sustainability

MDPI

Article

# Locally Available Organic Waste for Counteracting Strawberry Decline in a Mountain Specialized Cropping Area

**Sebastian Soppelsa [1], Luisa Maria Manici [2,*], Francesco Caputo [2], Massimo Zago [1] and Markus Kelderer [1]**

[1] Laimburg Research Centre, 39040 Ora-Auer, Italy; sebastian.soppelsa@laimburg.it (S.S.); massimo.zago@laimburg.it (M.Z.); markus.kelderer@laimburg.it (M.K.)
[2] Council for Agricultural Research and Economics (CREA), Research Center Agriculture and Environment, 40128 Bologna, Italy; francesco.caputo@crea.gov.it
* Correspondence: luisamaria.manici@crea.gov.it

**Abstract:** Crop decline caused by soil borne fungal pathogens affects specialized cropping systems such as fruit trees and strawberry. A study was carried out to investigate the effectiveness of pre-plant application of waste-derived biomasses in strawberry (*Fragaria* × *ananassa*) to reduce that phenomenon. A field experiment was carried out in an alpine strawberry specialized valley in South Tyrol (Italy), in a long term cultivated field selected for yield reduction over recent years. In July 2018, one month before strawberry transplanting, a field experiment with four soil treatments was set up: anaerobic digestate (solid fraction) of liquid manure, compost from anaerobic digestate of organic fraction of municipal solid waste (OFMSW), untreated control and Dazomet as chemical control. Plants were grown for two cycles (2019 and 2020). Dazomet always gave a significant (over 50%) increase in marketable yield per plant in both the years, anaerobic digestates did not improve strawberry production; compost from OFMSW gave phytotoxic effects in the first year, but improved strawberry yield like Dazomet in the second. Changes of rhizosphere bacterial populations and difference in root pathogen abundance, especially that of *Dactylonectria torresensis*, were correlated to the crop response to treatments. Findings suggest that waste-derived biomasses are a promising eco-friendly option for counteracting strawberry yield decline. Their positive impact was mostly linked to functional improvements induced by microbial variations. However, the use of such organic amendment requires careful evaluation of composition, doses and above all application times to reduce phytotoxic effects that in some cases can occur in the first months after application.

**Keywords:** root rot; soil borne pathogens; digestate; compost; *Pseudomonas*; *Dactylonectria torresensis*; crop decline

check for updates

**Citation:** Soppelsa, S.; Manici, L.M.; Caputo, F.; Zago, M.; Kelderer, M. Locally Available Organic Waste for Counteracting Strawberry Decline in a Mountain Specialized Cropping Area. *Sustainability* **2021**, *13*, 3964. https://doi.org/10.3390/su13073964

Academic Editors: Ugo De Corato and Marc A. Rosen

Received: 19 February 2021
Accepted: 26 March 2021
Published: 2 April 2021



## 1. Introduction

Crop decline, namely the gradual reduction of plant vigor and yield, which characterizes specialized cropping systems, is linked to the loss in soil biodiversity caused by the frequent return of the crop to the same plots and the increase in nonspecific fungal pathogens that saprophytically survive on plant residue [1,2]. This is a multiple cause phenomenon that involves reducing plant nutrients, growth promotion, and all ecosystem services commonly supported by a diverse and balanced soil microbiome [3]. Furthermore, rooting reduction caused by the complex of nonspecific (opportunistic) fungal pathogens selected by the target crop's continuous return increases yield losses [4]. Strawberry is mainly produced in specialized growing areas where this high value crop shows symptoms ascribable to "crop decline" after some repeated cultivations. It appears as a gradual reduction in yield and quality, reduced plant growth rate in post-transplant, collapse of plants during the fruit ripening stage, and generally reduced plant ability to counteract biotic and abiotic stress [5]. On the other hand, strawberry is one of the crops for which chemical fumigants, such as methyl bromide and chloropicrin have been mostly applied in the past [6]. Indeed, eco-friendly alternatives for controlling specific and opportunistic soil

borne pathogens, such as anaerobic soil disinfestation, soil solarization, biofumigation, etc., have been most widely tested on this crop over the last decades [7–9]. However, low input strategies for controlling soil-borne pathogens imply an optimal endowment of soil organic matter (SOM) content because organic matter is the matrix of the soil microbiome, which plays multiple roles in soil functioning. This includes mechanisms, such as antagonism, pathogens, growth promotion, and others involved in soil suppressiveness, which is the soil's natural ability to suppress pathogens and promote plant health [10,11]. On the one hand, the increase of microbial biomass in soil following organic amendment has been widely linked to the increase in soil health [12]; on the other, many more difficulties exist that link improvement to how chemical features, composition, and origin of the organic materials can affect the composition of soil microbial communities responsible for the beneficial or detrimental functional changes [13,14]. Among the studies performed on organic amendment application for improving soil health in strawberry, the most successful results were obtained in long term applications; whereas in the short term ones, they largely varied according to experimental field conditions, chemical properties, pH, origin and degradation degree of materials [15–18].

The use of digestates as soil amendments from the anaerobic digestion of organic raw materials such as manure, food waste, sewage sludge and other organic waste, is the recycling method closer to the ideal model of circular economy [19]. In Europe, Biogas and biomethane production has had a particular boost in the last 15 years, thanks also to European support policies such as the European Renewable Energy Directive of 2009 [20]. This has largely increased quantity of residual biomasses from anaerobic production of biogas to be handled. Digestates from biogas production have several known benefits such as high fertilization properties and soil enrichment with carbon [21,22]. Conversely, little is known about the effects of digestates in manipulating soil microbial communities and improving soil health; indeed, to date contrasting results have been observed due to the variability of digestate composition and to soil response according its original status [23].

Based on the above considerations, a field trial was set with focus on the response of specific functional microbial populations to the soil amendment with digestates. Based on the above considerations, a field trial was set with focus on the response of specific functional microbial populations to the soil amendment with digestates. This because decades of field trials essentially based on crop yield response to the use of digestates a have not been able to provide a clear answer on their usefulness for improving soil health due to the highly variable outputs. A field study has been carried out to estimate potentiality of locally available digestates for counteracting strawberry decline in an alpine specialized growing area (Martell Valley) located in a famous national park and as such highly interested in developing sustainable agro-techniques to integrate current practices. Indeed, innovations for the revival of strawberry cultivation are necessary in that valley as maintenance of SOM with animal manure and rotation with horticultural crops, after four decades, are not anymore sufficient to guarantee an economically competitive productive and qualitative standard of strawberry production.

## 2. Materials and Methods

### 2.1. Experimental Site

The field trial was performed in a mountain valley, the Martell Valley (South Tyrol, Italy), located in the largest protected natural environment in Italy and the whole alpine chain, the Stelvio National Park, whose climate is classified as Dfb (humid continental climate, or Boreal; no dry season; warm summer) according to Köppen-Geiger climates [24]. It is the highest altitude strawberry cultivation area in Europe (from 900 m up to 1800 m asl), where strawberries are harvested from the end of June to early August, thus covering the late fragment of the strawberry market in Italy. Therefore, this crop has become an essential part of the local economy since the end of the 1980s—beginning of the 1990s. The field selected for this trial has been cultivated with strawberry since the late 1980s with some breaks for other crops (savoy cabbage, cauliflower or chicory), but over recent years has

shown typical crop decline symptoms such as increasing collapsed plants at ripening stage and stunted plant growth with consequent reduction of strawberry production per plant.

