The temporal pattern of change in Olsen extractable P was more complicated. After a rise from the first to the second sampling, it abruptly declined, then rose again and finally smoothly declined to similar values with those of the first harvest.

Comparisons between sites in above-ground biomass production and total above-ground N and P content

The wet site was more productive than the dry site (Figure 2). Forbs (i.e. the non-legume dicots) contributed more than 65%, and grasses more than 50% in above-ground biomass of the wet and the dry site, respectively. Legumes had a very low abundance in both sites and abundance of sedges was remarkable only in the dry site. In either site forbs contributed more in above-ground total N or P content than in above-ground biomass of community because they had on

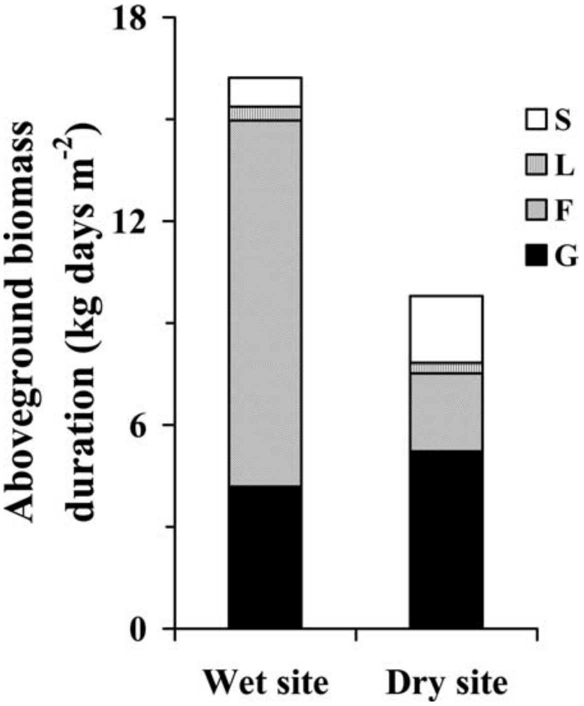

Figure 2. Means of aboveground biomass duration of whole communities and of component groups of species in the two sites (G, grasses; F, forbs; L, legumes; S, sedges).

average higher N and P concentrations in comparison to graminoides.

Weighted above-ground N and P concentrations and N:P ratios of groups and of individual species

Weighted above-ground N and P concentrations and N:P ratios varied greatly among species and were influenced by site (Table 3). Orthogonal comparisons in weighted N and P concentrations and N:P ratios for the sufficiently abundant and common species in the two sites revealed significant differences of means between either groups of species within sites or sites within groups of species (Figure 3). Among groups of species, legumes had the highest N concentration and grasses and sedges the lowest in both sites. Legumes again had the highest P concentrations in the wet site, followed by forbs, grasses and sedges, but forbs had the highest P concentrations in the dry site and sedges the lowest. Between sites, legumes had higher N and P concentrations in the wet site; all other groups had higher N concentrations in the dry site, and, except for sedges, higher P concentrations in the wet site. N:P ratios for all groups of species were higher on average in the dry site; in both sites, legumes had the highest ratio mean and forbs the lowest, while sedges had higher N:P ratio mean than grasses.

Table 4 gives the site means, averaged over species means, of non-legumes species in weighted N and P concentrations and N:P content ratios. Site means of N concentrations and N:P ratios were lower in the wet site, while site means of P concentrations were similar in the two sites. P concentrations of species within either site were most variable; N:P ratios between species were more variable than N concentrations in the wet site, but less variable in the dry site.

Mean community weight N concentrations, calculated by dividing community N duration content by community biomass duration, were 17.2 and 16.7 mg g^{-1}, for the wet and the dry site, respectively; respective community P concentrations were 1.97 and 1.47 mg g^{-1} and community N:P ratios were 8.73 and 11.36. Compared to site means of species N and P concentrations, community N and P concentrations were higher in the wet site, but lower in the dry site. In both sites, N:P ratio of community was lower than the site mean of N:P ratios of species.

N and P concentrations of species were lowered progressively during the growing period but N:P ratios of species showed much lower temporal variation (data not shown). However, the ranking of groups of species was the same for every of these variables within each site in the various harvests made. For each site, community N and P concentrations for every harvest, calculated by dividing total community nutrient content by total community biomass, tended to be linearly lowered during the growing period (data not shown); the slope of the linear regression between community N and P concentrations in the various harvests was lower in the wet site.

