

A CRITICAL TEST OF THE TWO PREVAILING THEORIES OF PLANT RESPONSE TO NUTRIENT AVAILABILITY¹

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Whereas the “law of the minimum” (LM) states that plant growth is limited by a single resource at any one time, the “multiple limitation hypothesis” (MLH) proposes that optimum plant behavior results from balancing resource costs and benefits so that all resources limit plant growth simultaneously. We tested the hypothesis that neither the LM nor the MLH account for plant responses to all mineral nutrients. Fronds of the aquatic plant *Lemna minor* were grown in nutrient solutions with increasing levels of four nutrients: nitrogen, phosphorus, potassium, and magnesium. Neither LM or MLH adequately predicted plant responses to all of these nutrients: 23 of the 60 responses analyzed were classified as belonging to the LM; 20 cases were classified as undefined; and 17 cases as MLH. The type of response strongly depended on the specific pair of nutrients considered. The validity of the MLH model would depend on the accompanying resource limiting plant growth and on the severity of the stress. We propose that a “nutrient-specific” analysis, considering the biology of each mineral nutrient rather than grouping plant resources as a whole, is more appropriate than general models in understanding plant responses to nutrient availability.

Key words: law of the minimum; *Lemna minor*; magnesium; multiple limitation hypothesis; nitrogen; phosphorus; plant resources; potassium.

At the beginning of the 19th century, Carl Sprengel published several works about the role of essential resources on plant growth. He affirmed that “. . . when a plant needs 12 substances to develop, it will not grow if any one of these is not available in a sufficiently large amount as required by the nature of plants” (Sprengel, 1828). Some years later, Justus Von Liebig re-elaborated Sprengel’s pioneering ideas and articulated the “law of the minimum” (Liebig, 1855; see also van der Ploeg, Bohm, and Kirkham, 1999). The law of the minimum states that plant growth is limited by a single resource at any one time. Only after the availability of that resource increases to the point of sufficiency can another resource enhance plant growth (Fig. 1). Mitscherlich (1909) enriched these theories by formulating the law of diminishing yield increments, which considers the limits of plant growth in the absence of resource limitations. According to this law, the yield response curves for a particular resource have a precise upper limit and are asymptotic. Liebig’s law and the Mitscherlich model have been widely accepted in modern agriculture (Cerrato and Blackmer, 1990; Paris, 1992). No great controversy between these theories exists, because Liebig refers to situations where plants are constrained by at least one limiting resource whereas Mitscherlich’s work mainly refers to the potential or maximum growth attainable by higher plants.

In contrast, Liebig’s law leads to divergent predictions compared to the current paradigm of plant resource use in ecology, the “multiple limitation hypothesis” (MLH), which is based on microeconomic analogies and cost-benefit analysis (Bloom, Chapin, and Mooney, 1985; Chapin et al., 1987; Gleeson and Tilman, 1992; see also Rastetter and Shaver, 1992; Van der Berg, 1998). According to the MLH, optimum plant adaptive behavior results from balancing resource costs and benefits in such a way that all resources limit plant growth simultaneously

(Bloom, Chapin, and Mooney, 1985). Consequently, “the greatest fitness for an individual plant results in a morphology and physiology for which no resource is taken up in excess” (Gleeson and Tilman, 1992, p. 1323). This theory proposes that if growth is limited by one resource, a plant should allocate more effort to acquiring the limiting resource and less to acquiring other resources. The resulting shift in the allocation of internal resources enhances the acquisition of the limiting resource, creating a dynamic balance in which growth is equally limited by all resources (Bloom, Chapin, and Mooney, 1985). One key assumption of the MLH is that resources can be substituted to a varying degree by each other through a common currency such as carbon (Bloom, Chapin, and Mooney, 1985). The typical examples of the MLH model are plant responses to growth constraints imposed simultaneously by aboveground (e.g., carbon or light) and belowground resources (e.g., nitrogen). By adjusting the root-to-shoot ratio, plants can balance the acquisition of carbon or nitrogen. An important feature of these morphological adjustments is that in both carbon-limited and nitrogen-limited plants, both carbon and nitrogen become effectively limiting (Chapin et al., 1987). In carbon-limited plants, reduced root growth limits nitrogen acquisition. In nitrogen-limited plants, reduced shoot growth limits photosynthetic carbon gain. Under these circumstances, the MLH predicts that carbon-limited plants would respond positively to increased nitrogen supply and nitrogen-limited plants would respond positively to increased carbon supply.

Liebig’s law and the MLH predict markedly different responses to the addition of a single resource. MLH plants should have a positive response to the addition of any individual resource at all levels (Fig. 1) (Gleeson and Tilman, 1992). From Fig. 1, it is clear that Liebig’s law and the MLH cannot be valid at the same time and in any specific case one should prevail over the other. As noted above, the MLH fits empirical observations of plant responses to the interactions of carbon, water, and nitrogen limitations. However, when interactions among diverse nutrient resources are considered, the situation is less clear. In the first place, there are 16 essential mineral nutrients and a limited number of plant allocation

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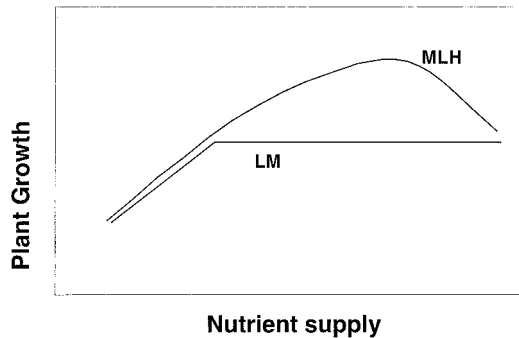


Fig. 1. Illustration of theoretical models of plant growth responses to increasing resource availability, showing predictions of the multiple limitation hypothesis (MLH) and the law of the minimum (LM).

strategies, most of which will affect the acquisition of multiple nutrients. For example, enhanced root growth would enhance the acquisition of all belowground resources, not only the limiting one. Positive correlations between the mechanisms of acquisition (which results in simultaneous uptake) will prevail and the likelihood of multiple limitations would diminish (Gleeson and Tilman, 1992). This presumption denotes why the MLH may not apply to mineral nutrition. Another difficulty is that mineral nutrients are very diverse, have specific roles in plant functioning and cannot be substituted for one another. Therefore, co-optimization of all 16 nutrients is complex, given the limited number of adaptations, especially morphological, that are possible.

