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ORIGINAL PAPER

Dosage-dependent shift in the spore community of arbuscular mycorrhizal fungi following application of tannery sludge

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Abstract The controlled disposal of tannery sludge in agricultural soils is a viable alternative for recycling such waste; however, the impact of this practice on the arbuscular mycorrhizal fungi (AMF) communities is not well understood. We studied the effects of low-chromium tannery sludge amendment in soils on AMF spore density, species richness and diversity, and root colonization levels. Sludge was applied at four doses to an agricultural field in Rolândia, Paraná state, Brazil. The sludge was left undisturbed on the soil surface and then the area was harrowed and planted with corn. The soil was sampled at four intervals and corn roots once within a year (2007/2008). AMF spore density was low (1 to 49 spores per 50 cm^3 of soil) and decreased as doses of tannery sludge increased. AMF root colonization was high (64%) and unaffected by tannery sludge. Eighteen AMF species belonging to six genera (*Acaulospora*, *Glomus*, *Gigaspora*, *Scutellospora*, *Paraglomus*, and *Ambispora*) were recorded. At the sludge doses of 9.0 and 22.6 Mg ha^{-1}, we observed a decrease in AMF species richness and diversity, and changes in their relative frequencies. Hierarchical grouping analysis showed that adding tannery waste to the soil altered AMF spore community in relation to the control, modifying the mycorrhizal status of soil and selectively favoring the sporulation of certain species.

Keywords Mycorrhizae · Spore density · Species diversity · pH · Phosphorus · Residue

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Introduction

Brazil is one of the world's leading leather producers, processing 44.4 million hides per year and generating exports valued at US$ 2.19 billion in 2007. However, since leather processing generates large amounts of liquid and solid waste, averaging 150–200 kg of sludge (dry weight basis) for every ton of processed leather (Pacheco 2005), adequate treatment and disposal of this waste has become a leading environmental concern.

One alternative for disposing of sludge involves applying it to agricultural soils, since tannery waste is nutrient rich and has the potential to neutralize soil acidity. This practice can boost soil fertility and plant nutrition (Alcântara et al. 2007), but it can also lead to unsuitable soil pH (Cavallet and Selbach 2008) as well as to an excess of soluble salts (Alvarez-Bernal et al. 2006) and chromium (Kamaludeen et al. 2003) in treated soils, jeopardizing the current and future sustainability of agriculture in treated areas.

Applying tannery waste to soils can change their physical (Menneer et al. 2001), chemical, and biological (Alvarez-Bernal et al. 2006) characteristics. Whether

healthy soils can be maintained under this practice for long periods is still on debate since tannery sludge has the potential to impair soil biological activity (Kamaludeen et al. 2003) and affect key nutrient-cycling processes like nitrification (Alvarez-Bernal et al. 2006).

Arbuscular mycorrhizal fungi (AMF) constitute an important group of soil microorganisms that establish symbiosis with most plants, improving their growth, besides other environmental and ecological benefits like soil aggregation, nutritional balance, and plant protection against biological and environmental stresses (Smith and Read 2008). AMF are unique and important components of the soil biota, and their occurrence, activity, and efficiency can be valuable indicators of soil quality and agricultural sustainability (Stenberg 1999). While some studies have examined AMF in areas polluted by tannery waste (Raman and Sambandan 1998; Khan 2001; Khade and Adholeya 2009), few have studied how the controlled application of tannery sludge in agricultural areas affects these soil microorganisms.

The objective of this study was to assess the effect of tannery sludge application on AMF spore density, richness, and diversity based on spore population, and on the mycorrhizal status of corn grown in the area. We tested the hypothesis that applying tannery sludge decreases spore density, species richness, and root colonization.

