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Colonization and community structure of root-associated microorganisms of *Sabina vulgaris* with soil depth in a semiarid desert ecosystem with shallow groundwater

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Abstract Arbuscular mycorrhizal fungi (AMF) have been observed in deep soil layers in arid lands. However, change in AMF community structure with soil depth and vertical distributions of the other root-associated microorganisms are unclear. Here, we examined colonization by AMF and dark septate fungi (DSF), as well as the community structure of AMF and endophytic fungi (EF) and endophytic bacteria (EB) in association with soil depth in a semiarid desert with shallow groundwater. Roots of *Sabina vulgaris* and soils were collected from surface to groundwater level at 20-cm intervals. Soil chemistry (water content, total N, and available P) and colonization of AMF and DSF were measured. Community structures of AMF, EF, and EB were examined by terminal restriction fragment length polymorphism analysis. AMF colonization decreased with soil depth, although it was mostly higher than 50%. Number of AMF phylotypes decreased with soil depth, but more than five phylotypes were observed at depths up to 100 cm. Number of AMF phylotypes had a significant and positive relationship with soil moisture level within 0–15% of soil water content. DSF colonization was high but limited to soil surface. Number of phylotypes of EF and EB were diverse even in deep soil layers, and the community composition was associated with the colonization and community composition of AMF. This study indicates that AMF species richness in roots decreases but is maintained in deep soil layers in semiarid regions, and change in AMF colonization and community structure associates with community structure of the other root-associated microorganisms.

Keywords Arbuscular mycorrhizal fungi · Endophytic bacteria · Endophytic fungi · Mu-us desert (China) · Semiarid land · Vertical distribution

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Introduction

Arid and semiarid lands are severe environments for plants. In dry ecosystems, plants extend their roots deeper to access water. In the tundra, boreal forests, and temperate grasslands, root systems are shallow, and up to 90% of the roots mass is confined to the top 30 cm of soil (Jackson et al. 1996; Robinson et al. 2003). However, in cold and warm desert environments, this proportion is reduced to about

50% (Jackson et al. 1996; Robinson et al. 2003). These patterns reflect interactions between temperature and water availability and are thought to enhance stress tolerance (Robinson et al. 2003). Another mechanism that plants use to adapt to stressful environments is symbiosis with root microorganisms. Mycorrhizal fungi are major mutualistic microorganisms that enhance nutrient uptake, plant growth, and stress tolerance to drought and salt (Smith and Read 2008) and play important roles in plant growth and survival even in arid and semiarid regions.

Generally, colonization by arbuscular mycorrhizal fungi (AMF) decreases with increased soil depth (Jakobsen and Nielsen 1983; Rillig and Field 2003). However, in arid land, Virginia et al. (1986) observed colonization by AMF even at depths of 4.5–4.8 m, indicating the importance of mycorrhizal study in deep soil layers. Oehl et al. (2005) reported high spore diversity in deep soil layers, but studies on AMF community structure have largely been restricted to the main rooting zone (10–30 cm). In addition, there are only a few studies examining AMF community in root in deep soil layers Dalpé et al. 2000; Oehl et al. 2005).

In addition to AMF, dark septate fungi (DSF), endophytic fungi (EF), and endophytic bacteria (EB) were often observed in plant roots in arid and semiarid regions (Barrow 2003; Taniguchi unpublished). Virginia et al. (1986) reported that the number of root nodules by nitrogen-fixing bacteria was highest at depths below 2.5 m with a maximum near 5 m, indicating that root-associated microorganisms might exist in deep soil layers. However, few studies have examined these endophytic microorganisms with soil depth, and the relationships of the root-associated microorganisms with host plant or AMF in deep soil layer are still not clear.

Sabina vulgaris Ant is an evergreen coniferous shrub. This species dominates in the Mu-us desert of Inner Mongolia, China, and uses relatively deep soil water as well as groundwater (Ohte et al. 2003), suggesting that *S. vulgaris* has roots around the groundwater level. AMF have been observed in deep soil layers in arid lands (Virginia et al. 1986), but it has also been reported that AMF colonization is negatively influenced by groundwater level in wetlands with annual precipitation exceeding 1,000 mm (Miller 2000). Additionally, the vertical distribution of AMF is unclear in arid or semiarid ecosystems with shallow groundwater.

In this study, we examined the effect of soil depth on the colonization and community structure of AMF, EF, and EB in a semiarid ecosystem. The relationships between soil chemistry (water content, total N, and available P) and colonization or community structure of root-associated microorganisms were also analyzed. In addition, we analyzed the relationships between each root-associated microorganism. Our hypotheses were that (1) diversity of AMF in roots decreases with soil depth as with the colonization, but the diversity is still high in deep soil layers, (2) change in water content with soil depth affects the colonization and community structure of AMF and the other root-associated microorganisms in semiarid regions, and (3) change in the colonization or community structure of AMF relates to the colonization and community structure of the other root-associated microorganisms.

