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Soil suppressiveness and functional diversity of the soil microflora in organic farming systems

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Abstract

Arable fields of 10 organic farms from different locations within the Netherlands were sampled in four subsequent years. The soil samples were analysed for disease suppressiveness against Rhizoctonia solani, Streptomyces scabies and Verticillium dahliae. Furthermore, a variety of microbial characteristics and chemical and physical soil properties were assessed. All these characteristics and different environmental factors were correlated by multivariate analyses.

Significant differences in soil suppressiveness were found for all three diseases. Suppressiveness against Rhizoctonia was more or less consistent between the sampled fields in 2004 and 2005. This suppressiveness correlated with higher numbers of Lysobacter and Pseudomonas antagonists, as well as fungal diversity in DGGE patterns. Furthermore, results of 2006 showed that one year of grass-clover clearly stimulated Rhizoctonia suppression. Also Streptomyces soil suppressiveness was consistent between 2004 and 2005, but it concerned other soils than the ones which were suppressive against Rhizoctonia. Streptomyces suppression correlated with higher numbers of antagonists in general, Streptomyces and the fungal/bacterial biomass ratio, but with a lower organic matter content and respiration. Soil suppressiveness against Verticillium was not consistent between the years and therefore probably not related to soil factors.

Introduction

Enhancement of disease suppressive properties of the soil is of great importance in organic as well as other sustainable agricultural systems. Disease suppressiveness enhances crop health and allows a reduction in pesticide use. However, not enough is known about the influence of agricultural practices and soil characteristics on disease suppressive properties of the soil and its relation with diversity, composition and activity of the soil microflora. Several techniques to characterise the microflora (composition, diversity, antagonistic properties) have been developed recently and applied to experimental fields to study the influence of crop rotation and agricultural practices (Garbeva et al 2004, 2006; Salles et al 2004).

In the present study, soil samples were from organic farms at different locations within the Netherlands, each with their own agricultural practices. The soil samples were analysed for disease suppressiveness, microbial characteristics, as well as chemical and physical properties. Our objective was to detect soils which differed in soil

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suppressiveness, and to identify soil factors as well as microbial characteristics which correlate with suppressiveness.

Materials and methods

Arable fields of 10 organic farms from different locations within the Netherlands were sampled in August of 2003, 2004, and 2005. All fields were cropped with grass-clover in 2003. In 2006, a selection of the previously analysed fields with the largest contrasts in disease suppression were sampled again and compared with a grass-clover field from the same organic farm. From each field four independent samples were taken. Several physical, chemical and biological parameters were analysed by external labs. Disease suppression and microbial characteristics were analysed as described below.

Three plant-pathogen systems were used to assess soil suppressiveness in a climate room: (1) *Rhizoctonia solani* AG2.2IIIB, a fungal pathogen causing root rot and damping off of sugar beet, (2) *Streptomyces scabies*, a bacterial pathogen causing scab on radish (as a model for potato), and (3) V*erticillium dahliae*, a fungal pathogen causing wilt in rape seed.

Molecular fingerprints of bacteria and fungi were prepared from the soil microbial communities using PCR-DGGE (polymerase chain reaction - denaturing gradient gel electrophoresis) (method as described in Garbeva et al 2006).

Soil bacteria were plated on an agar medium. Two days later *Rhizoctonia* was co-inoculated on these plates. The bacteria that inhibited hyphal growth of *Rhizoctonia* were selected and sequenced (part of 16S) for identification.

Multivariate analyses to correlate disease suppressiveness with environmental factors and soil characteristics were performed with the statistical program CANOCO (Ter Braak 1995).

Results

The soils differed substantially in organic matter content, clay content, pH, C/N ratio, type of manure applied, fungal and bacterial biomass (see Table 1).

Disease suppressiveness tests of 2003 had too large variations, and were optimized in the years after. Significant differences in soil suppressiveness were found for all three diseases in 2004 and 2005. The fields of the farms A, G, D were most suppressive against *Rhizoctonia*; most conducive were fields of E, F, I. The results of 2006 clearly showed the positive influence of grass-clover on disease suppression against *Rhizoctonia*.

The fields of the farms H, I, J were most suppressive against *Streptomyces* in 2004 and 2005; most conducive were fields of C, E, G. Grass-clover did not influence the suppressiveness against *Streptomyce*s (results of 2006). Soil suppressiveness against *Verticillium* was not consistent between the years and therefore probably not related to soil factors.

Every year between 80-150 isolates, which inhibited *Rhizoctonia,* were sequenced for identification. *Streptomyces*. *Lysobacter* and *Pseudomonas* were most common: resp. 41, 29 and 16 % of the isolates in 2004 and 34, 23 en 17 % in 2006. *Lysobacter* was mainly present in clay soils and was clearly stimulated by growing grass-clover.

