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# Combined Effects of Biochar and Inhibitors on Greenhouse Gas Emissions, Global Warming Potential, and Nitrogen Use Efficiency in the Tobacco Field

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**Abstract:** Biochar (BC), nitrification inhibitors (methyl 3-(4-hydroxyphenyl) propionate, MHPP), and urease inhibitors (n-butyl phosphorothioate triamine, NBPT) have emerged as effective soil greenhouse gas (GHG) mitigation strategies in agroecosystems. However, the combined use of BC and inhibitors in karst areas has no available data. Therefore, the combined effects of BC, MHPP, and NBPT on GHG emissions, global warming potential (GWP) and nitrogen use efficiency (NUE) in roasted tobacco cropping systems were studied to improve the understanding in climate mitigation. $CO_2$, $CH_4$, and $N_2O$ emissions from soils were measured using static chamber-gas chromatography. Results showed that the combined use of BC and inhibitors significantly increased soil total nitrogen, available potassium, electric conductivity, pH, and soil organic matter compared to the control. The combined use of BC and MHPP or NBPT significantly increased cumulative soil $CO_2$ emissions by 33.95% and 34.25%, respectively. The exponential–exponential function of soil $CO_2$ fluxes with soil moisture and temperature demonstrated good fit ($R^2$: 0.506–0.836). The combination of BC and NBPT increased the cumulative soil $CH_4$ emissions by 14.28% but not significantly compared to the fertiliser treatment. However, the combination of BC and MHPP resulted in a significant reduction in cumulative soil $CH_4$ emissions by 80.26%. In addition, the combined use of BC and MHPP or NBPT significantly reduced the cumulative soil $N_2O$ emissions by 26.55% and 40.67%, respectively. The inhibition effect of NBPT was better than MHPP. Overall, the combined use of BC and inhibitors significantly reduced the yield-scaled GWP, markedly increased crop yield and NUE, and mitigated climate change in the southwest karst region.

**Keywords:** biochar; nitrification inhibitor; urease inhibitor; greenhouse gas; global warming potential; nitrogen use efficiency



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## 1. Introduction

Terrestrial ecosystem emissions have dramatically increased atmospheric $CO_2$, $CH_4$, and $N_2O$, which contribute to global warming [1]. Since 2010, atmospheric greenhouse gas (GHG) concentrations have continued to increase; in 2017, the annual average concentrations of $CO_2$, $CH_4$, and $N_2O$ reached 410.53, 1853, and 328.9 $mm^3/m^3$, respectively [2]. Consequently, effective measures to change agricultural management programmes to mitigate GHG emissions are urgently needed.

The following effective field measures are currently recommended to reduce GHG emissions without decreasing crop yields: biochar (BC) amendments [3] and dual-inhibitor applications [4]. BC is a highly aromatic carbon sequestration material produced by pyrolytic carbonisation of biomass under anoxic or oxygen-limited conditions [5]. BC is

rich in N, P, and K and has high pH, high porosity, large specific surface area, high carbon content, high cation exchange capacity, and high thermal stability [6]. Some studies have shown that BC has a relatively low turnover rate and may survive in the soil for more than 100 years [7]. Therefore, if BC interacts with the soil for a long time, then it can be used as a means to mitigate climate change by enhancing soil carbon sequestration [8]. The carbon sequestration pathways of BC include the conversion of readily decomposable plant carbon to stable BC, increased capability of plants to capture atmospheric $CO_2$ due to increased biomass, and inhibition of decomposition of readily decomposable soil organic carbon [9]. Simultaneously, the application of BC may impact soil $CO_2$ emissions by changing soil structure, cation exchange, enzyme activity, and microbial communities [10]. Numerous studies have shown that soil GHG emissions are reduced with BC addition. Agegnehu et al. (2016) found that the BC treatment group had significantly lower GHG emissions than the control group [11]. Xie et al. (2013) found that replacing straw amendments with BC reduced $CH_4$ emissions and increased soil organic carbon storage [12]. However, several studies have shown that BC amendments are ineffective and may even promote GHG emissions. For example, Zhang et al. (2010) found that BC significantly increased $CH_4$ emissions [13]. In addition, the application of BC at high rates increased $CO_2$ and $N_2O$ emissions from forest soils [14]. The effect of BC on soil GHG emissions depends on many factors, such as feedstock, pyrolysis temperature, and BC application rate, according to a series of meta-analyses [15,16]. Furthermore, environmental conditions, such as soil texture, fertiliser application, and climate change, can affect GHG emissions from BC-applied soils [17].

Moreover, urease and nitrification inhibitors are frequently used in soil amendments. Studies have shown that nitrification inhibitors decrease $N_2O$ generation by directly lowering nitrification and indirectly reducing denitrification of $NO_3^-$ [18,19]. Urease inhibitors diminish the concentration of $NH_4^+$ in the soil and the likelihood of $N_2O$ volatilisation by retarding the conversion of $NH_4^+$ [20]. Zaman et al. (2008) observed that the application of inhibitors significantly reduced $N_2O$ emissions and $NO_3^-$ leaching [21]. A meta-analysis showed that nitrification inhibitors reduce direct $N_2O$ emissions by 44% [22]. However, inconsistent results have also been discovered with urease and nitrification inhibitors in the reduction of N losses. Lam et al. (2018) found that the application of urease inhibitors in acid soils increased $N_2O$ emissions by 17% [23]. In addition, the effects of urease and nitrification inhibitors on soil $CH_4$ emissions are variable because they may increase $CH_4$ emissions, enhance $CH_4$ oxidation, or have no impact at all [24,25]. These different results may be attributable to soil type, inhibitor type, inhibitor application rate, and application method [26,27]. Meanwhile, in crop nitrogen (N) transformation and utilisation, the use of nitrification and urease inhibitors significantly increased crop yields and promoted N uptake [28].

Studies recently found that the combined application of BC and inhibitors is more effective than single applications in mitigating GHG emissions and improving crop yields [29,30]. Most of these studies were conducted on agricultural land and focused on food crops, such as maize, rice, and wheat, with minimal research on cash crops, such as tobacco [31,32]. In southwest China, namely, the karst area, tobacco is a significant cash crop [33]. Karst areas are known to be highly susceptible to soil degradation caused by human activities [34], whilst the unique geological environment and climatic circumstances of karst areas constitute an important production prerequisite for high-quality tobacco. The application of BC and inhibitors to the soil can increase soil carbon sink, promote soil fertility, and mitigate climate change. Therefore, investigating the combined effects of BC, urease, and nitrification inhibitors on soil GHG fluxes in roasted tobacco cropping systems in karst areas is crucial. Given the uncertainty of the combined effects of BC, methyl 3-(4-hydroxyphenyl) propionate (MHPP), and n-butyl phosphorothioate triamine (NBPT) on GHG emissions from the soil, a full accounting of the global warming potential (GWP) of soil GHG emissions is necessary during assessment of their climate impact in karst areas. A field experiment was set up using static chamber-gas chromatography (GC) techniques in the roasted tobacco

cropping system in karst areas to simultaneously measure soil $CO_2$, $CH_4$, and $N_2O$ fluxes. This study aimed to understand the combined effects of BC and MHPP or NBPT on soil GHG emissions from roasted tobacco cropping systems in karst areas and those on crop production and N conversion.

## 2. Material and Methods

### *2.1. Experimental Site*

The field trial was conducted at the Pingba Tobacco Experimental Base of the Guizhou Academy of Tobacco Science (26°26′193″ N, 106°14′166″ E; 1391 m above sea level). The region has a typical subtropical humid monsoon climate with an annual average temperature of 14.5 °C and an annual average precipitation of 122 mm. Referring to the Genetic Soil Classification of China, the soil in this study was yellow soil. The soil texture of the site was clay loam. Table 1 lists the basic properties of the different treatments.

**Table 1.** Effect of BC, MHPP, and NBPT on soil properties.

| | T1 | T2 | T3 | T4 |
|---|---|---|---|---|
| pH | 6.58 ± 0.15 $^{b}$ | 6.47 ± 0.02 $^{b}$ | 7.04 ± 0.09 $^{a}$ | 6.94 ± 0.17 $^{a}$ |
| EC (μS/cm) | 151.73 ± 33.42 $^{c}$ | 388.67 ± 74.57 $^{b}$ | 881.67 ± 52.37 $^{a}$ | 895.00 ± 86.26 $^{a}$ |
| TC (g/kg) | 19.19 ± 0.89 $^{a}$ | 19.19 ± 1.77 $^{a}$ | 21.49 ± 2.60 $^{a}$ | 20.18 ± 2.34 $^{a}$ |
| TN (g/kg) | 1.76 ± 0.12 $^{b}$ | 1.83 ± 0.04 $^{b}$ | 2.26 ± 0.16 $^{a}$ | 2.07 ± 0.10 $^{a}$ |
| TP (g/kg) | 0.92 ± 0.06 $^{a}$ | 1.03 ± 0.05 $^{a}$ | 1.07 ± 0.02 $^{a}$ | 0.96 ± 0.03 $^{a}$ |
| TK (g/kg) | 13.83 ± 0.83 $^{a}$ | 14.37 ± 0.76 $^{a}$ | 12.17 ± 0.78 $^{b}$ | 11.67 ± 0.71 $^{b}$ |
| AN (mg/kg) | 123.08 ± 6.94 $^{b}$ | 146.43 ± 5.91 $^{a}$ | 143.89 ± 19.65 $^{ab}$ | 133.97 ± 11.61 $^{ab}$ |
| AP (mg/kg) | 13.14 ± 3.89 $^{b}$ | 23.94 ± 3.40 $^{ab}$ | 27.43 ± 9.72 $^{a}$ | 20.51 ± 5.44 $^{ab}$ |
| AK (mg/kg) | 289.29 ± 122.78 $^{b}$ | 467.88 ± 72.14 $^{b}$ | 1382.41 ± 328.31 $^{a}$ | 1042.48 ± 138.50 $^{a}$ |
| SOM (g/kg) | 31.20 ± 4.50 $^{b}$ | 29.66 ± 5.91 $^{b}$ | 45.23 ± 2.07 $^{a}$ | 41.48 ± 6.17 $^{a}$ |

T1, T2, T3, and T4 represent CK-, F-, F + BC + MHPP-, and F + BC + NBPT-amended soils, respectively. EC: electric conductivity; TC: total carbon content; TN: total nitrogen content; TP: total phosphorus content; TK: total potassium content; AN: rapid-acting nitrogen content; AP: effective phosphorus content; AK: rapid-acting potassium content; SOM: soil organic matter content. Different letters within the line indicate significant differences between treatments at $p < 0.05$.

### *2.2. Field Experiments*

The experiment was conducted in a completely randomised block design with three replicated four-treatment field trials. In April 2022, roasted tobacco-specific fertiliser, BC, nitrification inhibitor (MHPP), and urease inhibitor (NBPT) were uniformly mixed into the 0–20 cm soil layer and applied at 675 kg/ha, 20 t/ha, 13.5 kg/ha, and 337.5 g/ha, respectively. The treatment settings were as follows:

(1) T1: No fertiliser application (CK);
(2) T2: Single application of special fertiliser for roasted tobacco (F);
(3) T3: Special fertiliser for roasted tobacco (F) + biochar (BC) + nitrification inhibitor (MHPP);
(4) T4: Special fertiliser for roasted tobacco (F) + biochar (BC) + urease inhibitor (NBPT).

