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Responses of soil microbial catabolic diversity to arbuscular mycorrhizal inoculation and soil disinfection

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Abstract Although it is usually admitted that arbuscular mycorrhizal (AM) fungi are key components in soil bio-functioning, little is known on the response of microbial functional diversity to AM inoculation. The aims of the present study were to determine the influence of *Glomus intraradices* inoculum densities on plant growth and soil microflora functional diversity in autoclaved soil or non-disinfected soil. Microbial diversity of soil treatments was assessed by measuring the patterns of in situ catabolic potential of microbial communities. The soil disinfection increased sorghum growth, but lowered catabolic evenness (4.8) compared to that recorded in the non-disinfected soil (6.5). *G. intraradices* inoculation induced a higher plant growth in the autoclaved soil than in the non-disinfected soil. This AM effect was positively related to inoculum density. Catabolic evenness and richness were positively correlated with the number of inoculated AM propagules in the autoclaved soil, but negatively correlated in the non-disinfected soil. In addition, after soil disinfection and AM inoculation, these microbial functionality indicators had higher values than in the autoclaved or in the non-disinfected soil without AM inoculation. These results are discussed in relation to the ecological influence of AM inoculation, with selected fungal strains and their associated microflora on native soil microbial activity.

Keywords Arbuscular mycorrhizal fungi · Soil disturbance · Microbial catabolic diversity

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Introduction

Arbuscular mycorrhizal (AM) fungi are essential components of sustainable soil–plant systems (Schreiner et al. 2003) because of their role in increasing plant growth and nutrient uptake (Smith and Read 1997). The AM symbiosis influence the structure and functioning of surrounding bacterial communities (Marshner et al. 2001; Marshner and Timonen 2005), a microbial compartment which is commonly named "mycorrhizosphere" (Linderman 1988). Organic compounds produced by external mycelium can provide microsites for microbial colonisation and growth (Johansson et al. 2004), and it has been shown that the soil around fungal hyphae (the hyphosphere) supports different bacterial activities and bacterial community composition from those of the mycorrhizosphere (Andrade et al. 1998;

Mansfeld-Giese et al. 2002; Marshner and Timonen 2005). Microorganisms in the hyphosphere of AM fungi may affect the functions of the external AM mycelium such as nutrient and water transport from soil to the host plant (Bethlenfalvay et al. 1999; Mansfeld-Giese et al. 2002).

It has already been shown that AM inoculation of plants under semi-arid conditions is very efficient in establishing plants on disturbed soil (Estaun et al. 1997). Besides the influence of AM fungi on the plant growth, these fungal symbionts also improve physical, chemical and biological soil properties (Requena et al. 2001). The role of soil microbial diversity in the functioning of soils is largely unknown (Degens et al. 2001), but decreases in soil microbial diversity may alter the resistance of soils to stress or disturbance (Giller et al. 1997). It has been suggested that below-ground diversity is a major factor contributing to the maintenance of plant diversity and to ecosystem functioning (Van der Heijden et al. 1998). Although it is usually admitted that AM fungi are key ecological factor in governing the cycles of major plant nutrients, few studies on the response of microbial functional diversity (component of overall diversity in soil) to AM inoculation exist in the literature (Caravaca et al. 2003; Alguacil et al. 2005; Duponnois et al. 2005a, b).

In this study, we hypothesised that AM fungal inoculation will influence the functional diversity of soil microbial communities and that this impact will depend on the extent of AM soil colonisation and of loss of soil biological diversity. The functional diversity of microbial populations in bulk soil was evaluated by measuring catabolic response profiles (CRPs) according to Degens et al. (2000). In the present study, the soil sampling area did not support a vegetation cover during the dry season (November to May) and during the wet season; this area was dominated by a mixture of herbaceous species.

Materials and methods

Plant and fungal inoculum

Sorghum seeds (*Sorghum bicolor* L.) were surface-sterilized with 1% NaOCl for 15 min and rinsed with demineralised water. They were pre-germinated for 2 days in Petri dishes on humid filter paper at 25°C in the dark. The germinating seeds were used when rootlets were 1–2 cm long.