Disease suppressiveness of soils was correlated with all measured parameters by PCA (principal components analysis). Quantity of *Lysobacter* and *Pseudomonas* antagonists, as well as the number of fungal bands in the DGGE patterns, correlated with higher disease suppression against *Rhizoctonia* (Fig. 1 left).

Soil suppressiveness against *Streptomyces* was found in other soils (i.e. farm H, I, J) and correlating microbial characteristics were: quantity of antagonists in general, *Streptomyces*, fungal/bacterial biomass ratio (Fig. 1 right). On the other hand higher organic matter (os) and respiration (O2cons) correlated with lower suppressiveness.

Table 1. Physical, chemical and biological soil characteristics in 2004

farm	years organic	% clay <2 µm 1)	% org. matter 1)	pH-KCl 1)	C/N ratio 1)	fungal biomass 2)	bacterial biomass 2)	F/B ratio 2)	type of manure 3)		
									%SA	%LA	%P
A	1	11.5	1.7	7.4	13.6	32.4	112.4	0.30	0	0	100
B	13	13.8	1.6	7.3	12.4	29.7	93.1	0.33	98	0	2
C	2	2.0	10.2	5.2	26.1	14.1	26.3	0.61	100	0	0
D	2	11.8	2.9	7.5	18.4	20.0	106.3	0.20	28	30	42
E	12	27.5	6.8	7.2	11.6	18.4	137.7	0.14	50	50	0
F	14	2.0	2.8	4.7	18.9	12.8	39.9	0.39	57	24	18
G	8	19.0	10.3	6.8	13.4	16.8	100.3	0.18	2	89	9
H	3	20.5	2.9	7.4	12.2	25.4	131.0	0.19	62	38	0
I	7	29.3	1.9	7.4	15.7	13.9	77.3	0.18	20	66	14
J	10	1.8	1.9	5.4	21.3	11.8	15.6	0.86	50	50	0
LSD		2.1	1.7	0.4	3.4	7.9	26.0	0.24			

1) analysed by BLGG (Oosterbeek, NL)
2) analysed by J. Bloem (Alterra, Wageningen, NL) (Bloem et al, 2006)
3) values per farm applied; SA = soluble animal manure, LA = liquid animal manure, P = plant derived material

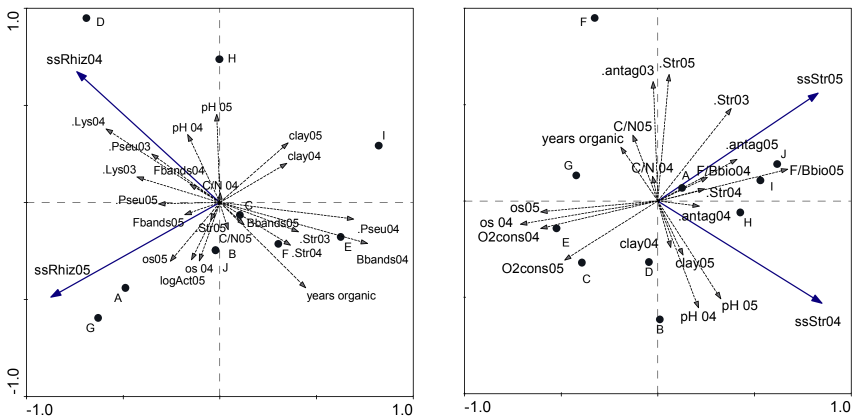

Fig. 1 Correlation between a selection of soil factors and soil suppressiveness against Rhizoctonia (ssRhiz, left) or Streptomyces (ssStr, right) analysed with PCA (principal components analysis). A-J presents the samples of the different organic farms. Arrows pointing in the same direction are positively correlated, and longer arrows have a stronger correlation.

Discussion

Soil suppressiveness appeared to differ significantly between the fields of the different organic farms. However, the suppressiveness of the fields was pathogen specific. Thus, none of the soils was suppressive for all of the pathogens tested. Consequently, different soil parameters correlated with the suppressiveness against each pathogen.

Most frequent occurring antagonists inhibiting *Rhizoctonia* appeared to be *Streptomyces*, *Lysobacter* and *Pseudomonas*. *Streptomyces* and *Pseudomonas* are known for there capacity to inhibit several fungal pathogens (Tuitert el al 1998; Whipps 2001). However, the antagonistic potential of *Lysobacter* is rather new (Folman et al 2003) and soil suppressiveness has never before been correlated with this genus. The presence of *Lysobacte*r was soil and probably crop dependent, since it occurred mainly in clay soils and was most abundant during and short after the grass-clover crop. Further molecular analysis of the soils will be performed to investigate the role of *Streptomyces, Lysobacter* and *Pseudomonas* composition and quantity in more detail.

Using the current approach, we could identify factors influencing disease suppression. Correlating all kinds of microbial characteristics will facilitate the development of microbial indicators for healthy soils and sustainable agricultural practices.

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