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Interactive effects of compost and biochar on nitrate leaching, microbial biomass and plant growth

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Research Article

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Posted Date: November 20th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11559118/v1

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Interactive effects of compost and biochar on nitrate leaching, microbial biomass and plant growth

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12 **Abstract**

13 Background and Aims: Nitrogen (N) losses through nitrate (NO_3^-) leaching reduce N fertiliser
14 efficiency and plant growth, while contributing to environmental pollution, highlighting the
15 need for improved soil management strategies.

16 Methods: In a controlled pot experiment, we investigated the effects of biochar and compost,
17 applied individually and in combination at 1.5% (m/m), on N leaching, soil properties,
18 microbial biomass, and plant growth (ryegrass, *Lolium perenne*) under contrasting N inputs (0
19 vs 50 mg N kg^{-1} or ~100 kg N ha^{-1}).

20 Results: As expected, NO_3^- was the dominant form of inorganic N loss across all treatments.
21 Compost significantly reduced NO_3^- leaching regardless of N addition, likely due to enhanced
22 microbial immobilisation, whereas biochar reduced N leaching only when no N was added.
23 Biochar increased total carbon (C), while compost increased soil N, dissolved organic C, and
24 microbial biomass C and N. Compost application negatively affected plant biomass and N
25 uptake, likely because of short-term microbial N immobilisation. However, combining biochar
26 with compost improved plant growth compared to compost alone, suggesting that biochar
27 mitigated adverse effects of compost. Conclusion: Overall, our findings demonstrate that
28 compost is more effective than biochar in reducing N leaching, while biochar can mitigate the
29 immobilisation effect of compost on plant performance. These results highlight the importance
30 of considering interactions between organic amendments and N availability to optimise plant
31 growth and reduce environmental impacts.

32

33 ***Keywords*** *: ^{15}N recovery, microbial immobilisation, nitrogen leaching, ryegrass, soil fertility*

34

Introduction

Nitrogen (N) is one the most important essential elements that drives primary productivity. Therefore, a large amount of N is applied in agricultural systems to ensure production. However, much of the applied N is lost by different pathways including volatilisation, leaching and denitrification. Among the various pathways of N loss, nitrate (NO_3^-) leaching is particularly important due to its high solubility, weak adsorption to soil reactive surfaces, and rapid mobility through the soil profile (Weil and Brady 2016; Bagheri et al. 2021; Wang et al. 2015). Consequently, NO_3^- readily moves beyond the root zone, reducing fertilizer N use efficiency and contributing to groundwater contamination and eutrophication of surface water bodies (Weitzman et al. 2022; Padilla et al. 2018). These issues highlight the importance of developing soil management strategies that enhance N retention in the soil while sustaining agricultural productivity.

Organic amendments are widely recognized as potential tools for improving soil quality and regulating nutrient cycling processes (Lal 2004; Ouyang et al. 2022; Lehmann and Joseph 2015). Among these, biochar and compost are particularly important due to their contrasting physicochemical properties and functional roles in soil systems. Biochar is a carbon (C)-rich, porous, and chemically stable material produced through pyrolysis of biomass. Its high surface area and stability enable improvements in soil physical structure, water retention, and nutrient dynamics through sorption processes and indirectly by microbial interactions (Sohi et al. 2010; Lehmann and Joseph 2015; Omondi et al. 2016). In contrast, compost is a biologically active organic amendment rich in labile C and nutrients, which stimulates microbial activity and accelerates nutrient cycling. Through its readily decomposable C fraction, compost can enhance microbial N immobilization, thereby temporarily reducing the availability of mineral N forms such as NO_3^- (Aoyama and Nozawa 1993; Wang et al. 2021; Geisseler et al. 2021).

Despite their recognised benefits, the effectiveness of biochar and compost in mitigating N losses remains highly variable across studies. Biochar has been shown to reduce NO_3^- leaching in some cases; however, its capacity to directly retain NO_3^- is limited due to the anionic nature of NO_3^- and the predominantly negative charge of soil and biochar surfaces (Yang et al. 2017; Borchard et al. 2019). Therefore, reductions in NO_3^- loss are often attributed to indirect mechanisms such as improved soil water retention, altered pore structure, and enhanced microbial immobilization of N (Lehmann et al. 2011; Clough et al. 2013). However, these effects are inconsistent, particularly under high N loading, where microbial and physicochemical retention processes may become saturated (Agegnehu et al. 2016; Liu et al. 2025).

In contrast, compost is generally more effective in reducing NO_3^- leaching due to its ability to stimulate microbial immobilization via labile C inputs (Manzoni et al. 2012; Kizewski et al. 2019; Cao et al. 2021). Nevertheless, this effect is strongly context-dependent and influenced by N availability, decomposition rates, and environmental conditions (Aytenew and Bore 2020; Hamada et al. 2024). Moreover, compost may also induce short-term constraints such as increased salinity or phytotoxicity, which can negatively affect plant growth despite improvements in soil nutrient status (Bernal et al. 2009). These contrasting outcomes highlight the complexity of compost-induced soil processes and the need for integrated assessment across chemical, microbial, and plant systems.

Recent studies suggest that combining biochar and compost may provide synergistic benefits by integrating the rapid nutrient release of compost with the stabilizing and sorptive properties of biochar (Steiner et al. 2010; Liu et al. 2012; Joseph et al. 2014). Such interactions may improve N retention by balancing mineralization and immobilization processes, while also enhancing soil structure and nutrient buffering capacity. However, empirical evidence remains

inconsistent, and the extent to which biochar can mitigate the negative effects of compost, particularly under varying N inputs, is not yet fully understood.

