



# Article Interaction of Biochar with Chemical, Green and Biological Nitrogen Fertilizers on Nitrogen Use Efficiency Indices

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Abstract: Chemical nitrogen (N) fertilizers are regarded as one of the environmental contaminants in addition to the necessity for fossil sources for their production. Conversely, it is impossible to neglect the supply of nitrogen needed as one of the essential ingredients for plant function. For organic agriculture, it is crucial to use alternative fertilizer management to reduce the harmful impacts and production costs of chemical fertilizers. In a one-year pot experiment, nitrate ( $NO_3$ ) leaching and nitrogen efficiency of wheat were examined in relation to biochar (B) mixed with urea (U), legume residues (L), and azocompost (A), which represent chemical, green, and biological sources of Nfertilizers, respectively. Control (no biochar, no fertilizer), U (46 kg ha<sup>-1</sup>), A (5 t ha<sup>-1</sup>), L (5 t ha<sup>-1</sup>), B (10 t  $ha^{-1}$ ), UB, AB, and LB were the experimental treatments. Grain yield of wheat was enhanced by 337% and 312% with UB and UL, respectively. The LB produced the highest grain N yield, with a rise of 8.8 times over the control. L had the highest N-use efficiency, with an increase of 149% over the control. The highest N-harvest index and N-recovery efficiency were obtained by using LB, with values of 91 and 70 %, respectively. Nitrate leaching occurred in the following order: U > Control  $\geq$  A  $\geq$  L > UB > AB  $\geq$  LB > B. Nitrogen is retained for the plant in the extensive specific surface of biochar when N-fertilizers are used in conjunction with them. This not only improves N-efficiency but also minimizes nitrogen loss through leaching. Additionally, the soil can benefit from the addition of leguminous organic fertilizer in a similar way as to urea fertilizer in terms of increasing wheat grain yield, particularly when combined with biochar.

Keywords: sustainable agriculture; grain yield; residual management; bread wheat; nitrogen fertilization

# 1. Introduction

Cereals, particularly wheat, are among the most important crops, and cereal grains play a significant role in the provision of crucial minerals, carbohydrates, and proteins throughout the world [1]. The quantitative and qualitative yield of wheat crops is decreased by a lack of soil organic matter, complete burning of straw residues, multiple crops in a single crop year, and lack of professional chemical fertilizer use [2]. One of the requirements of an appropriate agricultural operations plan is the optimal and balanced management of nutrients. In order to produce the product, the plant uses nutrients such as nitrogen (N), phosphorus (P), and potassium (K) along with energy from sunshine, water, and carbon dioxide. The most typical and pervasive nutrient limitation in cereals, notably in wheat, is nitrogen shortage in soil [3]. Each ton of wheat grain and straw results in the extraction of 20.4 and 7.2 kg of N from the soil, respectively [4]. As a result, the most common and practical method of increasing the N needed by wheat is the use of chemical



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). N-fertilizers such as urea and ammonium sulfate [5]. The disadvantages of using chemical N-fertilizers, such as the loss of nitrogen through leaching, groundwater pollution, the cost of fertilizer production, and the usage of fossil sources in the process, however, are always disregarded [6]. Proper crop residue management has been regarded in recent years as one of the best ways for sustainable agriculture and for a consistent supply of nutrients [7].

The use of legume residues as an organic fertilizer can, due to its abundance of nitrogen, be thought of as an alternative to N-containing chemical fertilizers [8]. The use of hairy vetch (*Vicia villosa* L.) as green manure mainly boosted soil nitrogen and improved the quantity of protein in the following wheat, according to a study on the impact of 13 tons per hectare of a vetch and oat mixture on the amount of absorbable nitrogen [9]. Addition of 15 tons per hectare of rye green manure to the soil decreased soil acidity, increased dissolved organic carbon, and increased the amount of P that can be absorbed by the soil [10]. Applying organic fertilizer (straw and green manure) to a soil boosted the rate of soil mass respiration, as well as nutrient absorption [11]. It has enhanced the minerals in barley and significantly impacted both microbial and enzymatic activity [8]. The proper crop residue management by the application of N-fertilizer or by the plowing of the legume increases productivity in the near term and improves nitrogen efficiency in the farming system [12]. The combined application of rice straw and legume was taken into consideration to boost soil nitrogen and increase yield of rice [13]. The rise of rhizobial and phosphate-soluble bacterial activity was attributed to legume cover crops [9].

