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Non-Chemical Soil Fumigation for Sustainable Strawberry Production in Southern Italy

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Abstract: In intensive strawberry production, monoculture is a common practice worldwide; however, prolonged replanting can cause plant disorders and jeopardize profitable cultivation of this highly valuable crop. To mitigate replanting problems, the strawberry industry is still highly dependent on chemical fumigation. Given the increasing regulatory restrictions and concerns about human and environmental risks from fumigants use, there is a growing interest in the adoption of effective, non-chemical alternatives. Two non-chemical soil fumigation practices, i.e., anaerobic soil disinfestation (ASD) and bio-fumigation with biocide plants (BIOFUM), were tested against chemical fumigation by chloropicrin + 1,3-dichloropropene mixture (STANDARD) and untreated (UNTREAT) control in a 2-year trial established in a commercial strawberry farm in Southern Italy (40°25′ N, 16°42′ E). Overall, the alternative practices provided consistently better results than UNTREAT; whereas, compared to STANDARD, their performance was significantly different in the two years: in 2018/19 season the alternative practices registered a 20% (ASD) and 39% (BIOFUM) marketable yield loss compared to STANDARD, while in the 2019/20 season yield differences were not significant. Although both practices appear promising as eco-friendly alternatives to chemical fumigation, in this short-term trial ASD performed better than BIOFUM both in terms of yield and fruit size, resulting in a more advanced stage for practical adoption.

Keywords: *Fragaria* × *ananassa*; replanting; soil borne pathogens; bare-root plants; ASD; biofumigation; dry matter; leaf area; marketable yield; fruit quality

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1. Introduction

Strawberry is a fruit crop of high economic value, with an annual production of 1,300,000 t [1] and a positive trend in production, cultivated surface, and consumption [2] in the European Union. In intensive strawberry production, repeated planting in the same site is a common practice worldwide [3–6]; however, prolonged monoculture is often the cause of plant disorders, such as stunted development, weak root system, and marketable yield reduction [7–12]. Such disorders are associated to the so-called 'soil sickness' syndrome, whose mechanisms are complex and not fully elucidated so far. Biotic and abiotic factors are implicated [4], and in strawberry a major role is played by the accumulation of soil-borne pests, mainly nematodes (such as *Meloidogyne* spp. and *Pratylenchus* spp.), and fungal pathogens (*Pythium* spp., *Phytophthora* spp., *Verticillium dahliae*, *Rhizoctonia solani*, *Fusarium* spp., *Macrophomina* spp.) [13–15].

The strawberry industry (fruit and nursery) in Europe relies strongly on soil chemical fumigation to mitigate replanting problems, and the chloropicrin and 1,3-dichloropropene (1,3 D) mixture is still widely applied for the control of fungal pathogens, nematodes and weeds [2,6,16]. Due to their potential adverse health effects on farmers and nearby populations, these two products have been excluded (in 2013 and 2010, respectively) from the list of chemical fumigants allowed in the EU; nevertheless, their use is still allowed, under limited authorizations, for specific agricultural sectors, including the strawberry industry [6,16]. However, because of increasing regulatory restrictions in the EU, the use of chemical fumigants in the near future is unclear and uncertain. In the absence of viable alternatives, the negative impact that further restrictions or phasing out of currently allowed fumigants would have on strawberry industry from a socio-economic point of view, is an issue of great concern [2]. Uncertainty and increasing concerns about human and environmental risks on fumigants use have raised a growing interest towards the implementation of non-chemical strategies [5,17].

Anaerobic soil disinfestation (hereafter ASD) is a promising alternative to chemical fumigation [5,18–20]. Key-elements of this technique are the incorporation of easily decomposable organic amendments in the soil, and the creation of anaerobic conditions through soil irrigation and sealing with totally gas-impermeable films. This amendment stimulates microbial respiration and oxygen consumption, soil tarping minimizes gas exchange between the air and the soil, and irrigation reduces the initial soil oxygen level and facilitates the diffusion of metabolites and volatile compounds through the soil during the treatment [21]. Although the mechanism of disease suppression is not fully understood, dramatic changes in the composition of indigenous microbial community in ASD-treated soil were observed, with a domination of facultative and obligate anaerobes [5,17,18,22,23], responsible for the production and release of volatile organic compounds (VOCs) that are toxic to a broad range of soilborne pests and pathogens [19,24,25]. On strawberry, the ASD technique has already being applied on a commercial basis in California [5,26], Florida [20], Japan [18] and The Netherlands [27]. Easily decomposable solid or liquid organic substrates of various origin (e.g., rice or wheat bran, mustard seed meal, composted poultry litter, sugarcane molasses, diluted ethanol, etc.) proved suitable [5,18–20]. On the other hand, the used C-source generates unique VOCs profiles [25], playing a crucial role in the disease control efficacy of ASD technique [17,19]. Other key factors in determining ASD effectiveness are the anaerobicity threshold, and the soil temperature and moisture [5,20,28].

Another 'natural' alternative to chemical fumigation is biofumigation (hereafter BIOFUM), a technique that exploits the typical defensive system of the Brassicaceae family plants [29]. Indeed, many species of this family produce and hold, in different plant organs, large amounts of glucosinolates (GSLs), a class of plant secondary metabolites [30]. In their native form, GLSs generally have a weak biological activity; however, when cells are disrupted, in the presence of water, GLSs come into contact with and are hydrolyzed by the enzyme myrosinase, releasing various bioactive breakdown products, mainly isothiocyanates (ITCs) and, in less quantity, nitriles, epithionitriles and thiocyanates, depending on the reaction conditions [30,31]. Isothiocyanate compounds have a high biocidal activity over a wide range of pests [32–34] and pathogens [29,35,36]. Commonly used biofumigant species, such as brown and white mustard, rocket or radishes, contain different GSLs (sometimes a mixture of GSLs), hence produce different ITCs; different cultivars or plant parts may also contain different GSLs quantity or profiles [37,38].

Conventional application of the BIOFUM practice involves the growing of biofumigant green manures selected for their rusticity, high biomass production and content of specific GLSs, the fine chopping of the mature plants followed by immediate tillage into the soil and the soil tarping with plastic film. Since the concentration of GLSs in the seed is much higher than in the other plant organs, the feasibility of exploiting the residual meals of *Brassica* seeds from the industrial process of oil extraction, as the basic material to perform BIOFUM,

has also been investigated [39]. Brassica-derived defatted seed meals have proved effective on various crops to control different pests and pathogens also without soil sealing [40,41].

