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Soil-plant factors, others than the type of salt-specific anions are affecting the mycorrhiza colonisation of some halophytes

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Abstract: The relationship between some soil physical-chemical characteristics, with more focus on the types of salt-specific anions and the colonisation parameters of the arbuscular mycorrhizal fungi (AMF) were examined on the most dominant halophytes, grown at four saline soils in Hungary. At site Z (Zám) mainly the chloride, at site Ny (Nyírőlapos) mainly the sulphate ions dominated in the soil samples, while at site A (Apajpuszta) and site Zsz (Zabszék) the carbonate anions were the most frequent. A large colonisation variability of the AM fungi were found in the four saline sites and the studied halophytes. Among the affecting soil-plant factors a strong host dependency was recorded with almost no mycorrhiza colonisation on *Puccinellia limosa* and the highest values at the *Plantago maritima* and *Aster tripolium*. As a function of the salt-levels a relative location of the halophytes could be found at each sites. The AMF colonisation intensity (M%) and functioning, measured as arbusculum richness (A%) was reduced with the overall increasing salinity, more particularly with the total cation-content of the soils. The deleterious effect of some other abiotic factors, i.e., the very poor or rich nutrient availability and the humus accumulation tended to be also negatively correlated by the mycorrhizal parameters.

Abbreviations: AMF – arbuscular mycorrhiza fungi; M% – mycorrhiza intensity of the root system, A% – arbusculum richness.

Introduction

Soils in which water-soluble salts play a dominant role determining their physical, chemical and biological properties belong to the salt-affected soils. Depending on the type of the salts, however, they can be very diverse in their appearance, morphology and in some other characteristics (Szabolcs 1998). The soil in general is said to be saline if the conductivity of soil saturation extract exceeds 4 dS m^{-1} or the concentration of salts is higher than 0,1%. Salt-affected soils cover roughly 10% of the surface of the continents (Szabolcs, 1989). More than a hundred countries have salt-affected soils occupying different proportions of their territory (particularly extended in dry arid and semi-arid areas (Al-Karaki, 2000).

On the saline or sodic areas both the macrobiota (halophytes) and the microbiota (rhizosphere-constituents) usually adapt to the particular stress conditions (Ruiz-Lozano and Azcon, 2000). The functional symbiosis between the two partners can reduce the imbalance of essential cations and anions, the toxicity of salt-specific ions, the unfavourable pH and soil structure (Hasegawa et al., 1986, Barea et al. 2002). Several bacterial or fungal representatives of the salt-adapted micro- or rhizobiota can tolerate the saline conditions as high, as 4-30% of NaCl concentration (Ruiz-Lozano et al. 1996; Zahran 1997, Biró et al. 2002). Adaptation is a complex phenomenon, during which the plant physiological status (i.e., respiration, water relations, root exudation of nutrients, compatible osmolytes) and as a consequence the rhizosphere components are also changing continuously (Schwarz and Gale 1984; Nagy et al. 1995; Murakeozy et al. 2002 and 2003). In the rhizosphere of halophytes the benefit of the symbiosis is the stress-alleviation during the salt stress (Ruiz-Lozano and Azcón 2000). Regarding the mycorrhiza colonisation a negative correlation was found between the salt concentration and mycorrhiza intensity or the spore numbers in the soils. Beside the reduced spore-germination and hyphal growth, the species diversity can be negatively affected by the large salt concentrations. The most common mycorrhiza type in the salt-affected soils is the *Glomus* spp. (Juniper and Abbott, 1993), i.e., the *G. geosporum-caledonium* clade (Landwehr et al. 2002), which was reported to be the most-adapted species to the highly saline conditions when considering only the total salt-levels. Less attention is given, however to the effect of various salt-specific aninons on the mycorrhiza formation, which has a greater versatility in Hungary (i.e., hydroclorides, carbonates, phosphates..etc.) than a previous only sulphatic German site (Landwehr et al. 2002). The root colonisation parameters, such as the mycorrhizal frequency (F%) and intensity (M%) of the most dominant halophytes, produced by the salt-adapted fungi was found to be quite strong at each studied sites in Central- and Eastern-Europe (Hildebrant 2000). The effect of salt stress is generally less harmful at those strongly mycorrhizal, than at the non-mycorrhizal hosts (Hirrel and Gerdemann, 1980; Al-Karaki 2000; Poss et al. 1985, Hartmund 1987). Plant families, such as the well-known, initially non-mycorrhizal Chenopodiaceae or Brassicaceae are reported to become also mycorrhiza-dependent especially at the multiple-stressed conditions (Hoefnagels et al. 1993; Regvar et al. 2003). The significantly overexpressed mycorrhizal colonisation and function was reported by Füzy et al. (2008) at the summer drought-stressed conditions on the basically salinic sites. The final values of the AMF symbiosis therefore is considered to be a consequence of several environmental factors in the multifactorial soil-plant-microbe-environmental systems. Question arise to study the interrelations between the most frequent abiotic (stress)factors and the mycorrhizal colonisations of the most dominant halophytes. The main characteristics of the selected sites are the particular differences in the type of salt-specific anions and the nutrient availabilities. The aim of our work is to estimate the dependencies and the most affecting factors of mycorrhizal colonisation of the dominant halophytes in the two major salt affected regions (Kiskunság and Hortobágy) in Hungary, with common and different values of some physical-chemical soil-properties.

