Biochar and DMPP Regulated Root-Zone CO2, N2O and CH4 Emissions of Soil-Ridged/Substrate-Embedded Cultivation Sweet Pepper in Chinese Solar Greenhouse

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Biochar and DMPP regulated root-zone CO_2, N_2O and CH_4 emissions of soil-ridged/substrate-embedded cultivation sweet pepper in Chinese solar greenhouse

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Abstract: Northern China is a major production area for off-season vegetables in Chinese solar greenhouse. Usually, greenhouse gas emission flux and coefficient in Chinese solar greenhouse are higher than those in the open field. The reason for this phenomenon is heavy nitrogen (N) fertilization (esp. chemical N and organic manure N) and frequent irrigation during year-round cultivation. A novel substrate cultivation method for vegetable production in Chinese solar greenhouse, called soil-ridged/substrate-embedded cultivation (SSC), was put forward to reduce environmental pollution and increase use efficiency of nutrients. To clarify the characteristics of SSC root-zone greenhouse gas emissions, and the regulation effects of biochar and DMPP addition, five treatments were designed in Chinese solar greenhouse under the same nitrogen application level, including soil-ridge cultivation (SC, as a control), SSC (peat: vermiculite: perlite (v/v=2:1:1), SSC-B50% (biochar: vermiculite: perlite,v/v=2:1:1), SSC-B25% (biochar: peat: vermiculite: perlite, v/v=1:1:1:1), and SSC-DMPP (SSC supplemented with 1% (w/w) DMPP of N fertilizer). Results showed that SSC improved fruit yield of sweet pepper of by 10.99% compared to SC. SSC-B50% and SSC-DMPP significantly improved sweet pepper growth compared to SSC. Moreover, SSC-DMPP increased sweet pepper yield by 10.30% compared to SSC treatment, while SSC-B50% and SSC-B25% treatments lowered the yield by 47.1% and 13.7% separately. Five treatments presented various root-zone temperature features. Also, substrate pH of SC, SSC-B50%, and SSC-B25% is alkaline, while SSC and SSC-DMPP treatments is acidic. Besides, the Global Warming Potential was significantly mitigated in the SSC cultivation compared with the SC. Similarly, the greenhouse gas intensity decreased from 0.074 to 0.038 kg CO_2-eq kg^{-1} yield. Compared with the SSC treatment, cumulative N_2O emissions were significantly reduced in the SSC-DMPP treatment. The greenhouse gas intensity also decreased from 0.038 to 0.033 kg CO_2-eq kg^{-1} yield. Thus, we concluded that SSC was a promising method characterized with reduced greenhouse gas emissions and increased fruit yield. Application of DMPP in SSC cultivation significantly reduced N_2O emissions. We recommend SSC method use in Chinese solar greenhouse with DMPP addition in substrate to optimize greenhouse gas mission.

Keywords: Biochar; CO_2; N_2O; Mitigation; Substrate-cultivation

1. Introduction

North China is a major production area for off-season vegetables using Chinese solar greenhouses (CSG). However, a series of agricultural resource and environmental problems, such as soil salinization, underground water pollution, soil continuous cultivation obstacles[1], and greenhouse gas (GHG) emissions etc. have occurred after the long-term soil cultivation in CSG. Recent data indicated that greenhouse vegetable production is one of the main sources of agricultural N_2O emissions[2,3]. In particular, vegetable production in CSG resulted in a higher level of GHG emissions (particularly N_2O) because of the overuse of N fertilizers, frequent irrigation, and intensive soil

cultivation year-round. This issue is increasingly becoming too important to ignore[4]. Thus, it is vital to explore some simple and effective ways to reduce agricultural resource and environment problems in CSG in North China to enable clean and sustainable greenhouse vegetable production.

Soilless culture constituted the main cultivation system primarily used in horticultural facilities in Europe and North America[5]. Studies have shown that soilless cultivation is the most effective way to overcome a series of problems arisen from soil cultivation, such as soil salinization and continuous cropping obstacles[6]. Furthermore, recent reports have also showed that soilless culture can decrease GHG emission, such as CO_2, N_2O, and CH_4[7-9]. Llorach-Massana et al.[7] estimated an emissions factor (EF) of 0.0072-0.0085 kg N_2O^{-1} per kg N^{-1} for substrate cultivation lettuce. Similarly, Daum and Schenk[8] experimentally assessed an EF of 0.004-0.016 kg N_2O^{-1} per kg N^{-1} for cucumbers grown in rockwool substrate. These studies had proven that soilless cultivation can produce an EF value that is 50% percent less than that accepted by the IPCC (2006)[10]. Furthermore, hydroponics that contain rockwool reduced CO_2 emissions by reducing the number of rhizosphere microorganisms[9]. However, the cultivation substrates usually perform poor in temperature stability, leading to large fluctuation in diurnal root-zone temperature of vegetables. Frequent occurrence of low- and high-temperature stresses in the CSG impacted the performance of substrate cultivation[11]. To overcome this issue, Fu et al.[12] invented a novel substrate-cultivation method, called as soil-ridged/substrate-embedded cultivation (SSC), to substitute for soil cultivation. SSC combines the best features of the root-zone temperature buffer capacity of soil cultivation and the high-yield performance of soilless cultivation [13]. Moreover, drip irrigation techniques and custom-made grooves with inserted plastic film were used to accurately control the amount and fates of water and fertilizer, preventing the downward leaching of nutrients. However, compared with soil cultivation, substrate cultivation has different properties and microbial populations in root zone[14]. Accordingly, the root-zone GHG emission characteristics of the SSC root zone may differ from those of soil cultivation. Clarifying the characteristics of GHG emissions of the SSC root zone, and developing effective mitigation pathway is important basis for the evaluation and large-sacle application of the SSC method. In addition, there is no research report on the effects of 3,4-dimethylpyrazole phosphate (DMPP) and biochar on GHG emissions of soilless crops in CSG, particularly SSC method.

In the intensive plant cultivation system of the CSG, the use of biochar as a growth substrate may have the benefit to replace non-renewable media, such as peat[15]. Furthermore, it may have the potential to reduce GHG emissions[16]. Biochar is a carbon-rich product produced by the pyrolysis of biomass with little or no oxygen[17]. Biochar improved plant growth had reported by changing the soil properties, such as pH, water retention, and porosity, and promoting microbial activity[18,19]. Numerous studies have highlighted the impacts of biochar on soil environment, crop yields and GHG emissions[20-22], but there are few studies on the use of biochar as a soilless crop cultivation substrate. Awad et al.[23]indicated that using a combination of rice husk biochar and perlite as a matrix increased the growth of leafy plants by approximately two-fold compared with the use of perlite alone. However, Daniele et al. [24] found that biochar only increased the biomass of green tomato, but not the tomato yield, and most of its quality indicators. Furthermore, 550℃ maple bark biochar had the highest inhibitory effect on CO_2 emissions, and reduced them by 50%. However, at 700℃, pine chip biochar stimulated CO_2 emission and increased its cumulative emissions[16]. Differences between reported GHG emission results may be due to differences in the physical and chemical properties of biochar, depending on the feedstock and the pyrolysis temperature used in the production process[16].

3,4-Dimethylpyrazole phosphate(DMPP) is one of the most effective nitrification inhibitors (NIs) [25]. It has been reported that DMPP has several obvious advantages over other currently used NIs. This compound is highly effective at inhibiting soil nitrification, reducing N_2O emissions, increasing N fertilizer efficiency and crop yields[25,26]. Previous studies have shown that the application of DMPP in intensive vegetable fields significantly reduced the cumulative soil N_2O emissions by 75%[27-30]. In addition, this inhibitor significantly increased the yield of corn, wheat, carrots, and lettuce[31,32]. However, to our knowledge, the growth of soilless crops supplied with nitrogen fertilizer combined with DMPP has not yet been reported. To maximize the effective use of N fertilizer, and to minimize

the negative environment impacts of greenhouse vegetable production, further research is urgently needed to determine use strategies of biochar and DMPP for SSC method management.

