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Maltose and Totally Impermeable Film Enhanced Suppression of Anaerobic Soil Disinfestation on Soilborne Pathogens and Increased Strawberry Yield

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Abstract: Anaerobic soil disinfestation (ASD) is widely used to control soilborne diseases in organic crop production. The effect of ASD used different sealed films on soilborne pathogens and strawberry growth was evaluated in two laboratory studies and two field trials. Under maltose as carbon sources, 28 °C temperature and 30% of soil moisture optimal conditions ASD decreased *Fusarium* spp. and *Phytophthora* spp. by 100%. ASD used maltose as an organic amendment and sealed with totally impermeable film (TIF) obtained the highest suppression (>96%) against *Fusarium* spp. and *Phytophthora* spp. (>91%). According to the laboratory results, the efficacy of ASD utilizing 6 or 9 t/ha maltose and sealing with TIF was evaluated and compared with reference treatment with chloropicrin (Pic) or solarization (Sol) in the field trials. Compared with the untreated soil, ASD treatments greatly reduced the pathogenic population of *Fusarium* spp. and *Phytophthora* spp., and successfully controlled the damage of fusarium wilt with evidence of lower mortality (6%). ASD significantly increased soil nutrition promoted plant growth and increased strawberry yield, which was similar as the Pic, but better than Sol treatment. The analyzed fungal and bacterial microbiota did not show significant differences in the taxonomic richness and diversity between the compared treatments. Nevertheless, the abundance of some bacterial and fungal taxa tended to change between treated. The evidence showed that adding maltose and sealing TIF for ASD has the potential to replace Pic for pathogen control in commercial strawberry production.

Keywords: anaerobic soil disinfestation; sealing tarps; soil physicochemical properties; soil bacterial and fungal community; strawberry fruit yield

1. Introduction

With the rapid development of the strawberry (*Fragaria* × *ananassa*) industry, China had 133,685 ha of land planted strawberries which was more than 25% of the world's strawberry production area in 2017 [1]. Attractive profits to strawberry producers have encouraged further production.

The continuous strawberry cultivation on the same land over many years has increased the prevalence of soilborne diseases caused by *Phytophthora* spp. and *Fusarium* spp., which now limit further expansion of the strawberry industry [2,3]. Fusarium wilt, a particularly serious strawberry soilborne disease caused by *Fusarium oxysporum f.* sp. *fragariae*, is present in the United States, China and many other countries [4,5]. Soilborne diseases cause more than 30% yield reduction and the lost yield depending on climatic and other conditions [6].

Soil disinfestation with broad-spectrum chemical fumigants such as chloropicrin (Pic), metam sodium (MS) can effectively control soilborne diseases caused by fungi, bacteria, nematodes, weeds and soil pests, leading to improved crop quality and yield [7,8]. However, these fumigants also pose a potential risk to the health of fumigators and the surrounding environment. Chloropicrin (Pic) and 1,3-dichloropropene(1,3-D) are facing increased regulatory pressure in the world. For example, Pic has been banned in Europe, the United States request barrier films and has expanded buffer zones with Pic application, while in China it is being phase-out [9,10]. In addition, they can also impact the microbial diversity of the soil, including beneficial microorganisms [8,10]. By modifying the microbial populations, these fumigants could interfere with the soil nitrogen cycle including mineralization [11] and nitrification reduction [12], which in turn affects crop fertilization, quality and yield [13]. These negative side-effects have encouraged the search for alternatives that growers can use to control soilborne diseases.

One alternative is anaerobic soil disinfestation (ASD) which is also known as bio-fumigation or no-chemical fumigant technology [14]. ASD works by mixing organic amendment into the soil, saturating the soil with water, and covering it with a plastic film to maintain anaerobic conditions for 3–5 weeks. ASD kills bacteria, oomycetes, fungi, nematodes and weeds in soil [15]. ASD has been tested on tomato, cucumber and strawberry crops in the United States, Japan, China. and the Netherlands [16].

Shennan et al. (2009) first evaluated ASD in California strawberries. ASD showed great promise for use in the control of soilborne pathogens and pests [15,17]. They identified several challenges to adopting the technique on a commercial scale, including optimum incorporation of the carbon source into the soil and the addition of sufficient water to generate the anaerobic conditions required to induce disease suppression [17]. The variety of materials and options for ASD applications, including carbon sources, type and temperature of soil and plastic tarp type, influence the efficacy of pathogen suppression and disease control [18,19]. The control effect of ASD on the target is very different, the effect on the pathogenic bacteria, nematode and weed were between 59–94%, 15–56% and 32–81%, respectively [14]. Higher amendment rates lead to higher suppression of the tomato or strawberry pathogens [15,20]. Covering the surface of the soil with a plastic film when using soil fumigation is typically a method to guarantee the efficacy and reduce the air exchange [21]. ASD with rice bran alone or preceded by incorporation of a Sudangrass cover crop and followed by drip application of molasses showed the effect on *Fusarium* and *Pythium ultimum* [22]. Plastic film on the surface of the soil serves several purposes, including optimum hydrothermal soil conditions, weed control, improvement of soil temperature [23,24]. Plastic film type such as polyethylene (PE) or totally impermeable film (TIF) showed the different efficacy on reduction emission rate of chemical fumigant (Telone C35) [25]. TIF treatments showed a better effect on the nematode with better reduction of fumigant emissions and saved 30% fumigant consumption [26].

ASD's mode of action is not fully understood. The main hypotheses include: (1) Metal ion generation, lower soil pH, lower oxidation–reduction and lower redox potential [14,25]; (2) Sterilization process productions such as volatile organic acids or fermentation acids and gas production will shift bactericidal and fungi community in the soil [3,17]; (3) Modification of the microbial species diversity and abundance. For example, ASD can change the soil bacteria microbial function such as denitrification, nitrogen fixation and the production of organic acids by fermentation [27].

The constituent factors of soil are very complicated. The relationship between soil microbial communities and soilborne diseases has been explained by many scientists, and the occurrence of

soilborne diseases is directly related to the rhizosphere microbial communities [28,29]. The study found that long-term continuous crops have formed 'fungal' soil, and the proportion of pathogenic fungi in the soil has increased, which is more likely to cause soilborne diseases [30]. At the same time, the structure and quantity of plant rhizosphere microbial communities have competition, parasitic and antagonistic relationships with the utilize of rhizosphere secretions. For example, in a healthy soil environment, the number of beneficial microorganisms such as *Trichoderma, Pseudomonas* and *Bacillus* will increase, and the growth of these beneficial microorganisms will inhibit the activity of pathogenic, thereby reducing the occurrence of soilborne diseases [28].

This is particularly important to evaluate the alternatives to Pic in the sustainable development of the strawberry production system. ASD as a feasible alternative has strong potential to be used in China. However, the potential effects in combination with optimizing impact factors such as carbon source and covering film are less well studied.

The specific objectives of this study were (1) to determine the optimal experimental conditions with orthogonal experimental design in the lab, and (2) to reveal the suitability of the films for ASD, and (3) to evaluate the efficacy of maltose as a carbon source when sealed with TIF on ASD control of soilborne diseases and assay the responses of strawberry yield performance, soil physical and chemical changes and soil bacterial and fungal microbial communities shift after fumigation.

2. Materials and Methods

2.1. Experiment Design in Laboratory

2.1.1. Soil Sample Collection

Soil samples (0–20 cm cultivated layer) were collected during the strawberry harvest in Beijing's Changping district (N40°12′27.74′′ N, 116°24′25.02′′ E). The soil was sandy loam (62% sand, 36% silt and 2% clay) with pH of 7.6. Soil organic matter, NH_4^+-N, NO_3^--N, P and K were 3.7, 0.8, 5.3, 48.2, 38.5 mg·kg^{-1} soil, respectively. The farm was continuously planting strawberries for more than 17 years and fusarium wilt and verticillium wilt infect the crops severely. In the lab, the soil was filtered through a 2 mm sieve and stored at 4 °C for later experiments.

