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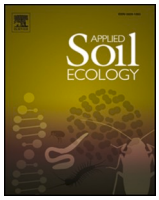

Short-term soil amendment by sewage sludge anaerobic digestate in a tomato monoculture suppresses Fusarium vascular wilt disease by changing the taxonomic characteristics of soil microbiota

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ABSTRACT

Digestate incorporation into topsoil determines soil fertility improvement by changing composition and structure of soil microbiota. However, how microbiota responds to short-term soil amendment by sewage sludge anaerobic digestate (SSD) for suppressing Fusarium vascular wilt disease is unknown. This study compares the effects of three SSD-based treatments to suppress *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) in a long-term cherry tomato monoculture under field condition in a 3-year trial. Three sampling sites with two application times (two bulk soils at 3–12 weeks after amendment and one tomato rhizosphere soil at 12 weeks post-amendment) were chosen. Three digestate typologies (liquid, centrifuged, and dried) having physicochemical features and heavy metals content below the legal limits were tested at 50 l, 3.5 kg and 2.5 kg $m^{-2}y^{-1}$ dose, respectively. Fusarium wilt disease was measured for three consecutive years by severity index and *Fol* abundance in tomato vascular tissue was assessed by ITS rDNA gene sequencing. *Fol* abundance and taxonomic structure of *Fusarium* community in the rhizosphere were determined at specie level at the end of the trial by ITS rDNA and *EF1α* rDNA genes sequencing, respectively. The taxonomic structure (α-, β-diversity) of soil bacterial community (SBC) was characterized from *phylum* up to genus level at the end of the trial in all the sites by 16S rRNA gene sequencing. The results showed that dry SSD reduced disease severity in field up to 18 % and *Fol* abundance in tomato up to 0.9 ITS copies g^{-1} tissue. *Fol* abundance was decreased in the rhizosphere up to 0.0027 ng μl^{-1} DNA, while the *Fusarium* community shifted between treated-soils and un-amended. The SBC composition (α-diversity) changed in the rhizosphere by applying dry digestate, while the SBC structure (β-diversity) shifted either among treatments or sites. Bacterial members related to *Fol* suppression (*Bacillus*, *Chitinophaga*, *Flavihumibacter*, *Flavobacterium*, *Pseudomonas* and *Terrimonas*) increased in the rhizosphere ($P < 0.01$, $P < 0.001$) more than in the bulk soils by applying both dewatered-SSDs. Thus, digestate soil amendment carried out for three consecutive years has affected tomato Fusarium wilt severity by changing the taxonomic characteristics of fusaria and bacteria communities of the amended soil.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an industrial crop consumed globally owing to multiple health benefits, ranked at the second place in cultivation only next to potato. About 65% yield loss and plant mortality of 50-60 % were observed from 2011 to 2013 in a cherry tomato (*L. esculentum* var. *cerasiforme*) monoculture of the South Italy due to Fusarium vascular wilt disease outbreak caused by *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) race 1. The disease causes severe wilting of the soil-grown tomato plants in a commercial area of approximately 5 ha. *Fol* is a soil-borne fungal pathogen causing productivity loss and detrimental effect on tomatoes quality that can be effectively controlled

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by resistant varieties, although this strategy determines unsustainable limitations (Sachdev and Singh, 2017). Soil chemical fumigation is another control strategy, since the pathogen survives in soil, crop-waste, and litter for longer time by resistant propagules (chlamydospores) becoming more virulent for tomato over time (Hassan, 2020). However, the use of methyl bromide, 1,3-dichloropropene, chloropicrin, and synthetic fungicides (Carbendazim, Benomyl, Curzate, and Nativo) were restricted, or even banned (Honaganahalli and Seiber, 1996; Aktar et al., 2009; Ahmad et al., 2021). Since the soil beneficial microbial communities protect susceptible hosts by activating biocontrol mechanisms (Castaño et al., 2013; Hussain et al., 2016; Ben Abdallah et al., 2016; Karthika et al., 2020), the adoption of disease suppressive soil (DSS) is among the most explored strategies to control *Fol* infection over time (Alabouvette, 1999; Sundaramoorthy and Balabaskar, 2013) and *F. oxysporum* f. sp. *radicis-lycopersici* (*Forl*) (Alabouvette et al., 1993) in many cropping systems (Mazzola, 2002; Schlatter et al., 2017). Several options were available for increasing soil suppression for longer time (Baker et al., 2020; De Corato, 2023). For instance, the virtuous reuse of agri-based organic wastes and residues into compost and bio-organic fertilizer (fortified-compost with bio-inoculant), here grouped under the term of organic amendments (OAs), is a viable option to design tailored DSS under the perspective of a circular economy. The exploitation of inducing-DSS by supplementation of compost and biochar-based compost (Azim et al., 2018; Bonanomi et al., 2020; Sayara et al., 2020) and crop residues (Yuan et al., 2021), or combined treatment of soil fumigation (Dazomet) and biological organic fertilizer for increasing the abundance of plant growth-promoting microorganisms (Lopes et al., 2021) to control Fusarium wilt of watermelon (Zhang et al., 2021), or combinations of 'microbial biocontrol agents (MBCAs)' and bio-fertilizer to manage soil-borne pathogens (Ruano-Rosa and Mercado-Blanco, 2015), were the most explored strategies chosen for manipulating the native soil microbiota into new and stable microbiome patterns for the biocontrol of fusaria-related wilts (Kinkel et al., 2011; De Corato, 2020).

Sewage sludge anaerobic digestate (SSD) is a biomass widely available, being provided by urban wastewaters (UWWs) treatment integrated with anaerobic digestion (AD) of sewage sludge (SS) to yield methane (De Corato et al., 2018). Although the global production of SS and SSD shows faster growing trend, SSD is bio-waste still too underexplored to increase and maintain soil suppression for longer time (De Corato, 2021). Though AD could be a better way by which SS can be recycled into SSD as a soil improver thanks to its appreciable content of organic matter and macro-micronutrients (Antonkiewicz et al., 2018), it is still currently disposed by incineration (Netherlands and Switzerland) or landfilling (Italy, Serbia, and Croatia) (Eurostat, 2017). Since SSD is classified as a putrescible waste which needs stabilization before being recycled as a soil amendment, many European frameworks were focused about its reuse in organic agriculture (European Council Directive, 1986; European Commission, 2017). Moreover, since SSD is a potential hazardous material due to high content of heavy metals and organic contaminants that might exceed the legal limits (Wang et al., 2008), its rational use in cropland needs a correct management (Milieu Ltd., 2008; Bianchini et al., 2016; Sharma et al., 2017). Many benefits were documented by using biosolids (SS and SSD) as soil amendments due to acceptable content of soil organic matter (SOM), total organic carbon (TOC), total nitrogen (TN), and available phosphorus (AP), without changing exchangeable potassium (EK), pH, and electrical conductivity (ECe) in biosolid-amended soil. From the one hand, biosolid enhances tomato growth and improves fruit-yield quality by restoring soil fertility (Eid et al., 2021), as well as several benefits on productivity and quality of the durum wheat and barley grains by using composted sludge were investigated (Pasqualone et al., 2014, 2016), as well as on soil quality by SSD-based compost (Alvarenga et al., 2015, 2016), and on productivity and quality of tomatoes and cucumbers in greenhouse by amending potting soil with crude SSD (Cristina et al., 2019, 2020). On the other hand, despite the existing indirect/direct links among soil microbial communities and biosolid-amended soils for suppressing *Forl* (Giannakis et al., 2021), the suppressive mechanisms are still poorly understood. In addition, composted sludge shows high attitude to suppress Fusarium wilt on tomato in association with *Trichoderma asperellum* and the nonpathogenic *F. oxysporum* Fo47 (Cotxarrera et al., 2002), while soil amended with digestate-based compost at 5 and 10 % dose suppresses *Fusarium solani* damping-off of tomato by increasing plant growth (Vitti et al., 2021).

The present study is aimed at evaluating the short-term effects of SSD in a long-term cherry tomato monoculture severely affected by Fusarium vascular wilt. According to Khatri et al. (2023), we have tested the following hypothesis: (i) short-term SSD soil supplementation changes the taxonomic characteristics of fusaria and bacterial communities, and (ii) soil microbiome disturbance drives *Fol* suppression by increasing abundance of bacterial members related to suppression.

2. Materials and methods

2.1. Experimental site and soil management

The experimental site was located in Foggia (41°27′37″N and 15°30′05″E), Apulia region (South of the Italy). It had a Mediterranean climate with a mean annual temperature of 15.9 °C and mean monthly temperatures ranging from 26.5 °C (August) to 9.0 °C (January) with a mean annual rainfall of 988 mm, and a relatively dry summer (<84 mm). The site was intensively cultivated for long time by cherry tomato monoculture. A field soil of 50 ha was chosen for this study, being characterized by sandy texture (60.5 % sand, 20.1 % silt, and 19.5 % clay), alkaline (pH = 7.3), with normal salinity (ECe = 0.4 dS m^{-1}), poor in SOM (1.64 %) and TOC (1.95 %), and with low content in total carbonates (2.6 %) and active carbonates (1.4 %). Table S1 shows texture, hydrological, and physicochemical characteristics of soil considered in this study.

In each growing season, cherry tomato plants cv Genio were sowed in paper-pot, grown up in a climatic room at standard condition, transplanted at 15 days-old in field at the planting density of 4 plants per square meter, drip irrigated, and grown from mid-January (planting season) to end-June (harvesting season) according to the standard agronomical practices adopted in this area. Soil received a mineral fertilization with 5 t NPK $ha^{-1}y^{-1}$ consisting of 100 kg ammonium nitrate, 80 kg mineral superphosphate, and 200 kg potassium sulphate. Cropland was kept without crop (fallow) from August to December after each tomato cultivation cycle.

2.2. SSD features, soil amendment, and experimental set-up

SSD derived from a UWWs treatment plant (Gi.Se.Co. Ltd) located in Monopoli (Apulia, Italy) designed for about 450,000 population equivalents and assembled with 4 anaerobic digesters of 5000 l each to yield methane (mean power of 1.8 MWh). The primary feedstock was a mix of 80 % sewage sludge, 15 % straw/vinasse, and 5 % a commercial starter fermented at 33 ± 3 °C for 25 days. The liquid fraction ('primary', labelled as CKBFp) was obtained by a solid-liquid separation plant after sedimentation and stored at room temperature for 5 days before freezing. Afterwards, primary SS slurry was further fermented in a completely mixed anaerobic reactor at 37 ± 2 °C for 40 days. The digestate was mechanically extruded by two pipelines obtaining another liquid fraction ('secondary', labelled as CKBFs) and a solid fraction which was dewatered by a decanter centrifuge ('centrifuged') and an oven for few minutes ('dry'). Three kinds of SSD were collected at the end of the process: (i) a liquid digestate labelled as 'CKBF' obtained by pooling CKBFp and CKBFs, (ii) a solid digestate fraction labelled as 'FBF' dewatered by centrifugation, and (iii) a solid digestate fraction labelled as 'FOF' dried at 200 °C. All digestate fractions were stored at room temperature for 7 days before freezing at −20 °C. Table S2 shows physicochemical characteristics of SSD tested in this study. SSD features

were within the cogent local limits for organic agriculture (Italian Decree Law No. 99, 1992; Italian Law No. 130, 2018) with exception for Cu, Zn, Ni and Cr (Italian Decree Law No. 75, 2010).