Materials and methods

Study site

Our study site was located in the Mu-Us desert, in the southern part of the Ordos plateau in Inner Mongolia, China. A detailed site description was provided by Ohte et al. (2003). Mean annual precipitation is 345 mm, and the mean annual temperature is 6.5ºC (Kobayashi et al. 1995). Most of the precipitation falls from July to September. The soil is mostly sandy and is easily transported by wind if ground cover is removed. Soil pH at a depth of 0–5 cm is approximately 8.6 (Hirobe et al. 2001). Yoshikawa et al. (1999) examined the groundwater table by digging holes and measuring the distances from ground to groundwater level. Groundwater depth in the interdune lowland is fairly shallow, ranging from about -1.5 to 0 m. This region is at the southern end of the cool-temperate steppe zone, and two shrub species, *Artemisia ordosica* Krasch. and *S. vulgaris*, dominate (Yamanaka et al. 1998). *S. vulgaris* has a prostrate life form, and patch size can be more than 15 m in canopy diameter.

Collection of *S. vulgaris* roots

We selected three mature *S. vulgaris* trees, which existed separately from other individuals. The three trees (S1, S2, and S3) were more than 30 m from one another. Tree heights were 1.5, 1.2, and 0.9 m, and radii were 9.0, 5.1, and 10.8 m for S1, S2, and S3, respectively. In the beginning of September 2008, we sampled the roots from each tree at three locations: 40 cm from the center of the main stem (CS), 150 cm inside from the edge of the prostrate branch (IP), and 150 cm outside from the edge of the prostrate branch (OP; Fig. S1). Soil samples containing roots were collected at each location as soil blocks (15×15×20 cm) or by using an auger (diameter=7 cm) from the surface to groundwater level at 20-cm depth intervals. In total, 27, 18, and 21 soil samples were

collected from S1, S2, and S3, respectively. The groundwater levels of S1, S2, and S3 were 160–180, 100–120, and 120–140 cm, respectively. Soil samples were used to measure soil chemistry. Roots were fixed in 70% ethanol, and the subsamples for molecular analysis were stored at -20ºC.

Colonization by AMF and DSF in *Sabina* roots

Roots from each sample were cut into 2.0- to 5.0-cm lengths, and 20–30 cm of *Sabina* roots was used for clearing and staining. The roots were cleared in 10% KOH at 121ºC for 20 min and diluted alkaline peroxide solution for 25 min at room temperature, then put in 2% HCl for 5 min, and stained in 0.05% trypan blue. Root sections (1.0 cm; total of 15–20 cm) were mounted on slides and observed under a light microscope (200–400×). Colonization rate was determined by the intersection method (Brundrett et al. 1996). A total of 100 intersections per sample was observed, and the colonization of AMF and DSF was determined based on the number of intersections with arbuscule and pigmented fungal structures, respectively. In this study, we considered dark hyphae and microsclerotia in roots as DSF.

Soil chemistry

Approximately 10.0 g of soil was used for the measurement of soil water content after drying at 70ºC for 24 h. Air-dried soil samples were sieved (<2 mm) and used for analyses of total carbon (C), nitrogen (N), and available phosphorus (P). About 1.0 g of each soil sample was dried at 60ºC for 3 days and ground with a mortar and pestle before analyzing total C and N using a CN coder (Macro Corder JM1000CN, J-Science Lab, Kyoto Japan). To determine available P, a 1.25 g soil sample was extracted with 25 ml of 0.5 M $NaHCO_3$, adjusted to pH 8.5, and the P concentration in the extracts was determined colorimetrically using a molybdate–ascorbic acid method (Olsen and Sommers 1982).

DNA extraction from roots

Roots were washed in an ultrasonicator and rinsed with 70% ethanol three times with vortexing. The roots were cut into 1.0 cm pieces, and 20–30 cm of roots were placed into 2.0 ml tubes. These roots were ground with a cell disruptor (Precellys 24, Bertin Technologies, Aix en Provence, France). The tubes with ground roots were subsequently filled with 700 μl of AP1 buffer from the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and 8 μl of 2-mercaptoethanol. Then deoxyribonucleic acid (DNA) was extracted following the manufacturer's instructions. After the DNA extraction, polyethylene glycol (PEG) precipitation was conducted with PEG 8000 solution [13% (w/v) PEG in 1.6 M NaCl]. The resultant DNA pellet was rinsed with 70% ethanol and dried. Each dried DNA pellet was dissolved in 100 μl of AE buffer from the DNeasy Plant Mini Kit.