Materials and methods

Analysis of soil parameters

Representative soil-samples were taken at a proximity of the randomly selected halophytes at each sites. Simultaneously the electric conductivity of the 0-20 and 0-40 cm soil-depths were estimated in the field with a four-electrode conductivity sensor SCT 12 apparatus (Martek Instruments Inc., Raleigh, NC, USA) and in the laboratory in a suspension of 1 g soil mixed with 2.5 mL water with a conductometer (Jeway 4020, Jeway Electric Co. Ltd.,Fuzhou, PR of China). Water-content, hygroscopicity, pore-distribution and plasticity index (KA) was estimated according to Buzás (1983). Among the chemical parameters the Tyurin-tal1Sampling

Soil and root-samples were randomly collected from the rhizosphere of main halophytes, such as the *Festuca pseudovina* (Hack.ex Wiesb.), *Artemisia santonicum* (L.), *Plantago maritima* (L.), *Puccinellia limosa* ((Schur.) Homberg.), *Aster tripolium* (Jacq.), *Lepidium crassifolium* (W. et K.) from 5-15 cm depth at four sampling sites, such as Nyírőlapos (Ny), Zám (Z), Apajpuszta (A) and Zabszék (Zsz) on the Hortobágy and the Kiskunság region in Hungary in April, 2001 and 2002. Regardi'E) as *Artemisio-Puccinellia limosae.* A concomittant measure of the salt-levels with a Martek SCT 12 conductimeter in the 20-40 cm layers of the soils was done for assessing the relative location of the selected halophytes at the particular salt-levels at the sites.

Estimation of mycorrhizal colonization values Root-samples of 1g fresh lateral roots of the main halophytes were randomly taken, rinsed in water and stored in a 70% alcohol before examining the AMF colonisation. After storage root-samples were cut to aproximately 1cm segments and boiled in a 15% KOH solution for 40 minutes and stained with aniline blue. A stereo, dissecting microscope was used to study the colonisation characteristics by the five-class system of Trouvelot et al. (1986). The presence and abundance of hyphae, vesicles and arbuscules were determined in 30 root segments for each plants. Frequency and intensity of mycorrhization (F%, M%) furthermore the absolute and relative arbusculum content (A%, a%) were estimated and calculated in the root-system of the studied host-plants.

Table 1. The minimum, maximum and the average values (*n*=6) of the measured main soil physical and chemical characteristics and the final judgement about the nutrient availability of four saline sites in Hungary. Salt-affected soils were sampled in the spring of 2001 at sites of Nyírőlapos (Ny), Zám (Z), Apajpuszta (A) and Zabszék (Zsz).

