Soil lead pollution modifies the structure of arbuscular mycorrhizal fungal communities

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ORIGINAL ARTICLE

Soil lead pollution modifies the structure of arbuscular mycorrhizal fungal communities

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Abstract

The impact of lead (Pb) pollution on native communities of arbuscular mycorrhizal fungi (AMF) was assessed in soil samples from the surroundings of an abandoned Pb smelting factory. To consider the influence of host identity, bulk soil surrounding plant roots soil samples of predominant plant species (*Sorghum halepense*, *Bidens pilosa*, and *Tagetes minuta*) growing in Pb-polluted soils and in an uncontaminated site were selected. Molecular diversity was assessed by sequencing the 18S rDNA region with primers specific to AMF (AMV4.5NF/AMDGR) using Illumina MiSeq. A total of 115 virtual taxa (VT) of AMF were identified in this survey. Plant species did not affect AMF diversity patterns. However, soil Pb content was negatively correlated with VT richness per sample. Paraglomeraceae and Glomeraceae were the predominant families while Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, and Gigasporaceae were less abundant. Acaulosporaceae and Glomeraceae were negatively affected by soil Pb, but Paraglomeraceae relative abundance increased under increasing soil Pb content. Overall, 26 indicator taxa were identified; four of them were previously reported in Pb-polluted soils (VT060; VT222; VT004; VT380); and five corresponded to cultured spores of *Scutellospora castaneae* (VT041), *Diversispora* spp. and *Tricispora nevadensis* (VT060), *Diversispora epigaea* (VT061), *Glomus proliferum* (VT099), and *Gl. indicum* (VT222). Even though AMF were present in Pb-polluted soils, community structure was strongly altered via the differential responses of taxonomic groups of AMF to Pb pollution. These taxon-specific differences in tolerance to soil Pb content should be considered for future phytoremediation strategies based on the selection and utilization of native Glomeromycota.

Keywords Heavy metal · Soil pollution · 18S rDNA · Arbuscular mycorrhizal fungi · Biodiversity

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Introduction

Soil is the most valuable ecosystem in the world (Pepper et al. 2009) and human activities over thousands of years have left a legacy of polluted soils worldwide (Swartjes 2011). The excessive concentration of pollutants is a real menace due to the potential off-site migration by erosion of contaminated dust or leaching into the groundwater and rivers (Järup 2003). Lead (Pb) is one of the most widespread metal contaminants in soil and Pb decontamination is crucial for the maintenance of environmental health and ecological restoration (Moosavi and Seghatoleslami 2013).

Increasing research is developing novel, science-based remediation methods such as microbial degradation or phytoremediation (Rodríguez-Eugenio et al. 2018). Phytoremediation represents a promising technological tool with low disruptive impact on nature. It consists of maintenance of adapted living plants, which tolerate and accumulate

heavy metals (HM), by developing an extensive root system, thus reducing leaking of HM into the groundwater as well as erosion (Ali et al. 2013). For instance, high Pb concentrations were recorded in roots and shoots of plants growing in Pb polluted soil in the proximity of a former battery recycling factory in Córdoba, Argentina (Salazar and Pignata 2014; Graziani et al. 2016; Salazar et al. 2016). The existence of a diverse array of tolerant plants represents a potential biological resource for soil phytoremediation strategies (Regvar and Vogel-Mikuš 2008; Bhargava et al. 2012). Meanwhile, in a phytoremediation protocol, the optimization of plant fitness and growth by native microorganisms, such as mycorrhizal fungi, can contribute to successfully decontaminating several HM out of soils by using plants (Gaur and Adholeya 2004; Chagnon and Brisson 2017; Chaturvedi et al. 2018; de Fátima Pedroso et al. 2018). Hence, identification of the symbiotic microbiota can contribute towards a successful plant-based bioremediation strategy.

Arbuscular mycorrhizal fungi (AMF, Glomeromycota, Tedersoo et al. 2018) are root-associated symbionts of more than 80% of all plants and they inhabit a wide range of soils including those contaminated with HM (Smith and Read 2008). Indeed, Sánchez-Castro et al. (2017) detected the presence of several AMF ribotypes, belonging mainly to Paraglomerales and Diversisporales, in extremely polluted soils (97,333 ppm Zn and 31,333 ppm Pb). In association with metal-tolerant plants, at elevated levels of contamination, AMF mediate the reduction of soil HM bioavailability by trapping the element in spores and mycelia (González-Chávez et al. 2009; Salazar et al. 2018). Moreover, the glycoprotein, "Glomalin," produced by AMF can immobilize Cd, Cr, Cu, Pb, and Zn (González -Chavez et al. 2004; Cornejo et al. 2008; Gil-Cardeza et al. 2014). In addition, intracellular hyphae are able not only to retain the pollutant but also to modulate the phytotoxicity through enzymatic mechanisms (Dehn and Schüepp 1990; Huang et al. 2017; Chen et al. 2018). The tolerance to HM stress differs between and within taxonomic groups of Glomeromycota communities (Pawlowska and Charvat 2004; Wong et al. 2007), influencing the bioremediation capacity of plants (Del Val et al. 1999; Hildebrandt et al. 2007). Furthermore, AMF isolates from metal-polluted soils exhibit better resistance to HM toxicity compared to isolates from unpolluted soils (González -Chavez et al. 2002; Sudová et al. 2008). For these reasons, a comparative analysis of AMF community structure in contaminated and uncontaminated soils is essential for the identification of metal-tolerant AMF species and development of efficient phytoremediation techniques.

