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Nutrient acquisition in mycorrhizal lettuce plants under different phosphorus and nitrogen concentration

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Abstract

This study aimed to determine the combined effects of N (1, 5 or 9 mM) and P (0.1 or 0.5 mM) fertilization on mycorrhizal and non-mycorrhizal lettuces. Plant growth, macro and micronutrient assimilation, and specific absorption rate (SAR) were measured. Arbuscular mycorrhizal (AM) colonization and extraradical mycelium development were also examined. The high availability of N and P in the soil reduced the content of macro and micronutrients in AM plants. At the lowest P application (0.1 mM), the AM colonization increased nutrient acquisition at all N levels tested. However, the highest application of N and P to the soil reduced the uptake of N, P, K, Mn and Zn in AM compared to non-AM lettuce plants. These fertilizer levels were also inhibitory to AM-intra and extraradical colonization. In contrast to non-AM plants, AM colonization increased SAR values at the lowest N and P levels for nearly all the nutrients, and decreased the amount of nutrients absorbed per unit of root mass at the highest N and P levels applied. Results demonstrated some negative effects of high N and P application in soil on the acquisition of N, P, K, Fe, Mn and Zn for mycorrhizal plants.

Keywords: Arbuscular mycorrhiza; N–P interaction; Nutrient assimilation; Specific absorption rate (SAR)

1. Introduction

In order to ensure the success of low agrochemical input, effective soil management and soil biology must be taken into account.

The cycling of macro and micronutrients in an ecosystem is influenced by multiple interactions involving soil microbial populations [10]. Arbuscular mycorrhizal (AM) associations play important roles in this nutrient cycling through their microbial activity and their involvement in plant nutrient acquisition [8,12]. The extraradical mycelium connects plant roots to the surrounding soil microhabitat increasing the soil volume exploited by host plants. This allows plants to survive in nutrient and/or water depleted zones [33]. Mycorrhizal hyphae transport mineral nutrients over greater distances from depleted zones than do roots [24]. Thus, under low nutrient conditions AM-colonized roots may have an enhanced uptake of relatively immobile macro and micronutrients [16,27,28,45]. In addition, mycorrhizal roots often have not only increased length but also modified root architecture [11]. Nevertheless, under high nutrients conditions, a reduced accumulation of minerals has been observed in AM plants [4,5,14,29,39,43,50].

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AM colonization can cause physiochemical or microbiological changes to the rhizosphere thus affecting root hyphae uptake of some nutrients [1,2,32]. Nitrogen (N) and phosphorus (P) levels are considered among the most important factors affecting AM association efficiency. Not only the level but also the balance between N and P fertilizer regimes are crucial in establishing an efficient symbiosis. Enhanced N acquisition in AM plants had been explained by an increased N demand in AM-colonized plants because of high P nutrition [17].

It is known that the development of mycorrhizal colonization and its effectiveness on plant growth is enhanced in poor soils. Brundrett et al. [13] showed that AM root colonization is increased at low and medium nutrient levels but reduced at high levels. Under agronomic practices, P limitation in soil is usually compensated by heavy P fertilization that reduces AM colonization and effectiveness. The concept of effectiveness is defined as the ability of an AM inoculum to increase plant nutrient uptake and plant growth. Thus, the effective use of AM inocula and resulting colonization would results in similar plant growth and yield and reduce the need for high levels of P application which historically results in soil and ground water contamination in many agricultural areas. Conventional high input agricultural systems tend to change by sustainable systems with a minimal environmental impact. The aim is to change to more sustainable forms of agriculture in which the results will depend on both the AM inoculum efficiency and soil nutrient availability. However, in order to optimize management of AM fungi/host plants under field conditions, more knowledge on the effect of agricultural practices is required.

Increased accessibility of soil nutrient pools to plants has been associated with AM colonization [15]. Heavy fertilization of N and P decreased mycorrhizal colonization, affecting AM functioning on mineral nutrient acquisition. Differences in the mycorrhizal behaviour in nutrient acquisition per root weight and colonized root weight are expected when the nutritive status in the growth medium changes.

