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Response of soil microbial communities to compost amendments

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Abstract

Soil organic matter is considered as a major component of soil quality because it contributes directly or indirectly to many physical, chemical and biological properties. Thus, soil amendment with composts is an agricultural practice commonly used to improve soil quality and also to manage organic wastes. We evaluated in laboratory scale experiments the response of the soilborne microflora to the newly created soil environments resulting from the addition of three different composts in two different agricultural soils under controlled conditions. At a global level, total microbial densities were determined by classical plate count methods and global microbial activities were assessed by measuring basal respiration and substrate induced respiration (SIR). Soil suppressiveness to *Rhizoctonia solani* diseases was measured through bioassays performed in greenhouses. At a community level, the modifications of the metabolic and molecular structures of bacterial and fungal communities were assessed. Bacterial community level physiological profiles (CLPP) were determined using Biolog™ GN microtiter plates. Bacterial and fungal community structures were investigated using terminal restriction fragment length polymorphism (T-RFLP) fingerprinting. Data sets were analyzed using analysis of variance and ordination methods of multivariate data. The impact of organic amendments on soil characteristics differed with the nature of the composts and the soil types. French and English spent mushroom composts altered all the biological parameters evaluated in the clayey soil and/or in the sandy silty clay soil, while green waste compost did not modify either bacterial and fungal densities, SIR values nor soil suppressiveness in any of the soils. The changes in bacterial T-RFLP fingerprints caused by compost amendments were not related to the changes in CLPP, suggesting the functional redundancy of soil microorganisms. Assessing the density, the activity and the structure of the soil microflora allowed us not only to detect the impact of compost amendment on soil microorganisms, but also to evaluate its effect at a functional level through the variation of soil disease suppressiveness. Differences in disease suppressiveness were related to differences in chemical composition, in availability of nutrients at short term and in microbial composition due to both incorporation and stimulation of microorganisms by the compost amendments.

Keywords: Compost amendments; Microbial communities; Bacteria; Fungi; Community level physiological profiles (CLPP); Terminal restriction fragment length polymorphism (T-RFLP); Soil suppressiveness; *Rhizoctonia solani*; Soil quality

1. Introduction

Soil amendment with compost is an agronomically interesting practice as well as an attractive waste management strategy. The addition of mature compost to soil favors plant development and improves soil quality, as well as having a suppressive effect on many diseases caused by soilborne plant pathogens (Cotxarrera et al., 2002; Erhart et al., 1999). Compost amendments therefore maintain and enhance the fertility and productivity of agricultural soils, allowing a sustainable land use.

Studies of the impact of composts on soil have mainly evaluated physical and chemical factors, potentially involved in plant productivity parameters. Evaluations performed both in microcosms and field experiments showed that organic amendments not only act by improving soil structure and as a source of nutrients, they can also strongly influence the soil microflora (Crecchio et al., 2001). The addition of good quality composts may increase global microbial biomass and enhance soil enzyme activity (Albiach et al., 2000; Perucci et al., 2000; Debosz et al., 2002), but little is known about the specific modifications received by the different components of the microbial communities (Kiikkilä et al., 2001; Chander and Joergensen, 2002).

Compost is effective in controlling diseases caused by soilborne pathogens, such as *Pythium, Phytophthora, Fusarium* spp. or *Rhizoctonia solani* both in fields and in

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potting mixtures in greenhouses (Schönfeld et al., 2003; Steinberg et al., in press). Disease suppressiveness was often related to biotic rather than abiotic factors (Reuveni et al., 2002; Garbeva et al., 2004). Compost amendments can modify the microbial community composition and as a result, enhance the competition and/or antagonism among microbes, leading to a decrease in plant pathogens activity (Hoitink and Boehm, 1999; Steinberg et al., 2004).

The impact of compost on both the structure and activity of bacterial and fungal communities and disease suppressiveness in soils was investigated. This study was undertaken with the objective of determining the effect of compost amendments on the soil biological properties at different levels of integration, including a global level, by measuring soil suppressiveness and total microbial densities and activities and a community level, by analyzing shifts in the metabolic and molecular structure of bacterial and fungal communities. Two soils with different characteristics were analyzed, as well as three composts from different origins.

2. Materials and Methods

2.1. Soils and organic amendments

The assays were performed with two soils, Dijon (D), a clayey soil, pH 7.4 and St Usage (SU), a sandy silty clay soil, pH 7.6. Soils were sampled from the top layer (0–20 cm) of a meadow grass (D) and a soil previously cultivated with maize (SU). Soils were manually treated to remove gravels and root and maize stubble debris and were stored at 4 °C until use. Three composts of different compositions were used: a green waste compost (GW) (Fouchange, France) and two spent mushroom composts (SMC), UK SMC (Wellesbourne, United Kingdom) and F SMC (France Champignon, Saumur, France) (Table 1). In addition, mixes of compost and soil were prepared (20–80%, v/v). Amended soil samples were mixed with a 3D rotary shaker and incubated in 40-l containers for 10 days at 20 °C at constant moisture of 75% of soil water holding capacity. Aliquots of each treatment were sieved (2 mm) and used immediately for the biological assays. Aliquots were also stored at −80 °C for further molecular analyses.

2.2. Soil suppressiveness to R. solani disease

The suppressiveness of unamended and amended soils to *R. solani* damping-off was assessed using the bioassay proposed by Camporota (1989). Briefly, a pathogenic strain (G6) of *R. solani* AG2-2 was grown on moistened barley grains (50 ml water/100 g barley grains, Ox Gall powder 1 g l^{-1}), previously autoclaved for 1 h at 110 °C, three consecutive days and supplied with antibiotics (streptomycin sulfate 0.5 g l^{-1}, chlortetracycline 1 g l^{-1} (Sigma-Aldrich, Saint Quentin Fallavier, France)). Inoculated barley was incubated for 3 weeks at 25 °C, with periodic shaking, air dried and grounded

Table 1
Physicochemical characteristics of soils, composts and soil–compost mixtures used