In this study, the AMF community structure was characterized in six sites with different levels of soil-Pb and one site with uncontaminated soil. To this end, we (i) determined the AMF diversity in the surrounding soil of three plant species cited as potential metal accumulators (*Sorghum halepense*, *Bidens pilosa*, and *Tagetes minuta*) present in both contaminates sites and one uncontaminated site; (ii) evaluated the correlation between soil-Pb contamination and sequence abundance of families of Glomeromycota; (iii) compared community structure between contaminated sites and one uncontaminated site; and (iv) identified indicator taxa of Pb-polluted situations. We hypothesized that soil-Pb pollution would represent a strong selection pressure, negatively affecting diversity patterns as well as altering the composition of native AMF communities. We expected to identify soil-Pb tolerant taxa which can be employed in a phytoremediation protocol.

Material and methods

Study area

The study was conducted in Bouwer, a village located 18 km away from Córdoba city, Argentina (31° 33′ 34.02″ S; 64° 11′ 9.05″ W). In this area, a battery recycling factory operated for two decades (1984 to 2005), but finally closed due to a lack of emission control and inadequate waste disposal that caused a severe accumulation of Pb over an extensive zone (Salazar and Pignata 2014). The soil is an Entic Haplustoll (USDA 2014), while the climate of the region is characterized by 15 °C mean annual temperature and 900 mm annual rainfall (Gorgas and Tassile 2002). As described by Salazar et al. (2018), six sites were selected. However, their selection was based on a gradient of increasing distance from the abandoned factory (S1 to S6), while in the present study, the sites were identified according to the level of total Pb content (μg^{-1}): Pb0 (14 ± 1), Pb1 (89 ± 6); Pb2 (365 ± 23); Pb3 (544 ± 33); Pb4 (965 ± 56); Pb5 (2938 ± 150); and Pb6 (16,186 ± 686). The uncontaminated site Pb0 was located 2.7 km away from the factory area. Its Pb content corresponds to the natural concentration of the element. Soil properties (i.e., organic matter, nitrogen, pH, electrical conductivity, and extractable phosphorus and potassium) can be found in Salazar et al. (2018).

Sampling procedure

In both contaminated and uncontaminated sites, during autumn of 2015, we sampled bulk soil surrounding plant roots of the three following dominant plants: *Bidens pilosa* L., *Tagetes minuta* L., and *Sorghum halepense* (L.) Pers, previously studied for Pb accumulation (Salazar and Pignata 2014; Cid et al. 2016; Sosa et al. 2016). The number of bulk soil samples surrounding plant roots varied according to the local availability of plants in each sampling site (Table 1).

The samples were collected using a stainless-steel shovel to remove entire plants and roots with their surrounding soil and were placed in plastic bags. In total, we sampled 24 plants

Table 1 Sampling design, arbuscular mycorrhizal virtual taxa (VT) richness, and sequencing intensity of bulk soil samples of different plant species from sites with increasing levels of Pb pollution

Pb level	Plant species	No. of samples	Total no. of VT	Mean no. of VT per sample	Mean no. of AMF reads per sample
0	*Sorghum halepense*	3	63	44.3	28,423
1	*Bidens pilosa*	1	49	49.0	32,608
	Sorghum halepense	1	44	44.0	29,661
	Tagetes minuta	3	68	52.6	17,168
2	*Bidens pilosa*	1	41	41.0	16,096
	Sorghum halepense	2	25	18.5	27,359
	Tagetes minuta	1	30	30.0	11,300
3	*Sorghum halepense*	1	40	40.0	30,123
	Tagetes minuta	1	57	37.0	14,822
4	*Bidens pilosa*	2	77	63.5	24,878
	Sorghum halepense	1	49	49.0	17,293
5	*Sorghum halepense*	3	52	32.6	17,599
	Tagetes minuta	1	38	38.0	61,046
6	*Bidens pilosa*	2	36	26.0	8744
	Sorghum halepense	1	18	18.0	47,688

with their corresponding soil samples. Once in the laboratory, the roots and soil were separated by shaking them in plastic boxes and sieving. Approximately 50 g of each soil sample was placed in a sealed plastic bag and maintained at − 20 °C for DNA extraction. Arbuscular mycorrhizal colonization was detected in all the studied plant species.

