Compost amendments enhance peat suppressiveness to *Pythium ultimum*, *Rhizoctonia solani* and *Sclerotinia minor*

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**Abstract**

Peat is the most common organic material used for the preparation of potting mix because of its homogeneous and favorable agronomic characteristics. However, this organic material is poorly suppressive against soilborne pathogens and fungicides are routinely used to manage damping-off diseases. In the present study, we investigated the suppressive capability of five compost – peat mixtures towards the plant pathogens *Pythium ultimum*, *Rhizoctonia solani* and *Sclerotinia minor* – *Lepidium sativum* pathosystems. For all organic media, 18 parameters were measured including enzymatic activities (glucanase, N-acetyl-glucosaminidase, chitobiosidase and hydrolysis of fluorescein diacetate), microbiological (BIOLOG EcoPlates™, culturable bacteria and fungi), and chemical features (pH, EC, total, extractable and humic carbon, total and organic N, NH4–N, total protein and water content). In addition, 13C-CPMAS-NMR spectroscopy was used to characterize the organic materials. Peat amended with composts reduced disease damping-off caused by *P. ultimum*, *R. solani* and *S. minor* in 60% of the mixtures and compost derived from animal manure showed the largest and most consistent disease suppression. Sterilization decreased or eliminated suppressiveness of 42.8% of the mixtures. The most useful parameters to predict disease suppression were different for each pathogen: extractable carbon, O-aryl C and C/N ratio for *P. ultimum*, alkyl/O-alkyl ratio, N-acetyl-glucosaminidase and chitobiosidase enzymatic activities for *R. solani* and EC for *S. minor*. Our results demonstrate that the addition of composts to peat could be useful for the control of soilborne pathogens.

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1. Introduction

Plants produced in container-media are susceptible to soilborne pathogens causing damping-off diseases, which cause large yield loss in nursery and soil-less systems. Increasing public concerns regarding food safety and environmental pollution forced the progressive restrictions of soil fumigants and fungicides, and encouraged the research of alternative methods for the control of such fungal diseases (Lazarovits, 2001). Exploitation of the disease suppressive properties of organic amendments has been proposed to integrate or replace traditional control strategies (Noble and Coventry, 2005). Peat is the most utilized organic substrate for the preparation of potting mix (Carlile, 2009) because of its positive agronomic characteristics such as constant chemical and physical properties, high water retention capacity, optimal porosity and controlled pH. However, peat is hardly ever suppressive against soilborne pathogens such as *Rhizoctonia solani* (Kühn) and *Pythium* spp. (Bonanomi et al., 2007), and fungicides are routinely used to manage damping-off diseases. Moreover, the use of peat for horticultural purpose will probably be discouraged because of its limited sustainability and negative impact on global climatic changes due to the production of greenhouse gases (Carlile, 2009). Therefore, the substitution of peat with other organic substrates is urgently needed. In this view, the use of composts appears to be a promising strategy as demonstrated by successful applications in United States and Netherlands (Veeken et al., 2005).

Compost is obtained by the biological decomposition of organic materials which determines their chemical stabilization and the sanitization from human and plant pathogens and weed seeds (Noble and Roberts, 2004). Composts amendment has been proposed to control diseases caused by soilborne diseases, and there are many examples of pathogens effectively controlled by the application of such organic amendments: *Macrophomina phaseolina* (Tassi) (Lodha et al., 2002), *R. solani* (Termorshuizen et al., 2007) and *Verticillium dahliae* (Kleb.) (Malandraki et al., 2008), several species of *Fusarium* (Borrero et al., 2004), *Pythium* (Scheuermann 2000), *Verticillium* (Borraro et al., 2008) and *Cercospora* species (Borraro et al., 2008).
et al., 2005) and Sclerotium (Coventry et al., 2005). However, far less attention has been paid to the effect of organic amendments on some important pathogens such as Sclerotinia spp. (Lumsden et al., 1983). The capability of composts to suppress plant diseases could be related to: enhanced activities of antagonistic microbes that, competing with pathogens for substrate resources, cause microbiostasis (Serra-Whitling et al., 1996); release of fungitoxic compounds during decomposition (Kuter et al., 1988); and the induction of systemic resistance in host plants (Zhang et al., 1996).

The combined application of peat and suppressive composts at relatively low dosages, ranging from 1% to 20% by volume, appears to be a promising perspective because it maintains the agronomic features of peat but, simultaneously, enhances the suppressive capability of the potting mixtures (Veeken et al., 2005; van der Gaag et al., 2007). However, inconsistent disease suppression has been often reported, and the difficulty in predicting compost suppressiveness seriously hindered their practical use (Bonanomi et al., 2010). Since growers require a consistent effect of composts, the possibility of predicting suppressiveness to pathogens is a central issue for researchers.

