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Effect of biochar on nitrogen retention in soil under corn plant inoculated with arbuscular mycorrhizal fungi

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ABSTRACT

Maintaining the levels of nitrogen in agricultural fields to ensure crop yield performance is challenging due to the complex dynamics of nitrogen transformation in soil. Nitrogen is mainly taken up by plant roots in the form of nitrate, but it is considered as an environmental pollutant that threatens human and animal health. Therefore, it is necessary to use adsorbent compounds to retain nitrate in the soil. The effectiveness of two types of biochar produced from rice husk (Br) and populous wood (Bp) and two arbuscular mycorrhizal fungi, namely *Funneliformis intraradices* (Mi) and *Funneliformis versiforme* (Mv), on nitrate leaching in soil was evaluated. The soil columns planted with corn were filled with an artificial sandy clay loam soil fertigated with urea fertilizer under glasshouse conditions. After nine weeks of growing the plants, a pulse of nitrogen (0.48 g urea per core) was added to the columns. One week after the addition of urea, the shoots of the plants were removed, and the columns immediately flushed with 500 ml of deionized water to leach the soil nitrogen from the columns. The results showed that the shoots' dry-weight increased significantly ($p \leq 0.05$) in almost all the treatments with the highest in the BrMi treatment when compared to the control (C). The nitrate concentration in the leachate decreased 79% (from 23.2 mg/l in C treatment to 4.2 mg/l in Bp treatment), but the nitrate concentration in the soil solution increased up to 6.7-fold (Bp was the highest), which suggested a high N retention by the biochars used. It was concluded that the application of biochar and mycorrhizal fungi could reduce nitrogen loss through this artificial sandy clay loam soil and may have some implications in environment conservation.

1. Introduction

Nitrogen (N), phosphorus (P), and potassium (K) are three soil nutrients that most often limit plant growth. In order to obtain high crop yields, high rates of inorganic N fertilizers are applied to agricultural fields worldwide. Despite the fact that N in the form of urea is widely used in Iran, its efficiency is not as high comparatively to some other countries [1]. The heavy application of N has raised some major concerns in the face of public opposition, mainly from health organizations and environmentalists [2]. The soluble fraction of N can be taken up by the plant or can easily be transferred out of the soil to natural water via leaching or runoff. Nitrogen in the forms of nitrate (NO_3^-) and nitrite (NO_2^-) is considered a pollutant and increases the risk of surface and groundwater contamination that threatens the environment as well as public health by entering the food chain [3-4]. In addition, the subsequent transformations of the mentioned N species lead to environmental losses through greenhouse gas emissions [5]. The use of biochar in soils is a useful method to reduce N leaching. Biochar is derived from the thermal decomposition of a wide range of carbon-rich biomass materials such as grasses, hard and softwoods and agricultural and forest residues [6]. Several

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studies have been performed on the effectiveness of biochar in soil fertility and crop productivity, reduction of greenhouse gas emission, and controlling the mobility of a variety of environmental pollutants such as heavy metals, pesticides, and other organic contaminants [7–10]. A few studies have investigated the ability of different biochars to retain nutrients in the soil, particularly phosphate, ammonium, and nitrate [11-12]. For example, Lehmann *et al.* [13] reported that the addition of biochar produced from secondary forest residuals significantly reduced the leaching of N fertilizer and increased plant growth and nutrition. According to Laird *et al.* [3], the addition of biochar produced from hardwood to typical Midwestern agricultural soil treated with swine manure significantly reduced N and P leaching by 11% and 69%, respectively. The influence of arbuscular mycorrhizal (AM) fungi inoculation on N leaching in agricultural soils amended with biochar has received almost no attention. The establishment of the mycorrhizal network offers a number of basic advantages for plants: increases the surface area for the absorption of nutrients, extends soil pores to improve the physical and biological properties of soils, and accesses forms of N and P that are unavailable to non-mycorrhizal plants that improves nutritional status [14]. Therefore, the objective of this work was to determine the effect of two biochar amendments on the leaching of nitrate and ammonium in soil columns planted with corn inoculated with AM fungi. Note that the native fungi were not eliminated in the studied soil because sterilization would eliminate the other soil microorganisms involved in N cycling and eventually change the biological and chemical properties of the soil.

