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Impact of $MgCl_2$ Modified Biochar on Phosphorus and Nitrogen Fractions in Coastal Saline Soil

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Abstract

Biochar application is claimed to improve nutrient availability in many problem soils; however, pristine biochars are often reported to produce inconsistent results. Therefore, appropriate biochar modification techniques are required to retain soil nutrients at an optimum level. To increase Nitrogen (N) and Phosphorus (P) availability in coastal saline soil, two slow pyrolyzed biochars viz domestic organic waste (DWB) and farmyard manure (FMB) were modified with $MgCl_2$. Ten different treatments comprising the biochars (pristine and modified) with and without the recommended fertilizer were applied (2% w/w) to the soil and incubated for ninety days. The soils were analyzed for pH, EC, available NH_4^+, NO_3^- and different phosphorus fractions sequentially extracted by NH_4Cl, $NaHCO_3$, NaOH, and HCl. During the incubation period, biochar treatments increased all phosphorus and nitrogen fractions than the control and recommended fertilizer treatment. The application of FMB significantly ($p < 0.05$) increased NH_4Cl, $NaHCO_3$, and NaOH extractable P fractions from DWB, while HCl soluble fraction was enhanced ($p > 0.05$) by DWB. The increased Al and/or Fe bound phosphate after 60 days of incubation had significant correlations to decreasing soil pH and $NaHCO_3$-P, indicating reduced availability with time. Further Mg modification slightly increased P availability only after 60 days of incubation. The modification also improved both nitrogen fractions but significantly ($p < 0.05$) increased the NO_3-N content which could be the result of electrostatic attraction between Mg^{2+} and NO_3^- ions. Overall, Mg-modified biochar may retain both phosphates and nitrates in soil. However, the magnitude of retention will vary depending on biochar type, nutrient species, and aging in soil.

Keywords

Biochar Application, Saline Soil, $MgCl_2$ Modification, Nutrient Availability, Phosphorus

1. Introduction

Biochar is defined as biomass carbon in a deliberately stabilized form, produced in the partial or total absence of oxygen through high-temperature pyrolysis [1]. In recent years, biochar has received much interest not only due to its potential to sequester carbon [2] but also for its ability to improve soil fertility [3] and crop yields [4]. Additionally, it has been demonstrated to amend many soil problems including low organic matter content, nutrient deficiency, low water holding capacity, acidity, and salinity [5] [6]; through changing soil physicochemical properties [7] [8]. To meet the challenges of global soil and food security, it is essential to bring these unproductive problem soils under sustainable cultivation systems [9]. Proper utilization of biochar could become a reasonable tool for ameliorating those soils.

Increasing soil salinity has become a crucial problem for agricultural production around the world. The situation is worse in countries where fresh water is scarcely available in the salt-affected regions. For instance, in Bangladesh agriculture is hindered by soil salinity in many coastal and offshore districts including Khulna, Barishal, Patuakhali, Satkhira, Pirojpur, Barguna, Bagerhat, Bhola, Cox's Bazar, Gopalganj, and Jhalkati. The salinity affected area is continuing to rise rapidly; from 8330 square km in 1973 to 10,560 square km in 2009 [10], threatening the food security of the whole coastal region. A similar situation prevails in many countries with coastline where saltwater intervenes land by tropical storm surge or by salty groundwater irrigation [11]. Innovative technologies are essential to amend this vast amount of coastal lands that could make those agronomically productive again.

Salt-affected soils can be amended by biochar application as biochar possesses high organic matter and nutrients content [12]. Biochars can provide nutrients especially cationic ones (e.g. K, Ca, Mg, Zn, Mn), can increase CEC and surface area, stabilize soil structure, replace Na from exchange sites with Ca [12], and ultimately improve saline soil's nutrient use efficiency [13]. However, the effect of biochar on macronutrients (especially P and N) of saline soil is not well understood yet.

In general, although the total phosphorus (P) and nitrogen (N) content in saline soil can be relatively high, their availability to plants is limited. In particular, much of the P (80%) becomes immobile and unavailable for plant uptake due to adsorption, precipitation, and leaching [14] [15]. Previously, biochar has shown the potential to increase P availability in these soils through direct P release or indirectly by improving P use efficiency [16]. However, the negative effect of biochar application on soil available P has also been reported in saline-sodic soil, especially with fertilization [17]. Thus, knowledge of various soil P fractions is essential for a better understanding of P transformation processes after biochar application. However, sorption of anionic nutrients (PO_4^-, NO_3^- etc.) by biochar is reported to be less effective due to the electrostatic repulsion between anionic molecules and negatively charged biochar. Therefore, an innovative and efficient way to enhance biochar's property to retain anionic nutrients will help to sustain the comprehensive nutrient supply in these salt-affected soils.

Extensive attention has recently been given to the modification of biochar with novel structures and surface properties which will improve the sorption properties and reduce anionic nutrient leaching [18]. Modifying biochar with metal oxides has recently being used to improve biochar's anion retention capacity [19]. Because some metal oxides have been seen to form positively charged functional groups on biochar surfaces which can increase PO_4^{3-} sorption capacity by a factor of 12 to 50 by pH-dependent binding [20]. Zhang *et al.* [21] compared the MgO modification of biochar produced from 5 different feedstocks and found increased adsorption capacity for PO_4^{3-} and NO_3^- which was attributed to the positive charge of the MgO that precipitated on the biochar surfaces. However, the MgO is a basic compound that might further increase the pH of alkaline saline soils after the combined application of biochar. Instead, we propose the modification by slightly acidic $MgCl_2$ which will be more appropriate in the saline condition. Additionally, most of the studies conducted so far used metal-modified biochar to quantify enhanced P sorption from water. However, the soil is a heterogeneous system and we should consider the effect of coexisting ions in soil or from fertilizer on the nutrient sorption capacity, and vice versa. To our knowledge, no previous study has focused on the effect of Mg-modified biochar on both P and N dynamics, which is essential before recommending the system for large-scale field application.

