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Soil environmental factors shape the rhizosphere arbuscular mycorrhizal fungal communities in South African indigenous legumes (Fabaceae)

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Abstract. *Alimi AA, Adeleke R, Moteetee A. 2021. Soil environmental factors shape the rhizosphere arbuscular mycorrhizal fungal communities in South African indigenous legumes (Fabaceae). Biodiversitas 22: 2466-2476.* The crucial role played by arbuscular mycorrhizal fungi (AMF) in the nutritional adaptation of plants to nutrient-deficient soils is well recognized. However, little is known about the diversity of AM fungal communities in the rhizosphere of indigenous legumes of South Africa. This study presents the first morphological characterization of the AM fungal diversity present in the rhizosphere of eleven indigenous legume species from two provinces in South Africa and correlates the diversity and community structure of AMF to soil physicochemical properties. Twenty AM fungal species belonging to ten genera were identified; *Glomus* and *Acaulospora* were the dominant genera, while *Acaulospora colombiana, A. mellea*, and *Claroideoglomus etunicatum* were the prevalent species. The AM fungal spore density, diversity, and community structure differed markedly among the legume species in both provinces. Correlation and canonical correspondence analyses revealed that the diversity and spatial structure of AM fungal communities were significantly influenced by soil properties. This study provides a theoretical insight into the future potential of using the dominant AM fungal species as inoculants for sustainable management of legumes and indicated that soil factors are important environmental determinants of AM fungal diversity and community structure.

Keywords: Arbuscular mycorrhizal fungi, community structure, diversity, indigenous legumes, soil factors

Abbreviations: AMF: Arbuscular mycorrhizal fungi, P: available phosphorus, K: available potassium, OC: organic carbon, NO_3^--N: nitrate, NH_4^+-N: ammonium, Cu: copper Mn: manganese, Zn: zinc BD: bulk density, SD: spore density, SR: species richness, IF: isolation frequency, RA: relative abundance, H: Shannon-Weiner diversity index, J: Pielou evenness index, CCA: canonical correspondence analysis

INTRODUCTION

Legumes (Fabaceae) constitute the second most important plant family after grasses (family Poaceae), contributing significantly to global food and nutritional security (Graham and Vance 2003). Additionally, legumes are considered essential service providers for natural and agroecosystems due to their ability to contribute to soil fertility through biological nitrogen fixation (Peoples and Craswell 1992; Cleveland et al. 1999). Legumes establish mutualistic relationships with arbuscular mycorrhizal fungi (AMF), which help them to meet their nutritional requirements for phosphorus (P) from soils with low P availability (Chalk et al. 2006) and promote their tolerance to several biotic and abiotic stresses (Smith et al. 2010). Such AM fungal-mediated supply of P is essential in driving the high P-demanding legume-rhizobia symbiosis, thus making legumes highly reliant on the arbuscular mycorrhizal association than other plant groups (Barea et al. 1987).

The AMF are ubiquitous in terrestrial ecosystems, where they form obligate symbiotic relationships with the roots of vast majority of land plants, including legumes (Brundrett and Tedersoo 2018). Nevertheless, it has been established that the diversity and structure of rhizosphere AM fungal communities vary in different plants, and that host plant species and soil characteristics are strong determinants of this differentiation (Eom et al. 2000; Johnson et al. 2004; Martínez-García et al. 2011; Santos-González et al. 2011; Liu et al. 2015).

South Africa is marked by acidic soils with very low nutrient concentrations, particularly phosphorus (Barnard and du Preez 2004; Hawkins et al. 2005). That notwithstanding, the country is characterized by a high diversity of indigenous legumes, which are widely distributed in different biomes (Trytsman et al. 2011). Under these soil nutrient-deficient conditions, it is envisaged that AMF are likely to play a vital role in the nutrition and adaptation of indigenous legumes to this environment. However, the diversity of indigenous AM fungal communities present in the rhizosphere of these

plants remains unexplored. Indeed, there is an urgent need for the establishment of conservation strategies as almost 30% of indigenous South African legumes are currently threatened due to several anthropogenic factors (Yahara et al. 2013).

One promising approach that is currently emphasized for the propagation and management of indigenous plant communities is the use of indigenous AMF from the soil (Hawley and Dames 2004; Fitzsimons and Miller 2010). Indigenous AMF enhances host plants' uptake of mineral nutrients and water from soils, improve soil aggregate stability through the release of glomalin into the soil, and are also well adapted to the biotic and abiotic stress conditions that characterize the local environments of their indigenous hosts (de Oliveira et al. 2017). The combination of these attributes can facilitate plant growth, performance, and survival in stressed environments, and hence may benefit sustainable management programs (Maltz and Treseder 2015). Consequently, the knowledge of the indigenous AM fungal diversity in the rhizosphere is a critical fundamental step towards the understanding of the functional roles of AMF in natural ecosystems. Such information would enable the identification and selection of efficient AM fungal species or species combinations that can be exploited as inoculants for the management of plants under both nursery and field conditions (Chen et al. 2018). Therefore, the aim of this study was firstly to assess the diversity of AM fungal communities present in the rhizosphere of eleven indigenous legumes from two South African provinces, using morphological method, and secondly to investigate the influence of soil properties on the diversity and spatial structure of AM fungal communities.

MATERIALS AND METHODS

Study area

The study was conducted in certain areas within the Gauteng and Mpumalanga Provinces (Figure 1). These provinces are located in the grassland biome of South Africa and have summer rainfall (Rutherford and Westfall 1986). Grasses dominate the vegetation of the study areas, but a variety of forbs and few trees are also present (Mucina et al. 2006; Lötter, 2014). There is a broad spectrum of soil types, including the red-yellow-grey latosol plinthic catena, black and red clays and solonetzic soils, freely drained latosols, and black clays (Rutherford and Westfall 1986). The Gauteng Province is situated on latitude 26.2708°S and longitude 28.1123°E, at an average altitude of 1,512 m above sea level. The average minimum and maximum temperature range from 10.2 °C to 24.8 °C, and annual precipitation averages 771 mm per year. Mpumalanga is positioned on latitude -29.8129°S and longitude 30.6364°E. The province has several distinctive physiographic regions; the Highveld, where the altitude ranges from 1,200-1,800 m above sea level in the west; the forested Drakensberg mountains rising to more than 2,300 m above sea level in the east; and the low-lying Lowveld in the northeast. Temperatures in Mpumalanga vary with altitude, from a mean of 10 °C in the Highveld and an average of 23 °C in the subtropical Lowveld. The annual rainfall increases from west to east, averaging 341-1933 mm.