Special fertiliser for roasted tobacco (N: $P_2O_5$: $K_2O$ = 10:10:25) was applied in all plots before the high row monopoly (30 cm). After sowing, uniformly sized tobacco seedlings (Yunyan87) were transplanted from the seedbed to the row monopoly. For tobacco transplantation, 1.1 m row spacing and 0.6 m row distance were adapted. A total of 60 plants were planted in each plot, which had an area of 39.6 $m^2$. Tobacco yields in the field trial were remarkably dependent on natural precipitation. The additional fertiliser (272 kg/ha) was applied 1 month after the basic fertiliser (675 kg/ha). Consistent management measures were applied during the growing season. Soil temperature and moisture sensors were placed at soil depths of 5, 10, and 20 cm, and the data were recorded with a logger (TR-6, Shuncoda, China). The entire growth cycle of roasted tobacco is approximately 120 days, which is divided into three stages: root extension period (REP, 30 days), vigorous period (VP, 30 days), and mature period (MP, 60 days).

*2.3. Gas Sampling and Measurements*

Soil GHG fluxes were measured using static chamber-GC techniques from May to August 2020. A chamber base (10 cm high, 30 cm diameter) with deep circular depressions was placed inside each area. These bases were kept in place for the duration of the experiment. When the opaque removable polyvinyl chloride (PVC) chambers (50 cm high) were put on the bases, the depressions were filled with water to prevent air leakage. The chamber has a thermometer that records the air temperature during sampling time. An electric fan was also installed in the chamber to ensure that the gases were well mixed during the sampling process. A 20 mL sample of gas was taken from each chamber at 0, 6, 12, and 18 min after each closure using a syringe, and then filled into evacuated 12 mL glass bottles. The samples were then analysed within 24 h after collection. Soil GHG emissions were assessed every 7 days. Measurements between 8:00 and 11:00 a.m. were selected to limit the daily volatility in flow patterns.

A modified GC (Agilent 7890), including a flame ionisation detector (FID) and an electron capture detector (ECD), was used to detect $CO_2$, $CH_4$, and $N_2O$ in soil [35]. $N_2$ was used as the carrier gas. Nitrous oxide was separated using two stainless-steel columns (column 1 with 1 m length and 2.2 mm i.d.; column 2 with 3 m length and 2.2 mm i.d.) fitted with 80–100 mesh Porapack Q. $CH_4$ and $N_2O$ were identified using FID and ECD, respectively. The oven, ECD, and FID were run at 55 °C, 330 °C, and 200 °C, respectively [36].

The GC was calibrated using three recognised reference gases to assure the precision and dependability of the continuous readings. If linear regression values of $R^2 > 0.90$ were not provided, then they were not included in the gas sample data set. Soil GHG emission fluxes were determined using the following equation [37]:

$$F_{GHG} = \frac{60}{100} \times \rho_0 \times H \times \frac{273}{273 + T} \times \left(\frac{dc}{dt}\right),$$

where $F_{GHG}$ is the flux of $CO_2$, $CH_4$, or $N_2O$ ($mg \cdot m^{-2} \cdot h^{-1}$); 60 and 100 are unit conversion factors for calculating GHG fluxes; $\rho_0$ is the density of $CO_2$, $CH_4$, or $N_2O$ in standard conditions (g/L); H is the height of the sampling chamber (cm); T is the temperature of the chamber (°C); dc/dt is the rate of change (μL/L) of the $CO_2$, $CH_4$, or $N_2O$ concentration in the sampling chamber over time t (min), with positive and negative values indicating emissions and absorption, respectively.

The emissions of $CO_2$, $CH_4$, and $N_2O$ are accumulated over two adjacent measurement periods during the growth cycle of roasted tobacco. These emissions are determined using the following equation:

$$E_{GHG} = \sum_{i=1}^{n} (F_i + F_{i+1})/2 \times (t_{i+1} - t_i) \times 24,$$

where $E_{GHG}$ is the cumulative emission of soil GHGs; $F_i$ and $F_{i+1}$ represent the soil GHG fluxes at sampling periods i and i + 1, respectively; $t_{i+1} - t_i$ is the interval (d) between the i-th and (i + 1)-th measurement times; n is the total number of gas samples collected.

The relationship between soil $CO_2$ emission fluxes and soil temperature and moisture can be fitted with the help of the following exponential–exponential function [38]:

$$F_{CO_2} = ae^{bW}e^{cT},$$

where a, b, and c are fitting constants; W is the soil volumetric water content (SVWC) (%); T is the soil temperature (°C).

The $N_2O$ emission factor ($EF_{N2O}$) (kg/ha) from the application of N fertiliser was estimated by applying the following equation:

$$EF_{N2O} = (EF - E_0)/N_{ap} \times 100\%,$$

where EF (kg/ha) is the cumulative $N_2O$ emission from the N fertiliser treatment; $E_0$ (kg/ha) is the cumulative $N_2O$ emission from the unfertilised treatment (T1); $N_{ap}$ is the amount of N fertiliser (kg N/ha) applied to N treatment plots.

### *2.4. Determination of Crop Yield, Nitrogen Use Efficiency, and GWP*

The yield of tobacco is the mass of dry matter collected during the growing season. Tobacco yield was assessed on three tobacco plants from each plot. Tobacco samples were divided into three parts: root, stem, and leaf. The samples were then washed with tap water, followed by deionised water. These samples were placed in an oven at 105 °C for 30 min and finally baked at 65 °C for 48 h to maintain a uniform weight. The yield was calculated by weighing the dried tobacco samples, and the total nitrogen (TN) content was assessed using an elemental analyser (Vario MACRO Analyser, Elementar Analysensysteme GmbH, Hanau, Germany). The amount of N uptake and the nitrogen use efficiency (NUE) in the crop were obtained by calculation.

The NUE is calculated as follows:

$$NUE = (N_{DMF} - N_{DMU})/N_{ap},$$

where $N_{DMF}$ is the N content of dry matter obtained from the fertilised treatment; $N_{DMU}$ is the N content of dry matter obtained from the unfertilised control treatment; $N_{ap}$ is the amount of N applied.

Cumulative emissions of $CO_2$, $CH_4$, and $N_2O$ were analysed to balance the net greenhouse effect. Cumulative GHG emissions were converted into $CO_2$ equivalents ($CO_2$-eq) using their specific GWP values. These emissions are an index defined as the cumulative radiative forcing caused by a unit quantity of current gas release and at a selected time frame in the future. The GWP (based on a 100 year time horizon) is 25, 298, and 1 for $CH_4$, $N_2O$, and $CO_2$, respectively. The following equation was used to estimate the GWP of different treatments [39]:

$$GWP = CO_2 \text{ emission} + CH_4 \text{ emission} \times 25 + N_2O \text{ emission} \times 298,$$

where GWP is expressed in kg $CO_2$-eq/ha; $CO_2$, $CH_4$, and $N_2O$ fluxes are expressed in kg/ha.

The yield-scaled GWP (t $CO_2$-eq/t) is calculated in accordance with the following method [40]:

$$Yield - scaled\ GWP = GWP/yield.$$

### *2.5. Soil Collection and Analyses*

Soil samples (0–20 cm) were collected 1 week before the construction of the plots and at the end of the field experiment. Five soil samples were collected from each plot and then mixed thoroughly to form a composite sample. Soil pH was determined with a pH meter in a soil–water suspension at a ratio of 1/2.5. Soil electric conductivity (EC) was assessed with a conductivity meter (Orion, CM-180) at a soil/water ratio of 1/2.5. Soil total carbon (TC) and TN were determined with an elemental analyser [41]. Soil total phosphorus (TP) and available phosphorus (AP) were measured using the $H_2SO_4$–$HClO_4$ ablation and $NaHCO_3$ extraction techniques, respectively. The NaOH fusion method and the $CH_3COONH_4$ extraction technique were used to estimate total potassium (TK) and available potassium (AK), respectively [42]. Soil available nitrogen (AN) was analysed by the alkaline solution diffusion method [43]. Soil $NH_4^+$ and $NO_3^-$ were extracted with 2 M KCl solution (1:5, *w/v*). $NH_4^+$ and $NO_3^-$ were determined by the flow analyser system [29]. Soil inorganic nitrogen (SIN) was determined by 1 M KCl extraction followed by Kjeldahl distillation in the presence of MgO + Devarda's alloy, followed by titration for TN [44]. Soil organic matter (SOM) was determined by oxidation with potassium dichromate.

### *2.6. Data Analyses*

All data are provided as the means ± standard error. One-way analysis of variance was used to evaluate the effects of N fertiliser, BC, MHPP, and NBPT on soil $CO_2$, $CH_4$, and $N_2O$ emissions and their related GWP, yield-scaled GWP, crop yield, $EF_{N2O}$, N uptake, and NUE. At the 0.05 significance level, the LSD test was used to evaluate the significance of the observed differences. The relationship between GHG fluxes and environmental factors was explored using correlation analysis. Multiple linear regression analysis was used to determine the significance of the fit of soil $CO_2$ emissions concerning soil temperature and water content at different depths. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

## 3. Results and Discussion

### *3.1. Effects of BC, MHPP, and NBPT on Soil Physicochemical Properties*

Results showed that the combined use of BC and MHPP or NBPT significantly increased TN by 13.11% and 23.50% ($p < 0.05$), respectively, whilst AK levels significantly increased by 1.23-fold to 1.95-fold compared to T2 (Table 1). Regarding TC, the mean values of T3 and T4 were 11.99% and 5.16% higher than T2 but insignificant, respectively. In addition, fertilisation treatments (T2, T3, and T4) all increased the mean values of AP concentrations in the soil. T2, T3, and T4 significantly increased the soil EC values by 1.56, 4.81, and 4.90 times compared to T1. The combined effect of BC and inhibitors significantly increased the mean soil pH by 7.26% to 8.81% due to the alkalinity of BC and the neutrality of the inhibitor. By contrast, no significant changes were observed in AN, TP, and TK levels in unamended and amended soils after BC and inhibitor addition. SOM levels were mainly associated with the combination of BC and inhibitors. No significant change was observed in T2 compared to the control, whilst SOM levels increased significantly by 44.97% and 32.95% in T3 and T4 treatments, respectively. The meta-analysis found that the application of BC and inhibitors had different effects on soil physicochemical properties, which were related to factors, such as feedstock, pyrolysis temperature, application rate, and soil texture [45]. Overall, the combination of BC and inhibitor significantly increased soil pH, EC, and SOM and improved soil fertility, which is consistent with the results of Singh et al. (2022) [46]. This combination also significantly increased TN, markedly reduced $NO_3^-$ leaching, and improved the effectiveness of N fertiliser [47].

