INCUBATION STUDIES OF BIOCHAR AND MANURE TO MITIGATE CARBON
DIOXIDE RELEASE AND NITROGEN DEFICIENCY IN SEMI-ARID SOILS

by

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STATEMENT BY AUTHOR

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ACKNOWLEDGEMENTS

Dedication

To My Parents: Miyuki and Kiyotaka Yamafuji

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ABSTRACT

Biochar (BC), produced through pyrolysis of organic residues, is increasingly being used as a beneficial soil amendment. We studied the effects of BC and animal manure additions on carbon dioxide (CO_2) release and nitrogen (N) dynamics in three semi-arid climate soils. The objective of this study was to understand how BC application modifies soil nitrogen dynamics and moderate the effects of manure application in semi-arid agricultural systems on different textured soils: the loamy sand (LS) soil, the silty loam (SL) soil, and the clay loam (CL) soil. We found the positive interaction of BC and manure with BC suppressing CO_2 emissions in manure amended soils. BC increased nitrogen mineralization in manure-amended soils towards the end of the incubation period 28 days. No significant N immobilization was observed in unamended soils. BC and manure soil additions reduced N deficiencies in all three soils. A second study focused on measuring carbon dioxide emissions from biochar-amended alkaline semi-arid soil. The objective of this study was to test if acidified and non-acidified BC released the same amount of CO_2. The results showed that the soil samples amended with acidified BC released more CO_2 than those amended with untreated BC with high alkalinity. It is postulated that untreated BC could absorb CO_2; whereas, acidified BC with no alkalinity could not. The LS soil amended with BC released less CO_2 than LS soil control perhaps due to the soil microbial activity inhibitory effects of the BC's residual water soluble polynuclear aromatic hydrocarbons. Thus, the interaction with BC and steer manure application could suppress the release of CO_2.

1. **INTRODUCTION**

1.1 Literature Review

1.1.1 Biochar

Biochar (BC) has started to attract attention of scientists internationally, primarily because of the discovery of Terra Preta soils with elevated fertility levels thought to come from repeated additions of charcoal by the local tribe in the Amazon River basin of upper Brazil, suggesting that "permanent or semi-permanent agriculture can itself create sustainably fertile soils" (Glaser et al., 2001). BC, produced by burning biomass residues in the absence or near-absence of air, is now widely accepted as a soil amendment in hot, wet regions of the world with low fertility soils (typically Oxisols) (Lehmann et al., 2011). However, BC could also be used on semi-arid agricultural systems with loamy sand soils, which have low water holding capacity (WHC) (Yu et al., 2013) and low nutrient contents (Sukartono et al., 2011). Since BC has a large surface area, is porous, and has a large WHC, when applied to soils, the roots of plants may increase around BC particles to absorb nutrients (Marris, 2006).

BC is known to decompose very slowly in soils (Lehmann et al., 2009). BC has the potential to increase the activity of soil microorganisms in agricultural soils (Lehmann and Marco, 2006) and can act as a habitat for populations of microorganisms that turn soil into spongy, dark material (Lehmann et al., 2006), creating an environment where aerobic microbes easily increase because of abundant air and water (Thies and Rillig, 2009). Adding BC into soils may facilitate plant root symbiotic microbes, as well as root nodule bacteria (Yoshizawa et al., 2006). Overall, biochar is thought to produce changes in soil that are beneficial to biological, physical and chemical properties (Ennis et al., 2012).

Using BC also has benefits for improving agricultural systems because BC application to the soils can reduce carbon dioxide (CO_2) release (Lehmann et al., 2006). BC still contains the large amount of carbon in the form of solid pyrolysis products rich in carbon and poor in oxygen, with smaller amounts of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC). Jones et al., (2011), observed that BC contains significant quantities of DOC and DIC as well. BC additions could physically change soil properties, including water holding capacity (WHC), bulk density, and porosity, but according to Jones et al., (2011) there should be no significant effect on CO_2 release. BC has the structure of highly aromatic organic material and includes approximately 75 % carbon concentration (Lehmann and Rondon, 2006). The soil microbial activity might be inhibited by effects of the BC's residual water soluble polynuclear aromatic hydrocarbons (Artiola et al., 2012).

1.1.2 Biochar and Manure Interaction in Soil

BC itself would not have large effect on carbon and nitrogen dynamics, but BC and manure interaction in soil would bring out large effects. In detail, leakage of nutrients was suppressed when BC was added to the soils using manure (Laird et al., 2010; Lehmann et al., 2003). The application of BC could change soil carbon and nitrogen dynamics (Rogovska et al., 2011, Clough et al., 2013). BC can be holding nutrients that already exist, decomposed materials of organic matter, and added manure over a long period of time (Clough et al., 2013). Since almost no nutrients are in BC, especially carbonized wood, if small amounts of manure are added, a possibly interaction between BC and manure would be present (Rogovska et al., 2011). BC has an effect to decrease the concentration of inorganic N in soils (Shenbagavalli and

Mahimairaja, 2012). BC has the ability to catalyze the decrease of NO_3^--N to N_2, so that it probably could influence denitrification (Shenbagavalli and Mahimairaja, 2012).

On the terrestrial environment, both arid regions and semi-arid regions have a great potential to store three fertilizer elements: nitrogen (N), phosphorous (P), and potassium (K) (Duan et al., 2006). Using manure could result in a productivity improvement, yet it could also increase pressure on the environment, including an increase in CO_2 (Flavel and Murphy, 2006) and ground water pollution due to leaching and salt damage (Clough et al., 2013).

When organic fertilizer is scattered in the field, mostly organic N in addition to NH_4 would be present. Ammonium nitrogen (NH_4^+-N) is produced by the decomposition of the dead bodies of animals or organic fertilizer including poultry manure and steer manure. However, it is still difficult for plants to absorb the condition of NH_4^+-N. A dynamic of N in the soils is divided into two absorption forms of NH_4^+-N and nitrate-nitrogen (NO_3^--N) which plants easily can uptake. The mineralization process in the part of N cycle is the conversion of an element from an organic form to an inorganic state as a result of microbial decomposition. Immobilization is the conversion of an element from the inorganic to the organic form that occurs by soil microorganisms decomposing plant residues. The change of NH_4^+-N to NO_3^--N in the soils is known as nitrification. NO_3^--N could be lost due to leaching and denitrification that is the biological conversion of nitrates into nitrogen gas which is then released into the atmosphere. Eventually, NO_3^--N is absorbed by plant's roots, utilized for photosynthesis, and becomes nutrients for plant growth. Reducing inorganic N means that BC could absorb NH_4^+-N from the soil solution (Lehmann et al., 2006). Therefore, BC could have an effect of decrease in ammonification on the soil due to adsorption (Gundale and DeLuca, 2006).

1.2 Thesis Format

      This thesis presents rationale for the current study, reports primary results, and discusses main conclusions. A more detailed description of methodologies and a more extensive discussion of the results can be found in Appendix A entitled "*Carbon and nitrogen mineralization after application of biochar and manure to semi-arid soils*" and Appendix B entitled "*Carbon dioxide emissions from biochar-amended alkaline semi-arid soil*". Both Appendix A and B are formatted in manuscript style for the submission to the journal Soil Science for publication. Samples used in this study were collected from April-July 2013 with the help of graduate students. All samples were processed in the Environmental Pedology Laboratory at The University of Arizona. Thesis drafting was conducted under the guidance of Dr. Craig Rasmussen.

2. CURRENT STUDY

We summarize the motivations and rationale to investigate the effect of biochar (BC) on the carbon and nitrogen cycle in manure amended semiarid agricultural soils in southern Arizona. A brief description and discussion of major findings is presented here with highly detailed methods, results, and discussion presented in Appendix A and B.

2.1 Rationale for Study

Quantifying BC characteristic, including chemical and physical properties is fundamental to fully understanding the mechanism of carbon and nitrogen dynamics in BC amended soils. Developing the ability to confidently predict soil response to BC over a range of different types of soil is needed to increase our understanding of the efficacy of BC amendment for improving agricultural soil productivity. In this study, we hypothesized that (1) the interaction with BC and steer manure application could suppress the release of carbon dioxide (CO_2) and increase the rate of nitrogen (N) mineralization, and (2) BC alkalinity released to solution facilitates partitioning of atmospheric CO_2 into solution phase carbonates, effectively leading to a drawdown or consumption of atmospheric CO_2.

Common agricultural practice in the semi-arid agricultural region is application of animal manure to soil as a readily available form of plant nutrients. Manure application could be associated with negative environmental effects including substantial gaseous and solution phase N losses. Application of BC with manure has the potential to mitigate these issues by acting as a rapid, short-term sink for N, and building soil stores of C and N. Biochar-nutrient dynamics in semi-arid agricultural system are poorly understood and represent an important knowledge gap

for understanding and improving nutrient management practices in this region. This knowledge gap is addressed in the first study summarize in this thesis.

Soil incubation experiments have demonstrated decreased carbon dioxide release in soils amended with BC relative to unamended soils. The reasons for the discrepancy are unclear and may include (i) toxicity effects on the extant microbial communities due to presence of minor amounts of polynuclear aromatic hydrocarbons in the char that suppress biological activity, or (ii) abiotic mechanisms whereby alkalinity released from the BC into soil solution chemically reacts with and partitions atmospheric CO_2 into solution phase carbonate species. This mechanistic ambiguity represents an important knowledge gap in understanding BC interactions in the environment. The second experiment summarized in this thesis directly tests the abiotic mechanism and aimed to determine the potential effects of BC alkalinity on atmospheric CO_2.

2.2 Summary of Results

2.2.1 Biochar and Manure Interaction Experiment

The CO_2 experimental data was separated into three groups. Each loamy sand (LS) soil treatments, silt loam (SL) soil treatments, and clay loam (CL) soil treatments included soil itself, soil + biochar, soil + manure, and soil + biochar + manure. Cumulatively, treatments in all soil groups, especially LS soil treatments in addition to manure were releasing more CO_2 than treatments without manure. Loamy sand soil + manure (LSM) and silty loam soil + manure (SLM) released significantly less CO_2 than loamy sand soil + biochar + manure (LSBM) and silty loam soil + biochar + manure (SLBM), respectively. After the amount of CO_2 produced by each treatment of three different groups was normalized with its respective total organic carbon (TOC), BC treatments, including loamy sand soil + biochar (LSB), silty loam soil + biochar

(SLB), and clay loam soil + biochar (CLB) show the least CO_2 release in each soil group. Moreover, LSBM, SLBM, and CLBM released significantly less CO_2 than LSB, SLB, and CLB, respectively.

The concentration of total inorganic nitrogen (TIN) and ammonium-N (NH_4^+-N) of all soil + manure (SM) and soil + biochar + manure (SBM) was significantly higher than all soils and with BC, and BC had no effect on soils. Effects of BC on manure were not found in both LS and SL groups but in CL group at the concentration of Nitrate-N (NO_3^--N). All SM treatments had positive effects on TIN mineralization at 14-0 and 28-0 days. We found some variations in each soil groups at NO_3^--N mineralization; BC did not have an effect on manure throughout the experiment.

2.2.2 Alkalinity Experiment

The experimental data was separated into two groups. BC treatments included DI water, BC and acidified BC. Loamy sand (LS) soil treatments included LS soil, LS soil + BC, and LS soil + acidified BC. We found two different conditions releasing CO_2 on each group. Cumulatively, BC evolved significantly less CO_2 than acidified BC. Amended LS soil with BC (LSB) and with acidified BC (LSAB) evolved significantly more CO_2 than LS; however, after each sample was normalized with its respective TOC, LS released most CO_2 of treatments. Moreover, LS released more CO_2 than both LSB and LSAB.

2.3 Summary and Conclusions

The summary of this incubation study yields that application of BC and manure interaction using semi-arid soils could effect on carbon and nitrogen dynamics.

2.3.1 Biochar and Manure Interaction Experiment

Our hypothesis that the interaction with BC and steer manure application could suppress the release of CO_2 was supported. In terms of TOC in each treatment, all samples adding BC released less CO_2 than other sample mixed due to BC's absorption power.

We hypothesized that the interaction with BC and steer manure application could increase nitrogen (N) mineralization. Total inorganic concentration in all soil + manure (SM) did not decrease between 0 and 28 days, which means that nitrification by nitrifying bacteria and N fixation or immobilization occurred at the same time. The depletion of NO_3^--N was shown at 14 days from the initial point in all groups due to denitrification, immobilization, or probably absorption by BC.

The BC and manure interaction experiment shows that in terms of each treatment size, soil + biochar + manure (SBM) released more CO_2 than soil + manure (SM) because of the presence of extra carbon, high nutrient level from manure, and microbes which act synergistically. However, in terms of TOC in each treatment, all samples adding BC released less CO_2 than other sample mixed. It means that BC originally contained much carbon inside.

At total inorganic nitrogen (TIN) concentration, mineralization was seen during the period of 28 days when each manure and BC + manure was mixed with soils. Adding manure shows the great response of variation besides adding BC. This is because manure contained excess of N, so that all microbes had great activity which could have mineralization and immobilization. In terms of NH_4^+-N, we found similar trend with TIN concentrations. The trend shows all manure treatments had the range of 80 to 100 mg N kg^{-1}, but all the other treatments had approximately 20 mg N kg^{-1}. All soil treatments adding manure and both BC and manure

had the effect on NO_3^--N compared with soils at 28 days. Overall, BC and manure soil additions had effect of reducing N deficiencies in all three soils type.

2.3.2 Alkalinity Experiment

Our hypothesis that alkalinity reduces measured CO_2 mineralization due to formation of carbonates in solution was supported. The mechanism of this suggestion was that BC still has a relatively large amount of alkalinity in it to acidified BC. This alkalinity could go into the solution immediately and the lower dissolved CO_2 in the water would then allow for more of the CO_2 in the headspace in the jar for incubation to dissolve into the water.

Acidified BC released more CO_2 than BC due to its alkalinity. This experiment yielded that the result supported our hypothesis that abiotic mechanisms, whereby alkalinity released from the BC into soil solution chemically reacts with and partitions atmospheric CO_2 into solution phase carbonate species. We found that the application of BC into the loamy sand (LS) soil could suppress CO_2 release, so that BC actually could exert a constraining influence over CO_2 release due to BC's adsorption power through its alkalinity. However, BC originally contained high ratio of the TOC; therefore, the amount of CO_2 release from BC was higher than LS soil. Soil respiration is related to the number of microbes. Microbes easily could grow in LSB under the biotic condition because BC can make a suitable environment for them.

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APPENDIX A

Carbon and nitrogen mineralization after application of

biochar and manure to arid soils

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Abbreviations:
BC (Biochar)
CAC (Campus Agricultural Center)
CEPM (Center for Environmental Physics and Mineralogy)
CL (Clay Loam Soil)
CLB (Clay Loam Soil + Biochar)
CLM (Clay Loam Soil + Manure)
CLBM (Clay Loam Soil + Biochar + Manure)
DI (Deionized)
IRGA (Infrared Gas Analyzer)
OM (Organic Matter)
PFW (Pine Forest Waste)
LS (Loamy Sand)
RRAC (Red Rock Agricultural Center)
LSB (Loamy Sand Soil + Biochar)
LSM (Loamy Sand Soil + Manure)
LSBM (Loamy Sand Soil + Biochar + Manure)
SBM (Soil + Biochar + Manure)
SL (Silt Loam Soil)
SLB (Silt Loam Soil + Biochar)
SLM (Silt Loam Soil + Manure)
SLBM (Silt Loam Soil + Biochar + Manure)
SM (Soil + Manure)
SRER (Santa Rita Experimental Range)
TIN (Total Inorganic Nitrogen)
TOC (Total Organic Carbon)
WHC (Water Holding Capacity)

Abstract

We studied the potential of biochar (BC) on carbon and nitrogen (N) mineralization dynamics of animal manure by incubating the loamy sand (LS) soil, silt loam (SL) soil, and clay loam (CL) soil during a period of 112 days. The objective of this study was to understand the potential of BC application to facilitate the soil nutrient dynamics and temper the negative environmental effects of manure application in semi-arid agricultural systems on different kinds of textured soils. We hypothesized that the interaction with BC and steer manure application could suppress the evolution of carbon dioxide (CO_2) and increase nitrogen (N) mineralization. In terms of total organic carbon (TOC) originally present in each sample, the addition of BC resulted in reduced CO_2 production. This was likely caused by both alkalinity and inhibition by polynuclear aromatic compounds inside the BC. We found the positive interaction of BC and manure because BC still could suppress CO_2 when manure was applied to the soil. N mineralization experiment shows that amendment of both BC and manure resulted in an increased N mineralization mostly at 28 days, but N immobilization occurred in unamended soils. Overall, BC and manure soil additions had effect of reducing N deficiencies in all three soils type. Thus, the interaction with BC and steer manure application could suppress the release of CO_2.

Introduction

Manure is a great fertilizer containing nitrogen (N), phosphorus (P), potassium (K) and other nutrients. Using manure could result in a productivity improvement, yet it could also increase pressure on the environment, including an increase in carbon dioxide (CO_2) (Flavel and Murphy, 2006) and ground water pollution due to leaching and salt damage (Clough et al.,

2013). However, leakage of nutrients was suppressed when biochar (BC) was added to the

agricultural soils using manure (Laird et al., 2010; Lehmann et al., 2003). BC might mitigate

potential negative impacts of steer manure application to soils, particularly focusing on carbon

mineralization and N dynamics. On the terrestrial environment, both arid regions and semi-arid

regions have a great potential to store three fertilizer elements: N, P, and K (Duan et al., 2006).