In this study, an experiment was conducted in CSG to investigate the characteristics of SSC root-zone GHG emissions, and the regulation effects of biochar and DMPP addition. The purpose of our research was (i) to quantify the characteristics of CO_2, N_2O and CH_4 emissions in the SSC root zone; and (ii) to evaluate the effects of sweet pepper yield and the GHG emissions from SSC in the root zone following the addition of DMPP and biochar. Finally, effective cultivation management strategies were put forward.

Our hypothesis were: a) SSC can mitigate GHG emissions in the root zone compared with SC; b) SSC with DMPP can further mitigate GHG emissions in the root zone; c)the ability of biochar to mitigate greenhouse gas emissions in the root zone will vary according to the proportion of biochar in the mixed matrix.

2. Materials and methods

2.1. Experiment site and plant material

Our investigation was performed in a CSG of Shunyi research station (40.09°N, 116.91°E) in the Institute of Agricultural Environment and Sustainable Development of the Chinese Academy of Agricultural Sciences, Beijing during October 2019 to March 2020. The soil texture is silt loam. The surface (0-20 cm) soil was with a contained 29.36 $g \cdot kg^{-1}$ organic matter, 3.76 $mg \cdot kg^{-1}$ NH_4^+-N, 136.85 $mg \cdot kg^{-1}$ NO_3^--N. The sweet pepper cultivar (Capsicum annuum L. Haifeng No.190) was utilized as a plant substance. The seeds were all germinated in a nursery tray accumulated with vermiculite and once-daily watered meticulously. On the 5th of October 2019, identical and vigorous seedlings with three perfect leaves were transplanted on ridges, with a distance of 30 cm apart, transplant seedlings into substrate or soil. The area of the test plot is 50 m^2.

2.2. Experimental design

There were four SSC treatments with different substrate composition and with supplemented with DMPP or not in this experiment. They are peat: vermiculite: perlite (v/v=2:1:1, SSC); biochar: vermiculite: perlite (v/v=2:1:1, SSC-B50%); biochar: peat: vermiculite: perlite (v/v=1:1:1:1, SSC-B25%); and SSC supplemented with 1% (w/w) DMPP of N fertilizer (SSC-DMPP). Soil-ridge cultivation was set as a control, three replicates were randomly designed for each treatment. DMPP was obtained from YC biotech companies in Jiangsu, China. DMPP is applied to the root zone with the nutrient solution. Biochar utilized for the substrate investigation was constructed from maize straw supplied by Sanju Biomass Novel Material Technology Company, Jiangsu province, China. The biochar was built in a vertical kiln at a pyrolysis temperature of about 450℃[33]. Initial biochar features were: Organic C content 413.00 $g \cdot kg^{-1}$, total N 7.97 $g \cdot kg^{-1}$, available P 80 mg/kg, pH (H_2O) 8.79, and surface area 2.995 m^2/g.

Construct an SSC ridge through the following steps. SSC ridge structure and seedlings plant were described by Fu et al. [13] as follows: specially made grooves of wire-mesh within a placed plastic film all-embracing substrate were inserted trimly on the ground with the north-south direction and subsequently was filled with homogeneous peat matrix, perlite, and vermiculite along with a volume ratio of 2:1:1. Moreover, in order to enhance the capacity of the buffer attributed to the root-zone temperature, the soil was stacked up and piled with considering both sides of the ridge related to the particular grooves of wire-mesh. Ultimately, the ridge of SSC was covered via plastic mulch, and also drip irrigation was implemented to provide water as well as fertilizing through nutrient solution. The characterization of wire-mesh groove in a U-shape is as follows: 10 cm width, 300 cm length, and 10 cm height. Further, the ridges distance, namely, 40, 20, and 10 cm were considered for bottom width, top width, and height, respectively, that was declared owing to the production requisiteness. The specification of SC was similar to the regular SSC ridges. All procedures were performed by three

replicates. The ridge length in the direction of the north to south remained 3.0 m, and ten sweet peppers are planted.

The implemented nutrient solution had the following composition (mmol·L^{-1}): 0.77 K_2SO_4, 0.1 KCl, 0.5 KH_2PO_4, 0.65 $MgSO_4 \cdot 7H_2O$, 1×10^{-3} H_3BO_3, 1×10^{-3} $MnSO_4 \cdot 4H_2O$, 1×10^{-4} $CuSO_4 \cdot 5H_2O$, 1×10^{-3} $ZnSO_4 \cdot 7H_2O$, 5×10^{-6} $(NH_4)_6MO_7 \cdot 4H_2O$, 0.1 EDTA-Fe, 3.39 $Ca(NO_3)_2 \cdot 4H_2O$[34]. Except for the SSC-DMPP treatment, the other four treatments are provided by a unified nutrient solution tank. SSC-DMPP treatment added DMPP with a nitrogen application rate of 1% and irrigated the plants together with the nutrient solution. The EC and pH of the nutrition materials have remained at 2.0 dS·m^{-1} and 6.5, respectively, via modifying the solution existed in the tank of nutrient solutions every 10 d.

Before transplanting seedlings, samples were collected in the soil or substrate of the cultivation area, mixed and stored at -20°C for analysis of their physical and chemical properties. Total N, available P, K contents of experimental soil, and substrate before cultivation were measured using the procedure detailed by Lu et al. [35], and the results are presented in Table 1.

Table 1. Total nitrogen, available phosphorus and potassium of experimental soil and substrate before cultivation (Mean ± standard error; n = 3).

Treatments	Total nitrogen (g/kg)	Available phosphorus (mg/kg)	Available potassium (g/kg)
SC[a]	0.96±0.01	4.26±0.01	0.46±0.03
SSC	6.54±0.32	5.34±1.08	0.52±0.02
SSC-B 50%	8.37±0.34	75.89±14.52	4.00±0.09
SSC-B 25%	7.74±0.15	26.41±3.51	2.76±0.02
SSC-DMPP	6.51±0.20	5.29±0.47	0.52±0.04

[a] SC：soil culture, as a control; SSC：peat, vermiculite, and perlite at the volume ratio (2:1:1，v/v)；SSC-B 50%: biochar, vermiculite, and perlite at the volume ratio (2:1:1，v/v); SSC-B 25%: biochar, peat, vermiculite and perlite at the same volume ratio (1:1:1:1); SSC-DMPP: peat, vermiculite, and perlite at the volume ratio (2:1:1，v/v), and DMPP with 1% nitrogen was applied. The same below.

When all considered procedures were fulfilled successfully, a drip irrigation system was implemented. The cultivation process was divided into three major steps, including vegetative growth, flowering, and ripening processes as the first to third steps. Afterward, the achieved seedlings (30 d after transplantation) were watered by using automatic systems via a nutrient solution at 10:00 within the vegetative growth, and also the volume of irrigation related to each seedling was around 20 ml at a precise time. At the second step (flowering), the sweet peppers were all irrigated by taking advantage of nutrient solution at 10:00, while the volume of irrigation for each plant was about 40 ml at a time. At the third step (ripening), planted sweet peppers were irrigated through nutrient solutions at 10:00, and 13:00, respectively, and also the volume of daily irrigation for each plant was around 80 ml. The irrigation amount was assigned according to the growth of sweet pepper.

2.3. Growth parameters measurement

Leaf chlorophyll content, plant height, and stem diameter were evaluated via chlorophyll meter (SPAD-502, Konica Minolta Sensing Co., Osaka, Japan), ruler, as well as vernier calliper at 30 d, 60 d, and 90 d after transplantation. The fruits of ripen sweet pepper were harvested on Jan. 15, 2020, Jan. 30, Feb. 15, Feb. 29, and Mar. 15. Five plants were randomly selected from each treatment, and the yield of pepper was measured by accumulative balance to get the yield of the plant. Total output calculations were based on planting density and area. After the sweet pepper fruits were harvested on March 15th, the plants were subjected to a seedling pulling treatment. The five plants tested were washed thoroughly with faucet water, rinsed well with deionized (DI) water, and next separated into stems and roots. Fresh masses of roots and shoots were measured. Plant organs were dried for at least 48 h at 105°C in a ventilated oven to determine their dry weights[36].

