UNIVERSIDADE DE BRASÍLIA
FACULDADE DE AGRONOMIA E MEDICINA VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA

CHANGES IN SOIL CARBON POOLS BY BIOCHAR APPLICATION: GLOBAL OVERVIEW AND LOCAL TRENDS

ALTERAÇÕES NOS COMPARTIMENTOS DE CARBONO DO SOLO PELA APLICAÇÃO DE BIOCHAR: VISÃO GLOBAL E TENDÊNCIAS LOCAIS

JHON KENEDY MOURA CHAGAS

TESE DE DOUTORADO EM AGRONOMIA

BRASÍLIA-DF

NOVEMBRO/2024

UNIVERSIDADE DE BRASÍLIA

FACULDADE DE AGRONOMIA E MEDICINA VETERINÁRIA

PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA

ALTERAÇÕES NOS COMPARTIMENTOS DE CARBONO DO SOLO PELA APLICAÇÃO DE BIOCHAR: VISÃO GLOBAL E TENDÊNCIAS LOCAIS

JHON KENEDY MOURA CHAGAS

ORIENTADOR: CÍCERO CÉLIO DE FIGUEIREDO

TESE DE DOUTORADO EM AGRONOMIA

BRASÍLIA-DF

NOVEMBRO/2024

UNIVERSIDADE DE BRASÍLIA

FACULDADE DE AGRONOMIA E MEDICINA VETERINÁRIA

PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA

ALTERAÇÕES NOS COMPARTIMENTOS DE CARBONO DO SOLO PELA APLICAÇÃO DE BIOCHAR: VISÃO GLOBAL E TENDÊNCIAS LOCAIS

JHON KENEDY MOURA CHAGAS

Tese de doutorado submetida ao Programa de Pós-Graduação em Agronomia da Faculdade de Agronomia e Medicina Veterinária da Universidade de Brasília (UnB), como parte das exigências para obtenção do título de Doutor em Agronomia - Produção Sustentável.

APROVADA POR:

____

CÍCERO CÉLIO DE FIGUEIREDO (ORIENTADOR)
Faculdade de Agronomia e Medicina Veterinária, Universidade de Brasília
e-mail: cicerocf@unb.br

____

GABRIELA BIELEFELD NARDOTTO (EXAMINADOR INTERNO)
Instituto de Ciências Biológicas, Universidade de Brasília
e-mail: gbnardoto@unb.br

____

FABIANA ABREU DE REZENDE (EXAMINADOR EXTERNO)
Embrapa Mandioca e Fruticultura
e-mail: fabiana.rezende@embrapa.br

____

CARLOS ALBERTO SILVA (EXAMINADOR EXTERNO)
Departamento de Ciência do Solo, Universidade Federal de Lavras
e-mail: csilva@ufla.br

BRASÍLIA-DF, 22 DE NOVEMBRO DE 2024.

FICHA CATALOGRÁFICA


```
         Chagas, Jhon Kenedy Moura
C433a       Alterações nos compartimentos de carbono do solo pela
         aplicação de biochar: visão global e tendências locais / Jhon
         Kenedy Moura Chagas. -- Brasília, 2024.
            164 p. : il

            Tese(Doutorado em Agronomia) -- Universidade de Brasília,
         2024.

            Orientador: Prof. Dr. Cícero Célio de Figueiredo.

            1. Biochar. 2. Sequestro de carbono no solo. 3. Biochar
         de lodo de esgoto. 4. Meta-análise. 5. Matéria orgânica do
         solo. 6. Solos tropicais. 7. Pirólise. 8. Agricultura
         sustentável. 9. Mudanças climáticas – Mitigação. I. Título.
         II. Figueiredo, Cícero Célio de, orient. III. Universidade de
         Brasília, Faculdade de Agronomia e Medicina Veterinária,
         Programa de Pós-Graduação em Agronomia.
                                                            631.41
```


REFERÊNCIA BIBLIOGRÁFICA

CHAGAS, J. K. M. **Alterações nos compartimentos de carbono do solo pela aplicação de biochar: visão global e tendências locais**. 2024. 164 f, il. Tese (Doutorado em Agronomia) – Faculdade de Agronomia e Medicina Veterinária, Universidade de Brasília, Brasília, 2024.

CESSÃO DE DIREITOS

NOME DO AUTOR: JHON KENEDY MOURA CHAGAS

TÍTULO DA TESE: ALTERAÇÕES NOS COMPARTIMENTOS DE CARBONO DO SOLO PELA APLICAÇÃO DE BIOCHAR: VISÃO GLOBAL E TENDÊNCIAS LOCAIS

GRAU: DOUTORADO ANO: 2024

Nome: Jhon Kenedy Moura Chagas

***E-mail*:** jhonkchagas@gmail.com

Dedico a Deus, a todos que me apoiaram nessa jornada e àqueles que, com persistência, nunca deixaram de perguntar "Quando você termina esse doutorado?"

AGRADECIMENTOS

Em primeiro lugar, agradeço a Deus pelo dom da vida e pelas maravilhas que opera nela.

À minha família: aos meus pais, Dorvalino e Josefa, cujo amor, trabalho, empenho, dedicação e paciência foram a base que sustentou este longo caminho; aos meus irmãos, Ismaell, Isabela e Jaime, obrigado por estarem sempre ao meu lado. Amo vocês! Aos demais familiares, que mesmo à distância, sempre me apoiaram e torceram por mim; e ao Igor, pela companhia e apoio nos últimos tempos.

À Universidade de Brasília, aos professores e servidores do programa de pós-graduação, e especialmente ao meu orientador, Prof. Dr. Cícero Célio de Figueiredo. São quase 10 anos de convivência, desafios e conquistas. Obrigado por responder às minhas mensagens em horários inusitados e por nunca desistir de me motivar.

Aos amigos da pós-graduação, Alyson, Ana Cláudia, Ana Clara, Andréa, Camila, Geovanna, Gustavo, Jóisman, Maycon, Nicole, Rhaila, Roberto, e Thais F., por tornarem os dias mais leves. Vocês provaram que a jornada é tão importante quanto o destino.

À Priscila, cuja habilidade para resolver os caprichos do analisador elementar foi imprescindível (e requisitada algumas vezes).

À Dra. Gabriela Nardoto, Dr. Vinícius Vasconcelos, Dra. Beata Madari e seus alunos, pelas valiosas contribuições e suporte nas análises.

À Lenine da Central Analítica do Instituto de Química, pelas análises de TGA que tanto enriqueceram este trabalho.

À Companhia de Saneamento Ambiental do Distrito Federal, por disponibilizar o lodo de esgoto para a produção do biochar.

A todos que contribuíram para a condução do experimento ao longo dos sete anos: a equipe do Laboratório de Estudos da Matéria Orgânica do Solo, os servidores da Fazenda Água Limpa e os alunos de iniciação científica e estagiários que participaram das análises laboratoriais. Este experimento de longa duração foi possível graças ao esforço conjunto de todos vocês.

Às equipes do Ibama e da Secretaria de Meio Ambiente de Cristalina, por me acompanharem nesta longa jornada.

E a todos que, direta ou indiretamente, contribuíram para a realização deste trabalho, o meu mais sincero agradecimento!

“We know more about the movement of celestial bodies than about the soil underfoot.”

(Leonardo da Vinci)

“*The beautiful thing about healthy soil is that it does not matter if you care about climate change, or if you care about the drought, or if you just care about healthy food, or if you want to see more biodiversity, restoring soil hits all of those targets. Almost everyone has some self-interest or desire for soil to be restored*.”

(Laura Tucker, Kiss the Ground)

“*Global sustainability is now the only avenue to future inclusive progress that can deliver the Sustainable Development Goals and the Paris climate agreement.”*

(Johan Rockstrom)

ABSTRACT

Biochar application has emerged as a promising strategy to enhance soil carbon (C) sequestration, improve soil health, and mitigate climate change. However, its effects on soil C pools vary widely due to differences in feedstock, pyrolysis conditions, application rates, soil types, and environmental factors. This study aimed to quantify the potential of biochars to alter soil C pools under different conditions through a global meta-analysis and to understand the direct and indirect contributions of sewage sludge biochar (SSB) to soil C storage in a seven-year field trial on a tropical Oxisol. In the first chapter, a meta-analysis of 586 paired comparisons from 169 studies worldwide was conducted. The results showed significant increases in total C (TC), organic C, microbial biomass C, labile C, and fulvic acid following biochar application, with factors such as biochar properties, soil characteristics, and experimental conditions influencing these effects. The second chapter detailed a seven-year field trial assessing the effects of SSB pyrolyzed at 300°C and 500°C on soil C pools and crop yield. SSB application increased soil TC and total nitrogen levels, enhanced the non-oxidizable organic C pool, and improved soil fertility. However, positive effects on crop yield declined over time without supplemental mineral fertilization. In the third chapter, the global meta-analysis findings were integrated with the local field trial results. The comparison revealed discrepancies, highlighting the importance of tailoring biochar applications to local conditions. Despite lower percent increases in soil C fractions in the field trial, the absolute TC gains were substantial, suggesting that SSB can effectively enhance soil C stocks in tropical soils when appropriately managed. This study confirms that biochar can enhance soil C sequestration, but its effectiveness is highly context-dependent, emphasizing the need to understand the factors influencing its impact on soil C pools for sustainable agriculture and climate change mitigation.

Keywords: biochar, soil carbon sequestration, sewage sludge biochar, meta-analysis, soil organic matter pools, tropical soils, pyrolysis temperature, sustainable agriculture, climate change mitigation

RESUMO

A aplicação de biochar surgiu como uma estratégia promissora para aumentar o sequestro de carbono (C) no solo, melhorar a saúde do solo e mitigar as mudanças climáticas. No entanto, seus efeitos nas frações de C do solo variam amplamente devido a diferenças na matéria-prima, condições de pirólise, taxas de aplicação, tipos de solo e fatores ambientais. Este estudo teve como objetivo quantificar o potencial dos biochars para alterar as frações do C do solo sob diferentes condições através de uma meta-análise global e compreender as contribuições diretas e indiretas do biochar de lodo de esgoto (SSB) para o armazenamento de C no solo em um estudo de campo de sete anos em um Latossolo tropical. No primeiro capítulo, foi realizada uma meta-análise com 586 comparações pareadas de 169 estudos conduzidos em todo o mundo. Os resultados mostraram aumentos significativos no C total (TC), C orgânico, C da biomassa microbiana, C lábil e ácido fúlvico após a aplicação de biochar, com fatores como propriedades do biochar, características do solo e condições experimentais influenciando esses efeitos. O segundo capítulo detalhou um ensaio de campo de sete anos avaliando os efeitos do SSB pirolisado a 300°C e 500°C nas frações de C do solo e na produtividade da cultura. A aplicação de SSB aumentou os níveis de TC do solo e nitrogênio total, aumentou a fração de C orgânico não oxidável e melhorou a fertilidade do solo. No entanto, os efeitos positivos na produtividade da cultura diminuíram ao longo do tempo sem a fertilização mineral suplementar. No terceiro capítulo, os resultados da meta-análise global foram integrados com os resultados do estudo de campo local. A comparação revelou discrepâncias, destacando a importância de adaptar as aplicações de biochar às condições locais. Apesar dos aumentos percentuais menores nas frações de C do solo no estudo de campo, os ganhos absolutos de TC foram substanciais, sugerindo que o SSB pode efetivamente aumentar os estoques de C no solo em solos tropicais quando adequadamente manejado. Este estudo confirma que o biochar pode aumentar o sequestro de C no solo, mas sua eficácia é altamente dependente do contexto, enfatizando a necessidade de compreender os fatores que influenciam seu impacto nas frações de C do solo para uma agricultura sustentável e mitigação das mudanças climáticas.

