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Decrease in Inorganic Nitrogen and Net Nitrogen Transformation Rates with Biochar Application in a Warm-Temperate Broadleaved Forest

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Abstract: Changes in soil nutrient dynamics after biochar application may affect indirect carbon sequestration through changes in plant productivity in forest ecosystems. In the present study, we examined the effects of woody biochar application on soil nitrogen (N) cycling over 8 months in a warm-temperate deciduous broad-leaved forest. Mineral soil samples were collected from the plots treated with different biochar applications (0, 5, and 10 Mg ha^{-1}), and the soil inorganic N concentration was measured. Net mineralization and nitrification rates were determined in each plot using the resin–core method. Soil temperature and water content did not change significantly, but the pH increased significantly following biochar application. Soil inorganic N concentrations (NH_4^+ and NO_3^-) and net N transformation rates (mineralization and nitrification rates) were significantly reduced. Microbial biomass and the nitrification ratio (the ratio of nitrification rate to mineralization rate) were unchanged, indicating that the decrease in soil inorganic N concentration was due to the reduced mineralization rate. Adsorption of substrates (from organic matter) by the applied biochar is the most likely reason for the reduction in the N mineralization rate. The results indicate that biochar application does not necessarily stimulate N transformation, which will affect indirect carbon sequestration.

Keywords: biochar; forest; field experiments; nitrogen; mineralization; nitrification; climate change; carbon sequestration; microbial biomass; soil

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1. Introduction

Biochar is a carbon-rich material produced by thermal degradation of biomass, such as wood, manure, or leaves, under oxygen limitation and at a relatively low temperature [1]. Few soil microorganisms produce the necessary enzymes to decompose biochar; thus, it is resistant to microbial degradation [1]. As a result, biochar carbon remains in the soil for a prolonged period (e.g., >1000 years) [2,3]. Therefore, it has been suggested that the conversion of industrial waste, such as lumber processing residues, into biochar, can aid in carbon sequestration and mitigation of climate change through a reduction in the atmospheric CO_2 concentration [4]. Woolf et al. [5] showed that sustainable global use of biochar can offset up to 12% of current anthropogenic CO_2-C equivalent emissions. In addition, biochar is generally known to improve certain physicochemical properties of soils, such as water retention and fertility, which is expected to increase plant photosynthesis and primary production of plants [6]. As a result, biochar application may promote CO_2 absorption from the air and therefore contribute to indirect carbon sequestration [6].

Forest ecosystems cover a large area of the Earth's surface and originally stocked vast amounts of organic carbon as biomass and soil organic carbon (SOC) [7]. Therefore, forests are considered to be promising sinks for carbon sequestration. In this context, Lehmann [4] proposed the novel idea that biological residues could be converted to biochar and applied to forest soils to promote global carbon sequestration. However, the initial focus of research on biochar application was agricultural lands and comparatively little attention has been paid to forest ecosystems as a target of biochar application [8,9]. In addition, few studies have applied biochar to forest ecosystems and conducted field measurements except, for example, studies of Scots pine forest in Finland [10,11], subtropical Moso bamboo forest in China [12,13], and temperate broad-leaved forest in Japan [14,15]. Given that incubation experiments are widely known to yield different results from field-based evaluations, even if the soils used were collected from the same site [16,17], it is important to measure the response of ecosystems in the field.

To consider indirect carbon sequestration through the stimulation of plant productivity following biochar application to a forest ecosystem, it is essential to clarify changes in soil nutrient dynamics consisting of fluxes and pools. Inorganic nitrogen (N; mainly NH_4^+ and NO_3^-) in soils is generally considered to be the main factor that limits the net primary production of forest ecosystems [18,19]. In particular, temperate forests are known to be nitrogen-limited [20], and Pregitzer et al. [21] reported a significant increase in net primary production after N fertilizer application in temperate forests. Because mineralization and subsequent nitrification are the primary sources of inorganic N in forest soils, it is essential to determine the effects of biochar application on these processes. Wildfire-produced biochar may enhance N mineralization and nitrification in a temperate forest [22]. However, the effects of biochar application for carbon sequestration on mineralization and nitrification in a forest ecosystem are less well studied, and even the few results obtained to date vary widely among case studies. For example, Gundale et al. [23] reported that biochar application enhances net N mineralization in Scots pine forest of Sweden. In contrast, Palviainen et al. [24] reported nonsignificant changes in N mineralization rates and nitrification rates in the mineral soil layer (0–10 cm depth) in Scots pine forest in Finland. Song et al. [25] reported that the gross nitrification rate in the topsoil layer (0–20 cm depth) decreased in response to biochar application in Moso bamboo forest. Thus, the effects of biochar application on mineralization and nitrification of forest soils in the field need to be examined in various forests. However, to our knowledge, there are no field studies on the effects of biochar application on nitrogen transformation rates in warm-temperate broad-leaved forests.