Lynch and Gonzalez (1993) observed that optimum use of primary resources, such as light and nitrogen, automatically determines the allocation patterns of a range of other resources (e.g., mineral nutrients such as calcium, magnesium, etc.), which otherwise may conceivably have other "optimum" allocations. Primary resources (presumably water, light, and nitrogen) may benefit from inherent mechanisms to integrate the principal resource constraints and when in short supply may affect growth through MLH-type responses. Lower priority nutrients, those not as universally limiting in plant evolution, would be required in rather fixed amounts defined by the growth rate and when in short supply may affect growth through the law of the minimum caused by physiological dysfunction, rather than the colimitation mechanisms proposed by Bloom, Chapin, and Mooney (1985). In this case, nitrogen would behave according to the MLH, but other nutrients may obey Liebig's law, and the interactions of multiple nutrient constraints are likely to be complex. Given the prevalence of multiple nutrient constraints in natural ecosystems (notably the acid soil complex characteristic of humid forests), this distinction is of considerable practical significance.

The objective of this work was to evaluate the validity of the Liebig and MLH theories under conditions of multiple nutrient constraints to plant growth. We tested the hypothesis that neither the law of the minimum nor the multiple limitation hypothesis account for plant responses to all mineral nutrients. In particular, we predict that plant responses to nitrogen availability will follow the MLH model, whereas Liebig's law is the prevailing model describing plant responses to the other nutrients. Our focus here is biomass responses of individual plants rather than physiological mechanisms or community responses, which are worthy topics beyond the scope of this study.

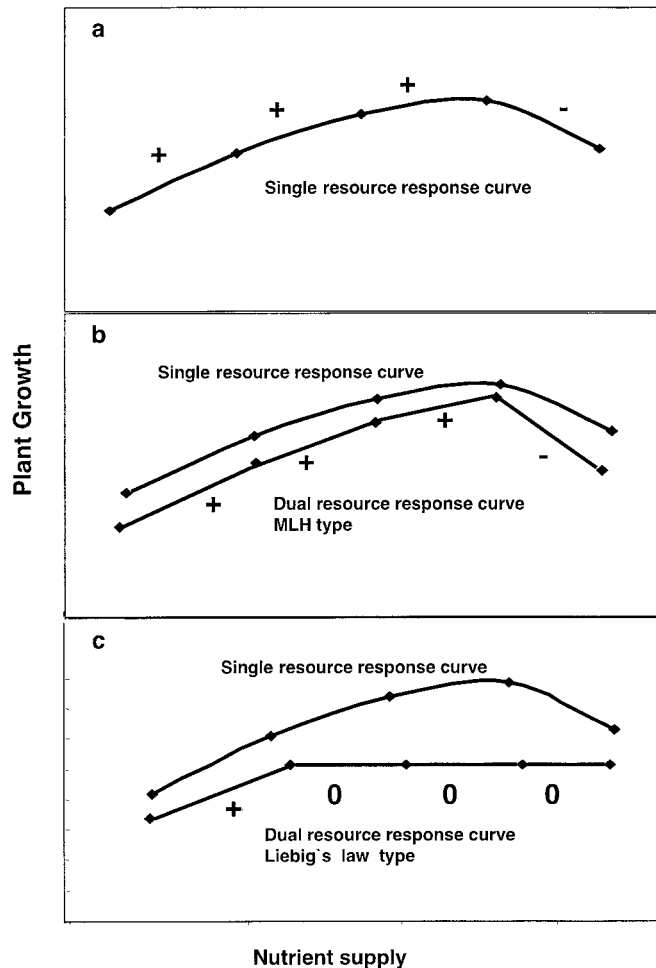


Fig. 2. Illustration of system used to classify growth responses to nutrient availability. (a) Growth response to increasing availability of a nutrient resource ("single-resource response curve"). One-way ANOVAs were performed for each one of the five doses of the nutrient, and the sign of the slope (positive, neutral, or negative) between successive levels of the "subject" nutrient was recorded. (b) Growth response to increasing availability of a nutrient resource under conditions of low availability of a second nutrient resource, according to the multiple limitation hypothesis (MLH). Note that positive responses to increasing nutrient availability occur in every case except at the level of the resource where no growth-promoting effect was observed in the single-resource response curve (a). For a response to be considered MLH, the sign of the slopes between successive levels of the subject nutrient must coincide with the signs of the slopes in the single-resource experiment for the respective nutrient. (c) Growth response to increasing availability of a nutrient resource under conditions of low availability of a second nutrient resource, according to the law of the minimum (LM; Liebig's law). Note that the curves saturate before reaching the level of the resource where no growth-promoting effect was observed in the single-resource response curve (a).

MATERIALS AND METHODS

Plant material and growth conditions—Plants of *Lemna minor* were obtained from the plant collection of the Department of Biology of the Pennsylvania State University, University Park, Pennsylvania, USA. *Lemna minor* is a floating plant that lives just below or at the surface of fresh water (Hillman, 1962). Roots of *L. minor* arise at the node just beneath the leaves. The individual structure of this plant is called a frond, which usually has a maximum of five leaves. Fronds produce new "daughter" fronds, which remain for some time attached to the "mother" frond (Hillman, 1962). The "daughter" fronds continue the cycle, and after a very short period of time they are