		D	D+GW	D+F SMC	D+UK SMC	SU	SU+GW	SU+F SMC	SU+UK SMC	GW	F SMC	UK SMC
Soil texture			Clayey				Sandy silty clay					
Mineral fraction	Clay	37.9	36.9	36.8	35.9	17.3	18.3	18.9	17.8	ND[a]	ND	ND
in soils < 2 mm (%)	Silt	45.2	42.8	43.4	43.4	49.8	49.6	49.7	49.7	ND	ND	ND
	Sand	16.9	20.3	19.9	20.7	32.9	32.1	31.4	32.5	ND	ND	ND
1 pH		7.4	7.6	7.6	7.5	7.6	7.7	7.6	7.6	7.8	7.8	7.5
2 Total $CaCO_3$(%)		5.1	7.4	6.9	5.7	0.6	1.6	0.9	0.7	22.8	6.9	5.3
3 P_2O_5 (g kg^{-1})		0.217	0.704	0.35	0.577	0.235	0.633	0.356	0.544	ND	ND	ND
4 CEC[b] (cmolc kg^{-1})		19.8	22.4	21.7	21.2	8.6	11.1	10	10.6	ND	ND	ND
5 CaO (g kg^{-1})		10.7	11.74	14.95	12.16	4.59	7.38	7.97	6.26	ND	ND	ND
6 MgO (g kg^{-1})		0.16	0.34	0.32	0.43	0.07	0.24	0.22	0.27	ND	ND	ND
7 K_2O (g kg^{-1})		0.33	1.65	1.05	0.72	0.24	1.59	1.05	0.59	ND	ND	ND
8 Organic Matter (%)		3.09	5.73	4.63	4.3	1.77	4.74	4.34	3.34	37.07	39.36	53.56
9 Organic C (%)		1.79	3.33	2.69	2.5	1.03	2.75	2.52	1.94	21.55	22.89	31.14
10 Organic N (%)		0.17	0.32	0.26	0.24	0.11	0.27	0.24	0.18	1.76	1.87	2.56
11 C/N[c]		10.74	10.28	10.34	10.24	9.69	10.35	10.42	10.73	12.28	12.28	12.16
12 NO_3^- (mg N kg^{-1} dw[d])		11.76	114.6	91.73	184.4	8.81	104.71	109.7	112.1	ND	ND	ND
13 $NH4^+$ (mg N kg^{-1} dw)		2.56	4.49	5.9	3.86	7.68	7.66	9.01	7.76	ND	ND	ND
14 Zn (mg kg^{-1})		3.7	8	6.2	9.6	2.9	6.9	4.7	9.3	ND	ND	ND
15 Fe (mg kg^{-1})		13.1	19.1	9.9	11.1	151.1	65.4	46.9	66	ND	ND	ND

Soils: Dijon (D) and St Usage (SU). Composts: green waste compost (GW), French spent mushroom compost (F SMC) and English spent mushroom compost (UK SMC).

[a] ND, not determined.

[b] CEC, cation exchange capacity.

[c] C/N, C organic/ N total.

[d] dw, soil dry weight.

in sterile conditions. Sets of 100 10-day-old pine seedlings (*Pinus nigra* var. austriaca) grown in steamed potting mixture were supplied with 300 ml of the different soils or soil–compost mixtures previously inoculated at 0.5% (w/v) with the barley formulated inoculum of *R. solani* AG2-2. For the controls, pine plantlets were grown in non-inoculated soils and soil–compost mixtures. The experiments were carried out in greenhouse at 25 °C and each treatment was replicated three times. Randomized complete block designs were used. The number of damped-off pine plantlets was noted after 3 days of incubation, every day up to 10 days. Area under disease progress curve (AUDPC) was calculated and disease suppression was expressed as follows: (100 − AUDPC of amended soil) × 100/AUDPC of non-amended soil.

2.3. Microbial densities

The densities of cultivable bacteria and fungi were estimated using a standard dilution-plating procedure. Five grams of soil, soil–compost mixture or compost, were suspended in 45 ml of sterile water and shaken for 20 min. Ten-fold dilutions were made. Bacteria were quantified on yeast peptone glucose agar (yeast extract 5 g l^{-1}, peptone 5 g l^{-1}, glucose 10 g l^{-1}, agar 15 g l^{-1}) supplied with cycloheximide (100 mg l^{-1}) and fungi were quantified in melting malt extract agar (malt 15 g l^{-1}, agar 10 g l^{-1}) supplied with antibiotics (citric acid 250 mg l^{-1}, chlortetracycline 50 mg l^{-1} and streptomycin 100 mg l^{-1}).

2.4. Microbial activity

Global microbial activity was assessed by measuring basal respiration (BR) through CO_2 production rate and substrate induced respiration (SIR) through CO_2 production rate after glucose addition. BR and SIR were determined in non-amended soils and soil–compost mixes following a procedure adapted from Carpenter-Boggs et al. (2000). Soil (50 g fresh weight) was placed in vials and water was added in the same quantity as glucose aqueous solution in further SIR measures. Vials were capped and incubated at 22 °C in the darkness. CO_2 was measured 8 h after water application using a Portable Micro Gas Chromatograph P200 (MTI Analytical Instruments). SIR was subsequently measured using the same soil samples. After leaving vials opened overnight, an aqueous solution of 30 g glucose l^{-1} was added to obtain a final concentration of 1.5 mg glucose g^{-1} dry soil. Vials were capped and CO_2 production was measured 8 h after glucose supply. Microliters of released CO_2 were calculated by using a calibration line prepared with known quantities of CO_2 and results were expressed as µl CO_2 g^{-1} dry soil h^{-1}. Three replicates per treatment were performed.

2.5. Community level physiological profiles of bacteria

Intensity and diversity of bacterial metabolism were evaluated by the technique proposed by Garland and Mills (1991) using Biolog GN microtiter plates. These 96 well microtiter plates contain 95 different carbon sources, a negative control and tetrazolium dye. Microorganisms were extracted from the non-amended and amended soils using a standard extraction method (Winding and Hendriksen, 1997). Each well of the microtiter plate was inoculated with 150 µl of diluted soil suspension, containing 50 µg/ml cycloheximide solution to prevent fungal development. Plates were incubated at 25 °C. Color formation was measured at 590 nm with a microtiter plate reader (Molecular Device). Readings were made at regular time intervals for 92 h. Average Well Color Development (AWCD) of each plate was calculated as the mean of the absorbance values for all 95 response wells per reading time. Three replicates per treatment were performed. Kinetics of AWCD was used to determine the speed and the level of development of the bacterial communities using the 95 provided substrates. The metabolic diversity of the bacterial communities was assessed by comparing the CLPPs of all replicates and all treatments at time 92 h. Normalization for the effect of putative variable inoculum densities between plates was achieved by dividing individual well responses by the AWCD of the corresponding microtiter plate prior to the multivariate analysis (Garland, 1997).