2. Materials and methods

2.1. Biochar and soil properties

The biochars were produced from rice husk and populous wood (designated hereafter as Br and Bp, respectively) by using a slow pyrolysis method as described by Zheng *et al.* [12]. They were exposed to a temperature of 500 °C for 12 h in a pyrolyzer and continuously flushed with 99% pure gaseous N_2. Detailed information on biochar preparation and characteristics and methods of analysis have been presented elsewhere [15-16]. Some properties of the biochar samples are presented in Table 1. All the biochars were gently crushed through sieves with a ceramic pestle so that the mesh size of the biochar particles fell between 2000 and 500 µm; they were briefly rinsed with double distilled water to remove ash.

Table 1. Some properties of the soil and biochars used in this study.

Parameters	Unit	Soil	Biochar produced from rice husk	Biochar produced from populous wood
EC*	dS/m	0.335	0.592	0.621
pH*	-	7.51	7.63	7.75
Clay	%	37.9	-	-
Silt	%	41.7	-	-
CEC	$Cmol_c$/ kg	15.3	-	-
Nitrate	mg/kg	0.63	N.D$	N.D
Ammonium	mg/kg	1.89	N.D	N.D
Total C	%	0.75	38.10	60.45
Total N	%	0.012	0.644	0.470
Total S	%	-	0.066	0.44
Total H	%	-	1.255	2.294

* Determined in 1:2.5 soil (biochar): water suspension

$Not detected

Surface soil (xeric haplocalcids, clay loam texture) was collected from a wheat field at a 0- 20 cm depth in the Bastam district of Shahrood, Iran. The soil was air-dried and ground to pass through a 2 mm sieve and thoroughly homogenized. The soil pH was determined in a 1:2.5 soil to water suspension. The total organic carbon (TOC) was measured using an oxidation method with potassium dichromate [17]. The total N content of the soil samples was determined using the Kjeldahl method. The total phosphate (TP) content was determined by spectrophotometry using phosphomolybdate blue [18]. The NO_3^-–N content in the soil and leachate were analyzed using spectrophotometric methods (Jenway 6305) with phenol di-sulfonic acid at 540 nm [19]. Particle size analysis was conducted by the hydrometer method. Some chemical and physical properties of the soil are presented in Table 1.

2.2. Experimental design

The greenhouse experiment was carried out in polyethylene columns (40 cm length×13 cm diameter) as described in Asghari and Cavagnaro [20]. Briefly, a thin layer (~2 cm) of acid-washed sand (~100 g) was placed on a layer of cotton mesh at the base of the columns with a cap (with a central hole15 mm in diameter) on the base. To each column, 3 kg of a soil: sand mixture (2:1 ratio, which the soil texture changed to sandy clay loam) was added to a final bulk density of 1.2 g/cm^3. A soil: sand mix was used in this experiment as it provides a very uniform mixture, under the same leaching conditions, and ready for the extraction of the roots and hyphae at the time of harvest [21-22]. The treatments (in three replicates) included a control without biochar, 1% (by weight) Br, and 1% Bp in which only half of each column was inoculated with *Funneliformis intraradices* (Mi) or *Funneliformis versiforme* (Mv) (purchased from the bio-tech Turan Company, Shahrood, Iran). The inoculum consisted of sand plus colonized root fragments, spores, and the external hyphae of the *Trifolium alexnaderinum* trap culture. It should be pointed out that mycorrhizal colonization was observed in the non-inoculated treatments, which was related to the population of native mycorrhizae in the soil studied (data not shown). The corn seeds (single cross 704) were surface sterilized (with sodium hypochlorite) and sown in polyvinyl columns in the greenhouse with natural light. The seedlings were thinned to two per column after about two weeks. The columns were irrigated (to weight) with deionized water every second day, to 80% of the field capacity, thereby ensuring that no water leached out of the columns during the plant growth phase of the experiment. Long Ashton nutrient solution was added (3 ml per pot) once per week for four weeks. After nine weeks, the pots were supplied with a pulse of N as urea (480 mg urea per core that was equivalent to 150 kg N/ha) dissolved in 25 ml deionized water. One week after the urea addition treatment, the shoots of the plants were removed (to eliminate water loss through transpiration); the columns were immediately flushed with 500 ml of deionized water to leach soil N from the columns according to Asghari and Cavagnaro [20], to simulate a large rainfall event. The leachates were collected from the columns until the cessation of leaching (24 h) and NO_3^- was determined colorimetrically, as described above. Soil subsamples were also collected from two layers (0–10 and 10-20 cm depths) and extracted using a 2 M KCl solution to determine the NO_3^- concentration in the soil solution. Different rates of 0.01 M NaOH or HCl solution were added to the suspensions obtained from each biochar to determine the point of zero charge by the potentiometric mass titration method [23]. The mixture was agitated for 24 h in a shaker to reach equilibrium. The pH value before and after the addition of the acid or base was recorded.