An excellent substitute for chemical N-fertilizers is azocompost, which is another source of organic fertilizers high in nitrogen [14]. Anabaena (Anabaena) is a genus of filamentous cyanobacteria, and they are known for nitrogen-fixing abilities, and they form symbiotic relationships with certain plants, such as azolla (Azolla pinnata), which is a small floating fern. Azolla is typically inoculated and produced as a cover crop for rice farming, where it is added as a top-dressing to the soil. Maintaining the azolla inocula between cropping seasons is a major constraint to its wider adoption by rice farmers. The problem of maintaining azolla vegetatively would be solved if mass quantities of spores could be acquired [15]. Anabaena-azolla association can be used as an effective substitute for chemical fertilizers because of its capacity to absorb and stabilize atmospheric nitrogen [14]. The green-blue algae anabaena-azollae is combined with organic materials, such as rice and wheat straw, in a warm atmosphere with adequate ventilation to support the growth of microorganisms to produce azocompost [15]. Azocompost is utilized as a biological fertilizer because of the amount of green matter it generates, nitrogen that can be absorbed from the atmosphere and added to the soil, and its potential suitability for the environment. They are thought to be super beneficial. Adding azocompost boosted rice output to the point that using 5 to 10 tons of azocompost per hectare is similar to using 30 to 60 kg of nitrogen per hectare [15]. As a result, using azocompost as green manure can significantly increase the soil's nitrogen content in addition to enhancing soil qualities.

Biochar, a stable carbon substance created by the pyrolysis process, has drawn special attention in a number of publications on plant nutrition because of its potential as an agricultural soil stabilizer [16–18]. Managing agricultural residues, enhancing the physicochemical characteristics of soil, lowering air, soil, and groundwater pollution, and enhancing plant growth are all made possible by the inherent properties of biochar, such as its high specific surface area, high porosity, and accessibility to nutrients, which make it an invaluable and multifaceted soil modifier [16,19,20]. However, the use of biochar in soil is influenced by soil characteristics and fertilizer management [21]. The use of biochar with organic fertilizer could mainly boost maize, peanut, and cowpea yields in a setting with depleted soil [22]. In order to increase maize production, the use of biochar on maize needs to be paired with the required amount of N, P, and K fertilizers as well as plant nutrition systems [18,23].

Studies on the nitrogen effectiveness of N-fertilizers both with and without application of biochar or with various doses of biochar were performed [22,24,25]. Nonetheless, according to the best of our knowledge, no studies have contrasted the impact of applying biochar and N-fertilizers from various sources, separately and together, on nitrate leaching and wheat's nitrogen use efficiency. Therefore, the main objectives of this study were the influences of (a) biochar, (b) different N-fertilizer source (urea, legume residues, and azocompost respectively as chemical, green, and biological N-source) and (c) mix of biochar and N-fertilizers on nitrogen use efficiency of wheat (*Triticum aestivum* L.) cultivar Arta and nitrate leaching.

# 2. Materials and Methods

### 2.1. Experimental Procedure

During the spring and summer of 2020, a pot experiment was carried out in a greenhouse at the Agricultural Technology and Natural Resources Development Center in Rasht, Iran (37°11′02.5″ N 49°39′36.6″ E). Average daily and night temperatures during the experiment were 24.3 and 13.1 °C, respectively. The mean value of relative humidity was 64%. Three replicates of the treatments were established in a completely randomized design. Bread wheat (Triticum aestivum L.) cultivar Arta was the focus of the investigation. Unbroken grains of the same size and color were washed in distilled water, disinfected for about 15 min in a solution of 10% sodium hypochlorite, and then dried by air. The grains were then planted in plastic pots that were 35 cm in height by 30 cm wide and held 6 kg of soil. The soil was classified as a *Ultisol* in soil taxonomy and had a loamy texture. At a depth of 30 mm, ten grains were sown in each pot. The following four materials were used based on optimal recommended amount in literature [2,6,9,12,22] to evaluate the treatments: urea (46% N) at a dose of 480 kg ha<sup>-1</sup> (equal to 1.15 g pot<sup>-1</sup>), legume residues (4.5% N) at a dose of 5 t ha<sup>-1</sup> (equal to 12.1 g pot<sup>-1</sup>), azcompost (5.25% N) at a dose of 4 t ha<sup>-1</sup> (equal to 9.66 g pot<sup>-1</sup>), and biochar (1.68% N) at a dose of 10 t ha<sup>-1</sup> (equal to 24.2 g pot<sup>-1</sup>). Therefore, the used treatments were as follows: Control (no fertilizer, no biochar), U (urea), L (legume residues), A (azocompost, B (biochar), UB (urea + biochar), LB (legume residues + biochar), and AB (azocompost + biochar). Three days before planting, the treatments were added to the soil. As a base fertilizer for wheat, P and K were applied at rates of 39.6 kg P ha<sup>-1</sup> (equal to 0.126 g P pot<sup>-1</sup>, in the form of calcium superphosphate) and at 99.6 kg K ha<sup>-1</sup> (equal to 0.313 g K pot $^{-1}$ , in the form of potassium chloride), respectively.