In recent years, commercial products useful to the application of both the ASD and BIOFUM techniques have been industrially developed, aiming to offer easier-to-use and more standardized C-source material and, possibly, more reproducible results. To evaluate the feasibility of ASD and BIOFUM techniques as non-chemical alternatives to chemical fumigation, we carried out a two-year trial in a highly productive strawberry-growing area strongly relying on fumigant agrochemicals to minimize soil replanting problems. Part of the results of the first-year trial were reported in [42].

2. Materials and Methods

The study was carried out for two consecutive strawberry crop seasons (2018/19 and 2019/20) in Scanzano Jonico (40°25′ N, 16°42′ E), in the coastal area of Basilicata region, Southern Italy. In this area, strawberry is mainly cultivated under plastic multi-tunnels to anticipate the harvest season, which usually ranges from January to May, and soil-borne pests and pathogens control is conventionally performed by pre-planting chemical fumigation using chloropicrin + 1,3-D mixture.

The two-season trial was performed in two distinct but adjacent land plots of the same commercial farm, both featured with sandy-clay loam texture (USDA classification), pH 7.7–7.9, active $CaCO_3$ < 0.5, total organic carbon 0.8–1.0%. In the previous 5 years, the two land plots had been cultivated with strawberries and annually fumigated with a mixture of chloropicrin (18 mL m^{-2}) and 1,3-D (20 mL m^{-2}), i.e., the standard farm fumigation practice. In both years, plot management followed the standard practices for multi-tunnel strawberry production in this area [43]; from mid-November until the end of harvest, plants were supplied with about 80 kg Ha^{-1} of N and P, and 73 kg Ha^{-1} of K through the drip irrigation system. Daily min and max air temperatures from October (planting) to May (end of harvest) were obtained from a meteorological station of the Basilicata Region located nearby the farm site [44], and the average mean monthly temperature was calculated.

2.1. Soil Treatments

In the 2018/2019 cropping season the following soil treatments were applied: (1) Untreated (UNTREAT) control; (2) Chemical fumigation (STANDARD) with a chloropicrin + 1,3-D mixture drip-injected [45], at the dose of 18 mL m^{-2} + 20 mL m^{-2}, respectively; (3) Biofumigation (BIOFUM) with defatted seed from Brassicaceae plants (commercial product: Biofence® pellets, Nutrien Italia S.p.A, Livorno, Italy [39], at the dose of 250 g m^{-2}) together with the growth promoters Kelidor G (inoculum of *Rhizophagus irregularis*, bacteria of the rhizosphere and *Trichoderma harzianum*, at the dose of 1 g m^{-2}), Fuspiù G (non-plant pathogenic oomycetes of the Nectriaceae family, at the dose of 2.5 g m^{-2}) and Radiforce WP (mycorrizial inoculum with a prevalence of *Thricoderma harzianum*, at the dose of 1 g m^{-2}) (Agrifutur s.r.l., Alfianello (BS), Italy); (4) Anaerobic Soil Disinfestation (ASD) (commercial product: Soil Resetting® granules, Thatchtec, Wageningen, The Netherlands, at the dose of 800 g m^{-2}). Soilresetting® is an easily decomposable, 100% plant-based organic matter product (https://thatchtec.nl/en/soil-resetting; accessed on 2 February 2021). Biofence® and growth promoters (BIOFUM) and Soil Resetting® (ASD) products were tilled into the soil at a 25–30 cm depth in mid-July 2018, then the treated plots were irrigated until field capacity. Immediately after watering, the ASD plots were tarped with a transparent, totally impermeable film (TIF, Agriplast, Vittoria, Italy), removed after three weeks; on the other hand, the BIOFUM plots were not tarped after watering. In mid-August, in all the treatment plots, 80 cm wide and 15 cm high beds were prepared; two irrigation drip lines were placed on top of the beds, which were mulched with 0.05 mm thick black polyethylene film; to allow complete weed control, interrows were mulched with black polypropylene sheets. At the beginning of September, the STANDARD plots were chemically fumigated through the drip irrigation system.

In the growing season 2019/20, the UNTREAT, STANDARD, BIOFUM and ASD treatments were applied following the same procedure and timing described above for 2018/19. A fifth treatment (ASD_mod) was added, consisting in making the following variation in the ASD application procedure: the incorporation of Soil Resetting® was carried out just before the preparation of the raised beds (21 August for all the treatments) and a double-layer plastic film (black polyethylene + transparent TIF) was used to mulch the beds. In ASD_mod plots, soil wetting was carried out through the drip irrigation lines directly under the mulch, irrigating the beds until field capacity.

In both years, Sabrosa* bare-root plants were transplanted in early October in double rows (300 per mulched bed), spaced 25 cm apart, at a density of 72 plants m^{-2}. The tunnels were covered with transparent polyethylene film at the beginning of November.

The experiment was designed as a randomized block with four replicates of 300 plants per treatment.

2.2. Plant Growth

In the 2018/2019 growing season, four randomly selected plants per treatment (one per replicate) were collected at each of the following three dates—24 January (15 days before the first picking date), 26 March (mid-harvest peak) and 1 May (end of harvest peak)—taking care that most of the root system was recovered. Sampling was repeated in the 2019/2020 season, and four plant treatment^{-1} were collected at each of the following four dates: 17 January (16 days before the first picking), 28 March (harvest peak), 7 May (end of harvest peak) and 25 May (harvest end). After washing the root system over a fine mesh sieve to eliminate soil residues, plants were divided into roots, crowns, and leaves (including petioles).

Total leaf area (LA, cm^2) of each plant was measured by using a LI-COR mod. LI-3000 area meter instrument (LI-COR, Lincoln, NE, USA). The fresh weight of roots, crown and canopy of each plant was recorded, then sub-samples of each plant part were bagged separately and dried to 60 °C until constant weight for the dry weight (DW) estimation.