Weighted N and P concentrations of non-legume species were highly and linearly related (Figure 4) and community concentrations were fitted closely to linear regression lines; the slope of the linear regression was significantly higher (8.42 vs. 4.29) for species of the dry site.

In either site, N:P ratios in above-ground biomass were much lower than the ratio 14:1 for the most examined species; only the *Trifolium* species in the wet site and *Carex caryophyllea*, *Trifolium heldreichianum* and *Lotus corniculatus* in the dry site had N:P ratios intermediate of 14:1 and 16:1.

Table 3. Mean squares from ANOVA for the effects of sites on N and P weighted concentrations of the 16 sufficiently abundant and common in both sites species

Source of Variation	d.f.	Nitrogen	Phosphorus	N:P
Sites	1	89.27*	0.85**	108.95***
Replications in sites	6	9.91	0.03	1.07
Species	15	92.09***	1.01***	32.71***
Sites x Species	15	14.01**	0.42***	12.82***
Error	90	5.62	0.05	1.28
CV (%)		13.06	12.85	10.43

$^{*}P > 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

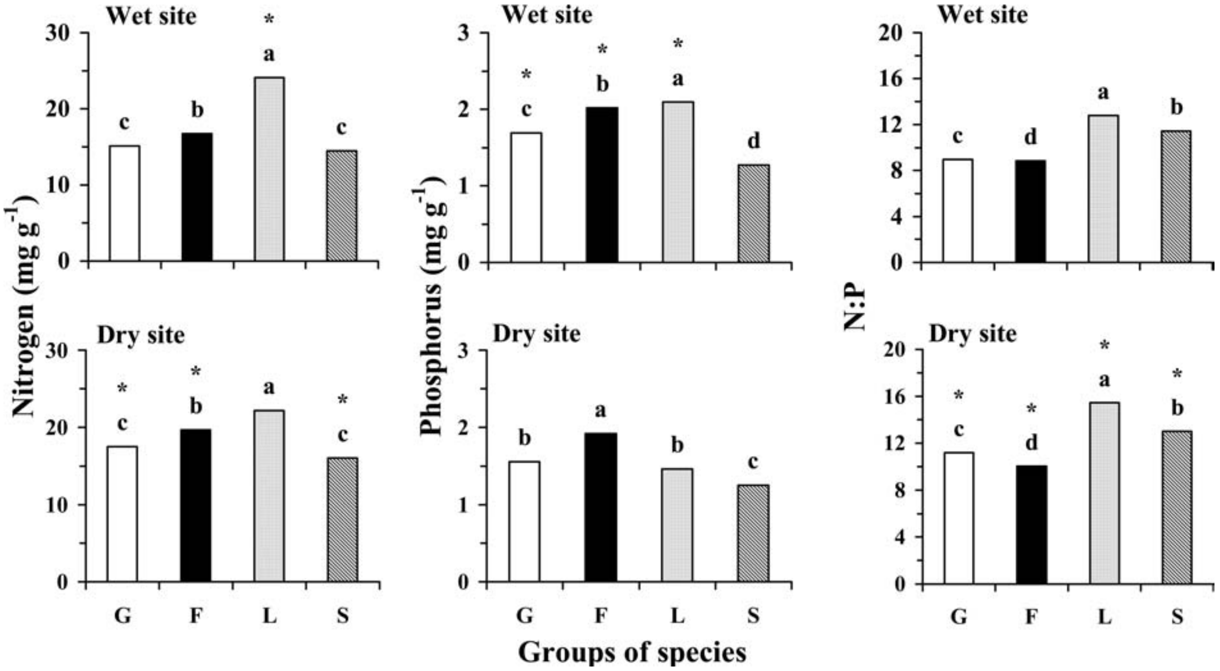

Figure 3. Means of weighted N and P concentrations and N:P ratios in aboveground biomass of the groups of species (G, grasses; F, forbs – non-legume dicots; L, legumes; S, sedges) in the two sites. Columns within each site with different letter indicate significant difference at $P < 0.05$. $*$ in column indicate greater value at $P < 0.05$ in comparison to respective column of the other site.