	Saline sites			
Soil factors	Site Ny	Site Z	Site A	Site Zsz
EC (dS m^{-1})	1.2 – 3.6 1.9	0.9 – 4.1 2.2	0.7 – 1.7 1.1	1.2 – 1.3 1.2
pH (H_2O - 1:2,5)	8.4 – 9.8 8.9	7.5 – 9.5 8.5	8.5 – 9.6 9.2	9.0 – 9.7 9.3
Humus (%)	1.9 – 2.5 2.1	2.5 – 4.5 3.4	0.9 – 2.0 1.5	0.7 – 1.2 0.9
$CaCO_3$ (%)	3.4 – 5.5 5.0	1.3 – 5.1 3.1	15.2 – 27.5 21.1	28.1 – 41.4 34.6
Higroscopicity (%)	0.9 – 1.8 1.3	1.4 – 2.4 2.0	0.6 – 1.0 0.9	0.5 – 0.7 0.6
P (P_2O_5-mg kg^{-1})	60 – 110 74	200 – 420 246	20 – 50 42	10 – 90 69
Total cations (me l^{-1})	41 – 88 62	48 – 90 67	14 – 39 28	37 – 66 52
Alkalinity - CO_3+HCO_3 (me l^{-1})	24.1 – 40.0 32.0	4.4 – 49.3 25.4	8.8 – 19.7 16.0	13.1 – 30.7 23.0
Na (me l^{-1})	37.4 – 83.9 57.2	32.0 – 78.3 49.6	11.0 – 35.3 23.8	33.5 – 64.3 47.8
K (me l^{-1})	0.9 – 1.3 1.0	0.6 – 2.5 1.2	0.2 – 0.8 0.3	0.3 – 1.5 0.6
Ca (me l^{-1})	1.5 – 2.7 1.9	2.5 – 10.2 5.1	2.2 – 3.1 2.1	0.9 – 4.5 2.0
Mg (me l^{-1})	1.5 – 2.6 2.0	1.4 – 4.7 3.0	0.7 – 3.0 1.2	0.7 – 4.5 1.7
Cl (me l^{-1})	0.0 – 11.8 4.6	9.8 – 52.9 28.5	0.0 – 4.9 2.7	4.9 – 16.7 10.9
SO_4 (me l^{-1})	3.6 – 35.2 20.2	3.5 – 6.3 4.7	2.4 – 12.8 5.7	2.6 – 7.8 4.7
Available nutrients*	good	very good	poor	very poor

*according to Buzás, 1983.

Statistical analysis

At least three repetitions have been done for each measurement. Statistical analysis of mycorrhizal colonization and arbuscule formation was conducted by one-way analysis using ANOVA at $LSD_{5\%}$. Correlation-regression studies were performed to estimate the interrelations between the most affecting biotic and abiotic factors at the sites. The correlation between mycorrhizal colonization and the environmental factors was examined by linear regression at the p 0.05 significance level.

Results

Site characteristics and the relative location of dominant halophytes

The main physical- and chemical characteristics of the studied sites are shown in the Table 1. The four Hungarian salt-affected sites have several common and distinct soil-characteristics. Differences were found mainly in the quality and quantity of the salt-specific anions, which have resulted

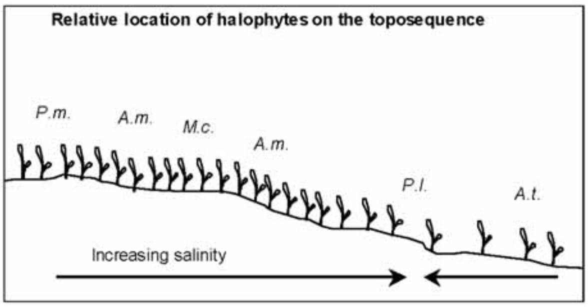

Figure 1. Relative location of the main halophytes on the toposequence along the increasing salinity of the soil, representing of all four studied sites. Further details for the potential salt-levels can be found in Table 1 and Figure 2. (Dominant halophytes are: *M.c.= Matricaria chamomilla*, *P.m.=Plantago maritima*, *A.m.=Artemisia maritima*, *A.t.=Aster tripolium*, *P.l.=Puccinellia limosa*).