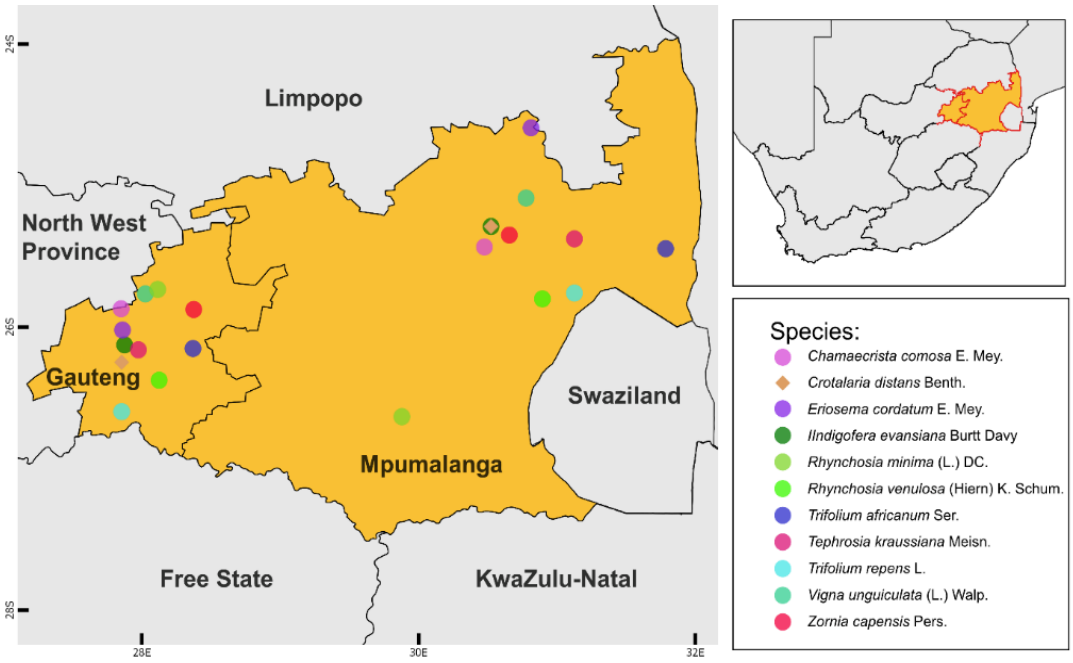

Figure 1. Map of South Africa showing the sampling points of legumes in each province

Procedures

Sampling

Sampling was conducted in February 2019. In each province, rhizosphere soils were aseptically collected from four individuals of each legume species at a depth of 0-20 cm using a soil auger (Eijkelkamp Soil and Water, EM Giesbeek, Netherlands). Additionally, cylindrical soil cores were collected per sample with a bulk density sampler (Eijkelkamp Soil and Water, EM Giesbeek, Netherlands), to calculate soil bulk density. Samples were kept in sterile Ziplock bags and transported to the laboratory. A subsample was stored at 4 °C prior to spore isolation, while the other fraction was air-dried, passed through a 2 mm sieve, and used for the determination of soil physicochemical properties.

Soil analysis

Selected physicochemical properties of soil were analyzed following standard protocols. Briefly, soil pH was determined from a 1:2.5 (w/v) soil suspension in 1N KCl. Available potassium (K) was assessed using the ammonium acetate method of Schollenberger and Simon (1945). Organic carbon (OC) was quantified using the Walkley-Black method (Walkley and Black 1934). Available phosphorus (P) was evaluated using the Bray 1 method (Bray and Kurtz 1945). Nitrate (NO_3^--N) was determined by the method of Sonneveld and Van den Ende (1971), while ammonium (NH_4^+-N) was estimated using the ammonia-selective electrode method (Banwart et al. 1972). Extractable micronutrients including copper (Cu), manganese (Mn), and Zinc (Zn) were obtained by acid digestion of soil (Jackson 1958). Bulk density (BD) was measured after drying soil samples for 48 hrs at 105 °C (ISO 2017) and soil particle size distribution was determined with the Bouyoucos hydrometer method (Bouyoucos 1962).

AM fungal spore isolation and identification

Spores were isolated from replicates of each legume species using the modified wet sieving and decanting method (Brundrett et al. 1996) and sucrose density gradient centrifugation method (Daniels and Skipper 1982). Summarily, 1000 ml of water was added to 100 g of air-dried soil. The mixture was stirred, and the resulting soil suspension was decanted through a series of 750, 250, 100, and 38 µm sieves arranged in decreasing order of mesh sizes. The materials retained in the 750 µm sieve was checked for large spores and sporocarps. Soil materials retained in the 250, 100, and 38 µm sieves were washed into centrifuge tubes. The tubes were centrifuged at 4,000 rpm for 5 mins. Thereafter, the supernatants were decanted, 50% (w/v) sucrose solution was added to the pellets, mixed gently, and centrifuged at 3,000 rpm for 1 min. The supernatants from each tube were then decanted into the 38 µm sieve and rinsed with distilled water several times to remove the sucrose solution. Recovered spores and sporocarps were washed into sterile Petri dishes and counted under the dissecting microscope at 50× magnification. Spore density (SD) was expressed as the total number of spores and sporocarps in 100 g of soil. For identification, spores were picked under the dissecting microscope with glass micropipette, mounted on slides with polyvinyl-lactic acid-glycerol (PVLG) or polyvinyl-lactic acid-glycerol mixed with Melzer's reagent (1:1 (v/v) and observed at 100-400× magnification. The spores were identified to the species level on the basis of shape, color, size, number of spore walls, and presence or absence of subtending hyphae using the taxonomic criteria described by Schenck and Perez (1990), together with the descriptions of reference cultures from the International Culture Collection of (vesicular) arbuscular mycorrhizal fungi INVAM (1997).