Aim of this study is to assess the capability of five composts to enhance the suppressiveness of peat-compost potting mixtures to three common soilborne pathogens: Pythium ultimum (Trow), R solani and Sclerotinia minor (Jagger). To identify the mechanisms that determine compost disease suppressiveness, the organic materials were chemically and biologically characterized by measuring 18 parameters including chemical, microbiological, and enzymatic ones.

2. Methods

2.1. Organic media

Two commercial peat potting mixes were used. Sample identified as P1 which consisted of 50% light and 50% dark Sphagnum peats and 0.5 kg/m² PG-Mix: 14–16–18 (N, P, K); and P2, composed of 70% light and 30% dark Sphagnum peats and 0.5 kg/m² PG-Mix: 14–16–18 (N, P, K). Five composts derived from different feedstock origin were collected at commercial Italian factories. The following materials were collected: (1) composted residues obtained from a viticulture and enological factory (hereafter C1); composted organic fraction of differentiated municipal bio-waste (C2); composted organic fraction of undifferentiated municipal bio-waste (C3); composted cow manure (C4) and, finally, a medium obtained from composting a mixture of 50% organic fraction of differentiated municipal bio-waste and 50% peat (C5). Samples were collected by sub-sampling 10 aliquots for each commercial product (stocks size ranged between ~100 and ~500 kg).

2.2. Damping-off disease bioassay

The suppressive capability of P1 amended with the five different composts and P2 not amended to P. ultimum, R. solani and S. minor damping-off was evaluated by pot assays using Lepidium sativum L. as host plant. P1 peat was amended with the five composts at two rates (10% and 20% v/v). Bioassays were carried out with L. sativum which is recognized as a sensitive and reliable plant test (Bonanomi et al., 2006; Erhart et al., 1999). Not amended P1 and P2 were used as controls because they are two common horticultural media. All bioassays were carried out with sterile (twice autoclaved) and not sterile medium.

Several pathogen isolates were obtained from diseased lettuce (Lactuca sativa L.) (S. minor), rocket (Eruca sativa L.) (P. ultimum), and cabbage (Brassica oleracea L.) (R. solani) and maintained on PDA medium. Isolates of each species were preliminarily tested for pathogenicity on lettuce, L. sativum, and showed very similar behavior. Therefore, one isolate for each species was used in the bioassays. Isolates were stored on the fungi collection of the Department of Arboriculture, Botany and Plant Pathology, University of Naples “Federico II”, Italy.

Pathogen inocula were prepared as follows: common millet seeds were placed in 0.5 l flasks, saturated with a PDB (potato dextrose broth) solution (1/10 w/w) and twice autoclaved. Flasks were inoculated with fungi cultured on PDA (potato dextrose agar) for 7 days, and incubated for 21 days at 20 °C. The resulting fungal millet inoculum was air-dried for 3 days, powdered in a mortar, and added at four levels to the potting mixtures. Pathogen inoculum was used at different concentrations (0%, 0.3%, 1% and 3% w/w, dry weight) to test the effect of different inoculum density and to avoid the “flattening effect” of the results often observed when only one concentration is used (Termorshuizen et al., 2007). In the controls, non-inoculated common millet preared as described above was added.

Pots (7 cm diameter and 100 ml volume capacity) were filled with the different organic mixtures and sown with 20 L. sativum seeds cv. Comune (Blumen), moistened to field capacity and arranged in greenhouse (25 °C) following a complete randomized design. Pot distribution was rearranged randomly every 2 days to avoid the effects of environmental heterogeneity in the greenhouse. After 7 days disease incidence was recorded as percentage of diseased plants. Damping-off percentage was calculated as described by Veeken et al. (2005):

$$\% DO = \frac{HPo - HPi}{HPo} \times 100\%$$

where HPo is the number of healthy plants in the non-inoculated control mixture and HPI is the number of healthy plants in the inoculated potting mixtures.

Globally, the experimental design included seven organic mixtures applied at two rates, both sterile and not sterile with four inoculum levels and five replications. The full experimental design was applied on the three pathogens for a total of 1680 pots and 33,600 sowed seeds. The experiment has been repeated twice.

2.3. Chemical analyses and 13C-CPMAS-NMR spectroscopy

Different nitrogen forms (total N, total organic N = TON, and NH₄-N) were determined according to the European directive (Regalement CE n.2003 of 13/10/2003), and different carbon forms (total organic carbon = TOC, total extractable carbon = TEC, and humic carbon = C₄) were determined according to the Italian directive (G.U. n.21 del 26/01/2001). Water content of organic media was determined after desiccation at 105 °C for 72 h. EC and pH were determined according to the official methods of the Italian National Society of Soil Science (Violante, 2000).