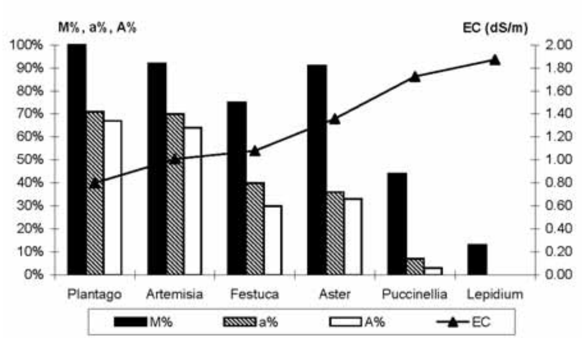

Figure 2. Colonisation values, i.e., the intensity (M%) and the relative and absolute arbusculum richness (a% and A%) of the arbuscular mycorrhizal fungi (AMF) in the rhizosphere of the most dominant halophytes, the *Plantago, maritima*, *Artemisia maritima*, *Festuca pseudovina*, *Aster tripolium*, *Puccinellia limosa* and *Lepidium crassifolium*, grown in the salt affected soils of the site A (Apaj), sampled in 2001. Electric conductivity (EC dS m^{-1}), i.e the salt levels of the soil-samples are also

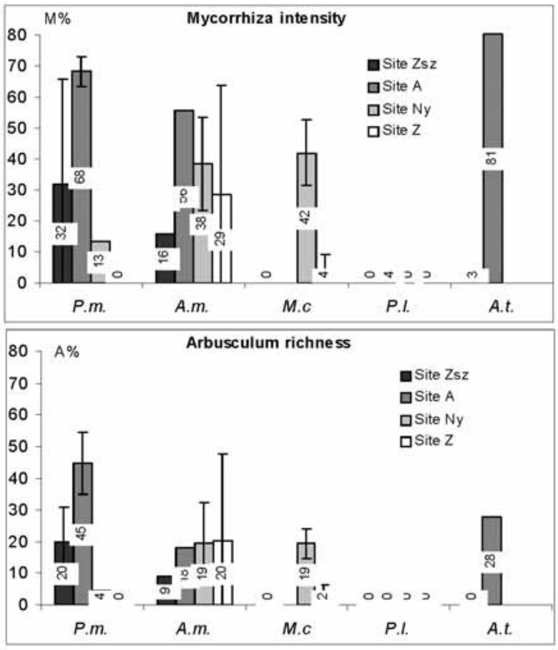

Figure 3. Intensity of mycorrhizal colonisation (M%) and the absolute arbusculum richness (A%) of the main halophytes (M.c.: *Matricaria chamomilla*, P.m.: *Plantago maritima*, A.m.: *Artemisia maritima*, A.t.: *Aster tripolium*, P.l.: *Puccinellia limosa*) at four salinic sampling sites (Zsz, A, Ny and Z) with different soil characteristics in Hungary. Roots of the halophytes were sampled in the spring of 2001. Mean values and the standard deviations (n=3).

the alterations in the salt-levels, measured as electric conductivity (EC). The greatest values of such salinity was recorded at sites Z (EC=0,9-4,1) and NY (EC=1,2-3,6), with the dominance of Cl^{-} (28,5 me l^{-1}) and SO_4^{2-} (20,2 me l^{-1}) anions, respectively. Beside this, sites A and Zsz are relatively rich in CO_3^{2-} and HCO_3^{-} (34,6 and 21,1 me l^{-1}) and the electric conductivity is quite low, the maximum levels of EC (dS m^{-1}) are not higher than 1,7 (site A) and 1,3 (site Zsz). The level of the salt therefore is a key-factor in the soils, which are determining the plant cover and the dominance of the particular plant species, tolerating the saline conditions. The relative locations of the studied dominant halophytes are shown in the Figure 1, with a general pattern of the studied plants at each sites, affecting therefore mainly from the measured average salt-levels, but seem to be not influenced from the types of the salt-specific anions. Regarding the plant-cover the same type of plant-community (the *Artemisio-Festucetum pseudovinae*) can be found at all the sites, except the site Z with the *Artemisio-Puccinellia limosae* plant community.

Biotic factors affecting the mycorrhiza colonisation

The impact of biotic environmental factors, more particularly the most affecting parameter, the type of the host-plants was also studied on the levels of the mycorrhiza colonisation. According to the Trouvelot et al. method (1986) the intensity (M%) and the arbusculum richness (A%) can be easily assessed and therefore the most frequently studied. As it is shown in the Figure 2 and the Figure 3, the host-plants (the macrosymbionts) are mainly determining the potential colonisation ratio of the mycorrhiza fungi (the microsymbiont). When comparing the colonisation values among the four sites (Fig. 3) the most uniform result of AM fungal symbiosis was found on the *Puccinellia limosa* (Schur) Holmgb, which plant was dominating on the most salinic areas of the four sampling sites. The maximum mycorrhization values of this plant, however was found to be very low or almost zero (at the studied sites). In case of the other four plants the colonisation was quite strong, at least at one or two sampling sites (for example *Matricaria chamomilla* = *Matricaria recutita* L. at site Ny or *Aster tripolium* L. subsp. Pannonicum (Jacq.) Soó, at site A). We found especially high values for the mycorrhiza colonisation parameters at site A (M%=60-