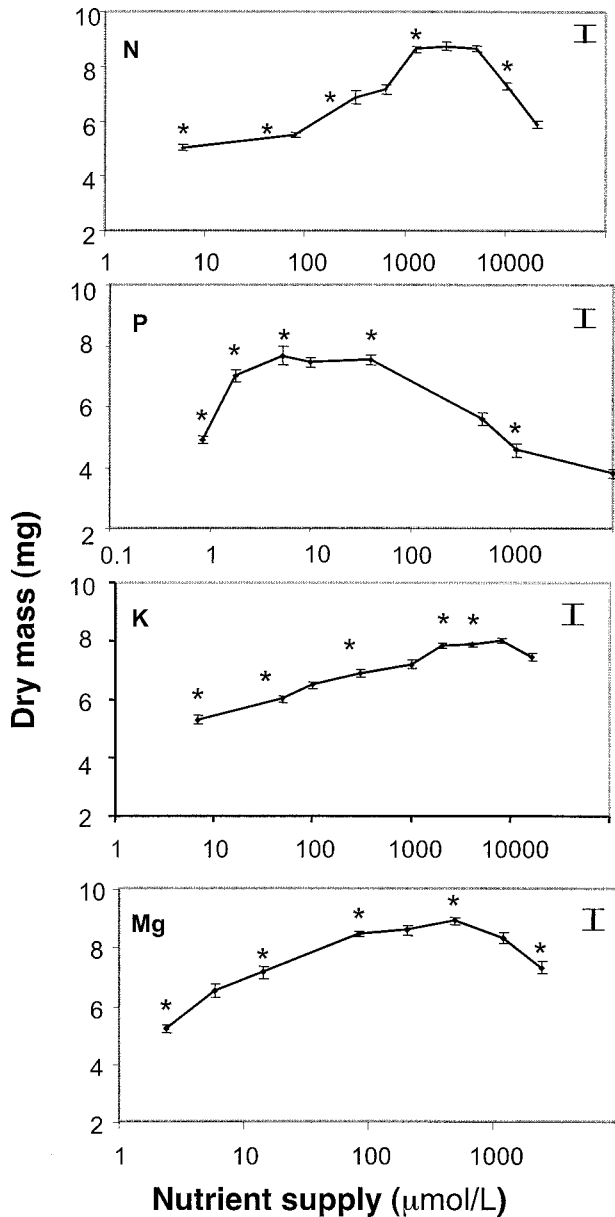


Fig. 3. Single-resource response curves for nitrogen, potassium, phosphorus, and magnesium. Bars on the right side indicate the critical value for comparison between means according to the LSD test. Asterisks indicate the nutrient supply levels selected for the dual-resource response curves.

able to produce new fronds. Under our growth conditions, plants completed several growth cycles and increased dry mass from 6 to 10 times in only 6 d. This extraordinarily high growth rate makes *L. minor* a suitable model for studies, like the present one, in which time scale is a key factor to interpret plant adaptations to changes in the availability of resources. Collected fronds were soaked in distilled water to remove nutrient residues from the original media. Five fronds were transplanted to 100-mL petri dishes filled with nutrient solution. A modified half strength Epstein's nutrient solutions adjusted to pH 4.5 was used. This consisted of P (40 µmol/L, alumina buffered), K (3000 µmol/L), NO₃ (7000 µmol/L), NH₄ (1000 µmol/L), Ca (2000 µmol/L), SO₄ (500 µmol/L), Mg (500 µmol/L), Cl (25 µmol/L), B (12.5 µmol/L), Mn (1 µmol/L), Zn (1 µmol/L), Cu (0.25 µmol/L), Mo (0.25 µmol/L), and EDTA-Fe (25 µmol/L). In each specific treatment, the salt containing the studied nutrient was replaced or adjusted accordingly. To maintain uniformity among the experimental units, all selected fronds had four leaves, making a

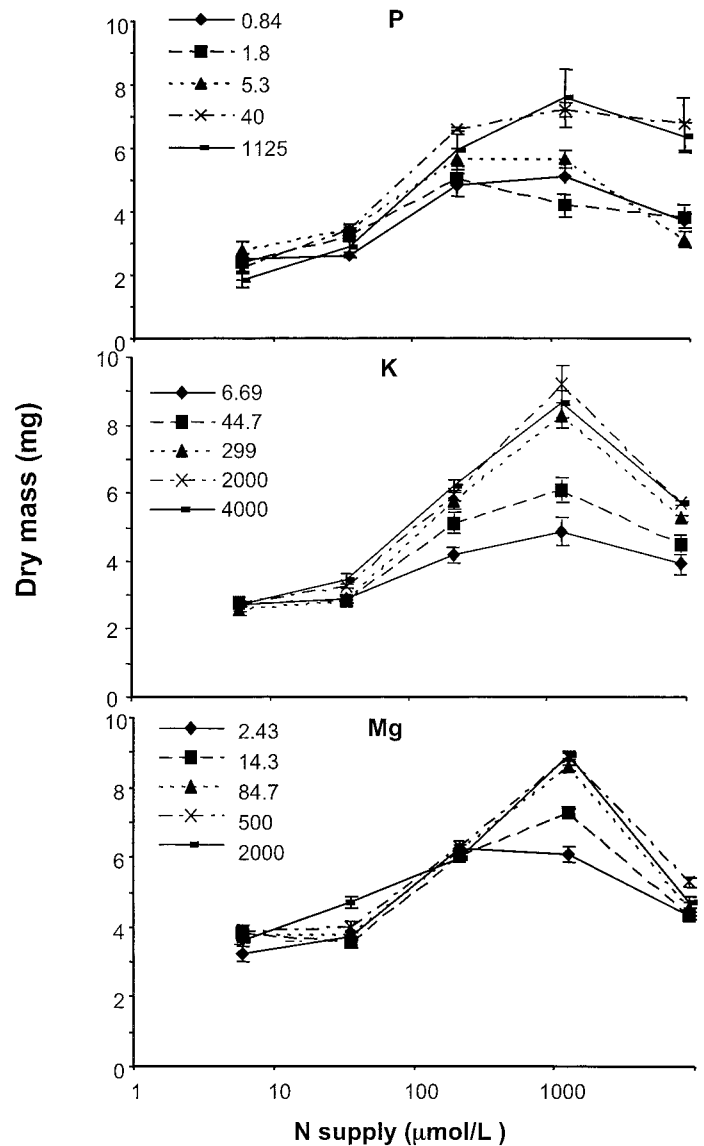


Fig. 4. Dual-resource response curves for nitrogen as the subject nutrient and phosphorus, potassium, and magnesium as accompanying nutrients. Values given for each curve represent the concentration of the accompanying nutrient in micromoles per liter.

total of 20 leaves per dish. The dishes were installed in a growth room with the following conditions: photosynthetic photon flux density, 150 µmol photosynthetically active radiation · m⁻² · s⁻¹ continuous light; temperature 25° ± 2°C; day and night and relative humidity, 65 ± 5%. We focused on four nutrients: nitrogen, phosphorus, potassium, and magnesium. In the first set of experiments, plant growth in response to varying availability of each of these nutrients was determined. Based on the resulting response curves, the interactions among the four nutrients (taken in pairs) were studied in the second set of experiments.

Single-resource enrichment experiments—The response curves to nitrogen, phosphorus, potassium, and magnesium were determined by adding increasing amounts of these nutrients to the aqueous media. The following concentrations of nutrients were chosen to evaluate the response curves: nitrogen, 6, 80, 320, 640, 1280, 2560, 5120, 10240, 20480 µmol/L nitrogen as ammonium nitrate; phosphorus, 0.84, 1.8, 5.3, 10, 40, 512, 1125, 10000 µmol/L phosphorus; potassium, 7, 50, 100, 300, 1000, 2000, 4000, 8000, 16000

TABLE 1. One-way ANOVAs for total biomass in the dual-resource experiments involving nitrogen; comparison of means according to the LSD method; and classification of the enrichment response curves as multiple limitation hypothesis (MLH) type, law of the minimum (LM) type, or undefined. The same letters in the same row means no statistical difference between treatments. The symbol below the letter represents the sign of the slope between the successive levels of the subject.

