


RESEARCH

Open Access



Synergistic effect of organic and inorganic fertilization on the soil inoculum density of the soilborne pathogens *Verticillium dahliae* and *Phytophthora* spp. under open-field conditions

Claudio Cocozza¹, Emad Abdelhameed Abdeldaym², Gennaro Brunetti^{1*} , Franco Nigro¹ and Andreina Traversa¹

Abstract

Background: The increasing demand of food causes an excessive exploitation of agricultural lands, often inducing phenomena of soil sickness accompanied by the development of soilborne diseases. The use of residual biomasses together with inorganic fertilizers can be considered a good agricultural practice for controlling the inoculum density of soilborne phytopathogens since soil conditioners can release inorganic nitrogen, polyphenols and fatty acids that, especially in vitro, have demonstrated various degree of suppressiveness against such pathogens. Further, soil organic amendments can also modify the population of soil culturable bacteria and fungi that, in turn, can affect the soilborne diseases in several ways. With this study, the authors aim to evaluate the impact of the synergistic application of different biomasses and inorganic fertilizers on the soil inoculum density of *Verticillium dahliae* and *Phytophthora* spp. during two potato cycles under open-field conditions. The biomasses used for the fertilization of the potato crop were olive pomace residues (OPR), composts from municipal solid wastes (CMW), spent mushroom compost (SMC), and livestock manure-based compost (BRX).

Results: The inoculum density of *Verticillium dahliae* appeared inhibited by BRX due to its low C/N ratio that caused a quicker release of inorganic nitrogen with respect to the others soil conditioners. In contrast, OPR was conducive to the aforementioned soilborne pathogen since that biomass was characterized by a very high percentage of unsaturated fatty acids that, rather, stimulate the inoculum density of *V. dahliae*. Finally, polyphenols did not influence the same pathogen because they apparently turned into no toxic compounds very quickly. The inoculum density of *Phytophthora* spp. was reduced equally by all the biomasses used in combination with the inorganic fertilizers, regardless of their composition and quantity, mainly because of the development of general microbial suppression. Therefore, the chemical characteristics of the soil conditioners apparently did not affect the inoculum density of *Phytophthora* spp.

*Correspondence: gennaro.brunetti@uniba.it

¹ Dipartimento Di Scienze del Suolo Della Pianta e Degli Alimenti, Università Degli Studi Di Bari "Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy

Full list of author information is available at the end of the article

Conclusions: The results of this work underline the behavioral diversity of the different pathogens towards the different means adopted. *Phytophthora* spp. are sensitive to any kind of biomasses combined with inorganic fertilizers while the inoculum density of *Verticillium dahliae* should be reduced using soil conditioners characterized by low C/N ratio and low quantity of unsaturated fatty acids.

Keywords: Olive pomace, Compost, Spent mushroom compost, Livestock manure-based compost, Mineral N, Fatty acids, Polyphenols, Total culturable bacteria, Total culturable fungi

Background

Soilborne diseases are responsible to the reduction of yield and quality of crops and contribute to the development of the soil sickness [1]. Since the use of chemicals (fumigants and fungicides) has been restricted, other sustainable approaches are necessary to control the soilborne pathogens and guarantee high yield and quality of crops [2].

Organic amendments have been tested to control many soilborne pathogens such as *Rhizoctonia solani* [3, 4], *Verticillium dahliae* [5, 6], *Fusarium* spp. [7, 8], *Phytophthora* spp. [9], *Pythium* spp. [9, 10], *Sclerotinia* spp. [4], Chilosi et al. [11]) and *Sclerotium cepivorum* [12]. However, organic soil conditioners can be suppressive to some pathogens and conducive to others. Vestberg et al. [13] tested 21 composts against the strawberry crown rot caused by *Phytophthora cactorum* and the cucumber wilt disease caused by *Pythium* spp., and found that only 7 composts showed suppressiveness capacity towards these pathogens. Mazzola et al. [14] reported an increase of *Pythium*-related disease on apple after the application of *Brassica napus* seed meal at different rates, and a decrease of *Rhizoctonia* spp. with the lowest dose of the same treatment. Gilardi et al. [15] tested two *Brassica carinata* residues, *Brassica juncea*-based biofumigation, compost, chicken manure and cattle manure towards *Fusarium oxysporum* on lettuce crop and found that *Brassica carinata* and compost reduced the pathogen, while the green manure of *Brassica juncea*, cattle and chicken manure provided only its partial control. Scheuerell et al. [16] tested 36 composts against *Pythium ultimum* and *Pythium irregulare* on cucumber and against *R. solani* on cabbage and found disease suppression in 49% of cases but the sole *R. solani* was stimulated in 14% of trials. Bonanomi et al. [17] evaluated the effects of several biomasses on soilborne pathogens and found that compost was the most suppressive material, with more than 50% of cases; peat was suppressive only in 4% of treatments, while crop residues were suppressive and conducive in 45% and 28% of the trials, respectively. Chilosi et al. [11] used a green compost mixed with peat to evaluate its suppressiveness towards many soil pathogens and found a significant reduction of root rot

by *Sclerotinia sclerotiorum*, no effect toward *Phytophthora nicotianae* and an increase of disease produced by *Rhizoctonia solani*. Koivunen et al. [18] conducted a study on the persistence of *Botrytis cinerea* in organic field soils and found that this pathogen was suppressed by the addition of manure-based compost but not by yard waste compost. Further, the lower dose of compost and the addition of crab meal, i.e., chitin, enhanced the suppressiveness towards the pathogen.

The effectiveness of soil organic conditioners in the reduction of soil pathogens is linked to some conditions such as the plant–pathogen relationship, the rate of application, the degree of maturity of composted amendments or the decomposition stage of not composted organic residues [19], and the colonization of compost by specific groups of antagonistic microorganisms (Chilosi et al. [11, 20]). In addition, the composition of the biomasses used as soil conditioners can influence their effects against soilborne pathogens. The abundance of chitins or chitin-derived C compounds in some matrices stimulates the proliferation of chitinolytic agents responsible to the degradation of fungal pathogen cell walls and to reduction of their pathogenicity, while labile C substrates, such as simple sugars and cellulose, can reduce the suppressiveness capacity of composts [21, 22]. The pH of the biomasses can influence the availability of nutrients and the activity of microorganisms, in this sense, in more alkaline conditions pathogens such as fungi lose the competition with bacteria and through a biocontrol mechanism their pathogenicity is reduced [23, 24]. A higher electrical conductivity (EC) has also been shown to reduce sporulation and virulence of pathogens [24]. The kind of nitrogen, especially ammonium and nitrate, present in inorganic fertilizers or released from the mineralization of biomasses can influence plant disease incidence [25]. Dixon [26] reported that the use of calcium cyanamide suppresses soil-borne pathogens encouraging the development of beneficial microorganisms due to the slow release of N and the ready availability of calcium. Tenuta and Lazarovits [27] and Postma et al. [28] found that the application of biomasses characterized by a high N content was effective in the eradication of *Verticillium microsclerotia*. Rousk and Bååth [29] found a higher suppression of nematodes

and pathogen fungi with the application of alfa residues, characterized by a low C/N ratio, with respect to barley straw showing high C/N ratio and this result was ascribed to a favored bacterial growth and activity. Lazarovits et al. [30] found that the addition of organic amendments rich in N reduced the populations of plant pathogens, and strongly increased the populations of soil bacteria. Other studies have demonstrated that some short chain saturated fatty acids (FA) show inhibitory effects on several plant pathogens [30–33]. Even polyphenols have antimicrobial effects towards different bacteria, yeast, and fungi [34] and may be released during the decomposition of organic amendments or produced by microorganisms implied in the same decomposition, even if they are easily broken down by specific bacteria, yeasts and fungi in a short time and incorporated into the soil humic fraction [35].