At the microbial level, soil processes are strongly regulated by C and N availability, commonly described through the framework of ecological stoichiometry (Sterner and Elser 2002; Cleveland and Liptzin 2007). Microbial growth and activity depend on the balance of these resources, and deviations from optimal C:N ratios can shift microbial processes between N immobilization and mineralization (Sinsabaugh et al. 2009; Mooshammer et al. 2014). Carbon-rich amendments such as compost can alleviate C limitation and enhance microbial N uptake, particularly with inorganic N addition, thereby altering biomass production and nutrient cycling dynamics (Blagodatskaya and Kuzyakov 2008; Bengtson et al. 2012; Manzoni et al. 2012). However, the extent to which these stoichiometric controls operate under combined amendment applications remains insufficiently understood.

These soil microbial processes ultimately influence plant growth and N uptake. Although organic amendments are generally expected to enhance soil fertility, their short-term effects on plant performance can be inconsistent. Compost application may reduce plant growth due to temporary N immobilization, elevated salinity, or phytotoxic compounds, whereas biochar may improve plant performance by enhancing soil physical conditions, nutrient retention, and water availability (Wong et al. 2022; Lehmann and Joseph 2015). The interaction between these amendments and N inputs is therefore critical in determining overall system productivity and N use efficiency.

Given these uncertainties, there is a clear need to evaluate how biochar and compost, applied individually and in combination, influence N dynamics, soil chemical properties, microbial N immobilization, and plant responses under contrasting N availability. In particular,

106 understanding the interactions between C inputs and N supply is essential for optimizing
107 amendment strategies that balance nutrient retention with crop productivity.

108 Therefore, this study investigated the effects of biochar and compost on N leaching, soil
109 chemical properties, microbial biomass, and plant growth under contrasting N inputs.
110 Specifically, we aimed to: (i) quantify the relative contributions of NO_3^- and ammonium (NH_4^+)
111 to N losses, (ii) determine how biochar and compost influence N leaching dynamics under
112 different N levels, and (iii) assess their impacts on microbial biomass and plant N uptake.

113 With the widely held notion that NO_3^- would be the dominant form of N loss due to its high
114 mobility in soil we hypothesized that (1) biochar's effectiveness in reducing NO_3^- leaching
115 would decline under high N availability; (2) compost would reduce NO_3^- leaching more
116 effectively than biochar through enhanced microbial immobilization; and (3) combined
117 application of biochar and compost would produce synergistic effects on plant N uptake and
118 overall N retention.

119 **Materials and methods**

120 ***Materials and experimental design***

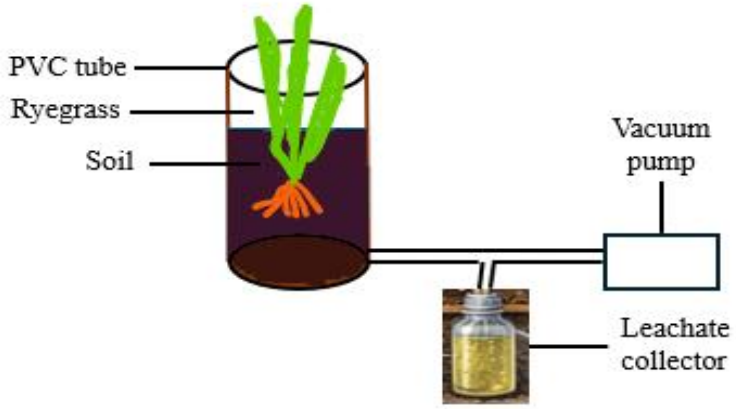
121 A pot experiment was conducted using a completely randomized design in a full factorial
122 scheme with three factors (biochar, compost, and N addition) and two levels for each factor,
123 i.e., with and without treatment application, comprising 8 treatment combinations and three
124 replicates per treatment, resulting in a total of 24 pots. The soil was collected from a non-
125 fertilized grassland to a depth of 20 cm at Lansdowne farm, Cobbitty, NSW, Australia
126 (34°01'25.1"S 150°39'46.8"E). The soil was classified as a Red Chromosol (or a Luvisol in the
127 World Reference Base) with 57.1 % sand, 11.6 % silt and17.9 % clay, a pH of 6.66, and total

C and N contents of 1.39 % and 0.11 %, respectively. Biochar was sourced from *Eucalyptus* feedstock, which had undergone pyrolysis in a continuous flow kiln at temperatures up to 550°C for 30-40 minutes. The biochar had a pH of 6.65, an electric conductivity (EC) of 47 µS cm^{-1}, and total C and N contents of 64.8% and 0.05%, respectively. We used sugarcane (*Saccharum officinarum*) compost, which was prepared following the procedure as described by Syaranamual et al. (2025). Briefly, after chopping sugar cane residue into small pieces (< 1 cm), the material was kept in a container at 20 °C and 60% moisture (w/w), and turned over weekly. The final compost had a pH of 7.11, and EC of 1365 µS cm^{-1}, and total C and N contents of 51.5% and 0.64%, respectively.

Pots (diameter: 11 cm, height: 15 cm) were filled with 1.4 kg soil that was previously mixed with 21 g biochar (1.5%) for the biochar only treatment, 21 g compost (1.5%) for the compost only treatment, and 10.5 g biochar and 10.5 g compost for the combined biochar and compost treatment. Biochar and compost were added on a dry weight basis. Each pots contained an outlet at the bottom for leachate collection (see below). We added the N fertilizer in the form of NH_4NO_3 at a rate of 50 mg kg^{-1} (~100 kg N ha^{-1}). To pots with N fertilization, we also added ^{15}N as $(NH_4)_2SO_4$ (99 atom%) at 1 mg N kg^{-1} in 10 ml of deionized water. After set-up, pots were left to equilibrate for 3 days at a moisture content of 70% water holding capacity (WHC), which was calculated following soil saturation and weighing soil after drainage. Thereafter, 0.15 g of ryegrass (*Lolium perenne*) seeds were sown in each pot. The pots were kept in a plant growth room under environmentally controlled conditions with 16 h day^{-1} of light, a temperature of 25°C (day) and 20°C (night), and the soil moisture at 30% gravimetric content (70% WHC). Because plants were growing fast, we decided to clip aboveground biomass after 21 days of growth, thereby simulating a grazing event. Pots were harvested after 42 days.