PCR and TRFLP analysis

AMF specific primers, fungal specific primers, and bacterial specific primers were used for the polymerase chain reaction (PCR) amplification. The large subunit recombinant ribonucleic acid (rRNA) gene of AMF was amplified with the primer pair of LR1 (van Tuinen et al. 1998) and FLR2 (Trouvelot et al. 1999) and a nested reaction with the AMF-specific primer pair FLR3 with a 5′ 6-FAM fluorescent label and non-labeled FLR4 (Gollotte et al. 2004). The internal transcribed spacer (ITS) region of EF was amplified with primers of ITS1F (Gardes and Bruns 1993) with a 5′ 6-FAM fluorescent label and a non-labeled ITS4 (White et al. 1990). The 16S ribosomal RNA gene of EB was amplified with 8f (Edwards et al. 1989) with a 5′ 6-FAM fluorescent label and non-labeled 1492r (Wilson et al. 1990). The PCR mix for AMF consisted of 1 μl (first PCR) or 0.5 μl (second PCR) of template DNA and 11.5 μl of PCR cocktail (AmpliTaq Gold 360, Applied Biosystems, Foster City, CA, USA) containing 0.3 μM of each primer and 0.5 μl of enhancer. The PCR mix for EF and EB consisted of 1 μl of template DNA (for EF) or 1 μl of 1/10 diluted template DNA (for EB) and 11.5 μl of PCR cocktail (AmpliTaq Gold 360) containing 0.5 μM of each primer and 0.5 μl of enhancer. The AMF thermal profile was as follows: 10 min at 95ºC for initial denaturation, 25 cycles (first PCR) or 30 cycles (second PCR) of 30 s at 95ºC for denaturation, 30 s of annealing at 58ºC, and 1 min at 72ºC for extension, with a 7 min final extension at 72ºC. The thermal profile for EF and EB was as follows: 10 min at 95ºC for initial denaturation, 35 cycles of 30 s at 95ºC for denaturation, 30 s of annealing at 55ºC (for EF) or 50ºC (for EB), and 1 min at 72ºC for extension, with a 7 min final extension at 72ºC.

All PCR products from each *Sabina* root were inspected with 0.7% agarose gel electrophoresis prior to treatment with restriction enzymes. PCR products were digested with *Taq*I or *Nde*II (Nippon Gene, Toyama, Japan) for AMF, *Alu*I or *Hin*fI (Nippon Gene) for EF, and *Msp*I or *Rsa*I (Nippon Gene) for EB. A 0.5 μl aliquot of the digested products was mixed with 9.0 μl of Hi-Di formamide and 0.5 μl of GeneScan 1200 LIZ (Applied Biosystems). After denaturing at 95ºC for 3 min, a terminal restriction fragment length polymorphism (TRFLP) analysis was conducted on an automated sequencer, ABI PRISM 3130 genetic analyzer (Applied Biosystems). Terminal restriction fragments (TRFs) generated by the sequencer were analyzed with GeneMapper 3.7 (Applied Biosystems). The

fragments were detected by peak height and the presence or absence of each TRF fragment was used for following analysis. Fragments smaller than 25 base pairs were removed from further analyses.

Cloning and sequencing analysis for TRF reference data of AMF

The large subunit rRNA gene of AMF was amplified with nine samples, including the TRFs of AMF that occurred frequently. The PCR products were divided into three groups and purified with the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA). Then the PCR products in the solution were cloned using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA). More than 30 colonies were picked from each group, and the transformed PCR products were amplified with non-labeled M13F and M13R primers and AmpliTaq Gold 360 (Applied Biosystems). The PCR products were digested with either *Taq*I or *Nde*II (Nippon Gene), and electrophoresis was conducted with the digested products on 1.5% agarose gels. The DNA of each RFLP type was amplified with labeled primers, and TRFLP was performed as described above. DNA coinciding with the TRFs was re-amplified and sequenced using the M13F primer. Sequences were analyzed using a 1310 Genetic Analyzer (Applied Biosystems). Sequences were compared against those in the public databases by BLASTN searches (http://blast.ddbj.nig.ac.jp/top-j.html). A phylogenetic analysis was conducted using the AMF sequences from this study and those of related species deposited in the DNA Data Bank of Japan (DDBJ), European Molecular Biology Laboratory (EMBL) Bank, and GenBank. Multiple alignments of the nucleotide sequences were generated using the ClustalX 1.81 program (Thompson et al. 1997). The alignments were then inspected and corrected manually. Phylogenetic analyses were performed using the neighbor-joining method with the PAUP 4.0b10 program (Swofford 2003). A bootstrap test was performed with 1,000 replications.

Data analyses

Relationships between environmental factors and AMF or DSF colonization, number of AMF phylotypes, number of EF phylotypes, and number of EB phylotypes were examined using linear and quadratic regression analyses. Soil depth was categorized as the following: *1* 0–20 cm, *2* 20–40 cm, *3* 40–60 cm, *4* 60–80 cm, *5* 80–100 cm, *6* 100–120 cm, *7* 120–

Table 1 The relationships between colonization of arbuscular mycorrhizal fungi (AMF), or dark septate fungi (DSF), or number of phylotypes of AMF, endophytic fungi (EF), or endophytic bacteria (EB), and each environmental factor