We aimed to clarify the impact of ligneous biochar application to the forest floor on net mineralization and nitrification rates in a temperate broad-leaved forest in Japan. A field experiment was designed in which biochar was applied to the forest floor at rates equivalent to 0, 5, and 10 Mg ha^{-1}. Net N mineralization and nitrification rates were evaluated using the field-based resin–core method [26]. In this approach, an intact soil core sandwiched between ion-exchange resin enables the estimation of net mineralization and nitrification rates in as close to the original field environment as possible without destruction of the soil structure and disturbance of water and gas flow.

2. Materials and Methods

2.1. Study Sites

A field experiment was conducted in a warm-temperate secondary deciduous forest in Honjo, Saitama Prefecture, Japan (36°12′ N, 139°10′ E). The forest was dominated by oak (*Quercus serrata* Thunb. ex Murray) of approximately 20 m height, and the forest floor was densely covered by *Pleioblastus chino* (Franch. et Sav.) Makino (an evergreen dwarf bamboo) approximately 0.5 m high. The tree density and aboveground biomass of this forest in 2015 were 765 stems ha^{-1} and 167 Mg ha^{-1}, respectively. The total basal area was 25.2 m^2 ha^{-1} of which *Q. serrata* occupied approximately 85%. A more-detailed description of the forest structure is provided by Ohtsuka et al. [14]. The study site has a warm-temperate monsoon

humid climate. Annual mean air temperature and precipitation are 15.0 °C and 1286.3 mm, respectively (1981–2010). The soil was originally derived from alluvion volcanic ash and is classified as Alic Hapludands [14].

2.2. Experimental Design

A total of 12 experimental plots (20 m × 20 m) were established at the study site. The plots were randomly assigned to the three treatments with different amounts of biochar applied (n = 4): 0 (control), 5, and 10 Mg ha^{-1} (plots C0, C05, and C10, respectively). Commercially available woody biochar (particle size < 5 mm) was spread manually on the surface of the organic layer on the forest floor in late November 2015. Shortly thereafter, in December, the applied biochar was covered by fresh litter from litterfall. The biochar used was commercially available and was prepared from broad-leaved and coniferous wood chips pyrolyzed at 600–700 °C (Shiratori Super MOKUTAN, grade C; Shiratori Mokuzai Kakoh Cooperative Society, Gifu, Japan). The carbon and N contents of the biochar were 71% and 0.78%, respectively.

The soil temperature (5 cm depth) and volumetric soil water content at approximately 0−5 cm depth were measured once a month from April to December 2016 using stick thermometers (TT-508; Tanita, Tokyo, Japan) and soil moisture sensors (ML3 ThetaProbe; Delta-T Devices, Cambridge, UK). At each measurement, temperature and water content were measured at 48 points per plot.

2.3. Net N Transformation Rates in the Field

The net mineralization and nitrification rates were measured using the field-based resin–core method [26]. An intact soil core placed in a cylinder was sandwiched between bags filled with ion-exchange resin, which traps NH_4^+ and NO_3^- entering and leaving the soil core. This structure prevents plant root penetration and therefore allows the absorption of inorganic ions by plant roots to be ignored. In addition, this structure permits exchange of gases and water with the enclosed soil, which maintains a relatively "open" soil system and therefore tracks N transformation under conditions similar to the original soil environment. Net N transformation (net mineralization and net nitrification) rates can be estimated based on the changes in the amount of inorganic N (NH_4^+ and NO_3^-) before and after incubation.