The AM fungus *Glomus intraradices*, Schenk and Smith (DAOM 181602, Ottawa Agricultural Herbarium) was used because of its high ability to form abundant internal vesicles (Duvert et al. 1990). It was propagated on leek (*Allium porrum* L.) for 12 weeks under greenhouse conditions in calcined clay (Plenchette et al. 1996). Leek plants were uprooted, gently washed, and colonised roots were hand-cut into 1–3 mm long pieces, bearing around 250 vesicles cm^{-1}, each considered as one propagule (Plenchette 2000; Duvert et al. 1990). To obtain a logarithmic scale of inoculum density (0, 1, 3, 10, 30, 100 propagules per 100 g soil), AM root pieces were counted under a dissecting microscope and, for each inoculum rate, the number was adjusted to 100 root pieces per 100 g of soil with non-mycorrhizal leek roots, prepared as above. Root pieces were then thoroughly mixed with the soil.

Experimental design and quantitative evaluation

Soil was collected in an experimental station localised at Gampella (20 km from Ouagadougou, Burkina Faso; 1°21′W, 12°25′N), crushed and passed through a 2-mm sieve. One part of the soil was subjected to autoclaving (Klein et al. 1986), and the other part was not (non-disinfected). The soil was filled into 0.5-l pots. Then, it was autoclaved for 60 min at 120°C. After cooling, it was subjected to a second disinfection cycle (120°C, 60 min) to ensure the entire elimination of the native microflora at the beginning of the experiment (Duponnois, unpublished data). Contaminant microorganisms will subsequently colonise the soil. The physico-chemical characteristics of the native soil were as follows: pH (H_2O) 5.9; clay (%) 8.75; fine silt (%) 4.75; coarse silt (%) 15.1; fine sand (%) 43.6; coarse sand (%) 27.8; total carbon (%) 2.04; total nitrogen (%) 0.03; Olsen phosphorus 3.85 mg/kg; total phosphorus 95.6 mg/kg. After autoclaving, they were as follows: pH (H_2O) 5.6; clay (%) 7.75; fine silt (%) 2.75; coarse silt (%) 17.1; fine sand (%) 42.6; coarse sand (%) 29.8; total carbon (%) 0.35; total nitrogen (%) 0.04; Olsen phosphorus 4.3 mg/kg; total phosphorus 96.8 mg/kg.

For each soil treatment and each inoculum density, plastic pots (5.5 cm diameter; 6 cm high) were filled with 100 g of soil containing the required number of AM propagules, and one pre-germinated seed of Sorghum was planted per pot. Pots were arranged in a randomised complete block design with eight replicates per treatment. They were placed in greenhouse conditions (30°C day, 20°C night, 10-h photoperiod) at IRD, Ouagadougou, and watered daily with deionised water without the addition of nutrients.

After 2 months of culturing, the plants were harvested, and the oven-dried weight (1 week at 65°C) of shoot was recorded. The soil of each pot was carefully mixed and kept at 4°C for further use. The root systems were gently washed, cleared and stained according to the method of Phillips and Hayman (1970). About 50 1-cm root pieces were observed per plant under a microscope (magnification, ×250). The extent of mycorrhizal colonisation was expressed as [the number of mycorrhizal root pieces]/[total

number of observed root pieces]×100. Remaining roots were oven-dried (1 week at 65°C) and weighed.