2.1.2. Orthogonal Experimental Design

Aim to find the best treatment combinations, we designed orthogonal experiments to obtain the best efficacy of soil disinfestation. The experiments were based on an orthogonal design L27 (3^{13}) to study three factors: (A) air temperature (14 °C, 20 °C, 28 °C), (B) soil moisture (10%, 20%, 30% of soil moisture) and (C) carbon sources (wheat bran, maltose, bio-char) (Table S1). The carbon sources' concentration added in the soil for ASD treatments was selected a 5% (*w/w*) level. We designed the control without organic amendment and with the same level of soil water content to calculate the efficacy reduction rate of the fungi pathogens. After the soil was prepared, 15 g soils were added in a 20-mL headspace vial and sealed with a cap immediately. The soils in the headspace vial were incubated at different temperatures for 21 days. After the incubation, the soils were mixed thoroughly and sampled from each replicate headspace vial. Each soil sample was stored at 4 °C for fungal pathogens isolated. Komada–Fusarium selective media for *Fusarium* spp. [31] and Masago selective media for *Phytophthora* spp. [32] were used to assess soil pathogens. The fungal isolation steps were described as step by step. First, 15 g mixed soil samples were weighed and transferred in an Erlenmeyer flask and 85 mL 0.7‰ sterilized agar water was added with the soil. Then we placed the Erlenmeyer flask on a shaker for 20 min (The rotation speed was 200 rpm). After this step, we prepared the soil suspension. Second, 1 mL of the soil suspension was added into 49 mL of the selected medium. After shaking and mixed completely, the mixture was poured evenly into three 90-mm culture dishes. Third, the fungi were cultured at 28 °C for 3 days, and the colonies of *Fusarium* spp. and *Phytophthora* spp. were counted for data analysis. The average values from the triplicate analyses are displayed

as CFU per gram soil. The effects of these factors and their interaction were evaluated by one-way ANOVA analysis method using SPSS statistical software.

2.1.3. Experimental for Cover Tarps

Pathogen efficacy was evaluated in ASD-fumigated soil amended with 5% (*w/w*) maltose. Mixtures of soil mixed with 5% maltose dose were removed into 550 mL Duran wide-neck glass flasks (Schott AG, Mainz, Germany). The maltose amended soil, or the no amendment soil was adjusted to 20% water content (gravimetric moisture content) for the following experiments. The quantity of the maltose–soil or no amendment soil was 300 g fresh weight for each flask. The flasks were sealed with different films had a randomly design with three replications (Table 1). The soil in the flasks was incubated at 28 °C for 21 days. After the experiments, the soils were mixed thoroughly and sampled from each replicate flask. Each soil sample was stored at 4 °C and isolated as the method described presently for fungal pathogens.

Table 1. Experimental treatments of the laboratory studies for virtually impermeable film (VIF), polyethylene (PE) and totally impermeable film (TIF).

Treatments *	Organic Amendment	Rate (%, *w/w*)	Film Type	Formulated Product Manufacturer
CK	–	–	–	
VIFCK	–	–	0.03 mm VIF	VIF: Guangdong Jinming Machinery Co., Ltd. China
VIFMal	maltose	5	0.03 mm VIF	
PECK	–	–	0.05 mm PE	PE: Shandong Longxin Plastic Industry Co., Ltd. China
PEMal	maltose	5	0.05 mm PE	
TIFCK	–	–	0.05 mm TIF	TIF: Shandong Longxin Plastic Industry Co., Ltd. China
TIFMal	maltose	5	0.05 mm TIF	
WaterCK	–	–	150 mL deionized water	
WaterMal	maltose	5	150 mL deionized water	

* Abbreviations: CK = control, no organic amendment; Mal = maltose.

2.2. Field Trials

2.2.1. Field Trials Design

The research was undertaken in greenhouses from July 2017 to June 2018 in two locations: Trial I (Daxing, 39°41′27.21″ N, 116°36′30.30″ E) and Trial II (Changping, 40°12′24.14″ N, 116°25′43.21″ E) in the district of Beijing, China. Trial I (Greenhouse A) and Trial II (Greenhouses B and C) had produced strawberries for more than 5 and 15 years, respectively. The greenhouses had no chemical fumigation of the two trial sites in 2016. The numbers of *Fusarium* spp. and *Phytophthora* spp. in the soil before disinfestation in two trial sites were higher than 7000 and 6000 CFU·g^{-1}, respectively. Both greenhouses had silty loam soil (Trial I: 57% sand, 38% silt and 5% clay; Trial II: 60% sand, 37% silt and 3% clay). Further details of the greenhouse trials are provided in Tables 2 and 3.

Table 2. Soil characteristics at the experimental sites.

Site	pH (1:2.5)	Organic Matter Content (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Available Phosphorus (mg kg^{-1})	Available Potassium (mg kg^{-1})	Oxidation-Reduction (mv)	Electrical Conductivity (μs cm^{-1})
Trial I	7.83	13.00	6.61	144.69	701.67	452.20	209.50	1222.33
Trial II	7.98	23.63	8.09	152.40	1259.00	912.90	208.50	949.67

Table 3. Treatments by location and duration.

Site	Strawberry Variety	Plot Area (m^2)	Disinfestation Date	Film Removal	Strawberry Transplant	End of the Trial
Trial I	*Red face*	2.8 × 9.5 = 26	2017/7/20	2017/8/12	2017/9/30	2018/6/4
Trial II	*Red face*	3.2 × 7.5 = 22	2017/7/27	2017/8/23	2017/8/28	2018/5/15

Experiment materials included maltose (purity 85%), TIF and PE were obtained from the same manufacturers described in Table 1. Pic (99.5% purity) was obtained from Dalian Lvfeng Chemical Co, Ltd., Dalian, China. The Pic injector machine was manufactured by Beijing Jiexi Agricultural Technology Co., Ltd., Beijing, China. Soil temperature and soil moisture were recorded using an XR-3200 data logger equipped with temperature and relative humidity sensors (Pace Scientific, Inc., Mooresville, NC, USA). Soil temperature was measured at 0, −5, −10, −15 and −20-cm-deep. The soil moisture was measured at −10-cm-deep only in the plot where soil temperature and moisture were monitored at 30 min intervals throughout all the ASD period.

The soil treatments were: (1) maltose 6 (Mal 6), consisting of 6 t/ha of maltose applied by drip irrigation using 50 t/ha and covered by TIF for three weeks; (2) maltose 9 (Mal 9) consisting of 9 t/ha of maltose applied by drip irrigation with 50 t/ha water and covered by TIF for three weeks; (3) Pic was injected by machine into the soil at the dosage of 0.375 t/ha and covered with PE for two weeks; (4) soil solarization (Sol) consisted of drip irrigation with 50 t/ha water followed by a cover of TIF for three weeks; (5) untreated control (CK) left the soil untreated (no water no film). All the treatments were replicated four times and randomly distributed at each site (Table 4).

Table 4. Anaerobic soil disinfestation treatments in the field trials.

Site	Treatments	Dose (t/ha)	Application Method	Water Consumption (t/ha)	Film Type (0.05 mm)	Cover Time (weeks)	Replicates
Trial I	Mal 6	6	Drip irrigation	50	TIF	3	4
	Mal 9	9	Drip irrigation	50	TIF	3	4
	Pic	0.375	Machine Injection	–	PE	2	4
	Sol	–	Drip irrigation	50	TIF	3	4
	CK	–	–	–	–	–	4
Trial II	Mal 6	6	Drip irrigation	50	TIF	3	4
	Mal 9	9	Drip irrigation	50	TIF	3	4
	Pic	0.375	Machine Injection	–	PE	2	4
	Sol [c]	–	Drip irrigation	50	TIF	3	4
	CK	–	–	–	–	–	4

Strawberry plants (cultivar '*Red face*') were grown on beds (30-cm-high and 50-cm-wide, each containing 2 rows of plants spaced 20 cm apart). The beds were spaced in rows of 0.7 m (from center-to-center).

2.2.2. Soil Sampling

Four soil samples were randomly taken from the soil (0–20 cm) in each plot. The area of each plot of Trial I and Trial II was 26 and 22 m^2, respectively. The soil sampled time was 30 days and 120 days after soil was treated in two trial sites. The soil samples were transferred to the laboratory in an icebox and stored at 4 °C for *Fusarium* spp. and *Phytophthora* spp. analysis. In addition, we selected and stored 100 g soil samples at room temperature for physicochemical analyses and 10 g soils stored at −80 °C for DNA extraction (except Mal 6 which was not properly stored and discarded) from each plot in Trial II, respectively. The methods to isolate and quantitatively measure the soil *Fusarium* spp. and *Phytophthora* spp. were the same as described in the laboratory studies.