All peats and composts were characterized by 13C Cross Polarization Magic Angle Spinning (CPMAS), Nuclear Magnetic Resonance (NMR) spectroscopy that allow a detailed characterization of the chemical composition of complex organic matter (Boehm et al., 1997; Kögel-Knabner, 2002). 13C NMR spectra were obtained in the solid state (CPMAS) under the same conditions in order to allow a quantitative comparison among spectra. Experiments were carried out with a Bruker AVANCE™ 300, equipped with a 4 mm Wide Bore MAS probe, operating at a 13C resonating frequency of 75.475 MHz. Samples (100–150 mg) were packed in 4 mm zirconia rotors with Kel-F caps and spun at 13 ± 1 kHz. To account for possible inhomogeneity of the Hartmann–Hahn condition at high rotor spin rates, a 1H ramp sequence was applied in CP experiments during a contact time (CT) of 1 ms. The 13C-CPMAS experiments were conducted collecting 6000 scans with 2266 data
points over an acquisition time of 25 ms, and a recycle delay of 2.0 s. The Bruker Topspin 1.3 software was used to collect and elaborate the spectra. All the free induction decays (FID) were transformed by applying a 4 k zero filling and a line broadening of 100 Hz. The areas for different $^{13}$C resonances were assigned according to Knicker and Lüdemann (1995) and Peuravuori and Pihlaja (1998) into seven integrating regions as follows: 188–165 ppm (aliphatic and aromatic carboxyl C, C in amide groups; carboxyl/amide), 164–142 ppm (oxygen substituted aromatic C from lignin and nonhydrolyzable tannins; phenolic, O-aryl), 141–112 ppm (unsubstituted and alkyl-substituted aromatic C; aryl), 111–93 ppm (anomeric C; di-O-alkyl), 92–67 ppm (oxidized and/or carbohydrate C; O-alkyl), 66–47 ppm (mainly methoxyl C; methoxyl/N-alkyl), 46–0 ppm (aliphatic C; alkyl). The area of each spectral region ($R_i$) was divided by the sum of all spectral areas, in order to obtain a relative percentage ($R_i$):

$$R_i = \left( \frac{A_{i,\text{abs}}}{\sum_i A_{i,\text{abs}}} \right) \times 100$$

The values were used as variables for further analysis. $^{13}$C NMR derived indices (CC/MC, alkyl/O-alkyl, (aryl + O-aryl)/O-alkyl) and the degree of hydrophobicity (HB/HI) of the substrates have been calculated according to Spaccini et al. (2000) and Almendros et al. (2001).

2.4. Microbiological, biochemical and enzymatic analyses

Total populations of culturable bacteria and fungi microbial were determined according to Larkin and Honeycutt (2006). Microbial activity was assessed by using the hydrolysis of fluorescein diacetate method (FDA) (Workneh et al., 1993). Total protein content and N-acetyl-glucosaminidase activity (NAGase), glucanolytic activity (glucanase), and chitobiosidase activity (Biase) were determined as follows. Peats and compost filtrates were obtained by suspending an aliquot of the organic material (100 g, dry weight) in sterile water (1 L) and incubated in a rotary shaker (140 rpm) at 25 °C. After 2 h, the suspensions were centrifuged at 16,000 g (Centrifuge Sorvall SC5C plus, USA) for 10 min at 20 °C. The supernatant was removed and concentrated to one-tenth of the initial volume in a rotary evaporator at 40 °C (Büchi Heating Bath B-490, Switzerland) and a vacuum membrane pump (Vacuubrand GMBH + CO, Germany). The concentrated sample was filtered through a 0.22-μm membrane filter (Millipore, Bradford, MA, USA), and stored at −20 °C with 20% glycerol until use. Filtrates were tested for the total protein concentration by colorimetric assay (Bio-Rad assay), for NAGase and glucanase activities as described by Napolitano et al. (2006), and for Biase activity as indicated by Harman et al. (1993).

BIOLOG® EcoPlates™ (BLG) provide a method for determination of community-level physiological profiling of microbial populations based on carbon substrate utilization (Biolog Inc., CA, USA). BLG consist of 96 wells containing 31 different carbon sources, and a blank in triplicate. As the carbon source is utilized the tetrazolium violet dye present in the wells is reduced developing a purple color. Absorbance readings were taken at 590 nm with a plate reader every 24 h for 96 h. Microbial activity in each microplate, expressed as average well-color development (AWCD) was determined as described by Gomez et al. (2006). Organic amendment suspensions (1 g of powdered dried material suspended in 20 ml of distilled water) were shaken (50 rpm) for 2 h at room temperature and then centrifuged at 800g for 2 min to extract the microbes. Supernatant was removed and stored at 4 °C, while the precipitate was processed by another extraction cycle. The second supernatant was added to the first one. Aliquots of 100 μl from this water microbial suspension, diluted at 10⁻² (dilution individuate by preliminary experiments), were inoculated into the microplates. The plates were incubated at 28 °C for 4 days.