Microbial functional diversity of soil treatments was assessed by measuring the patterns of in situ catabolic potential of microbial communities (Degens and Harris 1997). One gram of equivalent dry soil was moistened with each of the 29 substrates suspended in 2 ml sterile distilled water in 10-ml bottles (West and Sparling 1986). CO_2 production from basal respiratory activity in the soil samples was measured by adding 2 ml sterile distilled water to 1 g equivalent dry weight of soil. After the addition of the substrate solutions to soil samples, bottles were immediately sealed with a Vacutainer stopper and incubated at 28°C for 4 h in the darkness. The respired CO_2 in the headspace of each bottle was determined by taking a 1-ml syringe sample and by analysing CO_2 concentration using an infrared gas analyser (Polytron IR CO_2, Dräger™) in combination with a thermal flow meter (Heinemeyer et al. 1989). Carbon dioxide measurements were subtracted from the CO_2 basal production and were expressed as microgram of CO_2 per gram of soil per hour. Among 29 substrates, there were eight amino acids (L-cystein, L-serine, L-phenylalanine, L-asparagine, L-histidine, L-lysine, L-glutamic acid, L-tyrosine), two amines (L-glutamine, D-glucosamine), three carbohydrates (D-Glucose, D-mannose, sucrose), two amides (*N*-methyl-D-glucamine, succinamide) and 14 carboxylic acids (ascorbic acid, formic acid, gallic acid, fumaric acid, gluconic acid, quinic acid, malonic acid, α-ketoglutaric acid, α-ketobutyric acid, succinic acid, tartaric acid, uric acid, oxalic acid, malic acid). The amines and amino acids were added at 10 mM, whereas the carbohydrates were added at 75 mM and the carboxylic acids at 100 mM (Degens and Vojvodic-Vukovic 1999). Catabolic richness and catabolic evenness were calculated to evaluate the catabolic diversity of soil treatments. Catabolic richness, *R*, expresses the number of substrates used by microorganisms in each soil treatment. Catabolic evenness, *E*, represents the variability of a substrate among the range of tested substrates, and it was calculated using the Simpson–Yule index, $E = 1/p_i^2$ with p_i=[respiration response to individual substrates]/[total respiration activity induced by all substrates for a soil treatment] (Magurran 1988).

Statistical analysis

Data were treated with one-way and three-way analysis of variance (ANOVA). Means were compared using PLSD Fisher test ($p<0.05$). Mycorrhizal indexes were transformed by arcsin (sqrt) before statistical analysis.

Results

Plant growth (shoot and root biomasses) was significantly higher in the autoclaved soil than in the non-disinfected soil ($p<0.05$; Table 1). In both soil treatments, plant growth and mycorrhizal colonisation were significantly linked with the rates of AM inoculation (Table 1). In autoclaved soil, regression coefficients (r^2) were 0.976 ($p<0.001$) and 0.639 ($p<0.05$) for the shoot and root biomass, respectively, and

Table 1 Influence of AM inoculation at different rates on growth and mycorrhizal colonisation of sorghum plants after a 2-month culture in an autoclaved or non-disinfected soil

		AM inoculum rates (number of inoculated propagules per 100 g of soil)					
		0	1	3	10	30	100
Autoclaved soil	Shoot biomass (mg dry weight)	121.4 a[a]	128.3 ab	141.5 b	142.1 b	151.1 c	161.1 d
	Root biomass (mg dry weight)	34.1 a	46.9 b	57.4 c	57.6 c	57.1 c	59.7 c
	Total biomass (mg dry weight)	155.4 a	175.2b	198.3 c	199.7 c	208.2 c	220.8 d
	Root/shoot ratio	0.28 a	0.37 b	0.41 b	0.41 b	0.38 b	0.37 b
	Mycorrhizal colonisation (%)	0.0 a	5.0 b	21.7 c	30.1 c	28.3 c	51.7 d
Non-disinfected soil	Shoot biomass (mg dry weight)	68.7 a	71.4 ab	78.5 bc	81.4 c	79.4 bc	84.8 c
	Root biomass (mg dry weight)	26.1 a	26.4 a	27.2 a	28.4 a	29.0 a	29.7 a
	Total biomass (mg dry weight)	94.8 a	97.8 ab	105.7 b	109.8 b	108.4 b	114.4 b
	Root/shoot ratio	0.38 a	0.37 a	0.35 a	0.35 a	0.36 a	0.35 a
	Mycorrhizal colonisation (%)	0.0 a	3.3 a	18.3 b	16.7 b	30.0 c	46.7 d

[a] Data in the same line followed by the same letter are not significantly different according to the one-way ANOVA ($p<0.05$).

in non-disinfected soil, they were 0.815 ($p<0.001$) and 0.974 ($p<0.001$). These associations were also found with AM colonisation indexes ($r^2=0.974$, $p<0.001$ and $r^2=0.948$, $p<0.001$, in the autoclaved and non-disinfected soils, respectively).

Soil disinfection has significantly decreased the catabolic responses to glucamine, L-serine, L-tyrosine, D-glucosamine and α-ketogutaric, α-ketobutyric, oxalic, formic, succinic acids (Table 2). In contrast, soil disinfection increased catabolic responses to L-lysine, ascorbic and malonic acids (Table 2).