2.6. Molecular structure of bacterial and fungal communities

Bacterial and fungal community structures in non-amended soils, soil–compost mixtures and composts were investigated using T-RFLP fingerprinting (Liu et al., 1997). The total community DNA was extracted and purified using the method described by Edel-Hermann et al. (2004). Briefly, DNA was extracted from 1g of soil using a chemical extractant (sodium dodecyl sulfate) and a physical disruption (bead-beater), purified through polyvinylpolypyrrolidone and Sepharose 4B columns. Lysis buffer was modified (100 mM Tris–HCl, pH 8, 100 mM EDTA pH8, 1.5M NaCl, 0.5M Na_2HPO_4 pH8, and 1% w/v SDS) in the case of the F SMC compost in order to produce enough quantity of DNA suitable for PCR amplification. DNA extractions were performed in triplicate from independent samples.

Bacterial and fungal community structures were analyzed by T-RFLP of 16S and 18S rRNA genes, respectively. 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the primer 27F (AGAGTTTGATCCTGGCTCAG) (Edwards et al., 1989) labeled with the fluorescent dye D2 (Beckman Coulter, Fullerton, CA, USA) and the primer 1392R (ACGGGCGGTGTGTACA) (Braker et al., 2001). 18S rRNA gene was amplified by PCR using the primer nu-SSU-0817-5′ (TTAGCATGGAATAATRRAATAGGA) and the primer nu-SSU-1536-3′(ATTGCAATGCYCTATCCCCA) (Borneman and Hartin, 2000) fluorescently labeled with the dye D3 (Beckman Coulter). Both PCR amplifications and T-RFLP analyses were carried out using

the procedure described by Edel-Hermann et al. (2004), with the following differences concerning bacterial T-RFLP analysis: PCR amplifications were performed using 30 cycles, with an annealing temperature of 57 °C, and 150 ng of purified PCR products were digested with 5 units of the restriction enzyme *Hae*III, instead of 5 units of *Alu*I and 5 units of *Mbo*I utilized in the 18S T-RFLP analysis. Resulting fragments were analyzed through capillary electrophoresis sequencer CEQ™ 2000XL (Beckman Coulter). The resulting electrophoregrams were analyzed as previously described (Edel-Hermann et al., 2004). The communities were characterized by the sizes of the terminal restriction fragments (TRFs) and their intensity measured by the height of the peaks.

2.7. Statistical analysis

Data of bioassays, microbial densities, global microbial activity (BR and SIR) and speed and level of development of the bacterial communities (kinetics of AWCD) were compared by analysis of variance (ANOVA) and Student–Newman–Keuls tests using Statview (SAS Institute, Inc., version 5). Ordination analyses were performed using ADE-4 software (Thioulouse et al., 1997) to summarize multivariate data to a few variables or dimensions and to provide an arrangement of the treatments on the two first dimensions. The physicochemical characteristics (excluding the interdependent characteristics related to the soil texture) and the normalized CLPPs at time 92 h of unamended and amended soils were submitted to principal component analyses (PCA). T-RFLPs profiles were compared by correspondence analysis as previously described (Edel-Hermann et al., 2004). Finally, a possible correlation between 16S T-RFLP data and CLPP data was investigated by co-inertia analysis.

3. Results

3.1. Soil characteristics

The clayey soil of Dijon and the sandy silty soil of St Usage exhibited different physicochemical characteristics leading to a clear distinction between them after PCA analysis (Fig. 1a). This distinction was due to differences in content of total $CaCO_3$, CaO, $N–NH^{4+}$ and Fe and capacity of cation exchange (CEC), as shown by the correlation circle built from the data matrix (Fig. 1b). The addition of organic amendments into the soils altered their physicochemical properties in similar ways. Organic amendments modified the content of P_2O_5, K_2O, $N–NO_3$ and Zn and the percentage of organic matter, organic C and organic N, in both soils. Green waste compost caused the most important changes in both soils. Changes caused by UK and F SMC were similar in the two soils.