Variable	Model type	*a*	*b*	*c*	AIC	r^2	*P* value
Colonization of AMF							
Depth	Linear	91.93	−5.58		376.5	0.45	<0.001
Water content	Quadratic	68.02	2.14	−0.17	332.4	0.48	<0.001
Total N	Quadratic	60.50	134.63	−223.49	371.5	0.18	0.01
Available P	Quadratic	79.03	−48.01	57.73	403.3	0.08	0.17
Colonization of DSF							
Depth	Quadratic	52.56	−17.86	1.52	428.9	0.29	<0.001
Water content	Quadratic	−2.72	3.34	−0.14	384.5	0.07	0.22
Total N	Quadratic	−2.92	177.90	−282.05	393.5	0.21	0.007
Available P	Linear	8.44	18.78		441.9	0.03	0.28
Number of phylotypes of AMF							
Depth	Linear	13.07	−1.07		203.0	0.46	<0.001
Water content	Linear	7.22	0.29		184.5	0.14	0.02
Total N	Quadratic	6.99	21.5	−26.83	206.5	0.24	0.004
Available P	Quadratic	8.67	2.66	−0.28	231.3	0.03	0.57
Number of phylotypes of EF							
Depth	Linear	17.00	−1.07		237.0	0.28	<0.001
Water content	Linear	12.37	0.15		219.1	0.02	0.42
Total N	Quadratic	10.13	40.35	−67.48	226.7	0.25	0.004
Available P	Linear	13.36	−0.03		252.0	<0.01	0.98
Number of phylotypes of EB							
Depth	Linear	8.37	−0.08		199.6	<0.01	0.67
Water content	Linear	6.79	0.19		169.8	0.09	0.06
Total N	Linear	7.72	1.82		187.0	0.02	0.38
Available P	Linear	7.61	1.70		198.6	0.03	0.28

Linear ($y=a+bx$) and quadratic ($y=a+bx+cx^2$) regression analyses were performed. Results with smaller AIC are shown

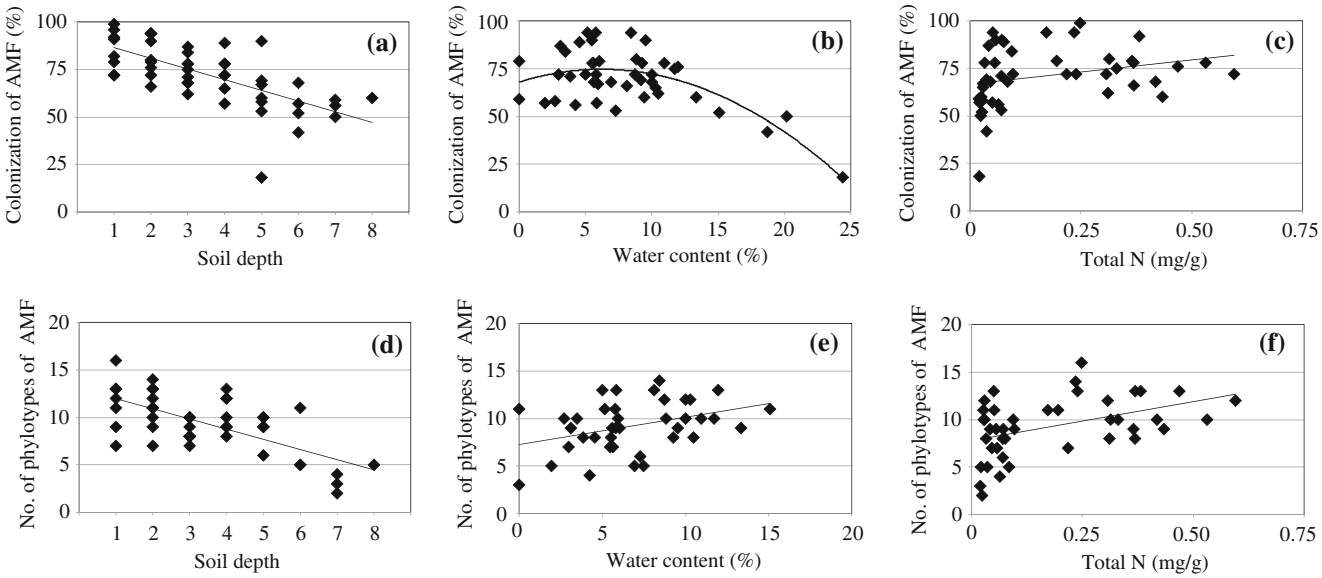

Fig. 1 The relationships between **a** colonization of arbuscular mycorrhizal fungi (AMF) and soil depth, **b** colonization of AMF and water content, **c** colonization of AMF and total N, **d** number of AMF phylotypes and soil depth, **e** number of AMF phylotypes and water content, **f** number of AMF phylotypes and total N. All linear and quadratic regressions were statistically significant (Table 1). Soil depth was categorized and regression analyses were performed (*1* 0–20 cm, *2* 20–40 cm, *3* 40–60 cm, *4* 60–80 cm, *5* 80–100 cm, *6* 100–120 cm, *7* 120–140 cm, *8* 140–160 cm)

140 cm, and *8* 140–160 cm. Water content in surface soil (0–20 cm) of S2 and S3 was removed from further analyses because it rained just before sampling S2 and S3, and surface soil water content was influenced by the rain. Additionally, the soil layers with high water content and total N in soil were also removed from the regression analysis as outliers. Different fits were compared using the Akaike information criteria (AIC) model selection technique (Burnham and Anderson 2002), and statistical models that minimized the AIC value are shown in Table 1.