2.2.3. Soil Physical and Chemical Properties

A model FP-640 flame photometer (Shanghai Instruments Group Co., Ltd., Shanghai, China) was used to determine potassium concentration [33]. A 1:2.5 soil to H_2O ratio suspension was used to measure pH, soil redox potential, soil conductivity (Shanghai Yidian Scientific Instrument Co., Ltd., Shanghai, China). We used a Futura^{TM} continuous flow analytical system (Alliance Instruments, Frépillon, France) to quantify ammonium nitrogen, nitrate–nitrogen and available phosphorus. Available-P was determined using the Olsen method and extracted with 0.5 mol L^{-1} $NaHCO_3$ solution [34]. The soil physical and chemical attributes were determined using the method described by Li et al. [35].

2.2.4. Strawberry Plant Growth, Mortality and Crop Yield

Strawberries plant growth, mortality and yield were determined in Trial II. Sixty randomly selected strawberries (in the middle of each plot) were selected to record the plant height, stem diameter and mortality from December 2017 to June 2018 in each plot.

Strawberry yield was measured daily from 1–28 February 2018 by randomly selected 2 rows of each plot in the greenhouse. The total crop yield over the month in all treatments were compared. Crop yield over more than a month was not undertaken because the available research funds were not possible to compensate the grower's lost income for more than one month.

2.2.5. DNA Extraction

A DNA extraction kit (Mo Bio Laboratories, Inc., Waltham, MA, USA) was used to extract and purify DNA from 0.25 g of soil following the manufacturer's protocol. After extraction, the quality and concentration of the DNA samples were measured using 0.1% (*w/v*) agarose gel electrophoresis and a NanoDrop^{TM} 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The DNA extract was further stored at −20 °C.

The V3-V4 region (universal primers 338F-806R) [36] and the internal transcribed spacer (ITS) region (ITS 1F-ITS2R) [37] were selected for bacteria and fungi, respectively. The bacterial V3-V4 and fungal internal transcribed spacer (ITS) hypervariable regions were amplified using conventional primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) 806R (5′-GGACTACHVGGGTWTCTAAT-3′) and ITS1F (5′-CTTGGTCATTTAGGAAGTAA-3′) - ITS2R (5′-GCTGCGTTCTTCATCATGATGC-3′), respectively. The Illumina adapters were 5′-AATGATACGGCGACCACCGAGATCTACAC-3′ and 5′-CAAGCAGAAGACGGCATACGAGATGCCGCATTCGAT-3′), respectively. Polymerase chain reaction (PCR) amplifications were carried out in triplicate for each sample following an already described protocol [8]. The sequencing of PCR products was carried out on using an Illumina MiSeq system at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) following the procedures described by Zhang et al. [8].

2.3. Statistical Analyses

2.3.1. Laboratory Studies

The efficacy of *Fusarium* spp. and *Phytophthora* spp. control was calculated using the following equation:

$$Y = \frac{X_1 - X_2}{X_1} \times 100\%$$

where Y is the efficacy against *Fusarium* spp. and *Phytophthora* spp., X_1 indicates the CFU colonies of pathogen present in the untreated control, and X_2 is the CFU colonies of the pathogen in the fumigant-treated plots.

2.3.2. Field Trials

The mortality rate of the strawberry was quantified using the following equation.

$$X = \frac{N_1}{N_1 + N_2} \times 100\%$$

where X indicates the mortality rate for strawberry (%), N_1 was the number of strawberry plants that died during the experiment, and N_2 was the number of strawberry plants that were alive.

The number of *Fusarium* spp. and *Phytophthora* spp. colonies from the field trials were transformed as necessary (log10 for large numbers [>100] and square root transformations for small numbers [<100]) before ANOVA analysis using SPSS (Windows v. 22.0 statistical software). Prior to analysis, the data expressed as percentages were arcsine transformed to homogenize variances. However, the data

are given as untransformed values. Duncan's new multiple range tests was used to determine any statistical differences ($p \leq 0.\ 05$) between the treatments.

2.3.3. High Throughput Sequencing Data Analysis

Post-sequencing analysis was performed using Quantitative insights into microbial ecology (QIIME I 1.9.1) open-source bioinformatics pipeline. The sequence obtained was clustered into operational taxonomy units (OTUs) according to the similarity level using UPARSE Pipeline v 7.0 at the 97% classification threshold. The effective sequences were normalized in order to compare all samples at the same sequencing depth. Based on the cluster analysis, the Chao, Shannon, coverage and Shannon evenness indices were calculated, using SPSS Windows v. 22.0 statistical software. The taxonomy of each OTU representative sequence was analyzed by the RDP classifier (http://rdp.cme.msu.edu/) against the 16S rRNA database on the Silva (Release128 http://www.arb-silva.de) and the fungal ITS Unite (release 7.0 http://unite.ut.ee/index.php) database. Duncan's new multiple range test determined any statistical differences. Bray–Curtis method was used to analyze principal coordinates analysis (PCoA). Adonis was used to test for differences between groups of microbial diversity, and the number of replacements was set to 999. The scientific analysis of differences between treatments were performed using the Kruskal–Wallis H test ($p \leq 0.05$), multiple test correction using falsely discovery rate (FDR).

3. Results

3.1. Laboratory Study

Orthogonal Experiments

Twenty-seven treatments based on an orthogonal design were carried out to assay the effect of ASD on *Fusarium* spp.; (FUS) and *Phytophthora* spp.; (PHY) (Table S2). The influence factors combined with ASD showed a variable effect against FUS with the following order: C (carbon source) > A (soil moisture) > B (air temperature). The influence factors combined with ASD showed a variable effect against PHY with the following order: A (soil moisture) > C (carbon source) > B (air temperature) (Table 5). Factor B, factor C against FUS and PHY and factor A × B against FUS were statistically significant at $p < 0.05$ according to the ANOVA test (Table 5). There were no statistical differences observed between factors A × C and fac- tor B × C. Since there was an interaction between factor A and factor B, we determined the optimal combination by multiple comparisons. The results of multiple comparison test showed the optimal combination influence factors for the control efficacy of FUS was optimal (97%) with 20 °C air temperature and 30% soil moisture (A2 × B3) in Table S3. The optimal carbon source for the control efficacy of fungal pathogens was maltose as a carbon source. According to our results, the optimal conditions were A2, B3 and C2 which used maltose (C2, carbon source), 20 °C (A2, air temperature) and 30% (B3, soil moisture) to ASD. Under these optimal treatment conditions ASD decreased FUS and PHY by 100%. Based on the laboratory results described above, 20% of soil moisture and maltose were chosen as the factors for the following efficacy assay of ASD sealed with different tarps.

Table 5. ANOVA test on the results of the effects of the soil treatments on the numbers of colony-forming units (CFU) of *Fusarium* spp.; (FUS) and *Phytophthora* spp.; (PHY).

Fungi	Source [a]	Sum of Squares	Degree of Freedom	Mean Square	F	Significant
FUS	A	621	2	310	2.43	
	B	1994	2	997	7.81	*
	C	3063	2	1531	12.00	*
	A × B	4176	4	1044	8.18	*
	A × C	864	4	216	1.69	
	B × C	677	4	169	1.33	
	Error	1021	8	128		
PFY	A	156	2	78	0.27	
	B	7339	2	3670	12.74	*
	C	3135	2	1568	5.44	*
	A × B	310	4	77	0.27	
	A × C	3910	4	978	3.39	
	B × C	2853	4	713	2.48	
	Error	2305	8	288		

[a] Abbreviations: A = air temperature, B = soil moisture, C = carbon source. In each column, the data are the means of three replications. A × B, A × C and B × C represent the interaction of A, B factors, A, C factors and B, C factors, respectively. * Significant ($p < 0.05$) by F-test 3.1.2. Experiments of cover tarps.

