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Arbuscular mycorrhizal fungal symbiosis with *Sorbus torminalis* does not vary with soil nutrients and enzyme activities across different sites

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Effects of soil chemical properties on arbuscular mycorrhizal fungal (AMF) symbiosis with wild service tree (*Sorbus torminalis* L. Crantz) were examined with the aim of assessing the root colonization rate at three forest sites in northern Iran. Soil characteristics including pH, available phosphorus (P), potassium (K), organic matter, total nitrogen, acid and alkaline phosphatase activities, $CaCO_3$, spore density (SD) and AMF colonization of soil and root samples were analyzed. The study sites were investigated in spring and autumn to highlight the effects of varying soil chemical properties on AMF colonization. K, pH, root colonization, SD and acid phosphatase activity showed no significant differences among sites and seasons, while total nitrogen, P, organic matter and alkaline phosphatase activities showed significant differences among sites and seasons. AMF colonization rate was more than 51% and 32% of roots in spring and autumn, respectively. No correlation between root colonization and soil chemical parameters in spring and autumn were detected. There was no correlation between percentage of AM root colonization and SD nor other soil parameters in spring and autumn. SD and $CaCO_3$ were significantly negatively correlated in spring and autumn. Despite differences in soil characteristics, results showed that SD and root colonization were not significantly different among sites. We concluded that wild service trees had strong symbiosis with AMF, while soil properties might not have a significant effect on the symbiosis. Therefore, the use of AMF colonized seedlings can be considered as an appropriate method for reforestation and conservation of this rare tree species.

Keywords: Arbuscular Mycorrhizae, Soil Nutrients, Colonization, Soil Enzyme, *Sorbus torminalis*

Introduction

Arbuscular mycorrhizal fungi (AMF) play a critical role in the early establishment of land plants (Redecker et al. 2000). They can improve the host plants growth (Wu et al. 2011), improve plant nutrient and water uptake, enhance plant tolerance to environmental stresses, including metal tolerance, and are abundant in the plant rhizosphere interface (Smith & Read 2008, Xu et al. 2008, Barea et al. 2011). AMF are important micro-organisms which contribute to plant diversity and ecosystem functions (Van der Heijden et al. 1998). About 80% of higher plants have associations with arbuscular mycorrhizae (Smith & Read 2008). These associations lead to better phosphorous (P) absorption (Rooney et al. 2011) and the fungi can supply up to 80% of P and 25% of nitrogen (N) to the host plants (Marschner & Dell 1994). In turn, the fungi are highly dependent on their host plants for carbon nutrition (Smith & Read 1997). P is an important element which is actively supplied to host plants by AMF. The amount of P influx into colonized roots can be three to five times higher than non-mycorrhizal roots (Smith et al. 2003).

Some soil characteristics have significant influences on the development of mycorrhizal fungi in roots, such as potassium (K), nitrogen (Treseder 2004), pH (Isobe et al. 2007), and compaction (Waltert et al. 2002), as well as climatic conditions and host plant effects (Kivlin et al. 2011). Increasing soil acidity or alkalinity was also a detrimental factor to AMF sporulation in field soils (Isobe et al. 2007). AMF also affect soil enzymes (Huang et al. 2009, Wu et al. 2011) by increasing activities such as dehydrogenase, phosphatase and urease (Huang et al. 2009).

Wild service tree (*Sorbus torminalis* L. Crantz) is widely distributed across Europe, north-western African forests, and south-western Asia (Nicolescu et al. 2009). It is also a rare, protected tree species found in the northern forests of Iran at elevations between 170 to 2700 m a.s.l., where it can reach up to 34 m in height (Pourmajidian 2000). Different species of the genus *Sorbus* are found in association with both arbuscular mycorrhizal and ecto-mycorrhizal fungi (Raspe et al. 2000).

