Fungi in Danish soils under organic and conventional farming

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Abstract

A multi-soil study was conducted in Denmark including 29 sites, 8 classified as 'Organic', 11 as 'Conventional with manure and synthetic fertilisers' and 10 as 'Conventional with synthetic fertilisers'. The variability of fungal abundance within the three farming systems and the long-term effects of different farming systems on fungal propagules in soil were evaluated.

Fungal abundance showed large variations within all three farming systems and this variability reduced the possibility to obtain general conclusions on fungal composition in soils under different farming systems. This was illustrated by the results on total propagule numbers of filamentous fungi and yeasts. Penicillium spp. and Gliocladium roseum were more abundant under organic than conventional farming, while Trichoderma spp. were most abundant in conventionally farmed soils with synthetic fertilisers. These results were not altered after adjusting for possible differences in basic soil properties like total-C and N, extractable P, CEC, base saturation and soil density. The paper discusses whether the differences in fungal abundance are characteristics of a farming system itself or associated with certain management factors being more prevalent in one farming system than the other.

Key words: Farming system, bioindicator, Penicillium, Gliocladium roseum, Trichoderma, Generalized-linear-mixed-models, Poisson-mixed-regression

Introduction

Organic farming is based on management principles assumed to enhance soil microbial biomass, diversity, and activity (Anderson and Domsch, 1989; Domsch et al., 1983;

Schnürer et al., 1985; Dick, 1992). Over the last decades, numerous studies have been undertaken to verify whether organically and conventionally cultivated soils actually differed with regard to soil microbial characteristics (Bolton et al., 1985; Doran et al., 1987; Fraser et al., 1988; Elmholt and Kjøller, 1989; Heinonen-Tanski, 1990; Sivapalan et al., 1993; Knudsen et al., 1995; Elmholt, 1996; Knudsen et al., 1999; Ryan, 1999; Jensen et al., 2000; Bullock et al., 2002; Mäder et al., 2002; Schjønning et al., 2002; Shannon et al., 2002). Most of these studies were based on limited numbers of soils and did not address the diversity of management options within each farming system. This aspect is, however, quite relevant. For instance, conventional farms with cattle production that use manure and diverse crop rotation are much more similar to organic farms than conventional farms based on monoculture and using synthetic fertilisers. For any pertinent comparison between organically and conventionally cultivated soils, it is of utmost importance to know how the conventional soil is chosen (Heinonen-Tanski, 1990; Schønning et al., 2002) and the degree of stochastic variability of the assessed indicator values.

Soils in the present study were sampled at several sites under organic and conventional management, both at commercial farms and research institutions. Sites were classified either as 'Organic', 'Conventional with animal manure and synthetic fertilisers' or 'Conventional with synthetic fertilisers'. The primary aims of the study were to i) address the variability of fungal abundance in soils under each farming system, ii) quantify the long-term effects of farming systems on soil fungal propagules and iii) identify fungal taxa differing between farming systems emphasizing Penicillium spp., Trichoderma spp. and Gliocladium roseum Bain.

Materials and Methods

The natural occurrence of fungi was monitored at 29 sites in Denmark, eight being organically cultivated (ORG) for at least eight years and 21 conventionally cultivated. Among the latter, 11 used a combination of animal manure and synthetic fertilizers (AM/SF) and 10 used SF only (SF). Three of the sites were located at research farms and 26 at commercial farms. Management data for each group are shown in Table 1.

Soil sampling was performed in spring 1996. At each of the 29 sites, nine soil cubes (8 x 11.5 x 6-13 cm deep) were taken on a 3 x 3 grid, 10 m distance between each grid point as described by Schjønning et al. (2002). The nine soil cubes from each site were placed in a plastic container to remain undisturbed and stored at 2^{o}C for maximum three months. Soil characteristics were assessed according to methods described in Hansen & Sørensen (1996) (Table 1).

In order to take into account possible effects of varying clay contents, soils were classified in two categories, according to clay contents, i.e. either ≤11% or >11% clay. The cut-off value defining the two categories was chosen to minimize the variance of the clay contents in each of the two categories using a cluster analysis algorithm (Mardia et al., 1979).