The effects of mycorrhizal colonization on acquisition of relatively immobile nutrients by mycorrhizal plants are still unclear and inconsistent experimental results have been attributed to variations in soil conditions and/or mycorrhizal development [15]. Root–AM colonization often results in an enhanced uptake of relatively immobile metal-micronutrients such as Cu, Zn and Fe. However, previous studies show that these micronutrients were lower in the shoots of mycorrhizal maize plants but if soil treatments inhibit root colonization, the uptake of nutrients could also be affected [31].

In the present study, we investigated how the interaction of N and P fertilization affects the macro and micronutrient acquisition in mycorrhizal and non-mycorrhizal lettuce plants.

2. Materials and methods

2.1. Experimental design

A $3 \times 3 \times 2$ factorial randomized block design including 3 N levels (1, 5 and 9 mM N), two P levels (0.1 and 0.5 mM P), and two mycorrhizal treatments (with or without AM inoculation) was used. Thus, there were 12 treatment combinations replicated five times.

2.2. Soil characteristics

The test soil used, collected from Granada province (Spain), was a 'reddish-brown calcareous' type (42.0% clay, 39.8% loam, 18.2% sand) at pH 7.4; 1.23% organic matter; 4.5 mg P kg^{-1} soil extractable with 0.5 M $NaHCO_3^-$ (Olsen P). Soil samples were sieved (4 mm pore size), diluted with quartz sand (2/3, v/v) and steam sterilized (100 °C for 1 h on three consecutive days), then re-inoculated with a soil filtrate containing the normal microbiota without AM propagules. Pots were filled with 800 g of sterilized soil/sand mixture.

2.3. Host plant and inoculation treatments

Surface sterilized seeds of lettuce (*Lactuca sativa* L.) were sown in the sterilized soil/sand mixture. The mycorrhizal inoculum used was stock culture *Glomus mosseae* isolate (112 BEG) (Nicol. & Gerd.) Gerd. & Trappe. Mycorrhizal inoculation was carried out by adding 10 g per pot of a mycorrhizal inoculum from our stock culture collection. These thoroughly mixed rhizosphere samples contained spores, hyphae and mycorrhizal root fragments (80% root colonization) were maintained in polyethylene bags at 4 °C for 3–6 months before to be applied to the corresponding pots.

2.4. Growth conditions

Plants (one per pot) were grown for 60 days under greenhouse conditions with temperatures ranging from 19 to 25 °C, a 16/8 h light/dark period, and a relative humidity of 70–90%. A photosynthetic flux density of 400–700 μmol mol^{-1} s^{-1} was applied as supplementary light when necessary.

The watering regime consisted of weighing each pot once a day and adding water to the weight corresponding to 100% of water holding capacity.

Three soil nitrate treatments were done by adding 30 ml per pot (5 ml × 6 times) of a NH_4NO_3 solution at 1 mM (14 mg N), 5 mM (70 mg N), or 9 mM (126 mg N). These soil N treatments, respectively, represented low, medium and high N fertilization rates for lettuce plants, as previously tested (unpublished data).

Phosphate was supplied as KH_2PO_4 at 0.1 mM (3.1 mg P) and 0.5 mM (15.5 mg P) in 30 ml per pot (5 ml × 6

times). Nitrate or phosphate was added on alternate days.

All the plants were fertilized (10 ml per pot week^{-1}) with a modified Hewitt (1952) solution (mg l^{-1}): K_2SO_4, 400; $CaCl_2$, 708; $MgSO_4 \cdot 7H_2O$, 368; FeEDTA, 25; SO_4Mn, 2.23; $CuSO_4 \cdot 5H_2O$, 0.24; $ZnSO_4 \cdot 7H_2O$, 0.29; H_3BO_3, 1.86; $NaMoO_4 \cdot 2H_2O$, 0.035.

2.5. Measurements

After harvest, shoot and root biomass were recorded and shoot tissues were analyzed for macro (N, P, K, Ca and Mg) and micronutrients (Fe, Cu, Mn and Zn). After acid digestion treatment, P in plant tissue was determined colorimetrically using the vanado-molybdate method, and Ca, Mg, Zn, Mn, Fe, K and Cu were quantified by atomic absorption spectroscopy.