2.3. Yield per Plant and Fruit Weight

In both crop seasons, the harvested fruit was separated into marketable and nonmarketable. Deformed, undersized (<22 mm of diameter) and diseased berries were considered as non-marketable (3% to 5% of the harvested fruit regardless of the treatments) and discarded without being recorded. At each picking date, the yield of the marketable fruit of each treatment and replicate was weighed, and the average fruit weight assessed on 20 randomly selected fruits. Total yield plant^{-1} (TY, g) was calculated by summing the marketable yield of each picking in each plot dividing by the number of plants. Average fruit weight (FW, g fruit^{-1}) was calculated according to the following formula:

$$\Sigma(\text{fw} \times \text{y}) \times \text{TY}^{-1} \quad (1)$$

where fw = average fruit weight in a picking date; y = yield at the same picking date; TY = Sum of yield in all picking dates.

2.4. Fruit Quality

In both crop seasons, fruit quality was measured on 10 fruits per replicate (40 treatment^{-1}) sampled from the marketable fruit harvested at four picking dates from March to April (during the harvest peak). On each fruit, skin color was measured on two opposite points by using the Minolta CR-300 (Minolta Co. Ltd., Tokyo, Japan) instrument. The CIE L* a* b* color scale values were also used to calculate the hue angle (h°), indicating color shade, according to:

$$\text{h}^\circ = \arctan\left[\text{b}^* \times \text{a}^{*-1}\right] \quad (2)$$

and chroma (C*), according to:

$$\text{C}^* = \left[\text{a}^{*2} + \text{b}^{*2}\right)]^{1/2} \quad (3)$$

L* represents the brightness, ranging 0 (black color) to 100 (white color); h° angle ranges 0° (red color) to 90° (yellow) and 180° (green); C* quantifies the intensity of h°, where higher values indicate saturation. For instrumental characterization of internal quality, flesh firmness was measured by using a Fruit Texture Analyzer (GUSS, Strand, South Africa), equipped with a 6 mm star-shaped probe. The soluble solid content (SSC) was measured by using an Atago (mod. DBX-55) digital refractometer on a drop of the total juice extracted from 10 fruits per replicate and expressed in °Brix. The titratable acidity (TA, mEq 100 g fresh weight) was measured by using a 702 SM Titrino automatic titrator (Metrhom, Herisau, Switzerland) on 5 g of the same juice, diluted with 25 mL distilled water and titrated with sodium hydroxide (NaOH) 0.1 N to pH 7.0.

2.5. Total Soil Microflora and Plant Mortality Assessment

In both years, composite soil samples of approximatively 1 kg were randomly collected at the onset (January) and at the end (May) of the harvest season in the first 20 cm depth of each plot and treatment. All soil samples were passed through a 10.0 mm sieve. Twenty-gram aliquots of the sieved soil mixture were suspended in 180 mL of sterile distilled water in 500 mL flasks and shaken for 30 min at 150 rpm. Potato-dextrose agar medium amended with 0.5 g L^{-1} streptomycin sulphate was used for total soil microflora (TSM) assessment [46]. In addition, selective media for *Pythium* spp., *Phytophthora* spp., *Rhizoctonia* spp. and *Macrophomina phaseolina* were used following the methods reported in [46,47], except for *Verticillium* spp., assessed through the real-time PCR method described in [48]. Ten- to 10,000-fold dilutions were made and plated on different media, four replicates per each plot and three replicates per medium and dilution were performed. Plates were incubated at room temperature and after 5–10 days colonies were counted. TSM content was estimated as number colony-forming units (CFU) g^{-1} dry soil. The presence of dead plants was annually monitored from January to May in all the treatments.

2.6. Statistical Analysis

The effect of treatments on plant growth, yield, fruit quality traits, and total fungal populations in the two years was assessed using analysis of variance (ANOVA). Data were first checked for normality and homogeneity of variance by applying Shapiro–Wilk and Levene's tests. Datasets satisfying the normality and homogeneity of variance assumption were then analyzed by ANOVA, followed by the post-hoc Tukey HSD test for mean separation at $p \leq 0.05$. CFU g^{-1} dry soil data, for which normality and homogeneity of variance could not be assumed, were LOG_{10}-transformed prior to ANOVA analysis. Statistics were performed by using the "Statistica" software package, version 6.0, Statsoft Inc.

3. Results and Discussion

The 2018/19 growing season was characterized by constantly lower temperatures from planting (October) to the harvest end (May) compared to 2019/20 season (Figure 1). Temperatures of the two years differed most in January (4.6 °C vs. 8.4 °C, respectively in 2019 and 2020) and February (7.6 °C vs. 10.2 °C), that is when, in strawberry winter planting systems with bare-root plants, plant production cycle starts [49].

3.1. Plant Growth Pattern and Dry Biomass Partitioning

Plant leaf (LA) and dry biomass (DW) data are reported in Table 1. In both years, LA and DW increased during the growing season and resulted to be highly correlated to each other in all the treatment plots ($r \geq 0.90$, Figure 2a,b). Soil pre-planting treatments significantly affected plant growth in both years (Table 1).

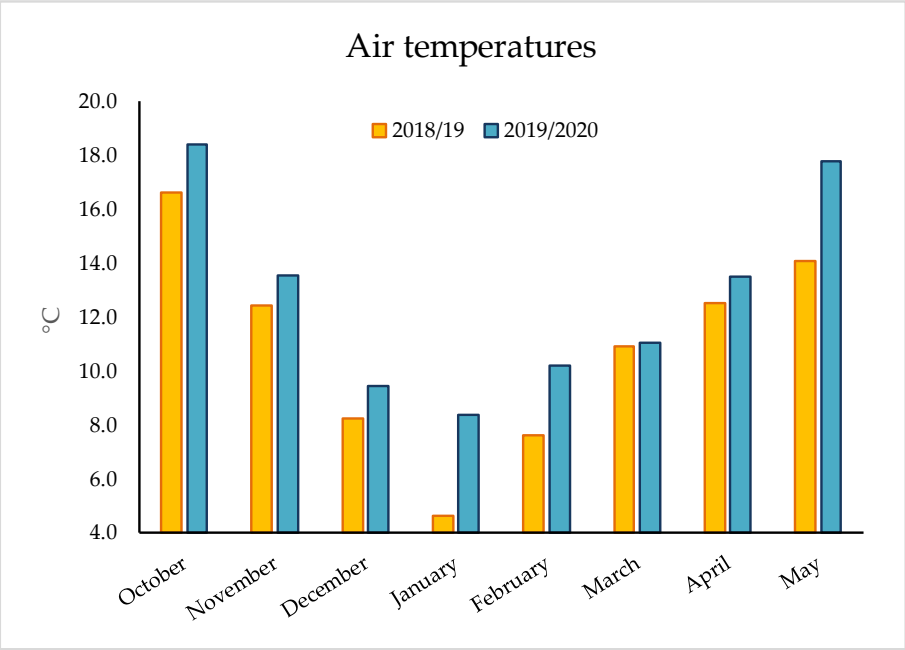

Figure 1. Mean monthly air temperatures from planting (October) to harvest end (May) in the 2018/19 and 2019/20 cropping seasons in Scanzano Jonico, Southern Italy. Data were obtained from a meteorological station located nearby the trial site belonging to the Basilicata Region (Centro Funzionale Decentrato (CFD), Regione Basilicata).