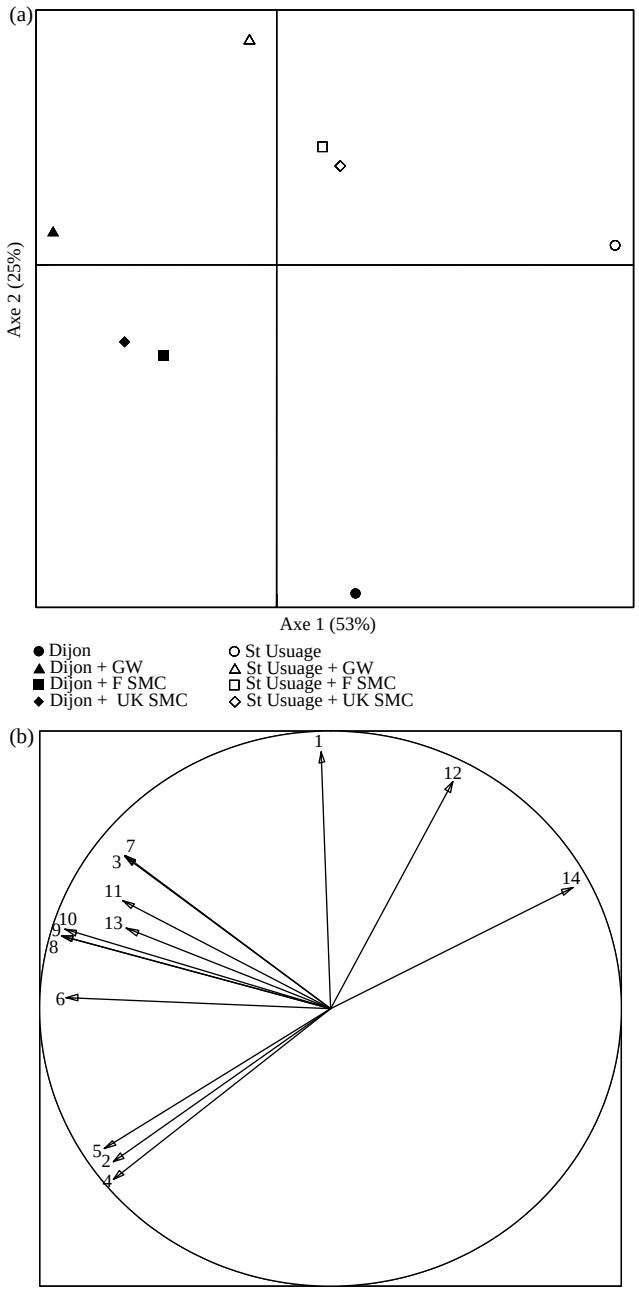

Fig. 1. (a) Principal component analysis (PCA) of the physicochemical characteristics of non-amended and amended soils. (b) Correlation circle of the PCA showing characteristics contributing to the distinction of the soils and their treatments. Numbers correspond to the characteristics indicated in Table 1.

3.2. Soil suppressiveness to R. solani disease

Bioassays with non-amended and amended soils were performed to evaluate the effect of compost addition on soil suppressiveness to *R. solani* damping-off. Non-amended soil of Dijon was very conducive to *R. solani* disease and 100% damping off was observed 10 days after the pathogen inoculation. The soil of St Usage was intermediate in suppressiveness and 60% damping off was observed at

the end of the bioassay. The F SMC significantly suppressed disease due to *R. solani* ($P<0.05$) when this compost was incorporated to the soil of Dijon, while the two other composts did not significantly alter disease suppression in this soil (Fig. 2). None of the three organic amendments caused any significant changes in disease suppression of the soil of St Usage.

3.3. Microbial densities

Compost amendments had different effects on soil microbial densities. Green waste amendment did not modify the densities of cultivable bacteria and fungi in the soils of Dijon and St Usage (Fig. 3). Conversely, UK SMC significantly increased the bacterial density in the soil of Dijon and the fungal density in the soil of St Usage. F SMC produced a significant increase in densities of both cultivable bacteria and fungi in both soils. Concerning the amendments, green waste was the compost with the lowest number of bacterial and fungal CFU, meanwhile F SMC had the highest microbial densities.

3.4. Microbial activity

Respiration rate in the soil of Dijon was very low compared to the respiration rate observed in the soil of St Usage, although the two soils had similar microbial densities (Fig. 4). Both SMC amendments produced a significant increase in CO_2 production rate (BR) in the two soils. Conversely, GW compost had no effect when added to the soil of Dijon, but it significantly increased the basal respiration in the soil of St Usage. In the same way, F SMC and UK SMC significantly increased SIR value in both soils while GW amendment caused no significant changes in any of the soils (Fig. 4b).

3.5. Community level physiological profiles of bacteria

The rate of substrate utilization by bacterial communities in the soil of Dijon (0.014 AWCD unit h^{-1}) was not modified after compost amendment, as shown by the progress curve of the AWCD during incubation time (Fig. 5). However, the maximum level of color development of the bacterial community (AWCD at the plateau) was

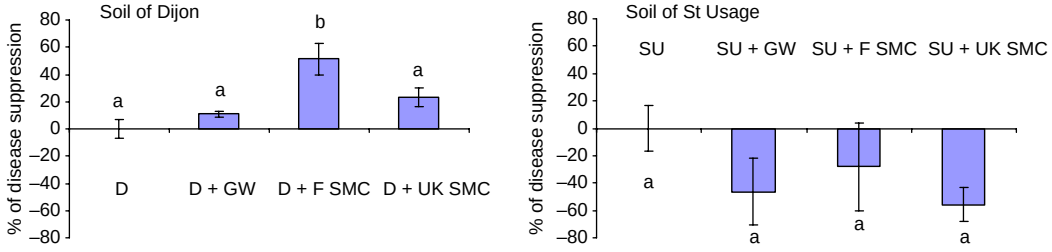

Fig. 2. *R. solani* disease suppression in non-amended and amended soils. Disease suppression is expressed as percentage of area under disease progress curve (AUDPC) reduction, when amended and non-amended soils were compared. Values are mean ± standard error ($n=3$). Different small letters indicate significant differences ($P< 0.05$). Soils: Dijon (D) and St Usage (SU); Composts: green waste (GW), French spent mushroom compost (F SMC), English spent mushroom compost (UK SMC).