Table 4. Site means, averaged over weighed N and P concentrations (in mg g^{-1}) and N:P ratios of the sufficiently abundant non-legume species (the 13 of Table 1 together with *Taraxacum officinale* of the wet site and *Cichorium intybus*, *Potentilla argentea* and *Teucrium chamaedrys* of the dry site) with SE and CVs of the means

	Wet site: $n = 14$			Dry site: $n = 16$		
	$N_{con.}$	$P_{con.}$	N:P	$N_{con.}$	$P_{con.}$	N:P
Mean	16.00	1.80	9.18	19.07	1.78	10.84
S.E.	0.64	0.12	0.44	0.91	0.10	0.32
CV (%)	15.00	24.47	17.96	19.17	21.89	11.90

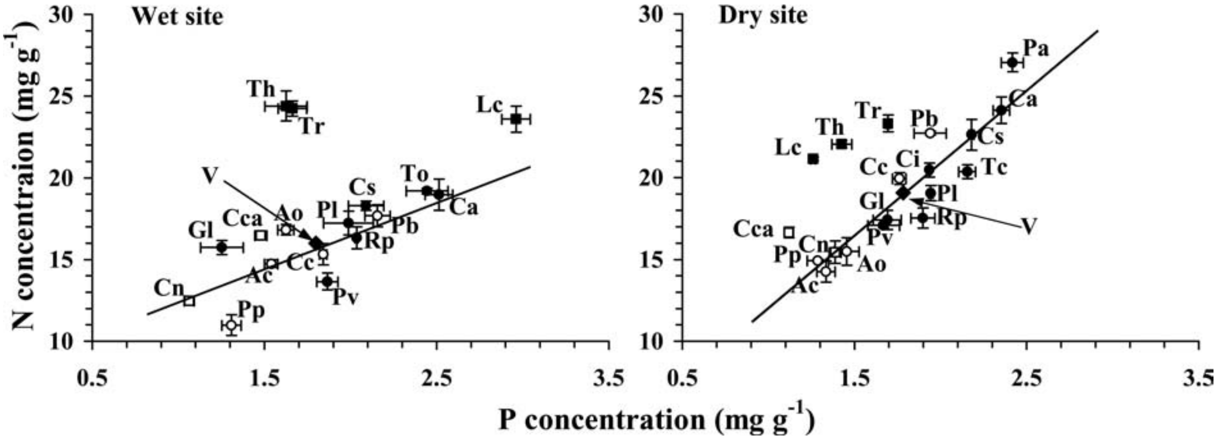

Figure 4. Relationship between N and P weighted concentrations of species in the two sites. Linear regression lines for non-legume species were: for wet site $N_{conc} = 8.27 + 4.29 \cdot P_{conc}$; for dry site $N_{conc.} = 4.06 + 8.42 \cdot P_{conc}$. Both were significant at $P < 0.001$. Error bars show ± 1 SE; $n = 4$. Abbreviations of species are shown in Table 1. Additional species: *Taraxacum officinale* (To) in the wet site and *Cichorium intybus* (Ci), *Potentilla argentea* (Pa) and *Teucrium chamaedrys* (Tc) in the dry site. Arrows indicate the position of communities weighted concentrations (filled rhomb with V). (○, grasses; □, sedges; ●, forbs; ■, legumes).

Fertilization experiment and species responses to nutrient additions

ANOVA analyses revealed significant effects of N in the wet site and of both N and P in the dry site for above-ground plant production. Between sites, community production for all nutrient treatments was greater in the wet site. In the wet site, N addition increased significantly the biomass of whole community, total grasses and total forbs (Figure 5) by favoring mostly the most abundant forb species *Cerastium semidecandrum*. In the dry site, addition of either nutrient increased the biomass of whole community. Addition of N increased biomass of grasses, by favoring mostly the most abundant species *Agrostis capillaris*; both nutrients favored the growth of *Galium lucidum*, the most abundant forb in the site (data not shown).

Although ANOVA analysis did not reveal significant responses of most species to N and P additions, the ratio of above-ground biomass of species in the N addition treatment and the control was positively and significantly correlated with weighted N concentration of species for the wet site (Figure 6) and when data of both sites were included ($r = 0.63$, $P < 0.001$). The same trend was evident between the ratio of above-ground biomass of species in the P addition treatment and the control and weighted P concentration of species for the dry site and when data from both sites were included ($r = 0.42$, $P < 0.05$).

Changes in species biomass after N or P enrichment did not correlate with species N:P ratios in most of cases (Figure 7). N:P ratio and species enhancement were positively related, only when the dry site was enriched with N.