Adding additional nutrients into the soils is a great benefit to plant growth. Since steer manure

contains various abundant nutrients, especially N, adding it into the soils would achieve the

desired effect because N is one of the most important nutrients for plant growth.

The application of BC could change soil carbon and nitrogen dynamics (Rogovska et al.,

2011, Clough et al., 2013). Since BC contains only a small quantity of minerals, it is not as

useful as manure. However, the important point is that at the time of adding BC into the soils, it

can exist stably in the ground for a long period of time. BC can be holding nutrients that already

exist, decomposed materials of organic matter, and added manure over a long period of time

(Clough et al., 2013). Since almost no nutrients are in BC, especially carbonized wood, if small

amounts of manure are added, a possibly interaction between BC and manure would be present

(Rogovska et al., 2011).

When organic fertilizer is scattered in the field, mostly organic N in addition to NH_4

would be present. Ammonium nitrogen (NH_4^+-N) is produced by the decomposition of the dead

bodies of animals or organic fertilizer including poultry manure and steer manure. However, it is

still difficult for plants to absorb the condition of NH_4^+-N. A dynamic of N in the soils is divided

into two absorption forms of NH_4^+-N and nitrate-nitrogen (NO_3^--N) which plants easily can

uptake. The mineralization process in the part of N cycle is the conversion of an element from an

organic form to an inorganic state as a result of microbial decomposition. Immobilization is the

conversion of an element from the inorganic to the organic form that occurs by soil microorganisms decomposing plant residues. The change of NH_4^+-N to NO_3^--N in the soils is known as nitrification. NO_3^--N could be lost due to leaching, denitrification that is the biological conversion of different enzymes that stepwise reduce nitrate to nitrogen gas under anaerobic conditions, and runoff but not NH_4^+-N because of its ionic bond. Eventually, NO_3^--N is absorbed by plant's roots, utilized for photosynthesis, and becomes nutrients for plant growth.

The objective of this study was to understand how the coupled application of manure and BC to agricultural soils could modify soil carbon and nitrogen dynamics and mitigate the environmental effects of manure application on different textured soils: loamy sand (LS), silty loam soil (SL), and clay loam soil (CL). We hypothesized that the interaction with BC and steer manure application could suppress the evolution of carbon dioxide (CO_2) and increase nitrogen (N) mineralization.

Materials and Methods

Both carbon dioxide (CO_2) and nitrogen (N) mineralization experiments were conducted in parallel at the Center for Environmental Physics and Mineralogy (CEPM) laboratory at the University of Arizona in Tucson, Arizona. Loamy sand (LS) surface soil was sampled from the top 0 to 15 cm of soil at The University of Arizona Red Rock Agricultural Center (RRAC) approximately 55 km northwest of Tucson, Arizona (Artiola et al., 2012). Silt loam surface soil was sampled from the top 0 to 15 cm of soil at Santa Rita Experimental Range (SRER) approximately 35 km south of Tucson, Arizona. Clay loam surface soil was sampled from the top 0 to 15 cm of soil at University of Arizona Campus Agricultural Center (CAC) approximately 7 km north of Tucson, Arizona. The loamy sand soil was characterized as Denure

soil series, course-loamy, mixed, superactive, hyperthermic, Typic Haplocambid (Soil Survey

Staff, 2006). Silt loam soil was characterized as Comoro series, fine-silt, mixed, superactive,

calcareous, thermic, Typic Torrifluvent (Soil Survey Staff, 2006). Clay loam soil was

characterized as Glendale soil series, fine-loamy over sandy-skeletal, mixed, superactive,

calcareous, thermic, Typic Torrifluvent (Soil Survey Staff, 2006). All soils were placed in the

dark at room temperature at the laboratory after collection, and air-dried and sieved to < 2 mm

fraction before starting the incubation process. We prepared manure named Grow King Steer

Manure as a commercial product. The experiment included twelve treatments with four times

replicates: (1) loamy sand (LS) soil, (2) silt loam (SL) soil, (3) clay loam (CL) soil, (4) loamy

sand soil + biochar (LSB), (5) silt loam soil + biochar (SLB), (6) clay loam soil + biochar (CLB),

(7) loamy sand soil + manure (LSM), (8) silt loam soil + manure (SLM), (9) clay loam soil +

manure (CLM), (10) loamy sand soil + biochar + manure (LSBM), (11) silt loam soil + biochar +

manure (SLBM), (12) clay loam soil + biochar + manure (CLBM). The physical and chemical

properties of each soil, biochar and manure are shown in Table 1.

Pine forest waste (PFW) woodchips derived BC 1-3 mm size fraction, was used for this

incubation experiment in all the batch experiments. The BC was produced using a 55,000-BTU

wood gas Mega stove in batch mode and slow pyrolysis with a BC interparticle temperature of

450 °C to 500 °C and a yield of 18 % to 20% by mass (Artiola et al., 2012). The BC alkalinity

was measured using a hot sulfuric acid digestion followed by back titration and reported as

$CaCO_3$ (Table 1).

The water holding capacity (WHC) for each of the incubation samples was determined

before the experiments. A 150-300 mL beaker was topped with a small funnel lined with

Whatman No. 42 filter paper. Approximately 10.0 g of sample was weighed out and placed into

the funnel, held by the filter paper. The beaker was filled with DI water to allow the filter paper

in the funnel to be submerged. Once saturated, the water from the beaker was removed, and the

sample was allowed to drip back into the beaker overnight. After collecting and weighing

subsample of the sample, it is placed in 105 $^{\circ}$C oven overnight to dry. Dry sample was weighed

and exact mass was recorded and used to calculate the gravimetric water content at field

capacity.

30.0 g each of LS soil, SL, and CL, 2 % of BC by soil weight, and 3.5 % of manure by

soil weight were measured into a labeled sample cup, and the mass of dry soil added was

recorded. Each sample was placed into a small plastic container and in turn placed inside a

Mason jar (16 oz) in preparation for the incubation experiment. The Mason jar lids were fitted

with septa hot glued on the top and bottom and each jar was air leak tested before the

experiment.

Using the prerecorded dry masses and field capacity moisture data, the amount of water,

needed to wet each particular sample to 60 % volumetric water content, was calculated. The

wetted samples in plastic containers were placed in the Mason jars with about 1.50 mL DI water

added to the bottom of each jar to keep soil moisture content constant. Between CO_2

measurements the jars were kept in a dark cabinet at a temperature of 21 $^{\circ}$C.

The CO_2 concentrations of the jars were measured using an Infra-Red Gas Analyzer

(Qubit CO_2 Analyzer, model S-151; Qubit Systems, Kingston, Ontario, Canada). Pure nitrogen

gas was used as the carrying gas at a rate of 125 mL / min for the instrument. The infrared gas

analyzer (IRGA) station works with the data logger "Logger Pro 3.4.2". The program graphed

out the results of each measurement over time in a curved peak form, using ppm units as the CO_2

concentration. A peak integration function was then used to calculate the area under the peak of

each measurement. The standard measurements for a calibration curve for 0.2 mL, 0.4 mL, 0.6

mL, 0.8 mL, 1 mL, 2 mL, and 3 mL of CO_2 respectively, were taken to calculate exact CO_2

concentrations from the integral data of the measurement. The concentration of the standard CO_2

gas used in our laboratory is 1% so 1.0 mL volume is contained 1% CO_2. The goal was to

measure the samples while their CO_2 concentrations were between 0.2 % and 3.0 %. The

measurement range of this instrument is 0-2000 ppm. To measure a container, the headspace was

mixed with a syringe, and then 1.0 mL sample was collected for CO_2 analysis. After sampling

the CO_2 in the headspace, there were uncapped and purged with a stream of air.

N mineralization experiment included the same materials and treatments as CO_2

experiment with three replicates. Total inorganic nitrogen (TIN) in internal N-cycling processes

was analyzed using aerobic laboratory incubation method (Hart et al., 1994). In this method, all

treatments using 10.0 g dry soil were weighed and put into Ziploc bag with adding the specific

amount of water that is needed to wet all samples to 60 % volumetric water content. All samples

were incubated for in the dark area with room temperature. We examined TIN from each

treatment sacrificed and extracted with 100 mL of 2 M KCl at 0, 14, and 28 days using

colorimetric method. N mineralization was calculated followed the basic formula:

$$N_{min} = (NH_4^+\text{-}N_f + NO_3^-\text{-}N_f) - (NH_4^+\text{-}N_i + NO_3^-\text{-}N_i) \qquad (Eq.\ 1)$$

where NH_4^+-N_f is final ammonium-N value, NO_3^--N_f is final nitrate-N value, NH_4^+-N_i is initial

ammonium-N value, and NO_3^--N_i is initial nitrate-N value.

All experiments were performed in at least 4-6 replicates. Statistical analysis for the

cumulative CO_2 –C for this experiment was performed using CoStat Statistical Software, 2-way

completely randomized analysis of variance (ANOVA) ($p < 0.05$), comparing and ranking means using the Student-Newman-Keuls test.

Results

Biochar and Manure Interaction Experiment

The carbon dioxide (CO_2) experimental data was separated into three groups. Each loamy sand (LS) soil treatments, silt loam (SL) soil treatments, and clay loam (CL) soil treatments included soil itself, soil + biochar, soil + manure, and soil + biochar + manure. Among line plots, CO_2 evolution from all soil treatments shows dramatic decreases during a period of the first 14 days. The amount of CO_2 evolution at the first day was significantly higher than at any point and stayed constant after 21 days (Fig. 1 a, b, and c).

Cumulatively, all treatments in each soil treatment in addition to manure evolved more CO_2 than all treatments without manure (Fig. 2). LS soil treatments evolved significantly more CO_2 than both SL soil and CL soil treatments (Fig. 2 a). Both CLM and CLBM show a rapid ascent after 49 days (Fig. 2 b and c). Figure 3 shows that loamy sand soil + manure (LSM) and silt loam soil + manure (SLM) evolved significantly less CO_2 than loamy sand soil + biochar + manure (LSBM) and silt loam soil + biochar + manure (SLBM), respectively. However, clay loam soil + manure (CLM) evolved significantly more CO_2 than clay loam soil + biochar + manure (CLBM). There was no significant difference between soil with biochar (BC) and without BC in all soil treatments.

After the amount of CO_2 produced by each treatment was normalized with its respective total organic carbons (TOC) (Table 1), rank orders of treatments were changed (Fig. 4 a, b, and c). There was no significant difference between SL soil and CL soil treatments but LS soil

treatment. At the end of the incubation period, Figure 6 shows that cumulative C - CO_2 evolution from LS soil treatments was significantly higher than from other soil treatments. In addition, there was significant difference between CL soil and CLM, yet there was no significant difference between soil and SM in LS soil and SL soil treatments. BC treatments, including loamy sand soil + biochar (LSB), silt loam soil + biochar (SLB), and clay loam soil + biochar (CLB) shows the least CO_2 evolution in each soil treatment: however, there was no significant difference between soil and soil with BC in SL soil and CL soil treatments. Moreover, CO_2 evolution from LSBM, SLBM, and CLBM was significantly less than LSB, SLB, and CLB, respectively.

The concentration of total inorganic nitrogen (TIN) and ammonium-N (NH_4^+-N) of all soil + manure (SM) and soil + biochar + manure (SBM) was significantly higher than all soils and with BC between 0 to 28 days (Fig. 7 a and b, Fig. 8 a and b, and Fig. 9 a and b). Moreover, there was no significant difference between soil and with BC in all soil treatments because BC originally contained negligible amount of N. There was significant difference of TIN concentration between SM and SBM at 14 and 28 days. There was significant difference of NH_4^+-N concentration between SM and SBM at 28 days. The depletion of both TIN and NH_4^+-N concentrations would not be shown between 0 and 28 days.

Nitrate-N (NO_3^--N) concentration of both LSM and LSBM gradually increased with the time; however, both LS soil and LSB decreased overall (Fig. 7 c). In SL soil treatments, NO_3^--N concentration of both SLM and SLBM shows the same trend as LS soil treatments, yet only SL soil continuously decreased (Fig. 8 c). CL soil treatments had the highest concentration in soil treatments (Fig. 9 c). Overall, effects of BC to increase N concentration on manure were not found in both LS soil and SL soil treatments but in CL soil treatments.

In terms of N mineralization, all figures indicate that each measurement time of the experiment was subtracted from the previous measurement time (Fig. 10, 11, and 12). At 28-14 days, significant TIN loss was shown between CL soil and CLM; however, there was no significant difference among all other treatments (Fig. 10 a, 11 a, and 12 a). LSM, SLM, and CLM had 10 mg N kg^{-1} which was approximately the same NH_4^+-N mineralization at 14-0 days; however, LSBM, SLBM, and CLBM shows NH_4^+-N loss (Fig. 10 b, 11 b, and 12 b). SLM, CLM, SLBM, and CLBM had significant increase of NO_3^--N mineralization compared to SL soil and CL soil at 28-14 days, yet LSB, LSM, and LSBM had no significant increase compared to the LS soil but had a tendency toward the increase (Fig. 10 c, 11 c, and 12 c).

We examined that the different patterns of graphs that shows the comparison of each measurement time to the initial. All SM treatments had positive effects on TIN mineralization at 14-0 and 28-0 days (Fig. 13 a, 14 a, and 15 a). NH_4^+-N mineralization of all soil treatments had similar trends with TIN mineralization (Fig. 13 b, 14 b, and 15 b).

NO_3^--N mineralization shows that BC did not have effect on all soils at 28-0 days besides CLB at 28-0 days (Fig. 13 c, 14 c, and 15 c). The rate fluctuated increase and decrease at 28-0 days. Although we found some variations in each soil treatments; BC did not have an effect on manure because of no statistically significant difference among all treatments of each treatment.

Discussion

Biochar and Manure Interaction Experiment

In terms of CO_2 evolution, the amount of CO_2 evolved implies the microbial biomass (Heinemeyer et al., 1989). Since there was no significant difference of CO_2 evolution between the soil and the soil with biochar (BC), the effect of BC evolving CO_2 could not be found on all

soils because enough nutrients was not presenting in treatments. We assume that microbes in soil + biochar + manure (SBM) increased rather than soil + manure (SM) in both LS soil and SL soil treatments maybe due to synergistic of microbial activity, extra carbon, and high nutrient from manure contained originally. Since BC has a large surface area, is porous, and has a water holding capacity (WHC), when applied to soils, the roots of plants may increase around BC particles to absorb nutrients (Marris, 2006). Therefore, BC facilitated the effect of manure that releases CO_2 greatly in comparison to the soil. Microbes increased because of large effects from manure which could provide nutrients; hence, adding manure into the soil resulted in the existence of the majority of microbes. Nevertheless, the opposite result was shown that CLBM evolved significantly less CO_2 than CLM. This is probably because CAC where we collected CL soil has been previously fertilized; hence, such fertilizer in CL soil could enhance BC's ability that could suppress CO_2 evolution. Continuing to investigate the interaction between BC and CL may provide clues to this discrepancy in CO_2 evolution between SBM and SM.

LS soil treatments significantly evolved more CO_2 in other soil treatments, and the SL soil and the CL soil had almost had same amount of CO_2 evolution, probably because soils had differences of air permeability, pH, and conditions. Since the LS soil has higher air permeability rather than the SL soil and the CL soil, CO_2 gas might be easily exchanged. The LS soil had relatively low pH (6.8) to the CL soil and the SL soil (7.9), which means that lower pH still could release more CO_2. Soil condition was not optimal in three systems for optimal mineralization since it had lots of variables (Bowden et al., 1998), especially for the density difference of microbial or heterotrophic bacteria in terms of CO_2 evolution. When abiotic condition and biotic condition was focused on CO_2 evolution, the first 3 days shows most CO_2 evolved due to the combination of the abiotic condition, which chemically evolved CO_2, and the

biotic condition, which biologically evolved CO_2 (Jones et al., 2011). We tried to remove the

first 3 days' data and focused on microbial metabolism; however, cumulative CO_2-C evolution

shows there was no big difference among treatments. Further investigation is needed to better

clarify the difference CO_2 evolution among the LS soil, the SL soil, and the CL soil.

The rank order of SM and SBM was completely switched the position with unamended

soil by normalizing with the TOC because each treatment originally contained different amounts

of the TOC (Table 1). SM had higher evolution of CO_2 in SL soil and CL soil treatments but not

in LS soil treatment because microbial activity in soil became more active due to manure's

nutrients. Soils in semi-arid regions usually contain less than 1 % of the TOC (Artiola et al.,

2012). Amended soils with BC evolved significantly less CO_2 than unamended soils because

microbial activity in the soils was probably suppressed by the presence of minor amounts of

polynuclear aromatic hydrocarbon in BC. Moreover, we also found the positive interaction

between BC and manure because SBM evolved significantly less CO_2 than SM. Thus, we

suggest that BC could suppress CO_2 evolution on the soils and manure.

We hypothesized that the concentration of ammonium-nitrogen (NH_4^+-N) in all soil +

manure (SM) would dramatically decrease with the time due to immobilization or nitrification.