Fungal analyses

One soil core (1 cm diameter, 7 cm long) was drawn from each of the nine soil cubes per site. Three such core samples were combined to represent one row in the nine-point grid. From each of the three replicate samples, a portion of soil was homogenised in a stomacher for 15 sec in dilution medium containing water with 0.85% (w/w) NaCl and 1% (w/w) peptone l^{-1} (approx. 1:10 on dry weight basis, i.e. Dilution 10^{-1}). This initial 10^{-1} dilution was further diluted ten-fold using the NaCl-peptone dilution medium

(Dilution 10^{-2}). V8-juice agar (V8; Diener, 1955) was used to assess the total abundance of yeast fungi and filamentous fungi as well as for the specific detection of Trichoderma spp. and G. roseum. Dichloran-Glycerol (18%) Agar (DG18; OXOID CM729; Hocking and Pitt, 1980) was used to assess the xerophilic fungi. Both media were amended with 50 ppm chloramphenicol and 25 ppm chlortetracycline to inhibit bacterial growth. Dilution 10^{-2} was used for plating (0.1 ml, two Petri dishes per replicate sample, amount of soil per Petri dish 0.217 – 1.26 mg (average 0.56 mg)). For filamentous fungi and yeasts, V8 and DG18 plates were incubated at 20°C in the dark for five days. The presence/absence of the genus Trichoderma and the species G. roseum, respectively, were assessed on V8 after a further two days at 20°C in 12h near-UV/12h darkness.

Statistical analyses

The counts per Petri dish were modelled as a function of the amount of soil amended to a Petri dish by applying the following model: Denote by Y_{fsrp} the colony count in the p^{th} Petri dish of the r^{th} replicate of s^{th} site under farming system f. The variable Y_{fsrp} was assumed to be conditionally Poisson distributed, given two normally distributed random components Z and U, representing site and replicate sample within site, respectively. The conditional expectation of Y_{fsrp} , given $Z=z_s$ and $U=u_{sr}$, was

$$\alpha_f \, g + (\gamma_1 + \ldots + \gamma_k) \, g + \beta \, g^2 + (z_s + u_{sr}) \, g \,, \qquad (1)$$

where g was the amount of soil amended. The fixed effect α_f was the fungal abundance (CFU mg^{-1} soil) at a site with farming system f, adjusted by the fixed effects of k additional explanatory variables given in the term $(\gamma_1 + \ldots + \gamma_k)\, g$ in (1). These are indicating variables for the quartiles of the following soil characteristics: total C, total

N, extractable P, CEC, base saturation and soil density. Estimates of α_f based on (1) were adjusted estimates, those without the adjustment given by $(\gamma_1 + \ldots + \gamma_k)\, g$, were crude estimates. The term, $\beta\, g^2$, corrects for possible non-linearity of the curve relating the amount of amended soil to the expected number of CFU. Details on the model can be found in Labouriau and Elmholt (2000), and a similar model was applied by Elmholt et al. (1999).

G. roseum and Trichoderma spp. colonies could not be identified and enumerated on DG18 due to lack of sporulation. Although the colonies could not be enumerated on V8 either due to overcrowding, G. roseum or Trichoderma spp. could be identified and their occurrence was modelled by a binomial regression for correlated measures, using generalized estimating equations (GEE) (Fahrmeir and Tutz, 1994; Liang and Zeger, 1986; Liang and Zeger, 1989).

Results

The three farming systems were comparable in terms of soil characteristics. A Kruskal-Wallis test (Table 1) showed no statistically significant difference in clay contents, total C and N, CEC, base saturation or bulk density. The total amount of extractable P was significantly higher under AM/SF than under ORG and SF. Using a Fisher exact test, no statistically significant difference among the farming systems were detected in the proportion of soils with low clay contents.

In total, V8 yielded 35-123 colonies of filamentous fungi per Petri dish (mean 74, SD 19, median 71, n=149) and 3-149 yeast colonies (mean 24, SD 20, median 18, n=152) and DG18 32-130 filamentous fungi (mean 75, SD 20, median 73, n=174) and 0-105 (mean 30, SD 19, median 27, n=174) Penicillium spp. The colony counts were converted to colony forming units (CFU) mg^{-1} oven-dry soil. A comparison of V8 with

DG18 gave for both media 151 CFU of filamentous fungi mg^{-1} (SD 55 and 50, respectively). Fungal abundance was highly variable for all three farming systems and for all fungal groups, the range for filamentous fungi on DG18 being 91-227 CFU mg^{-1} for ORG soils, 59-267 CFU mg^{-1} for AM/SF soils, and 88-233 CFU mg^{-1} for SF soils (Figure 1).