Hence, we hypothesized that the positive charges on biochar surface brought by $MgCl_2$-impregnation will increase the phyto-availability of phosphorus and nitrogen. Therefore, the present study focused on the effects of biochar, Mg-modified biochar, and co-application of fertilizer on different phosphorus and nitrogen fractions and investigated the mechanism that can alter the availability of these nutrients. The major findings of the paper include:

- Mg-modification initially reduced labile P in the saline soil, and then increased gradually after 60 days of incubation.
- Increasing Al/Fe phosphate formation in the soil was correlated to declining $NaHCO_3$ extractable P and soil pH.
- Modified biochar could retain NO_3-N in soil and has the potential to reduce leaching loss.

2. Methods and Materials

Saline soil samples were collected for the laboratory-based incubation study from the Ramgoti soil series situated at Kalapara, Patuakhali (Latitude—21˚53'26.3"N, Longitude—90˚8'9.5"E); a coastal region of Bangladesh. Samples were collected from surface soil to a depth of 0 - 15 cm by composite soil sampling method as described in the soil survey manual by USDA [22]. After collection, soil samples were air-dried at room temperature, sieved through 2 mm and stored for further incubation.

2.1. Biochar Production and Modification

Feedstocks for biochar production were collected from the daily kitchen waste of a students' residence at the University of Barisal and farmyard manure from Char-Aicha village (Latitude—22˚75'11.31"N, Longitude—90˚38'18.16.5"E), Barishal Bangladesh. Collected feedstocks were appropriately dried and cut into small pieces for pyrolysis. A locally designed aluminum kiln with a temperature monitor was used for pyrolysis. The pyrolysis was done for 1.5 hours on a gas stove and the production temperature was controlled between 350˚C - 400˚C after reaching the maximum temperature. The produced biochars were sieved through 2 mm and stored in a plastic container for further analysis and use.

Mg modification of biochar was done by the chemical modification process according to [23]. In short, biochars were dipped in $MgCl_2$ solution with a mass-to-volume ratio of 1:5 for 3 h in an oven at 95˚C. After cooling, the solution was filtered and wet biochars were kept in the oven for 12 hours at 120˚C. Finally, the unmodified and modified biochars produced from farmyard manure and domestic organic waste were designated as farmyard manure biochar (FMB), domestic organic waste biochar (DWB), modified farmyard manure biochar (MoFMB), modified domestic organic waste biochar (MoDOB) respectively.

2.2. Experimental Setup

Ten treatment combinations namely control, recommended fertilizer only, two different biochars (pristine and modified) with and without recommended fertilizer doses were applied in the soil. Biochars were added at a rate of 2% dry weight basis and incubated at 50% of maximum water holding capacity of soil at 25˚C of temperature for 90 days. A total of 60 pots were prepared for three separate data recording times (30, 60 and 90 days). The incubation study was arranged in a completely randomized design with two replicates and investigated for changes in soil pH, EC, nitrogen and phosphorus fractions after every 30 days. The applied fertilizer dose was suggested by SRDI (Soil Resource Development Institute, Bangladesh) online fertilizer recommendation system [24] for local high yielding Boro rice as Boro is commonly cultivating in saline soils of Bangladesh.

2.3. Phosphorus Fractionation

The fractionation of P in the soil was carried out by following the sequential extraction analysis proposed by Hedley *et al.* [25] and modified by Chen *et al.* [26].

The different phosphorus fractions were extracted by the following sequential extraction procedure (**Figure 1**):

- Labile phosphorus extracted by 1 M NH_4Cl (1:10).
- Exchangeable phosphorus by 0.5 M $NaHCO_3$ (pH 8.5; 1:10).
- Al and Fe phosphates by 0.1 M NaOH (1:10).
- Ca-phosphate by 1 M HCl (1:10).

2.4. Nitrogen Fractionation

After each incubation period, soil sub-samples were analyzed for nitrogen availability for both ammonium and nitrate form. To investigate that, samples were extracted by 1N potassium chloride.

2.5. Physical Analysis

The yielding capacity of produced biochars at production temperature was calculated by equation described by Naeem *et al.* [27]. The water holding capacity of soil and biochars was analyzed by the method of ASTM Committee D-18 on Soil and Rock [28]. The particle size analysis of the soil samples was determined by the hydrometer method [29].

2.6. Chemical Analysis

The pH of the soil and biochar samples was analyzed by pH meter (HACH HQ30D) with a sample to water ratio of 1:2.5 and 1:10 respectively. To determine the electronic conductivity of soil and biochar samples, a 1:5 and 1:10 solid to solution ratio was used followed by 1 hour of shaking. The soil and biochar samples were also analyzed for cation exchange capacity [30], organic carbon content [31], available potassium and calcium [32]. The total nutrient content of the samples was determined after digesting the sample with H_2SO_4 (for N) and HNO_3-$HClO_4$ (for P, K, Mg) method. Vanadomolybdate yellow color method