Nutrient ^a	Level	F	P	Nitrogen level					Classification	
				1	2	3	4	5		
N					0	+	+	–		
	P	1	26.1	0.00	C	C	A	A	B	Undefined
		2	10.0	0.000	D	CD	A	AB	BC	LM
	3	32.6	0.00	B	B	A	A	B	LM	
		4	28.9	0.00	B	B	A	A	A	LM
5	26.0	0.00	B	B	A	A	A	Undefined		
K	1	18.7	0.00	C	C	B	A	B	MLH	
	2	41.6	0.00	C	C	B	A	B	MLH	
	3	120.6	0.00	C	C	B	A	B	MLH	
	4	130.5	0.00	C	C	B	A	B	MLH	
	5	114.4	0.00	D	C	B	A	B	MLH	
Mg	1	68.7	0.000	C	C	A	A	B	LM	
	2	152.4	0.00	D	D	B	A	C	MLH	
	3	210.0	0.00	D	D	B	A	C	MLH	
	4	204.4	0.00	D	D	B	A	C	MLH	
	5	365.5	0.00	D	C	B	A	C	MLH	
Totals										
MLH									9	
LM									4	
Undefined									2	

^a In these treatment, N is the subject nutrient; P, K, and Mg are the accompanying nutrients.

μmol/L potassium as potassium sulfate; and magnesium, 2.4, 5.9, 14.3, 84.7, 206, 500, 1215, 2430 μmol/L magnesium as magnesium sulfate. These phosphorus levels were provided by P-loaded alumina, which provided a buffered concentration of the element in the aqueous solution (Lynch et al., 1990).

Each nutrient level was replicated five times, making a total of 170 experimental units. Plants were harvested after 6 d of growth. At harvest, images of the petri dishes were taken with a digital camera (Pixera, Optical Apparatus, Los Gatos, California, USA) and then scanned and analyzed with DT-Scan (Delta-T Devices, Cambridge, UK) to calculate leaf area. Total biomass (roots plus shoots) was obtained after drying the harvested material 2 d at 60°C in an electric oven.

Dual-resource enrichment experiments—The four nutrients were combined in pairs to evaluate six interactions: N × P, N × K, N × Mg, P × K, P × Mg and K × Mg. From the single-resource enrichment experiments, five representative doses were chosen for each nutrient. These five doses were selected to include the whole range of the response curve: deficiency, optimum, and supraoptimal, which decreased growth compared to the optimum level. In the case of potassium, this last level was not attained. The doses of nitrogen, potassium, and magnesium were adjusted to fit a log scale, assuming a linear relation between successive points of the single resource response curve. The selected levels were nitrogen, 5.9, 35.6, 213, 1280, and 9000 μmol/L; phosphorus, 0.84, 1.8, 5.3, 40, and 1125 μmol/L; potassium, 6.69,

44.7, 299, 2000, and 4000 μmol/L; and magnesium, 2.4, 14.3, 84.7, 500, and 2000 μmol/L.

The interaction between each pair of nutrients was evaluated by combining the five doses of each one of them. Each pair of nutrients constituted an individual experiment composed of 25 treatments (5 levels of the first nutrient × 5 levels of the second nutrient). The total number of experimental units was 750 (6 pair of nutrients × 25 treatments × 5 replicates). Plants were harvested after 6 d of growth. Total biomass (roots plus shoots) was obtained after drying the harvested material 2 d at 60°C.

Statistical analysis and determination of the type of response—Five replicates were employed in all cases. The nutrient interactions evaluated in the dual-resource enrichment trials were separated into four groups, one for each nutrient. Each group included the combination of a “subject” nutrient with three “accompanying” nutrients. The subject nutrient was represented in the x-axis and the accompanying nutrient in the y-axis. This grouping system allowed a better visualization of the plant responses to each nutrient. Plant responses to increasing nutrient levels were classified as “Liebig,” “MLH,” or “undefined” according to the shape of the enrichment curve. Each one of the five curves of the plots corresponding to the dual-resource experiments was classified into these categories. To determine the type of response of each curve, a simple classification system was made based on the results of the single-resource enrichment experiments (Fig. 2). Each curve was compared

TABLE 2. One-way ANOVAs for the total biomass in the dual-resource experiments involving phosphorus; comparison of means according to the LSD method; and classification of the enrichment response curves as multiple limitation hypothesis (MLH) type, law of the minimum (LM) type, or undefined. The same letters in the same row means no statistical difference between treatments. The symbol below the letter represents the sign of the slope between the successive levels of the subject.

Nutrient ^a	Level	F	P	Phosphorus level					Classification
				1	2	3	4	5	
P	N	1.8	0.19	AB	+	+	0	–	LM
		3.6	0.03	B	0	A	AB	D	Undefined
		3.3	0.04	B	+	A	A	AB	Undefined
		12.5	0.00	BC	0	0	0	0	Undefined
		12.9	0.00	B	0	B	A	A	Undefined
K	1	0.007	5.2	C	0	B	AB	A	Undefined
		9.9	0.00	C	0	0	0	0	Undefined
		5.9	0.01	C	0	B	BC	A	Undefined
		4.0	0.02	C	0	A	AB	AB	Undefined
		19.9	0.00	C	0	A	AB	D	Undefined
Mg	1	12.7	0.00	A	0	B	B	B	LM
		4.7	0.02	A	–	0	0	0	LM
		1.45	0.26	A	–	0	0	–	Undefined
		3.41	0.04	B	0	A	A	A	Undefined
		8.6	0.00	B	0	A	A	B	Undefined
Totals									
MLH								0	
LM								3	
Undefined								12	

^a In these treatments, P is the subject nutrient; N, K, and Mg are the accompanying nutrients.

to the curve of the subject nutrient in the single-resource experiment. One-way ANOVAs and LSD tests were performed for each dose of the accompanying nutrient, and the sign of the slope (positive, neutral, or negative) between successive levels of the subject nutrient was noted. An MLH response curve must have a positive slope at all levels of the subject nutrient except at the level where no promoting effect on growth was observed in the single-resource experiments. In other words, the sign of the slopes between the successive levels of the subject nutrient must coincide with the signs of slopes in the single-resource experiment for the respective subject nutrient for a response to be considered as MLH. A Liebig response curve must have a flat section before reaching the level of maximum growth for the subject nutrient, as determined in the single-resource experiment. This flat section would be represented by neutral or negative slopes at doses of the subject nutrient that had a positive sign (i.e., doses that increased plant growth) in the single-resource experiment. The five points corresponding to the second nutrient should be distributed according to the relative response in plant growth in the single resource experiment of this second resource. When a curve could not be clearly classified as MLH or Liebig, it was described as “undefined.” In our view, this approach is an objective, quantifiable way to evaluate the interactions between two nutrients, taking into account plant responses to each of them in the absence of other constraints.