The roots were washed under a cold water stream. Samples of washed roots were cut into approximately 1 cm-segments, cleared with 10% KOH in boiling water bath for 15 min, and stained with trypan blue for mycorrhizal colonization estimation [38]. The percentage of mycorrhizal root length was calculated by a gridline intersect technique [19]. The extraradical mycelium was determined by a modified and combined method according to Jones et al. [25] and Newman [36].

Mycorrhizal dependency (MD), or response to mycorrhizal colonization, was calculated by using the following formula

$$\mathrm{MD} = \frac{(\mathrm{DRYM_{AM}}) - (\mathrm{DRYM_{NON\text{-}AM}})}{\mathrm{DRYM_{AM}}} \times 100 \quad (1)$$

where DRYM is dry mass of mycorrhizal (AM) or non-mycorrhizal (non-AM) plants.

Specific absorption rate (SAR) is defined as the amount of nutrients absorbed per unit of root mass [20] and calculated as follows

$$\mathrm{SAR} = \frac{\text{Plant nutrient } (\mu g)}{\text{Root mass } (mg)} \quad (2)$$

All the data were statistically analyzed by an analysis of variance after arcsin transformation. When a significant ($P < 0.05$) treatment effect was found, the mean values were compared using the Duncan test ($P < 0.05$).

3. Results and discussion

Key factors in the context of sustainable systems are the microbial communities which regulate nutrient cycling. AM fungal species are important members of such microbial communities. However, the optimal functioning of AM symbiosis depends on soil fertilizer level [43,44].

In the present study, the shoot mass was not influenced by AM colonization at the highest N and P levels (Table 1). As P and N availability in soil increased (0.5 mM P combined to 5 or 9 mM N), the AM and non-AM plants had similar shoot and root masses. MD was the highest when plants were fertilized with the highest N and the lowest P levels that could be considered as unbalanced nutrient conditions (Table 2).

Differences in nutrient shoot contents (nutritional plant status) between mycorrhizal and non-mycorrhizal plants depended on quantities of P and N applications to the growth medium (Figs. 1 and 2).

Root mycorrhizal colonization had no effect on Mg, Cu, Fe and S contents of the shoot when the growth medium was fertilized with 0.5 mM P and 9 mM N. Such high levels of added N and P reduced the N, P, K, Mn and Zn shoot contents in AM compared to non-AM plants (Figs. 1 and 2). Colonization by *G. mosseae* did not affect P, K, Ca, Cu, Fe and S quantities in the shoot when plants were supplied with the same P level (0.5 mM), but the N level applied was 5 mM. Under such fertilizer conditions, (0.5 mM P and 5 mM N) only Mg content was increased whereas N, Mn and Zn contents were reduced in AM plants. When plants were fertilized with the same P level but with lower N nearly all the macro and micronutrients (P, K, Ca, Mg, Cu, Fe, Mn, Zn and S) were enhanced by AM colonization. N was the only exception (Figs. 1 and 2). Although plant nutrient accumulation is often shown to be positively influenced by mycorrhizal symbiosis, its effect not only disappeared but it was reduced when a quantity of 0.5 mM P was combined to N levels of 5 or 9 mM (Figs. 1 and 2).

Under a low P fertilization (0.1 mM), mycorrhizal colonization by *G. mosseae* contributed, in general, to a greater accumulation of macro and micronutrients (except N and Fe), independently of the N level applied. However, this mycorrhizal colonization was not as effective, in terms of nutrient acquisition, mainly when P was increased in the soil (Figs. 1 and 2).

Moreover, 0.5 mM P reduced N uptake in AM plants, independently of the N level added in the medium. In contrast to previous studies [23,46,47], AM colonization did not positively affect N accumulation in lettuce plants. When no P was added, a positive effect of mycorrhizal colonization on N shoot accumulation had been determined under a range of N sources and/or amounts in soil and water content in the medium ([6,7,46,47]).