Nonmetric multidimensional scaling (NMDS) with Euclidean distance was conducted on binary data generated from TRFLP for AMF, EF, and EB. Then Pearson's correlation analyses or Spearman's rank correlation analyses were conducted between the ordination score of the first and second axes of the NMDS ordination and environmental factors. To reveal the relationships between each microorganism, Pearson's correlation analyses were conducted using the data of colonization, number of phylotypes, and ordination scores of the first and second axes of each microorganism. All

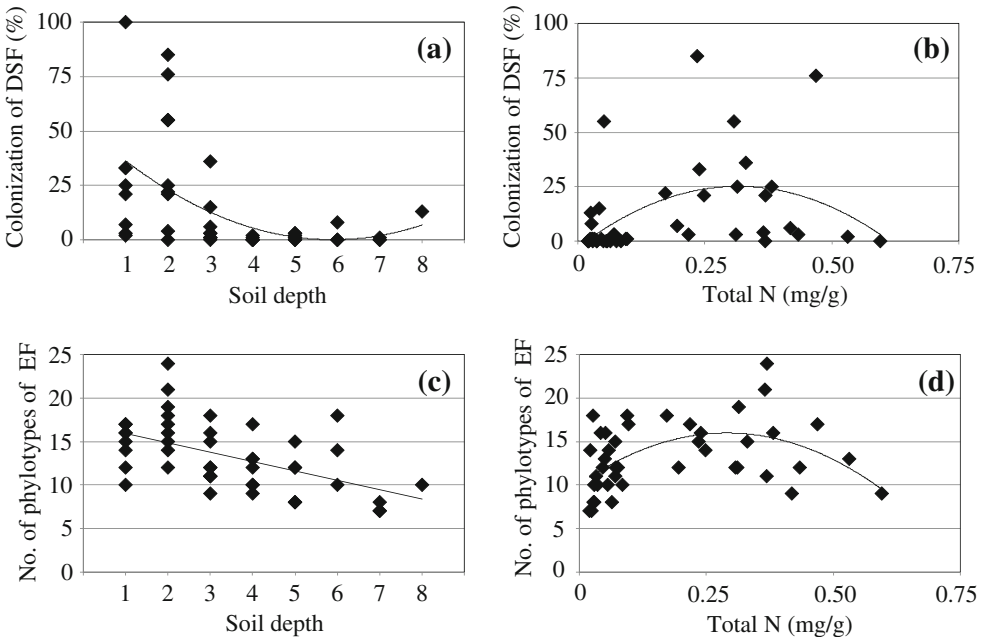

Fig. 2 The relationships between **a** colonization of dark septate fungi (DSF) and soil depth, **b** colonization of DSF and total N, **c** number of endophytic fungi (EF) phylotypes and soil depth, and **d** the number of EF phylotypes and total N. All linear and quadratic regressions were statistically significant (Table 1). Soil depth was categorized and regression analyses were performed (*1* 0–20 cm, *2* 20–40 cm, *3* 40–60 cm, *4* 60–80 cm, *5* 80–100 cm *6* 100–120 cm, *7* 120–140 cm, *8* 140–160 cm)

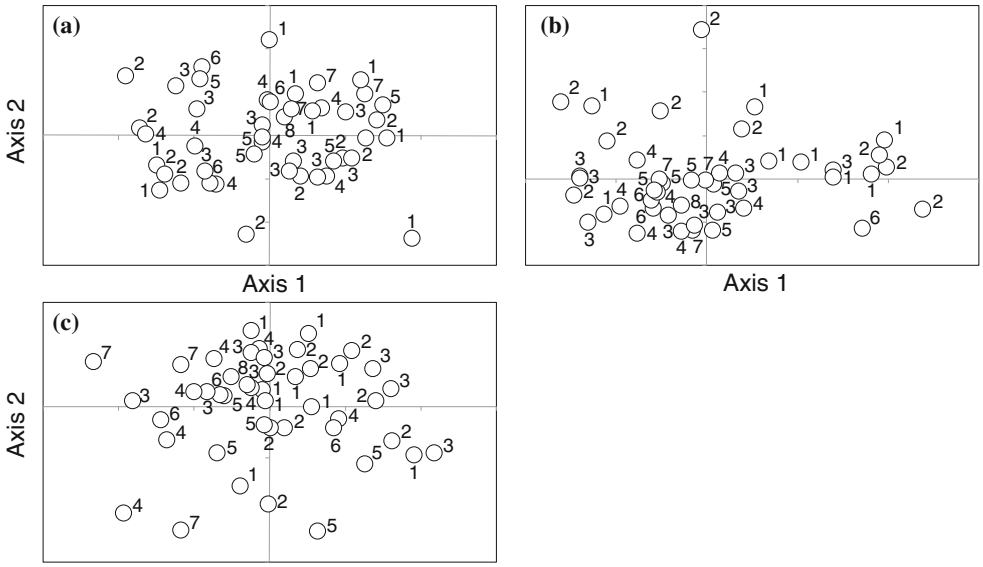

Fig. 3 NMDS plots of community composition for **a** arbuscular mycorrhizal fungi, **b** endophytic fungi, and **c** endophytic bacteria in *Sabina vulgaris* roots. Numbers represent soil depth (*1* 0–20 cm, *2* 20–40 cm, *3* 40–60 cm, *4* 60–80 cm, *5* 80–100 cm, *6* 100–120 cm, *7* 120–140 cm, *8* 140–160 cm)