2.3.1. Installation and Recovery of Resin Cores

One resin core was placed at a randomly selected point in each of the 12 experimental plots. The organic soil layer, including the applied biochar, was removed carefully and the top of the mineral soil layer (A layer) was exposed. Then, a polyvinyl chloride cylinder (inner diameter 5.6 cm, height 5 cm) was inserted vertically and removed to obtain the soil core (0–5 cm depth of the A layer). Bags made from non-woven polyethylene cloth containing approximately 20 g (wet weight) ion-exchange resin (Amberlite MB-1; Organo, Tokyo, Japan) were attached to the top and base of the soil core. The column was backfilled to the original point from which it was collected and the organic soil layer, including the biochar, was returned on top of the column carefully to minimize disturbance. A separate soil core was collected in the same manner near the location where the resin core was backfilled and was used as a control soil sample.

Resin cores were installed in April and August 2016, and recovered in August and December 2016, respectively. The net N transformation rates during April–August and August–December were determined. The collected soil and resin bags were separately stored at 4 °C until analyzed.

2.3.2. Quantification of NH_4^+ and NO_3^- in Soil and Resin

Inorganic N (mainly NH_4^+ and NO_3^-) was extracted from the soil and resin using 2 M KCl with a 1:10 ratio of soil (or resin) to 2 M KCl. Soil or resin suspended in 2 M KCl was shaken for 1 h at 200 rpm at room temperature. The suspension was filtered with filter paper (No. 2; ADVANTEC, Tokyo, Japan) and the filtrate was used for further analysis.

Ammonium (NH_4^+) was quantified using the indophenol method [27]. Sodium phenol nitroprusside (1.0 mL) and 1.0 mL sodium hypochlorite solution were added to 2.0 mL of the sample. After standing at room temperature for 45 min, the absorbance was measured using a spectrophotometer (UV-1800; SHIMADZU, Tokyo, Japan) at a wavelength of 635 nm. Nitrate (NO_3^-) was measured using a partial modification of the Cataldo method [28]. An aliquot (0.5 mL) of the sample was evaporated to dryness and 0.4 mL of 5% salicylic acid–sulfate solution was added and stirred. After standing at room temperature for 20 min, 10 mL of 2 M sodium hydroxide was added. After standing for a further 20 min, the absorbance was measured at a wavelength of 410 nm using a spectrophotometer.

2.3.3. Calculation of Net N Mineralization and Nitrification

The net amount of mineralized N was calculated by subtracting the total amount of NH_4^+ and NO_3^- in the control soil from the total amount of NH_4^+ and NO_3^- in the core soil and lower resin after incubation. Similarly, the net nitrification was determined as the difference in the amount of NO_3^- between the control soil and the resin core (core soil and lower resin) after incubation. The net mineralization or nitrification rates were determined by dividing the net changes in inorganic N (NH_4^+ and NO_3^-) or NO_3^- by the number of incubation days and the rates were expressed on a per area basis (μg N m^{-2} day^{-1}).

2.4. Soil Analyses

Soil samples (0–5 cm depth of the A layer) were collected monthly from April to December 2016, using a 100 cc stainless steel soil corer. Soil samples were collected from five points per plot at each sampling date and mixed in one bag to obtain one composite sample for soil analyses per plot. To clarify the seasonal changes in the pool size of inorganic N in the soil, the NH_4^+ and NO_3^- concentrations in the field soil samples were determined in the same manner described in Section 2.3.2 (Quantification of NH_4^+ and NO_3^- in soil and resin).

The soil pH (H_2O) (fresh soil:water = 1:5, *w*/*w*) was measured for the samples collected in June, September, and December 2016, with a pH meter. The soil samples were freeze-dried, passed through a 2 mm mesh sieve, ground into a powder with a mortar, and the total C and N content was determined using a CN analyzer (Sumigraph NC-22; Sumika Chemical Analysis Service, Tokyo, Japan).