Soil received annually SSD supplementation three months before tomato transplanting in the field (mid-October) with 50 l CKBF, 3.5 kg FBF, and 2.5 kg FOF $m^{-2}y^{-1}$ dose. Such amount approximately provided the optimal TN rating (from 80 to 90 kg N $ha^{-1}y^{-1}$) that resulted below the recommended dose for the Apulian area (170 kg N $ha^{-1}y^{-1}$) according to the EU-Nitrate Directives (Monteny, 2001) and Italian legislation for the production and utilization of digestate in agriculture (Mi.P.A.A.F., 2016). Both dewatered digestates (FBF and FOF) were manually incorporated into topsoil at the maximum depth of 60 cm, while liquid digestate (CKBF) was dispensed onto soil surface by watering and then mixed with topsoil. The experimental trial was set-up for three consecutive tomato cropping cycles, beginning from the first planting (mid-January 2014) to the last harvesting (end-June 2017). We monitored about 160 m^2 of a field soil by an experimental design comprising four treatments (three thesis amended with digestate and one non-amended serving as control) arranged in a completely randomized blocks scheme of 12 plots (Fig. S1A) measuring 10 m^2 each with 40 plants (Fig. S1B).

2.3. Pythopathological analyses

Phytopathological analyses were carried out at Department of Bioenergy, Biorefinery, and Green Chemistry of ENEA along the trial.

2.3.1. Scoring the tomato Fusarium vascular wilt severity in field soil

Symptoms over whole tomato plants were annually monitored in field from the mid-January 2014 to the end-June 2017 beginning from the mildest symptom rating (<10 % foliar yellowing) to the severe ones (plant death). Disease rating was established 90-days after emergence by estimating the Fusarium wilt severity degree with a visual rating scale of five classes from 0 to 4 (Bora et al., 2004). Each plant was uprooted and lower stem and tap root were longitudinally sectioned for vascular tissue examination. Single plant was scored by: 0 = healthy plant or with asymptomatic leaves without discoloured stems, 1 = leaf symptoms in <25 % of the whole plant with leaves that appeared yellow or showed irregularity shaped yellow spots and stems with <25 % of vascular discoloration, 2 = leaf symptoms ranging from 26 to 50 % of the whole plant with the edges of leaves rolls up with scorch symptoms and stems with 26–50 % of vascular discoloration, 3 = leaf symptoms from 51 to 75 % of the whole plant with leaf margin rolls and wilting symptom and stems with 51–75 % of vascular discoloration, and 4 = plant death or with leaf symptoms in >76 % of the whole plant and stems with 76–100 % of vascular discoloration. The 'Disease Severity Index' (DSI) was calculated on 40 plants for each plot by formula (Akkopru and Demir, 2005):

$$DSI = \frac{\Sigma\ (\text{Number of plants scored} \times \text{corresponding disease severity score})}{\text{Total number of plants} \times \text{the highest disease severity score}} \times 100$$

2.3.2. Assessing the F. oxysporum abundance in tomato vascular tissue

Abundance of *F. oxysporum* in stem vascular tissue was annually assessed on 40 plants from each plot after DSI% calculation. Tomato plant was individually uprooted and leaves discarded. Stem was cut off at soil level, sterilized in 2 % NaClO for 4 min, and rinsed in sterile distillate water twice. Fresh tissue samples of 0.35 g taken from each plant were used for genomic DNA extraction and further amplification by Real-Time quantitative PCR (qPCR). Total DNA was extracted in triplicate from tissue pieces grounded into fine powder with liquid nitrogen using pestle and mortar (Dellaporta et al., 1983). Extraction was performed by 20 min incubation at 65 °C, DNA pellet re-dissolved in TE with 200 mg RNase ml^{-1}, and DNA quantified using a UV–Vis spectrophotometer (NanoDrop ND-1000; Thermo-Fisher Scientific™ Inc., MI, Italy). PCR-amplification of the ITS rDNA gene region was performed with the *Fol*-specific primer pair sp1-2f:sp1-2r to yield amplicons (Table S3). Data were pooled and expressed as ITS rDNA copies g^{-1} tissue.

2.3.3. Testing the pathogenicity of Fol isolates from the tomato rhizosphere soil

Pathogenicity of *F. oxysporum* colonies from the rhizosphere was bioassayed on tomato plants for confirming their identity as *Fol*. A total of 60 wilted plants were annually and randomly selected from the field (5 plants per plot), uprooted, and adhering soil analyzed on *Fusarium*-selective media (Komada, 1975). *Fusarium* colonies were individually transferred onto potato dextrose agar media (PDA; Sigma-Aldrich, Milan, Italy) and morphologically characterized on basis of the biometric characters of conidiophores and macro-microconidia using a photomicroscope (BX60; Olympus, Milan, Italy) (Nelson et al., 1983; Carlile et al., 2001). A total of 300 colonies of *F. oxysporum* were picked (5 colonies per plant) and tested for the pathogenicity characters on tomato seedling cv Supermarmande by standard procedures.

2.4. Soil microbiome analyses

Soil microbiome analyses were carried out at Department of Soil, Plant, and Food Science of University of Bari at the end of the trial.

2.4.1. Soil sampling

Soil samples were collected at the end-June 2017 from two bulk soils at 3 and 12 weeks after SSD amendment and one rhizosphere soil 12 weeks post-amendment (Fig. S1C). Bulk soils were sampled (0–60 cm) by 0.8 cm × 2 cm soil cores following a randomly 9-point scheme by a hand auger (6 cm diameter) by selecting random sub-samples that were bulked yielding an approximate amount of 2.8 kg bulk soil for each plot and sampling site (Violante, 2000). A total of 24 composite samples (4 treatments × 2 bulk soils × 3 replicates) were collected. Rhizosphere soil was sampled by a randomly 9-point scheme from individually selected tomato plants to make random sub-samples. Each plant was uprooted, and root samples undergone to vigorous shaking to remove soil particles that were tightly the roots. Rhizosphere soil was scraped off with sterile forceps and sub-samples pooled by yielding approximately 500 g soil per plot. A total of 12 composite samples (4 treatments × 1 rhizosphere soil × 3 replicates) were collected. The samples were placed in plastic bag, kept on ice during transportation to the laboratory to maintain the field condition, homogenized, sieved (<2 mm) at field moisture, and stored at −20 °C for a maximum of two weeks. Soil aliquots of 20 g were stored in 50 mL sterile vials at −80 °C until DNA extraction.

2.4.2. F. oxysporum abundance

Abundance of *F. oxysporum* was determined in triplicate on aliquots of 0.5 g fresh soil by qPCR amplification of the ITS rDNA gene region with the universal fungal primer pair ITS1F:ITS4R for yielding amplicons (Table S3). *Fusarium* genomic DNA was extracted using NucleoSpin® Kit for soil (Machery-Nagel; Dueren, Germany) according to manufacturer's instructions and stored at −20 °C until qPCR. Quality

and concentration of DNA were determined on samples diluted at least 10 ng μl^{-1} and standard curve with 10-fold dilutions of the corresponding PCR product was generated. After checking quality of the PCR products by 2 % agarose gel electrophoresis in TAE buffer, the amplicons were purified with the Agencourt AMPure XP system and quantified by a UV–Vis spectrophotometer (NanoDrop ND-2000). Pooled data were expressed as *F. oxysporum* ITS rDNA quantity (ng μl^{-1}).

2.4.3. Characterization of the fusaria and bacterial communities

2.4.3.1. DNA extraction and PCR amplification. To characterize the *Fusarium* community in rhizosphere, genomic DNA was extracted in triplicate according to the procedures described. To characterize the bacterial community in bulks and rhizosphere soils, genomic DNA was extracted in triplicate on aliquots of 0.5 g fresh soil using the commercial kit Fast DNA Spin Kit for Soil (MP Biomedicals; CA, United States) combined with the Fast Prep System (BIO-101) homogenizer according to manufacturer's instructions. DNA was quantified by a NanoDrop ND-2000 UV–Vis spectrophotometer and stored at −20 °C until qPCR and Illumina sequencing.

To amplify *the Fusarium EF1α* rDNA gene region with the specific primer pair *EF1f*:*EF1r* to yield amplicons (Table S3), an array of 12 DNA samples were analysed. After the first round of PCR (Table S3), the amplification products were run on 2 % agarose gel electrophoresis in TAE to determine the appropriate dilution used for the second round (Table S3) with 1 μl of diluted PCR products. Amplicons of the second PCR round were purified with the Agencourt AMPure XP system and quantified with Quant-iT PicoGreen® dsDNA Assay Kit (Invitrogen Life Technologies; Carlsbad, CA, USA). To amplify the V4-V5 hypervariable region of the bacterial 16S rRNA gene with the universal bacterial primer pair 515F:907R to yield 292 bp amplicons (Table S3), an array of 36 DNA samples were analysed. Quality of qPCR-amplification was verified by 1.5 % agarose gel electrophoresis in TAE. Amplicons were quantified using a fluorometer (Qubit 2.0, Invitrogen Life Technologies). Pooled data were sent to Illumina sequencing's company (IGA Technology Services s.r.l., Udine, Italy).

2.4.3.2. Illumina MiSeq sequencing and rDNA libraries. The purified *Fusarium* amplicons were pooled in equimolar concentrations and final concentration of the *EF1α* rDNA amplicons was determined using a qPCR Illumina library quantification kit. Amplicons library were mixed with 10 % PhiX control and run on Illumina MiSeq (PE300 v3 chemistry strategy) according to Illumina's protocols. Raw sequence data were available at the National Center for Biotechnology Information (NCBI) in the Sequence Read Archive (SRA, http://www.ncbi.nlm.nih.gov/sra) under SRA accession number SRX492514-64 (BioProject PRJNA837364).