Palavras-chave: biochar, sequestro de carbono no solo, biochar de lodo de esgoto, meta-análise, frações da matéria orgânica do solo, solos tropicais, temperatura de pirólise, agricultura sustentável, mitigação das mudanças climáticas

LIST OF FIGURES

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

^{14}C – radiocarbon

4p1000 – 4 per 1000 Initiative

ABC – Low Carbon Emission Agriculture, *Agricultura de Baixa Emissão de Carbono*, in Portuguese

AIC – Akaike's information criterion

ANOVA – analysis of variance

BIC – Bayesian information criterion

BPCA – benzene polycarboxylic acid

C – carbon

CAESB – Environmental Sanitation Company of the Federal District, *Companhia de Saneamento Ambiental do Distrito Federal*, in Portuguese

$CC_{y,t}$ – organic carbon content on a dry weight basis for biochar type t in year y

CI – confidence interval

CI_{PC} – confidence interval for the percent change

CS – carbon stability

DFFIT – difference in fit

DOC – dissolved organic carbon

DTA – differential thermal analysis

dTG – differential thermogravimetry

EOOC – easily oxidizable organic carbon

FA – fulvic acid

Fcp – elemental carbon content

FTIR – Fourier transform infrared spectroscopy

GHG – greenhouse gas

GS – growing season

HA – humic acid

HU – humin

LC – labile carbon

MAC – mineral–associated carbon

MBC – microbial biomass carbon

$M_{y,t}$ – biochar mass

NMR – nuclear magnetic resonance

NOC – non–oxidizable carbon

NPK – nitrogen, phosphorus, and potassium (mineral fertilization)

ns – non-significant

OC – organic carbon

PC – particulate carbon

Pc – percent change

POXC – $KMnO_4$-oxidizable carbon

PRde – permanence factor

PV – pore volume

r – repetition

REML – restricted maximum likelihood

RR – response ratio

SCS – soil carbon stock

SD – standard deviation

SE – standard error

SEM – standard error of the mean

SOC – sloil organic carbon

SOM – soil organic matter

SS – sewage sludge

SSA – surface specific area

SSB – sewage sludge biochar

SSB300 – sewage sludge biochar pyrolyzed at 300°C

SSB500 – sewage sludge biochar pyrolyzed at 500°C

TC – total carbon

TGA – thermogravimetric analysis

TML – thermal mass loss

TN – total nitrogen

TN – total nitrogen

TSF – thermostable fraction

V4 – phenological stage of four developed leaves

V6 – phenological stage of six developed leaves

WL – weight loss

$\delta^{13}C$ – stable carbon isotope ratio

$\delta^{15}N$ – stable nitrogen isotope ratio

TABLE OF CONTENTS

1. INTRODUCTION

Despite being discussed for a long time, the climate change issue is increasingly contemporary. Therefore, because environmental responsibility is global, international agreements have been signed to mitigate climate change (Brazil, 2017). In addition, reducing greenhouse gas (GHG) emissions is coupled with using carbon (C) sequestration technologies, i.e., removing C from the atmosphere. If appropriately adopted, both approaches can jointly play a key role in mitigating the global climate crisis (Fuss et al., 2014).

Various technologies have been adopted across the world to increase C sequestration in the soil and mitigate GHG emissions (Smith, 2016). Biochar is the solid product of pyrolysis, the thermochemical conversion process of biomass in the absence or limited presence of oxygen (Sohi, 2012). Biochar as a soil amendment has been proposed as a global strategy to mitigate climate change (Lehmann et al., 2021) and contribute to food security (Joseph et al., 2021). An interesting review lists C sequestration as the main benefit of biochar to soil ecosystem services (Blanco-Canqui, 2021). According to Lehmann et al. (2021), biochar systems have the potential to offset 2.4 to 6.3 Pg yr^{-1} of CO_2-equivalent emissions globally by i) reducing C mineralization and non-CO_2 emissions compared to non-pyrolyzed feedstocks, ii) avoiding fossil fuel emissions, including those associated with biomass transport and application/disposal, iii) promoting plant growth and thus C storage in plant biomass, and iv) reducing mineralization of soil organic matter (SOM).

Several feedstocks can be used in biochar production (Tomczyk et al., 2020). However, in the circular economy scenario, locally available sustainable sourced feedstocks are preferable (Hu et al., 2021). Sewage sludge (SS) is a waste of limited disposal, and its reuse is still neglected worldwide. Alternatively, when applied to soil, SS biochar can sequester C without changing current land use (Racek et al., 2020).

The content and stability of the C that makes up biochar depends on several aspects, including the feedstock and pyrolysis temperature (Adhikari et al., 2024; Li et al., 2019). Many independent studies have evaluated the effects of biochar on soil C pools (Figueiredo et al., 2019b; Huang et al., 2018; Yang et al., 2018; Zhao et al., 2020). However, studies focused on an integrated comprehension of these pools are still lacking, and they can elucidate the impact of biochar use on SOM transformation and quality.

In general, the soil comprises two major C pools: labile and stable or non-labile (Dynarski et al., 2020). These pools play distinct roles in the soil C dynamics. The C in labile pools (e.g., microbial biomass C, potassium permanganate oxidizable C) is more rapidly cycled and is used as an energy source by the soil microbiota. On the other hand, C stored in stable pools (e.g., inert C, humin) can remain in the soil for thousands of years, immobilizing C that might otherwise be in the atmosphere (Strosser, 2011). As most studies with biochar do not provide long-term results and do not discriminate between these pools, quantifying soil C in its totality, in some cases soil C immobilization due to biochar application requires confirmation.

Finally, due to environmental ubiquity, thinking globally and acting locally is necessary. In this study, we initially quantified the effects and identified the factors modulating biochar-induced changes in soil C pools by analyzing studies conducted across multiple sites and conditions worldwide. Additionally, we addressed efforts to sequester C in the soil using biochar under local conditions. Subsequently, the results of a long-term local study were evaluated and compared to global findings.

2. OBJECTIVES

2.1. Main objective

To assess the changes in C sequestration in different SOM pools promoted by biochar application, and to establish links between global results and those from a seven-year field trial of sewage sludge biochar (SSB) application on an Oxisol.

2.2. Specific objectives

1. To quantify the potential of biochar to alter soil C fractions under a wide variety of biochar, soil, and experimental conditions through a global meta-analysis.
2. To assess the dynamics of C in labile and stable SOM pools over seven years following SSB application, and to evaluate the direct and indirect contributions of SSB to soil C stocks.
3. To integrate insights on the effects of biochar on soil C pools from global and local perspectives by comparing global meta-analysis data with local field trial results, offering a comprehensive analysis of biochar use for soil carbon management.

3. HYPOTHESIS

1. Biochars from different feedstocks produced under distinct pyrolysis conditions affect soil C pools differently, and factors such as biochar application rate, soil C content, climate, duration, and type of experiment are critical in influencing these effects.
2. SSB can sequester C into stable SOM pools over an intermediate period due to its low C/N ratio; at the applied biochar rate, the direct contribution of C from SSB to total soil C content is negligible, but the indirect contribution through stimulation of plant biomass production significantly increases soil C stocks.
3. Integrating global meta-analysis data with local field trial results will reveal that biochar's effects on soil C pools are context-specific, and that localized field data are essential to validate and refine global findings for effective soil C management practices.

4. LITERATURE REVIEW

4.1. Strategies to boost soil C sequestration

The anthropic contribution to global warming is unequivocal (IPCC, 2021). In light of this, global commitments are being signed to mitigate the ongoing climate change. Recently, ambitious voluntary goals were signed in the Paris Agreement, and Brazil is one of the parties. This agreement aims to limit global warming to 2°C compared to pre-industrial levels, aiming to achieve a maximum of 1.5°C (Brazil, 2017). Thus, its focus is on reducing greenhouse gas (GHG) emissions. In this sense, at COP26 in 2022, Brazil reaffirmed its commitment to reduce GHG emissions in 37% by 2025 and 50% by 2030, both compared to 2005 levels. In addition, Brazil's long-term goal is to achieve climate neutrality by 2050 (Brazil, 2022a).

Furthermore, in parallel, C sequestration strategies such as the “4 per 1000 Initiative” (4p1000) have been adopted to offset the inevitable GHG emissions (UNFCCC, 2021). This approach focuses on sequestering C in the soil, the second largest reservoir on the planet (Lal, 2010). Therefore, to enable nations to meet these goals, developing, validating, improving, and disseminating effective and sustainable C sequestration technologies is crucial.

In this context, Brazil’s booming agribusiness plays a key role in implementing both approaches. By adopting sustainable and validated practices and technologies, it will be possible to contribute to the voluntary or regulated C and methane credit market. Thus, stakeholders will be encouraged to pursue climate goals and Brazilian agribusiness will continue to move towards sustainability.

Brazil's environmental agenda includes the Low Carbon Emission Agriculture Plan (Plan ABC[1], 2010-2020) and the Sectoral Adaptation Plan for a Low Carbon Agriculture for Sustainable Development (Plan ABC^{+}, 2020-2030), sectoral policies for mitigation and adaptation to climate change that aim to consolidate a low C economy in agriculture. The strategies of these plans focus on the restoration of degraded pastures, the integration of crop-livestock-forest, agroforestry systems, no-tillage systems, biological N fixation, forest planting and animal waste treatment (Brazil, 2021, 2012). The Brazilian government announced the National Fertilizer Plan in 2022 to reinforce this goal. It foresees the adoption of new materials and new sources of raw materials in the fertilizer industry to reduce external dependency, in addition to the incorporation of the circular economy concept and access to the C market by the Brazilian plant nutrition industry (Brazil, 2022b). Thus, agro-industrial and urban wastes, such

[1] *Agricultura de Baixo Carbono*, in Portuguese

as sewage sludge (SS), are promoted as a potential source to supply nutrients and C to the soil simultaneously.

4.2. Sewage sludge generation and disposal: current scenario

Population growth (UN, 2019), improved access to sanitary sewerage (JMP, 2021) and stricter environmental regulations result in increased SS production. This solid waste generated in wastewater treatment plants is composed of water, microorganisms, organic materials, and sedimented minerals (Zhang et al., 2017). Thus, SS is an important source of organic matter and nutrients (Kacprzak et al., 2017). Furthermore, in activated sludge wastewater treatment plants, approximately 50% of the operating costs are associated with sludge management (Collivignarelli et al., 2015).

Worldwide, an estimated 984.6 million m^3 of wastewater is produced daily, equivalent to 49.0 m^3 yr^{-1} per capita (Jones et al., 2021). In the Federal District of Brazil, from 2015 to 2021, on average 338 m^3 of SS were generated daily, of which less than half (44%) was disposed of (CAESB, 2022). There are several research approaches focused on minimizing SS generation in treatment plants. However, the paradigm shift on this topic has made it possible to recognize multiple possibilities for SS disposal. Hence, from a circular economy perspective, it is necessary to dispose of this waste properly (Awasthi et al., 2022).