Arbuscular mycorrhizal fungal diversity and community studies

Species richness (SR), isolation frequency (IF), relative abundance (RA) were used to evaluate the structure of AM fungal communities in the rhizosphere of different legumes. These indices were computed as follows: Species richness (SR) was measured as the total number of identified AMF species per soil sample. Isolation frequency (IF) = (the number of samples in which a particular AMF species was observed/the total number of samples) × 100, where AMF species were classified into the following groups according to Zhang et al. (2004): dominant (IF > 50%), most common (IF 31%-50%), common (IF 10%-30%), and rare (IF < 10%). Relative abundance (RA) = (the number of spores of a particular genus)/total number of identified spores) × 100. The AM fungal species diversity in different legumes was assessed by the Shannon-Weiner diversity index, H (Shannon 1948) using the formula, $H = -\Sigma^k_i=1(Pi \ln Pi)$; Pi is the relative abundance of each identified AMF species per sample and is calculated by the formula $Pi = ni/N$, where ni represents the number of individuals of a species and N is the total number of individuals of all species. Species evenness was obtained by Pielou evenness index, J (Pielou 1966) using the formula, J = H/log(S), where H is the value obtained for Shannon-Weiner index and S is the species richness.

Statistical analysis

Statistical analyses were performed using R statistical software version 4.0.0. (R Core Team, 2020). Significant differences in SD, SR, H, J, and soil properties among plants within and between provinces were tested using Two-way ANOVA followed by Tukey HSD (Tukey Honest Significant Differences). The data were tested for normality and transformation was attempted if necessary, prior to analysis of variance. Significant means were compared using Least Significant Difference (LSD) at the 5% probability level ($P < 0.05$). Pearson correlation analysis was used to test for the relationship between AM fungal spore density, diversity indices, and soil properties. The canonical correspondence analysis (CCA) was employed to evaluate the influence of soil parameters on the spatial structuring of AMF communities. For the CCA analysis, soil and AMF community data were log-transformed using an automatic forward and backward Stepwise model in the vegan package. Test for significance of the environmental (constraining) variables were checked using a permutation test.

RESULTS AND DISCUSSION

Soil properties

Results of the rhizosphere soil physicochemical parameters of studied plants from the two provinces are shown in Table 1. Generally, the soils were acidic (pH < 7) and low in nutrients. The mean soil pH ranged from 4.38-5.87 in Gauteng and 4.48-5.84 in Mpumalanga. The textural classification of rhizosphere soils of legumes from Gauteng was largely sandy-loam, while in Mpumalanga, the rhizosphere soils were characterised by a broad spectrum of textural types including sandy-loam, sandy clay, sandy clay loam, loam, clay, loamy sand, and clay loam. Except for bulk density, all the measured soil parameters varied significantly ($P < 0.05$) among plants both within and between provinces.

Arbuscular mycorrhizal fungal spore density, diversity, and community structure

The mean AM fungal spore density isolated from the rhizosphere of studied plants ranged from 306-812 in Gauteng and 284-759 in Mpumalanga (Table 2). In both provinces, the highest and lowest average SD was observed in *Trifolium repens* and *Tephrosia kraussiana*, respectively. Spore density differed significantly ($P < 0.05$) among legume species within provinces, but no significant difference ($P > 0.05$) was observed in SD of the same legume species between provinces. Of all the diversity parameters (SR, H, and J) investigated, only SR and H differed significantly ($P < 0.05$) among legumes within provinces, but no significant difference ($P > 0.05$) was found between provinces (Table 2).

Twenty species of AMF were identified in the rhizosphere of legumes in both provinces (Figure 2). These species are distributed within ten genera. *Glomus* was the predominant genus (represented by seven species), *Acaulospora* (three species), *Rhizophagus* and *Septoglomus* (two species each), and *Claroideoglomus*, *Entrophospora*, *Funneliformis*, *Sieverdingia*, *Gigaspora*, and *Scutellospora* (one species each). Based on IF, *Acaulospora colombiana*, *Claroideoglomus etunicatum*, *A. mellea*, *Sieverdingia tortuosa*, *Glomus ambisporum*, and *Rhizophagus intraradices* were the dominant species in the rhizosphere of legumes in Gauteng, whereas *A. mellea*, *C. etunicatum*, *G. ambisporum*, *G. magnicaule*, *A. colombiana*, and *Septoglomus constrictum* were the dominant species in Mpumalanga. *Acaulospora colombiana*, *A. mellea*, and *C. etunicatum* were the most frequent species since they were present in all samples, with the highest IF of 100%. *Gigaspora decipiens* and *Scuttelospora biornata* were the rare AM fungal species in the rhizosphere of legumes in both provinces.

The AM fungal community structure and their relative abundance varied among legumes within and between provinces. In Gauteng, the genus *Glomus* was the most abundant in the rhizosphere of *C. distans*, *I. evansiana*, *T. africanum*, *T. repens*, and *Z. capensis*; *Acaulospora* exhibited higher abundance in *C. comosa*, *R. minima*, and *V. unguiculata*; *Septoglomus* was the most abundant genus in *E. cordatum and R. venulosa*, while the genus *Funneliformis* had higher abundance in *T. kraussiana*. In Mpumalanga, *Glomus* was the most abundant genus in *C. comosa*, *R. minima*, and *T. africanum*, whereas genus *Acaulospora* was the most abundant in *C. distans*, *E. cordatum*, *I. evansiana*, *R. venulosa*, *T. krausiana*, *T. repens*, *V. unguiculata*, and *Z. capensis*.

Influence of soil factors on AM fungal diversity and community structure

Pearson correlation analysis showed that SD, SR, H, and J were positively correlated with soil pH; SD was negatively correlated with NO_3 but positively correlated with Mn; SD and SR were negatively correlated with P but positively correlated with K. Also, a significant positive correlation was found between SR, H, J, and BD (Table 3). The step-wise model for the CCA triplot revealed that soil properties significantly ($P = 0.001$) influenced the structure of AM fungal communities (Figure 3). The first and second CCA axes showed that more than 50% of the total variability in AMF community structure was explained by soil variables. According to the CCA results, the distribution of *G. delhiense*, *G. tubaeforme*, *G. sinuosum*, *G. taiwanensis*, *G. decipiens*, and *S. biornata* were significantly associated with K and Zn, the distribution of *S. deserticola* was correlated with BD, the distribution of *A. colombiana* and *G. magnicule* were related with P and NH_4, whereas the distribution of *R. intraradices*, *G. rubiforme*, *R. irregularis*, *E. infrequens*, *F. geosporum*, and *S. constrictum* were least influenced by soil properties.