2.4. Root-zone temperature, substrate EC and pH determination

The root-zone temperature was recorded every 10 min by the CR1000 data collector and T-type thermocouple wire produced in the United States. Root-zone temperature measurement was performed in the vertical profile at a depth of 5 cm. According to the different growth stages of sweet peppers, we continuously selected Nov. 7-11, 2019, Dec. 7-11, and Jan. 7-11, 2020 for data analysis.

Soil and substrate samples were collected before seedling transplantation and after harvest, and fresh samples were dried. Unless otherwise specified EC and pH measurements were carried out in triplicate. EC and pH were assessed as defined by Lu et al.[35] via adding the 10 g of soil and substrate in 25 mL DI water. Then, after 1.5 h of stirring, pH (PHS-2F, Shanghai INESA Scientific Instrument Co., Ltd., China) and EC (DDSJ-308F, Shanghai INESA Scientific Instrument Co., Ltd., China) were evaluated on the passing of filtration using a filter paper with VWR grade 413 (5μm).

2.5. Gas sampling and determination

Greenhouse gas(GHG) fluxes were evaluated *via* the method of the static chamber[37]. Before gas extraction, One PVC collar was inserted in per plot, and the top side of the collar possessed a groove, loaded with water 5 cm high in order to seal the flange of a 10cm × 10cm × 40cm gas-sampling chamber. A fan was fixed on the top of the box to mix the gas, and the gas sampling tube and temperature probe were respectively placed inside the box. Briefly, gas samples were collected between 10:00 and 11:00 in the early hours of each sampling day. The utilized lid apparatus in the top-side has a gas sampling three-way valve connected to the gas sample bag, five 100 mL headspace samples were taken using a 150 mL gas sample bag at 0, 10, 20, 30, and 40 min after the shutting of the chamber. Then, a 100ml gas-tight syringe was used to transfer 40 mL of gas in the gas sample bag to a special vacuum glass bottle, which was instantly taken to the laboratory for further investigation. The concentration of existed gas in the specimen was studied comprehensively in 24 h after sampling procedure, an Agilent 7890A gas chromatograph provided with a flame ionization detector (FID) for CO_2 and CH_4 detection as well as an electron capture detector (ECD) in order to detect N_2O detection[38]. The carrier gas in ECD and FID were Ar-CH_4 and High-purity N_2, respectively. The gas specimen was separated *via* columns made of stainless steel and packed with Porapak Q (80/100 mesh. The oven temperature was adjusted at 55℃, and the temperature of the FID and ECD was fixed at 330℃ and 200℃, respectively. The gas chromatography configurations defined by Xiang et al. (2015)[38] were implemented for the analysis of gas concentration. GHG fluxes were assessed *via* utilizing the linear increases within gas concentration with time. Specimen sets were denied unless they created a linear regression value of $R^2 \geq 0.90$[39].

2.6. Data analysis and statistics

The flux of Gas emissions ($mg \cdot m^{-2} \cdot h^{-1}$) was measured using the following equation [38]:

$$F = \rho \times \frac{V}{A} \times \frac{dc}{dt} \times \frac{273}{273+T} \times \frac{P}{P_0} \quad (1)$$

Where F is indicated in $mg \cdot m^{-2} \cdot h^{-1}$; ϱ states the gas density at the standard state, V(m^3) represents the chamber volume, A (m^2) states the area of the pot, dc/dt is the modification of gas concentration with time, and T (°C) is the average temperature inside the considered chamber within the sampling period, P_0(mm·Hg) represents standard atmospheric pressure, $P/P_0 \approx 1$.

The seasonal cumulative gas emissions (E, kg N_2O-N ha^{-1}) were calculated regarding to the equation characterized utilizing the Eq. (2) [38]:

$$E = \sum_{i=1}^{n} \frac{Fi+Fi+1}{2} \times (t_i + 1 - t_i) \times 24 \quad (2)$$

In this equation, F_i and F_{i+1} state the N_2O (or CH_4, CO_2) efflux at the $_i$th and ($_{i+1}$)th assessment time (mg N/C m^{-2} h^{-1}), respectively, t_{i+1}-t_i is the interval between the $_i$th and ($_{i+1}$)th assessment time (*d*), and n is the total assessment time.

In order to realize the climatic effects of the vegetable fields systems under the addition of DMPP and biochar, we present GWP to determine greenhouse impacts applying the equation altered from Zhang et al.[37].

$$\mathrm{GWP}(\mathrm{kg}\ CO_2 - \mathrm{equivalent}\ \mathrm{ha}^{-1}) = 25 \times \mathrm{GWP}_{(CH_4)} + 298 \times \mathrm{GWP}_{(N_2O)} + \mathrm{GWP}_{(CO_2)} \quad (3)$$

The greenhouse gas intensity (GHGI) is the other concept that make a connection between biochar and DMPP to GWP, which was measures *via* dividing GWP by the yield from vegetable [40].

$$\mathrm{GHGI} = \frac{\mathrm{GWP}}{\mathrm{grain}\ yield}(kg\ CO_2 - \mathrm{equivalent}\ \mathrm{ha}^{-1}\ vegetable\ yield\ yr^{-1}) \quad (4)$$

Statistical analyses were carried out utilizing SPSS 25.0 for Windows (SPSS China, Beijing, China). A one-way ANOVA was used to analyze the effects of the DMPP, substrate type on vegetable yield, plant height, SPAD, stem diameter, intensity over the full experimental period. Data from the incubation study were analyzed according to a randomized complete block design with three replicates.A repeated measure univariate ANOVA was also used to test the effect of biochar and DMPP on the cumulative CO_2, CH_4 and N_2O emissions.The variance homogeneity was verified by a graphical analysis of the residuals and no transformation was necessary. The significant differences between means were established using Tukey's test at $p < 0.05$.

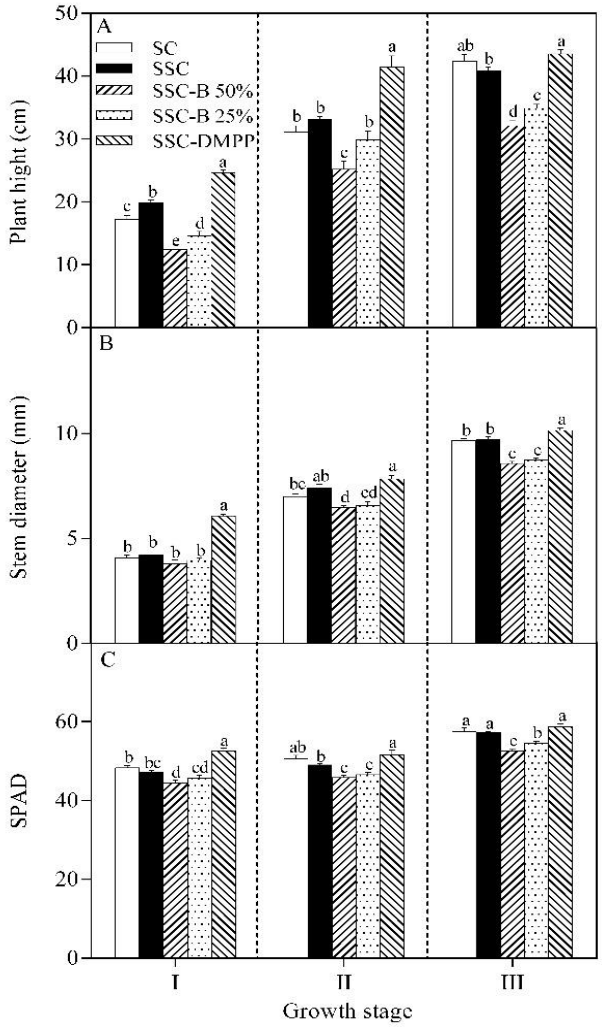
3. Results

3.1. Morphological parameters and SPAD at 30 d, 60 d, 90 d after transplantation of sweet pepper

Fig. 1 (A) showed the effects of biochar and DMPP addition on plant height of SSC sweet pepper in three growth stages. It can be seen from the figure that the plant height is increasing with the growth period, and there are significant differences in the plant height of sweet peppers with different treatments at the same stage ($P < 0.05$). From the vegetative growth to the ripening stage, the plant height was always highest in SSC-DMPP treatment, and it was significantly higher than the other four treatments in the first two stages. In the ripening stage, the SSC-DMPP treatment changed from vegetative growth to reproductive growth, and its growth rate was relatively lagging, while the growth rate of SC treatment in the flowering to ripening stages accelerated, and its plant height in the ripening stage was not statistically different from SSC-DMPP treatment. SSC-B50% and SSC-B25% of biochar treatments grew slowly in the growing season of sweet pepper, and was consistently significantly lower than SC treatment.