Nowadays, there are multiple possibilities for the disposal and reuse of SS, including incineration, landfilling, reuse in agriculture, reuse in civil construction, recovery of degraded areas, composting, absorbent material, pyrolysis, gasification, among others (Awasthi et al., 2022; Collivignarelli et al., 2019). Regarding agricultural reuse, the amount of N and P in the sewage could replace 25% and 15% of the total N and P fertilizers used worldwide, respectively (Andersson et al., 2020). However, its disposal may be limited due to the organic and inorganic contaminant content (Kacprzak et al., 2017).

The Brazilian Cerrado region is characterized by naturally low soil fertility (Lopes and Guilherme, 2016). However, recent studies have demonstrated the potential of SS application to enhance soil nutrient availability in this region (Amorim Júnior et al., 2021; Prates et al., 2020). Furthermore, the application of SS has been found to positively impact soil structure by promoting aggregate formation and improving water infiltration rates (García-Orenes et al., 2005; Nicholson et al., 2018; Tsadilas et al., 2005). Given the frequent occurrence of prolonged droughts in the Cerrado (Hofmann et al., 2021), such improvements in soil structure are of great significance. However, it is important to consider the potential effects of SS reuse on soil ecological processes and biodiversity in this region. Although SS application and disposal benefits in Cerrado soils are evident (Prates et al., 2022), addressing challenges related to SS quality, contaminant removal, and biodiversity preservation is crucial. Therefore, establishing appropriate regulations and implementing advanced treatment technologies are vital to ensure the safe and sustainable reuse of SS in the Cerrado.

4.3. Sewage sludge biochar: a sustainable alternative for SS use

The pyrolysis of some materials generates a solid product called biochar (Sohi, 2012). It differs from other types of charcoal in that it is intended for soil amendment or C sequestration (Novotny et al., 2015). Its properties and applications depend on the type of material, temperature, residence time, heating rate and pyrolysis atmosphere (Goldan et al., 2022; Li et al., 2019). So, several feedstocks can be pyrolyzed, even SS (Figueiredo et al., 2018). As a thermal processing, the pyrolysis of SS can eliminate pathogens, reduce the availability of contaminants and also reduce the final volume (Chagas et al., 2021b; Fathi Dokht et al., 2017; Paz-Ferreiro et al., 2018).

Furthermore, when applied to the soil, sewage sludge biochar (SSB) presents multiple agro-environmental benefits. SSB can act as a source of nutrients (Faria et al., 2018), improve soil physicochemical (Fathi Dokht et al., 2017) and biological properties (Figueiredo et al., 2019a), reduce GHG emissions (Ibrahim et al., 2017), increase crop yield (Chagas et al., 2021a), and sequester C in the soil (You et al., 2019).

4.4. Biochar as a technology for soil C sequestration

The Amazonian Dark Earths found in the Brazilian Amazon are one of the prominent global examples of anthropic soil C sequestration. Their formation is intimately linked to the pyrolysis of materials by indigenous peoples (Glaser, 2007). Since then, the discussion about technologies that could analogously increase soil fertility and accumulate C has intensified (Verheijen et al., 2010). However, it is necessary to keep in mind the difference between biochar, which is artificially produced and intended for land application, and other charcoals (such as black C from wildfires) and the unintentional creation of the Amazonian Dark Earths (Novotny et al., 2015).

In this sense, in contrast to combustion/incineration, pyrolysis of an organic feedstock retains much of the C originally present in the raw biomass (Zhang et al., 2015). Additionally, this process is capable of making the organic compounds more aromatic and, therefore, recalcitrant (Wang et al., 2016). Thus, when biochar is applied to the soil there is a direct C input through organic matter.

Stoichiometrically, the total soil C content change is directly proportional to the C content added through biochar. This property is a function of the feedstock, dose applied, biochar C content and pyrolysis conditions (Tomczyk et al., 2020; Wei et al., 2019). Thus, biochars obtained from lignocellulosic feedstocks tend to have more C than those with high ash content. Also, although there is generally a loss of C with increasing pyrolysis temperature, it becomes more stable (Li et al., 2019). Koyama and Hayashi (2019) evaluated the application of rice husk biochar pyrolyzed at 350-400°C (359 g kg^{-1} C) at doses of 10, 20 and 40 t ha^{-1} in single and successive applications for two years. They also assessed the application of 20 t ha^{-1} of unpyrolyzed rice husk. The soil TC content increased linearly with the application dose and the number of applications, increasing from 7.93 to 9.53 g kg^{-1} C for each 1 kg m^{-2} C applied via biochar. Furthermore, biochar showed a residual effect compared to rice straw application.

Besides the direct effect mentioned, biochar also indirectly increases the soil C content by stimulating biomass production by crops (Jeffery et al., 2017; Liu et al., 2013). Another indirect contribution of biochar to soil C stock is the protection of native SOM by several mechanisms (Fatima et al., 2021; Joseph et al., 2021), such as adsorption, physical protection, aggregates formation and stabilization. Finally, a reduction of GHG emissions may occur due to the liming effect of biochar, the release of toxic compounds, the interaction with soil microbiota, the interaction with dissolved organic C, among others (Cayuela et al., 2014). Meta-analysis of 105 studies showed that biochar, as a physical additive in solid wastes composting, has an efficiency of ~63% in reducing GHG emissions during composting (Cao et al., 2019).

Despite this effect on total soil C, it remains to clarify how biochar contributes to distinct C pools accumulation in the soil. Biochar affects soil C pools differently according to biochar and soil properties. Biochars produced at high temperatures generally have a greater content of highly stable C (Domingues et al., 2017). On average, this pool immobilizes C in the soil for 250 to 660 years (Abney and Berhe, 2018). Thus, its long-lasting and unequivocal contribution can raise the soil C saturation limit (ceiling concentration) to levels higher than those obtained with non-pyrolysed biomass (Gross et al., 2021). Furthermore, the pyrogenic C in biochar reacts more slowly to soil management (Cooper et al., 2020), which helps maintain the added soil C level.

Applying biochars pyrolyzed at low temperatures and derived from feedstocks rich in volatile materials can contribute mostly to the labile pools of the SOM (Figueiredo et al., 2019b; Li et al., 2020). Thus, this possibility can be analyzed from two points of view: nutrient cycling will be stimulated (Anderson et al., 2011); and mineralization of the native SOM may occur, known as the positive priming effect (Wang et al., 2016). Therefore, to avoid deleterious effects on SOM, it is necessary to know and apply biochars with a C balance (labile and recalcitrant) appropriate to the need of use.

Given this, it is necessary to carefully evaluate the different C pools of soils amended with biochar. It is also essential that this evaluation be done in long-term studies (Poggere et al., 2022) to validate biochar under certain use conditions as a possible technology for C credit certification. Long-term studies have been conducted in distinct climate regions, soil types, and using contrasting feedstocks. As a result, different levels of C sequestration are obtained. Therefore, it is necessary to summarize their results, seek a general understanding and explore further issues. Thus, studies with continuous and repeated applications are required, but assessing the dynamics of soil C after ceasing the application of biochar (residual effect) is necessary. Furthermore, applying mineral fertilizer, especially N, during the residual effect period is another aspect that can alter the mineralization rate of the applied C; hence assessment is required.

It is important to highlight that, in general, producing and applying biochar is an attempt to locally reproduce the effects/properties of the Amazonian Dark Earths in a relatively short period of time. However, the results of field studies with biochar are limited to a little over a decade and have not yet reached the time scale that would allow them to be compared to Amazonian Dark Earths regarding soil C sequestration.

4.5. Methods for soil organic matter fractionation

Soil is inherently a complex and dynamic system (Turner, 2021), and its organic matter is no different in this regard. The complexity of SOM is reflected in the diversity of fractionation methods developed and currently used. Such methods are based on the chemical composition, stability, and location of the SOM, and many of them are designed to isolate functional pools that vary in stability for use in modeling SOM dynamics (von Lützow et al., 2007). However, these approaches are challenging, especially in disturbed systems such as agricultural lands, and may not reflect exactly what was designed in the conceptual model (Duddigan et al., 2019).

This review intends to give a general overview of some available fractionation methods for SOM studies. In general, SOM fractionation procedures are divided into chemical and physical methods. The physical fractionation methods are based on the premise that the spatial distribution and interaction between soil particles control the dynamics of SOM. They usually comprise fractionation by particle size or density (Christensen, 2001; von Lützow et al., 2007).

In natural environments, most SOM is typically associated with mineral soil particles (Sokol et al., 2022), allowing reliable results through physical fractionation. However, biochar in soils can lead to significant amounts of C being either free or present within unstable aggregates, resulting in incorrect allocation to short turnover time pools. Independent field trials by Paetsch et al. (2017) and Grunwald et al. (2017) showed that, after one year, 52% and 80% of the applied biochar were found in the free particulate organic matter fraction and free light fraction, respectively. This suggests that the stability of C in these fractions is higher than in soils without biochar, and they serve different ecological functions. Therefore, caution is necessary when interpreting physical fractionation results in biochar-amended soils. Adding biochar to soil generally increases the proportion of particulate C relative to the total C content. With its slow decomposition rate compared to other organic residues, biochar accumulates as larger solid particles, increasing the particulate C fraction.

Chemical fractionation methods are based on solubility, hydrolysability, and resistance to oxidation or destruction of the mineral phase (von Lützow et al., 2007). They usually to estimate the C in the SOM pools including active, slow and passive. Such methods focus on the chemical stability of SOM, but usually do not consider its availability to decomposers.

The SOM active pool, also called the labile pool, has a turnover time of days to a few years. This pool comprises materials of recent origin, usually high in nutrients and energy value, root exudates, and microorganisms (Wander, 2004). It is often procedurally represented by the fractions $KMnO_4$-oxidizable C (POXC), microbial biomass C (MBC), dissolved organic C (DOC), hot water soluble C, among others. Fulvic acid is considered the most labile fraction of humic substances due to the prevalence of simpler compounds (Sherrod et al., 2019). These fractions are used as indicators of management-induced changes in SOM as well as soil quality indicators (Bongiorno et al., 2019). Moreover, despite being the most dynamic, this pool slightly contributes in percentage to soil C sequestration.

The SOM slow or intermediate pool has a few years to decades turnover time. It comprises partially decomposed residues, microbial by-products of the active pool and humified materials. This pool is essential for soil C sequestration but is also strongly influenced by management practices. The fractions related to this pool are generally associated with physical protection of the SOM (Wander, 2004), so chemical fractionation may not be the best approach to assess this pool. Some authors state HA and easily oxidizable organic C as fractions that comprise this pool.

The Walkley-Black method was developed to quantify soil organic C (SOC) (Nelson and Sommers, 1996). However, the main known limitation of this acid digestion with $K_2Cr_2O_7$ is its inefficiency in oxidizing recalcitrant forms of C, such as pyrogenic C (black C) (Benbi and Nisar, 2020). Thus, it is necessary to use a correction factor to estimate the SOC. Therefore, the easily oxidizable organic C is determined when the factor is not applied. Theoretically, only the slow pool is accounted for if the labile fraction is deducted from the easily oxidizable organic C.

Sherrod et al. (2019) assessed the relationship between humic substance fractions and other SOM pools in contrasting US soils. They found a moderate correlation (r=0.4985) between HA and the slow pool (considered as the particulate organic matter C from physical fractionation). Hence, it cannot be statistically concluded whether the HA fraction properly represents the slow pool.

Conceptually, the methods for determining the non-labile pool (also called passive, recalcitrant, inert or stable pool) include fractions whose half-life ranges from decades to centuries due to strong biochemical stability or restricted availability (Wander, 2004), including pyrogenic C (black C, charcoal) and humin fractions.

