

Effect of Anaerobic Soil Disinfestation with Yeast Amendment on Weed Control and Strawberry Yield

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Abstract. Anaerobic soil disinfestation (ASD) is a promising alternative to chemical fumigation for managing soilborne pathogens and weeds in strawberry production. Field trials were conducted to evaluate ASD using brewer's spent grain (BSG) and yeast inoculation in an annual hill plasticulture system. This study compared ASD treatments at full and half carbon (C) amendment rates with and without yeast, a fumigation treatment with chloropicrin plus 1,3-dichloropropene, and nontreated controls. Application of ASD was performed for 21 days, and soil temperature and anaerobic conditions were monitored. Weed density, strawberry yield, and fruit quality were assessed. The results indicated that ASD significantly reduced weed density compared with nontreated plots in both growing seasons. Although strawberry yield was not significantly different between ASD-treated and nontreated plots in the first season, ASD-treated plots exhibited higher yields in the second season. Yeast addition enhanced crop yield in ASD-treated plots, suggesting that it can improve ASD effectiveness. These findings support the potential of yeast-amended ASD as a viable strategy for integrating weed and soilborne disease management while reducing chemical fumigant use.

In Virginia, strawberries are mostly grown using an annual hill plasticulture (AHP) production system, with strawberry plants transplanted in early fall (Christman and Samtani 2025). The lack of rotation with other crops, including cover crops, at many small-acreage strawberry farms can degrade soil health and increase pest pressure in the long-term.

Competition between strawberries and annual weeds can result in loss of yield and reduced net return. Troublesome annual weeds include common chickweed (*Stellaria media* L. Vill.), redroot pigweed (*Amaranthus retroflexus* L.), and Shepherd's purse (*Capsella bursa-pastoris* L. Medik.), biennials include wild carrot (*Daucus carota* L.), and perennials include dandelion (*Taraxacum officinale* L. Weber ex F.H.Wigg.), quackgrass (*Elymus repens* L. Gould), white clover (*Trifolium*

repens L.), and yellow nutsedge (*Cyperus esculentus* L.) (Melanson et al. 2024).

Weed control is essential in strawberry plasticulture systems; however, limited herbicide options, largely because of low economic incentives for chemical companies to register products for specialty crops, make weed control a persistent and nationwide challenge (Fennimore and Doohan 2008). Currently registered herbicides have significant limitations. Oxyfluorfen and flumioxazin have a 30-d pre-plant application period and there are crop phytotoxic risks with herbicides such as flumioxazin and napropamide (Fennimore and Doohan 2008; Melanson et al. 2024). Therefore, the need to develop reliable alternative weed control practices are increasing.

Anaerobic soil disinfestation (ASD) has demonstrated efficacy in suppressing many soilborne pests across a diversity of cropping systems and environments (Shennan et al. 2014, 2018; Shrestha et al. 2018a; Rosskopf and Di Gioia 2023). The method involves using several tons of decomposable organic materials per hectare, irrigating to field capacity, and covering with polyethylene film to block gas diffusion and create an anaerobic condition. Under anaerobic conditions, the carbon (C) source is decomposed by facultative and obligate anaerobic microorganisms. These microorganisms could produce toxic or suppressive compounds such as organic acids, aldehydes, alcohols, metal ions, and volatile organic acids (Huang et al. 2015).

In general, the types and rates of C sources are essential components of ASD. Carbon sources should contain sufficient labile C and have a moderate ratio of C to nitrogen (N) to support soil microbial growth (Momma 2008; Rosskopf and Di Gioia 2023). Brewer's spent grain (BSG) is a solid byproduct generated from the beer-brewing process. The BSG represents approximately 85% of the total byproducts of brewing. The main composition of BSG includes exhausted grain husks obtained after mashing and lautering. Additionally, BSG could be a potential source of C for ASD because of three reasons. First, BSG is currently available free of material costs because BSG is a waste of beer production. The number of draft breweries in Virginia and neighboring states is increasing. Second, BSG can produce ethanol when mixed with distiller's yeast (*Saccharomyces cerevisiae* Meyen ex E.C. Hansen) (Liguori et al. 2015), and ethanol is a useful C source for ASD (Momma et al. 2013). Because of ethanol's high cost, using low-cost BSG to produce bioethanol under field conditions could be feasible. Moreover, Horita and Kitamoto (2015), Liu et al. (2023) and Shirane et al. (2023) showed that organic residue from bioethanol fermentation had the potential to enhance ASD treatment. Third, fresh BSG has an optimal C:N ratio of approximately 14:1, which could reduce the N inputs from fertilizer. The recommended C:N ratio ranges from 10:1 to 35:1 (Shennan et al. 2018). Liu et al. (2020, 2023) evaluated the effect of several C sources mixed with ethanol and yeast on weed control under controlled environment conditions, which indicated the potential of a yeast amendment in ASD to enhance the suppression effect of ASD. No research of the performance of ASD using BSG and yeast under field conditions has been reported.

Using low C rates for ASD has been identified as a challenge by various researchers who have explored the relationship between C input and pest suppression. The efficacy of various organic amendments is influenced by their C:N ratio, with different C sources exhibiting varied impacts on disease suppression outcomes (Daugovish et al. 2021; Hewavitharana et al. 2021; Shrestha et al. 2018a, 2018b). The C:N ratio of organic amendments has a strong influence on the efficacy of ASD that affects microbial activity and the production of bioactive compounds. For example, amendments with a lower C:N ratio (e.g., 10:1–20:1), such as soybean meal, tend to promote more rapid decomposition and production of organic acids, thus enhancing pathogen suppression, suppressing weed growth, and improving overall soil health (Di Gioia et al. 2025; Nagargade et al. 2018; Osipitan et al. 2022). In contrast, amendments with a higher C:N ratio may lead to slower microbial activity and reduced efficacy, thereby decreasing their effectiveness for weed suppression over time (Braun et al. 2019; Hewavitharana et al. 2021; Prasanna and Sharma 2023; Shrestha et al. 2018a). Recent studies further demonstrated that amendments

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with low C:N ratios (e.g., soybean meal at approximately 6:1) maintain higher levels of nitrate N during and after ASD, leading to improved crop performance, whereas high C:N sources (e.g., molasses at approximately 57:1) can immobilize N and reduce crop yield potential (Di Gioia et al. 2025). These studies demonstrated that selecting C sources with optimal C:N ratios is critical for achieving effective anaerobic conditions and pest control. For instance, Shrestha et al. (2018a, 2018b) discussed how varying C:N ratios in amendments such as molasses and rice bran could significantly alter pest suppression abilities in crops like tomato and strawberry. The implication is clear that optimum C input is fundamental for achieving anaerobic conditions that are conducive to pathogen control.

Moreover, as indicated by multiple studies, the integration of organic amendments with lower C rates can still facilitate effective anaerobic conditions, which are critical for the deployment of ASD across diverse agricultural landscapes (Liu et al. 2023; Vecchia et al. 2020). Results from field trials indicated that adjustments of C rates can lead to significant variations in pathogen suppression, underscoring the need for tailored C management strategies based on specific cropping systems and regional challenges (Shi et al. 2019; Shrestha et al. 2016; Singh et al. 2022). Without dedicated research focused on low C rates applied under authentic field conditions, our understanding of the full potential of ASD remains incomplete. An increased focus on low C rates in ASD is essential not only because of its identified challenges but also because it aligns with broader sustainability goals in agriculture. These goals include promoting soil health and encouraging the use of locally available organic materials. By incorporating amendments such as BSG or rice bran, ASD stimulates beneficial microbial activity under anaerobic conditions, thus suppressing soilborne pathogens while minimizing environmental impacts (Daugovich et al. 2021; Shennan et al. 2018). This approach supports long-term agroecosystem resilience through reduced chemical inputs, improved soil biodiversity, and enhanced nutrient cycling (Roskopf and Di Gioia 2023; Shrestha et al. 2016). Moreover, ASD enables the

repurposing of agricultural byproducts, thus contributing to circular economy practices and lowering production costs for growers (Poret-Peterson et al. 2019). As such, ASD represents a viable strategy for sustainable crop production in specialty crop systems, particularly when combined with complementary approaches such as crop rotation, grafting onto resistant rootstocks, biological control agents, and genotype-specific cultivar selection. While studies have demonstrated synergistic benefits in yield, pathogen suppression, and economic returns through such integrations (Chattha et al. 2025; Donahoo et al. 2021; Khadka and Miller 2021; Zavatta et al. 2014), further research is needed to evaluate the consistency, scalability, and long-term impacts of these combined strategies across diverse crops, environments, and management systems. The objectives of this study were to evaluate weed density, crop nitrogen status, and strawberry quality and yield following ASD using BSG and yeast inoculation in open-field production and determine whether yeast could enhance ASD effectiveness when ASD is applied at a half C rate.