Microbial biomass was measured using the adenosine triphosphate (ATP) method [29,30]. A portion of the collected soil was freeze-dried and stored at −80 °C. Freeze-dried soil samples (1.0 g, dry weight) placed in 50 mL plastic centrifuge tubes were extracted by addition of 10 mL trichloroacetic acid extract solution (a mixture of 0.5 M trichloroacetic acid and 0.25 M disodium hydrogen phosphate) followed by immediate ultrasonic homogenization for 2 min (VP-30S; TAITEC, Tokyo, Japan). Tubes were kept in ice-cold water during this procedure. After centrifugation of the soil suspensions at 7000 rpm for 20 min at 4 °C (CR20G; HITACHI, Tokyo, Japan), 10 μL of the clear supernatant was carefully transferred to a 1.5 mL microtube, to which 990 μL of 0.025 M HEPES buffer (pH 7.0) was added (100-fold dilution), and the mixture was vortexed. Samples (100 μL) were dispensed into microplates and used for ATP determination. Sample ATP concentrations were quantified based on the luminescence intensity generated by the luciferin–luciferase enzyme method using the ATP standard reagent kit (AF-2A1; DKK-TOA, Yamagata, Japan) and the ATP assay reagent kit (AF-3L1; DKK-TOA, Yamagata, Japan). Luminescence intensity was measured with a microplate luminometer (GloMax96; Promega, Madison, WI, USA). The ATP concentration in the soil was converted to microbial biomass (mg C g^{-1}) using the factor of 11 μmol ATP (g biomass C)$^{-1}$ [30].

2.5. Statistical Analysis

One-way content, soil pH, soil total C and N concentrations, and net N transformation (mineralization and nitrification) rates during each incubation period with biochar treatment as a factor. In addition, two-way ANOVA was used for analysis of the inorganic N

pool and microbial biomass data with sampling timing (month) and biochar treatment as factors. The significance of differences among treatments was tested using Tukey's test. All statistical analyses were conducted using the statistical computing language R (version 4.3.0) [31] analysis of variance (ANOVA) was used to analyze the data for soil temperature, soil water.

3. Results

3.1. Soil Environment

All variables tended to increase after biochar application, but only soil pH and soil carbon/nitrogen (C/N) ratio showed significant differences among treatments (Table 1).

Table 1. Mean (±SD) soil physicochemical properties for three biochar application treatments during the experimental period.

Treatment	Soil Temperature (°C)	Water Content (%)	pH	Total Carbon (mg g^{-1})	Total Nitrogen (mg g^{-1})	Soil C/N
C0	17.9 ± 5.86 ^{a}	21.6 ± 4.06 ^{a}	4.67 ± 0.07 ^{a}	76.8 ± 15.2 ^{a}	4.75 ± 0.89 ^{a}	16.2 ± 0.13 ^{a}
C05	18.0 ± 5.93 ^{a}	22.5 ± 3.99 ^{a}	4.80 ± 0.08 ^{b}	78.7 ± 20.0 ^{a}	4.83 ± 1.15 ^{a}	16.3 ± 0.27 ^{a}
C10	18.2 ± 5.85 ^{a}	23.1 ± 3.85 ^{a}	4.82 ± 0.13 ^{b}	87.7 ± 20.7 ^{a}	5.25 ± 1.23 ^{a}	16.7 ± 0.16 ^{b}

Note: C/N: carbon/nitrogen ratio; C0, C05, and C10: 0 (control), 5, and 10 Mg biochar ha^{-1}, respectively. Means followed by different lowercase letters within a column differ significantly ($p < 0.05$; Tukey's test).

The pH of soil treated with biochar (C05 and C10) was significantly higher than that of the C0 soil ($p < 0.05$). The soil C/N ratio tended to increase with biochar application and that of the C10 treatment was significantly higher than those of C0 and C05 ($p < 0.05$) (Table 1).

3.2. Net N Transformation Rates

3.2.1. Mineralization

From April to August, net N mineralization rates in the plots treated with biochar (C05 and C10) were significantly lower than those of the control (C0; $p < 0.01$), and they were 54.9% and 47.3% of the control, respectively (Figure 1a).

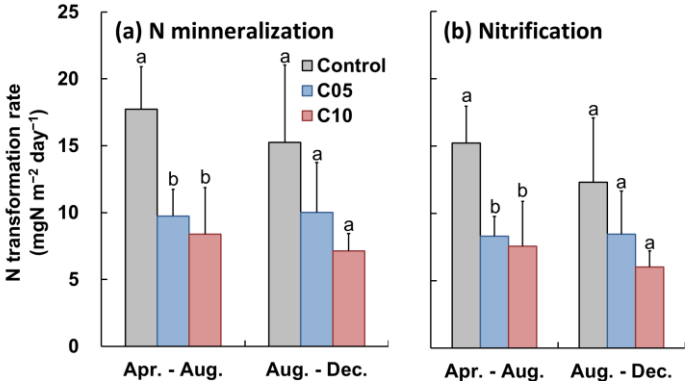

Figure 1. Net nitrogen (N) mineralization rate (**a**) and net nitrification rate (**b**) for three biochar application treatments in two experimental periods. Bars and error bars indicate means ± SD ($n = 4$). Different lowercase letters above bars indicate a statistically significant difference between treatments ($p < 0.01$). C0, C05, and C10: 0 (control), 5, and 10 Mg biochar ha^{-1}, respectively.

