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Biochar Effects on Mineral Nitrogen Leaching, Moisture Content, and Evapotranspiration after ^{15}N Urea Fertilization for Vegetable Crop

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Abstract: Globally, mineral nitrogen (N) losses as nitrate leaching (NL) are a substantial portion of applied fertilizer and cause surface and sub-surface water contamination. To precisely measure NL and its interlink parameters, biochar soil amendment was tested in this study. Three treatments—biochar (BC), without biochar (WB) with ^{15}N urea (300 kg/ha), and control (no fertilization)—were tested in soil-filled lysimeters (circular PVC (Polyvinyl Chloride) tank of 30 cm diameter and 35 cm height) equipped with moisture content sensors and weighing assembly for the consecutive two cropping of *Brassica Camprestis Var. Chinensis*. The ^{15}N-urea in the first season and the poultry manure in the second season were applied, but the fate of the ^{15}N was examined in leachate, dry matter, and soil. As compared to WB, BC significantly decreased mineral N leaching, including nitrate levels (35%), increased electrical conductivity (68.5%), and water availability (20% inches per foot), while there was a non-significant increase in biomass per plant (2.84%), evapotranspiration (8.33%), dry matter (6.89%), and a decrease in mean leachate volume (7.63%). Moreover, BC accumulated values were higher than WB, as N uptake (38%), water use efficiency (12.24%), maximum fresh weight (11.4%), and soil N retained (185%) after cropping. The soil pH, the bulk density, and the total nitrogen were changed but presented non-significant differences. Therefore, biochar can increase soil N retention and available water to improve water use efficiency and decrease potential N leaching.

Keywords: soil nitrate; lysimeter; water use efficiency; dry matter; soil pH; soil electrical conductivity

1. Introduction

Worldwide, nitrogenous fertilizers are abundantly used to enhance crop production in agricultural sectors, but lower nitrogen (N) use efficiencies in almost all agricultural land originate from nitrogen losses in the form of ammonia volatilization, nitrate leaching, and emission of greenhouse gases (GHG). On the one hand, active N threatens the environment in the form of worsened changes in precipitation and temperature patterns, and on the other hand, higher nitrate concentrations in surface and subsurface waters put pressure on water resources of the globe. Higher nitrate in potable water has been generating problems after agricultural modernization such as blue baby syndrome in humans [1], poisoning in animals [2], and harmful effects on plant bodies in certain conditions [3]. In China, nitrate contamination has been affecting not only shallow groundwater (unconfined aquifers) but also deep groundwater (confined aquifers), which may take a long time for recovery [4]. Nitrate pollution was recently measured in the whole of China, and it was found to have caused adverse effects in adult males, children, and infants in Shaanxi and Shandong provinces [3]. Therefore, the Chinese government has to take management measures to protect the groundwater from further dreadful conditions, because a considerable portion of the population depends on the groundwater resources for drinking, livestock farming, and other uses.

Total N losses vary from region to region depending on fertilizer type [5], fertilizer dose [6], soil characteristics [7], water content availability [8], climate [9], and management practices [10]. In any agricultural region, management practices cover seedbed preparation, time of plantation, fertilization, harvest, irrigation, vegetation type and rotation, and protected or non-protected agricultural practices. However, the best management practice is to utilize all the influential parameters to control nitrate leaching sustainably [9] and without compromise to yield reduction, because the world population is becoming enlarged with expectedly high demands of food, fiber, and bioenergy [9], especially in large, highly populated countries such as China. In the last few decades, the Chinese government has ensured surplus water and fertilizer availability to farmers on time to enhance crop production, which might boost agricultural production and endorse N losses as nitrate leaching [11]. Many researchers reported nitrate leaching losses in different parts of the country of up to 50% of applied fertilizer and 30–35% nitrogen utilization efficiency. China has a large population (about 22% of the world population) and 7% of the total world agriculture land (approximately 135 million ha [12]), wherein 19.4% (26 million ha) is degraded and polluted [13]. Thus, sustainable development in the arable land in China might be faced with multiple challenges, including nitrate leaching and maximizing production to feed the future population [14]. Many remedial measures have been taken, such as covering crops, nitrogen inhibitors, and biochar additions, but a sustainable solution still has yet to be implemented. However, reduction in N losses has been verified in many cases; Quemada et al. [15] analyzed a 40% reduction in nitrate leaching because of improved fertilizer and water management. Biochar (BC) application in soil to control N losses as well as carbon sequestration is becoming a popular method in the agricultural sectors throughout the world [16,17] because it synergizes climate mitigation, soil conditioning, and safe environmental waste management [18]. Moreover, BC improves soil hydraulic properties, as it holds more water to deliver for crop production [17], increases soil respiration, and upgrades recalcitrance in soil [19]. However, the aspect of biochar that may increase crop yield is not fully understood because of controversial results from different studies. For example, Kammann et al. [20] found a substantial increase in quinoa (*Chenopodium quinoa L.*) yield in a pot experiment, whereas Rogovska et al. [21] found no increase in maize yield in a subtropical region, and some studies negatively described a reduction in yield [22]. Hence, biochar amendment in a particular soil–water–plant system should be analyzed under climatic conditions, water availability, and temperature to fully understand its benefits.

Global warming alarmingly generates soil aridity/water scarcity problems in China due to shifts in severe hydrological, meteorological, and agricultural drought as well as increases in the frequency of extreme precipitation events [23]. Consequently, these postulating sequences might probe the risk of surface flooding and subsurface nitrate leaching, because water has a positive relation with soil nitrification processes [24] and high nitrate solvent capacities. In particular, heavy rains in monsoon season after dry and hot summers cause severe nitrate leaching losses in agriculture sectors of China [25]. N losses in a particular farmland system can be monitored in many ways, but isotopic pool dilution (IPD) is considered a powerful tool for determining the N dynamics in a soil–plant–water system [26]. Water and nitrogen are inter-related and are integral factors related to crop yield, soil fertility, and nutrient leaching from an agriculture soil [6]. Some studies illustrated that crop yield was increased directly with excess availability of water and nitrate, but after exceeding a certain adequate limit, it may have damaging effects on the ecosystem. Therefore, moisture content must be taken into consideration while monitoring N transformation and leaching in any soil–water–plant system [27] with the help of different methods and devices. Different types of moisture sensors are available in different markets, which may be installed in soil during pot, lysimeter, or field experiments for instant and quick data acquisition and manipulation, but their accuracy is always questionable because of different technical and non-technical issues. It is better to calibrate the moisture sensor before and after installation in soil [28]. This study was conducted in an open greenhouse that had similar conditions as a field, but protections were applied in severe weather conditions such as freezing temperature. Such types of greenhouses are commonly used in Beijing for vegetable production.

