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Mineral Nutrient Limitations of Calcifuge Plants in Phosphate Sufficient Limestone Soil

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Twelve species of calcifuge plants were grown in an Ordovician-limestone soil with and without phosphate amendment, as well as in an acid silicate soil of their natural habitat. Phosphate treatment of the limestone soil raised the P concentrations of the plant biomasses to levels within sufficiency ranges reported for cultivated plants and productivity usually increased two- to five-fold. Out of twelve species studied, *Scleranthus perennis* was unable to survive in the limestone soil unless treated with phosphate, whereas growth and general performance of *Galium saxatile* was impaired by phosphate additions. Biomass dilution effects on micro-nutrients, but usually not on macronutrients, were recorded as a result of the phosphate treatment. Dilution of Mn was most distinct and Fe was least distinct. However, no foliar symptoms clearly assignable to Mn deficiency were observed. Symptoms of foliar chlorosis, reminiscent of Fe deficiency, developed in *Galium saxatile*, *Carex pilulifera* and *Veronica officinalis*. In *C. pilulifera*, but not in *V. officinalis*, chlorosis was accompanied by decreasing foliar Fe concentrations.

Key words: Calcifuge plants, limestone soil, calcareous, mineral nutrition, phosphorus, iron, manganese, copper, zinc, limiting nutrients.

INTRODUCTION

Inability to solubilize and render phosphate available was recently demonstrated as a key factor excluding calcifuge vascular plants from limestone soils (Tyler, 1992, 1994). Both total inorganic and organic phosphorus may occur in similar amounts in limestone and silicate topsoils, but exchangeable phosphate in limestone soils is usually $< 0{\cdot}01$ μmol g^{-1}, compared to 0·10–1·0 μmol g^{-1} in most silicate soils (Tyler and Olsson, 1993; Tyler, unpubl. res.). Native inorganic phosphorus in calcareous soils is mainly present as an insoluble apatite-like calcium phosphate. Some low-molecular organic acids, in particular oxalic acid, may solubilize appreciable amounts of calcium phosphate. Recently, evidence of highly differing organic acid exudation patterns and rates as mechanisms involved in calcifuge and calcicole behaviour was demonstrated with a large selection of plants (Ström, Olsson and Tyler, 1994; Tyler and Ström, 1995).

However, a few calcifuge species tested so far have not responded with increased growth or survival to soluble phosphate amendment of limestone soils. A few other species, while increasing their growth rates considerably, suffered from chlorosis or displayed other morphological divergences. Several other calcifuges developed normally following phosphate amendment of limestone soil.

The occurrence of chlorosis in native plants growing on limestone sites was already observed by Grime and Hutchinson (1967). This is a well-known problem with many crops cultivated in calcareous soils and is usually interpreted as Fe deficiency (reviewed by, e.g. Kinzel, 1982; Bergmann, 1988). Similar studies have not been performed with wild-growing plants. Also other micronutrients, such as Mn, Zn, and Cu, are quite sparingly soluble in soils of a high pH and might become growth-limiting, e.g. under conditions of macronutrient sufficiency or excess, as demonstrated in certain crops.

Elements not instantaneously limiting growth are usually taken up in some excess by plants, compared to calculated or demonstrated demands. This principle has been widely elucidated in studies of cultivated plants (e.g. Bussler, 1970; review by Bergmann, 1988) and luxury or excess uptake of several mineral nutrients, related to soil chemical properties, has also been demonstrated in wild-growing species (e.g. Tyler, 1976). Elements that are becoming markedly diluted in an expanding biomass constitute potential limitations to further growth.

The objective of this study was to investigate the uptake of mineral nutrients in above-ground biomass of 12 wild-growing vascular plant species that are frequent on acid silicate soils but are never or only exceptionally found on calcareous soils. Uptake was studied with both untreated and phosphate treated limestone soil, as well as with a non-calcareous silicate soil. Adult field-plant material was used, because several of the species included in the study proved unable in previous experiments to develop beyond the seedling stage in limestone soils not treated with phosphate. It is hypothesized that micronutrients (Fe, Mn, Zn or Cu) will become limiting in limestone soils supplied with phosphate, as evidenced by decreasing biomass concentrations or by display of chlorotic or other deficiency symptoms at increasing growth rates.