### *2.2. Preliminary Soil Health Evaluation by Greenhouse Bio-Assay*

In early April 2019, nine soil samples were randomly collected from the 0–25 cm topsoil layer along the diagonal of the field to obtain a total of about 40 kg of soil. Soil samples were mixed, and a 500 g subsample was taken for physico-chemical analysis. Soil analyses (soil texture, pH and chemical soil features) were performed according to the periodically updated VDLUFA method book (https://www.vdlufa.de/Methodenbuch/index.php?option=com_content&view=article&id=7&Itemid=108&lang=de&lang=en, accessed on 15 March 2021), German version.

Soil was then divided into two parts, one of which was divided into four bags, watered up to field capacity and subjected to a thermal sanitization treatment as described in Manici et al. [25]. Heat-treated soil was kept in the air at 20 ± 4 °C for a week to achieve a humidity similar to untreated soil. Original and heat-treated soil were divided into twelve pots each filled with approximately 1.5 kg of soil, they were arranged according to a randomized design with three replicates of four plants each. Pots were then planted with strawberry frigoplants $A^{++}$ (cv. Elsanta) which were grown for 75 days in greenhouse at 20 to 24 °C with periodical watering. Fruits were harvested weekly from the first fruit harvest. At the end of the trial, the above ground part of each plant was divided from the roots and processed to estimate dry matter of the above ground part per plant.

Roots were washed under running water and processed for fungal endophytes isolation as described in Manici et al. [25]. Root infection frequency was inferred from 48 root segments (12 per plant) per treatment for a total of 288 analyzed root segments (144 original soil, 144 heat treated soil).

### *2.3. Field Trial*

#### 2.3.1. Pre-Plant Treatments and Field Trial

A field trial over two growing seasons (2019 and 2020) was conducted in the previously selected strawberry field located at Gand, Martell (46°55′ N; 10°78′ E; 1.361 m asl). The impact on soil health of two organic amendments (compost from digestate and separate solid fraction of digestate) compared to untreated and a chemically sanitized soil product based on Dazomet was tested. Compost was obtained from anaerobic digestion of organic fraction of municipal solid waste (OFMSW) by Bio Energia Trentino S.r.l. (Faedo, Trento, Italy). The chemical composition of the Compost treatment was pH 8.1, dry matter 76.1%, organic matter 33.1% FM (fresh matter), nitrogen 1.42% FM (Supplementary Material S1). The application dosage was 23.0 t $ha^{-1}$ of treated surface, which in strawberry crop corresponds to 36.4% of cultivated surface. Digestate consisted of separate fraction of digestate from anaerobic digestion of liquid manure, from the local biogas plant of Aldein (Aldein, Bolzano-Bozen, Italy). The digestate treatment was characterized by pH 8.1, dry matter 19.9%, organic matter 13.4% FM, nitrogen 0.94% FM (Supplementary Material S1). The application dosage was 34.5 t $ha^{-1}$ of treated surface, which in strawberry crop corresponds to 36.4% of cultivated surface. The final nitrogen amount provided with both compost and digestate was approximately the same, 325 kg $ha^{-1}$ of total nitrogen. The heavy metal content for both products was within legal limits of Italian law (D. Lgs. 29 April 2010, n. 75). Physico-chemical analysis of products were performed at the Laboratory for Soil and Plant Analysis at the Laimburg Research Centre, according to the methodology described in the official VDLUFA guidelines (Verband der Deutschen LandwirtschaftlichenUntersuchungs- und Forschungsanstalten) [26]. Dazomet was used as positive control (99%, Basamid® Granulat, Certis Europe, Saronno, Italy; at a dosage of 255 kg $ha^{-1}$ of effective treated surface).

In mid-July 2018, soil was tilled and raised beds were built. A furrow over each raised bed was made and organic amendments (compost and digestate) were incorporated at a depth of 25–30 cm. Afterwards, furrows were closed and raised beds were covered with white plastic mulch film in order to avoid weed growth and keep fruit clean. Three weeks

after soil treatments, plants were transplanted in a staggered double row, with 15 cm intra-row and intra-plant spacing. Fresh plug plants were from the Società Agricola Salvi Vivai s.s. (Ferrara, Italy) and cultivar Elsanta was chosen as it is known to be highly susceptible to soil-borne pathogens. Mineral fertilization was performed in both 2019 and 2020 by NPK fertigation as follows: twice a week for 2 weeks at the early vegetative stage with a dosage of 10 kg ha$^{-1}$ of 20–20–20; twice for week for 4 weeks since early fruit production with dosage of 10 kg ha$^{-1}$ of 15–5–30. During the growing season, strawberry crop was protected according to standard pest control protocol for conventional production. Plants were covered with frost protection fleece to protect them during the frost period from November to April. The experiment setup was organized as a completely randomized block design with three replicates per treatment. Each replicate corresponded to a whole row of 150 plants.

#### 2.3.2. Evaluation of Strawberry Production

Ripe strawberry fruits were harvested every three to four days, starting from the first week of July to the end of the month for both years. Harvested fruits were classified into marketable (diameter > 25 mm) and non-marketable (small, deformed, and diseased). Results are reported as marketable yield (g plant$^{-1}$). Fruit quality traits were assessed at third picking time. The soluble solid content (Brix) was determined with a refractometer (RFM840, Bellingham-Stanley Ltd., Kent, UK). The titratable acidity, expressed as percentage of citric acid, was measured with a titrator (Flash Automatic Titrator, Steroglass, Perugia, Italy). Fruit firmness is expressed as Durofel Index (DI) (Agrosta® Winterwood, Agrosta Sàrl, Serqueux, France); The color was measured with a colorimeter (Minolta, model CR-400, Tokyo, Japan). Values are presented as Color Index [CI = (1000 × a)/(L × b)], with higher CI value indicating a more intense red color in the fruit [27]. These parameters were evaluated on a subsample of 12 randomly chosen plants in each row/replicate.

### *2.4. Soil Sampling and Molecular Analysis*

#### 2.4.1. Rhizosphere Soil Sampling

Soil sampling for evaluating rhizosphere microbial communities was performed at the productive stage of strawberry (crown and fruit development) in mid-June 2019 and 2020, 11 (2019) and 23 (2020) months after soil treatments respectively. Four plants per replicate were collected for each treatment. Rhizosphere soil adhering to roots was sampled after having gently shaken the roots of each replicate of 4 plants to remove any loose matter. In that way, three replicates of rhizosphere soil sample from each treatment were obtained. A subsample of 25 g of soil was taken, air dried at room temperature for 12 h and stored in 50 mL sterile vials at −80 °C until processed for soil DNA extraction.

#### 2.4.2. Soil DNA Extraction

Total genomic DNA was extracted from 0.25 g of rhizosphere soil (dry weight) using the PowerSoil DNA Isolation kit according to manufacturer instructions (MoBio Laboratories, Carlsbad, CA, USA). Quantification and quality control of DNA were performed using Infinite 200 NanoQuant (Trading AG, Switzerland) and DNA was stored at −20 °C until use. Three DNA extractions per sample were performed for PCR and PCR-DGGE.