RESULTS

Single-resource enrichment experiments—Figure 3 shows the biomass accumulation of *L. minor* in response to increas-

ing levels of nitrogen, phosphorus, potassium, and magnesium. Plants of all experiments attained similar biomass at the minimum level (around 5 mg/dish). A decrease in biomass accumulation at the maximum level was observed in the nitrogen, phosphorus, and magnesium enrichment experiments, constituting a point of inversion in the yield response curves. In the case of potassium, this point of inversion was not reached, and the highest level did not increase the biomass accumulation. Because of the high correlation between biomass accumulation and leaf number ($r^2 = 0.993$) and leaf area ($r^2 = 0.992$), results of these two parameters are not shown.

Dual-resource enrichment experiments—*Nitrogen as the subject nutrient*—The single-resource enrichment experiment for this element showed a positive growth response when increasing nitrogen levels from level 1 to level 4 and a decrease at level 5 (Fig. 3). Consequently, in the dual-resource experiments, an MLH plant response should show a constant increase in biomass accumulation from level 1 to level 4 of nitrogen and a decrease at level 5. In the experiment N × P, Liebig-like plant responses were observed at phosphorus levels 2, 3, and 4; in all of them the fourth level of nitrogen caused no increment on plant growth. The interaction of ni-

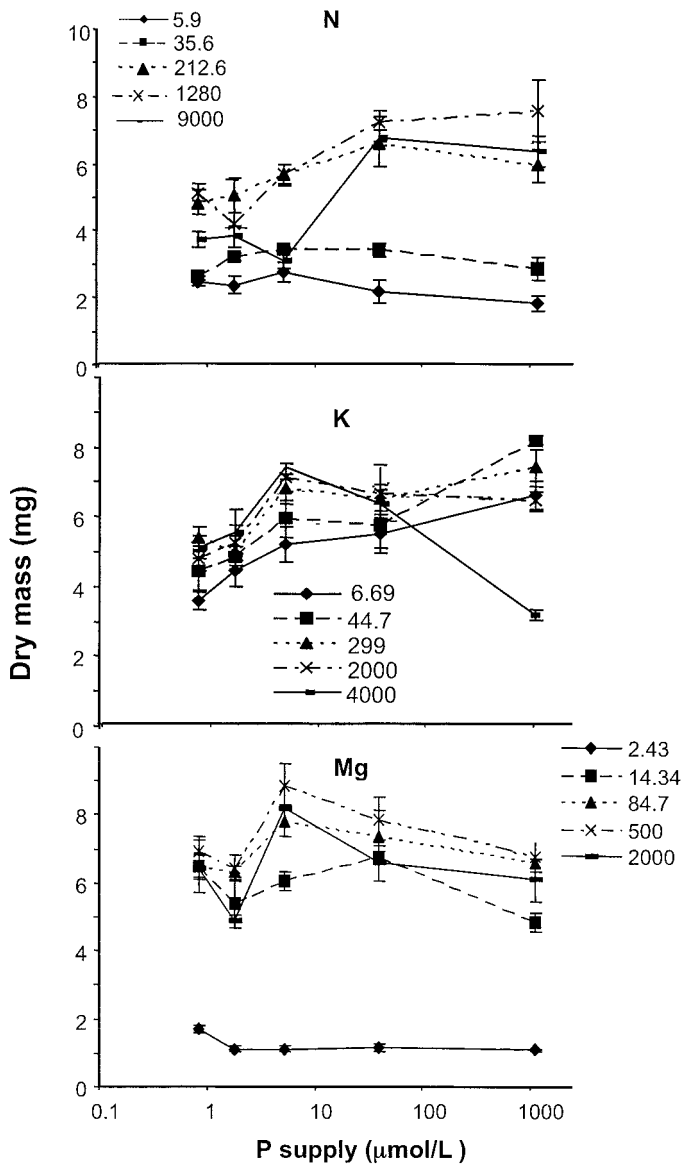


Fig. 5. Dual-resource response curves for phosphorus as the subject nutrient and nitrogen, potassium, and magnesium as accompanying nutrients. Values given for each curve represent the concentration of the accompanying nutrient in micromoles per liter.

trogen (as a subject nutrient) with potassium showed a nearly ideal MLH plant response (Fig. 4; Table 1). Positive responses to nitrogen addition were verified at all potassium levels until nitrogen level 4. A dramatic decrease in growth was observed at nitrogen level 5. In the experiment $N \times Mg$, four MLH plant responses were observed (at levels 2, 3, 4, and 5 of magnesium) and only at the lowest level of magnesium was the response classified as Liebig.

Phosphorus as the subject nutrient—The single-resource enrichment experiment for this element indicated (a) a positive growth response to increasing phosphorus availability from level 1 to level 3 (positive slope section); (b) no difference between levels 3 and 4 (flat section of the curve); and (c) a decrease in growth at level 5 (section with negative slope) (Fig. 3). According to these results, in the dual-resource ex-

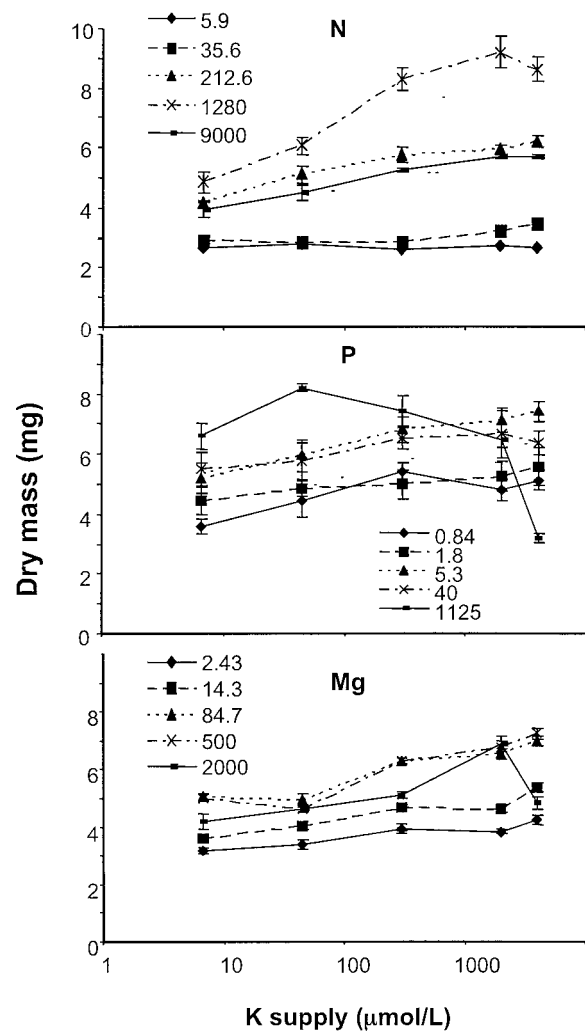


Fig. 6. Dual-resource response curves for potassium as the subject nutrient and nitrogen, phosphorus, and magnesium as accompanying nutrients. Values given for each curve represent the concentration of the accompanying nutrient in micromoles per liter.