Materials and Methods

Experimental design. Field trials were conducted at the Hampton Road Agricultural Research and Extension Center, Virginia Beach, VA, USA (36°9'N, 76°2'W) during the 2018–19 and 2019–20 growing seasons. Both trials were arranged in a randomized complete block design with four blocks. In the 2018–19 growing season, three blocks had a history of prior strawberry cultivation, and one block was under grassy vegetation that was mowed on regular basis as needed. The soil type at the site was tetotum loam (sandy loam, moderately well-drained, deep; parent material: loamy, fluvial, and marine sediments) (US Department of Agriculture Natural Resources Conservation Service 2006). Preplant soil tests were conducted in both seasons by sending soil samples to the Virginia Tech. Soil Testing laboratory in late July. The soil test report provided recommendations for pH adjustment. The soil pH was adjusted to 6.2 by broadcasting limestone at 898 kg·ha⁻¹ in early Aug 2018. There were

seven treatments in both growing seasons, with each replicate comprising a bed with a length of 10.7 m, width of 0.8 m on the bed tops, and height of 0.15 m. The beds were oriented north–south. All seven treatments (Table 1) were randomized in each block. After treatment completion in both growing seasons, the center 4.6 m length of each bed was used for strawberry plug transplanting and data collection (Fig. 1). Within this section, weed monitoring window with a length of 1.5 m and width of 0.8 m was established on the bed top and covered with a clear tarp to assess weed emergence without interference from strawberry plants.

Samples of BSG (Commonwealth Brewing Company, Virginia Beach, VA, USA) were sent to the University of Tennessee (NC soil analyzer; CE Elantech, Lakewood, NJ, USA) for C:N ratio determination in late Jul 2018. The recommended rate of the C source in ASD is 4 mg C·g⁻¹ soil (Shennan et al. 2018). The BSG had a C:N ratio of 14:1, which indicated that BSG would provide 271 kg·ha⁻¹ organic N to the soil at the 4 mg C·g⁻¹ soil rate. The total N from BSG was much higher than the soil test recommendation and the recommendation from the regional production guide (Poling et al. 2005). Thus, to reduce the N added to the soil, the BSG was mixed with pelleted paper mulch (C:N ratio 57:1; Lebanon Seaboard Corporation, Lebanon, PA, USA). However, three-times more paper mulch than BSG was present in the mixture when the N rate was 78 kg·ha⁻¹, which was the N rate recommended by the soil test. Because our study primarily focused on the effect of BSG, there was a need to increase the BSG volume at least equal to the paper mulch volume. The C:N ratio of BSG (14:1) was much lower than that of paper mulch (57:1). Thus, when the C source mixture was at a constant C rate, the proportion of BSG increased compared with paper mulch, which also increased the total N rate. To reach a balance between the BSG volume and total N rate, the N rate used in this study for all plots was 117 kg N·ha⁻¹. With that N rate, 60% (w/w) fresh BSG was in the mixture with the full C rate and 97% (w/w) fresh BSG was in the mixture with the half C rate. Synthetic preplant N fertilizers (urea 46–0–0; PCS Sales, Inc., Northbrook, IL,

Table 1. Treatment list including the rates of carbon (C) sources in the bedded area and nitrogen (N) rate from both C sources and fertilizer.

Treatments ¹	Yeast (kg dry matter·ha ⁻¹)	BSG (kg dry matter·ha ⁻¹)	Paper mulch (kg dry matter·ha ⁻¹)	N from C sources (kg dry matter·ha ⁻¹)	N from synthetic fertilizer (kg·ha ⁻¹)	Total C (kg·ha ⁻¹)	Total N (kg·ha ⁻¹)
ASD, C source full without yeast	0	4,082	12,330	117	0	6,700	117
ASD, C source full with yeast	10	4,082	12,330	117	0	6,700	117
ASD, C source half without yeast	0	6,080	1,570	117	0	3,350	117
ASD, C source half with yeast	5	6,080	1,570	117	0	3,350	117
Nontreated control without yeast	0	0	0	0	117	0	117
Nontreated control with yeast	10	0	0	0	117	0	117
1,3-D + Pic	0	0	0	0	117	0	117

¹The rates of C sources and yeast were as follows: full rate 4 mg C·g⁻¹ soil, 6.7 t C·ha⁻¹, yeast full rate 10 kg·ha⁻¹; half rate 2 mg C·g⁻¹ soil, 3.35 t C·ha⁻¹, yeast half rate 5 kg·ha⁻¹; and nontreated control with yeast 10 kg·ha⁻¹. The rate of 1,3-D + Pic was 196 kg·ha⁻¹. The BSG had 44% C and 3.7% N base on dry matter. The BSG was applied was fresh matter with 69% moisture content (w/w). The weight of fresh BSG for the C full rate was 13,169 kg·ha⁻¹, and that for the C half rate was 19,614 kg·ha⁻¹. The paper mulch applied was dry matter, which had a 40% C rate and 0.7% N rate.

ASD = anaerobic soil disinfestation; BSG = brewer's spent grain.

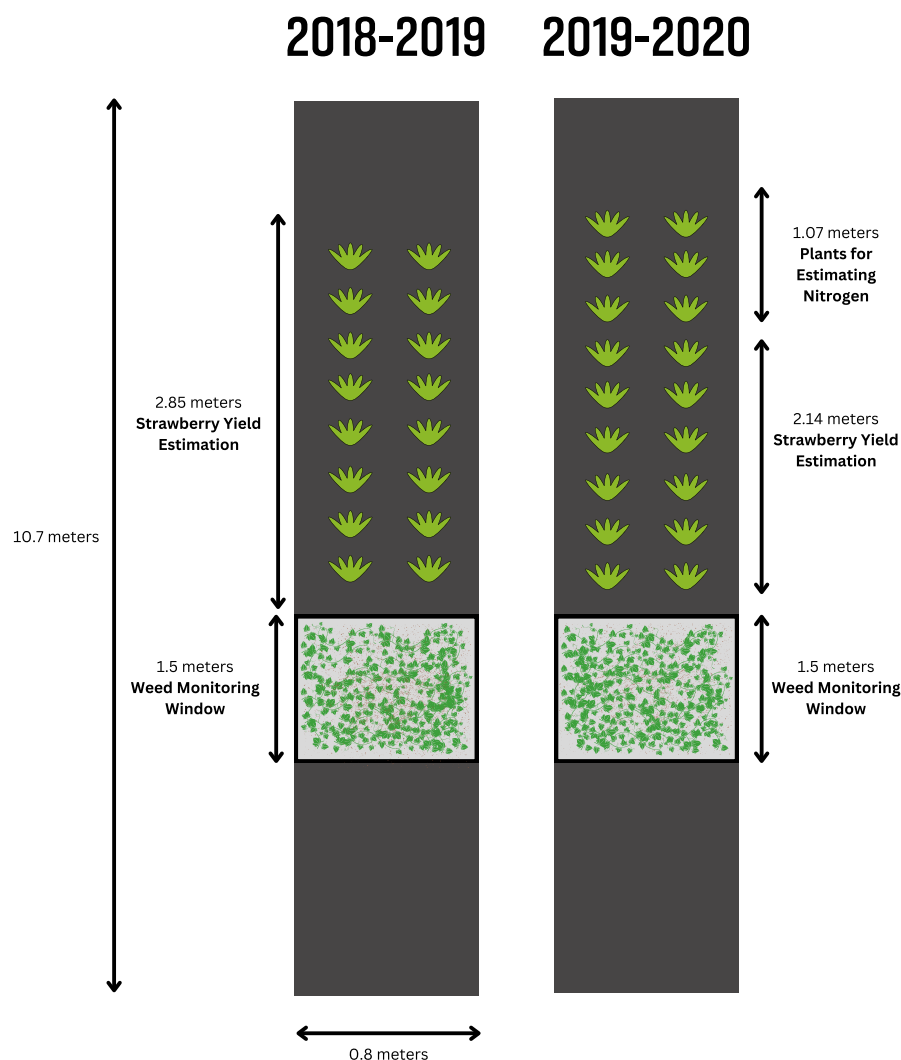


Fig. 1. An illustration (not to scale) of the weed monitoring window, crop yield, and nitrogen estimation per replicate in the two growing seasons. No strawberry crop planting was conducted within the weed window section. Illustration credit: Alana Martin.