The influence of AM inoculation in individual catabolic responses also depended on soil treatments. In the autoclaved soil, AM inoculation significantly increased the catabolic responses to sucrose, glucamine, L-serine, L-phenylalanine, L-tyrosine, D-glucosamine, ascorbic, malic and uric acids, decreased responses to quinic and gallic acids, whereas in the non-disinfected soil, fungal inoculation significantly increased catabolic responses to L-cystein, L-serine, L-lysine and tartaric acid and significantly decreased catabolic responses to glucamine, L-histidine and D-glucosamine (Table 2).

Changes in CRP's in response to the increased rates of AM inoculations are given in Fig. 1. For both soil treatments, highest respiration responses were recorded after adding ketoglutaric and ketobutyric acids to the soils (Fig. 1). Increasing the number of AM propagules in the autoclaved soil significantly enhanced catabolic responses to sucrose, succinamide, glucamine, L-cystein, L-serine,

Table 2 Significance of autoclaving, *G. intraradices* inoculation (AM inoc.) and intensity of inoculation (AM intensity) on individual catabolic responses resulting from the addition of simple organic substrates to the soil

Substrates	Treatments				
	Autoclaving	AM inoc.		Intensity of AM inoc.	
		Autoclaved soil	Non-disinfected soil	Autoclaved soil	Non-disinfected soil
Carbohydrates					
D-Glucose	NS*	NS	NS	NS	NS
D-Mannose	NS	NS	NS	NS	NS
Sucrose	NS	(+)**	NS	(+)**	NS
Amides					
Succinamide	NS	NS	NS	(+)**	NS
Glucamine	(−)***	(+)**	(−)**	(+)**	NS
Amino acids					
L-Cystein	NS	NS	(+)**	(+)**	NS
L-Serine	(−)**	(+)**	(+)**	(+)**	(+)**
L-Phenylalanine	NS	(+)**	NS	(+)**	NS
L-Asparagine	NS	NS	NS	NS	NS
L-Histidine	NS	NS	(−)**	NS	(−)***
L-Lysine	(+)**	NS	(+)**	NS	(+)**
L-Glutamic Ac	NS	NS	NS	NS	(+)**
L-Tyrosine	(−)***	(+)**	NS	(+)**	(+)**
Amines					
L-Glutamine	NS	NS	NS	NS	NS
D-Glucosamine	(−)**	(+)***	(−)**	(+)**	(−)**
Carboxylic acids					
α-Ketobutyric acid	(−)***	NS	NS	(+)***	(−)***
α-Ketoglutaric acid	(−)**	NS	NS	NS	NS
Fumaric acid	NS	NS	NS	NS	(+)***
Oxalic acid	(−)**	NS	NS	(+)**	NS
Tartaric acid	NS	NS	(+)***	(+)***	NS
Gluconic acid	NS	NS	NS	NS	NS
Ascorbic acid	(+)**	(+)**	NS	(+)**	(+)***
Malonic acid	(+)***	NS	NS	NS	(+)**
Malic acid	NS	(+)***	NS	(+)***	NS
Quinic acid	NS	(−)***	NS	(−)***	(+)**
Formic acid	(−)***	NS	NS	NS	(−)***
Gallic acid	NS	(−)**	NS	NS	NS
Succinic acid	(−)***	NS	NS	NS	NS
Uric acid	NS	(+)***	NS	(+)***	NS

*Not significant at $p<0.05$
**Significant at $p<0.01$
***Significant at $p<0.05$
(+) Significant increase at $p<0.05$
(−): Significant decrease at $p<0.05$
NS Not significant