periment an MLH plant response should show a constant increase in dry mass accumulation from level 1 to level 3 and a decrease at level 5.

The $P \times N$ experiment showed clear Liebig responses at nitrogen level 1 (Fig. 5). At nitrogen levels 3, 4, and 5, the patterns of the curves indicated some deviation from the typical MLH or Liebig behavior, so were classified as undefined. In the $P \times K$ experiment, the shape of the curves generally resembled an MLH response. However, because of large variability many of the differences among phosphorus levels were not statistically significant. In any case the signs of the successive slopes coincided in full with the ones found in the single-resource experiment, so they were defined as having an undefined response (Fig. 5; Table 2). No uniform pattern was observed in the $P \times Mg$ experiment. Liebig responses were observed at magnesium levels 1 and 2, whereas magnesium levels 3, 4, and 5 were classified as undefined.

Potassium as the subject nutrient—A positive response in plant growth when increasing potassium levels from level 1 to level 4 was observed in the single-resource enrichment exper-

TABLE 3. One-way ANOVAs for total biomass in the dual-resource experiments involving potassium; comparison of means according to the LSD method; and classification of the enrichment response curves as multiple limitation hypothesis (MLH) type, law of the minimum (LM) type, or undefined. The same letters in the same row means no statistical difference between treatments. The symbol below the letter represents the sign of the slope between the successive levels of the subject.

Nutrient ^a	Level	F	P	Potassium					Classification																																							
				1	2	3	4	5																																								
K	N	0.3	0.85	A	+	+	+	0																																								
				A	A	A	A	A	LM																																							
				0	0	0	0	0																																								
				B	B	B	A	A	LM																																							
				0	0	+	0	0																																								
P	2.55	0.08	0.08	B	B	A	AB	AB	LM																																							
				0	+	0	0	0																																								
				A	A	A	A	A	LM																																							
				0	0	0	0	0																																								
				A	BC	AB	AB	A	LM																																							
Mg	10.9	0.00	0.00	A	+	0	0	0																																								
				A	A	A	A	A	LM																																							
				0	0	0	0	0																																								
				B	A	AB	B	C	Undefined																																							
				+	0	0	0	—																																								
Totals	MLH	31.5	0.00	C	C	AB	B	A	Undefined																																							
				0	+	0	0	0																																								
				D	C	B	B	A	Undefined																																							
				0	+	0	0	+																																								
				C	C	B	AB	A	LM																																							
Totals	LM	33.1	0.00	C	0	+	0	0																																								
				0	+	0	0	0																																								
				D	D	C	B	A	LM																																							
				0	0	0	0	—																																								
				D	BC	B	A	B	Undefined																																							
Totals	Undefined	63.1	0.00	D	0	0	0	—																																								
				0	0	0	0	—																																								
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				0	0	0	0	—																																								
				D	BC	B	A	B	Undefined																																							
Totals	Undefined	25.0	0.00	D	+	0	+	—																																								
				+	0	0	+	—																																								
				D	D	C	B	A	LM																																							
				0	0	0	0	—																																								
				D	BC	B	A	B	Undefined																																							
Totals	MLH	35.2	0.00	B	A	AB	B	C	Undefined																																							
				0	+	0	0	—																																								
				0	0	0	0	—																																								
Totals	LM	35.2	0.00	B	A	AB	B	C	Undefined																																							
				0	+	0	0	—																																								
				0	0	0	0	—																																								
Totals	Undefined	35.2	0.00	B	A	AB	B	C	Undefined																																							
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LM									11																																							
Undefined									4																																							

^a In these treatments, K is the subject nutrient; N, P, and Mg are the accompanying nutrients.

iment, and no significant difference between levels 4 and 5 was observed (Fig. 3). In consequence, in the dual-resource experiments an MLH plant response should have a constant increase in dry mass accumulation from potassium level 1 to level 4 and no difference between levels 4 and 5.

Most observed responses to this nutrient belonged to the Liebig type (Fig. 5; Table 1). In the K × N experiment, responses to nitrogen levels 1, 3, 4, and 5 were Liebig type, whereas the response to the second level of nitrogen was undefined (Fig. 6; Table 3). In the K × P experiment, five Liebig-like responses were identified. In the K × Mg experiment, the shape of the response curves also resembled the Liebig type. However, the positive response to the higher level of potassium did not allow classifying the magnesium levels 1 and 2 as Liebig. Only magnesium levels 3 and 4 completed all the requirements to be classified as the Liebig type.

Magnesium as the subject nutrient—The single-resource enrichment experiment for this element showed a positive growth response when increasing magnesium levels from level 1 to level 3, no difference between levels 3 and 4 and a small decrease in plant growth at level 5 (Fig. 3). In the dual-resource experiment an MLH plant response should show a con-

stant increase (i.e., a positive slope) in dry mass accumulation from level 1 to level 3 and a decrease at level 5.

The response to this element did not show a definite tendency between the MLH and the Liebig-type responses (Fig. 7; Table 4). The type of responses depended upon the accompanying nutrient considered. The Mg × N experiment showed a definite Liebig response at nitrogen levels 1, 2, and 3 and an MLH-type response at level 4. A mix of the MLH- and Liebig-like responses were found in Mg × P interactions. Typical Liebig responses were observed at phosphorus concentrations 1, 2, and 4, and MLH responses were found at phosphorus concentrations 3 and 5. The five curves of the Mg × K experiment showed an MLH-type response.