The estimated abundance of filamentous fungi (on V8 and DG18) and yeast fungi (on V8) are shown in Table 2 stratified according to high and low soil clay contents. There was no statistically significant difference among farming systems nor any statistically significant interaction between soil characteristics and farming systems.

Crude and adjusted estimates of Penicillium spp. on DG18 (Table 2) differed statistically significantly among farming systems and soil clay contents ($P<0.01$), ORG soils with low clay contents having more Penicillium spp. than conventional soils and ORG soils with high clay content. There was no significant difference between AM/SF and SF. Essentially the same results were obtained for adjusted estimates with no statistically significant interaction between soil characteristics and farming systems. The abundance of Penicillium spp. did not vary as a function of the number of years under organic farming, as exemplified by the farms under 7-9 years of ORG farming, showing values of 13, 59, 109 and 161 CFU mg^{-1} soil, respectively. The two random components related to site and replicate within site varied to the same extent in all models fit. For the model for crude estimates of Penicillium spp. in soils with low clay contents for instance, the estimates of the variance of the random components related to site and replicate within site were 153 (95% Wald CI 64-745) and 181 (95% Wald CI 114-328), respectively.

Trichoderma spp. and G. roseum were assessed as the number of Petri dishes in which they were detected and the statistical analysis was based on estimated probabilities of observing a colony in soil suspensions from each of the three farming systems following a correction for the amount of soil. The p-value for jointly testing equality of probability of observing Trichoderma spp. or G. roseum among the three farming systems was 0.004 and <0.001, respectively, showing a statistically significant effect of farming system on both taxa. Table 3 presents the results for pairwise comparisons between farming system for Trichoderma spp. and G. roseum. The comparison shows a significantly higher probability of isolating G. roseum from ORG soils and a significantly higher probability of isolating Trichoderma spp. from SF than ORG and AM/SF soils. Similar results were obtained with models in which the basic soil characteristics were included as explanatory variables in addition to farming system. In this case, the p-value for jointly testing equality of probability of observing Trichoderma spp. or G. roseum among the three farming systems was 0.004 and <0.001, respectively, and no statistically significant interaction between soil characteristics and farming systems was detected.

Discussion

Earlier work at four organically cultivated farms detected long-term effects on some fungal groups but stressed that a broad range of soils was needed to validate any difference (Elmholt, 1996). The present study included a large number of sites. As summarized by Parkinson (1994), every facet of dilution plating as a method for isolating fungi from soil has been subjected to close scrutiny. There is general agreement that the majority of fungal colonies originates from spores or other propagules and not from hyphae. In consequence, the method is suited to assess the soil

contents of species culturable on the nutrient agar in choice. Although V8 is regarded a general medium, a completely non-selective medium does not exist (Parkinson, 1994), and differences between farming systems might exist in fungi that grow on neither V8 nor DG18. Several V8 plates had to be discarded due to fast-growing Mucor and Mortierella spp. resulting in more missing data using V8 than DG18 on which these fungi grow slower due to lower a_w. DG18 data were therefore used when possible, i.e. for total filamentous fungi and Penicillium spp. The number of fungal colonies varied considerably from 35 to 123 filamentous fungi per V8 agar plate. Competition was stronger in crowded plates and this may lead to underestimating fungal abundance, an effect compensated for by proper statistical methods (Elmholt et al., 1999; Labouriau and Elmholt, 2000).

There was large variation within each farming system (Figure 1) as is known from other multi-soil studies (Stenberg et al., 1998; Emmerling et al., 2001). Fungal 'hot-spots' were not observed in this study, probably due to the sampling procedure. This was confirmed by the estimates of the variance of the random components associated with site and replicate within site, respectively, being of the same order of magnitude in all the models fit. This point is important in analysing Penicillium spp. because it rules out the possibility that the distinctly high abundance observed in some soils was due to hot spots.

Variability in fungal abundance may be caused by climate, soil type, management, and sampling methodology. The 29 samples ranged from loamy sands to sandy loams and were representative of agricultural soils in Denmark. The clay content is a soil characteristic that is related to the inherent properties of the soil rather than to management effects. The median soil clay content was not significantly different for the

three farming systems (Table 1) and the proportion of soils with low clay contents did not differ between farming systems. However, a strong interaction between farming system and clay contents was detected. Since fungal abundance is known to be susceptible to clay contents (Stenberg et al., 1998; Emmerling et al., 2001; Knudsen et al., 2002), the analyses were therefore stratified according to clay contents. The estimated effects of farming system and clay contents were essentially the same in the crude and the adjusted analyses; therefore, other factors than those included in in adjusted estimates must determine the differences in fungal abundance between farming systems.