Equimolar concentrations of the purified 16S rRNA amplicons were submitted to paired-end sequencing (2 × 300 bp) and run on Illumina library. Raw sequence data were deposited at the NCBI under SRA accession number SRX492543-24 (BioProject PRJNA837385).

2.4.3.3. EF1α rDNA sequence processing. Paired reads from Illumina sequencing were processed with Quantitative Insights into Microbial Ecology (QIIME) using the pipeline v1.9.1. (Caporaso et al., 2010). The fungal sequences were aligned by MAFFT v7.304 (Yamada et al., 2016). A *EF1α* rDNA table of 12 samples showing the number of filtered sequences after quality control passage and good's coverage was generated. Each representative sequence was assigned to Operational Taxonomic Units (OTUs) using the UCLUST algorithm and the UNITE database v7.1 (https://unite.ut.ee/) for fungal sequences (Abarenkov et al., 2010) by picking at 97 % similarity cut-off. To compare and plot memberships of the samples at specie level, the Principal Coordinates Analysis (PCoA) based on Bray-Curtis distance was performed to cluster the *Fusarium* community among treatments by the ade4 package software v1.7-17. Ellipses at 95 % confidence level were plotted around the samples. The Similarity Percentage (SIMPER) analysis was performed using the PAST software (Hammer et al., 2001) to identify the *Fusarium* species (OTUs%) responsible for differences between treatments.

2.4.3.4. 16S rRNA sequence processing. Raw reads from Illumina sequencing were processed by MOTHUR software v1.39.5 (Schloss et al., 2009). Following the Standard Operating Procedure (SOP, http://www.mothur.org/wiki/MiSeq_SOP), the raw sequences were first denoised, trimmed, aligned, and classified by the SILVA bacterial taxonomy database (Quast et al., 2013). The sequences were analyzed using the QIIME pipeline v1.9.1. following the instructions for Illumina 16S rRNA analyses (www.qiime.org). Sequences were truncated based on the PHRED algorithm if their quality dropped below 20 bp over a sliding window of 25 bp and low-quality sequence reads with <75 % of their original length were trimmed. The filtered sequences were obtained with a 15 bp minimal overlapping length and <10 % mismatches and assembled to generate consensus tags using FLASH (Mago and Salzberg, 2011). Paired end FASTQ files were merged using the PEAR and CLC software (CLC Bio; Aarhus, Denmark) for quality and length trimming. The sequences were grouped into OTUs at 97 % similarity cut-off using the UPARSE pipeline v10.0.1090 (Edgar, 2013). A representative bacterial sequence number from each OTU was aligned using PYNAST in SILVA database v1.19 (https://www.arb-silva.de/) by which each sequence was assigned using the BLAST algorithm (Edgar, 2010). Replications, chimaeras, singletons, chloroplasts, viruses, and mitochondria were discharged from the analysis using the UCHIME algorithm (Edgar et al., 2011). A 16S rRNA table of 36 samples showing the number of filtered sequences after quality control passage and good's coverage was generated. The sequences were assigned to the most dominant bacterial OTUs within the cluster by the RDP classifier (Wang et al., 2007). High quality sequences were clustered into OTUs from *phylum* to genus level. Alpha-diversity and Beta-diversity were inferred by sequence processing. The α-diversity indices measuring the observed OTUs number, Chao1 richness, and Shannon diversity were determined by rarefaction curves using the QIIME software package. The β-diversity structure was evaluated by Non-metric Multi-Dimensional Scaling (NMDS) analyses, and a matrix was generated using non-metric distance for comparing and plotting samples between treatments and sampling sites. Ellipses at 95 % confidence level were plotted around the grouped samples. The SIMPER analysis was performed using the PAST software to identify the bacterial genera (OTUs) responsible for differences between treatments.

2.5. Statistical analyses

Statistics were plotted by the IBM SPSS software v23.0 (SPSS Inc.; Chicago, IL, USA). Analyses were performed using the SAS software package (SAS Institute; Charlotte, NC, USA) to evaluate difference between treatments at each sampling site by Analyses of Variance (ANOVA). When the tests were significant at $P < 0.05$ level, post-hoc comparisons were made by Tukey Honest Significant Differences (Tukey HSD test). The normality assumption and homogeneity of the variance were checked by the Shapiro–Wilk and Bartlett tests, respectively. The DSI% data were transformed into Bliss angular values ($\arcsin\sqrt{\%}$) to normalize distribution before analyses, while the ITS rDNA copies g^{-1} tissue data were submitted to logarithmic transformation to satisfy the assumption of normality. Once homogeneity of variance was verified, the values were pooled and means undergone to ANOVA over all variables to assess effects between treatments. One-way ANOVA was used to compare differences for DSI%, *F. oxysporum* DNA quantity in tomato (ITS copies g^{-1} vascular tissue) and *F. oxysporum* DNA quantity in rhizosphere (ITS ng μl^{-1}), the α-diversity indices of SBC (OTUs number, Chao1 richness, and Shannon diversity), relative abundances of the *Fusarium* species and bacteria genera (OTUs %). Means judged

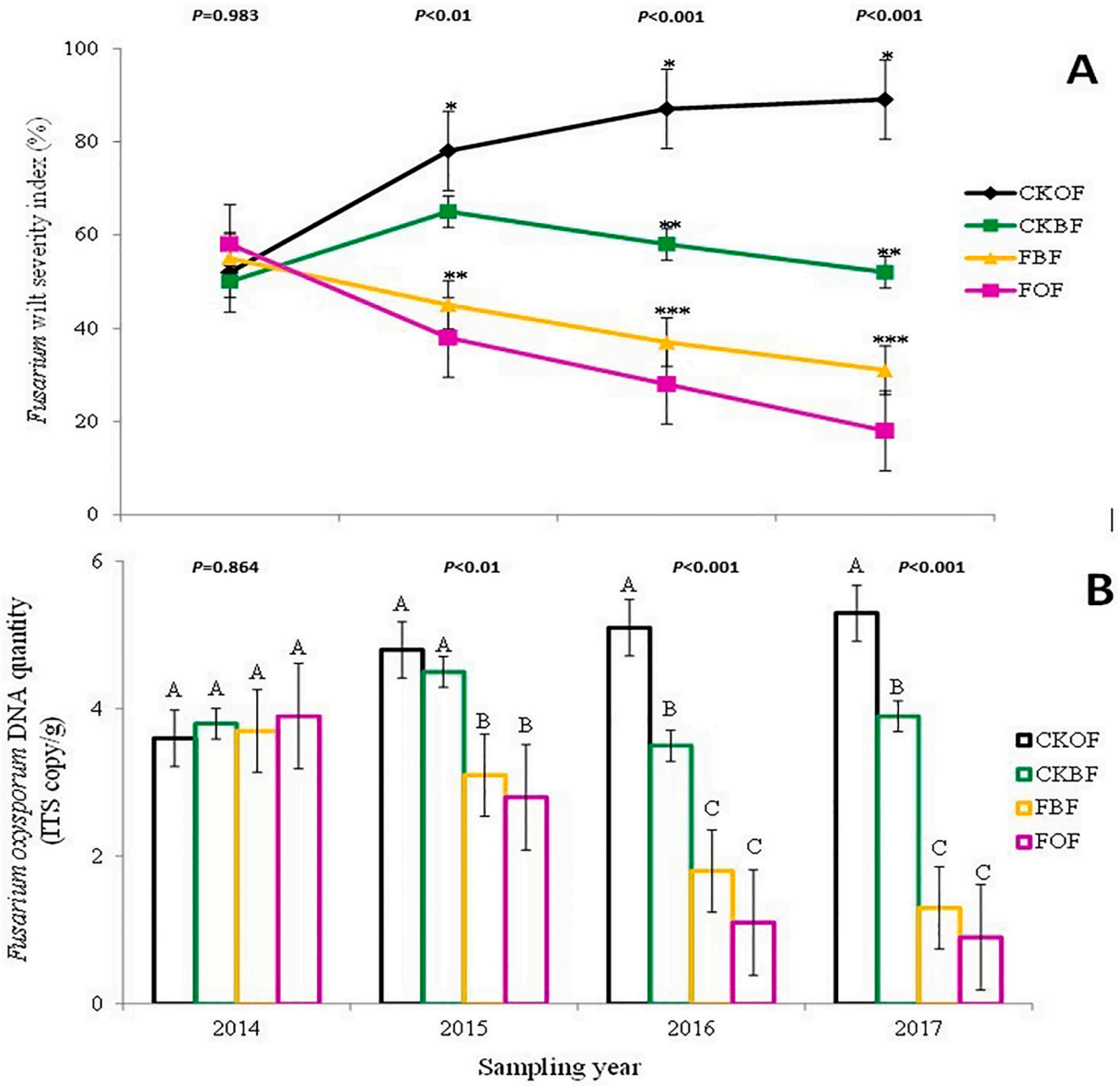

Fig. 1. Effect of SSD soil amendment on the Fusarium wilt severity (DSI %) in field soil and *Fusarium oxysporum* DNA abundance (ITS rDNA copy number g^{-1}) in vascular tissue of tomato in a 3-year trial.
(A) Graphical plot showing the DSI % pattern assessed on 40 plants taken from each plot. Values are pooled means of three replicates where *P*-values represent the results of one-way ANOVA by comparing averages between treatments. Different number of asterisks represents significant difference according to the Turkey HSD post-hoc test ($P < 0.05$). Bar indicates the standard error (SE) of the mean ($n = 3$).
(B) Bar plot showing the ITS copies g^{-1} values measured on 0.35 g fresh tissue per plant taken from 40 plants of each plot. Different letter for each year indicates significant differences based on the Turkey's HSD post-hoc test ($P < 0.05$). Bar indicates the SE of the mean ($n = 3$).

significantly different were separated by the Turkey's HSD post-hoc test at $P < 0.05$ level. The correlation method based on Spearman's rank analyses to find significant correlations ($P < 0.05$) among the abundance of *F. oxysporum* and the relative abundances of fusaria species and bacterial genera in bulks and rhizosphere soils between treatments was applied.

3. Results

3.1. Effect of SSDs amendment on the Fusarium wilt severity and Fol abundance in tomato

Fusarium wilt severity index in field and *Fol* abundance in tomato vascular tissue are shown in Fig. 1. The DSI % values (Fig. 1A) showed that no difference among all treatments was found in 2014 ($F = 0.18$, $P = 0.983$). While two grouped treatments were found in 2015 ($F = 8.75$, $P < 0.01$) of which the first one included CKOF and CKBF and the second one FBF and FOF. Instead, three grouped treatments were differenced both in 2016 and 2017 ($F = 36.38$, $P < 0.001$), where the first one comprised CKOF, the second one CKBF, and the last group included FBF and FOF. The lowest DSI% was 18 % by amending soil with dry digestate (FOF) when compared to 89 % for un-amended soil (CKOF) after 3-years of digestate soil amendment.