The roots were washed of all the remaining soil with deionized water. For determining mycorrhizal association, a certain amount of the fresh root was rinsed in formalin +acetic acid +alcohol solution, cleared with hot KOH and acidified with HCl. Lacto glycerol was used to stain the roots, and lactic acid + glycerol was applied to remove the extra color [24]. Mycorrhizal colonization of the corn roots were determined using the gridline intersect method [25] to confirm mycorrhizal associations. In this method, 10 pieces of the root were spread out in a petri-dish marked with gridlines. The presence or absence of fungal infection was noted at each point where the roots intersected a line under light microscopy at ×40 magnification. The shoot and root plant materials were dried at 60 °C, ground to a fine powder, and the concentration of N was determined by dry combustion [26]. The data were analyzed by a one-way ANOVA procedure using SPSS software. Mean comparisons were carried out by Least Significant Difference (LSD; $P \leq 0.05$).

3. Results and discussion

3.1. Plant growth

Plant analysis indicated that all the treatments except Bp and BrMv (rice husk biochar + *Funneliformis versiforme* fungus inoculation) significantly ($p \leq 0.05$) increased the shoots dry weight (Figure 1). The highest level of shoot dry weight (12.6 g/core) was found in BrMi (rice husk biochar + *Funneliformis intraradices* fungus inoculation), which was about 4.35 fold when compared to the control (2.9 g/core). The shoot dry weight tended to be relatively greater in BrMi treatment than BpMi (9.8 g/ core), though there was no significant difference ($p \leq 0.05$) between Br and Bp and between Mi and Mv. Several studies have shown that applying biochar leads to better growth of the plants, probably through altering many soil chemical, physical, and biological properties such as soil pH, cation exchange capacity (CEC), moisture content and microbial activity, thereby improving the root uptake of nutrients [5,14,27]

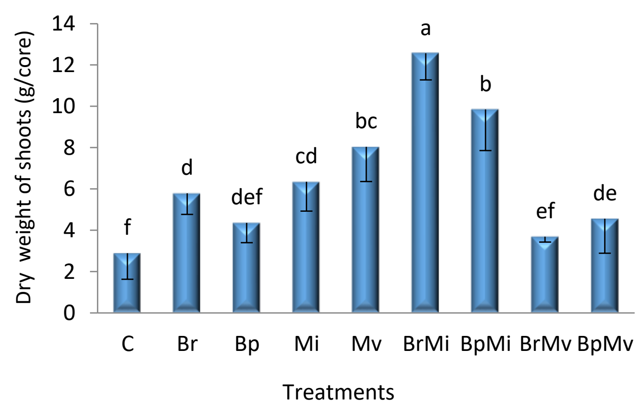

Fig. 1. Mean of shoot dry weight of corn grown in soil columns with different treatments, C: control, Br: rice husk biochar, Bp: populous wood biochar, Mi: *Funneliformis intraradices* fungus, Mv: *Funneliformis versiforme* fungus. Mean values followed by different letters are significantly different ($p \leq 0.05$) by LSD's test. Error bars indicates the standard deviation of three replicates.