Several methods can be used to determine pyrogenic C, including oxidation with hydrogen peroxide (H_2O_2) (Jackson, 1958), which removes SOM accessible to exoenzymes, and the quantification of benzene polycarboxylic acids (BPCA) (Glaser et al., 1998), degradation products of polyaromatic compounds. Comparing several methods, Helfrich et al. (2007) concluded that the method with H_2O_2 was among the most efficient for extracting stabilized SOM. Gerke (2019) recently stated that methods of determining pyrogenic C such as BPCA overestimate their soil contents by accounting for C comprising humic substances.

Fractionation of SOM into humic substances results in fractions that are independent of each other in terms of solubility in acidic and alkaline media. In summary, the organic matter is operationally fractionated into: fulvic acid (FA), soluble in alkaline and acidic media; humic acid (HA), soluble in alkaline and insoluble in acidic media; humin, insoluble in alkaline media (Stevenson, 1994). Gray and brown humic acids can also be determined depending on the fractionation procedure used. There are many discussions about the ecological function of FA and HA (Gerke, 2018). However, regarding humin, scientific knowledge tends to converge toward a consensus. Humin is a more stable SOM fraction, and some of the C in biochar is quantified in this fraction due to its intrinsic insolubility (Hayes et al., 2017). Hence, evaluating the humin fraction as a C reserve pool in biochar amended soils may be interesting.

As with physical methods, no established set of chemical fractionation methods accurately represent the SOM pools in terms of their stability and ecological function. Thus, combined methods can be used alternatively. However, the fractions usually overlap, even among chemical extractions (Sherrod et al., 2019). Therefore, this strategy is also not effective in representing the SOM pools. Another common approach is to combine prior physical fractionation with chemical methods (von Lützow et al., 2007). As density or particle size-based methods are not the most suitable for biochar-amended soils, this strategy is challenging to implement in such cases.

Methods for soil organic matter fractionation encompass various chemical and physical techniques, each focusing on different aspects of SOM. However, it is crucial to consider additional analytical approaches to understand the continuum of SOM components comprehensively. Thermogravimetric analysis (TGA) is a widely employed technique to study the thermal behavior of various materials, including biochar (Patel et al., 2019). TGA measures the mass change of a sample as it is heated under controlled conditions, providing insights into its composition and thermal properties. In the context of soils amended with SSB, TGA has been utilized to investigate the pyrolysis behavior, stability, and decomposition kinetics of biochar, as well as its influence on soil characteristics. By assessing the thermal stability of SOM, TGA serves as a complementary method to chemical, physical, and biological fractionation techniques, enabling a comprehensive understanding of the continuum of SOM components (Plante et al., 2009). Previous research has utilized differential thermogravimetry (dTG) and differential thermal analysis (DTA) techniques to analyze sandy loam soil amended with SSB (Gascó et al., 2012). TGA offers several advantages, including simplicity, rapidity, minimal sample size, and limited sample preparation requirements (Plante et al., 2009). However, one of the limitations of this method is that it is strongly influenced by soil management (Tokarski et al., 2020). Notably, in soils with biochar, TGA thermograms exhibit distinct peaks at high temperatures associated with recalcitrant organic matter, such as polycyclic aromatic hydrocarbons.

Finally, when evaluating SOM fractions, it is desirable to have soil TC data available. This allows the C extracted in the other fractions to be validated and relativized. Therefore, in biochar amended soils, the fractionation method must be carefully chosen (Cooper et al., 2020). Moreover, since there is no consensus in the literature, the aim here is not to choose which set of fractions best characterizes the SOM pools and their dynamics, but to try to relate the results of established methods.

4.6. Distinguishing black carbon from biochar in Cerrado soils: challenges and analytical techniques

In the vast Cerrado biome, black C derived from natural vegetation fires plays a crucial role in soil dynamics and ecosystem functioning. Covering approximately 2 million km², the Cerrado is characterized by a mosaic of vegetation types, including forestlands, shrublands, and grasslands (IBGE, 2019). It represents the most extensive tropical savannah region in the Neotropics, harboring a rich diversity of plant and animal species (Colli et al., 2020).

Frequent wildfires in the Cerrado, driven by seasonal climate and vegetation dynamics (Durigan, 2020), lead to the incomplete combustion of native vegetation and the formation of black C. Comprising charred plant residues, black C is intricately linked to the fire regime and ecological dynamics of the Cerrado (de Oliveira et al., 2022). Native black C serves as a long-term storage form of C in the soil due to its resistance to microbial degradation, enhancing its ability to sequester C and mitigate GHG emissions (Bird et al., 2015; Preston and Schmidt, 2006).

In contrast to native black C, biochar is a carbon-rich material intentionally produced through the pyrolysis of organic feedstocks and added to soils as an amendment. In recent years, there has been growing interest in applying biochar in agricultural systems of the Cerrado region (Faria et al., 2018; Madari et al., 2017; Silva et al., 2017). The importance of distinguishing between natural black C and biochar lies in their different roles in the environment. While both contribute to C sequestration, biochar is often intentionally added to soils to improve fertility and enhance C storage (Chagas et al., 2021a; Wang et al., 2023). On the other hand, black C is a byproduct of natural and anthropogenic fires and can have varying impacts on soil properties and processes.

Several methodologies have been developed to differentiate between natural black C and biochar in soils. These include the use of molecular markers (Kaal et al., 2012), isotopic signatures (Singh et al., 2012), multi-elemental analysis (Freddo et al., 2012), and advanced spectroscopic techniques (De la Rosa et al., 2008).

Molecular markers such as levoglucosan and BPCA provide insight into pyrogenic C sources. Levoglucosan is a specific molecular marker that can be degraded over time (Kaal et al., 2012). While BPCA patterns indicate black C sources, overlap with other soil organic matter fractions complicates definitive attribution (Glaser et al., 1998). Given their variable alteration and non-unique formation, molecular markers alone cannot unambiguously identify black C sources in soils.

Isotopic analysis of radiocarbon (^{14}C) and stable C isotopes ($\delta^{13}C$) can help distinguish some biochar from black C. However, overlapping radiocarbon contents make biochar and black carbon from recent wildfires difficult to distinguish. Although stable isotope signatures reflect sources such as C3 vs. C4 plants, feedstock variability, pyrolysis effects, decomposition, aging, and selective preservation can obscure original signatures (Ascough et al., 2010; Hammes et al., 2007). Thus, isotopes alone may not definitively identify black C origins in soils.

Advanced spectroscopic techniques, such as nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FTIR), can provide detailed information on the chemical structure of pyrogenic C. However, these techniques have limitations regarding lack of specificity, interpretation complexity, and the requirement for specialized equipment and expertise (De la Rosa et al., 2008; Singh et al., 2016).

Current methods for quantifying and characterizing pyrogenic C in soils, such as the BPCA method (Glaser et al., 1998), thermochemical analysis, and spectroscopic techniques, do not specifically differentiate between black C and biochar. Moreover, these methods can be influenced by other soil constituents, leading to potential over- or underestimation of black C or biochar content (Gerke, 2019; Hammes et al., 2007).

The challenges in distinguishing between natural black C and biochar arise from their similar formation processes and resulting properties. Both forms are characterized by a highly condensed aromatic structure, making them resistant to decomposition and enabling long-term C storage (Kopecký et al., 2021; Zhang et al., 2015). Furthermore, they can share physical properties such as color and particle size, complicating differentiation (Singh et al., 2012).

A multi-technique approach combining microscopy, spectroscopy, and isotopic methods appears promising for robust discrimination between biochar and native black C in Cerrado soils (Schmidt et al., 2001). As biochar use increases in the biome, accurately distinguishing these black C forms will be crucial for understanding their impacts.

4.7. “Think globally, act locally”

Globalization has reaffirmed the perception of an interconnected and borderless environment, hence ubiquitous. In 1915, Patrick Geddes used the expression “Think globally, act locally” in the context of urban planning (Geddes, 1915). It emphasizes the importance of considering the global context even in small local actions, as their combined impact can be significant.

This approach requires the development of local climate agendas that align with the specificities of each region and contribute to the achievement of the Sustainable Development Goals outlined in the 2030 Agenda (Moallemi et al., 2019). Achieving this requires innovative governance based on the scrutiny of consolidated scientific evidence.

Meta-analysis, a powerful tool, enables the synthesis of extensive global research. Meta-analysis informs local decision-makers by identifying broader patterns and relationships, revealing hidden effects and applying rigorous methodologies (Shelby and Vaske, 2008). For example, meta-analysis can evaluate the modulators of C sequestration in soil due to biochar amendment (Fagard et al., 1996), providing valuable insights for climate crisis mitigation and contributing to global goals such as the 4p1000 Initiative and the Paris Agreement (Lehmann et al., 2021).

Integrating global research into local strategies exemplifies the essence of “think globally, act locally”. It harmonizes science, policy, and practice across scales to address environmental challenges such as climate change. Implementing evidence-based local solutions is critical to achieving international sustainability goals and fostering the widespread adoption of low-carbon practices.

Local decision-makers can use the outcomes of meta-analyses to tailor biochar implementation, considering optimal feedstocks, pyrolysis conditions, soil types, and more, significantly impacting global climate and development goals. Furthermore, collective local actions wield substantial global influence, playing a pivotal role in achieving climate and development goals. Knowledge exchange between local and global scales further enhances understanding, as local monitoring data can continuously update meta-analyses and models.

In summary, integrating global research through meta-analysis empowers localized strategies, bridging local knowledge with global sustainability aspirations. This dynamic fusion enables effective responses to environmental challenges and facilitates the transition to a sustainable future.

4.8. References

Abney, R.B., Berhe, A.A., 2018. Pyrogenic carbon erosion: implications for stock and persistence of pyrogenic carbon in soil. Front Earth Sci (Lausanne) 6, 1–16. https://doi.org/10.3389/feart.2018.00026

Adhikari, S., Moon, E., Paz-Ferreiro, J., Timms, W., 2024. Comparative analysis of biochar carbon stability methods and implications for carbon credits. Science of The Total Environment 914, 169607. https://doi.org/10.1016/j.scitotenv.2023.169607

Amorim Júnior, S.S. de, Pereira, M.A. de S., Lima, P. de M., Marishigue, M., Guilherme, D. de O., Magalhães Filho, F.J.C., 2021. Evidences on the application of biosolids and the effects on chemical characteristics in infertile tropical sandy soils. Clean Eng Technol 4, 100245. https://doi.org/10.1016/j.clet.2021.100245

Anderson, C.R., Condron, L.M., Clough, T.J., Fiers, M., Stewart, A., Hill, R.A., Sherlock, R.R., 2011. Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia (Jena) 54, 309–320. https://doi.org/10.1016/j.pedobi.2011.07.005

Andersson, K., Rosemarin, A., Lamizana, B., Kvarnström, E., McConville, J., Seidu, R., Dickin, S., Trimmer, C., 2020. Sanitation, Wastewater Management and Sustainability: from Waste Disposal to Resource Recovery, 2nd ed. United Nations Environment Programme and Stockholm Environment Institute, Nairobi and Stockholm.