The *F. oxysporum* abundance trend in tomato stem vascular tissue (Fig. 1B) showed that the lowest quantity of *F. oxysporum* genomic DNA was 0.9 ITS rDNA copies g^{-1} tissue at the end of the trial in soil supplemented with dry digestate in comparison to 5.3 ITS rDNA copies g^{-1} in those un-amended ($F = 12.48$, $P < 0.001$). The repeated application of both dewatered SSDs reduced disease severity <31 % (Fig. 1A) and pathogen abundance <1.3 ITS rDNA copies g^{-1} tissue (Fig. 1B). Liquid

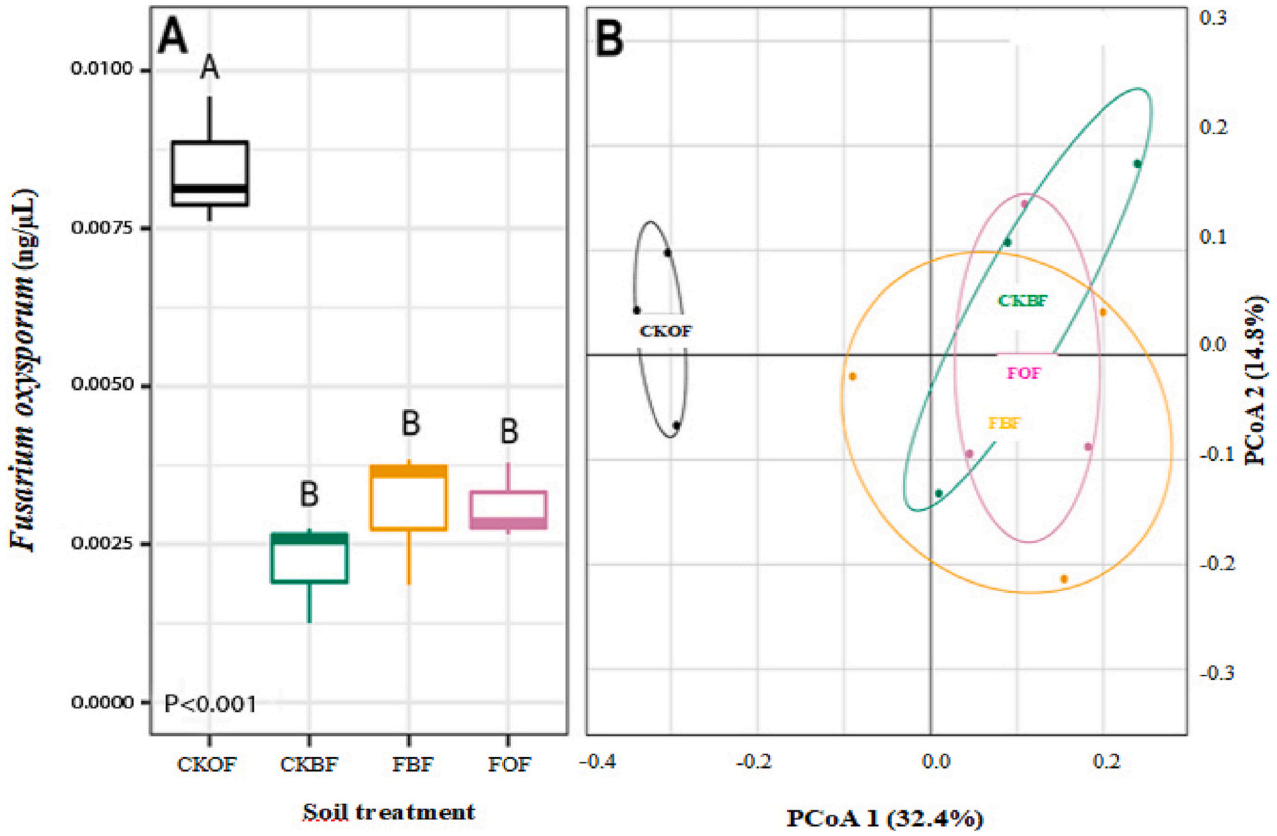

Fig. 2. Effect of SSD soil amendment on the abundance of *F. oxysporum* and *Fusarium* community structure in the tomato rhizosphere at the end of the trial based on the ITS rDNA and *EF1α* rDNA genes sequencing, respectively.
(A) Box plot showing *F. oxysporum* DNA quantity (y-axis) among treatments (x-axis). Values are measured in triplicate ($n = 3$) on 0.5 g fresh soil for each plot and expressed as ng μl^{-1}, where the *P*-value ($P < 0.001$) represents one-way ANOVA by comparing averages between treatments. Different letters indicate significant differences according to the Turkey's HSD post-hoc test ($P < 0.05$).
(B) Principal Coordinates Analyses (PCoA) plot showing the *Fusarium* community structure based on Bray-Curtis distance, where the 3 dots for each treatment represent the sub-samples.

digestate showed reduction of 52 % and 3.9 ITS copies g^{-1} for disease severity and pathogen abundance, respectively.

3.2. Effect of SSDs amendment on the F. oxysporum abundance and pathogenicity of Fol isolates in the soil

The effect of SSDs amendment on the relative abundance of *F. oxysporum* in the rhizosphere is showed in Fig. 2A. The highest *F. oxysporum* DNA quantity resulted be 0.0079 ng μl^{-1} in the un-amended soil in comparison to those amended (from 0.0025 to 0.0038 ng μl^{-1}) with difference highly significant ($F = 28.6$, $P < 0.001$), instead difference between treatments was not found ($F = 0.21$, $P = 0.76$). Over a total of 300 *F. oxysporum* colonies *in-vivo* tested for the pathogenicity attributes, 45 % of them induced wilting symptoms of severity = 4 on tomato plant-test, 32 % of severity = 3, 12 % of severity = 2, and the remaining 11 % of severity = 1. No plant without symptoms after artificial inoculation with the *Fol* isolates was found.

F. oxysporum DNA quantity in bulk soils was lower at 3 weeks post-amendment (0.0049 ng μl^{-1}; $F = 18.4$, $P = 0.013$) and 12 weeks post-amendment (0.0041 ng μl^{-1}; $F = 22.3$, $P = 0.005$) than in un-amended soil (0.0089 ng μl^{-1}) without difference among treatments (meanly, $F = 0.13$, $P = 0.89$).

3.3. Effect of SSDs amendment on soil fusaria community

A table of good quality *EF1α* rDNA sequences from 12 soil samples was generated (Table S4). A total of 57,634 sequences were attributed to >19 *Fusarium* species. The average sequence number per sample was 4803 (standard deviation = 43,82). Each sample was rarefied up to 30,000 *EF1α* sequences (un-shown data). The sequences left after rarefaction represented a coverage of 99.3 ± 0.2 %.

The filtered *EF1α* sequences revealed modification of *Fusarium* abundance in tomato rhizosphere by digestate supplementation (Table S5). Indeed, relative abundance of *F. oxysporum* decreased from 61.37 % in un-amended soil up to 11.74 %, 16.63 % and 15.49 % ($F = 12.5$, $P < 0.001$) in soil amended with CKBF, FBF and FOF, respectively. While 5 *Fusarium* species as *F. avenaceum* ($F = 5.16$, $P = 0.031$), *F. culmorum* ($F = 5.52$, $P = 0.028$), *F. equiseti* ($F = 9.82$, $P = 0.002$), *F. graminearum* ($F = 8.78$, $P = 0.004$) and *F. proliferatum* ($F = 5.92$, $P = 0.023$) increased in the relative abundance. On the other side, 10 fusaria species (*F. acuminatum*, *F. crockwellense*, *F. langsethiae*, *F. poae*, *F. sambucinum*, *F. solani*, *F. sporotrichioides*, *F. tabacinum*, *F. tricinctum and F. verticillioides*) were not changed ($P > 0.05$) in the relative abundance.

The SIMPER analyses showed the main fusaria species responsible for differences among treatments (Table 1). *F. oxysporum* showed the highest dissimilarity contribution (34.45 %), followed by *F. equiseti* (31.57 %), *F. graminearum* (22.25 %), *F. proliferatum* (20.87 %), *F. avenaceum* (19.75 %) and *F. culmorum* (11.23 %). Other values ranging from about 1.63 % (*F. sporotrichioides*) to <1 % were associated to OTUs whose dissimilarity contribution was lesser than the fusaria species afore cited.

Analysis of *EF1α* data explained a significant amount of variation in the fusaria community structure between 12 samples ($F = 8.69$, $P < 0.01$). The PCoA (Fig. 2B) clustered the un-amended soil samples from

Table 1
Similarity percentage (SIMPER) analysis showing the main *Fusarium* species (OTUs) responsible for differences between treatments (**in bold**) in tomato rhizosphere soil at the end of the trial.

Fusarium spp.	Dissimilarity contribution (%)	Cumulative (%)	Treatment[a]			
			CKOF	CKBF	FBF	FOF
F. acuminatum	0.28	98.68	51	39	45	47
F. avenaceum	**19.75**	**73.22**	**76**	**342**	**383**	**366**
F. crockwellense	1.23	93.89	18	13	16	15
F. culmorum	**11.23**	**79.65**	**25**	**192**	**173**	**187**
F. equiseti	**31.57**	**48.67**	**78**	**425**	**515**	**480**
F. graminearum	**22.25**	**57.74**	**18**	**331**	**318**	**392**
F. langsethiae	0.01	99.98	0	1	1	1
F. oxysporum	**34.45**	**34.45**	**580**	**192**	**123**	**204**
F. poae	0.05	99.94	5	1	2	2
F. proliferatum	**20.87**	**62.32**	**14**	**175**	**142**	**196**
F. sambucinum	0.25	96.16	18	27	22	38
F. solani	0.32	90.62	51	49	65	57
F. sporotrichioides	1.63	90.19	31	23	27	18
F. tabacinum	0.03	99.91	0	4	2	2
F. tricinctum	0.07	99.96	0	2	3	1
F. verticillioides	0.08	99.94	0	1	2	2

Value is the *EF1α* rDNA sequences number associated to each OUT by pooling data.
[a] CKOF = non-amended soil, CKBF = soil amended with liquid digestate, FBF = soil amended with centrifuged digestate, FOF = soil amended with dry digestate.

Table 2
Spearman's correlation rank between the relative abundances of *Fusarium* spp. at specie level and *Fusarium oxysporum* in tomato rhizosphere of a soil amended with digestate (CKBF, FBF and FOF) and un-amended (CKOF) at the end of the trial.