DISCUSSION

Our results support our general hypothesis that neither Liebig's law nor the multiple limitation hypothesis adequately account for plant responses to all mineral nutrients. Plant responses to some nutrients followed Liebig's law, responses to other nutrients followed the multiple limitation hypothesis, and in some cases neither paradigm correctly predicted plant responses. Twenty-three of the 60 responses analyzed were clas-

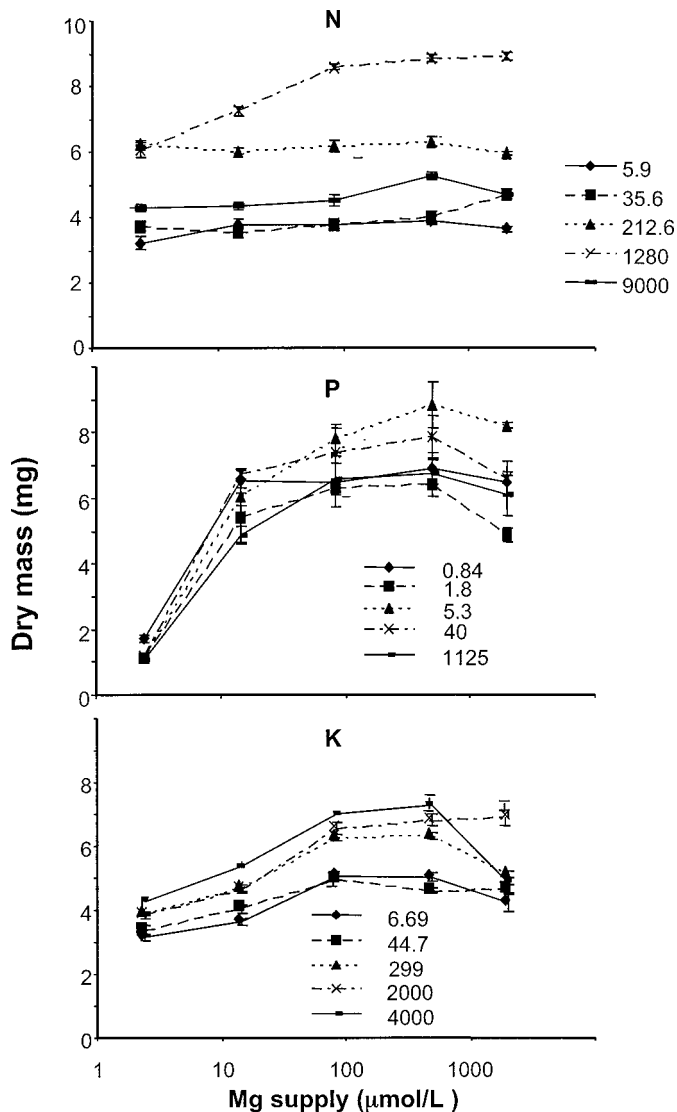


Fig. 7. Dual-resource response curves for magnesium as the subject nutrient and nitrogen, phosphorus, and potassium as accompanying nutrients. Values given for each curve represent the concentration of the accompanying nutrient in micromoles per liter.

sified as belonging to the Liebig model, 20 cases were classified as undefined, and 17 cases as MLH. Although these figures suggest that Liebig was the predominant model describing plant responses to nutrient availability, a closer analysis reveals that the type of response strongly depended on the specific pair of nutrients varied. Our approach permitted two different perspectives for each dual resource experiment, depending on which nutrient was considered the subject and which one the accompanying resource. Data from each experiment were separated into two groups, one for each nutrient as “subject,” which allowed for analysis of the responses to each nutrient individually. The analysis, summarized in Tables 1–4, demonstrated that the regulation of plant effort invested in the capture of essential resources is made on a “resource-by-resource” basis, instead of by a global plant strategy, valid for all nutrients. Therefore, it would not be appropriate to classify a plant as Liebig or MLH, because plants can have Liebig and MLH responses at the same time. Plant responses to nu-

trient availability depended on the specific nutrient and on the occurrence and severity of simultaneous constraints to plant growth.

Nitrogen was the only nutrient showing a predominance of MLH curves (9 out of 15 cases; Table 1). These MLH cases were found when the accompanying nutrients were either potassium or magnesium (5 and 4 cases, respectively), indicating that plant capacity to increase growth in response to nitrogen was somewhat independent of the supply of these nutrients. Even under the stress imposed by shortages of either potassium or magnesium, *L. minor* was able to benefit from increases in nitrogen supply. Responses to nitrogen availability suggest that nitrogen has a preferential status among mineral nutrients, consistent with the fact that it is the mineral nutrient required in largest amounts by plants and that its availability is sub-optimal in most environments. Nitrogen is tied into plant growth allocation by direct involvement with plant growth regulators and by acting as a plant growth regulator itself (Marschner, 1995). The fact that no MLH cases were found when phosphorus was the accompanying nutrient of nitrogen denotes that the preferential status of nitrogen among mineral nutrients is not universal. We would not expect nutrients other than phosphorus to block MLH responses to nitrogen. Like nitrogen, phosphorus availability is a primary constraint in plant evolution and regulates many plant processes (Lynch and Deikman, 1998), including biomass allocation patterns (e.g., Cakmak, Hengeler, and Marschner, 1994; Rubio and Lavado, 1999; Nielsen, Eshel, and Lynch, 2001) that influence the acquisition of carbon and other resources. However, results summarized in Table 2 and published literature indicates that some roles of phosphorus compounds are controlled by other mineral nutrients (Fisher, Hausen, and Hodges, 1970; O’Neill and Spanswick, 1984; Marschner, 1995), suggesting that growth responses to phosphorus interact with other nutrients. This dependence appears to be strong enough to prevent phosphorus from rendering MLH responses.

Besides nitrogen, the only nutrient that showed MLH-type responses was magnesium. This nutrient showed a relative balance of MLH and Liebig responses (8 and 5 cases, respectively; Table 4). Most of the MLH cases observed for magnesium occurred in the experiment with potassium, in which all curves were classified as MLH (Table 4). Potassium behaved as a typical Liebig nutrient (Fig. 6; Table 3), showing a predominance of the Liebig model (11 cases) over the undefined group (4 cases). Whenever limitations imposed by the availability of other nutrients existed, plants could not enhance growth despite an increasing supply of potassium. This is not surprising, because potassium is not directly linked to biomass allocation patterns (Ingestad and Agren, 1991; Cakmak, Hengeler, and Marschner, 1994) and its sufficiency does not necessarily imply a promotion in the acquisition of other mineral nutrients.