The purpose of the study was to evaluate the soil–water–plant relationship in the context of mineral nitrogen (nitrate and ammonium ions) and—more precisely and accurately—in the presence and the absence of biochar as well as no biochar and fertilization for *Brassica Camprestis Var. Chinensis* cultivation in two consecutive cropping seasons (April to July and August to November). The comparative analyses among the treatments were made for the parameters encompassing total nitrate and ammonia leaching, total N uptake, biomass per plant, evapotranspiration, and water use efficiency. Moreover, the soil properties, including pH, Electrical Conductivity (EC), bulk density, total nitrogen, moisture content, and tracer nitrogen values after the first and the second harvest, were also monitored in consecutive two seasons.

2. Materials and Methods

2.1. Site Details

The case study was conducted at the greenhouse in the Institute of Agriculture and Sustainable Development in Haiden District that is in a suburb of Beijing, China. It has a population of 20 million people and a vegetable consumption of about 11 million tons annually. A substantial portion of vegetable production (approximately 67%) is produced in protected greenhouses [29]. Beijing has a typical monsoon climate with hot summers and cold winters with an average 447–580 mm rainfall and 10–12 °C average annual temperature. Hot and dry summer spells make it essential to irrigate adequately for the optimum production. In addition, vegetable production in the winter season is not possible without a protected greenhouse environment. Temperature and precipitation graphs present the heavy rainfall occurrence during the cropping season with the number of dry spells, as shown in Figure 1.

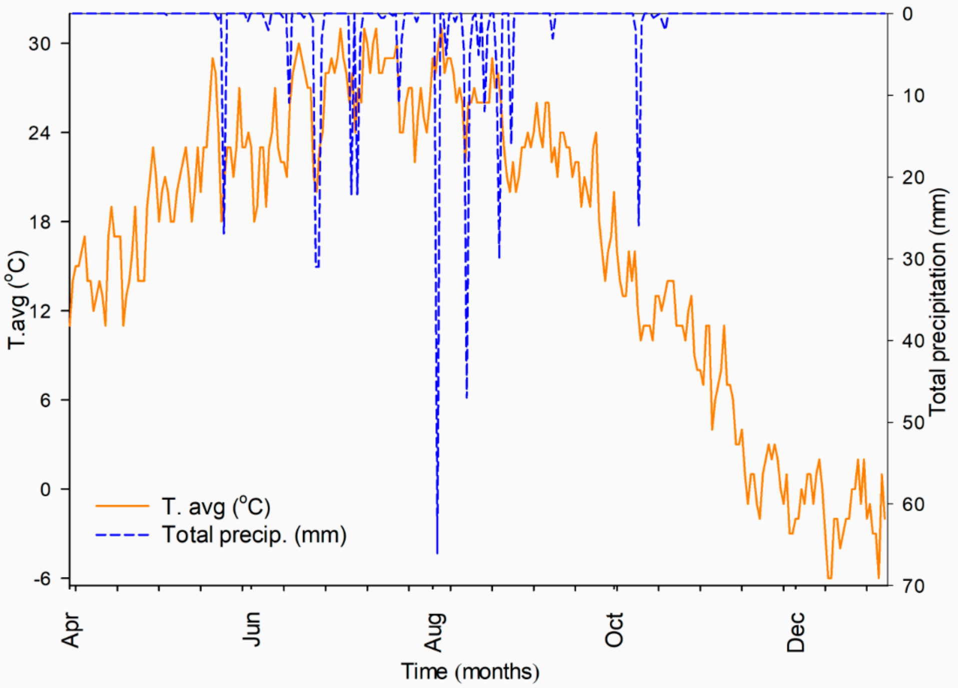

Figure 1. Daily mean temperature and precipitation data during the two cropping seasons.

2.2. Experiment Design

This experiment was conducted in 9 soil filled columns that had 30 cm diameters and 35 cm heights with a leachate collection assembled at the bottom. The vegetable, *Brassica Camprestis Var. Chinensis*, was cropped in two consecutive seasons (April to July and September to December). Three treatments with 3 repetitions—no biochar and no fertilization (CK), biochar and fertilization (BC), and fertilization without biochar addition (WB)—were tested for total mineral N leaching (nitrate and ammonium ions), total label N leaching, total nitrogen utilized by plant, total and tracer nitrogen retained in soil after every season, evapotranspiration (ET), water use efficiency (WUE), total water volume leached, water holding in terms of inches per foot (IPF), growth rate as fresh plant weight after specified time interval (30, 45, 60, and 90 days), biomass per plants (kg), and N leaching rate (mg/L). These parameters were analyzed for the treatments CK, BC, and WB before the time of transplantation in the first season to the end of the second season at harvesting time. For the fertilizer treatments (WB and BC), ^{15}N urea at the rate of 300 kg N/ha per lysimeter was applied in the first season, whereas the poultry manure (1.98% nitrogen, 0.83% phosphorus, and 1.53% potassium) at the rate of 300 kg N/kg was used in the second season. Soil was collected from Shunyi District in March 2017 that was covered after harvesting in December 2016. Each soil filled column weighed 33 kg and was the same for all treatments. The soil properties measured before use in the experiment were included: soil texture was 45.9% sand, 36.9% silt, and 17.2% clay, 1.46 g/cm^3 bulk density, 1.07 g N/kg total nitrogen, 8.15 pH, and 115 µS/cm (0.115 dS/m) EC. Furthermore, labeled urea 98.5% enrichment, moisture sensor, poultry manure biochar at the rate of 20 ton/ha, and irrigation at the rate 1 inch per week were used in the experiment. Decagon (ECH$_2$O) was installed in each lysimeter as the device to measure the soil moisture content in term of IPF.

Poultry manure biochar (1.95% nitrogen, 0.83% phosphorus, and 1.53% potassium) was prepared from the mixture of chicken litters and straw at the slow pyrolysis (10 °C min^{-1}) up to the temperature of 450 °C. The biochar prepared from the poultry manure usually had the potential to increase organic carbon, electrical conductivity, and soil pH. The resultant poultry manure prepared had pH 8.85, EC 12.3 dS/m, and Cation Exchange Capacity (CEC) 740 mol$_c$/kg. Vegetable seeds were germinated in soaked cotton wool spread in a seed starting tray and were transplanted in the lysimeters after being placed in a controlled environment for 10 days at 25 °C. Fifteen plants were transplanted in each lysimeter and were kept 1–2 inches apart from each other. Lysimeters were set in open greenhouse premises where no side walls or shelters persisted in the summer to provide obstruction from light and air circulation. These types of greenhouses are common in Beijing, where the transparent shelters are placed only in the months of November and December to protect the crops from the severe winter conditions.