Materials and methods

Study area and experimental set-up

The experiment was carried out in an agricultural landscape in the municipality of Rolândia, Paraná state, in Southern Brazil (23°17′S 51°29′W, 650 m a.s.l.). The local climate is classified by Köppen as Cfa, with mean annual precipitation of 1,600 mm falling mostly between September and March. The study area has been managed for more than 10 years under the no-till system with rotating crops of soybean or corn in the austral summer and wheat or oats in winter. The soil is very clayey and classified as Rhodic Kandiudult (US Soil Taxonomy). Before the onset of the experiment, the top 0–10 cm of soil had the following characteristics: pH 5.5 in 0.01 mol l^{-1} $CaCl_2$ (1:2.5, soil/solution v/v), 24.3 gkg^{-1} of total C, 5.7 gkg^{-1} of total N, cation exchange capacity of 119.6 $mmol_c$ dm^{-3}, base saturation of 65%, available phosphorus of 34 mg dm^{-3} (resin) (Raij et al. 2001), and soil texture of 74% clay, 6% silt, and 20% sand (Camargo 1986).

The experiment was arranged in a complete randomized block design with four replicates. Tannery sludge was applied in July 2006 and August 2007 at rates of 0, 3.4, 13.5, 23.6, and 33.7 Mg ha^{-1} (dry weight basis) in the first year and 0, 2.3, 9.0, 15.8, and 22.6 Mg ha^{-1} (dry weight basis) in the second year. These amounts were calculated based on the total N contained in tannery sludge and are equivalent to 0, 120, 480, 840, and 1,200 kg ha^{-1} of total N. Corn crops require 120 kg ha^{-1} of N for high yields (IAPAR 2003).

In order to mimic the management techniques adopted by various Brazilian tanneries, sludge was applied to the soil surface in plots of 90 m^2 at natural humidity and mixed into the soil by harrowing at 0–20 cm of depth 89 days after the first application and 87 days after the second application. Corn was planted soon thereafter, when we installed a sixth treatment, referred to hereafter as the agronomic treatment (AT), which received 40 kg ha^{-1} of N at planting and 80 kg ha^{-1} of N at the seedling stage (six to eight leaves totally expanded) in the form of urea.

Tannery sludge

The tannery sludge was obtained from the Curtume Vanzella Ltda. (municipality of Rolândia, Paraná state, Brazil) and was an equal mix (1:1 v/v) of liming sludge generated in the unhairing and liming stages of leather processing, and primary sludge from the tannery's wastewater treatment plant, consisting of the precipitation of processing effluents, with the exception of those containing chromium. The physical–chemical characterization of the sludge is shown (Table 1). Further details on procedures of analyses are given in Nakatani et al. (2011).

Soil and root samplings

During the second year of the experiment, soil samples were collected at 12, 136, and 271 days after the second application of sludge for spore counting and chemical analysis, except day 12 for the AT treatment, since it was established only at the corn sowing date. We collected nine subsamples per plot from the top 10 cm of soil and combined them to form a composite sample. These samples were oven-dried (40°C for 48 h) for chemical analysis or stored at 4°C for AMF spore counts.

At the end of the corn flowering period (177 days after the second sludge application), soil and corn roots were sampled for assessment of AMF spore diversity and root colonization. Soil and fresh roots were taken from five 1-m-long transects and 0–10 cm depth along both sides of the central line in each plot, ca. 10 cm from the sowing line, corresponding to 27 plants per plot.

Soil chemical analyses

Available P (resin) was determined in the extracts by molecular absorption spectrophotometry (Raij et al. 2001).