of these data analyses, except for the AIC, were performed using SPSS ver. 16.0J (SPSS Japan, Tokyo, Japan). The AIC was computed manually using the following formula:

$$\mathrm{AIC} = n[\ln\{2\pi(\mathrm{RSS})/n\} + 1] + 2p$$

where RSS is the residual sum of squares between each model prediction and the observation, n is the number of observations, and p is number of total parameters in the model.

Results

Colonization and phylotype richness of AMF, EF, and EB

Colonization of *S. vulgaris* by AMF was very high and more than 70% in the surface soil layer (Fig. 1a). AMF colonization decreased with soil depth and had a significant and negative relationship with soil depth (Table 1, Fig. 1a; r^2=0.45, P<0.001). AMF colonization was also significantly associated with water content (r^2=0.48, P<0.001) and total N (r^2=0.18, P=0.01) in soil (Table 1, Fig. 1b, c). AMF colonization decreased when water content was high and increased linearly with the increase in soil total N.

In some surface soil layers, colonization of DSF was considerably high (Fig. 2a). DSF colonization had significant relationships with soil depth and total N in soil (r^2=0.29, P<0.001 for soil depth, r^2=0.21, P=0.007 for soil N; Table 1, Fig. 2a, b).

From the TRFLP analyses, we detected 47, 118, and 52 phylotypes for AMF, EF, and EB, respectively. The number of AMF phylotypes decreased with soil depth and had a negative relationship with soil depth (r^2= 0.46, P<0.001; Table 1, Fig. 1d). The number of AMF phylotypes also had significant relationships with water content (r^2=0.14, P=0.02) and total N (r^2=0.24, P= 0.004; Table 1, Fig. 1e, f). The number of AMF phylotypes increased slightly with the increase in water content when water content was within 0–15%. The number of AMF phylotypes also increased with the increase in total N. The number of EF phylotypes decreased with soil depth and had a significant negative relationship with soil depth (r^2=0.28, P<0.001; Table 1, Fig. 2c). Total N also had a significant association with the number of EF phylotypes (r^2=0.25, P=0.004; Table 1, Fig. 2d). The number of EB phylotypes was not

Table 2 Correlation coefficients between environmental factors and first or second axis ordination score for NMDS

	Correlation analyses	
	Axis 1	Axis 2
Arbuscular mycorrhizal fungi		
Depth	−0.02	0.22
Water content	−0.31	−0.17
Total N	−0.11	0.05
Available P	−0.05	0.20
Endophytic fungi		
Depth	−0.22	-0.55^{***}
Water content	0.44^{***}	−0.07
Total N	0.45^{***}	0.22
Available P	0.36^{*}	0.07
Endophytic bacteria		
Depth	-0.46^{***}	−0.21
Water content	0.07	-0.37^{**}
Total N	0.06	0.22
Available P	0.04	0.13

Pearson's correlation analyses were conducted for soil chemistry (*P< 0.05; **P<0.01; ***P<0.001). Spearman's rank correlation analyses were conducted for soil depth (*P<0.05; *P<0.01; ***P<0.001)

significantly associated with soil depth (r^2=0.004, P=0.67; Table 1). The relationship between the number of EB phylotypes and water content was not significant (r^2=0.09, P=0.06; Table 1).