SVWC increased significantly with rising soil depth during the roasted tobacco growth cycle ($p < 0.05$, Table 2). Compared to the control, T2, T3, and T4 treatments increased the SVWC at a depth of 5 cm by an average of 4.39%, 2.12%, and 4.09%, respectively, during the root extending period. T2, T3, and T4 treatments increased the SVWC at 10 cm depth by an average of 11.54%, 6.90%, and 5.16%, respectively. Moreover, soil water content at 5, 10, and 20 cm was significantly different in either treatment. At the vigorous period, the combined effect of the BC and inhibitors on SVWC was consistent with the root extending period but varied relatively minimally (from 0.38% to 9.06%) compared to the control. T3 and T4 treatments increased the SVWC at 5 cm depth by an average of 6.47% and 2.64% compared to the control. Meanwhile, T3 and T4 treatments increased the SVWC at 20 cm depth by an average of 1.83% and 0.38%, respectively. Notably, the combination of BC and inhibitors had a larger effect on soil water content at 5 and 10 cm than at 20 cm. At the mature period, T3 and T4 treatments reduced SVWC at 5, 10, and 20 cm depths compared to the control (with T3 at −3.22%, −1.70%, and −3.24% and T4 at −5.24%, −0.16%, and −5.94%, respectively).

Throughout the roasted tobacco growth cycle, SVWC showed remarkably similar trends with transplanting time for the different treatments (Figure 1). The mean SVWC at 5 cm was 16.37% ± 0.93%, 16.92% ± 0.87%, 16.61% ± 0.94%, and 16.35% ± 0.74% for T1, T2, T3 and T4, respectively. Moreover, although not dramatically, soil temperature declined with soil depth and was negatively linked with SVWC (R: −0.39 to −0.50, $p < 0.05$). The mean 5 cm soil temperature for T1, T2, T3, and T4 was 25.50 ± 0.68 °C, 24.42 ± 0.78 °C, 24.15 ± 0.75 °C, and 24.24 ± 0.55 °C, respectively, whilst the mean 20 cm soil temperature was 23.42 ± 0.52 °C,

22.60 ± 0.57 °C, 22.37 ± 0.51 °C, and 22.64 ± 0.44 °C, respectively. At the mature period, soil temperatures for T3 and T4 treatments at different depths were significantly lower than for T1, which may have been due to the combination of BC and inhibitors [48,49]. However, as the application rate of BC was low, the difference in soil temperature between treatments during the root extension and vigorous periods was insignificant.

**Table 2.** Effect of BC, MHPP, and NBPT on soil moisture and temperature at various depths during the roasted tobacco growth cycle.

| Growth Periods | Treatment | SVWC5 | SVWC10 | SVWC20 | ST5 | ST10 | ST20 |
|---|---|---|---|---|---|---|---|
| REP | T1 | 13.20 ± 0.99 $^{aC}$ | 17.24 ± 1.03 $^{aB}$ | 23.79 ± 1.42 $^{aA}$ | 25.54 ± 0.89 $^{aA}$ | 24.06 ± 0.72 $^{aAB}$ | 22.52 ± 0.64 $^{aB}$ |
| | T2 | 13.78 ± 0.91 $^{aC}$ | 19.23 ± 0.90 $^{aB}$ | 25.75 ± 1.16 $^{aA}$ | 24.85 ± 1.14 $^{aA}$ | 22.69 ± 0.88 $^{aAB}$ | 21.53 ± 0.73 $^{aB}$ |
| | T3 | 13.48 ± 1.03 $^{aC}$ | 18.43 ± 1.15 $^{aB}$ | 24.26 ± 1.08 $^{aA}$ | 24.12 ± 0.96 $^{aA}$ | 22.62 ± 0.76 $^{aA}$ | 21.17 ± 0.67 $^{aA}$ |
| | T4 | 13.74 ± 0.88 $^{aC}$ | 18.13 ± 0.84 $^{aB}$ | 23.32 ± 0.94 $^{aA}$ | 24.91 ± 0.70 $^{aA}$ | 23.24 ± 0.64 $^{aA}$ | 22.02 ± 0.66 $^{aA}$ |
| VP | T1 | 20.85 ± 1.14 $^{aC}$ | 25.85 ± 1.17 $^{aB}$ | 31.11 ± 2.67 $^{aA}$ | 24.81 ± 0.41 $^{aA}$ | 23.61 ± 0.37 $^{aA}$ | 22.58 ± 0.22 $^{aA}$ |
| | T2 | 22.74 ± 1.24 $^{aC}$ | 26.73 ± 0.99 $^{aB}$ | 33.13 ± 2.09 $^{aA}$ | 24.28 ± 0.50 $^{aA}$ | 22.90 ± 0.35 $^{aA}$ | 22.04 ± 0.29 $^{aA}$ |
| | T3 | 22.20 ± 1.15 $^{aC}$ | 27.58 ± 1.33 $^{aB}$ | 31.68 ± 2.37 $^{aA}$ | 23.94 ± 0.57 $^{aA}$ | 22.63 ± 0.43 $^{aAB}$ | 21.68 ± 0.32 $^{aB}$ |
| | T4 | 21.40 ± 1.03 $^{aC}$ | 26.91 ± 1.06 $^{aB}$ | 31.23 ± 2.29 $^{aA}$ | 23.83 ± 0.54 $^{aA}$ | 22.90 ± 0.36 $^{aA}$ | 22.38 ± 0.28 $^{aA}$ |
| MP | T1 | 15.84 ± 0.75 $^{aC}$ | 18.85 ± 0.73 $^{aB}$ | 22.22 ± 0.78 $^{aA}$ | 25.93 ± 0.69 $^{aA}$ | 25.24 ± 0.61 $^{aA}$ | 24.68 ± 0.64 $^{aA}$ |
| | T2 | 15.48 ± 0.60 $^{aC}$ | 17.97 ± 0.57 $^{aB}$ | 20.84 ± 0.81 $^{aA}$ | 24.18 ± 0.69 $^{bA}$ | 23.93 ± 0.62 $^{bA}$ | 23.80 ± 0.63 $^{bA}$ |
| | T3 | 15.33 ± 0.71 $^{aC}$ | 18.53 ± 0.86 $^{aB}$ | 21.50 ± 1.01 $^{aA}$ | 24.32 ± 0.71 $^{bA}$ | 24.14 ± 0.57 $^{bA}$ | 23.76 ± 0.51 $^{bA}$ |
| | T4 | 15.01 ± 0.43 $^{aC}$ | 18.82 ± 0.65 $^{aB}$ | 20.90 ± 0.98 $^{aA}$ | 23.99 ± 0.43 $^{bA}$ | 23.61 ± 0.28 $^{bA}$ | 23.28 ± 0.37 $^{bA}$ |

SVWC5, SVWC10, and SVWC20 represent the soil volumetric water content at 5, 10, and 20 cm depths, respectively; ST5, ST10 and ST20 represent the soil temperature at 5, 10, and 20 cm depths, respectively. Different lowercase letters within a column indicate significant differences between treatments at $p < 0.05$. Different capital letters within a row indicate significant differences between soil depths at $p < 0.05$.

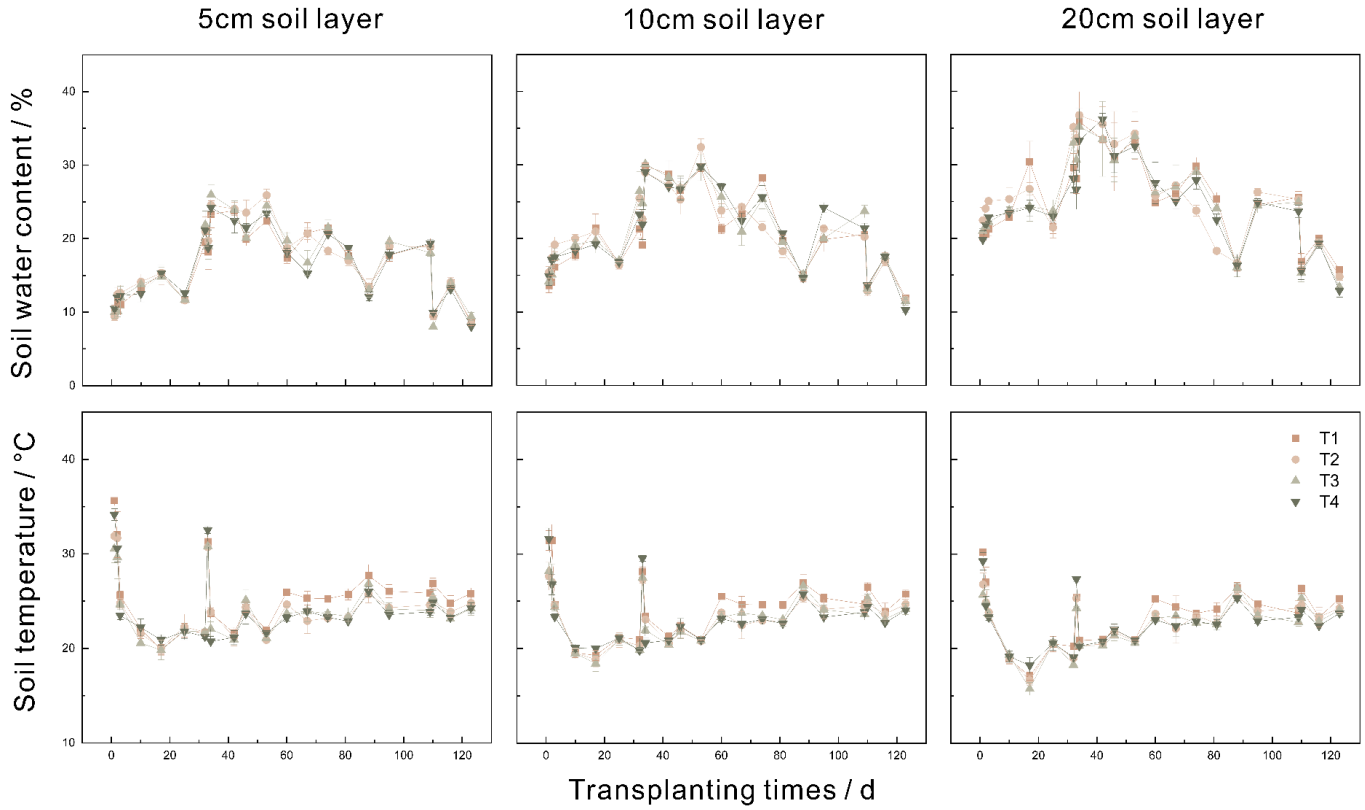


**Figure 1.** Changes in SVWC and soil temperature at three depths (5, 10, and 20 cm) for different treatments during the roasted tobacco growth cycle.

### *3.2. Effects of BC, MHPP, and NBPT on Soil $CO_2$ Emission*

In roasted tobacco cropping systems, soil $CO_2$ fluxes showed similar seasonal dynamics between treatments after the application of BC, MHPP, and NBPT (Figure 2a). Fertiliser application significantly increased soil $CO_2$ emissions compared to the control. Soil $CO_2$ emissions were significantly increased in T3 and T4 treatments compared to T2, but no significant difference was observed between the two treatments. At present, studies conducted on the combined use of BC and inhibitors have mainly focused on $N_2O$, with few studies on $CO_2$. Suggestions indicate that BC, as an exotic carbon source, has a portion of its active carbon pool [50]. In addition, the soil has a portion of the active carbon pool, and the combination of BC and inhibitors may lead to the decomposition of the respective active carbon pools of BC and soil. BC promotes soil $CO_2$ emissions [51], and MHPP or NBPT may promote or inhibit these emissions. Notably, the combined use of BC and inhibitors contributed significantly to high soil $CO_2$ fluxes.