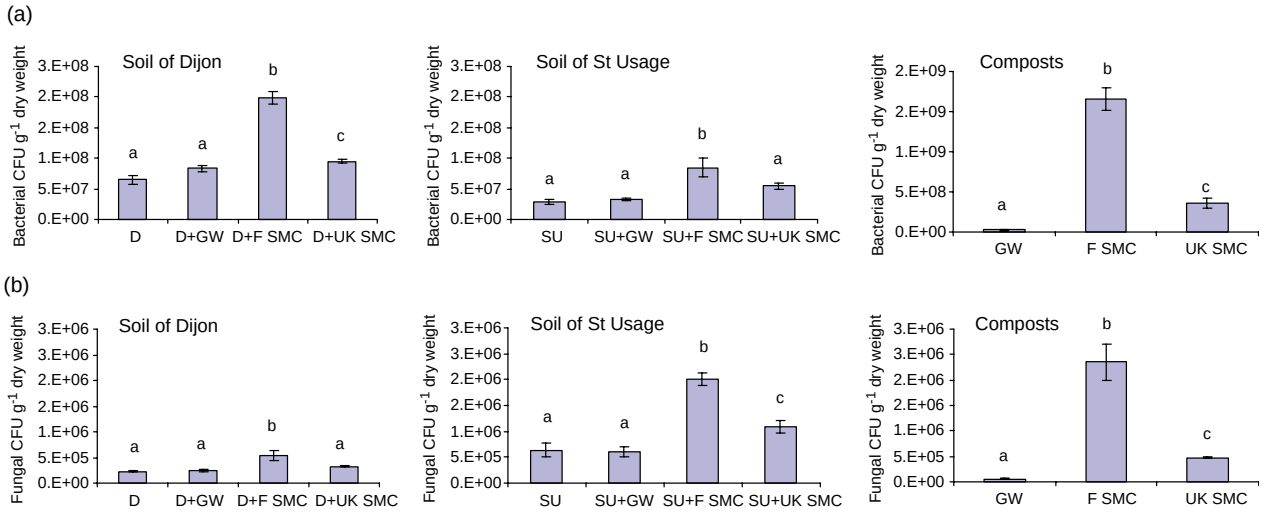

Fig. 3. Bacterial and fungal densities in non-amended soils, amended soils and composts. Values are means ± standard error ($n=3$). Different small letters on top of the bars indicate significant differences ($P< 0.05$). Soils: Dijon (D) and St Usage (SU); Composts: green waste (GW), French spent mushroom compost (F SMC), English spent mushroom compost (UK SMC).

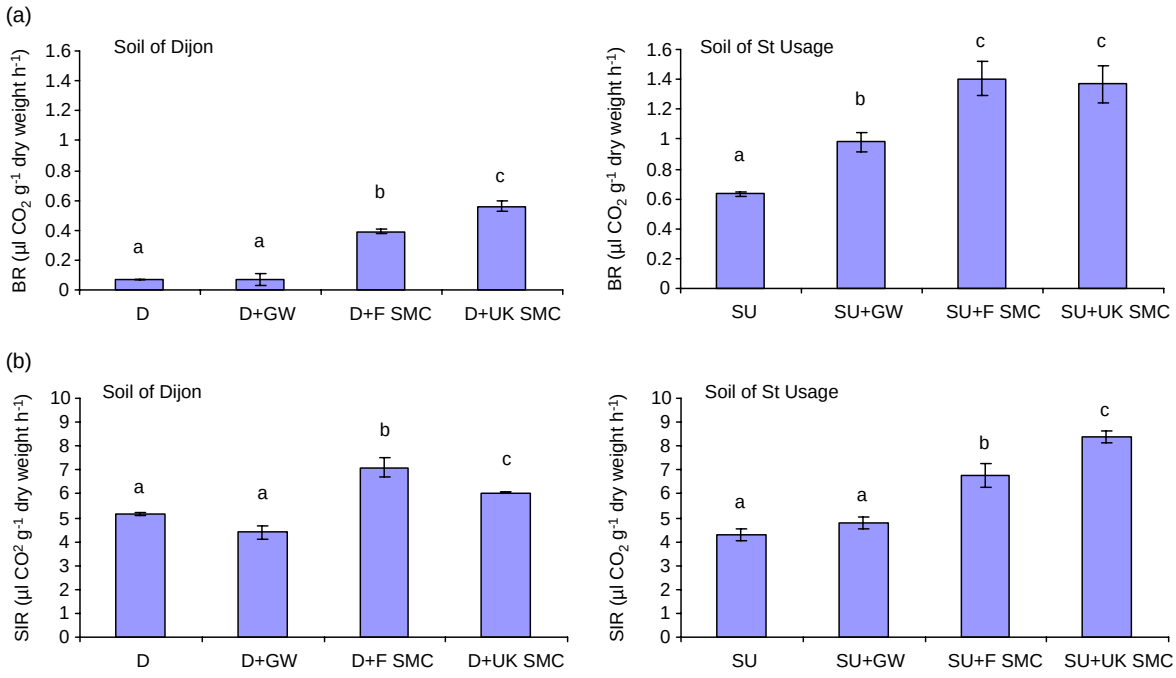

Fig. 4. Basal respiration (BR) and substrate induced respiration (SIR) in non-amended and amended soils. Values are mean ± standard error ($n = 3$). Different small letters on top of the bars indicate significant differences ($P < 0.05$). Soils: Dijon (D) and St Usage (SU); Composts: green waste (GW), French spent mushroom compost (F SMC), English spent mushroom compost (UK SMC).

significantly higher in Dijon-UK SMC mixture (1.11 AWCD) than in the non-amended soil (0.94 AWCD). In the soil of St Usage, GW and UK SMC addition significantly increased the rate of substrate utilization (from 0.016 AWCD unit h^{-1} to 0.026 and 0.024 AWCD unit h^{-1}, respectively), as well as the level of maximum color development (from 1.1 AWCD in non-amended soil to 1.65 in GW–soil mixture and 1.53 AWCD in UK SMC-soil mixture). The CLPP of the soilborne bacteria from non-amended and amended soils were analyzed by PCA (Fig. 6). CLPP of bacteria from the soil of Dijon was different from the CLPP exhibited by bacteria from the soil of St Usage. The three compost amendments modified substrate metabolism of the bacterial communities of the soil of Dijon whereas only F SMC affected substrate metabolism of the bacterial communities in the soil of St Usage. The shifts observed in overall substrate utilization could not be attributed to changes in any particular Biolog substrate.

3.6. Molecular structure of bacterial and fungal communities

Compost amendments produced considerable changes in bacterial community structure as illustrated in Fig. 7 with the soil of Dijon amended with F SMC. The disappearance of peaks and the occurrence of others in the TRF fingerprints of the amended soil compared to the TRF fingerprints of the non-amended soil indicated alterations of the abundance ratios of the bacterial groups harbouring the DNA sequences revealed by these peaks. The correspondence analysis performed with non-amended and amended soils confirmed that the bacterial community structure was modified by the addition of composts in both soils (Fig. 8). However, in both soils, the most important shift was produced by the