Ascough, P.L., Bird, M.I., Meredith, W., Wood, R.E., Snape, C.E., Brock, F., Higham, T.F.G., Large, D.J., Apperley, D.C., 2010. Hydropyrolysis: Implications for Radiocarbon Pretreatment and Characterization of Black Carbon. Radiocarbon 52, 1336–1350. https://doi.org/10.1017/S0033822200046427

Awasthi, M.K., Singh, E., Binod, P., Sindhu, R., Sarsaiya, S., Kumar, A., Chen, H., Duan, Y., Pandey, A., Kumar, S., Taherzadeh, M.J., Li, J., Zhang, Z., 2022. Biotechnological strategies for bio-transforming biosolid into resources toward circular bio-economy: A review. Renewable and Sustainable Energy Reviews 156, 111987. https://doi.org/10.1016/j.rser.2021.111987

Benbi, D.K., Nisar, S., 2020. Developments in measurement and modelling of soil organic carbon, in: Ghosh, P.K., Mahanta, S.K., Mandal, D., Mandal, B., Ramakrishnan, S. (Eds.), Carbon Management in Tropical and Sub-Tropical Terrestrial Systems. Springer Singapore, Singapore, pp. 379–402. https://doi.org/10.1007/978-981-13-9628-1_23

Bird, M.I., Wynn, J.G., Saiz, G., Wurster, C.M., McBeath, A., 2015. The pyrogenic carbon cycle. Annu Rev Earth Planet Sci 43, 273–298. https://doi.org/10.1146/annurev-earth-060614-105038

Blanco-Canqui, H., 2021. Does biochar improve all soil ecosystem services? GCB Bioenergy 13, 291–304. https://doi.org/10.1111/gcbb.12783

Bongiorno, G., Bünemann, E.K., Oguejiofor, C.U., Meier, J., Gort, G., Comans, R., Mäder, P., Brussaard, L., de Goede, R., 2019. Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. Ecol Indic 99, 38–50. https://doi.org/10.1016/j.ecolind.2018.12.008

Brazil, 2022a. Brazil First Nationally Determined Contribution - Second update [WWW Document]. United Nations Framework Convention on Climate Change (UNFCCC). URL https://unfccc.int/NDCREG (accessed 1.24.23).

Brazil, 2022b. Decreto No 10.991, de 11 de março de 2022. Institui o Plano Nacional de Fertilizantes 2022-2050 e o Conselho Nacional de Fertilizantes e Nutrição de Plantas. Diário Oficial da União, de 11/03/2022, Seção 1, Edição Extra, p.1-2, Brasília. (in Portuguese)

Brazil, 2017. Decreto No 9.073, de 05 de junho de 2017. Promulga o Acordo de Paris sob a Convenção-Quadro das Nações Unidas sobre Mudança do Clima, celebrado em Paris, em 12 de dezembro de 2015, e firmado em Nova Iorque, em 22 de abril de 2016. Diário Oficial da União, de 06/06/2017, p.3, Brasília. (in Portuguese)

Brazil. Ministry of Agriculture, Livestock and Supply, 2021. Plano setorial para adaptação à mudança do clima e baixa emissão de carbono na agropecuária com vistas ao desenvolvimento sustentável (2020-2030): visão estratégica para um novo ciclo. MAPA, Brasília. (in Portuguese)

Brazil. Ministry of Agriculture, Livestock and Supply, 2012. Plano Setorial de Mitigação e Adaptação às Mudanças Climáticas para Consolidação da Economia de Baixa Emissão de Carbono na Agricultura: plano ABC. MAPA/ACS, Brasília. (in Portuguese)

Cao, Y., Wang, X., Bai, Z., Chadwick, D., Misselbrook, T., G. Sommer, S., Qin, W., Ma, L., 2019. Mitigation of ammonia, nitrous oxide and methane emissions during solid waste composting with different additives: A meta-analysis. J Clean Prod 235, 626–635. https://doi.org/10.1016/j.jclepro.2019.06.288

Cayuela, M.L., van Zwieten, L., Singh, B.P., Jeffery, S., Roig, A., Sánchez-Monedero, M.A., 2014. Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. Agric Ecosyst Environ 191, 5–16. https://doi.org/10.1016/j.agee.2013.10.009

Chagas, J.K.M., Figueiredo, C.C. de, Paz-Ferreiro, J., 2021a. Sewage sludge biochars effects on corn response and nutrition and on soil properties in a 5-yr field experiment. Geoderma 401, 115323. https://doi.org/10.1016/j.geoderma.2021.115323

Chagas, J.K.M., Figueiredo, C.C. de, Silva, J. da, Shah, K., Paz-Ferreiro, J., 2021b. Long-term effects of sewage sludge–derived biochar on the accumulation and availability of trace elements in a tropical soil. J Environ Qual 50, 264–277. https://doi.org/10.1002/jeq2.20183

Christensen, B.T., 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. Eur J Soil Sci 52, 345–353. https://doi.org/10.1046/j.1365-2389.2001.00417.x

Colli, G.R., Vieira, C.R., Dianese, J.C., 2020. Biodiversity and conservation of the Cerrado: recent advances and old challenges. Biodivers Conserv 29, 1465–1475. https://doi.org/10.1007/s10531-020-01967-x

Collivignarelli, M.C., Canato, M., Abbà, A., Carnevale Miino, M., 2019. Biosolids: what are the different types of reuse? J Clean Prod 238, 117844. https://doi.org/10.1016/j.jclepro.2019.117844

Collivignarelli, M.C., Castagnola, F., Sordi, M., Bertanza, G., 2015. Treatment of sewage sludge in a thermophilic membrane reactor (TMR) with alternate aeration cycles. J Environ Manage 162, 132–138. https://doi.org/10.1016/j.jenvman.2015.07.031

Companhia de Saneamento Ambiental do Distrito Federal (CAESB), 2022. Performance indicators report: 2021 results [*Relatório de indicadores de desempenho: resultados 2021*]. CAESB, Brasília. (in Portuguese)

Cooper, J., Greenberg, I., Ludwig, B., Hippich, L., Fischer, D., Glaser, B., Kaiser, M., 2020. Effect of biochar and compost on soil properties and organic matter in aggregate size fractions under field conditions. Agric Ecosyst Environ 295, 106882. https://doi.org/10.1016/j.agee.2020.106882

De la Rosa, J.M., Knicker, H., López-Capel, E., Manning, D.A.C., González-Perez, J.A., González-Vila, F.J., 2008. Direct Detection of Black Carbon in Soils by Py-GC/MS, Carbon-13 NMR Spectroscopy and Thermogravimetric Techniques. Soil Science Society of America Journal 72, 258–267. https://doi.org/10.2136/sssaj2007.0031

de Oliveira, E.A., Feldpausch, T.R., Marimon, B.S., Morandi, P.S., Phillips, O.L., Bird, M., Murakami, A.A., Arroyo, L., Quesada, C.A., Marimon-Junior, B.H., 2022. Soil pyrogenic carbon in southern Amazonia: Interaction between soil, climate, and above-ground biomass. Frontiers in Forests and Global Change 5, 880963. https://doi.org/10.3389/ffgc.2022.880963

Domingues, R.R., Trugilho, P.F., Silva, C.A., Melo, I.C.N.A. de, Melo, L.C.A., Magriotis, Z.M., Sánchez-Monedero, M.A., 2017. Properties of biochar derived from wood and high-nutrient biomasses with the aim of agronomic and environmental benefits. PLoS One 12, e0176884. https://doi.org/10.1371/journal.pone.0176884

Duddigan, S., Shaw, L.J., Alexander, P.D., Collins, C.D., 2019. A comparison of physical soil organic matter fractionation methods for amended soils. Appl Environ Soil Sci 2019, 1–12. https://doi.org/10.1155/2019/3831241

Durigan, G., 2020. Zero-fire: Not possible nor desirable in the Cerrado of Brazil. Flora 268, 151612. https://doi.org/10.1016/j.flora.2020.151612

Dynarski, K.A., Bossio, D.A., Scow, K.M., 2020. Dynamic Stability of Soil Carbon: Reassessing the "Permanence" of Soil Carbon Sequestration. Front Environ Sci 8, 514701. https://doi.org/10.3389/fenvs.2020.514701

Fagard, R.H., Staessen, J.A., Thijs, L., 1996. Advantages and disadvantages of the meta-analysis approach. J Hypertens 14, S9–S13. https://doi.org/10.1097/00004872-199609002-00004

Faria, W.M., Figueiredo, C.C. de, Coser, T.R., Vale, A.T., Schneider, B.G., 2018. Is sewage sludge biochar capable of replacing inorganic fertilizers for corn production? Evidence from a two-year field experiment. Arch Agron Soil Sci 64, 505–519. https://doi.org/10.1080/03650340.2017.1360488

Fathi Dokht, H., Movahedi Naeini, S.A., Dordipour, E., De Jong, L.W., Hezarjaribi, E., 2017. Effects of sewage sludge and its biochar on soybean yield in fine-textured loess soil. Environmental Health Engineering and Management 4, 81–91. https://doi.org/10.15171/EHEM.2017.12

Fatima, S., Riaz, M., Al-Wabel, M.I., Arif, M.S., Yasmeen, T., Hussain, Q., Roohi, M., Fahad, S., Ali, K., Arif, M., 2021. Higher biochar rate strongly reduced decomposition of soil organic matter to enhance C and N sequestration in nutrient-poor alkaline calcareous soil. J Soils Sediments 21, 148–162. https://doi.org/10.1007/s11368-020-02753-6

Figueiredo, C., Lopes, H., Coser, T., Vale, A., Busato, J., Aguiar, N., Novotny, E., Canellas, L., 2018. Influence of pyrolysis temperature on chemical and physical properties of biochar from sewage sludge. Arch Agron Soil Sci 64, 881–889. https://doi.org/10.1080/03650340.2017.1407870

Figueiredo, C.C. de, Farias, W.M., Coser, T.R., Monteiro de Paula, A., Sartori da Silva, M.R., Paz-Ferreiro, J., 2019a. Sewage sludge biochar alters root colonization of mycorrhizal fungi in a soil cultivated with corn. Eur J Soil Biol 93, 103092. https://doi.org/10.1016/j.ejsobi.2019.103092

Figueiredo, C.C. de, Farias, W.M., Melo, B.A. de, Chagas, J.K.M., Vale, A.T., Coser, T.R., 2019b. Labile and stable pools of organic matter in soil amended with sewage sludge biochar. Arch Agron Soil Sci 65, 770–781. https://doi.org/10.1080/03650340.2018.1524577

Freddo, A., Cai, C., Reid, B.J., 2012. Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar. Environmental Pollution 171, 18–24. https://doi.org/10.1016/j.envpol.2012.07.009

Fuss, S., Canadell, J.G., Peters, G.P., Tavoni, M., Andrew, R.M., Ciais, P., Jackson, R.B., Jones, C.D., Kraxner, F., Nakicenovic, N., Le Quéré, C., Raupach, M.R., Sharifi, A., Smith, P., Yamagata, Y., 2014. Betting on negative emissions. Nat Clim Chang 4, 850–853. https://doi.org/10.1038/nclimate2392

García-Orenes, F., Guerrero, C., Mataix-Solera, J., Navarro-Pedreño, J., Gómez, I., Mataix-Beneyto, J., 2005. Factors controlling the aggregate stability and bulk density in two different degraded soils amended with biosolids. Soil Tillage Res 82, 65–76. https://doi.org/10.1016/j.still.2004.06.004

Gascó, G., Paz-Ferreiro, J., Méndez, A., 2012. Thermal analysis of soil amended with sewage sludge and biochar from sewage sludge pyrolysis. J Therm Anal Calorim 108, 769–775. https://doi.org/10.1007/s10973-011-2116-2

Geddes, P., 1915. Cities in Evolution. Williams and Norgale, Londres.

