# Sand mining disturbances and their effects on the diversity of arbuscular mycorrhizal fungi in a riparian forest of Iran Postprint

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## Abstract

The major objective of this study was to evaluate the effects of sand mining disturbances on the diversity of arbuscular mycorrhizal fungi (AMF). In addition, the proportional changes in the diversityof AMF to the distances from riverbanks were assessed.

## Full Text

## Preamble

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**Sand mining disturbances and their effects on the diversity of arbuscular mycorrhizal fungi in a riparian forest of Iran**

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## Abstract

The major objective of this study was to evaluate the effects of sand mining disturbances on the diversity of arbuscular mycorrhizal fungi (AMF). In addition, the proportional changes in AMF diversity relative to distance from riverbanks were assessed. For this purpose, the riparian forest of the Maroon River in

Iran was divided into three locations with a 200-meter wide zone in between. The locations studied were designated as Distance I (riverbank), Distance II (intermediate), and Distance III (farthest from riverbank). In each distance, 10 individuals of each species—*Tamarix arceuthoides* and *Populus euphratica*—were randomly selected. Simultaneously, soil and root samples were collected from the rhizosphere of the studied tree species. Results indicated that a total of 13 AMF species were observed in the *T. arceuthoides* rhizosphere, while 19 AMF species were recorded in the *P. euphratica* rhizosphere, belonging to six genera and six families. Among these AMF species, *Glomus segmentatum*, *G. geosporum*, *G. rubiforme*, *G. nanolumen*, *G. spinuliferum*, *Claroideoglomus drummondii*, *Gigaspora gigantea*, and *Acaulospora paulinae* appeared only in the *P. euphratica* rhizosphere, while *G. multiforum* and *Claroideoglomus claroideum* were observed only in the *T. arceuthoides* rhizosphere. Moreover, Distance II had the fewest AMF species in both rhizospheres, as well as the lowest spore density and root colonization rate. Our results are significant in that they provide a list of resistant AMF species that could be used in biodiversity conservation.

**Keywords:** arbuscular mycorrhizal fungi; disturbance; riparian forest; sand mining; Maroon River

## 1 Introduction

Riparian forests maintain a chain of important ecological functions, including water purification, flood control, and natural habitat and biodiversity conservation (Burton, 2006; Harris and Kocher, 2007; Strasser et al., 2014). Nevertheless, they are among the most endangered ecosystems in the world (Tockner and Stanford, 2002). Khuzestan, a southwestern province of Iran, is well known as a home to major rivers and abundant fresh water resources. The littoral lands beside Khuzestan' s riverbanks are covered by riparian forests, mostly composed of *Populus euphratica* and *Tamarix arceuthoides* (Browicz, 1977). These riparian forests, with high floral diversity (Sakio and Tamura, 2008), are crucial for ecological health in this arid area (Isebrands and Richardson, 2014). In spite of the importance of these riparian forests, little is known regarding their ecological information.

Several factors cause the degradation of these vulnerable ecosystems, including the invasion of exotic plant species (Naiman et al., 2000), environmental pollution (Tockner and Stanford, 2002), and sand mining (Tockner and Stanford, 2002; Lawal et al., 2011; Sreebha and Padmalal, 2011; Bravard et al., 2013). It is noteworthy that sand mining has been identified as among the most devastating factors due to over-exploitation of sand and gravel worldwide. Researchers have found that sand mining may result in the loss of riparian forest (Padmalal et al., 2008), depletion of vegetation, water pollution (Byrnes and Hiland, 1995; Ashraf et al., 2011), landscape disturbance (Lawal et al., 2011), and many other negative effects (Ashraf et al., 2011). However, very little information is available on the effect of sand disturbances on soil microorganisms, such as the diversity of arbuscular mycorrhizal fungi (AMF) in these ecosystems.