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MATERIALS AND METHODS

The soils used were a rendzic leptosol (pH-KCl 7·4), approx. 10 cm deep, from the Ordovician limestone 'alvar' of Öland (56° 25′ N; 16° 30′ E) and the top 10 cm (A_h horizon) of a dystric cambisol (pH-KCl 4·3), developed from a silicate-rock moraine in Scania (55° 57′ N; 13° 50′ E), south Sweden. Both soils were light-textured and organic matter content varied from 10 to 12% dry weight. Exchangeable phosphate in the limestone soil was $\leqslant 0{\cdot}01$ μmol g^{-1}, in the silicate soil $0{\cdot}80 \pm 0{\cdot}05$ μmol g^{-1} dry weight. Concentrations of total P in soils from the same sites, however, do not differ appreciably according to previous studies [Tyler and Olsson (1993), where details of soil analytical methods are given]. Iron exchangeable by 0·1 M $BaCl_2$ was $< 0{\cdot}1$ μmol g^{-1} in the limestone and $5{\cdot}6 \pm 1{\cdot}2$ μmol g^{-1} dry weight in the silicate soil.

Soils were sieved (6 mm) before further treatment. Water-soluble $CaHPO_4 . 2H_2O$, 5 mmol l^{-1} of soil, was carefully mixed into half of the limestone soil volume used as the phosphate treatment.

Materials of adult, well-developed plants were collected in the silicate soil area on 31 May 1995. After storage overnight in moistened paper bags at 8 °C, equal-sized specimens were cut down to approx. 30 mm shoot (leaf) and approx. 40 mm root length and planted in polythene vessels with bottom drainage, 1 l soil per plant. Six species of grasses and sedges, and six herbaceous species (see Table 1) were tested, six replicates in each soil and treatment. During the experiment soil moisture was allowed to fluctuate between 50 and 60% of the water-holding capacity (which corresponded to 30·6–36·7% dry weight of the silicate soil and 20·2–24·2% dry weight of the limestone soil). Losses were compensated by H_2O addition at the soil surface. The experiment was conducted in a greenhouse, where temperature was kept at 14–16 °C at night and 20–25 °C by day. Additional light, 70 W m^{-2}, was provided by high-pressure sodium lamps during daytime if ambient light was < 100 W m^{-2}.

The experiment started on 1 Jun. 1995. Ten species were harvested after 30 d (1 Jul.) and two slow-growing species after 50 d (20 Jul.). Only in one species (*Rumex acetosella*) was the flowering stage attained. One of the species, *Veronica officinalis*, was run in duplicate sets and cropped on both occasions. Before cropping, morphological differences among biomasses from the different soils and treatments were recorded. Immediately following cropping, the above-ground biomass was rinsed in H_2O spray for 1 min and dried at 85 °C for 3 d. After determination of dry weight, the biomasses of all species (except *Galium saxatile*, which was considered too low for analysis) were wet digested (conc. HNO_3) for complete destruction of organic matter. Excess acid was evaporated to 2 ml, H_2O added to 25 ml and the solutions analysed for K, Mg, Ca, Mn, Fe, Zn, S, and P by plasma emission spectrometry, and for Cu by flame atomic absorption spectrometry.

The soils of the growing vessels were liberated from roots by sieving (6 mm) and extracted in 0·1 M $BaCl_2$ solution for analysis of ammonium and nitrate by flow injection technique according to Ruzika and Hansen (1981) and for exchangeable K by AAS after addition of 1000 ppm Cs l^{-1} as CsCl to warrant uniform excitation level of the K atoms.

Biomass produced was calculated as mg dry weight per plant and reported as means ± confidence limits ($P < 0{\cdot}05$) for each species and treatment. Concentrations of elements in the biomass were calculated as μmol g^{-1} dry weight. Significance of the difference between treatment means was calculated by Tukey test. The shares of different cationic elements of the molar cation sums were calculated as the ratios formed by these shares in the biomasses of the phosphate amended (P+) and the unamended (P−) limestone soil.

RESULTS

The availability of nitrogen and potassium

As the N mineralization rate may sometimes limit primary productivity in soil experiments, the exchangeable NH_4^+ and

TABLE 1. *Above-ground biomass produced during the experiment, mg dry weight per plant. Means ± confidence limits (P < 0·05); n = 6*

Species	Duration (d)	Silicate soil	Limestone soil (P−)	Limestone soil (P+)
Grasses/sedges				
Agrostis capillaris L.	30	242 ± 42	100 ± 16	502 ± 87
Carex pilulifera L.	30	208 ± 37	68 ± 10	142 ± 48
Deschampsia flexuosa (L.)T	30	133 ± 25	87 ± 24	139 ± 39
Holcus mollis L.	30	100 ± 19	55 ± 19	190 ± 22
Luzula campestris (L.) DC.	30	223 ± 64	63 ± 26	160 ± 42
Luzula pilosa (L.) Willd.	50	365 ± 89	83 ± 24	203 ± 100
Herbs				
Galium saxatile L.	30	82 ± 39	40 ± 18	19 ± 9
Potentilla argentea L.	30	165 ± 54	72 ± 24	256 ± 66
Rumex acetosella L.	30	305 ± 62	137 ± 41	473 ± 107
Scleranthus perennis L.	50	241 ± 105	no surv.	272 ± 126
Veronica officinalis L.	30	110 ± 31	57 ± 22	147 ± 22
Veronica officinalis L.	50	465 ± 75	136 ± 42	574 ± 53
Viscaria vulgaris Bernh.	30	440 ± 54	353 ± 69	795 ± 215