2.3. Experiment Procedure

Soil was filled up to 32 cm in height with a weigh of 33 kg, and a moisture content sensor was installed about 5 cm above the bottom in all of the lysimeters. Each soil filled column was calibrated before applying fertilization and plantation according to the procedure explained by [28]. Leachate volume collected during calibration was measured and analyzed for nitrate and ammonium concentration. Columns were placed in an open place for 7 days, and the moisture content was determined every day. Thereafter, topsoil up to 15 cm was stirred and mixed with biochar in 3 columns. Every column was transplanted with 15 plants in each season. In the first season, labeled urea fertilizer in water solution form was applied in 6 columns. Single super phosphate (SSP) and potassium sulphate were also applied in all columns at the rate of 150 kg P/ha and 300 kg K/ha, respectively.

Every day, moisture content and weight were measured at a fixed time. Irrigation was scheduled as 500 ml in two days by a sprinkler. Plant dry matters and fresh weights for each column were prepared 4 weeks after the transplantation and 2 weeks thereafter up to the harvest in both seasons. Therefore, plant dry matter and plant fresh weight samples were prepared in each season five times. Leachate was collected after a heavy rainfall or after a week as it assembled in the collection chamber.

After harvest, columns were kept in an open environment for about 45 days, and processed farmyard manure was applied a week prior to plantation. The same schedule for irrigation and preparation of the dry matter was followed in the second season. After the first and the second harvest, soil samples were taken with the help of a plunger of 2.54 cm diameter and 30 cm depth to determine the soil nitrate and the ammonia level.

2.4. Laboratory Analysis

Lachat Flow Injection (LFI) equipment based on the Flow Injection Analysis procedure invented by Růžičkaand Hansen was used to analyze mineral nitrate and ammonia concentration in soil as well as in leachate samples. Soil samples were prepared for LFI as soil and a KCl 2M solution in a ratio of 1:5 and were placed in a mixer for 30 minutes at a speed of 40 rpm. Leachate samples were filtered and passed directly to LFI for testing. Samples were prepared for soil and leachate to determine the ^{15}N nitrate and the ^{15}N ammonia as guided by Coplen et al. [30] and Goeges and Dittert [31], respectively. Plant dry matter or biomass were prepared as the plant was unplugged from roots, washed in de-ionized water, oven dried at 70 °C for 24 hours, crushed, ground, and forwarded for ^{15}N and total N analysis. The total biomass and the biomass per plant were weighted for all treatments. Isoprime 100 IRMS United Kingdom was used to conduct all the isotopic tests. Digital EC meter and pH were used to measure the EC and the pH according to the Rayment [32] procedure for soil samples after calibration.

Fresh plant weight was taken as the plant was removed from the soil, washed off, blotted gently with a soft paper towel to remove access water, and thereafter weighted. Wet fresh biomass was taken separately for each plant and the number of plants taken out each time to monitor the plant growth for all the lysimeters. Every day, IPF values were taken by sensor, thus there were three values for each treatment, and thereby an average value was considered as a single day reading for a treatment. Equations (3) and (4) were used to calculate the ET and the WUE. The vegetative growth above soil surface produced in a lysimeter was used to calculate the biomass per plant and the maximum plant fresh weight. In each season, plants were unplugged 5 times as 3 plants from a single lysimeter on each harvest date. In both seasons, the last 3 plants were harvested 90 days after the transplantation in lysimeters, when the plants in all the treatments gained their maximum vegetative growth before the reproductive stage according to the seed providing company (Beijing Advanced Seed Co., Beijing, China).

2.5. Calculations

Nitrogen has two stable isotopes with the atomic masses 14 (^{14}N) and 15 (^{15}N). The difference in the isotopic composition in atmosphere and other substances (soil, plants, etc.) is used to trace the source. The measurement of the stable nitrogen isotopic ratio is referred to as δ and is expressed in terms of thousandths (‰);

$$\delta 15\mathrm{N} = \{(\mathrm{R}_{\mathrm{sample}} - \mathrm{R}_{\mathrm{reference}})/\mathrm{R}_{\mathrm{reference}}\} \times 1000 \text{ (Coplen et al., 2011]} \tag{1}$$

where $R = {}^{15}N/{}^{14}N$

R reference may be taken as the natural atmospheric concentration (0.3663) or may use the control crop natural isotopic ratio. In this study, the CK crop, the soil, the plant, and the leachate were taken as reference isotopic ratios. Therefore,

Water use efficiencies for all lysimeters were found by Equations [33] and [34]:

$$\begin{aligned} &Total\ 15N\ concentration\ absorbed\ by\ plant,\ soil\ or\ leachate \\ &\quad = \left(\%\,\frac{\delta 15\mathrm{N}}{1000}\right) \times (Total\ N\ in\ soil,\ or\ plant\ or\ leachate) \end{aligned} \tag{2}$$

$$\text{Water Use Efficiency} = (\text{Dry matter/ET}) \tag{3}$$

The concentrations of N in the extracts were determined by an automated total organic carbon/total nitrogen analyzer (Multi N/C, 3100/HT1300, Analytik Jena, Germany).

Evapotranspiration was measured with the help of weighing lysimeters with moisture content sensors [35]:

$$\Delta W = P + I + ET \pm \Delta S \Rightarrow ET = = P + I - \Delta W \pm \Delta S \quad (4)$$

where ΔW, P, I, ET, and ΔS refer to changes in lysimeter weight, precipitation, irrigation, evapotranspiration, and ΔS change in moisture content, respectively. A decagon sensor was used to measure moisture content in term of IPF and analog digital number (ADC). Before fertilization and cultivation, sensors were calibrated for IPF and Percentage (PCT) to the corresponding lysimeter weight and the known moisture content.

2.6. Statistical Analysis

F and T-tests with one-way Anova were applied to determine the significant variations between the initial values of soil pH, EC, bulk density, and total nitrogen (N) taken before fertilization and after the first and the second harvests for CK, WB, and BC treatments. Similarly, one-way Anova was applied to calculate the variation between the total N and the ^{15}N in dry matter, leachate, and soil with ammonium and nitrate ion. In the post hoc test, multiple comparisons all pair-wise among the treatments in the two seasons were made by the Scheffe test. Furthermore, single plant maximum growth curve and leachate volume for cumulative values, total yield, ET, water availability in IPF, and WUE were also compared between treatments as well as seasonal variation in the form of tables and graphs with SEM (standard error of mean) by the computer programs MS Office 13 (Microsoft Cooperation, Washington, DC, USA), Statistix 8.1 (Analytical Software, Tallahassee, FL, USA) SigmaPlot (Systat Software Inc., San Jose, CA, USA) and GraphPad Prism (GraphPad Software Company, San Diego, CA, USA).