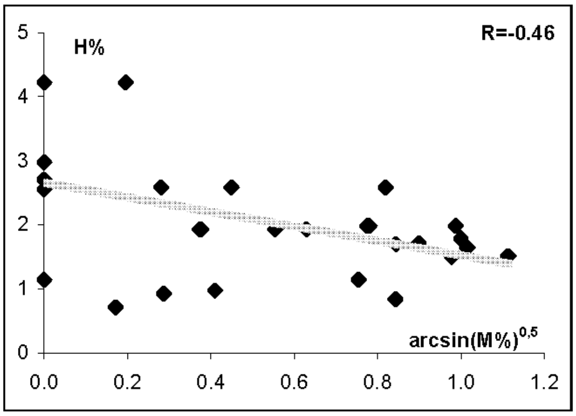

Figure 4. Negative correlation (R=-0.46) between the humus accumulation (%) and the arc-sin transformed data of the mycorrhizal intensity (M%) of the five studied halophytes (*Matricaria chamomilla*, *Plantago maritima*, *Artemisia maritima*, *Aster tripolium* and *Puccinellia limosa*), grown in four saline soils (sites Zsz, A, Ny and Z) in Hungary (n=28). The correlation is significant (P=0.5 level).

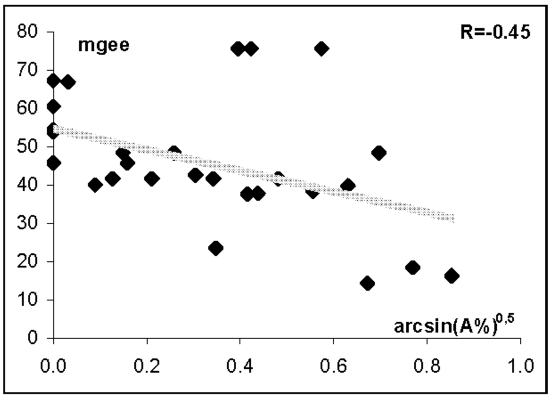

Figure 5. Negative correlation (R=-0.45) between the salinity, represented as the total cationic activity (mgee) and the arc-sin transformed data of the arbusculum richness (A%) found at the five studied halophytes (*Matricaria chamomilla*, *Plantago maritima*, *Artemisia maritima*, *Aster tripolium* and *Puccinellia limosa*) at the four saline soils (sites Zsz, A, Ny and Z) in Hungary (*n*=28). The correlation is significant (P=0.5 level).

80) on three model plants, such as the *Plantago maritima* L., the *Artemisia maritima* = *Artemisia santonicum* L. and the *Aster tripolium*) (Figure 2). At site Ny the AMF values are lower (M%=10-40), but still considerable at the most dominanum content, i.e thy mycorrhiza functioning (A%) and the mycorrhizal intensity (M%).

Abiotic factors affecting the mycorrhiza colonisation

According to Table 1, a large variability of other studied physico-chemical parameters can be realised among the four sites. The relatively less fluctuation was found in the pH levels. The average pH values are similar at each sites: Zsz=9,3 (9,0-9,7), A=9,2 (8,5-9,6), Z=8,5 (7,5-9,5) and Ny=8,9 (8,4-9,8). There are however significant differences among the four sites regarding the humus % and the available macro-nutrients, such as the phosphorous (P) and the potassium (K) in the soils. Referring to the categorisation of Buzas (1983) the highest level of those nutrients can be found at the site Z, while an average, good level is measured at site Ny, poor at site A and very poor at the site Zsz (Table 1). Such availability of the nutrients seem to be a crucial factor in the development of the mycorrhiza symbiosis. Those nutrient levels are showing a strong positive correlation with the humus (H%) accumulation values of the saline soils. According to the Figure 3, the mycorrhiza colonisation is reduced or diminished at site Z, where the highest nutrient availability (and humus accumulation, H% = 2,5-4,5) was found. Mycorrhiza symbiosis was especially strong at site A with a poorly available nutrients and humus content (H% = 0,9-2,0). The very poor situations at site Zsz (H% = 0,7-1,2), however were highly reducing the AMF symbiosis of the studied hosts. The negative correlation tendency of the accumulating humus content on the mycorrhiza colonisation is shown on the Figure 4.