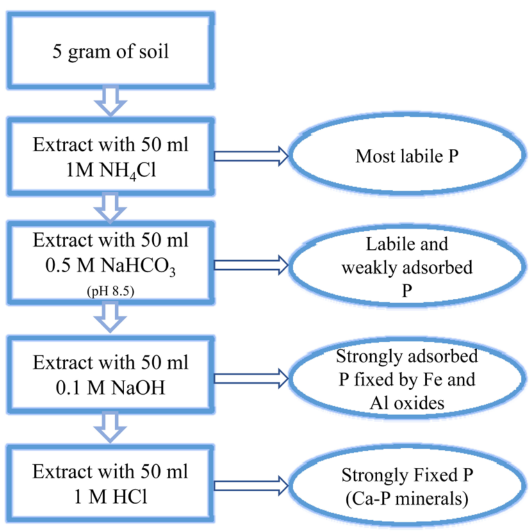

Figure 1. Sequential fractionation scheme for phosphorus.

was applied for measuring total phosphorus of soil by a spectrophotometer wavelength ranging from 400 to 490 nm as described by Jackson [30]. The total magnesium content in the soil, feedstock and biochars was determined by Atomic Absorption Spectrophotometer (AAS) with adequate dilution. To determine the available phosphorus in soil and in sequential extracts, the ascorbic acid blue color method was used [33]. Total and ammoniacal and nitrate nitrogen in the samples were determined after distillation as described in [34] with and without Devarda's Alloy (a reducing agent).

2.7. Statistical Analysis of Data

The impact of biochar type, modification, incubation period and fertilizer treatments on nitrogen and phosphorus fractions were analyzed by one and two-way ANOVA with the help of STATA version 14.0. Correlations among the fractions and soil pH and EC were also determined. Significant variations were further analyzed by the Tukey pair-wise comparison test at 5% significance level.

3. Results and Discussion

The coastal saline soil was found marginally alkaline in reaction (pH 7.61) and saline in nature with electrical conductance of 4.98 mS/cm which represents salinity class of S2 (slightly saline) as suggested by SRDI [10]. The organic carbon content of the soil was very low (0.37%) having a loamy textural class with moderate clay content (21.00%). The maximum water holding capacity was recorded as 70%. The cation exchange capacity (CEC) of the soil was found 12.25 me/100g soil. Phyto-availability of nitrogen, phosphorus and potassium were low; 20.58 ppm, 8.33 ppm and 0.03% respectively. The total nitrogen, phosphorus, potassium and magnesium content were found 0.04%, 0.03%, 0.12% and 0.11% (**Table 1**) correspondingly.

Biochar yielding capacity of farmyard manure was 6% higher than domestic organic waste. The pH of the produced biochars was exceedingly alkaline in nature (>9.0). Both cation exchange capacity and Electrical conductance were found higher in domestic organic waste biochar (**Table 1**). Additionally, the water holding capacity (360%) and organic carbon content (44.81%) of domestic organic waste biochar were considerably higher than the farmyard manure biochar. Total P and K content of DOW biochar were also higher than its counterpart (**Table 1**). The results indicate that, in general, DOW biochar has better agronomic values than farmyard manure biochar which is in line with the findings of Piash *et al.* [35].

The ANOVA test showed that all the parameters tested were significantly affected by treatments and as well as incubation period; except NH_4-N and NO_3-N by the incubation period (**Table 2**). Conversely, all the parameters were remarkably influenced by the interaction of both the factors except NH_4-N. The change of pH, EC, NH_4Cl-P, HCl-P, NH_4-N and NO_3-N was substantially affected by the treatment than incubation period, accounting for 83.61%, 80.32%,

Table 1. Physicochemical properties of soil and pristine biochars.

Properties	Soil (Ramgoti Series)	Farmyard Manure Biochar	Domestic Organic Waste Biochar
Textural Class	Loam	-	-
Yielding capacity (%)	-	48.56	42.51
Water holding capacity (%)	70	240	360
Total Organic C (%)	0.37	40.36	44.81
Total N (%)	0.04	0.24	0.17
Total P (%)	0.03	4.83	4.88
Total K (%)	0.12	0.11	0.51
Total Mg (%)	0.11	0.92	0.80
NH_4OAc extractable K (%)	0.03	-	-
NH_4OAc extractable Ca (%)	0.25	0.35	0.4
KCl extractable N (mg/kg)	20.58	-	-
$NaHCO_3$ extractable P (mg/kg)	8.33	-	-
pH	7.61	9.12	9.84
EC (mS/cm)	4.98	1.23	4.28
CEC (me/100g soil)	12.25	19.6	24.5

Table 2. Summary of ANOVA test for parameters measured.

Parameter	Source of variations														
	Treatment					Period					Treatment x Period				
	SS	df	MS	F	Pr > F	SS	df	MS	F	Pr > F	SS	df	MS	F	Pr > F
pH	5.454	9	0.606	91.849	0.0001	1.069	2	0.535	81.048	0.0001	0.716	18	0.04	6.031	0.0001
EC	23.02	9	2.557	55.009	0.0001	5.638	2	2.819	60.631	0.0001	6.934	18	0.385	8.286	0.0001
NH_4Cl-P	1.578	9	0.175	464.4	0.0001	0.347	2	0.173	458.99	0.0001	0.523	18	0.029	76.95	0.0001
$NaHCO_3$-P	3.411	9	0.379	2201.8	0.0001	7.909	2	3.954	22975	0.0001	1.515	18	0.084	488.9	0.0001
NaOH-P	1.42	9	0.158	184.8	0.0001	2.261	2	1.13	1323.6	0.0001	1.943	18	0.108	126.4	0.0001
HCl-P	0.929	9	0.103	56.68	0.0001	0.025	2	0.013	6.922	0.0020	2.377	18	0.132	72.51	0.0001
NH_4-N	0.128	9	0.014	106.58	0.0001	0.006	2	0.003	20.775	0.0001	0.007	18	0.000	3.025	0.0010
NO_3-N	0.346	9	0.038	384.46	0.0001	0.001	2	0.001	7.3	0.0010	0.041	18	0.002	22.52	0.0001

81.97%, 97.38%, 95.52% and 99.71%, respectively (Table 2). On the other hand, release of $NaHCO_3$-P and NaOH-P were more affected by incubation period than the treatments applied, accounting for 69.87% and 61.42%, respectively (Table 2).