The soil culturable microorganisms represent only a small part of the microbial community, but they are involved in the active and ongoing processes and are sensitive to soil management [36]. In addition, they include important genera of bacteria and fungi (e.g., *Bacillus*, *Pseudomonas*, *Streptomyces*, *Trichoderma*) that feature plant growth promoting activities, produce secondary metabolites useful for crop protection, show antagonistic features [37]. According to the review by Bonanomi et al. [38], total culturable bacteria and, with a similar pattern but a lower extent, total culturable fungi, appear the best microbiological parameters in terms of suppression indices with all kind of organic amendments and soil pathogens, and their increase is strictly related to the level and the transformation processes of the organic matter [39]. In particular, fluorescent pseudomonads, sporogenous bacteria and *Trichoderma* spp. show the highest percentage of positive correlation (73, 60% and 56%, respectively), with no cases of negative correlation with suppressiveness.

It is noteworthy that almost all the aforementioned researches have been conducted in vitro and not in less

predictable and more complex open-field conditions where other advanced investigation techniques, such as the next generation sequencing, show a low reliability [40]. Therefore, with the present study, the authors aimed to evaluate the effects of different biomasses, abundant in Mediterranean region and therefore easily available, on the soil inoculum density (ID) of *Verticillium dahliae* and *Phytophthora* spp. during two potato crop cycles inserted in a wider crop rotation. In particular, the authors investigated how the selected biomasses influenced the soil-borne pathogens ID through their composition and input of polyphenol and fatty acids, their impact on the soil culturable bacteria and fungi and their effects on the mineral forms of nitrogen.

Methods

Site description, treatments, and soil sampling

The trend of the soil ID of *Verticillium dahliae* and *Phytophthora* spp. was investigated in open field at a private farm located in Polignano a Mare (Bari), Italy. Since the farm produces many vegetables, the authors investigated the two crop cycles of potato of the following crop rotation: spinach, potato, head cabbage, potato, celery leaves.

The experimental field was divided into 15 randomized plots (5 × 5 m), deriving from three replicates of the following treatments: (1) olive pomace residues (OPR, 50 t ha⁻¹) arising from the mechanical extraction of olive oil of two phase mills; (2) compost deriving from composting of the organic fraction of municipal solid and pruning residues (CMW, 20 t ha⁻¹); (3) spent mushroom compost (SMC, 30 t ha⁻¹); (4) compost deriving from composting of cow, horse and poultry manures (BRX, 1.5 t ha⁻¹); (5) control without any application of soil conditioners (CON). Only the potato crop was treated with the previous organic soil amendments, while details of the entire crop rotation are reported in Table 1. The amendments have been incorporated into the soil by rotavation at about 20 cm soil depth. The dose of each amendment incorporated was established according

Table 1 Fertilization and sampling scheme of the experiment

Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Red	Blue		Orange				Purple					Red	Yellow		Orange		Purple							Red	Black	
T0			T1	T2					T3	T4			T5	T6			T7	T8								
Spinach			First potato crop					Head cabbage			Second potato crop			Celery leaves												

Red: the entire experimental field was fertilized with 1.5 t BRX ha⁻¹. Blue: fertigation of the entire experimental field with 50 kg urea phosphate ha⁻¹ at the beginning of September, and with 150 kg urea ha⁻¹ at the end of September. Orange: application of CMW, SMC, OPR and BRX according to the "Materials and methods" section. Purple: the entire experimental field was fertilized with 1 t 15:15:15 ha⁻¹. Yellow: fertigation of the entire experimental field with 150 kg ammonium sulphate ha⁻¹. Red and black: the entire experimental field was fertilized with 800 kg BRX ha⁻¹ and 400 kg calcium cyanamide ha⁻¹. Green: crop cycles

T0: Chemical analyses, total soil culturable fungi and bacteria, population density of *V. dahliae* and *Phytophthora* spp. before application of biomasses; T1: all microbial analyses after treatments; T2: total soil culturable fungi and bacteria; T3: all analyses at harvest; T4: chemical analysis before the application of biomasses; T5 and T6: total culturable soil fungi and bacteria; T7: all microbial analyses at harvest; T8: check of the residual effect of biomasses on the population density of *V. dahliae* and *Phytophthora* spp

to the local good agricultural practices, except for BRX that was incorporated at the label dose. Further details of the experimental site, amendments and treatments are reported elsewhere [1].

Each plot was sampled for chemical analyses by collecting five soil cores at a depth of 20 cm using an auger and a W sampling scheme. Analogously, each plot was sampled at a depth where *V. dahliae* and *Phytophthora* spp. may be more abundant (8–15 cm) for quantifying their ID, keeping samples in refrigerator at 5 °C for 3 days maximum till microbial analyses.

Chemical analyses of organic amendments and soils

Soil chemical analyses were performed in accordance with the methods by Page et al. [41]. The lipidic fraction of the biomasses was extracted using petroleum ether as a solvent in a Soxhlet apparatus and quantified. The fatty acids (FA) composition was determined as follows: the extracted phase was solubilized using 250 μL of H_2SO_4 1% v/v methanol solution and put in 1.5 mL centrifuge tubes. Samples were heated at 50 °C overnight. Two hundred and fifty μL of heptane were then added to each sample and mixed. After stratification, the upper phase was collected in amber glass vials and used for gas chromatographic analysis. A HP 5890 gas chromatographer equipped with a HP 88 column 100 m \times 0.25 mm id \times 0.2 film thickness was used. Fatty acid methyl esters were identified by external standards injection and expressed as percentage of the lipidic fraction [42].

The total polyphenols content of each soil conditioner was determined using the slightly modified Folin–Ciocalteu method described by Gambacorta et al. [43]. The amount of total phenols was expressed as gallic acid equivalents (GAE, mg gallic acid kg^{-1} sample) through the calibration curve of gallic acid.

Microbial analyses

The population of the soil culturable fungi and bacteria was determined according to the slightly modified protocol reported by De Mastro et al. [36]. In details, 10 g of each soil sample were suspended in 90 mL of sterile phosphate buffer (0.1 M, pH 6.8) and shaken vigorously at 270 rpm for 1 h. Then, 100 μL from several tenfold serial dilutions (10^{-1} – 10^{-6}) of each sample were spread onto 5 plates of (i) 0.1% tryptic soy agar (TSA) for total bacterial counts and (ii) potato dextrose agar (PDA) with 50 mg chlortetracycline and 1 mL L^{-1} tergitol for total fungal counts. Bacterial and fungal plates were incubated at 28 °C and 25 °C, respectively, for a week, and colonies were counted and expressed as $\log(\text{CFU} + 1) \text{ g}^{-1}$ of dry soil (CFU = Colony Forming Unit).

The inoculum density (ID) of *V. dahliae* microsclerotia was estimated according to the protocol of Harris

et al. [44], with some modifications. Briefly, soil samples were thoroughly mixed, air dried at room temperature for 30–40 days, carefully crumbled, and sieved with 2-mm openings. Twenty-five grams of soil were placed in 250 mL flasks and made up to 100 mL volume with sterile distilled water. Then, samples were agitated on a gyratory shaker, at 240 rpm for one hour, and the suspension was washed under running tap water through nested sieves with 160- μm and 25- μm openings. The residue collected on the 25- μm sieve was washed into a 50-mL beaker to obtain a final volume of 20 mL. Aliquots of 2-mL soil suspension under continuous stirring were individually distributed, by using a wide orifice pipette, on the surface of 10 Petri dishes containing a semiselective medium [45]. The inoculated petri dishes were incubated in dark room and at room temperature for 10–14 days. Then, the surface of incubated selective medium was gently washed under quite stream of fresh water to remove soil particles. All washed plates were incubated again at room temperature for 15–20 days. The ID of *V. dahliae*, determined by recording the colonies arising from microsclerotia in each plate under a 20 \times magnification stereomicroscope, was calculated as number of microsclerotia per gram of dried soil (mscl g^{-1}).