Biochar was produced from wheat straw using slow pyrolysis at laboratory scale. The desired peak pyrolysis temperature was 450 °C with a heating rate of 5 °C min<sup>-1</sup> and residence time of 2 h, which was then allowed to cool down naturally. Azocompost, manufactured from a combination of *Anabaena azollae* algae and wheat straw, was obtained from the Agricultural Jihad Organization of Iran. Additionally, after being crushed, a mixture of hairy vetch (*Vicia villosa* L.) residues, a typical legume feed for animals, were applied as nitrogen green N-fertilizer.

Soil sample was collected from 20 cm depth, and some properties were analyzed before the experiment by the following methods: soil pH and electrical conductivity (EC) in a 1:1 (*w:v*) soil to water ratio; organic carbon (OC) by wet oxidation [26]; total nitrogen (N) by Kjeldahl [27]; and CEC by ammonium acetate extraction [18]. Available K was analyzed using a 5:50 ratio of soil:ammonium acetate (NH<sub>4</sub>OA<sub>c</sub>)-buffered solution at pH 7, in which the basic cations adsorbed in soil were replaced by NH<sub>4</sub><sup>+</sup> ions [28] and measured by spectroscope (ICP-OES, PerkinElmer). Available P was determined by Olsen method using spectrophotometry [10]. Table 1 lists the properties of the materials and soil that were utilized in the experiment.

The functional groups in the biochar used in this study were analyzed using the Fourier transform infrared (FTIR) spectrophotometer in the mid-infrared region, from 4000 to 400 cm<sup>-1</sup>. The results of the FTIR spectra are shown in Figure 1. Overall, the spectra of the biochar display a large peak at 3430 cm<sup>-1</sup>. This peak is related to O–H stretching vibrations of the surface hydroxyl groups. The peaks at 1618–1629 cm<sup>-1</sup> could represent the ketonic C=O group. The peak at 1564 cm<sup>-1</sup>, attributed to the C–O+ stretching vibrations of the oxonium groups, contributes to the protonation on the surface of biochar. The peak at 1400 cm<sup>-1</sup> could be ascribed to aromatic C–H symmetric stretching vibrations, while the peak at 700–800 cm<sup>-1</sup> could be ascribed to aromatic C–H out-of-plane bending vibrations.

	pН	EC (ds cm <sup>-1</sup> )	CEC (cmolc kg <sup>-1</sup> )	Organic Carbon (%)	Available P (mg kg <sup>-1</sup> )	Available K (mg kg <sup>-1</sup> )	Total N (%)	C/N Ratio	SSA (m <sup>2</sup> g <sup>-1</sup> )	Ash (%)
Soil Azocompost	5.11 6.23	0.16 0.32	23.2	2.61 11.8	2.15 25.6	3.23 184.1	1.17 5.25	- 1.24	-	-
Legume residues	6.81	0.51	-	17.6	178.4	242.4	4.50	3.91	-	-
Biochar	9.37	1.21	165.1	42.6	9.02	25.53	1.68	25.3	129	17.5

Table 1. Chemical characteristics of soil and applied materials.

EC: electric conductivity; CEC: cation exchange capacity; P: phosphorus; K: potassium; N: nitrogen; SSA: specific surface area.



Figure 1. Fourier transform infrared (FTIR) spectra of wheat straw biochar.

## 2.2. Sampling and Measurements

The experiment was run between 10 April and 26 September 2020. During the growth season, weeds were physically removed, and no pests or illnesses were observed. Wheat was harvested when it reached to the ripening stage at physiological maturity (i.e., Z93 scale based on Zadoks growth scale [29]). The SPAD 502 chlorophyll meter (Konica Minolta Inc., Tokyo, Japan) was used to measure the crop nitrogen status from flag leaves. The SPAD measurement was conducted at jointing and filling stages (i.e., Z18 and Z92 scales respectively, based on Zadoks growth scale [29]). In order to measure the nitrogen concentration and yield for straw and grain, the plants were cut from the soil surface at harvest time. After harvesting, the samples from each treatment were dried individually for 48 h at 75 °C to measure dry weight. For the determination of the plant nitrogen concentration, one gram per sample was digested with sulfuric acid, salicylic acid, and oxygenated water. The digests were measured using the Kjeldahl method [27]. The formulas listed in Table 2 were used to calculate nitrogen efficiency indices. Each week during the trial (23 times in total), deionized water was used in irrigation to keep the soil moisture to field capacity. The irrigation was continued until flowering stage (i.e., Z75 based on Zadoks growth scale [29]). After each irrigation, the pots' drain water was collected. The leachate samples were filtered through disposable 0.45 mm pore-size filters (Whatman, Clifton NJ, USA) for analyzing pH, EC and nitrate. The pH and EC of leachate (1:5 v/v) were measured in deionized water using a pH meter (Mettler Toledo Delta 320) and an electrical conductivity meter (DDS-307A), respectively. The nitrate concentration of the leachate was measured using spectrophotometry.