Discussion

Productivity and species composition of sites

The results of this work indicate that water, not only as resource but also as factor influencing the physicochemical characteristics of soil, affects the productivity and the species composition of plant communities. Although soil in both sites has been developed from the same parental rock, the long-term higher water availability in the wet site resulted in greater accumulation of organic matter and consequently in higher availabilities of N and P. The higher soil P availability and the greater P uptake by plants in the wet site may be due to various processes. First, the greater soil water increased the P diffusion coefficient, since diffusion is the main process of P movement from soil to root surface (Fitter and Hay, 1987). Second, waterlogging changed ferric phosphates into more soluble ferrous compounds (Patric and Mahapatra, 1968). Third, the flush of P from organic pool must be greater in the wet site (Willet, 1989), since organic content of soil was higher. Forth, waterlogging increased P uptake per unit of root biomass and changed root morphology for more favorable nutrient uptake (Rubio et al., 1997). Fifth, the greater root biomass and activity accelerates the weathering of the parent rock and the consequent release of P.

The differences between sites in availabilities of soil resources are reflected in primary productivity and species composition. The greater soil water content and the higher N and P availabilities of the wet site resulted in greater above-ground biomass and favored mostly the growth of forbs, which dominated in this site because they require adequate soil water (Smedley et al., 1991) and high N and P availabilities, as indicated by their relatively high biomass N and P concentrations. On the other hand, forbs were restricted by grasses in the less fertile dry site. Koutroubas et al. (2000) provided evidence of increasing competitive ability of forbs against grasses with increasing P availability and Mamolos and Veresoglou (2000) reported dramatic changes in species composition after P enrichment, which favored the species with the highest P tissue concentration.

N and P concentrations in above-ground biomass and N:P ratios

Above-ground N and P concentrations were substantially varied among species and between sites as Güsewell and Koerselman (2002) showed and they were progressively lowered during the growing period, (Chapin, 1980; Van der Linden, 1980, 1986; Veresoglou, 1983), because of the dilution effect. However, the ranking of species groups in either nutrient concentration within each site did not change in the various harvests made.

As confirmed by other works, i.e. in Veresoglou (1983), de Mars et al. (1996) and Thompson et al. (1997), forbs had generally higher N and P concentrations than grasses and sedges. As expected, legumes had the highest N concentrations among the species groups, because they are able to fix atmospheric N. Probably promoted by greater water and P availability in the wet site, legumes had the highest P concentrations among the species groups and in comparison to dry site higher N concentrations.

Weighted community N and P concentrations were higher in the wet site in comparison to dry site because of the greater N and P availability of soil. Similarly, grasses, forbs and legumes had higher P concentrations in the wet site, but all groups, except legumes, had lower N concentration. These lower N concentrations could by explained by the higher dilution of N to greater produced biomass in the wet site, caused probably by the lack of other limiting factors; in the dry site, this dilution was probably constrained by effects of limiting factors, like P and possibly water.