Discussion

Most members of the family Fabaceae form AM symbiosis, although mycorrhizal dependency varies among species (Trappe 1987). Similar to findings from previous studies (Wang et al. 2004; Wang and Jiang 2015; Toh et al. 2018), there was a significant difference in spore density among legume species in this study. This may be due to the composition and proportion of root exudates secreted by different host plants (Carrenho et al. 2002; Jones et al. 2004). In natural ecosystem, different legumes produce different types of flavonoids and strigolactones, which act as chemo-attractants for AMF to the roots, and ultimately influence AMF growth and spore production (D'arcy-Lameta 1988; Steinkellner et al. 2007). Other factors like differences in sporulation patterns among AM fungal taxa and environmental influences can equally contribute to variation in AMF spore populations (Anderson et al. 1984; Addy et al. 1994; Eom et al. 2000).

Table 1. Physico-chemical properties of soil in the rhizosphere of legumes in both provinces

Gauteng	Soil properties	*Chamaecrista comosa*	*Crotalaria distans*	*Eriosema cordatum*	*Indigofera evansiana*	*Rhynchosia minima*	*Rhynchosia venulosa*	*Trifolium africanum*	*Tephrosia kraussiana*	*Trifolium repens*	*Vigna unguiculata*	*Zornia capensis*
	pH (KCl)	5.40 ± 0.09^{ab}	5.48 ± 0.11^{ab}	4.38 ± 0.19^{b}	4.85 ± 0.34^{ab}	4.95 ± 0.29^{ab}	5.35 ± 0.12^{ab}	5.38 ± 0.11^{ab}	5.41 ± 0.17^{ab}	5.58 ± 0.12^{ab}	5.87 ± 0.58^{a}	5.52 ± 0.34^{ab}
	NO_3 (mg kg^{-1})	8.26 ± 0.05^{c}	8.68 ± 0.10^{c}	16.10 ± 0.12^{b}	6.45 ± 0.12^{d}	0.29 ± 0.03^{h}	3.77 ± 0.12^{f}	5.14 ± 0.07^{e}	28.40 ± 0.42^{a}	8.51 ± 0.09^{c}	8.48 ± 0.26^{c}	1.46 ± 0.06^{g}
	NH_4 (mg kg^{-1})	1.35 ± 0.02^{f}	1.96 ± 0.02^{d}	5.79 ± 0.11^{a}	1.16 ± 0.02^{fg}	0.96 ± 0.02^{g}	3.61 ± 0.09^{b}	1.73 ± 0.01^{e}	2.65 ± 0.02^{c}	2.53 ± 0.03^{c}	2.18 ± 0.01^{d}	3.64 ± 0.01^{b}
	P (mg kg^{-1})	1.14 ± 0.03^{gh}	3.33 ± 0.03^{e}	4.77 ± 0.02^{d}	2.46 ± 0.06^{f}	1.24 ± 0.03^{gh}	7.12 ± 0.02^{b}	1.53 ± 0.24^{g}	5.44 ± 0.04^{c}	1.02 ± 0.02^{h}	9.30 ± 0.14^{a}	2.07 ± 0.01^{f}
	Organic C (%)	0.96 ± 0.02^{h}	1.00 ± 0.01^{h}	3.26 ± 0.01^{c}	1.50 ± 0.01^{g}	2.27 ± 0.01^{e}	2.66 ± 0.01^{d}	3.45 ± 0.01^{b}	0.73 ± 0.01^{i}	4.04 ± 0.04^{a}	0.65 ± 0.02^{j}	2.00 ± 0.01^{f}
	K (mg kg^{-1})	94.40 ± 0.25^{h}	130.00 ± 0.02^{f}	175.00 ± 0.41^{d}	224.00 ± 3.21^{c}	188.00 ± 0.26^{d}	233.00 ± 0.13^{c}	282.00 ± 7.24^{b}	155.00 ± 5.77^{e}	394.00 ± 3.25^{a}	113.00 ± 0.12^{g}	153.00 ± 0.05^{e}
	Cu (mg kg^{-1})	3.37 ± 0.01^{a}	0.97 ± 0.01^{g}	1.18 ± 0.04^{f}	0.25 ± 0.02^{i}	1.75 ± 0.02^{e}	0.77 ± 0.03^{h}	1.85 ± 0.01^{d}	2.06 ± 0.02^{c}	2.26 ± 0.02^{b}	0.81 ± 0.02^{h}	1.15 ± 0.02^{f}
	Mn (mg kg^{-1})	84.40 ± 0.25^{a}	40.10 ± 0.07^{e}	35.60 ± 0.63^{h}	11.40 ± 0.03^{j}	46.30 ± 0.35^{c}	36.50 ± 0.30^{gh}	42.30 ± 0.06^{d}	37.80 ± 0.10^{f}	52.50 ± 0.28^{b}	27.20 ± 0.05^{i}	37.80 ± 0.07^{fg}
	Zn (mg kg^{-1})	4.72 ± 0.03^{e}	3.93 ± 0.02^{f}	8.58 ± 0.02^{c}	0.76 ± 0.01^{i}	1.95 ± 0.02^{h}	12.30 ± 0.05^{b}	4.05 ± 0.02^{f}	7.44 ± 0.03^{d}	12.00 ± 0.03^{b}	3.45 ± 0.02^{g}	14.50 ± 0.26^{a}
	BD (gcm^{-3})	1.45 ± 0.05^{ns}	1.55 ± 0.04^{ns}	1.53 ± 0.06^{ns}	1.51 ± 0.02^{ns}	1.50 ± 0.03^{ns}	1.59 ± 0.09^{ns}	1.59 ± 0.08^{ns}	1.45 ± 0.12^{ns}	1.53 ± 0.10^{ns}	1.51 ± 0.01^{ns}	1.53 ± 0.07^{ns}