Table 1. Total leaf area (LA, $cm^2 \cdot plant^{-1}$) and dry weight (DW, g $plant^{-1}$) of cv. Sabrosa* plants developed under different soil pre-planting treatments in the 2018/2019 and 2019/2020 seasons in Scanzano Jonico, south of Italy.

	2019			2020			
	24 January	**26 March**	**1 May**	**17 January**	**28 March**	**5 May**	**25 May**
				Total Leaf Area (LA, cm^2)			
UNTREATED	168.3 ^{d}	823.8 ^{d}	991.3 ^{c}	389.0 ^{c}	1030.8 ^{c}	1279.9 ^{c}	1553.8 ^{c}
BIOFUM	349.2 ^{c}	1348.8 ^{c}	1676.8 ^{b}	527.8 ^{bc}	1810.3 ^{b}	2097.0 ^{b}	2423.3 ^{b}
ASD	614.5 ^{b}	1724.3 ^{b}	2019.5 ^{b}	730.4 ^{ab}	2004.5 ^{b}	2272.8 ^{ab}	2690.4 ^{ab}
ASD_mod	-	-	-	852.9 ^{a}	1984.8 ^{b}	2321.3 ^{ab}	2701.2 ^{ab}
STANDARD	747.5 ^{a}	1940.0 ^{a}	2306.3 ^{a}	880.8 ^{a}	2315.3 ^{a}	2690.6 ^{a}	2955.3 ^{a}
				Total Plant dry Weight (DW, g)			
UNTREATED	6.4 ^{d}	13.8 ^{d}	18.4 ^{d}	8.0 ^{b}	15.8 ^{d}	20.4 ^{c}	29.4 ^{c}
BIOFUM	10.2 ^{c}	22.3 ^{c}	28.6 ^{c}	10.9 ^{b}	29.1 ^{c}	34.6 ^{b}	42.1 ^{b}
ASD	16.3 ^{b}	28.7 ^{b}	44.3 ^{b}	15.8 ^{a}	28.9 ^{b}	41.9 ^{ab}	51.5 ^{ab}
ASD_mod	-	-	-	16.2 ^{a}	32.1 ^{ab}	43.6 ^{ab}	54.2 ^{ab}
STANDARD	19.5 ^{a}	34.1 ^{a}	50.9 ^{a}	17.0 ^{a}	35.7 ^{a}	46.4 ^{a}	59.5 ^{a}

Within the same sampling date, different superscript letters indicate significant differences among treatments ($p \leq 0.05$, Tukey HSD test).

In the 2018/19 cropping season, starting from the first sampling date, the plants in the UNTREATED plots had the lowest LA and DW values, resulting in being constantly the smallest; whereas, the plants in the chemically-fumigated plots (STANDARD) were the largest. Plant development in the ASD and BIOFUM plots was intermediate, with the former recording larger LA and DW values than the latter (Table 1). Reduced plant growth is a well-known phenomenon in strawberry replanting conditions when no soil disinfection treatments are implemented [3,8,9,14,50]. In our trial conditions, at the final sampling date (1 May 2019), the whole plant DW in the UNTREAT plots was only 36%, 41% and 64% of plant DW in the STANDARD, ASD and BIOFUM treatment plots, respectively. Similar

differences were found when comparing LA values of plants grown in the UNTREAT plots to those of the other soil treatments at the same date (Table 1).

In the four treatments, while leaf and crown DW increased during the sampling period (Figure 3a,b), root DW decreased (Figure 3c). Specifically, root DW decreased continuously in the plants of UNTREAT and BIOFUM plots, while in ASD and STANDARD plots values were stable in the first two sampling dates and decreased significantly in the sampling of 1 May.

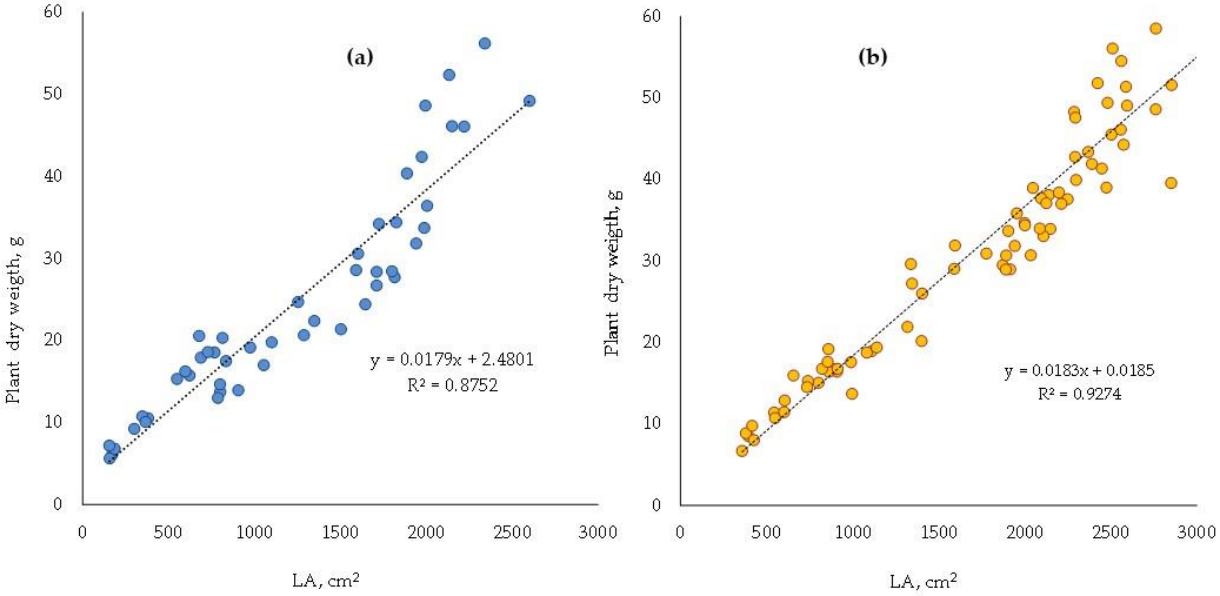

Figure 2. Relationship between plant leaf area (LA, cm^2) and dry weight (DW, g) in the first (**a**) (left) and the second-year (**b**) (on the right) trials.