However, we assume that nitrification, N fixation, or immobilization probably occurred at the

same time so it shows no difference. BC could have an effect of decrease in ammonification on

the soil due to adsorption (Gundale and DeLuca, 2006). The concentration of NH_4^+-N in all

treatments slightly increased between 0 and 28 days probably because of mineralization by

microbial decomposition; however, the point that how nitrification or mineralization more

sensitive to adverse soil conditions was unclear. Both SL soil and CL soil treatments show

effects of BC on the SM that BC could decrease the concentration of both NH_4^+-N and nitrate-

nitrogen (NO_3^--N) at 28 -14 days. The depletion of NO_3^--N was shown at 14 days from the initial point in all treatments due to denitrification, immobilization, or probably absorption by BC. BC has the ability to catalyze the decrease of NO_3^--N to N_2, so that it probably could influence denitrification (Shenbagavalli and Mahimairaja, 2012). For other reason, denitrification probably happened because degradation rate of manure was high. As manure was decomposed, microbes used oxygen to convert organic matter; hence, depleting atmosphere inside the bag of oxygen occurred. It has to be replaced by oxygen defusing. If the rate of the biological activity inside of bag is too high, then oxygen diffusion would not keep up and inside of bag became anaerobic. Higher CO_2 evolution and oxygen consumption indicates that microbial activity is also high. We have not run the experiment to detect the level of nitrous oxide and adsorption power of each sample, especially BC because the potential of BC could have the adsorption of inorganic N (Yao et al., 2012). Therefore, immobilization could be one of the answers to decrease NO_3^--N as the widely accepted theory. NO_3^--N level increased at 28 days because of mineralization and nitrification. All CL soil treatments ranked in the top of the concentration rates was because we could suggest that CAC field where we sampled CL soil has been fertilized previously, thus high level of NO_3^--N was detected from CL soil.

Positive value indicates N mineralization, and negative value indicates N immobilization (Hart et al. 1994). Since soil + biochar + manure (SBM) in all soils treatments had less TIN and NH_4^+-N mineralization than soil + manure (SM) had, we suggest that the manure facilitates immobilization of TIN in the soil, and BC had the effect to suppress immobilization on SM. Manure also had the short term effect to enhance mineralization of NH_4^+-N at 14-0 days, yet BC suppressed mineralization in SM. Therefore, we assume that it is probably because nutrients of NH_4^+-N from manure are originally contained. The result from CO_2 experiment shows that LS

soil treatments evolved more CO_2 than the other soil treatments; therefore, the variation of immobilization rate may be related to the number of microbes in the soil. Since SLM, CLM, SLBM, and CLBM had less NH_4^+-N mineralization, it shows immobilization effect on NO_3^--N mineralization. Therefore, although NH_4^+-N was converted to NO_3^--N, the proportion of immobilization and denitrification was higher. Overall change in NO_3^--N mineralization, we found that mineralization facilitates in amended soils with BC, especially in SBM; however, immobilization occurred in unamended soils. CL soil treatments show great response due to the fertilizer in CAC.

On the different patterns of N mineralization in terms of the comparison to the initial time, all treatments of TIN and NH_4^+-N mineralization in all soil treatments lost their effectiveness over time, which means that all treatments eventually immobilized by microbes. Although SM still had the effect of mineralization at 14-0 and 28-0 days, the effect did not last until the end of this experiment. Overall change in TIN mineralization, most negative effect was found on CL soil treatments but least negative effect on SL soil treatments. We found the fluctuation of NO_3^--N mineralization increased and decreased at 28-0 days because nitrification and immobilization was alternated.

Conclusion

The current biochar (BC) and manure interaction experiment shows that in terms of each treatment size, soil + biochar + manure (SBM) evolved more CO_2 than soil + manure (SM) by the presence of extra carbon, high nutrient level from manure, and microbes which act synergistically. However, in terms of total organic carbon (TOC) in each treatment, all samples adding BC released less CO_2 than other sample mixed. It means that BC originally contained

much carbon inside. On the nitrogen mineralization experiment, at total inorganic nitrogen (TIN) concentration, adding manure and both BC and manure into the soils occurred mineralization during the period of 28 days. In terms of soil type, each soil had different time lag occurring mineralization and immobilization. Adding manure shows the great response of variation besides adding BC. This is because manure contained excess of N, so that all microbes had great activity that could mineralize and immobilize. In terms of NH_4^+-N, we found similar trend with TIN rates. All soil treatments adding manure and both BC and manure had the effect on NO_3^- compared with soils at 28 days. BC and manure soil additions reduced N deficiencies in all three soils.

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Figure Captions

Fig 1: CO_2 evolution from (a) loamy sand (LS) soil treatments including LS soil, LS soil + biochar (LSB), LS soil + manure (LSM), and LS soil + biochar + manure (LSBM), (b) silt loam (SL) soil treatments including, SL soil, SL + biochar (SLB), SL + manure (SLM), and SL + biochar + manure (SLBM), and (c) clay loam (CL) soil treatments including CL soil, CL + biochar (CLB), CL + manure (CLM), and CL + biochar + manure (CLBM) for the period of 112 days.

Fig 2: Cumulative CO_2 - C evolution from (a) loamy sand (LS) soil treatments including LS soil, LS soil + biochar (LSB), LS soil + manure (LSM), and LS soil + biochar + manure (LSBM), (b) silt loam (SL) soil treatments including, SL soil, SL + biochar (SLB), SL + manure (SLM), and SL + biochar + manure (SLBM), and (c) clay loam (CL) soil treatments including CL soil, CL + biochar (CLB), CL + manure (CLM), and CL + biochar + manure (CLBM) for the period of 112 days.

Fig 3: Cumulative CO_2 - C evolution from all treatments including biochar, manure, and both biochar and manure with each of loamy sand (LS) soil, silt loam (SL) soil, and clay loam (CL) soil for the period of 112 days. n = 4, error bar = 1 S.D. For each parameter, different letters indicate treatment means significantly different at P < 0.05.

Fig 4: CO_2 evolution normalized total organic carbon from (a) loamy sand (LS) soil treatments including LS soil, LS soil + biochar (LSB), LS soil + manure (LSM), and LS soil + biochar + manure (LSBM), (b) silt loam (SL) soil treatments including, SL soil, SL + biochar (SLB), SL + manure (SLM), and SL + biochar + manure (SLBM), and (c) clay loam (CL) soil treatments including CL soil, CL + biochar (CLB), CL + manure (CLM), and CL + biochar + manure (CLBM) for the period of 112 days.

Fig 5: Cumulative CO_2 - C evolution normalized with total organic carbon from (a) loamy sand (LS) soil treatments including LS soil, LS soil + biochar (LSB), LS soil + manure (LSM), and LS soil + biochar + manure (LSBM), (b) silt loam (SL) soil treatments including, SL soil, SL + biochar (SLB), SL + manure (SLM), and SL + biochar + manure (SLBM), and (c) clay loam (CL) soil treatments including CL soil, CL + biochar (CLB), CL + manure (CLM), and CL + biochar + manure (CLBM) for the period of 112 days.

Fig 6: Cumulative CO_2 - C evolution normalized with total organic carbon from all treatments including biochar, manure, and both biochar and manure with each of loamy sand (LS) soil, silt loam soil (SL), and clay loam soil (CL) for the period of 112 days. n = 4, error bar = 1 S.D. For each parameter, different letters indicate treatment means significantly different at P < 0.05.

Fig 7: Concentration of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of loamy sand (LS) soil treatments at 0, 14, and 28 days.

Fig 8: Concentration of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of silt loam (SL) soil treatments at 0, 14, and 28 days.

Fig 9: Concentration of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of clay loam (CL) soil treatments at 0, 14, and 28 days.

Fig 10: Mineralization of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of loamy sand (LS) soil treatments. Each time of measurement was subtracted from previous time of measurement.

Fig 11: Mineralization of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of silt loam (SL) soil treatments. Each time of measurement was subtracted from previous time of measurement.

Fig 12: Mineralization of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of clay loam (CL) soil treatments. Each time of measurement was subtracted from previous time of measurement.

Fig 13: Mineralization of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of loamy sand (LS) soil treatments. Each time of measurement was subtracted from initial time of measurement.

Fig 14: Mineralization of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of slity loam (SL) soil treatments. Each time of measurement was subtracted from initial time of measurement.

Fig 15: Mineralization of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of clay loam (CL) soil treatments. Each time of measurement was subtracted from initial time of measurement.
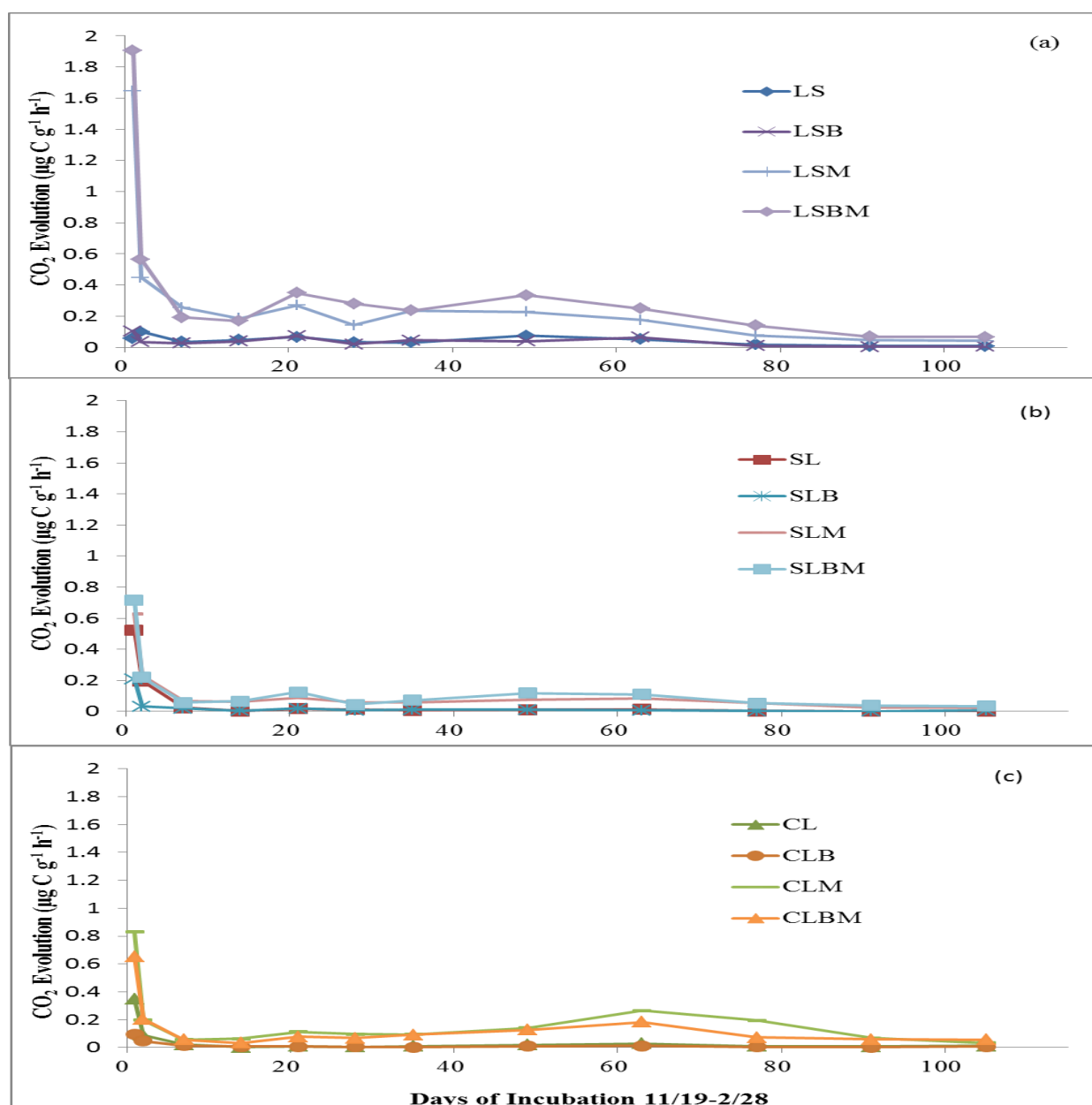
Fig 1: CO$_2$ evolution from (a) loamy sand (LS) soil treatments including LS soil, LS soil + biochar (LSB), LS soil + manure (LSM), and LS soil + biochar + manure (LSBM), (b) silt loam (SL) soil treatments including, SL soil, SL + biochar (SLB), SL + manure (SLM), and SL + biochar + manure (SLBM), and (c) clay loam (CL) soil treatments including CL soil, CL + biochar (CLB), CL + manure (CLM), and CL + biochar + manure (CLBM) for the period of 112 days.
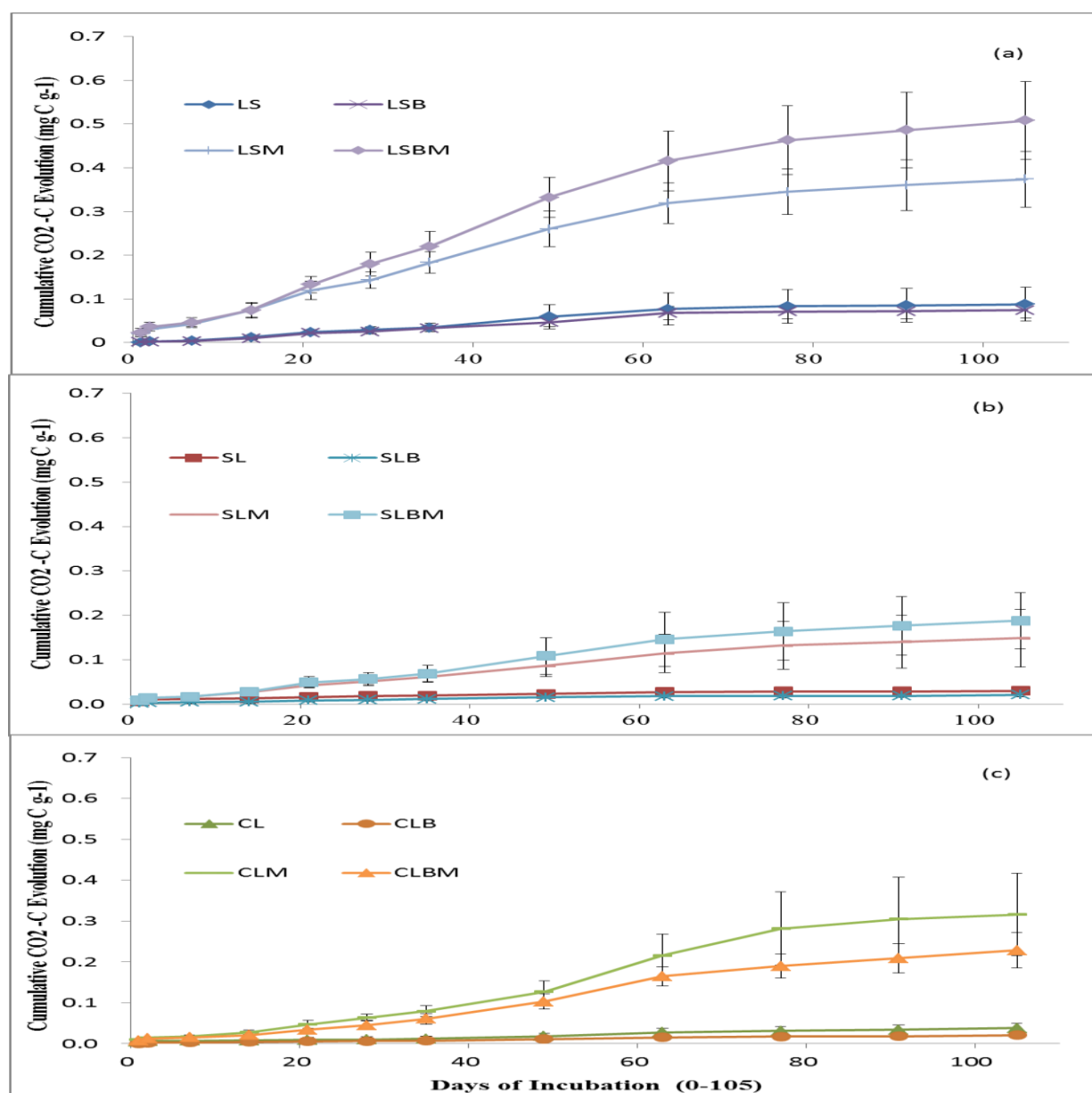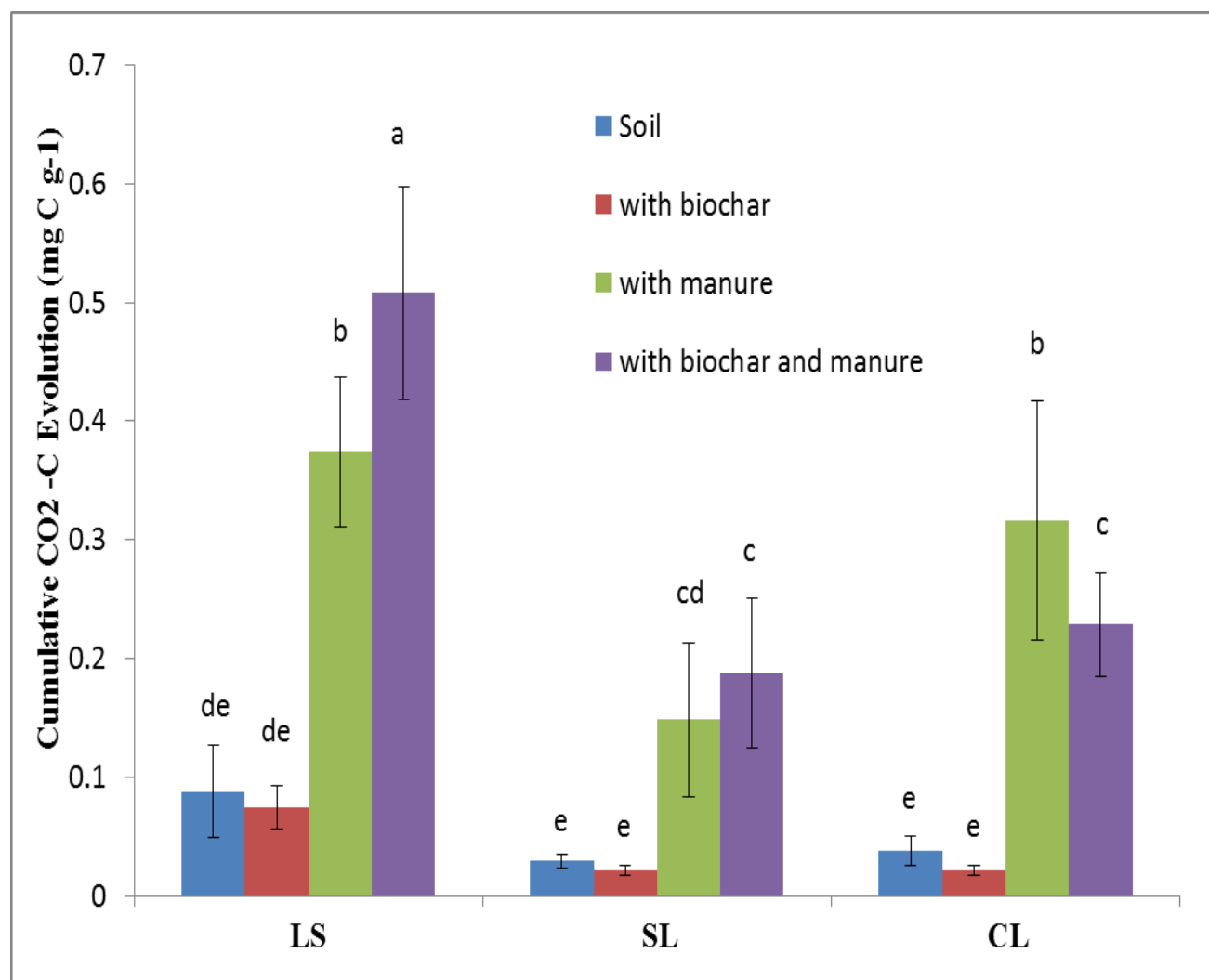
Fig 2: Cumulative CO_2 - C evolution from (a) loamy sand (LS) soil treatments including LS soil, LS soil + biochar (LSB), LS soil + manure (LSM), and LS soil + biochar + manure (LSBM), (b) silt loam (SL) soil treatments including, SL soil, SL + biochar (SLB), SL + manure (SLM), and SL + biochar + manure (SLBM), and (c) clay loam (CL) soil treatments including CL soil, CL+ biochar (CLB), CL + manure (CLM), and CL + biochar + manure (CLBM) for the period of 112 days.
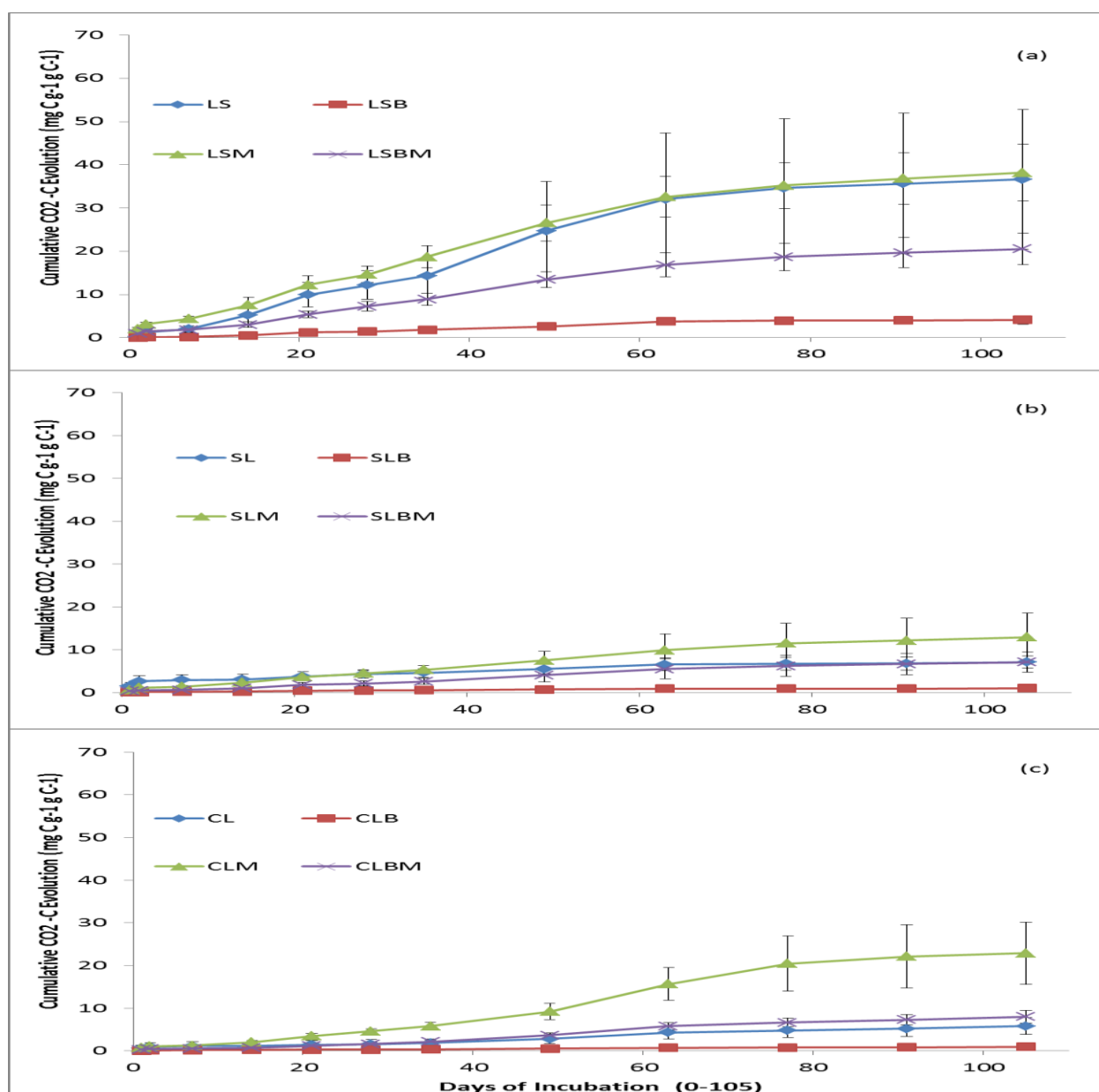
Fig 3: Cumulative CO_2 - C evolution from all treatments including biochar, manure, and both biochar and manure with each of loamy sand (LS) soil, silt loam (SL) soil, and clay loam (CL) soil for the period of 112 days. n = 4, error bar = 1 S.D. For each parameter, different letters indicate treatment means significantly different at $P < 0.05$.