2.5. Statistical analyses

Three-way ANOVA was applied for each pathogen to test the effects of compost type, application rate and sterilization on seedling damping-off. For this analysis we used the average damping-off assessed at different inoculum concentrations (0%, 0.3%, 1% and 3%). Percentage data of damping-off were arc sine to satisfy the assumption of normality. The sterilization effect of organic substrates was evaluated by the ratio of damping-off incidence in sterile/not sterile medium. Values above 1 of this ratio indicate loss of suppressiveness with sterilization, 1 no effect and values below 1 an increase of suppressiveness with sterilization. One-way ANOVA was used to test difference in chemical, microbiological and enzymatic characteristics among the organic substrates. Finally, the relationships among all substrate parameters and damping-off incidence were assessed using a regression analysis.

3. Results

3.1. Damping-off disease incidence

All pathogens incited high damping-off incidence on P1 and P2, with no statistically significant differences between the two materials (Fig. 1). Damping-off of L. sativum caused by all pathogens was significantly affected by compost type and sterilization, with a significant interaction between compost type and sterilization (Table 1). Since no significant dose effect (10% vs 20% w/w) was observed (Table 1), only data at 20% were reported. Compared to P1, all five composts significantly reduced P. ultimum incidence, three composts (C2, C3 and C4) controlled S. minor, and two composts (C1 and C4) suppressed R. solani (Fig. 1). Four composts showed multi-suppressive properties, i.e. were capable to control more
than one pathogen. Specifically, C5 suppressed only S. minor, C2 and C3 controlled P. ultimum and S. minor, C1 suppressed P. ultimum and R. solani and C4 was able to control all the pathogens (Fig. 1). Compost sterilization (heat treatment) reduced mixture suppressiveness in 9 of 21 cases, whereas an increase of disease suppression was not reported (Fig. 2). The magnitude of the sterilization effect was larger for C4 and C5 composts towards R. solani and for C3 towards S. minor (Fig. 2). All composts were not phytotoxic at the application rate used because not significant growth reduction was observed in the amended but non-inoculated controls (data not shown).

3.2. Chemical analyses and 13C-CPMAS-NMR spectroscopy

Total N and TON were generally larger for composts compared to peats (Table 2). Among composts, C4 showed the highest and C5 the lowest amount of N and TON. The concentration of NH4−N was rather high in C1 and C4 samples. C/N ratio was generally lower for composts, ranging from 9 to 19, compared to peats (Table 2). Peats were subacid, while all composts, with the exception of C5, have pHs above 9. Electrical conductivity (EC) of all samples was relatively low, but composts displayed higher values compared to peats (Table 2). The amount of humic carbon (Ca) and the degree of humification assessed by the DH and HR indexes reported variable values among composts and peats (Table 2).

The 13C-CPMAS-NMR analysis revealed distinct differences among the organic materials, especially between composts and peats. Peats have a more evident aliphatic alkyl-C region (0–46 ppm, characteristic of lipids), and a slightly lower level of the O-alkyl-C regions (67–92 and 93–111 ppm, mainly associated with sugars and polysaccharides). Moreover, the regions associated with methoxyl C (66–47 ppm) and with carboxyl C (188–165 ppm) were less developed in peats compared to composts, except for C5 (Table 3). As a consequence of these differences, peats have a higher alkyl/O-alkyl ratio and hydrophobicity index (HB/HI), with the exception of compost C3 (Fig. 3; Table 3). The differences of 13C NMR spectra among composts were less clear-cut. Samples C2 and C3 have more developed alkyl-C region, whereas the O-alkyl-C regions were larger in C5 and smaller in C3 (Table 3). The C3 sample reported the largest aryl region and the higher HB/HI index.

3.3. Microbiological, biochemical and enzymatic analyses

Populations of culturable bacteria and fungi were higher in C2 and C3 composts and lowest in C1 sample (Table 2). C2 and C3 were derived from the organic fraction of municipal bio-wastes. P1 and P2 reported intermediate values of fungal and bacterial populations (Table 2).

Enzymatic activities and protein content of P1 and P2 were comparable, with the exception of the Biase activity that was higher for P1 (Fig. 4). In contrast, composts showed a large variability for these parameters. In detail, Biase activity was low for all composts except for C4 that has a very high value (Fig. 4). The same pattern was found for NAGase activity (Fig. 4). FDA activity was higher for C5 followed by C3 and C4, while the lowest values were observed for C2 and C1 composts (Fig. 4). Glucanase activity was highest in the two peats and C4, and lowest in C3 and C1 (Fig. 4). The protein content was low for the two peats and C5, and high for C4 and C1 composts.