TABLE 2. *Concentrations (μmol g^{-1} dry weight) of mineral elements in above-ground biomass produced in the silicate soil. Means ± confidence limits (P < 0·05); n = 6*

	K	Mg	Ca	Mn	Fe	Zn	Cu	S	P
Agrostis capillaris	698±13	77±2	78±4	5·7±0·1	1·4±0·1	1·2±0·1	0·28±0·04	75±5	57±2
Carex pilulifera	678±82	59±3	95±7	3·1±0·1	1·5±0·1	0·8±0·1	0·18±0·01	82±7	49±7
Deschampsia flexuosa	417±7	46±6	53±6	4·5±0·6	1·2±0·1	0·6±0·1	0·31±0·05	53±1	40±1
Holcus mollis	808±13	107±7	121±19	7·2±0·3	1·4±0·1	0·8±0·0	0·59±0·06	70±1	61±1
Luzula campestris	632±7	57±3	108±3	3·8±0·2	3·2±0·1	1·5±0·1	0·27±0·01	75±8	65±2
Luzula pilosa	706±6	69±4	103±2	7·2±0·1	2·6±0·2	1·8±0·1	0·20±0·01	42±1	41±2
Potentilla argentea	394±19	109±4	230±3	2·5±0·5	4·4±0·5	2·1±0·3	0·17±0·01	61±1	40±5
Rumex acetosellsa	1207±58	134±6	174±30	9·8±0·3	2·1±0·1	1·3±0·1	0·18±0·00	67±6	63±2
Scleranthus perennis	844±88	173±3	155±4	5·2±0·3	1·6±0·1	3·1±0·1	0·13±0·00	66±2	82±2
Veronica officinalis	859±32	55±5	164±2	1·8±0·1	5·5±0·5	1·3±0·1	0·19±0·00	69±2	78±5
Viscaria vulgaris	1046±19	214±19	276±12	8·4±0·1	3·1±0·1	2·0±0·2	0·19±0·01	63±6	66±2

Veronica officinalis grown for 50 d.

TABLE 3. *Mean concentrations of mineral elements in above-ground biomass produced during the experiment with the unamended (P−) and the phosphate amended (P+) limestone soil, calculated as percentage of concentration in biomass produced with the silicate soil*

		K	Mg	Ca	Mn	Fe	Zn	Cu	S	P
Agrostis capillaris	P−	90*	104	258*	72*	144*	69*	98	101	93
	P+	92*	97	289*†	17*†	93†	79*	80*†	121*†	216*†
Carex pilulifera	P−	51*	69*	306*	86	95	65*	133*	63*	34*
	P+	107†	93†	263*†	50*†	57*†	94†	153*†	106†	378*†
Deschampsia flexuosa	P−	78*	109	314*	59*	179*	80*	88	64*	80*
	P+	86*†	100†	339*	49*†	160*	84	75*	78*†	99†
Holcus mollis	P−	90*	56*	160*	34*	95	67*	87	85*	67*
	P+	92*	96†	253*†	20*†	146*†	112*†	61*†	120*†	192*†
Luzula campestris	P−	79*	95	319*	100	88	72*	105	70*	74*
	P+	133*†	101	260*†	75*†	83	116*†	91†	89†	140*†
Luzula pilosa	P−	71*	104	340*	78*	105	72*	116*	69*	54*
	P+	81*	95	442*†	48*†	86*†	61*†	102	101†	284*†
Potentilla argentea	P−	84*	95	170*	148*	132*	84	108	77*	124
	P+	98	90	176*	189*†	123	51*†	78*†	97†	191*†
Rumex acetosella	P−	59*	57*	245*	43*	163*	107	121	75*	29*
	P+	90*†	73*†	381*†	26*†	118	90†	85*†	178*†	223*†
Scleranthus perennis	P−					no survival				
	P+	86	79*	307*	84*	89	24*	82*	95	143*
Veronica officinalis	P−	71*	49*	208*	163*	134*	85*	83*	74*	55*
	P+	79*	101†	264*†	106†	94†	49*†	81*	108†	136*†
Viscaria vulgaris	P−	92*	47*	147*	53*	43*	43*	73*	75*	38*
	P+	107†	48*	164*	49*	54*†	41*	68*	86*†	215*†
All species	P−	76*	79*	247*	84	118	74*	101	75*	65*
	P+	96†	88	285*	65*	100	73*	87	107†	202*†