Fig 4: CO$_2$ evolution normalized total organic carbon from (a) loamy sand (LS) soil treatments including LS soil, LS soil + biochar (LSB), LS soil + manure (LSM), and LS soil + biochar + manure (LSBM), (b) silt loam (SL) soil treatments including, SL soil, SL + biochar (SLB), SL + manure (SLM), and SL + biochar + manure (SLBM), and (c) clay loam (CL) soil treatments including CL soil, CL + biochar (CLB), CL + manure (CLM), and CL + biochar + manure (CLBM) for the period of 112 days.
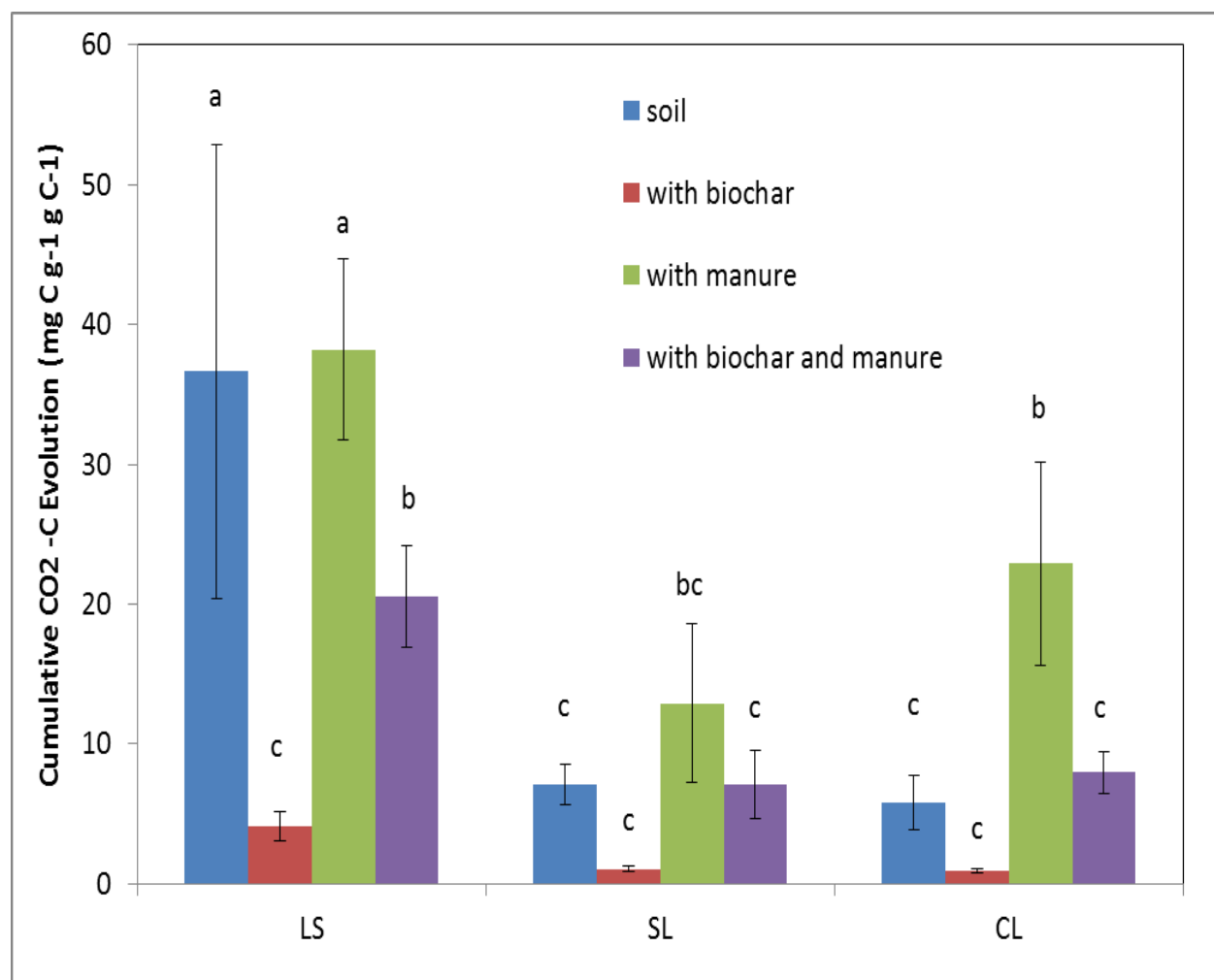
Fig 5: Cumulative CO_2 - C evolution normalized with total organic carbon from (a) loamy sand (LS) soil treatments including LS soil, LS soil + biochar (LSB), LS soil + manure (LSM), and LS soil + biochar + manure (LSBM), (b) silt loam (SL) soil treatments including, SL soil, SL + biochar (SLB), SL + manure (SLM), and SL + biochar + manure (SLBM), and (c) clay loam (CL) soil treatments including CL soil, CL + biochar (CLB), CL + manure (CLM), and CL + biochar + manure (CLBM) for the period of 112 days.