As described prior, treatments had significant ($p < 0.01$) effect on changing soil pH, non-modified domestic organic waste biochar significantly increased the pH of the soils regardless of fertilization. In general, modification of biochar significantly ($p < 0.05$) reduced soil pH than their non-modified counterparts

(Table 3). Liu and Zhang [36] disclosed that pH decrease can be caused by combining cation and carbonates in biochar to slightly soluble compounds that resist hydrolysis while reducing hydroxyl content. Therefore, Mg modification can cause a decrease in soil pH. The incubation period had no significant effect on soil pH. However, after starting the experiment, pH markedly increased by biochar application from the background value (7.61) and then started to decrease gradually after 30 days (Table 3).

Application of the altered farmyard biochar treatment without fertilization could significantly ($p < 0.01$) increase the EC of the soil. $MgCl_2$ modification also had significant ($p < 0.01$) ability to increased soil salinity. However, the increase in soil EC was lower than the initial value (4.98 mS/cm) of the soil, measured right after collecting from the field. Neither the incubation time nor the fertilization had a considerable effect on soil EC.

3.1. Influence of Biochar, Biochar Modification and Fertilization on the Phosphorus Fractions of the Saline Soil

3.1.1. Influence on Most Labile NH_4Cl Extractable Fraction

As shown in Figure 2, NH_4Cl extractable phosphorus fraction in the saline soil was significantly affected by the biochar and fertilizer treatments ($p < 0.05$). In general, farmyard manure biochar had a better effect in terms of increasing the most labile phosphorus content in the soil. Additionally, the co-application of fertilizer with non-modified biochars demonstrated the best results to increase NH_4Cl extractable phosphorus fraction throughout the whole incubation period. Phosphorus availability was considerably lower in domestic organic waste biochar treated soils compared to the farmyard manure biochar treatments despite having more total phosphate in it. This might be due to having more Ca-bound phosphate in the treatment.

Table 3. Changes in soil pH and EC after application of different treatments.

Treatment	Soil pH			Soil EC		
	30 days	60 days	90 days	30 days	60 days	90 days
Cont.	7.53^{a}	7.77^{bc}	7.78^{bcd}	3.22^{a}	2.91^{ab}	3.20^{ab}
Fert.	7.74^{ab}	7.75^{bc}	7.65^{ab}	3.53^{ab}	2.90^{ab}	2.78^{b}
FMB	8.21^{abc}	7.98^{cd}	7.67^{ab}	2.81^{a}	2.78^{a}	3.47^{ab}
FMB_Ft	8.13^{abc}	8.08^{ad}	7.72^{abc}	3.04^{a}	2.91^{ab}	3.86^{ab}
MoFMB	7.72^{a}	7.68^{bc}	7.52^{a}	4.46^{b}	4.16^{cd}	4.67^{a}
MoFMB_Ft	7.84^{abc}	7.64^{b}	7.54^{a}	3.65^{ab}	4.58^{d}	4.49^{a}
DWB	8.46^{bc}	8.30^{a}	8.04^{ef}	3.55^{ab}	3.61^{abc}	4.73^{a}
DWB_Ft	8.51^{c}	8.38^{a}	8.16^{f}	3.69^{ab}	3.84^{bcd}	4.39^{ab}
MoDWB	8.22^{abc}	8.15^{ad}	7.99^{def}	3.94^{ab}	3.92^{cd}	4.17^{ab}
MoDWB_Ft	8.24^{abc}	8.11^{ad}	7.93^{cde}	3.67^{ab}	3.54^{abc}	4.86^{a}

*Different small letters denote significant difference among the treatments at an incubation period.

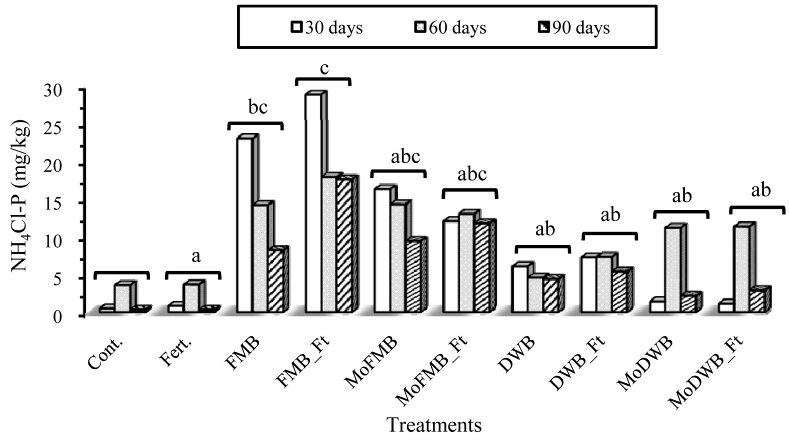

Figure 2. Impact of farmyard manure (FMB) & domestic organic waste (DWB) biochar, Mg modification (Mo) and fertilizer application (Ft) on NH_4Cl extractable phosphorus fraction. Bars with different small letters denote significant difference among the treatments.

After 60 days of incubation, NH_4Cl available phosphorus diminished in most of the treatments, however dramatic increase was shown by both fertilized and non-fertilized modified domestic organic waste biochar treatments. This might be due to some interacting effect between Ca and Mg to release some labile PO_4^- in soil. Rangwaswamy [37] also demonstrated that the incorporation of biochar in soil significantly and slowly increased the loosely bound phosphorus.