No significant difference in mineralization rate was observed between the C05 and C10 treatments ($p > 0.05$). From August to December, no significant difference in the rate was detected among treatments, but the rate tended to decrease in the plots treated with biochar (C05 and C10). The rates in C05 and C10 were 65.7% and 46.8% of the control, respectively.

3.2.2. Nitrification

Similar to the mineralization rate, the net nitrification rates from April to August in the plots treated with biochar (C05 and C10) were significantly lower than those of the control (Figure 1b), and they were 54.6% and 49.7% of the control, respectively. Although a trend for the rate to decrease with biochar application was observed from August to December (the rates in C05 and C10 were 68.8% and 48.9% of the control, respectively), the differences were not significant.

3.2.3. Nitrification Ratio

The nitrification ratio, which indicates the proportion of the mineralized N that was further transformed to NO_3^- (i.e., nitrified), was determined by dividing the net nitrification rate by the net mineralization rate. The mean (±SD) ratios in the C0, C05, and C10 treatments were, respectively, 86.0% ± 4.8%, 85.9% ± 4.2%, and 89.1% ± 4.3% from April to August, and 80.1% ± 7.7%, 84.2% ± 2.4%, and 84.4% ± 5.4% from August to December. No significant difference in the nitrification ratio was observed among the treatments in both experimental periods.

3.3. Pool Sizes of Extractable NH_4^+ and NO_3^-

Average (±SD) NH_4^+ concentrations in the C0, C05, and C10 treatments during the experiment period were 8.4 ± 3.0, 6.7 ± 3.5 and 5.3 ± 2.7 μg N g^{-1}, respectively. The concentration tended to decrease with biochar application and that in the C10 treatment was significantly lower than that of the control ($p < 0.05$) (Figure 2a).

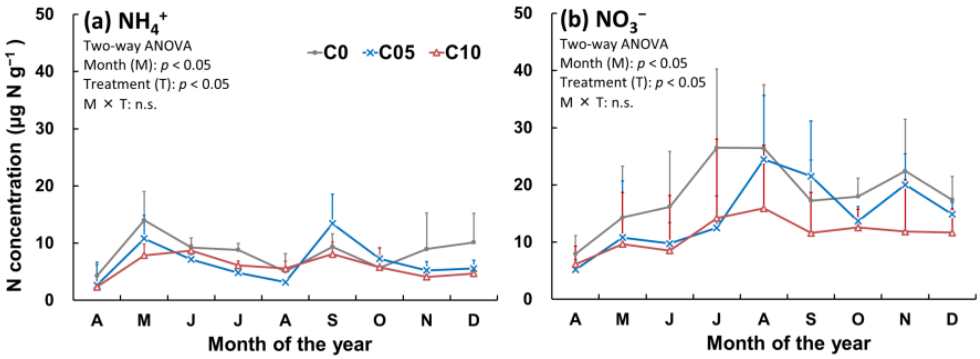

Figure 2. Ammonium (NH_4^+) (**a**) and nitrate (NO_3^-) (**b**) concentrations in the soil for three biochar application treatments. Values and error bars are means ± SD ($n = 4$). n.s.: not significant. C0, C05, and C10: 0 (control), 5, and 10 Mg biochar ha^{-1}, respectively.

Average (±SD) NO_3^- concentrations during the experimental period tended to decrease with biochar application, i.e., C0 (18.5 ± 5.92 μg N g^{-1}), C05 (14.8 ± 6.19 μg N g^{-1}), and C10 (13.7 ± 6.73 μg N g^{-1}). The NO_3^- concentration in C10 was significantly lower than that of C0 ($p < 0.01$) (Figure 2b).

3.4. Microbial Biomass

Average (±SD) microbial biomass during the experimental period in C0, C05, and C10 was 0.13 ± 0.026, 0.13 ± 0.029, and 0.14 ± 0.043 mg C g^{-1}, respectively (Figure 3). No significant differences among the treatments were observed.