The concentration of nitrogen in the plant shoot ranged from 1.7 % in Mv and BrMi treatments to 2.6 % in the C treatment (Figure 2). Nevertheless, there was no significant difference ($p \leq 0.05$) in the nitrogen concentration among the treatments. It should be pointed out that the nitrogen uptake (calculated by multiplying dry weight of shoot [g shoot/core] with nitrogen concentration [g N/ g shoot]) was the highest (0.21 g N/ core) in the BrMi and the lowest (0.07 g N/core) in the C. The nitrogen uptake was calculated by multiplying the dry weight of the shoot (g shoot/core) with the nitrogen concentration (g N/ g shoot).

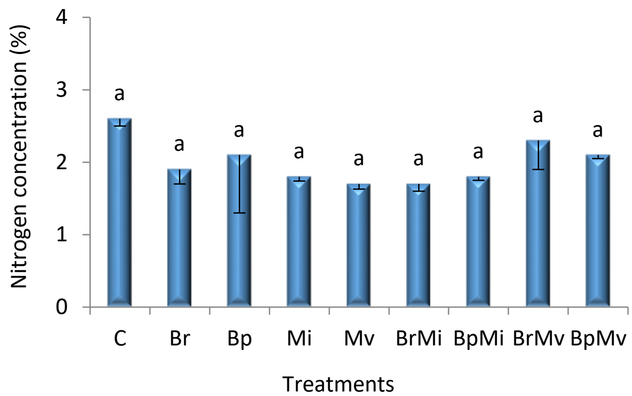

Fig. 2. Mean of total N concentration in the shoots of corn grown in soil columns with different treatments, C: control, Br: rice husk biochar, Bp: populous wood biochar, Mi: *Funneliformis intraradices* fungus, Mv: *Funneliformis versiforme* fungus. Mean values followed by different letters are significantly different ($p \leq 0.05$) by LSD's test. Error bars indicates the standard deviation of three replicates.

3.2. Nitrate loss

All the treatments, especially biochar combined with mycorrhizal fungi (i.e., BrMi, BrMv, BpMi, and BpMv), significantly ($p \leq 0.05$) decreased the NO_3^- concentration in the leachate collected from the soil columns at the end of the experiment when compared to C (Figure 3). The decreased rates in the BrMi, BpMi, BrMv, and BpMv were 63%, 68%, 79%, and 78%, respectively. Nevertheless, the NO_3^- concentration at the 0-10 cm depth of the soil columns was significantly higher in all the treatments compared to C, indicating the retention of nitrate by the AM inocula and biochars application (Figure 4). The significant increases ($p \leq 0.05$) of nitrate concentration at the 10-20 cm depth were only observed in the Mv, BpMi, and BrMi treatments (Figure 5).

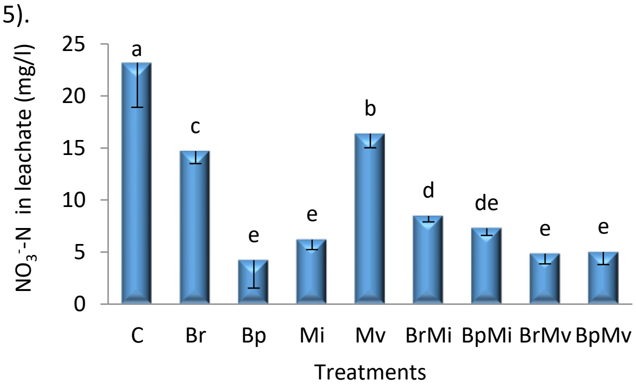

Fig. 3. Nitrate concentration in the leachate. C: control, Br: rice husk biochar, Bp: populous wood biochar, Mi: *Funneliformis intraradices* fungus, Mv: *Funneliformis versiforme* fungus. Mean values followed by different letters are significantly different ($p \leq 0.05$) by LSD's test. Error bars indicates the standard deviation of three replicates.