Nitrogen Index	Formula	Reference
Nitrogen use efficiency (g $g^{-1}$ )	$NUE = rac{Grain \ yield}{N_{supply}}$	[30]
Nitrogen uptake efficiency (g $g^{-1}$ )	$NUpE = rac{N_{shoot}}{N_{supply}}$	[30]
Nitrogen utilization efficiency (g $\mathrm{g}^{-1}$ )	$NUtE = rac{Grain \ yield}{N_{shoot}}$	[31]
Nitrogen physiological efficiency (g $g^{-1}$ )	$\begin{array}{l} NPE = \\ \frac{Grain \ yield \ at \ N_x - Grain \ yield \ at \ N_0}{N_{shoot} \ at \ N_x - N_{shoot} \ at \ N_0} \end{array}$	[31]
Nitrogen agronomic efficiency (g $g^{-1}$ )	$\begin{array}{c} NAE = \\ \frac{Grain \; yield \; at \; N_x - Grain \; yield \; at \; N_0}{N_x} \end{array}$	[30]
Nitrogen harvest index (%)	$NHI = rac{N_{grain}}{N_{shoot}}  imes 100$	[32]
Nitrogen recovery efficiency (%)	$\frac{NRE}{\frac{N_{shoot} \ at \ N_x - N_{shoot} \ at \ N_0}{N_{supply}} \times 100$	[32]
Soil nitrogen dependent rate (%)	$SNDR = rac{N_{shoot} \ at \ N_0}{N_{shoot} \ at \ N_x}  imes 100$	[31]

Table 2. Used formulas for nitrogen indices calculations.

 $N_{supply}$ : nitrogen content in the soil + nitrogen content in the fertilizer;  $N_{shoot}$ : total nitrogen yield in the aboveground harvested plant;  $N_{grain}$ : nitrogen yield in the grain;  $N_x$ : N fertilized treatment x;  $N_0$ : control without nitrogen fertilization.

#### 2.3. Data Analysis

The statistical analysis of the effects fertilizers on nitrogen use efficiency were performed in a completely randomized design with three replicates. The triplicate data of nitrate leaching and plant growth characteristics were subjected to analysis using the two-way ANOVA test, performed on all data using the IBM SPSS Statistics (Version 24), statistical analysis program. Treatment means were separated using the least significant difference test. The least significant difference (LSD) test was used to compare mean differences that were statistically significant at p < 0.05. Excel 2020 was used to draw all the figures.

#### 3. Results

The concentrations of leaf chlorophyll can be reflected in SPAD values. Figure 2 displays the SPAD values of the flag leaves. When wheat was in the jointing stage, the SPAD values were determined. N-fertilizers considerably (p < 0.05) boosted these values. The SPAD values in each of the three types of N-fertilizers were significantly increased by the addition of biochar. Similar results in flag leaf SPAD values were seen while wheat was in the grain filling stage.

Adding U, A and L together with B increased N supply compared to the control by 78–81%. The combination of U, A and L together with B significantly increased the N supply compared to U, A and L applications alone, with no differences between U, A and L either when applied alone or together with B.

Grain and shoot yield and N yields were significantly different between treatments (p < 0.05) (Table 3). All treatments increased the grain yield considerably compared to the control. The UB and LB treatments had the highest grain yield of all the treatments, with 68.37 and 64.40 g pot<sup>-1</sup>, respectively, and no significant difference between them. The L treatment produced the maximum grain yield when compared to N-treatments without biochar. Combined fertilizers and biochar induced excellent product in overall shoot yield, and the highest shoot yield was associated with UB with a 142% increase over control. Additionally, there was no significant difference between treatments using untreated biochar.



**Figure 2.** Soil and plant analyzer development (SPAD) values of flag leaves determined during the (**a**) jointing stage and (**b**) grain filling stage of wheat plants in soil treated with different N-fertilizers and biochar. Significant differences of means are shown by different lowercase letters.