By the end of the incubation period (90 days), all treatments reduced the amount of NH_4Cl extractable phosphorus fraction though the co-application of farmyard manure biochar and fertilizer sustained to have comparatively higher amount. It is notable that, non-fertilized treatments of farmyard biochar almost halved the labile phosphorus fraction.

Fertilized treatments increased the availability of labile P compared to non-fertilized treatments as expected. Similarly, Amaizah *et al.* [38] found that the content of water-soluble phosphorus increased with the soil treated with fertilization with mineral phosphorus as compared with the control. This increase might be caused by the accumulation of Phosphorus on biochar surfaces and free spaces while applied in soil [39].

In general, the application of modified biochar treatments showed reduced P availability than their non-modified counterparts. Some studies reported that, Mg modified Biochar showed better phosphorus adsorption in saline soils and the maximum P adsorption capacity was 1.46 times higher than Biochar [40]**.** In this study, the NH_4Cl was unable to extract the Mg adsorbed P fractions on the modified biochar surfaces. Electrostatic attraction between phosphates and Mg might have promoted the adsorption of P on biochar, subsequently preventing the leaching of P [41].

Tukey pairwise comparison test confirms that application of farmyard manure biochar with fertilizer increase this fraction than all domestic organic waste treatment at 5% confidence level. Between two biochars, farmyard manure biochar provided better result than domestic organic waste biochar. Glaser and Lehr [42] also reported similar information from the meta-analysis and revealed that biochars derivative from animal residues show a positive response to plant-available Phosphorus than other biochar in biochar-treated soils. Since phosphorus content depends on soil pH and farmyard manure biochar treated soils showed comparatively lower pH (7.5 - 8.2) than domestic organic waste biochar (7.9 - 8.5), this might be a probable reason.

3.1.2. Influence on Labile and Phytoavailable $NaHCO_3$ Extractable Fraction

Biochar treatments increased the $NaHCO_3$ extractable phosphorus content in soils significantly ($p < 0.05$). In general, the phytoavailable P content in the treatments followed the order of FMB_Ft > FMB > MoFMB_Ft > MoFMB > DWB_Ft > DWB > MoDWB_Ft > MoDWB > Fert. > Control. Farmyard manure biochar with fertilizer showed the best result according to Tukey pairwise comparison test at 5% confidence level.

$NaHCO_3$ extractable phosphorus fraction was significantly ($p < 0.05$) affected by the incubation period. The trend saw a slight increase at 60 days and then a steep fall after 90 days of incubation (**Figure 3**). The data illustrated that between 30 to 60 days, phosphorus availability peaks (average-33.72 mg/kg), except control and the only fertilizer treatment which saw a declining trend. Based on the data, it can be said that the biochar created condition or dissolved itself to prolong the availability of phosphate in saline soil. The rise in phosphorus availability reported in this study is comparable to those reported by Opala *et al.* [43] and who claimed microbially mediated mineralization of soil organic Phosphorus to form inorganic Phosphorus for the increase with time (60 days). Liu *et al.* [44] suggested the relative abundance and distribution of phosphate-solubilizing bacteria (*i.e. Thiobacillus*, *Pseudomonas* and *Flavobacterium*)

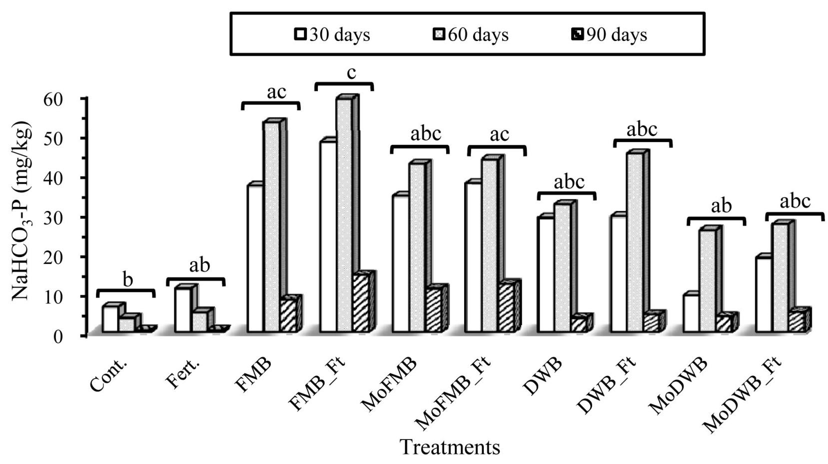

Figure 3. Impact of farmyard manure (FMB) & domestic organic waste (DWB) biochar, Mg modification (Mo) and fertilizer application (Ft) on $NaHCO_3$ extractable phosphorus fraction. Bars with different small letters denote significant difference among the treatments.

after biochar application as responsible for increasing available phosphorus. Besides Taghavimehr [45] informed that significant increases in the concentration of Phosphorus in salt-affected soils because of 1) biochar induced generation of dissolved organic carbon (DOC) that blocks the sorption sites on clay particles to diminish Phosphorus adsorption by soil colloids, and 2) the release of humic substances and their influence on Phosphorus availability by minimizing the formation of calcium phosphate crystal phases. Additionally, the decline in NH_4Cl extractable fraction suggests that those might have converted from very labile phosphate to exchangeable phosphates which are still bioavailable for plant use.