AMF are soil-borne microbial communities that form mutualistic symbioses with terrestrial plants (Smith and Read, 2008). They belong to the fungal phylum Glomeromycota (Schüßler et al., 2001). These fungi are known to be important for the stability and productivity of ecosystems (van der Heijden et al., 2008). Furthermore, they are crucial for vegetation establishment (Caravaca et al., 2003; Quoreshi, 2008). Yet, it should be pointed out that these fungal communities are likely to be affected by vegetation, soil $CaCO_3$, land use, and disturbance (Jha et al., 1992; Lambin et al., 2003; Oehl et al., 2011; Moradi et al., 2015). Therefore, these fungal communities can be used as soil quality indicators (Oehl et al., 2011; Verbruggen et al., 2012).

Recently, researchers have found that AMF can form mutualistic symbioses with some riparian plant species, such as *P. euphratica* and *Tamarix* spp. (Yang et al., 2008). The infection rate and status of AMF in riparian forest species were affected by soil chemical properties (Shi et al., 2006; Yang et al., 2008; Moradi et al., 2017). Furthermore, it has been demonstrated that AMF spore density, root colonization, and diversity differ in these riparian forests (Beauchamp et al., 2005; Wang et al., 2010; Lehnhoff et al., 2012; Yang et al., 2013). Therefore, we hypothesize that the responses of AMF in different riparian forests to sand mining disturbances are species-dependent. The aim of this study is to evaluate the effect of sand mining and truck movements on the diversity of AMF in a riparian forest dominated by *P. euphratica* and *T. arceuthoides* in Khuzestan, Iran.

## 2.1 Study area

This study was conducted in the riparian forest of the Maroon River in Behbahan within Iran's southern Khuzestan Province. The study site was located at 32°38' 53"–30°39' 30" N and 50°09' 30"–50°10' 25" E with an average elevation of 250–300 m a.s.l. (Fig. 1 [Figure 1: see original paper]). The average annual precipitation and mean annual temperature of the site were 350 mm and 24°C, respectively. This site was typically dominated by *P. euphratica* and *T. arceuthoides* with floral coverage by other species like *Lycium* sp. and *Vitex pseudonegundo* to a lesser degree.

## 2.2 Sampling method

The riparian forest along the width direction was divided into three zones with a 200-meter wide zone in between. The locations studied were designated as Distance I (riverbank), Distance II (intermediate), and Distance III (farthest from riverbank). The reason behind this division was that sand mining and truck movements took place within Distance II. To differentiate the distance effect from the disturbance effect, we left 50 meters as a buffering zone between each of the studied distances. On the other hand, we left 50 meters before and after sand mining locations as a buffering zone (Fig. 1). Since the dominant species in this riparian forest were *P. euphratica* and *T. arceuthoides*, we randomly selected 10 trees from each species per studied distance (60 samples total, 30 samples for each species). Rhizosphere samples were taken from a depth of 15 cm after litter removal in spring (Moradi et al., 2015). These soil samples were used for spore extraction and determination of soil physical-chemical properties.

**Fig. 1** Location of study site and sampling design

## 2.3 AMF spore extraction and identification

Spores were extracted from the soil by wet sieving and decanting (Gerdemann and Nicolson, 1963), followed by centrifugation in water. Sieve sizes ranged from 38 to 500 µm. Five grams of rhizosphere soil from the studied plant species were used for spore extraction. All extracted spores were mounted on slides stained with PVLG (polyvinyl alcohol lactic acid glycerol), as well as PVLG with Melzer reagent. The identification procedure was based on observation of morphological features like spore walls, colors, and sizes using a stereomicroscope (Olympus CH-2) and in accordance with Schenck and Perez (1988).

## 2.4 Soil analysis

All soil samples were air-dried and passed through a 2-mm sieve. Soil phosphorus (P) (Olsen et al., 1954), exchangeable potassium (K) (Morwin and Peach, 1951), nitrogen (N) (Bremner and Mulvaney, 1982), organic carbon (OC) (Walkley and Black, 1934), pH (deionized water suspension of 1:2.5), and texture (hydrometric) were determined. Soil moisture content was determined using oven drying at 105°C for 24 h.

## 2.5 Diversity indices

AMF diversities were evaluated using the Shannon-Weiner index of diversity (H ), evenness, Simpson’ s index of dominance, and Margalef’ s richness index (Eqs. 1-4), where Pi is the relative abundance of each identified species per sampling site.