The ID of *Phytophthora* spp. was assessed using a selective medium (Masago et al. [46]) with minor modifications. Briefly, 10 g of 2-mm mesh sieved soil were suspended in 90 mL of water in an Erlenmeyer flask, and vigorously shaken, using a magnetic stirrer. One-milliliter aliquots of soil suspension were transferred with a wide orifice pipette to the surface of 6 Petri dishes containing the selective medium and spread by shaking the dish. After 24 h of incubation at 20 °C, soil was removed from the plates with a gentle stream of running water and incubated again at 20 °C for additional three days. Colonies showing the morphology of *Phytophthora* spp. were then counted to give the ID expressed as propagules per gram (ppg) of dry soil. Soil moisture was determined by desiccating 20 g of soil for 24 h at 110 °C.

Statistical analysis

Each treatment was performed in three biological replicates. Experimental data were tested against the normal distribution using the Shapiro–Wilk's test together with their homoscedasticity by means of the Levene test, then it was performed the analysis of variance (ANOVA) followed by the Tukey HSD test.

Results

Chemical results

Table 2 shows the main soil parameters at the beginning of the trial. Noteworthy are the textural class (silt clay

Table 2 Main soil properties at the beginning of the trial

Soil parameter	Unit	Average value
USDA Textural class		Silt clay loam
pH _{H2O} (1:2.5)		7.7 ± 0.07
pH _{KCl} (1:2.5)		7.1 ± 0.03
EC (1:2)	dS m ⁻¹	0.36 ± 0.03
OC	g kg ⁻¹	9.71 ± 0.37
TN		1.28 ± 0.05
Ca _{exc}		3.84 ± 0.36
K _{exc}		1.20 ± 0.07
Mg _{exc}		0.63 ± 0.03
Na _{exc}		0.08 ± 0.01
C/N		7.6
P _{ava}	mg kg ⁻¹	127 ± 6.0

EC electrical conductivity, OC organic carbon, TN total nitrogen

loam), the relatively low organic carbon content and the high concentration of available phosphorous.

No significant difference was observed in the soil N-NH₄⁺ concentration at the end of each potato crop regardless of the specific treatment (Table 3) although the quantity of N added with each amendment differed substantially [1]. In contrast, the soil nitrate content changed significantly among treatments: in fact, the BRX amended plots showed the highest values and OPR and CON the lowest ones (Table 3).

Table 4 shows the content of polyphenols in each soil conditioner and the quantity supplied to the soil through the treatments. Considering the quantity of soil conditioner used, OPR added the highest amount of polyphenols to the soil, i.e., about 6.5 and 12 kg ha⁻¹ in the first and the second year of trial, respectively. Conversely, BRX added the lowest amount, about 0.4 and 0.45 kg ha⁻¹ the first and the second year of trial, respectively, even if that soil conditioner was the richest in polyphenols content among all the biomasses tested.

Table 5 reports the lipidic content of each soil conditioner, the percentage of FA of the lipidic fraction, the quantity of lipids and fatty acids added to soil during the trial. The highest quantity of FA has been supplied by OPR (about 2.2 and 1.9 t ha⁻¹ the first and second year,

Table 3 Ammonium and nitrate content in soils at different sampling times

	CON	CMW	BRX	OPR	SMC
NH ₄ ⁺ (mg kg ⁻¹)					
T0	5.0 ± 0.6	4.8 ± 0.2	4.7 ± 0.5	5.1 ± 0.3	5.5 ± 0.7
T3	6.6 ± 0.6	7.9 ± 1.6	10.0 ± 4.3	7.7 ± 0.2	7.2 ± 0.3
T4	5.3 ± 0.3	5.4 ± 0.0	5.8 ± 0.0	5.8 ± 0.3	5.8 ± 0.6
T7	7.7 ± 0.4	7.0 ± 1.3	7.0 ± 0.3	7.1 ± 0.2	7.3 ± 1.4
NO ₃ ⁻ (mg kg ⁻¹)					
T0	33.2 ± 1.6	31.1 ± 0.9	30.7 ± 1.3	35.7 ± 0.6	34.6 ± 1.1
T3	37.5 ± 1.4 de	43.2 ± 6.0 cd	83.1 ± 9.2 a	32.1 ± 1.9 ef	43.1 ± 5.7 cd
T4	39.4 ± 4.2 d	39.6 ± 2.8 d	45.5 ± 1.7 c	34.8 ± 0.3 e	52.0 ± 5.6 bc
T7	40.7 ± 2.6 d	51.0 ± 8.5 bc	77.3 ± 3.6 a	45.0 ± 3.0 c	63.3 ± 12.3 b

T0, T3, T4, T7: sampling times (see Table 1 for details)

BRX cow, horse and poultry manures-based compost, OPR olive pomace residues, SMC spent mushroom compost, CMW compost obtained from the organic fraction of the municipal solid wastes

The values in each column followed by a different letter are significantly different according to Tukey's HSD test ($p < 0.05$)

respectively) since this biomass is still characterized by a certain amount of olive oil and was used in high quantity (50 t ha⁻¹). In contrast, SMC showed the lowest content of lipidic fraction (on average 0.24%) and the lowest percentage of FA (on average 35% of the lipidic fraction), therefore, even if it was added in relatively high quantity (30 t ha⁻¹), the amount of FA provided was the lowest (about 10 and 13 kg ha⁻¹ the first and the second year, respectively). The FA composition among biomasses varied: OPR presented the lowest number of FA, mainly oleic (about 63% of all FA), palmitic (17%), linoleic (9%) and vaccenic (2%), whereas BRX showed the highest number of FA, with oleic (33%), palmitic (26%), stearic (14%) and linoleic (5%) being the most represented acids. CMW and SMC contained almost the double FA than OPR but their composition was different, the former showed mostly oleic (25%), palmitic (19%), stearic (6%), elaidic (4%), myristic (3.6%), caprylic (2.8%) and lauric (2.5%) acids, whereas the latter was composed mainly by palmitic (7.8%), caproic (5%), stearic (3%) and oleic (3%) acids, with the remaining ones showing roughly similar concentration.

Table 4 Polyphenols content in the different organic matrices added to soil at each treatment

Organic matrices	First year				Second year			
	CMW	BRX	OPR	SMC	CMW	BRX	OPR	SMC
Total polyphenols content (mg kg ⁻¹)	78.65	307.48	200.68	100.66	96.64	349.26	371.48	177.32
Total polyphenols added (kg ha ⁻¹)	1.33	0.40	6.42	2.38	1.63	0.45	11.89	1.35

BRX cow, horse and poultry manures-based compost, OPR olive pomace residues, SMC spent mushroom compost, CMW compost obtained from the organic fraction of the municipal solid wastes

Table 5 Content of lipidic fraction and fatty acids in organic matrices and their quantity added with each treatment

Organic matrices parameters	First year				Second year			
	CMW	BRX	OPR	SMC	CMW	BRX	OPR	SMC
Lipidic fraction content (%)	2.48	2.19	7.34	0.23	2.12	2.36	6.42	0.25
Lipids added (kg ha ⁻¹)	420	28.4	2347	30.3	358	30.6	2054	33.5
Fatty acids (% of the lipidic fraction)	74.6	89.8	93.8	33.2	77.7	85.9	91.1	37.5
Saturated fatty acids (% of the fatty acids)	52.1	49.6	19.7	79.4	50.7	51.5	20.9	83.3
Unsaturated fatty acids (% of the fatty acids)	47.9	50.4	80.3	20.6	49.3	48.5	79.1	16.7
Fatty acids added (kg ha ⁻¹)	313	25.5	2201	10.1	278	26.3	1871	12.6