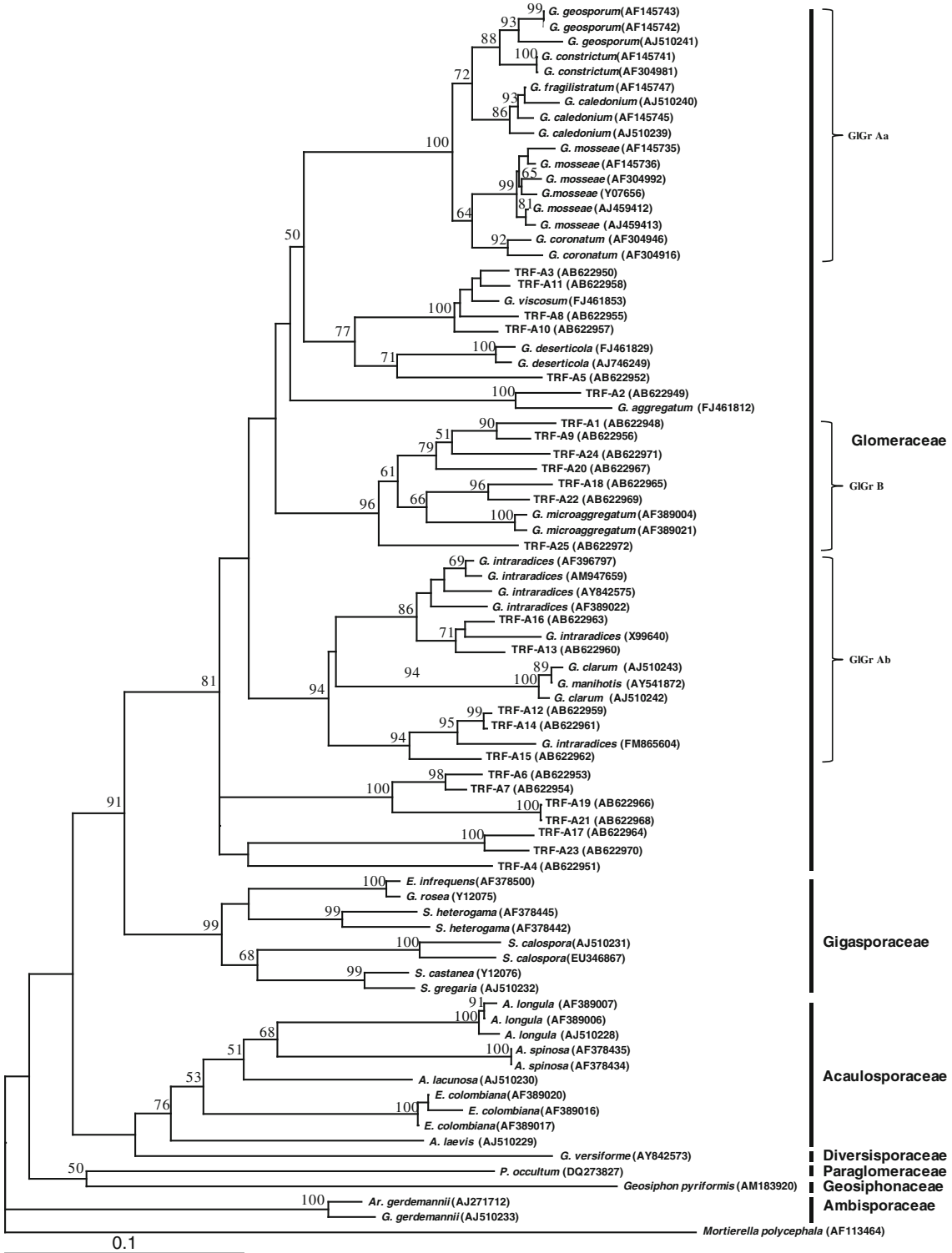

Fig. 4 A neighbor-joining tree was constructed using the PAUP 4.0b10 program, representing relationships between sequences in this study and sequences derived from public databases. Numbers at nodes indicate percent bootstrap support (1,000 replications). The works of Schüßler (2001) and Schwarzott et al. (2001) were referred to the taxonomic names and groupings

Table 3 Correlation coefficients between colonization of arbuscular mycorrhizal fungi (AMF) and dark septate fungi (DSF), and number of phylotypes of AMF, endophytic fungi (EF), and endophytic bacteria (EB)

	Colonization of DSF	No. of AMF Phylotypes	No. of EF Phylotypes	No. of EB Phylotypes
Colonization of AMF	0.40**	0.47***	0.31*	0.04
Colonization of DSF		0.55***	0.39**	−0.03
No. of AMF Phylotypes			0.45**	−0.07
No. of EF Phylotypes				0.22

Pearson's correlation analyses were conducted (*P<0.05; **P<0.01; ***P<0.001)

Community composition of AMF, EF, and EB

The NMDS analysis revealed that AMF community composition had no relationship with soil depth (Fig. 3a) and that the correlation coefficient between the NMDS analysis ordination score and depth was not significant (r=-0.02 and 0.22 in the first and second axes, respectively, P>0.05).

EF community composition differed between upper and deep soil layers. Samples from the upper soil layers are shown in the upper part of Fig. 3b. The second axis ordination score had a significant correlation with soil depth (r=-0.55, P<0.001; Table 2). The first axis ordination score was significantly correlated with water content (r=0.44, P<0.001), total N (r=0.45, P<0.001), and available P (r=0.36, P<0.05) in soil.

In the NMDS analysis of EB, samples from deeper soil layers were distributed in the second and third quadrants (Fig. 3c). The first axis ordination score was significantly correlated with soil depth (r=-0.46, P<0.001; Table 2), whereas the second axis score was significantly correlated with water content (r=-0.37, P<0.001).

We identified 26 TRF sequences from the cloning and sequencing analysis of AMF. All of the sequences belonged to Glomeromycota. The phylogenetic analysis revealed that all of these species belonged to the genus *Glomus* (Fig. 4).

Relationships between AMF, EF, and EB

AMF colonization was significantly correlated with DSF colonization (r=0.40, P<0.01; Table 3) and number of phylotypes of AMF (r=0.47, P<0.001) and EF (r=0.31, P<0.05). Number of AMF phylotypes was also correlated with DSF colonization (r=0.55, P<0.001) and number of EF phylotypes (r=0.45, P<0.01). DSF colonization was correlated with number of EF phylotypes (r=0.39, P<0.01). Ordination score of axis 2 of AMF was significantly correlated with axes 1 ordination score of EF (r=-0.37, P<0.05) and EB (r=-0.31, P<0.05; Table 4).