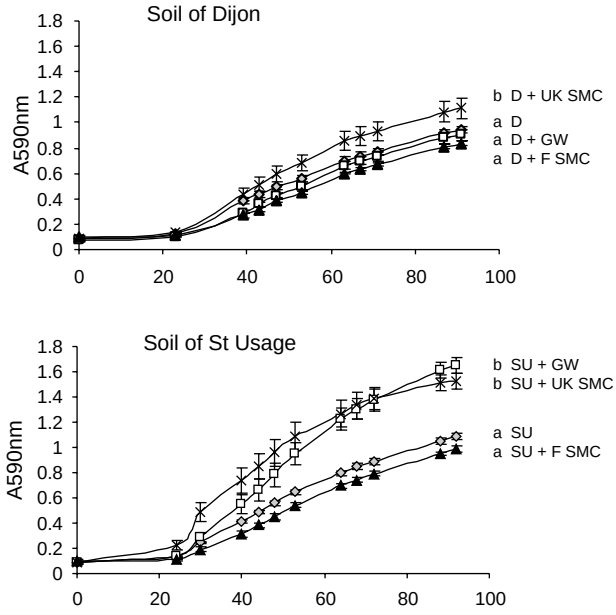

Fig. 5. Kinetics of the average well color development (AWCD) of bacterial communities originating from the non-amended and amended soils. Values are mean ± standard error ($n = 3$). Different small letters at the right end of each kinetics indicate significant differences ($P < 0.05$). Soils: Dijon (D) and St Usage (SU); Composts: green waste (GW), French spent mushroom compost (F SMC), English spent mushroom compost (UK SMC).

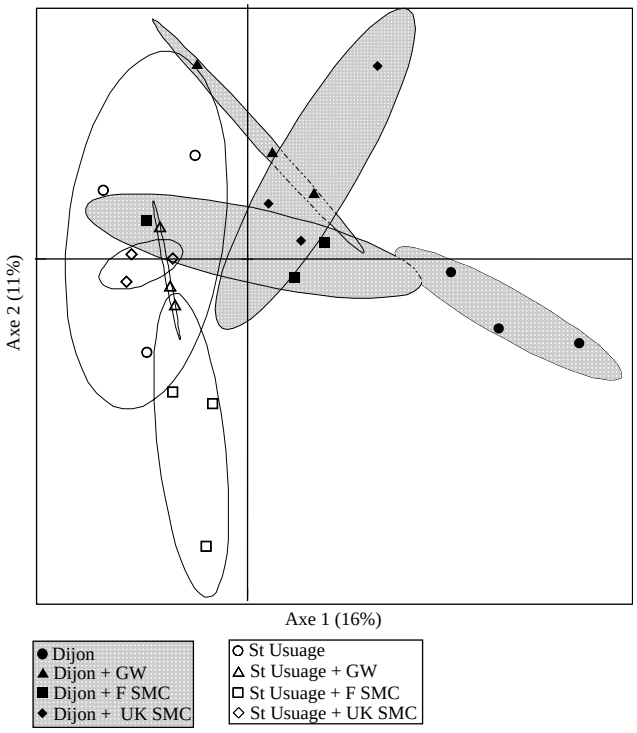

Fig. 6. Principal component analysis of the community level physiological profiles (CLPP) of non-amended and amended soils. Ellipses represent 90% confidence limits. Soils: Dijon (D) and St Usage (SU); Composts: green waste (GW), French spent mushroom compost (F SMC), English spent mushroom compost (UK SMC).

introduction of F SMC. In the soil of St Usage, the bacterial community structure was altered in a similar way by the two SMC amendments. Finally, the bacterial community structures of the two soils remained distinguishable whether they were amended or not.

All amendments produced shifts in the fungal community structures of both soils, with the exception of GW compost in the soil of Dijon (Fig. 9). When a shift was induced, the composts modified fungal community structures in different ways in the soil of Dijon as well as in the soil of St Usage. The fungal community structures of both soils amended with F-SMC were not different from each other, indicating that the same microorganisms could have been stimulated or incorporated by the compost in both soils.

Finally, these analyses indicated that changes in the microbial community structure induced by compost amendments were related both to the soil and to the type of organic amendment used.

4. Discussion

All the soil biological properties evaluated in this study were modified after compost addition in microcosms. Modifications varied depending on the soil and the organic matter applied. Three composts from different origins and two soils differing in physicochemical characteristics were used. The same amendment caused different effects in the soil of Dijon and in the soil of St Usage, and different amendments added to the same soil caused different effects. All the composts produced shifts in the bacterial and/or fungal community structures. Depending on the amended soil, these modifications could be followed or not by an increase in microbial densities and/or activities.

Compost amendments did not have any lethal effect on the soil microflora since the numbers of cultivable bacteria and fungi remained unchanged after GW amendment and it was increased after the addition of SMC amendments. Spent

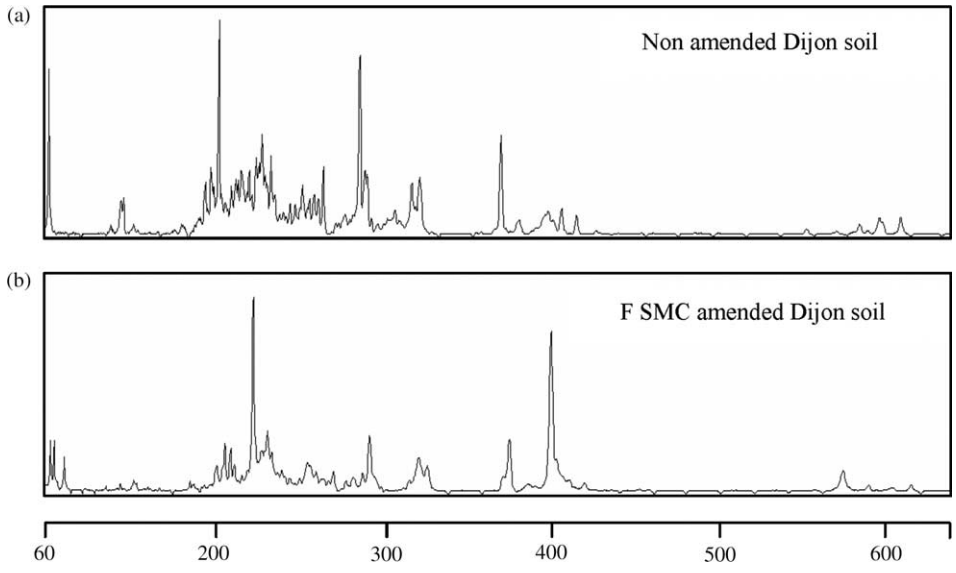

Fig. 7. T-RFLP profiles of bacterial communities in (a) the non-amended soil of Dijon and (b) the soil of Dijon amended with F SMC. X-axis, fragment size in bp.