It can be seen from Fig. 1(B) that the stem diameter of sweet peppers at different growth stages that have been treated with SSC-DMPP to be the highest, and SSC-B50% is the lowest, and there are significant differences between the two treatments ($P < 0.05$).

SPAD value indirectly evaluates the chlorophyll content of sweet pepper leaves. It can be seen from Fig. 1(C) that the SPAD value of each treatment did not show an increasing trend in the first two stages, only the chlorophyll content of the sweet pepper leaves increased during the ripening stage. In addition, the chlorophyll content of sweet peppers in different growth stages has been the highest in SSC-DMPP treatment, and was significantly higher than the other four treatments in the vegetative growth period. Compared with SC treatment, SSC treatment had no marked difference in chlorophyll content during the whole growth stage. The two treatments of SSC-B50% and SSC-B25% significantly reduced the chlorophyll content.



Note: The cultivation period was divided into three growth stages (I, vegetative growth; II, flowering; III, ripening). Different growth stages, for sweet pepper, different letters indicates the difference significance among treatments at 0.05 level, and during each growth stages are presented as mean values (± standard error, n=5).

Figure 1. Effects of biochar and DMPP addition on plant height (A), stem diameter (B) and SPAD (C) of SSC sweet pepper in three growth stages.

3.2. Biomass and fruit yield of sweet pepper

Table 2 reports a analysis of the sweet pepper plant and the root system through destructive methods after the sweet pepper fruit is harvested. Different treatments significantly affected the root and shoot fresh and dry weight and yield of sweet pepper plants. Compared with SC treatment, 25% SSC and SSC-B treatment significantly reduced the fresh weight of plants, and 50% SSC-B treatment significantly reduced the dry weight of plants. In addition, compared with SC treatment (about 4.55 kg·m-2), SSC treatment increased fruit yield by 10.99%, and SSC-DMPP treatment increased fruit yield by 22.42%. However, SSC-B 50% and SSC-B 25% reduced the yield of sweet pepper by 40.00% and 3.74%, respectively.

Table 2. Biomass and fruit yield from SSC sweet pepper supplemented biochar and DMPP (Mean ± standard error; n = 5).

	Fresh weight(g)			Dry weight(g)			Fruit yield per plant(kg)	Fruit yield ($kg \cdot m^{-2}$)
	Shoot	Root	Total	Shoot	Root	Total		
SC	610.7±18.3a	59.1±1.9b	669.8±20.1a	60.0±1.5ab	13.8±0.9ab	73.6±1.1ab	0.76±0.02c	4.55
SSC	542.8±12.1c	62.6±1.4b	605.4±12.0c	54.0±1.3bc	14.7±1.4ab	68.7±2.5bc	0.84±0.02b	5.05

SSC-B 50%	581.3±2.6ab	69.7±1.4a	651.0±2.7ab	51.6±3.4c	12.3±0.9b	63.9±4.2c	0.46±0.01d	2.73
SSC-B 25%	561.8±10.5bc	62.1±1.0b	623.8±11.0bc	54.7±1.6bc	14.1±1.1ab	68.8±2.2bc	0.73±0.01c	4.38
SSC-DMPP	605.3±10.0a	69.7±2.4a	675.0±9.0a	61.8±1.1a	16.0±0.8a	77.8±1.0a	0.93±0.01a	5.57

Note：Lowercase indicates the difference significance among treatments at 0.05 level. The same as below.

3.3. Root-zone temperature at different growth stages of sweet pepper

As shown in Table 3-5, with the continuous growth of sweet pepper, the various indexes of root-zone temperature in each treatment gradually decreased. The root-zone temperature reached the highest on November 7-11, and the lowest on January 7-11. Whether it is the root-zone temperature during the day or night, the difference between every two months reaches about 5°C. The highest root-zone temperature varies between November and December. The lowest root-zone temperature in November is 10°C higher than that in December. The lowest temperature in the root-zone gradually decreases to about 3°C every two months. In the same month, the differences of various indicators between different treatments were all between 0-3°C.

Table 3. Root-zone temperature of sweet pepper from soil cultivation and SSC supplemented with biochar and DMPP on Nov.7-11, 2019 .

Treatments	Average daytime temperature	Average nighttime temperature	Average highest temperature	Average lowest temperature	Difference of average highest and lowest temperature
SC	24.05	21.23	28.05	17.92	10.13
SSC	25.99	18.94	30.76	15.56	15.20
SSC-B 50%	25.27	18.94	29.84	15.52	14.32
SSC-B 25%	24.22	21.23	27.84	17.82	10.03
SSC-DMPP	25.89	19.12	30.40	15.66	14.75

Table 4. Root-zone temperature of sweet pepper from soil cultivation and SSC supplemented with biochar and DMPP on Dec.7-11, 2019.

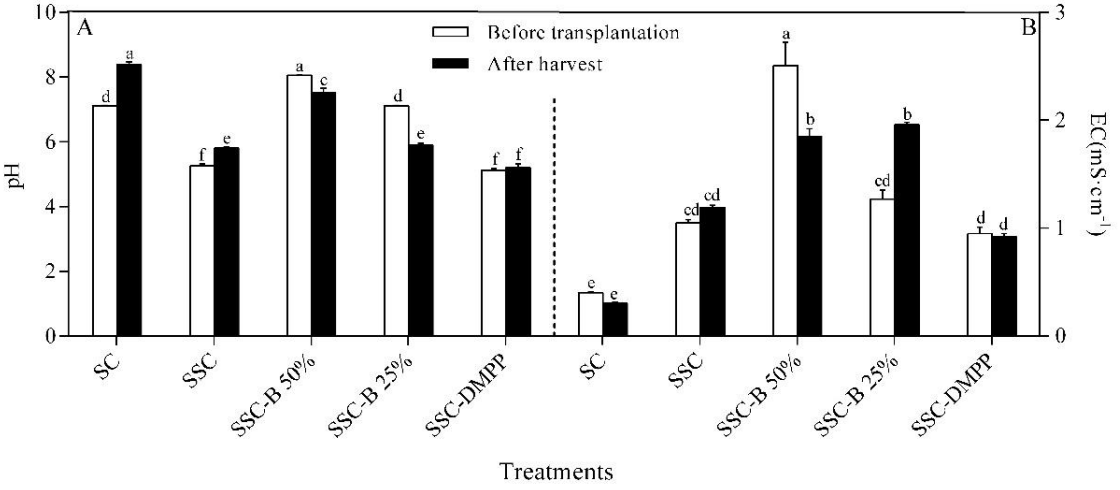
Treatments	Average daytime temperature	Average nighttime temperature	Average highest temperature	Average lowest temperature	Difference of average highest and lowest temperature
SC	17.33	17.31	19.94	13.44	6.50
SSC	17.07	16.14	21.55	14.40	7.15
SSC-B 50%	19.05	16.80	22.81	13.29	9.52
SSC-B 25%	17.66	17.02	21.68	13.86	7.82
SSC-DMPP	16.87	16.33	20.82	13.42	7.40

Table 5. Root-zone temperature of sweet pepper from soil cultivation and SSC supplemented with biochar and DMPP on Jan.7-11, 2020.