Gerke, J., 2019. Black (pyrogenic) carbon in soils and waters: a fragile data basis extensively interpreted. Chemical and Biological Technologies in Agriculture 6, 13. https://doi.org/10.1186/s40538-019-0151-6

Gerke, J., 2018. Concepts and misconceptions of humic substances as the stable part of soil organic matter: a review. Agronomy 8, 76. https://doi.org/10.3390/agronomy8050076

Glaser, B., 2007. Prehistorically modified soils of central Amazonia: a model for sustainable agriculture in the twenty-first century. Philosophical Transactions of the Royal Society B: Biological Sciences 362, 187–196. https://doi.org/10.1098/rstb.2006.1978

Glaser, B., Haumaier, L., Guggenberger, G., Zech, W., 1998. Black carbon in soils: the use of benzenecarboxylic acids as specific markers. Org Geochem 29, 811–819. https://doi.org/10.1016/S0146-6380(98)00194-6

Goldan, E., Nedeff, V., Barsan, N., Culea, M., Tomozei, C., Panainte-Lehadus, M., Mosnegutu, E., 2022. Evaluation of the use of sewage sludge biochar as a soil amendment - a review. Sustainability 14, 5309. https://doi.org/10.3390/su14095309

Gross, A., Bromm, T., Glaser, B., 2021. Soil organic carbon sequestration after biochar application: a global meta-analysis. Agronomy 11, 2474. https://doi.org/10.3390/agronomy11122474

Grunwald, D., Kaiser, M., Junker, S., Marhan, S., Piepho, H.-P., Poll, C., Bamminger, C., Ludwig, B., 2017. Influence of elevated soil temperature and biochar application on organic matter associated with aggregate-size and density fractions in an arable soil. Agric Ecosyst Environ 241, 79–87. https://doi.org/10.1016/j.agee.2017.02.029

Hammes, K., Schmidt, M.W.I., Smernik, R.J., Currie, L.A., Ball, W.P., Nguyen, T.H., Louchouarn, P., Houel, S., Gustafsson, Ö., Elmquist, M., Cornelissen, G., Skjemstad, J.O., Masiello, C.A., Song, J., Peng, P., Mitra, S., Dunn, J.C., Hatcher, P.G., Hockaday, W.C., Smith, D.M., Hartkopf-Fröder, C., Böhmer, A., Lüer, B., Huebert, B.J., Amelung, W., Brodowski, S., Huang, L., Zhang, W., Gschwend, P.M., Flores-Cervantes, D.X., Largeau, C., Rouzaud, J.-N., Rumpel, C., Guggenberger, G., Kaiser, K., Rodionov, A., Gonzalez-Vila, F.J., Gonzalez-Perez, J.A., de la Rosa, J.M., Manning, D.A.C., López-Capél, E., Ding, L., 2007. Comparison of quantification methods to measure fire-derived (black/elemental) carbon in soils and sediments using reference materials from soil, water, sediment and the atmosphere. Global Biogeochem Cycles 21, GB3016. https://doi.org/10.1029/2006GB002914

Hayes, M.H.B., Mylotte, R., Swift, R.S., 2017. Humin: its composition and importance in soil organic matter, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, Burlington, pp. 47–138. https://doi.org/10.1016/bs.agron.2017.01.001

Helfrich, M., Flessa, H., Mikutta, R., Dreves, A., Ludwig, B., 2007. Comparison of chemical fractionation methods for isolating stable soil organic carbon pools. Eur J Soil Sci 58, 1316–1329. https://doi.org/10.1111/j.1365-2389.2007.00926.x

Hofmann, G.S., Cardoso, M.F., Alves, R.J. V., Weber, E.J., Barbosa, A.A., Toledo, P.M., Pontual, F.B., Salles, L. de O., Hasenack, H., Cordeiro, J.L.P., Aquino, F.E., Oliveira, L.F.B., 2021. The Brazilian Cerrado is becoming hotter and drier. Glob Chang Biol 27, 4060–4073. https://doi.org/10.1111/gcb.15712

Hu, Q., Jung, J., Chen, D., Leong, K., Song, S., Li, F., Mohan, B.C., Yao, Z., Prabhakar, A.K., Lin, X.H., Lim, E.Y., Zhang, L., Souradeep, G., Ok, Y.S., Kua, H.W., Li, S.F.Y., Tan, H.T.W., Dai, Y., Tong, Y.W., Peng, Y., Joseph, S., Wang, C.-H., 2021. Biochar industry to circular economy. Science of The Total Environment 757, 143820. https://doi.org/10.1016/j.scitotenv.2020.143820

Huang, R., Tian, D., Liu, J., Lv, S., He, X., Gao, M., 2018. Responses of soil carbon pool and soil aggregates associated organic carbon to straw and straw-derived biochar addition in a dryland cropping mesocosm system. Agric Ecosyst Environ 265, 576–586. https://doi.org/10.1016/j.agee.2018.07.013

Ibrahim, M., Li, G., Khan, S., Chi, Q., Xu, Y., 2017. Biochars mitigate greenhouse gas emissions and bioaccumulation of potentially toxic elements and arsenic speciation in Phaseolus vulgaris L. Environmental Science and Pollution Research 24, 19524–19534. https://doi.org/10.1007/s11356-017-9605-1

Instituto Brasileiro de Geografia e Estatística (IBGE), 2019. Biomas e sistema costeiro-marinho do Brasil: compatível com a escala 1:250 000. IBGE, Rio de Janeiro. (in Portuguese)

IPCC, 2021. Summary for Policymakers, in: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J.B.R., Maycock, T.K., Waterfield, T., Yelekçi, O., Yu, R., Zhou, B. (Eds.), Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Switzerland, p. 40.

Jackson, M.L., 1958. Soil chemical analysis. Prentice Hall Inc., Englewood Cliffs.

Jeffery, S., Abalos, D., Prodana, M., Bastos, A.C., van Groenigen, J.W., Hungate, B.A., Verheijen, F., 2017. Biochar boosts tropical but not temperate crop yields. Environmental Research Letters 12, 053001. https://doi.org/10.1088/1748-9326/aa67bd

Joint Monitoring Programme for Water Supply Sanitation and Hygiene (JMP), 2021. Progress on household drinking water, sanitation and hygiene 2000-2020: five years into SDGs. WHO/UNICEF, Geneva.

Jones, E.R., van Vliet, M.T.H., Qadir, M., Bierkens, M.F.P., 2021. Country-level and gridded estimates of wastewater production, collection, treatment and reuse. Earth Syst Sci Data 13, 237–254. https://doi.org/10.5194/essd-13-237-2021

Joseph, S., Cowie, A.L., Van Zwieten, L., Bolan, N., Budai, A., Buss, W., Cayuela, M.L., Graber, E.R., Ippolito, J.A., Kuzyakov, Y., Luo, Y., Ok, Y.S., Palansooriya, K.N., Shepherd, J., Stephens, S., Weng, Z. (Han), Lehmann, J., 2021. How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. GCB Bioenergy 13, 1731–1764. https://doi.org/10.1111/gcbb.12885

Kaal, J., Martínez Cortizas, A., Reyes, O., Soliño, M., 2012. Molecular characterization of Ulex europaeus biochar obtained from laboratory heat treatment experiments – A pyrolysis–

GC/MS study. J Anal Appl Pyrolysis 95, 205–212. https://doi.org/10.1016/j.jaap.2012.02.008

Kacprzak, M., Neczaj, E., Fijałkowski, K., Grobelak, A., Grosser, A., Worwag, M., Rorat, A., Brattebo, H., Almås, Å., Singh, B.R., 2017. Sewage sludge disposal strategies for sustainable development. Environ Res 156, 39–46. https://doi.org/10.1016/j.envres.2017.03.010

Kopecký, M., Kolář, L., Váchalová, R., Konvalina, P., Batt, J., Mráz, P., Menšík, L., Hoang, T.N., Dumbrovský, M., 2021. Black Carbon and Its Effect on Carbon Sequestration in Soil. Agronomy 11, 2261. https://doi.org/10.3390/agronomy11112261

Koyama, S., Hayashi, H., 2019. Effects of single and successive applications of rice husk charcoal on paddy soil carbon content and rice productivity during two cropping seasons. Soil Sci Plant Nutr 65, 196–202. https://doi.org/10.1080/00380768.2019.1579042

Lal, R., 2010. Managing soils and ecosystems for mitigating anthropogenic carbon emissions and advancing global food security. Bioscience 60, 708–721. https://doi.org/10.1525/bio.2010.60.9.8

Lehmann, J., Cowie, A., Masiello, C.A., Kammann, C., Woolf, D., Amonette, J.E., Cayuela, M.L., Camps-Arbestain, M., Whitman, T., 2021. Biochar in climate change mitigation. Nat Geosci 14, 883–892. https://doi.org/10.1038/s41561-021-00852-8

Li, S., Harris, S., Anandhi, A., Chen, G., 2019. Predicting biochar properties and functions based on feedstock and pyrolysis temperature: a review and data syntheses. J Clean Prod 215, 890–902. https://doi.org/10.1016/j.jclepro.2019.01.106

Li, X., Wang, T., Chang, S.X., Jiang, X., Song, Y., 2020. Biochar increases soil microbial biomass but has variable effects on microbial diversity: A meta-analysis. Science of The Total Environment 749, 141593. https://doi.org/10.1016/j.scitotenv.2020.141593

Liu, X., Zhang, A., Ji, C., Joseph, S., Bian, R., Li, L., Pan, G., Paz-Ferreiro, J., 2013. Biochar's effect on crop productivity and the dependence on experimental conditions - a meta-analysis of literature data. Plant Soil 373, 583–594. https://doi.org/10.1007/s11104-013-1806-x

Lopes, A.S., Guilherme, L.R.G., 2016. A career perspective on soil management in the Cerrado region of Brazil, in: Sparks, D.L. (Ed.), Advances in Agronomy, v.137. Academic Press, Cambridge, pp. 1–72. https://doi.org/10.1016/bs.agron.2015.12.004

Madari, B.E., Silva, M.A.S., Carvalho, M.T.M., Maia, A.H.N., Petter, F.A., Santos, J.L.S., Tsai, S.M., Leal, W.G.O., Zeviani, W.M., 2017. Properties of a sandy clay loam Haplic Ferralsol and soybean grain yield in a five-year field trial as affected by biochar amendment. Geoderma 305, 100–112. https://doi.org/10.1016/j.geoderma.2017.05.029

Moallemi, E.A., Malekpour, S., Hadjikakou, M., Raven, R., Szetey, K., Moghadam, M.M., Bandari, R., Lester, R., Bryan, B.A., 2019. Local Agenda 2030 for sustainable development. Lancet Planet Health 3, e240–e241. https://doi.org/10.1016/S2542-5196(19)30087-7

Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter, in: Sparks, D.L., Pag, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (Eds.), Methods of Soil Analysis. Part 3. Chemical Methods. Soil Science of America and American Society of Agronomy, Madison, pp. 961–1010. https://doi.org/10.2136/sssabookser5.3.c34

Nicholson, F., Bhogal, A., Taylor, M., McGrath, S., Withers, P., 2018. Long-term effects of biosolids on soil quality and fertility. Soil Sci 183, 89–98. https://doi.org/10.1097/SS.0000000000000239

Novotny, E.H., Maia, C.M.B. de F., Carvalho, M.T. de M., Madari, B.E., 2015. Biochar: pyrogenic carbon for agricultural use - a critical review. Rev Bras Cienc Solo 39, 321–344. https://doi.org/10.1590/01000683rbcs20140818