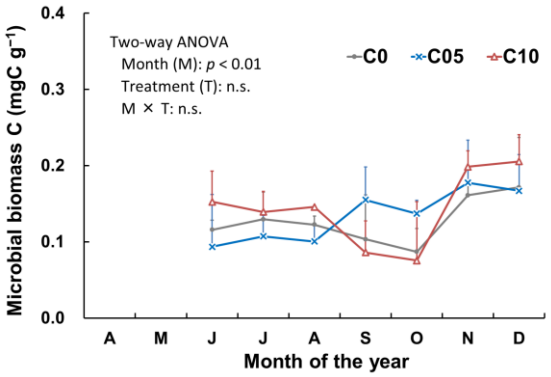

Figure 3. Soil microbial biomass for three biochar application treatments. Values and error bars are means ± SD (n = 4). n.s.: not significant. C0, C05, and C10: 0 (control), 5, and 10 Mg biochar ha^{-1}, respectively.

4. Discussion

4.1. Net N Transformation Rates

The net mineralization rate in the mineral soil layer (A layer) was reduced to 46.8–65.7% of the control by biochar application in this study (Figure 1), which contrasted with the findings of certain previous studies. In boreal forests, for example, biochar applications comparable to those in the present study (5 or 10 Mg ha^{-1}) had no significant effect on the mineralization rate [24]. Gogoi et al. [22] reported that wildfire-produced biochar enhances N mineralization in temperate forest ecosystems. Zhang et al. [32] conducted a meta-analysis of field studies of biochar application with tillage, irrigation, and fertilization and concluded that biochar significantly enhances N mineralization and nitrification. Thus, biochar effects on N mineralization vary markedly depending on the raw materials, pyrolysis temperature, period after application, and biochar C/N ratio [33]. There are three major possible causes for the reduction in the mineralization rate observed in the present study.

1. Acceleration of immobilization

Even persistent biochar contains a labile carbon fraction [34] that could be used by microorganisms for their growth (increase in biomass, immobilization). This microbial N immobilization may contribute to a reduction in the net N mineralization rate after biochar application, as discussed in some previous studies [34,35]. In particular, it has been suggested that the application of biochar with a high C/N ratio, such as beech wood biochar, promotes N immobilization owing to the increase in the amount of carbon available for microbes in the soil [36]. The more N that is immobilized, the more soil inorganic N that will be incorporated into the microbial biomass, which results in a decrease in the net (apparent) rate of mineralization. However, in the present study, no significant difference in microbial biomass was observed between treatments with and without biochar application throughout the experimental period (Figure 3). Thus, it is unlikely that N immobilization was promoted by biochar application. Instead, the mineralization and nitrification rates both decreased without any change in microbial biomass, suggesting that the mineralization activity (i.e., mineralization rate per unit microbial biomass) of the microbial community decreased.

2. Changes in microbial communities

Changes in soil physicochemical properties in response to biochar application alter the microbial community structure [34,37,38]. For example, several studies have shown that the fungal/bacterial biomass ratio (F/B) changes after biochar application [39,40]. Fungi have lower N requirements than bacteria, and there is a relationship between the F/B and C/N ratios of the soil [41]. In the present study, the soil C/N ratio increased significantly following biochar application in the C10 treatment (Table 1), which may have affected the F/B ratio. According to Li et al. [8], the mechanisms of changes in the F/B ratio after

biochar application may include the following. (1) The colonization by fungi of micropores in the biochar may be prohibited because the typical size of fungal hyphae is generally larger than bacterial cells. As a result, bacteria may be better protected from grazing than fungi. (2) Bacterial growth may be promoted by biochar application because bacteria prefer a neutral or alkaline soil pH. (3) Fungi may have the ability to colonize lower-quality carbon materials, such as charcoal, which contains a higher percentage of aromatic carbon compounds. These microbial community changes may have affected various soil microbial processes, including N mineralization and nitrification.