Treatments	Average daytime temperature	Average nighttime temperature	Average highest temperature	Average lowest temperature	Difference of average highest and lowest temperature
SC	15.26	13.16	18.28	10.35	7.93
SSC	16.20	12.57	20.45	9.79	10.66
SSC-B 50%	15.49	12.82	18.63	10.19	8.44
SSC-B 25%	15.63	14.02	19.44	11.02	8.42
SSC-DMPP	15.82	12.94	18.73	10.11	8.63

3.4. Substrate electric conductivity and pH

As shown in Fig. 2, SC, SSC-B50%, and SSC-B25% treatments are an alkaline cultivation media, while SSC and SSC-DMPP treatments are an acidic cultivation media. After the sweet pepper was harvested, the pH value of SC, SSC and SSC-DMPP treatment increased, while the pH value of SSC-B50% and SSC-B25% treatment decreased. Different from pH value, the (electric conductivity)EC value of substrate cultivation treatment was significantly higher than that of soil (P<0.05), and the EC background value of SSC-B50% treatment was the highest. After the sweet pepper is harvested, the EC value of SSC-B25% treatment is the highest. Besides, the EC value of SSC-DMPP treatment was significantly lower than that of SSC treatment(P < 0.05).



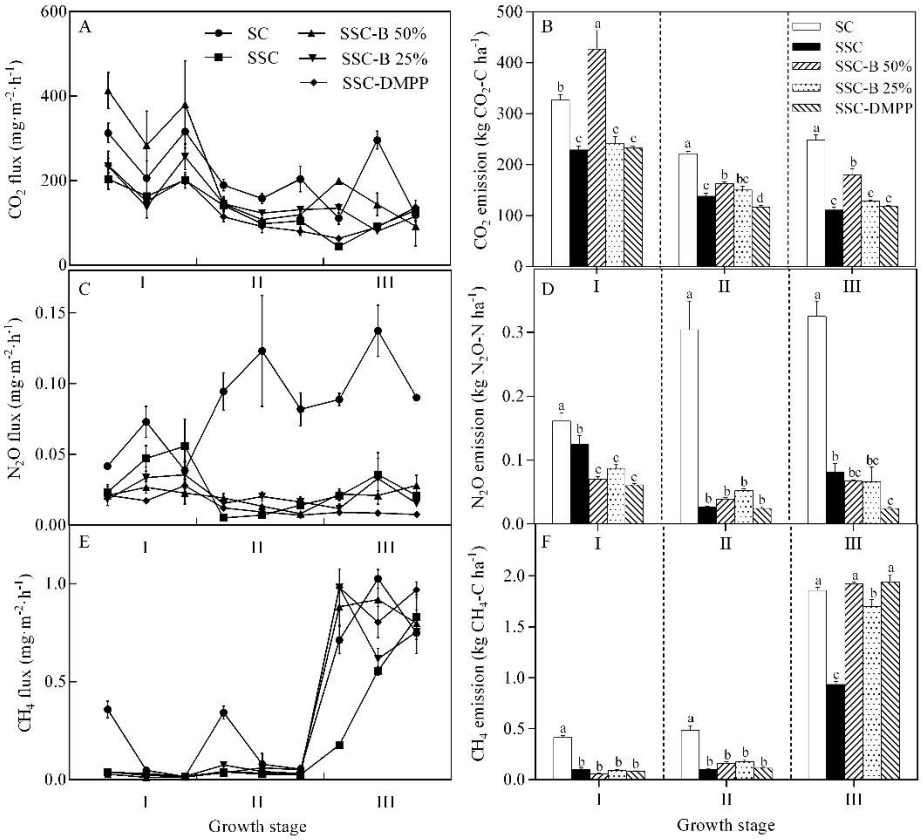
316

317 Note: Different letters indicates the difference significance among treatments at 0.05 level. The pH and EC of sweet
318 pepper plants before transplantation and after transplanting were determined.

319 **Figure 2.** Changes of pH and EC of soil and substrate before transplantation and after harvest of sweet
320 pepper.

321 *3.5. Root-zone CO_2, N_2O and CH_4 emissions at three growth stages*

322 Among the three stages, the CO_2 emission in vegetative growth period was the highest, and all
323 treatments showed a trend of gradual decline with the delay of growth period, as shown in Fig. 3A.
324 The CO_2 emissions of different treatments in the same growth stage were significantly different ($P <$
325 0.05, Fig. 3B). In the first four weeks after transplantation (growth stage I), SSC-B50% treatment had
326 the highest CO_2 emissions (approximately 427 kg CO_2-C ha^{-1}), which was significantly higher than
327 other treatments. SSC treatment has the lowest CO_2 emissions (approximately 229 kg CO_2-C ha^{-1}),
328 which is not significantly different from SSC-B25% and SSC-DMPP treatments. In the latter two stages,
329 the CO_2 emissions of SC treatment are the highest and are significantly higher than the other several
330 treatments. The CO_2 emissions of SSC, SSC-B25%, and SSC-DMPP treatment are about half that of SC
331 treatment.
332 In contrast, the N_2O emissions of each treatments are significantly different at the same stage (P
333 <0.05, Fig. 3C), but there are differences from the CO_2 emissions model. Unlike the CO_2 emissions, the
334 SC treatment was significantly higher than the other four treatments throughout the growth stage and
335 showed a gradual upward trend as the growth period delayed(Fig. 3C). However, the N_2O emissions
336 of SSC, SSC-B50%, SSC-B25%, and SSC-DMPP treatments remained low, and the four treatments had
337 the highest N_2O emissions in the first 4 weeks after transplantation (growth stage I). In the four weeks
338 after transplantation, compared with SSC treatment (approximately 0.125 kg N_2O-N ha^{-1}), SSC-B50%,
339 SSC-B25%, and SSC-DMPP treatments all significantly reduced N_2O emissions, and there was no
340 significant differences in N_2O emissions during the entire growth period among the three
341 treatments(Fig. 3D).
342 The CH_4 emissions fluctuated greatly during the cultivation period (Fig. 3E), while the CH_4
343 emissions between the treatments were significantly different at the same stage ($P < 0.05$). In the first
344 two growth stages, except for the SC treatment, the CH_4 emissions of the other four treatments did not
345 exceed 0.2 kg CH_4-C ha^{-1}. But in the ripening period, except for the SSC treatment, the CH_4 emissions
346 of the other four treatments all exceeded 1.5 kg CH_4-C ha^{-1}(Fig. 3F).



Note: The cultivation period was divided into three growth stages (I, vegetative growth; II, flowering; III, ripening). Different growth stages, for sweet pepper, different letters indicates the difference significance among treatments at 0.05 level, and during each growth stages are presented as mean values (± standard error, n=3).

Figure 3. Effects of Biochar and DMPP on CO_2 (A-B), N_2O (C-D) and CH_4 (E-F) dynamics and cumulative emissions of SSC sweet pepper during three growing stages

3.6. Cumulative CO_2, N_2O, and CH_4 emissions and their GWP, GHGI

The cumulative N_2O emissions in the control was significantly higher than those in the SSC, SSC-B50%, SSC-B25%, and SSC-DMPP treatments, which was up to 0.79 kg N_2O-N ha^{-1} (Table 6). The cumulative N_2O emissions of SSC treatment are only 29.0% of SC treatment. And compared with the cumulative N_2O emissions processed by SSC treatment, the cumulative N_2O amount was not significantly affected by biochar addition, that is, biochar amendment resulted in 21.7% and 13.0% decrease in the SSC-B 50% and SSC-B 25% treatments. However, SSC-DMPP significantly reduced N_2O cumulative emissions by 52.2%. The cumulative CO_2 emissions amount was significantly resulted in 40.1% decrease in the SSC treatment as compared with the SC treatment. Compared with SSC treatment, SSC-B50% treatment significantly increased the cumulative CO_2 emissions, while SSC-B25% and SSC-DMPP treatments had unsignificant difference. SSC treatment has the lowest cumulative CH_4 emissions. SSC-B50%, SSC-B25%, and SSC-DMPP treatments all significantly increased the cumulative CH_4 emissions. SSC treatment significantly mitigated the GWP compared with the SC treatment. While the GWP was not significantly affected by SSC-B25% and SSC-DMPP as compared with the SSC treatment.