Paetsch, L., Mueller, C.W., Rumpel, C., Angst, Š., Wiesheu, A.C., Girardin, C., Ivleva, N.P., Niessner, R., Kögel-Knabner, I., 2017. A multi-technique approach to assess the fate of biochar in soil and to quantify its effect on soil organic matter composition. Org Geochem 112, 177–186. https://doi.org/10.1016/j.orggeochem.2017.06.012

Patel, S., Kundu, S., Halder, P., Rickards, L., Paz-Ferreiro, J., Surapaneni, A., Madapusi, S., Shah, K., 2019. Thermogravimetric analysis of biosolids pyrolysis in the presence of mineral oxides. Renew Energy 141, 707–716. https://doi.org/10.1016/j.renene.2019.04.047

Paz-Ferreiro, J., Nieto, A., Méndez, A., Askeland, M., Gascó, G., 2018. Biochar from biosolids pyrolysis: a review. Int J Environ Res Public Health 15, 956. https://doi.org/10.3390/ijerph15050956

Plante, A.F., Fernández, J.M., Leifeld, J., 2009. Application of thermal analysis techniques in soil science. Geoderma 153, 1–10. https://doi.org/10.1016/j.geoderma.2009.08.016

Poggere, G., Santana, M.J.B., Barbosa, J.Z., Corrêa, R.S., Melo, L.C.A., 2022. A data synthesis on the biochar properties and implications for air, soil, and water quality in Brazil. Environmental Quality Management (early view). https://doi.org/10.1002/tqem.21875

Prates, A.R., Coscione, A.R., Teixeira Filho, M.C.M., Miranda, B.G., Arf, O., Abreu-Junior, C.H., Oliveira, F.C., Moreira, A., Galindo, F.S., Sartori, M.M.P., He, Z., Jani, A.D., Capra, G.F., Ganga, A., Nogueira, T.A.R., 2020. Composted sewage sludge enhances soybean production and agronomic performance in naturally infertile soils (Cerrado region, Brazil). Agronomy 10, 1677. https://doi.org/10.3390/agronomy10111677

Prates, A.R., Kawakami, K.C., Coscione, A.R., Filho, M.C.M.T., Arf, O., Abreu-Junior, C.H., Oliveira, F.C., Moreira, A., Galindo, F.S., He, Z., Jani, A.D., Capra, G.F., Ganga, A., Nogueira, T.A.R., 2022. Composted sewage sludge sustains high maize productivity on an infertile Oxisol in the Brazilian Cerrado. Land (Basel) 11, 1246. https://doi.org/10.3390/land11081246

Preston, C.M., Schmidt, M.W.I., 2006. Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. Biogeosciences 3, 397–420. https://doi.org/10.5194/bg-3-397-2006

Racek, J., Sevcik, J., Chorazy, T., Kucerik, J., Hlavinek, P., 2020. Biochar – Recovery Material from Pyrolysis of Sewage Sludge: A Review. Waste Biomass Valorization 11, 3677–3709. https://doi.org/10.1007/s12649-019-00679-w

Schmidt, M.W.I., Skjemstad, J.O., Czimczik, C.I., Glaser, B., Prentice, K.M., Gelinas, Y., Kuhlbusch, T.A.J., 2001. Comparative analysis of black carbon in soils. Global Biogeochem Cycles 15, 163–167. https://doi.org/10.1029/2000GB001284

Shelby, L.B., Vaske, J.J., 2008. Understanding meta-analysis: a review of the methodological literature. Leis Sci 30, 96–110. https://doi.org/10.1080/01490400701881366

Sherrod, L.A.A., Vigil, M.F.F., Stewart, C.E.E., 2019. Do fulvic, humic, and humin carbon fractions represent meaningful biological, physical, and chemical carbon pools? J Environ Qual 48, 1587–1593. https://doi.org/10.2134/jeq2019.03.0104

Silva, I.C.B. da, Basílio, J.J.N., Fernandes, L.A., Colen, F., Sampaio, R.A., Frazão, L.A., 2017. Biochar from different residues on soil properties and common bean production. Sci Agric 74, 378–382. https://doi.org/10.1590/1678-992x-2016-0242

Singh, B., Fang, Y., Johnston, C.T., 2016. A Fourier-Transform Infrared Study of Biochar Aging in Soils. Soil Science Society of America Journal 80, 613–622. https://doi.org/10.2136/sssaj2015.11.0414

Singh, N., Abiven, S., Torn, M.S., Schmidt, M.W.I., 2012. Fire-derived organic carbon in soil turns over on a centennial scale. Biogeosciences 9, 2847–2857. https://doi.org/10.5194/bg-9-2847-2012

Smith, P., 2016. Soil carbon sequestration and biochar as negative emission technologies. Glob Chang Biol 22, 1315–1324. https://doi.org/10.1111/gcb.13178

Sohi, S.P., 2012. Carbon storage with benefits. Science (1979) 338, 1034–1035. https://doi.org/10.1126/science.1225987

Sokol, N.W., Whalen, E.D., Jilling, A., Kallenbach, C., Pett-Ridge, J., Georgiou, K., 2022. Global distribution, formation and fate of mineral-associated soil organic matter under a changing climate: A trait-based perspective. Funct Ecol 36, 1411–1429. https://doi.org/10.1111/1365-2435.14040

Stevenson, F.J., 1994. Humus Chemistry: Genesis, Composition, Reactions, 2nd ed. Wiley, New York.

Strosser, E., 2011. Methods for determination of labile soil organic matter: An overview. J Agrobiol 27, 49–60. https://doi.org/10.2478/s10146-009-0008-x

Tokarski, D., Wiesmeier, M., Doležalová Weissmannová, H., Kalbitz, K., Scott Demyan, M., Kučerík, J., Siewert, C., 2020. Linking thermogravimetric data with soil organic carbon fractions. Geoderma 362. https://doi.org/10.1016/j.geoderma.2019.114124

Tomczyk, A., Sokołowska, Z., Boguta, P., 2020. Biochar physicochemical properties: pyrolysis temperature and feedstock kind effects. Rev Environ Sci Biotechnol 19, 191–215. https://doi.org/10.1007/s11157-020-09523-3

Tsadilas, C.D., Mitsios, I.K., Golia, E., 2005. Influence of biosolids application on some soil physical properties. Commun Soil Sci Plant Anal 36, 709–716. https://doi.org/10.1081/CSS-200043350

Turner, B., 2021. Soil as an archetype of complexity: a systems approach to improve insights, learning, and management of coupled biogeochemical processes and environmental externalities. Soil Syst 5, 39. https://doi.org/10.3390/soilsystems5030039

United Nations Framework Convention on Climate Change (UNFCCC), 2021. “4 per 1000” initiative (4p1000) [WWW Document]. URL https://www.4p1000.org/ (accessed 4.19.21).

United Nations (UN). Department of Economic and Social Affairs. Population Division, 2019. World Population Prospects 2019 [WWW Document]. URL https://population.un.org/wpp/ (accessed 11.27.19).

Verheijen, F., Jeffery, S., Bastos, A.C., van der Velde, M., Diafas, I., 2010. Biochar application to soils: a critical scientific review of effects on soil properties, processes and functions. Publications Office, Luxembourg. https://doi.org/10.2788/472 von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. Soil Biol Biochem 39, 2183–2207. https://doi.org/10.1016/j.soilbio.2007.03.007

Wander, M., 2004. Soil Organic Matter Fractions and Their Relevance to Soil Function, in: Magdoff, F., Weil, R.R. (Eds.), Soil Organic Matter in Sustainable Agriculture. CRC Press, Boca Raton, p. 412. https://doi.org/10.1201/9780203496374.ch3

Wang, J., Xiong, Z., Kuzyakov, Y., 2016. Biochar stability in soil: meta-analysis of decomposition and priming effects. GCB Bioenergy 8, 512–523. https://doi.org/10.1111/gcbb.12266

Wang, L., Deng, J., Yang, X., Hou, R., Hou, D., 2023. Role of biochar toward carbon neutrality. Carbon Research 2, 2. https://doi.org/10.1007/s44246-023-00035-7

Wei, S., Zhu, M., Fan, X., Song, J., Peng, P., Li, K., Jia, W., Song, H., 2019. Influence of pyrolysis temperature and feedstock on carbon fractions of biochar produced from pyrolysis of rice straw, pine wood, pig manure and sewage sludge. Chemosphere 218, 624–631. https://doi.org/10.1016/j.chemosphere.2018.11.177

Yang, X., Wang, D., Lan, Y., Meng, J., Jiang, L., Sun, Q., Cao, D., Sun, Y., Chen, W., 2018. Labile organic carbon fractions and carbon pool management index in a 3-year field study with biochar amendment. J Soils Sediments 18, 1569–1578. https://doi.org/10.1007/s11368-017-1874-2

You, J., Sun, L., Liu, X., Hu, X., Xu, Q., 2019. Effects of sewage sludge biochar on soil characteristics and crop yield in loamy sand soil. Pol J Environ Stud 28, 2973–2980. https://doi.org/10.15244/pjoes/93294

Zhang, J., Lü, F., Zhang, H., Shao, L., Chen, D., He, P., 2015. Multiscale visualization of the structural and characteristic changes of sewage sludge biochar oriented towards potential agronomic and environmental implication. Sci Rep 5, 9406. https://doi.org/10.1038/srep09406

Zhang, Q., Hu, J., Lee, D.J., Chang, Y., Lee, Y.J., 2017. Sludge treatment: Current research trends. Bioresour Technol 243, 1159–1172. https://doi.org/10.1016/j.biortech.2017.07.070

Zhao, C., Zhang, Y., Liu, X., Ma, X., Meng, Y., Li, X., Quan, X., Shan, J., Zhao, W., Wang, H., 2020. Comparing the Effects of Biochar and Straw Amendment on Soil Carbon Pools and Bacterial Community Structure in Degraded Soil. J Soil Sci Plant Nutr 20, 751–760. https://doi.org/10.1007/s42729-019-00162-4

CHAPTER I

BIOCHAR INCREASES SOIL CARBON POOLS: EVIDENCE FROM A GLOBAL META-ANALYSIS

5. BIOCHAR INCREASES SOIL CARBON POOLS: EVIDENCE FROM A GLOBAL META-ANALYSIS[2]

Abstract

Biochar is a carbon-rich material that increases soil C sequestration and mitigates climate change. However, due to the variability of experimental conditions, types of biochar and soil, the influence of biochar on the accumulation of different soil carbon fractions remains unclear. Therefore, a meta-analysis was performed that included 586 paired comparisons obtained from 169 studies conducted in various countries around the globe. The data set average showed significant relative increases of 64.3, 84.3, 20.1, 22.9 and 42.1% for total C (TC), organic C, microbial biomass C, labile C and fulvic acid, respectively. The dissolved organic C, humic acid and humin fractions showed no significant variations. The relative increase in TC was favored by increasing biochar rates applied to fine-textured soils with low C content in temperate climate regions seen through short-term experiments conducted under controlled conditions. This behavior was different for each soil C fraction. Therefore, variations between experimental conditions, types of biochar and soil show that it is necessary to consider multiple factors when choosing the conditions of biochar use to maximize C sequestration in the soil and/or the increase of labile C fractions in the soil.