USA) at the aforementioned rate were only applied to nontreated control plots and plots treated with 80% chloropicrin plus 20% 1,3-dichloropropene (1,3-D + Pic) at 196 kg·ha⁻¹. The fumigant 1,3-D + Pic, is widely used in commercial strawberry production in the southeast United States because of its broad-spectrum efficacy against soilborne pathogens and weeds (Samtani et al. 2010; Triky-Dotan et al. 2016; Villarino et al. 2021; Yan et al. 2017). When applied under virtually impermeable films (VIF) at the recommended rates (e.g., approximately 157 kg·ha⁻¹), 1,3-D + Pic effectively reduces pathogen incidence while minimizing crop phytotoxicity, environmental, and human exposure risks (Samtani et al. 2017). Phosphorus and potassium were not needed, as determined by the soil test. The amendments (C sources, yeast, or fertilizers) (Table 1) were broadcast manually before final bedding on 30 Aug in both growing seasons. The amendments were incorporated to a depth of 15 cm by a machine that cultivated, shaped beds, installed drip tapes, and laid plastic film in one pass. All beds, including nontreated controls, were covered with a 0.03-mm VIF

(TriEst Ag Group, Inc., Greenville, NC, USA). A 0.38 mm single drip line with 30.5 cm emitter spacing (Chapin; Jain Irrigation, Inc., Watertown, NY, USA) was used to saturate the soil during ASD treatment and irrigate and fertigate the beds during the growing season. The drip line was approximately 5 cm under the bed surface.

On 5 Sep 2018 and 2019, ASD treatments were initiated by irrigating the beds to maintain the soil moisture content at field capacity (23%) (Elmore et al. 1997). The soil moisture before ASD initiation was approximately 13% in both seasons. The soil moisture was measured by the Field Scout TDR 100 soil moisture meter (Spectrum Technologies, Inc., Aurora, IL, USA). The TDR meter was used periodically over the 21 d ASD period to determine the irrigation times and durations. Redox potential (Eh) sensors (ORP sensor; ORP2000 Extended Life ORP Sensor; Sensorex, Garden Grove, CA, USA) were installed at the center of the bed at a depth of 15 cm to determine soil anaerobic conditions. The sensors were connected to an automatic data logging system (CR-1000; Campbell Scientific, Logan, UT, USA). Soil temperature sensors (U12

Deep Ocean Temperature Data Logger; Onset, Bourne, MA, USA) were installed simultaneously and at the same depth as the ORP sensors. Because limited sensors were available, ORP sensors and temperature sensors were installed only in half of the four blocks for both trials. Each of the treatments except 1,3-D + Pic had two plots containing sensors. The 1,3-D + Pic plots had no sensors. Both sensors recorded readings every 60 min for the duration of the treatments.

Because of the landing of Hurricane Florence in Sep 2018, all the sensors and data loggers were temporarily removed from beds from 12 Sep 2018 to 15 Sep 2018. During the following 2019–20 growing season, some hurricane precautions such as waterproof sealant for dataloggers and portable batteries were applied before hurricane Dorian landed in Sep 2019. Thus, the recordings during the 2019–20 growing season were not interrupted. The 1,3-D + Pic was applied at 196 kg·ha⁻¹ on 7 Sep in both growing seasons. On 26 Sep 2018 and 27 Sep 2019, the 21 d ASD period was ended by punching planting holes and removing the sensors. Before punching holes for transplanting strawberries, Italian ryegrass [*Lolium perenne* ssp. *multiflorum* (Lam.) Husnot] was seeded at 28 kg·ha⁻¹ as a cover crop in furrows between strawberry beds on 21 Sep 2018 and 26 Sep 2019. Italian ryegrass was mowed as needed throughout the strawberry growing season.

After completing preplant treatments, ‘Ruby June’ strawberry plugs (Aarons Creek Farms, Buffalo Junction, VA, USA) were transplanted in all beds on 9 Oct in both growing seasons in two rows per bed with 36 cm in-row spacing. There were 16 plugs per bed in the 2018–19 growing season and 18 plugs per bed (12 plugs used for harvest and fruit quality data collection and 6 plugs used for N estimation only) in the 2019–20 growing season. For both growing seasons, typical postplanting fertilization, pest, and irrigation management practices for conventional strawberries in the region (Poling et al. 2005) were conducted unless otherwise stated.

Weed density. The weed monitoring window had a length of 1.5 m and width of 0.8 m on the bed top and was covered with a clear tarp to assess weed emergence without interference from strawberry plants. The weed monitoring window was outside the bed section used for strawberry plug transplanting, thus ensuring accurate weed density measurements. The weed viewing window was established on 2 Oct 2018 and 4 Oct 2019. The weed viewing window was created by replacing the black 0.03 mm VIF tarp (TriEst Ag Group, Inc., Greenville, NC, USA) with a 0.025 mm clear tarp (Robert Marvel Plastic Mulch, LLC, Annville, PA, USA) to separate the treatment effect from the suppressive effects of the black plastic tarp. The dates for weed evaluation were chosen when the emerging weeds canopy in most window areas covered approximately 50% of the window area. On each evaluation date, the emerging weeds in the window area were identified and counted by species. Both the weed counts by species for each treatment from each evaluation date and the total counts for each treatment from each evaluation

date were summed separately. The summed total counts were recorded as cumulative weed density. After each evaluation, all counted weeds in the window area were carefully removed by hand to keep the entire weed plant, including the shoot and root. Fresh biomass and dry biomass of the harvested weeds were recorded by replicates. In the 2018–19 growing season, weeds were counted 13 weeks after transplanting (WAT) on 10 Jan 2018, 18 WAT on 14 Feb 2019, and 24 WAT on 28 Mar 2019. In the 2019–20 growing season, weeds were counted 13 WAT on 6 Jan 2020, 19 WAT on 19 Feb 2020, and 25 WAT on 31 Mar 2020. Emerged weeds from the bed shoulders were not included in the weed count but were hand-weeded after each weed counting date.

Crop stand and health index. In both growing seasons, crop stand and health were noted monthly from November to June. Strawberry plants in a bed were evaluated for crop vigor and health using a scale of 0 (all plants in a plot are dying) to 10 (all plants are vigorous and have no disease).

Nitrogen estimation. To estimate the N content in leaves during the early harvest season and determine the plant nutrient status, leaf tissue and petiole samples were collected on 12 Apr 2019 and 6 Apr 2020. The samples collected were the 25 most recently mature trifoliate leaves with the associated petiole for each treatment. These samples were sent to the North Carolina Department of Agriculture and Consumer Services (Raleigh, NC, USA) for a nutrient analysis. Additionally, in the 2019–20 growing season, six whole plants per plot were collected on 23 Jun and 24 Jun 2020 for the biomass analysis. Each plant was divided into six components (leaves, petioles, crown, root, flower, and berries). Any soil debris on the roots was removed carefully by hand and then gently rinsed with tap water. Both the fresh biomass and dry biomass of all six plant components were recorded.

Yield, fruit size, fruit firmness, and total soluble solids content. Strawberry fruits were harvested starting on 16 Apr 2019 and 12 Apr 2020 and through 14 Jun 2019 and 18 Jun 2020. The fruits were hand-picked twice per week, and the harvested fruits were sorted into marketable and nonmarketable categories. The nonmarketable fruits included fruits that weighed less than 10 g and those that were diseased, rotten, deformed, overripe, or misshapen (Johnson et al. 2005; US Department of Agriculture Agricultural Marketing Service 2006). The diseased berries included those infested with anthracnose or botrytis. Anthracnose fruit rot, primarily caused by *Colletotrichum acutatum*, is characterized by circular, sunken, dark brown to black lesions that often produce orange to salmon-colored spore masses (acervuli) under moist conditions (Louws and Cline 2014). Botrytis fruit rot (gray mold), caused by *Botrytis cinerea*, appears as soft water-soaked lesions that rapidly become covered with fluffy gray spore masses that typically initiate from injured, overripe, or senescing fruit tissue (Louws and Ridge 2014). Postharvest data parameters included the fruit diameter, fruit firmness, fruit

total soluble solids (TSS) content, and pH of the fruit juice. Fruit diameter was recorded using a Vernier caliper scale on five marketable fruits collected randomly from each replicate once per week. Additionally, during the 2019–20 growing season, those fruits were weighed. At every other harvest date, five marketable fruits from each replicate were randomly tested for firmness using a tabletop fruit texture analyzer (GS-15 Fruit Texture Analyzer; QA Supplies, Norfolk, VA, USA). The same five fruits were tested for TSS using a refractometer (MA871 Refractometer; Milwaukee, Rocky Mount, NC, USA); they were tested for pH using a pH tester electrode (combo pH/conductivity/TDS tester; HANNA Instruments, Smithfield, RI, USA).