In this study, we hypothesized that AMF colonization of specific plants can vary significantly between sites with different soil characteristics. A comprehensive study was conducted to determine how AMF colonization of a wild service tree (*Sorbus torminalis*) was influenced by soil nutrients and enzyme activities at three different sites. A better understanding of AMF relationships with the wild service tree and its role in nutrient absorption and soil enzymes will enable better management of the tree stands in terms of nutrient uptake and more vigorous tree growth.

Materials and methods

Study sites and sampling

The study was conducted in a natural mixed forest of northern Iran consisting of *Fagus orientalis* Lipsky*, Carpinus betulus* L., and *Acer cappadoeicum* Gled along with a minor population of *Sorbus torminalis*. According to differences in aspect, slope, elevation, precipitation and some soil edaphic features, three sites were selected for this study: Kheiroud, Lalis, and Takrin (Tab. 1).

Five *S. torminais* trees were randomly se-

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Tab. 1 - Basic information of the study sites.

Site	Aspect	Elevation (m a.s.l.)	Latitude N	Longitude E	Slope (%)	Rainfall (mm)	Soil Texture	Temperature (°C)		
								Min	Max	Mean
Kheiroud	S	1000	36° 40′	51° 43′	30-70	1457	clay	7	25	16
Lalis	N-NW	1700	36° 32′	51° 23′	0-60	731	clay	-10	29	10
Tarkin	N	1500	36° 28′	51° 51′	30-80	1085	clay-silt	-13	32	10

lected from each of the three sites. Five root and soils samples were collected at the crown projection area at the bottom of each tree in spring and autumn. Fine roots and soil samples were collected to a depth of 15 cm after removal of forest litter and organic horizon. Samples were bulked, air-dried, sieved (< 2 mm), sealed in plastic bags, and stored at 4 °C until further analyses.

Soil chemical parameters

Soil pH was measured in a soil:deionized water suspension of 1:2.5. Due to their alkaline nature, P content in the soil samples was determined using the method developed by Olsen et al. (1954).

Soil K was extracted using the neutral ammonium acetate method (Morwin & Peach 1951) and was quantified using a flame photometer. Soil organic carbon (%) was determined according to the Walkley & Black (1934) method. In addition, soil organic carbon was converted to soil organic matter (OM, %) using a factor of 1.724. Total nitrogen (TN, %) was determined using the conventional standard Kjeldhal procedure (Bremner & Mulvaney 1982).

The method of Ohlinger (1996) was used to measure acid and alkaline phosphatase activities using *p*-nitrophenyl as substrate. Analyses were conducted in duplicate with one non-substrate control. Results were expressed as μg *p*-nitrophenol g^{-1} h^{-1} at 37 °C.

AMF root colonization and spore density

The five root samples from each site were carefully washed to remove soil and organic particles. Root samples were then cut into pieces of one cm and preserved in FAA buffer (5 ml formalin: 5 ml acetic acid in 90 ml of 70% alcohol). Roots were stained using 0.05% trypan blue in lactophenol (Phillips & Hayman 1970) and mounted on a petri dish with grid-line intersects. Root length colonization was measured under a dissecting microscope (Olympus CH2) using the method proposed by Giovannetti & Mosse (1980) and intraradical status of hyphae, arbuscules and vesicles was used to determined root length colonization.

AMF spores were extracted by wet sieving (sieves of size 500 and 38 μm), and decanting (Gerdemann & Nicolson 1963), followed by centrifugation in water. For estimation of the AMF spore density (SD) in soil surrounding each host tree, 15 g sub-samples of soils in two replications were analyzed, and all spores were counted. Average of the two sub-samples was taken and expressed as number of AMF spores per gram soil sample (g^{-1}).

Data analysis

Statistical analysis was conducted using the package SPSS® v. 16 for Windows. Data was subjected to one-way analysis of variance followed by Tukey's test ($\alpha = 0.05$) to detect differences in variable means among the three sites. Student *t* test was used to determine acid and alkaline phosphatase activity differences between seasons. The relationship between soil chemical characteristics and AMF colonization were determined by Pearson's correlation coefficients. Magnitude of correlations were interpreted according to Guilford's rule-of-thumb (Guilford 1973) as follows: (i) $r < 0.20$: negligible correlation; (ii) $0.20 < r < 0.40$: weak correlation; (iii) $0.40 < r < 0.70$: moderate correlation; (iv) $0.70 < r < 0.90$: strong correlation; (v): $r > 0.90$: very strong correlation.