Mycorrhizal contribution to P uptake in lettuce plants was the highest with the applied combination of 0.5 mM P and 1 mM N. In the case of K, the highest mycorrhizal effect was observed with a ratio of 0.1 mM P and 1 mM N. For Cu, Fe, Zn and S, this effect was enhanced in the conditions of 0.1 mM P and 9 mM N. This range of mycorrhizal responses on nutrient acquisition according

Table 1
Effect of N and P fertilization (combination of N and P levels) in non-mycorrhizal (Control) or mycorrhizal (*G. mosseae*) lettuce shoot and roots

	0.1 mM P			0.5 mM P		
	1 mM N	5 mM N	9 mM N	1 mM N	5 mM N	9 mM N
(A) Shoot (g)						
Control	8.2 f	8.7 f	11.1 cd	7.8 f	22.3 b	29.3 a
G. mosseae	10.3 d	13.5 c	19.3 b	10.6 cd	22.3 b	27.9 a
(B) Root (g)						
Control	7.7 c	5.6 d	6.8 c	6.9 c	10.6 b	14.1 a
G. mosseae	6.8 c	7.4 c	10.1 b	7.4 c	13.2 ab	16.4 a

For each nutrient, values not sharing a letter in common differ significantly ($P < 0.05$) from each other.

Table 2
MD of lettuce plants in relation to N and P levels in the medium

P levels (mM)	N Levels		
	1 mM	5 mM	9 mM
0.1	20.4	35.5	42.5
0.5	26.4	–	–

to particular ratios of N and/or P cannot be attributed to a common cause for all the nutrients, as it could be the case for the extraradical mycelium development.

The suppression of extraradical hyphal development occurring in soil with high N and P levels, also seen in soil with high micronutrients levels [31], cannot explain our results since under such conditions no AM effect should be expected. Such a negative effect may be explained by the direct influence that these nutrients have on changing the rhizosphere microbial population in the AM root environment.

The length of AM mycelium was used as a sensitive marker of the physiological state of AM colonization. In fact, the extraradical mycelium was at its maximum with the supply of 0.1 mM P and 9 mM N (Table 3). Under conditions of N and P abundance, lettuce plants appeared to be independent of AM colonization for the uptake of macro and micronutrients since AM colonization was strongly reduced. Similarly, the beneficial effect of mycorrhizal inoculation on Cu and Zn uptake was eliminated by adding micronutrients to soil [31]. In the present study, a similar absence of AM effect was observed on S, Cu, Fe, Ca and Mg uptake, and a detrimental AM effect on N, P, K, Mn and Zn uptake was found depending on the amount of N and/or P in the medium.

The decrease in Fe, Mn and Zn contents in mycorrhizal plants is surprising since their mobility is low, and their root uptake limited [9,10]. Thus, the uptake of these micronutrients ought to be the lowest in non-AM plants since AM colonization provides a large surface area for the absorption of immobile nutrients enhancing such values over non-mycorrhizal roots only when AM colonization was well developed.

Results from this study suggest that the availability of some micronutrients in soil was reduced as an effect of physico-chemical changes in AM rhizosphere of high N and P supplied plants. A binding/immobilization capacity of AM roots for these microelements under such growth conditions could occur, as described for heavy metal contaminated soils [30,48].

Under conditions of the lowest N and P levels (0.1 mM P and 1 mM N), there was higher (P, K, Ca, Mg, Zn and S), similar (N, Cu and Fe), or lower (Mn) nutrient uptake in AM than non-AM plants. In the case of P, K, Ca, Mg, Cu, Zn and S, the mycorrhizal colonization also improved the uptake of these nutrients when lettuce plants were grown in the lowest P level (0.1 mM), independently of the N level supplied. But when the level of P increased to 0.5 mM and of N over 1 mM (until 5 mM), a reduced effect (N, Mn and Zn) or no effect (P, K, Ca, Cu, Fe and Zn) was found on the absorption of these nutrients by AM plants. When 0.5 mM P was applied and N raised from 1 to 9 mM, the nutrient uptake values were reduced by AM colonization.