The interactions of potassium \times nitrogen and potassium \times magnesium were noteworthy. These cases serve as an example to analyze how different mechanisms can occur simultaneously. When either nitrogen or magnesium were considered as the subject nutrient and potassium the accompanying nutrient, all the resulting curves were classified as MLH. When potassium was considered as the subject nutrient in the same experiment, no MLH curves were found in any case. Normally, we would expect MLH responses to override Liebig responses, so if we have both an MLH nutrient (typically nitrogen) and a Liebig nutrient (in this case potassium) together, we would expect

TABLE 4. One-way ANOVAs for total biomass in the dual-resource experiments involving magnesium; comparison of means according to the LSD method; and classification of the enrichment response curves as multiple limitation hypothesis (MLH) type, law of the minimum (LM) type, or undefined. The same letters in the same row means no statistical difference between treatments. The symbol below the letter represents the sign of the slope between the successive levels of the subject.

Nutrient ^a	Level	F	P	Magnesium					Classification
				1	2	3	4	5	
Mg N	1	3.37	0.04	B	+	+	0	—	LM
	2	7.5	0.00	BC	C	BC	B	A	Undefined
	3	1.6	0.24	A	A	A	A	A	LM
	4	71.7	0.00	C	B	A	A	A	MLH
	5	8.5	0.00	B	B	B	A	B	Undefined
P	1	22.4	0.0	B	A	A	A	A	LM
	2	43.8	0.00	D	BC	AB	A	C	LM
	3	64.9	0.00	C	B	A	A	A	MLH
	4	32.7	0.00	B	A	A	A	A	LM
	5	32.4	0.00	C	B	A	A	AB	MLH
K	1	30.0	0.00	C	C	A	A	B	MLH
	2	17.1	0.00	C	B	A	A	A	MLH
	3	112.0	0.00	D	C	A	A	B	MLH
	4	62.1	0.00	C	B	A	A	A	MLH
	5	57.8	0.00	D	B	A	A	C	MLH
Totals									
MLH									8
LM									5
Undefined									2

^a In these treatments, Mg is the subject nutrient; N, P, and K are the accompanying nutrients.

plants to respond to the MLH nutrient, and this may affect acquisition of the Liebig nutrient as well. But the opposite is not likely to occur, thus responses to additions of the Liebig nutrient will not be noticeable when the MLH resource is in low supply. In an extreme case, if deficiency of a Liebig nutrient is severe enough, we would expect this deficiency to limit growth responses to an MLH nutrient as well. The divergent responses in the potassium × magnesium experiment are accounted for in a particular way. Whereas potassium cannot be replaced by other cations in its role in the cytosol and chloroplasts, the large amounts of potassium required in the vacuoles for osmotic functions can be accomplished by other cations, such as magnesium (Wyn-Jones, Brady, and Speirs, 1979). The replacement of potassium by magnesium could allow plants to partially override potassium deficiencies and to respond positively to magnesium enrichments in growing media poor in potassium, leading to MLH responses. In contrast, potassium cannot replace magnesium due to the more specific functions of magnesium, which cannot be accomplished by any other cation. Besides some circumstantial positive feedback like the MLH responses found for magnesium when interacting with potassium, negative correlations between uptake of ions of the same electrical charge should be expected, es-

pecially for cations. This is because the number of binding sites is small compared to the concentration of competing ions and the selectivity of these sites is limited (Marschner, 1995), and as a result, a large supply of a given cation will depress the uptake of other cations. It is reasonable to predict that greater biomass allocation to roots will increase the uptake of most nutrients (Gleeson and Tilman, 1992). However, it is not correct to assume that positive correlations among nutrients for uptake is a general process in plant nutrition.

In the first published works about the multiple limitation hypothesis (Chapin et al., 1987; Gleeson and Tilman, 1992), nitrogen was the only mineral nutrient used to demonstrate the validity of the MLH model. The long-standing observation that nitrogen fertilizers may overstimulate shoot growth, inducing deficiencies of other nutrients, is consistent with a regulatory role for nitrogen in plant biomass allocation. Our proposal that ecophysiological strategies inherently favor a subset of prioritized resources is consistent with the hypothesis that European forest decline may be exacerbated by tree strategies to maximize nitrogen and carbon acquisition to the detriment of calcium and magnesium nutrition (Schulze, 1989). Results discussed by Chapin et al. (1987) and Tilman and Wedin (1991) are supportive of the inclusion of water, carbon, and

nitrogen into this group. Our results are partially consistent with this perspective for nitrogen, since this element showed MLH-type curves in the potassium and magnesium experiment but not in the experiment with phosphorus (Fig. 1). Thus, the applicability of the MLH model, even for primary resources, would depend on the "accompanying" resource that could be limiting plant growth. A second group of resources would be constituted by resources (including mineral nutrients other than nitrogen) that, although being essential, do not have a regulatory role in biomass allocation. These are not able to increase plant growth if other nutrients are in short supply, i.e., they are not likely to have MLH-type responses, unless specific substitution processes are applicable, as suggested by the potassium \times magnesium interaction in this study.

In our dual resource experiments, the lack of a uniform pattern of response among nutrients is an indicator of the complexity inherent in physiological responses to nutrient limitations and the difficulty of articulating general, simplified models to predict plant responses to nutrient deficits. No simple general models can account for our observations. The situation is even more complex in nature, where plants often confront multiple nutrient limitations. Nutrient availability varies widely among both aquatic and terrestrial ecosystems and through time, and the most frequent situation is that plants have to confront multiple nutrient deficits concurrently. Vegetation continually adjusts its uptake capacity to compensate for changes in nutrient availability in the plant environment (Rastetter and Shaver, 1992). Under these circumstances, optimum uptake of plant nutrients should not be expected in nature. The fact that plant uptake not only reflects the metabolic role of the nutrients but also reflects physical similarities among nutrients at the binding sites (Marschner, 1995) makes plants unable to exclude unnecessary ions from uptake and determines that plants absorb excesses of nutrients.

The interconvertibility of plant resources has been noted as an important factor determining that plants can act as optimal foragers and render MLH-type responses (Bloom, Chapin, and Mooney, 1985). The example mentioned by these authors is the substitution of nitrogen by carbon in nitrogen-stressed plants, through the use of sugars instead of amino acids as osmoticants. In the field of plant nutrition, substitution does not seem a common phenomenon among essential nutrients, because each nutrient has specific roles in plant functioning. Although some cases of substitutions among mineral nutrients exist, these cases are exceptional. On the other hand, since several MLH responses were found, our results do not unequivocally support Liebig's law, which states that only one resource limits growth. The lack of a uniform pattern of response indicates that neither the MLH nor the law of the minimum have universal validity. We conclude that a "nutrient specific" analysis that considers the complexity of the mineral nutrients and their interactions will lead to deeper understanding of plant responses to nutrient availability.

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