The results in the second-year trial showed both similarities and differences compared to the previous year regarding the effect of treatments on plant growth.

As in the 2018/2019 growing season, plant DW and LA increased during the season (Table 1) and resulted to be highly correlated to each other ($r \geq 0.90$, Figure 2b) in all the treated plots. Since the sampling of January, and similarly to the first year results, the plants of the UNTREAT plots were the smallest (either in terms of LA and DW values) compared to the other treatments, including the ADS_mod which was introduced in the second-year trial. Among the soil treatments, the STANDARD was the best in terms of whole plant development although, unlike the first-year trial, growth differences with ASD were not statistically significant. Overall, plant growth in BIOFUM plots was lower than in STANDARD, statistically similar to ASD and ASD_mod plots, and higher than in the UNTREAT plots (Table 1).

At the end of the 2020 cropping season (sampling of 25 May), average plant DW in the UNTREAT plots was only 50%, 54%, 57%, and 70% of plant DW in the STANDARD, ASD_mod, ASD and BIOFUM treatment plots, respectively. Similar differences were found comparing the average LA values of plants of the different treatments at the same date (Table 1).

Also, in the second-year trial, leaf and crown DW tended to increase across the sampling dates (Figure 4a,b), while root DW tended to decrease (Figure 4c). However, differently from the first year, the root DW did not record significant differences among treatments at the end of the harvest season (Figure 4c, samplings of 7 and 25 May). Finally, some root growth recovery, although statistically not significant, was noticed at the last sampling date in all treatments. This pattern was not observed in the first-year trial, where plant sampling ended about a month before the harvest end.

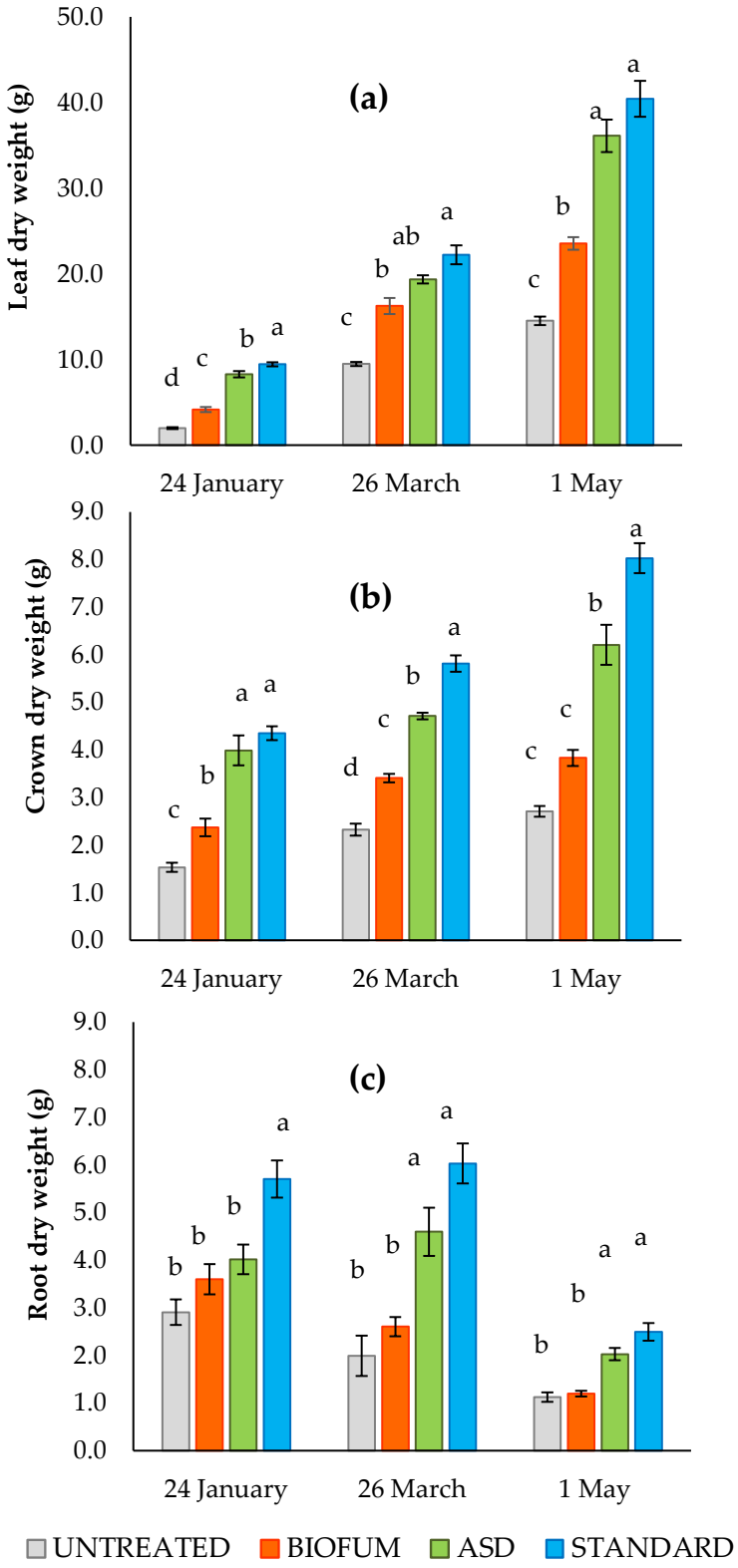

Figure 3. Effect of different soil pre-plant treatments on the leaf (**a**) (top), crown (**b**) (middle), and root (**c**) (below) dry weight of Sabrosa* plants during the 2018/19 cropping season (first year trial). Bars are average ± standard error. Within the same sampling date, bars marked by different letters are significantly different ($p \leq 0.05$, Tukey HSD test).