Fusarium spp[a]	*Fusarium* relative abundance (OTUs%)				Correlation coefficient	Adjusted significance
	CKOF	CKBF	FBF	FOF	(r-value)	(*P*-value)
F. avenaceum	1.08 ± 0.6	15.62 ± 1.7	11.21 ± 1.1	13.63 ± 1.7	−0.811	0.017*
F. culmorum	7.65 ± 0.8	17.55 ± 2.7	19.55 ± 2.7	18.81 ± 2.7	−0.420	0.037*
F. equiseti	<1	30.82 ± 1.6	29.83 ± 1.7	23.23 ± 1.1	−0.982	<0.001**
F. graminearum	<1	37.23 ± 2.7	31.57 ± 9.4	34.41 ± 8.5	−0.975	<0.001**
F. proliferatum	1.65 ± 0.5	13.14 ± 1.5	15.49 ± 1.5	10.74 ± 1.5	−0.852	0.015*

[a] Fusaria associated to Fusarium head blight (FHB) of durum wheat kernels.
* Significant correlation ($0.01 < P < 0.05$).
** Highly significant correlation ($0.001 < P < 0.01$, $P < 0.001$).

the SSD-amended ones along the two axes of plot box, thereby showing as fusaria community shifted after 3-years of digestate supplementation. Although a separation in ordination space between the control soil and the digestate-amended soils was found, it is noticeable to remark that no difference in *Fusarium* abundance ($F = 0.15$, $P = 0.91$), nor richness ($F = 0.03$, $P = 0.93$), nor diversity ($F = 0.22$, $P = 0.89$) was found since ellipses of the digestate-amended soils were overlapped.

The Spearman's rank correlation test (Table 2, Table S6) showed the relative abundance of *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. graminearum* and *F. proliferatum* that were negatively correlated ($P < 0.05$) with the abundance of *F. oxysporum* in the rhizosphere.

3.4. Effect of SSDs amendment on soil bacteria community

3.4.1. 16S rRNA sequence output, quality control, and sequence rarefaction

A table of good quality 16S rRNA sequences from 36 soil samples was generated (Table S7). A total of 7,837,391 sequences were attributed to >28 taxonomic groups of which 92 % to bacteria and the remaining 8 % to other groups (*Archaea*, *Eukaryota*, *Holozoa* and *Nucletmycea*). The average sequence number per sample was 217,705 (SD = 143,44). To obtain an equivalent sequencing depth for further analysis of α-diversity, each sample was rarefied up to 25,000 16S sequences (Fig. S2). The sequences left after rarefaction represented a coverage of 97.3 ± 0.7 %.

3.4.2. Taxonomy

The filtered 16S sequence analyses revealed strong modification of bacterial community composition. Taxonomic classification allowed identification of 23 genera (Table S8A) of which 13 of them significantly varied in the relative abundance in at least one site by digestate application (*Bacillus*, *Chitinophaga*, *Dyadobacter*, *Ferruginibacter*, *Flavihumibacter*, *Flavisolibacter*, *Flavobacterium*, *Gaiella*, *Gemmata*, *Gemmatimonas*, *Olivibacter*, *Pseudomonas* and *Terrimonas*); while the remaining ones (*Arthrobacter*, *Chryseobacterium*, *Leifsonia*, *Luteimonas*, *Mucilaginibacter*, *Othaekwangia*, *Pedobacter*, *Pirellula*, *Rhodanobacter* and *Sediminibacterium*) were unchanged. Particularly, increase in the rhizosphere (Table S8B, Fig. S3) was found for *Bacillus* ($F = 20.3$, $P < 0.001$), *Chitinophaga* ($F = 6.14$, $P = 0.016$), *Flavihumibacter* ($F = 5.36$, $P = 0.023$), *Flavobacterium* ($F = 6.03$, $P = 0.018$), *Olivibacter* ($F = 16.59$, $P < 0.001$), *Pseudomonas* ($F = 20.94$, $P < 0.001$) and *Terrimonas* ($F = 24.8$, $P < 0.001$). Instead, decrease was dominant for *Dyadobacter* ($F = 5.26$, $P = 0.046$), *Gaiella* ($F = 21.03$, $P < 0.001$) and *Gemmatimonas* ($F = 7.89$, $P = 0.011$). Digestate supplementation increased ($P < 0.05$) the relative abundance of *Bacillus* ($F = 20.4$, $P < 0.001$), *Ferruginibacter* ($F = 6.12$, $P = 0.039$), *Flavisolibacter* ($F = 5.89$, $P = 0.043$) and *Pseudomonas* ($F = 17.65$, $P < 0.001$) in the bulk soil at 12 weeks post-amendment (Table S8B); or *Bacillus* ($F = 20.5$, $P < 0.001$), *Ferruginibacter* ($F = 5.45$, $P = 0.041$) and *Flavisolibacter* ($F = 5.63$, $P = 0.047$) in the bulk soil 3 weeks after amendment (Table S8B).

The SIMPER analyses highlighted the main bacterial genera responsible for differences among treatments in the sampling sites (Table 3). *Bacillus* and *Pseudomonas* showed the highest dissimilarity contribution (31.78 % and 23.57 %, respectively), followed by *Flavihumibacter* (8.25 %), *Olivibacter* (7.62 %), *Chitinophaga* (7.06 %), *Flavobacterium* (6.75 %) and *Terrimonas* (5.98 %). Other values ranging

Table 3
SIMPER analysis showing the main bacterial genera (OTUs) responsible for differences between treatments (**in bold**) in bulks and rhizosphere soils at the end of the trial.

Bacteria genus	Dissimilarity contribution (%)	Cumulative (%)	Treatment[a]			
			CKOF	CKBF	FBF	FOF
Arthrobacter	0.13	100	25	34	24	19
Bacillus	**31.78**	**31.23**	**2660**	**3392**	**9723**	**10,214**
Chitinophaga	**7.06**	**61.71**	**2408**	**2576**	**5785**	**6437**
Chryseobacterium	0.21	99.81	112	104	155	197
Dyadobacter	1.81	88.85	166	142	44	98
Ferruginibacter	0.78	93.54	87	102	195	134
Flavihumibacter	**8.25**	**53.74**	**236**	**342**	**3883**	**3166**
Flavisolibacter	0.68	94.96	112	107	185	154
Flavobacterium	**6.75**	**64.65**	**0**	**34**	**53**	**2047**
Gaiella	0.45	97.16	178	147	147	158
Gemmata (Gemm-1)	0.32	98.62	72	137	165	151
Gemmatimonas	1.63	90.19	68	43	37	58
Leifsonia	0.23	99.71	32	24	30	29
Luteimonas	0.27	99.46	253	242	234	198
Mucilaginibacter	0.18	99.74	35	41	24	22
Olivibacter	**7.62**	**59.49**	**840**	**703**	**3087**	**4647**
Othaekwangia	0.48	97.87	13	14	15	25
Pedobacter	0.22	99.78	123	134	105	191
Pirellula	0.17	99.88	232	248	224	275
Pseudomonas	**23.57**	**46.67**	**1268**	**1475**	**7715**	**8180**
Rhodanobacter	0.28	99.19	208	221	243	257
Sediminibacterium	0.15	99.93	232	184	198	232
Terrimonas	**5.98**	**73.53**	**26**	**32**	**2985**	**2437**

[a] Value of each treatment is the 16S rRNA sequences number associated to each OTU by pooling data of bulk and rhizosphere soils. Comparison between sites through all pairwise comparisons (e.g. CKOF-bulk soil3-CKOF-bulk soil12, CKOF-bulk soil3-CKOF-rhizosphere, CKOF-bulk soil12-CKOF-rhizosphere, etc.) are not displayed.

from about 1.81 % to <1 % were associated to OTUs whose dissimilarity contribution was lesser than the genera afore mentioned.

3.4.3. Alpha- and beta-diversity

Digestate supplementation altered bacterial composition (α-diversity) (Fig. 3). There was a significant effect of treatment in the rhizosphere on OTUs number ($F = 5.88$, $P < 0.05$), richness ($F = 22.12$, $P < 0.001$), and diversity ($F = 10.28$, $P < 0.01$). Post-hoc comparisons showed that OTUs number, richness, and diversity were significantly higher in soil amended with dry digestate than in soil amended with centrifuged and liquid digestate and those un-amended. There was a significant effect on richness ($F = 7.69$, $P < 0.05$) in bulk soil at 3 weeks post-amendment. Post-hoc comparisons showed that richness was significantly lower in soil amended with dry digestate than those un-amended. Analyses of 16S sequence from the bulk soil samples at 12 weeks after amendment showed no significant effect of digestate on bacterial OTUs number ($F = 0.03$, $P = 0.438$), richness ($F = 0.01$, $P = 0.097$), and diversity ($F = 0.05$, $P = 0.416$).

Analysis of 16S data explained a significant amount of variation in SBC structure (β-diversity) between 36 samples ($F = 25.68$, $P < 0.001$) (Fig. 4). Digestate shifted bacterial structure either among treatments or sampling sites. Ordination plots showed separation between the un-amended soil and those digestate-amended, as well as among the soils amended with liquid digestate and dry digestate, instead soil amended by centrifuged digestate was overlapped with both (Fig. 4A). Comparisons among sites showed separation in ordination space between the bulk soil samples collected 3 weeks after amendment and rhizosphere, while the bulk soil samples collected 12 weeks post-amendment were overlapped with both (Fig. 4B).

3.4.4. Correlation between the abundances of bacterial members associated to fusaria suppression and F. oxysporum

The Spearman's rank correlation test showed (Table 4, Table S9) that the relative abundance of bacterial members associated to *Fusarium* suppression such as *Bacillus*, *Chitinophaga*, *Flavihumibacter*, *Flavobacterium*, *Olivibacter*, *Pseudomonas* and *Terrimonas* were highly and negatively correlated ($0.001 < P < 0.01$ and $P < 0.001$) with the abundance of *F. oxysporum* in the rhizosphere.

4. Discussion

This study evaluated the effects of three slurry-based digestate typologies on taxonomic characteristics of the fusaria and bacteria communities in the digestate-amended soil to effectively suppress fusaria-related wilt of tomato. Results showed that digestate have potential to shift the microbiota structure with short-term effects by inducing fusaria wilt suppression. Notably, there was no difference in the tomato emergence rates between treatments at the beginning of the growth stage since all seedlings had 100 % emergence (data not shown) indicating that SSD has no phytotoxic effects on tomato in field. Thus, our study demonstrated that digestate changes the soil microbiome structure by inducing fusaria suppression in the absence of potential detrimental effects associated to tomato cultivation. Thus, we assumed such practice as environmentally safe and agriculturally sustainable (Barzee et al., 2019).