Veronica officinalis grown for 50 d.
* Concentration differs ($P < 0·05$) from the silicate soil.
† Concentration differs ($P < 0·05$) from the unamended limestone soil.

the NO_3^- concentrations of the cultivation soils of all vessels were determined at the end of the experiment. Both the (P−) and the (P+) limestone soils contained considerable amounts of NO_3^-, 6·4±0·3 and 4·1±0·4 μmol g^{-1} dry weight, respectively, a difference expectable from the probability that less N would have been absorbed from the (P−) soil as a consequence of poor plant growth. Both limestone soil treatments were very low in exchangeable NH_4^+, 0·05±0·00 μmol g^{-1}, as expected from the usually high nitrification rate in soils of low acidity. In the silicate soil less mineral N remained, though probably still sufficient for normal growth, proportions of NO_3^- and NH_4^+ being crudely equal (0·46±0·05 and 0·56±0·05 μmol g^{-1}, respectively).

The great demand for K in plant growth may also sometimes limit primary production in pot experiments of excessive duration. Exchangeable K, however, did not differ appreciably between the (P−) and (P+) limestone soils, being 1·19±0·02 and 1·04±0·03 μmol g^{-1}, respectively, at the end of the experiment. In the silicate soil 0·71±0·04 μmol g^{-1} was measured. Consequently, neither

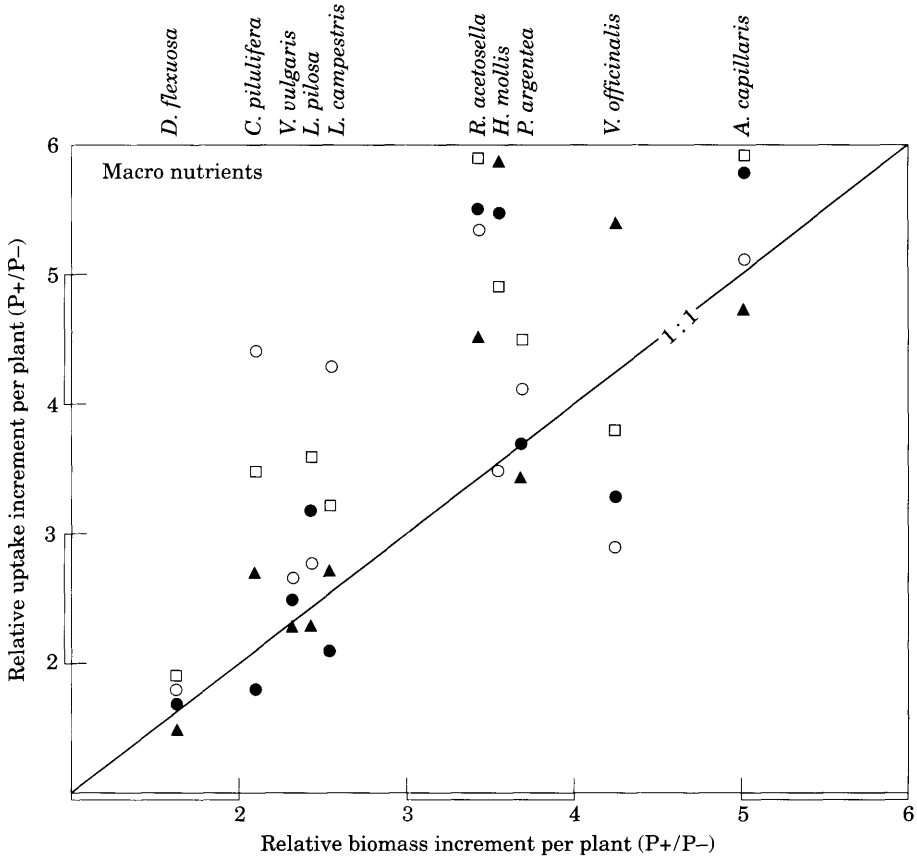

FIG. 1. Relations between mean relative uptake increment per plant of macro nutrients and mean biomass increment per plant caused by a phosphate addition to the limestone soil. A position below the 1:1 line indicates a dilution of the nutrient in the biomass. ●, Ca; ▲, Mg; ○, K; □, S.

N nor K would have imposed limitations to plant growth in any of the limestone soil treatments and no severe limitations to growth in the silicate soil.