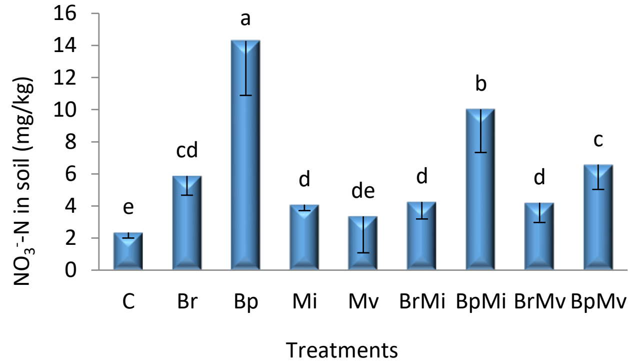

Fig. 4. Nitrate concentration collected from 0-10 cm core depth. C: control, Br: rice husk biochar, Bp: populous wood biochar, Mi: *Funneliformis intraradices* fungus, Mv: *Funneliformis versiforme* fungus. Mean values followed by different letters are significantly different ($p \leq 0.05$) by LSD's test. Error bars indicates the standard deviation of three replicates.

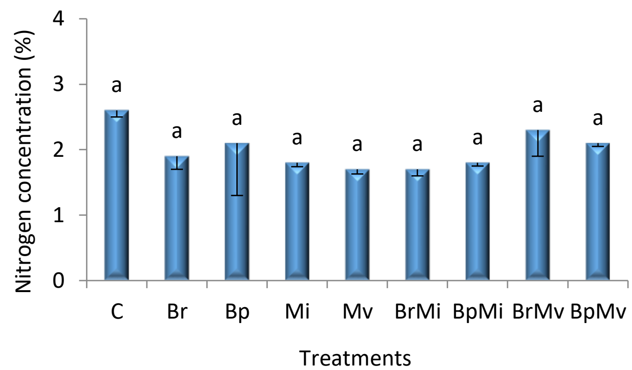

Fig. 5. Nitrate concentration collected from 10-20 cm core depth. C: control, Br: rice husk biochar, Bp: populous wood biochar, Mi: *Funneliformis intraradices* fungus, Mv: *Funneliformis versiforme* fungus. Mean values followed by different letters are significantly different ($p \leq 0.05$) by LSD's test. Error bars indicates the standard deviation of three replicates.

The decrease in the NO_3^- concentration by biochar application in the leachate can be mainly attributed to the increase of pH value in the soil columns. As shown in Figure 6, the pH value in Br and Bp treatments increased 0.12 and 0.33 units, respectively, when compared to C. It implies that the biochar application enhanced the pH-dependent negative charges, thereby increasing the CEC in the soil. Some reports confirm that a biochar-induced increase in soil CEC leads to more retention of NH_4^+ [28-29]. Dempster et al. [28] also suggested that the decreases in N lost via leachate by biochar application are attributed to an inhibition of the nitrification rate. Also, biochar can reduce the volume of soil leachate (Figure 7), probably through improving soil structure and soil water retention, thereby decreasing the nitrification rate (microbial transformation of NH_4^+ to NO_3^-). It should be pointed out that the rate of this process is affected by some factors such as moisture and available sources of oxygen and carbon, which all influence microbial activity. Aeration plays a significant role in the nitrification process because almost all known nitrifiers are metabolically active in aerobic conditions [30]. Additionally, the metabolic activities of microorganisms involved in nitrification are affected by soil moisture content. Soil moisture regulates the diffusion of O_2 in the soil microenvironment. Numerous studies have shown that the activity of nitrifiers is greatest when the soil moisture content increases from wilting point to field capacity. As moisture content increases beyond field capacity, it leads to a lack of O_2 needed for respiration by nitrifiers [31-32]. Therefore, the increases in NH_4^+ adsorption and/or in soil moisture content may be the main reasons for the decreased nitrification rate when biochar is added. Because the leachate volume decreased about 26 and 21% with the Br and Bp treatments, respectively, indicating extremely high water retention. Biochar additions also influence the chemical properties of the soil, thereby possibly decreasing the nitrification rate [34]: changes in pH, electrical conductivity (EC), cation exchange capacity (CEC), nutrient levels [33], and consequently nutrient sorption efficiency. For instance, Yang et al. [35] found that biochar amendment limited nitrification of NH_4^+ into NO_3^- in two acid soils due to the chemical adsorption by biochar. Wang et al. [36] reported that the reduced nitrification rate by the addition of peanut shell biochar was mainly due to phenolic compounds present in the biochar. However, some studies indicated that incorporating biochar within soil increased the nitrification rate, mainly through improving the soil physical properties [11,37].