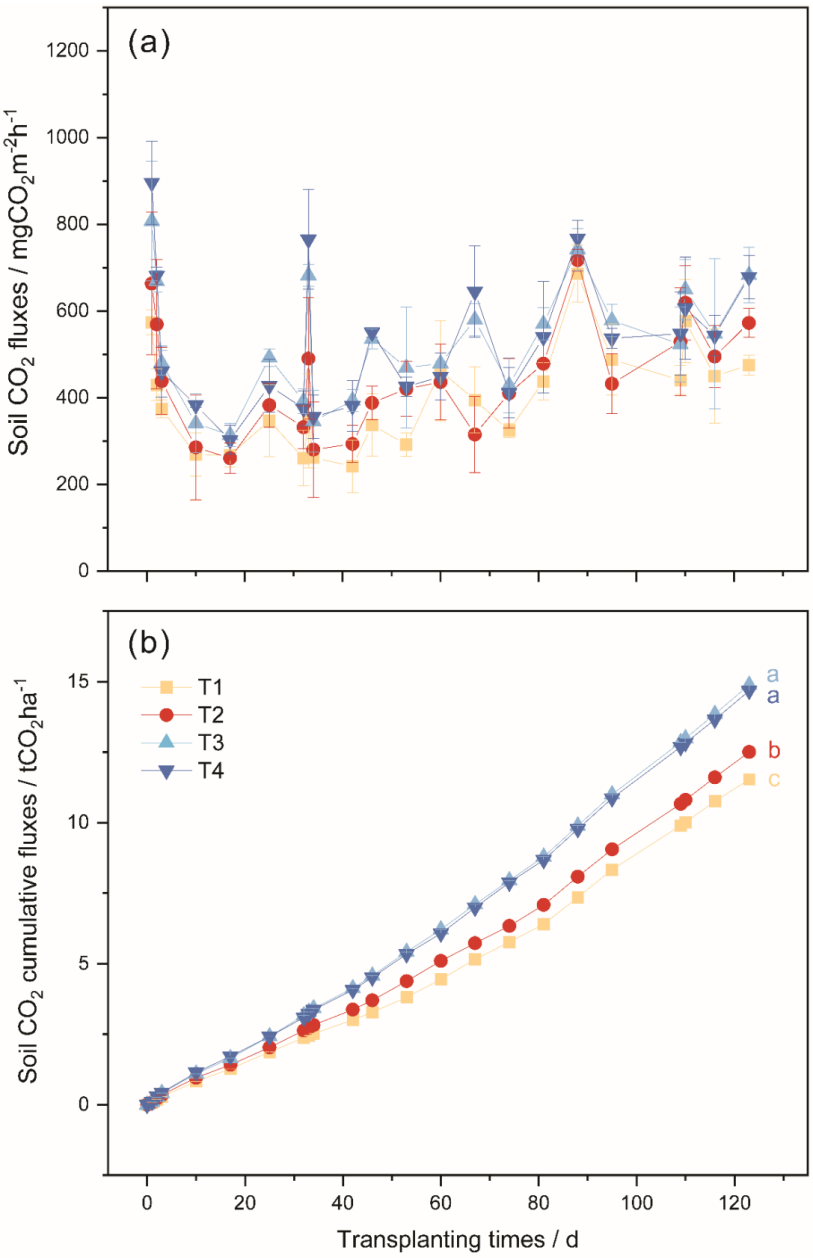


**Figure 2.** Changes in soil $CO_2$ flux (**a**) and cumulative $CO_2$ flux (**b**) for different treatments during the roasted tobacco growth cycle. Different letters indicate significant differences at $p < 0.05$.

Results showed that soil $CO_2$ fluxes varied significantly with transplanting time for the treatments from T1 to T4, with average daily $CO_2$ emission fluxes of 397.03, 445.82, 531.83, and 533.03 mg $CO_2 \cdot m^{-2} \cdot h^{-1}$ at 120 days after transplanting. Meanwhile, the average daily $CO_2$ emission fluxes at 30 days after transplanting were 360.21, 418.76, 499.29, and 503.70 mg $CO_2 \cdot m^{-2} \cdot h^{-1}$ for treatments from T1 to T4, respectively (Figure 2b). For the majority of the roasted tobacco growth cycle, $CO_2$ emissions from soils with the application of BC and inhibitors were significantly higher than the control whilst those from soils with BC and inhibitors were occasionally lower than those from the control. No significant difference was observed in average daily $CO_2$ emissions between T3 and T4 treatments. Compared to the control, the average daily soil $CO_2$ fluxes were substantially larger in T2, T3, and T4 treatments, increasing by 12.29%, 33.95%, and 34.25%, respectively ($p < 0.05$). Cumulative $CO_2$ emissions from the control (11.54 ± 0.00 t $C \cdot ha^{-1}$) were significantly lower than those from T2 (12.51 ± 0.00 t $C \cdot ha^{-1}$), T3 (14.89 ± 0.00 t $C \cdot ha^{-1}$), and T4 (14.68 ± 0.00 t $C \cdot ha^{-1}$) treatments. However, no significant difference in cumulative $CO_2$ emissions was observed between T3 and T4 treatments, suggesting that the combined effect of BC and MHPP was not significantly different from that of BC and NBPT.

Correlation analysis results revealed a strong and positive correlation between soil $CO_2$ emission fluxes and soil pH but not soil EC. Soil pH is considered to be the important factor influencing $CO_2$ emissions, and the combined application of BC and inhibitors significantly enhanced soil pH and increased soil $CO_2$ emission fluxes [52]. This finding is also consistent with that of Li et al. (2021), wherein the combined use of BC and inhibitors raised soil pH, and soil $CO_2$ emission fluxes were associated with changes in soil properties [53]. Sheng et al. (2016) also found that the carbon sequestration potential of BC in soils decreased with soil pH, especially in the short term [54]. Notably, soil $CO_2$ emission fluxes are also related to soil temperature and moisture. Significantly negative correlations existed between soil $CO_2$ emission fluxes and SVWC (Figure 3a). The linear function described a variation in $CO_2$ fluxes from 25.0% to 36.0%, with larger correlation coefficients at 20 cm depth than at 5 and 10 cm depth. In addition, soil $CO_2$ fluxes were significantly positively correlated with soil temperature at different soil depths, with the linear function explaining 39.7% to 51.8% of the variation in $CO_2$ fluxes. Moreover, the correlation coefficients for 20 cm depth were larger than those for 5 and 10 cm depths. The exponential–exponential function accounted for 50.6% to 70.1%, 61.1% to 78.8%, and 78.6% to 83.3% of the variance in soil respiration at the 5, 10, and 20 cm soil depths, respectively, when soil $CO_2$ emission fluxes were evaluated by soil temperature and moisture (Table 3). $R^2$ values were larger in the 5 and 10 cm soil layers for T3 and T4 treatments than for T1 and T2. This condition indicates that an exponential model effectively explained soil respiration by soil temperature and moisture after the application of BC and inhibitors. Furthermore, the association of soil respiration with soil temperature and moisture in the 20 cm soil layer did not differ significantly between the treatments. Overall, soil moisture and temperature conditions may be the main variables influencing soil $CO_2$ emissions from agroecosystems in karst areas [38,55].

**Table 3.** Exponential–exponential functions of soil respiration with soil moisture and temperature for different treatments at various soil depths after the application of BC, MHPP, and NBPT.

| Treatments | Soil Depths | | |
|---|---|---|---|
| | 5 cm | 10 cm | 20 cm |
| T1 | $F = 199.14e^{-0.023W}e^{0.040T}$ ($R^2 = 0.506$) | $F = 196.96e^{-0.022W}e^{0.045T}$ ($R^2 = 0.611$) | $F = 197.55e^{-0.024W}e^{0.054T}$ ($R^2 = 0.826$) |
| T2 | $F = 180.91e^{-0.020W}e^{0.049T}$ ($R^2 = 0.517$) | $F = 108.09e^{-0.019W}e^{0.076T}$ ($R^2 = 0.727$) | $F = 83.60e^{-0.013W}e^{0.087T}$ ($R^2 = 0.836$) |
| T3 | $F = 136.46e^{-0.012W}e^{0.063T}$ ($R^2 = 0.701$) | $F = 106.27e^{-0.010W}e^{0.077T}$ ($R^2 = 0.788$) | $F = 88.77e^{-0.005W}e^{0.084T}$ ($R^2 = 0.786$) |
| T4 | $F = 147.53e^{-0.012W}e^{0.059T}$ ($R^2 = 0.689$) | $F = 105.32e^{-0.010W}e^{0.076T}$ ($R^2 = 0.763$) | $F = 90.74e^{-0.011W}e^{0.088T}$ ($R^2 = 0.835$) |