Statistical analysis. Data were analyzed using JMP version 14 (SAS Institute Inc., Cary, NC, USA). Before the analysis of variance (ANOVA), data were checked for normality and homogeneity of variance assumptions. The temperature data were averaged for the 21 d ASD treatment duration, and maximum and minimum temperatures achieved during the treatment period were recorded. The cumulative Eh was calculated based on the hourly average Eh and absolute value of the difference between each hourly average Eh and the calculated critical Eh (CEh; the Eh value below which an anaerobic status is considered), and it was summed over the entire 21 d ASD period. The CEh was calculated using the following formula: $CEh = 595mV - 60mV \times \text{soil pH}$ (Rabenhorst and Castenson 2005; US Department of Agriculture Natural Resources Conservation Service 2010). The soil temperature and cumulative Eh data were analyzed using a two-way ANOVA (treatments \times growing seasons). The data were analyzed separately for each growing season if the treatment \times growing season interaction was significant ($P < 0.05$). The data were pooled over growing seasons if the treatment main effect was significant ($P < 0.05$) and treatments \times growing seasons interaction was not significant ($P > 0.05$). The multiple comparisons were conducted using the protected Fisher's least significant difference ($P < 0.05$).

The weed density data did not meet the assumptions of normality, and the rank transformation was used. Then, the transformed data were analyzed using a two-way ANOVA. Nontransformed means were presented. The strawberry health index, fruit yield, fruit firmness, fruit size, and fruit TSS data were analyzed using a method similar to that used to analyze temperature and Eh data using a two-way ANOVA. The fruit firmness and fruit size data were averaged for each replicate by each evaluation date for each growing season. The percentage of marketable yield (marketable yield rate) data were transformed using $\log(x + 1)$ for the analysis, and only the nontransformed data were presented.

Results

Temperature and cumulative redox potential. The temperature and Eh data were not collected for plots treated with 1,3-D + Pic.

There was no significant main effect of treatment for high temperatures at a depth of 15 cm over the two growing seasons (Table 2). The soil temperature varied significantly between the two growing seasons ($P < 0.05$). The average soil temperature during the ASD treatment period was higher in the 2019–20 growing season (28.6 °C) compared with that in the 2018–19 growing season (30.0 °C), indicating season-to-season variability in environmental conditions affecting ASD efficacy. Many soil organisms, including weeds, are negatively affected at over 40 °C (Stapleton and DeVay 1995). Thus, the cumulative hours during which the temperature was higher than 40 °C were calculated. In the 2018–19 growing season, ASD-treated beds at the full rate with yeast had the greatest number of hours comprising temperatures above 40 °C, which included significantly more plots than those treated with ASD at the half rate with or without yeast or nontreated control plots with or without yeast. In the 2019–20 growing season, plots treated with ASD at a full rate with yeast had a significantly greater number of hours comprising temperatures above 40 °C compared to that of the other treatments.

The cumulative Eh data had no significant ($P > 0.05$) treatment \times growing season interaction. Thus, the cumulative Eh data were pooled over two growing seasons. Plots treated with all four ASD treatments had significantly higher cumulative Eh compared to that of the two nontreated control plots (Table 2). No significant differences in the cumulative Eh were found among ASD treatments.

Weed density. In both growing seasons, the dominant broadleaf weeds species were carpetweed (*Mollugo verticillate* L.), Carolina geranium (*Geranium carolinianum* L.), cudweed (*Gnaphalium* spp.), henbit (*Lamium amplexicaule* L.), and white clover (*Trifolium repens* L.). The dominant grass weed species were bermuda grass (*Cynodon dactylon* L. Pers.) and crabgrass (*Digitaria sanguinalis*). Shepherd's purse (*Capsella bursa-pastoris* L. Medik.) and yellow nutsedge (*Cyperus esculentus* L.) were the dominant weed species only in the 2018–19 growing season. Shepherd's purse and yellow nutsedge were rarely observed in the 2019–20 growing season (Tables 3 and 4). In the 2018–19 growing season, ASD-treated plots had a significantly lower density of Shepherd's purse (Table 3).

Carpetweed density was lowest in 1,3-D + Pic treated plots, followed by ASD treatments (Table 3). Carolina geranium density was lowest in ASD-treated plots with the full C rate with and without yeast and ASD-treated plots at the half C rate with yeast. The yeast amendment significantly reduced the Carolina geranium density for plots treated with ASD at the full rate compared with that without yeast. The 1,3-D + Pic treatment was ineffective for controlling Carolina geranium. For cudweed, the plot treated with all ASD treatments and 1,3-D + Pic had a significantly lower density than that of the two nontreated control plots. Henbit density was lowest in plots treated with ASD and 1,3-D + Pic. However, henbit density in the nontreated control plots with yeast

Table 2. High soil temperature at the 15-cm depth for 21-d period of anaerobic soil disinfestation (ASD) with different carbon (C) dose rates and yeast amendment.

Treatment ⁱ	High temp (°C)		Hours >40 °C		Cumulative Eh (Vhr) 2019–20
	2018–19	2019–20	2018–19	2019–20	
ASD, C source full without yeast	45.9 ⁱⁱⁱ	45.0	14 ab	15 b	80 a
ASD, C source full with yeast	46.1	45.3	21.5 a	34 a	57 a
ASD, C source half without yeast	41.0	41.9	3.5 b	6 b	81 a
ASD, C source half with yeast	42.0	44.0	7 b	14 b	74 a
Nontreated control without yeast	34.3	43.9	0 b	11 b	15 b
Nontreated control with yeast	33.4	44.0	0 b	16 b	13 b
LSD	N/A	N/A	11.43	12.8	28.1
CV (%)	N/A	N/A	61.0	32.1	21.0
<i>P</i> treatment effect ⁱⁱ	N.S. ^{iv}		0.0014		0.0024
<i>P</i> season effect	0.0047		0.0011		N.S.
<i>P</i> treatment × season	0.018		0.018		N.S.

ⁱThe rates of C sources and yeast were as follows: full rate 4 mg C·g⁻¹ soil, 6.7 t C·ha⁻¹, yeast full rate 10 kg·ha⁻¹; half rate 2 mg C·g⁻¹ soil, 3.35 t C·ha⁻¹, yeast half rate 5 kg·ha⁻¹; and nontreated control with yeast 10 kg·ha⁻¹.

ⁱⁱGrowing season × treatment interaction was significant for high temperature and hours >40 °C. For cumulative Eh, only the treatment main effect was significant, and the data were pooled over the two growing seasons.

ⁱⁱⁱMeans in the same column with the same letters are not significantly different based on Fisher's least significant difference at $\alpha = 0.05$. Columns without letters indicate that no significant differences were detected among treatments ($P > 0.05$).

^{iv}N.S. indicates not significant ($P > 0.05$).

CV = coefficient of variation; Eh = redox potential; LSD = least significant difference; *P* = probability value.

was similar to that in the plots with ASD treatments. White clover density was lowest in plots treated with ASD and 1,3-D + Pic. The overall cudweed, henbit, and white clover density values for all treatments in the 2018–19 growing season were significantly higher than those in the 2019–20 growing season (Fig. 2). For bermuda grass (Table 4), plots treated with ASD without yeast at both rates and 1,3-D + Pic had a significantly lower density than that of the two nontreated plots; however, there was no significant difference among plots with all ASD treatments. The yeast amendment did not affect weed density in plots with the nontreated control. For large crabgrass, only plots treated with

ASD at a full rate without yeast and 1,3-D + Pic had significantly lower weed density than that of the two nontreated plots (Table 4). For yellow nutsedge, only plots treated with ASD at a half rate with yeast and 1,3-D + Pic significantly reduced weed density.

In the 2018–19 season, cumulative weed density was highest in the nontreated control, at 283 and 223 plants per m²; however, plots treated with ASD and 1,3-D + Pic had significantly lower weed counts ($P < 0.05$). Weed dry biomass with the ASD treatment with the full C rate was higher than that with 1,3-D + Pic treatment. In the 2019–20 growing season, plots treated with all ASD treatments and 1,3-D + Pic had a significantly

lower density of cumulative weed density, fresh weed biomass, and dry weed biomass compared with those of plots with nontreated control without yeast (Table 5). Regarding cumulative density, plots treated with ASD with yeast at both rates had a significantly lower density than that of plots treated with 1,3-D + Pic and those treated with ASD without yeast. These results indicate that ASD treatments, particularly with yeast amendments, contributed to overall weed suppression by reducing both weed density and biomass across multiple species.

Crop stand counts and crop health. The strawberry stand counts had no two-way or main effect significance ($P > 0.05$) (data not shown). For the plant health index (Table 6), the treatment main effect and growing season effect were significant ($P < 0.05$). There was no significant difference among plots treated with all ASD treatments and 1,3-D + Pic. The plots with nontreated control without yeast had a significantly lower health index than plots treated with all ASD treatments and 1,3-D + Pic, while plots with nontreated control with yeast had a health index similar to that of plots treated with ASD at a full C rate without yeast and with 1,3-D + Pic. The main reason for the lower index of the nontreated plots was the less vigorous or small size of crops. Strawberry plant health indices also differed significantly between the two growing seasons. The growing season effect and treatment main effect were significant to the health index ($P < 0.005$). The plant health index was higher in the 2019–20 season (8.8), suggesting more favorable conditions or better crop performance compared with that the 2018–19 season (7.8).