Table 1 Physical and chemical attributes of the tannery sludge used in the experiment

Attribute	1st application	2nd application
pH[a]	12.7	9.7
Electrical conductivity (dS m^{-1})[a]	29.5	16.6
Total solids, at 65°C (g kg^{-1})	53.3	55.4
Volatile solids (g kg^{-1})	442	554
Neutralization power ($CaCO_3$ g kg^{-1})	262	361
Organic C (g kg^{-1})	308	321
Total N (g kg^{-1})	35.7	53.2
N–NH_4^+ (g kg^{-1})	20.4	21.9
N–NO_3^- (g kg^{-1})	0.2	0.2
C/N ratio	8.7	6.0
Ca (g kg^{-1})	78.9	88.0
Mg (g kg^{-1})	0.7	1.0
K (g kg^{-1})	0.1	3.3
P (g kg^{-1})	3.9	3.8
S (g kg^{-1})	36.1	43.0
Na (g kg^{-1})	10.0	66.9
Mn (mg kg^{-1})	2,858	3,340
Fe (mg kg^{-1})	408	1,249
B (mg kg^{-1})	4.5	5.6
Zn (mg kg^{-1})	43.3	73.0
Cu (mg kg^{-1})	4.5	16.0
Mo (mg kg^{-1})	3.3	<0.5[b]
Al (mg kg^{-1})	2,257	13,440
Pb (mg kg^{-1})	<1.0[b]	9.3
Ni (mg kg^{-1})	3.0	7.8
Cr (mg kg^{-1})	1,613	580

Results expressed on a dry basis, after drying at 65°C for 48 h

[a] Measured in *in natura* samples

[b] Concentrations below the detection limit, including As, Cd, Hg, and Se

Soil acidity (pH) was measured via potentiometry in 0.01 mol l^{-1} $CaCl_2$ at a soil/solution ratio of 1:2.5. Electrical conductivity (EC) was measured via conductivimetry in an aqueous solution at a soil/water ratio of 1:2. Mineral nitrogen (N–NH_4^++N–NO_3^-) was extracted with KCl (2 mol l^{-1}) at the ratio of 1:10 (m/v). N–NH_4^+ was determined using an analytical system of flow injection analysis (FIA) with a spectrophotometric reading at 605 nm (Kamogawa and Teixeira 2009) and N–NO_3^- by ultraviolet spectrophotometry, with readings at 220 nm and 275 nm (APHA 2005). Some soil chemical characteristics at each sampling date are shown in Table 2.

Soil P and pH increased after the addition of tannery sludge in all sampling periods. Levels of mineral N and EC of soils increased with increasing doses of sludge. These increases presented the highest values shortly after application (12 days) and decreased at the later sampling periods.

AMF spore counts, richness, and root colonization

AMF spores were extracted by wet sieving 50 cm^3 of soil (Gerdemann and Nicolson 1963) followed by centrifuging in water at 3,000 rpm for 3 min. The pellet was resuspended in a 50% sucrose solution and centrifuged again at 2,000 rpm for 2 min. Spores were counted and separated by morphotype at 40× magnification (Leica MZ 125, Switzerland), then mounted on slides with polyvinyl alcohol-lacto-glycerol and Melzer's reagent for a microscopic examination of structural phenotypic characteristics and for taxonomic identification. AMF species were identified by one of us (SLS) following Schenck and Pérez (1990) and comparisons with morphological descriptions of species presented at INVAM webpage (http://invam.caf.wvu.edu). Based on identification data, we determined Shannon's diversity index, species richness, and absolute and relative frequency of AMF families for each treatment. Shannon's index was based on the number of species registered in each treatment. Species richness was calculated as the total number of species detected in each treatment.

Mycorrhizal root colonization was assessed using 1 g of fine roots treated as follows: clearing by heating in 10% KOH at 90°C for 30 min, acidification with 1% HCl for 2 min, and staining with "pen ink" at 90°C for 30 min (Vierheilig et al. 1998). We quantified the percentage of colonization with a dissecting microscope (40× magnification) (Leica MZ 125, Switzerland) using the grid-line intersect method (Giovannetti and Mosse 1980).