Evenness (E) was calculated by Equation 2: where Hmax = $\log_2(S)$, and S is the total number of species.

Simpson’ s index of dominance (D) was calculated by Equation 3: where ni is the number of spores of a species and N is the total number of identified spore samples.

Margalef’ s richness index (Dm) was calculated by Equation 4: where S is the number of species.

## 2.6 Data analysis

All data were subjected to normality tests and analyzed using one-way ANOVA to determine any differences between diversity indices and physical-chemical soil properties within the designated distances. If data were significantly different, pairwise comparison was conducted using Duncan’ s post hoc analysis. Moreover, Pearson’ s correlation coefficient was used to determine any significant correlation between soil parameters and AMF diversity indices. All analyses were performed using SPSS 16.0 software. Furthermore, principal component analysis (PCA) was performed to identify the most important factors affecting AMF diversity. To evaluate the effects of soil physical-chemical properties, we applied redundancy analysis (RDA) with Monte Carlo (unrestricted 999 permutations) to verify the relationship between AMF diversity indices and soil physical-chemical properties. This analysis was performed using CANOCO for Windows version 4.5. Additionally, PC-ORD for Windows (V.5) (McCune and Mefford, 1999) was used to determine keystone species in the study area.

## 3.1 AMF diversity

The results indicate that 21 AMF species belonging to six genera and six families were found in the riparian forest (Table 1 ). Thirteen species were observed in the *T. arceuthoides* rhizosphere and 19 in the *P. euphratica* rhizosphere. As shown in Table 1, *Glomus segmentatum*, *G. geosporum*, *G. rubiforme*, *G. nanolumen*, *G. spinuliferum*, *Claroideoglomus drummondii*, *Gigaspora gigantea*, and *Acaulospora paulinae* appeared only in the *P. euphratica* rhizosphere, while *G. multiforum* and *C. claroideum* occurred only in the *Tamarix* sp. rhizosphere (Table 1). *Glomus* was the most frequent genus observed in stands of *T. arceuthoides* and *P. euphratica*, consistent with the findings of Börstler et al. (2008). It should be noted that some AMF species are plant species-specific to *T. arceuthoides* and *P. euphratica*. The reason for this may be related to host plant health and/or soil conditions that affect AMF diversity (Chaudhary et al., 2008). Another reason may be genetic differences among AMF species, since such differences could affect the survival or functionality of AMF and their host plants (Colard et al., 2011).

The *T. arceuthoides* rhizosphere had 9, 6, and 11 AMF species detected in Distances I, II, and III, respectively, while the *P. euphratica* rhizosphere had 11, 9, and 13 species, indicating that Distance III (the farthest research zone from the riverside) had the highest AMF diversity, while Distance II (the zone severely disturbed by sand mining) had the fewest. Moreover, *G. geosporum* and *G. rubiforme* were AMF species detected only in the *P. euphratica* rhizosphere within Distance II, suggesting that the tolerance of these AMF to disturbance may be useful for future restoration activities (Lara-Pérez et al., 2014).

We also found that *F. badium*, *F. constrictum*, and *C. etunicatum* were AMF species whose frequencies remained unaffected by distance from the riverside or sand mining location. This result may indicate that these fungi have adapted well to anthropogenic disturbances. In contrast, distance from both the riverside and sand mining had negative effects on the frequencies of *F. mossea*, *G. gibbosum*, *G. nanolumen*, *G. spinuliferum*, *C. drummondii*, *A. trappei*, and *P. fransiscana* (Table 1). These AMF may serve as indicators of land degradation

(Kennedy and Papendick, 1995). Based on this study, a list of AMF species can be proposed for both disturbed and non-disturbed distances, which may be helpful for site restoration (Symanczik et al., 2014).

*F. badium* and *G. gibbosum* were identified as keystone indicator species at Distance III, while no keystone species were found at the other two distances in the *P. euphratica* stand (Table 2 ). It is well documented that AMF are keystone soil microorganisms for biodiversity conservation (O' Neill et al., 1991; Power and Mills, 1995; Piraino et al., 2002). The lowest frequency of *G. gibbosum* at Distance II (the severely disturbed distance) indicated that the negative effects of sand mining on keystone species are rather pronounced. Our results are significant in that they provide a list of resistant AMF species that could be used in biodiversity conservation.