BRX cow, horse and poultry manures-based compost, OPR olive pomace residues, SMC spent mushroom compost, CMW compost obtained from the organic fraction of the municipal solid wastes

Total culturable bacteria and fungi

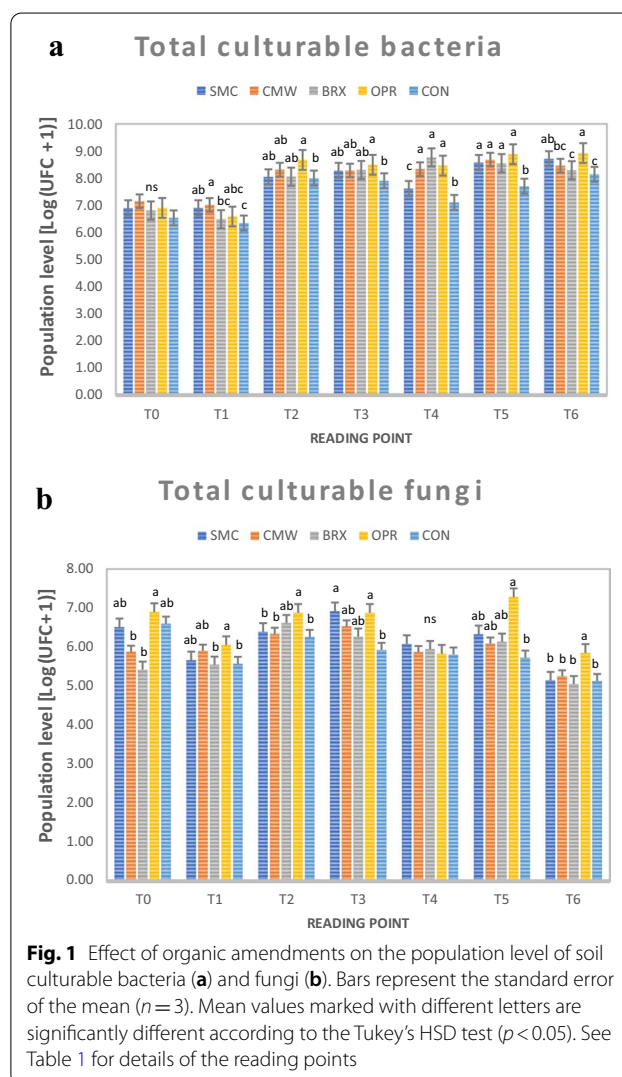
Figure 1 shows the trend of soil culturable bacteria (SCB) and fungi (SCF), during the trial. One month after the first organic amendment (T1), control plots had the lowest SCB population with respect to the treated soils, showing the effect of the biomasses on these microorganisms. After that, OPR was the only soil conditioner that induced always a significantly higher SCB population, probably due to the largest amount applied over the course of the two crop cycles (Fig. 1a). A similar trend was observed for the SCF population: at T1, control and BRX plots showed the lowest SCF population, but in the following sampling point OPR induced the highest population level of SCF, with significantly different value than the control (Fig. 1b).

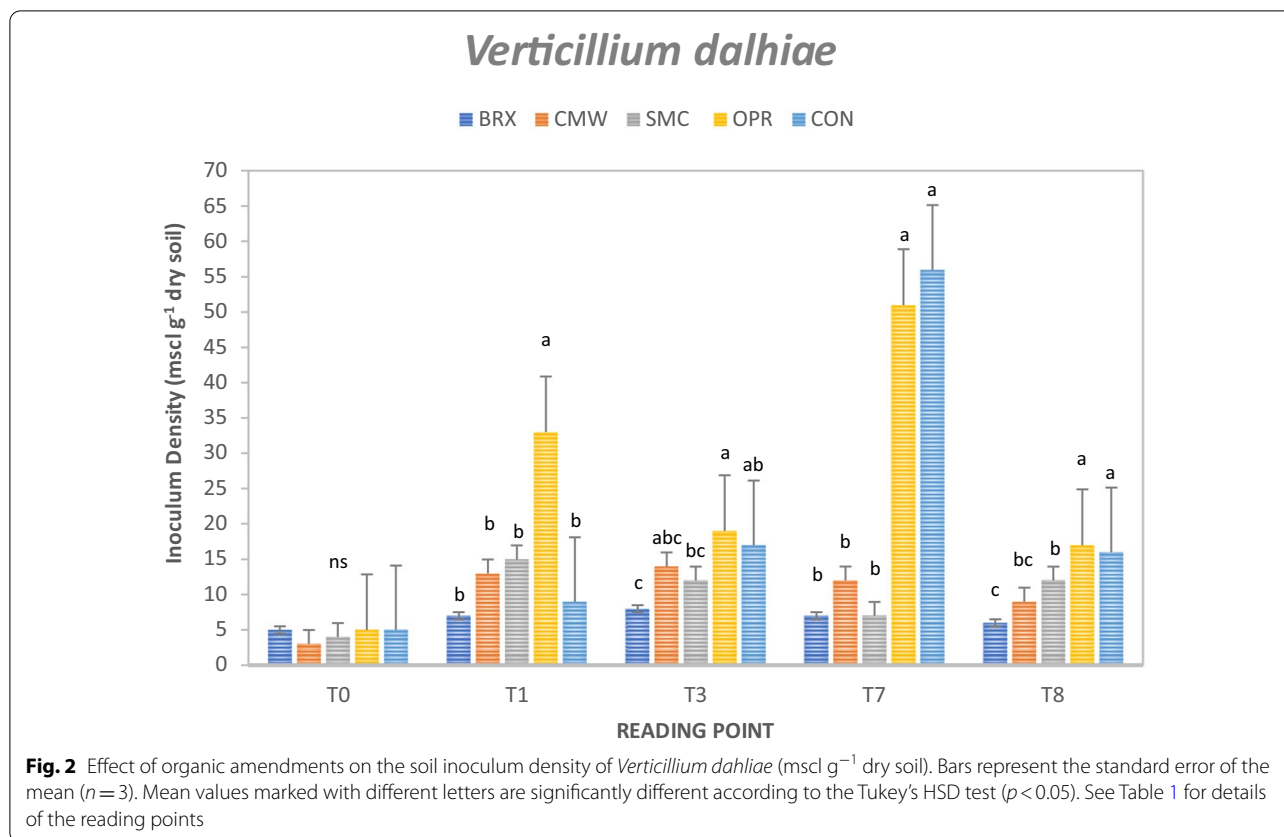
Inoculum density of soil pathogens

The ID of *V. dahliae* during the trial is reported in Fig. 2. At the beginning of the experiment (T0), before the incorporation of the biomasses, the ID was similar among all plots. One month later, the number of microsclerotia increased significantly in plots treated with OPR remaining lower and similar in all other treatments and CON. At the end of the first potato cycle (T3), OPR plots showed the highest value of *V. dahliae* microsclerotia while BRX was the treatment with the lowest content of the pathogen. At the end of the second potato cycle (T7), OPR and CON plots resulted in the highest number of microsclerotia with respect to the other treatments, and the same trend was observed at the last sampling too. Two months later the synergistic application of a calcium cyanamide-based fertilizer (400 kg ha⁻¹) and BRX (800 kg ha⁻¹) to all plots (T8, Table 1), the ID was still significantly lower in BRX plots with respect to OPR and CON.

The BRX application, above all, was the treatment that markedly reduced the ID of *V. dahliae*, whereas OPR was the worst, due to the similar results with CON, rather it was even conducive at the beginning of the trial, one

month after the first application of the biomasses (T1). The CMW and SMC treatments showed roughly the same trend, with results closer to BRX than OPR.