3. Results

3.1. Evapotranspiration and Inches Per Foot

A total of 181 days ET and IPF were determined for all treatments under different climatic conditions, as presented in Figure 2. In the first cropping season, there was nearly the same trend of ET in all treatments with a little increase in BC in the first season, while in the second season, BC treatment showed a little decrease, which was not statistically significant as compared to WB and CK (as shown in Table 1). This variation in the results was not due to the poultry addition in the second season in BC, because WB was also treated with the same. However, it might have been due to the combined action of poultry manure and biochar in the second season. It was evaluated from the accumulative ET mean value differences for the two seasons that ET increased 1.08% and 5.08% in WB and BC then CK, respectively. Moreover, maximum and minimum ET were observed in BC as compared to WB and CK. Mean daily ET in the second season was observed to be lower than the first season because temperature dropped after the month of October.

In both seasons, the water availability in terms of inches per foot was significantly higher in BC as compared to WB and CK. The standard deviation in the first season was more than in the second season, which might have been due to the higher evapotranspiration rate in the first season, as given in Table 1. The maximum values of IPF were observed in the BC treatment, as shown in Figure 2.

Table 1. Mean values with standard deviation for the parameters measured in each sampling date for the treatments; CK (control), WB (without biochar), and BC (biochar added) for the two cropping season of the *Brassica Camprestis Var. Chinensis* plantation in lysimeters. The small letters (a, b, c) present the level of significance among the treatments in a season according to the pairwise Scheffe test.

Properties	Seasons	CK	WB	BC	Properties	CK	WB	BC
TN in DM (mg)	*Season-1*	326 ± 257a	470 ± 404a	529 ± 435a	***^{15}N in DM (mg)***		85 ± 68.7a	116 ± 91.3a
	Season-2	193 ± 181a	360 ± 479a	369 ± 512a			19.8 ± 24.5a	28.7 ± 34.3a
ET (mm)	*Season-1*	8.76 ± 4.32a	8.46 ± 3.97a	9.32 ± 5.02a	***IPF***	2.31 ± 0.75b	2.54 ± 0.49b	3.08 ± 0.65a
	Season-2	3.54 ± 2.55a	3.65 ± 2.49a	3.53 ± 2.53a		2.39 ± 0.34b	2.51 ± 0.35b	3.01 ± 0.40a
TN Nitrate (mg)	*Season-1*	20.6 ± 8.2c	48.5 ± 24.5a	35.1 ± 18.4b	***^{15}N Nitrate (mg)***		41.22 ± 20.5a	30.8 ± 18.20b
	Season-2	14.4 ± 7.9c	50.6 ± 14.4a	30.13 ± 7.1b			28.7 ± 10.64a	13.9 ± 3.2b
TN Ammonia (mg)	*Season-1*	2.23 ± 1.84b	5.21 ± 3.2a	6.18 ± 3.06a	***^{15}N Ammonia (mg)***		3.90 ± 2.7b	4.9 ± 2.33a
	Season-2	1.99 ± 1.44b	4.84 ± 2.35a	5.74 ± 3.92a			1.6 ± 1.8b	3.36 ± 2.9a
Total volume (L)	*Season-1*	1.00 ± 0.96a	0.9 ± 1.04a	0.86 ± 0.91a	***Dry weight (DW) (g)***	62.9 ± 3.09b	79.5 ± 2.75ab	87.4 ± 11.02a
	Season-2	0.67 ± 0.31a	0.61 ± 0.33a	0.48 ± 0.24a		44.45 ± 10.35b	61.95 ± 3.65ab	66.12 ± 6.81a
Biomass per plant (g)	*Season-1*	9.90 ± 1.71b	14.26 ± 0.98a	14.93 ± 1.92a	***Maximum WUE***	6.05 ± 0.95a	5.88 ± 1.09a	6.93 ± 1.14a
	Season-2	10.89 ± 2.2b	14.97 ± 1.92a	15.1 ± 2.06a		12.47 ± 2.69a	11.59 ± 5.25a	18.84 ± 5.30a
TN Nitrogen leached (mg)	*Season-1*	12.32 ± 3.05b	29.00 ± 13.23a	22.27 ± 9.67a	***^{15}N Nitrogen leached (mg)***		24.35 ± 11.19a	19.27 ± 9.72a
	Season-2	8.87 ± 4.76c	29.94 ± 7.85a	19.37 ± 4.02ba			16.4 ± 4.1a	9.39 ± 2.62b

TN: total nitrogen, DM: dry matter, ET: evapotranspiration, IPF: inches per foot, WUE: water use efficiency.