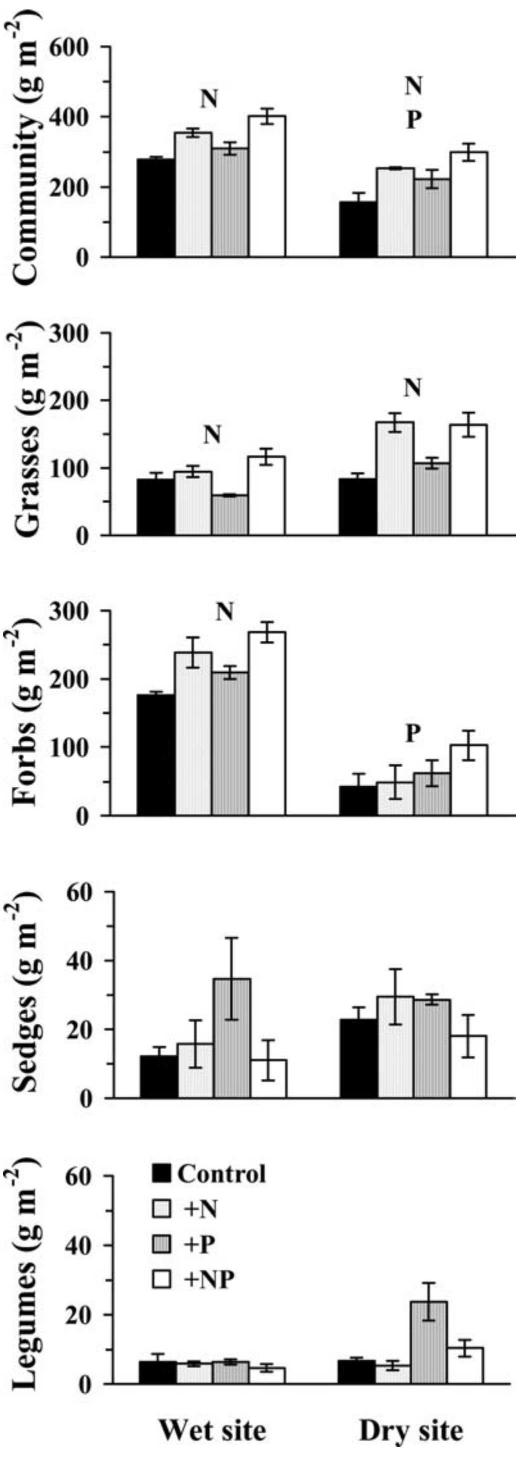

Figure 5. Effects of N and P fertilization on the aboveground biomass of whole community and of total grasses, forbs, sedges and legumes of the two sites in 1997. N and P indicate significant effects of nitrogen, phosphorus, respectively, at $P < 0.05$, at least. Error bars show ± 1 SE; $n = 4$.

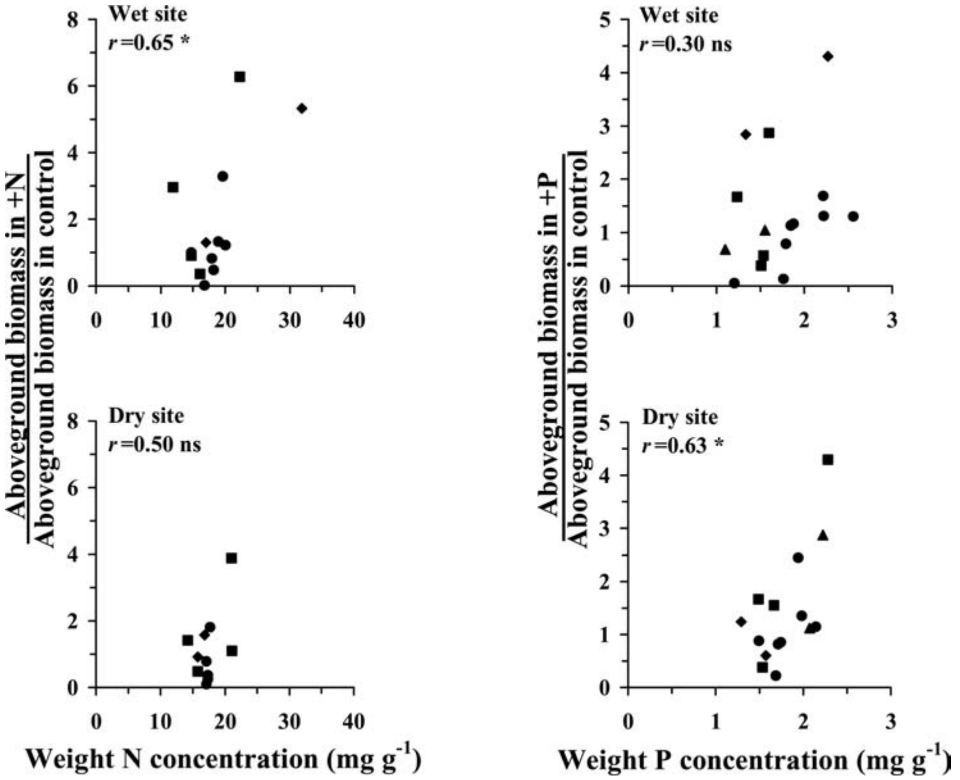

Figure 6. Relationships between the ratio of aboveground biomass in the +N (or the +P) treatment and the control of the sufficiently abundant non-legume (or all) species and weighted aboveground N (or P) concentrations of species for the wet and the dry site. ns $P > 0.05$; $^*P < 0.05$. (■, grass species; ♦, sedge species; ● forb species; ▲, legume species).

This agrees with the finding of Koerselman and Meuleman (1996) that N and P availability mainly affects biomass production and the species composition of the vegetation rather than biomass N and P concentrations. This, also, is probably a reason explaining why biomass nutrient concentrations of species do not always reflect nutrient availability as measured in soil (Güsewell and Koerselman, 2002). However, the weighed community N concentration was higher in the wet site because of the much greater contribution of forbs, which concentrated more N in comparison to grasses and sedges.