Based on the kinetics of color development in BLG plates, all composts reported higher AWCD values compared to peats (Fig. 4), with the highest values for C1. The maximum AWCD values showed by P1 and P2 after 92 h of incubation were only half of that of the compost with the lowest value (C5).

Table 1

<table>
<thead>
<tr>
<th>Effect</th>
<th>Pythium ultimum</th>
<th>Rhizoctonia solani</th>
<th>Sclerotinia minor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P-value</td>
</tr>
<tr>
<td>Compost type</td>
<td>4</td>
<td>44.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Application rate</td>
<td>1</td>
<td>1.3</td>
<td>0.25</td>
</tr>
<tr>
<td>Sterilization</td>
<td>1</td>
<td>26.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Compost type × application rate</td>
<td>4</td>
<td>2.1</td>
<td>0.10</td>
</tr>
<tr>
<td>Compost type × sterilization</td>
<td>4</td>
<td>2.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Application rate × sterilization</td>
<td>1</td>
<td>1.7</td>
<td>0.18</td>
</tr>
<tr>
<td>Compost type × application rate × sterilization</td>
<td>4</td>
<td>2.0</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* Compost type (C1–C5), application rate (10% and 20%) and sterilization (sterile and not sterile medium) are the main factors of the three analyses. P-values <0.05 in bold.

b df: degree of freedom.
3.4. Relation among substrate parameters and seedling damping-off

The whole cross-correlation matrix, being composed of 38 parameters and indexes, produced 703 Pearson correlation coefficients (data not shown). Herein, only the most relevant results are described.

Concerning chemical parameters, the different nitrogen forms (N total and TON) were positively auto-correlated and showed strong positive correlations with pH, BLG AWCD (Pearson coefficients: 0.84; P < 0.05) and the methoxyl C region of $^{13}$C NMR spectra (Pearson: 0.95; P < 0.01). The C/N ratio reported strong negative correlations with pH (Pearson: −0.93; P < 0.01), BLG AWCD (Pearson: −0.96; P < 0.01) and the carboxyl C region of $^{13}$C NMR spectra (Pearson: −0.94; P < 0.01). Total extractable carbon (TEC) was negatively correlated with C/N ratio (Pearson: −0.74; P < 0.01), directly correlated with pH (Pearson: 0.75; P < 0.05) and the carboxyl C region of $^{13}$C NMR spectra (Pearson: 0.87; P < 0.05). Populations of culturable bacteria and fungi were positively auto-correlated (Pearson: 0.97; P < 0.01), but only statistically not significant correlations were found with other parameters. The
four enzymatic activities were not auto-correlated, with the exception of Biase and NAGase that reported a strong positive correlation (Pearson: 0.98; \( \rho < 0.01 \)). In addition, Biase and NAGase activities were positively correlated with the amount of NH\(_4\)-N (Pearson: 0.95; \( \rho < 0.05 \)). FDA activity reported only not significant correlations with other parameters. Finally, glucanase activity reported only a positive correlation with the di-O-alkyl-C region of \(^{13}\text{C}\) NMR spectra (Pearson: 0.80; \( \rho < 0.05 \)).

Damping-off incidences caused by the three pathogens (\( P. \text{ultimum} \), \( R. \text{solani} \) and \( S. \text{minor} \)) were not auto-correlated (Table 4). \( P. \text{ultimum} \) damping-off in non-sterile medium was positively correlated with C/N ratio and with the O-aryl C region of \(^{13}\text{C}\) NMR spectra (Table 4). Interestingly, this last correlation was also observed in sterile medium. Moreover, \( P. \text{ultimum} \) disease in not sterile medium was negatively correlated with pH, TEC and the carboxyl C region of \(^{13}\text{C}\) NMR spectra (Table 4). Damping-off incidence caused by \( R. \text{solani} \) in not sterile medium was negatively correlated with Biase and NAGase activities, NH\(_4\)-N and total protein content (Table 4). In contrast, a significant positive correlation was found in not sterile media between damping-off incidence and the alkyl/O-alkyl ratio. This correlation was lost in sterile medium (Table 4). Finally, damping-off incidence caused by \( S. \text{minor} \) in non-sterile medium was negatively correlated with substrate EC (but not in sterile medium) and positively correlated with the O-aryl C region of \(^{13}\text{C}\) NMR spectra (Table 4).

4. Discussion

Peat amended with composts reduced disease damping-off caused by \( P. \text{ultimum} \), \( R. \text{solani} \) and \( S. \text{minor} \) in 9 out of 21 of the compost–peat combinations tested. This is an encouraging result (for \( P. \text{ultimum} \) all mixtures were suppressive) and indicates that some of these composts could be used to create suppressive substrates by partially substituting peat in the potting medium.