Changes to the pH of the rhizosphere, which can be dependent on soil nutrient levels could also be involved in absorption/assimilation aspects. A direct influence of applied nutrients that may enhance the rhizosphere microbial population in the environment of AM roots and could explain negative AM effects on nutrient acquisition. Mycorrhizal colonization may also alter chemical properties of the rhizospheric soil [37,42]. Vaast and Zasosky [49] showed that exchangeable acidity decreased in the AM rhizosphere due to a more alkaline pH. In the present study, the nutrient contents of N, P, K, Fe and Zn, in the combined 0.5 mM P/9 mM N fed-AM plants, were lower than those of their non-AM counterparts. This could be due to a greater or a more metabolically active microbial population which requires these essential nutrients from the near-root environment [34]. This may explain the lower standing nutrient status of the plant (Figs. 1 and 2).

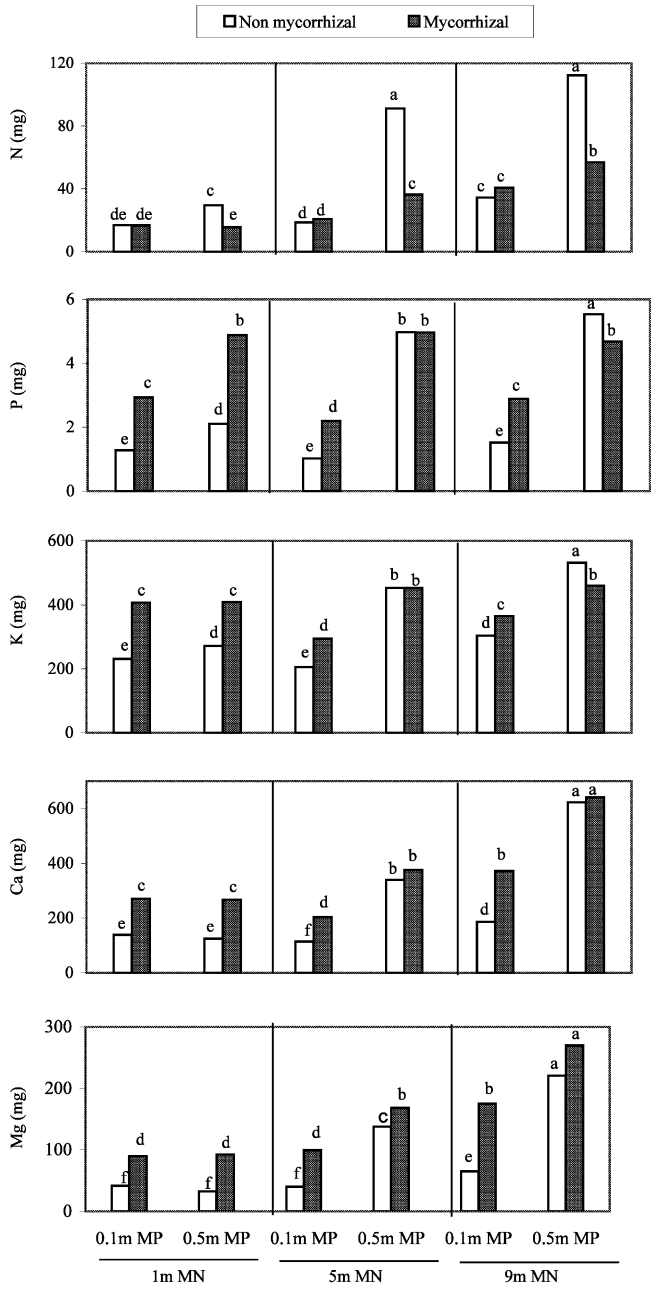

Fig. 1. Effect of N and P fertilization (combination of N and P levels) on macronutrients content in mycorrhizal and non-mycorrhizal lettuce plants. For each nutrient, values not sharing a letter in common differ significantly ($P < 0.05$) from each other.