Fig 6: Cumulative CO_2 - C evolution normalized with total organic carbon from all treatments including biochar, manure, and both biochar and manure with each of loamy sand (LS) soil, silt loam soil (SL), and clay loam soil (CL) for the period of 112 days. n = 4, error bar = 1 S.D. For each parameter, different letters indicate treatment means significantly different at $P < 0.05$.
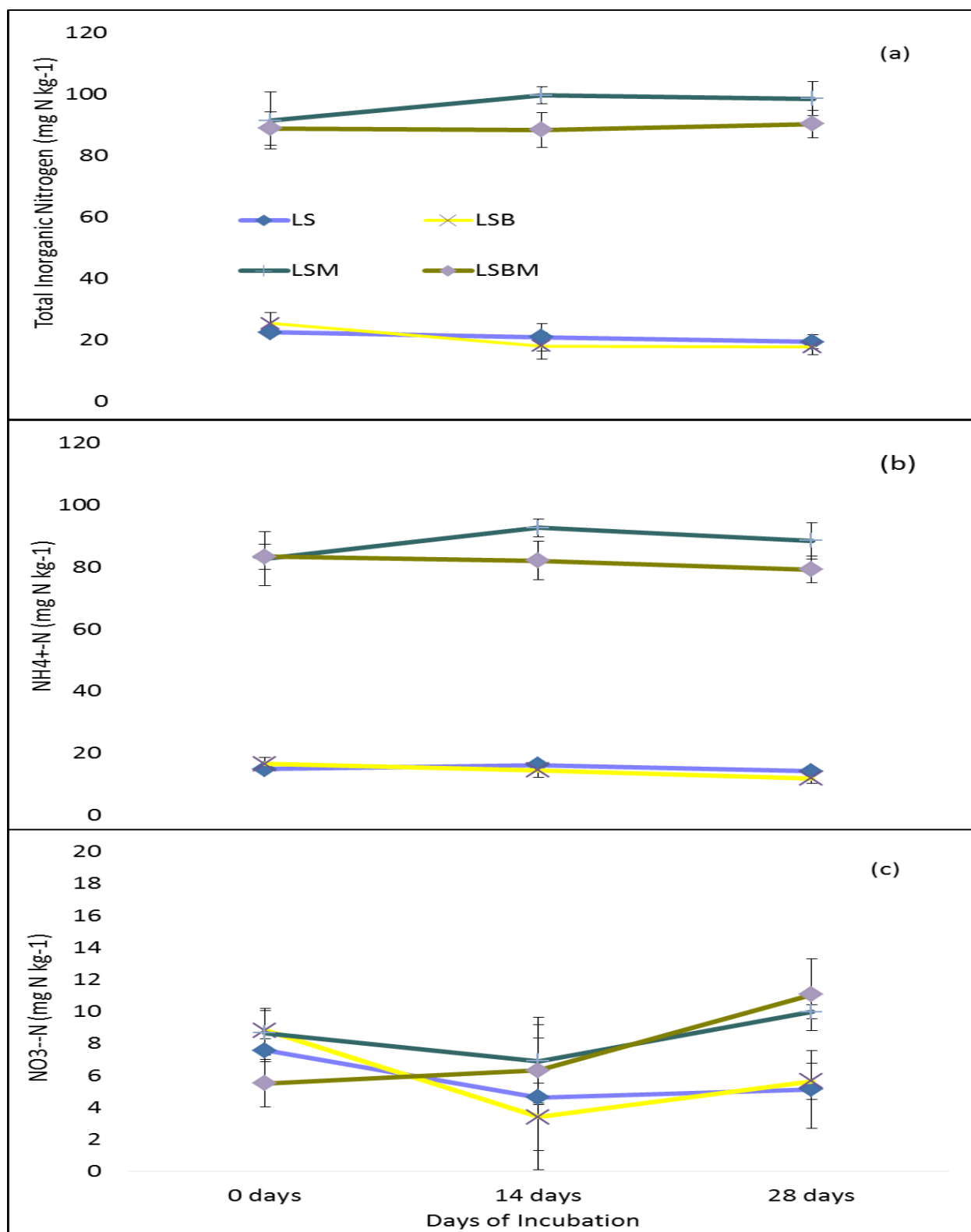
Fig 7: Concentration of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of loamy sand (LS) soil treatments at 0, 14, and 28 days.
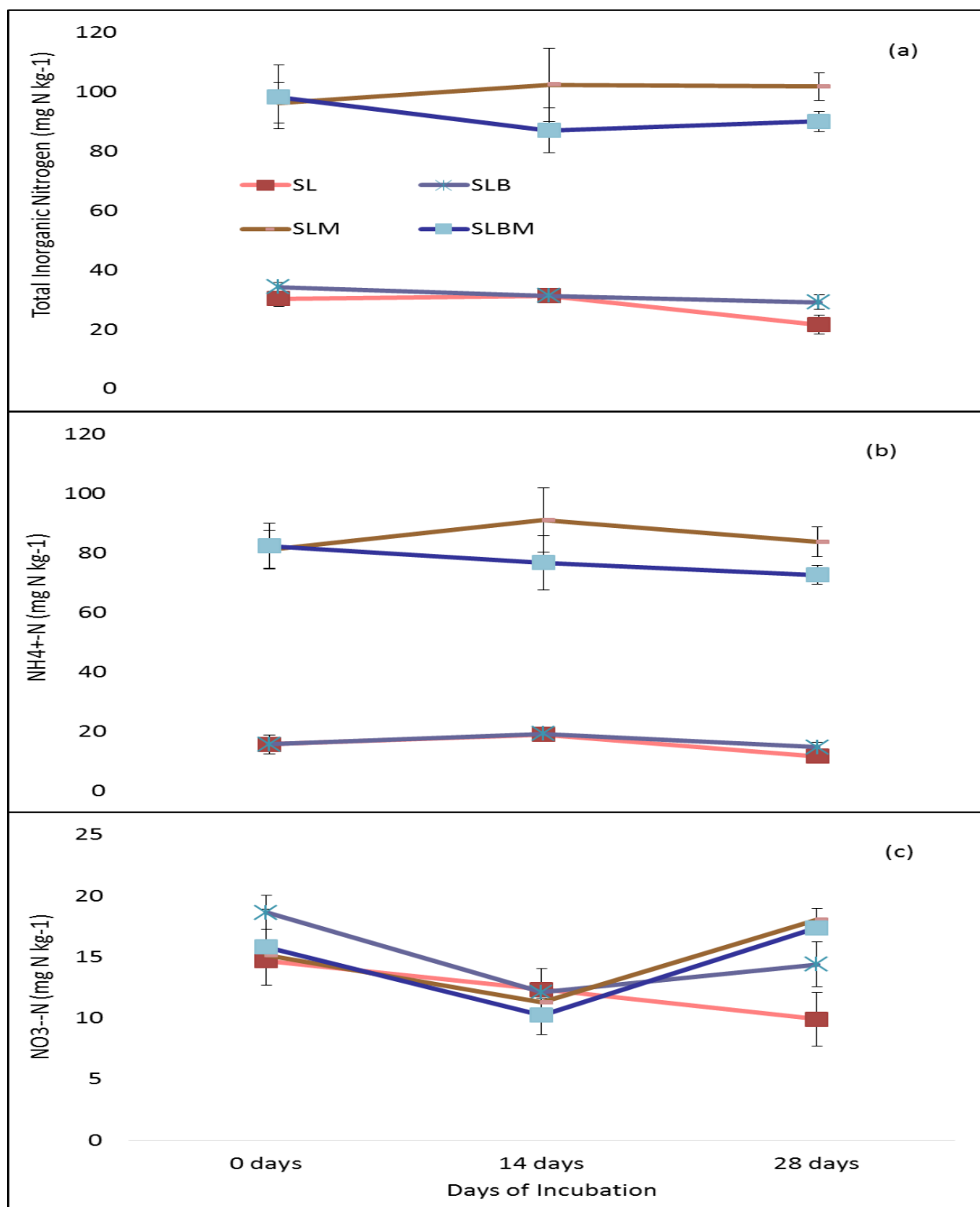
Fig 8: Concentration of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of silt loam (SL) soil treatments at 0, 14, and 28 days.
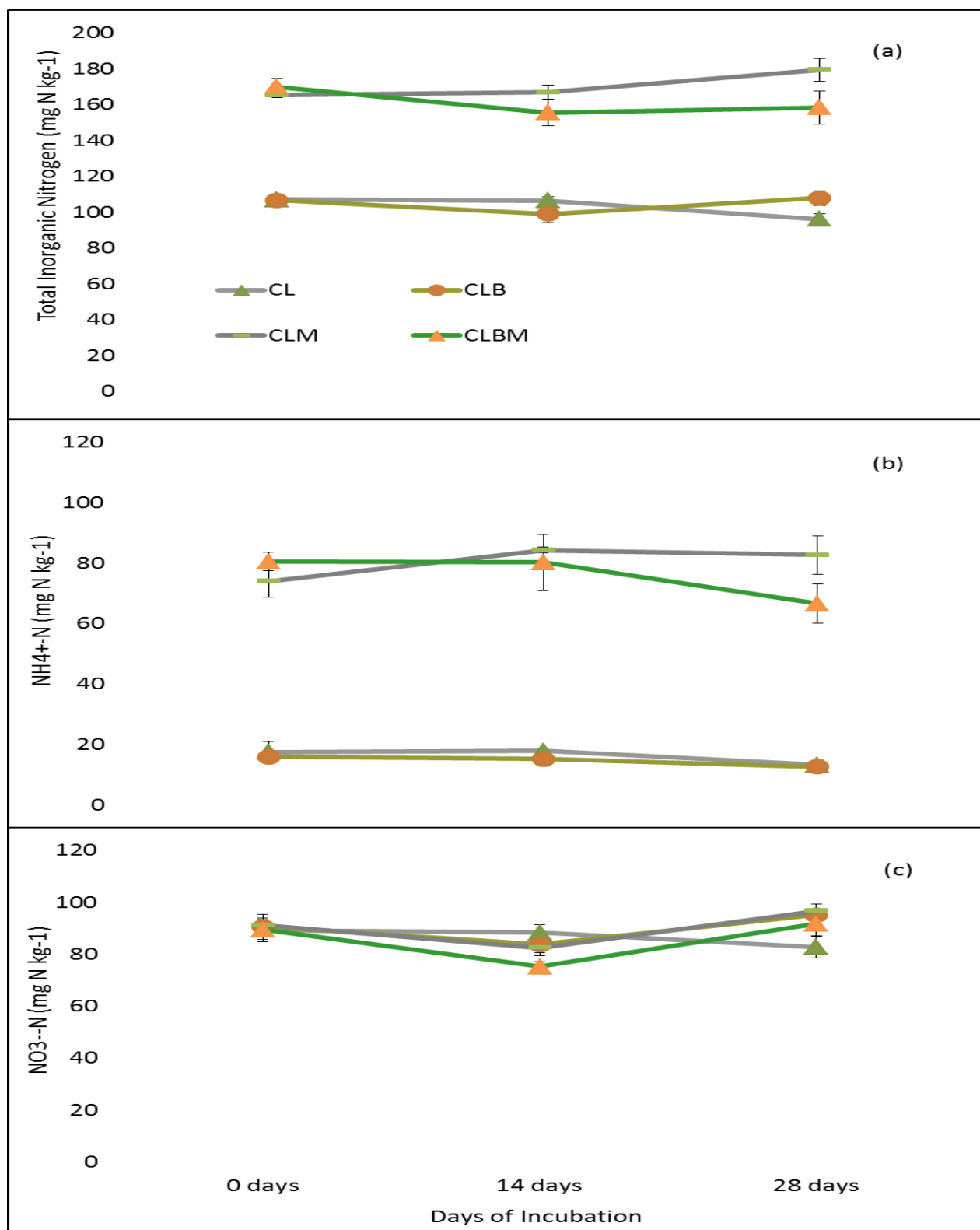
Fig 9: Concentration of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of clay loam (CL) soil treatments at 0, 14, and 28 days.
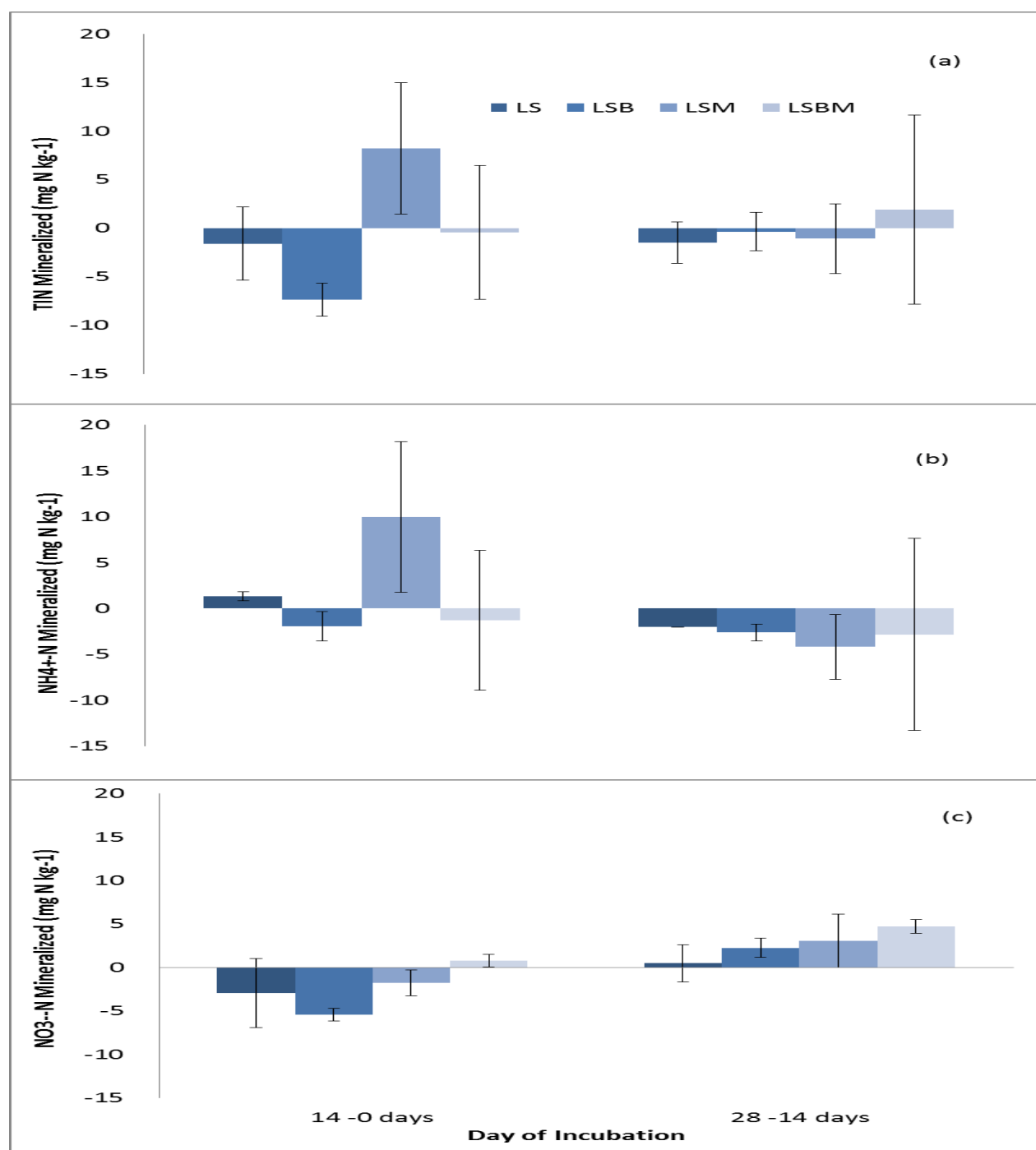
Fig 10: Mineralization of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of loamy sand (LS) soil treatments. Each time of measurement was subtracted from previous time of measurement.
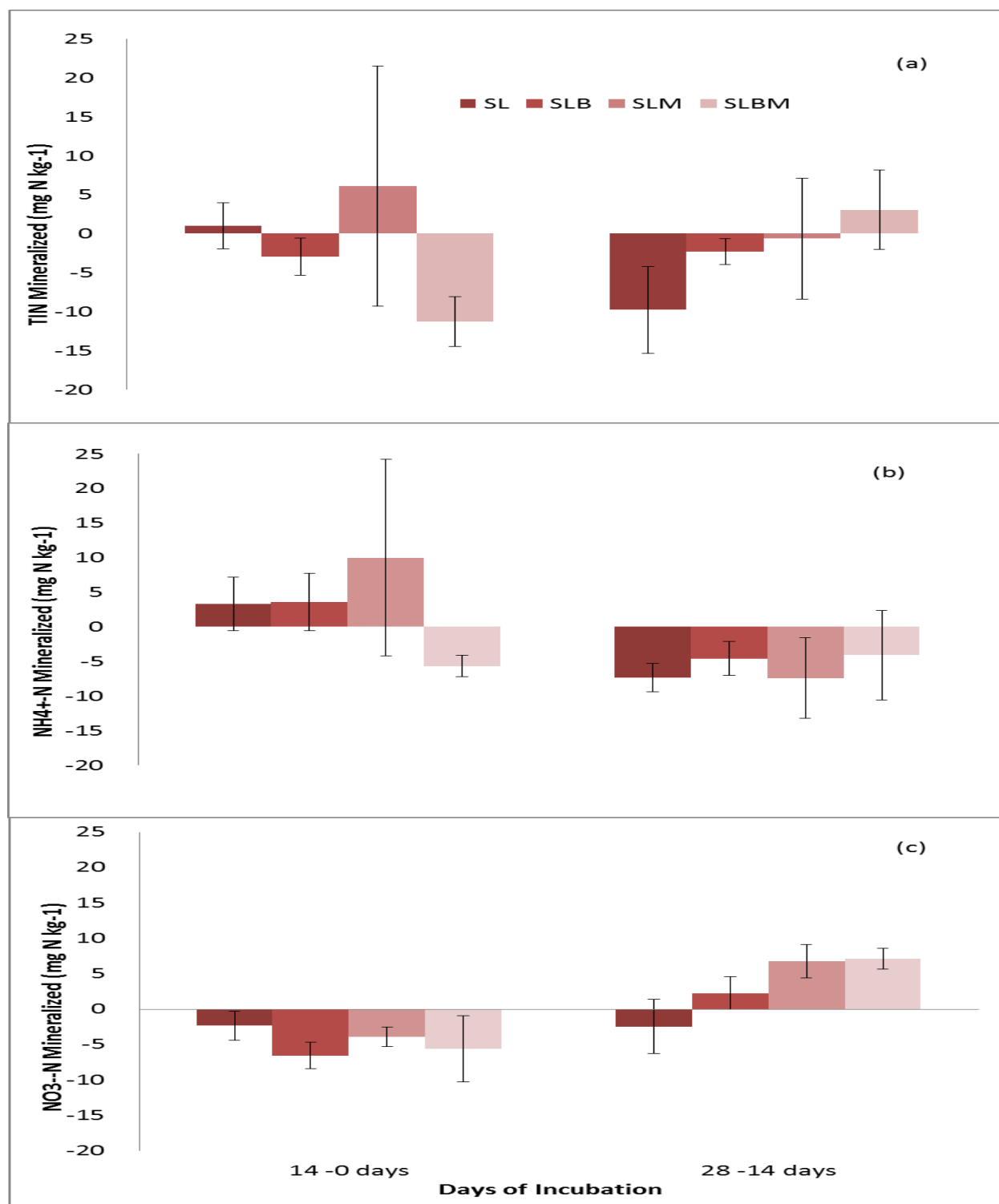
Fig 11: Mineralization of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of silt loam (SL) soil treatments. Each time of measurement was subtracted from previous time of measurement.

Fig 12: Mineralization of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of clay loam (CL) soil treatments. Each time of measurement was subtracted from previous time of measurement.
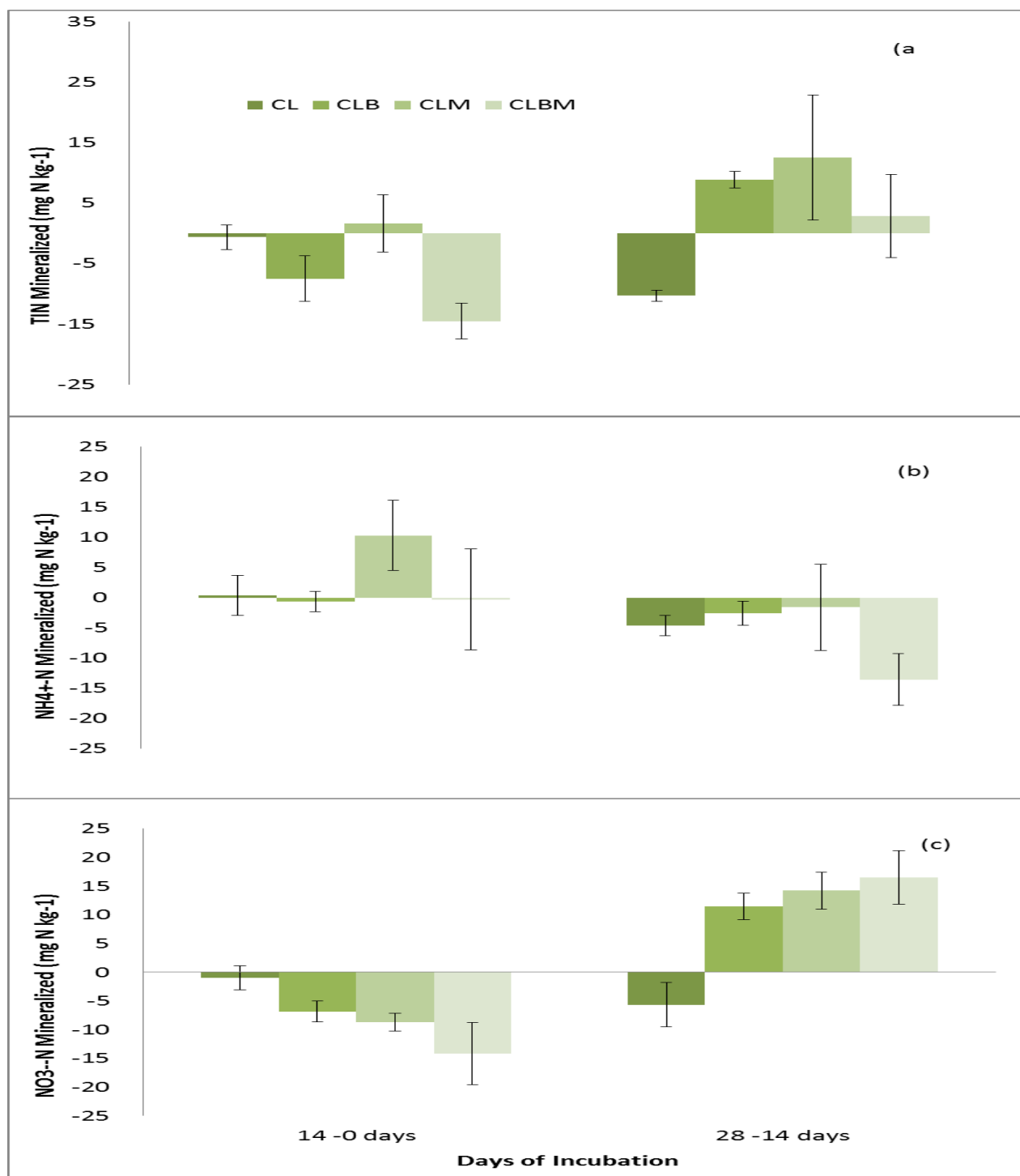
Fig 13: Mineralization of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of loamy sand (LS) soil treatments. Each time of measurement was subtracted from initial time of measurement.