After 60 days of incubation, all biochar treatments saw a sharp decline; however, it was still better than the sole fertilizer application (**Figure 3**). Xu *et al.* [17] suggested that, the decline in the $NaHCO_3$ extractable phosphorus fraction might be due to the conversion of labile P to nonlabile inorganic or organic Phosphorus. Our data also supports the fact as the amount of NaOH extractable P fraction has substantially increased by the end of the incubation period (90 days). This implies the fact that the phytoavailable phosphates might have converted to Al and/or Fe phosphates. In contrast, the decline may be attributed to biochar induced enhancement in pH that led to precipitation of applied phosphorus in soil, thus rendering it unavailable for plant uptake.

The modification of biochar didn't have any significant positive or negative effects on phosphorus availability. However, modification reduced the availability in most of the cases up to 60 days of incubation. After that, modified biochars treatments had better availability of phosphorus than their non-modified counterparts, except the MoFMB_Ft treatment. This trend suggests that modification might harm Phyto availability of phosphorous within a shorter period of time, however, could be effective for longer periods. Studies reveal that altered biochar attracts Phosphate ion by electrostatic attraction resulting in increased phosphorus availability in soil [46]. Electrostatic attraction occurred between P and Mg-biochar, which is a positively charged surface, thus increasing the P adsorption capacity of Mg-biochar.

Overall results imply the fact that biochar treatments irrespective of modification or fertilization could make soil phosphorus available for plants up to a certain period. This trend of phosphorus availability for considerable period is beneficial for crop fertilization, as most of the phosphorus fertilizer is applied at the very beginning of the cropping season. For example, Urea is applied in three steps after Boro rice transplanting and the Phosphorus fertilizer is applied during the field preparation. Phosphorus loss occurred by various reasons and plant doesn't get the Phosphorus when it needs. Rice Plant needs phosphorus from the growing phase to mature phase (around 60 days).

3.1.3. Influence on Al and Fe-Bound NaOH Extractable Fraction

By the end of the incubation period, NaOH extractable phosphorus fraction in biochar treated soils (average-72.32 mg/kg) increased significantly ($p < 0.05$)

than earlier periods. Average NaOH-P in biochar threated soils are 55.75 mg/kg after 30 days and 53.50 mg/kg after 60 days, whereas soils without biochar treatment had much lower (**Figure 4**). This trend implies the fact that the amount of Al and Fe bound phosphate fraction increased significantly after 60 days of incubation. The decreasing trend of phyto-available phosphorus ($NaHCO_3$-P) after 60 days of incubation (**Figure 3** and **Figure 4**) might have contributed to the increasing Al and Fe bound phosphates, making them unavailable for plants. Considerable correlation was found between increasing NaOH-P and decreasing $NaHCO_3$-P for farmyard manure biochar applied treatments, however, not for the domestic organic waste biochar treated soils. Modification of the biochars decreased the amount of NaOH extractable P, however, the effect was nonsignificant ($p = 0.14$).

The increasing amount of NaOH-P might also be attributed to decreasing pH of the soil (**Table 3**) as decreasing the pH increases the availability of Al^{3+} and Fe^{2+} ions which could fix more phosphates. In this study, significant ($p < 0.05$) correlation was found between decreasing pH and increasing NaOH extractable phosphorus content.

3.1.4. Influence on Ca-Bound HCl Extractable Fraction

Effect of various treatments on HCl extractable phosphorus fraction was found statistically significant ($p < 0.05$). Among all treatments, the introduction of Domestic Organic Waste biochar with Fertilizer (average 268.27 mg/kg) showed the highest HCl extractable P-fraction (According to Tukey pairwise comparison test at 5% confidence level). In comparison with control, all treatment significantly increased HCl extractable phosphorus fraction. Tukey pairwise comparison test also confirms that the incorporation of modified farmyard manure biochar & domestic organic waste biochar displayed better results than all treatments (at 5% confidence level).

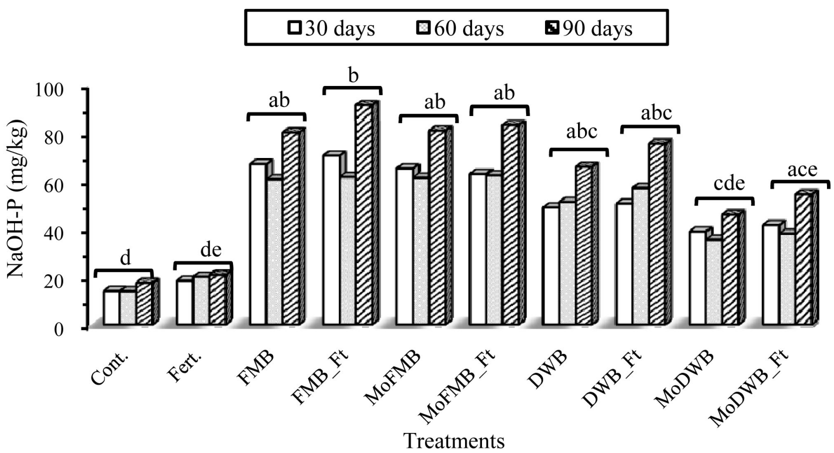

Figure 4. Impact of farmyard manure (FMB) & domestic organic waste (DWB) biochar, Mg modification (Mo) and fertilizer application (Ft) on NaOH extractable phosphorus fraction. Bars with different small letters denote significant difference among the treatments.

HCl soluble phosphorus fraction does not radically change within the incubation period. At 30 days all the treatments increased HCl extractable phosphorus content than the control. Increases in HCl extractable phosphorus fraction may be due to increased ionic strength and Ca concentration in soil solutions after biochar application, which can increase P adsorption [47]. After 60 days of incubation, most of the treatments saw a slight decline in the content of HCl extractable fraction, however, the content increased again by the end of the incubation period (90 days). The decrease after 60 days can be attributed to occlusion of HCl extractable phosphorus fraction within the organic matrix [48] or dominant nature of $CaCO_3$ [49].