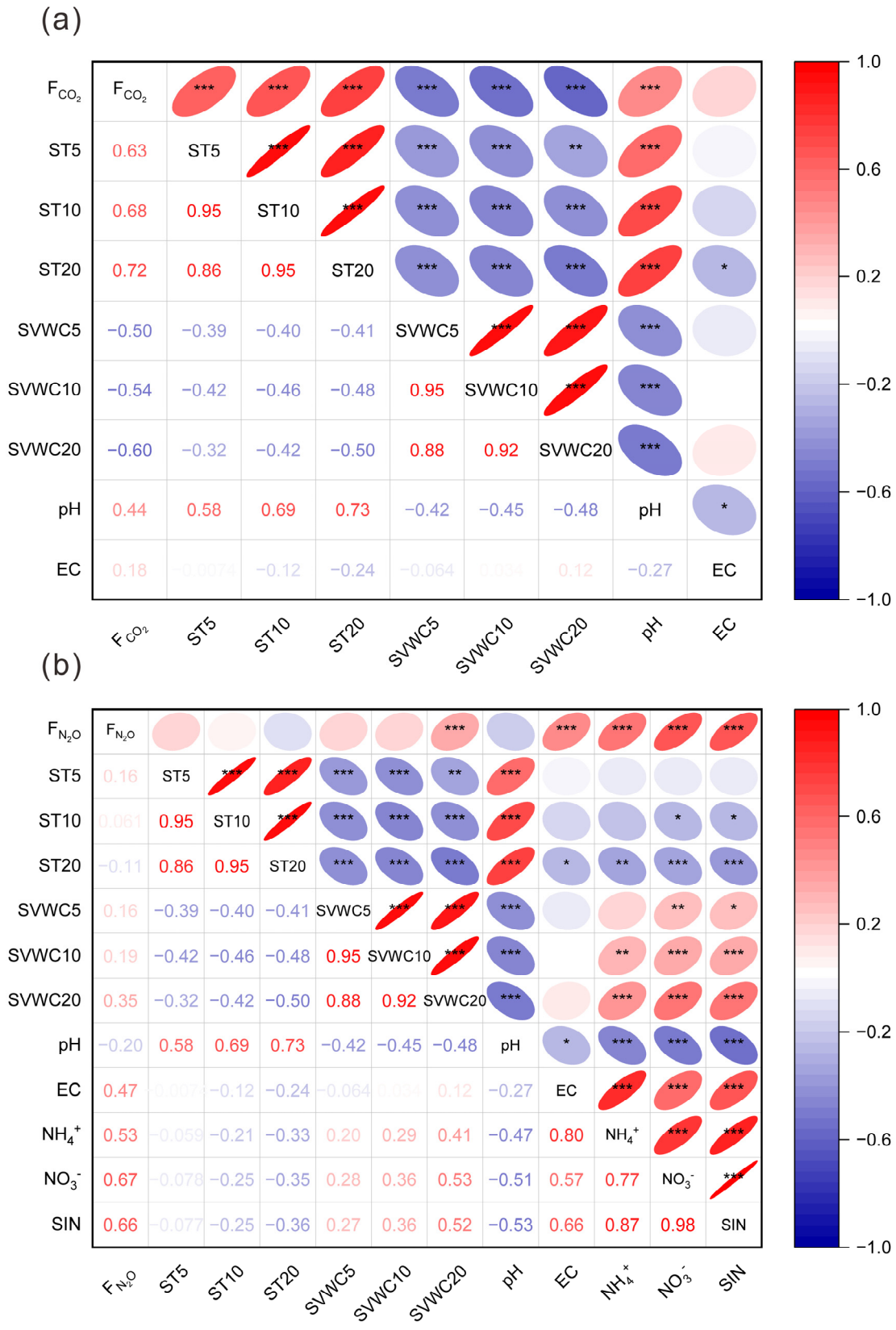


**Figure 3.** Correlation analysis of soil $CO_2$ (**a**) and $N_2O$ (**b**) fluxes with environmental factors. $FCO_2$ and $FN_2O$ represent the fluxes of $CO_2$ and $N_2O$, respectively. SIN: soil inorganic nitrogen. Asterisks indicate statistical significance with significance levels of * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$ for $p$ values.

### *3.3. Effects of BC, MHPP, and NBPT on Soil $CH_4$ Emission*

Throughout the roasted tobacco growth cycle, $CH_4$ fluxes in all field treatments showed similar seasonal variations, demonstrating the occurrence of positive fluxes (showing release) and negative fluxes (showing uptake) (Figure 4a). Linear regression analysis revealed that soil $CH_4$ fluxes were positively correlated with SVWC, particularly at 5 cm (Figure 5). Soil was converted from a $CH_4$ sink to a $CH_4$ source by increasing soil moisture, whilst the combination of BC and inhibitors only enhanced or diminished the $CH_4$ source sink effect under different soil moisture conditions. Under high soil moisture conditions, additional anaerobic zones are formed in the soil, and methanogenic bacteria are active. Simultaneously, the organic carbon added by BC provides a rich and effective substrate for methanogenic bacteria; thus, $CH_4$ emissions are high [56]. On the contrary, under low soil moisture conditions, the anaerobic zone is small, and the oxidative zone is large. Methane-oxidising bacteria are remarkably active, and most of the $CH_4$ produced is oxidised by methane-oxidising bacteria, even absorbing $CH_4$, creating a negative flux [57]. In this study, the mean daily $CH_4$ emission fluxes at 120 days after transplanting for the treatments from T1, T2, T3, and T4 were −0.88, 0.04, −2.02 and 2.48 μg $CH_4 \cdot m^{-2} \cdot h^{-1}$, respectively. Regarding cumulative soil $CH_4$ emissions, compared to the control with 66.16 ± 20.05 g $CH_4 \cdot ha^{-1}$, T4 and T2 treatments were the highest at 120.38 ± 21.20 and 105.34 ± 17.40 g $CH_4 \cdot ha^{-1}$, respectively, followed by T3 at 20.79 ± 19.27 g $CH_4 \cdot ha^{-1}$, indicating that fertiliser application significantly increased cumulative soil $CH_4$ emissions ($p < 0.05$, Figure 4b). In addition, a significant difference in cumulative soil $CH_4$ emissions was observed between the T3 and T4 treatments. The plots with BC and NBPT had a 14.28% increase in mean cumulative soil $CH_4$ emissions; however, the plots with BC and MHPP had a significant 80.26% reduction. Thus, the combined effect of BC and inhibitors had differences in influencing $CH_4$ emissions.

On the one hand, BC application was found to increase soil $CH_4$ uptake significantly [58]. However, Yu et al. (2013) discovered that the effect of BC application on $CH_4$ emissions depended on soil moisture, reducing $CH_4$ emissions at low and medium soil moisture levels but stimulating $CH_4$ production at the highest soil moisture levels [56]. On the other hand, inhibitors had contradictory effects on $CH_4$ emissions, including inhibition [59], promotion [60], and no impact [61]. In addition, for T2, the high ammonium content during fertiliser hydrolysis may have increased the abundance of methanogenic bacteria, which may have indirectly promoted soil $CH_4$ production when C was taken up by microorganisms as a substrate [28]. Similar to the findings of Li et al. (2009), the combined application of BC and MHPP reduced $CH_4$ emissions by 80.26% compared to T2 [59]. An increase in root biomass was observed for T3 treatment, which is consistent with the results of Xu et al. (2002) [62]. An increase in crop root biomass can markedly raise oxygen availability at the inter-root level [63], which, in turn, inhibits methanogenic activity [64,65] and increases methanotroph activity [66,67]. Therefore, the combined application of BC and MHPP in this research likely inhibited $CH_4$ generation and increased $CH_4$ oxidation by raising crop biomass and oxygen availability, resulting in a decrease in $CH_4$ emissions. By contrast, the effects of BC and NBPT may have been different. NBPT had a considerable inhibitory effect on $CH_4$ oxidation in T4 due to the high ammonium retention after fertiliser application, resulting in an increase in nitrification relative to methane-oxidising bacteria and an overall decrease in $CH_4$ oxidation. At this time, nitrification is less efficient than $CH_4$ oxidation by methane-oxidising bacteria [68]. Consequently, the combined use of BC and NBPT may enhance $CH_4$ emissions, mostly due to their positive effects on crop production, increasing C input to the soil through BC and root secretions [69]. The net impact of BC in combination with MHPP or NBPT on $CH_4$ emissions depends mainly on the final outcome of their synthesis, oxidation, and transport processes.

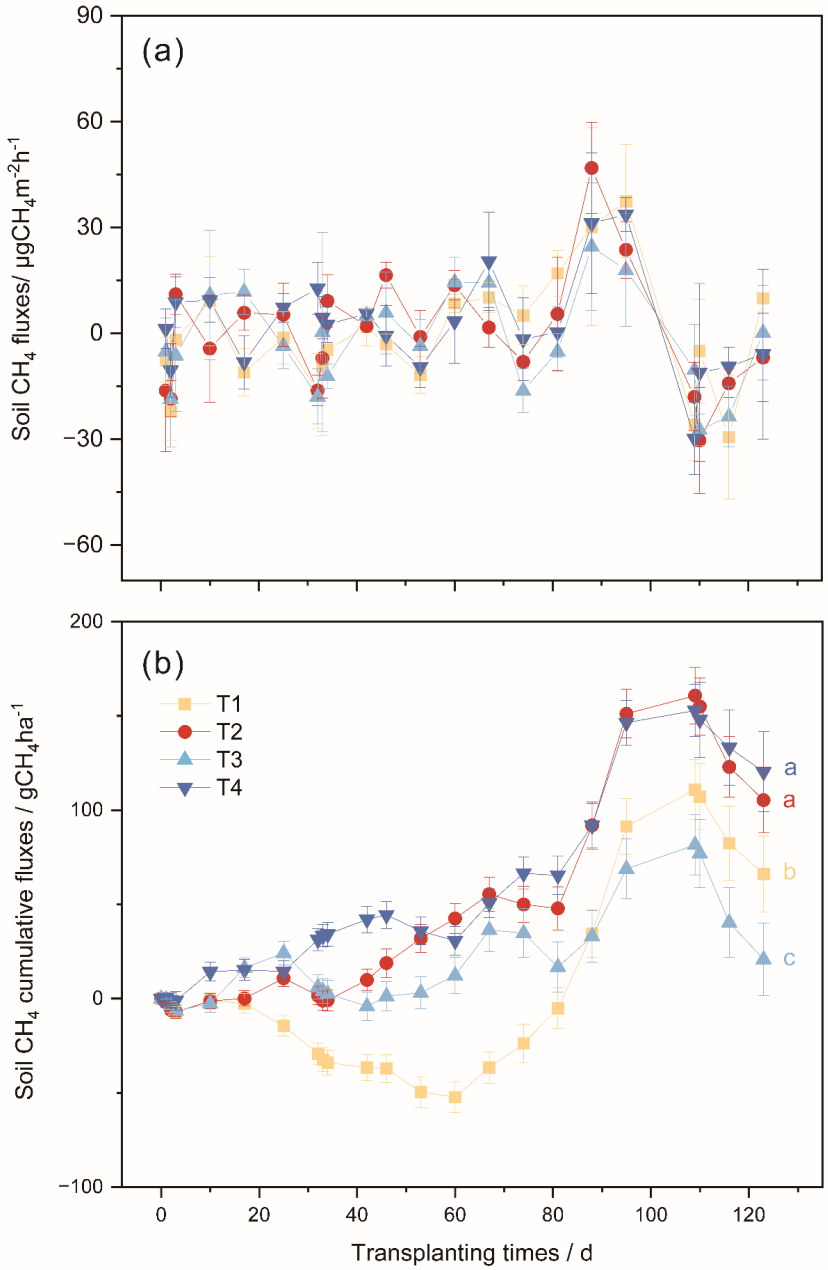


**Figure 4.** Changes in soil $CH_4$ flux (**a**) and cumulative $CH_4$ flux (**b**) for different treatments during the roasted tobacco growth cycle. Different letters indicate significant differences at $p < 0.05$.