#### 2.4.3. Quantitative PCR (qPCR)

The response to soil treatments in strawberry preplant by *Cylindrocarpon*-like fungi (mainly represented by *Dactylonectria torresensis*) and *Pseudomonas* spp. were evaluated using quantitative polymerase chain reaction (qPCR). *Dactylonectria torresensis* Cyl 64 isolate (CBS-KNAW culture collection, accession n. CBS 133999) and *P. chlororaphis* (DSMZ accession n. 6508) were taken as references for relative quantification. Resulting amplicons were purified using the PureLink Quick PCR Purification Kit (Invitrogen) and quantified by Infinite 200 NanoQuant (Trading AG, Switzerland). The gene copy number calculation was obtained using the formula: gene copy/μL = DNA [ng/μL] × 6.02 × 1023/base

pairs × 660 × 109. Purified amplicons were serially diluted 10-fold and four replicates were used for standard curve generation for quantification of unknown samples. The slope of the standard curves was used to calculate qPCR reaction efficiency.

PCR reaction of total soil DNA amplification, obtained using the PCR conditions already described in Caputo et al. [28], were performed using the primer pair Mac1/Macpa2 [29] and Ps-f/Ps-r and PsF/518r respectively [30].

qPCR assays were carried out using Rotor-Gene SYBR® Green PCR Kit (Qiagen, Hilden, Germany) on a QIAGEN Rotor-Gene Q (Corbett Rotor-Gene 6000) according to manufacturer instructions. Two technical replicates were performed for 3 identical independent runs, to assess reproducibility of the assays. Briefly, 1x Rotor-Gene SYBR® Green PCR Master Mix was used in a final reaction volume of 25 µL, with a final primer concentration of 1 µM and 2.5 µL of template. After an initial PCR activation step at 95 °C for 5 min, cycling conditions consisted in 5 sec denaturation at 95 °C, and 40 cycles of combined annealing extension at 65 °C for 10 s. Post-amplification melting curve analysis was performed to verify specificity and identity of qPCR products, with a ramp from 55 °C to 99 °C, rising by 1 °C each step. Results were analyzed with the Rotor-Gene 6000 Series Software 1.7 program. Sterile water was used as no-template control in each run. Results were expressed as pg $\mu L^{-1}$.

#### 2.4.4. Bacterial Community DGGE Fingerprinting (PCR-DGGE)

PCR-DGGE fingerprinting of total bacterial soil communities was performed using two sets of primers 63f and 518r [31] as described in Caputo et al. [28]. Three PCR samples (200–250 ng) per treatment were loaded on polyacrylamide gels. The DGGE analysis was repeated twice to confirm the pattern. The resulting presence-absence banding pattern of 2019 and 2020 were subjected to one-way non-parametric multivariate analysis of variance (AMOVA).

### *2.5. Statistical Analysis*

Crop production data were analyzed with Statgraphics centurion version 18.1.01 software (Statgraphics Technologies, Inc. The Plains, VA, USA). Strawberry productive and qualitative parameters were subjected to two-way analysis of variance (ANOVA) and, for variables which were significantly different, the mean separation test using Fisher's least significant difference (LSD) procedure was applied. Those analyses were performed after the Levene's test to assess the equality of variances. The fruit quality features were compared between 2019 and 2020 and between the treatments in each of the year using the Kruskal–Wallis test for nonparametric data.

Presence–absence data matrices from DGGE fingerprints were subjected to one-way non-parametric multivariate analysis of variance (npMANOVA) using Euclidean distance measure. In addition, unweighted pair-group average (UPGMA) dendrograms using Euclidean distance were inferred. Multivariate analysis was conducted with PAST vers. 3.24 [32]. PAST was also used to estimate mean and standard deviation of the Chao 2 Diversity index of bacterial communities using bootstrap replicates.

## 3. Results

### *3.1. Original Soil Features*

Original soil at the beginning of the trial was characterised by a SOM content largely above 2% (Table 1); which is considered the limit for fertility in top soils [33]. Moreover, based on mineral nitrogen and Olsed-P supply, mineral nutrients were not a limiting fertility factor.

**Table 1.** Original soil features of strawberry experimental field.

| Soil Texture [1] | SOM (%) | pH | C/N | Mineral N [2] (mg kg$^{-1}$) | Olsen-P (mg kg$^{-1}$) |
|---|---|---|---|---|---|
| silt-loam | 3.3 | 6.9 | 7 | 8 | 28 |

[1] Soil texture classified using the Soil Triangle Hydraulic Properties Calculator [34]. [2] ($NH^{+4}$ + $NO^{-3}$).

### 3.2. Soil Healthevalution by Greenhouse Bio-Assay

Among the vegetative and productive parameters assessed to estimate strawberry growth in the in-pot assay, only the vegetative one (above-ground plant biomass) significantly differed ($p < 0.01$) in heat-treated as compared original soil. Plant biomass of strawberry plants resulted 59% higher in heat-treated as compared original soil; also, ripe fruit weight per plant was higher in heat-treated than original soil (34%), but not in a significant way.

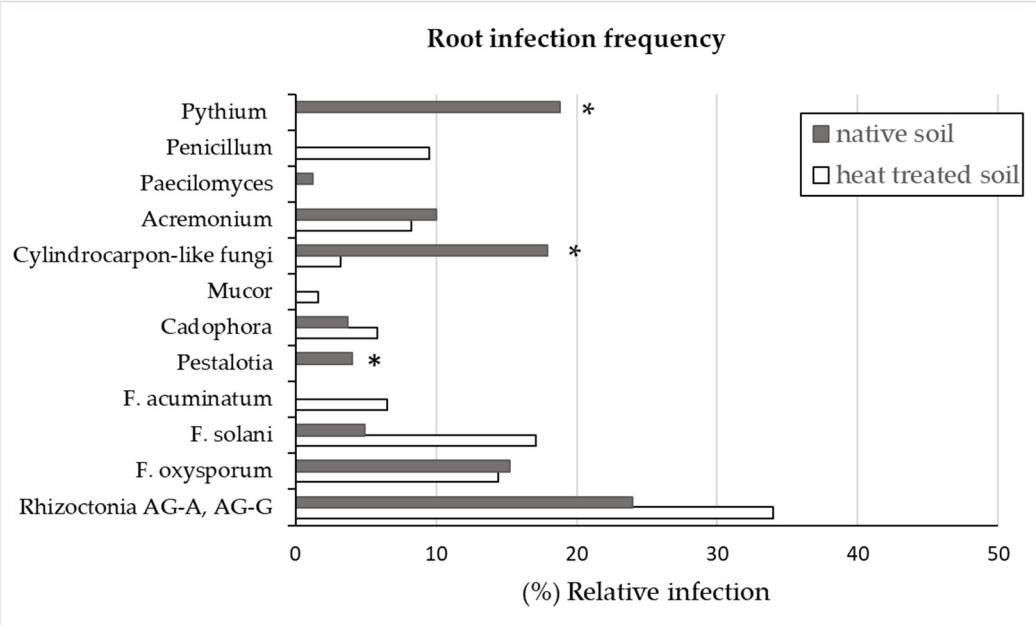
Root colonization frequency by fungal endophytes was higher ($p < 0.05$) in original soil than in heat-treated (accounting for 26 and 18%, respectively). However, the most interesting finding was the correlation between plant growth (estimated in terms of above-ground plant biomass) and root infection frequency by each root colonizing fungal specie. The largest part of fungal species isolated from roots showed a low correlation with plant growth (Table 2) and three species (i.e., two *Fusarium* spp. and *Cadophora* sp.) showed a high positive correlation, which suggested a beneficial relationship with the host plant of those two groups of fungal endophytes (Table 2).