In the *T. arceuthoides* stand, *G. gibbosum* and *C. etunicatum* were considered keystone species in Distance I and Distance II, respectively (Table 3 ). This finding is consistent with Piraino et al. (2002), who provided evidence that *C. etunicatum* occurred in highly disturbed environments. Different keystone AMF species found in *P. euphratica* and *T. arceuthoides* stands reflect not only differences in responses to plant species but also differences in AMF symbiosis responses to anthropogenic activity. The changes in AMF species due to anthropogenic regimes suggest that AMF are good indicators of soil quality changes, consistent with the findings of Verbruggen et al. (2012).

## 3.2 Principal component analysis (PCA)

The PCA results for *T. arceuthoides* indicated that the first and second axes explained 48.80% and 23.46% of total variance, respectively (Fig. 2 [Figure 2: see original paper]). Furthermore, Monte Carlo permutation tests on redundancy analysis (RDA) showed that soil sand, silt, moisture, clay, and phosphorus (P) are the most important factors affecting AMF diversity in *T. arceuthoides* (Table 4 ). The PCA result for *P. euphratica* revealed that the first and second axes explained 69.27% and 19.71% of total variance, respectively (Fig. 3 [Figure 3: see original paper]). Monte Carlo permutation tests on RDA indicated that the most significant factors affecting AMF diversity in *P. euphratica* stands are soil moisture, clay, and pH (Table 5 ). Our results are consistent with Deepika and Kothamasi (2015). These results imply that soil property changes caused by anthropogenic activity can result in significant AMF changes, and that sand mining disturbance affects AMF both directly and indirectly by altering soil conditions or host plant root status.

**Fig. 2** Principal component analysis of AMF diversity indices and soil physical-chemical properties in *T. arceuthoides* rhizosphere. SD, spore density; D, Simpson' s index of dominance; Ric, richness; Dm, Margalef' s richness index; Col, colonization; H , Shannon-Weiner index of diversity; N, nitrogen; OC, organic carbon; K, potassium; P, phosphorus; Moi, moisture; Eve, evenness.

**Fig. 3** Principal component analysis of AMF diversity indices and soil physical-chemical properties in *P. euphratica* rhizosphere. SD, spore density; D, Simpson' s index of dominance; Ric, richness; Dm, Margalef' s richness; Col, colonization; H , Shannon-Weiner index of diversity; E, evenness; N, nitrogen; OC, organic carbon; K, potassium; P, phosphorus; Moi, moisture.

## 3.3 Spore density (SD) and root colonization rate

The SD associated with the *T. arceuthoides* rhizosphere in Distance II, where sand mining and truck movements occurred, had the highest significant value (Table 6 ). Nevertheless, no significant difference was observed in SD values for *P. euphratica* (Table 7 ). The higher SD in Distance II may be explained by soil compaction that reduced root growth (Wallace, 1987; Trejo et al., 2016). In this study, *P. euphratica* and *T. arceuthoides* showed different SD and root colonization rates, reflecting the fact that different plant species respond differently to these factors (Li et al., 2007).

Furthermore, a high level of symbiosis was observed between AMF and *P. euphratica*, consistent with the findings of Wang et al. (2010). However, our results are not in accordance with Yang et al. (2013), who reported significantly lower SD in *P. euphratica* rhizospheres. The reason for the significantly higher SD in the current study may be attributable to higher AMF diversity compared to Yang et al. (2013). The minimum and maximum colonization rates for *T. arceuthoides* and *P. euphratica* were observed at Distances II and III, respectively (Table 6), providing clear evidence of the negative effect of sand mining and truck movement on root colonization rates at Distance II. Changes in SD and root colonization may be induced by soil physical-chemical changes caused by sand mining activity. This study provides a much-improved understanding of the impact of sand mining on AMF diversity, colonization, and SD.