Results

Soil N, P, K, pH and OM

Average values of the soil nutrients at the three sites are presented in Tab. 2. Pearson's correlation coefficients (*r*) for each feature in spring and autumn are presented in Tab. 3 and Tab. 4.

The value of total nitrogen in the soil was significantly higher for Tarkin than for the other areas in both seasons ($p < 0.01$ - Tab. 2). The mean values of total nitrogen as a critical soil component in Kheiroud and Lalis were in the same range with no significant differences between them. The values of total nitrogen between the spring and autumn seasons in all sites were not significantly different.

The P showed no significant differences among Kheiroud and Takrin during spring and autumn seasons. On the other hand, significant differences among these two sites and Lalis were observed ($p < 0.05$). In general, the highest amount of P was observed in the autumn samples from Lalis.

The mean values of exchangeable K for the sites ranged between 312.23 (Kheiroud) and 566.41 (Lalis) in spring, and between 311.73 (Kheiroud) and 532.05 (Lalis) in autumn. This represents 81.4% and 70.7% differences between minimum and maximum values of K in spring and autumn, respectively. No significant differences were found between K values observed in the Kheiroud and Takrin sites compared to Lalis. In addition, no significant differences were evident between spring and autumn for all sites.

Soil pH was a significant factor in the three sites during spring and autumn. The lowest pH in this study was 6.15 at Lalis and the highest was 8.75 at Kheiroud.

Concerning OM, Tarkin had the highest values during spring and autumn. There was a significant difference among values for Tarkin and Lalis in the spring. In autumn however, significant differences ($p < 0.05$) were observed among Tarkin and Kheiroud (Tab. 3).

Root length colonization and SD

The average colonization rates for all sites

Tab. 2 - Mean soil parameters in different seasons at the three study sites. (TN): total nitrogen; (P): phosphorous; (K): potassium; (OM): organic matter; (RC): root colonization; (SD): spore density; (A.P): acid phosphatase; (Al.P): alkaline phosphatase. (*): μg p-nitrophenol g^{-1} h^{-1}. Means within columns (in separate sites) followed by the same letters were not significantly different (p>0.05) after Tukey's test.

Site	Season	TN (%)	P (ppm)	K (ppm)	pH	OM (%)	RC (%)	SD (N/g)	A.P*	ALP*
Kheiroud	Spring	0.25^{a}	7.16^{a}	312.23^{a}	7.99^{a}	8.29^{ab}	59.0^{a}	69^{a}	499.51^{a}	627.76^{ab}
	Autumn	0.22^{a}	8.64^{a}	311.70^{a}	7.93^{a}	8.13^{a}	38.5^{a}	81^{a}	554.96^{a}	608.24^{a}
Tarkin	Spring	0.44^{b}	10.02^{ab}	315.60^{a}	7.69^{a}	12.72^{a}	60.0^{a}	56^{a}	825.06^{a}	837.77^{b}
	Autumn	0.43^{b}	9.46^{a}	342.10^{a}	7.62^{a}	14.15^{b}	42.0^{a}	79^{a}	891.62^{a}	843.00^{a}
Lalis	Spring	0.18^{a}	12.22^{b}	566.40^{a}	7.32^{a}	6.64^{b}	61.0^{a}	50^{a}	597.95^{a}	408.94^{a}
	Autumn	0.28^{a}	14.66^{b}	532.10^{a}	7.05^{a}	9.52^{ab}	44.5^{a}	77^{a}	872.85^{a}	563.00^{a}

during the spring and autumn seasons are presented in Tab. 2. Over all the sites, AMF colonization rates in spring and autumn showed an average of about 60 and 41.6%, respectively. Root colonization in spring and autumn ranged between 51.0-68.5% and 32.4-55.3%, respectively. No significant differences among the three sites were detected (p > 0.05), but root colonization was significantly different between the two seasons in each site (p < 0.01).