Discussion

Colonization, phylotype richness, and community composition of AMF

AMF colonization of *S. vulgaris* was very high, but decreased with soil depth. However, AMF colonization was almost maintained at more than 50% even at the depth of 160 cm. Zajicek et al. (1986) reported that AMF colonization declined with depth, but AMF colonization was observed at depths up to 210 cm. Our result agreed with the result of Zajicek et al. (1986).

Oehl et al. (2005) have reported that number of AMF spores decreased with increasing soil depth, but there was a large diversity of AMF species even in the deep soil layers (50–70 cm) in agroecosystems. In addition, Dalpé et al. (2000) examined the viable AMF propagules of *Faidherbia albida* in deep (1.5–34 m) and surface (0.15 m) soils and showed that at least one AMF species distributed at both depths in four harvesting sites located in semiarid and more humid areas. In our study, AMF phylotype richness in root decreased with soil depth, but more than five phylotypes

Table 4 Correlation coefficients between first or second axis ordination score for the NMDS of arbuscular mycorrhizal fungi (AMF), endophytic fungi (EF), and endophytic bacteria (EB)

	AMF-Axis 2	EF-Axis 1	EF-Axis 2	EB-Axis 1	EB-Axis 2
AMF-Axis 1	−0.002	−0.19	−0.12	−0.11	0.18
AMF-Axis 2		−0.37*	0.04	−0.31*	0.14
EF-Axis 1			0.02	−0.10	−0.07
EF-Axis 2				0.17	0.11

Pearson's correlation analyses were conducted (*P<0.05; **P<0.01; ***P<0.001)

were observed at depths up to 100 cm. It supports the result of Dalpé et al. (2000) and Oehl et al. (2005), suggesting that the propagules of some AMF species are viable in deep soil layers and the AMF species richness in roots is also maintained.

AMF colonization was significantly associated with water content in soil. AMF colonization clearly decreased when water content was high, indicating AMF colonization was decreased by groundwater. The number of AMF phylotypes increased with moisture level within 0–15% of soil water content. This increase in phylotype richness with an increase in water content agrees with the results of Beauchamp et al. (2006). They examined AMF community structure in a semiarid riparian ecosystem and showed that soil moisture had a significant and positive relationship with AMF species richness. In dry ecosystems, AMF richness may increase with increases in soil water. In our study, AMF phylotype richness also had a positive relationship with soil N. Alguacil et al. (2009) reported that AMF is more diverse after applying an adequate amount of an organic substance. Decrease in N source in soil with soil depth might be one of the reasons why AMF phylotype richness decreased with soil depth. In our study site, water content and nitrogen were limited and might affect AMF species richness. In addition, fine root biomass of *S. vulgaris* decreased with soil depth (Taniguchi unpublished). The reduction of root biomass might affect not only the decrease in AMF colonization but also phylotype richness with soil depth.

In this study, at least 55% (26/47 TRFs) of AMF species were thought to be *Glomus* species. Allen et al. (1995) reported that the majority of AMF spores detected in association with *Artemisia* in a semiarid region in the western United States were Glomaceae, which agreed with our results.

Colonization and community structure of DSF, EF, and EB in deep soil, and the relationships with AMF

DSF colonization was high but limited in surface soil. DSF was rarely observed below a depth of 80 cm. This distribution had a weak but significant relationship with total N. Menkis et al. (2004) isolated DSF from decayed coarse roots, and DSF reportedly produces several enzymes including polyphenol oxidases (Currah and Tsuneda 1993; Fernando and Currah 1995). The DSF species in our study may use organic substances in the soil and the DSF distribution might have a relationship with soil N. The number of EF phylotypes decreased with soil depth, but more than six phylotypes were detected even at the groundwater level depth. Our study showed that EF also exists in deep soil layers in a semiarid region.

A number of EB phylotypes were distributed in deep soil layers and did not change at groundwater level soil depth, although the species composition correlated with soil depth. The regression analysis showed that the number of EB phylotypes was not significantly related with soil water content. Therefore, EB might be more stable than AMF and EF in various environmental conditions.

Correlation analyses revealed that AMF colonization was associated with DSF colonization and the number of AMF and EF phylotypes. In addition, ordination score of AMF from NMDS analyses was significantly correlated with that of EF and EB. These results indicated that change in colonization and community structure of AMF could associate with the colonization and community structure of EF and EB.

In this study, we revealed that colonization and phylotype richness of AMF and EF decrease with soil depth at semiarid region with shallow groundwater. However, not only AMF but also EF and EB phylotype richness were high in deep soil layers, and the change in AMF colonization and community structure could associate with the colonization and community structure of EF and EB. For EF and EB, we did not examine the sequences and the functions were not clear. Further research on the function of AMF, EF, and EB and the relationship between AMF and the other root-associated microorganisms in deep soil layers will clarify the importance or role of the root-associated microorganisms in dry ecosystems.

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