Soil DNA extraction, PCR, and sequencing

For each sample, the soil was thoroughly mixed and 0.5 g of the homogenized sample was used to extract DNA using the Power Soil DNA isolation kit (MO BIO Lab. Inc., USA), according to the manufacturer's instructions. One microliter of DNA template, containing 30 nanograms of DNA, was added to a 40-μl (final volume) PCR mix containing 25.6 μl of MQ water, 4 μl of 10 × buffer, 1.5 μl dNTPs (2.5 mM), 1.5 μl of reverse and forward primers (10 mM), 4 μl $MgCl_2$ (50 mM), 0.5 μl BSA (10 mg/ml), and 0.4 μl BIOTAQ polymerase (5 U/μl). The primers AMV4.5NF and AMDGR (Sato et al. 2005) were used to amplify a part of the SSU (approximately 300 bp), using the following PCR conditions: an initial cycle of 95 °C for 5 min; then 37 cycles of 95 °C for 20 s, 56 °C for 30 s, 72 °C for 1.5 min; and a final cycle of 72 °C for 7 min. Primers were labeled with sample-specific multiplex identification DNA-tags. A negative control, consisting of MQ water instead of DNA, was subjected to the PCR under the same experimental conditions and revealed on a gel to be amplicon-free. The PCR products were assessed for size distribution and for DNA concentration using a Bioanalyzer 2100 (Agilent Technologies Inc., Santa Clara, CA, USA). The products were cleaned using 0.99 Ampure beads (Beckman Coulter, Beverly, MA, USA) to remove short fragments. The amplicons were diluted with MQ water to produce the same DNA concentration for each sample in the final pool before being sequenced. Paired-end Illumina MiSeq sequencing was carried out at Base Clear (Leiden, The Netherlands).

Bioinformatics

Two-directional reads were assembled to contigs using the Mothur v. 1.32.1 (Schloss et al. 2009). The primers were removed, and poor-quality ends were trimmed based on a 0.02 error probability limit with Geneious Pro 8.1 (BioMatters, New Zealand). Subsequently, the sequences were filtered using USEARCH v.8.0 (Edgar 2010) and the following settings—all sequences were truncated to 200 bp, and the sequences with an expected error > 1 were discarded. The remaining sequences were collapsed with USEARCH into unique sequence types on a per-sample basis while preserving read counts and excluding singletons. These sequences served as the input for OTU clustering at a 97% sequence similarity following Öpik et al. (2010), using USEARCH, while simultaneously removing putatively chimeric sequences. Representative sequences of the OTUs were subjected to a similarity search against the Maarj*AM* database (Öpik et al. 2010) using USEARCH v.8.0. OTUs that did not have at least an 80% similarity over a minimum of 180 bp to any Glomeromycota sequence in the Maarj*AM* database were excluded from further analysis. A set of representative sequence reads was deposited in the NCBI nucleotide collection (accession numbers MK125545–MK127534).

Statistical analysis

Sequencing efficacy was assessed with rarefaction analysis using the rarefy function from R package VEGAN (Oksanen

2013; R Development Team 2018). Sampling effort was estimated using the specaccum function in VEGAN. Then, the data matrix was standardized by rarefaction to the median read count per sample (23,290 reads). This approach has been shown to represent an optimal approach for reducing bias due to differences in sample size while retaining information (de Cárcer et al. 2011). Generalized Linear Mixed Models (GLMMs) were used to test for differences in AMF VT richness per sample among Pb levels and plant species identity using glmer function of R package lme4 implemented in Infostat software (Di Rienzo et al. 2018). When significant differences were observed, mean comparisons were performed by DGC test (DGC: Di Rienzo-Guzmán-Casanoves) ($p < 0.05$) using Infostat software (Di Rienzo et al. 2002, 2018). The Spearman test was used to detect correlations between observed VT richness and family abundances with Pb concentration using the Infostat software (Di Rienzo et al. 2018). While the GLMM test allowed the comparisons of mean values, the correlation was performed in order to detect whether the reduction in VT richness was significantly associated with Pb content.

The effect of soil Pb level, soil properties, and plant species on AMF community composition was assessed by permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) using the Adonis function with 9999 permutations based on a presence-absence matrix. Variation in AMF taxonomic community composition was visualized by non-metric multidimensional scaling (three-dimensional NMDS, with 50 iterations) using the metaMDS function and Bray-Curtis distance from R package VEGAN. We plotted ellipses representing communities belonging to the different Pb levels with the ordiellipse function using the standard deviations of weighted averages. Function envfit from VEGAN was used to plot vectors of the most significant variables (p max = 0.05) onto the NMDS ordination diagram using the presence-absence matrix. To identify AMF taxa associated with a particular Pb situation, we used indicator species analysis (Dufrêne and Legendre 1997) as implemented in the function indVal from R package LABDSV (Robert 2012) using the rarefied and normalized matrix (i.e., relative abundance matrix). Only those VT with an indicator value of at least 0.25 and p value < 0.05 were considered.