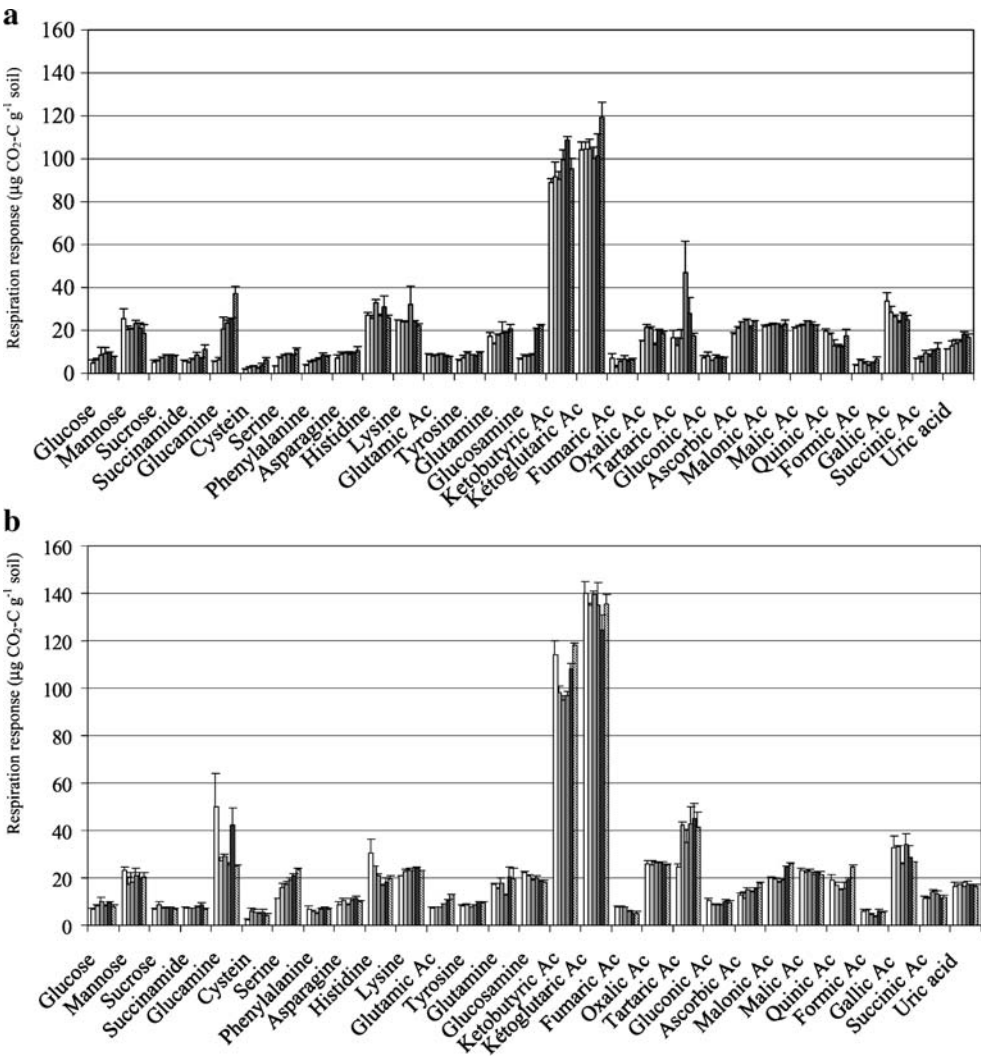

Fig. 1 Catabolic response profiles in autoclaved soil (**a**) or in non-disinfected soil (**b**) after treatment with increasing levels of AM inoculations. (*empty*, 0 inoculum piece (control); *white*, one inoculum piece; *grey*, three inoculum pieces; *dark grey*, ten inoculum pieces; *black*, 30 inoculum pieces; *striped*, 100 inoculum pieces. Error bars represent standard errors (n=8)

L-phenylalanine, L-tyrosine, D-glucosamine and α-ketobutyric, oxalic, tartaric, ascorbic, malic and uric acids, whereas it only decreased catabolic responses to quinic acid (Table 2) and, in the non-disinfected soil, it increased catabolic responses to L-serine, L-lysine, L-glutamic acid, L-tyrosine and fumaric, ascorbic, malonic and quinic acids, whereas it decreased catabolic responses to L-histidine, D-glucosamine, α-ketobutyric and formic acids (Table 2).