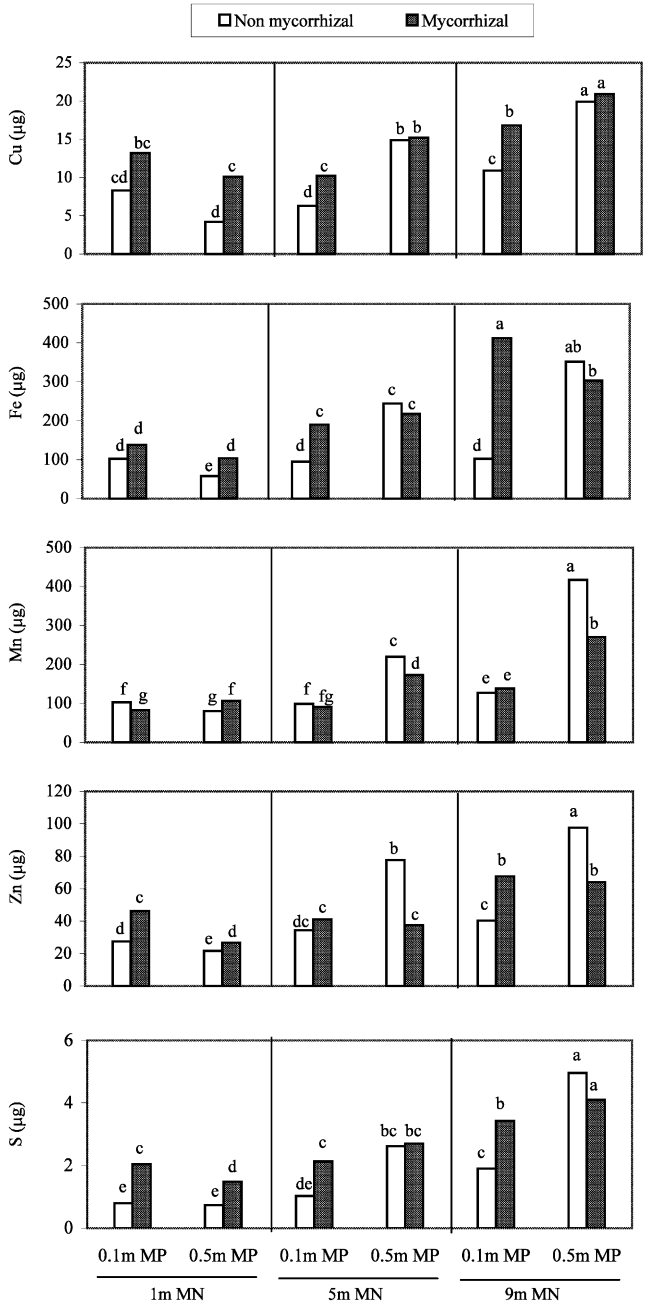

Fig. 2. Effect of N and P fertilization (combination of N and P levels) on micronutrients content in mycorrhizal and non-mycorrhizal lettuce plants. For each nutrient, values not sharing a letter in common differ significantly ($P < 0.05$) from each other.

In the medium containing the low level of P (0.1 mM), the increasing N supply did not change the positive effect of AM colonization on plant macro and micronutrient uptake. High levels of P (0.5 mM) and of N (9 mM) led to significantly increased nutrient contents in non-AM plants. However, in the case of AM plants these fertilizer levels did not affect the macro and micronutrient contents.

The greatest increases in Ca and Mg shoot contents were observed when the P (0.5 mM), and N (9 mM) were applied. A similar tendency was evidenced for non-AM and AM plants.