Table 6. Cumulative N_2O, CO_2 and CH_4 emissions of biochar and DMPP applied in the three growth stages of sweet pepper (Mean ± standard error; n = 3).

Treatments	N_2O emission (kg N_2O-N ha^{-1})	CO_2 emission (kg CO_2-C ha^{-1})	CH_4 emission (kg CH_4-C ha^{-1})	GWP (kg CO_2-eq ha^{-1})	GHGI (kg CO_2-eq kg^{-1} yield)

SC	0.79±0.06a	798±21a	2.76±0.04a	3387±64a	0.074
SSC	0.23±0.03b	478±2b	1.14±0.02d	1899±17c	0.038
SSC-B 50%	0.18±0.01bc	770±46a	2.14±0.01b	2978±168b	0.109
SSC-B 25%	0.20±0.02b	520±13b	1.96±0.07c	2069±55c	0.047
SSC-DMPP	0.11±0.01c	468±5b	2.14±0.07b	1839±21c	0.033

Note：Lowercase indicates the difference significance among treatments at 0.05 level. The same as below.

$$\mathrm{GWP}\ \ (\mathrm{kg}\ \ CO_2 - \mathrm{equivalent}\ \ \mathrm{ha}^{-1}) = 25 \times \mathrm{GWP}_{(CH_4)} + 298 \times \mathrm{GWP}_{(N_2O)} + \mathrm{GWP}_{(CO_2)}$$

$$\mathrm{GHGI} = \mathrm{GWP/grain}\ \ yield\,(kg\ \ CO_2 - \mathrm{equivalent}\ \ \mathrm{ha}^{-1}\ \ vegetable\ \ yield\ \ yr^{-1})$$

4. Discussion

4.1. Effects of adding biochar and DMPP into SSC substrate on growth and yield of sweet pepper

SSC is a novel substrate cultivation method for fruit and vegetable production in CSG with significant practical advantages that can solve a series of environmental problems caused by the production of fruits and vegetables[13]. Compared with SC treatment, that of SSC improved the yield of peppers by 10.99%, although it did not facilitate the growth of plants. The reason for this result could be that the SSC model takes full advantage of the high yield of soilless culture and is more conducive to promoting the reproductive growth of crops[41]. In addition, as a root-limited cultivation and plastic film barrier, SSC can effectively retain nutrients in the zone of root growth, which is convenient for sweet peppers. However, soil cultivation cannot maintain nutrients in the root zone, which then easily diffuse into the surrounding areas, resulting in insufficient nutrients in the root zone, thus, weakening its reproductive growth[13].

In this study, biochar was tested as a cultivation substrate. Compared with the SSC treatment, the two treatments with the addition of biochar resulted in poorer growth during the three stages of growth of sweet pepper (Fig.1). In fact, the low temperature pyrolysis method for making biochar may have created toxic compounds that inhibit plant growth[42]. In addition, biochar with highly variable amounts of volatile matter will also inhibit plant growth in the short term owing to the immobilization of nitrogen[42]. However, the biomass accumulation of the SSC-B50% treatment increased significantly by 7.60% compared with the SSC control. In terms of biomass accumulation, the reason why biochar-containing substrates perform better may be owing to the interaction between biochar and plant nutrition, which is based on what was proposed in previous studies with vegetable crops[15]. It is worth noting that the higher accumulation of biomass that was found in sweet peppers grown on biochar did not lead to enhancements in yield. In fact, compared with treatment with peat, the plants grown in biochar media exhibited different trends of the distribution of organs, which aids in the development of accumulation of plant biomass instead of reproductive organs[15]. Furthermore, the allelopathic effect of biochar-derived hydrocarbons or toxic levels of heavy metals can also have deleterious effects on yields[42]. There are fewer studies on the use of biochar as a soilless culture, and more reports explain the mechanism for the positive impact on crop yields[23,43]. Therefore, insights into the causes of negative effects of biochar on yield are necessary.

Applications with DMPP fertilizer promoted the growth of sweet peppers (Fig. 1). In many experiments with vegetables, when fertilizer is applied in concert with DMPP, NH_4^+-N is supplied to crops at a higher rate over a longer time. Plants will absorb a large amount of NH_4^+-N, which will reduce the concentration of NO_3^- [32]. In addition, DMPP-induced partial NH_4^+-N may decrease the pH and increase microbial activity in the rhizosphere[32]. This lower pH should improve the ability of plants to absorb other nutrients, particularly micronutrients and lay a foundation for crops to absorb the nutrients needed to grow and improve their quality[32]. This could be confirmed in this experiment (Fig. 2). More importantly, the application of DMPP not only increased the quality of the plants' dry matter but also significantly increased the yield of sweet peppers by 10.30% compared with the SSC treatment. The results of applying DMPP to promote vegetable yield have been reported in previous studies[32]. This could be because the application of DMPP reduces the leaching of nitrate nitrogen, maintains the efficiency of fertilizer of the nutrient solution, and ensures that there is sufficient fertility in the substrate during the latter period of nutrient absorption of sweet peppers, thereby increasing

their yield[31,32]. Therefore, fertilizers combined with DMPP may improve the yields of agricultural crops and promote the green, clean and sustainable development of agriculture.

4.2. Effects of adding biochar and DMPP into SSC substrate on root-zone GHG emissions

An increasing number of studies have shown that GHG emissions from horticultural products are mostly because of the cultivation procedure, rather than the industrial products of materials attained from cultivation: viz., biocides, fertilizer, electricity, etc. [7,9,44]. Our research concentrated on the cultivation procedure and compared CH_4, CO_2, and N_2O emissions from soil cultivation and substrate cultivation. The results show that the rhizosphere regions GHG emissions of SSC treatment are significantly reduced compared to SC treatment (Fig. 3). The total GHG emissions of SC and SSC are 3387 and 1899 kg CO_2-eq ha^{-1}, respectively. This explains that the SSC procedure indicated a positive impact on global warming. In fact, the soil includes a huge pool of carbon and abundant 16S rRNA genes. Therefore, a greater amount of CH_4 and CO_2 emissions from soil could be easily described as a great number of microorganisms and also resulted in a high rate of microbial soil respiration [9,45]. Interestingly, the N_2O emissions in the soil come from nitrification and denitrification reactions. However, the inert conditions of substrates used in soilless culture and their poor water retention capacity inhibit the growth of microbial populations, making denitrification may be the main mechanism [7,46]. But the denitrification route is further limited by the high porosity of the substrate, which leads to the aerobic environment[46]. Therefore, soilless cultivation may alleviate N_2O emissions.

Generally, peats in enhanced decomposition release less amount of CO_2 and are rich in recalcitrant C[47]. In the present study, both SSC-B50% and SSC-B25%, which were replaced by peat increased the cumulative emissions of CO_2 and CH_4 ($P < 0.05$). Although we did not observe the total C content in the mixed matrix, the cumulative CO_2 emissions of SSC-B50% treatment was significantly higher than that of SSC-B25% treatment. The increase in CO_2 emissions caused by the addition of biochar may be because of: (i) abiotic release of inorganic C [48]; (ii) microbial decomposition of more unstable biochar components[49]. Moreover, there was evidence that a strong correlation was seen between the cumulative emissions of CO_2 and CH_4, merely anaerobic methanogenic archaea utilize CO_2 as an energy resource, releasing CH_4[16]. Especially when the rhizosphere regions were at a neutral to alkaline pH, the addition of biochar will enhance CH_4 emissions[50]. In contrast, the addition of biochar mitigating N_2O emissions (Table 2). The mitigation of N_2O by biochar addition was related to several potential mechanisms: (i) Inhibit denitrification by improving rhizosphere regions aeration; (ii) Increase pH to promote complete denitrification; (iii) decrease in soil N availability for microbial activities through immobilization or sorption; (iv) induce the toxic impacts on microorganisms involved in cycle of N[51]. Overall, Biochar completely replaces peat and may lead to increased emissions of CO_2 and CH_4, but the partial addition of biochar into the peat matrix seems to be a fascinating practice for developing clean and green production. Future investigation should survey the impact of the increment of various rates of biochar to peat matrix on yields of myriad CSG crops as well as GHG emissions during production.