Table 3 Regression analysis between the number of inoculated AM propagules and the mean catabolic responses with each category of organic substrates (carbohydrates, amides, amines, amino acids, organic acids) in the autoclaved and non-disinfected soils

Substrate category	Equation	r^2	p
Non-disinfected soil			
Carbohydrates	$y = 11.96 - 0.034x^{(1)}$	0.0002	0.95
Amides	$y = 22.57 - 2.50x$	0.064	0.31
Amines	$y = 18.49 - 0.01x$	0.0001	0.99
Amino acids	$y = 11.99 + 0.83x$	0.41	0.0045
Organic acids	$y = 31.88 + 0.88x$	0.085	0.24
Autoclaved soil			
Carbohydrates	$y = 11.83 + 0.37x$	0.019	0.58
Amides	$y = 5.51 + 8.93x$	0.79	0.0001
Amines	$y = 10.14 + 5.56x$	0.73	0.0001
Amino acids	$y = 11.13 + 1.06x$	0.32	0.015
Organic acids	$y = 26.78 + 1.52x$	0.32	0.015

Responses to the amino acid group were positively linked with the intensity of AM propagules added to the non-disinfected soil, whereas no significant links were found with the other substrate groups (Table 3). In the autoclaved soil, catabolic responses to amide, amine, amino acid and carboxylic acid groups were positively linked with the number of AM propagules (Table 3).

After 2 months of culturing, in the treatments without AM inoculation, catabolic evenness and richness were significantly higher in the non-disinfected soil (6.5 and 21, respectively) than in the autoclaved soil (4.8 and 14.2, respectively; Fig. 2). Inoculation with increased AM propagules significantly decreased catabolic diversity in non-disinfected soil, whereas it increased it in autoclaved soil (Fig. 2).

Discussion

Soil sterilization not only eliminates root pathogens but also changes soil chemical properties, and can result in an initial nutrient flush and the temporary absence of nutrient immobilisation into the soil microbial biomass as well, eliminating root pathogens (Bever et al. 1997; Troelstra et al. 2001). These phenomena could explain the unexpected increased plant biomass in autoclaved soil.

Soil microbial activities include decomposition, nutrient transformations, plant growth promotion/suppression and modification of soil physical processes (Giller et al. 1997; Wardle et al. 1999). Part of this microbial functional diversity can be estimated by measuring catabolic response profiles (Degens and Harris 1997; Degens et al. 2000) and, subsequently, by calculating catabolic evenness, a component of microbial functional diversity defined as the uniformity of used substrate (Degens et al. 2001). It has been found that catabolic diversity in soils with low catabolic evenness was less resistant to stress or disturbance than in soils with high catabolic evenness (Degens et al. 2001). The soil used in the present study had a catabolic evenness of 6.5, which is very low in comparison to those recorded in temperate soils (Stevenson et al. 2004). This depleted microbial functional diversity has been recorded in a previous study with another sandy soil collected in Burkina Faso (Duponnois et al. 2005a). For instance, catabolic evenness ranged from 18.2 (soils under crops) to 21.4 (soils under indigenous vegetation) in New Zealand (Stevenson et al. 2004). Soil disinfection induced a depletion of soil organic C and had a lower catabolic evenness (4.8) than that recorded in the non-disinfected soil. This result is in accordance with previous studies from which it was concluded that the relationship exists between organic C pools and microbial catabolic diversity in soils (Degens et al. 2000). According to Degens et al. (2000), the theoretical catabolic evenness of the soil used in the present study would be 17.1 (y=1.3 log$x$+15.4 with y=catabolic evenness and x=total organic C, mg/cg soil). The large discrepancy between this tropical soil and temperate soils used in most studies could be explained by the influence of plant cover on the multiplication of soil microorganisms. Degens et al. (2000) have determined the effects of land