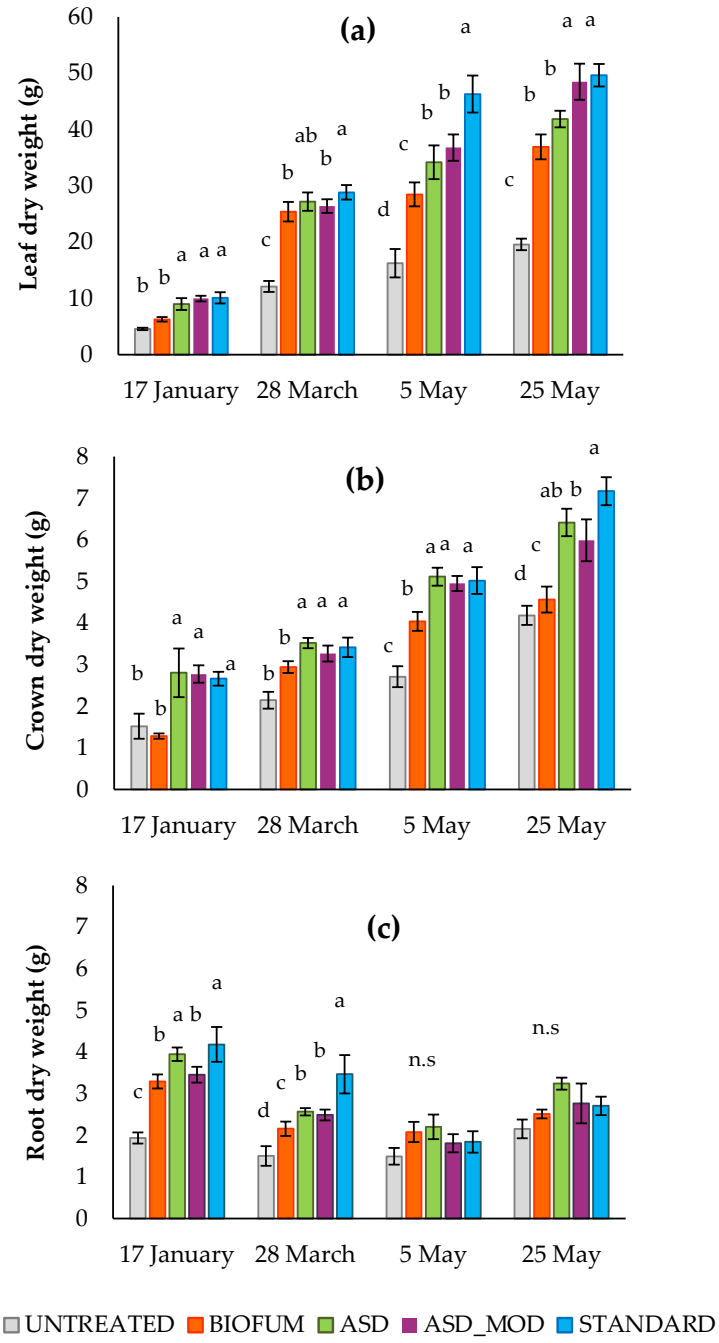

Figure 4. Effect of the different soil pre-plant treatments on the leaf (**a**) (top), crown (**b**) (middle), and root (**c**) (below) dry weight of Sabrosa* plants during the 2019/20 cropping season (second year trial). Bars are average ± standard error. Within the same sampling date, bars marked by different letters are significantly different ($p \leq 0.05$, Tukey HSD test); n.s. = non significant ($p \leq 0.05$).

The leaf, crown and root biomass growth patterns observed in this study were consistent with those observed in a study carried out in North Carolina on bare-root plants of three strawberry cultivars grown in a similar winter planting system [51]. In the North Carolina study, regardless of the cultivars, the root biomass development was the largest from planting (October) until February–March, and rapidly decreased once the plants started to produce flowers and fruits, resulting in being very reduced at the end of the cropping season. The crown and, to a much greater extent, the leaf biomass, conversely, increased throughout the harvest period. In our study, in both years, the soil pre-planting treatments significantly influenced the amount of plant biomass, but did not alter the pattern of leaf,

crown and root development along the season. These patterns, indeed, seem to be typical of the cultivated strawberry plant and little influenced by different cultivar [51] or plant type (i.e., bare-root vs. plug plants) [52].

3.2. Harvest Season Pattern and Yield Performance

The 2018/2019 harvest season lasted 111 days and was completed in 20 pickings (Figure 5a). Harvest frequency, i.e., the interval time between one picking and the following, was usually of 4–5 days. In all treatments, most of the crop (≥75% out of total yield plant^{-1}) was harvested between 25 March and 1 May pickings (37 days). The 2019/20 cropping season was similar for duration and number of pickings to the previous one (Figure 5b); however, the harvest curve pattern in the second year was more gradual and without the sharp harvest peak of the previous year, presumably due to the more regular and favorable climatic conditions before and along the harvest period.