Results

In this study, we successfully sequenced AMF genomic DNA of 24 soil samples from the immediate vicinity of the root systems of three plant species growing under increasing levels of Pb pollution (Table 1). A total of 602,740 reads carried a correct bar code (the correct primer sequence) and received a hit (similarity ≥ 97%) against a virtual taxon (VT) from the MaarjAM database (Öpik et al. 2010). The rarefaction analysis revealed that this sequencing effort was sufficient to detect representative AMF richness values in the studied samples given the asymptotic rarefaction curves (Fig. S1a). The species accumulation curves suggested that some undetected taxa remained in all Pb situations, particularly in Pb3, where only two samples were obtained (Fig. S1b).

AMF VT richness

Overall, 115 VT of AMF were detected (Table 1S), ranging from 18 to 63.5 VT per sample (Fig. 1). Plant species identity did not affect VT richness per sample (GLMMs, $F_{2,21} = 1.49$, $p > 0.05$). *Bidens pilosa*, *T. minuta*, and *S. halepense* exhibited a mean richness of 44.83 (± 5.5 SE), 43.25 (± 5.5), and 34.92 (± 3.9) VT per sample, respectively. However, the observed VT richness significantly differed according to the concentration of Pb in soil (GLMMs, $F_{6,17} = 9.02$, $p < 0.001$) (Fig. 1). Indeed, there was a negative correlation between the level of Pb in soil and the mean number of VT per sample (Spearman, − 0.43; $p < 0.05$). No significant correlation was detected between organic matter content, pH, N, extractable P and VT richness.

AMF community composition

We found a significant effect of soil Pb on the taxonomic composition of AMF communities (PERMANOVA, $R^2 = 0.55$, $p < 0.001$; Table 2). The identity of plant species did not affect the structure of AMF communities (PERMANOVA, $R^2 = 0.10$, $p > 0.05$; Table 2). Among soil properties, Pb concentration (PERMANOVA, $R^2 = 0.167$, $p < 0.001$), pH (PERMANOVA, $R^2 = 0.081$, $p < 0.05$), organic matter content (PERMANOVA,

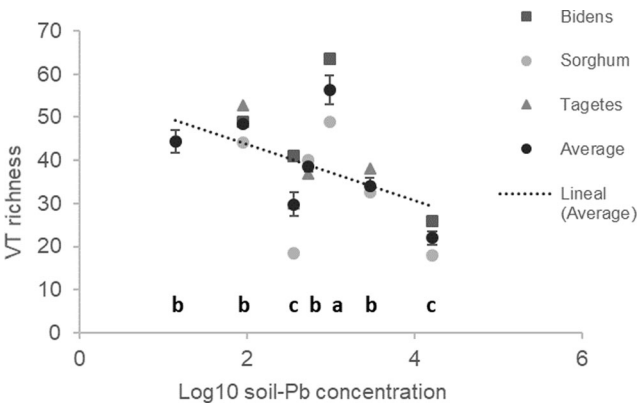

Fig. 1 Richness of AMF virtual taxa (VT) per sample in relation to log-transformed values of soil-Pb concentration. Soil Pb concentration was log10 transformed (μg g^{-1}): Pb0 = 1.15 (14 ± 1); Pb1 = 1.95 (89 ± 6), Pb2 = 2.56 (365 ± 23); Pb3 = 2.76 (544 ± 33); Pb4 = 2.98 (965 ± 56); Pb5 = 3.47 (2938 ± 150); and Pb6 = 4.21 (16,186 ± 686). The mean richness differed significantly among soil Pb level (generalized linear mixed model (GLMM), $p < 0.002$). Letters indicate significant differences in richness among the averaged values of Pb levels ($p < 0.05$, DGC test). Bars represent standard error (n detailed in Table 1)

$R^2 = 0.083$, $p < 0.01$), and extractable phosphorus (PERMANOVA, $R^2 = 0.07$, $p < 0.05$) had a significant effect on AMF community composition (Fig. 2). Fungal communities from heavily Pb-contaminated soils (Pb5 and Pb6) were located separately on the ordination biplot alongside the vector of soil Pb concentration. Samples from Pb1 and Pb4 formed a distinct cluster, opposite the highest levels of Pb, while Pb0 and Pb3 communities were near the vector of soil phosphorus. Finally, the Pb2 situation was most closely associated with soil pH (Fig. 2).