	Sand (%)	74.00 ± 3.06^{ac}	76.00 ± 3.00^{ac}	40.00 ± 3.61^{d}	73.00 ± 0.44^{ac}	78.00 ± 1.25^{a}	74.00 ± 1.53^{ac}	65.00 ± 2.52^{c}	38.00 ± 1.73^{d}	74.00 ± 1.15^{ac}	66.00 ± 1.73^{bc}	77.00 ± 1.83^{ab}
	Silt (%)	14.00 ± 1.15^{ab}	10.00 ± 1.15^{b}	12.00 ± 1.53^{ab}	15.00 ± 1.53^{ab}	8.00 ± 1.53^{b}	13.00 ± 1.53^{b}	20.00 ± 2.65^{a}	12.00 ± 2^{ab}	14.00 ± 2.08^{ab}	13.00 ± 2.00^{ab}	10.00 ± 1.53^{b}
	Clay (%)	12.00 ± 1.53^{b}	14.00 ± 1.53^{b}	48.00 ± 4.16^{a}	12.00 ± 1.15^{b}	14.00 ± 3.06^{b}	13.00 ± 1.15^{b}	15.00 ± 2.52^{b}	50.00 ± 2.89^{a}	12.00 ± 2.65^{b}	21.00 ± 3^{b}	13.00 ± 1.53^{b}
	Textural Class	SaLm	SaLm	Cl	SaLm	SaLm	SaLm	SaLm	SaCl	SaLm	SaClLm	SaLm
Mpumalanga	pH (KCl)	5.84 ± 0.20^{a}	5.61 ± 0.10^{ab}	5.39 ± 0.17^{ac}	5.35 ± 0.13^{ac}	5.39 ± 0.17^{ac}	4.64 ± 0.28^{bc}	5.63 ± 0.23^{ab}	4.61 ± 0.25^{bc}	4.61 ± 0.05^{bc}	4.48 ± 0.22^{c}	5.37 ± 0.36^{ac}
	NO_3 (mg kg^{-1})	4.31 ± 0.13^{g}	6.28 ± 0.16^{e}	11.10 ± 0.08^{c}	10.90 ± 0.05^{c}	5.37 ± 0.05^{f}	8.99 ± 0.16^{d}	3.22 ± 0.06^{h}	19.50 ± 0.28^{a}	3.68 ± 0.07^{gh}	13.90 ± 0.18^{b}	0.60 ± 0.13^{i}
	NH_4 (mg kg^{-1})	3.46 ± 0.01^{b}	2.60 ± 0.03^{c}	17.80 ± 0.13^{a}	3.47 ± 0.01^{b}	1.28 ± 0.01^{ef}	2.73 ± 0.10^{c}	1.50 ± 0.01^{de}	1.23 ± 0.01^{f}	1.42 ± 0.01^{df}	1.25 ± 0.02^{f}	1.67 ± 0.01^{d}
	P (mg kg^{-1})	3.06 ± 0.02^{f}	4.45 ± 0.02^{e}	2.02 ± 0.02^{g}	1.04 ± 0.03^{h}	5.21 ± 0.04^{d}	11.20 ± 0.03^{a}	2.33 ± 0.06^{g}	8.64 ± 0.12^{b}	2.14 ± 0.05^{g}	6.20 ± 0.18^{c}	3.04 ± 0.01^{f}
	Organic C (%)	1.55 ± 0.02^{e}	2.59 ± 0.03^{c}	5.34 ± 0.02^{a}	5.35 ± 0.02^{a}	1.54 ± 0.02^{e}	4.77 ± 0.01^{b}	1.89 ± 0.01^{d}	1.57 ± 0.01^{e}	1.00 ± 0.03^{g}	1.96 ± 0.05^{d}	1.36 ± 0.01^{f}
	K (mg kg^{-1})	131.00 ± 0.42^{e}	163.00 ± 0.10^{d}	233.00 ± 0.06^{ac}	247 ± 3.31^{ab}	135.00 ± 14.60^{e}	163.00 ± 0.05^{d}	223.00 ± 2.81^{bc}	218.00 ± 8.77^{c}	258.00 ± 3.43^{a}	103.00 ± 0.52^{f}	138.00 ± 0.06^{de}
	Cu (mg kg^{-1})	0.28 ± 0.01^{h}	1.77 ± 0.01^{e}	3.73 ± 0.03^{b}	1.06 ± 0.03^{g}	11.60 ± 0.03^{a}	3.18 ± 0.03^{c}	1.57 ± 0.01^{f}	0.21 ± 0.02^{h}	0.24 ± 0.02^{h}	2.44 ± 0.07^{d}	1.03 ± 0.04^{g}
	Mn (mg kg^{-1})	25.40 ± 0.03^{e}	53.00 ± 0.06^{c}	84.40 ± 0.12^{b}	131.00 ± 0.42^{a}	25.60 ± 0.03^{e}	29.40 ± 0.05^{d}	25.40 ± 0.06^{e}	1.08 ± 0.11^{i}	13.10 ± 0.06^{h}	16.10 ± 0.02^{g}	18.10 ± 0.09^{f}
	Zn (mg kg^{-1})	2.09 ± 0.04^{e}	6.11 ± 0.04^{c}	13.40 ± 0.05^{a}	6.15 ± 0.02^{bc}	1.34 ± 0.01^{g}	3.68 ± 0.02^{d}	6.26 ± 0.02^{b}	0.67 ± 0.01^{i}	1.07 ± 0.01^{h}	1.48 ± 0.01^{f}	1.46 ± 0.01^{fg}
	BD (gcm^{-3})	1.49 ± 0.12^{ns}	1.51 ± 0.03^{ns}	1.59 ± 0.04^{ns}	1.55 ± 0.02^{ns}	1.58 ± 0.06^{ns}	1.60 ± 0.03^{ns}	1.48 ± 0.05^{ns}	1.54 ± 0.07^{ns}	1.57 ± 0.09^{ns}	1.53 ± 0.06^{ns}	1.60 ± 0.08^{ns}
	Sand (%)	75.00 ± 0.84^{ab}	75.00 ± 1.73^{ab}	52.00 ± 1.73^{c}	68.00 ± 0.49^{b}	67.00 ± 2.30^{b}	36.00 ± 1.53^{d}	68.00 ± 3.32^{b}	50.00 ± 2.89^{c}	83.00 ± 2.31^{a}	34.00 ± 2.39^{d}	33.00 ± 1.33^{d}
	Silt (%)	11.00 ± 1.15^{cd}	13.00 ± 2.52^{cd}	10.00 ± 1.53^{cd}	14.00 ± 2.31^{cd}	10.00 ± 2.00^{cd}	42.00 ± 3.06^{a}	20.00 ± 4.62^{bc}	8.00 ± 1.53^{cd}	6.00 ± 1.15^{d}	27.00 ± 3.61^{b}	19.00 ± 2.00^{bc}
	Clay (%)	14.00 ± 2.00^{b}	12.00 ± 1.15^{b}	38.00 ± 3.00^{a}	18.00 ± 2.08^{b}	23.00 ± 1.73^{b}	22.00 ± 2.31^{b}	12.00 ± 2.00^{b}	42.00 ± 1.15^{a}	11.00 ± 1.53^{b}	39.00 ± 4.04^{a}	48.00 ± 4.16^{a}
	Textural Class	SaLm	SaLm	SaCl	SaLm	SaClLm	Lm	SaLm	Cl	LmSa	ClLm	Cl