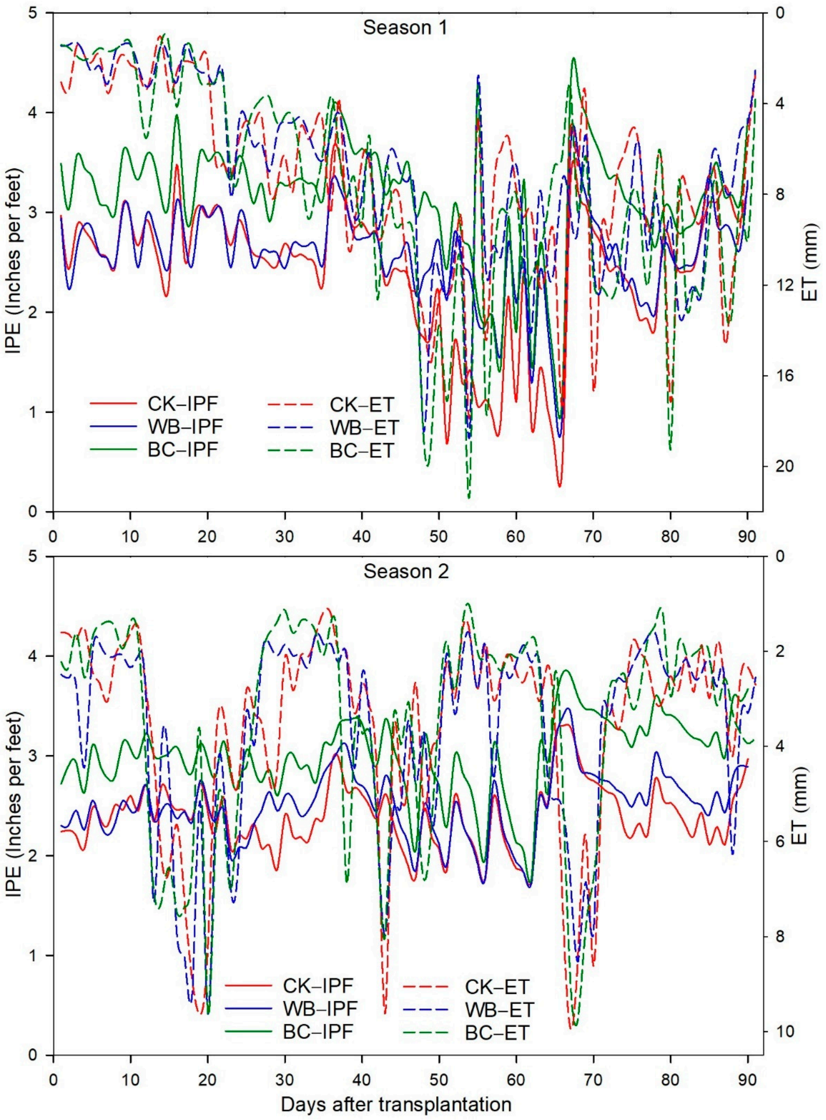

Figure 2. Mean values for IPF and ET for each day in two cropping seasons presenting peak ET in the first season in BC and lower in all treatments at different times in the second season, while IPF showed maximum values in the second season in BC and minimum in the first season in CK. In all treatments, maximum ET was observed in the first season whereas it was IPF in the second season.

3.2. ^{15}*N and Total N Uptake*

In the two cropping seasons, 10 plant extracts were taken and analyzed for total N and ^{15}N concentrations for the seasonal difference and total uptakes, as shown in Figure 3. Non-significant increases in N uptake were observed in BC and WB in both seasons as compared to CK. Overall total N uptakes in the two seasons in WB and BC were 34–37% and 44–52% more than CK, respectively. WB and BC consumed more total N as compared to CK by10–22% and 19–26% in the first season and 56–70% and 76–87% in the second season, respectively. BC consumed more ^{15}N than WB by 13–23% more in the first, while it was 50–137% in the second and 38% overall, but non-significant differences were calculated. Therefore, BC treatment in our study absorbed more ^{15}N and total N as compared to WB and CK (as given in Table 1), but the results were non-significant. In the first season, more total and ^{15}N were consumed by all treatments than in the second season because of the lower biomass production in the second season and the difference in fertilization (poultry manure application) in the second season. The ^{15}N was applied in the first season, but it was consumed in the second season, which presented the lasting effects of urea fertilization up to the second season, as shown in Figure 3.

As BC absorbed more ^{15}N in the second season than WB, the biochar amendment increased the lasting time and the quantity in our study.

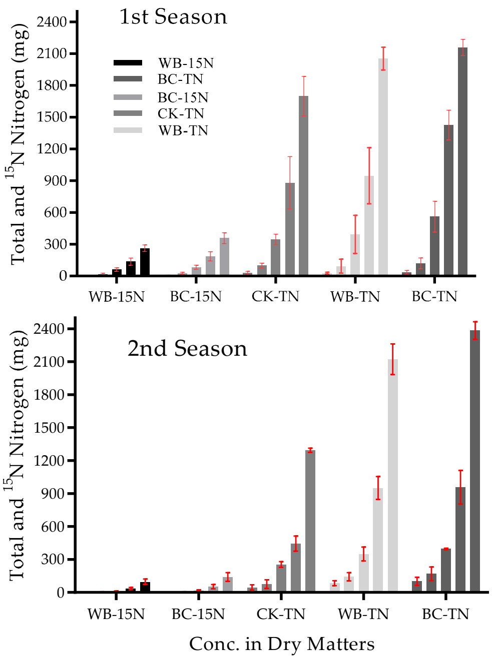

Figure 3. In each season, five dry matter samples were prepared, and accumulative values with error bars [standard mean of error (SEM)] presented for the BC-15N and the WB-15N (^{15}N in biochar and without biochar treatments), CK-TN, WB-TN, and BC-TN (total nitrogen in control, biochar, and without biochar treatments). BC accumulative total and trace nitrogen consumption was more than other treatments. ^{15}N uptake was not different in BC and WB up to the third dry matter (50 days after fertilization) but thereafter exceeded in BC and lasted up to the end of the second cropping season.

3.3. Total Leachate Volume, Total and ^{15}N Nitrate

A total of nine leachates were collected with different amounts of leachate volumes during two cropping seasons, as presented in Figure 4b. Total leachate volumes were 8.3% and 0.99% less accumulated in BC and WB than CK, but there were no significant variations among the treatments, as given in Table 1. Leachate volume collected from BC was approximately 7.63 (mean value) less than WB. Moreover, more leachate volume was collected in the first season as compared to the second season in all treatments.

Total N nitrate leached from the fertilizer treatments was higher by 2.16–2.95 times and 1.23–1.59 times CK from the WB and the BC in the first season, whereas it was higher by 3.13–4.04 and 1.45–1.69 times in the second season, respectively. WB treatment conduced significantly more total N nitrate leaching than BC in both seasons, as given in Table 1. The total N nitrate leached down from the first season in all treatments was higher than from the second season, which might have been due to the poultry manure in the second season in the fertilized treatments and no addition of N for the control while cropping the second time.

Total ^{15}N nitrate leached in BC was 26% and 69% less in the first and the second season as compared to WB, respectively. Moreover, overall, 35% less ^{15}N nitrate leached in BC. Total nitrate collected in leachates was 73% and 49.5% of the applied ^{15}N contributed in WB and BC, respectively. Thus, the nitrate leached from the applied ^{15}N urea and previous stock in BC was less than in WB.