**Table 2.** Functional classification of fungal endophytes based on Pearson correlation (r) values between root infection frequency and plant biomass of strawberry plants grown in the in-pot bioassay of soil health.

| Fungal Species | r | Plant Relationship |
|---|---|---|
| *Rhizoctonia* sp. AG-A & AG-G | 0.24 | Neutral [1] |
| *F. oxysporum* | −0.06 | neutral |
| *F. solani* | 0.64 | beneficial |
| *F. acuminatum* | 0.71 | beneficial |
| *Pestalotia longisetula* | −0.72 | pathogenic |
| *Cadophora* sp. | 0.51 | beneficial |
| *Mucor hiemalis* | 0.34 | neutral |
| *Cylindrocarpon*-like fungi | −0.78 | pathogenic |
| *Acremonium* sp. | 0.03 | neutral |
| *Paecilomyces lilacinus* | −0.34 | neutral |
| *Penicillum* spp | 0.34 | neutral |
| *Pythium* sp. | −0.62 | pathogenic |

[1] Functional classification of fungal species based on Pearson correlation values: from −0.5 to 0.5—neutral; ≥0.5—beneficial; <−0.5—pathogenic.

Conversely, *Pythium* sp., *Cylindrocarpon*-like fungi (which were mainly represented by *Dactylonectria torresensis)* and *Pestalotia longisetula* showed a highly negative Pearson correlation with plant growth. These findings suggest the pathogenicity role of the latter fungal species, shown in Figure 1, from which it is possible to observe a dramatic reduction of root infection by those pathogens in strawberry plants grown in heat-treated soil (Figure 1).




* Pathogenic

**Figure 1.** Fungal root endophytes in strawberry plants grown on original and heat-treated soil in the in-pot test carried out for preliminary evaluation of the soil health status in the field selected for this trial. Full name of fungi is reported in Table 2.

### *3.3. Strewberry Yield in Response to Soil Treatment in Pre-Plantof Field Trial*

#### 3.3.1. Strawberry Yield Response

Marketable yield was the only strawberry parameter significantly affected by pre-plant treatments in this trial. It did not differ between years (Table 3), but it differed significantly between treatments ($p < 0.001$) in both years. However, as suggested by the significant interaction between the two factors of variability (year × treatment, Table 3), the strawberry yield response to the treatments varied over the years. Digestate of liquid manure never increases strawberry production as compared to untreated control over the years (Figure 2). Compost from OFMSW gave a dramatic yield reduction (−41%) as compared to control in 2019 (Figure 2, 2019); whilst it gave the best production performance in 2020, when it showed a significant yield improvement compared to the untreated control, with yield values similar to those observed for Dazomet (Figure 2, 2020). The yield response to Compost from OFMSW in 2019 was conditioned by the severe phytotoxic effect observed immediately after transplant, in September 2018, and during the growing stage in spring 2019 (Figure 3). Finally, soil treatment with Dazomet in pre-plant gave the best strawberry yield across both the years of trial (Figure 2, 2019 and 2020). Fruit quality traits such as acidity, sugars, firmness and fruit external color differ significantly between the year; whilst they did not fifer significantly between treatments in any of the two years of the trial (Table 4).

#### 3.3.2. Correlation between Crop Yield and Quantitative Microbial Changes in Soil

*Pseudomonas* quantity in rhizosphere was not correlated with strawberry yield (r: −0.07 and 0.18 in 2019 and 2020, respectively). On the contrary, *D. torresensis* overall resulted negatively correlated with strawberry yield, thus confirming its role of pathogen as expected based on the preliminary test (Table 2). In 2019, the correlation between yield and *Cylindrocarpon*-like fungi was null, but net of the anomalous value of Compost treatment showing phytotoxic effect (Figure 2, 2019 and Figure 3). *Cylindrocarpon*-like fungi (*D. torresensis*) negatively correlated with marketable yield (r: −0.43). A significant yield increase in plots treated in pre-plant with Dazomet and Compost was observed in

2020, when the highest (r: −0.95) negative correlation between yield and *D. torresensis* was recorded (Figure 2, 2020). The latter finding supported the hypothesis that Compost was able to suppress *Cylindrocarpon*-like fungi during the second growing cycle.

**Table 3.** Two-way ANOVA of strawberry production per plant (fruit weight) in two growing cycles of the crop (2019 and 2020). Factor of variability: Year (Y) and Treatment (T).

| Factors | DF | *p*-Value |
|---|---|---|
| Year (2019–2020) | 1 | Ns [1] |
| Treatment | 3 | 0.0007 |
| Y × T | 3 | 0.0002 |
| **Year** | **count** | **Average (g)** |
| 2019 | 12 | 134.6 [2] A |
| 2020 | 12 | 152.4 A |

[1] not significant. [2] means followed by a different letter differ significantly. according to Fisher's LSD procedure, at a 95% confidence level.

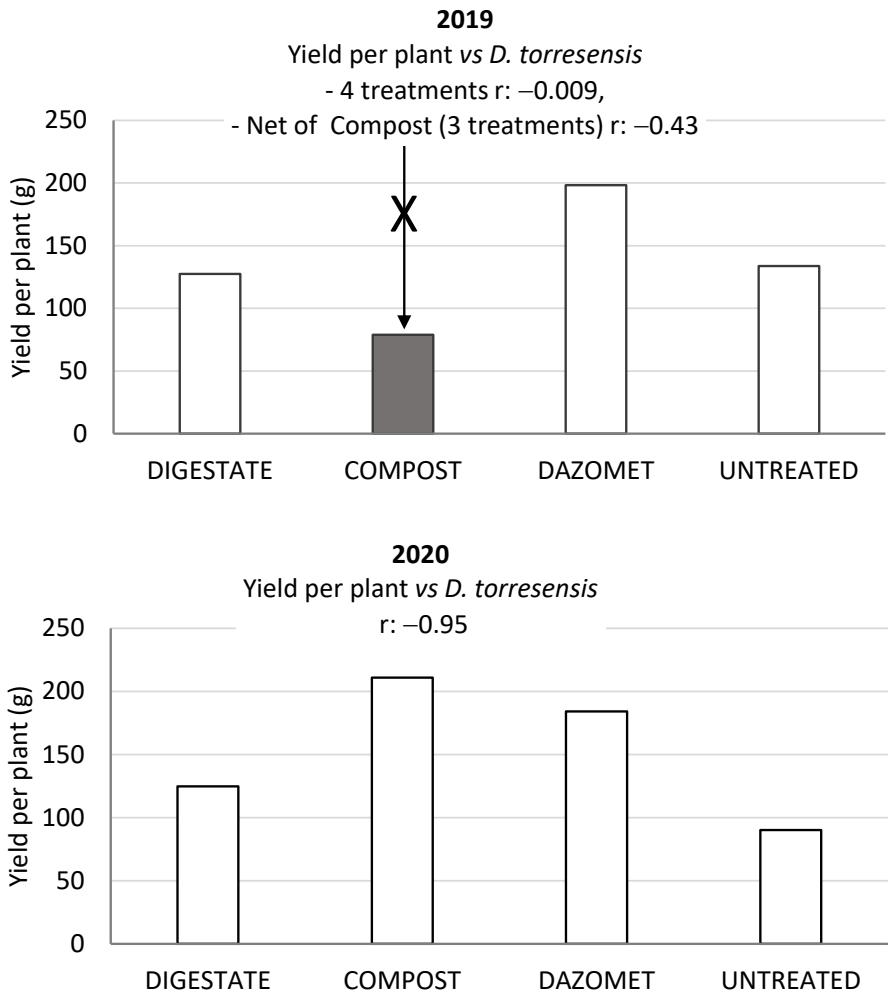



**Figure 2.** Means of strawberry yield per plant (weight) in each of the two growing cycles of the crop (2019 and 2020). r: Pearson correlation between strawberry yield and *D. torresensis* quantity in rhizosphere soil. Treatments: COMPOST from digestate of OFMSW (fraction of municipal solid waste), DIGESTATE from liquid manure, UNTREATED and DAZOMET. Different letters on top of columns indicate significant differences according to Fisher's LSD procedure, at a 95% confidence level.