Management likely accounts for much of the observed fungal variability. ORG soils had in particular a diverse crop rotation with high frequencies of mixtures with grasses and legumes while varying forms of manure were used within each farming system (Table 1). Heinonen-Tanski (1990) found higher variability in soil planted to leys ascribing this to the root environment being more heterogeneous than in a cereal crop. To cope with the multitude of different soils, sampling was only performed once at each site. This calls for careful choosing of sampling time, as varying temperature and water and nutrient availability cause large seasonal fluctuations in fungal populations (Elmholt and Kjøller, 1989; Sivapalan et al., 1993; Elmholt, 1996; Bullock et al., 2002). Therefore samples were taken in spring when water contents were close to field capacity, a strategy also followed by Emmerling et al. (2001). Furthermore, only such fields were selected in which minimum four months had elapsed after ploughing and addition of animal manure. The sampling depth of 6-13 cm ensured that the soil had not been disturbed by seedbed preparation and sowing operations or had been enriched in

total C due to OM accumulation in the soil surface layer. Finally, pastures had been ploughed under at least 18 months before sampling.

Fungal variability within ORG soils was not smaller than within other soils (Figure 1) though the conventionally cultivated soils cover a broader range of management options, including pesticides and synthetic fertilizers. Carter et al. (2004) stated that farming practices 'fall along a continuum rather than into discrete groups' and that some overlap may occur. Resulting from this, soil attributes like fungal abundance and composition can be expected to fall along a continuum too as clearly demonstrated in this study. The large variation within each farming system inevitably reduces the possibility to discriminate between farming systems, and Dick (1992), Ryan (1999) and Carter et al. (2004) concluded that consistent long-term effects of ORG farming were difficult to confirm. In accordance with this, the present study found no significant differences in terms of broad taxonomic groups like yeasts and filamentous fungi. Filamentous fungi are known to be rather insensitive to farming system as assessed by dilution plating (Bolton et al., 1985; Fraser et al., 1988; Elmholt, 1996; Shannon et al., 2002; Bullock et al., 2002). Fungal hyphal length was proposed instead (Elmholt and Kjøller, 1987; Shannon et al., 2002), but direct microscopy is extremely laborious and less suited for multi-soil studies.

Stockdale et al. (2002) concluded that the same nutrient cycling processes operate in organically and conventionally cultivated soils and that nutrient pools are essentially the same. However, the relative importance and rates of the processes may differ and this may be reflected in terms of structural differences in some soil microbiota (Elmholt and Kjøller, 1989; Knudsen et al., 1995; Sivapalan et al., 1993; Bullock et al., 2002; Mäder et al., 2002; Shannon et al., 2002). All these studies were based on one or a few sites

and on very specific management conditions. The present study included a range of soils with high variability and yet some structural differences were found. The abundance of Penicillium spp. was significantly affected by the farming system and the clay contents as found earlier by Elmholt and Kjøller (1989), Sivapalan et al. (1993), Knudsen et al. (1995) and Elmholt (1996). However, the positive relationship between duration of ORG farming and Penicillium abundance as detected by Elmholt (1996) was not confirmed. Rather management factors like the high frequency of crop mixtures with legumes and grasses in ORG farming (Table 1) could be associated with significantly higher abundances of Penicillium.

G. roseum was significantly more abundant in ORG than in other soils regardless of fertilizer systems as suggested by Elmholt and Kjøller (1989). The present study showed a significantly higher probability of detecting Trichoderma spp. in SF soils than other soils (Table 3), in opposition to Bullock et al. (2002), who showed organic fertilisers to increase Trichoderma populations. Increases in the reproductive capacity of a taxon as revealed by an increase in CFU, might well give the species a selective advantage. Thus the increase in Penicillium spp. and G. roseum in ORG soils and of Trichoderma spp. in SF soils deserves considerations as to whether enrichment of these fungi is actually a desirable change of direction for the soil mycobiota. For both Penicillium and Trichoderma additional information at species level should be gained, whereas Knudsen et al. (1995) demonstrated that all G. roseum isolates from SF and ORG soils were antagonists of Fusarium culmorum (W.G.Sm.) Sacc., a property that makes a high reproductive capacity of G. roseum desirable.