Since the 1970s, many studies demonstrated that compost application effectively controls many soilborne fungal pathogens (Noble and Coventry, 2005; Bonanomi et al., 2007). However, the majority of these early studies examined the capability of one or few composts to specifically suppress one pathogen (Trillas-Gay et al., 1986; Spring et al., 1980). Only more recent investigations assessed the suppressiveness of many composts towards different pathogens. For instance, Scheuerell et al. (2005) compared 36 compost types in three different pathosystems and Termorshuizen et al. (2007) applied 18 composts in seven pathosystems. These studies reported that disease suppressiveness was rather variable across different composts, and pathogen species. For instance, Termorshuizen et al. (2007) found that two different isolates of the fungus \( R. \text{solani} \), belonging to the same anastomosis group (AG2), assayed separately against cauliflower and pine on the same compost gave contrasting responses. In addition, variability in growth medium suppressiveness could be relevant also between materials obtained with similar composting processes and feedstock origins (Veeken et al., 2005). In a recent review, Bonanomi et al. (2010) found that organic amendments were suppressive to different pathogens only in few cases, whereas in the majority of the experiments analyzed showed that a material suppressive to one pathogen was ineffective or even conducive to other pathogens. Results of the present study also suggest that compost suppressiveness is often pathogen specific. In fact, only compost C4 significantly suppressed all pathogens, whereas C1 suppressed \( P. \text{ultimum} \) and \( R. \text{solani} \) but not \( S. \text{minor} \), C2 suppressed \( P. \text{ultimum} \) and \( S. \text{minor} \) but not \( R. \text{solani} \) and, finally, C5 effectively controlled \( P. \text{ultimum} \) but not \( R. \text{solani} \) and \( S. \text{minor} \). All these observations suggest that the identification of a compost that can be suppressive to all or many pathogens probably is not achievable. However, the practical use of compost suppressive to a specific pathogen could be possible if its specific suppressive properties can be accurately predicted. Unfortunately this is a difficult task.

In our study \( L. \text{sativum} \) damping-off caused by \( P. \text{ultimum} \) was suppressed by C2 and C3 composts, independently from sterilization treatment. This suggests that the biotic component of these composts plays a limited role, if any, in pathogen suppression. Similarly Pascual et al. (2002) reported that the humic fraction of a
municipal solid waste was effective in controlling \textit{P. ultimum} on \textit{Pisum sativum}, also in sterile conditions. In contrast with C2 and C3 samples, composts C1, C4 and C5 lost part of their suppressiveness after sterilization, suggesting that their biotic component played a significant role. The control of \textit{Pythium} has been often related to the general suppression model, which supposes that a broad variety of microbial species creates a competitive environment for pathogens (Baker and Cook, 1974). In this context, a promising predicting parameter was the FDA activity that in several studies resulted positively correlated with \textit{Pythium} suppressiveness (Chen et al., 1988; Stone et al., 2001). However, this model was not supported by Erhart et al. (1999), and this study also showed that FDA activity was not correlated with \textit{P. ultimum} suppression. The significant negative correlation between TEC and pH with \textit{P. ultimum} damping-off could be explained because the conductive peats have lower pH and TEC values compared to suppressive composts. In a previous study, Boehm et al. (1997), the conductive peats have lower pH and TEC values compared to suppressive composts (open bars) and the two peats (dark gray bars). Bars indicate +1 standard deviation, lowercase lettering indicates significant differences (One-way ANOVA: \(P < 0.05\), Duncan test).

In general term, \textit{R. solani} is considered to be the most problematic pathogen since its control with composts is a rare phenomenon (Krause et al., 2001; Scheuerell et al., 2005; Bonanomi et al., 2010). Scheuerell et al. (2005), for instance, found that only 17% of the assayed composts (\(n = 36\)) suppressed \textit{R. solani}, and that none of the 15 physical, chemical and biological measured parameters provided significant correlations with disease incidence. In this study, the two suppressive composts, C1 and C4, preserve or entirely lost their suppressiveness after sterilization, respectively, suggesting that the underlying mechanisms of pathogen suppression were different. The significant negative correlation between NAGase and Biase with damping-off is due to the C4 sample which had the highest enzymatic activities. Several studies demonstrated that \textit{R. solani} control is partially due to the specific action of such extracellular lytic enzymes produced by antagonists microbes (Jung et al., 2003). The specificity of this mechanism is consistent with the model of specific suppression proposed for \textit{R. solani} (Hoitink and Boehm, 1999). In contrast with C4 sample, the mechanism(s) that suppress \textit{R. solani} in C1 compost is probably different. In fact, this material had very low NAGase and Biase activities, but had the highest total protein and NH\textsubscript{4}–N content. This might explain the highly significant negative correlation between damping-off and total protein and NH\textsubscript{4}–N content. However, NH\textsubscript{4}–N has no fungitoxic properties as does NH\textsubscript{3} (Tenuta and Lazarovits, 2004) and, consequently, a direct role in \textit{R. solani} suppression seems unlikely. Interestingly, we found a significant positive correlation in not sterile medium between damping-off incidence and the alkyl/O-alkyl ratio, correlation that was lost in sterile medium. The alkyl/O-alkyl ratio is considered as a sensitive index of the stabilization and humification of organic matter. An increase of this ratio is interpreted as the result of a progressive degradation of carbohydrate and a corresponding increase of microbial derived lipid bio-macromolecules (Almendros et al., 2000). As this index increases, the bio-availability of carbohydrates, such as cellulose, progressively decreases because they are consumed by microbes and the remaining fraction is protected from decomposition, being enclosed in lignin which is more resistant to microbial decomposition (Berg and McClaugherty, 2003). The limited carbohydrate bio-availability