Morphology and quantity of biomass produced

Biomass produced in the unamended (P−) limestone soil during the experiment was, on average, approx 40% of the biomass produced in the silicate soil, but varied from 0 to 80% among the different species (Table 1). *Scleranthus perennis* did not even survive in the (P−) limestone soil and growth was very poor in *Luzula campestris* and *L. pilosa*. Relative growth of *Viscaria vulgaris* (80%) and *Deschampsia flexuosa* (65%) was best among the species tested, probably due to a larger amount of transplanted original biomass able of translocating resources to new growth. An almost complete inability of *V. vulgaris* to develop from seeds in limestone soils has previously been established (Tyler and Olsson, 1993).

Addition of soluble phosphate to the limestone soil had a drastic positive effect on the production of above-ground biomass in most of the species, increasing 2·5–5-fold compared to the (P−) limestone soil. *Galium saxatile* was the only apparent exception, having an even poorer growth in the (P+) limestone soil (Table 1). Biomass produced on the (P+) limestone soil was larger (in *Agrostis capillaris* and *Holcus mollis* much larger) than in the silicate soil, with the exception of *Galium saxatile*, the two *Luzula* species and *Carex pilulifera*.

No apparent deficiency symptoms, including necroses or colour deviations compared to plants under field conditions, were observed in plants grown in the silicate soil. This was also the case with *Agrostis capillaris*, *Deschampsia flexuosa*, *Potentilla argentea* and *Viscaria vulgaris* cultivated in both (P−) and (P+) limestone soil. *Holcus mollis* displayed no symptoms in the (P+) soil but developed a violet colour of leaf apices in the (P−) soil. The leaves of *Rumex acetosella* rapidly turned purple-scarlet in the (P−) but not in the (P+) soil where, however, leaves and inflorescences grew somewhat paler green than in the silicate soil. Signs of foliar chlorosis in the limestone soils were observed with *Luzula pilosa* and *L. campestris*, with the latter also exhibiting necroses in the (P−) soil, where growth was poor. Distinct foliar chloroses, most marked in the young leaves, were observed in *Galium saxatile*, *Carex pilulifera* and *Veronica officinalis*, mainly in the (P+) soil.

In summary, the various calcifuge species tested behaved differently as to growth rate and foliar morphology. Whereas all species, except *Galium saxatile*, reacted positively with an often vigorous growth on phosphate amendment of the limestone soil, species differed greatly with respect to foliar symptoms. In species inclined to chlorosis, symptoms in the

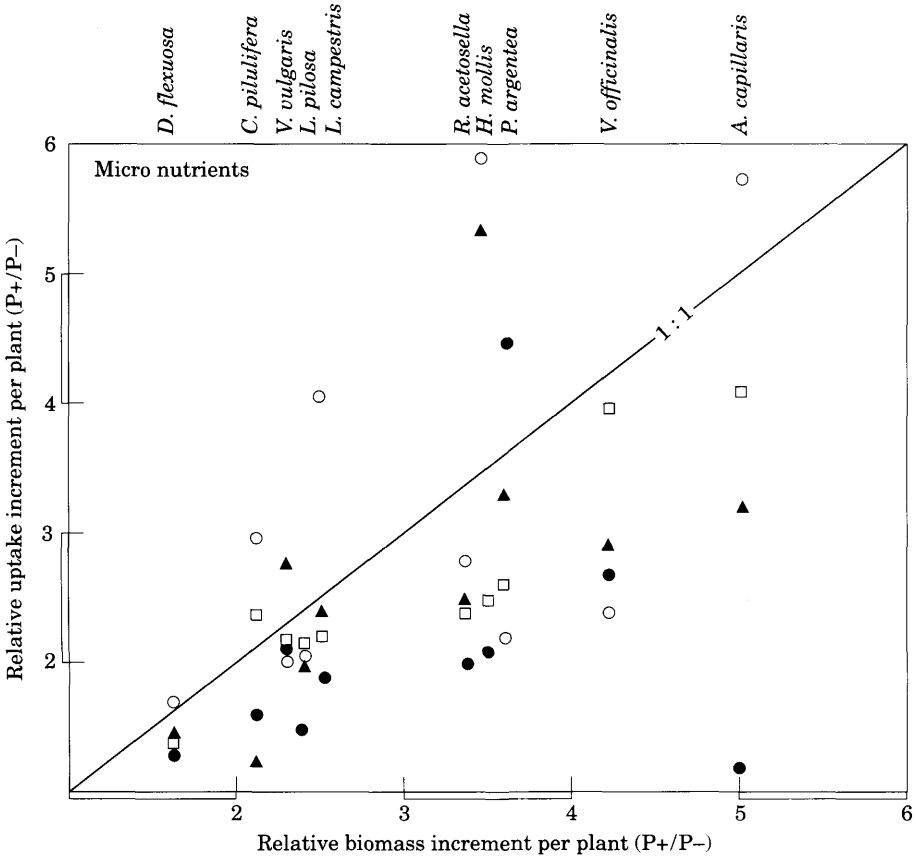

FIG. 2. As for Fig. 1, but micro nutrients. ●, Mn; ▲, Fe; ○, Zn; □, Cu.

expanding biomass were strengthened by the phosphate addition. The red or violet discoloration with two of the species in the (P−) soil did not appear at phosphate treatment, nor did the necroses observed in one species.