Fig 14: Mineralization of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of slity loam (SL) soil treatments. Each time of measurement was subtracted from initial time of measurement.

Fig 15: Mineralization of (a) total inorganic nitrogen (TIN), (b) ammonium (NH_4^+-N), and (c) nitrate (NO_3^--N) of clay loam (CL) soil treatments. Each time of measurement was subtracted from initial time of measurement.

TABLE

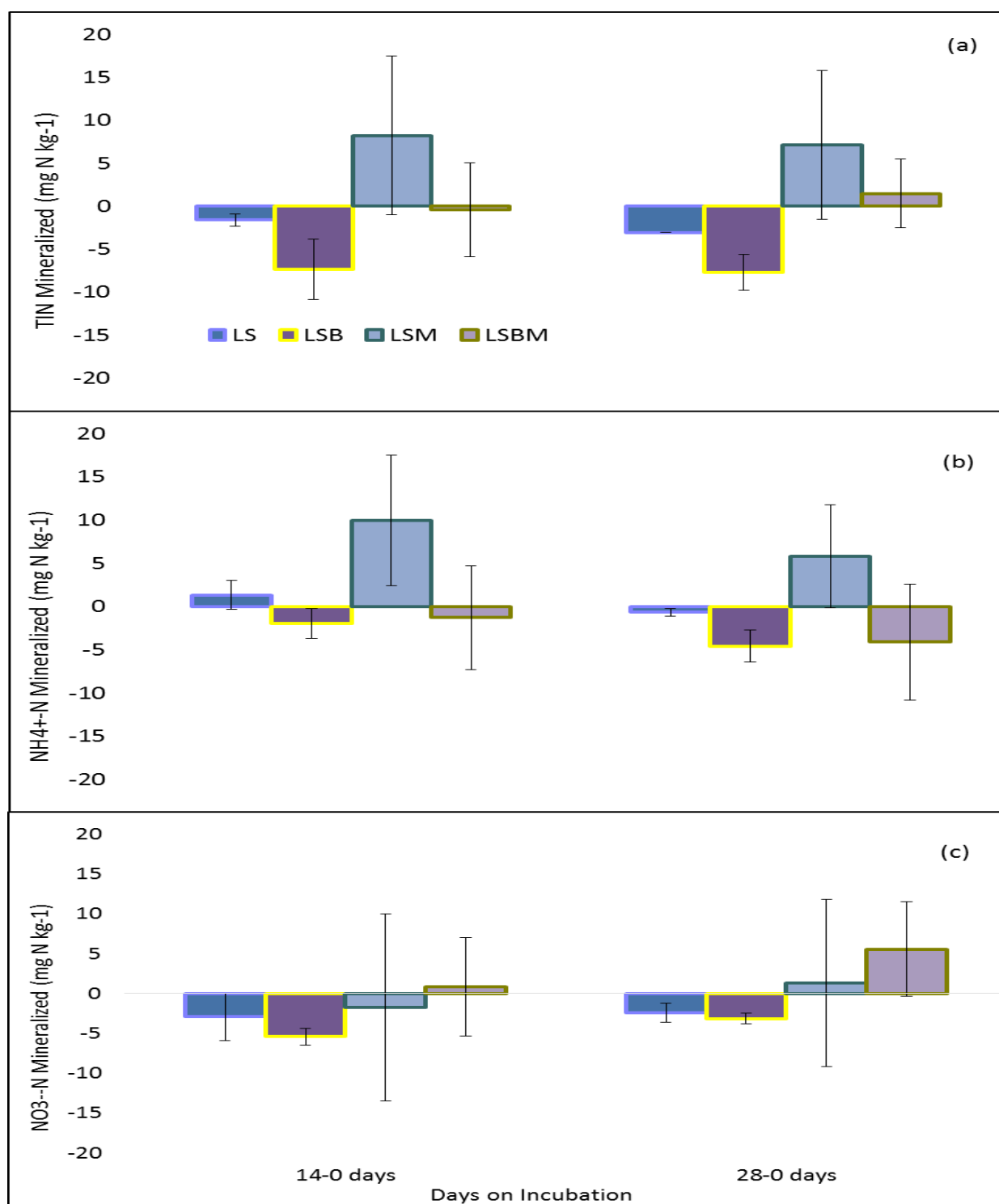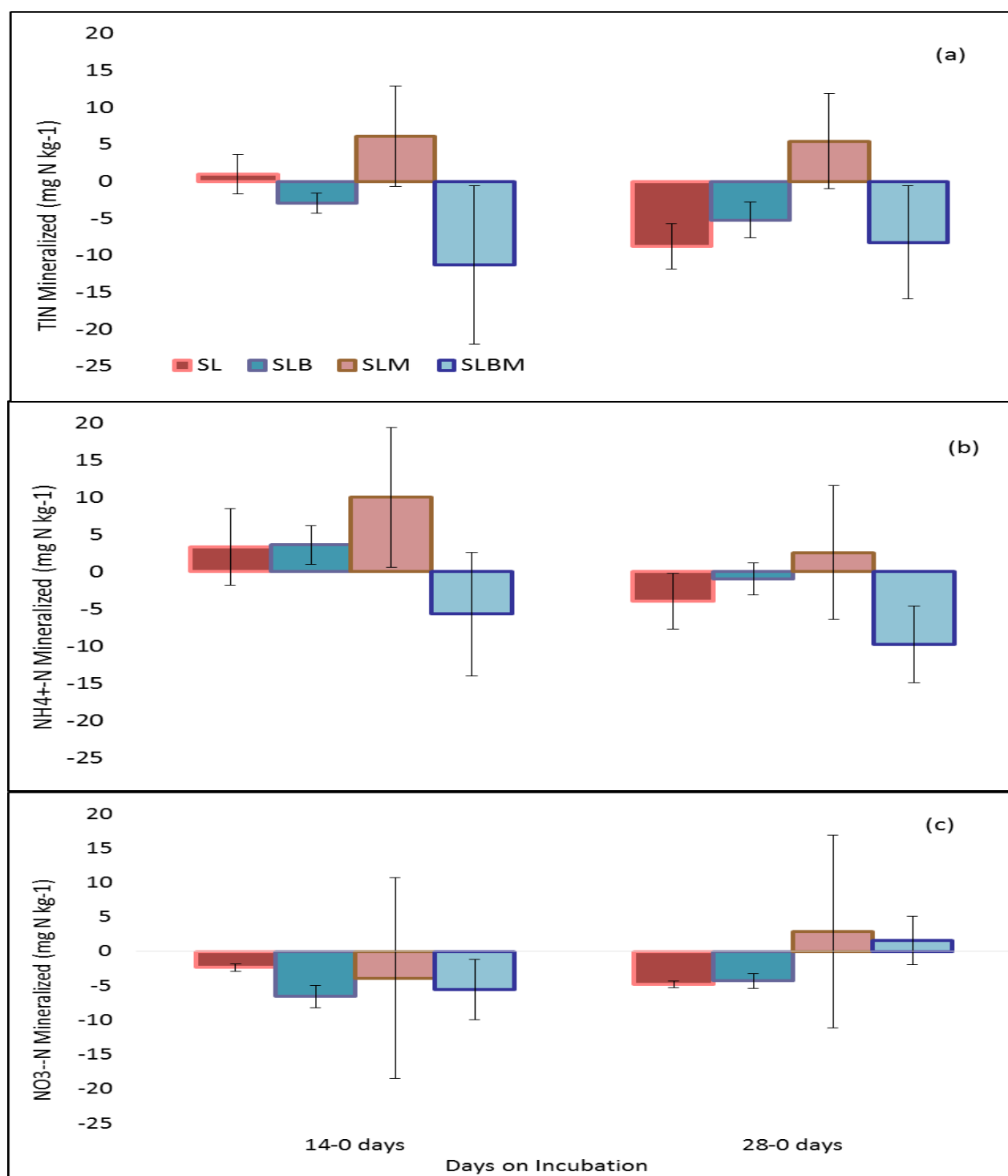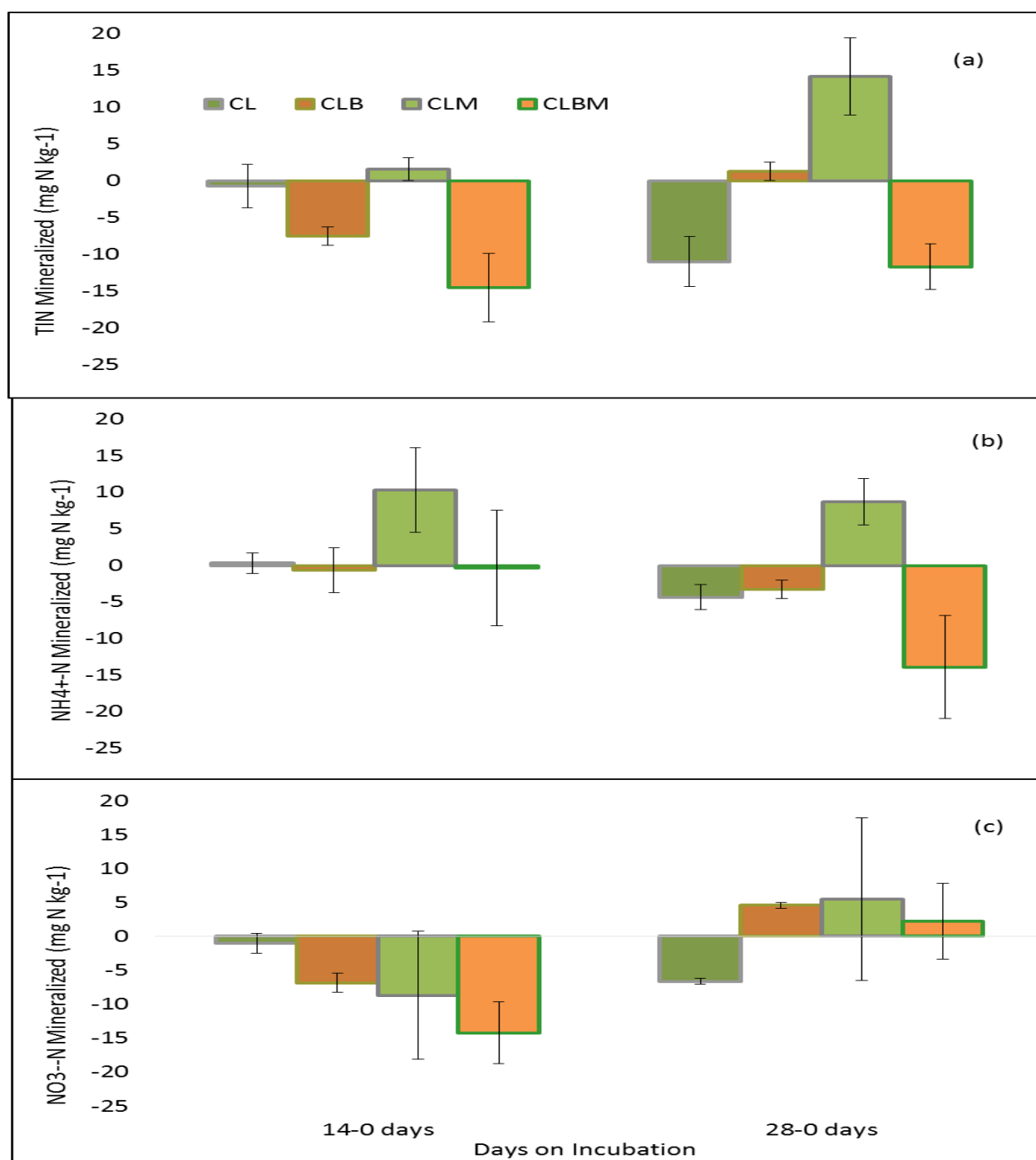List of Tables:

Table 1: Physical and chemical properties of the soils, biochar, and manure

TABLE 1. Physical and chemical properties of the soils, biochar, and manure

	Red Rock Soil	SRER Soil	CAC Soil	Biochar	Manure
Texture	loamy sand	silt loam	clay loam	N/A	N/A
pH	6.83	7.94	7.88	8.89	8.33
Water Holding Capacity (%)	22.86	50.65	46.93	287.6	277.45
Total C (%)	0.3	0.6	1.7	79.844	79.7
Total Organic C (%)	0.24	0.415	0.655	79.7	79.7
Total Inorganic N (%)	0.043	0.0084	0.0465	> 0.0001	1.099
NO_3^--N (mg N kg-1)	7.6	14.7	89.3	> 0.0001	N/A
NH_4^+-N (mg N kg-1)	15	15.67	18	> 0.0001	N/A

APPENDIX B

Carbon dioxide emissions from biochar-amended alkaline semi-arid soil

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Abbreviations:
BC (Biochar)
CEPM (Center for Environmental Physics and Mineralogy)
DIC (Dissolved Inorganic Carbon)
DI (Deionized)
DOC (Dissolved Organic Carbon)
IRGA (Infrared Gas Analyzer)
OM (Organic Matter)
PFW (Pine Forest Waste)
LS (Loamy Sand)
LSAB (Loamy Sand Soil + Acidified Biochar)
RRAC (Red Rock Agricultural Center)
LSB (Loamy Sand Soil + Biochar)
TOC (Total Organic Carbon)
WHC (Water Holding Capacity)

Abstract

Biochar (BC), produced through pyrolysis of organic residues, is increasingly being used as a beneficial soil amendment. We studied effects of BC on carbon dioxide (CO_2) evolution in semi-arid climate soil. BC in the soils is not easily decomposed by microbes; hence, carbon does not return to the atmosphere due to carbon cycle mechanisms, which means that the carbon capture and storage by BC can contribute to reduce CO_2. We came up with two research questions: (1) could the application of BC to the soils in semi-arid agricultural systems suppress CO_2 evolution? (2) Would CO_2 production be different between both amended and unamended soils and untreated and acidified BC? The hypothesis was that BC alkalinity released to solution facilitates partitioning of atmospheric CO_2 into solution phase carbonates, effectively leading to a drawdown or consumption of atmospheric CO_2. This experiment directly tested the abiotic mechanism and determined the potential effects of BC alkalinity on atmospheric CO_2 using both untreated and acidified BC. The results show that the soil samples amended with acidified BC evolved more CO_2 than those amended with untreated BC with high alkalinity. It is postulated that untreated BC could absorb CO_2; whereas, acidified BC with no alkalinity could not. The loamy sand (LS) soil amended with BC (LSB) evolved less CO_2 than the LS soil control perhaps due to the soil microbial activity inhibitory effects of the BC's residual water-soluble polynuclear aromatic hydrocarbons. Thus, alkalinity reduces measured CO_2 mineralization due to formation of carbonates in solution.

Introduction

A significant knowledge gaps remains as to how the application of biochar (BC) to arid and semi-arid soils where carbonates may precipitate and accumulate as solid state carbonates in

the soil will affect the soil carbon cycle. The carbon cycle is important in ecosystems because all living things are made of carbon in one way or another. The Earth has hyper-arid, arid, and semi-arid soils, which cover approximately 4%, 15%, and 12% of soil, respectively of the land surface (FAO Conservation Guide, 1989). The arid and semi-arid regions have a great potential for plant growth although they are limited by water shortage and plant nutrients. Therefore, understating mechanisms that can improve agricultural production in such environments is important.

BC has started to attract attention of scientists internationally, primarily because of the discovery of Terra Preta soils with elevated fertility levels thought to come from repeated additions of charcoal by the local tribe in the Amazon River basin of upper Brazil suggesting that "permanent or semi-permanent agriculture can itself create sustainably fertile soils" (Glaser et al., 2001). BC, produced by burning biomass residues in the absence or near-absence of air, is now widely accepted as a soil amendment in hot, wet regions of the world with low fertility soils (typically Oxisols) (Lehmann et al., 2011). However, BC could also be used on semi-arid agricultural systems with loamy sand soils, which have low water holding capacity (WHC) (Yu et al., 2013) and low nutrient contents (Sukartono et al., 2011). Since BC has a large surface area, is porous, and has a large WHC, when applied to soils, the roots of plants may increase around BC particles to absorb nutrients (Marris, 2006).

BC is known to decompose very slowly in soils (Lehmann et al., 2009). Hence, unlike plant and animal residues, most of the BC carbon does not return to the atmosphere. Therefore, BC soil amendments are considered a form of carbon capture, which contribute to reducing atmospheric carbon dioxide (CO_2). BC also has the potential to increase the activity of soil microorganisms in agricultural soils (Lehmann and Marco, 2006) and can act as a habitat for populations of microorganisms that turn soil into spongy, dark material (Lehmann et al., 2006),

creating an environment where aerobic microbes easily increase because of abundant air and water (Thies and Rillig, 2009). BC can also absorb nutrients and water that otherwise would be leached below the soil root zone. Since charcoal is produced at temperatures above $400^{\circ}C$, no organic matter (OM) is left that can be used as a food for saprophytic microbes as plant tissues and cells are carbonize completely forming a high porosity structure. Adding BC into soils may facilitate plant root symbiotic microbes, as well as root nodule bacteria (Yoshizawa et al., 2006). Overall, BC is thought to produce changes in soil that are beneficial to biological, physical and chemical properties (Ennis et al., 2012).