ASD covered with three different plastic films both exhibited significantly reduced *Fusarium* spp. and *Phytophthora* spp. in soil with organic amendments than in the soil without organic amendment (Table 6). The rates of reduction of *Fusarium* spp. and *Phytophthora* spp. by at least 84% and 87%, respectively. TIFMal and VIFMal treatments showed excellent efficacy on soilborne pathogens, and the effect showed no significant difference between these two treatments. However, WaterMal treatment significantly increased the CFU of *Fusarium* spp., also the rate of suppression on *Phytophthora* spp. was 51%. These results suggested that ASD using maltose as a carbon source requires plastic film covering measures to ensure the efficacy of soilborne diseases.

Table 6. Effects of the soil treatments on the numbers of colony-forming units per gram (CFU·g^{-1}) of *Fusarium* spp.; (FUS) and *Phytophthora* spp.; (PHY) on selective media after fumigation.

Treatments	Rate (%, *w/w*)	Tarp Kind	FUS		PHY	
			CFUg^{-1} *	% Reduction	CFU g^{-1}	% Reduction
CK	–	–	11,960 ± 339 b	–	14,234 ± 320 a	–
VIFCK	–	0.05 mm VIF	14,400 ± 200 ab	–	16,767 ± 306 a	–
VIFMal	5	0.05 mm VIF	442 ± 31 d	96.3	675 ± 146 d	91.0
PECK	–	0.05 mm PE	18,700 ± 700 ab	–	21,600 ± 541 a	–
PEMal	5	0.05 mm PE	1912 ± 20 c	84.0	1925 ± 357 c	86.5
TIFCK	–	0.03 mm TIF	17,967 ± 404 ab	–	17,867 ± 729 a	–
TIFMal	5	0.03 mm TIF	58 ± 8 d	99.5	100 ± 26 d	99.3
WaterCK	–	150 mL deionized water	15,155 ± 751 ab	–	14,810 ± 834 a	–
WaterMal	5	150 mL deionized water	34,696 ± 1175 a	–	6970 ± 485 b	51.0

* Values are means ± SD ($N = 3$). Means followed by the same letter are not significantly different ($p = 0.05$). Duncan's new multiple range tests was used to determine any statistical differences ($p \leq 0.05$) between the treatments.

In the laboratory studies, the treatments covered with plastic or water, but without organic amendment showed no efficacy against *Fusarium* spp. and *Phytophthora* spp. compared with the untreated control. These results show that the use of maltose as a carbon source is necessary to ensure the effectiveness of ASD.

Based on the laboratory results, we selected ASD used maltose as a carbon source and sealed with TIF for the field trials.

3.2. Field Trials

3.2.1. Soil Temperature and Moisture

The average, maximal and minimal soil temperatures were 36 °C, 78 °C, 23 °C and 38 °C, 61 °C, 24 °C in two trial sites, respectively (Table 7). The average soil temperature decreased as the soil depth increased, with the lowest temperature value 23 °C appears at the −20 cm below the surface of the Trial I. Active accumulated temperatures were calculated between 987–1016 °C and 1007–1060 °C in Trial I and Trial II, respectively. The average soil moisture during ASD disinfestation was 36% and 37% at the Trial I and Trial II, respectively.

Table 7. Soil temperature and soil moisture during anaerobic soil disinfestation.

Item	Soil Temperature at Different Depths (°C)					Soil Moisture (%)
	−20 cm	−15 cm	−10 cm	−5 cm	0 cm	
Trial I						
Accumulated temperature *	1016	1022	1031	1048	987	–
Average value	36.3	36.5	36.8	37.4	35.2	36.2
Highest value	42.9	45.0	46.6	52.0	78.0	41.9
Lowest value	27.9	25.8	24.3	24.1	23.4	28.6
Trial II						
Accumulated temperature *	1007	1019	1039	1060	1011	–
Average value	37.1	37.6	38.3	39.1	37.3	36.8
Highest value	44.5	48.8	49.5	54.3	61.2	43.4
Lowest value	32.4	31.5	30.0	29.4	24.2	30.5

* Accumulated temperature means average temperature per day × number of days (duration of anaerobic soil disinfestation (ASD) disinfestation).

3.2.2. Soilborne Fungal Pathogens

After 30 days of soil disinfestation, the colonies of *Fusarium* spp. and *Phytophthora* spp. were significantly lower in ASD, Pic and Sol soil treated plots than in the untreated soil at trial I and Trial II (Table 8). At least 89% (Trial I) and 75% (Trial II) reduction in *Fusarium* spp. and 85% (Trial I) and 70% (Trial II) reduction in *Phytophthora* spp. were observed by ASD soil treatments, respectively. The efficacies of ASD against *Fusarium* spp. and *Phytophthora* spp. were higher than the Sol treatments in the two trials. The highest reductions in *Fusarium* spp. and *Phytophthora* spp. by at least 99% and 99% were observed in Pic soil treated plots. There was no significant difference between the Mal 6 treatments and Mal 9 treatments in suppression soilborne fungi, except Mal 6 treatments showed a lower reduction in *Fusarium* spp. than Mal 9 in the Trial I (Table 8).

Notably, after 120 days of soil fumigation and with the strawberry cultivation, the number of *Fusarium* spp. and *Phytophthora* spp. colonies recovered in all soil treated plots and have no significant difference t compared with the control (Table 8).

3.2.3. Soil Physicochemical Parameters

Compared with the control, Mal 6, Mal 9, Pic and Sol significantly increased NH_4^+-N (between 15–29 mg·kg^{-1}) and electrical conductivity (948–1115 μs·cm^{-1}) in soil, but significantly decreased NO_3^--N by 32–66 (Table 9). Compared with the control, the concentrations of available P significantly decreased by 25% in Mal 9. Compared with the control, Mal 9 significantly increased the concentrations of available K while Pic treatment significantly reduced it. At the same time, the soil pH showed a slightly decreased from 8.1 to 7.5 after the Mal 6 and Mal 9 treatments (Table 8).

Table 8. Effects of the soil treatments on the numbers of colony-forming units per gram (CFU·g^{-1}) of *Fusarium* spp.; (FUS) and *Phytophthora* spp.; (PHY)on selective media after fumigation in field trials.

Site	Treatments	FUS (30 d)		PHY (30 d)		FUS (120 d)		PHY (120 d)	
		CFU·g^{-1}	% Reduction	CFU·g^{-1}	% Reduction	CFU·g^{-1}	% Reduction	CFU·g^{-1}	% Reduction
Trial I 2017–2018	Mal 6	830 ± 154 c	88.9	1040 ± 98 c	84.6	2095 ± 128 a		3288 ± 240 a	
	Mal 9	681± 95 c	89.3	532 ± 102 d	92.3	2254 ± 340 a		3173 ± 386 a	
	Pic	33 ± 8 d	99.4	126 ± 30 e	98.2	1630 ± 230 a		3007 ± 392 a	
	Sol	226 ± 477 b	77.7	3045 ± 128 b	66.6	1541 ± 285 a		2319 ± 284 a	
	CK	10,255 ± 743 a		8803 ± 468 a		899 ± 64 a		2029 ± 312 a	
Trial II 2017–2018	Mal 6	422 ± 84 bc	75.2	458 ± 87 bc	69.7	1486 ± 108 a		2196 ± 114 a	
	Mal 9	275 ± 46 c	81.7	255 ± 42 c	75.4	1448 ± 56 a		1668 ± 86 a	11.3
	Pic	14 ± 4 d	99.1	8 ± 3 d	99.1	641 ± 74 a	24.9	1353 ± 164 a	28.0
	Sol	629 ± 112 b	69.7	677 ± 88 b	57.8	942 ± 48 a		1549 ± 35 a	17.6
	CK	2375 ± 340 a		2743 ± 324 a		853 ±102 a		1880 ± 58 a	

CK = untreated—no film or water; Mal6 = ASD achieved with 6 t/ha of maltose applied by drip irrigation and covered by totally impermeable film (TIF) for three weeks; Mal 9 = ASD achieved with 9 t/ha of maltose applied by drip irrigation and covered by TIF for three weeks; Pic = chloropicrin applied by machine injection at a dose of 0.375 t/ha and covered by polyethylene film for two weeks; Sol = soil solarization with 50 t/ha water by drip irrigation and covered with TIF for three weeks. Means (N = 4). Means followed by the same letter are not significantly different (p = 0.05) according to the LSD test.