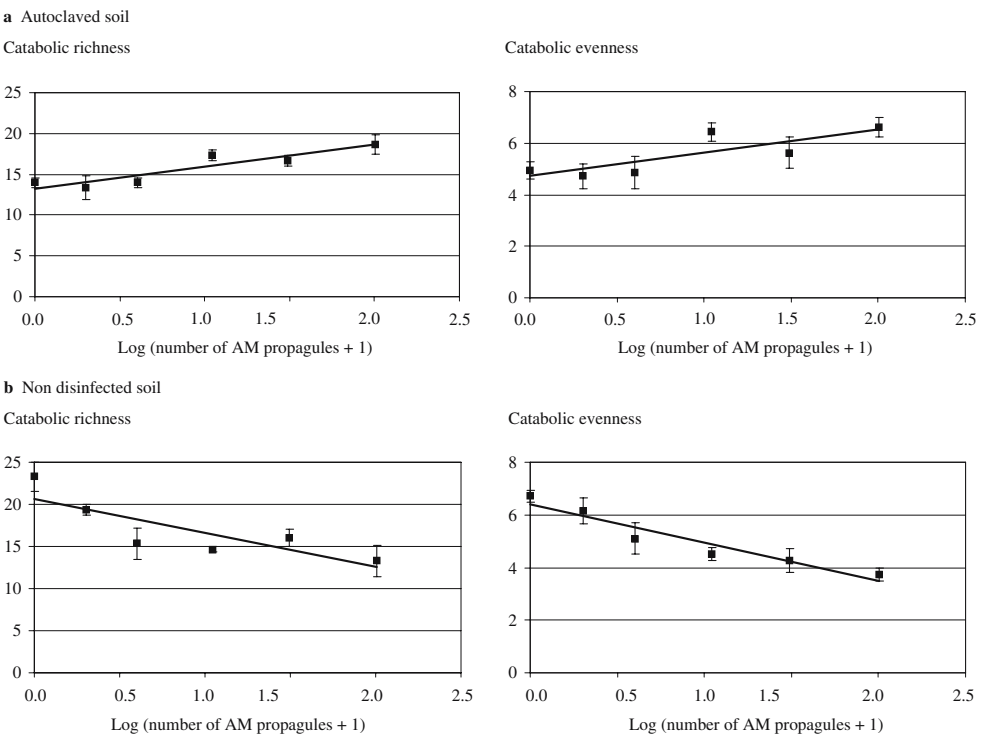

Fig. 2 The effect of AM inoculum intensities on catabolic richness and catabolic evenness of the autoclaved soil (**a**) (Regression curves are y=13.24+2.67x; r^2=0.84; p<0.0001 and y=4.71+0.91x; r^2=0.68; p<0.05, respectively) and of the non_disinfected soil (**b**) (Regression curves are y=20.67−4.06x; r^2=0.69; p<0.05 and y=6.40−1.46x; r^2=0.93; p<0.0001)

uses on microbial catabolic diversity and the relationships between these effects and pools of organic C in soils. The range of soils with varying land uses was sampled from areas with different vegetation types (long-term non-arable horticulture, commercial *Pinus radiata* plantation, etc.). It is well known that plant roots positively influence the multiplication of soil microflora. The low levels of microbial activities observed in bulk soils, free of roots in the present study, are probably due to the fact that the soil sampling area did not support a vegetation cover during the dry season (November to May) and, during the wet season, this area was dominated by a mixture of herbaceous species.

In Sahelian areas, desertification is a result of anthropogenic impacts that induce subsequent loss of major physico-chemical and biological soil properties (soil structure, plant nutrient availability, organic matter content, microbial activities; Piéri 1991). In particular, it has already been shown that AM fungal inoculation of plants is very efficient under semi-arid conditions (Estaun et al. 1997; Duponnois et al. 2001a). In both soil treatments (autoclaving or non-disinfection), the positive fungal effect was correlated with the number of infective AM propagules inoculated into the soil, and it suggests that AM effect is dependent on mycorrhizal soil infectivity (Sylvia 1990; Duponnois et al. 2001b). However, *G. intraradices* inoculation involved higher stimulation of plant growth in autoclaved than in non-disinfected soil. Microbial interactions and native AM fungi may decrease the promoting effect of the introduced AM fungal isolate on the plant growth (Duponnois et al. 2005b). A significant positive effect was recorded at three-propagule inoculum rate. This result showed that this AM isolate was very competitive against native microflora as has been demonstrated in previous studies (Duponnois et al. 2005b; Sanon et al. 2006).