When AM and non-AM plants were grown at the highest P (0.5 μM) and N (5 and 9 mM) AM plants showed specific mineral acquisition over non-AM plants. However, for some nutrients (Ca, Mg, Cu, Fe and S), there were no significant AM effect and, for others (N, P, K, Mn and Zn) a negative effect was observed. This may indicate the lack of mycorrhizal benefit at these high fertilizer levels in which the

Table 3
Mycorrhizal colonization (% and total of mycorrhizal root length) and extraradical mycelium production (cm g^{-1} soil) in lettuce plants in relation to N and P levels in the medium

P levels	N levels								
	1 mM			5 mM			9 mM		
	AM			AM			AM		
	Ex. myc	%	Total	Ex. myc	%	Total	Ex. myc	%	Total
0.1 mM	7.7±1	41.3±8	452.8±50	4.2±0.6	36.9±10	431.5±60	7.8±1.1	30.7±6	535.0±30
0.5 mM	2.9±0.5	13.8±5	223.7±30	1.7±0.6	1.9±0.5	25.8±11	1.2±0.5	0.5±0.2	8.3±3

For each nutrient, values not sharing a letter in common differ significantly ($P < 0.05$) from each other.

mycorrhizal roots were not significantly increased, and the extraradical mycelium were highly reduced. This could explain why the surface area of AM roots did not seem particularly effective in nutrient acquisition. High N and P levels, that have negative effects on AM colonization and extraradical mycelium development, might to consequently affect the mycorrhizal effectiveness on nutrient uptake.

The availability of some micronutrients (Mn and Fe) in a medium would depend on soil pH as well as on the oxidation/reduction potential. Acidification of the rhizosphere is a typical response of plants to P deficiency [32], even when plants are supplied with NO_3^-. Thus, plants grown with 0.1 mM P may produce a stronger acidification than plants grown with 0.5 mM P. Differences in rhizosphere pH and in P content between mycorrhizal and non-mycorrhizal plants grown with different N and P combinations may be a mechanism by which the micronutrient availability in soil is indirectly limited.

Acquisition of Mn was reduced in AM plants as a consequence of changes in populations of Mn-related rhizobacteria [3,4,39]. Similarly, S and N cycling by microorganisms was also an effect of AM colonization [1,2].

The enhanced plant uptake of most of the macro and micronutrients due to AM colonization at the lowest N and P levels was higher than the higher N level input to the non-AM plants. Results from this study show a negative influence of AM fungus on mechanisms associated with mineral nutrition of plants when grown in a high macronutrient level medium. This suggests an altered root activity. The altered nutrient accumulation in shoots possibly by a process of sequestering on or in roots had been suggested [30]. Allocation of photosynthates to roots is modified depending on plant nutrition, thus contributing to a greater nutrient scavenging ability of roots, benefiting or not the AM plants growing under limited or sufficient nutrient input conditions, respectively.

The negative impact of high N and P levels on mycorrhizal root colonization has been extensively reported. Muthukumar and Udaiyan [35] also found that root colonization was unaffected by increasing P when plants were N deficient. The suppression of external mycelium observed in soil with high N and P levels, as Gryndler et al. [22] observed, may reduce the potential of mycorrhizal plants for micronutrient uptake. Nevertheless, the lower nutrient uptake by AM plants under such conditions was unexpected. In principle, AM plants having a developed extraradical mycelium could potentially absorb more micronutrients than AM plants with less developed extraradical hyphae. However, the negative impact of AM colonization found in the present study cannot be explained by this mechanism. A possible explanation for the decreased nutrient uptake in AM plants might be linked to the process of rhizospheric changes caused by the increasing level of N and P in the growth medium.

The negative mycorrhizal effect found on N, P, K, Mn and Zn contents in AM plants at high N and P levels suggests a more biological than chemical modification at the rhizosphere level. Nutrient availability and quantitative or qualitative changes in root exudate production can affect rhizosphere microbial populations that have particular nutrient requirements [18,21]. In the present study, the enhancement of K, Ca and Mg in AM plants depended on both N and P levels, principally Raju et al. [40,41] reported that the acquisition of the same three minerals was decreased or similar to that of non-AM plants when P was added.