Data analyses

Spore count data were square root transformed $(X+0.5)^{1/2}$ and submitted to univariate statistical analysis, using ANOVA for each sampling time, following a complete randomized block design, and mean comparisons by Student's t test, in which the treatments were contrasted with the absolute control (dose 0). Statistical analysis of Shannon's diversity index was carried out via a t test using standard procedures in the software PAST (Hammer et al. 2001). We calculated Spearman's correlation coefficients between spore count data and soil chemical data based on replicate data. Species-level diversity data for AMF were also analyzed via hierarchical grouping, based on occurrence of species, by the simple matching method with Ward's algorithm and Euclidean distance as the measurement unit. Statistical procedures were performed with the software STATISTICA (StatSoft, Inc. 2001) unless indicated elsewhere.

Table 2 Soil chemical attributes in an agricultural area after tannery sludge application (0 to 22.6 Mg ha^{-1}) in 3 sampling dates, at Rolândia, Paraná, Brazil

Attributes	Sampling date	Tannery sludge dose (Mg ha^{-1})					AT
		0	2.3	9.0	15.8	22.6	
Available P (mg dm^{-3})	12	24.3±1.7	24.3±2.8	32.3±2.9	35.8±2.2	43.8±4.3	–
	136	25.8±2.6	28.5±2.6	35.3±3.5	42.5±4.4	58.0±6.4	27.5±3.3
	271	22.0±2.4	29.0±6.4	26.7±1.2	49.3±4.5	58.0±10.9	29.5±6.0
Soil pH	12	5.3±0.1	5.6±0.0	5.7±0.1	5.8±0.1	6.0±0.1	–
	136	5.2±0.0	5.5±0.1	5.4±0.1	5.5±0.2	5.9±0.1	5.3±0.2
	271	5.0±0.1	5.4±0.1	5.3±0.1	5.5±0.2	5.8±0.1	5.1±0.2
EC (dS m^{-1})	12	1.5±0.2	4.1±0.4	9.1±1.2	13.8±1.5	21.2±2.9	–
	136	1.6±0.1	2.0±0.2	2.2±0.2	3.1±0.4	3.9±0.3	1.9±0.3
	271	1.6±0.3	1.8±0.2	2.0±0.2	2.4±0.2	2.7±0.1	1.6±0.2
Mineral N (mg kg^{-1})	12	18.0±5.8	49.2±8.7	111.1±37.0	136.5±34.9	207.2±49.3	–
	136	17.0±1.6	19.2±1.9	21.1±1.3	27.0±3.0	33.3±2.5	20.7±3.9
	271	9.7±1.1	11.8±2.9	14.5±1.5	16.5±2.3	17.7±3.7	13.5±2.6

Values represent the average followed by standard deviation (n=4)

AT agronomic treatment—corresponds to 120 kg ha^{-1} of N as urea, *EC* electrical conductivity

Results

AMF spore density

The application of tannery sludge decreased AMF spore density especially in the first and third sampling dates (Fig. 1). The observed spore density ranged from one to 49 spores per 50 cm^3 of soil, in which the highest average spore counts were observed in the control (no sludge). In the first sampling time, all treatments with tannery sludge caused reduction in the density of spores as compared to the absolute control (dose 0), while in the third sampling time, only the treatment with the smallest dose did not differ from the control. The agronomic treatment (N as urea) showed no effects on the density of spores in relation to the absolute control. Spearman's correlation coefficients showed significant negative relationship (P<0.1) between spore density and soil P content (−0.70), pH (−0.38), EC (−0.36), and mineral N (−0.21).