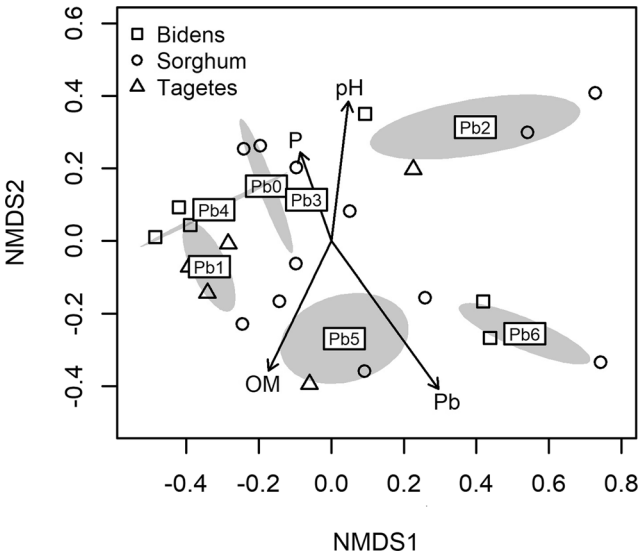

Fig. 2 NMDS plot of variation in bulk soil AMF communities based on the Bray-Curtis dissimilarity between samples (stress = 0.12). Ellipses indicate one SD around group centroids (Pb levels). Arrows represent statistically significant fitted vectors ($p < 0.05$) of environmental variables on the ordination plot where arrow points indicate the direction of the gradient, and the length represents the correlation between ordination and the variables: extractable phosphorus (P, μg g^{-1}), pH, total Pb concentration (Pb, μg g^{-1}), and organic matter content (OM, %)

Proportional read abundance of Glomeromycota families

The proportions of VT reads belonging to the AMF families under increasing Pb levels are shown in Fig. 3. The absolute sequence number per family is presented in Fig. S2.

Altogether, eight families of Glomeromycota were identified. Paraglomeraceae was predominant in both uncontaminated and contaminated soils, its relative abundance per sample ranged from 69% in Pb0 to 95% in Pb6. Glomeraceae reached 28% in Pb0 and 3% in Pb6. Noteworthily, Gigasporaceae exhibited read abundances of 0.01% in Pb0 but increased to 21.3% in Pb6 (Table S1). Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, and Diversisporaceae were detected in rather low proportions. Furthermore, Acaulosporaceae was not detected in Pb5 and Pb6 (Fig. 3, Table 1S). Finally, the relation between relative abundances of AMF families and soil Pb concentration was estimated (Fig. 4). We found that the proportion of VT reads of Acaulosporaceae and Glomeraceae was negatively affected by soil Pb. However, the abundance of Paraglomeraceae soared under increasing levels of the contaminant (Fig. 4). None of the other families showed significant responses to Pb.

Indicator taxa

Considering the significant effect of soil Pb concentration on community composition, we investigated whether some VT were indicators of a particular Pb concentration level. Overall, the analysis resulted in 26 AMF indicator taxa (Table 3). They corresponded to 22.6% of total VT accounting for 14,620 reads (i.e., 3.2% of the total reads). Seven taxa were identified from the uncontaminated site (Pb0); they belonged to Diversisporaceae (VT058), Glomeraceae (VT209; VT248; VT312; VT125), and Acaulosporaceae (VT024, VT227); and 19 taxa were indicators of Pb-polluted sites. Seven taxa belonging to Gigasporaceae (VT041), Diversisporaceae (VT060; VT061), Glomeraceae (VT096; VT121; VT120), and Archaeosporaceae (VT004) were indicators of the lowest level of Pb-pollution (Pb1). Glomeraceae taxa were found as indicators of Pb2 (VT206); Pb3 (VT392; VT331), Pb4 (VT126; VT399; VT099; VT222; VT288); and Pb6 (VT280). Additionally, one taxon of Diversisporaceae (VT380) and Claroideoglomeraceae (VT276) for Pb4 and one of Gigasporaceae (VT318) for Pb6 were significant indicator taxa.

Table 2 Variation in the AMF community composition (PERMANOVA analysis) from bulk soil samples in relation to plant species and Pb level

Model	Df	SS	MS	Pseudo-F	R^2	P value
Plant species	2	0.27915	0.13958	12.468	0.10614	0.206
Residuals	21	235.097	0.11195	0.8938		
Total	23	263.012				
Pb level	6	14.614	0.243561	35.427	0.55563	**0.**001
Residuals	17	11.688	0.068751	0.4443		
Total	23	26.301				

Discussion

High AMF richness but negatively correlated with Pb level

This study of Glomeromycota diversity under increasing levels of Pb pollution shows that AMF can persist and maintain relatively high diversity. The presence of AMF in soils contaminated with HM, including Pb, was observed earlier