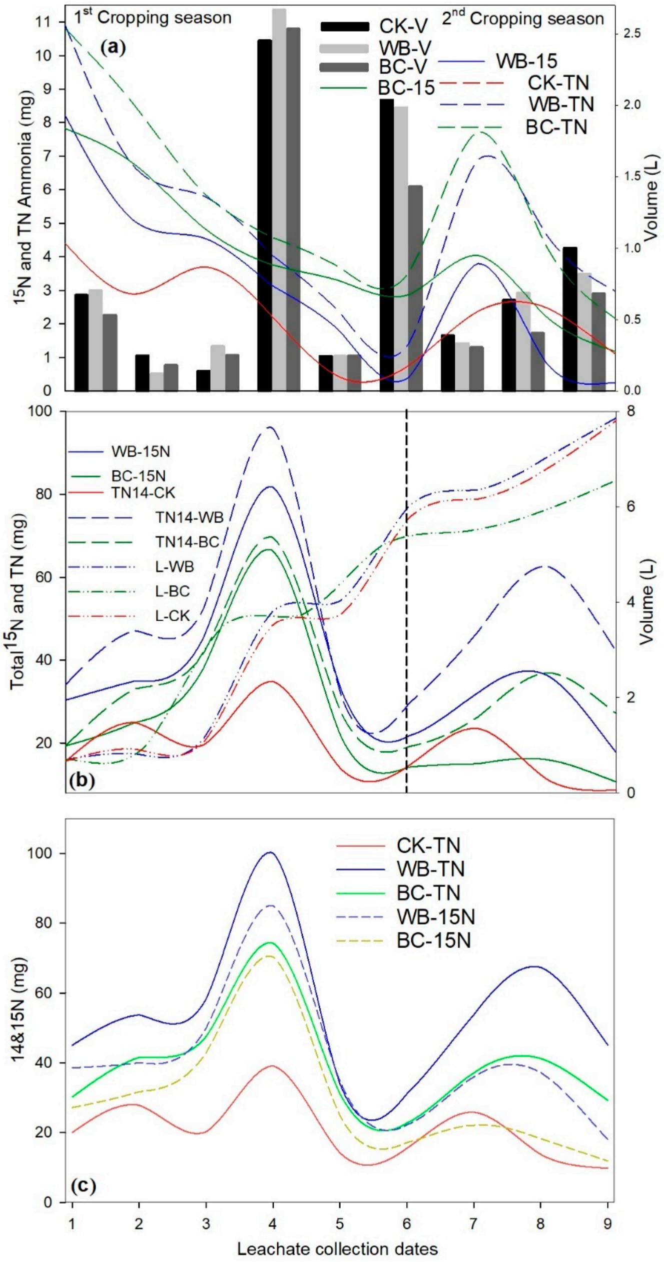

Figure 4. (**a**) Different ^{15}N (WB-15 and BC-15) and total N (CK-TN, WB-TN, and BC-TN) ammonia mean levels and leachate mean volumes (CK-V, WB-V, and BC-V) measured on each event as well as cumulated values in lysimeter study against the days after plantation as *x*-axis for the two cropping seasons of *Brassica Camprestis Var. Chinensis* in Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences Beijing China. Maximum ammonia levels (labeled and non-labeled) were collected in the first season for all treatments. Moreover, higher ammonia concentrations were collected in BC (biochar added) as compared to WB (without biochar added) and CK (control). (**b**). The mean value of total N (^{14}N +^{15}N) nitrate, ^{15}N nitrate, accumulative volume of leachate in without biochar (L-WB) with biochar (L-BC) and control (L-CK). The maximum total and the ^{15}N nitrate levels were analyzed in the fourth leachate (maximum volume in all treatments) collected after heavy rain (66 mm). WB presents the more nitrate leaching (total and tracer) as compared to BC and CK in both seasons. Moreover, total volumes collected in all treatments were recorded to be not significantly different. (**c**). Total mineral N leached in control (CK-TN), biochar added (BC-TN), and without biochar (WB-TN) as well as ^{15}N leached (WB-15N and WB-15N). Overall, mineral N was leached more in WB as compared to BC in all nine leachates.

3.4. Total and Tracer Ammonia in Leachate

Different amounts of leachate volumes collected at different events with different ammonium ion concentrations were observed, as shown in Figure 4a. Maximum total N ammonia was leached down in the BC as 2.68–2.93 times CK overall, 2.42–2.74 times CK in the first season, and 2.53–3.17 times CK in the second season, while in WB, it was 2.26–2.47 overall, 2.23–2.37 in the first season, and 2.04–2.72 times CK in the second season. Total ammonia leached down was 18% (accumulative mean value) more in BC as compared to WB. Similarly, ^{15}N ammonia was also 41% more in BC as compared to WB. In the first season, 26% more ^{15}N ammonia leached in BC than in WB, and it was 110% more in the second season. Moreover, more total and ^{15}N ammonia were leached in the first season as compared to the second season, as given in Table 1.

3.5. Water Use Efficiency, ^{15}N and Total N Uptake

Non-significant and lower water use efficiency was observed in all treatments for both cropping seasons, and accumulative values for BC were more than WB and CK. Moreover, nearly the same WUE accumulative values were observed in WB and CK. All treatments had lower WUE in the first cropping season than in the second, as presented in Figure 5. However, BC showed a 23–40% increase in accumulative WUE in two seasons as compared to CK. In two cropping seasons, it was analyzed that BC consumed 38.459% and WB consumed 27.714% of the applied ^{15}N urea in dry matters. Moreover, BC consumed 7.4% and 42% more total N as compared to WB and CK in two cropping seasons. In the second season, increased WUE favored the N uptake in the last observation as compared to the other observations for all the treatments. The maximum WUE (mean values) was calculated to be higher in BC as compared to other treatments, but it was not significant. For all treatments, WUE was higher in the second season than in the first season, as given in Table 1. Moreover, accumulative mean values of WUE for two seasons in BC were 12.24% higher than in WB but presented non-significant results.