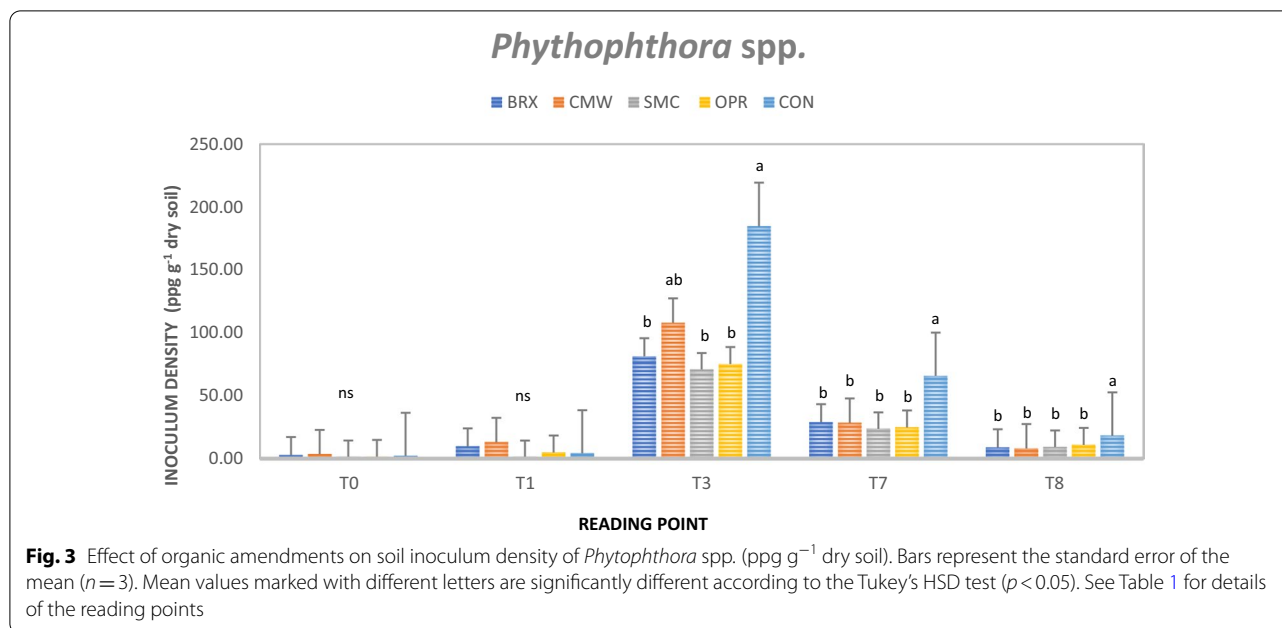
A significant negative correlation has been found between *V. dahliae* ID and soil nitrate concentration ($r = -0.991$, $p = 0.001$) or biomass pH ($r = -0.975$; $p = 0.02$), while the EC of each organic matrix was also negatively correlated to the ID of the pathogen, but not significantly ($r = -0.827$; $p = 0.173$). Significant positive correlations have been found between the total polyphenols or the quantity of lipids supplied with each soil conditioner and the ID of *V. dahliae* ($r = 0.994$, $p = 0.006$ and $r = 0.991$, $p = 0.009$, respectively).

The trend of *Phytophthora* spp. ID was different from that of *V. dahliae*. At T0 and T1, no differences were observed among all treatments probably due to the lack of specific host plant to parasitize (the first transplantation occurred three months later) and the low winter temperature that could have affected the surviving rate of the pathogens (Fig. 3). At the end of the first and second crop cycle (T3 and T7), the effects of biomasses on the ID of *Phytophthora* spp. were evident since the control showed a significantly higher ID than the plots treated with biomasses. No significant correlation was observed between the growth of *Phytophthora* spp. and each single chemical parameter ($r = 0.461$, $p = 0.539$; $r = 0.473$, $p = 0.527$; $r = -0.384$, $p = 0.523$; $r = -0.435$, $p = 0.565$;

$r = -0.278$, $p = 0.722$ for pH, EC, nitrate, polyphenols and lipids, respectively).

Discussion

The higher nitrate content recorded at the end of each potato crop cycle is the results of the fertilization of potato and, since all plots received the same amount of inorganic fertilizers, the differences among treatments were possibly due to the quality of each biomass, rather than the quantity applied, that could have played a role in the mineralization and release of the mineral N. In particular, at the end of the experiment, soil nitrate content increased by about 152% after the amendment with BRX, while it increased only by 26% after the amendment with OPR. This result is apparently associated to the C/N ratio of biomasses, since it can influence their mineralization and the development of the beneficial microbial community [27, 29, 30]. BRX and OPR showed C/N ratio of 12.3 and 92.4, respectively [1], and since a positive correlation between N assimilation into microbial biomass and the C/N ratios of soil conditioners exists [47], the former soil conditioner induced the soil microbial community to immobilize less N and increased the rate of nitrification. The faster mineralization of BRX, as indicated by



the higher soil nitrate content (Table 2) at the end of each crop cycle (T3 and T7, Table 1) and the negative correlation between *V. dahliae* ID and soil nitrate concentration ($r = -0.991$, $p = 0.001$), determined a better control of this pathogen. The reduction of *V. dahliae* ID observed with the application of BRX may be caused by toxic substances, such as ammonia and nitric acid that can disrupt the membranes of microsclerotia and that are released especially when the C/N ratio of the medium is low [27]. In addition, OPR showed higher *V. dahliae* ID because that biomass was characterized by the lowest values of pH (5.7–6.3) and EC (0.9–1 dS m⁻¹) among the biomasses studied [1], while suppressiveness is related to alkaline pH and high EC value [24].

Other parameters generally responsible to lower pathogens ID are the amount and composition of SCB and SCE, influenced by the nature of amendments. Enwall et al. [48], Pezzolla et al. [49] and Ye et al. [47] reported that the application of organic amendments influences the soil microbial biomass due to the introduction of exogenous microorganisms and available organic compounds. In particular, the quantity applied and the presence of lignocellulosic residues, deriving from the ground olive kernels, can be the reason for the positive effect of OPR on the SCB and SCF populations (Fig. 1). Our results are in accord with another study reporting an increase of bacterial and fungal populations in the soil after the amendment with OPR [50], and a high soil microbial population increases the competition for nutrients among microorganisms and enhances the antibiotic-producing bacteria [51]. Although the OPR

application resulted in higher SCB and SCE, it was the worst biomass in controlling the ID of *V. dahliae*, suggesting that the control of this pathogen was mainly due to the release of mineral N rather than the stimulation of beneficial microorganisms. With regards to *Phytophthora* spp., Bonanomi et al. [38] demonstrated that the C/N ratio was not indicative of suppressiveness for those soil-borne pathogens, and the microbiostasis was the mechanisms against phytopathogens with propagules less than 200 μm diameter as *Pythium* spp. and *Phytophthora* spp. In our study, the higher SCB and SCF registered in the amended plots with respect to the control ones (Fig. 1) could be a marker indicating an increase of antagonistic microbial population able to control *Phytophthora* spp. and leading to similar effect of the different soil conditioners, as reported elsewhere [9, 20].

The content of polyphenols added with the different amendments was higher for OPR with respect to the other soil conditioners, especially with respect to BRX (Table 4). El Abbassi et al. [52] reported many previous studies in which some polyphenols, especially those deriving from cinnamic and benzoic acids from biomasses such as OPR, inhibit different pathogenic fungi, but in our study positive correlation has been found among the ID of *V. dahliae* and the content of polyphenols added ($r = 0.994$, $p = 0.006$). In the open-field conditions, the apparent stimulation of the pathogen can be the result of a fast degradation/mineralization of the in vitro active antifungal polyphenols into new molecules less or no toxic implied in the formation of soil humic substances [35, 53]. In fact, previous studies reported that

polyphenols persist temporarily in soils due to the degradation by fungi, bacteria and yeasts [53–57], and monomers can be adsorbed by the soil colloids, or recombined to form new polyphenols.