Concentrations of N and P of species were positively correlated. The linear correlation coefficients ($r = 0.79$ for the wet site and 0.90 for the dry site, when legumes were excluded) were almost the highest, when they are compared with those found in surveys of vascular plants in wetlands and in terrestrial ecosystems (i.e. in Bedford et al., 1999; Garten, 1976; Grime et al., 1997; Güsewell and Koerselman, 2002; Thompson et al., 1997), The existence of positive correlation between N and P concentrations for non-legume species grown in the same site justifies the almost lower variation in N:P ratios of species in comparison to variations in N or P concentrations. Legumes were deviated greatly from the other species in N:P ratios, because being able to use another source of N, i.e. atmosphere, can enhance N uptake and consequently N:P ratios, as we found in this study. Other possible sources of variation in N:P ratios could arise by the differences between (a) species in their ability to associate with mycorrhizae, because the mycorrhizae species exploit more efficiently the soil P (Fitter, 1990) and are therefore expected to have lower N:P ratios, especially in P deficient soils, (b) shallow- and deep-rooted species, since the supply N:P ratio of soil could vary with the soil depth, and (c) by differences between species in accumulation of compounds such as those used for osmotic adjustment, defense, and storage (Lambers et al., 1998).

Responses of species to N and P additions

Short-term responses were evident on whole communities, species groups and dominant species after N addition in the wet site and N and P in the dry

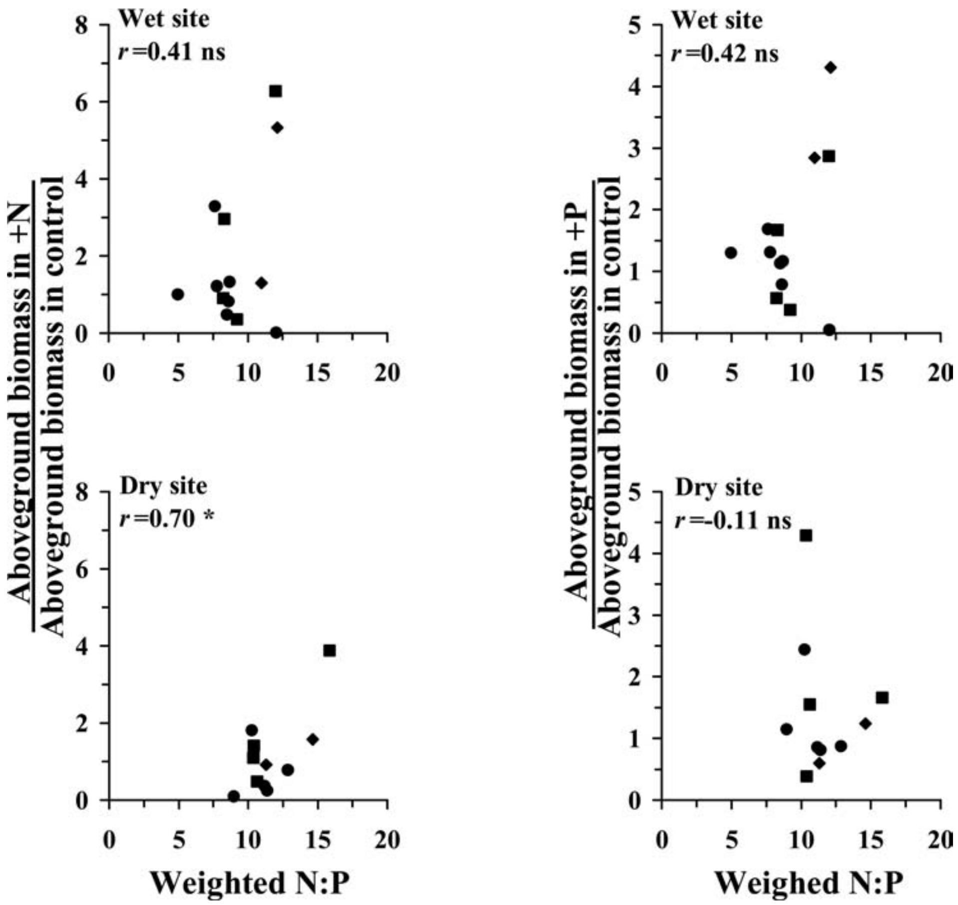

Figure 7. Relationships between the ratio of aboveground biomass in the +N (or the +P) treatment and the control of the sufficiently abundant non-legume species and weighted aboveground N:P ratios of species for the wet and the dry site. ns $P > 0.05$; $^*P < 0.05$. Symbols as in Figure 6.