**Figure 3.** Spring 2019, strawberry vegetative stage at first growing cycle. Plants showing homogeneous vegetative growth reduction in one of three replicates (row below the white arrows) treated with COMPOST from OFMSW in pre-plant.

**Table 4.** Difference in fruit quality features based on the Kruskal–Wallis test for nonparametric data. Below, significance of difference in fruit quality between the two growing cycles (2019 and 2020) and between the four treatments in each of trial years (2019 and 2020).

| | Citric Acid (%) | Sugar (Brix) | Firmness (Durofel Index) | Color Index |
|---|---|---|---|---|
| 2019–2020 | * [1] | *** | *** | *** |
| 2019 | | | | |
| Untreated | 70 | 6.77 | 37.23 | 74.51 |
| Dazomet | 82 | 6.93 | 35.50 | 70.74 |
| Compost | 73 | 6.33 | 34.53 | 79.97 |
| Digestate | 81 | 7.24 | 35.23 | 85.45 |
| Total mean | 77 | 6.82 | 35.63 | 77.67 |
| | ns | ns | ns | ns |
| 2020 | | | | |
| Untreated | 62 | 9.03 | 43.97 | 49.63 |
| Dazomet | 66 | 7.96 | 42.37 | 48.21 |
| Compost | 70 | 8.72 | 41.40 | 49.88 |
| Digestate | 77 | 9.63 | 48.77 | 48.14 |
| Total mean | 69 | 8.83 | 44.13 | 48.97 |
| | ns | ns | ns | ns |

[1] * $p < 0.05$; *** $p < 0.001$; ns not significant.

### 3.3.3. Qualitative Changes of Soil Bacterial Communities

Total bacteria and *Pseudomonas* communities differed significantly between soil treatments in 2019, 13 months after soil treatments, while in 2020, 23 months after soil treatments, they did not (Table 5). UMPGA dendrograms grouped the communities of both total bacteria and *Pseudomonas* according to soil treatments in 2019, which did not occur in 2020 (Figure 4). In addition, in 2019 both bacterial communities showed a larger genetic distance from one another than in 2020. Findings of both AMOVA and UMPGA clustering clearly showed that all soil treatments were able to affect rhizosphere bacterial composition up to 13 months after application. On the contrary, that effect was not recorded in 2020 when bacterial communities in untreated and treated soil showed much lower distance in genetic composition as compared to the previous year (Figure 4).

**Table 5.** One way non parametric multivariate analysis of variance (npMANOVA) of difference in composition of total bacterial and *Pseudomonas* spp. communities between soil treatments.

| | 2019 | 2020 |
|---|---|---|
| Total bacteria | ** | ns |
| *Pseudmonas* spp. | * | ns |

** $p < 0.01$, * $p < 0.05$; ns: not significant.

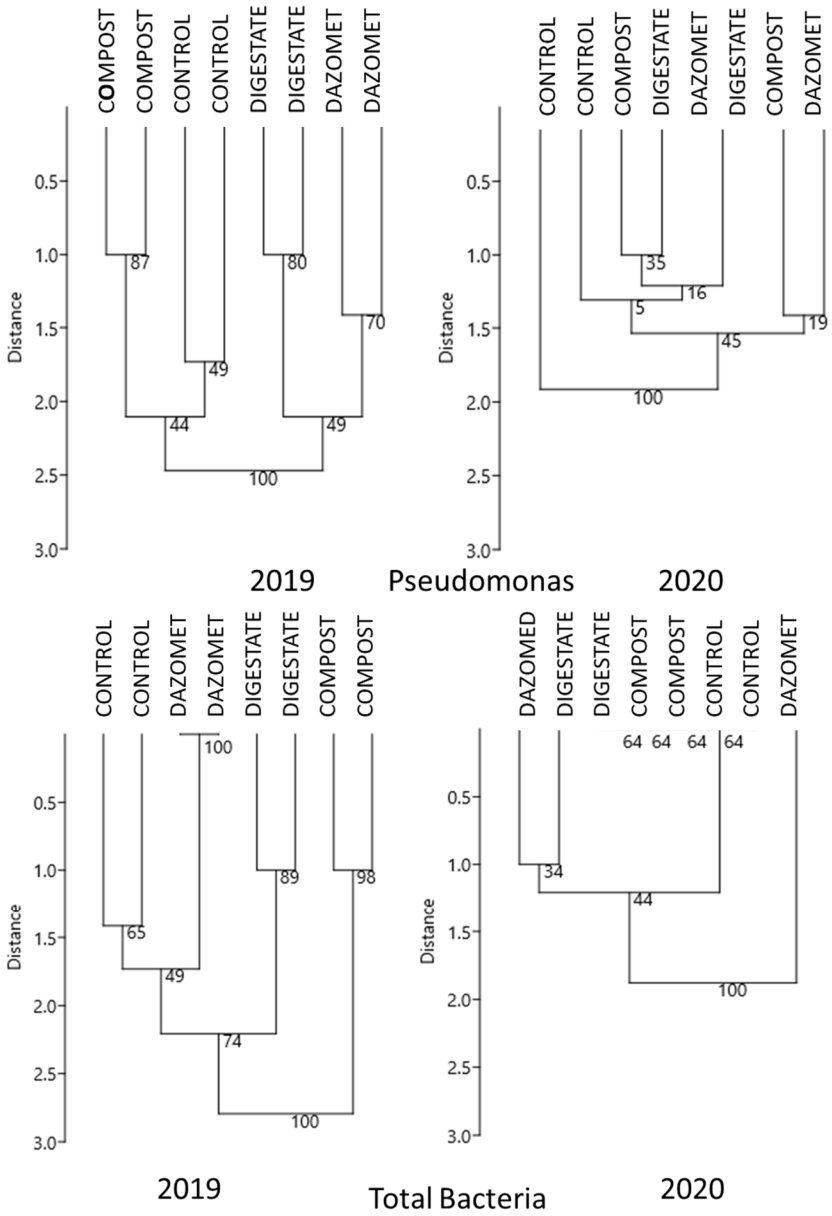



**Figure 4.** Paired Groups (UMPGA) of total bacteria and *Pseudomonas* composition in the rhizosphere of strawberry plants in the first (2019) and second year (2020) during ripening stage. Two replicates per treatments were considered using Euclidean distance. Bootstrapping 1000.

Chao 2 diversity of total bacteria and Pseudonomonas communities intreated soils (Compost, Digestate and Dazomet) decreased from the first to the second year, though only *Pseudomonas* diversity differed significantly between 2019 and 2020 (Figure 5). Interestingly, total bacteria and *Pseudomonas* communities in untreated controls did not differ in the Chao2 diversity index between 2019 and 2020. The findings suggested that soil treatments in pre-plant generally increased bacterial diversity up to the end of the first strawberry

crop cycle, i.e., 13–14 months after soil treatment (2019); while bacterial diversity evolved towards the lower values (Chao 2 values around 8.4) of the native soils.