Crop yield, fruit size, fruit firmness, and total soluble solids. The total yields were calculated by marketable yield added to nonmarketable yield. The nonmarketable yield was primarily from fruit rot diseases (anthracnose

Table 3. Broadleaf weed density in 2018–19 and 2019–20 growing seasons.

Treatment ⁱ	Weed density (plants·m ⁻²)						Shepherd's purse	
	Carpetweed	Carolina geranium	Cudweed	Henbit	White clover		2018–19	2019–20
ASD, C source full without yeast	22 b ⁱⁱⁱ	13 cde	21 b	3 bc	44 bc		2 cd	0
ASD, C source full with yeast	16 b	8 f	16 b	2 bc	33 c		4 bc	0
ASD, C source half without yeast	13 b	17 bcd	17 b	3 bc	37 c		2 c	0
ASD, C source half with yeast	10 b	12 def	22 b	3 bc	38 c		32 ab	0
Nontreated control without yeast	29 a	33 a	52 a	18 a	67 a		50 a	0
Nontreated control with yeast	33 a	23 abc	47 a	17 ab	59 ab		41 a	0
1,3-D + Pic	0 c	25 ab	14 b	0 c	32 c		0 d	0
LSD	10.95	4.58	12.56	7.83	8.50		17.5	N/A
CV (%)	32.7	19.0	34.6	39.7	42.7		40.8	N/A
<i>P</i> treatment effect ⁱⁱ	<0.0001	<0.0001	<0.0001	0.0129	0.0003		0.0079	
<i>P</i> season effect	N.S. ^{iv}	N.S.	<0.0001	0.0075	0.01		0.0042	
<i>P</i> treatment × season	N.S.	N.S.	N.S.	N.S.	N.S.		0.0028	

ⁱThe rates of C sources and yeast were as follows: full rate 4 mg C·g⁻¹ soil, 6.7 t C·ha⁻¹, yeast full rate 10 kg·ha⁻¹; half rate 2 mg C·g⁻¹ soil, 3.35 t C·ha⁻¹, yeast half rate 5 kg·ha⁻¹; and nontreated control with yeast 10 kg·ha⁻¹.

ⁱⁱGrowing season × treatment interaction was significant for Shepherd's purse. For carpetweed and Carolina geranium, only the treatment main effect was significant, and data were pooled over the two growing seasons. For cudweed, henbit, and white clover, the treatment effect and growing season effect were significant; therefore, the only treatment main effect is presented in this table, and the season effect is presented in Fig. 2.

ⁱⁱⁱMeans in the same column with the same letters are not significantly different based on Fisher's least significant difference at $\alpha = 0.05$. Columns without letters indicate that no significant differences were detected among treatments ($P > 0.05$).

^{iv}N.S. indicates not significant ($P > 0.05$).

ASD = anaerobic soil disinfestation; C = carbon; CV = coefficient of variation; LSD = least significant difference; *P* = probability value.

Table 4. Weed density for grasses and yellow nutsedge in 1.5-m lengths of windows for the 2018–19 and 2019–20 growing seasons after preplant treatments.

Treatment ⁱ	Bermuda grass	Weed density (plants·m ⁻²)			
		Large crabgrass		Yellow nutsedge	
		2018–19	2019–20	2018–19	2019–20
ASD, C source full without yeast	2 bc ⁱⁱⁱ	6 cd	5	5 ab	0
ASD, C source full with yeast	3 ab	8 bc	2	2 ab	0
ASD, C source half without yeast	2 bc	12 ab	4	3 ab	0
ASD, C source half with yeast	2.5 abc	10 abc	6	2 bc	0
Nontreated control without yeast	16 a	17 ab	2	9 a	0
Nontreated control with yeast	13 ab	18 a	7	8 a	1
1,3-D + Pic	0 c	0 d	2	0 c	0
LSD	7.42	3.82	N/A	2.75	N/A
CV (%)	22.5	36.5	N/A	19.6	N/A
<i>P</i> treatment effect ⁱⁱ	0.04	<0.0001		0.0006	
<i>P</i> season effect	N.S. ^{iv}	<0.0001		<0.0001	
<i>P</i> treatment × season	N.S.	0.002		0.0015	

ⁱThe rates of C sources and yeast were as follows: full rate 4 mg C·g⁻¹ soil, 6.7 t C·ha⁻¹, yeast full rate 10 kg·ha⁻¹; half rate 2 mg C·g⁻¹ soil, 3.35 t C·ha⁻¹, yeast half rate 5 kg·ha⁻¹; and nontreated control with yeast 10 kg·ha⁻¹.

ⁱⁱGrowing season × treatment interaction was significant for large crabgrass and yellow nutsedge. For bermuda grass, only the treatment effect was significant; therefore, the data were pooled over the two growing seasons.

ⁱⁱⁱMeans in the same column with the same letters indicate no differences with Fisher's least significant difference at $\alpha = 0.05$. Columns without letters indicate that no significant differences were detected among treatments ($P > 0.05$). The weed data comes from a 1.5 × 0.8 m⁻² fixed quadrat window and was converted to m².

^{iv}N.S. indicates not significant ($P > 0.05$).

ASD = anaerobic soil disinfestation; C = carbon; CV = coefficient of variation; LSD = least significant difference; *P* = probability value.

and botrytis) and rot caused by excessive moisture on the beds. Some of the nonmarketable fruit yield was caused by undersized fruits (weight <10 g). The growing season × treatment interaction was significant for marketable yield, total yield, monthly total yield, and marketable yield rate (Tables 7 and 8). In the 2018–19 growing season, there were no significant differences among plots treated with ASD treatments and nontreated control for both marketable and total yields (Table 7). Plots treated with 1,3-D + Pic had the highest marketable yield. Yield of ASD with the half C rate without yeast was not different from that with 1,3-D + Pic. In the 2019–20 growing season, plots treated with ASD treatments and 1,3-D + Pic provided significantly higher marketable and total yields for the

whole harvest season than those plots with both nontreated controls. The ASD with the full C rate with yeast treatment had the highest marketable yield. The treatment main effect was not significant for the marketable yield rate (marketable rate = marketable yield/total yield × 100%). Plots with ASD treatments at both rates with yeast had marketable and total yields comparable to those of plots with 1,3-D + Pic, while plots treated with ASD without yeast had a significantly lower yield than that of plots treated with 1,3-D + Pic. Thus, in the 2019–20 growing season, the yeast amendments significantly enhanced the marketable and total yields for plots treated with ASD treatments. These trends were also found for the monthly total yield in May and June during the 2019–20 growing

season, when an increase in yield was recorded for ASD with yeast treatments compared with that for ASD without yeast treatments (Table 8). The weekly total yields were variable for each week in both growing seasons, which indicated that the distribution of strawberry yield was not uniform over the harvest season. There was a significant difference in average fruit weight in 2019–20 growing season (Table 9), and plots treated with ASD with yeast had significantly higher fruit weight than ASD without yeast or with nontreated controls. In both growing seasons, the fruit size, fruit firmness, and TSS were not significantly different among treatments (Supplemental Table 1).

Leaf nitrogen content. The leaf N content, expressed as the N index, varied by treatment and season (Table 10). In the 2018–19 growing season, strawberry plants with the 1,3-D + Pic treatment had the highest N index, which was classified as sufficient based on leaf tissue nutrient guidelines for strawberry crops. A sufficient N index typically corresponds to 2.0% to 3.0% total N content in recently mature strawberry leaves during early fruiting (North Carolina Department of Agriculture and Consumer Services 2020; Poling et al. 2005). The N indices below this range are considered low, indicating potential nutrient deficiency. The N content in the ASD treatment plants at both half and full C rates remained low, while the nontreated control without yeast was sufficient. The addition of yeast to ASD did not consistently improve the leaf N content. In the 2019–20 growing season, the soil fumigation treatment had the highest N index (sufficient), while the N content in ASD treatments ranged from low to sufficient. The full-rate ASD without yeast had strawberry plants with sufficient leaf N, whereas the same treatment with yeast remained low. The soil fumigation treatment consistently resulted in the highest N index, highlighting its role in soil conditioning and nutrient availability.