Mineral nutrient concentrations

The concentrations of mineral nutrients in the above-ground biomasses differed to a great extent among soils and treatments (Tables 2 and 3). In the (P−) limestone soil a much higher Ca concentration of the biomasses was accompanied by lower or slightly lower concentrations of most other elements in the silicate soil, markedly so for P, though usually not for Fe. In *Agrostis capillaris*, *Deschampsia flexuosa*, *Potentilla argentea*, *Rumex acetosella* and *Veronica officinalis* the biomass concentrations of Fe were actually higher ($P < 0.05$) in the (P−) limestone soil than in the silicate soil, whereas they were lower only in *Viscaria vulgaris*.

Biomass concentrations of P were on average three times as high in the (P+) as in the (P−) limestone soil. Concentrations and uptake of K and S were mostly also higher in the (P+) soil than in the (P−) soil, though interspecies differences were evident (Table 3; Fig. 1). Biomass concentrations of Mg in the (P−) limestone soil were similar to or lower ($P < 0.05$) than in the silicate soil (Table 3). Phosphate amendment tended to stimulate the uptake of Mg more than the biomass production, particularly evident for *Veronica officinalis*, *Carex pilulifera* and *Holcus mollis* (Fig. 1). In none of the species, was any dilution effect observed on biomass Mg as a result of the increased biomass production in the (P+) soil.

The concentrations of Fe tended to be slightly lower in the biomasses of the (P+) soil, though not consistently; significantly ($P < 0.05$) mainly in *Carex pilulifera* and *Agrostis capillaris*. On average, biomass concentrations of Fe in the (P+) limestone soil were similar to those in the silicate soil. Several species reduced their concentrations of Mn considerably when grown in the (P+) compared to the (P−) limestone soil (Fig. 2). This was particularly evident in *Agrostis capillaris* which diluted biomass Mn at almost the same rate as biomass increased. Also with the other species tested (except *Potentilla argentea* and *Viscaria vulgaris*) Mn concentrations of the biomass were lower ($P < 0.05$) in the (P+) than in the (P−) limestone soil, indicating a dilution effect of this element. Compared to the plants cultivated in the silicate soil, Mn concentrations in the biomass from the (P+) limestone soil were particularly low (17–26%) in *Agrostis capillaris*, *Holcus mollis* and *Rumex aetosella*. These species had a high productivity in the (P+) soil and did not develop any apparent morphological aberrations.

Concentrations of Zn in the biomass were usually slightly lower in both limestone soil treatments than in the silicate soil (Table 3), most markedly lower for *Viscaria vulgaris* and (in the P+ treatment) for *Scleranthus perennis*. A dilution effect in the (P+) treatment was observed with

TABLE 4. *Shares of different cationic elements of the molar cation sums, calculated as the ratios of the shares in biomasses of phosphate amended (P+) and unamended (P−) limestone soil*

	K	Mg	Ca	Fe	Mn	Zn	Cu
Agrostis capillaris	0·99	0·92	1·09	0·62	0·23	1·11	0·79
Carex pilulifera	1·38	0·88	0·57	0·40	0·38	0·96	0·76
Deschampsia flexuosa	1·02	0·84	1·01	0·83	0·76	0·97	0·79
Holcus mollis	0·87	1·47	1·35	1·32	0·51	1·41	0·61
Luzula campestris	1·29	0·82	0·62	0·73	0·57	1·22	0·66
Luzula pilosa	0·97	0·78	1·11	0·70	0·52	0·71	0·75
Potentilla argentea	1·08	0·88	0·97	0·87	1·18	0·57	0·68
Rumex acetosella	1·01	0·85	1·03	0·48	0·39	0·55	0·46
Veronica officinalis	0·94	1·78	1·07	0·59	0·55	0·49	0·82
Viscaria vulgaris	1·02	0·90	0·98	1·09	0·81	0·83	0·81
All species	1·04	0·94	0·94	0·72	0·59	0·82	0·71

Veronica officinalis grown for 50 d.