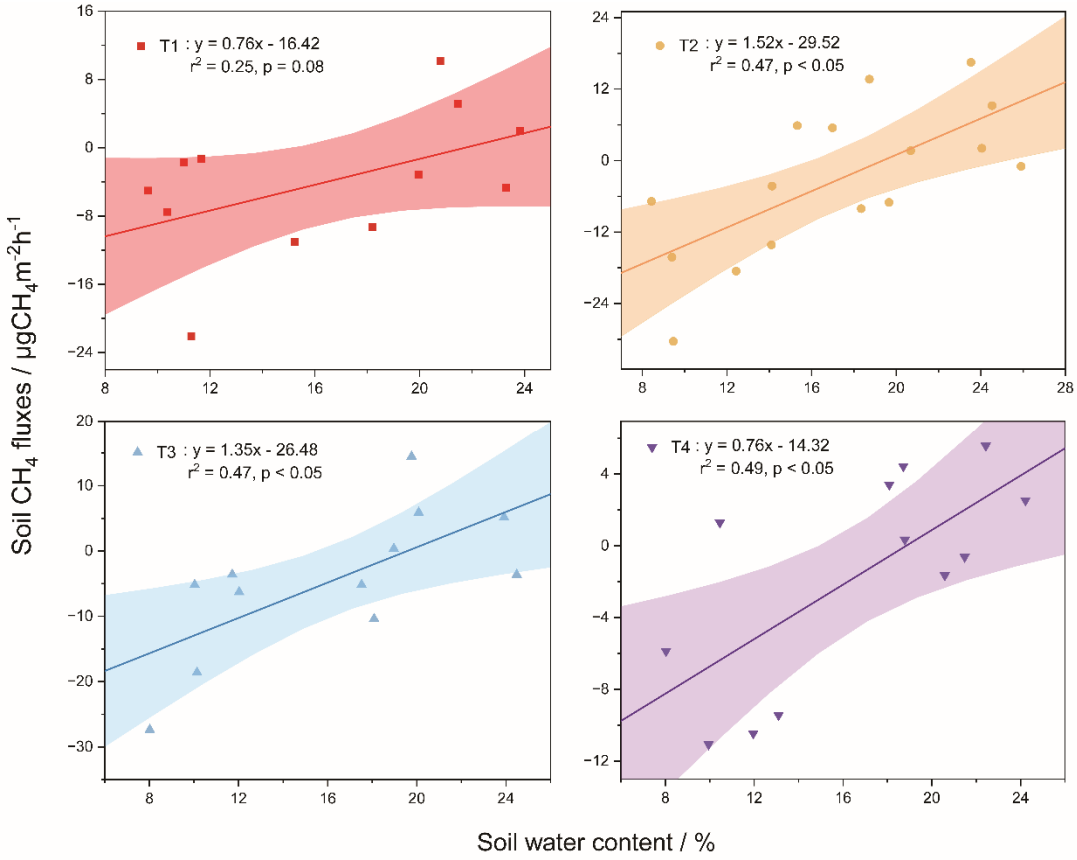


**Figure 5.** Linear regression of soil $CH_4$ emission fluxes against SVWC for each field treatment during the roasted tobacco growing cycle. The coloured areas indicate the 95% confidence interval of the regression line.

### *3.4. Effects of BC, MHPP, and NBPT on Soil $N_2O$ Emission*

Notably, a trade-off connection was discovered between soil $N_2O$ emissions and the existence of BC, MHPP, and NBPT. The combination of BC and MHPP revealed that NBPT reduced soil $N_2O$ emissions compared to T2, which is consistent with past field observations in non-karst areas [70,71]. Seasonal fluctuations in soil $N_2O$ fluxes throughout the roasted tobacco growth cycle depended mainly on N fertiliser application and were also influenced by SVWC (Figure 6a). The mean daily $N_2O$ emission fluxes for treatments from T1, T2, T3, and T4 were 54.98, 350.26, 274.77, and 232.45 μg $N_2O \cdot m^{-2} \cdot h^{-1}$, respectively, at 120 days after transplanting. Cumulative soil $N_2O$ emissions for T2, T3, and T4 treatments were 7.72 ± 0.00, 5.67 ± 0.00, and 4.58 ± 0.00 kg $N_2O \cdot ha^{-1}$, which were 6.49, 4.50, and 3.45 times higher than the control, respectively (Figure 6b). Moreover, cumulative soil $N_2O$ emission fluxes were significantly reduced by 26.55% and 40.67% for the combination of BC and MHPP or NBPT, respectively, compared to the plots with fertiliser application. The combined effect of BC and NBPT was more effective than the combination of BC and MHPP in suppressing soil $N_2O$ emissions. In addition, correlation analysis revealed a positive connection between soil $N_2O$ fluxes and 20 cm SVWC but not significantly with soil temperature. Notably, soil $N_2O$ fluxes had significant positive correlations with EC, $NH_4^+$, $NO_3^-$, and SIN (Figure 3b). In all field treatments, soil $N_2O$ fluxes increased with rising SIN content, and the linear function depicted the change in soil $N_2O$ fluxes from 53.0% to 76.0% (Figure 7). Combining the effects of BC and inhibitor combination on soil physicochemical properties, BC had high N content and NBPT contained amino groups, but MHPP did not contain amino groups. Moreover, for the sample experimental site, the amount of BC applied was substantially higher than the amount of both inhibitors. Therefore, the combination of BC and inhibitors significantly increased TN. The BC and

NBPT combination increased soil TN to a larger extent than BC and MHPP. However, $NH_4^+$, $NO_3^-$, and SIN were more effective than TN in indicating $N_2O$ emissions. SIN contains two main components, namely, $NH_4^+$ and $NO_3^-$, which are important substrates for nitrification and denitrification, respectively. A significant correlation was also observed between the dynamics of $N_2O$ emission fluxes and those of $NH_4^+$ and $NO_3^-$ concentrations. In particular, $NO_3^-$ and SIN had a considerable impact on controlling soil $N_2O$ emissions in karst areas [72,73].

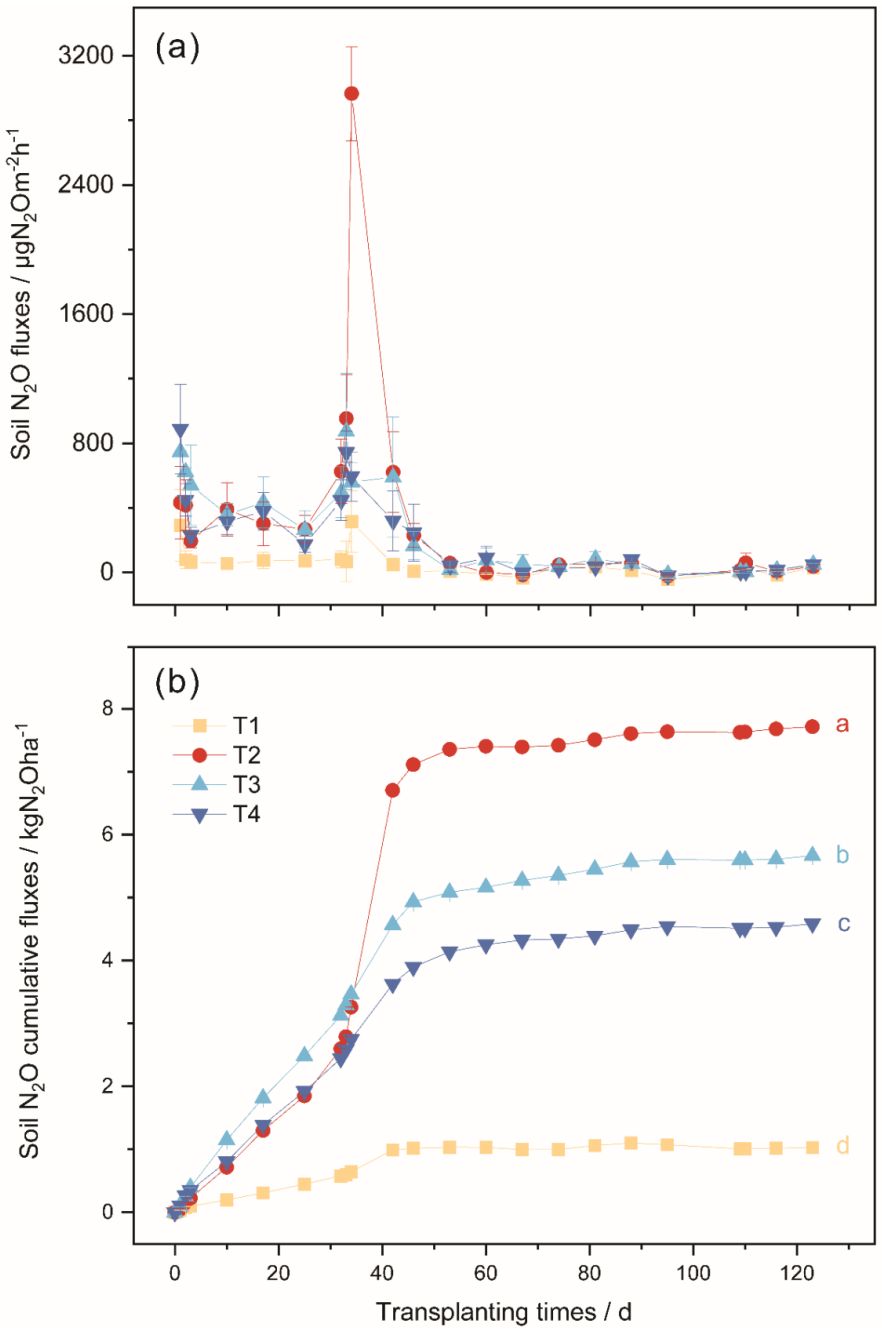


**Figure 6.** Changes in soil $N_2O$ flux (**a**) and cumulative $N_2O$ flux (**b**) for different treatments during the roasted tobacco growth cycle. Different letters indicate significant differences at $p < 0.05$.

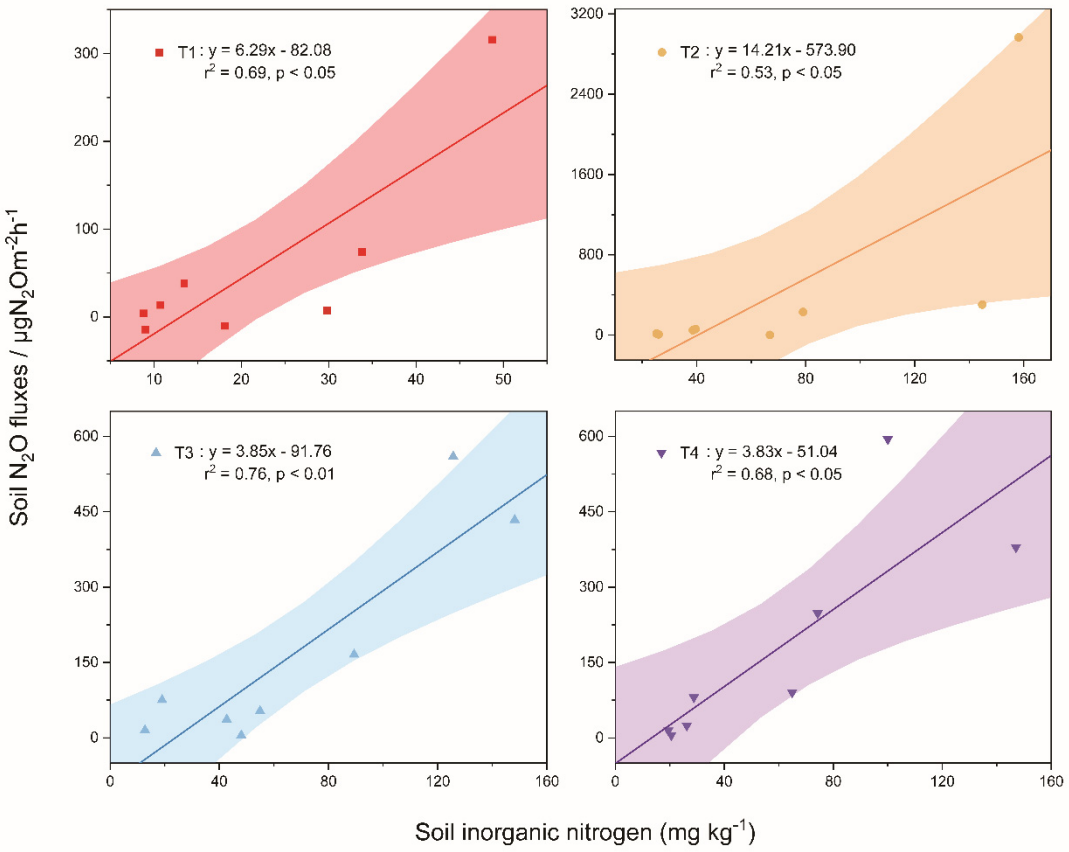


**Figure 7.** Linear regression of soil $N_2O$ emission fluxes with soil inorganic N for each field treatment during the roasted tobacco growing cycle. The coloured areas indicate the 95% confidence interval of the regression line.