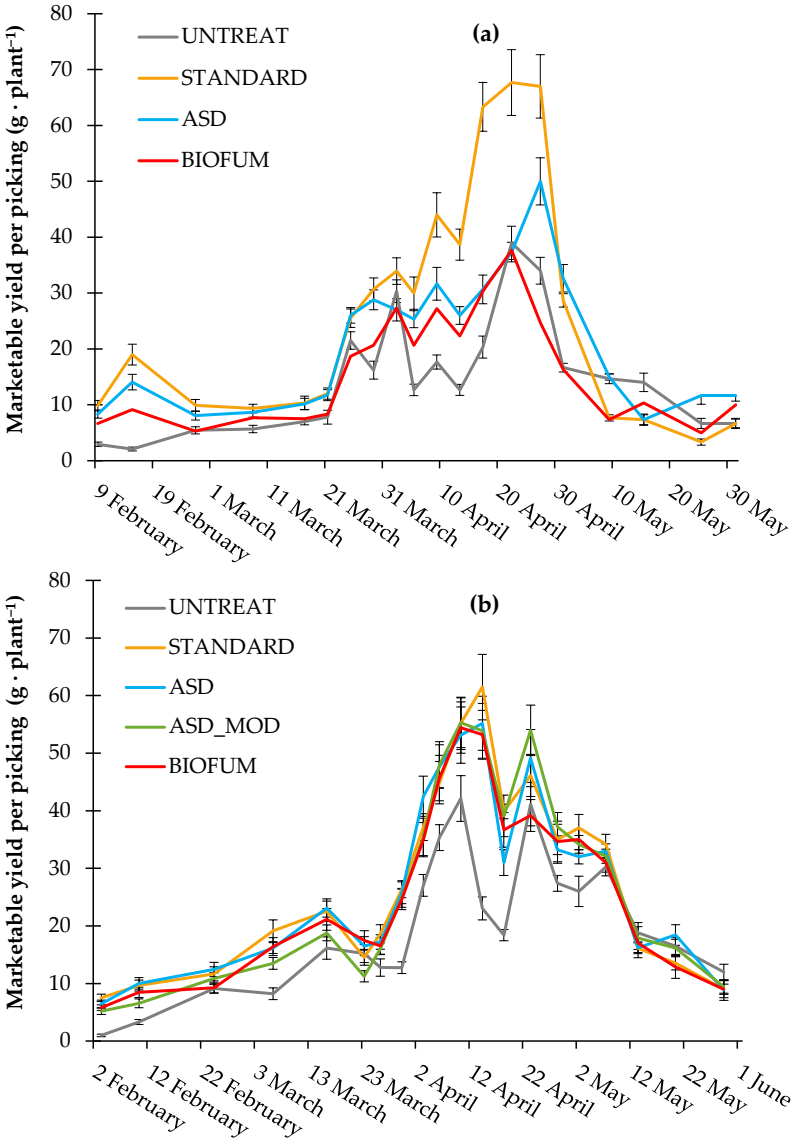

Figure 5. Patterns of the harvest seasons 2018/2019 ((**a**) above, adapted from [42]) and 2019/2020 ((**b**) below) of Sabrosa* under different soil pre-planting treatments. Average yield plant^{-1} ± standard error.

Yield data in the two cropping seasons are reported in Table 2. While in the STANDARD plots the yield performance was similar in the two years, the yield plant^{-1} of the

other treatments was considerably higher in the second year, with differences ranging from 100 ÷ 120 g plant^{-1} in the UNTREAT and ASD plots to 200 g plant^{-1} in BIOFUM plots.

Table 2. Total yield and main fruit qualitative characteristics of Sabrosa* under different soil pre-planting treatments in the 2018/19 and 2019/20 cropping seasons. In both cropping seasons, weight, flesh firmness, SSC, TA, and L*, Chroma (C*) and Hue (°h) values are the mean of four sampling dates from March to April (during the harvest peak).

Harvest Season 2019									
Treatment	**Total Yield**		**Fruit Weight**	**Flesh Firmness**	**SSC**	**TA**	**L***	**C***	**°h**
	g plant^{-1}	**t Ha^{-1}**	**g**	**g**	**°Brix**	**mEq 100 g FW^{-1}**			
UNTREATED	294.0 ^{c}	21.2 ^{c}	24.5 ^{c}	589.4 $^{n.s.}$	8.7 $^{n.s.}$	10.7 $^{n.s.}$	37.9 $^{n.s.}$	44.5 $^{n.s.}$	27.7 $^{n.s.}$
STANDARD	524.9 ^{a}	37.8 ^{a}	29.3 ^{a}	581.0	8.0	9.9	38.0	45.2	28.6
BIOFUM	323.5 ^{c}	23.3 ^{c}	26.0 ^{b}	623.0	8.6	10.6	37.9	44.5	26.3
ASD	422.2 ^{b}	30.4 ^{b}	27.7 ^{ab}	629.0	8.3	10.7	38.1	44.3	26.9
Harvest Season 2020									
Treatment	**Total Yield**		**Fruit Weight**	**Flesh Firmness**	**SSC**	**TA**	**L***	**C***	**°h**
	g plant^{-1}	**t Ha^{-1}**	**g**	**g**	**°Brix**	**mEq 100 g FW^{-1}**			
UNTREATED	396.5 ^{b}	28.4 ^{b}	22.6 ^{c}	646.1 $^{n.s.}$	8.0 $^{n.s.}$	10.8 $^{n.s.}$	37.6 $^{n.s.}$	44.4 $^{n.s.}$	30.4 $^{n.s.}$
STANDARD	559.6 ^{a}	40.3 ^{a}	28.4 ^{a}	601.0	7.5	9.1	38.1	45.0	29.1
BIOFUM	523.4 ^{a}	37.7 ^{a}	25.6 ^{b}	599.0	7.8	10.0	36.9	43.6	29.7
ASD	548.0 ^{a}	39.5 ^{a}	27.0 ^{a}	610.0	7.7	9.8	37.5	44.0	30.2
ASD_mod	539.7 ^{a}	38.9 ^{a}	27.4 ^{a}	624.0	7.9	10.5	37.9	44.6	30.2

Within the same column and year, different superscript letters indicate significant differences among treatments ($p \leq 0.05$, Tukey HSD test); 'n.s.' indicates no significant difference. Data of harvest season 2019 are from [42].

Yield performance was significantly influenced by soil treatments (Table 2). In the first year of trials, the STANDARD plots were the most productive while the UNTREAT were the least, although not significantly different from the BIOFUM plots (Table 2); yield ·plant^{-1} of the ASD plots was lower than the STANDARD and higher than the BIOFUM and UNTREAT. In the absence of any soil treatment (UNTREAT), the yield loss was 44% as compared to the STANDARD. The two alternative techniques limited but not eliminated production losses vs. the STANDARD plots, with a significant yield reduction of 20% (ASD) and 38% (BIOFUM).

In the 2019/20 cropping season, the UNTREAT plots were the least productive (Table 2), with a 30% yield loss compared to the STANDARD plots, which—although lower than in the previous year—is still an important difference. Interestingly, and differently from the previous year results, the yield performance of the BIOFUM and ASD plots was statistically not different from that of the STANDARD plots (Table 2). The better yield results of the non-chemically-treated plots in the second-year trial are presumably due to the more favorable climatic pattern, which prevented from the onset of replant-related stress conditions affecting plant productivity, as it had occurred in the first year. Finally, the similar yield values in the ASD and ASD_mod plots are a very promising result, considering that the latter treatment allows a simplification in the application of the ASD technique.