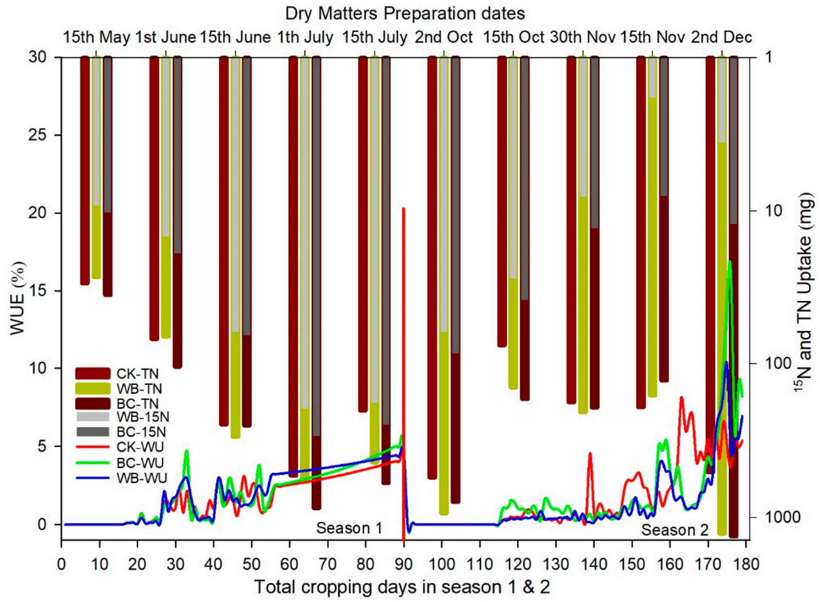

Figure 5. Mean water use efficiency (dry matter/ET) as WB-WU, BC-WU, and CK-WU, total N (^{15}N +^{14}N) uptake (CK-TN, WB-TN, and BC-TN) and labeled N (^{15}N) uptake (WB-^{15}N and BC-^{15}N) in our study conducted in IEDA, CAAS Beijing China for the plantation of *Brassica Campestris Var. Chinensis* in two consecutive croppings in weighing lysimeters for WB (without biochar plus ^{15}N-Urea), BC (biochar added plus ^{15}N-urea), and control (no biochar and urea). Highest total N was consumed in all treatments when WUE was higher at the end of the cropping season. BC presented higher total N, labeled N, WUE, and dry matter, as observed from different peaks at different times during the cropping seasons.

3.6. Plant Growth Curve

Plant growth rate was not constant and fluctuated with respect to the growth stage and the season. To monitor average growth rate per unit day, the whole mean fresh weight for treatment was plotted against time (days), as shown in Figure 6. A significant increase in growth rate (dry weight) was noticed in BC and WB as compared to CK. Highest mean growth rate for a single plant was recorded in BC and WB as 39% and 26.4% in the first season, whereas it was 61.1% and 39.3% more than CK in the second season, respectively. The mean maximum single plant fresh weight was 11.4% higher in BC than in WB for the two seasons. Similarly, BC produced more growth rate of a single plant than WB, but the increase was non-significant. The dry matter produced during the two seasons in BC was 6.89% greater than in WB. Similarly, the biomass per plant in BC was approximately 2.84% (mean value) increased from WB in two seasons. Moreover, the significant difference in the biomass per plant was produced in the fertilized treatments as compared to the control (given in Table 1).

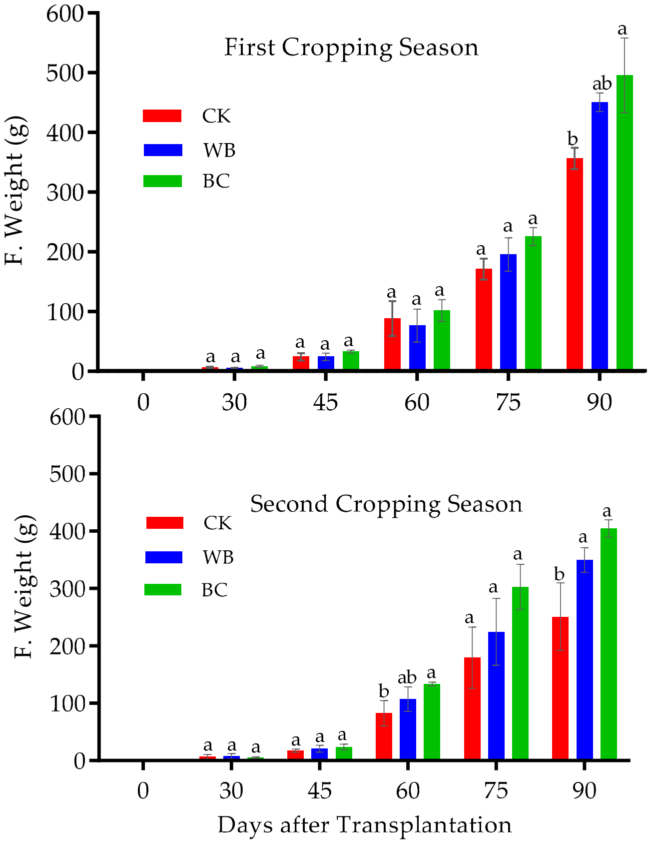

Figure 6. Presents the mean value with error bars (SEM) for the cumulative dry weight (DW) growth in grams (g) against days after transplanting in two cropping seasons while cropping *Brassica Camprestis Var. Chinensis* in lysimeter experiments for BC (biochar added and ^{15}N-urea), WB (without biochar added and ^{15}N-urea), and control (no added biochar as well urea). The small letters (a, b, c) present the level of significance among the treatments in a season according to the pair-wise Scheffe test.

3.7. Total and Labeled N in Leachate

Significant mineral nitrogen including tracer was leached down in WB and thereafter in BC as compared to CK in the second season, whereas a significant difference was found in the fertilized (WB and BC) and the control (CK) in the second season. All leachates somehow carried mineral nitrogen concentrations and contained more than 10 mg/l in almost all treatments, as shown in Figure 4c. Moreover, there was a significant difference in ^{15}N leached down in WB as compared to BC in the second season, but not in the first season. Total mineral N values leached down in WB and BC were

2.63–2.80 and 1.69–2.01 times CK, respectively. Total ^{15}N mineral leached down from the WB in two seasons was calculated to be 10.37% of the applied ^{15}N labeled fertilizer, whereas it was 7.60% for BC.

3.8. Soil Properties

The recorded values for the soil parameters, including pH, EC, and bulk density, were not statically varied after the first and the second harvest, except soil EC. Nonetheless, soil pH and bulk densities showed more variations in BC as compared to WB and CK, as presented in Table 2. Soil total nitrogen in CK was significantly decreased after the first and the second season, while it was not significantly varied in WB and BC. ^{15}N retention in BC soil was found to be twice greater than in WB after the first harvest and 1.85 times (mean value) greater after the second harvest.

Table 2. Mean values with standard deviation for the soil characteristics of CK (control), WB (without biochar), and BC (biochar added) before and after the first and the second harvest of the *Brassica Camprestis Var. Chinensis* plantation in lysimeters. The small letters (a, b, c) present the level of significance among the treatments according to the pair-wise Scheffe test.