Urease inhibitors reduce the effectiveness of substrates for nitrification, which is dominant in agricultural dry-crop soils, by inhibiting the active site of the urease molecule and, thus, delaying urea hydrolysis [26,74]. Nitrification inhibitors directly inhibit the nitrification process in soils by limiting the oxidation of $NH_4^+$ to $NO_2^-$ by ammonia-oxidising bacteria, thereby slowing the formation of $NO_3^-$ in soils [75]. This phenomenon lowers the availability of $NO_3^-$ to denitrifying bacteria [76]. The inhibition of soil $N_2O$ emissions by the combined use of BC and MHPP or NBPT is evident in the unique geological background conditions of karst (Table 4). Recent studies revealed numerous reasons for the reduction in $N_2O$ emissions due to the application of BC, MHPP, and NBPT. Shen et al. (2014) observed that BC addition increased $N_2O$ emissions by 13–82% [77]. According to Troy et al. (2013), the stimulatory impact of BC on $N_2O$ emissions was related to an increase in denitrification caused by BC-derived unstable organic carbon in significant concentrations [78]. Liu et al. (2019) discovered that the application of BC improved the abundance of $NH_3$-oxidising bacteria and accelerated nitrification, providing denitrifying bacteria with the capability to produce $N_2O$ and $NO_3^-$ [79]. Thus, BC addition promoted $N_2O$ emissions, which may be due to increased nitrification and denitrification processes. Furthermore, according to the meta-analysis, the application of inhibitors reduced $N_2O$ emissions by 33–58% [22]. Xia et al. (2017) found that $N_2O$ emissions were reduced by 31% after the application of urease inhibitors [80], which suggested that the reduction in nitrite effectiveness with the addition of nitrification inhibitors inhibited the soil nitrification process and reduced $N_2O$ emissions. In addition, the combined effect of BC and inhibitors may markedly reduce the supply of $NO_3^-$, thereby decreasing the activity of denitrifying bacteria and inhibiting $N_2O$ formation [81]. Chen et al. (2019) discovered that the decrease in $N_2O$ emissions under the combination of BC and inhibitors is caused by the increased abundance of nosZI, which

accelerates the conversion of $N_2O$ to $N_2$ [82]. Results revealed that the combined use of BC and inhibitors was highly effective in reducing $N_2O$ emissions and boosting crop yield and NUE [31]. Li et al. (2022) found that the combined application of BC and inhibitors could further reduce soil $N_2O$ emissions, but the addition of BC had the potential to reduce the effectiveness of inhibitors. Thus, the mitigating effect of the combination of BC and inhibitors needs further study [29].

**Table 4.** Cumulative $N_2O$ flux (kg/ha) and $N_2O$ emission factor ($EF_{N2O}$) (%) for the different treatments during the roasted tobacco growth cycle.

| Growth Periods | T1 | T2 | T3 | T4 |
|---|---|---|---|---|
| | | Cumulative $N_2O$ flux (kg/ha) | | |
| REP | $0.58 \pm 0.00^{c}$ | $2.60 \pm 0.00^{b}$ | $3.13 \pm 0.00^{a}$ | $2.44 \pm 0.00^{b}$ |
| VP | $0.42 \pm 0.00^{d}$ | $4.79 \pm 0.00^{a}$ | $2.15 \pm 0.00^{b}$ | $1.88 \pm 0.00^{c}$ |
| MP | $0.03 \pm 0.00^{b}$ | $0.32 \pm 0.00^{a}$ | $0.39 \pm 0.00^{a}$ | $0.26 \pm 0.00^{a}$ |
| Total | $1.03 \pm 0.00^{d}$ | $7.72 \pm 0.00^{a}$ | $5.67 \pm 0.00^{b}$ | $4.58 \pm 0.00^{c}$ |
| | | $EF_{N2O}$ (%) | | |
| REP | / | $1.35 \pm 0.00^{b}$ | $1.70 \pm 0.00^{a}$ | $1.24 \pm 0.00^{b}$ |
| VP | / | $2.08 \pm 0.00^{a}$ | $0.82 \pm 0.00^{b}$ | $0.69 \pm 0.00^{b}$ |
| MP | / | $0.14 \pm 0.00^{a}$ | $0.17 \pm 0.00^{a}$ | $0.11 \pm 0.00^{a}$ |
| Total | / | $3.17 \pm 0.00^{a}$ | $2.20 \pm 0.00^{b}$ | $1.69 \pm 0.00^{c}$ |

Different letters within a row indicate significant differences between treatments at $p < 0.05$.

$EF_{N2O}$ varied considerably between treatments throughout the growth cycle of roasted tobacco (Table 4). $EF_{N2O}$ varied from 0.11% to 2.08% at different growth stages, with T2 treatment having the largest $EF_{N2O}$ in the vigorous period and T3 and T4 treatments with the largest $EF_{N2O}$ in the root extension period. Except for T1, all three treatments applied basal fertiliser before the root extension period, and the cumulative $N_2O$ emissions of treatments from T2 to T4 did not change significantly during the root extension period. This finding may be attributed to the partially developed root system and the insufficient root exudates, resulting in the absence of significant differences under the combined effect with microorganisms. Thus, the combined effect of BC and inhibitors was insignificant [83]. $EF_{N2O}$ generally decreased over time. However, similar to $EF_{N2O}$, cumulative $N_2O$ emissions from T2 treatment were largest at this time, which was due to secondary fertilisation during the vigorous period. Compared to T2, cumulative $N_2O$ emissions were substantially low in T3 and T4 treatments, suggesting that the combination of BC, MHPP, and NBPT played a significant suppressive role. No significant difference in $EF_{N2O}$ was observed between treatments during the mature period. Throughout the roasted tobacco growth cycle, treatments with the addition of BC and MHPP or NBPT had substantially lower mean $EF_{N2O}$ with significant reductions in $EF_{N2O}$ of 30.60% and 46.69%, respectively, compared to treatments with only N fertiliser applied. This difference was mainly observed during the vigorous period, wherein no significant difference was found during the root extension and mature periods. Furthermore, $EF_{N2O}$ was larger in T3 treatment than in T4, suggesting that the combined effect of BC and NBPT was superior to the BC and MHPP combination considering $N_2O$ emission inhibition [84,85].

### *3.5. GWP and Crop Yield Response to BC, MHPP, and NBPT*

The combined effects of BC, MHPP, and NBPT on crop yields in roasted tobacco cropping systems are of concern as the demand for crop production in karst areas increases, and the accompanying changes in GWP could provide an important indication of climate change mitigation. Significant differences ($p < 0.05$) in GWP were found between treatments throughout the roasted tobacco growing cycle. The mean GWP values were 11.85, 14.81, 16.58, and 16.05 t $CO_2$-eq/ha for T1, T2, T3, and T4, respectively. T4 treatments had substantially larger mean GWP than T1 and T2 treatments. However, no significant difference was observed between T3 and T4 ($p < 0.05$), which was related to the contribution

of $CO_2$, $N_2O$, and $CH_4$ to the mean GWP of the roasted tobacco growing cycle. The four treatments were dominated by the contribution of $CO_2$, which ranged from 84.46% to 97.39%. The contribution of $N_2O$ was considerably reduced in T3 and T4 treatments using inhibitors compared to T2, with 10.19% and 8.51%, respectively. By contrast, the contribution of $CH_4$ to the mean GWP was negligible compared to $CO_2$.

Similar to earlier studies [86–88], the combined use of BC and inhibitors played an important role in increasing crop yield. The mean crop yield was 1.96, 3.62, 4.83, and 5.03 t for T1, T2, T3, and T4, respectively. Compared to T1, the addition of BC and inhibitors significantly increased tobacco biomass by 146.43% and 156.63% in T3 and T4 treatments, respectively. This result is comparable to the average increase ($\leq$10% to $\geq$200%) observed by meta-analysis [89,90]. No significant difference in crop yield was found between T3 and T4 treatments. By contrast, T2 treatment with fertiliser application only increased tobacco biomass by 84.69%. These results may be attributed to the high nutrient content of BC and its rapid dissolution in the soil solution, thus increasing soil fertility, especially AN, AP, AK, and SOM contents. Consequently, the inhibitor increased the effectiveness of SIN, plant N uptake, and N fertiliser. Therefore, the combination of BC and inhibitors can promote tobacco growth and increase biomass.

Notably, the yield-scaled GWP value is a key indicator [40]. Over the roasted tobacco growth cycle, mean yield-scaled GWP values were 6.04, 4.10, 3.43, and 3.19 t $CO_2$-eq/t for T1, T2, T3, and T4, respectively (Table 5). Compared to the fertilised treatment, the mean yield-scaled GWP values were significantly reduced by 16.34% and 22.20% for the T3 and T4 treatments, respectively, but were not significantly different. The combined use of BC and inhibitors increased mean GWP throughout the roasted tobacco growth cycle but reduced the mean yield-scaled GWP. By contrast, the latter is more important than the former [91]. The NBPT outperformed the MHPP considering mean yield-scaled GWP in roasted tobacco cropping systems. Therefore, the combined application of BC and inhibitors should be actively promoted to increase crop yield whilst reducing the yield-scaled GWP and mitigating global warming [26].