3.3. Fruit Weight and Other Quality Traits

Data of fruit weight and other relevant qualitative traits of the two cropping seasons are reported in Table 2. The average fruit weight was higher in 2018/19 than in 2019/20, with a difference ranging from 0.9 g in the ASD and STANDARD plots up to 1.9 g in the UNTREAT plots. The larger fruit load could partly explain the lower average fruit weight in the cropping season 2019/20, despite the more favorable weather trend and the greater canopy development in the second- vs. the first-year trial (Table 1). Soil treatments significantly affected the average weight of the fruit. In the first-year trial, average fruit

weight in the UNTREAT plots was the lowest and, compared to the other treatments, the loss in weight ranged from 6% (BIOFUM) to 16% (STANDARD) (Table 3). In the ASD-treated plots, average fruit weight was intermediate between the STANDARD and the BIOFUM treatments, and statistically not different from any of them (Table 3). The lowest fruit weight in the UNTREAT plots was confirmed in the second cropping season (Table 3), with a loss ranging from 9% (vs. BIOFUM) to 20% (vs. STANDARD). Average fruit weight in the ASD plots was statistically similar to that in the STANDARD plots, also when the ASD technique was applied directly in bed (ASD_mod treatment). The smaller fruit size in the BIOFUM than in the STANDARD plots was confirmed also in the second year of evaluation (Table 2). Compared to the UNTREAT, the alternative techniques (including the ASD_mod) had an ameliorative effect on this important commercial parameter. In both years, the average fruit weight in the STANDARD plots was in the same range of fruit size of the other parts of the farm cultivated with Sabrosa* and chemically fumigated (data not shown). Finally, in both years, the different soil treatments did not significantly affect the other fruit quality parameters: flesh firmness, soluble sugar content, titratable acidity, brightness (L), chroma and hue.

Table 3. Effect of the different soil pre-planting treatments on total microflora content in the 2018/19 and 2019/20 cropping seasons.

Total Microflora Content				
	2018/19		2019/20	
	January	May	January	May
Treatment	Log_{10} CFU g^{-1} dry Weight Soil			
UNTREAT	4.64 ^{bA}	4.84 ^{bA}	5.32 ^{aA}	5.33 ^{aA}
STANDARD	4.42 ^{bA}	3.81 ^{cB}	4.95 ^{bA}	5.04 ^{bA}
BIOFUM	4.68 ^{bB}	5.81 ^{aA}	4.98 ^{bA}	4.98 ^{bA}
ASD	5.32 ^{aA}	5.13 ^{bA}	5.44 ^{aA}	5.32 ^{aB}
ASD_mod	-	-	5.30 ^{aA}	5.16 ^{abB}

Within the same column, different superscript lower-case letters indicate significant differences among treatments, whereas different superscript upper-case letters within treatment and year indicate significant differences between January and May samplings ($p \leq 0.05$, Tukey HSD test).

3.4. Total Soil Microflora and Plant Mortality Assessment

At the beginning of the first harvest season (January 2019), TSM content was the highest in ASD compared to the other treatment plots (Table 3). In the interval time between January and May samplings, TSM content increased significantly in BIOFUM plots and decreased in STANDARD plots, so that the microflora concentration in May was the highest in BIOFUM, the lowest in STANDARD, and intermediate in ASD and UNTREAT plots. In the second-year trial, the TSM content in January was the highest in ASD, ASD_ mod and UNTREATED plots, and the lowest in STANDARD and BIOFUM plots, resulting rather stable in the interval time between January and May 2020 soil samplings, except for ASD and ASD_mod plots, where TSM content decreased significantly (Table 3). In the two years of trials, soil microflora concentration did not record a consistent upward or downward trend along the cropping season and/or across treatments and did not reflect plant growth and yield performances of the different treatments.

The percentage of dead plants was below 1% in both years and no visual disease symptoms were observed across treatments, including the UNTREAT control. Analyses of soil samples confirmed that, no matter the treatments and the sampling dates, the content of the root pathogens *Verticillium* spp., *Rhizoctonia* spp., *Macrophomina phaseolina*, *Phytophthora* spp. and *Pythium* spp. was always below the limit of detection (LOD). The lack of a strong pressure of lethal pathogens in the trial conditions, i.e., soils chemically fumigated for several consecutive years, could explain the very high plant survival percentage in our trials.

In previous studies on strawberry, the lower vegetative growth and productivity observed in non-fumigated vs. fumigated plots in the absence of plant mortality was explained as the effect of sub-lethal and competitive soil organisms [8,50]. We cannot exclude that also soil-borne nematodes played a role in plant growth and yield performance reduction in the non-fumigated vs. the STANDARD plots, and to a different extent depending on the treatment. However, since no specific investigation on nematodes was carried out in the present trial, any possible effect of these parasites on plant performance in the different treatments cannot be quantified and falls, indistinctly, into the negative effect exerted by 'sub-lethal and competitive soilborne organisms'.

4. Conclusions

The two-year test showed that, even in the absence of a strong pressure from lethal soilborne pathogens, strawberry replanting with no soil disinfection treatments strongly reduced plant growth, yield and fruit size in comparison to soil fumigation with chloropicrin and 1,3 D, the commercial practice in the area where we carried out the trial. In the trial conditions of ASD by using Soil Resetting® granules and BIOFUM with Biofence® pellets techniques provided significant improvement with respect to the untreated control and, although less performant than STANDARD chemical fumigation in mitigating the replant-related effects, show potential as non-chemical alternatives to chemical fumigation. Considering the yield and fruit quality performance in the two years of trial, the ASD technique looks more promising than the BIOFUM. However, the soil sealing with clear TIF film in the traditional application of the ASD treatment is perceived as a limiting factor to the practical adoption of this technique. Interestingly, the new procedure implemented and tested in the second-year trial allowed a simplification in the ASD application, without penalizing crop performance. BIOFUM and ASD_mod techniques, as applied in our study, can be easily integrated into the strawberry farming practice, and their implementation does not imply additional costs compared to STANDARD fumigation, as in ASD_mod application, or even allows a saving of up to 30% in the case of BIOFUM.

Further investigations are needed to ensure better and more consistent results over the years. Long-term trials where treatments are consecutively applied on the same plots might allow an evaluation of the cumulative effect of the alternative techniques on soil biota evolution and crop response. Finally, large-scale testing/demonstration involving growers' associations, including the organic sector, are required to shift from the experimental to the applicative phase and to help evaluating whether these practices are economically sustainable and adoptable in the commercial practice.

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