	Table 3. Nitrogen supp	y, yields and nitroge	n yields as affected b	y treatments and N s	supply
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Traatmanta	N Supply (g pot <sup>-1</sup> )		Yield (g	; pot <sup>-1</sup> )	N Yield (g pot <sup>-1</sup> )		
ileatilients -	Fertilizer	Supply <sup>1</sup>	Grain	Shoot	Grain	Shoot	
Control	-	1.17 c	15.63 e	58.90 e	0.16 e	0.21 e	
U	0.53 b	1.70 b	54.07 c	120.4 c	0.87 c	1.07 c	
А	0.51 b	1.68 b	52.23 c	114.9 c	0.78 c	1.02 c	
L	0.54 b	1.71 b	58.27 b	114.7 c	0.99 c	1.27 c	
В	0.41 b	1.58 b	35.83 d	79.50 d	0.47 d	0.61 d	
UB	0.94 a	2.11 a	68.37 a	141.9 a	1.37 ab	1.57 a	
AB	0.91 a	2.08 a	56.13 b	119.1 c	1.23 b	1.45 b	
LB	0.95 a	2.12 a	64.40 ab	131.6 b	1.55 a	1.70 a	

<sup>1</sup> Supply: nitrogen content in the soil + nitrogen content in the fertilizer. Control: no fertilizer, no biochar; U: urea; L: legume residues; A: azocompost; B: biochar, UB: urea + biochar; LB: legume residues + biochar; AB: azocompost + biochar. Significant differences between treatments are shown in lower case letters (p < 0.05).

The nitrogen yield of grain and shoot increased significantly as a result of all treatments compared to the control, and LB performed the best among treatments with grain and shoot nitrogen concentrations of 1.55 and 1.70 g pot<sup>-1</sup>, respectively. Furthermore, there was a significant difference in the amounts of both grain and shoot N yields between fertilizers that were treated with biochar (UB, AB, and LB) and those that were untreated with biochar (U, A, and L), with higher values for UB, AB, and LB.

The nitrogen indices impacted by various fertilizer management had changed significantly (p < 0.05) (Table 4).

The NUE in the L treatment was 34.01 g grain  $g^{-1} N_{supply}$  higher than in the other treatments except for UB (NUE: 32.44 g grain  $g^{-1} N_{supply}$ ). The NUE between U and its corresponding treatment (UB) did not significantly differ, although the NUE of A and L were significantly higher than their corresponding treatments, AB and LB, respectively.

The NUpE was highest in the treatments with fertilizers and biochar as well as L. The NUpE with LB was significantly higher than other treatments with 0.8 g  $N_{shoot}$  g<sup>-1</sup>  $N_{supply}$ . While there was no significant difference between fertilizers treated with biochar, L showed a significantly higher NUpE than U and A.

Treatments	NUE	NUpE	NUtE	NPE	NAE	NHI	NRE	SNDR
			(g g <sup>-1</sup> )				(%)	
Control	13.65 d	0.18 d	76.00 a	-	-	76.19 d	-	-
U	31.78 b	0.63 b	50.63 c	44.41 b	71.70 b	81.31 c	50.42 d	19.67 b
А	31.15 b	0.61 b	51.33 c	44.92 b	71.53 b	76.47 cd	48.15 d	20.65 b
L	34.01 a	0.74 a	45.88 d	39.91 c	77.83 a	77.95 cd	61.85 b	16.54 c
В	22.74 c	0.39 c	58.46 b	49.32 a	48.93 c	77.04 d	25.56 e	34.28 a
UB	32.44 ab	0.75 a	43.50 d	38.49 c	55.90 c	87.26 b	64.61 b	13.37 d
AB	26.95 c	0.70 a	38.64 e	32.32 d	43.99 d	84.82 b	59.66 c	14.46 d
LB	30.38 b	0.80 a	37.92 e	32.54 d	51.01 c	91.17 a	70.22 a	12.37 d
LB	26.95 c 30.38 b	0.70 a 0.80 a	зв.64 е 37.92 е	32.32 d 32.54 d	43.99 d 51.01 c	84.82 b 91.17 a	59.66 C 70.22 a	14.46 d 12.37 d

Table 4. Changes in nitrogen indices in different treatments.

NUE: nitrogen use efficiency; NUpE: nitrogen uptake efficiency; NUtE: nitrogen utilization efficiency; NPE: nitrogen physiological efficiency; NAE: nitrogen agronomic efficiency; NHI: nitrogen harvest index; NRE: nitrogen recovery efficiency; SNDR: soil nitrogen dependent rate; Control: no fertilizer, no biochar; U: urea; L: legume residues; A: azocompost; B: biochar, UB: urea + biochar; LB: legume residues + biochar; AB: azocompost + biochar. Significant differences between treatments are shown in lower case letters (p < 0.05).