AM fungi modify root functions (i.e. root exudation) and selectively influence rhizosphere populations (Andrade et al. 1998). As microbial communities around AM roots differ from those of the rhizosphere, this microbial compartment is commonly named "mycorrhizosphere" (Linderman 1988). The mycorrhizosphere is usually divided in two different zones, one under the dual influence of root and fungal partners (mycorrhizosphere) and the other subjected to the AM hyphae (the mycosphere and hyphosphere; Andrade et al. 1998; Marschner and Baumann 2003; Marshner and Timonen 2005). In addition, fungal activities could also modify the functioning of microbial communities (Schreiner et al. 2003; Duponnois et al. 2005b; Sanon et al. 2006; Ouahmane et al. 2006). In the present study, it has been recorded that catabolic evenness and richness were positively correlated with the number of AM propagules in an autoclaved soil and negatively correlated in the same non disinfected soil. In non-disinfected soil, the introduced inoculum of *G. intraradices* induced disequilibria in microbial functionalities. Hence, it suggested that AM inoculation of the non-autoclaved soil might increase the susceptibility of soil microflora to stress and disturbance (Degens et al. 2001). Although *G. intraradices* inoculation had stimulated plant growth, this fungal inoculant had not improved soil microbial attributes contributing to soil quality. In contrast, after soil disinfection and AM inoculation, catabolic evenness and richness were significantly higher than in the control (non-inoculated soil) and in the non-disinfected soil without AM inoculation.

Root pieces used as inoculum were probably colonised with microbial populations qualitatively and quantitatively different from those associated with non-mycorrhized root pieces. Hence, the introduction of mycorrhizosphere microflora could also contribute to the enhancement of microbial activity in disinfected soil. The lack of appropriate control (soil inoculation with filtered AM root washings) does not allow to determine the direct effect of AM fungal inoculation on soil microbial diversity from that induced by the mycorrhizosphere populations.

Chitin is one of the most abundant polysaccharides on earth and occurs in many groups of organisms, mainly the fungi, arthropods and marine invertebrates (Hunt 1970), and its hydrolysate monomer is D-glucosamine (Flannery et al. 2001). It indicates that among methods for monitoring fungal biomass in solid-state fermentation (enzymatic activity, respiration rate, etc.), glucosamine can be considered as a good biomass indicator (Desgranges et al. 1991). Catabolic response to glucosamine increased with the number of inoculated AM propagules, whereas it decreased in the non disinfected soil, suggesting that the fungal biomass was larger in the autoclaved soil than in the non-disinfected one, and this greater fungal colonisation involved a higher effect on the microbial functionalities.

The r–K strategy suggests that there are differences between microorganisms in their ability to exploit and survive in different environment (Andrews and Harris 1986). Generally, r-strategists are considered to dominate disturbed environments, and K-strategists are characteristics of stable environments (Pianka 1970). It has been reported that rhizosphere soils contain a greater proportion of culturable bacteria that were r-strategists and were, therefore, able to respond and grow quickly in response to easily available nutrients (Sarathchandra et al. 1997). In our study, increasing the number of AM propagules in the autoclaved soil significantly enhanced catabolic responses to compounds that have been reported in root exudates (Curl and Truelove 1986; Gebre and Tschaplinski 2002). Hence, *G. intraradices* inoculation associated with its mycorrhizosphere microflora has probably stimulated the multiplication of contaminant r-strategist microorganisms (i.e.

Bacillus spp., *Pseudomonas* spp.) probably coming from the irrigation tap water. This positive AM fungal effect in microbial functionalities was related with the extent of AM fungal colonisation of soil. In contrast, *G. intraradices* inoculation in non-disinfected soil could decrease soil microbial catabolic diversity by inhibiting the multiplication of r-strategist microorganisms. It has been reported that populations of total culturable bacteria decreased in mycorrhizal-inoculated rhizospheres (Vazquez et al. 2000).

The present study confirms that the AM symbiosis contributes to soil microbial diversity and that this effect is largely dependent on the extent of AM fungal establishment in roots and soil. It shows that controlled AM inoculation could be of particular relevance in disinfected soils, by not only stimulating plant growth, but also improving soil catabolic evenness and, consequently, increasing the resistance of microbial community to stress and disturbance.

In non-disinfected soil, AM inoculation had opposite effects, and it reduced the soil catabolic evenness. These results question the ecological influence of inoculation with selected AM fungal strains and associated microflora on the native soil microbial diversity. In field experiments, the impact of AM inoculation has mainly been evaluated by measuring plant growth, but rarely by studies of soil microbial characteristics. As AM fungal inoculation of plants could not only favour plant establishment (Jasper 1994) but also affects microbiological activities contributing to soil quality, soil microbial modifications induced by the introduced AM inoculum should be considered in revegetation strategies (Franson and Bethlenfalvay 1989).

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