The objective of this research was to test how removing alkalinity from BC affects CO_2 mineralization in water and soil. We hypothesized that BC alkalinity released to solution facilitates partitioning of atmospheric carbon dioxide into solution phase carbonates, effectively leading to a drawdown or consumption of atmospheric CO_2.

Materials and Methods

This experiment was conducted at the Center for Environmental Physics and Mineralogy (CEPM) laboratory at the University of Arizona in Tucson, Arizona. Loamy sand surface soil was sampled from the top 0 to 15 cm of soil at University of Arizona Red Rock Agricultural Center (RRAC) approximately 55 km northwest of Tucson, Arizona (Artiola et al., 2012). This soil was characterized as Denure soil series, course-loamy, mixed, superactive, hyperthermic, Typic Haplocambid (Soil Survey Staff, 2006). The soil was placed in the dark at room temperature at the laboratory after collection, air-dried, and sieved to < 2mm before starting the incubation process. The experiment included six treatments each replicated four times: (1) DI water, (2) biochar (BC), (3) acidified BC, (4) loamy sand (LS) soil, (5) loamy sand soil+ biochar

(LSB), and (6) loamy sand soil + acidified biochar (LSAB). The physical and chemical properties of LS and BC are shown in Table 1.

Pine forest waste (PFW) woodchips derived BC 1-3 mm size fraction, was used for this incubation experiment in all the batch experiments. The BC was produced using a 55,000-BTU wood gas Mega stove in batch mode and slow pyrolysis with a BC interparticle temperature of 450 °C to 500 °C and a yield of 18 % to 20% by mass (Artiola et al., 2012). The BC alkalinity was measured using a hot sulfuric acid digestion followed by back titration and reported as $CaCO_3$ (Table 1).

A 0.1N sulfuric acid solution was used to acidify BC and raise its pH to 5.0. The acid solution was added to BC and water mixture to bring to pH 5.0. Enough acid to neutralize BC alkalinity (assuming that BC has approximately 2.5-% total alkalinity (as CaCO3)) was used. Each beaker was filled with 1:10 ratio of BC and DI water, and the other beaker contained 1:10 ratio of BC and sulfuric acid. Each beaker was placed on a stir plate with the pH electrode inserted into the solution, and a moderate stirring rate was maintained with the stir plate and mixing rod. The pH of the BC-water suspensions was measured every 2 or 3 days for a period of 44 days (Figure 1). The average total amount of 0.1 N sulfuric acid needed to bring the BC-water mixture to pH 5.0 and remain there was 2.31 ml of 0.1N sulfuric acid per gram of BC. Therefore, the PFW BC released 1.15% alkalinity (as $CaCO_3$) after 44 days. Nonetheless, it was assumed that the BC had on average 2.5 % total alkalinity (as $CaCO_3$) and a BC batch sufficient to conduct the incubation experiments was acidified with sulfuric and allowed to stabilize for 44 days. The pH of BC used in the incubation experiments was 2.2.

Loamy sand (LS) soil inoculum solution was added to the BC incubation vessels. The LS soil extract was prepared following a method by Wagai and Sollins (2002). Briefly, 10.0 g of

sieved moist soil are mixed with 50.0 mL DI water and shaken at slow speed for half an hour then left at room temperature overnight. The soil slurry was filtered through a 5-micrometer membrane to obtain the filtrate used as inoculum for the CO_2 emissions incubation studies.

The water holding capacity (WHC) for each of the incubation samples was determined before the experiments. A 150-300 mL beaker was topped with a small funnel lined with Whatman No. 42 filter paper. Approximately 10.0 g of sample was weighed out and placed into the funnel, held by the filter paper. The beaker was filled with DI water to allow the filter paper in the funnel to be submerged. Once saturated, the water from the beaker was removed, and the sample was allowed to drip back into the beaker overnight. After collecting and weighing subsample of the sample, it is placed in 105 ^{o}C oven overnight to dry. Dry sample was weighed and exact mass was recorded and used to calculate the gravimetric water content at field capacity.

20.0 g of LS soil and 10.0 g of each BC and acidified BC were prepared. Two soil:biochar mixtures were also prepared using 18.0 g of LS soil mixed with 2.0 g of BC or acidified BC,. Each sample was placed into a small plastic container and in turn placed inside a Mason jar (8 oz) in preparation for the incubation experiment. The Mason jar lids were fitted with septa hot glued on the top and bottom and each jar was air leak tested before the experiment.

Using the prerecorded dry masses and field capacity moisture data, the amount of water, including 1 mL of inoculum needed to wet each particular sample to 60 % volumetric water content, was calculated. The wetted samples in plastic containers were placed in the Mason jars with about 0.75 mL DI water added to the bottom of each jar to keep soil moisture content

constant. Between CO_2 measurements the jars were kept in a dark cabinet at a temperature of 21 $^{\circ}$C.

The CO_2 concentrations of the jars were measured using an Infra-Red Gas Analyzer (Qubit CO_2 Analyzer, model S-151; Qubit Systems, Kingston, Ontario, Canada). Pure nitrogen gas was used as the carrying gas at a rate of 125 mL / min for the instrument. The infrared gas analyzer (IRGA) station works with the data logger "Logger Pro 3.4.2". The program graphed out the results of each measurement over time in a curved peak form, using ppm units as the CO_2 concentration. A peak integration function was then used to calculate the area under the peak of each measurement. The standard measurements for a calibration curve for 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1 mL, 2 mL, and 3 mL of CO_2 respectively, were taken to calculate exact CO_2 concentrations from the integral data of the measurement. The concentration of the standard CO_2 gas used in our laboratory is 1% so 1.0 mL volume is contained 1% CO_2. The goal was to measure the samples while their CO_2 concentrations were between 0.2 % and 3.0 %. The measurement range of this instrument is 0-2000 ppm. To measure a container, the headspace was mixed with a syringe, and then 1.0 mL sample was collected for CO_2 analysis. After sampling the CO_2 in the headspace, there were uncapped and purged with a stream of air.

All experiments were performed in at least 4-6 replicates. Statistical analysis for the cumulative CO_2 –C for this experiment was performed using CoStat Statistical Software, 2-way completely randomized analysis of variance (ANOVA) ($p < 0.05$), comparing and ranking means using the Student-Newman-Keuls test.

Results

The experimental data was separated into two groups. Biochar (BC) treatments included DI water, BC and acidified BC. Loamy sand (LS) soil treatments included the LS soil, LS soil + BC, and LS soil + acidified BC. CO_2 evolution of BC treatments show the largest CO_2 gas emissions in the first day (Figure 2a) and quickly decreasing during the next four days from the first day (Figure 2b) and remained constant after 5 days to the end of the incubation period of 230 days. During the first 4 days, acidified BC evolved the largest amount of CO_2 remaining above the other treatments until day 70. LS soil treatments (Figure 3a) has similar trends, with the LS soil mixed with acidified BC initially releasing more CO_2 than the LS soil mixed with regular BC or the control (LS) during the first 5 days (Figure 3b).

Overall, the cumulative amounts of CO_2-C evolved for the two sets of experiments and all treatments show that there were statistically differences among all treatments and controls. However, each set of experiments show that there was no statistically difference between BC and acidified BC in BC treatments (Figure 4), and there was statistically difference among LS soil treatments (Figure 5). For example, the BC samples treated only with soil inoculum released up to 4 times more carbon than LS soil treatments and acidified BC released significantly more carbon, than the non-acidified BC. However, LS + biochar (LSB) evolved significantly more CO_2 evolution than LS + acidified biochar (LSAB). Also, the addition of BC materials to the LS soil doubled its carbon emissions.

The amounts of CO_2 produced by each sample also statistically analyzed after they were normalized with respect to the total organic carbon (TOC) initially measured each sample (Table 1), rank orders of each treatment were changed. Acidified BC still evolved more CO_2 than BC all of the time because both had the same amount of the TOC. The LS soil control shows the highest

CO_2 evolution of all LS soil treatments at all times. Cumulatively, the normalized CO_2 evolution from the LS soil plus BC (acidified or not) was 20 times lower than the control (Figure 6 and 7).

Discussion

The incubation data indicates that the additions of biochar (BC) to the loamy sand (LS) soil reduced soil carbon emissions significantly. Therefore, it also has benefits for improving agricultural systems because BC application to the soils can reduce CO_2 evolution (Lehmann et al., 2006). Although overall CO_2 emissions increased after BC applications, there may have been due to the addition of a small fraction of bioavailable (water soluble) organic carbon (OC) present in BC. Thus, when the TOC in each sample was considered, BC suppressed CO_2. The observed long-term CO_2 emission trends observed in the BC amended soils, suggest that the pine forest waste (PFW) BC used in these incubation studies has a very recalcitrant form carbon to a soil.

Given the very large CO_2 emissions during the first 4 days, we can assume that these were associated with abiotic processes such as degassing and/or changes in water pH that resulted in the formation of carbonates. Subsequent and nearly constant CO_2 evolutions were biologically induced due to microbial respiration, but small and constant. The microbial respiration rates were probably slow and did not produce much CO_2 because nitrogen was not added to the incubation bottles. The acidified BC evolved more CO_2 than BC under the abiotic condition because BC still has a large amount of alkalinity in it. When some of this alkalinity when into solution it raised to pH in the water allowing for more of the CO_2 in the headspace in the jar to dissolve into the water forming bicarbonate. Therefore, the headspace in the jar of the BC treatment had less CO_2 than acidified BC. In other words, BC was a sink, not a source of CO_2.

In detail, alkalinity in the form of Calcium oxide and Calcium hydroxide reduces measured CO_2 mineralization due to the formation of carbonates in solution that then reduces dissolved CO_2. Therefore, it draws CO_2 out of the atmosphere into the solution to balance atmospheric pCO_2. Atmospheric pCO_2 goes up as BC is mineralized and forcing more CO_2 into solution. Carbonate formation then serves as a sink for CO_2 mineralized from BC. Acidified BC should have allowed more CO_2 in the headspace since there was less alkalinity present to trap the excess of CO_2. Therefore, loamy sand soil + acidified biochar (LSAB) evolved more CO_2 than the LS soil and loamy sand soil + biochar (LSB) during the initial incubation period. Jones et al. (2011), reported similar trends and concluded that a combination of the abiotic condition, which chemically evolved CO_2, and the biotic condition, which biologically evolved CO_2 were involved in the evolution of CO_2 from biochar-amended soils.

BC would contain the large amount of carbon, probably because of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC). Jones et al., (2011), observed that BC contains significant quantities of DOC and DIC as well. We assume that microbes might consume organic carbon in the soils to produce more organic carbon to the atmosphere; therefore, numbers of microbes were probably reduced until reaching optimal numbers. DOC and DIC in samples were maybe decreasing because microbes would be consuming it.

When the relative organic carbon emissions (as CO_2-C) were compared in the BC treatments and LS soil treatments, the LSB treatment evolved more CO_2 than BC. BC additions could physically change soil properties, including water holding capacity (WHC), bulk density, and porosity, but according to Jones et al., (2011) there should be no significant effect on CO_2 evolution. Our data strongly suggest that BC suppressed CO_2 emissions in the LS soil. Given that

this soil has little alkalinity of its own and not significant acidity to consume the excess alkalinity found in BC that in turn became a sink for CO_2.

The rank order of LSB was switched the position with the LS soil by normalizing with the TOC because each sample originally contained different amounts of the TOC. Soils in semi-arid regions usually contain less than 1 % of the TOC (Artiola et al., 2012). Since both BC and acidified BC for this experiment contained approximately 80% of the TOC, BC still evolved less CO_2 than acidified BC, but the LS soil evolved most CO_2 of LS soil treatments. BC has the structure of highly aromatic organic material and includes approximately 75 % carbon concentration (Lehmann and Rondon, 2006), toxicity effected the extant microbial communities due to the presence of polynuclear aromatic hydrocarbons in BC may have suppressed biological activity (Artiola et al., 2012). Thus, we suggest that microbial metabolism to release CO_2 could decrease.

Conclusion

This study has demonstrated carbon mechanisms by which application of biochar (BC) could result in reducing CO_2 evolution using the incubation method. First, acidified BC evolved more CO_2 than BC due to its alkalinity. This experiment yielded that the result supported our hypothesis that abiotic mechanisms, whereby alkalinity released from the BC into soil solution chemically reacts with and partitions atmospheric CO_2 into solution phase carbonate species. We found that the application of BC into the loamy sand (LS) soil could suppress CO_2 evolution, so that BC could exert a constraining influence over CO_2 evolution due to BC's adsorption power through its alkalinity or polynuclear aromatic hydrocarbon inside BC. However, BC originally contained high ratio of the TOC; therefore, the amount of CO_2 evolution from BC was higher

than the LS soil. CO_2 evolution is related to the number of microbes (Sakamoto and Oba, 1994).

Microbes easily could grow in loamy sand soil + biochar (LSB) under the biotic condition

because BC can make a suitable environment for them.

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Figure Captions

Fig 1: pH measurement of two biochar samples for a period of 44 days. Sample 1 was mixed with DI water at pH 8.89 initially. Sample 2 was mixed with sulfuric acid at pH 1.92 initially.

Fig 2: CO_2 evolution from three different samples of water + inoculum, biochar, and acidified biochar for the period of (a) 231 days and (b) 4 days from the beginning.

Fig 3: CO_2 evolution from three different samples of loamy sand soil, loamy sand soil + biochar, and loamy sand soil + acidified biochar for the period of (a) 231 days and (b) 4 days from the beginning.

Fig 4: Cumulative CO_2 - C evolution from all samples of water + inoculum, biochar, acidified biochar for the period of 231 days. Means $\pm$ SE of four replicates. For each parameter, different letters indicate treatment means significantly different at $P < 0.05$.

Fig 5: Cumulative CO_2 - C evolution from all samples of loamy sand soil, loamy sand soil + biochar, and loamy sand soil + acidified biochar for the period of 231 days. Means $\pm$ SE of four replicates. For each parameter, different letters indicate treatment means significantly different at $P < 0.05$.

Fig 6: Amount of CO_2 normalized with total organic carbon from all samples of water + inoculum, biochar, acidified biochar for the period of 231 days. Means $\pm$ SE of four replicates. For each parameter, different letters indicate treatment means significantly different at $P < 0.05$.