Table 9. Physicochemical properties of the soil recorded 30 days after the soil treated in Trial II.

Treatments	NH_4^+-N (mg·kg^{-1})	NO_3^--N (mg·kg^{-1})	Available P (mg·kg^{-1})	Available K (mg·kg^{-1})	Organic Matter (mg·kg^{-1})	Electrical Conductivity (µs·cm^{-1})	pH (1:2.5)
Mal 6	28.56 ± 1.5 a	64.85 ± 1.2 e	889 ± 36.4 a	813 ± 70 b	19.03 ± 1.6 a	948 ± 49 b	7.58 ± 0.2 ab
Mal 9	15.12 ± 1.2 cd	84.05 ± 8.8 d	693 ± 7.7 b	1150 ± 41 a	19.67 ± 1.0 a	1171 ± 47 a	7.54 ± 0.2 ab
Pic	18.76 ± 2.3 bc	123.95 ± 4.1 b	800 ± 78.2 ab	409 ± 37 c	13.42 ± 1.4 b	1115 ± 103 ab	7.41 ± 0.2 b
Sol	23.64 ± 3.1 ab	103.81 ± 4.0 c	906 ± 57.2 a	725 ± 90 b	16.18 ± 3.0 ab	1144 ± 47 ab	7.58 ± 0.1 ab
CK	12.30 ± 1.0 d	182.91 ± 4.1 a	931 ± 29.0 a	800 ± 71 b	16.43 ± 0.2 ab	561 ± 52 c	8.06 ± 0.2 a

Values are means ± SD (N = 4). Means followed by the same letter are not significantly different (p = 0.05) according to the LSD test. CK = untreated—no film or water; Mal 6 = ASD achieved with 6 t/ha of maltose applied by drip irrigation and covered by TIF for three weeks; Mal 9 = ASD achieved with 9 t/ha of maltose applied by drip irrigation and covered by TIF for three weeks; Pic = chloropicrin applied by machine injection at a dose of 0.375 t/ha and covered by polyethylene film for two weeks; Sol = soil solarization with 50 t/ha water by drip irrigation and covered with TIF for three weeks. Data were analyzed for ANOVA. Duncan's new multiple range tests was used to determine any statistical differences ($p \leq 0.05$).

3.2.4. Strawberry Growth, Mortality and Yield

The strawberry plant height and stem diameter with Mal 9 and Pic treatments were significantly higher than in the untreated control plots. The plant height and stem diameter have no significant difference between Mal 6, Pic, Sol and the CK treatments on 28 December 2017. However, by 4 May 2018, Mal 6, Mal 9 and Pic treatments showed significantly increased strawberry plant height and stem diameter, compared to CK. Mal 9 observed the highest strawberry plant height (21–25% more than the CK) and stem diameter (30–25% more than the CK) over that time, and there were no significant differences in these parameters between the Mal 6, Mal 9 and Pic treatments (Table 10).

The strawberry plants in the control plots suffered the highest mortality (11.3%) in Trial II. The lowest mortality was obtained with Mal 9 (4.5%), followed by Pic (5.0%), Mal 6 (6.0%) and Sol (6.8%). Mal 9 and Pic significantly reduced plant mortality toward the end of the experiment (Table 9).

The yield of strawberry varied with fumigation treatments (Table 9). Mal 6, Mal 9 and Pic treatments significantly increased the strawberry yield in Trial II. The CK had the lowest yield (2.35 t/ha) when compared to the other treatments. Mal 9 had a most powerful yield (5.66 t/ha) and had no significant difference with Mal 6 and Pic treatments. Sol treatments showed a lower strawberry yield than the soil fumigation plots, and the yield higher compared with the CK.

Table 10. Strawberry growth, mortality and yield at Trial II.

Treatments	Plant Height (cm)	Stem Diameter (mm)	Plant Height (cm)	Stem Diameter (mm)	Mortality (%)	Yield (t/ha)
Date	**28 December 17**	**28 December 17**	**4 May 18**	**4 May 18**	**5 June 18**	**February 2018**
Mal 6	18.91 ± 0.4 bc	21.37 ± 1.0 b	37.20 ± 0.9 a	36.48 ± 0.6 a	6.00 ± 0.4 ab	5.10 ± 0.3 a
Mal 9	21.99 ± 0.6 a	27.61 ± 1.8 a	35.78 ± 0.5 a	35.76 ± 1.9 a	4.50 ± 0.5 b	5.66 ± 0.2 a
Pic	21.36 ± 1.3 ab	24.11 ± 2.1 ab	36.15 ± 0.8 a	34.87 ± 0.9 a	5.00 ± 0.7 b	5.49 ± 0.1 a
Sol	19.59 ± 0.9 b	21.92 ± 0.9 b	29.35 ± 1.7 b	30.86 ± 1.7 b	6.75 ± 0.5 ab	3.48 ± 0.1 b
CK	18.16 ± 0.7 c	21.26 ± 0.5 b	28.53 ± 1.9 b	28.72 ± 0.9 b	11.25 ± 0.8 a	2.35 ± 0.1 c

Means (n = 4). The yield of the strawberry was monitored from 1 to 28 February 2018. CK = untreated—no film or water; Mal 6 = ASD achieved with 6 t/ha of maltose applied by drip irrigation and covered by TIF for three weeks; Mal 9 = ASD achieved with 9 t/ha of maltose applied by drip irrigation and covered by TIF for three weeks; Pic = chloropicrin applied by machine injection at a dose of 0.375 t/ha and covered by polyethylene film for two weeks; Sol = soil solarization with 50 t/ha water by drip irrigation and covered with TIF for three weeks. Data were analyzed for ANOVA. Duncan's new multiple range test was used to determine any statistical differences ($p \leq 0.05$).

3.2.5. Microbiota Analysis by High Throughput Sequencing (HTS)

The HTS of the 16S and ITS amplicons yielded a total of 951,418 and 1051,353 reads, respectively. The reads were clustered into 9650 and 1768 operational taxonomy units (OTUs) for bacteria and fungi, respectively. The rarefaction curves of all samples slowly reached a plateau, indicating effective sequencing of bacterial and fungal community diversity.

Alpha Diversity Analysis

The alpha diversity of bacterial and fungal indices of Chao (from 3860 to 4603; from 298 to 433), Shannon (from 5.99 to 6.42; from 1.94 to 2.82), coverage (from 0.980 to 0.981; from 0.998 to 0.999) and Shannon evenness (from 0.75 to 0.79; from 0.36 to 0.49) were not significantly different between the 5 conditions, respectively (Table 11).

Table 11. Analysis of alpha diversity index among different treatments at the operational taxonomic unit level [a].

Treatments	Chao	Shannon	Coverage	Shannon Evenness
		Bacteria		
Mal 6	4419 ± 148 a	6.42 ± 0.1 a	0.980 ± 0.001 a	0.79 ± 0.01 a
Mal 9	3860 ± 273 a	5.99 ± 0.2 a	0.981 ± 0.001 a	0.76 ± 0.01 a
Pic	4304 ± 471 a	6.04 ± 0.4 a	0.980 ± 0.002 a	0.75 ± 0.03 a
Sol	4603 ± 446 a	6.40 ± 0.2 a	0.980 ± 0.002 a	0.79 ± 0.01 a
CK	4149 ± 171 a	6.31 ± 0.1 a	0.980 ± 0.002 a	0.79 ± 0.01 a
		Fungi		
Mal6	433 ± 32.8 a	2.78 ± 0.39 a	0.998 ± 0.0001 a	0.47 ± 0.06 a
Mal9	298 ± 53.5 a	1.94 ± 0.36 a	0.999 ± 0.0003 a	0.36 ± 0.06 a
Pic	342 ± 31.9 a	2.55 ± 0.35 a	0.999 ± 0.0002 a	0.45 ± 0.06 a
Sol	363 ± 56.6 a	2.82 ± 0.37 a	0.999 ± 0.0004 a	0.49 ± 0.06 a
CK	378 ± 63.9 a	2.46 ± 0.29 a	0.998 ± 0.0003 a	0.44 ± 0.04 a

Means (n = 4, Mal6 use the average value as the fourth replicate for each index), Data were analyzed for ANOVA. Duncan's new multiple range test was used to determine any statistical differences ($p \leq 0.05$).