Crop biomass. Dry biomass was only measured during the 2019–20 growing season to evaluate plant response to soil amendment treatments. Dry biomass accumulation varied among treatments, with significant differences observed in leaves, petioles, crown, and root biomass (Table 11). The 1,3-D + Pic treatment resulted in the highest biomass across all plant parts, with significantly greater dry weight in leaves, petioles, and roots compared with those in all other treatments. Among the ASD treatments, there were no differences. The ASD with the full C rate and without yeast as well as the ASD with the half C rate and with yeast also showed moderate biomass accumulation, but it remained significantly lower than that with the 1,3-D + Pic treatment. No significant differences were observed in flower biomass and berry biomass among treatments. This suggests a positive association between biomass accumulation and yield performance under effective ASD or fumigant treatments. Although biomass data were not collected in the 2018–19 season, yield trends in that season did not differ significantly across treatments, indicating a more limited

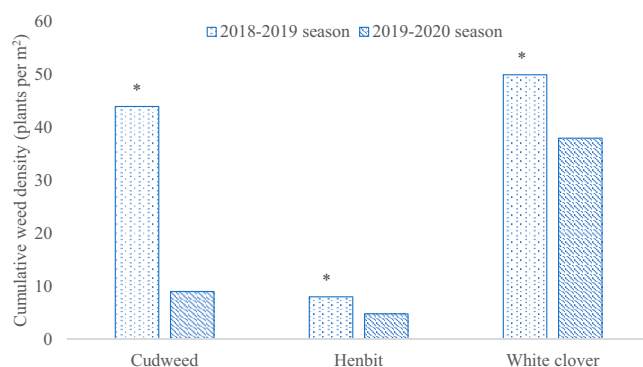


Fig. 2. Cumulative weed density for cudweed, henbit, and white clover averaged for all treatments in the 1.5-m length of the weed-viewing window for the 2018–19 and 2019–20 growing seasons. Significant differences among growing season within each species are indicated by an asterisk above the bars based on Fisher's least significant difference ($\alpha = 0.05$). The growing season effect and treatment main effect were significant for cudweed, henbit, and white clover ($P < 0.005$). The treatment main effect is presented in Table 3.

Table 5. Cumulative weed density and cumulative fresh biomass and dry biomass in 1.5-m lengths of windows for the 2018–19 and 2019–20 growing seasons after preplant treatments.

Treatment ⁱ	Cumulative weed density (plants·m ⁻²) ⁱⁱ		Cumulative fresh biomass (g·m ⁻²)		Cumulative dry biomass (g·m ⁻²)	
	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20
ASD, C source full without yeast	113 c ⁱⁱⁱ	68 bc	3,045 ab	919 bc	2,553 ab	604 b
ASD, C source full with yeast	133 bc	44 d	3,381 abc	761 c	2,851 ab	449 b
ASD, C source half without yeast	119 c	76 b	2,821 abc	1,021 b	2,396 abc	662 b
ASD, C source half with yeast	139 bc	57 cd	2,630 bc	955 bc	2,185 bc	615 b
Nontreated control without yeast	283 a	148 a	4,336 a	2,006 a	3,605 a	1,667 a
Nontreated control with yeast	223 ab	135 a	5,294 a	1,642 a	4,298 a	1,318 a
1,3-D + Pic	60 d	67 bc	1,874 c	1,008 b	1,453 c	657 b
LSD	90.91	26.55	2210.53	446.99	1797.34	437.05
CV (%)	35.2	21.3	29.0	25.6	28.5	34.8
<i>P</i> treatment effect	<0.0001		<0.0001		<0.0001	
<i>P</i> season effect	<0.0001		<0.0001		<0.0001	
<i>P</i> treatments × seasons	0.0001		0.02		0.02	

ⁱThe rates of C sources and yeast were as follows: full rate 4 mg C·g⁻¹ soil, 6.7 t C·ha⁻¹, yeast full rate 10 kg·ha⁻¹; half rate 2 mg C·g⁻¹ soil, 3.35 t C·ha⁻¹, yeast half rate 5 kg·ha⁻¹; and nontreated control with yeast 10 kg·ha⁻¹.

ⁱⁱGrowing season × treatment interaction was significant for cumulative weed counts and cumulative fresh biomass and dry biomass.

ⁱⁱⁱMeans in the same column with the same letters are not significantly different based on Fisher's least significant difference at $\alpha = 0.05$.

The weed data comes from a 1.5 × 0.8 m⁻² fixed quadrat window and was converted to m².

ASD = anaerobic soil disinfestation; C = carbon; CV = coefficient of variation; LSD = least significant difference; *P* = probability value.

plant response. Therefore, the enhanced biomass and yield observed in 2019–20 may reflect both treatment effects and favorable environmental conditions.

Discussion

The mean soil temperature during the ASD period in the 2018–19 growing season was significantly higher than that in the

Table 6. Strawberry plant health index for the entire growing seasons.

Treatment ⁱ	Health index ⁱⁱ
ASD, C source full without yeast	8.4 ab ⁱⁱⁱ
ASD, C source full with yeast	8.6 a
ASD, C source half without yeast	8.6 a
ASD, C source half with yeast	8.5 a
Nontreated control without yeast	7.9 c
Nontreated control with yeast	8.0 bc
1,3-D + Pic	8.3 abc
LSD	0.566
CV (%)	16.4
<i>P</i> treatment effect	0.0086
<i>P</i> season effect	<0.0001
<i>P</i> treatment × season	N.S. ^{iv}

ⁱThe rates of C sources and yeast were as follows: full rate 4 mg C·g⁻¹ soil, 6.7 t C·ha⁻¹, yeast full rate 10 kg·ha⁻¹; half rate 2 mg C·g⁻¹ soil, 3.35 t C·ha⁻¹, yeast half rate 5 kg·ha⁻¹; and nontreated control with yeast 10 kg·ha⁻¹.

ⁱⁱThe crop health indicates by index from 0 (all plants are dead) to 10 (all plants are vigorous and no disease). The evaluation was based on all strawberry plants in the 3.6 m bed length. The growing season effect and treatment main effect was significant for health index ($P < 0.005$). Thus, the treatment's main effect was present in this table.

ⁱⁱⁱMeans in the same column with the same letters are not significantly different based on Fisher's Least Significant Difference at $\alpha = 0.05$.

^{iv}N.S. indicates not significant ($P > 0.05$).

ASD = anaerobic soil disinfestation; C = carbon; CV = coefficient of variation; LSD = least significant difference; *P* = probability value.

2019–20 growing season. That difference was consistent with the air temperature data retrieved from the US Department of Agriculture Natural Resources Conservation Service, National Water and Climate Center (US Department of Agriculture Natural Resources Conservation Service 2020) for the nearest station at Norfolk International Airport. The average air temperature in Sep 2018 was 26.1 °C, while it was 24.5 °C in Sep 2019. The highest soil temperature from ASD treatments was approximately 10 °C higher than the highest air temperature in Sep 2018 and Sep 2019. This difference in maximum soil temperatures between the soil and the air was observed in our previous greenhouse trials (Liu et al. 2020). The difference in the cumulative numbers of hours with soil temperatures above 40 °C may have caused the significant growing season × treatment interaction, especially in the nontreated control plots (Table 2). Although the cumulative number of hours in both growing seasons when the mean soil temperature was above 40 °C may not have been enough to eliminate some soilborne pests on its own, the black tarp may have enhanced weed suppression in the cropping area by blocking the necessary light for weed germination and growth (Johnson and Fennimore 2005). The cumulative number of hours during which the mean soil temperature was between 30 and 35 °C in the 2019–20 growing season was greater than that in the 2018–19 growing season (data not shown). The temperature ranged from 30 to 35 °C and was likely an ideal temperature range for yeast metabolism and fermentation (Feldman 2012). The greater number of hours in 2019–20 during which the mean soil temperature ranged from 30 to 35 °C may have led to more metabolites produced from yeast fermentation. This difference in yeast activities could explain the better performance of yeast in the 2019–20 growing season compared to that in the 2018–19 growing season. For example, plots treated with ASD with yeast had

significantly lower total weed density than ASD without yeast in the 2019–20 growing season, but there was no significant difference in total weed density for plots treated with ASD either with or without yeast in the 2018–19 growing season.

Inconsistent weed control by ASD has been reported (Guo et al. 2017; Shrestha et al. 2016); however, in this study, although the weed control effect from ASD varied for different weed species, ASD consistently suppressed several weeds, including carpetweed, Carolina geranium, cudweed, henbit, and white clover. Compared with the research that had inconsistent weed control, this study had several different factors that may have resulted in differences. The different factors included different locations and soil types, different C sources and C:N ratios, and different soil temperatures. For example, Guo et al. 2017 reported inconsistent yellow nutsedge, grass, and broadleaf weed control, which had a sandy soil type and molasses as C sources. In this study, we used sandy loam soil and BSG mixed with paper mulch as C sources. The interactions between the C source, C:N ratio, and yellow nutsedge tuber suppression were also reported (Shrestha et al. 2018b). A trend of a relatively lower amendment C:N ratio (10:1 or 20:1) leading to lower soil pH, which may indicate higher organic acid contents, was observed (Shrestha et al. 2018). It is possible that organic acids have potential impacts on weed suppression. The weed density in this trial was relatively low, especially in the 2019–20 growing seasons. Other studies indicated that ASD could effectively control weeds when their density is low (Di Gioia et al. 2016; Guo et al. 2017). Thus, the efficacy of ASD with yeast for weed control needs to be evaluated under high weed density field conditions. For the weed species that ASD did not consistently suppress, a trend of all ASD treatments numerically reducing all weed counts when weed density was higher than 10 counts/m² was observed. The weed density data in this study were collected

Table 7. Effect of treatment and growing season on marketable, nonmarketable, and total yields during the harvest period in 2018–19 and 2019–20 growing seasons in Virginia Beach, VA, USA.