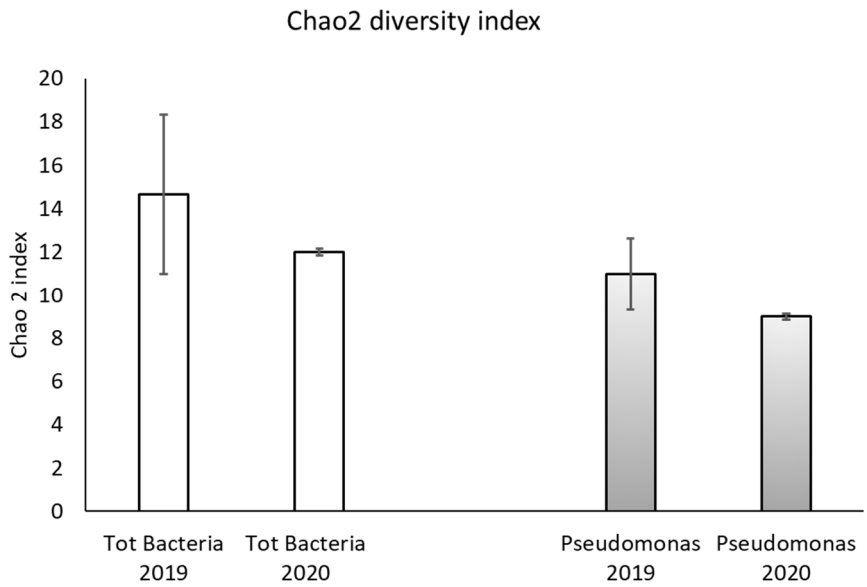



**Figure 5.** Mean Chao 2 diversity index of total bacteria and *Pseudomonas* spp. communities in treated soils (DAZOMED, COMPOST, DIGESTATE). Controls, not included in this comparison, did not differ in Chao2 diversity index between 2019 and 2020 accounting for a lower value (about 8.4). Bars represent standard deviation inferred with bootstrap replicates.

## 4. Discussion

Findings of this study supported effectiveness of waste organic materials such as digestates as organic amendment for increasing the natural ability of soil to control soil borne pathogens. This is consistent with the current great interest in recycling organic wastes to improve the soil quality on farmlands [35]. However, despite the limited number of digestates tested in this study, the already known issues concerning the use of these materials in agriculture were highlighted. They were: phytotoxicity or simply plant development inhibition in the short period after digestate incorporation into the soil and large variability of the effectiveness of increasing soil suppressiveness toward the complex of fungal pathogens responsible for root development reduction.

The highest strawberry production in soil treated with chemical fumigant was consistent with the results of the preliminary evaluation of the soil health status which gave an increase of 51% in plant growth in sanitized soil as compared to native soil. Furthermore, the highest strawberry field performance with Dazomet confirmed the role of root rot fungal pathogens that had accumulated in the soil following the frequent return of strawberry on the same field for decades. Finally, in line with the preliminary in pot assay and with literature [36–38], *D. torresensis* (*Cylindrocarpon*-like fungi) resulted one of the main fungal pathogens associated with yield reduction in strawberry.

Compost from anaerobic digestate of organic fraction of municipal solid waste (OFMSW), gave severe growth reduction in the first growing cycle (2019), but it resulted the best soil treatment along with Dazomet in 2020. That yield improvement in 2020 was mainly related to the lower inoculum of *Cylindrocarpon*-like fungi in those two treatments. Besides the negative correlation between strawberry yield and *D. torresensis* over the two growing cycles, the major role of the root fungal pathogens on yield losses was consistent with the no-limiting nutrient content for the crop. Indeed, in addition to a good endowment of organic matter and mineral nutrients on native soil, strawberry crop had supply of N-P-K through fertigation in the most demanding crop phases.

The above argument might, therefore, provide an explanation for the low effectiveness of Digestate from liquid manure. Although Digestate added to the soil the same N rate of

Compost, strawberry yield in this treatment never differed from untreated control. The latter finding suggests that microbial factors rather than nutrient availability acted as plant growth promoters in the Compost treatment during the second growing cycle (2020), after the initial toxic effect was overcome. Part of those microbial factors were elucidated; molecular investigations into bacterial communities showed that pre-plant soil treatments acted as disturbance on soil-resident communities by increasing diversity, whilst quantity of functional populations such ad *Pseudomonas* were not affected. Therefore, microbial disturbance may be one of the effects of soil treatment with waste derived materials such as digestates. However, the microbial changes observed in all treatments resulted beneficial only in Dazomet and in Compost. The effectiveness of the chemical treatment was probably due mainly to the reduction of *Cylindrocarpon* inoculum. However, it is well known that any type of sterilization (chemical or physical) of intensively cultivated soils reduces soil borne pathogens and promotes re-colonization by indigenous communities with a soil restoration-like effect [25,39]. Conversely, the notable increase of strawberry yield in the Compost treatment may be explained primarily by the bacterial opportunities induced by this treatment. Undoubtedly, the impact of this organic waste on soil deeply differed from the null impact on crop yield by Digestate. On the other hand, Compost caused a dramatic growth reduction of strawberry plants in the first growing cycle, suggesting a strong impact on chemical and microbial soil properties in the short period. Precisely that soil disturbance seems have positively modified microbial communities in the medium period, leading to an improved suppressiveness towards soil borne pathogens during the second growing cycle. Supporting this observation, *D. torresensis* inoculum appeared highly reduced in Compost treated soil.

Indeed, one of the main items of the European Green Deal to reach climate neutrality within 2050 is restoring biodiversity and reducing pollution. As far as the latter item, anaerobic digestion of liquid manure for biogas production represented since early 2000 a solution to the limits imposed by the European Nitrate Directive 91/676/CEE [40] and further updates to reduce the nitrate pollution in the European areas of intensive agricultural and livestock activity.

Differences in disease suppressiveness exerted by organic amendments of different origin and chemical features have been often related to a different impact on microbial populations [23,41–43]. The current challenge is using digestates and other organic waste as soil amendment aiming at harnessing the potential of indigenous soil microbes. However, the microbial populations with greater capacity to increase soil suppressiveness have not been identified so far due to the variability of agro-environments in which encouraging results have been obtained and the complexity of the mechanisms involved in the relationship between plant and microorganisms. The null correlation between *Pseudomonas* and strawberry yield in this study is an example of this. In fact, *Pseudomonas* spp. have often been identified as important beneficial components of soil suppressiveness [44], especially towards the opportunistic pathogens accumulated with monoculture, so much so such bacterial group has been often suggested as an indicator of soil health [45,46]. On the other hand, chemical and microbiological properties of the suppressive composts may differ substantially, and measurements of microbial populations and activity have not been so far predictive of the level of disease suppression in all composts [43].

A recent survey has shown that several farmlands close to biogas plants, which had been repeatedly amended with digestate in the last decade, showed improved soil suppressiveness towards the root rot agent of maize in an in-pot test at the same condition. That effect was linked to a series of microbial changes able to reduce root infection by fungal pathogens in maize. Those microbial changes varied amongst experimental sites suggesting that many mechanisms were involved in suppressiveness towards root pathogens and that they differed between sites [47].

## 5. Conclusions

Findings of this study, once again, showed that organic wastes are promising for increasing soil fertility. Organic waste input is interesting not only for the possibility to increase N availability for plants and soil organic matter content in the medium-long period [48], but also for its ability to induce beneficial microbial changes in the short period. The latter property become particularly interesting in soils affected by crop decline linked to microbial diversity decline caused by monocropping or intensive cropping systems.