Principal Coordinate Analysis

Principal coordinate analyses using bacterial and fungal compositions are shown in Figure 1. The contribution of the bacterial community composition in the two principal coordinates was 34% and 20% (Figure 1a). The soil samples' bacterial communities from greenhouse A were located separately in the second quadrant and the soil samples from greenhouses B and C were mainly located separately in the fourth and third quadrants. The Adonis result showed no significant difference between the ASD, Sol, Pic and CK treatment groups (p = 0.672).

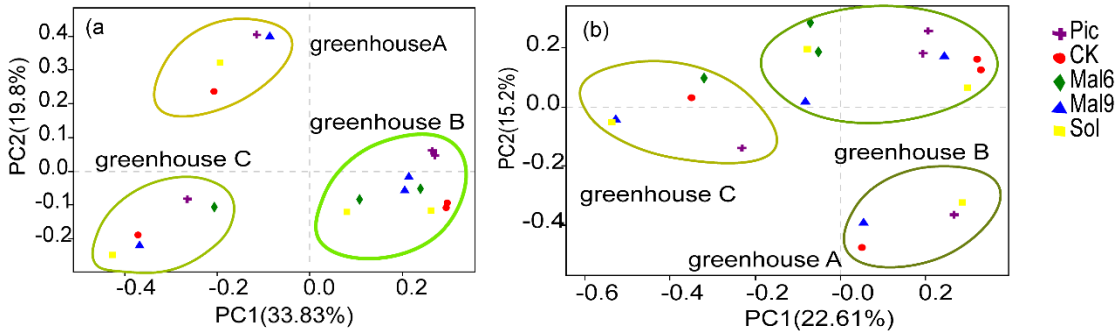

Figure 1. Principal coordinate analysis diagram of bacteria (**a**) and fungi (**b**) after treatments at the OTU level (based on Bray–Curtis method. CK = untreated—no film or water; Mal6 = ASD achieved with 6 t/ha of maltose applied by drip irrigation and covered by TIF for three weeks; Mal9 = ASD achieved with 9 t/ha of maltose applied by drip irrigation and covered by TIF for three weeks; Pic = chloropicrin applied by machine injection at a dose of 0.375 t/ha and covered by polyethylene film for two weeks; Sol = soil solarization with 50 t/ha water by drip irrigation and covered with TIF for three weeks.

The contribution of fungal community composition in the two principal coordinates was 23% and 15% (Figure 1b). The soil samples' fungal community from greenhouse A were located separately in the fourth quadrant, and the soil samples from greenhouse B and C were mainly located separately in the first and second quadrants.

Microbiota Composition

Compared with the blank control, ASD treatments changed the soil bacterial and fungal microbial community composition, but the species composition was not significantly different at the level of species taxonomy after FDR verification.

The bacterial OTUs were assigned into 38 Phyla, 111 Classes, 322 Orders, 576 Families and 1222 Genera. The different treatments showed similar 16S rRNA profiles at the Class level (Figure 2a). Bacilli, Actinobacteria, Alphaproteobacteria, Gammaproteobacteria, Gemmatimonadetes and Anaerolineae bacteria were the most important and accounted for more than 60% of the sequences. The fungal OTUs can be assigned to 12 Phyla, 30 Classes, 67 Orders, 149 Families and 280 Genera. The different treatments showed similar 16S rRNA profiles at the Class level (Figure 2b). Sordariomycetes, Eurotiomycetes, unclassified_k_Fungi, unclassified_p_Ascomycota, Agaricomycetes and Dothideomycetes were the most important and accounted for more than 80% of the reads.

Without FDR correction, the relative abundance of bacteria class (Figure 3a) Clostridia (p = 0.04932) and Halanaerobiia (p = 0.01196) have significantly increased in ASD, Pic and Sol treatments compared with CK. At the genus level (Figure 3c), Mal 6, Mal9 and Pic significantly increased the bacterial genera *Clostridium_sensu_stricto_1* (p = 0.01657), *Azoarcus* (p = 0.04073), *Clostridium_sensu_stricto_12* (p = 0.009159), however Mal 6, Mal9 and Pic significantly decreased the bacterial genera *unclassified_c__Gammaproteobacteria* (p = 0.01601), *Niastella* (p = 0.01706), *Solirubrobacter* (p = 0.04582), *Reyranella* (p = 0.03405), compared with control. Nevertheless, all these differences were no longer significant when applying the false discovery rate correction. The relative abundance of the fungi *Cladosporium* (p = 0.02461) treated with soil fumigation increased compared with CK, the number of fungi *Gibberella* (p = 0.01978) in all soil treatments decreased significantly compared with control (Figure 3d). However, these differences were no more significant when applying FDR correction. The relative abundance of sequences assigned to *Fusarium* sp. treated with Mal9, Pic and Sol slightly decreased compared with CK, but the observed differences were not significant.

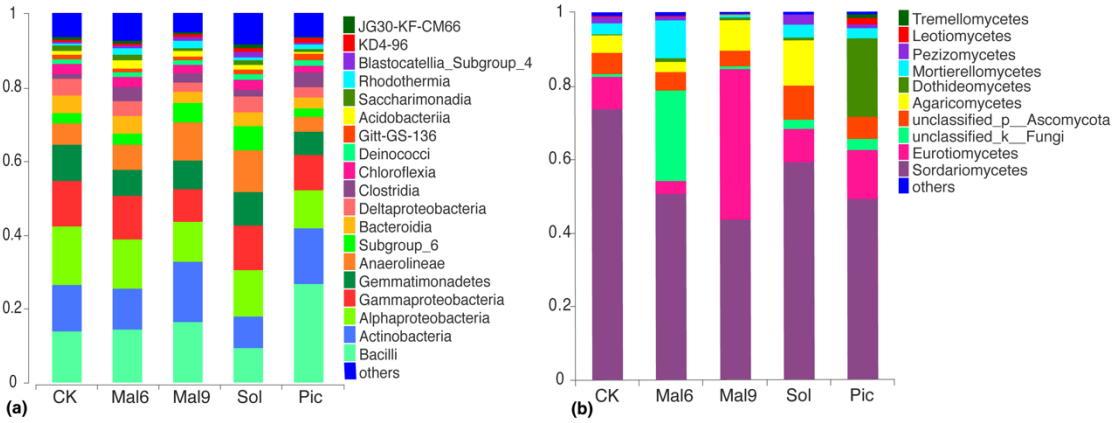

Figure 2. Soil bacteria (**a**) and fungi (**b**). Class compositional response to the different treatments. CK = untreated—no film or water; Mal6 = ASD achieved with 6 t/ha of maltose applied by drip irrigation and covered by TIF for three weeks; Mal9 = ASD achieved with 9 t/ha of maltose applied by drip irrigation and covered by TIF for three weeks; Pic = chloropicrin applied by machine injection at a dose of 0.375 t/ha and covered by polyethylene film for two weeks; Sol = soil solarization with 50 t/ha water by drip irrigation and covered with TIF for three weeks.