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Enzymatic activities (glucanase; \textit{N}-acetyl-glucosaminidase, NAGase; chitobiosidase, Biase; and hydrolysis of fluorescein diacetate, FDA), total protein content and metabolic activity measured by Biolog EcoPlate (average well-color development, AWCD) of the five composts (open bars) and the two peats (dark gray bars). Bars indicate +1 standard deviation, lowercase lettering indicates significant differences (One-way ANOVA: \(P < 0.05\), Duncan test).}
\end{figure}
Table 4

| Correlation matrix between chemical, microbiological, 13C-CPMAS-NMR derived and enzymatic parameters of the five composts and the two peats and damping-off incidence. Values are Pearson coefficients; statistical significances are from regression analysis (underline, \( P < 0.05 \); bold, \( P < 0.01 \)).

<table>
<thead>
<tr>
<th></th>
<th>Pythium unsterile</th>
<th>Pythium sterile</th>
<th>Rhizoctonia unsterile</th>
<th>Rhizoctonia sterile</th>
<th>Sclerotinia unsterile</th>
<th>Sclerotinia sterile</th>
</tr>
</thead>
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<td>-0.35</td>
<td>-0.32</td>
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<td>-0.67</td>
<td>-0.31</td>
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<td>0.35</td>
<td>0.23</td>
<td>0.30</td>
<td>0.41</td>
<td>0.04</td>
<td>0.14</td>
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<td>N total</td>
<td>-0.54</td>
<td>-0.34</td>
<td>-0.69</td>
<td>-0.59</td>
<td>-0.25</td>
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<tr>
<td>NH(_4)-N</td>
<td>-0.33</td>
<td>-0.08</td>
<td>-0.86</td>
<td>-0.49</td>
<td>-0.12</td>
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<td>TON(^a)</td>
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<td>-0.38</td>
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<td>-0.59</td>
<td>-0.24</td>
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<tr>
<td>TOC(^b)</td>
<td>0.69</td>
<td>0.55</td>
<td>0.17</td>
<td>-0.01</td>
<td>-0.06</td>
<td>-0.04</td>
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<td>TEC(^c)</td>
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<td>0.89</td>
<td>0.22</td>
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<td>-0.38</td>
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<td>-0.54</td>
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<td>-0.47</td>
<td>0.28</td>
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<td>0.25</td>
<td>0.04</td>
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<td>DHA(^f)</td>
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<td>0.51</td>
<td>-0.28</td>
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<td>0.30</td>
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<td>-0.15</td>
<td>-0.16</td>
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<td>C/N</td>
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<td>0.62</td>
<td>0.42</td>
<td>0.26</td>
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<td>Alkyl</td>
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<td>0.32</td>
<td>0.69</td>
<td>0.36</td>
<td>-0.14</td>
<td>-0.10</td>
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<td>Methoxy</td>
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<td>-0.63</td>
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<td>-0.08</td>
<td>-0.50</td>
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<td>Di-O-alkyl</td>
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<td>0.15</td>
<td>-0.30</td>
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<td>0.83</td>
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<td>0.91</td>
<td>0.08</td>
<td>-0.16</td>
<td>0.78</td>
<td>0.70</td>
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<td>-0.15</td>
<td>0.02</td>
<td>-0.42</td>
<td>-0.23</td>
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<td>Alkyl/O-alkyl</td>
<td>0.48</td>
<td>0.15</td>
<td>0.76</td>
<td>0.25</td>
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<tr>
<td>CC/MC(^h)</td>
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<td>0.09</td>
<td>0.48</td>
<td>0.76</td>
<td>0.26</td>
<td>-0.04</td>
</tr>
<tr>
<td>(Aryl + O-aryl)/O-alkyl</td>
<td>0.61</td>
<td>0.56</td>
<td>0.17</td>
<td>-0.36</td>
<td>0.40</td>
<td>0.83</td>
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<td>HB/HF(^i)</td>
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<td>0.17</td>
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<td>0.46</td>
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<tr>
<td>Protein</td>
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<td>-0.86</td>
<td>-0.81</td>
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<td>Glucanase</td>
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<td>0.34</td>
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<td>NAGase</td>
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<td>-0.74</td>
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<td>Biase</td>
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<td>-0.75</td>
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<td>FDA</td>
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<td>-0.57</td>
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<td>Rhizoctonia unsterile</td>
<td>0.71</td>
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<td>Rhizoctonia sterile</td>
<td></td>
<td>0.38</td>
<td>-0.50</td>
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<tr>
<td>Sclerotinia unsterile</td>
<td></td>
<td>0.77</td>
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<tr>
<td>Sclerotinia sterile</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Total organic nitrogen.
\(^b\) Total organic carbon.
\(^c\) Total extractable carbon.
\(^d\) Humic carbon.
\(^e\) Humification index = TEC – CH/CHA.
\(^f\) Humification degree = 100 × CH/TEC.
\(^g\) Humification ratio = 100 × CH/TOC.
\(^h\) CC/MC (Spaccini et al., 2000).
\(^i\) HB/HF, hydrophobic index (Spaccini et al., 2000).