Linking chemical properties of composts with beneficial microbial changes induced by organic waste as way to improve soil suppressiveness is the current challenge. The number of studies into recycling organic wastes in the form of fertilizer or organic amendment has been increasing in response to the European Waste Framework Directive of 2008 [49]. Many of these deal with the efficient use of digestates from anaerobic production of biogas [22,47,50] because they represent the last ideal segment of circular economy. The large number of case studies performed so far show variable advantages for crop health with great difference even between digestates of the same origin and in the same cropping system, such as in this study. Therefore, meta-analysis could at this point help to identify some factors useful for setting a digestate-use strategy aimed at increasing soil suppressiveness.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/su13073964/s1, Table S1: Chemical composition of Digestate (Digestate of liquid manure) and Compost (Compost from OFMSW) treatments.

**Author Contributions:** Conceptualization, L.M.M. and M.K.; data curation, S.S.; formal analysis, L.M.M.; field investigation, S.S., and M.Z.; methodology in molecular analysis, F.C.; supervision, M.Z. and M.K.; writing—original draft, L.M.M.; writing—review and editing, S.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Italian Ministry of Agriculture, Food and Forest Policy (MiPAAF) under the project AGROENER (D.D. n. 26329, 1 April 2016) http://agroener.crea.gov.it/ accessed on 15 March 2021.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** The authors would like to extend a special thanks to Martina Boschiero and Martin Thalheimer for their input and support in the early stages of developing this research.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Broeckling, C.D.; Broz, A.K.; Bergelson, J.; Manter, D.K.; Vivanco, J.M. Root exudates regulate soil fungal community composition and diversity. *Appl. Environ. Microbiol.* **2008**, *74*, 738–744. [CrossRef] [PubMed]
2. Eisenhauer, N.; Lanoue, A.; Strecker, T.; Scheu, S.; Steinauer, K.; Thakur, M.P.; Mommer, L. Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Sci. Rep.* **2017**, *7*, 44641. [CrossRef] [PubMed]
3. Lemanceau, P.; Maron, P.A.; Mazurier, S.; Mougel, C.; Pivato, B.; Plassart, P.; Ranjard, L.; Revellin, C.; Tardy, V.; Wipf, D. Understanding and managing soil biodiversity: A major challenge in agroecology. *Agron. Sustain. Dev.* **2014**, *35*, 67–81. [CrossRef]
4. Panth, M.; Hassler, S.C.; Baysal-Gurel, F. Methods for Management of Soilborne Diseases in Crop Production. *Agriculture* **2020**, *10*, 16. [CrossRef]
5. Koike, S.T.; Gordon, T.R.; Daugovish, O.; Ajwa, H.; Bolda, M.; Subbarao, K. Recent Developments on Strawberry Plant Collapse Problems in California Caused by *Fusarium* and *Macrophomina*. *Int. J. Fruit Sci.* **2013**, *13*, 76–83. [CrossRef]
6. López-Medina, J.; López-Aranda, J.M.; Medina, J.J.; Miranda, L.; Flores, F. Chemical and non-chemical alternatives to methyl bromide fumigation of soil for strawberry production. *J. Hortic. Sci. Biotechnol.* **2003**, *78*, 597–604. [CrossRef]
7. Pinkerton, J.N.; Ivors, K.L.; Reeser, P.W.; Bristow, P.R.; Windom, G.E. The use of soil solarization for the management of soilborne plant pathogens in strawberry and red raspberry production. *Plant Dis.* **2002**, *86*, 645–651. [CrossRef]
8. Shennan, C.; Muramoto, J.; Koike, S.; Baird, G.; Fennimore, S.; Samtani, J.; Bolda, M.; Dara, S.; Daugovish, O.; Lazarovits, G.; et al. Anaerobic soil disinfestation is an alternative to soil fumigation for control of some soilborne pathogens in strawberry production. *Plant Pathol.* **2018**, *67*, 51–66. [CrossRef]

9. Camprubí, A.; Estaún, V.; Bakali, M.A.; Garcia-Figueres, F.; Calvet, C. Alternative strawberry production using solarization, metham sodium and beneficial soil microbes as plant protection methods. *Agron. Sustain. Dev.* **2007**, *27*, 179–184. [CrossRef]
10. You, M.P.; Sivasithamparam, K. Changes in microbial populations of an avocado plantation mulch suppressive of Phytophthora cinnamomi. *Appl. Soil Ecol.* **1995**, *2*, 33–43. [CrossRef]
11. Mazzola, M. Mechanisms of natural soil suppressiveness to soilborne diseases. *Antonie Van Leeuwenhoek* **2002**, *81*, 557–564. [CrossRef] [PubMed]
12. Bongiorno, G.; Postma, J.; Bünemann, E.K.; Brussaard, L.; de Goede, R.G.M.; Mäder, P.; Tamm, L.; Thuerig, B. Soil suppressiveness to *Pythium ultimum* in ten European long-term field experiments and its relation with soil parameters. *Soil Biol. Biochem.* **2019**, *133*, 174–187. [CrossRef]
13. Bonilla, N.; Cazorla, F.M.; Martínez-Alonso, M.; Hermoso, J.M.; González-Fernández, J.J.; Gaju, N.; Landa, B.B.; de Vicente, A. Organic amendments and land management affect bacterial community composition, diversity and biomass in avocado crop soils. *Plant Soil* **2012**, *357*, 215–226. [CrossRef]
14. Franke-Whittle, I.H.; Juárez, M.F.D.; Insam, H.; Schweizer, S.; Naef, A.; Topp, A.R.; Kelderer, M.; Rühmer, T.; Baab, G.; Henfrey, J.; et al. Performance evaluation of locally available composts to reduce replant disease in apple orchards of central Europe. *Renew. Agric. Food Syst.* **2020**, *34*, 543–557. [CrossRef]
15. Wang, S.Y.; Lin, S.S. Composts as soil supplement enhanced plant growth and fruit quality of strawberry. *J. Plant Nutr.* **2002**, *25*, 2243–2259. [CrossRef]
16. Lloyd, M.; Kluepfel, D.; Gordon, T. Evaluation of Four Commercial Composts on Strawberry Plant Productivity and Soil Characteristics in California. *Int. J. Fruit Sci.* **2016**, *16*, 84–107. [CrossRef]
17. Millner, P.D.; Ringer, C.E.; Maas, J.L. Suppression of strawberry root disease with animal manure composts. *Compost Sci. Util.* **2004**, *12*, 298–307. [CrossRef]
18. Fang, X.; You, M.P.; Barbetti, M.J. Reduced severity and impact of Fusarium wilt on strawberry by manipulation of soil pH, soil organic amendments and crop rotation. *Artic. Eur. J. Plant Pathol.* **2012**, *134*, 619–629. [CrossRef]
19. Fagerström, A.; Seadi, A.; Rasi, T.; Briseid, S. *The Role of Anaerobic Digestion and Biogas in the Circular Economy*; IEA Bioenergy: Cork, Ireland, 2018; Volume 37, ISBN 978-1-910154-46-5.
20. European Union. *Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the Promotion of the Use of Energy from Renewable Sources and Amending and Subsequently Repealing Directives 2001/77/EC and 2003/30/EC*; European Commission: Bruxelles, Belgium, 2009.
21. Barzee, T.J.; Edalati, A.; El-Mashad, H.; Wang, D.; Scow, K.; Zhang, R. Digestate biofertilizers support similar or higher tomato yields and quality than mineral fertilizer in a subsurface drip fertigation system. *Front. Sustain. Food Syst.* **2019**, *3*, 58. [CrossRef]
22. Tambone, F.; Scaglia, B.; D'Imporzano, G.; Schievano, A.; Orzi, V.; Salati, S.; Adani, F. Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. *Chemosphere* **2010**, *81*, 577–583. [CrossRef]
23. Podmirseg, S.M.; Waldhuber, S.; Knapp, B.A.; Insam, H.; Goberna, M. Robustness of the autochthonous microbial soil community after amendment of cattle manure or its digestate. *Biol. Fertil. Soils* **2019**, *55*, 565–576. [CrossRef]
24. Rubel, F.; Brugger, K.; Haslinger, K.; Auer, I. The climate of the European Alps: Shift of very high resolution Köppen-Geiger climate zones 1800–2100. *Meteorol. Z.* **2017**, *26*, 115–125. [CrossRef]
25. Manici, L.M.; Caputo, F.; Rossi, A.; Topp, A.R.; Zago, M.; Kelderer, M. Thermal disturbance of fertile soils to search for new biological control options in strawberry crops affected by yield decline. *Biol. Control* **2018**, *126*, 65–73. [CrossRef]
26. Hoffmann, G. *Methodenbuch, Band 1, Die Untersuchung von Böden, vierte Auflage,1. Teillieferung, 2. Teillieferung 1997*; VDLUFA-Verlag: Darmstadt, Germany, 1997.
27. Tessmer, M.A.; Appezzato-da-Glória, B.; Antoniolli, L.R. Influence of growing sites and physicochemical features on the incidence of lenticel breakdown in "Gala" and "Galaxy" apples. *Sci. Hortic.* **2016**, *205*, 119–126. [CrossRef]
28. Caputo, F.; Nicoletti, F.; De Luca Picione, F.; Manici, L.M. Rhizospheric changes of fungal and bacterial communities in relation to soil health of multi-generation apple orchards. *Biol. Control* **2015**, *88*, 8–17. [CrossRef]
29. Alaniz, S.; León, M.; Vicent, A.; García-Jiménez, J.; Abad-Campos, P.; Armengol, J. Characterization of Cylindrocarpon species associated with black foot disease of grapevine in Spain. *Plant Dis.* **2007**, *91*, 1187–1193. [CrossRef] [PubMed]
30. Widmer, F.; Seidler, R.J.; Gillevet, P.M.; Watrud, L.S.; Di Giovanni, G.D. A highly selective PCR protocol for detecting 16S rRNA genes of the genus Pseudomonas (sensu stricto) in environmental samples. *Appl. Environ. Microbiol.* **1998**, *64*, 2545–2553. [CrossRef]
31. El Fantroussi, S.; Verschuere, L.; Verstraete, W.; Top, E.M. Effect of phenylurea herbicides on soil microbial communities estimated by analysis of 16S rRNA gene fingerprints and community-level physiological profiles. *Appl. Environ. Microbiol.* **1999**, *65*, 982–988. [CrossRef] [PubMed]
32. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol. Electron.* **2001**, *4*, 1–9. [CrossRef]
33. Zdruli, P.; Jones, R.J.A.; Montanarella, L. *Organic Matter in the Soils of Southern Europe*; Office for Official Publications of the European Communities: Luxembourg, 2004; Volumw EUR 21083.
34. Saxton, K.E.; Rawls, W.J.; Romberger, J.S.; Papendick, R.I. Estimating Generalized Soil-water Characteristics from Texture1. *Soil Sci. Soc. Am. J.* **1986**, *50*, 1031. [CrossRef]