Treatment ⁱ	Marketable yield (g·plant ⁻¹)		Nonmarketable yield (g·plant ⁻¹)	Total yield (g·plant ⁻¹)		Marketable yield rate (%) ⁱⁱⁱ	
	2018–19	2019–20		2018–19	2019–20	2018–19	2019–20
ASD, C source full without yeast	397 b ⁱⁱ	410 c	78 b	488 b	474 c	81 ab	86 a
ASD, C source full with yeast	361 b	684 a	76 b	446 b	752 a	81 ab	91 a
ASD, C source half without yeast	419 ab	418 c	84 b	530 ab	475 c	79 ab	88 a
ASD, C source half with yeast	327 b	572 b	80 b	420 b	639 b	76 b	90 a
Nontreated control without yeast	297 b	226 d	64 b	379 b	270 d	78 b	84 ab
Nontreated control with yeast	319 b	287 d	66 b	414 b	323 d	77 b	89 a
1,3-D + Pic	536 a	559 b	139 a	663 a	710 ab	81 ab	79 ab
LSD	65.86	95.77	37.3	78.71	110.20	6.74	8.12
CV (%)	31.6	39.5	16.1	30.1	38.6	15.0	15.9
P treatment effect	<0.0001		<0.0001	<0.0001		N.S.	
P season effect	0.0127		0.0006	N.S.		<0.0001	
P treatment × season	0.0019		N.S. ^{iv}	0.0052		0.04	

ⁱ The rates of C sources and yeast were as follows: full rate 4 mg C·g⁻¹ soil, 6.7 t C·ha⁻¹, yeast full rate 10 kg·ha⁻¹; half rate 2 mg C·g⁻¹ soil, 3.35 t C·ha⁻¹, yeast half rate 5 kg·ha⁻¹; and nontreated control with yeast 10 kg·ha⁻¹.

ⁱⁱ Means in the same column with the same letters are not significantly different based on Fisher's least significant difference at $\alpha = 0.05$.

ⁱⁱⁱ Marketable yield rate = marketable yield/total yield.

^{iv} N.S. indicates not significant ($P > 0.05$).

ASD = anaerobic soil disinfestation; C = carbon; CV = coefficient of variation; LSD = least significant difference; P = probability value.

from the areas covered by clear tarp. However, the annual plasticulture strawberry production system typically uses a black tarp, which provides more weed suppression than a clear tarp (Johnson and Fennimore 2005). However, clear tarp warms soil more than black tarp; furthermore, conventional strawberry plasticulture with a clear tarp could provide higher yield than that with a black tarp (Johnson and Fennimore 2005). The combination of ASD and solarization using a clear tarp may have the potential to provide adequate weed control as well as higher yield compared with ASD with a black tarp (Butler et al. 2014b). The anaerobic condition is an essential factor in ASD, and the volatile organic compounds produced during the ASD period were related to soilborne pest control (Hewavitharana et al. 2014; Mowlick et al. 2012). The plastic film that highly reduces gas emission from soil may also lead to better ASD performance. Song et al. (2020) evaluated ASD with VIF, polyethylene (PE),

and totally impermeable film and found that totally impermeable film had the highest suppression against *Fusarium* spp. and *Phytophthora* spp.

The cumulative Eh observed in this study indicated significantly lower (more anaerobic) conditions in ASD-treated plots compared with the untreated control. This finding aligns closely with previous ASD studies, which consistently reported significant reductions in soil Eh during the treatment periods, suggesting that enhanced anaerobic conditions were successfully established (Liu et al. 2020; Muramoto et al. 2008; Shrestha et al. 2018). In our trials, however, despite significant differences in cumulative Eh between ASD-treated and nontreated plots, no significant differences in cumulative Eh were observed among different ASD treatments, which concurred with previous studies that indicated that beyond achieving anaerobic conditions, further reductions in Eh may not directly

correlate with improved weed suppression (Liu et al. 2020). Specifically, Liu et al. (2020) demonstrated that although cumulative Eh was consistently lower in ASD treatments with various C sources compared with that in nontreated plots, the magnitude of Eh reduction alone did not directly correlate with the weed suppression efficiency. The cumulative Eh in this study was higher for plots treated with ASD treatments than for nontreated plots, while that difference did not lead to significant suppression on large crabgrass and yellow nutsedge in the 2018–19 growing season. Yellow nutsedge is a troublesome weed species, but it had a very low weed density in this study. Some studies that had relatively high yellow nutsedge density and significant yellow nutsedge suppression showed no strong correlation between cumulative Eh and yellow nutsedge suppression (Muramoto et al. 2008; Paudel et al. 2020; Shrestha et al. 2018b). These results are consistent with the findings of Liu et al. (2020), who demonstrated that ASD effectively suppressed weeds such as common chickweed (*Stellaria media*) and white clover despite minimal differences in cumulative Eh among the ASD treatments and nontreated control. Additionally, Liu et al. (2020) observed reductions of weed emergence ranging from 50% to 100% across multiple weed species, including redroot pigweed (*Amaranthus retroflexus*) and common chickweed, suggesting broad-spectrum weed suppression independent of small variations in the cumulative Eh. Such patterns reinforce the concept that ASD efficacy not only depends on the anaerobic conditions but also depends heavily on other soil biochemical factors. Some other factors involved in ASD may have greater influence, such as volatile fatty acid accumulation, C source, and decomposition of weed propagules of microbial communities (Guo et al. 2018; Shennan et al. 2018; Shrestha et al. 2018). The microbial community may also enhance the nutrient availability in the soil by increasing the rate of C source decomposition (Guo et al.

Table 8. Monthly cumulative yield for the 3-month harvest period.

Treatment ⁱ	April total yield (g·plant ⁻¹)		May total yield (g·plant ⁻¹)		June total yield (g·plant ⁻¹)	
	2018–19	2019–20	2018–19	2019–20	2018–19	2019–20
ASD, C source full without yeast	119	125 b ⁱⁱ	337 b	228 c	32	120 b
ASD, C source full with yeast	115	187 a	309 b	372 a	22	192 a
ASD, C source half without yeast	115	125 b	381 ab	238 c	28	112 b
ASD, C source half with yeast	79	154 ab	292 b	313 b	23	170 a
Nontreated control without yeast	86	51 c	264 b	160 d	30	58 c
Nontreated control with yeast	106	67 c	290 b	188 cd	18	67 c
1,3-D + Pic	120	148 ab	514 a	379 a	30	182 a
LSD	N/A	94.11	160.33	91.90	N/A	47.58
CV (%)	N/A	57.5	36.4	37.4	N/A	44.1
P treatment × season	0.045		0.0031		<0.0001	

ⁱ The rates of C sources and yeast were as follows: full rate 4 mg C·g⁻¹ soil, 6.7 t C·ha⁻¹, yeast full rate 10 kg·ha⁻¹; half rate 2 mg C·g⁻¹ soil, 3.35 t C·ha⁻¹, yeast half rate 5 kg·ha⁻¹; and nontreated control with yeast 10 kg·ha⁻¹.

ⁱⁱ A column without any letter means there is no treatment effect ($P > 0.05$) based on Fisher's least significant difference at $\alpha = 0.05$. Means in the same column with the same letters are not significantly different based on Fisher's least significant difference at $\alpha = 0.05$. Columns without letters indicate that no significant differences were detected among treatments ($P > 0.05$).

ASD = anaerobic soil disinfestation; C = carbon; CV = coefficient of variation; LSD = least significant difference; P = probability value.

Table 9. Average fruit weights during the 2019–20 growing season.

Treatment ⁱ	Avg fruit wt (g)
ASD, C source full without yeast	30.0 cd ⁱⁱ
ASD, C source full with yeast	34.7 a
ASD, C source half without yeast	29.5 cd
ASD, C source half with yeast	32.7 ab
Nontreated control without yeast	28.3 d
Nontreated control with yeast	30.8 cd
1,3-D + Pic	30.9 bc
LSD	3.52
CV (%)	29.7
P	0.0012

ⁱThe rates of C sources and yeast were as follows: full rate 4 mg C·g⁻¹ soil, 6.7 t C·ha⁻¹, yeast full rate 10 kg·ha⁻¹; half rate 2 mg C·g⁻¹ soil, 3.35 t C·ha⁻¹, yeast half rate 5 kg·ha⁻¹; and nontreated control with yeast 10 kg·ha⁻¹.