4.1. Impacts of SSDs amendment on the tomato Fusarium wilt disease and soil Fusarium community

The main impacts of SSDs amendment on tomato Fusarium wilt and on soil *Fusarium* spp. community were: (i) reduction of Fusarium wilt severity in cropland and decrease of *Fol* DNA abundance in tomato, (ii) decrease of *Fol* abundance in rhizosphere and maintenance of the pathogenicity traits of *Fol* isolates, and (iii) change of taxonomic composition of *Fusarium* spp. community in the rhizosphere.

As regards the first impact, both the dewatered digestates seemed be more effective to suppress *F. oxysporum* wilt than the liquid ones after only one cycle of amendment. Moreover, the rates of the Fusarium wilt severity and those of the pathogen abundance in tomato tissue seemed be similar. This empirical observation was statistically checked by 'ad-hoc' Pearson correlation test showing significant ($P < 0.001$) and positive correlations between these two parameters for each treatment (unshown data). As well, pathogenicity of *F. oxysporum* colonies isolated from xylem of the tomato plants grown in field was also verified by

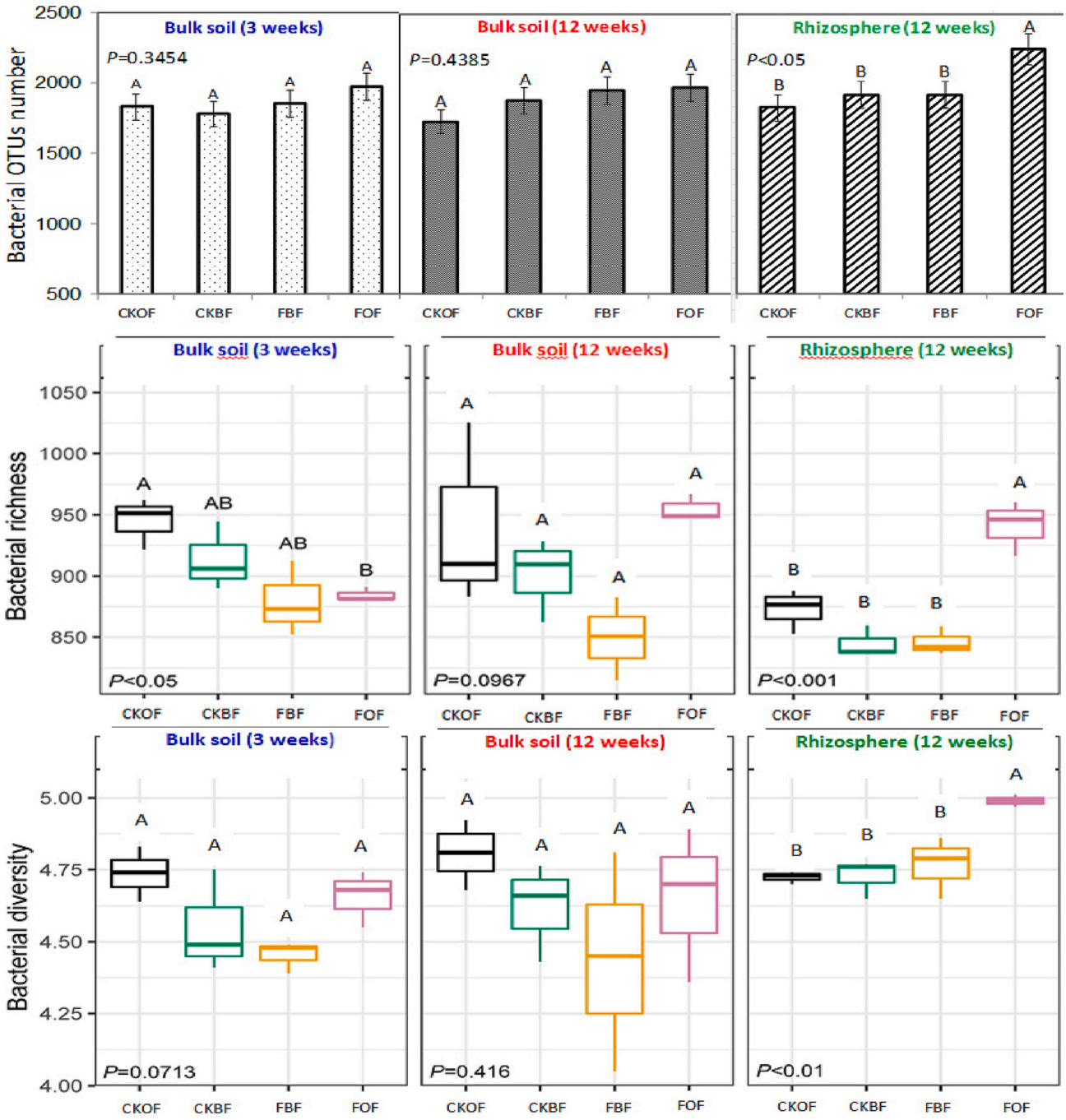

Fig. 3. Effect of SSD soil amendment on the α-diversity of soil bacterial community (SBC) at the end of the trial. Bar plot of the observed OTUs number and box plot of Chao1 richness and Shannon diversity (y-axis) between treatments (x-axis). The α-diversity indices are measured in triplicate ($n = 3$) on 0.5 g fresh soil for each plot basing on 16S rRNA gene sequencing. *P*-values represent the results of one-way ANOVA by comparing averages among treatments. Different letters in each box indicate significant differences among treatments according to the Turkey's HSD post-hoc test ($P < 0.05$).

bioassays. However, to the best of our knowledge, there is very little information about the suppressive efficacy of digestate from sewage sludge against soil-borne vascular pathogens, as well as on the progression rate of the disease symptoms over time in the digestate amended-cropland. However, it is plausible hypothesize that, albeit liquid digestate bring the same amount of nutrients and other substances of the dewatered ones at the doses here used, CKBF is quickly lost by percolation more than FBF and FOF, thereby reducing its suppressiveness. As well, the observed progressing rate between the Fusarium wilt severity and pathogen abundance *in planta* has confirmed *F. oxysporum* as the main etiological agent of the fusarium wilt outbreaks here observed, and that digestate supplementation has reduced the inoculum density over time.

As regards the second impact, the main benefit was related to the reduced abundance of *Fol* in rhizosphere, being decreased without difference by application of all digestate typologies by reaching an acceptable soil inoculum density below the value of 0.004 ng μl^{-1} after three amendment cycles. Such density value is considered as a "critical inoculum" to start Fusarium wilt infection in field (Srinivas et al., 2019). However, *F. oxysporum* colonies isolated from rhizosphere have consistently caused severe wilting symptoms on tomato plant-test even after digestate supplementation, thereby confirming their pathogenicity

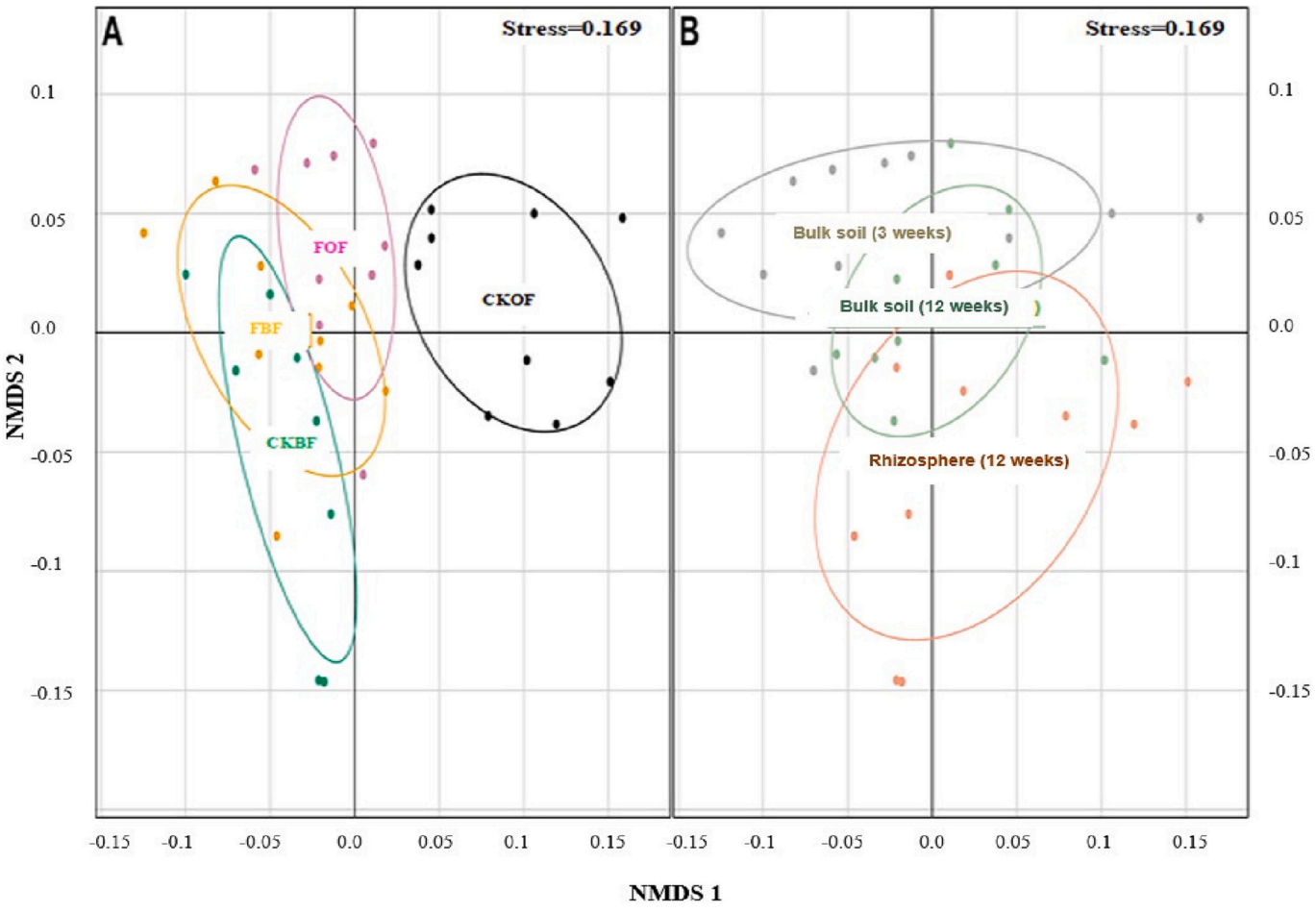

Fig. 4. Effect of SSDs soil amendment on the β-diversity of SBC at the end of the trial. Beta-diversity is measured in triplicate ($n = 3$) on 0.5 g fresh soil for each plot based on 16S rRNA sequencing.
(A) Non-metric Multi-Dimensional Scaling (NMDS) plot showing soil samples separated between treatments, where the 9 dots per treatment (3 replicates × 3 sampling sites) represent the sub-samples.
(B) NMDS plot showing soil samples separated among sites, where the 12 dots per site (3 replicates × 4 treatments) are the sub-samples.