In the present study, the biomass that added the higher quantity of lipids was OPR. Its lipidic fraction was mainly constituted by unsaturated FA (about 80%, Table 5), and by a relatively high content of the saturated palmitic acid (about 400 and 350 kg ha⁻¹ added the first and second year, respectively), and that could have promoted the *V. dahliae* ID. In fact, even if fatty acids are considered capable of inhibiting or disrupting biofilm formation by various microbial pathogen [58] or interfering with fungal sphingolipid biosynthesis [59], several in vitro studies have shown that saturated short chain fatty acids are the most efficient in the suppression of pathogens. Kumar et al. [58] demonstrated that capric acid and undecylic acid, 10 and 11-carbon saturated fatty acids, respectively, killed the pathogen *Candida albicans* even at low concentrations. Liu et al. [32], in a study conducted in vitro on three phytopathogenic fungi, found an inhibitory effect on spore germination with the addition of caproic, caprylic, or capric acid at very low doses (1 μm L⁻¹), or lauric or palmitic acid at a higher dose and only on some pathogens. In the same study no inhibitory effect was observed with the addition of oleic, linoleic or myristic acid. Undecylic acid, together with the myristic one, were identified as antifungal agents towards a series of pathogens such as *Trichoderma*, *Aspergillus*, *Candida*, *Fusarium*, *Pythium*, *Rhizoctonia* [60]. In contrast, laboratory tests have demonstrated that unsaturated fatty acids stimulated the germination of *Pythium ultimum* [61], and had a sporogenic effect on *Aspergillus* spp. [31, 62]. Harman et al. [63] found that palmitic acid stimulated the germination of *Fusarium solani* together with oleic and linoleic acids. In addition, a previous study [64] has demonstrated that linoleic and oleic acids were substrates utilized by fungi for the production of oxylipins that regulated the fungal development. The significantly higher values of microsclerotia observed in OPR plots can be presumably ascribed to the quality of FA that did not control the pathogen. This behavior was particularly evident at T7, when plots treated with OPR, applied twice during the trial, showed a peak of microsclerotia similar to CON and over seven times higher than BRX. The second soil conditioner in terms of quantity of lipids added was CMW, and its FA were almost equally divided between unsaturated and saturated (Table 4), among the latter ones, the relatively high content of myristic, lauric and caprylic acids could have promoted its good results in controlling *V. dahliae*.

Conclusions

The application of different biomasses with the same inorganic fertilization affected differently the ID of *V. dahliae* and *Phytophthora* spp. in open field. With respect to the former pathogen, the mineral N can be considered the most important parameter for its control, therefore one should select for that aim biomasses characterized by less recalcitrant organic matter and low C/N ratio, since their organic N can mineralize easier.

Soil conditioners characterized by large quantity of unsaturated fatty acids and applied at high rate per hectare can be conducive to *V. dahliae* and can stimulate the population level of both culturable fungi and bacteria. Soils already infested by *V. dahliae* or cultivated with crops susceptible to that pathogen should not be amended with this type of biomasses.

The polyphenols content of each soil conditioner apparently does not play an important role in controlling *V. dahliae* since they should be quickly transformed into no toxic compounds shortly after the amendment.

Phytophthora spp. are always susceptible to the synergistic application of inorganic fertilizers and biomasses regardless of the composition and the quantity of the latter. The reasons could be ascribed to an increase of beneficial microorganisms able to control these pathogens, rather than chemical parameters; however, more studies in open fields are necessary to confirm these conclusions.

Abbreviations

OPR: Olive pomace residues; CMW: Composts from municipal solid wastes; SMC: Spent mushroom compost; BRX: Livestock manure-based compost; CON: Control; FA: Fatty acids; PDA: Potato dextrose agar; TSA: Tryptic soy agar; CFU: Colony forming units; ANOVA: Analysis of variance; GAE: Gallic acid equivalents; ID: Inoculum density, that is the part of the pathogen population that gives the infection; SCB: Culturable bacteria; SCF: Culturable fungi.

Acknowledgements

Not applicable

Authors' contributions

CC, GB and FN conceived and designed the experimental strategies and manuscript. EAA performed all experiments and analyses. CC and AT have organized all data to insert in the publication. CC, GB and FN have supervised all experiments and analyses. CC, AT and FN have performed the statistical analysis. CC and AT have written the original draft of manuscript. CC, GB and FN have revised and validated the results. All authors read and approved the final manuscript.

Funding

This research received no external funding.

Availability of data and materials

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This manuscript is an original research and has not been published or submitted in other journals.

Consent for publication

All authors agreed to publish in the journal.

Competing interests

The authors declare that they have no competing interests.

Author details

¹ Dipartimento Di Scienze del Suolo Della Pianta e Degli Alimenti, Università Degli Studi Di Bari "Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy. ² Faculty of Agriculture, Department of Vegetable Crops, Cairo University, Giza, Egypt.