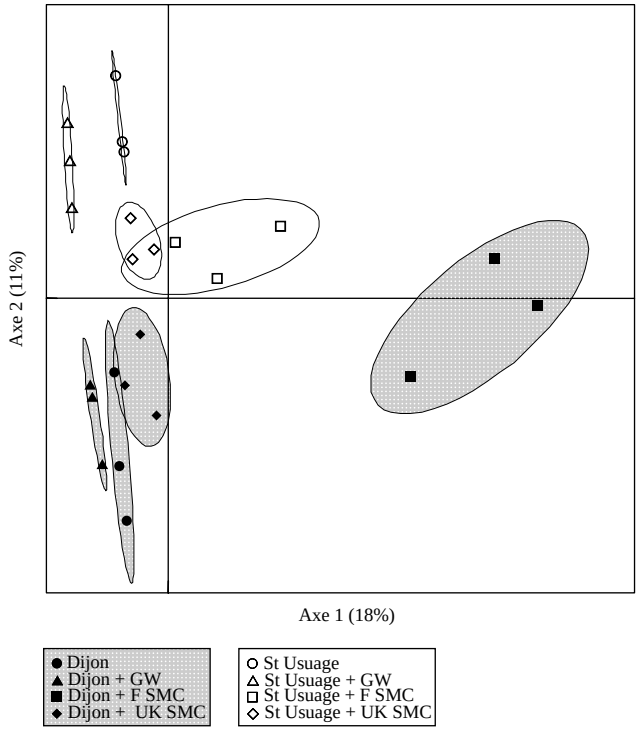

Fig. 8. Correspondence analysis of 16S T-RFLP data sets from non-amended and amended soils. Ellipses represent 90% confidence limits. Soils: Dijon (D) and St Usage (SU); Composts: green waste (GW), French spent mushroom compost (F SMC), English spent mushroom compost (UK SMC).

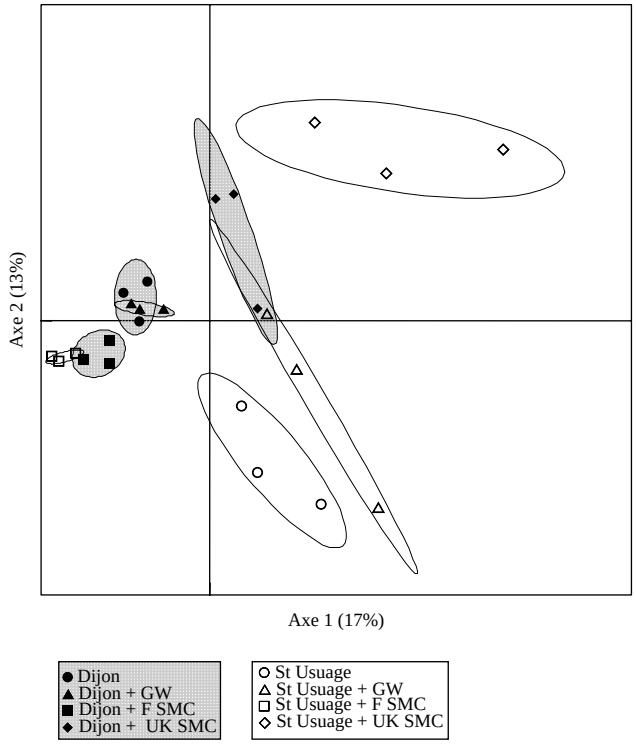

Fig. 9. Correspondence analysis of 18S T-RFLP data sets from non-amended and amended soils. Ellipses represent 90% confidence limits. Soils: Dijon (D) and St Usage (SU); Composts: green waste (GW), French spent mushroom compost (F SMC), English spent mushroom compost (UK SMC).

mushroom composts enhanced microbial densities in soils through either a stimulation of the soil microflora, or an input of the compost microflora or a combination of both. To clarify this point, an arithmetic density of bacteria and fungi in each soil–compost mixture (80–20) could be easily calculated based on the known number of bacteria and fungi in the natural soils and pure composts. Observed soil–compost mixtures densities higher than calculated densities would indicate a stimulation of the microflora. Such conclusions could not be drawn from the density data since similar values were obtained for observed and calculated densities. The height of the peaks revealed by the TRF profiles indicated the relative abundance of microbial groups harbouring identical terminal restriction fragments. Concerning the bacterial community structures in non-amended and F SMC-amended soil of Dijon, a big increase in the relative intensity of two peaks was produced as a consequence of the amendment. The peaks at 217 bp and 401 bp changed from a mean value in triplicate samples of 3 and 0% in the non-amended soil, to 12 and 10% in the amended soil, respectively. Taking into account that these peaks represented 8 and 17% of the total fluorescence in TRF fingerprints of the F SMC, F SMC amendment would have increased the number of cultivable bacteria in the soil of Dijon as a result of the input of its own microflora. Similar situations were observed in the other treatments, as well as in fungal communities, where the main increase in relative fluorescence was related to peaks also presents in compost microflora. However, certain soil microflora stimulation was also detected since relative fluorescence of some peaks was increased after compost amendment although these peaks were not detected in compost microflora. The comparison of the TRF profiles of non-amended and amended soil samples and compost samples suggested that shifts induced in the microbial community structures and in the microflora densities were mainly due to the amendment of the soil with new community members originating from the compost, although there was also a stimulation of the soil microflora.

Composts are an important source of nutrients usable by the microorganisms. As a consequence, composts amendments generally enhance the development of the microflora and increase the global activity of the soils (Bailey and Lazarovits, 2003). In our study, the two spent mushroom composts increased the global activity in the two soils while green waste compost only increased the global activity in the soil of St Usage. These different behaviors are due to the fact that GW compost is basically constituted by plant material, being decomposed with difficulty at short term and acting mainly as a long term source of nutrients. The presence of slowly decomposers in the soil of St Usage would explain the increase in global activity observed after GW amendment.