When considering the increasing salinity in the soils, a reduced mycorrhiza colonisation was found at the five dominant halophytes, shown in the Figure 2, as the host- and saline-dependency of mycorrhizal fungi. Correlation-regression analysis was performed to study the interrelations between the total cations and anions in the soils and/or the mycorrhizal colonisation values of the selected halophytes. There were 28 data-pairs evaluated from the four study-sites, sampled in April of 2001. At the 5% of significance level a negative effect of total cationic activities were found on the AMF colonisation parameters, among them mainly on the arbusculum richness (A%) of the halophytes (Figure 5). In case of the m

Among the abiotic environmental factors it is the salt, which might have the greatest influence on the symbiosis with the arbuscular mycorrhizal fungi. We found extra high values for the mycorrhiza colonisation at the site A. The 50–80% values for AMF colonisation (M%) is not general in the salt affected sites (Hildebrandt, 2000), more particularly, if we consider the early spring sampling time. At the beginning of the vegetation period, the mycorrhization rates are generally far from the potential maximum values (Giovanetti 1985, Mohammad 1998). Comparing the soil parameters of the two sampling sites (site A with the greatest colonisation values and site Z with almost no mycorrhization), there are considerable differences in the ion rates (Na^+, Cl^-, total cation content) and electric conductivity, wich values may negatively affect the spore germination (Juniper, 1993), the hifa elongation (McMillen, 1998; Juniper, 1993) and the root-colonisation (Hirrel, 1980; Cantrell, 2001) of the mycorrhizal fungi. The nutrient content is also quite different at those two sites (available nutrient is poor at site A, but it is quite high at site Z). This fact can also explain the differences, found between the sites in the mycorrhizal colonisation, because the high nutrient content, especially the available phosphor can repress the functioning of the symbiosis (Aliasgharzadeh, 2001). We could positively correlate the characteristics of the site Ny with this mycorrhiza phenomenon. The AMF colonisation on the other hand was found to be lower at the site Zsz, than it was predicted on the basis of the nutrient availabilities and the salt-levels. At the very low soil-nutrient status the beneficial effect of the AM fungi might be more efficiently developed, in this case however, a negative influence could be recorded. The sampling area of the site Zsz situated at a lake-side position, which resulted a greater water fluctuation and as a consequence the most negative mycorrhizal levels (data not shown). The oxygen necessity of the mycorrhizal fungi is known to be crucial in those water-fludied soils, as it was reported by Juniper (1993). Beside the extrem-high water-levels the very low water content, the permanent drought can be also initiative for the mycorrhiza formation. As it was reported in a previous study, among the permanent salinic conditions it is the water-stress, which can determine and enhance the apparent effectiveness of the halophytes at the summer-stressed conditions (Füzy et al. 2008).

Beside the effect of those abiotic environmental factors the colonisation values of the AM fungi are crucially depending on the particular host-plants. In this respect there are typical non-mycorrhizal plant-families, such as the Brassicaceae or Chenopodiaceae (Regvar et al. 2003). Host plants are also known with especially low colonisation values, like the *Puccinellia limosa* or extra high values, like the *Aster tripolium* (Carvalho, 2003). Naturally we observed low mycorrhizal colonisation values (M% and A%) at each main halophytes. The fact, however, that a host-plant is belonging to a certain plant species, does not exactly determine the final ratio of the AMF colonisation. The strong mycorrhization is considered to be a potential, which can be reached as a maximum value among the particular soil-plant-environmental conditions, where the macro- and microsymbionts are able to develop a joint surviving strategism, reported previously by Biró et al. (2005). The found site-specificities in the mycorrhiza colonisation is being a final outcome of the interacting biotic and abiotic environmental factors, in which the salt-levels (the salt-specific cations), and the water-dependent nutrient-availability (the humus-accumulation ratio in %) can play a crucial role in the development and functioning of the arbuscular mycorrhizal symbiosis.

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