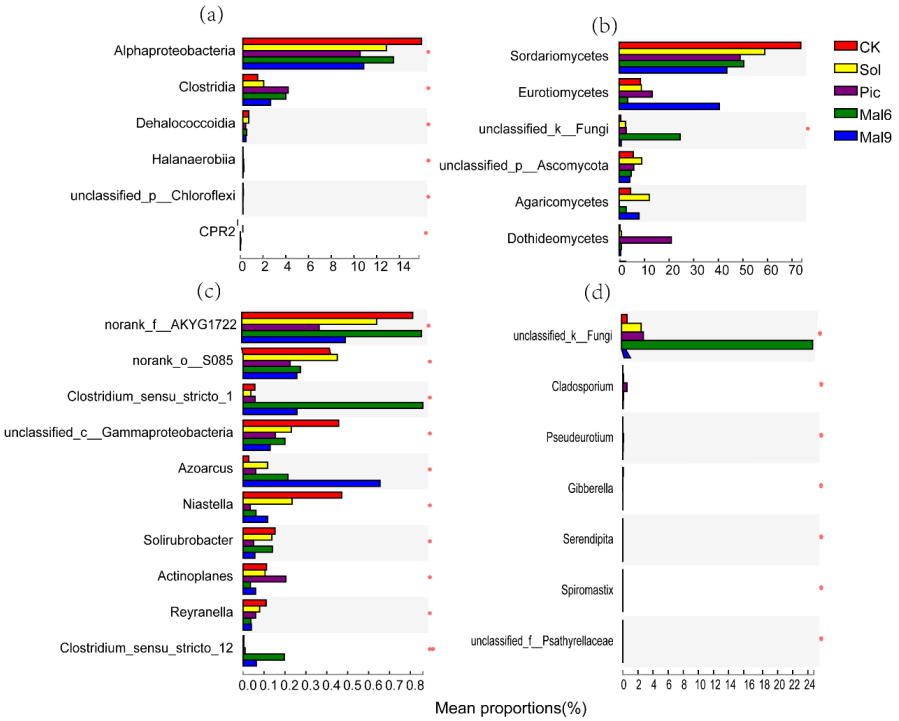

Figure 3. Significantly analysis of relative abundance of bacterial class (**a**), fungal class (**b**), bacterial genus (**c**) and fungal genus (**d**) histogram of soil microbial order exposed between different treatments: CK = untreated—no film or water; Mal6 = ASD achieved with 6 t/ha of maltose applied by drip irrigation and covered by TIF for three weeks; Mal9 = ASD achieved with 9 t/ha of maltose applied by drip irrigation and covered by TIF for three weeks; Pic = chloropicrin applied by machine injection at a dose of 0.375 t/ha and covered by PE for two weeks; Sol = soil solarization with 50 t/ha water by drip irrigation and covered with TIF for three weeks. The number of asterisks indicated a significant difference between treatments according to a Kruskal–Wallis H test. False Discovery Rate was used for the CI calculation adjustment ($p < 0.05$).

4. Discussion

4.1. Effect of ASD on Soil Fungal Pathogenic Microorganisms

The efficacy of ASD against a specific target pathogen varies based on three key parameters: C-source used, tarp type and soil temperature [18]. The selection of the type of carbon source is especially important for ASD, but anaerobic conditions alone in the soil are not sufficient to inhibit soil pathogenic microorganisms [38]. In our field trials, maltose as a carbon source for ASD disinfestation effectively reduced the colonies of *Fusarium* spp. and *Phytophthora* spp. when examined 30 days after the start of the treatment, compared with control (Table 8). The effect of ASD on *Fusarium* spp. was equivalent to Pic. maltose is a sugar mill byproduct, readily available in China and widely used as an excellent liquid carbon sources for ASD [14]. The field study showed ASD used maltose as carbon source and combined with PE provided an alternative to methyl bromide soil fumigation for control *Phytophthora capsica*, *Fusarium oxysporum f.* sp. *lycopersici*, nematodes (*Meloidogyne incognita)* and weed biomass in bell-pepper–eggplant double crop production system [39,40]. Liu et al. reported that glucose as a carbon source for ASD could significantly decreased *F. oxysporum* populations after 16 days soil treated by the laboratory experiments and the greatest decrease in *F. oxysporum* populations was found on the 4th day in the glucose treatment [41]. In Florida, ASD using molasses, 50 mm of irrigation water was as effective as using 1,3-D fumigation to control *F. oxysporum* and *M. phaseolina* [42]. The colonies of *Fusarium* spp. and *Phytophthora* spp. at longer time (120 days after soil fumigation) showed higher number in ASD and Pic treatments compared to the control (Table 8). Liu's plot test showed that the number of *Fusarium* in the soil recovered after 90 days with watermelon cultivation after RSD disinfestation [43]. The reduction of oxygen diffusion by the polyethylene film in combination with soil moisture created anaerobic conditions in the soil [44].

The cover tarps play an important role to increase soil temperature and control pathogens in the ASD progress [20]. TIF showed excellent low-permeability for the fumigants [23]. TIF greatly reduced the commercial fumigant dimethyl disulfide emissions to less than 2% of the total amount application [21]. The physical characteristic of TIF effectively prevents the exchange of oxygen and evaporation of moisture which is another barrier to oxygen diffusion, creates and maintains strict anaerobic conditions under the film, and promotes the growth of anaerobic microorganisms. Atsuko Ueki et al. observed that obligate anaerobic bacteria from the class *Clostridia* became the dominant bacteria in the soil after biologic soil disinfestation (BSD), and the released of enzymes with antipathogenic activity in the soil could effectively inhibit soilborne fungal pathogens [45]. Lactic acid bacteria quickly become the dominant bacteria with the critical anaerobic condition in the ASD process [46], and they inhibited the growth of *Aspergillus nidulans, Penicillium funiculosum* and *Fusarium poae* [47].

We also examined the treatments covered with plastic or water and without maltose amendment showed no efficacy against fungal pathogens (Table 6). As the same results showed that while anaerobic conditions only (without C amendment) was not as effective as ASD [14].

Soil temperature is one of the important factors that influences the efficacy of ASD. Similarly, the Sol with the higher temperature in Trial I showed better control effect of *Fusarium* spp. and *Phytophthora* spp. than Trial II (Table 7). At higher soil temperatures (>35 °C), the inhibitory effect on pathogens of ASD improves compared with medium (16 to 35 °C) and low (<16 °C) soil temperatures [14]. Laboratory experiments showed that under anaerobic conditions, the growth of *Fusarium oxysporum f. sp. fragariae* was greatly inhibited and the fungus growth and survival were suppressed with increasing temperature at 10–30 °C [48]. In field trials with 280–300 h of soil temperatures above 30 °C at 20 cm depth of the soil, *F. oxysporum* was consistently suppressed by ASD [48].

4.2. Effect of ASD on the Soil's Physicochemical Properties

The soil's physicochemical properties and the microbiota can impact the efficacy of ASD disinfestation against soil pathogens [14]. Moreover, the effectiveness of ASD depends on both

physicochemical [18] and biologic shifts in soils [49], which may relate to carbon substrates used to induce ASD [50]. Compared with control, ASD significantly increased NH_4^+-N, but reduced the soil's NO_3^--N (Table 9). The same test results are expressed in Liu's report [41]. These changes in the soil's physicochemical parameters can be related to the composition and structure changes of the soil microbial community [27]. Butler et al. reported that ASD-treated soils showed lower oxidation–reduction potential than the control treatments [20]. ASD can significantly increase the content of ammonium nitrogen in the soil and greatly reduce the content of nitrate nitrogen, which indicates that the ASD process will affect the mineralization of soil nitrogen. The mineralization rate is reduced when the microbial biomass in the soil is killed by disinfestation [11]. Under high-carbon and low-oxygen conditions, it promotes anaerobic denitrification and accelerates the consumption of nitrate. Earlier studies also showed that the fumigant dimethyl disulfide and allyl isothiocyanate fumigation treatment stimulated the growth of anaerobic denitrification bacteria and promoted the denitrification process to consume nitrate [51,52].

The previous study showed that using 2% (*w/w*) glucose as carbon source for ASD significantly increased soil OM [41]. Our results showed that compared with the control, Mal 9 can significantly increase the OM value. Because of Liu's indoor experiment added carbon concentration is higher than our field trials, and the trend is more intense.

4.3. Effect of ASD on Strawberry Fruit Yield

The combination of increased soil nutrients and better soil physicochemical properties encouraged the growth of strawberry plants and promoted high fruit yield (Table 9). Our experiment showed ASD added maltose can significantly increase the yield of strawberries by 141% compared to the control while achieving similar performance (+4%) compared to Pic. Meta-analysis showed a 6% higher increase yield for strawberries plant in ASD-treated soil than the chemical fumigation, and a 30% increase compared to untreated soil [14]. There could be many reasons for the increased responses of strawberry yield in our research compared to the meta-analysis. A meta-analysis including 123 publications reported that comparing ASD with the untreated control, the application rate, type of carbon sources can have a varied impact on crop yield [14]. In our study, the yield of strawberry fruit with Mal 9-treated plots was 11% higher than Mal 6, but the difference was not significant. Based on the literature, ASD used maltose as a carbon source not only reduced the number of pathogenic microorganisms that are transmitted by the soil, but also increased the utilization rate of NPK (Table 9). The soils in our research were sandy loam, and the ASD treatment was for three weeks at a relatively high temperature. This combination of soil type, high temperature promotes the control of soil pathogens [7,15].