Note: Values are given as means ± SEM. Means across rows and columns without a common superscript alphabet letters statistically differ ($P < 0.05$) according to two-way ANOVA and the TUKEY test. ns, not significant; NO_3, nitrate; NH_4, ammonia; P, available phosphorus; K, available potassium; Cu, copper; Mn, manganese; Zn, zinc; BD, Bulk density; Texture: SaLm, Sandy Loam; Cl, Clay; SaCl, Sandy Clay; SaClLm, Sandy Clay Loam; Lm, Loam; ClLm, Clay Loam; LmSa; Loamy Sand.

Table 2. Spore density and diversity indices of AMF in different legumes in both provinces

Provinces	Indices	*C. comosa*	*C. distans*	*E. cordatum*	*I. evansiana*	*R. minima*	*R. venulosa*	*T. africanum*	*T. kraussiana*	*T. repens*	*V. unguiculata*	*Z. capensis*
Gauteng	SD	541.00± 42.10^{a}	554.00± 43.30^{a}	384.00 ± 26.35^{ba}	519.00 ±25.20 ^{aa}	478.00 ± 35.30^{ba}	426.00 ± 34.90^{ba}	786.00 ± 29.24^{c}	306.00 ± 35.50^{d}	812.00 ± 45.60^{c}	433.00 ± 33.30^{ba}	569.00 ± 53.10^{a}
	SR	8.00 ± 1.31^{a}	9.00 ± 1.15^{a}	6.00 ± 1.35^{ac}	11.00 ± 1.73^{a}	5.00 ± 1.43^{ac}	8.00 ± 2.31^{a}	12.00 ± 1.05^{b}	4.00 ± 0.98^{ac}	18.00 ± 2.89^{b}	7.00 ± 1.55^{a}	8.00 ± 1.73^{a}
	H	1.82 ± 0.59^{a}	2.06 ± 0.54^{a}	1.70 ± 0.52^{a}	2.33 ± 0.30^{a}	1.57 ± 0.55^{ab}	1.99 ± 0.58^{a}	2.32 ± 0.20^{ac}	1.38 ± 0.54^{b}	2.72 ± 1.10^{c}	1.89 ± 0.57^{a}	2.06 ± 0.27^{a}
	J	0.87 ± 0.08^{ns}	0.94 ± 0.05^{ns}	0.95 ± 0.03^{ns}	0.97 ± 0.01^{ns}	0.98 ± 0.01^{ns}	0.98 ± 0.02^{ns}	0.93 ± 0.02^{ns}	0.99 ± 0.01^{ns}	0.94 ± 0.03^{ns}	0.97 ± 0.02^{ns}	0.99 ± 0.06^{ns}
Mpumalanga	SD	508.00± 33.84^{a}	581.00± 30.00^{a}	419.00 ± 24.00^{ba}	557.00 ± 28.30^{ba}	459.00 ± 23.38^{ba}	460.00 ± 23.60^{ba}	738.00 ± 37.80^{c}	284.00 ± 26.20^{d}	759.00 ± 34.30^{c}	389.00 ± 53.30^{ba}	514.00 ± 28.08^{a}
	SR	6.00 ± 1.53^{a}	6.00 ± 0.58^{a}	4.00 ± 0.76^{a}	7.00 ± 1.15^{a}	4.00 ± 0.53^{a}	8.00 ± 2.31^{a}	15.00 ± 1.27^{b}	5.00 ± 0.58^{a}	14.00 ± 2.31^{b}	9.00 ± 0.99^{a}	10.00 ± 2.08^{a}
	H	1.75 ± 0.44^{a}	1.76 ± 0.56^{a}	1.37 ± 0.57^{ab}	1.93 ± 0.58^{a}	1.31 ± 0.49^{ab}	2.02 ± 0.56^{a}	2.61 ± 0.18^{c}	1.54 ± 0.55^{ab}	2.50 ± 0.29^{c}	2.20 ± 0.44^{a}	2.20 ± 0.42^{a}
	J	0.98 ± 0.05^{ns}	0.98 ± 0.09^{ns}	0.99 ± 0.04^{ns}	0.99 ± 0.02^{ns}	0.95 ± 0.02^{ns}	0.97 ± 0.01^{ns}	0.96 ± 0.01^{ns}	0.96 ± 0.03^{ns}	0.95 ± 0.01^{ns}	0.96 ± 0.09^{ns}	0.95 ± 0.01^{ns}

Note: Values are means ± SEM. Means across rows and columns without a common superscript alphabet letters statistically differ ($P < 0.05$) based on two-way ANOVA and the TUKEY test. ns, not significant; SR, H, and J are the species richness, Shannon-Weiner diversity index, and Pielou evenness index, respectively.