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Figure captions

Figure 1

Variability in abundance of filamentous fungi in 29 Danish soils shown according to farming system and clay content. The result for each soil is shown as mean CFU mg^{-1} dry soil with SD (n=3). ORG = organically cultivated; AM = animal manure; SF = synthetic fertilizers. Open bars are soils with clay contents <11%, filled bars soils with clay contents ≥11%.

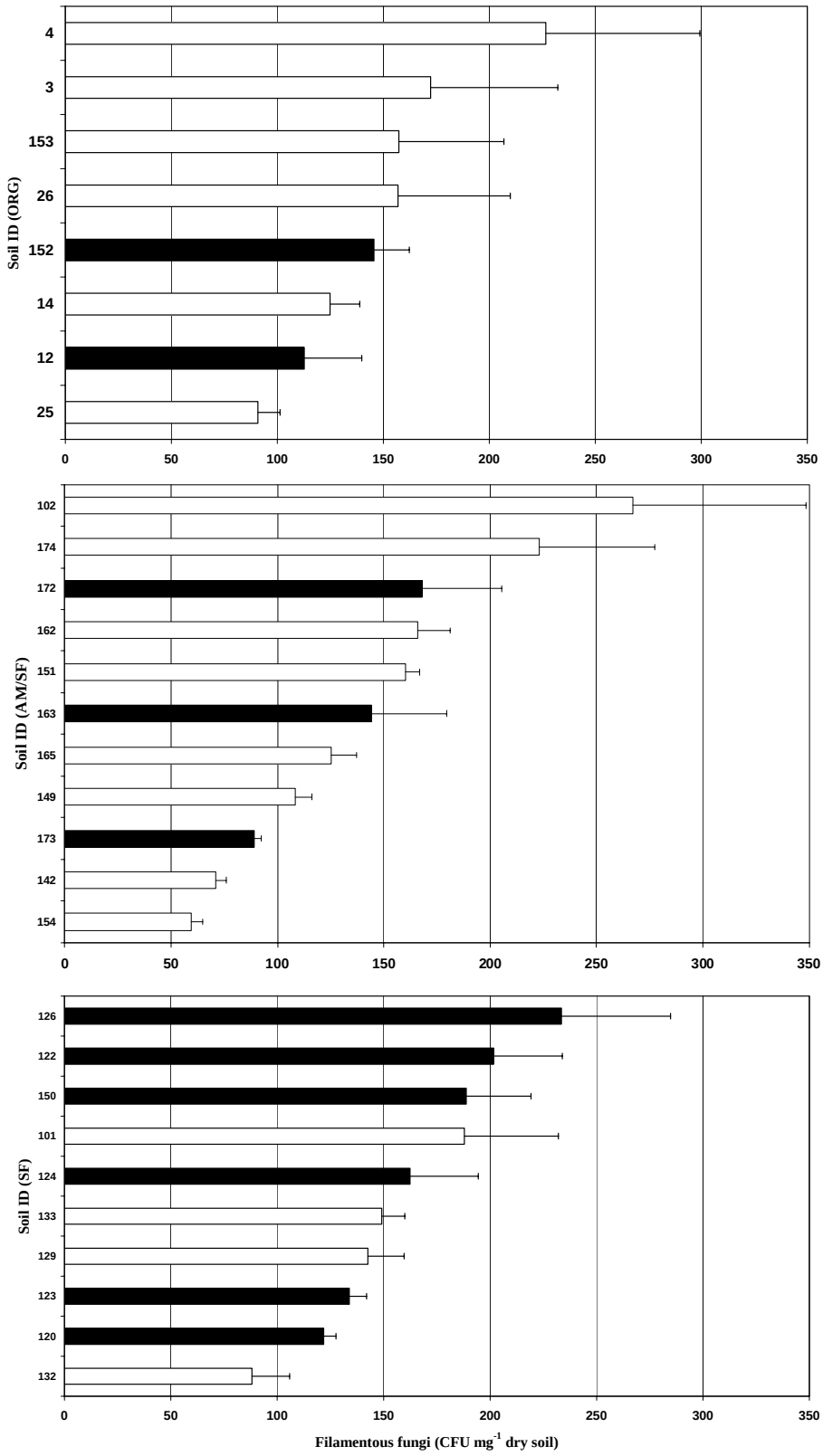

Figure 1. Elmholt & Labouriau

Table 1. Management and soil characteristics (mean values for the sampled sites in each group with SD).