Fig 7: Amount of CO_2 normalized with total organic carbon from all samples of loamy sand soil, loamy sand soil + biochar, and loamy sand soil + acidified biochar for the period of 231 days. Means $\pm$ SE of four replicates. For each parameter, different letters indicate treatment means significantly different at $P < 0.05$.
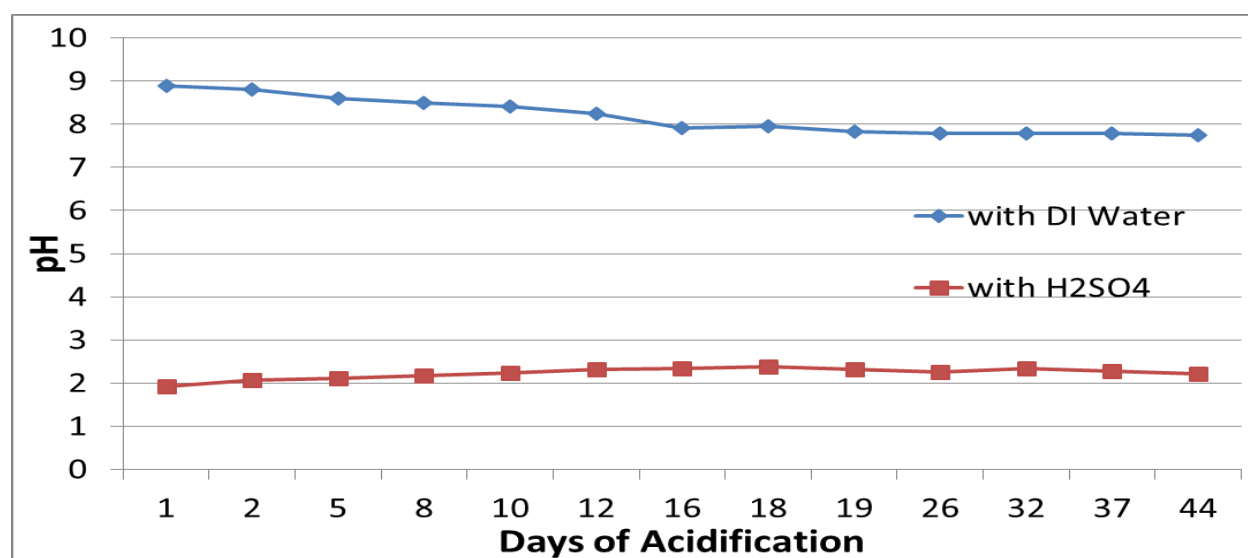
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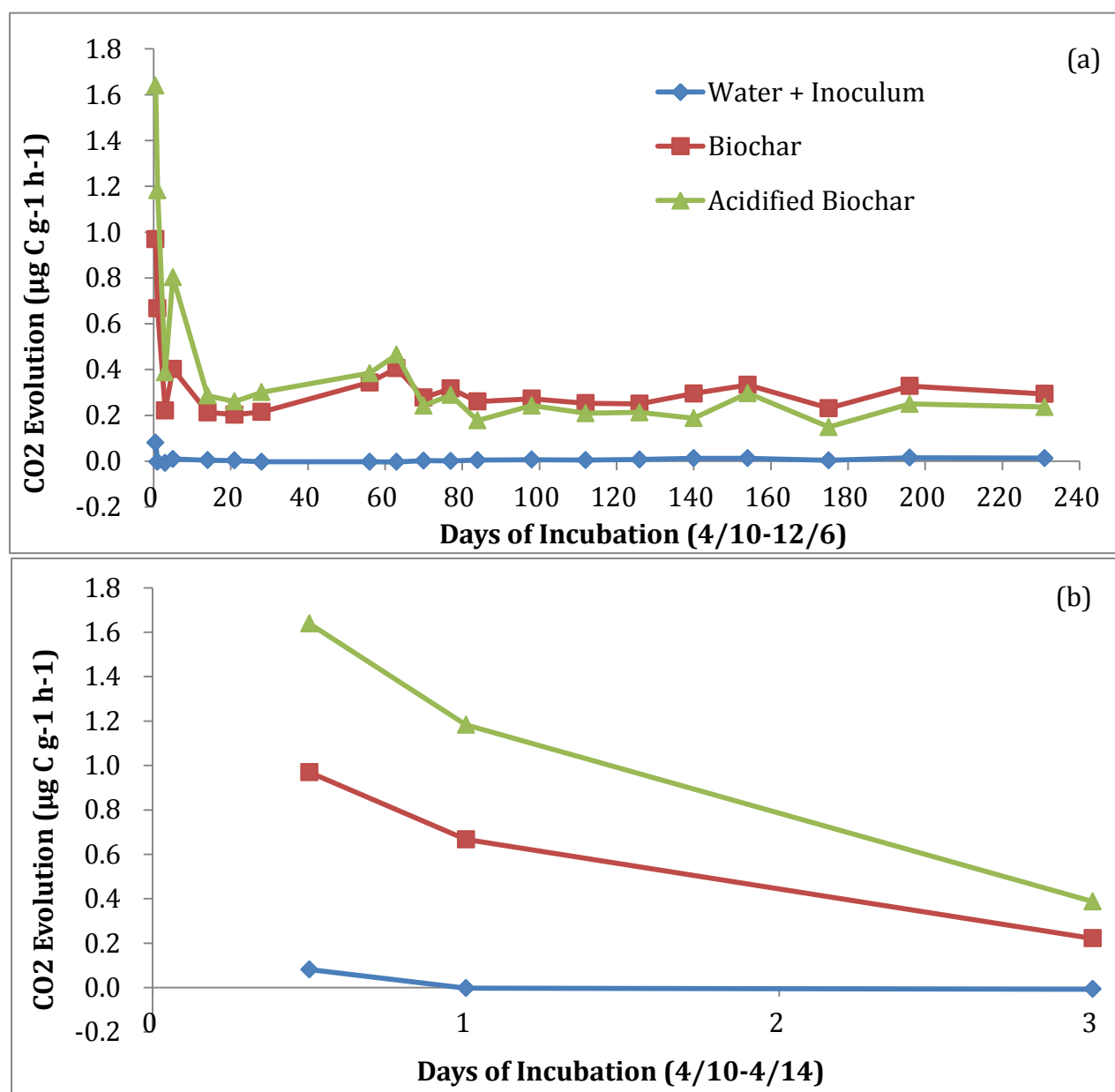
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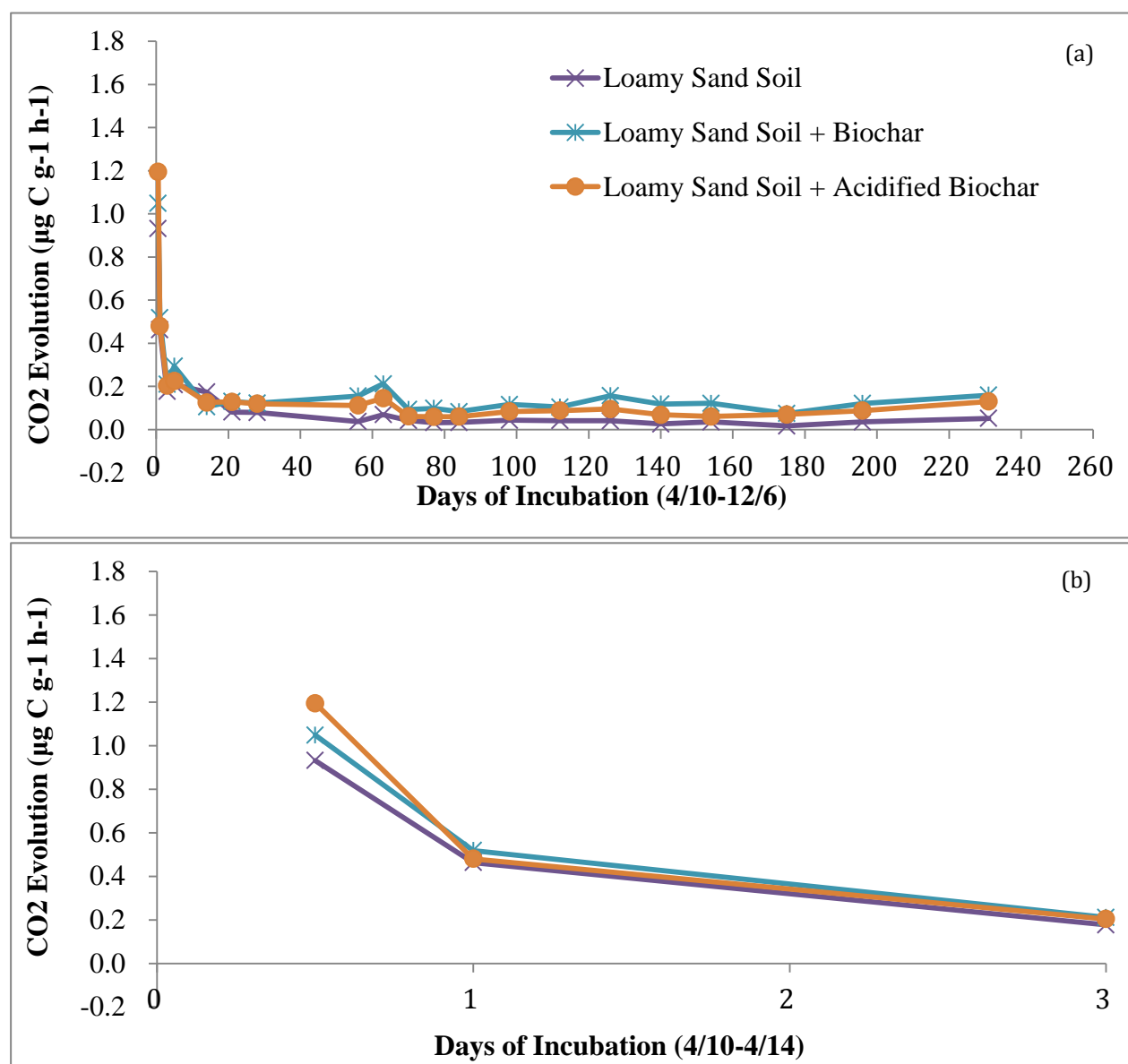biochar for the period of (a) 231 days and (b) 4 days from the beginning.

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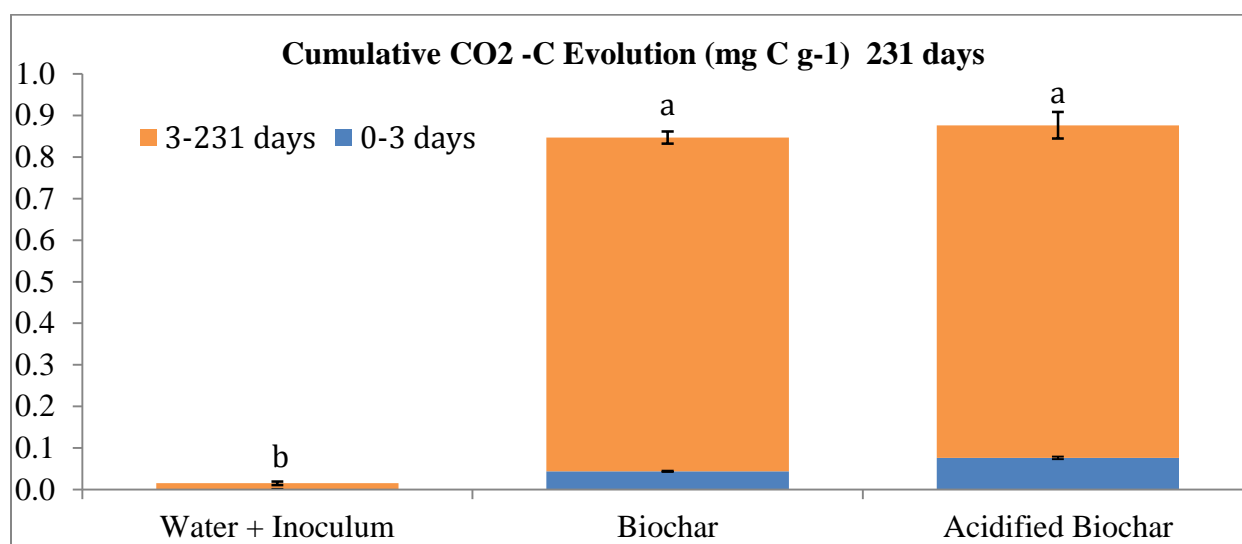
Cumulative CO2 -C Evolution (mg C g-1) 231 days

Fig 4: Cumulative CO_2 - C evolution from all samples of water + inoculum, biochar, acidified biochar for the period of 231 days. Means $\pm$ SE of four replicates. For each parameter, different letters indicate treatment means significantly different at $P < 0.05$.
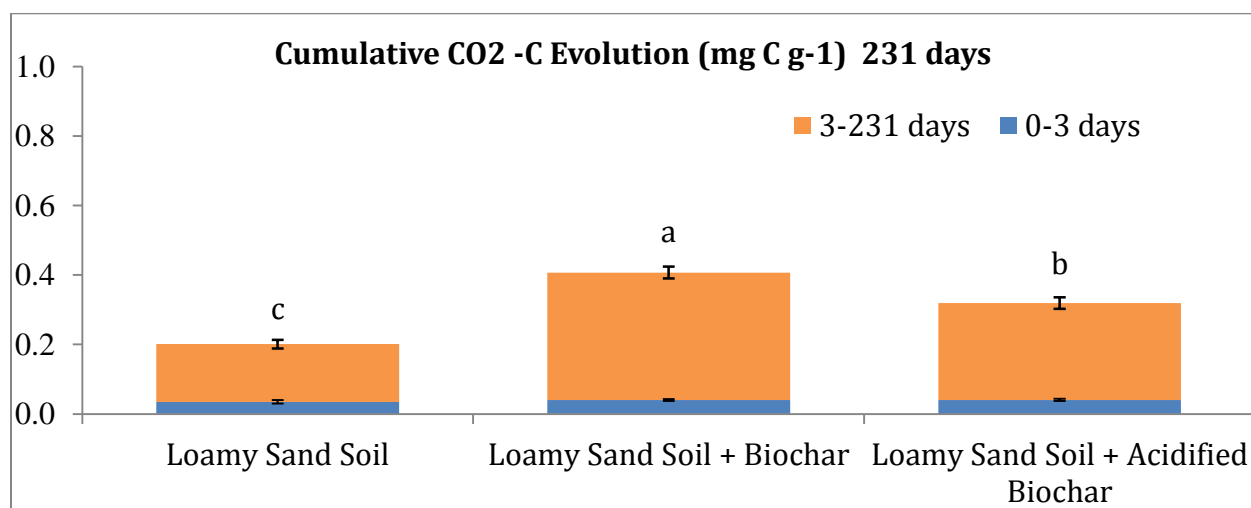
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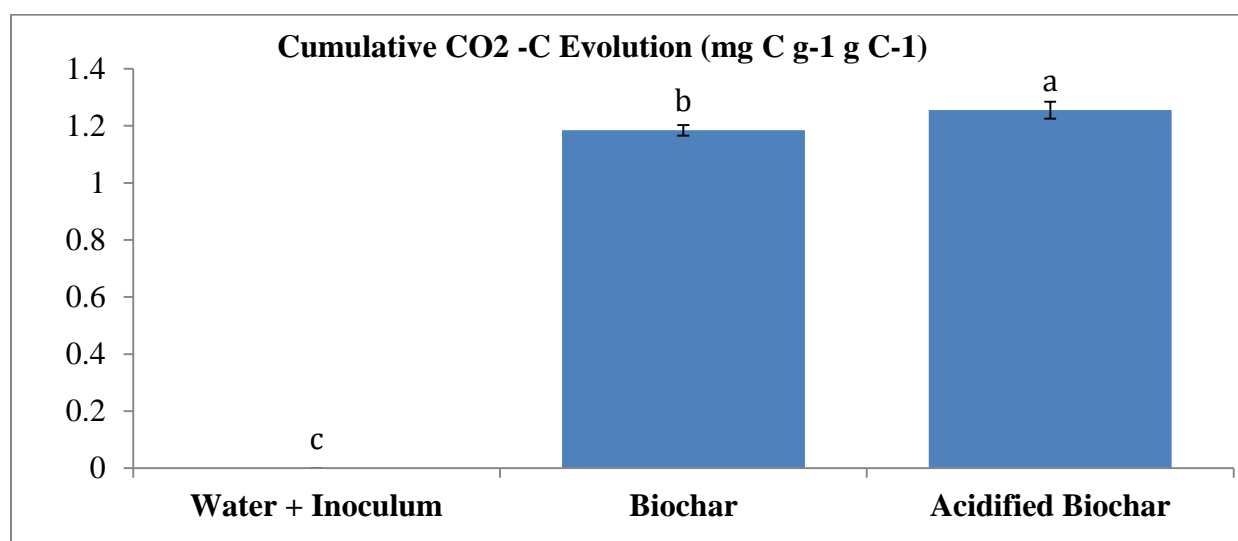
Cumulative CO2 -C Evolution (mg C g-1 g C-1)

Fig 6: Amount of CO_2 normalized with total organic carbon from all samples of water + inoculum, biochar, acidified biochar for the period of 231 days. Means $\pm$ SE of four replicates. For each parameter, different letters indicate treatment means significantly different at $P < 0.05$.

Cumulative CO2 -C Evolution (mg C g-1 g C-1)

Fig 7: Amount of CO_2 normalized with total organic carbon from loamy sand soil, loamy sand soil + biochar, and loamy sand soil + acidified biochar for the period of 231 days. Means $\pm$ SE of four replicates. For each parameter, different letters indicate treatment means significantly different at $P < 0.05$.
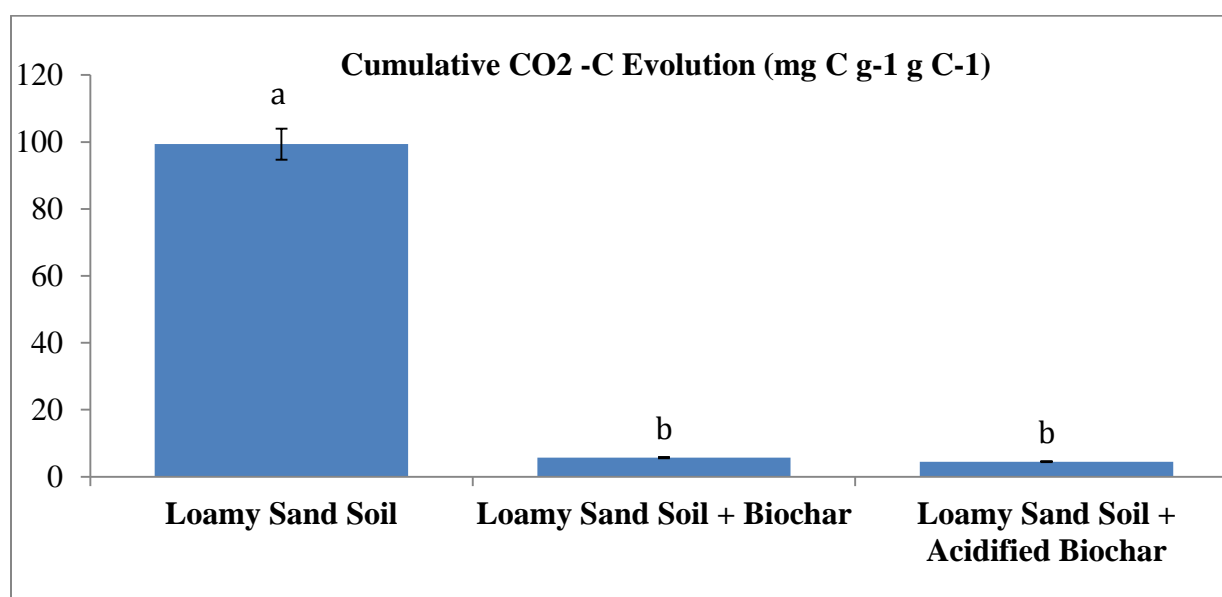
Tables

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TABLE 1. Physical and chemical properties of the soils and biochar for this experiment.

	Red Rock Soil	Biochar	Acidified Biochar	Loamy sand soil + Biochar	Loamy sand soil + Acidified Biochar
Texture	loamy sand	N/A	N/A	N/A	N/A
pH	6.83	8.89	2.22	7.4	5.61
Water Holding Capacity (%)	8.77	287.6	277.45	N/A	N/A
Total C (%)	0.3	79.844	79.7	N/A	N/A
Total Organic C (%)	0.21	79.7	79.7	N/A	N/A