**Table 5.** The contribution of the GHGs, crop yield, and yield-scaled GWP for different treatments during the roasted tobacco growth cycle.

| | T1 | T2 | T3 | T4 |
|---|---|---|---|---|
| $CO_2$ (kg/ha) | 11,536.58 ± 0.23 $^{c}$ | 12,507.42 ± 0.33 $^{b}$ | 14,886.81 ± 0.64 $^{a}$ | 14,683.24 ± 0.50 $^{a}$ |
| $CH_4$ (g/ha) | 66.16 ± 20.05 $^{b}$ | 105.34 ± 17.40 $^{a}$ | 20.79 ± 19.27 $^{c}$ | 120.38 ± 21.20 $^{a}$ |
| $N_2O$ (g/ha) | 1030.54 ± 0.02 $^{d}$ | 7716.30 ± 0.07 $^{a}$ | 5666.31 ± 0.04 $^{b}$ | 4583.23 ± 0.03 $^{c}$ |
| GWP ($tCO_2$-eq/ha) | 11.85 ± 0.00 $^{c}$ | 14.81 ± 0.00 $^{b}$ | 16.58 ± 0.00 $^{a}$ | 16.05 ± 0.00 $^{a}$ |
| $CO_2$ contribution (%) | 97.39 ± 0.00 $^{a}$ | 84.46 ± 0.00 $^{c}$ | 89.81 ± 0.00 $^{b}$ | 91.47 ± 0.00 $^{b}$ |
| $CH_4$ contribution (%) | 0.01 ± 0.00 $^{a}$ | 0.02 ± 0.00 $^{a}$ | 0.00 ± 0.00 $^{a}$ | 0.02 ± 0.00 $^{a}$ |
| $N_2O$ contribution (%) | 2.59 ± 0.00 $^{d}$ | 15.53 ± 0.00 $^{a}$ | 10.19 ± 0.00 $^{b}$ | 8.51 ± 0.00 $^{c}$ |
| Crop yield (t/ha) | 1.96 ± 0.15 $^{c}$ | 3.62 ± 0.14 $^{b}$ | 4.83 ± 0.35 $^{a}$ | 5.03 ± 0.24 $^{a}$ |
| Yield-Scaled GWP ($tCO_2$-eq/t) | 6.04 ± 0.00 $^{a}$ | 4.10 ± 0.01 $^{b}$ | 3.43 ± 0.00 $^{c}$ | 3.19 ± 0.00 $^{c}$ |

The contribution of $CO_2$ refers to the contribution of $GWP_{CO2}$ to $GWP_{GHG}$; that of $CH_4$ and $N_2O$ is the same as above. Different letters within a row indicate significant differences between treatments at $p < 0.05$.

### *3.6. Effects of BC, MHPP, and NBPT on N Uptake and NUE*

The net utilisation of N fertiliser currently used in the field is remarkably low and is accompanied by serious environmental problems, such as GHG emissions and N leaching. The ideal way to improve net utilisation is to use N boosters in conjunction with controlled N fertiliser use [92]. The main N boosters are MHPP and NBPT. MHPP prolongs the retention of ammonium N in the soil whilst NBPT allows urea to remain in the soil as $NH_4^+$ [93], thereby increasing the NUE of fertiliser and reducing the environmental impacts of N fertiliser runoff [94].

Throughout the roasted tobacco growth cycle, compared to T2 (Table 6), the combination of BC and inhibitors significantly increased N uptake by 33.52% and 39.11% in T3 and

T4 treatments, respectively. Meanwhile, the combination of BC and inhibitors significantly increased NUE by 19.85% and 23.16% in T3 and T4 treatments, respectively. By contrast, no significant difference in N uptake and NUE was observed between T3 and T4 treatments. No significant changes in NUE and N uptake were found between treatments during the root extension period. On the contrary, NUE and N uptake were substantially larger in T3 and T4 treatments compared to T2 during the vigorous and mature periods. This phenomenon may be attributed to the partially grown root system during the root extension period. Upon its entrance into the vigorous and mature period, the root system is fully developed and can produce sufficient root secretions, which can have an inhibitory effect on nitrification and benefit N uptake by the crop, thereby increasing NUE [95]. Therefore, the combined effect of BC and inhibitors can significantly increase plant N uptake and NUE.

**Table 6.** N uptake and NUE in different treatments during the roasted tobacco growth cycle.

| Growth Periods | Treatment | Dry Weight Accumulation (kg/ha) | Amount of N Uptake (kg/ha) | NUE (%) |
|---|---|---|---|---|
| REP | T1 | 198.38 ± 29.15 $^{b}$ | 6.84 ± 1.01 $^{b}$ | / |
| | T2 | 503.91 ± 27.74 $^{a}$ | 17.38 ± 0.96 $^{a}$ | 7.03 ± 0.03 $^{a}$ |
| | T3 | 527.21 ± 84.35 $^{a}$ | 18.19 ± 2.91 $^{a}$ | 7.56 ± 0.58 $^{a}$ |
| | T4 | 428.48 ± 54.22 $^{a}$ | 14.78 ± 1.87 $^{a}$ | 5.29 ± 0.29 $^{b}$ |
| VP | T1 | 353.03 ± 8.23 $^{c}$ | 12.18 ± 0.32 $^{b}$ | / |
| | T2 | 453.03 ± 13.02 $^{b}$ | 15.63 ± 0.42 $^{b}$ | 1.64 ± 0.00 $^{b}$ |
| | T3 | 842.47 ± 15.12 $^{a}$ | 29.07 ± 0.48 $^{a}$ | 8.02 ± 0.02 $^{a}$ |
| | T4 | 847.47 ± 11.12 $^{a}$ | 29.24 ± 0.40 $^{a}$ | 8.10 ± 0.00 $^{a}$ |
| MP | T1 | 1408.18 ± 107.87 $^{c}$ | 48.58 ± 3.72 $^{c}$ | / |
| | T2 | 2659.23 ± 100.99 $^{b}$ | 91.74 ± 3.48 $^{b}$ | 20.49 ± 0.03 $^{b}$ |
| | T3 | 3458.60 ± 255.84 $^{a}$ | 119.32 ± 8.83 $^{a}$ | 33.59 ± 0.54 $^{a}$ |
| | T4 | 3754.34 ± 176.94 $^{a}$ | 129.52 ± 6.10 $^{a}$ | 38.43 ± 0.25 $^{a}$ |
| Total | T1 | 1959.60 ± 149.14 $^{a}$ | 67.61 ± 5.15 $^{c}$ | / |
| | T2 | 3616.16 ± 140.84 $^{a}$ | 124.76 ± 4.86 $^{b}$ | 27.14 ± 0.03 $^{b}$ |
| | T3 | 4828.28 ± 354.16 $^{a}$ | 166.58 ± 12.22 $^{a}$ | 46.99 ± 0.75 $^{a}$ |
| | T4 | 5030.30 ± 243.29 $^{a}$ | 173.55 ± 8.39 $^{a}$ | 50.30 ± 0.34 $^{a}$ |

Different letters within a column indicate significant differences between treatments at $p < 0.05$.

A study showed that concentrations of the $NH_4^+$ form of mineral N were higher than $NO_3^-$ a few days after the application of BC, resulting in increased nutrient effectiveness, N uptake, and crop yield. The retention of ammonium N in the soil due to BC addition not only provides environmental benefits through reduced $N_2O$ emissions and $NO_3^-$ leaching [96] but also agronomic benefits through increased NUE, especially in soils with low N [97]. N retention by BC is associated with its pore structure and microporous electrostatic interaction [98]. Furthermore, a high pyrolysis temperature leads to the large $NO_3^-$ adsorption capacity of BC [99]; functional groups on BC may improve its $NO_3^-$ adsorption capacity by extending its soil contact time [49,100]. N taken by BC may be gradually released for crop absorption over time [101]. Several studies have shown that BC amendments may indirectly influence plant N absorption by modifying the N conversion efficiency of the soil and positively impacting crop yields [102,103]. Concerning inhibitors, Drulis et al. (2022) found that the use of urease inhibitors reduced nutrient leaching, improved NUE, and significantly increased crop yields [104]. Moreover, MHPP may limit the activity of ammonia-oxidising archaea (AOA) and ammonia-oxidising bacteria (AOB), as well as their associated enzymes, thereby delaying the oxidation of $NH_4^+$ to $NO_3^-$ and lowering $NO_3^-$ accumulation and leaching losses whilst maintaining high soil $NH_4^+$ concentrations [105,106]. Thus, nitrification inhibitors can similarly significantly increase NUE and crop yield [107]. In addition, studies have shown that the combined use of BC and inhibitors performed well considering yield enhancement, efficient N use, and reduction in N losses [31,108]. He et al. (2022) discovered that the combined application of

BC and inhibitors significantly improved plant N absorption and crop yield by decreasing residual inorganic N concentrations and $N_2O$ losses [32], which is consistent with the obtained results. He et al. (2018) found that the combined application of BC and inhibitors had no effect on NUE in the first year but increased significantly in the second year [109]. Therefore, the combined application of BC and MHPP or NBPT may be an effective practice to increase crop NUE, and this combination may lead to high fertiliser utilisation and reduce the amount of applied N fertiliser [110].

## 4. Conclusions

A comprehensive analysis of GHG emissions, GWP, and NUE of roasted tobacco cropping systems in the southwest karst area was conducted in this study to verify the combined effects of BC, MHPP, and NBPT. Considering soil physicochemical properties, the results showed that the combined use of BC and MHPP or NBPT significantly increased TN by 23.50% and 13.11%, respectively, whilst AK levels significantly increased by 1.95-fold and 1.23-fold compared to T2, respectively. Moreover, soil EC increased significantly by 1.56, 4.81, and 4.90 times in T2, T3, and T4 compared to T1. The combination of BC and inhibitors significantly increased the mean soil pH by 7.26% to 8.81%, whilst SOM levels were significantly increased by 44.97% and 32.95%, respectively. Considering soil GHG, the average daily soil $CO_2$ fluxes were substantially larger in T2, T3, and T4 treatments compared to the control, increasing by 12.29%, 33.95%, and 34.25%, respectively. The cumulative $CO_2$ emissions of the treatments with the addition of BC and inhibitors were significantly higher than the control by 27.21% to 29.03%. The exponential–exponential function of soil $CO_2$ emission fluxes with soil moisture and temperature was well fitted, and $R^2$ reached 0.506 to 0.836. The combination of BC and NBPT increased the cumulative soil $CH_4$ emissions by 14.28% compared to the fertiliser treatment. However, the combination of BC and MHPP significantly reduced the cumulative soil $CH_4$ emissions by 80.26%. Thus, the combined effect of BC and inhibitors on $CH_4$ emissions depends mainly on the results of their synthesis, oxidation, and transport processes. Furthermore, the combination of BC and MHPP or NBPT resulted in a significant reduction in cumulative soil $N_2O$ emissions by 26.55% and 40.67%, respectively. Notably, $NO_3^-$ and SIN are of considerable importance for limiting soil $N_2O$ emissions in karst areas. Throughout the roasted tobacco growth cycle, the combination of BC and MHPP or NBPT significantly reduced $EF_{N2O}$ by 30.60% and 46.69%, respectively, compared to the fertiliser treatment. With the combination of BC and inhibitors, the GWP increased significantly by 8.37% to 11.95%, with the contribution of $CO_2$ dominating the GWP ranging from 84.46% to 97.39%. Meanwhile, the crop yield increased significantly by 146.43% and 156.63% compared to the control. However, the mean yield-scaled GWP values were significantly reduced by 16.34% and 22.20% for T3 and T4 treatments, respectively, compared to the fertiliser treatment. In addition, N uptake was markedly enhanced by BC and inhibitor application, resulting in a significant increase in NUE of 19.85% to 23.16%, and reducing the residual inorganic N concentration and $N_2O$ loss in the soil. Therefore, the combined use of BC and inhibitors in tobacco fields in the southwest karst region is an effective option for improving crop yield and mitigating climate change.

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