An important feature of compost amendments is their effectiveness in controlling diseases caused by many

soilborne plant pathogens. *R. solani* damping-off has been suppressed by the addition of composts to soil or potting mixture in several occasions (Diab et al., 2003; Alabouvette et al., 2004; Mazzola, 2004). In an attempt to determine the consequence of the soil microflora modifications produced by compost amendments on the soil suppressiveness, we decided to compare the suppressive character of non-amended soil and soil–compost mixtures towards *R. solani* diseases. The bioassay showed a significant increase in soil suppressiveness of the soil of Dijon when it was amended with F SMC. In the other treatments no significant changes were observed. These results agree with the fact that suppression of *R. solani* by compost is a variable phenomenon less consistent than suppression of *Pythium* spp. and other pathogens (Craft and Nelson, 1996). Whereas most composts naturally suppressed *Pythium* and *Phytophthora* root rots, only about 20% of the compost tested naturally suppressed *R. solani* damping-off (Tuitert et al., 1998; Hoitink and Boehm, 1999).

Contrary to what was shown previously for soil suppressiveness to fusarium wilts in laboratory scale experiments (Höper et al., 1995), it was not possible to relate the enhancement in soil suppressiveness with the modifications of the density, the activity and the structure of soil microflora caused by F SMC amendment in the soil of Dijon. In the same way, the differences in physicochemical characteristics and biological parameters found between the soil of Dijon and the soil of St Usage did not justify the unique suppressive effect of F SMC in Dijon soil. This situation corresponds to a mechanism of specific suppression, where antagonistic populations have been stimulated by compost addition, rather than a mechanism of general suppression.

In the course of this study, we showed important differences between the effect of SMC and GW compost on soils characteristics. SMC altered all the biological parameters evaluated in the soil of Dijon and/or in the soil of St Usage, while GW compost did not modify soil suppressiveness, bacterial and fungal densities and SIR values in none of the soils. These different behaviors could be related to differences in chemical composition, in availability of nutrients at short term and in microbial composition.

The non-amended soils differed in the level of global activity, metabolic diversity and bacterial and fungal community structures. However, and contrary to what was observed with the physicochemical characteristics, the modifications produced by compost amendments on the biological properties in the two soils were distinct. Therefore, the study of the impact of compost amendments on soil characteristics through biological parameters allowed for the detection of specific shifts which were not detectable through physicochemical parameters. Although obtained through experiments at a laboratory scale ours *results* indicate the necessity of combining several approaches as well as the complementarity of the information that they provide.

Assessing the density, the activity and the structure of soil microflora allowed us to detect the impact of compost amendment on soil microorganisms and to evaluate its repercussion at a functional level through the variation of soil disease suppression. The use of global approaches (microbial densities and activities) and specific ones (bacterial metabolic activity and molecular structure of bacterial and fungal communities) permitted us to characterize the soil microbial communities at different levels. Biolog analysis showed the impact of compost amendment on bacterial metabolism, which not always agreed with the changes detected on global metabolism. Actually, global soil activity represents the metabolism of the whole soil microflora but not the specific activity of its different components. As a consequence, the enhancement of global activity after compost addition did not necessary implies an increase in bacterial activity, but may reveal the result of an increase in fungal activity and/or of other soil components of the microbiota.

Compost amendments caused changes in bacterial community structures, which were not directly related with changes in soil CLPP. The co-inertia analysis performed with 16S T-RFLP data and Biolog CLPP of non-amended and amended soil mixtures showed no correlation between both parameters (data not shown). This result may be explained by the functional redundancy of soil microorganisms, since one function can be carried out by a range of different microorganisms and changes in bacterial community structure do not necessary lead to changes in enzyme activities (Marschner et al., 2003). Furthermore, the patterns of substrate utilization only indicate functional aspects of the cultivable fraction of the soil community that grows on the various carbon sources in the Biolog wells (Widmer et al., 2001)

The comparison of the T-RFLP fingerprints among the different treatments revealed a big increase in the relative intensity of several peaks in the soil of Dijon after F SMC amendment. As these peaks could correspond to putative antagonistic microorganisms responsible for the reduction in *R. solani* damping-off observed for the soil of Dijon amended with F SMC, we attempted to identify them by comparing their length with TRF sizes predicted for known microorganisms, using the T-RFLP analysis program (TAP) tool of the Ribosomal Database Project (RDP) web site (Marsh et al., 2000). However, this analysis revealed that many TRF may be assigned to several bacterial species. As an example, the TAP tool proposed 19 different bacterial species for the TRF of 217 bp which was enhanced by F SMC in the soil of Dijon. Concerning fungal TRFs, two peaks of 196 and 327 bp clearly differentiated Dijon-F SMC mixture from the rest of the treatments, but so far no tool equivalent to TAP is available for their identification. Therefore, we will use a cloning and sequencing strategy for the identification of putative antagonistic microorganisms

related with the compost addition to the soil. The holistic approach used is wiser than the laborious and not always fruitful strategy of screening hundreds of randomly isolated microorganisms antagonistic towards *R. solani* because it focused on the components of the indigenous microflora co-acting within their own environment. Besides the disease severity due to *R. solani* activity, we evaluated in this study the consequences of organic amendments on the whole microflora of different soils. Such an approach is needed to evaluate what can be considered as the side effects of an agricultural practice. Organic wastes are more and more important in our society and they are all different in nature. All of them can not be incinerated and their use as composts in agriculture appears as a good way to recirculate this organic matter but we must be aware that we have to be cautious with their use because they are different and soils harbour different microflora.

The results of this study conducted in microcosms demonstrate that compost amendments strongly influence soil biological properties at a short term, at a global level as well as at a community level. Modifications depended on both the organic matter utilized and the amended soil. In many of the studies claiming that composts can suppress plant diseases, suppressiveness of one type of compost has been tested against one pathogen. Using three different composts in two different soils, we showed that suppression of *R. solani* diseases will be likely better predicted based on soil–compost mixes than on pure composts. But a wide range of pathosystems need to be tested to ascertain that general soil suppressiveness operating through biological mechanisms will be stimulated thanks to the enhanced activity of the resident soil microbial communities resulting from organic amendments.

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