Concerning AMF spore numbers, results showed that there was no significant difference in SD among sites during the spring and autumn seasons (Tab. 2).

Soil acid and alkaline phosphatase

As shown in Tab. 2, acid phosphatase activity showed no significant difference among the sites during spring and autumn, but alkaline phosphatase activity showed a significant difference among Lalis and Tarkin in the spring (*p<0.05*). In autumn, there were no significant differences among the sites (Tab. 2). Moreover, no significant differences were observed between acid and alkaline phosphatase activities in spring and autumn (Tab. 4).

The degree of variation observed indicates that the studied parameters were not equally variable among the sites (Tab. 2).

Correlation of soil chemical properties, SD and root length colonization

The Pearson's correlation coefficients between soil chemical properties, SD and root length colonization are presented in Tab. 5.

There was a significant and positive relationship between K and P in autumn (r =0.670; *p<0.01*) with moderate magnitude (Tab. 3), while there was no correlation between them in spring (*p>0.05* - Tab. 6).

There was a non-significant decrease in pH values from Kheiroud to Lalis (Tab. 2, *p>0.05*).

A negative correlation with moderate magnitude was observed between pH and soil P in spring (r=0.571, Tab. 1). However, this correlation was not significant in autumn (*p>0.05*).

There was a significant negative correlation between SD and $CaCO_3$ in spring and autumn (Tab. 6 and Tab. 3). However, root colonization was not significantly correlated with soil chemical and physiological parameters (Tab. 5).

No significant correlations between SD and other soil parameters between spring and autumn seasons were detected except for $CaCO_3$. As shown in Tab. 6 and Tab. 3, there was significant positive correlation between AMF spore density and slope in both spring and autumn seasons. No significant differences were observed between SD and elevation (Tab. 6 and Tab. 3).

The correlation coefficient between soil al-

Tab. 3 - Correlation coefficients between soil parameters in autumn. (TN): total nitrogen; (P): phosphorous; (K): potassium; (OM): organic matter; (SD): spore density; (A.P): acid phosphatase; (Al.P): alkaline phosphatase; (Elev): elevation; (*): p<0.05. (**): p<0.01.

Parameter	P	OM%	pH	TN%	K	$CaCO_3$	A.P	Al.P	SD	Elev	Slope%
P	1	-	-	-	-	-	-	-	-	-	-
OM%	.161	1	-	-	-	-	-	-	-	-	-
pH	-.080	-.032	1	-	-	-	-	-	-	-	-
TN%	.179	.844**	.090	1	-	-	-	-	-	-	-
K	.670**	.089	-.050	.168	1	-	-	-	-	-	-
$CaCO_3$	-.362	-.422	.470	-.424	-.367	1	-	-	-	-	-
A.P	.457	.739**	-.514*	.603*	.384	-.553*	1	-	-	-	-
Al.P	.104	.730**	.549*	.741**	-.035	.077	.233	1	-	-	-
SD	-.030	.091	-.434	.030	.048	-.577*	.181	-.368	1	-	-
Elev	.594*	.312	-.416	.280	.487	-.537*	.574*	.055	.420	1	-
Slope%	-.340	.376	-.113	.447	-.249	-.501	.162	.068	.752**	-.018	1

Tab. 4 - Acid and alkaline phosphatase differences between seasons. Values are means ± SE. Means were not significantly different between spring and autumn (p > 0.05) after Student's *t* test.

Season	Acid phosphatase (A.P)	Alkaline phosphatase (Al.P)
Spring	640.83 ± 56.90	624.82 ± 61.45
Autumn	773.14 ± 68.01	671.41 ± 54.37

Tab. 5 - Correlation coefficients between root colonization and soil parameters in spring and autumn. (TN): total nitrogen; (P): phosphorous; (K): potassium; (OM): organic matter; (SD): spore density; (A.P): acid phosphatase; (Al.P): alkaline phosphatase; (Elev): elevation.