Table 4
Spearman's correlation rank showing the highly-significant correlations[a] between the relative abundances of bacteria at genus level and *Fusarium oxysporum* in three sampling sites of a soil amended with digestate (CKBF, FBF and FOF) and un-amended (CKOF) at the end of the trial.

Sampling site		Bacteria genus	Bacteria relative abundance (OTUs%)				Correlation coefficient	Adjusted significance
			CKOF	CKBF	FBF	FOF	(r-value)	(*P*-value)
Bulk soil	(3 weeks post-amendment)	* *Bacillus*	2.65 ± 0.4	1.98 ± 0.2	8.06 ± 2.0	8.34 ± 2.1	−0.965	<0.001
	(12 weeks post-amendment)	* *Bacillus*	2.54 ± 0.5	2.76 ± 0.5	8.09 ± 2.0	9.76 ± 2.8	−0.982	<0.001
		**Pseudomonas*	<1	<1	8.23 ± 2.6	7.75 ± 1.8	−0.978	<0.001
Rhizosphere soil	(12 weeks post-amendment)	* *Bacillus*	<0.1	<0.1	13.78 ± 4.9	12.32 ± 4.8	−0.987	<0.001
		** *Chitinophaga*	2.13 ± 0.1	<1	13.01 ± 2.7	14.86 ± 4.1	−0.898	0.002
		** *Flavihumibacter*	<0.1	<0.1	2.03 ± 0.4	1.83 ± 0.5	−0.816	0.003
		** *Flavobacterium*	0.00	<0.1	1.47 ± 0.3	2.86 ± 0.3	−0.791	0.005
		Olivibacter	<1	<0.1	1.63 ± 0.1	3.37 ± 0.7	−0.973	<0.001
		**Pseudomonas*	0.00	2.24 ± 0.5	10.49 ± 4.6	10.74 ± 4.2	−0.982	<0.001
		** *Terrimonas*	<1	<1	1.25 ± 0.1	1.44 ± 0.2	−0.968	<0.001

[a] [a] $0.001 < P < 0.01$ and $P < 0.001$.
* Taxa known as a biocontrol agent to tomato Fusarium wilt.
** Taxa highlighted as a potential sensitive biomarker for inducing potential fusaria suppression.

attributes. Moreover, it is interesting to pay attention about the same efficacy of all SSD typologies for reducing the pathogen inoculum abundance, since such data seems be contradictory, or however ambiguous, with the previous findings. Indeed, the effectiveness of liquid digestate in reducing inoculum abundance in the same manner of the dewatered ones remains still unexplainable for us because liquid digestate appeared to be less suppressive than others SSD by observing the Fusarium wilt severity trend in cropland and *F. oxysporum* abundance in tomato at the end of the trial. To the best of our knowledge, there is little information about the suppressive effect of SSDs in reducing the potential inoculum density of fungal vascular pathogens for allowing any comparison with the literature data. It is very interesting to notice that suppressive effect of digestate is mainly related to dramatic reduction of the pathogen abundance rather than to own reduced pathogenicity. On the other side, a similat suppressive response is consistently documented by authors that amended conductive or weakly suppressive soils with on-farm green compost (Pane et al., 2020), biological organic fertilizer, and Brassicaceous seed meal for reducing soil inoculum density of *Phytophthora capsici* on *Cucurbita pepo* (Bellini et al., 2020), *Verticillium dahliae* on bell pepper (Tubeileh and Stephenson, 2020) and eggplant (Kanaan et al., 2018), *Fol* and *V. dahliae* on tomato (Antoniou et al., 2017), *F. oxysporum* f. sp. *capsici* on pepper (Ren et al., 2018), and *Fusarium oxysporum* f. sp. *niveum* on watermelon (Zhang et al., 2021).

As regards the third impact, another fundamental effect due to SSDs soil treatment regarded the shift of *Fusarium* community structure in the rhizosphere which was evaluated by a novel metabarcoding approach to investigate *Fusarium* species composition in soil samples (Cobo-Díaz et al., 2019). Indeed, the fusaria-related community resulted be deeply changed after only one amendment cycle of 3-yr due to dramatic reduction of the *F. oxysporum* inoculum. First, some fusaria species as *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. graminearum* and *F. proliferatum* seemed to have replaced *F. oxysporum* in the relative abundance. It is remarkable to notice that such fusaria species are associated to Fusarium head blight (FHB) of durum wheat kernels in different Italian cultivation areas, including the South of Italy. Besides to infect and damage wheat heads in field, such fusaria species biosynthesizes toxic secondary metabolites known as deoxynivalenol (Beccari et al., 2020). Although FHB is caused by a complex fusaria consortium, *F. graminearum in sensu stricto* is predominant in most areas of the world. Abundance of such *Fusarium* species in tomato rhizosphere in the absence of durum wheat can be explained by "soil legacy effect" resulting from an antecedent soil disturbance that may be a result of more else changes in land use or land cover or in soils with historically suppressive or conductive property (Bakker et al., 2018). Indeed, the soil under study has been continuously cultivated with durum wheat and barley from the 1980s until 1995 until severe epidemic outbreaks of FHB have forced farmers to change cropland from extensive farming systems based on cereal cultivation into intensive tomato cultivation. Digestate treatment, by reducing dramatically *Fol* inoculum with short-term effects, has probably shifted the *Fusarium* community in tomato rhizosphere towards a previous microbiological state which was characterized by the dominant fusaria species related to FHB due to soil-borne legacy effects (Jurburg et al., 2017). Interestingly, greater abundance of *F. equiseti* in the SSD-amended soil fusaria community should be noticed, since *F. equiseti* is a potential MBCA able to cover the crucial role of inducing-suppressive soil to Fusarium wilt of tomato either alone or in association with the nonpathogenic *F. oxysporum* Fo47 (Fuchs et al., 1997; Larkin and Fravel, 1998). The reduction of disease rating against *Forl* induced by *F. equiseti* GF191 was consistently high since it probably acts as a plant growth-promoting fungi. Thus, we can hypothesize that the highest abundance of *F. equiseti* may have contributed to reduce *Fol* inoculum in SSDs-amended soil, although we do not have carried out 'ad hoc' experiments for confirming this hypothesis.

4.2. Impacts of SSDs amendment on tomato Fusarium wilt suppression: drivers and mechanisms of action

Our findings showed that digestate soil application has reduced the fusaria-related wilt severity of tomato by altering the taxonomic composition of SBC according to Andrés et al. (2011). In this study, two differential impacts were implicated in tomato Fusarium wilt suppression: (i) the shifted composition and diversity of SBC in bulk and rhizosphere soils, and (ii) the increased relative abundance overall in rhizosphere of potential MBCAs of which five of them are noticed as potential sensitive biomarkers for inducing fusaria suppression.

As regards the first impact, the shifted taxonomic structure of SBC in rhizosphere more than in bulk soil might explain the most probable mechanisms of action related to *Fol* suppression. The clearest differences in SBC composition resulted to be consistent between un-amended soil and the amended ones with both dewatered digestates in rhizosphere more than in bulk soil. Supplementation with both dewatered digestates improved natural property of soil to effectively suppress *Fol* in the rhizosphere more than in the bulk soil inducing higher turnover in bacterial composition than the liquid ones. Such response indicated that short-term soil amendment with dewatered digestates has regulated faster variation in bacterial composition overcoming the natural soil resilience. Indeed, despite the SBC is expected to be resilient to external disturbance given its own high taxonomic diversity and functional redundancy (Dungan et al., 2003; Reeve et al., 2010; Jurburg and Salles, 2015), its structure was quickly reshaped by solid digestate, according to microbial diversity associated with Fusarium wilt of banana by *F. oxysporum* f. sp. *cubense* (Zhou et al., 2019). Conversely, liquid digestate has triggered only a weak disturbance to which the SBC resulted be more resilient rendering this approach ineffective in fusaria controlling. However, certain disturbances that are caused by repeated applications of digestate are still lesser known in literature than the traditional OAs to explain the drastic effects observed on taxonomy and functional traits of SBC. Ecological theory predicted that the initial soil disturbance induced by OAs affected the primary stability (resilience) of SBC, thus rendering it most susceptible towards further soil manipulation (Griffiths and Philippot, 2013). Such hypothesis indicates that certain disturbances may strongly change the soil microbial structure by short-time direct effects mainly due to competition for nutrient or release of antimicrobial compounds, but only for preparing the soil to further microbiome reshaping triggered by agricultural practices such as crop rotation, solarization, soil amendment with cow dong, biofumigation, etc. with suppressive effect over time (Jurburg et al., 2017).