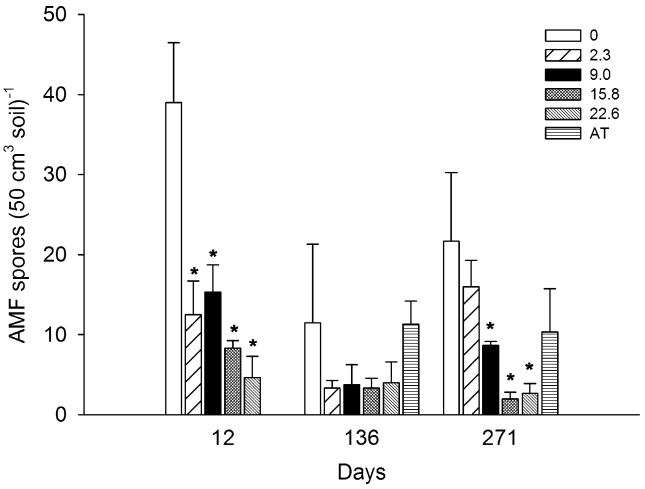

Fig. 1 Arbuscular mycorrhizal fungi spore densities in an agricultural soil under varying doses of tannery sludge (0 to 22.6 Mg ha^{-1}) at three sampling dates (12, 136, and 271 days after the second sludge application), in Rolândia, Paraná, Brazil. *Bars* indicate the standard deviation (n=4). *Asterisk* indicates significant difference from the control at each sampling date (P<0.05, t test)

Root colonization by AMF

No significant effect of tannery sludge application was observed on percentage of root colonization by AMF in corn plants (F=0.78, P>0.05). The overall root colonization level was 64%, ranging from 56% to 74% (data not shown).

AMF spore diversity

In the soil samples used to assess the diversity of AMF species, we also observed low spore counts and declining spore density with increasing doses of tannery sludge (F=5.11, P<0.01). The average counts varied from seven to 32 spores per treatment in 50 cm^3 of soil. Overall, 18 fungal species were identified belonging to the following genera: *Acaulospora* (six species), *Glomus* (five), *Gigaspora* (three), *Scutellospora* (two), *Paraglomus* (one), and *Ambispora* (one) (Table 3). While the genus *Acaulospora* accounted for the largest number of species, the genus

Table 3 Total number of identified spores of arbuscular mycorrhizal fungi (AMF) per treatment and average spore counting per family in an agricultural soil in the second year of the experiment under varying doses of tannery sludge (0 to 22.6 Mg ha^{-1}), in Rolândia, Paraná, Brazil

Species	Tannery sludge dose (Mg ha^{-1})					AT
	0.0	2.3	9.0	15.8	22.6	
Gigaspora decipiens Hall and Abbott	1	1	2	3	10	9
Gigaspora albida cf	0	0	0	0	0	2
Gigaspora sp1	5	0	0	0	0	1
Scutellospora pellucida (Nicol. and Schenck) Walker and Sanders	14	2	1	0	0	21
S. cerradensis Spain and Miranda	21	13	23	10	7	30
Acaulospora scrobiculata Trappe	38	14	5	4	1	8
Ac. mellea Spain and Schenck	1	1	0	1	0	1
Ac. morrowiae Spain and Schenck	0	0	0	0	0	3
Ac. spinosa Walker and Trappe	3	0	0	0	0	0
Ac. tuberculata Janos and Trappe	0	0	0	0	1	0
Acaulospora sp1	0	3	0	0	1	2
Glomus etunicatum cf	9	4	1	1	0	0
Glomus sp1	20	1	1	1	3	1
Glomus sp2	3	0	0	0	0	0
Glomus sp3	0	0	2	2	3	1
Glomus sp4	0	0	1	1	2	1
Paraglomus laccatum (Błaszk.) Renker, Błaszk. and Buscot	1	3	2	3	0	3
Ambispora appendicula (Schenck and Smith) Walker, Vestberg and Schuessler	1	3	0	0	0	1
Non-identified	9	5	4	1	0	7
Individuals	126	50	42	27	28	91
Richness of identified species	12	10	9	9	8	14
Shannon's diversity index	2.03	2.00	1.60*	1.94	1.74*	2.03
Family (average per family±SD)						
Gigasporaceae	10.3±7.1	4.0±3.7	6.5±5.0	3.3±2.1	4.3±4.8	15.8±9.7
Acaulosporaceae	10.5±9.7	4.5±5.8	1.3±1.0*	1.3±1.0*	0.8±1.0*	3.5±3.9
Glomeraceae	8.0±9.1	1.3±1.9*	1.3±1.0*	1.3±1.0*	2.0±1.4*	0.8±1.0*
Paraglomeraceae	0.3±0.5	0.8±1.5	0.5±1.0	0.8±1.0	0.0±0.0	0.8±1.5
Ambisporaceae	0.3±0.5	0.8±1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.3±1.5