site. In the wet site P was not found limiting because of greater mineralization rates in a wet environment with a relatively high organic matter content of soil. So, possible changes after P addition were not detectable as significant by ANOVA. On the other hand, ANOVA indicated that N was limiting in the wet site, since most groups, as well as the dominant species *Cerastium semidecandrum* responded to N addition. In the dry site, ANOVA showed that primary production and the dominant forb in the site *Galium lucidum* increased by addition of either nutrient, grasses and the dominant grass *Agrostis capillaris* were favored by N addition and forbs and legumes by P addition.

At the species level, a significant increase in biomass due to N and/or P fertilization is far less common than at the community level (Güsewell et al., 2003), because of high spatiotemporal variation in the relative abundance of species within mixed vegetation and to species responses to interspecific competition. However, species with higher N or P concentrations tended to be enhanced in both sites by addition of N or P, respectively.

Güsewell et al. (2003) stated that the N:P ratio appeared to be a better predictor of responses than individual nutrient concentrations. Therefore, significant relationships can be potentially found between N:P ratios of plant populations and their short-term responses to N or P fertilization in the direction that N addition will tend to enhance those species with high ratios, while P addition will do those species with low ratios. This seems not to be the case sometimes, when we suppose that N and P concentrations of species are indeed good predictors of short-term responses to N or P fertilization and they are also positively related, as we found in this work. If we assume that N and P concentrations of species are linearly related, i.e. (N concentration) $= a + b \cdot$ (P concentration), then N:P $=$ b $-$ a/(P concentration), it follows that N:P ratios will tend to be similar for all species when the intercept a is close to 0; they will be negatively related to P concentrations, and also to N concentrations since N and P concentrations are positively related, if the intercept a is positive, and they will be positively related with either P or N concentrations, when the intercept a is negative. The last case may occur when N:P ratios are extremely high, indicating P deficiency. However, in this case N will be not limiting and species will not respond to N enrichment, but the species with higher P concentrations, i.e. the species with higher N:P ratios, are expected to enhance more their biomass after P addition.

In our data, intercepts of linear regressions are positive in both sites and therefore N:P ratios of species tended to be inversely related to either N or P concentrations of species. For this reason, changes in species biomass after N or P enrichment were positively related to N or P concentrations of species, respectively, but they often tended to be negatively related to N:P ratios.

If biomass changes of species after N or P enrichment were predictable by N:P ratios of species in the way proposed by Koerselman and Meuleman (1996) and Güsewell et al. (2003), P enrichment would favor more the species with higher N:P ratios. For our data, P enrichment would favor more sedges than forbs, i.e. the species with lower P concentrations. It follows that further P limitation would favor more the species with the higher P concentrations. This contradicts statements proposing that, in nutrient competition, the most competitive species for a particular limiting nutrient are those that lower mostly the concentration of this nutrient in the soil solution (Tilman, 1982); these species tend to have the lowest tissue concentrations of this limiting nutrient (Tilman, 1990) and use more efficiently this nutrient. Confirming these statements, which are included in the of ‘resource ratio model’ of Tilman (1997), Mamolos et al. (1995b) and Koutroubas et al. (2000) found that species with lower concentrations of the limiting nutrients tended to dominate in plant communities. By working in the two sites of our study, Vasilikos (2001) also found similar results but only for the dry site.

When soil is enriched with the limiting nutrients, the species with higher concentrations are expected to be favored proportionally more (Mamolos and Veresoglou, 2000; Mamolos et al., 1995b; Tilman et al., 1999; Wells et al., 1986). This happens because either these species should free from the constraints of competing for the previous limiting nutrients (Tilman et al., 1999) or being faster growing species respond more to changes in nutrient availabilities (Chapin et al., 1986; Grime et al., 1997). The later reason seems to be more plausible, since the growth of species with the higher nutrient concentrations enhanced more after N and P enrichment even in the wet site where species composition was found not to be determined by the efficiency in the use of the limiting nutrient (Vasilikos, 2001).