An increasing trend in root colonization rate with increasing distance from the river was observed in this study, probably indicating that soil conditions improved for AMF symbiosis farther from the river. The high level of root colonization in this study is in agreement with the findings of Wang et al. (2010).

## 3.4 Diversity indices

The *T. arceuthoides* stand had the lowest AMF diversity at Distance II (Table 6), while the *P. euphratica* stand had the highest AMF diversity at Distance III (Table 7). Although Shannon-Weiner indices of diversity (H ) and Simpson' s index of dominance (D) displayed no significant differences among the three designated distances, the lowest significant D was observed in Distance II (Tables 6 and 7). The reason for this is that D is affected by species richness (Magurran, 2004), and higher species richness results in higher D values (Mahecha-Vásquez et al., 2017). As the lowest species richness was observed in Distance II, the lowest D was also observed in this zone (Stürmer and Siqueira, 2011). These results suggest that the impact of sand mining was severe enough to negatively affect AMF diversity in riparian forests. Sand mining resulted in changes in

AMF diversity and composition, and this knowledge is crucial for understanding mycorrhizal function in these ecosystems (Soka and Ritchie, 2014a, b).

In addition, distance from the local river also affected AMF diversity, with Distance III (the farthest from the river) hosting the highest AMF diversity. This may be due to the least negative effects of natural phenomena among the three distances.

## 3.5 Correlation between soil physical-chemical properties and AMF diversity indices

Pearson' s correlation between soil factors and AMF diversity indices at the *P. euphratica* stand revealed that AMF richness had a positive correlation with soil P and a negative correlation with pH. However, evenness had a significant negative correlation with richness, Simpson' s index of dominance (D), and Margalef' s richness index (Dm). Furthermore, soil moisture had significant negative correlations with root colonization and AMF richness, while having a negative significant correlation with spore density (SD) (Table 8 ). For the *T. arceuthoides* stand, AMF richness had a significantly positive correlation with root colonization (RC). Soil phosphorus (P) had a significantly positive correlation with D and a significantly negative correlation with Shannon–Weiner indices of diversity (H ). Margalef' s richness index (Dm) was significantly correlated with root colonization. Also, soil moisture had a significantly positive correlation with root colonization (RC) and a significantly negative correlation with SD (Table 9 ). These results indicate the importance of better soil conditions for higher AMF diversity (Mirzaei and Moradi, 2017). In this study, Distance III with better soil conditions and less anthropogenic activity had higher AMF diversity.

Several factors have been reported to affect AMF diversity, including environmental (Moradi et al., 2015) and soil conditions (Yang et al., 2011). In the present study, sand mining and truck movement were documented to result in AMF variations. Soil P and pH also affected AMF diversity, consistent with other studies (Treseder, 2004; Yoshimura et al., 2013). Similar to previous studies highlighting the use of AMF species as soil bio-indicators (Fokom et al., 2012; Vasconcellos et al., 2016), the present study has demonstrated that AMF diversity could be a key factor for understanding soil quality. Furthermore, it should be reiterated that because fungi are able to adapt to local habitats and are important for biodiversity conservation, AMF species should be carefully identified for each specific region (Symanczik et al., 2014).

Unlike other studies reporting that soil compaction did not affect AMF (Miransari, 2013; Thorne et al., 2013), the present study demonstrated that sand mining and truck movement negatively affected AMF diversity and SD by reducing fine roots (Waltert et al., 2002). As shown earlier, sand mining caused approximately 24.4% and 10.8% reductions in SD in *P. euphratica* and *T. arceuthoides*, respectively. These results are in accordance with Jasper et al. (1991).

## 4 Conclusions

The present study found that disturbances from sand mining and truck movements in riparian forests negatively affected AMF diversity and root colonization rates. Although the study site was only 400 meters long, the results displayed high spatial variation in AMF diversity, suggesting that riparian forests are rather sensitive to anthropogenic activities. Furthermore, this study shows that soil moisture, clay, silt, sand, and P are among the soil factors that can significantly change AMF communities in the rhizospheres of both *P. euphratica* and *T. arceuthoides*. In addition, distance from the local riverbank also contributed to variation in AMF diversity.

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*Note: Figure translations are in progress. See original paper for figures.*