AT agronomic treatment—corresponds to 120 kg ha^{-1} of N as urea

*$P<0.05$, values differ from the control (zero tannery sludge) by *t* test

SD standard deviation

Scutellospora dominated the community, accounting for 39% of the total spores.

The control and agronomic treatments showed the highest species richness (12 and 14 species, respectively) as well as the highest diversity index scores (2.03 in both treatments) (Table 3). Student's *t* tests of these index scores revealed that the plots that had received tannery sludge equivalent to 9.0 and 22.6 Mg ha^{-1} presented a significantly lower diversity of AMF species than the control (Table 3).

In treatments which received high doses of tannery sludge (9.0, 15.8, and 22.6 Mg ha^{-1}), 48–62% of the total spore density belonged to the family Gigasporaceae. Conversely, spore counts were evenly distributed among three families in the control treatment: Gigasporaceae, Acaulosporaceae, and Glomeraceae accounted for 33%, 33%, and 25% of total spores, respectively (Fig. 2). The relative density of Acaulosporaceae decreased from 36% at the smallest dose (2.3 Mg ha^{-1}) to 11% at the highest dose (22.6 Mg ha^{-1}), while the relative density of Glomeraceae increased from 10% to 29% in the same treatments.

The hierarchical grouping analysis of AMF species shows that spore community of treatments that received tannery sludge was different from the control and AT

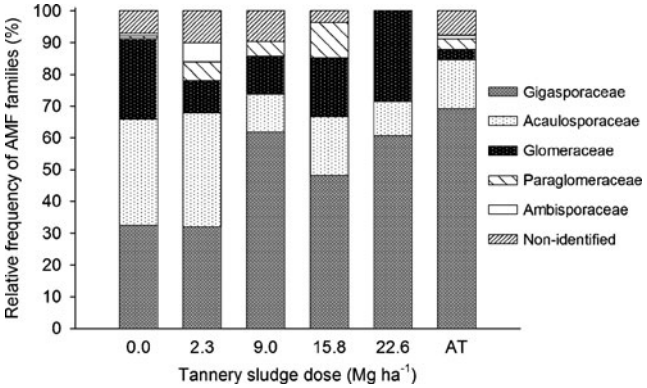

Fig. 2 Relative frequency of families of arbuscular mycorrhizal fungi, based on spores occurrence, in an agricultural soil under varying doses of tannery sludge (0 to 22.6 Mg ha^{-1}), in Rolândia, Paraná, Brazil. *AT* agronomic treatment, corresponds to 120 kg ha^{-1} of N in the form of urea

treatments (Fig. 3). The two smallest sludge doses were grouped together in the same branch of the figure, as well as the highest doses were grouped in the same sub-branch.

Discussion

AMF spore densities similar to those observed in this study have been recorded in areas polluted with tannery waste. Khade and Adholeya (2009) reported that spore counts ranged between 4 and 38 per 50 cm^3 of soil in a tannery sludge contaminated site. Conversely, Raman and Sambandan (1998) reported higher spore densities (up to 130 per 50 cm^3 of soil) in a tannery effluent polluted area. One possible cause of the low density observed in our study is the high natural soil fertility of the area, which was detected before the experiment was initiated. It is known that AMF are inhibited by high nutrient availability, especially by phosphorus (Jansa et al. 2006). Bhadalung et al. (2005) documented a decrease in spore density following fertilization with N and P as ammonium sulfate and triple superphosphate, respectively. Indeed, we detected a negative relationship between spore density and some soil nutrients, like phosphorus and mineral nitrogen. Therefore, sludge application may affect fungal sporulation by increasing the amounts of these nutrients in soil.