152 ***Soil leachates collection***

153 Soil leachate was collected at week 3 and week 6 after sowing. For each event, we added 210
154 ml of deionized water (simulating 100 mm rain events) to the top of the pot. We collected
155 leachates by connecting 250 ml plastic bottles to the outlet at the bottom of the pots and by
156 applying a -20 kPa suction using a vacuum pump, thereby mimicking the matric potential of
157 the soil at field capacity (Fig. 1).



158

159 **Fig. 1**. Schematic diagram of the leachate collection.

160 The volume of leachates was recorded and then analysed for NO_3^--N and NH_4^+-N on a flow
161 injection analyser using FIAlyzer-1000 System from FIA lab (Fluidics Intelligently
162 Automated, Seattle, WA, USA).

163 Total amounts of leached NO_3^--N and NH_4^+-N (μg pot^{-1}) were calculated by multiplying
164 concentrations with the volume of leachates for each sampling date. The pH and EC were
165 analysed using a pH meter (Metro-pH 320; Mettler-Toledo Instruments Ltd.) and EC meter
166 (Metro-pH 320; Mettler-Toledo Instruments Ltd.), respectively.

167

Soil sampling and analysis

Pots were harvested 42 days after sowing, and the soil was mixed to homogenize and then was sieved (2 mm) to remove roots. The pH and EC of the fresh sieved soil were determined in a soil slurry at a soil:water ratio of 1:5 (w/v) using a 5 g sample mixed with 25 ml of deionised water, which was stirred and left for 30 min at room temperature (22 °C) before measurement on a pH and EC meter.

The total carbon (TC) and total nitrogen (TN) were determined on a CHN analyzer (vario MACRO cube, Elementar Australia Pty Ltd, Sydney, NSW, Australia). Microbial biomass C (C_{mic}) and N (N_{mic}) at harvesting was measured using the fumigation-extraction method (Vance et al. 1987). Briefly, 10 g of fresh soil was fumigated with chloroform in a desiccator for 72 hours. Both the fumigated and 10 g of non-fumigated soil were shaken with 40 ml of 0.05 M K_2SO_4 for 1 hour, centrifuged for 5 minutes at 1100 *g*, and filtered through Whatman No. 42 filter paper. We analysed the extracts for total organic C and N on a TOC/TN-analyser (Shimadzu TOC-V csh, TNM-1, Kyoto, Japan). The C_{mic} and N_{mic} were calculated by subtraction of the C and N in the fumigated and non-fumigated samples, divided by a conversion factor of 0.45 for C (Beck et al. 1997) and 0.54 for N (Brookes et al. 1985), respectively. The total organic C and total N in the non-fumigated samples were regarded as dissolved organic C (DOC) and total dissolved N (TDN). Soils were further analysed for ^{15}N on an isotope ratio mass spectrometer (IRMS, HS2022, Sercon Limited, UK).

Plant sampling and analysis

On the harvest day, plants were sampled by separating the aboveground (shoot) and belowground (root) components. Shoots were dried in a drying oven at 60 °C until a constant weight was achieved, after which they were weighed. Roots were carefully retrieved from the

soil, washed to remove adhering soil particles, and then dried and weighed following the same procedure as for the shoots. Shoots from the first clipping and the harvest day, and roots were subsequently cut into small pieces and finely ground separately. The prepared samples were analysed for N concentration using a CHN analyzer. For ^{15}N determination, samples were analysed on the IRMS.

^{15}N recovery calculations

We calculated ^{15}N recovery in plant shoots, roots and soil ($^{15}N_{rec}$ in µg pot^{-1}) using a linear mixing model:

$$^{15}N_{rec} = N_{pool} \frac{atom\%\,^{15}N_{fert} - atom\%\,^{15}N_{nonfert}}{atom\%\,^{15}N_{added} - atom\%\,^{15}N_{nonfert}}$$

where N_{pool} is the N pool in plant shoots, roots and soil (in µg pot^{-1}), and atom%$^{15}N_{fert}$, atom%$^{15}N_{nonfert}$, and atom%$^{15}N_{added}$ are the ^{15}N atom% of plants, roots or soil in N fertilized pots, nonfertilized pots, and the ^{15}N label added, respectively.

Statistical analysis

We used three-way analysis of variance (ANOVA) to asses main and interactive effects of biochar, compost, and N addition for parameters from first and second leaching (NH_4^+, NO_3^-, pH and EC), soil properties after harvest (TC, TN, total C:N, DOC, TDN, microbial biomass C (C_{mic}), microbial biomass N (N_{mic}), microbial biomass C:N ratio ($C:N_{mic}$), pH and EC) as well as for plant parameters (shoot, root, and total biomass, and their N content). We used two-way ANOVA to test the main effect of biochar and compost on ^{15}N recovery in N fertilized pots only. If the ANOVA indicated statistical significance ($p < 0.05$), the Tukey's HSD test was used

as the *post hoc* tests to determine the different means among treatments. All statistics were done in JMP (version 19).