Management system [1]		**ORG**	**AM/SF**	**SF**	p-value [2]
Number of sites		8	11	10	
Soil characteristics	Clay (< 0.002 mm) (%)	10 (3)	9 (4)	13 (3)	0,12
	Total C (%)	1.68 (0.44)	1.58 (0.17)	1.42 (0.26)	0,20
	Total N (%)	0.16 (0.04)	0.14 (0.02)	0.14 (0.02)	0,53
	Extractable P (Olsen P) (mg kg^{-1}soil)	3 (1)	4 (1)	3 (1)	0,03
	CEC (meq 100 g $soil^{-1}$)	14.1 (2.6)	14.0 (4.1)	13.8 (2.3)	0,80
	Base saturation (%)	69 (14)	68 (16)	71 (12)	0,86
	Bulk density (g cm^{-3}soil)	1.46 (0.11)	1.46 (0.08)	1.52 (0.07)	0,13
Crop frequencies [3]	Monoculture crops	0,33	0,79	0,88	
	Cereals	0,27	0,52	0,59	
	Crop mixtures	0,67	0,21	0,12	
	Legumes	0,65	0,16	0,09	
	Grasses	0,56	0,21	0,21	
Fertilizer type	Synthetic	-	+	+	
	Slurry	+	+	-	
	FYM	+	+	-	
	Liquid manure	+	+	-	
	Composted FYM	+	-	-	
	Deep litter	+	-	-	
Pesticides		-	+	+	

[1] ORG = organically cultivated; AM = animal manure; SF synthetic fertilizer

[2] p-value for a Kruskal-Wallis test for equality of medians among the three farming systems

[3] Informations based on sampling year plus five preceding years for each site

Table 2. Estimated numbers of fungi (CFU mg^{-1} dry soil) under three farming systems and according to clay contents.

Farming system [1]	Clay content (%)	Filamentous fungi (V8)		Filamentous fungi (DG18)		Yeast fungi (V8)		Penicillium spp. [3]	
		Crude estimates [2]	Adjusted estimates [2]	Crude estimates [2]	Adjusted estimates [2]	Crude estimates [2]	Adjusted estimates [2]	Crude estimates [2]	Adjusted estimates [2]
AM/SF	≥ 11	221	194	216	182	52	58	76 [a]	76 [a]
AM/SF	< 11	219	218	210	200	58	56	67 [a]	70 [a]
SF	≥ 11	222	202	240	222	36	40	85 [a]	82 [a]
SF	< 11	234	241	232	226	56	55	76 [a]	78 [a]
ORG	≥ 11	206	174	196	176	58	48	65 [a]	69 [a]
ORG	< 11	221	212	218	203	37	42	186 [b]	163 [b]
p-value [4]		0,90	0,56	0,17	0,26	0,69	0,84	< 0.01	<0.01

[1] ORG = organically cultivated; AM = animal manure; SF = synthetic fertilizer

[2] The 'crude model' uses farming system as sole variable, while the 'adjusted model' uses farming system and the basic soil characteristics listed in Table 1 (total C, total N, Olsen-P, CEC, base saturation, and bulk density)

[3] Within row results with the same letter are not significantly different at the 1% level

[4] p-value for equality of abundances among all six combinations of farming system and clay contents

Table 3. Predicted probability of detecting a colony of Gliocladium roseum or Trichoderma spp. in a Petri dish for each of the three farming systems

Fungal taxon	**Farming system** [1)]	**Predicted probability of detection** [2)]	**p-values for pairwise comparisons** [3)]		
			AM/SF	SF	ORG
Gliocladium roseum	AM/SF	0,37	-		
	SF	0,46	0,336	-	
	ORG	0,70	0,005	0,030	-
Trichoderma spp.	AM/SF	0,35	-		
	SF	0,67	0,001	-	
	ORG	0,26	0,348	0,001	-

[1)] ORG = organically cultivated; AM = animal manure; SF = synthetic fertilizer

[2)] Predictions based on the average amount of soil amended to a Petri dish, i.e. 0.56 mg

[3)] Pairwise comprison between farming systems according to the GEE binomial model for correlated measures.