The NUtE in the control was 76 g grain  $g^{-1}$  N<sub>shoot</sub> higher than all treatments. The lowest NUtE had AB and LB. Other treatments showed intermediate values.

The NPE in B treatment showed the highest value with 49.32 g grain  $g^{-1}$  N<sub>shoot</sub>. Additionally, fertilizers treated with biochar, such as UB, AB, and LB, had considerably lower NPE values than their corresponding treatments without biochar.

The NAE of L treatment was higher than others with 77.83 g grain yield  $g^{-1} N_x$ . Moreover, there was a significant difference in the corresponding treatments, with higher values in treatments without biochar.

The lowest NHI value was in the control, and all fertilization treatments had a positive effect. The highest NHI was obtained by LB with 91%. Furthermore, the percentage of NHI for treatments that contain biochar was significantly higher than treatments that did not contain biochar.

The NRE of LB treatment was highest with 70.22 %. In addition, fertilizers treated with biochar had higher values than those without biochar.

The SNDR was significantly higher in B with value of 34.28% higher than other treatments. Treatments containing biochar had a lower SNDR than their corresponding treatments. The SNDR was compared to the control higher in B, similar in A but lower in all other treatments.

The significant changes of pH and EC in the leachates from soil under various treatments at the end of the experiment are shown in Figure 3 (p < 0.05). In treatments with biochar, the leachate pH was higher. At the end of the experiment, leachate pH increased by 3.6 units on average in the biochar treatments compared to the control. Similar to leachate pH, leachate EC increased by adding biochar. Leachate EC increased by 160%, 88%, and 133%, respectively, in the biochar-contained treatments UB, AB, and LB compared to untreated biochar treatments U, A, and L.

The cumulative nitrate leaching significantly differed between the treatments as shown in Figure 4 (p < 0.05). The U treatment produced the most nitrate leaching, 33% more than the control. There was no significant difference between treatments A and L and the control, while treatment B resulted in a significant decline in nitrate leaching that was lower than all treatments. In addition, UB, AB, and LB showed intermediate nitrate values between treatments.



**Figure 3.** Influence of treatments on pH (**a**) and electrical conductivity (EC) (**b**) of leachates from the soil at the end of the experiment. Significant differences of means are shown by different lowercase letters.



**Figure 4.** Changes in nitrate leaching status with applying different N-source treatments. Significant differences of means are shown by different lowercase letters.

### 4. Discussion

As the original soil contained far less total nitrogen, it is clear that treatments receiving additions produced significantly higher grain and shoot yields and nitrogen yields. As a result, every addition served as a potential source of nitrogen for plant growth. Furthermore, the ability to increase the yields and N yields was convincingly demonstrated when using organic N-fertilizers as much as urea. Legume residues in this study had much more P and K than other added materials [9]. Organic residues can enhance the amount of P and K that is available to plants by decreasing its surface absorption and can boost soil microbial biomass that improves the soil's ability to transfer nutrients [10]. The proportion of soluble  $Mn^{2+}$  in soil has increased when legume straw was added as a type of green manure during crop rotation [33]. Additionally, the presence of legume residues in the soil accelerates the microbial breakdown of organic molecules, creates reduction conditions, and supplies the electrons needed to convert certain nutrients into absorbable forms [34]. Zinc is complexed by organic acids produced during the decomposition of legume residues, which helps the plant absorb zinc [35]. Furthermore, rotating nitrogen-fixing and leguminous plants enhances soil nitrogen by absorbing elements from the soil's lower layers and reintroducing them to the production cycle [33]. Furthermore, the addition of straw and the use of legumes as organic fertilizer both increase the activity of rhizobium and vesiculararbuscular mycorrhiza in the root environment and the rate at which nutrients are absorbed

from the soil [9]. This is why the addition of legume residues was not significantly different from urea treatment in terms of grain and shoot N yields.

Adding biochar to three different N-fertilizers enhanced grain yield and increased grain nitrogen yield. It is possible to describe the causes of this effectiveness using a few mechanisms. At first, biochar has a high specific surface area because of its porous structure, which considerably promotes the development of nitrogen-fixing bacteria in the soil [36]. The study's biochar had a comparatively large specific surface area. Biochar may form biogeochemical interfaces (BGIs) because of its high porosity and variety of functional groups [37]. In order to enable the development of extremely varied bacterial communities, the compositional variability of BGIs may diversify the specialized microhabitats. Additionally, as biochar has a high cation exchange capacity and many functional groups with a negative charge on its surface, there is a higher chance that plants will be able to absorb nitrogen in the form of ammonium, which has a significantly positive impact on plant development [17,19]. The abundance of negative functional groups on the surface of biochar is clearly supported by the FTIR spectra, particularly the wavelength of 3420 cm<sup>-1</sup>, which corresponds to hydroxyl groups.