35. Alvarenga, P.; Palma, P.; Mourinha, C.; Farto, M.; Dôres, J.; Patanita, M.; Cunha-Queda, C.; Natal-da-Luz, T.; Renaud, M.; Sousa, .P. Recycling organic wastes to agricultural land as a way to improve its quality: A field study to evaluate benefits and risks. *Waste Manag.* **2017**, *61*, 582–592. [CrossRef] [PubMed]
36. Manici, L.M.; Caputo, F.; Baruzzi, G. Additional experiences to elucidate the microbial component of soil suppressiveness towards strawberry black root rot complex. *Ann. Appl. Biol.* **2005**, *146*, 421–431. [CrossRef]
37. Sung, J.-M.Y.-N.S.-S. Identification of Cylindrocarpon destructans Associated with Root Rot Disease of Strawberry. *Korean J. Mycol.* **1985**, *13*, 179–183.
38. Weber, R.W.S.; Entrop, A.P. Dactylonectria torresensis als wichtigste Komponente der Schwarzen Wurzelfäule an Erdbeeren und Himbeeren in Norddeutschland. *Erwerbs-Obstbau* **2017**, *59*, 157–169. [CrossRef]
39. Van der Voort, M.; Kempenaar, M.; van Driel, M.; Raaijmakers, J.M.; Mendes, R. Impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression. *Ecol. Lett.* **2016**, *19*, 375–382. [CrossRef] [PubMed]
40. The Council of European Communities. *Council Directive of 12 December 1991 Concerning the Protection of Waters against Pollution Caused by Nitrates from Agricultural Sources (91/676/EEC)*; European Commission: Bruxelles, Belgium, 1991.
41. De Corato, U. Soil microbiome manipulation gives new insights in plant disease-suppressive soils from the perspective of a circular economy: A critical review. *Sustainability* **2021**, *13*, 1688. [CrossRef]
42. Pérez-Piqueres, A.; Edel-Hermann, V.; Alabouvette, C.; Steinberg, C. Response of soil microbial communities to compost amendments. *Soil Biol. Biochem.* **2006**, *38*, 460–470. [CrossRef]
43. Craft, C.M.; Nelson, E.B. Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass caused by Pythium graminicola. *Appl. Environ. Microbiol.* **1996**, *62*, 1550–1557. [CrossRef] [PubMed]
44. Expósito, R.G.; de Bruijn, I.; Postma, J.; Raaijmakers, J.M. Current insights into the role of Rhizosphere bacteria in disease suppressive soils. *Front. Microbiol.* **2017**, *8*, 2529. [CrossRef]
45. De Souza, J.T.; Weller, D.M.; Raaijmakers, J.M. Frequency, diversity, and activity of 2,4-diacetylphloroglucinol-producing fluorescent Pseudomonas spp. in Dutch take-all decline soils. *Phytopathology* **2003**, *93*, 54–63. [CrossRef] [PubMed]
46. Chin-A-Woeng, T.F.C.; Bloemberg, G.V.; Lugtenberg, B.J.J. Phenazines and their role in biocontrol by Pseudomonas bacteria. *New Phytol.* **2003**, *157*, 503–523. [CrossRef]
47. Manici, L.M.; Caputo, F.; Cappelli, G.A.; Ceotto, E. Can repeated soil amendment with biogas digestates increase soil suppressiveness toward non-specific soil-borne pathogens in agricultural lands? *Renew. Agric. Food Syst.* **2020**, 1–12. [CrossRef]
48. Barłóg, P.; Hlisnikovský, L.; Kunzová, E. Effect of Digestate on Soil Organic Carbon and Plant-Available Nutrient Content Compared to Cattle Slurry and Mineral Fertilization. *Agronomy* **2020**, *10*, 379. [CrossRef]
49. UEE Directive 2008/98/EC on Waste and Repealing Certain Directives. Available online: https://www.bmu.de/en/law/directive-200898ec-on-waste-and-repealing-certain-directives/ (accessed on 9 February 2021).
50. Nkoa, R. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: A review. *Agron. Sustain. Dev.* **2014**, *34*, 473–492. [CrossRef]