probably impairs the ability of \textit{R. solani}, a species capable of exploiting cellulose as a carbon source \cite{Papavizas1970}, to incite the disease. The bio-availability of carbohydrate is considered crucial for suppression of \textit{Pythium} \cite{Hoitink1999} and \textit{R. solani} \cite{Tuitert1998}.

Compared to \textit{Pythium} spp. and \textit{R. solani}, fewer studies have investigated the possibility of control \textit{S. minor} with composts \cite{Bonanomi2007}, and only a few report a significant disease suppression \cite{Lumsden1983, Lumsden1986}. Consequently, very little information is available to predict disease incidence by this pathogen. \textit{Lumsden et al.} \cite{Lumsden1986} reported that \textit{S. minor} suppression after compost application was positively correlated with FDA activity, but \textit{Bonanomi et al.} \cite{Bonanomi2008} reported an opposite trend after soil amendment with plant residues \cite{Medicago sativa L. straw}. In this work, the FDA activity was not related to damping-off incidence. However, for all suppressive composts (C2, C3 and C4) part of their suppressiveness was lost after sterilization, suggesting a significant role of the biotic component. Of the 18 parameters assessed to characterize the different compost, only two showed a marginally significant correlation with \textit{S. minor} damping-off: the \textit{O}-aryl region of the 13C-CPMAS-NMR spectra and substrate EC. It is noteworthy, that the negative correlation between damping-off and EC was lost in sterile medium. This result suggests a high sensitivity of \textit{S. minor} to substrate salinity, but only in the presence of a native microbial community capable to compete with the pathogen. However, it is known that
compost chemistry and microbiology can be altered substantially during autoclaving treatment with changes in labile organic matter and mineral nitrogen forms (Tilston et al., 2002).

Finally, it is interesting to note that the information gathered from the carbon-source utilization profile of the BLG method, though being capable of distinguishing between peats and composts could not discriminate suppressive from non-suppressive composts for any of the studied pathogens. In this context, it is important to recall that while it is important to identify specific characteristics consistently associated with suppressive composts, it is equally important, as summarized in this work, to recognize variables unrelated to disease suppression (Table 4).

5. Conclusions

Peat amendments with compost have great potential for the control of soilborne pathogens, but their inconsistent performances still limit their wide use. There is no doubt that the beneficial effects of compost amendments far outweigh their detrimental effects. However, as long as the impact of this technique on disease suppression remains unpredictable, farmers may be justified in ignoring it as a tool for soilborne pathogen control. A strong effort has been made in the last decade to search for reliable indicators of compost suppressiveness (Bonanomi et al., 2010). Recent studies addressed this topic by characterizing a large number of compost samples for chemical, biochemical and microbiological features searching for parameters consistently correlated with disease suppression (e.g. Erhart et al., 1999; Scheuerrrell et al., 2005; Termorshuizen et al., 2007). This work provides some indication for the prediction of suppressiveness of different composts for P. ultimum (e.g. TEC, O-aryl C), R. solani (alkyl/O-alkyl ratio; NAGase and Biase enzymatic activities) and S. minor (EC). However, it is evident that any one variable alone cannot be a reliable and consistent parameter for predicting suppressiveness of all different OM amendments versus all soilborne pathogens. This likely occurs because the mechanisms of disease suppression are different and there may be many variables that need to be simultaneously monitored. This picture is further complicated by the large variability observed among and even within compost types and the often reported different response among pathogen isolates of the same species (Termorshuizen et al., 2007). Future studies are required to evaluate the reliability of the parameters proposed as indicators of suppressive compost, and to identify general principles capable to provide guidelines to predict the impact of compost amendment on soilborne diseases.

Acknowledgments

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References