Received: 30 December 2020 Accepted: 18 March 2021

Published online: 05 May 2021

References

- Abdeldaym EA, Traversa A, Cocozza C, Brunetti G. Effects of a two-year application of different residual biomasses on soil properties and potato yield. *Clean*. 2018. <https://doi.org/10.1002/clean.201800261>.
- Lelario F, Scrano L, De Franchi S, Bonomo MG, Salzano G, Milan S, Milella L, Bufo SA. Identification and antimicrobial activity of most representative secondary metabolites from different plant species. *Chem Biol Technol Agric*. 2018. <https://doi.org/10.1186/s40538-018-0125-0>.
- Pan X, Mihail JD, Kremer RJ, Xiong X. Fungistatic effect of a chicken manure-based organic fertilizer for suppression of a soilborne pathogen *Rhizoctonia Solani* Kühn. *J Soil Plant Biol*. 2019. <https://doi.org/10.33513/JSPB/1901-07>.
- Pane C, Piccolo A, Spaccini R, Celano G, Vilecco D, Zaccardelli M. Agricultural waste-based composts exhibiting suppressivity to diseases caused by the phytopathogenic soil-borne fungi *Rhizoctonia solani* and *Sclerotinia minor*. *Appl Soil Ecol*. 2013. <https://doi.org/10.1016/j.apsoil>.
- Avilés M, Borrero C. Identifying characteristics of *Verticillium* wilt suppressiveness in olive mill composts. *Plant Dis*. 2017. <https://doi.org/10.1094/PDIS-08-16-1172-RE>.
- Lang J, Hu J, Ran W, Xu Y, Shenet Q. Control of cotton *Verticillium* wilt and fungal diversity of rhizosphere soils by bio-organic fertilizer. *Biol Fertil Soils*. 2012. <https://doi.org/10.1007/s00374-011-0617-6>.
- Castaño R, Borrero C, Avilés M. Organic matter fractions by SP-MAS 13C NMR and microbial communities involved in the suppression of *Fusarium wilt* in organic growth media. *Biol Control*. 2011. <https://doi.org/10.1016/j.biocontrol.2011.05.011>.
- Pane C, Celano G, Piccolo A, Vilecco D, Spaccini R, Palese AM, Zaccardelli M. Effects of on-farm composted tomato residues on soil biological activity and yields in a tomato cropping system. *Chem Biol Technol Agric*. 2015. <https://doi.org/10.1186/s40538-014-0026-9>.
- De Corato U, Viola E, Arcieri G, Valerio V, Zimbardi F. Use of composted agro-energy co-products and agricultural residues against soil-borne pathogens in horticultural soil-less systems. *Sci Hortic*. 2016. <https://doi.org/10.1016/j.scienta.2016.07.027>.
- Vestberg M, Kukkonen S, Parikka P, Yu D, Romantschuk M. Reproducibility of suppression of *Pythium wilt* of cucumber by compost. *Agric Food Sci*. 2014. <https://doi.org/10.23986/afsci.41471>.
- Chilosi G, Aleandri MP, Bruni N, Tomassini A, Torresi V, Muganu M, Paolucci M, Vettraino A, Vannini A. Assessment of suitability and suppressiveness of on-farm green compost as a substitute of peat in the production of lavender plants. *Biocontrol Sci Technol*. 2017. <https://doi.org/10.1080/09583157.2017.1320353>.
- Coventry E, Noble R, Mead A, Whipps JM. Suppression of Allium white rot (*Sclerotium cepivorum*) in different soils using vegetable wastes. *Eur J Plant Pathol*. 2005. <https://doi.org/10.1007/s10658-004-1420-0>.
- Vestberg M, Kukkonen S, Rantala S, Prochazka P, Tuohimetsä S, Setälä H, Romantschuk M, Kurola J, Yu D, Parikka P. Suppressiveness of Finnish commercial compost against soil borne disease. *Acta Hortic*. 2011. <https://doi.org/10.17660/ActaHortic.2011.891.5>.
- Mazzola M, Granatstein DM, Elfving DC, Mullinix K. Suppression of specific apple root pathogens by *Brassica napus* seed meal amendment regardless of glucosinolate content. *Phytopathology*. 2001. <https://doi.org/10.1094/phyto.2001.91.7.673>.
- Gilardi G, Pugliese M, Gullino ML, Garibaldi A. Effect of different organic amendments on lettuce fusarium wilt and on selected soilborne microorganisms. *Plant Pathol*. 2016. <https://doi.org/10.1111/ppa.12460>.
- Scheuerell SJ, Sullivan DM, Mahaffee WF. Suppression of seedling damping off caused by *Pythium ultimum*, *Pythium irregulare*, and *Rhizoctonia solani* in container media amended with a diverse range of Pacific Northwest compost sources. *Phytopathology*. 2005. <https://doi.org/10.1094/phyto-95-0306>.
- Bonanomi G, Antignani V, Pane C, Scala F. Suppression of soilborne fungal diseases with organic amendments. *J Plant Pathol*. 2007;89:311–24.
- Koivunen EE, Tully KL, Swett CL. Co-managing soil and plant pathogens: effects of organic amendments on soil fertility and fungal pathogen survival. *Plant Soil*. 2018. <https://doi.org/10.1007/s11104-018-3779-2>.
- Janvier C, Villeneuve F, Alabouvette C, Edel-Hermann V, Manteille T, Steinberg C. Soil health through soil disease suppression: which strategy from descriptors to indicators? *Soil Biol Biochem*. 2007. <https://doi.org/10.1016/j.soilbio.2006.07.001>.
- De Corato U. Disease-suppressive compost enhances natural soil suppressiveness against soil-borne plant pathogens: A critical review. *Rhizosphere*. 2020. <https://doi.org/10.1016/j.rhisph.2020.100192>.
- Hadar Y, Papadopoulou K. Suppressiveness of composts: microbial ecology links between abiotic environments and healthy plants. *Annu Rev Phytopathol*. 2012. <https://doi.org/10.1146/annurev-phyto-081211-172914>.
- Kavroulakis N, Ntougias S, Besi MI, Katsou P, Damaskinou A, Ehaliotis C, Zervakis GI, Papadopoulou KK. Antagonistic bacteria of composted agro-industrial residues exhibit antibiosis against soil-borne fungal plant pathogens and protection of tomato plants from *Fusarium oxysporum* f.sp. *radicis-lycopersici*. *Plant Soil*. 2010. <https://doi.org/10.1007/s11104-010-0338-x>.
- Brady N, Weil RR. The nature and properties of soils. New York: Prentice Hall, Upper Saddle River; 2008.
- Cotxarrera L, Trillas-Gay MI, Steinberg C, Alabouvette C. Use of sewage sludge compost and *Trichoderma asperellum* isolates to suppress *Fusarium wilt* of tomato. *Soil Biol Biochem*. 2002. [https://doi.org/10.1016/S0038-0717\(01\)00205-X](https://doi.org/10.1016/S0038-0717(01)00205-X).
- Ghorbani R, Wilcockson S, Koocheki A, Leifert C. Soil management for sustainable crop disease control: a review. *Environ Chem Lett*. 2008. <https://doi.org/10.1007/s10311-008-0147-0>.
- Dixon GR. Managing clubroot disease (caused by *Plasmodiophora brassicae* Wor.) by exploiting the interactions between calcium cyanamide fertilizer and soil microorganisms. *J Agric Sci*. 2017. <https://doi.org/10.1017/S0021859616000800>.
- Tenuta M, Lazarovits G. Ammonia and nitrous acid from nitrogenous amendments kill microsclerotia of *Verticillium dahliae*. *Phytopathology*. 2002. <https://doi.org/10.1094/phyto.2002.92.3.255>.
- Postma J, Schilder MT, Bloem J, van Leeuwen-Haagsma WK. Soil suppressiveness and functional diversity of the soil microflora in organic farming system. *Soil Biol Biochem*. 2008. <https://doi.org/10.1016/j.soilbio.2008.05.023>.
- Rousk J, Bååth E. Fungal and bacterial growth in soil with plant materials of different C/N ratios. *FEMS Microbiol Ecol*. 2007. <https://doi.org/10.1111/j.1574-6941.2007.00398.x>.
- Lazarovits G, Conn KL, Potter J. Reduction of potato scab, verticillium wilt, and nematodes by soy meal and meat and bone meal in two Ontario potato fields. *Can J Plant Pathol*. 1999. <https://doi.org/10.1080/0706669909501170>.
- Aly A, Hussein E, Omar M, El-Abbasi I, Abd-Elsalam K. Effect of fatty acid content on the level of cottonseed colonization by fungi. *Biol Lett*. 2011. <https://doi.org/10.2478/v10120-011-0013-9>.
- Liu S, Weibin R, Jing L, Hua X, Jingan W, Yubao G, Jingguo W. Biological control of phytopathogenic fungi by fatty acids. *Mycopathologia*. 2008. <https://doi.org/10.1007/s11046-008-9124-1>.
- Walters D, Raynor L, Mitchell A, Walker R, Walker K. Antifungal activities of four fatty acids against plant pathogenic fungi. *Mycopathologia*. 2004. <https://doi.org/10.1023/B:MYCO.0000012222.68156.2c>.
- Plumed-Ferrer C, Väkeväinen K, Komulainen H, Rautiainen M, Smeds A, Raitanen J-E, Eklund P, Willför S, Alakomi H-L, Saarela M, von Wright A. The antimicrobial effects of wood-associated polyphenols on food pathogens and spoilage organisms. *Int J Food Microbiol*. 2013. <https://doi.org/10.1016/j.jifoodmicro.2013.04.001>.