In this study, application of different amounts of tannery sludge had no effect on the percentage of root colonization of corn plants. Ortega-Larrocea et al. (2007) also observed that sewage sludge applied to the soil had no effect on root colonization. This result is puzzling since addition of tannery sludge increased soil nutrient availability and this is well known to decrease root colonization (Treseder 2004). Two hypotheses could explain these results. The first explanation is that the season in which we measured root colonization was favorable for spore germination and rapid colonization of new roots, which resulted in decreased spore density in the soil (Khade and Adholeya 2009). The argument in favor of the second explanation is that the percentage of root colonization rates maintained their high values because sampling was done during the flowering stage of the corn plants. Gavito and Varela (1993) documented higher levels of mycorrhizal colonization in corn during the flowering stage, and a decrease following maturity and senescence. However, it is noteworthy that other studies on plants growing in areas polluted with tannery waste have also reported high levels of AMF root colonization: up to 64% in *Prosopis juliflora* (Raman and Sambandan 1998), up to 80% in *Acacia arabica* (Khan 2001), and up to 100% in *Parthenium* sp. (Khade and Adholeya 2009).

The control and agronomic treatments showed higher species richness than the treatments that received tannery sludge, what may reflect the history of the area, which had been under no-tillage and crop rotation with grain and legume crops for more than 10 years prior to the experiment. This type of soil management is known to favor the richness and consequently the diversity of AMF (Oehl et al. 2003; Castillo et al. 2006). However, after the soil received tannery sludge, some changes in the richness of species and Shannon's diversity index were observed, usually a decrease with increasing sludge doses. Lower AMF diversity was also observed by Khade and Adholeya (2009) and Khan (2001) in areas polluted by tannery waste, when compared to control sites. The results of this study thus support Khan's (2001) hypothesis that the application of tannery sludge exerts selective pressure on AMF, favoring the sporulation of some taxa and inhibiting others.

Most studies to date have recorded the number of AMF species in areas polluted by tannery waste and, to our

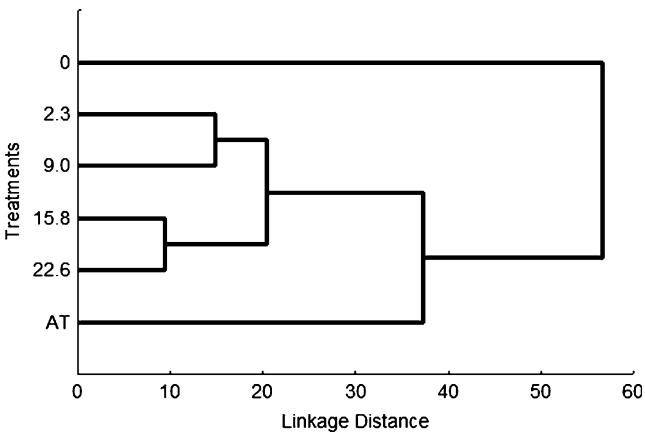

Fig. 3 Hierarchical clustering analysis of arbuscular mycorrhizal fungi based on the distribution of species in an agricultural soil under varying doses of tannery sludge (0 to 22.6 Mg ha^{-1}) in Rolândia, Paraná, Brazil. *AT* agronomic treatment, corresponds to 120 kg ha^{-1} of N in the form of urea

knowledge, this is the first study reporting changes in the diversity of the AMF spore community in areas that received controlled applications of tannery sludge. Selvaraj et al. (2005) recorded nine AMF species and three genera in an area polluted by industrial effluents from tanneries in India. Raman and Sambandan (1998), also in India, at three sites of tannery effluent polluted soils, found 15 AMF species and four genera. In our study, we identified 13 AMF species and six genera considering only the soil that received tannery sludge. Moreover, the AMF species richness may have been underestimated since trap cultures with different host plants in the greenhouse might have revealed additional AMF species (Moreira et al. 2007). Nevertheless, the possibility of some of the AMF species identified in the field to disappear in trap pots must also be recognized.