Mal 6, Mal 9 and Cip could significantly increase the strawberry economic income by 12,183, 14,664 and 13,910 \$ ha^{-1} in February for growers. The cost of Mal 6, Mal 9 and Cip were 5520, 6138 and 4411 \$ ha^{-1}, respectively (the cost of TIF is 4285 \$ ha^{-1}, the cost of PE is 1654 \$ ha^{-1}, the cost of Mal is \$ 206 ton^{-1} and the cost of Cip is 2748 \$ ha^{-1}). The net increased income from strawberry production was \$ 6663, \$ 8526 and \$ 9499 with Mal 6, Mal 9 and Cip soil fumigation in February, respectively. We use the highest average price of 'Hongyan' strawberry (4.43 \$ ha^{-1}) which based on the Beijing Xinfadi agricultural product market price forecast in February 2018 (http://www.xinfadi.com.cn) to calculate the strawberry output value. This shows that the economic benefits brought to farmers by using ASD disinfestation are obvious. However, the cost of the disinfestation of ASD is higher than the cost of chemical fumigant chlorinated bitter, it is a sterilization method that is worthy of popularization and application in organic planting parks. In particular, the net benefit of high-concentration maltose ASD treatment is close to that of chloropicrin.

4.4. Effect of ASD on Soil Bacterial and Fungal Communities

Alteration of the soil microbiome was proposed to play a role in disease suppression with ASD [16]. The crop cultivation history, disease types, soil temperature and locations, etc. have different effects

on the composition and structure changes of soil fungi and bacteria after ASD disinfestation [17]. ASD disinfestation can change the soil's physicochemical parameters and microbial composition [19,53].

The alpha diversity indices in our research showed that there was no significant difference in the richness, diversity and evenness of soil bacterial and fungal communities between the ASD treatments and the control. Compared with field trials, pathogens are more inhibited in experiments such as potting and other laboratory media. It may be due to the decrease of indoor soil heterogeneity and the decrease of other soil microbial populations [17,34,49]. Our results from the principal coordinate analysis showed that after ASD disinfestation 30 d, the bacterial and fungal communities were mainly clustered by the same trial site, and then slightly clustered by treatments. This indicated that the location had a greater impact in soil microbial community composition compared to the different soil disinfestation treatments. Poret-Peterson et al. [27] obtained a significant, but minimal differences in community composition between ASD alternative substrates (molasses, mustard seed meal and tomato pomace) treatments and different trials and the soil samples were taken at 4 weeks for trial 1 and 5 weeks for trial 2.

Thirty days after ASD disinfestation, there was no significant difference in relative abundance for fungal and bacterial species between different treatment groups. The Adonis test was not significant between groups ($p = 0.617$). Importantly, the False Discovery Rate correction was applied during the analysis which has not been the case in previous studies highlighting significant changes in the microbiota. The main driver of microbial community was the location as observed in other studies. Another study indicated that the soil samples quantity and location were significantly affects the bacterial and fungal communities structure through richness, uniformity, diversity and dispersion between treatments or even replicates [54].

However, our HTS results also have many similarities with the literature reports, including the detection of taxonomic and relative abundance change at different levels of bacterial and fungal communities. In this experiment, Firmicutes are the main affected species of ASD, contributing a relative abundance of 18–19%. Studies have revealed that Firmicutes, Clostridia and Bacillus are prominent in microbial communities during ASD [14].Firmicutes constitute the core sharing community in the soil and contribute about 22% relative abundance. The results showed that ASD with maltose as carbon inputs change soil bacteria community composition toward to be diazotrophic and anaerobic [27]. Poret-Peterson's [27] study of maltose as a carbon source significantly altered the composition of anaerobic bacteria in the soil. In our study, maltose as a carbon source for ASD can increase Clostridia abundance by 88–190% (although not significant) compared with control, which is the most highly responsive taxa and shared core genera of ASD soil communities in the field trials belonged to the phyla of Firmicutes [27]. *Clostridium* are considered as anaerobic microorganisms, *Clostridium* well known for the antagonistic effect on plant pathogens, such as *F. oxysporum* f. sp. *Lycopersici* [44]. *Clostridium* is mainly associated with a variety of metabolic functions, such as fermenting sugar, starch, pectin and cellulose [55]. Many species of *Clostridium* have been reported to produce organic acids by anaerobic digestion of organic matter, such as butyric acid and acetic acid [16]. *C. beijerinckii* isolated from ASD soil and was examined have the abilities to suppress the spinach wilt disease pathogen (*F. oxysporum f.* sp. *spinaciae*) as a representative soilborne fungal plant pathogen [56]. Research reports that *Bacilli* and *Gammaproteobacteria* have denitrification capabilities [57,58]. However, (Figure 2a) showed that *Gammaproteobacteria* did not differ significantly between treatments, Pic and Sol significantly changed the abundance of *Bacilli*, while Mal6 and Mal9 had little effect on *Bacilli*. Further difference analysis showed (Figure 3c) that all treatments except Pic significantly increased the abundance of nitrogen-fixing bacteria *Azoarcus*, especially Mal9 increased by 23 times, indicating that ASD treatments stimulated the growth of *Azoarcus* [58].

The ASD or Sol treatment increased the proportion of Basidiomycete and reduced the relative abundance of the phylum Ascomycota and class Sordariomycetes in the soil. The relative abundance of the phyla Ascomycota increased as N-fertilizer addition increased [59]. Lauber et al. reported that the abundance of Sordariomycetes was significantly positively correlated with the extractable phosphorus

concentration, suggesting that possible effects of a higher phosphorus concentration may be needed for its growth [60]. Initial microbiota was the key driver of the reassembled fungal community as the environmental factors during ASD. The relative abundances of fungal order Sordariales was negatively correlated with disease incidence and positively associated with ASD-conditioned soil environment [61]. We observed a reduction of *Gibberella* and *Fusarium* relative abundance after soil fumigation in strawberry soils, but an increase in the relative abundance of *Cladosporium* spp. after ASD treatments. *Gibberella/Fusarium* is a species-rich group spreading all over the place. As a producer of plant pathogens and/or mycotoxins that contaminate human or animal food, it has severe adverse consequences for the agricultural economy [62]. *Cladosporium* spp. showed a high incidence on against whiteflies and aphids species tested (81% of the total isolated species) [63].

5. Conclusions

The laboratory studies indicated that ASD was very effect against *Fusarium* spp. and *Phytophthora* spp. The best mitigation conditions were created by higher air temperature and soil moisture, and the addition of maltose as a carbon source. The research indicates the necessity of sealed films for ASD added maltose, and TIF showed better inhibition for pathogens. The use of maltose as a carbon source and sealed with TIF for ASD showed high efficacy against *Fusarium* spp. and *Phytophthora* spp. in two field trials. In addition, compared with the control treatments, ASD can promote plant growth, reduce plant mortality and significantly increase the yield of strawberries. Furthermore, there was no significant difference between the use of a conventional pesticide and ASD. The microbiota analysis showed that there were no significant overall differences in bacterial and fungal community diversity between the treatments and control.

In general, our studies suggest that amendment maltose at 6 or 9 t/ha for ASD showed great application potential as an alternative for chloropicrin against the parasitic pathogens in developing countries. In particularly, maltose could be easily applied as a carbon source for ASD in the greenhouse by a drip irrigation system.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/12/13/5456/s1, Figure S1: Influencing factor level trend chart. Trend graph of ASD control effect against *Fusarium* spp. (left) and *Phytophthora* spp(right). with different influencing factors; Table S1: Orthogonal design factors and levels used to optimize a combination for enhanced effects of fungal pathogens; Table S2: Effects of the soil treatments on the numbers of colony-forming units (CFU) of *Fusarium* spp. (FUS) and Phytophthora spp. (PHY) on selective media in soil after fumigation based on the Orthogonal design shown in Table S1; Table S3: The effects of ASD treatments on the numbers of colony-forming units (CFU) of *Fusarium* spp. (FUS) of A×B combinations under multiple comparison test results.

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