35. Sierra J, Martí E, Garau MA, Cruañas R. Effects of the agronomic use of olive oil mill wastewater field experiment. *Sci Tot Environ*. 2007. <https://doi.org/10.1016/j.scitotenv.2007.01.009>.
36. De Mastro F, Traversa A, Brunetti G, Debiase G, Cocozza C, Nigro F. Soil culturable microorganisms as affected by different soil managements in a two year wheat-faba bean rotation. *Appl Soil Ecol*. 2020. <https://doi.org/10.1016/j.apsoil.2020.103533>.
37. Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moëgne-Loccoz Y. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil*. 2009. <https://doi.org/10.1007/s11104-008-9568-6>.
38. Bonanomi G, Antignani V, Capodilupo M, Scala F. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biol Biochem*. 2010. <https://doi.org/10.1016/j.soilbio.2009.10.012>.
39. Weller DM, Raaijmakers JM, McSpadden Gardener BB, Thomashow LS. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu Rev Phytopathol*. 2002. <https://doi.org/10.1146/annurev.phyto.40.030402.110010>.
40. Rilling JI, Acuña JJ, Nannipieri P, Cassan F, Maruyama F, Jorquera MA. Current opinion and perspectives on the methods for tracking and monitoring plant growth-promoting bacteria. *Soil Biol Biochem*. 2019. <https://doi.org/10.1016/j.soilbio.2018.12.012>.
41. Page AL, Miller RH, Keeney DR. Methods of soil analysis. Part 2—Chemical and Microbiological Properties. 2nd ed. Madison: Agronomy Society of America; 1982.
42. Gambacorta G, Sinigaglia M, Schena A, Baiano A, Lamacchia C, Pati S, La Notte E. Changes in free fatty acid and diacylglycerol compounds in short-ripening dry cured sausage. *J Food Lipids*. 2009. <https://doi.org/10.1111/j.1745-4522.2009.01128.x>.
43. Gambacorta G, Faccia M, Triani A, Lamacchia C, Tommaso G. Phenolic composition and antioxidant activity of Southern Italian monovarietal virgin olive oils. *Eur J Lipid Sci Technol*. 2012. <https://doi.org/10.1002/ejlt.201200043>.
44. Harris DC, Yang JR, Ridout MS. The detection and estimation of *Verticillium dahliae* in naturally infested soil. *Plant Pathol*. 1993. <https://doi.org/10.1111/j.1365-3059.1993.tb01496.x>.
45. Huisman OC, Ashworth LJ. Quantitative assessment of *Verticillium albo-atrum* in field soils: procedural and substrate improvements. *Phytopathology*. 1974. <https://doi.org/10.1094/Phyto-64-1043>.
46. Masago H, Yoshikawa M, Fukada M, Nakanishi N. Selective Inhibition of *Pythium* spp. on a medium for direct isolation of *Phytophthora* spp. from soils and plants. *Phytopathology*. 1977. <https://doi.org/10.1094/Phyto-67-425>.
47. Ye J, Perez PG, Zhang R, Nielsen S, Huang D, Thomas T. Effects of different C/N ratios on bacterial compositions and processes in an organically managed soil. *Biol Fertil Soils*. 2018. <https://doi.org/10.1007/s00374-017-1246-5>.
48. Enwall K, Nyberg K, Bertilsson S, Cederlund H, Stenström J, Hallin S. Long-term impact of fertilization on activity and composition of bacterial communities and metabolic guilds in agricultural soil. *Soil Biol Biochem*. 2007. <https://doi.org/10.1016/j.soilbio.2006.06.015>.
49. Pezzolla D, Marconi G, Turchetti B, Zadra C, Agnelli A, Veronesi F, Onofri A, Benucci GN, Buzzini P, Albertini E, Gigliotti G. Influence of exogenous organic matter on prokaryotic and eukaryotic microbiota in an agricultural soil. A multidisciplinary approach. *Soil Biol Biochem*. 2015. <https://doi.org/10.1016/j.soilbio.2014.12.008>.
50. Federici E, Massaccesi L, Pezzolla D, Fidati L, Montalbani E, Proietti P, Nasini L, Regni L, Scargetta S, Gigliotti G. Short-term modifications of soil microbial community structure and soluble organic matter chemical composition following amendment with different solid olive mill waste and their derived composts. *Appl Soil Ecol*. 2017. <https://doi.org/10.1016/j.apsoil.2017.06.014>.
51. Alabouvette C, Hoper H, Lemanceau P, Steinberg C. Soil suppressiveness to diseases induced by soil-borne plant pathogens. In: Stotzky G, Bollag J, editors. *Soil biochemistry*. New York: Marcel Dekker; 1996. p. 371–413.
52. El-Abbassi A, Saadaoui N, Kiai H, Raiti J, Hafidi A. Potential applications of olive mill wastewater as biopesticide for crops protection. *Sci Total Environ*. 2017. <https://doi.org/10.1016/j.scitotenv.2016.10.032>.
53. Mekki A, Dhouib A, Sayadi S. Polyphenols dynamics and phytotoxicity in a soil amended by olive mill wastewaters. *J Environ Manage*. 2007. <https://doi.org/10.1016/j.jenvman.2006.05.015>.
54. Di Serio MG, Lanza B, Mucciarella MR, Russi F, Iannucci E, Marfisi P, Madeo A. Effects of olive mill wastewater spreading on the physico-chemical and microbiological characteristics of soil. *Int Biodeter Biodegr*. 2008. <https://doi.org/10.1016/j.ibiod.2008.03.006>.
55. Ergül FE, Sargin S, Öngen G, Sukan FV. Dephenolisation of olive mill wastewater using adapted *Trametes versicolor*. *Int Biodeter Biodegr*. 2009. <https://doi.org/10.1016/j.ibiod.2008.01.018>.
56. Kachouri S, Halaoui S, Lomascolo A, Asther M, Hamdi M. Decolourization of black oxidized olive mill wastewater by a new tannase-producing *Aspergillus flavus* strain isolated in soil. *World J Microbiol Biotechnol*. 2005. <https://doi.org/10.1007/s11274-005-6810-8>.
57. Kissi M, Mountadar M, Assobhei O, Gargiulo E, Palmieri G, Giardina P, Sannia G. Roles of two white-rot basidiomycete fungi in decolourization and detoxification of olive mill waste water. *Appl Microbiol Biotechnol*. 2001. <https://doi.org/10.1007/s002530100712>.
58. Kumar P, Lee J-H, Beyenal H, Lee J. Fatty acids as antibiofilm and antivirulence agents. *Trends Microbiol*. 2020. <https://doi.org/10.1016/j.tim.2020.03.014>.
59. Li XC, Jacob MR, ElSohly HN, Nagle DG, Smillie TJ, Walker LA, Clark AM. Acetylenic acids inhibiting azole-resistant *Candida albicans* from *Pentagonia gigantifolia*. *J Nat Prod*. 2003. <https://doi.org/10.1021/np030196r>.
60. Pohl CH, Kock JLF, Thibane VS. Antifungal free fatty acids: A Review. In: Méndez-Vilas A, editor. *Science against microbial pathogens: communicating current research and technological advances*. Badajoz: Formatex Research Center; 2011. p. 61–71.
61. Ruttledge TR, Nelson ER. Extracted fatty acids from *Gossypium hirsutum* stimulatory to the seed-rotting fungus *Pythium ultimum*. *Phytochemistry*. 1997. [https://doi.org/10.1016/S0031-9422\(97\)00265-3](https://doi.org/10.1016/S0031-9422(97)00265-3).
62. Calvo AM, Hinze LL, Gardner HW, Keller NP. Sporogenic effect of polyunsaturated fatty acids on development of *Aspergillus* spp. *Appl Environ Microbiol*. 1999. <https://doi.org/10.1128/AEM.65.8.3668-3673.1999>.
63. Harman GE, Mattick LR, Nash G, Nedrow BL. Stimulation of fungal spore germination and inhibition of sporulation in fungal vegetative thalli by fatty acids and their volatile peroxidation products. *Can J Bot*. 1980. <https://doi.org/10.1139/b80-188>.
64. Herman RP. Oxylin production and action in fungi and related organisms. In: Rowley AF, Kuhn H, Schewe T, editors. *Eicosanoids and related compounds in plants and animals*. London: Portland Press; 1998. p. 115–32.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen® journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)