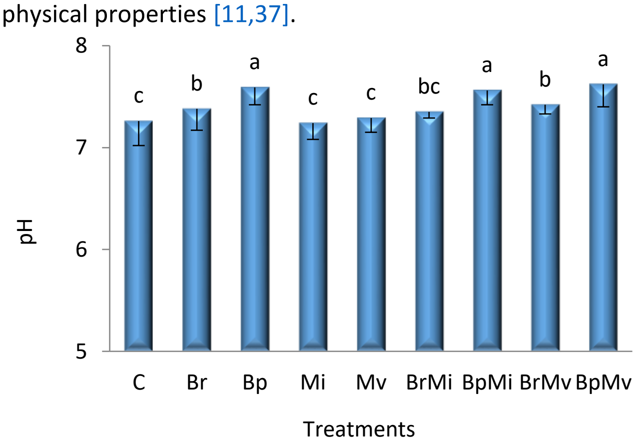

Fig. 6. The soil pH value in the treatments. C: control, Br: rice husk biochar, Bp: populous wood biochar, Mi: *Funneliformis intraradices* fungus, Mv: *Funneliformis versiforme* fungus. Mean values followed by different letters are significantly different ($p \leq 0.05$) by LSD's test. Error bars indicates the standard deviation of three replicates.

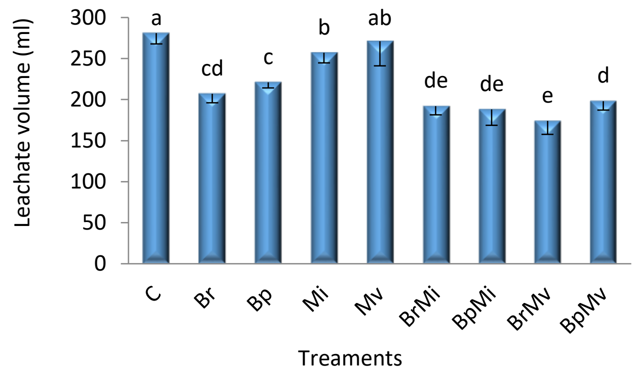

Fig. 7. Volume of leachate collected from the soil columns irrigated with 500 ml deionized water. C: control, Br: rice husk biochar, Bp: populous wood biochar, Mi: *Funneliformis intraradices* fungus, Mv: *Funneliformis versiforme* fungus. Mean values followed by different letters are significantly different ($p \leq 0.05$) by LSD's test. Error bars indicates the standard deviation of the three replicates.