The SAR capacity often increases as nutrient availability declines or as shoot growth rate and nutrient demand increase [26]. An efficient nutrient usage may reduce SAR. For most of the nutrients, SAR increased in non-AM plants as much as fertilization (N and P) increased. By contrast, in AM plants, the highest SAR values were determined at the lowest fertilization levels for most of the nutrients, with few exceptions (N, Mg, Fe) observed. For nearly all the nutrients, the lowest SAR in AM plants was determined at the highest N and P levels (9 mM N and 0.5 mM P). Major differences in SAR were noted for nutrients such as Fe, Zn and S, when P increased from 0.1 to 0.5 mM, under any N

Table 4
SAR (μg mg^{-1}) macronutrients (N, P, K, Ca and Mg) in mycorrhizal (M) and non-mycorrhizal (−) lettuce plants as affected by N and P levels in the medium

Mycorrhizal treatment	1 mM N		5 mM N		9 mM N	
	0.1 mM P	0.5 mM P	0.1 mM P	0.5 mM P	0.1 mM P	0.5 mM P
N						
−	14.80 c	26.00 b	23.10 b	37.60 a	37.1 a	43.50 a
+M	15.60 c	13.40 c	9.20 d	17.30 bc	23.7 b	17.30 bc
P						
−	1.12 d	1.85 c	1.27 cd	2.05 bc	1.6 c	2.14 bc
+M	2.72 b	4.21 a	1.90 c	2.36 bc	1.7 c	2.42 c
K						
−	202.20 b	238.30 b	253.30 b	187.00 bc	326.4 a	206.20 b
+M	377.20 a	352.50 a	253.40 b	215.50 b	210.9 b	139.80 c
Ca						
−	121.90 c	109.50 c	141.30 bc	140.30 bc	200.3 ab	241.20 a
+M	250.20 a	230.20 a	175.60 b	178.80 b	215.0 ab	194.70 ab
Mg						
−	36.70 c	28.60 c	49.40 b	56.00 b	69.8 ab	85.60 a
+M	82.90 a	79.60 a	85.70 a	80.40 a	101.3 a	82.00 a

For each nutrient, values not sharing a letter in common differ significantly ($P < 0.05$) from each other.

level; such differences were the strongest for mycorrhizal plants (Tables 4 and 5).

The present study has shown that mycorrhizal plants, at the highest N and P levels supplied, have a decreased amount of nutrients absorbed per unit of root mass. A higher retention of absorbed mineral nutrients in the root zone would result in a lower amount transported to the shoots. Root–shoot transport changes have been attributed to ionic regulation differences. These factors can also be involved in the mycorrhizal effect tested at the highest N and P level application. Low-input systems are more dependent on AM fungi than the conventionally managed, higher input systems. Results suggest that the beneficial mycorrhizal effect on plant

Table 5
SAR (μg mg^{-1}) of elements (Cu, Fe, Mn, Zn and S) in mycorrhizal (M) and non-mycorrhizal (−) lettuce plants as affected by N and P levels in the medium

Mycorrhizal treatment	1 mM N		5 mM N		9 mM N	
	0.1 mM P	0.5 mM P	0.1 mM P	0.5 mM P	0.1 mM P	0.5 mM P
Cu						
−	0.0073 b	0.004 c	0.0080 b	0.006 b	0.012 a	0.008 b
+M	0.0122 a	0.009 b	0.0090 b	0.007 b	0.010 b	0.006 b
Fe						
−	0.0895 d	0.051 e	0.1160 cd	0.100 d	0.216 b	0.136 c
+M	0.1280 c	0.089 d	0.1630 bc	0.104 cd	0.274 a	0.092 d
Mn						
−	0.0903 bc	0.070 c	0.1210 b	0.090 bc	0.137 ab	0.162 a
+M	0.0768	0.092 bc	0.0780 c	0.082 c	0.080 c	0.082 c
Zn						
−	0.0240 b	0.019 c	0.0420 a	0.030 b	0.043 a	0.038 a
+M	0.0427 a	0.023 b	0.0350 a	0.018 c	0.039 a	0.019 c
S						
−	0.0007 c	0.001 c	0.0013 b	0.001 bc	0.002 a	0.002 a
+M	0.0019 a	0.001 b	0.0018 a	0.001 bc	0.002 a	0.001 b

For each nutrient, values not sharing a letter in common differ significantly ($P < 0.05$) from each other.

nutrition was only evidenced under limited levels not only of P but also of N and that the fertilizer application can reduce and even eliminate any mycorrhizal benefit.

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