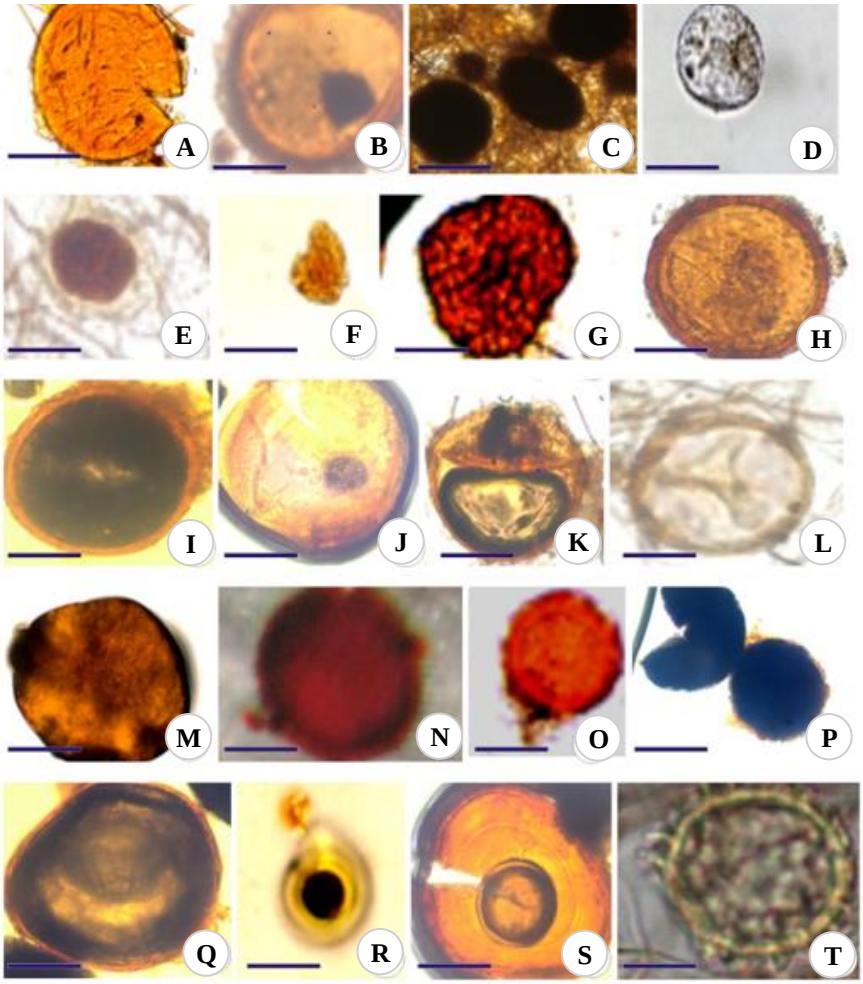

Figure 2. Diversity of AM fungal species identified in the rhizosphere soils of legumes in both provinces. A. *Glomus magnicaule* B. *Glomus delhiense* C. *Glomus ambisporum* D. *Glomus tubaeforme* E. *Glomus rubiforme* F. *Glomus sinuosum* G. *Glomus taiwanense* H. *Acaulospora colombiana* I. *Acaulospora mellea* J. *Acaulospora tuberculata* K. *Rhizophagus intraradices* L. *Rhizophagus irregularis* M. *Septoglomus deserticola* N. *Septoglomus constrictum* O. *Claroideoglomus etunicatum* P. *Entrophospora infrequens* Q. *Funneliformis geosporum* R. *Gigaspora decipiens* S. *Scutellospora biornata* T. *Sieverdingia tortuosa*. All scale bars = 50 µm except; B = 35 µm and G = 30 µm.

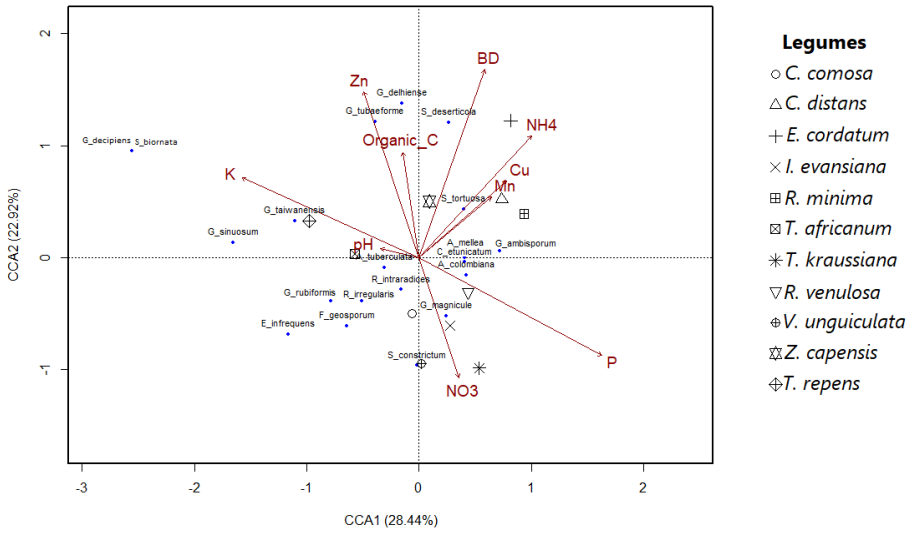

Figure 3. The canonical correspondence analysis (CCA) showing the relationship between AMF communities and soil properties. NO_3, nitrate; NH_4, ammonia; P, available phosphorus; K, available potassium; Cu, copper; Mn, manganese; Zn, zinc; BD, Bulk density.

Table 3. Correlation analysis between AM fungal spore density, diversity indices, and soil properties

Parameters	SD	SR	H	J
pH	0.32*	0.35**	0.44***	0.41**
NO_3	-0.4***	-0.23	-0.13	0.10
NH_4	-0.07	-0.17	-0.09	0.20
P	-0.59***	-0.30*	-0.12	0.05
Organic C	0.13	0.05	0.01	0.07
K	0.54***	0.49***	0.20	0.01
Cu	-0.09	-0.18	-0.18	-0.05
Mn	0.25*	-0.14	-0.13	0.12
Zn	0.11	-0.01	-0.02	0.14
BD	0.03	0.31*	0.5***	0.38**
Sand	0.13	-0.08	-0.02	0.13
Silt	-0.2	-0.04	0.03	0.05
Clay	0.01	0.15	0.14	0.01

Note: NO_3, nitrate; NH4, ammonia; P, available phosphorus; K, available potassium; Cu, copper; Mn, manganese; Zn, zinc; BD, Bulk density; SD, spore density; SR, spore richness; H, Shannon-Weiner diversity index; J, Pielou evenness index; *Significant at $P < 0.05$; **Significant at $P < 0.01$; *** Significant at $P < 0.001$.