ⁱⁱMeans followed by different letters within a column were statistically different using Fisher's least significant difference at $\alpha = 0.05$.

ASD = anaerobic soil disinfestation; C = carbon; CV = coefficient of variation; LSD = least significant difference; P = probability value.

2018). This may be the reason why ASD with yeast had a higher yield than ASD without yeast.

A significant crop yield increase resulting from ASD treatments was obtained in this study, but only in the 2019–20 growing season. The promotion of yield with ASD was also reported by other researchers who used ASD for strawberries (Song et al. 2020), eggplant, bell pepper (Butler et al. 2014a), and tomato (Di Gioia et al. 2016). However, ASD does not consistently enhance the yield. The marketable and total yields in this study did not increase in the 2018–19 growing season. McCarty et al. (2014) showed ASD did not increase yield for either tomato or bell pepper. There are many possible reasons for the yield increase. The application rate and type of C used for ASD could affect the crop yield (Shrestha et al. 2016). The suppression of disease and weeds could affect crop yield. The application of C sources could also increase the soil fertility level and influence the crop yield (Guo et al. 2017). For example, ASD

could greatly increase ammonium N while reducing nitrate N in the soil (Liu et al. 2016). The leaf tissue observed in this study also showed a similar trend, with leaves from ASD treatments showing less nitrate N than the nontreated control and plots treated with 1,3-D + Pic. The increase in the utilization rates of N, phosphorus, and potassium by ASD was also reported (Song et al. 2020).

In both growing seasons, the leaf tissue analysis was conducted during Apr 2018 and Apr 2019, and several nutrients were analyzed, such as N, phosphorus, and potassium. No synthetic fertilizer was applied to any ASD treatments in either growing season. Although the available N from the ASD C sources were the same as the available N from the synthetic fertilizer applied to the nontreated control and 1,3-D + Pic groups (Table 1), the available N from the organic ASD C sources would likely not be fully decomposed or mineralized during the growing season. The result of the leaf tissue analysis supported this assumption (Table 10).

In this study, ASD did not significantly affect nonmarketable yield (Table 7) because the nonmarketable fruits were mostly attributable to fruit rot diseases (anthracnose and botrytis) and environmental factors, which were unlikely to have been influenced by ASD. Thus, it indicated that the total yield (marketable yield + nonmarketable yield) increase caused by ASD was not the main result of ASD reducing nonmarketable yield. The yield increase may be related to the increased average fruit weight. There was a significant difference in average fruit weight (Table 9), and plots treated with ASD with yeast had significantly higher fruit weight than plots treated with ASD without yeast or with nontreated controls. However, the differences in fruit size, firmness, and soluble solid were not significant (Supplemental Table 1). Thus, higher fruit weight may result from higher fruit water content or fruit dry matter content such as a higher proportion of cell wall content. The dry matter content is likely to be higher using organic fertilization rather than inorganic fertilization (Reganold et al. 2010). In this study, yeast activities in soil may change soil fertility

and provide conditions similar to organic fertilization, which may contribute to the higher dry matter content. Therefore, additional research of whether yeast impacts soil fertility, bud number, fruit number, and fruit nutrient concentrations is needed to verify yield effects and explore the mechanisms of ASD for yield increase. An economic analysis of ASD for strawberry field production is also necessary.

The significant increase in dry biomass observed under the 1,3-D + Pic treatment is consistent with findings in the literature that highlight the efficacy of chemical fumigation to enhance plant growth by mitigating soil-borne pathogen pressure and improve nutrient availability. Chemical fumigation has been extensively used in strawberry production to manage soilborne diseases like *Verticillium dahliae*, which has been shown to enhance root health and overall plant vigor (Shennan et al. 2018). The observations of increased biomass across leaves, petioles, and roots confirmed that fumigation not only fosters vegetative growth but also enhances nutrient uptake efficiency, which is corroborated by related studies that demonstrated the link between reduced pathogen loads and improved plant growth metrics (Shennan et al. 2018). The absence of significant differences among the ASD treatments implied that the benefits observed in biomass accumulation were more significantly driven by the C source used rather than the yeast addition itself, thus aligning with prior research that identified organic amendments as key contributors to improved soil health via shifts in microbial communities and suppression of pathogens through the production of organic acids (Testen et al. 2021; Vincent et al. 2022). Importantly, the varying efficacy of these ASD mechanisms for biomass accumulation seemed to be influenced by environmental conditions and specific characteristics of the organic amendments used (Testen et al. 2021). Furthermore, it was noted that the nontreated control that lacked both yeast and fertilizer showed the lowest biomass for petioles and crowns, thus emphasizing the critical role of soil treatments in facilitating optimal plant development within strawberry production systems (Shennan et al. 2018). These empirical findings advocate for the adoption of ASD as a viable nonchemical alternative to traditional soil fumigation practices and highlight the necessity for further studies to optimize ASD treatment parameters to maximize plant growth and yield sustainably. Future research endeavors should aim to elucidate how different C sources, microbial dynamics, and environmental contexts affect biomass outcomes to refine soil management strategies in agriculture.

Conclusions

Similar to a fumigant standard treatment, ASD using BSG as a source of C as well as a yeast amendment demonstrated the potential to control certain weed species and enhance cumulative strawberry yield compared with a nontreated control. However, the effect was not consistent across the two growing seasons.

Table 10. Nitrogen content in leaves of 30-week-old plants expressed by the nitrogen index.

Treatment ⁱ	Nitrogen index value ^{ii,iii}	
	2018–19	2019–20
ASD, C source full without yeast	42 (L)	51 (S)
ASD, C source full with yeast	42 (L)	48 (L)
ASD, C source half without yeast	42 (L)	43 (L)
ASD, C source half with yeast	46 (L)	46 (L)
Nontreated control without yeast	54 (S)	46 (L)
Nontreated control with yeast	51 (S)	55 (S)
1,3-D + Pic	69 (S)	62 (S)

ⁱThe rates of C sources and yeast were as follows: full rate 4 mg C·g⁻¹ soil, 6.7 t C·ha⁻¹, yeast full rate 10 kg·ha⁻¹; half rate 2 mg C·g⁻¹ soil, 3.35 t C·ha⁻¹; yeast half rate 5 kg·ha⁻¹; and nontreated control with yeast 10 kg·ha⁻¹.

ⁱⁱThe nitrogen index is made by comparing the nitrogen concentration to the established sufficiency range for nitrogen in strawberry and translated to a numerical index between 0 and 124.

ⁱⁱⁱLetters in parentheses indicate interpretive groupings of sufficiency indices: L = low index range of 25–49; S = sufficient index range of 50–74.

ASD = anaerobic soil disinfestation; C = carbon.

Table 11. Dry biomass accumulation in the 2019–20 growing season.

Treatment ⁱ	Dry biomass (g·plant ⁻¹)					
	Leaves	Petioles	Crown	Root	Flowers	Berries
ASD, C source full without yeast	155.0 b ⁱⁱ	97.5 b	17.3	63.3 b	3.5	19.8
ASD, C source full with yeast	214.8 b	124.8 b	24.5	76.0 b	3.8	17.3
ASD, C source half without yeast	181.3 b	108.3 b	16.0	89.5 b	4.0	14.0
ASD, C source half with yeast	188.8 b	118.5 b	20.8	73.8 b	3.0	25.0
Nontreated control without yeast	189.0 b	86.5 b	19.0	73.5 b	2.5	15.5
Nontreated control with yeast	148.5 b	78.3 b	18.5	69.0 b	3.3	9.3
1,3-D + Pic	399.5 a	252.8 a	49.5	140.3 a	4.0	29.3
LSD	118.53	82.85	N/A	42.91	N/A	N/A
CV (%)	51.4	55.1	N/A	41.9	N/A	N/A
P	0.005	0.008	0.055	0.03	0.49	0.61

ⁱThe rates of C sources and yeast were as follows: full rate 4 mg C·g⁻¹ soil, 6.7 t C·ha⁻¹, yeast full rate 10 kg·ha⁻¹; half rate 2 mg C·g⁻¹ soil, 3.35 t C·ha⁻¹, yeast half rate 5 kg·ha⁻¹; and nontreated control with yeast 10 kg·ha⁻¹.

ⁱⁱMeans followed by different letters within a column were statistically different using Fisher's least significant difference at $\alpha = 0.05$. Columns without letters indicate that no significant differences were detected among treatments ($P > 0.05$).

ASD = anaerobic soil disinfestation; C = carbon; LSD = least significant difference; P = probability value.

The variability in effects across growing seasons suggested that more replicated trials are necessary to verify the effects of BSG and yeast.

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