Root colonization	pH	P	OM%	TN%	$CaCO_3$	K	A.P	Al.P	SD	Elev	Slope%
Spring	-.425	.201	-.114	-.126	-.207	.155	-.025	-.240	-.090	.158	-.015
Autumn	-.146	.216	.091	.025	-.326	-.145	.011	-.006	.043	.402	.090

Tab. 6 - Correlation coefficients between soil parameters in spring. (TN): total nitrogen; (P): phosphorous; (K): potassium; (OM): organic matter; (SD): spore density; (A.P): acid phosphatase; (Al.P): alkaline phosphatase; (Elev): elevation; (*): p<0.05; (**): p<0.01.

Parameter	pH	P	OM%	TN%	K	$CaCO_3$	A.P	Al.P	SD	Elev	Slope%
pH	1	-	-	-	-	-	-	-	-	-	-
P	-.571*	1	-	-	-	-	-	-	-	-	-
OM%	.320	.008	1	-	-	-	-	-	-	-	-
TN%	.254	-.009	.977**	1	-	-	-	-	-	-	-
K	-.345	.506	.036	-.284	1	-	-	-	-	-	-
$CaCO_3$	.484	-.462	-.298	-.297	-.352	1	-	-	-	-	-
A.P	-.103	.165	.776**	.747**	.523	-.496	1	-	-	-	-
Al.P	.563*	-.220	.884**	.882**	-.240	.091	.612*	1	-	-	-
SD	-.152	-.060	.210	.235	.130	-.515*	.172	-.040	1	-	-
Elv	-.498	.628*	-.016	-.032	.481	-.511	.314	-.220	.230	1	-
Slope%	-.052	-.113	.450	.560*	-.253	-.513	.291	.272	.814**	-.018	1

kaline phosphatase and pH was positive and significant in the spring with $r = 0.563$ ($p < 0.05$ - Tab. 3). In addition, the soil acid and alkaline phosphatase in relation to OM and TN were significantly correlated ($p < 0.01$). The magnitude of correlations between these factors was very high (Tab. 6 and Tab. 3).

Discussion

The significant effect of AMF on plant growth and leaf mineral concentrations has been previously documented (Motosugi et al. 2002). Our result showed that despite differences in soil properties in the studied sites, no significant differences in SD and root length colonization were observed. In a similar study on *Araucaria angustifolia*, Moreira et al. (2006) showed similar rates of root colonization in spring. A reasonable colonization rate is usually about 40% (Thonar et al. 2011). In the present study, based on observation of vesicle, arbuscule and mycelia in roots, AMF colonization rates were more than 51% and 32% of roots in spring and autumn, respectively. These results confirm a high level of symbiosis between wild service trees and AMF in spring. As mentioned earlier, the highest colonization rate was observed in spring. This response was similar to several earlier reports (Rodriguez-Echeverria et al. 2008, Closa & Goicoechea 2011).

Forest plantation management could greatly benefit from the use of wild service tree with high levels of root colonization by AMF, in particular in arid environments (Liu et al. 2007). Indeed, previous reports have demonstrated the tolerance of wild service tree to arid environmental conditions (Paganová 2008). Additionally, no significant correlations between root colonization and soil chemical parameters in spring and autumn were observed, consistently with several previous findings (Matevz et al. 2013, Gai et al. 2012, Closa & Goicoechea 2011, Rodriguez-Echeverria et al. 2008).