Results

Leachate pH and nutrient concentrations

We evaluated the effects of biochar, compost, and N application on NH_4^+, NO_3^-, pH, and EC for the two leaching events separately. We observed much larger NO_3^- leaching than NH_4^+ (Fig. 2). During the first leaching event, both biochar and compost significantly decreased and N addition increased NO_3^- leaching (Table, 1, Fig. 2). Biochar addition only reduced NO_3^- leaching without N addition, while compost addition reduced NO_3^- leaching both with and without N addition. During the second event, leaching of NO_3^- was smaller, and although similar patterns were observed as during the first leaching event, none of the treatments were significant (Table 1, Fig. 2).

Compost significantly increased, N addition significantly decreased, and biochar had no effect on pH during the first leaching event (Table 1, 2). During the second leaching event, an increase in pH with compost addition and a decrease in pH with biochar addition only occurred without N addition causing significant C×N and B×N interactions (Table 1). Biochar significantly reduced EC during the first and second leaching event, whereas compost increased EC without N addition, but less so with N addition (Table 2) resulting in significant C×N interactions (Table 1).

232 **Table 1**. ANOVA p values of the main and interactive effects of biochar (B), compost (C) and nitrogen addition (N) on ammonium (NH_4^+), nitrate

233 (NO_3^-), pH and electric conductivity (EC) at first and second leaching.

Source	NH_4^+ 1st ($\mu g\ pot^{-1}$)	NH_4^+ 2nd ($\mu g\ pot^{-1}$)	NO_3^- 1st ($\mu g\ pot^{-1}$)	NO_3^- 2nd ($\mu g\ pot^{-1}$)	pH 1st	pH 2nd	EC 1st ($\mu S\ cm^{-1}$)	EC 2nd ($\mu S\ cm^{-1}$)
Biochar (B)	0.42	**0.03**	**0.001**	0.15	0.11	**0.05**	**0.02**	**0.007**
Compost (C)	0.09	0.76	**<.0001**	0.12	**0.01**	0.15	0.24	**<.0001**
B×C	0.15	0.71	**0.0004**	0.21	0.06	0.12	0.16	**0.03**
Nitrogen (N)	0.11	**0.04**	**<.0001**	0.54	**0.04**	**0.0001**	**0.005**	**0.002**
B×N	0.19	0.07	**0.001**	0.80	0.76	**0.04**	0.88	0.07
C×N	**0.03**	0.19	**<.0001**	0.28	0.60	**0.004**	**0.01**	**0.001**
B×C×N	0.51	0.29	**0.001**	0.64	0.64	0.36	0.59	0.13

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Table 2. Leachate pH and EC during 1st and 2nd leaching event (mean±se, n = 3). Different letters behind values indicate differences among biochar, compost, and nitrogen treatments (posthoc Tukey test, $p < 0.05$).

Biochar	Compost	Nitrogen	pH 1st	EC 1st (µS cm^{-1})	pH 2nd	EC 2nd (µS cm^{-1})
No	No	No	5.49±0.93^{ab}	390±85^{ab}	6.84±0.30^{b}	87±2^{bc}
Biochar	No	No	5.33±0.60^{ab}	248±51^{b}	6.51±0.05^{b}	47±2^{c}
No	Compost	No	5.49±0.50^{ab}	653±50^{ab}	8.21±0.07^{a}	737±88^{a}
Biochar	Compost	No	7.00±0.15^{a}	323±36^{ab}	6.94±0.45^{b}	354±137^{b}
No	No	Nitrogen	4.52±0.15^{b}	974±281^{a}	6.33±0.19^{b}	88±32^{bc}
Biochar	No	Nitrogen	4.47±0.10^{b}	911±68^{ab}	6.47±0.34^{b}	77±19^{bc}
No	Compost	Nitrogen	5.20±0.28^{ab}	761±258^{ab}	6.11±0.27^{b}	255±16^{bc}
Biochar	Compost	Nitrogen	6.17±0.45^{ab}	290±38^{ab}	6.00±0.11^{b}	170±16^{bc}

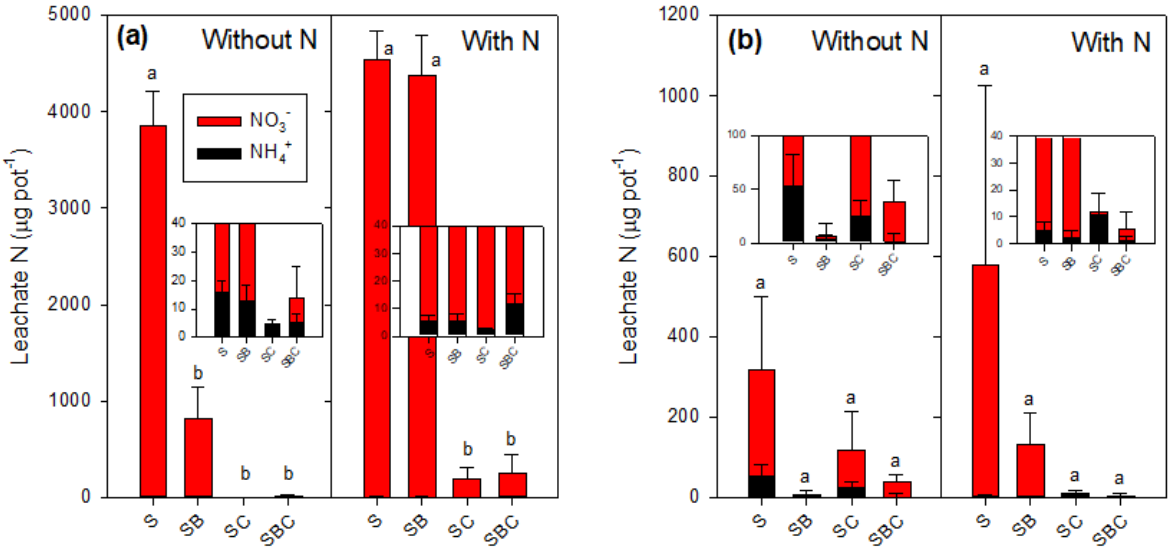


Fig. 2. Leaching of ammonium (NH_4^+) and nitrate (NO_3^-) during (a) the first and (b) the second leaching event in response to biochar, compost, and nitrogen (N) treatments. The left and right subpanels represent N leaching without and with N application, respectively. Inserts show results for low concentrations in detail. Error bars represent se. Treatment abbreviations: S: soil only, SB: soil with biochar, SC: soil with compost, SBC: soil with biochar and compost. Different letters above bars indicate significant differences in total leachate N among treatments (posthoc Tukey HSD test, $p < 0.05$).