Notably, DMPP treatment with 1% pure nitrogen reduced potential N2O emissions by a further 52.2% compared to SSC treatment (Table 6). This was also found in our previous experiments. This could be elucidated through the following description: (i)DMPP straightly repress N_2O emissions from nitrification-mediated pathways *via* hindering the metabolic activity and growth of AOB and feasibly AOA[52]; (ii)The increasing of DMPP significantly improved the affluence of both nosZI-N_2O and nosZII-N_2O reducers, and the ability of N_2O reduction to N_2 was enhanced, thus reducing N_2O emissions. The impact of DMPP on CO_2 and CH_4 emissions is still controversial[53]. Although, in this experiment, the addition of DMPP reduced the cumulative CO_2 emissions, and it promoted CH_4 emissions. DMPP can reduce the release of CO_2, which may be due to the reduction of organic carbon mineralization and carbon decomposition in the rhizosphere[54]. For the increase of CH_4 emissions, we cannot exclude the increase of rhizosphere microorganisms or N application[9]. In fact, the specific micro-mechanism of how DMPP affects CO_2 and CH_4 is still unclear, which needs further study and discussion.

4.3. Effects of the root-zone environment on N_2O emissions and plant growth

N_2O emissions from the root zone are not only affected by N fertilization but also by factors, such as temperature, pH and EC[8,9]. Although our experiments showed that the temperature of root zone gradually decreased with the growth period, the N_2O emissions did not decrease as a result. Interestingly, the N_2O emissions of soil cultivation increased in the latter two periods compared with the first period. The reasons for this phenomenon could include the following: (i) the root zone temperature is maintained at 10~30℃, which is conducive to nitrification and denitrification to produce N_2O, and (ii) we provided abundant NO_3^- substrates for the production of N_2O by denitrification. Thus, this promotes the production of N_2O emissions. However, the role of temperature in the nitrification and denitrification process in the root zone is more complicated. Future research should comprehensively consider the interactive effects of temperature and other control factors.

The optimal pH in the root zone of most substrates ranges from 5.5 to 6.5. Since the nutrient solution of each plant in the root zone is limited, the risk of exceeding or falling below this value will increase. The growth of most plants is restricted when they are exposed to external pH levels > 7 or < 5 [55]. This experiment showed that alkaline biochar as a cultivation substrate has a negative impact on the growth of sweet pepper plants. In addition, pH is an important and complex influencing factor in the process of producing N_2O emissions in the root zone. It affects N_2O emissions by directly or indirectly affecting the activities of microorganisms involved in the nitrogen conversion process and the activities of enzymes at different stages of action[56]. Our results show that soil cultivation provides a more suitable pH value for nitrification and denitrification and promotes the emissions of N_2O. The EC is considered to be one of the most important characteristics in soilless culture[57]. Typically, the EC value is used to evaluate the growth and yield of plants. If the EC is too low, the supply of some nutrients to the crop may be inadequate. Similarly, when the EC is too high, the plants are exposed to salinity[58]. In summary, it is very important to determine the substrate ratio suitable for the growth of sweet pepper, because it not only affects the yield but also affects the production of GHG emissions. The ratio of biochar to peat merits further testing and exploration.

5. Conclusions

In this study, the SSC method significantly increased the fruit yield of sweet pepper by 10.5% compared to SC. SSC-B50% and SSC-DMPP plants had a significant increase in root and shoot fresh weight compared with SSC treatment. SSC-DMPP treatment increased the yield of sweet pepper per plant by 10.7%, but SSC-B50% and SSC- B 25% treatments reduced by 45.2% and 13.1%, respectively. Furthermore, SSC significantly reduced the cumulative emissions of CO_2, N_2O, and CH_4 compared to SC. The SSC-DMPP treatment significantly reduced the cumulative N_2O emissions by reducing the N_2O emissions during the vegetative growth period and fruiting period of the sweet pepper, compared with the SSC treatment. However, the SSC-B50% treatment increased the CO_2 and CH_4 emissions, thereby significantly improved GWP. This study shows that SSC reduce N_2O emissions when compared to conventional crops, making soilless crops an attractive practice for reducing GHG emissions. It should be emphasized that the results of this study are based on the parameters described here, such as substrate type, fertilizer and irrigation systems, but it does not provide a single GHG emission result for all soilless crops. We hope that the results of this study will help to provide a new way of thinking about greenhouse vegetable cultivation.

Author contributions: Baoshi Li: Conceptualization, Methodology, Formal analysis, Investigation, Writing-Original Draft. Wenke Liu: Conceptualization, Resources, Project administration, Writing-Review & Editing, Supervision. Qi Wang: Conceptualization, Methodology, Formal analysis.

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Figures

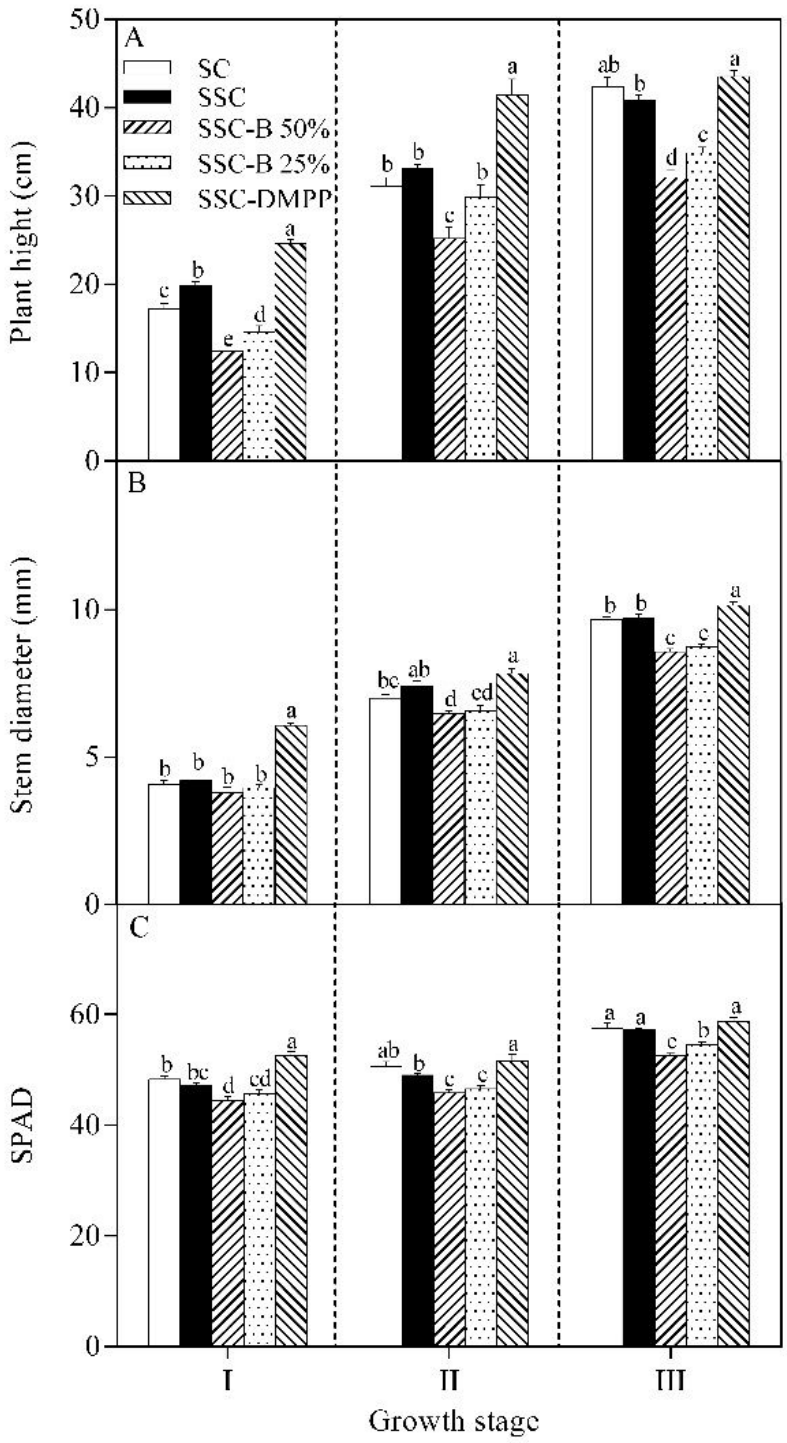

Figure 1

Effects of biochar and DMPP addition on plant height (A), stem diameter (B) and SPAD (C) of SSC sweet pepper in three growth stages.