As regards the second impact, a primary goal of this study was to know the effects of digestate on taxonomic characteristics of SBC and evaluate the impacts on fusaria suppressiveness. In this regard, in order to study differences about SBC taxonomic composition induced by compost (SSc and MSWc) and digestate, we have previously performed an additional experiment detailed in Supplementary data#2 (Fig. A). The samples from the unamended soil (CKOF) and the compost-amended soils were mostly colonized by *Chlorobi*, *Spirochaetes*, *Verrucomicrobia*, *Cyanobacteria*, *Deinococcus-Thermus*, *Actinobacteria*, and *Chlamydiae*. The samples from SSD-amended soils (CKBF, FOF and FBF) were instead dominated by *Firmicutes*, γ-*Proteobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Actinobacteria*, *Planctomicetes*, *Acidobacteria. Chloroflexi*, *Nitrospirae*, *Candidate divisions* (OD1, TM7 and OP10) and Bacteria_unclassified. In our study, digestate supplementation has increased abundance of some bacterial members with biocontrol potentiality to fusaria-related wilts. Specific changes in the soil bacterial microbiome composition might explain the most probable mechanisms of action related to *Fol* suppression since the relative abundance of *Bacillus*, *Chitinophaga*, *Flavihumibacter*, *Flavobacterium*, *Olivibacter*, *Pseudomonas* and *Terrimonas* were highly-negatively correlated with the *F. oxysporum* abundance in the rhizosphere. In literature is consistently reported that soil microbiota contributes to tomato Fusarium wilt suppression more than the soil physicochemical features shifting and selecting fewer

microbial groups functioning as specific MBCAs (Meghvansi and Varma, 2015; Cha et al., 2016; De Corato, 2020). From the one hand, bacterial members belonging to *Bacillus* and *Pseudomonas* were known to be strong suppressors of *F. oxysporum* in soil amended with compost, biochar (Hassan, 2020), and biogas slurries from pig manure and vinasse (Li et al., 2022). The *Bacillus* (Firmicutes or Bacillota) and *Pseudomonas* (γ-Proteobacteria or γ-Pseudomonadota) genera are well known for their crucial roles in disease suppression by production of versatile secondary metabolites (Ortiz and Sansinenea, 2022). Enrichment of *Bacillus* by dewatered digestates resulted be very consistent in all the sampling sites. Our findings were very interesting given that *Bacillus* was noticed as the top-MBCA genus capable to effectively suppress Fusarium wilt on tomato in plant growth media (Borrero et al., 2013) and in fusaria-diseased soil (Yuan et al., 2020). As well, *Bacillus* is often strongly associated with soil suppressiveness thanks to crop growth promotion and high antifungal activity (Lee et al., 2021) since it can produce antimicrobial compounds (Ortiz and Sansinenea, 2022). Similarly, the increased abundance of *Pseudomonas* in bulk and rhizosphere soils 12 weeks post-amendment with dewatered digestates indicated strong contribution of Pseudomonadota in suppression, since the *Pseudomonas* genus is considered the main responsible for suppressing Fusarium wilt of banana (Fu et al., 2020). Particularly, fluorescent pseudomonads are noticed as one of the primary MBCA inducing Fe-competition in soil by siderophores production that allow a conductive soil to become induced-suppressive soil for controlling Fusarium wilt of tomato (Larkin and Fravel, 1998; Haas and Défago, 2005; Lemanceau and Alabouvette, 2008). Indigenous *Pseudomonas* populations can fight against other microbial competitors of soil by serving as signalling-molecule for ecological interactions to enhance plant disease suppression by bio-organic fertilizers (Tao et al., 2020). On the other hand, five bacteria taxa belonging to Bacteroidota were negatively correlated with the *F. oxysporum* abundance in rhizosphere. Thus, we assumed such taxa as a potentially sensitive biomarker members in inducing fusaria suppression. Among them, *Chitinophaga sanctii* produces potentially resistant substances named elansolid (Steinmetz et al., 2011) capable to suppress pathogenic soil-borne fungi. *Chitinophaga* can exhibit fungicidal activities and can induce antagonistic activity towards other pathogenic fungal taxa (Chapelle et al., 2016). *Flavihumibacter petaseus*, belonging to the *Chitinophagaceae* family, has potential ability to suppress fungal vascular pathogens (Zhang et al., 2010). *Flavobacterium* is found to be suppressive to *F. oxysporum* in pot experiments (Kwak et al., 2018). The *Terrimonas* genus exhibits negative relationships with the apple replant disease syndrome (Yim et al., 2017), shows greater abundance in the rhizosphere of healthy *Panax notoginseng* (Wu et al., 2015), and results consistently noticed among the key groups that likely confer soil suppressiveness against disease-causing *F. oxysporum* on banana (Fu et al., 2020) and tomato (Ou et al., 2019). Thus, Bacteroidota includes potential biomarkers in fusaria-related wilt suppression in agreement with previous studies (Khatri et al., 2023). In contrast, although *Olivibacter* (Bacteroidetes) was negatively and highly correlated to *Fol* abundance in soil, no evidence on own potential role to suppress wilt-causing *F. oxysporum* was found in literature. However, advanced approaches such as ALDEx2, LEfSe analysis (LDA values >4), the random forest model, metastat and metagenomeSeq should be implemented in further study to identify new potential microbial biomarkers discriminating the bacterial community between treatments.

Our findings agree with surveys reporting how short cycles of soil amendment in field with anaerobic digestate of dairy manure have showed benefits in suppressing soil-borne pathogens by increasing total abundance of *Bacillus* and *Pseudomonas* in digestate-amended soil (Pan et al., 2018). Interestingly, authors have tried to suppress soil-borne disease of maize and alpine strawberry in North of the Italy by applying digestate from agri-based wastes and related co-products. Manici et al. (2020) used digestate of animal manure, agricultural waste, food waste feedstock, dairy manure, and energy crops in intensive fertile lands of the Emilia-Romagna region, whenever repeated maize cultivation caused progressive decline of the soil natural suppression. They concluded that soil capacity to support maize biomass production was principally related to natural ability of the digestate-amended soil to reduce root infection by specific action of saprophytic and opportunistic consortia of *Actinomycetes* and *Pseudomonas*, being resulted dominant in the digestate-amended soil than in the non-amended ones. Soppelsa et al. (2021) found similar behaviour in soil amended with digestate of liquid manure associated to composted digestate from organic fraction of MSW in a specialized area of the South Tyrol (Alto Adige region) for alpine strawberry cultivation to control strawberry root by *Dactylonectria torresensis*, the main fungal pathogen associated to strawberry decline. They concluded that SBC associated to strawberry rhizosphere was changed in abundance, structure, and diversity after digestate supplementation, being correlated to the pathogen reduction and positive crop response to digestate treatment in comparison with two control soils (non-amended and chemically treated).

To decipher drivers underlying the suppressive effect against tomato Fusarium wilt, we can claim soil microbiota as the main factor involved since neither nutrient content, nor total bacteria abundance, nor biological parameters of soil were correlated to *F. oxysporum* abundance in the rhizosphere, as resulting in Supplementary data#2 (Figs. B, C, D and Table A). Nonetheless, the enhanced soil nutrient content (SOM, TOC, TN, and AP) from manure and swine slurry in soil application represents an exogenous carbon source that provides suitable microhabitat for the growth and colonization of microbiota (Suleiman et al., 2016; Ye et al., 2021) that induces substantial change in soil of the physicochemical properties, enzymatic activities, and bacteria community structure (Hu et al., 2018; Liu et al., 2021). A survey performed in the South of China forestlands indicated that SS soil application altered composition and co-occurrence pattern of the SBC. The change in the relative abundance of bacteria (where *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* were increased, while *Acidobacteria*, *Chloroflexi* and *Verrucomicrobia* were decreased) was attributed to increase in the content of available nutrients (AP, EK) and heavy metal (Cd) and soil pH rather than to the exogenous microbiota (Zhao et al., 2021). In contrast, Li et al. (2022) claimed the crucial role of bacterial communities inhabiting the biogas slurry from pig manure and vinasse (*Actinomycetes*, *Bacillus* and *Pseudomonas*) in suppressing Fusarium wilt of cucumber by *F. oxysporum* f. sp. *cucumerinum* in pot experiments. Thus, we can suppose that microbiota coming from the complex evolution of the biological processes in anaerobically digested wastewater sludge (Mironov et al., 2020) could determine high magnitude disturbance of the native microbiota triggering competition for nutrient (by Pseudomonadota) or release of antifungal compounds (by Bacillota) or still lesser studied other biocontrol mechanisms (by Bacteroidota). Thus, the direct input of exogenous microbiota from the soil-incorporated SSD might play a primary role to alteration of microbial community structure assembly (Sun et al., 2015). Although this study has overlooked the microbial communities structure inhibiting in the SSDs, this aspect will be studied in-depth by selecting, characterizing, and testing one-each-one all bacteria members living in the biogas slurries which have potentiality to fusaria wilt suppression of tomato.

5. Conclusions and future perspectives

Soil amendment is an eco-friendly and non-chemical approach effective for reshaping the soil microbiota by reducing long-term monoculture problems by introduction or activation of MBCAs for soil-borne disease control. This study has showed some benefits that might derive by soil supplementation with dry SSD, without using neither chemical fumigants/fungicides nor suppressive compost, accordingly with the virtuous reuse of biosolids in a circular economy system. Our earlier results (Supplementary data #2) showed that: (i) the suppressive effect against *Fol* in potting soil is evident when considering disease severity (or gravity) index rather than disease incidence rate; (ii)

albeit the soil content of SOM, TOC, TN and AP increased after 12 weeks post-amendment, such differences does not correlate to *Fol* abundance in potting soil; (iii) SSD capability to reduce *Fol* inoculum does not relate neither to total bacteria abundance nor biological activity; and (iv) soil bacterial community (SBC) drives *Fol* suppression rather than soil fungal community. Our findings have demonstrated that dry SSD application has reduced wilt severity up to 18 % and *Fol* abundance in tomato up to 0.9 ITS copies g^{-1} tissue. In rhizosphere, *Fol* abundance decreased up to 0.0027 ng μl^{-1} DNA, while the *Fusarium* community shifted between treated-soils and the un-amended ones by replacing the *Fol* isolates with other fusaria species related to wheat kernel disease. Bacteria microbiome composition changed in rhizosphere applying dry digestate, while the SBC structure shifted among treatments and sampling sites. Bacterial members potentially related to *Fol* suppression belonging to Bacillota, Bacteroidota and Pseudomonadota were increased in the rhizosphere more than in the bulk soil. Thus, we can conclude that microbiota represents the main soil factor involved in fusaria wilt suppression of tomato. Since digestate sounds as a profitable option for cropland fertilization in organic farming systems, the approach here studied should be further performed by combining digestate with low-disturbing on-farm green compost or bio-organic fertilizer to reshape soil microbiome under long-term condition. To brother point of view, future studies should be implemented to combine dry SSD (powdered or pelletized) with pelletized green compost (PGC) since soil microbiome becomes the key-driver for fusaria wilt suppression. Indeed, in order to shift a soil with disease-conductive property into the suppressive ones, the combined application of dry SSD with PGC may be better option to reduce, at least partially, the severity degree of Fusarium vascular disease in comparison to the two treatments alone. Thanks to high disturbance magnitude triggered by digestate that acts as a strong suppressor with short-term effects, followed by PGC that instead acts either as a plant bio-stimulant or as a suppressor with long-term effects for determining a slow reshaping of soil microbiome, could be reached good benefits over time in sustainable agroecosystems.

Authors approval

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Ethics approval

The authors declare of have read, understood, and approved the ethics guidelines.

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Ugo De Corato: conceptualization and ideas, supervision, investigation, methodology and experimental design, laboratory experiments, data curation, formal analysis and writing of the original paper.

Fernando Antonio Cancellara: data set elaboration, graphical and figure editing.

Giovanni Lacolla and Davide Caranfa: phytopathological assessment and soil analyses.

Giovanna Cucci: literature review and critical revision of the final paper.

Declaration of competing interest

The authors declare that there are no any actual or potential competing interests including any financial, personal or other relationships with other people or organizations.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

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