Modified application of biochars regardless of fertilizer application overall decreased the total HCl extractable phosphate content throughout the incubation period. However, it was not significantly proven as modification of FMB increased the HCl soluble phosphorus (**Figure 5**). The decrease may be due to the Mg induced biochar replaced Ca to form $(Mg(H_2PO_4)_2)\cdot xH_2O$ or $(MgHPO_4)\cdot xH_2O$, which might become unavailable through HCl extraction. Fertilizer application non-significantly increased HCl extractable phosphorus fraction among the treatments. Xu *et al.*, [17] also found HCl-PO_4 was not affected significantly affected by fertilizer application.

Among the biochars, DWB application had more HCl extractable phosphates than Farmyard manure, though the difference was not significant. This phenomenon could be the result of inherent higher available Ca of DWB (**Table 1**) which could precipitate as Ca-PO_4 [50] making phosphate inaccessible in soil.

3.2. Influence of Biochar, Biochar Modification and Fertilizer Application on Nitrogen Fractions of Soil

3.2.1. Influence on Ammonium (NH_4^+) Fractions

Effect of biochar treatments on ammonium-N content was way better than the control. Among the biochar treatments, the sole usage of farmyard manure and

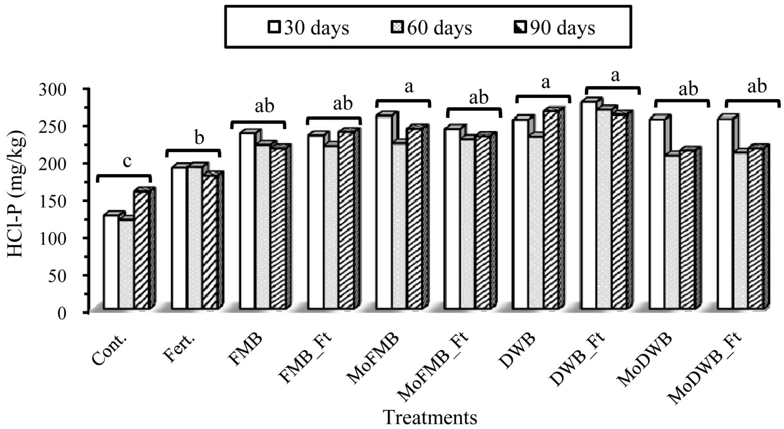

Figure 5. Impact of farmyard manure (FMB) & domestic organic waste (DWB) biochar, Mg modification (Mo) and fertilizer application (Ft) on HCl extractable phosphorus fraction. Bars with different small letters denote significant difference among the treatments.

domestic organic waste biochar was not significantly better than the recommended fertilizer application, however, the rest of the treatments including the Mg modified biochars without fertilizer was significantly superior (**Figure 6**). This study agrees with Yao *et al.* [51] and against the findings of Shenbagavalli and Mahimairaja [52]; who found both NH_4^+-N and NO_3-N content decreased after biochar application. The increase might be due to biochar's ability to decrease N losses to atmosphere as NH_3 through increased adsorption of ammonium onto biochar particles [53]. Yao *et al.* [51] demonstrated that biochar can also reduce the leaching of ammonium by 14%. Overall, modification improved the ammonium content in soil, though it wasn't significantly proven.

Application of fertilizer was significantly better ($p < 0.01$) than the non-fertilized treatments. Biochar treatments further supported to sustain the NH_4^+ content in soil. Ammonium content was not significantly changed with the days of incubation. However, after 60 days of incubation, all the treatments saw a slight rise in ammonium content except the control and modified application of DWB.

Application of farmyard manure biochar was capable to enhance (non-significantly) ammonium content than domestic organic waste biochar. This might be due to farmyard manure biochar's greater total nitrogen content. Nguyen *et al.* [54] claimed that high cation exchange capacity (CEC) of biochar preserves more soil NH_4-N, however, in this study, farmyard manure biochar had lower cation exchange capacity than domestic organic waste biochar (**Table 1**). Gong *et al.* [55] demonstrated that the cation exchange of Mg^{2+} was the dominant mechanism for NH_4-N adsorption onto the $MgCl_2$ modified biochar. Chen *et al.* [56] also reported significant adsorption of NH_4^+ by biochar in a sandy soil with pH ranging from 7 to 8. By the end of the incubation, the results revealed that pH range after the application of farmyard manure biochar to the soil ranged from 7.5 to 7.7 (**Table 2**) which was in between the pH range demonstrated by [56]. The mechanisms attributed to adsorption of NH_4^+ onto biochar surfaces include the presence of acidic functional groups [57], NH_4^+ reacting with

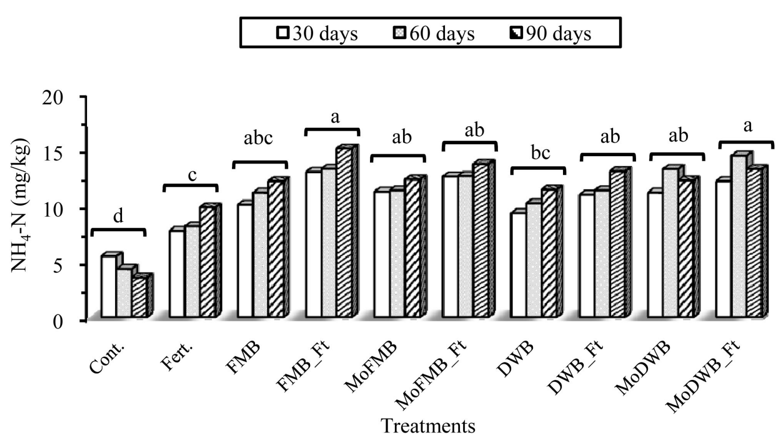

Figure 6. Impact of biochar, Mg modification (Mo) and fertilizer application (Ft) on NH_4^+ fraction. Bars with different small letters denote significant difference among the treatments.

carboxyl groups to form amides and amines [58], and NH_4^+ attraction to negatively charged surfaces [59].