Veronica officinalis and *Potentilla argentea* whereas Zn uptake seemed to be stimulated by the P amendment in *Carex pilulifera*, *Holcus mollis* and *Luzula campestris* (Fig. 2). The biomass concentrations of Cu in the limestone soil treatments on average differed little from those of the silicate soil, though slight dilution effects were recorded with some species in the (P+) treatment.

A different mode of assessing biomass dilution effects on elements was also tested. The share taken by a particular element out of the total molar sum of mineral elements was calculated for the cation elements, and differences between treatments were expressed as the ratio between these concentration shares in the biomasses of the (P+) and the (P−) limestone soil treatments (Table 4). A ratio < 1 indicates a dilution of the element in the molar sum of cations in the biomass of the (P+) soil. As K constitutes a major part of the molar cation sum (Table 2), data obtained with this mode of calculation is also rather closely based on the K concentrations of the biomass.

With few exceptions, only minor deviations from 1 were calculated for the cationic macronutrients (K, Mg, Ca). On the contrary, ratios < 1 were the rule for the micronutrients, in particular for Mn (Table 4). The dilution effect caused by phosphate treatment and/or the accompanying biomass increment was more pronounced with Mn than with any of the other elements in eight of the species studied, *Potentilla argentea* being the main exception. However, dilution effects, though less pronounced or marginal, were also recorded with Cu in all species, with Fe in seven and with Zn in five species.

DISCUSSION

There are apparent difficulties in defining deficiency levels of mineral nutrients in plants and soils of natural habitats, in spite of the fact that there is a wealth of data concerning crops and other economically important plants. Sufficiency/deficiency concentrations of elements in biomass of cultivated plants are not easily applicable to other species, as ambient biomass concentrations vary considerably among plants growing under seemingly similar conditions, a fact which does not necessarily reflect differing physiological demands. However, a general comparison with the sufficiency concentration data for mineral nutrients in the biomass of cereals, forage grasses and dicotyledon crops, reported by Bergmann (1988), reveals that concentrations measured in the present study, with very few exceptions, are well within the ranges stated, also in the limestone soil. The four micronutrients were usually in the upper half of the sufficiency ranges. But there is one real exception: the biomass concentrations of P were generally below the sufficiency ranges indicated, particularly in the untreated (P−) limestone soil but even in the silicate soil. The phosphate treatment of the limestone soil raised the biomass P concentrations to well within the sufficiency range but usually not to the upper half of this range, as reported for the foliar biomass of numerous crops.

The species tested in these and previous studies differ to some extent regarding their ability to grow in limestone soils. The stage of development is also of importance, establishment from seeds being particularly sensitive to low phosphate availability (Tyler, 1992). Among the 12 calcifuges tested in this study only four produced less aboveground biomass in the (P+) limestone than in the silicate soil: *Galium saxatile* (23%), *Luzula pilosa* (56%), *Carex pilulifera* (68%), and *Luzula campestris* (72%). Production was consistently lower in the (P−) than in the silicate soil, including one complete failure (*Scleranthus perennis*). As evident with *Veronica officinalis* a generally longer duration of the experiment would have produced even greater differences among treatments and soils in disfavour of the (P−) limestone soil.

One basic assumption in foliar analysis adopted in this study is that non-limiting essential elements are absorbed from soil in some excess compared to physiological demands. This assumption is founded on the generalization that increasing concentrations or fluxes of an ionic element to the rhizophere of plants usually increases the uptake rate of the element by the plants. It is certainly possible to find several exceptions to this rule, but numerous observations support a general validity of this assumption. As a consequence, decreasing concentrations of a mineral nutrient in the tissues of growing plants should mirror an inability of the plant to maintain the uptake rate of the nutrient. The relationship between this relative concen-

tration decrease in the biomass and the decreasing distance in concentration to a deficiency level is, however, not absolute, as consumption of non-limiting elements above the physiological demands may also be variable and not necessarily reflect the degree of excess.

Manganese, the element found to display the greatest concentration decreases in biomass produced on the (P+) limestone soil, is known to be taken up in great excess by some plants on acid soils and may there be released from the foliage in considerable quantities under ambient conditions (Bergkvist, 1987*a*, *b*). On calcareous soils, however, where concentrations of soil solution and exchangeable Mn are very low, this element sometimes decreases to deficiency levels in certain crops. The limited capacity of most of the calcifuges studied to absorb more Mn from the (P+) than from the (P−) limestone soil, in order to keep pace with the growth rate, might be an indication of an approaching state of deficiency, when phosphate is eliminated as the growth-limiting element.