It should be pointed out that the nitrate concentration at the 0-10 cm depth was significantly ($p \leq 0.05$) higher in the Br and Bp treatments than in the C, which may be partly attributed to the nitrate adsorption on the positively charged sites of the biochars. Some researchers also report that biochar may adsorb nitrate from the soil, depending on some of the chemical properties of biochar, such as anion exchange capacity (AEC) [12]. Nitrate retention in the Bp was much greater than the Br, probably because of a higher AEC in the Bp. On the other hand, the zero point of charge (the point at which the net surface charge is zero) determined from potentiometric mass titration curves [38] was predicted to be pH 7.31 and 9.08 for Br and Bp, respectively (Figure 8); the pH of the soil increased from 7.26 in the control to 7.38 and 7.59 with the Br and Bp treatments, respectively (Figure 6), indicating more positive charges in the Bp than that of the Br. It is worth noting that N present in the urea fertilizer used in the study is mineralized to NH_3, then protonated chemically to ammonium (NH_4^+), which is then converted into NO_3^- via NO_2^- in the nitrification process. Nitrate (NO_3^-) is very mobile and can be readily leached below the root zone of plants. Therefore, NH_4^+ loss from the soil is much lower than NO_3^-, since NH_4^+ adsorption mainly occurs by negatively charged soil colloids [20-39].

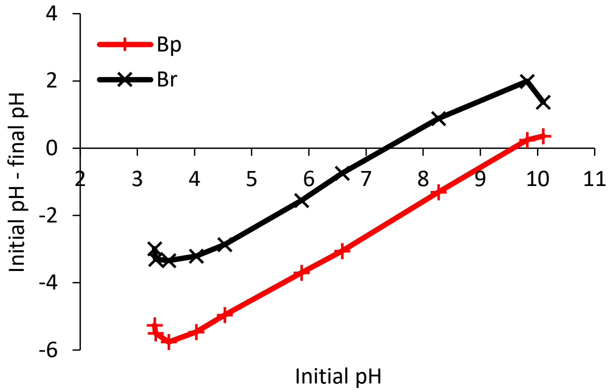

Fig. 8. The potentiometric mass titration curves for zero point charge (ZPC) determination of biochars produced from rice husk (Br) and populous wood (Bp). The intersection point of the curve initial pH minus finial pH versus initial pH is considered as the amount of point zero charge.

Arbuscular mycorrhizal fungi (AMF) could help plants acquire more nutrients by forming associations with plant roots to access a larger volume of soil [40] and, as a consequence, increase the shoot dry weight. Inoculation of AM to the plant roots, however, reduced nitrate loss from the soil columns when compared to the roots which received no AM inoculation. Enhanced plant N uptake, changes in microbial activities involved in N cycling, and a reduction in the leachate volume [21,41] have been reported as the mechanisms involved in the decrease in nitrate loss from soil columns inoculated with AM. The leachate content (Figure 7) and the concentration of total N in the plant shoots *(*Figure 2) have not been significantly ($p \leq 0.05$) changed by the AM inoculations, but the N uptake in the Mi and Mv treatments relative to the C increased about 52% and 82%, respectively. Therefore, it seems that the significant reduction of nitrate loss in this study may be mainly due to either a higher efficiency of the AM to retain nitrate or the N absorbed by the AM treated plants. However, the nitrate concentration in the Mi (at the 0-10 cm depth) and Mv (at the 10-20 cm depth) was significantly ($p \leq 0.05$) higher than in the C.

4. Conclusions

Biochar and AM treatments significantly lowered ($p \leq 0.05$) the nitrate concentration in the leachate, when compared to the control. The decreased nitrate concentration may be mainly attributed to a higher CEC and lower nitrification rate, though the highly porous structure of biochar leads to an increase in soil aeration and, as a consequence, improves nitrifier microbial activities. However, the increased retention of water in the biochar treatments leads to a decline in the nitrification process. Moreover, if the conditions are not favorable for nitrification in soil with higher CEC and organic matter, they will generally retain greater amounts of NH_4^+ over a longer period in comparison with the soil with lower CEC and organic matter. However, high water content in the soil columns treated with biochar will result in a more conducive environment for denitrifiers, which are active under anaerobic conditions. Thus, we believe that the application of both biochar and mycorrhiza together may be of practical importance in organic and sustainable agriculture.

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