It is also notable that, at the end of the incubation period all the farmyard manure biochar treatments had an increasing trend in terms of ammonium availability. However, fertilization was the main factor to improve the ammonium content rather than the modification.

3.2.2. Influence on Nitrate (NO_3^-) Fraction

A significant number of studies has proved biochar to be able to reduce the nitrate leaching from soil [60] [61] and retain those in the pores [62]. However, several studies claim biochar to reduce about 10% NO_3^- content from soil [54]. Therefore, the effect of modification on nitrate concentration is very important to be revealed. Additionally, biochar type and fertilization have a significant effect on soil inorganic nitrate availability [54].

In this study, the combined application of fertilizer and modified domestic organic waste biochar found out to be the best treatment to enhance the nitrate content in the soils (**Figure 7**). Besides, modified DWB was significantly (p < 0.05) better treatment than its non-modified counterpart. Analysis of variance confirmed the fact that modification of biochars had significant effect on retaining the NO_3^- content in the soil. According to [46] Mg modified biochar produced positive charge outside the biochar surface and increased NO_3^- content through physical adsorption.

In case of biochar type, domestic organic waste biochar increased NO_3^- content significantly compared to farmyard manure biochar. Kameyama *et al.* [60] attributed base functional groups that adsorbed NO_3^- as a reason. Domestic organic waste biochar had high pH and base forming cations than farmyard manure biochar (**Table 1**).

Application of fertilizer had better effect, not significant though. The incubation period had very little effect on the nitrate content of the saline soil. After 60

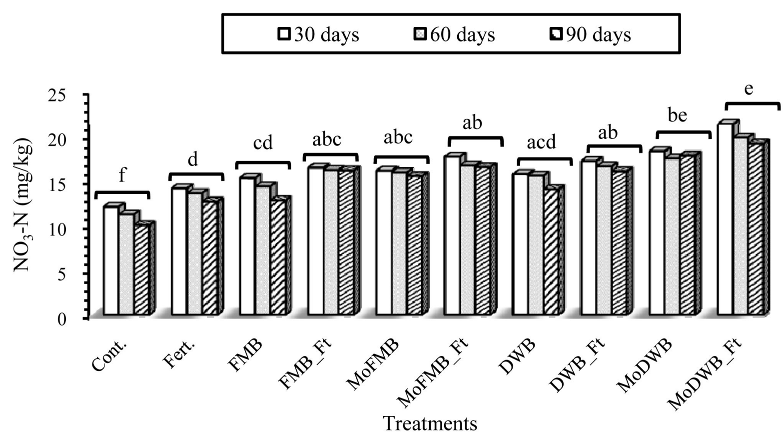

Figure 7. Impact of biochar, Mg modification (Mo) and fertilizer application (Ft) on NO_3^- fraction. Bars with different small letters denote significant difference among the treatments.

days of incubation, nitrate content demonstrated a decreasing trend. The study had comparable results to Kameyama *et al.* [60] who found increased saturated hydraulic conductivity when biochar was applied at a rate of $p \geq 5\%$, which led to enhanced leaching of NO_3^-. It is also possible that some of the NO_3-N might have lost through microbial denitrification [63] as well.

4. Conclusions

Biochar is believed to be an environment friendly and cost-effective soil amendment for different problem soils. Nutrient retention capacity of pristine biochar is still a matter of concern for less-fertile soils. Therefore, different modification techniques are continuously being introduced to strengthen the biochar's potential to be widely used as an agricultural amendment. This fractionation study in the coastal saline soil revealed that Ca-Phosphates were the predominant fraction in the applied biochars and $NaHCO_3$ extractable phosphorus fraction in biochar treatments was significantly ($p < 0.05$) affected by incubation time. The declining trend of this fraction after 60 days of incubation was correlated to the increasing amount of NaOH-soluble fraction significantly only in farmyard manure biochar treated soils. This kind of fractionation study helps to properly understand the interactive transformation of nutrients in different soil environments. The overall results imply that: 1) plant-available phosphates can transform to Al or Fe phosphates after 60 days of biochar application making them hardly available if soil pH decreases, 2) Mg modification had significant ($p < 0.05$) ability to retain NO_3-N, confirming the potential of the modification to reduce leaching, 3) Modified domestic waste biochar application with fertilizer proved out to be the best treatment for reducing nitrate loss.

Such findings suggest that the raw and modified biochar can have substantial effect on both the phosphorus and nitrogen availability in the saline soil irrespective of the biomass used for biochar preparation. Positive effect of Mg-modification on N retention was confirmed whereas P availability ($NaHCO_3$-P) was increased only after 60 days. Therefore, further intensive research is needed to reveal the $MgCl_2$ modified biochar's chemical or mineralogical mechanisms to alter phosphorus and nitrogen fractions.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of Data and Material

All data generated and/or analyzed during the current study are available from the corresponding author upon request.

Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Sharmin Jannat Lutfunnahar. The first draft of the manuscript was written jointly by Sharmin Jannat Lutfunnahar and Mahmudul Islam Piash. Final revision of the manuscript was performed by M Hasinur Rahman. All authors commented on and edited the previous versions of the manuscript. All authors have read and approved the final manuscript.

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