Soil properties

At harvest, across all other treatments, biochar addition insignificantly increased total C and C:N, and C:N_{mic} in soil, but decreased DOC, TDN, and EC (Table 3, 4, Fig. 3). Across all other treatments, compost addition increased soil TN, DOC, C_{mic}, N_{mic}, and EC, and decreased total C:N and C:N_{mic}. The combined application of biochar and compost significantly influenced multiple soil properties, including TC, TN, microbial biomass, pH, and EC, demonstrating strong interaction effects. Nitrogen addition mainly decreased labile pools such as DOC and TDN and slightly modified pH and EC. The C_{mic} increased with compost addition when no N was applied, but compost had no effect on C_{mic} when N was applied (Fig. 3). On the other hand,

259 the $C:N_{mic}$ was not affected by compost without N, but compost addition decreased the $C:N_{mic}$

260 with N addition (Fig. 3) resulting in significant C×N interactions (Table 3).

Table 3. ANOVA p values of the main and interactive effects of biochar (B), compost (C) and nitrogen addition (N) on soil properties after harvest: Total carbon (TC), total nitrogen (TN), total C:N, dissolved organic carbon (DOC), total dissolved nitrogen (TDN), microbial biomass carbon (C_{mic}), microbial biomass nitrogen (N_{mic}), microbial biomass carbon to nitrogen ratio ($C:N_{mic}$), pH and EC.

Source	TC (%)	TN (%)	Total C:N	DOC (mg kg^{-1})	TDN (mg kg^{-1})	C_{mic} (mg kg^{-1})	N_{mic} (mg kg^{-1})	$C:N_{mic}$	pH	EC (µS cm^{-1})
Biochar (B)	**<.0001**	0.09	**<.0001**	**0.04**	**0.001**	0.18	0.91	**0.05**	0.10	**<.0001**
Compost (C)	0.084	**<.0001**	**<.0001**	**0.001**	0.06	**<.0001**	**<.0001**	**0.001**	0.14	**<.0001**
B×C	**<.0001**	**0.01**	**<.0001**	0.07	0.13	**0.01**	**0.001**	**0.01**	0.17	**0.0003**
Nitrogen (N)	0.24	0.35	0.40	**0.03**	**0.0004**	0.12	0.67	**0.002**	**0.01**	**0.02**
B×N	0.75	0.15	0.70	0.56	0.22	0.72	0.06	0.03	0.05	0.89
C×N	0.24	0.66	0.16	0.11	**0.004**	**0.001**	0.09	**<.0001**	0.14	0.20
B×C×N	0.25	0.19	0.52	0.40	0.11	0.53	**0.04**	0.19	0.24	0.18

Table 4. Total soil C (TC), total N (TN), pH and EC, soil ^{15}N recovery, total C:N, dissolved organic C (DOC), and total dissolved N (TDN) (mean±se, n = 3). Different letters behind the value indicate differences among biochar, compost, and nitrogen treatments (posthoc Tukey test, $p < 0.05$).

Biochar	Compost	Nitrogen	TC (%)	TN (%)	pH	EC (µS cm^{-1})	Soil ^{15}N rec (µg pot^{-1})	Total C:N	DOC (mg kg^{-1})	TDN (mg kg^{-1})
No	No	No	1.39±0.03^{d}	0.11±0.002^{bc}	6.66±0.01^{ab}	137±4^{c}		12.7±0.5^{c}	37±1^{abc}	7.45±0.37^{a}
Biochar	No	No	2.58±0.16^{a}	0.12±0.001^{bcd}	6.70±0.04^{ab}	138±6^{c}		22.4±1.2^{a}	39±4^{abc}	4.57±0.86^{b}
No	Compost	No	1.67±0.08^{cd}	0.13±0.002^{a}	6.75±0.003^{a}	213±15^{a}		13.1±0.4^{c}	46±2^{a}	4.61±0.14^{b}
Biochar	Compost	No	1.95±0.06^{c}	0.12±0.003^{ab}	6.71±0.02^{ab}	156±3^{bc}		16.0±0.5^{b}	39±1^{abc}	3.87±0.20^{b}
No	No	Nitrogen	1.34±0.01^{d}	0.11±0.002^{bcd}	6.63±0.03^{b}	139±1^{c}	111±20^{a}	11.9±0.1^{c}	33±2^{bc}	3.95±0.26^{b}
Biochar	No	Nitrogen	2.35±0.08^{ab}	0.11±0.001^{cd}	6.69±0.02^{ab}	124±5^{c}	270±133^{a}	21.3±0.6^{a}	30±4^{c}	3.00±0.74^{b}
No	Compost	Nitrogen	1.62±0.03^{cd}	0.13±0.002^{a}	6.63±0.02^{b}	188±7^{ab}	448±40^{a}	12.9±0.1^{c}	45±1^{ab}	4.41±0.35^{b}
Biochar	Compost	Nitrogen	2.00±0.09^{bc}	0.12±0.002^{abc}	6.69±0.02^{ab}	144±3^{c}	273±137^{a}	16.7±0.5^{b}	38±1^{abc}	3.40±0.13^{b}