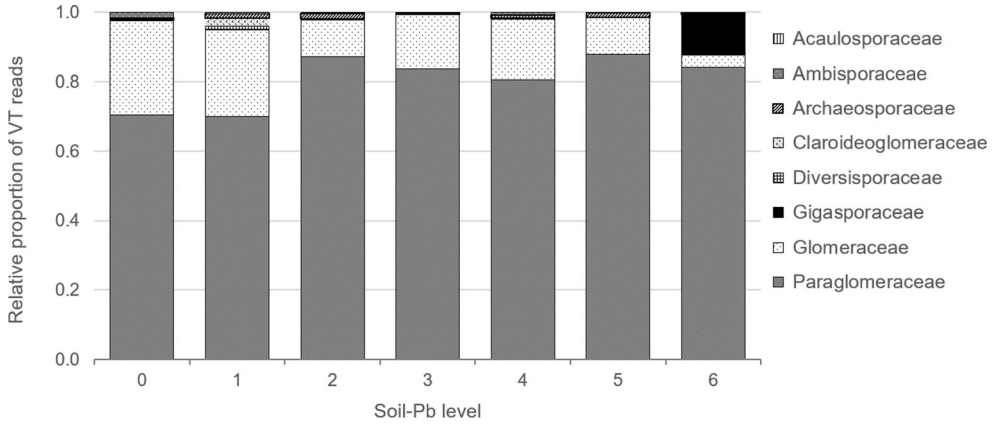

Fig. 3 Relative abundance of reads of detected families of Glomeromycota in sites with increasing levels of soil Pb pollution. Soil Pb content was (μg g^{-1}): Pb0 = 1.15 (14 ± 1); Pb1 = 1.95 (89 ± 6), Pb2 = 2.56 (365 ± 23); Pb3 = 2.76 (544 ± 33); Pb4 = 2.98 (965 ± 56); Pb5 = 3.47 (2938 ± 150); and Pb6 = 4.21 (16,186 ± 686)

(e.g., Abdel-Azeem et al. 2007; Long et al. 2010; Turrini et al. 2018). Moreover, Sánchez-Castro et al. (2017) detected Glomeromycota ribotypes under high contents of Zn and Pb, demonstrating the ability of AMF to survive under extreme levels of heavy metal contamination. In this study, high AMF VT richness was observed in the sampled area. The identification of 115 VT represents a valuable contribution to the local dataset of sequences from native AMF in a relatively understudied biogeographic region. With a comparable methodology, in a proximate area of Córdoba province, García de León et al. (2018) found 81 VT in soybean fields. Therefore, the finding of high VT richness suggests that a substantial number of AMF species persist in Pb-polluted soils. Considering the AMF obligate biotrophic life history, the maintenance of vegetal cover could have contributed to the preservation of this component of the soil biota (Klironomos and Hart 2002).

Despite the generally high AMF diversity, the negative correlation with Pb concentration indicates that certain species do not tolerate elevated concentrations of the contaminant. Pawlowska and Charvat (2004) examined in vitro effects of Cd, Pb, and Zn on critical life stages of selected species of Glomeromycota. They concluded that while certain AMF species can survive in HM-contaminated environments, other species are more sensitive to metal stress due to HM interference in spore germination and presymbiotic hyphal extension. Numerous surveys found differential sensitivity in polluted soils (e.g., Hassan et al. 2011; Krishnamoorthy et al. 2015; Lopes Leal et al. 2016); however, further studies should determine the long-lasting consequences of AMF species loss in ecosystem functioning of HM-polluted soils. It is worthwhile to highlight that the current study was carried out 10 years after contamination ceased.

Soil Pb content induced changes in AMF community composition

Former works have revealed varying composition of AMF communities between uncontaminated and HM-contaminated soils (Vallino et al. 2006; Zarei et al. 2008, 2010). A similar