Usually, spore density of AMF can be related to root colonization (Muthukumar et al. 2003), nutrient status (Mendoza et al. 2002), elevation (Gai et al. 2012), pH and soil P (Li et al. 2005, Minggui et al. 2012). However, in the present study no relationship between the percentage of AM colonization and spore density was found, according to previous literature reports (Becerra et al. 2009). Similar results were observed for other soil parameters with negative correlations between spore density and $CaCO_3$ in spring and autumn. Due to the same root colonization rates in the studied sites, no significant correlation between root colonization and spore density was expected. Trees in Kheiroud were located in areas with high pH and $CaCO_3$ which negatively influences AMF and spore density. Similar results were reported by Labidi et al. (2011). As stated by Mendoza et al. (2002), the SD level is related to nutrient status, especially nitrogen content. While a direct correlation between N and SD was not observed in this study, both N and SD were positively correlated with slope across all sites.

According to Yong et al. (2007), we found that root AMF colonization was affected by environmental conditions and host plants. Our results showed that available soil P in spring was significantly different among Lalis and Kheiroud, while in autumn Lalis was significantly different from the other two sites. Since there was no significant difference in root colonization among sites, soil available P differences can be attributed to the soil pH, that was the lowest in both seasons in Lalis compared to Tarkin and Kheiroud. Furthermore, pH had a significant negative correlation with available P. Zhao et al. (2011) stressed that available P increases with decreasing pH. It should be noted that in calcareous soil, P is retained by Ca ions and in acid soils it is retained by Fe and Al ions (Hisinger 2001).

There was a significant difference in total nitrogen among Tarkin and the other two sites for both seasons. In addition, the results indicated that the organic matter, the most important nitrogen source (Gairola et al. 2012), was higher in Tarkin than in the other sites. Moreover, we observed a significant correlation between total nitrogen and the organic matter, according to Gairola et al. (2012).

Factors such as temperature, moisture content (Kirschbaum 1995, Kane et al. 2005), elevation (Dai & Huang 2006), precipitation and geographical aspects (Griffiths et al. 2009) significantly affect soil OM content. For example, it is known that OM soil levels in north-facing slopes (as in Tarkin) are higher as a consequence of its lower decomposition rate (Quideau 2002). Since Kheiroud, Tarkin and Lalis are located in south, north, and northwest slopes, respectively, we hypothesize that the aforementioned differences in total nitrogen between Tarkin and the other two sites may be attributed to the higher amount of organic matter in Tarkin (as a consequence of its aspect) rather than other factors.

Several authors have reported that soil K content is influenced by elevation, total nitrogen, and organic matter (Basumatary & Bordoloi 1992, Gairola et al. 2012). In the present study, no significant differences among sites and no significant correlations between K and soil chemical properties were detected, according to previous findings by Paudel & Sah (2003).

It is reported that AMF colonization could result in higher acid and alkaline phosphatase activities, leading to higher P, N and K content in plant leaves (Amaya-Carpio et al. 2009). However, in the present study we did not found significant differences among sites in autumn as for such enzyme activities. On the other hand, alkaline phosphatase activity in Lalis was significantly different from that in Tarkin in spring. Such difference could be attributed to the effect of soil pH. Indeed, it has been documented that acid phosphatase activity is higher in acidic soils, while alkaline phosphatase activity was higher in calcareous or neutral soils (Dick & Tabatabai 1984). The importance of soil availability of N, pH, moisture content and total soil carbon in acid and alkaline activities has also been suggested (Dick et al. 2000, Sardans et al. 2008, Hamman et al. 2008, Hrynkiewicz et al. 2009). In our study, there were significant correlations between the above enzyme activities and OM, TN, pH and $CaCO_3$. Highest acid and alkaline phosphatase activity from Tarkin in both seasons was likely due to the higher amount of OM and TN in this site.

Conclusion

Wild service tree is a rare species in the northern forest of Iran. This species is important due to its economic and ecological value. Our results revealed that different soil chemical properties did not have significant effect on root colonization and spore density in the studied sites. Despite the strong differences in site conditions, our results revealed that up to 68% of roots of wild service trees could be colonized by AMF. Since this species is tolerant to direct sunlight and short-time water deficits, the use of wild service trees colonized by AMF could help in afforestation plans, particularly in arid areas.

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