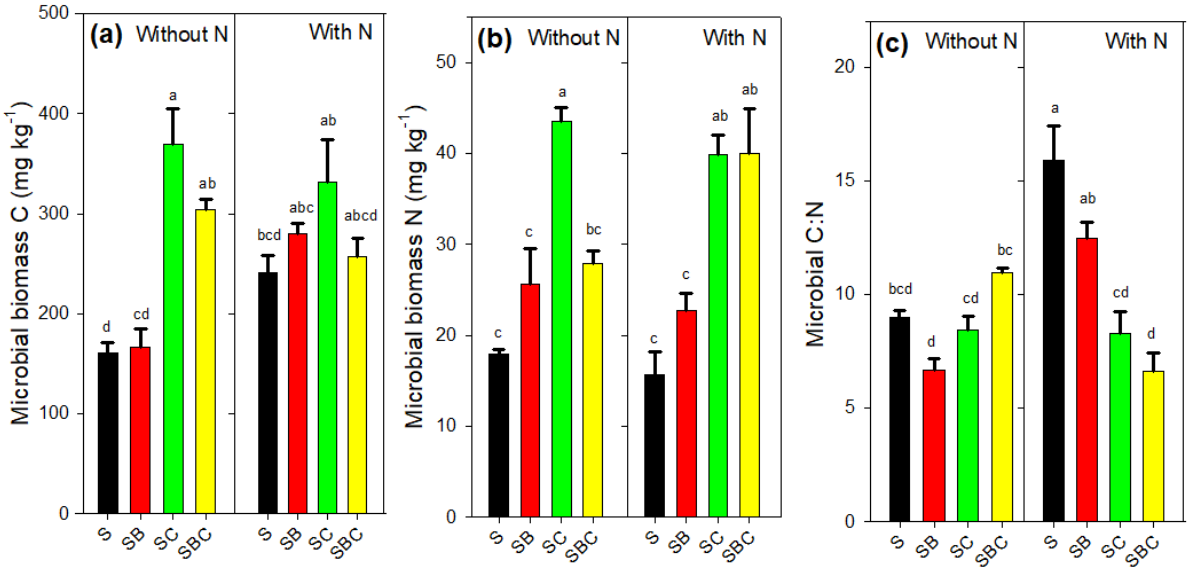


Fig. 3. Microbial biomass carbon (C) (a), microbial biomass nitrogen (N) (b), and the ratio of microbial biomass C:N (microbial C:N) (c) in response to biochar, compost, and N addition. The left and right panels represent without and with N application, respectively. Error bars represent se. Treatment abbreviations: S: soil only, SB: soil with biochar, SC: soil with compost, SBC: soil with biochar and compost. Different letters above bars indicate significant differences among treatments (posthoc Tukey test, $p < 0.05$).

Plant performance and ^{15}N recovery

Not surprisingly, N addition caused large increases in shoot and total plant biomass, as well as in plant N content (Table 5, 6, Fig. 4a). Compost decreased shoot and total plant biomass, their N content and ^{15}N recovery (Table 7, Fig. 4b), whereas biochar alone had no significant effect on plant biomass or ^{15}N recovery. However, across the N treatment, biochar improved shoot and total plant biomass when combined with compost (significant B×C interaction, Table 5, Fig. 4a). The ^{15}N recovery in soil and total ^{15}N recovery were not affected by biochar and compost (Table 7).

288 **Table 5**. ANOVA p-values of the main and interactive effects of biochar (B), compost (C) and nitrogen addition (N) on shoot, root, and total

289 biomass, and their N content.

Source	Shoot biomass ($g\ pot^{-1}$)	Root biomass ($g\ pot^{-1}$)	Total biomass ($g\ pot^{-1}$)	Shoot N ($mg\ pot^{-1}$)	Root N ($mg\ pot^{-1}$)	Total plant N ($mg\ pot^{-1}$)
Biochar (B)	0.92	0.45	0.95	0.80	0.86	0.84
Compost (C)	**<.0001**	0.21	**<.0001**	**<.0001**	**0.004**	**<.0001**
B×C	**0.02**	0.88	**0.02**	0.17	0.08	0.19
Nitrogen (N)	**<.0001**	0.57	**<.0001**	**<.0001**	**<.0001**	**<.0001**
B×N	0.94	0.88	0.97	0.89	0.30	0.89
C×N	0.32	**<.0001**	0.81	**0.02**	0.15	**0.04**
B×C×N	0.85	0.64	0.94	0.80	0.43	0.78

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Table 6. Shoot biomass, root biomass, and total biomass (mean±se, n = 3). Different letters behind the value indicate differences among biochar, compost, and nitrogen treatments (posthoc Tukey test, $p < 0.05$).

Biochar	Compost	Nitrogen	Shoot biomass (g pot^{-1})	Root biomass (g pot^{-1})	Total biomass (g pot^{-1})
No	No	No	1.60 ± 0.13^{bc}	0.19 ± 0.02^{ab}	1.78 ± 0.14^{ab}
Biochar	No	No	1.39 ± 0.06^{cd}	0.20 ± 0.01^{a}	1.59 ± 0.07^{bc}
No	Compost	No	0.22 ± 0.03^{f}	0.06 ± 0.01^{c}	0.28 ± 0.03^{f}
Biochar	Compost	No	0.39 ± 0.08^{ef}	0.08 ± 0.02^{bc}	0.48 ± 0.09^{ef}
No	No	Nitrogen	2.23 ± 0.02^{a}	0.09 ± 0.02^{abc}	2.32 ± 0.04^{a}
Biochar	No	Nitrogen	2.01 ± 0.16^{ab}	0.11 ± 0.01^{abc}	2.12 ± 0.16^{ab}
No	Compost	Nitrogen	0.67 ± 0.10^{ef}	0.18 ± 0.02^{ab}	0.85 ± 0.10^{de}
Biochar	Compost	Nitrogen	0.88 ± 0.16^{de}	0.18 ± 0.05^{ab}	1.06 ± 0.18^{cd}

299 **Table 7**. ANOVA p values of the main and interactive effects of biochar (B) and compost (C) on ^{15}N recovery in shoot, root, and total biomass in

300 treatments with N addition.