Soil Properties	*Treatments*	*Before Plantation*	*After 1st Harvest*	*After 2nd Harvest*
pH	*CK*	7.95 ± 0.079a	8.07 ± 0.148a	8.09 ± 0.107a
	WB	7.99 ± 0.081a	8.03 ± 0.095a	8.03 ± 0.105a
	BC	7.96 ± 0.098a	8.01 ± 0.137a	8.01 ± 0.096a
EC (μS cm^{-1})	*CK*	87 ±11.35a	92 ± 23.06b	88 ± 26.90b
	WB	93 ± 12.67a	93 ± 21.79b	115 ± 27.89b
	BC	91 ± 8.01a	151 ± 15.27a	205 ± 50.7a
Bulk Density (g cm^{-3})	*CK*	142.56 ± 0.136a	142.5 ± 0.601a	142.80 ± 0.696a
	WB	142.95 ± 0.085a	142.8 ± 0.416a	142.75 ± 0.505a
	BC	142.94 ±0.198a	143.00 ± 0.776a	142.53 ± 0.275a
Total nitrogen (gN kg^{-1})	*CK*	1.081 ± 0.006a	1.071 ± 0.012b	1.062 ± 0.012b
	WB	1.079 ± 0.016a	1.082 ± 0.014a	1.075 ± 0.017a
	BC	1.074 ± 0.015a	1.085 ± 0.016a	1.079 ± 0.018a
^{15}N nitrogen (%)	*WB*	100	20.90 ± 3.00b	10.5 ± 2.9b
	BC	100	43.56 ± 15.5a	19.4 ± 3.03a

4. Discussion

Many researchers reiterated the fact that biochar amendment in soil increased the soil water holding property [17,36] to provide more moisture for evaporation, transpiration, and evapotranspiration in soil and plant systems. Biochar modified soil to be a spongy nature to store more water at field capacity and to withhold more moisture at wilting points [37]. Thereby, in this study, biochar added soil presented peak values for ET and IPF. *Brassica Camprestis Var. Chinensis* belongs to the *Brassica Camprestis* family, which is considered to consume the highest N concentration (approximately 1500 to 4000 mg N/kg^{-1}) in nitrate form substantially [38]. In this study, total and tracer N in BC were consumed more than in WB in all treatments. Many studies revealed that biochar addition enhanced the N uptake, as Huang et al. [39] found a 23–27% increase in N fertilizer uptake in rice plants, and similar results were also reported by Awad et al. [40]. Nonetheless, few reports also elaborated that biochar had no effect on the overall N uptake; for example, Jones et al. [41] performed a three-year field trial to examine the agronomic effects of biochar including N uptake but found no increase in crop uptake. Huang et al. [42] found no significant increase in N uptake during a four-year experiment. Few researchers added the negative N uptake with biochar addition [43–45]. These contrasting results evaluate the complex nature of biochar and its interaction with a soil medium. The effect of poultry manure biochar on soil pH, EC, CEC, organic carbon, and nutrient availability depend upon the chemical composition of poultry manure and the temperature conditions in the pyrolysis process; for example, biochar composed above 400 °C has a better capacity to supply a quick release of nutrients as compared to biochar prepared at lower temperatures [46]. However, we found total N contents of

2.3–8.06% in the dry matters of the whole plant body at different growth stages with no significant difference in any treatment except lower N content in CK.

The biochar has a porous structure and a higher surface area, which can improve the soil ability to absorb and retain more water. Hence, the potential of biochar in reducing the volume of the solution percolated through the soil must be pursued [36,47,48], but conflicting results have been found [49,50]. In this study, we found inconsistent results showing that cumulative mean volume collected in leachates from BC was lower than other treatments with a non-significant difference.

The *Brassica Camprestis* family of vegetables consumes a higher level of nitrogen in nitrate form ranging from 1500 to 4000 mg N/kg [38]. Every soil has more or less nitrate concentration, which is the product of the soil mineralization process of organic matter and applied N fertilizers. Soil mineralization depends upon water content [51], temperature [52], organic matter [53], soil C:N ratio [54], and aerobicity [55]. Therefore, the urea mineralization in soil and the conversion into nitrate forms may take different amounts of time, which might be the reason for maximum nitrate and N levels in the fourth leachate. The second reason may have been the heavy rainfall event, which caused the generation of the maximum leachate volume from all the treatments. As demonstrated in many studies [56,57], maximum nitrate leaching occurred after the heavy rainfall events, particularly in the summer season. In our study, biochar addition decreased the nitrate and the total mineral N leaching but not the ammonium ion levels, as accumulative values were found to be a little bit more than others. The biochar addition can modify the soil characteristics (physical, chemical, and biological) and decrease nitrate leaching [described by other studies [58–60], whereas few researchers found contradictory results [61,62]. Nonetheless, biochar added soil can flush higher nitrate up to 90% of the total mineral N [58] and higher concentrated ammonium ion solution because of its absorption and desorption effects [63,64], which were also described in previous studies as they were observed in our experiment.

The peak water use efficiency was developed in the BC with overall non-significant differences in other treatments in our study. Uzoma et al. [65] found that three biochar treatments increased water use efficiency by about six, 91, and 139% as compared to the control in sandy soil, which supports our findings. During the summer season, frequent and more water had to be applied to maintain optimum moisture content in all treatments, which caused an increase in evaporation from soil and ET. Therefore, it might be inferred that there was lower WUE in the first cropping season.

Growth rates in WB and BC were higher as compared to CK, presented by the difference of N fertilizer addition on crop growth and yield. The single plant growth curve in BC presented more elevation as compared to WB, and maximum plant growth was observed in the summer season in our study. Different studies found similar results; Awad et al. [40] in a greenhouse study reported that the significant growth in shoot, leaves, and number of leaves was observed in biochar added soil. Jones et al. [41] noticed that biochar amendment increased the foliar mass in a three-year field study.

It was observed that tracer ^{15}N was present in almost all leachates collected from treated lysimeters, and the levels decreased after fertilizer application up to the first harvest and thereafter increased again. This fluctuation in N levels in leachate was surprising and somewhat conflicting but was observed in almost all treatments. Johnson et al. [66] recorded soil mineral decline from 200 ppm at day 20 after fertilization to 10 ppm within 161 days in a lab experiment. Similarly, Ferchaud et al. [67] analyzed that ^{15}N urea applied in one season was retained in the soil and was utilized in the next cropping season. After the first experiment, soil columns were placed in an open environment under the hot summer conditions for about 45 days. Many researchers [68,69] reported that a dry summer can accumulate nitrate in the surface soil, and subsequent rainfall might create peak nitrate leaching from the root zone. Therefore, peak N leaching developed in two cropping seasons might be due to these aforementioned reasons.

5. Conclusions

Biochar composed of poultry and straw at 650 °C pyrolysis has the ability to provide maximum mineral N to plant uptake, reduce nitrate leaching, and promote plant growth rate. It can exceed the residence time of nitrogen in soil medium of depths of 30 cm, which can be utilized in upcoming croppings. Biochar added to soil presented significantly higher water availability [IPF] in two cropping seasons and showed peak ET levels without significant differences. Hence, biochar addition is helpful not only to control the N leaching but also to provide optimum water availability in the cases of wet and dry conditions. In this study, urea volatilization and N_2O emission were not determined, thus further investigation is suggested in this regard.

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