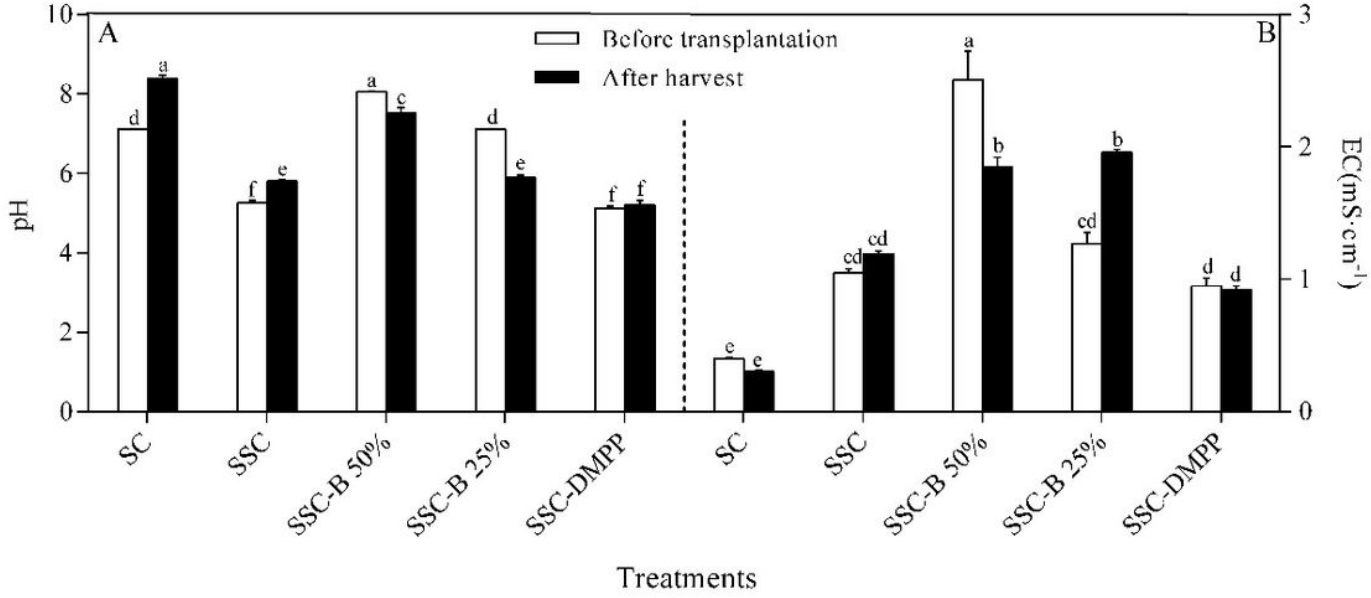

Figure 2

Changes of pH and EC of soil and substrate before transplantation and after harvest of sweet pepper. Note: Different letters indicates the difference significance among treatments at 0.05 level. The pH and EC of sweet pepper plants before transplantation and after transplanting were determined.

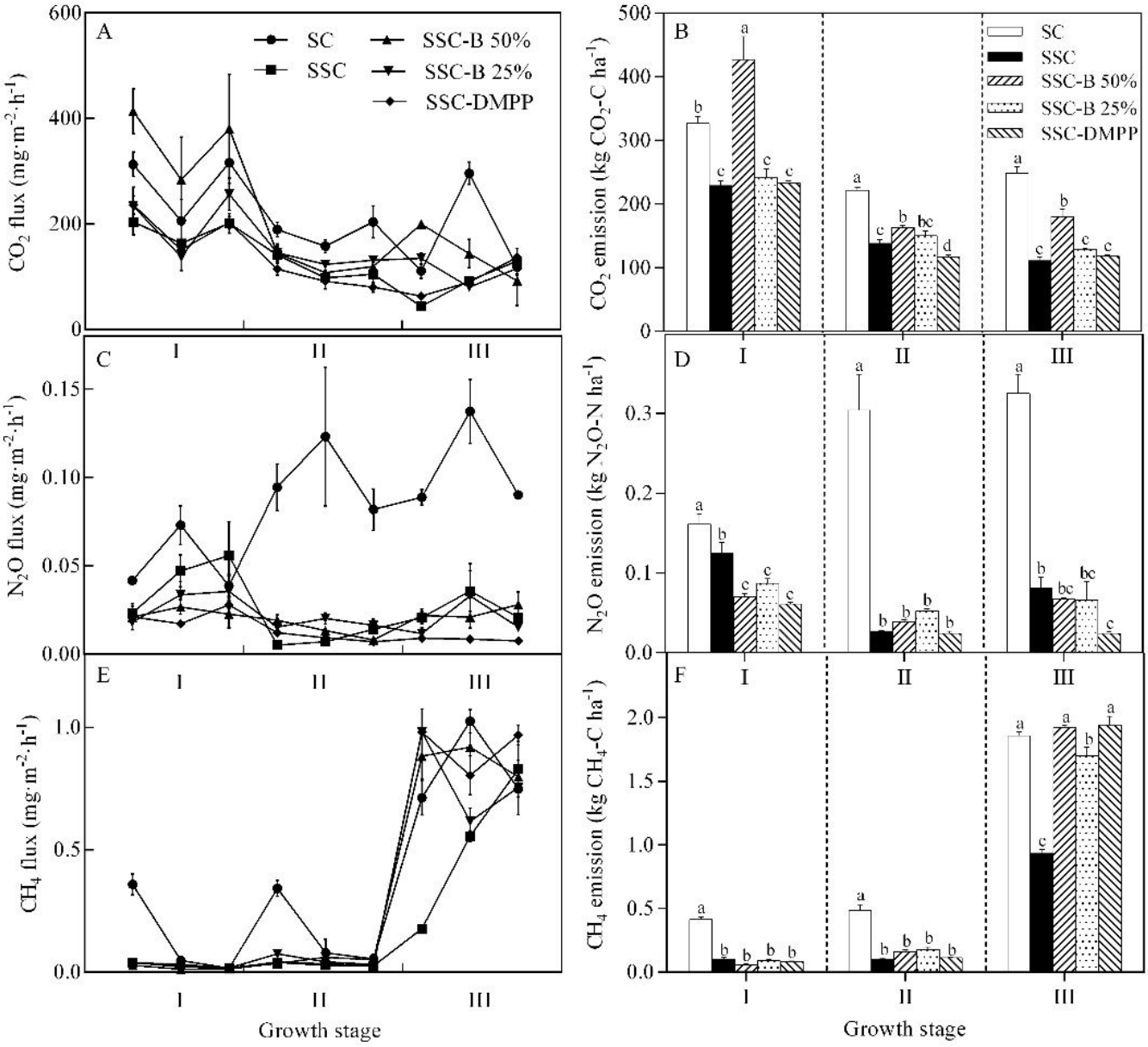

Figure 3

Figure 3. Effects of Biochar and DMPP on CO2 (A-B), N2O (C-D) and CH4 (E-F) dynamics and cumulative emissions of SSC sweet pepper during three growing stages Note: The cultivation period was divided into three growth stages (I, vegetative growth; II, flowering; III, ripening). Different growth stages, for sweet pepper, different letters indicates the difference significance among treatments at 0.05 level, and during each growth stages are presented as mean values (± standard error, n=3).

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