Arbuscular mycorrhizal fungal spores possess key morphological features that enable species-level identification, and many studies have inferred the community structure of AMF based on spore communities (Singh et al. 2008; Wang et al. 2019). In this study, we reported for the first time, the diversity of AM fungal communities in the rhizosphere of eleven indigenous legumes of South Africa using spore morphology. A total of 20 AM fungal species were detected. This diversity is relatively higher than what has been previously reported in other host plants in this region (Gaur et al. 1999; Straker et al. 2010) and in different leguminous plants from other semi-arid ecosystems (Dalpé et al. 2000; Feitosa de Souza, 2016; Choosa-Nga et al. 2019).

Furthermore, the results showed that *Glomus* was the predominant genus, followed by *Acaulospora*. Previous researchers have reported the dominance of these genera in other legumes (Songachan and Kayang 2013; Choosa-Nga et al. 2019). The dominance of *Glomus* and *Acaulospora* may be attributed to their small spore morphology, ease of sporulation, and wider adaptability to different plants and environmental conditions (Hepper 1984). More so, the fact that these genera propagate mostly by spores, which are considered highly resistant propagules under harsh environmental conditions (Lennon and Jones 2011), could also support their dominance in the rhizosphere of examined plants.

The genera *Gigaspora* and *Scutellospora* were recorded in low numbers in this study. Species of the family Gigasporaceae are largely established through other propagules such as hyphae and mycelial fragments (Hart and Reader 2002). Moreover, studies have indicated that species of Gigasporaceae are more prevalent in sandy soils such as dunes (Lee and Koske 1994). Hence, the rarity of these genera may reflect their low competitive capacity and narrow adaptability to this environment. This study revealed that *A. colombiana*, *A. mellea*, and *C. etunicatum* were the most frequent AM fungal species. *Acaulospora* species have greater survival capacity in low soil pH since their occurrence is principally associated with acidic soils (Morton 1986; Straker et al. 2010). *Claroideoglomus etunicatum* is a cosmopolitan species in diverse ecotypes (Becker and Gerdemann 1977). Perhaps, the ability of these species to adapt well in vast environments is responsible for their commonality to all the legumes.

The AM fungal community structure varied in different legume species, which accords with the findings of Choosa-Nga et al. (2019) and Olubode et al. (2020). The dissimilarity may be owing to host preferences for different AM fungal species (Castillo et al. 2016; Wang et al. 2019), as well as the potential influence of soil properties (Sweeney et al. 2021). Preferential associations among host-AMF pairs have been attributed to the functional diversity among AM fungal species; whereby host plants filter and reward the most beneficial AM fungal partners in their local environments with more carbon (Bever et al. 2009; Kiers et al. 2011). The differential abundance of members of the families Glomeraceae and Acaulosporacea in the rhizosphere of different legumes may be an indication of their functional significance. Hence, this finding suggests the possibility of utilizing the abundant AM fungal species in the rhizosphere soils of respective legumes as inoculum for management purposes.

Soil environmental factors have been widely acknowledged as key environmental determinants of AMF diversity and community structure, particularly at local scales (Jansa et al. 2014; Alguacil et al. 2016). In this study, AM fungal SD and diversity indices were significantly correlated with soil pH. pH is one of the most important environmental variables that shape AM fungal diversity and community structure by regulating the availability of nutrients and ions in the soil for plant uptake (Coughlan et al. 2000). Furthermore, since AM symbiosis is generally triggered by soil nutrient-limiting conditions; expectedly, AMF spore population and species richness should be inversely related to soil P and N levels (Read 1991). Thus, the lower P and N concentrations in the rhizosphere soils of studied legumes may have contributed to the significant negative correlation observed in this study.

Excessive soil compaction adversely affects plant root growth and AMF sporulation (Nadian et al. 1998; Yano et al. 1998). Soil bulk density is one of the most frequently used measures of compaction. Although the critical value of bulk density for inhibiting root growth differs with soil type, bulk density values greater than 1.6 g/cm^3 appear to restrict root growth and affect AMF activities (McKenzie et al. 2004). The values of bulk density recorded in the rhizosphere of legumes in this study fall within this range, and hence may account for the positive relationship observed between diversity indices, AM fungal communities, and BD. Significant positive association was also recorded between SD, SR, AM fungal communities and soil Mn and Zinc contents. These micronutrients play important roles in legume metabolic process, most

especially in nitrogen fixation and their uptake can be positively impacted by the presence of AMF (Weisany et al. 2013; Lehmann and Rillig 2015).

The stimulatory effects of soil K on AM fungal attributes have been reported (Furlan et al. 1989). The high soil K concentration recorded in the rhizosphere of studied legumes could have promoted AMF spore production, and in turn, enhanced AMF species richness and community structure. While plant-AMF symbiosis is in response to soil nutrient limitations, variation in the structure of rhizosphere AMF communities may result from complex interactions between biotic and abiotic factors (Dumbrell et al. 2010). The distribution of *R. intraradices*, *G. rubiforme*, *R. irregularis*, *E. infrequens*, *F. geosporum*, and *S. constrictum* were least affected by soil properties, thereby implying the role of other factors (presumably host plants, climate, or dispersal capabilities of AM fungal species) in shaping the structure of these species.

In conclusion, this study reveals the dominance and abundance of *Glomeraceae* and *Acaulosporaceae* in the rhizosphere of the examined legumes. This suggests that genera of both families may be particularly important in the establishment and adaptation of legumes to this environment. Accordingly, they could be the most promising genera for use as inoculants to further investigate the ecological impacts of the symbiotic interactions between AMF and legumes. Spore density and species richness were highest in *Trifolium* species, indicating their rhizosphere soils as a great source of inoculum. Overall, this study provides a valuable contribution to the biodiversity of AMF present in semi-arid soils and demonstrated the effect of soil environmental factors on AMF diversity and community structure.

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