With exception of species like legumes that have some special characteristics, our data indicate that N and P concentrations of species grown in the same site are positively related and N:P ratios appear to depend on the particular context in which species have growth (Güsewell et al., 2003), especially when the soil is shallow, as in this work, where species can not be differentiated in the distribution of roots with soil depth. This confirms the results of other works (i.e. Braakhekke and Hoftman, 1999; Güsewell and Koerselman, 2002) and contradicts predictions of ‘resource ratio model’ of Tilman (1997) that plants tend to take up nutrients in the proportions needed for growth, independently of the supply ratio. However, the short-term responses of species after N or P enrichment seem to be predicted better by absolute N and P concentrations than by biomass N:P ratios.

N:P ratios as predictors of N and or P limitation

Although N:P ratios of species are not reliable predictors of short-term responses of species in plant communities, there is strong evidence that these to a large extend are determined by the relative supplies of N and P, and therefore they can be used to detect nutrient deficiency experienced by plant species. Without other limiting factors, high N:P ratios would indicate P limitation, while low N:P ratios would indicate N limitation. For wetland plant communities, threshold N:P ratios, indicating N- or P-limitation, are between 14 and 16 (Güsewell et al., 2003; Koerselman and Meuleman, 1996; Olde Venterink, 2000). These ratios however vary greatly in the various vegetation types and they seem to be generally lower in grasslands than in wetlands (Tessier and Raynal, 2003), as our results indicate; the wet site had N:P ratio of 8.73, on community level, and was N limited, while the dry site had N:P ratio of 11.36 and was co-limited by N and P. Therefore the threshold N:P ratio to N- or P- limitation must be close to that found in the dry site. This threshold N:P ratio is much lower than those found in European wetlands and seems to be close to that found in savannas (Ludwig et al., 2001).

Conclusions

The results of our work indicate that the long-term intermittent water saturation of soils in dry areas of high altitude in temperate regions modifies the soil characteristics of natural plant communities, by increasing organic matter content and the availability of usually limiting nutrients, N and P. These changes, in combination with the adequate water availability, increase primary productivity and shift the composition of species from the dominance of graminoids in the dry sites to dominance of forbs in the wet sites. The facts that graminoids are less sensitive to drought than forbs and, having generally lower N and P concentrations, exploit more efficiently these nutrients when they are scarce, explain the dominance of graminoids in the dry site. This seems to be consistent with the 'ratio resource model' of Tilman (1997) that states that abundance of species in low-productive soils is regulated by competition-driven nutrient depletion. On the other hand forbs dominated in the wet site, because they have properly grown in a wetter soil that contains more available N and P. This furthermore is confirmed by the fact that the species with the higher N and P concentrations tended to enhance their biomass after both sites were enriched with N and P, respectively.

In either studied site, the shallow soil prevented the species from exploiting different soil layers, imposing therefore the co-existing species to grow in the same ecological context. Since N and P concentrations species were positively related, variation in biomass N:P ratios of non legume species was relatively low. This is consistent with statements that plants tend to take up nutrients in proportions determined by supply ratios and contradicts the predictions of 'ratio resource model' of Tilman (1997). However, in this study N and P concentrations of species, rather N:P ratios, were found better predictors of species responses to N and P enrichment.

N:P ratios of whole vegetation in non-fertilized plots were 8.73 for the wet and 11.36 for the dry site. These values, together with the responses of plants to N and P fertilization, indicate that the threshold N:P ratios to N- or P- limitation in herbaceous grasslands must be lower than those found in wetlands. Threshold N:P ratios vary among vegetation types and assessments of plant N:P ratios using experimental N and P additions for grasslands are rare (Tessier and Raynal, 2003). More experimental work is needed to be done in the various types of vegetation types of grasslands to isolate critical N:P ratios indicative of N or P limitation for use in ecosystem and natural resource management. These critical N:P ratios could vary in the various types of grasslands. For example, lowland grasslands are expected to have lower critical N:P ratios than upland and alpine grasslands, because species in low altitudes have generally lower N concentrations (Körner, 1989).

Acknowledgements

We thank Dr S. Güsewell for critical reading of the manuscript, K. Athanasiadis, C. Anagnostopoulos and P. Skenteridis for their assistance in experimentation and D. Ellis for the linguistic corrections, and two anonymous reviewers for their valuable comments. This research was funded by Ministry of Industry, Energy and Technology of Greece.

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Section editor: H. Lambers