3. Adsorption of substrate by biochar

Biochar can adsorb proteins and free amino acids, which are substrates for microbial N mineralization [36]. Berglund et al. [16] reported that biochar application alone did not change the rate of mineralization, but the addition of biochar in combination with glycine increased the mineralization rate. Furthermore, a relatively long-term (250–500 days) study by Zimmerman et al. [42] led to the hypothesis that the carbon mineralization of organic matter is reduced by biochar application because the soil biota and its extracellular enzymes lose access to the organic matter captured in the pores of the biochar (termed encapsulation). In forest soils, the main source of organic N is litterfall from tree species, which occurred soon after biochar application in the present study. Therefore, the organic N readily available to the microorganisms, such as soluble proteins and amino acids in litter leachate or derived from the initial decomposition of flesh plant litter, may have been adsorbed onto the fresh biochar, resulting in lower organic-form N (substrate) in the treated soil than in the control plots.

As well as the N mineralization rate, the nitrification rate was reduced to 48.9%–68.8% of the control by biochar application in this study (Figure 1). However, the nitrification ratio (ratio of nitrification rate to mineralization rate) did not change significantly; thus, it is unlikely that the activity of nitrifying bacteria decreased. Rather, the decrease in the nitrification rate may have resulted from a decrease in the amount of ammonia (the substrate for nitrification) in the soil caused by depression in N mineralization. Microbial biomass and activity increase with elevation in soil pH in acidic soils [43]. In the present study, however, the nitrification rate did not increase significantly despite a significant increase in soil pH (C0: 4.67; C10: 4.82) after biochar application (Table 1). Similar to the present study, Palviainen et al. [24] reported no significant change in nitrification rates in boreal forest in response to biochar application, which the authors attributed to an increase in pH (from 4.1 to 4.3) insufficient to increase nitrification rates. It has also been noted that the nitrification rate may be reduced by nitrification inhibitors, such as α-pinene and polycyclic aromatic hydrocarbons, that are included in the biochar [44]. Therefore, a negative effect caused by nitrification inhibitors on the nitrification rate may mask the increase in the nitrification rate owing to the increase in pH, resulting in no apparent change in the net nitrification rate.

4.2. Pool Sizes of NH_4^+ and NO_3^-

The NH_4^+ and NO_3^- concentrations in the soil were reduced to 63.4%–79.4% and 73.9%–80.0% of the control by biochar application, respectively (Figure 2). It is natural to assume that the depression in net mineralization and nitrification (Figure 1) contributed to the decrease in NH_4^+ and NO_3^- concentrations in the soil. However, there are other possible reasons for the decrease in inorganic N pool size in the soil.

For example, plant N requirements may have been amplified by biochar application. Tanazawa et al. [15] noted that biochar application increases the leaf N concentration and photosynthesis of *Q. serrata* at a similar study site to the present one. Furthermore, Ohtsuka et al. [14] observed that biochar application significantly increases the number of reproductive organs of *Q. serrata* at the same site as the present study. These results indicate that more N was absorbed by the plants after biochar application. The detailed mechanism of this result is not yet fully understood, but possible physiological factors

include increased Mg and S supply, reduced nutrient loss from leaching, and reduced aluminum toxicity after biochar application [14,15].

An additional possible reason for the decrease in inorganic N pool size in the soil is the adsorption of inorganic N in litter leachate by the biochar (which was produced at 600–700 °C from woody materials). Biochar can adsorb various ions, including NO_3^- and NH_4^+ [45]. In addition, biochar prepared under a temperature exceeding 600 °C adsorbs NO_3^- more effectively [46]. Furthermore, woody biochar has a high adsorption capacity owing to the larger surface area [47]. Although we currently lack information on the amount of inorganic N that can be adsorbed by the biochar used in the present study, especially in a field environment, the possibility of inorganic N adsorption by the biochar cannot be ruled out and should be investigated in future studies.

5. Conclusions

To the best of our knowledge, this is the first field-based study to report reduced N mineralization and nitrification rates following biochar application in a warm-temperate broad-leaved forest ecosystem. The reduction in the N mineralization rate and inorganic N pool size in soils is likely to affect plant photosynthesis and growth. Thus, nitrogen dynamics will need to be considered when biochar is applied in warm-temperate broad-leaved forests for carbon sequestration. Furthermore, soil nitrogen dynamics responded differently to biochar application, even when the same amount of biochar was applied [24], underscoring the importance of considering differences in biochar characteristics (raw material, pyrolysis temperature, C/N, etc.) and site characteristics (climate, dominant tree species, soil type, etc.). In addition, the physicochemical properties of biochar change over time [3], and biochar is known to settle downward in the soil profile over time [48]. Therefore, the long-term impact of biochar application on inorganic N fluxes and pools should be investigated.

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