Source	Shoot ^{15}N rec ($\mu g\ pot^{-1}$)	Root ^{15}N rec ($\mu g\ pot^{-1}$)	Total biomass ^{15}N rec ($\mu g\ pot^{-1}$)	Soil ^{15}N rec ($\mu g\ pot^{-1}$)	Total ^{15}N rec ($\mu g\ pot^{-1}$)
Biochar (B)	0.61	0.78	0.62	0.94	0.86
Compost (C)	**<.0001**	0.80	**<.0001**	0.12	0.67
B×C	0.49	0.61	0.51	0.13	0.13

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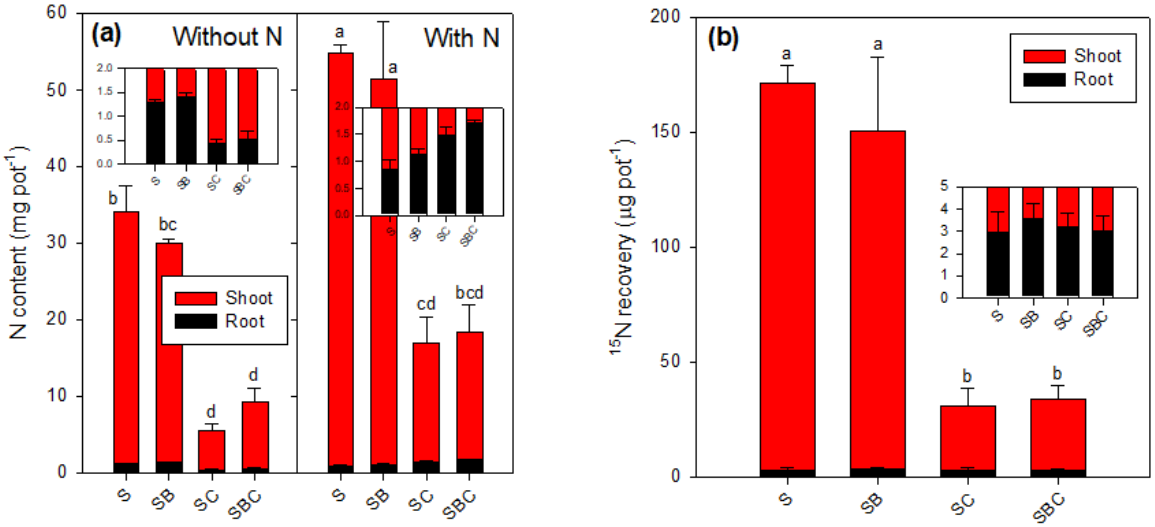


Fig. 4. Plant nitrogen (N) content in shoots and roots in response to biochar, compost and N treatments (a) and ^{15}N recovery in shoots and roots in response to biochar and compost in treatments with N addition (b). Inserts show results for low contents in detail. Error bars represent se. Treatment abbreviations: S: soil only, SB: soil with biochar, SC: soil with compost, SBC: soil with biochar and compost. Different letters above bars indicate differences in total plant N content and ^{15}N recovery among treatments (posthoc Tukey test, $p < 0.05$).

Discussion

Nitrogen leaching dynamics

Nitrate was the dominant form of N loss across all treatments, with NO_3^- leaching greatly exceeding NH_4^+ leaching. This pattern reflects the inherent mobility of NO_3^- in soils due to its limited interaction with negatively charged surfaces, whereas NH_4^+ is retained via cation exchange (Cameron et al. 2013; Di and Cameron 2002).

Amendment effects on NO_3^- leaching were transient and dependent on N availability. Biochar reduced NO_3^- leaching only in the absence of added N, supporting our first hypothesis, suggesting that its capacity to mitigate NO_3^- losses is constrained under conditions of high mineral N supply. Because biochar has limited capacity to adsorb NO_3^- on its surfaces (Yang

et al. 2017; Borchard et al. 2019), the reduced NO_3^- leaching without N addition may have arisen from indirect effects, including enhanced soil water retention (Clough et al. 2013; Lehmann and Joseph 2015), which this effect of biochar would be less effective when soil NO_3^- concentrations are high caused by N fertilization. It has also been suggested that biochar could stimulate microbial immobilization of N (e.g., Chen et al. (2021), but that is considered to be specific to individual soil-biochar combinations (Clough et al. 2013). Microbial biomass N was not affected by biochar in our study, suggesting that biochar effects on microbial immobilization were small.

In contrast, compost consistently reduced NO_3^- leaching irrespective of N addition, indicating a stronger control via microbially mediated immobilization, and confirming our second hypothesis. The supply of labile C from compost likely promoted rapid microbial uptake of mineral N, thereby reducing its susceptibility to leaching (Amlinger et al. 2007; Bernal et al. 2009). This was further supported by the significant increases in microbial biomass N (Table 3, Fig. 3b).

The absence of treatment effects during the second leaching event indicates that leaching losses were dominated by an initial flush of soluble N following amendment application. This temporal dynamic is consistent with the rapid depletion of mineral N pools after organic amendments reported in similar systems (Agegnehu et al. 2016).