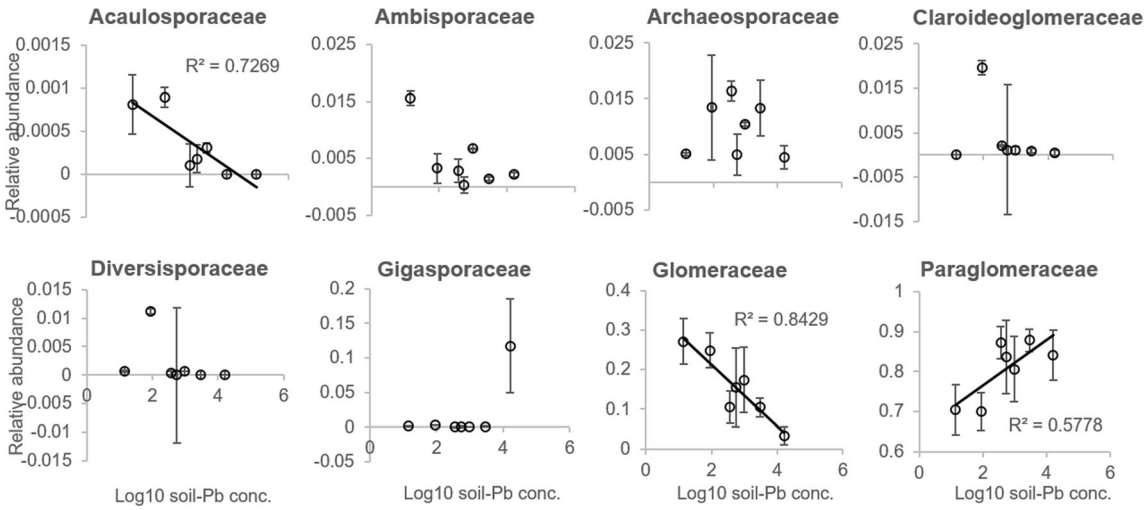

Fig. 4 Relationship between log-transformed values of Pb concentration ("*x*" axes) and the relative abundance of VT reads of detected families of Glomeromycota ("*y*" axes). Soil Pb concentration was log10 transformed (μg g^{-1}): Pb0 = 1.15 (14 ± 1); Pb1 = 1.95 (89 ± 6), Pb2 = 2.56 (365 ± 23); Pb3 = 2.76 (544 ± 33); Pb4 = 2.98 (965 ± 56); Pb5 = 3.47 (2938 ± 150); and Pb6 = 4.21 (16,186 ± 686). Bars represent SE (*n* is detailed in Table 1). Only significant relations are shown (lm, $p < 0.05$)

Table 3 Indicator AMF virtual taxa (VT, according to Maarj*AM* database) from sites with increasing levels of soil-Pb pollution

AMF VT	Family	Pb level	Indicator_value	probability
VT058	Diversisporaceae	0	10.00	0.001
VT209	Glomeraceae	0	0.666	0.037
VT024	Acaulosporaceae	0	0.666	0.037
VT227	Acaulosporaceae	0	0.651	0.035
VT248	Glomeraceae	0	0.636	0.029
VT312	Glomeraceae	0	0.623	0.028
VT125	Glomeraceae	0	0.424	0.044
VT041	Gigasporaceae	1	0.573	0.047
VT060	Diversisporaceae	1	0.773	0.012
VT061	Diversisporaceae	1	0.700	0.050
VT096	Glomeraceae	1	0.604	0.031
VT121	Glomeraceae	1	0.600	0.04
VT120	Glomeraceae	1	0.600	0.024
VT004	Archaeosporaceae	1	0.582	0.013
VT206	Glomeraceae	2	0.680	0.043
VT392	Glomeraceae	3	0.722	0.008
VT331	Glomeraceae	3	0.715	0.005
VT126	Glomeraceae	4	10.000	0.004
VT399	Glomeraceae	4	10.000	0.002
VT099	Glomeraceae	4	0.666	0.038
VT380	Diversisporaceae	4	0.666	0.039
VT222	Glomeraceae	4	0.658	0.001
VT288	Glomeraceae	4	0.623	0.039
VT276	Claroideoglomeraceae	4	0.569	0.036
VT318	Gigasporaceae	6	0.972	0.005
VT280	Glomeraceae	6	0.661	0.024

pattern was observed in our study as well, without a significant effect attributed to plant species. This result confirms the toxicity of Pb to many species of Glomeromycota. Furthermore, changes in physical and chemical soil properties can be triggered by high HM contents (Igwe et al. 2005). In the present study, the vectors fitted onto the ordination plot show that the incidence of soil pH and P availability contrasted with that of Pb and organic matter content. Therefore, it can be inferred that the alteration of AMF community structure was caused not only by the Pb toxicity per se, but also by the chemical modification of the soil environment under increasing Pb pollution. Hence, multiple restrictive conditions may have become a crucial challenge to sensitive species.

AMF families showed differential sensitivity to Pb

Metal stress not only induces the disappearance of less tolerant species, but also promotes species that are more tolerant (Del Val et al. 1999; Krishnamoorthy et al. 2015). In our study, Paraglomeraceae predominance was followed by Glomeraceae, although both families presented opposite responses to increasing soil Pb concentration. Paraglomeraceae is a relatively new family within Glomeromycota, identified by molecular assessments (Morton and Redecker 2001). It has been frequently found in local studies based on molecular taxonomy of AMF (Grilli et al. 2015; García de León et al. 2018; Faggioli et al. 2019), and in assessments of HM-pollution (Turnau et al. 2001; Sánchez-Castro et al. 2012; Lopes Leal et al. 2016). For instance, Lopes Leal et al. (2016) found that the relative abundance of *Paraglomus occultum* spores was 86% in a HM-contaminated soil, and 26% after the rehabilitation of the contaminated area. Furthermore, based on molecular techniques, Sánchez-Castro et al. (2017) identified one particular *Paraglomus* taxon in highly Zn- and Pb-contaminated soils, suggesting that this species should be considered for bioremediation purposes.