Shifts in the composition and structure of the AMF community have been found in studies on other types of waste (Stahl and Williams 1986; Del Val et al. 1999; Faryal et al. 2007). It is difficult to link general soil characteristics to AMF species occurrence, but the most possible causes are shifts in soil chemical attributes caused by the applied waste materials. The sludge used in our experiment was alkaline, so that applying increasing amounts of tannery sludge increased soil pH. Changes in pH affect several other soil properties, like the availability of elements, and these changes directly or indirectly determine the distribution of AMF species (Coughlan et al. 2000). In Brazil, species belonging to the family Acaulosporaceae are typically found in low-pH soils (Moreira et al. 2007); therefore, an increase in soil pH is likely to be the reason for the decrease of the relative frequency, but also, more importantly, in absolute frequency of this family along with increasing sludge doses.

Ezawa et al. (2000) showed that the AMF spore community was influenced by the levels of available phosphorus in soils following application of farmyard manure (400 $t\,ha^{-1}\,year^{-1}$), chemical fertilizer (370 kg of N $ha^{-1}\,year^{-1}$, as urea and 420 kg $ha^{-1}\,year^{-1}$ of P_2O_5, as magnesium phosphate), and alfalfa meadow (*Medicago sativa* L.). In that study, species of *Glomus* and *Acaulospora* were observed only at the highest phosphorus levels. In our study, we found an increase in the density of spores for the species *Gigaspora decipiens*, *Glomus* sp3, and *Glomus* sp4, along the tannery sludge doses, and consequently increasing P levels in soil, while in general the *Acaulospora* species decreased.

When the relative spore density is considered, Gigasporaceae was the dominant family in the highest sludge dose. This observation agrees with Khan (2001) who detected *Gigaspora* spp. being dominant in areas polluted with tannery waste. Nevertheless, this greater dominance in our study was due to only one species (*G. decipiens*) that increased with the doses of tannery sludge, showing a tolerance to stressing factors apparently negative to the other species, even within the same family. Such behavior, although less evident, seems also to occur for *Glomus* sp3 and *Glomus* sp4. It is known that different species, and even isolates from the same species, have different tolerance levels to stressing conditions, but the mechanisms involved are not clear (Leyval et al. 1997).

The decline in AMF diversity may possibly promote the selection for species with low symbiotic efficiency, which might compromise plant growth (Johnson 1993) since adverse conditions may favor the fungal species with the highest survival rates and not the highest efficiency (Kiers et al. 2002). According to Khade and Adholeya (2009), changes in soil AMF diversity caused by anthropogenic disturbance could cause a long-term reduction in beneficial mycorrhizal symbioses since reestablishment and/or adaptation of the AMF population is slow. These statements highlight the need for additional long-term studies on the application of tannery sludge in agricultural areas, so that this practice may not impair the biological health of soils. In the specific case of AMF, it is important to determine doses of tannery sludge that do not reduce the diversity and mycorrhizal inoculum potential, and to assess the symbiotic efficiency of AMF species exposed to these types of effluents.

Our results support the hypothesis that applying tannery sludge to agricultural soil decreases AMF spore density and species richness. However, this practice did not affect mycorrhizal percentage of root colonization rates, indicating that mycorrhizal infective capacity was not impaired in this soil, thus in disagreement with our hypothesis. Decrease in sporulation density in soil does not necessarily mean that the mycorrhizal community is being impaired since the levels of root colonization remained stable. However, it is very important to consider the species richness and diversity whenever applications of high doses of tannery sludge are planned.

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