Changes in soil C and N pools after harvest

Biochar and compost induced contrasting post-harvest effects on C and N pools, reflecting their divergent roles in C stabilization and nutrient turnover. Biochar increased total C while reducing DOC and TDN, indicating the direct contribution of biochar in enhancing stabilization of organic matter, while it also may have reduced availability of labile substrates. By promoting

sorptive protection of organic compounds and limiting their microbial accessibility through organo-mineral interactions, this could further enhance soil organic matter stabilization (Liang et al. 2006; Lehmann et al. 2011).

In contrast, compost increased total N, DOC, and microbial biomass C and N, confirming its function as a readily decomposable substrate that stimulates microbial activity and accelerates nutrient cycling (Amlinger et al. 2007; Bernal et al. 2009). The decline in soil C:N ratio further supports increased N availability and enhanced decomposition rates.

The combined application of biochar and compost resulted in significant interaction effects across multiple soil properties, including TC, TN and microbial C and N (Table 3), where increases in TC with biochar were less strong when combined with compost, while increases in TN, microbial C and N with compost were less strong when combined with biochar (Table 4, Fig. 3), indicating that biochar and compost moderate each other's effects on soil C stabilization and release of nutrients from compost. While this result likely stemmed from the fact that we added half the amount of each amendment in the combined treatment thereby matching the amount added in the single applications, it also illustrates that combined applications of biochar and compost may promote a more regulated nutrient supply by coupling rapid mineralization with sorptive retention (Agegnehu et al. 2017).

Nitrogen addition decreased DOC and TDN (Table 3, 4), likely reflecting increased assimilation by plants and microorganisms. This is consistent with evidence that N enrichment can enhance the incorporation of labile C and N into biomass pools (Kuzyakov and Xu 2013).

Microbial biomass responses indicate that resource stoichiometry strongly regulated microbial dynamics in this system. Compost increased C_{mic} under no-N conditions (Fig. 3), suggesting that microbial growth was primarily C-limited. However, this effect was not observed under N

addition, indicating a shift in limiting factors as N availability increased. The reduction in microbial C:N ratio following compost application in the presence of N suggests enhanced N assimilation and tighter coupling of C and N cycling within microbial biomass (Mooshammer et al. 2014; Cleveland and Liptzin 2007). These findings support the concept that microbial responses are governed by the balance of resource inputs rather than the supply of individual nutrients. The significant compost × N interaction on microbial C:N further underscores the importance of stoichiometric constraints in regulating microbial processes and highlights the need to consider coupled C–N dynamics in soil amendment strategies.

Plant growth, N uptake, and ^{15}N recovery

Our third hypothesis that combined application of biochar and compost would produce synergistic effects on plant N uptake and overall N retention was not supported. Plant responses confirmed that growth was N-limited, as evidenced by substantial increases in biomass and N uptake following N addition. In contrast, compost reduced plant biomass, N uptake, and ^{15}N recovery (Table 5, 6, Fig. 4), indicating a short-term negative effect on plant performance. This response was likely caused by increased microbial N immobilization, but other factors, such as phytotoxicity, and increased salinity associated with compost application may also have played a role (Bernal et al. 2009; Selim et al. 2012). These processes may temporarily reduce plant-available N despite increases in total soil nutrient pools.

Biochar alone did not significantly affect plant growth; however, its combined application with compost enhanced plant biomass production, suggesting that biochar alleviated the negative effects of compost. This may occur through sorption of inhibitory compounds, improved nutrient retention, and enhanced soil physical conditions (Lehmann and Joseph 2015; Agegnehu et al. 2017). The lack of significant effects on total ^{15}N recovery indicates that

amendments primarily influenced N partitioning between plant and soil pools rather than total system N retention.

Conclusion

The study demonstrated that NO_3^- was the dominant pathway of N loss, confirming its high mobility in soil systems. Among the amendments tested, compost was more effective than biochar in reducing NO_3^- leaching, primarily through enhanced microbial immobilization driven by labile C inputs. In contrast, biochar reduced NO_3^- leaching only under low N supply, indicating that its effectiveness is limited when mineral N availability is high.

Biochar and compost exerted contrasting effects on soil properties and nutrient dynamics. Biochar contributed to C stabilisation and reduced EC, whereas compost increased soil N availability, DOC, and microbial biomass, but also elevated salinity. These changes highlight the different functional roles of the two amendments in regulating soil processes.

Despite improving soil nutrient pools, compost negatively affected plant growth and N uptake in the short term, likely due to microbial immobilisation and potential phytotoxic or salinity effects. However, combining biochar with compost partially alleviated these negative impacts and improved plant biomass compared to compost alone, indicating that biochar can buffer adverse effects of the compost.

Overall, the results emphasise that amendment effectiveness depends strongly on N availability and interactions between C and N inputs. While compost is effective for reducing N losses, its short-term impacts on plant performance must be considered. The combined application of biochar and compost offers a more balanced strategy to enhance nutrient retention while maintaining plant productivity, although clear synergistic effects on N use efficiency were not

observed. These findings highlight the importance of integrated soil amendment strategies that account for both biogeochemical processes and plant responses to optimize agricultural sustainability and minimize environmental N losses.

Author contributions

SS, SM, and FAD designed the study. SM and FAD supervised the experiment. SS conducted the lab work and collected the data. SS, FAD, and SM performed the data analysis and interpreted the results. SS wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

Funding Declaration

SS receives a scholarship from the Indonesia Endowment Fund for Education Agency (LPDP).

Data Availability

Data will be available upon request from the corresponding author.

Competing Interests

Authors declare no competing interests

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