However, all the cationic micronutrients studied tended to be diluted in the biomass of most or several of the species in the (P+) soil, but usually less than for Mn. Though conflicting evidence exists, phosphate may act antagonistically on several transition and heavy metal ions, both chemically in the soil and physiologically in the plant, also reported for soils of a high pH (Booss, Kolesch and Höfner, 1982; Kolesch, Oktay and Höfner, 1984). One probable reason for the dilution effect on these micronutrients is therefore a phosphate uptake from the (P+) soil slightly in excess of the demands of these plants of nutrient-poor soils This may accelerate an approaching deficiency of micronutrients in phosphate-treated soil.

Least pronounced was the dilution effect in Fe, an element often proposed to constitute a main limitation to growth or general performance of calcifuges on calcareous soils. The only apparent example among the ten species analysed for tissue concentrations of elements was *Carex pilulifera*, which actually developed severe chlorosis when growth was accelerated by phosphate. This chlorosis was particularly pronounced in the youngest parts of the foliar biomass. Symptoms were not consistent with the pattern in graminids known to characterize deficiency of Mn, an element which was also appreciably diluted in the biomass of chlorotic *Carex pilulifera*. The chlorotic symptoms which developed in *Veronica officinalis* were, however, not accompanied by any Fe dilution in above-ground biomass of the (P+) soil, in spite of a vigorous growth.

In a previous report (Tyler, 1994), evidence of an inability to solubilize enough Fe from a limestone soil was given for *Galium saxatile*, the survival of which was very poor or failed in both untreated and phosphate treated soil unless repeatedly supplied with Fe(III)citrate. In the present study, poor growth was obtained with this species in the (P−) limestone soil and an even poorer growth, combined with chlorosis, in the (P+) soil. The availability of phosphate is apparently not the primary growth-limiting factor in *Galium saxatile*, whereas there is evidence from the previous study that Fe may be the element in this species.

Increased growth due to reversal of the phosphate limitations did not, or only marginally, cause dilution of the tissue Fe concentrations in most of the tested plants. However, possible Fe limitations may develop on the tissue level, due to immobilization of Fe in non-metabolic species (Nelson, Wallace and Brown, 1982; Chaney, 1984). This is not consistently due to the availability of soil Fe but may be a consequence of the high Ca^{2+} or HCO_3^- concentrations of the limestone soil environment, sometimes combined with a generally reduced root activity (Scholl, 1979; Bergmann, 1988).

The ecological importance of the partial chlorosis observed in the (P+) treatment with a few of the species, deserves more consideration. *Veronica officinalis* was almost able to maintain the higher productivity rate in the (P+) soil compared to the natural silicate soil between the first (30 d) and the second (50 d) sampling, in spite of clear signs of intercostal foliar chlorosis. Partial chlorosis of plants growing in open-field conditions at high illumination rates would be of minor importance, whereas chlorosis in plants growing on the forest floor would be at a competitive disadvantage. The three species which developed apparent chlorosis in this study (*Carex pilulifera*, *Veronica officinalis*, and *Galium saxatile*) have all rather wide field amplitudes in respect to illumination climate. It might be hypothesized that chlorosis caused by tissue Fe immobilization, or some other mechanism of Fe deficiency related to soil chemistry, would be most harmful in sites with low levels of incident light, e.g. in forests.

CONCLUSIONS

Calcifuge vascular plants are unable to develop successfully in limestone soil owing to deficient capacity in rendering enough phosphate available for uptake. There may be a few exceptions, notably *Galium saxatile*, where soil conditions apparently related to the Fe nutrition may constitute primary limitations.

It was hypothesized that micronutrients such as Fe, Mn, Zn, and Cu will become limiting if the phosphate status of a limestone soil is improved to sufficiency. Decreasing biomass concentrations (dilution) of micronutrients, possibly combined with foliar symptoms of deficiency, would be anticipated.

Results obtained may be summarized as follows. (*a*) Phosphate treatment of the limestone soil raised the P concentrations of the plant biomasses to levels within sufficiency ranges reported for cultivated plants and productivity usually increased two- to five-fold. Out of the 12 species studied, *Scleranthus perennis* was unable to survive in the limestone soil unless phosphate treated, whereas growth and general performance was impaired by phosphate only in *Galium saxatile*. (*b*) Dilution effects on the micronutrients (but usually not on the macronutrients) were recorded as a result of the phosphate treatment, being most distinct in Mn and least distinct in Fe. However, no foliar symptoms clearly assignable to Mn deficiency were observed. (*c*) Symptoms of foliar lime chlorosis reminiscent of Fe deficiency developed in a few of the species (*Carex pilulifera*, *Veronica officinalis* and *Galium saxatile*). In *Carex pilulifera*, but not in *V. officinalis*, was chlorosis accompanied by decreasing foliar Fe concentrations.

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