Providing nitrogen as urea had compared to legume residues a lower impact on the grain yield, causing a smaller influence on NUE, NUPE and NRE as already reported [38,39]. Our findings showed that legume treatments (L and LB) increased some nitrogen utilization efficiency parameters compared to treatments using urea (U and UB). Numerous studies revealed that adding urea fertilizer with a high nitrogen concentration does not ensure that the plant will consume the nitrogen and that a large segment of that nitrogen is lost to leaching or immobilization in the soil [40–42]. Urea fertilization tends to lower the NHI, which further reduces N-efficiency parameters such as NUPE [32].

Fertilization treatments had an impact on the N efficiency indices focused on N uptake and N recovery because the effects on biomass yields and N yields differed between fertilization treatments. Denitrification is one of the efficient ways to lower nitrogen uptake efficiency (NUpE), which is what leads to the development of non-aerobic conditions as a result of soil compaction [43]. The absorption efficiency declines as N-fertilizer usage rises [31]. Furthermore, optimum N-fertilizer management is an approach for boosting nitrogen efficiency [12]. The grain-to-biomass ratio, which ultimately depends on genetic availability and nutrient intake, particularly nitrogen, has also been reported to be a factor in NHI [44]. Up to a certain rate, more nitrogen leads to more aboveground biomass and a higher N uptake, but the N transfer to the grain might not be as high as the N uptake. The lower NHI in UB compared to LB might be explained due to this.

The enhanced soil physicochemical characteristics, water-holding capacity, and nutrient availability inside the biochar may be responsible for the higher NUE of wheat in response to the addition of biochar [13,24]. Applications of biochar to soils can boost agricultural output [23]. There are a number of reasons why biochar can be assumed to boost agricultural output. Studies in the lab and in the field have demonstrated that adding biochar to salt-affected soils significantly reduced salt stress and enhanced plant development directly by releasing vital macro- and micronutrients including Ca, K, N, P, and Zn that helped counteract the negative impacts of salts [18,22,25]. Additionally, biochar can enhance the number of soluble soil nutrients and increase the plant-available water content [45]. Furthermore, a rise in grain yield has a direct impact on how efficiently nitrogen is consumed [24]. Biochar-containing treatments had a higher grain yield than treatments without biochar, but this was achieved with much more N supply; consequently, the nitrogen use efficiency was lower

This explains why biochar-containing treatments had a higher impact than fertilizers without biochar on enhancing nitrogen use efficiency, whereas biochar enhanced the NUE and NUtE of wheat grown on a tropical soil [46].

The quantity of biomass yield per unit of N-fertilizer used defines NAE, which stands for agronomic N-efficiency [31].

An improvement of NAE is achieved by a decrease in nitrogen loss to the environment and an increase in N uptake; thereby, the drawbacks of fertilizer use are prevented [30]. The results of the current study revealed that NAE in the U treatment was much lower than in the L treatment. Crop management strategies including quantity, time, positioning, and N source can increase NAE's ability to identify the economic benefits of mineral N fertilization [47].

NRE is a crucial indicator of NUE that shows how well the provided nitrogen is absorbed and used by the plant [30], whereas NUtE measures a plant's ability to convert the nitrogen it takes up from the soil into grain [32]. As expected, the values of NRE and NUtE decreased as N-fertilizer was applied. This may be related to the effects of N application characteristics such as timing, location, method, climate, cultivar, form, and quantity of N, plant density, and biotic and abiotic challenges [47,48]. The ratio of yield increases with N treatment to total plant N absorption increases with N application was characterized as physiological N usage efficiency (NPE), and it represented the effectiveness with which N was utilized by the plant [32,34,49]. In this study, the NPE of plants declined significantly with increased N availability after various biochar treatments. The wheat plant's production increased every gram of N stored, but dropped with an increase in N supply, and complied with the reward-descending rule [12]. The ratio of total plant N uptake without N application to total plant N uptake with N application is known as the "soil N dependent rate" (SNDR), which measures the contribution of soil N to plant N nutrition [30]. In this trial, as N supply increased with N fertilization, the soil N-dependent rate generally declined significantly compared to the control (except for A and B). This showed that as N availability increased, wheat growth's reliance on soil N is reduced, and its reliance on fertilizer N is increased [50]. N-fertilizers significantly increased the growth-promoting effects on wheat. It proved that the more soil N was present, the more soil N was available. These clearly illustrated how important soil N enrichment was to wheat during the growing season. For a high yield and high N use efficiency, it was also necessary to sustain the high contribution of soil N to wheat growth and to promote soil fertility [51].