On the other hand, differential sensitivity of Glomeraceae species in HM-contaminated soils has been reported. For instance, *Funneliformis mosseae* is frequently found in sites with a high level of Pb pollution (Whitfield et al. 2004; Vallino et al. 2006; Hassan et al. 2011), while *Rhizophagus intraradices* is detected in non-contaminated and moderately contaminated habitats (Turnau et al. 2001) but not under high concentrations of Pb (Wong et al. 2007; Zarei et al. 2008). It is noteworthy that understanding the causes and consequences of changing

abundances of predominant families of Glomeromycota is critical to design phytoremediation strategies mediated by native microbiota.

Certain species of AMF can be dominant in contaminated soils but rare or absent in non-polluted situations. In this sense, Vallino et al. (2006) detected Gigasporaceae associated with the roots of *Solidago gigantea* growing in soils with the highest Pb content but not in sites with lower Pb concentration. Krishnamoorthy et al. (2015) studied AMF communities in the surroundings of a smelter in South Korea; even though several HM were studied, Gigasporaceae were associated with high soil Pb content. Similarly, we found that Gigasporaceae accounted for 20% of abundance at the highest level of Pb contamination (Pb6), but less than 1% in the remaining surveyed sites. Earlier, in the same study area, Salazar et al. (2018) quantified a considerable Pb accumulation in Gigasporaceae spores. Possibly, the ability to persist in Pb-contaminated soils, coupled with an aptitude to retain the pollutant, represents valuable competitive advantages of these members of Glomeromycota.

The generalized low abundances of Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, and Diversisporaceae may indicate a poor competitive capacity as constituents of native microbiota (Powell et al. 2009; Chagnon et al. 2013). In addition, the significant negative relation between soil Pb level and abundance of Acaulosporaceae revealed its extreme sensitivity to Pb stress (Wu et al. 2010). Nevertheless, some reports inform about possible biases of sequencing and bioinformatics concerning less abundant species (Lindahl et al. 2013). Next generation sequencing (NGS) techniques offer unprecedented insights into fungal community ecology, making the comparison of AMF communities truly feasible worldwide (Öpik et al. 2010; Bahram et al. 2015) and challenging the taxonomy of fungi from environmental datasets (Hibbett and Taylor 2013; Öpik et al. 2013). Even though NGS information may not be necessarily correlated with biomass allocation and morphospecies diversity (Alkan et al. 2004; Lindahl et al. 2013), NGS can inform on quantitative differences within and among fungal communities (Egan et al. 2018).

Nineteen AMF taxa were indicators of soil Pb pollution

Using indicator species analysis, the sites with Pb-pollution soil were characterized by a set of 19 AMF taxa. In general, they are reported in several continents and biomes in the Maarj*AM* database (date of access 04 March 2019). Interestingly, four taxa have been previously recorded in Pb-polluted soils. Hassan et al. (2011) found VT060 and VT222; Long et al. (2010) detected VT004; and Alguacil et al. (2011) recorded VT380. In addition, five indicator taxa of Pb-polluted sites correspond to cultured spores of *Scutellospora castaneae* (VT041 in Pb1); *Diversispora celata*, *Diversispora eburnea*, *Diversispora aurantia*, *Tricispora nevadensis* (VT060 in Pb1); *Diversispora epigaea* (VT061 in Pb1); *Glomus proliferum* and *Glomus indicum* (VT099 and VT222 in Pb4). The remaining taxa originated from environmental samples (i.e., roots and soil) and are not directly associated with identified morphospecies (Maarj*AM* database). Given that species isolated from HM-contaminated sites have shown higher capacity to take up or accumulate the pollutant than those from uncontaminated sites (Sudová et al. 2008; Salazar et al. 2018), the identification of indicator VT associated with cultural strains is a promising result for future studies testing the tolerance of AMF species to Pb pollution.

Conclusions

Pb contamination is one of the environmental factors able to alter AMF community composition. Even though a considerable number of taxa can survive under such stressful conditions, our results emphasize the effect of Pb pollution on AMF richness and on the abundance of certain families of Glomeromycota. The differential taxonomic response to soil Pb concentration suggests that some species were more affected than others by the surrounding conditions. These results should be considered for future phytoremediation strategies based on the selection and utilization of native AMF species that exhibit tolerance to soil Pb pollution. Likewise, the capacity of tolerant taxa not only to uptake or accumulate Pb but also to interact with natural vegetation deserves further investigation.

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