The SPAD data as a reflection of chlorophyll content demonstrated that plant development and increasing N-availability increased total chlorophyll in biochar-treated treatments. Boosting the supply of N can help plants maintain high chlorophyll contents throughout both the jointing and filling stages [22]. There is a favorable correlation between leaf N content, SPAD values and yield parameters [52].

Increased nutrient availability for plants in soil is mostly a result of biochar's high pH and alkaline properties [18]. The employed soil in the current study was naturally acidic, and biochar amendments frequently result in increases in pH and CEC of acidic soils [52]. Addition of biochar, which naturally contains ash, increases the amount of free bases in the soil [17]. This might raise the pH and make more nutrients for plant growth readily available [22]. By consuming protons during the decarboxylation of organic anions (i.e., ash alkalinity) from the additional biochar, the pH of the soil and leachate both increased [18]. The ash percentage in biochar used in this study, which included several mineral ions, including calcium, magnesium, potassium, and sodium, may be responsible for the increase in leachate EC [53]. Therefore, it should be highlighted that this could raise the risk of salinization [54]. However, agricultural management practices do not involve applying biochar to soil in large quantities. In this study, biochar treatments led to an increase in leachate EC as the mineral ions dissolved and leached in water. Additionally, biochar can improve nutrient retention, a benefit that results from the biochar itself rather than the ash [52]. The addition of biochar to soils raised the pH and CEC, leading to noticeably higher yields of maize (Zea mays L.) [18]. Additionally, the addition of biochar to fertilizer additions increased rice (Oryza sativa L.) yields more than fertilizer additions alone did [55].

The use of biochar demonstrated its capacity to bind nitrate in soil that has been fertilized with various kinds of N. Applying N-fertilizers containing biochar reduced the quantity of nitrate leaching as compared to their respective treatments, which is consistent

with the findings of a prior study [56]. One of the proposed explanations is that hydrated asymmetric nitrate ions are physically trapped inside the pores of the biochar particles as the mass solution flows into the particles [57]. Another explanation is the interaction of positively charged cationic salts or functional groups on the surface of the biochar with negatively charged nitrate [58]. The amount of nitrate adsorbed by freshly created biochar is also relatively little, which suggests that the capability of biochar to adsorb nitrate may be constrained [37]. Nevertheless, the anion exchange capacity may lead to nitrate sorption [58]. The biochar's FTIR showed a strong band at 1583 cm<sup>-1</sup> due to oxonium functional groups (Figure 1), which can help the biochar to bind nitrate [59]. Additionally, biochar can be used to efficiently increase the nitrogen use efficiency of fertilizers in loamy soil and decrease nitrate loss [24]. High cation exchange capacity, improved soil water holding capacity, and microbial immobilization of nitrogen as a result of biochar application are likely drivers of nitrogen uptake and storage in soil [18,19]. Treatments using biochar could better preserve nitrate, thereby reducing its loss by leaching. The high degree of specific surface area of biochar particles can be used to explain this lower leaching [57]. This might be explained by the biochar material's ability to boost the cation and anion exchange capacities (CEC, AEC) of soil [56]. As the soil's ability to hold water also improves in biochar-amended soil, another possible explanation is the physical retention of accessible N dissolved in the soil solution. Increased soil aggregation and a higher water holding capacity were linked to improved nitrogen retention in the biochar-treated soil [57]. The number of pores, the distribution of pore sizes, and the particular surface area of the soil all had an impact on how the soil aggregated [22]. Additionally, it has been noted that the composition of the bacterial community involved in N cycling changed when biochar treatments were applied. As a result, biochar may have had an impact on the microbial modification of N, which may be another method for reducing N leaching [43].

#### 5. Conclusions

As the plant will always need nitrogen, it is crucial to test various N-fertilizers and management strategies in order to maximize nitrogen efficiency and reduce environmental concerns. Definitely, biochar can make a considerable contribution to raising the effectiveness of N-fertilizers, particularly organic ones. This ability is a result of biochar's remarkable adsorption qualities, which include a high specific surface area, a high cation exchangeable capacity, and a variety of surface functional groups that improve the absorption of soluble forms of essential plant nutrients from the soil, including nitrogen. Due to its high nitrogen content, urea fertilizer is the most widely utilized source of nitrogen in modern agriculture. Meanwhile, leaching and mineralization in the soil have a substantial negative impact on how effectively urea fertilizer removes nitrogen from soils. The plant's requirement for growth can be met by organic leguminous fertilizer with a lower nitrogen percentage than urea, particularly when mixed with biochar.

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