Keywords: pyrolysis, carbon sequestration, organic carbon, microbial biomass carbon, labile carbon, humic substances

Resumo

O biochar é um material rico em carbono (C) que aumenta o sequestro de C no solo e mitiga a mudança climática. Entretanto, devido à variabilidade de condições experimentais, tipos de biochar e de solo, a influência do biochar no acúmulo de diferentes frações de C no solo permanece incerta. Para tal, foi realizada uma meta-análise que incluiu 586 comparações pareadas obtidas a partir de 169 estudos conduzidos em vários países ao redor do mundo. A média do conjunto de dados mostrou aumentos relativos significativos de 64,3, 84,3, 20,1, 22,9 e 42,1% para o C total (CT), C orgânico, C da biomassa microbiana, C lábil, C e ácido fúlvico, respectivamente. As frações de C orgânico dissolvido, ácido húmico e humina não mostraram variações significativas. O aumento relativo no CT foi favorecido por doses crescentes de biochar aplicadas a solos de textura fina com baixo teor de C em regiões de clima temperado, por meio de experimentos de curto prazo conduzidos sob condições controladas. Este comportamento foi diferente para cada fração de C do solo. Portanto, variações entre condições experimentais, tipos de biochar e solo mostram que é necessário considerar múltiplos fatores ao escolher as condições de uso do biochar para maximizar o sequestro de C no solo e/ou o aumento de frações lábeis de C no solo.

Palavras-chave: pirólise, sequestro de carbono, carbono orgânico, carbono da biomassa microbiana, carbono lábil, substâncias húmicas

[2] Published at Journal of Environmental Management 305 (2022) 114403 [doi:10.1016/j.jenvman.2021.114403]

5.1. Introduction

Soil is the second-largest carbon (C) reservoir on the planet, accounting for approximately 2500 Gt C (Lal, 2010). Therefore, small changes in soil C stocks can considerably impact the concentration of this element in the atmosphere and influence ongoing climate change (Smith, 2012). In this sense, the “4 per 1000” initiative (4p1000) launched in 2015 during COP 21 (UNFCCC, 2021) reported that the annual increase of 0.4% in soil C stock can offset the annual increase in greenhouse gas emissions and contribute to food security. Furthermore, there is a consensus that numerous technologies and management practices can be applied in agroecosystems to achieve this goal (Paustian et al., 2016, 2019; Stockmann et al., 2013; Zomer et al., 2017).

Pyrolysis is an ancient technique of thermochemical conversion of biomass under limited oxygenation (Laird et al., 2009). Using this process a stabilized solid product rich in C is obtained, which, when applied to soil, has the potential to offer several benefits to agriculture and the environment (Wang et al., 2020b; Ghosh and Maiti, 2020), especially for the sequestration of C. Furthermore, because of its high concentration of stable C (60-80% C), biochar is considered the leading soil amendment to rapidly increase soil C sequestration and thereby help mitigate global climate change (Ventura et al., 2019). The adoption of biochar technology can sequester 1.8 Gt of CO_2 yr^{-1} (Woolf et al., 2010). The quantity and quality of C in the biochar are influenced by the raw material and the pyrolysis conditions, among other factors (Tomczyk et al., 2020; Wei et al., 2019). When biochar from agro-industrial and urban residues is applied to the soil, it becomes more stable/recalcitrant because the pyrogenic C compounds from biochar are retained in the soil for a much longer period than would occur with the direct disposal of these non-pyrolyzed residues in the soil or landfills (Kuzyakov et al., 2014; Wang et al., 2016). Biochar, prepared from *Calotropis procera*, applied at 60 t ha^{-1} increased the C-stock of an 8-year old reclaimed mine spoil by 45% and increased the recalcitrant C by 67% (Ghosh and Maiti, 2021). Similarly, the organic carbon content was increased 2.9 times when Lantana biochar was used as an amendment for the mine spoil reclamation (Ghosh et al., 2020). The pyrogenic C of biochar influences the quality of soil organic matter (SOM) and can make it less subject to losses resulting from inadequate management practices (Cooper et al., 2020).

SOM pools present different turnover dynamics. For example, labile SOM fractions have the most rapid turnover rates, whereas the inert and the humic SOM fractions are more resistant to microbial decomposition (Yang et al., 2009). Thus, biochar application to the soil has direct and indirect effects on different SOM pools, altering several biochar functions such as C sequestration, nutrient supply and heavy metal retention. Biochars pyrolyzed at low temperatures are rich in nutrients and hold a large quantity of volatile compounds, which can increase the labile fractions of SOM and alter the soil microbiota and nutrient cycling (Figueiredo et al., 2019; Liu et al., 2016). On the other hand, biochars pyrolyzed at high temperatures may favor stable SOM fraction (e.g., black carbon) (Amoakwah et al., 2020; Figueiredo et al., 2019; Wang et al., 2016). Meta-analyses have shown that biochars can increase crop development and productivity (Jeffery et al., 2017; Liu et al., 2013), and greater input of C into the soil via crop residues can be obtained as a result of higher yields.

Fractionation of the SOM is essential to understand the dynamics of C in the soil due to its heterogeneous and complex chemical composition (Chan et al., 2002). The SOM can be divided into several fractions that are generally grouped according to their composition, degree of chemical stability and location in the soil structure (Duddigan et al., 2019; von Lützow et al., 2007). Therefore, the choice of SOM fractionation method is crucial when biochars were applied, as these materials have predominantly recalcitrant C compounds (Cooper et al., 2020).

In the last two decades there has been a growing number of studies showing the effect of biochar application on soil C fractions. However, due to multiple experimental conditions, types of biochar and soils, the results vary considerably. It is necessary to elucidate which are the determining factors in the dynamics of C fractions in the soil. Meta-analysis is a valuable tool to synthesize these results. Quantifying changes in soil C fractions due to the application of biochar can contribute to guiding the production and application of biochar to achieve pre-established goals. Additionally, such information can encourage disseminating this technology for agronomic and environmental purposes. Some previous meta-analyses evaluated the changes promoted by biochar amendment on some SOM fractions (Bai et al., 2019; Liu et al., 2016; Zhou et al., 2017). However, these studies left some knowledge gaps that are worth addressing. In these studies, the C determination method was not clearly stated, and it is not possible to distinguish between total C and organic C, for example. Furthermore, at most, two C pools were evaluated, which limits the use of these results in modelling soil C quality and sequestration. This meta-analysis study will clarify the effect of biochar from a wide variety of feedstock and pyrolysis conditions on different pools of soil C across the globe. Furthermore, it will be possible to point out the biochar potential to mitigate greenhouse gas emissions. The objective of this study was to quantify the potential of biochar to alter soil C fractions under a wide variety of biochar, soil and experimental conditions through a global meta-analysis.

5.2. Methods

5.2.1. Data source

The Web of Science database (main collection) was searched for articles published prior to February 10, 2021 in peer-reviewed journals. The following terms were searched for in the title, abstract and keyword fields: biochar AND soil AND (carbon OR C OR "organic matter") NEAR/4 (sequest* OR stor* OR stoc* OR accumul*); ("sewage sludge" OR biosolid*) AND biochar AND (carbon OR "organic matter") AND soil. In the end, 169 articles were selected for data extraction and inclusion in the meta-analysis because they met the following criteria: i) randomized experimental design; ii) explicit number of repetitions; iii) presence of treatments with and without biochar (control) under the same experimental conditions; and iv) evaluation of at least one soil C fraction whose method of determination was clearly presented.

5.2.2. Data collection

The results collected from the articles included the mean, standard deviation (SD) and the number of repetitions (r) for the following soil C fractions: total C (TC), determined in an elemental analyzer or by combustion; organic C (OC), estimated by wet oxidation with $K_2Cr_2O_7$ (e.g., Walkley-Black and Tyurin methods); labile C (LC), determined by oxidation with $KMnO_4$; dissolved organic C (DOC), extracted in water, KCl or K_2SO_4; microbial biomass C (MBC), fumigated or irradiated samples and extracted with K_2SO_4 or by the substrate-induced respiration method; and humic substances (fulvic acid - FA, humic acid - HA - and humin), extracted according to their solubility in acidic and alkaline media. The Plot Digitizer 2.6.9 software (https://sourceforge.net/projects/plotdigitizer) was used for data extraction when the results were presented only in figures. In total, 586 paired comparisons (with and without biochar) were obtained for the different SOM fractions evaluated.

When available, the standard error (SE) was converted to SD using the equation $SD = SE\sqrt{r}$. If the SD or SE was not informed, the SD was calculated from the coefficient of variation of known data (Bai et al., 2019). When multiple soil layers were evaluated, the 0-20 cm layer was preferred, and the weighted average was calculated if it was subdivided. If multiple assessments were performed over time, only the last one was included.

In addition to the C fractions, the following information was extracted from the article: i) location (latitude and longitude), ii) soil (pH, texture and total C content), iii) biochar (raw material, pyrolysis temperature, total C content and applied rate), and iv) experimental conditions (type of experiment and duration). The climate zone of the study site was defined based on the Köppen-Geiger climate classification (Beck et al., 2018). The biochar rate applied to the soil was converted from t ha^{-1} to percentage using density and depth of the soil layer. When soil density was not indicated, it was considered to be 1 g cm^{-3}. The average was used in cases where the pyrolysis temperature was informed as a range of values. To standardize the information, the soil texture was defined based on the sand, silt and clay fractions using the USDA soil classification system.

5.2.3. Data categorization

According to the raw material, the biochars were grouped into: animal manure (from poultry, cattle, pigs and chicken litter), crop residues (lignocellulosic plant waste, such as straw, bark, stalks, cobs, bagasse), greenwaste (grasses, leaves, fresh plants), sewage sludge, wood (wood and bamboo) and mixtures (including other raw materials and combinations of multiple raw materials). Regarding the pyrolysis temperature, the biochars were grouped into: low (≤350 °C), medium (350–600 °C) and high (≥600 °C). As for the biochar C content, these were categorized into three groups: ≤35%, 35–65% and ≥65%. The biochar rates applied to the soil were subdivided into: low (≤1%), medium (>1 and ≤2%), high (>2 and ≤5%) and very high (>5%). Three groups of soil texture were defined: coarse (loamy sand, sand and sandy loam), medium (loam, sandy clay loam, silt and silt loam) and fine (clay, clay loam, sandy clay, silty clay and silty clay loam). Soils were categorized into acidic (pH < 6.5), neutral (6.5–7.5) and alkaline (pH > 7.5). The C content in the soil was classified as: low (≤1%), medium (>1 and ≤2%) and high (>2%). Regarding the duration of the experiment, these were grouped into: ≤3 months, >3 months and ≤1 year, >1 and ≤ 2 years, and >2 years.

5.2.4. Meta-analysis

The response ratio (RR) was calculated as the log-transformed ratio of means for each paired comparison (Hedges et al., 1999), according to equation (1).

$$\mathrm{RR} = \ln\left(\frac{\mathrm{X_T}}{\mathrm{X_C}}\right) \tag{1}$$

where X_T and X_C are means of the biochar treatment and the control, respectively. Values of RR=0, >0 and <0 indicate that the biochar application did not alter, increase or decrease the soil C content, respectively.

From the RRs, a random-effects meta-analysis balanced by the inverse of variance was conducted (Viechtbauer, 2010). Thus, the mean effect size (RR_+) was obtained for each C fraction and the different subgroups. Outliers were identified for each C fraction through the leave-one-out method using the DFFITS indicator. This indicator iteratively quantifies in units of standard deviation how much the model estimate is changed after excluding the i-th paired comparison. Therefore, 10, 4, 4, 1, 2 and 1 paired comparisons were removed for the TC, OC, DOC, MBC, LC and humin fractions, respectively. The heterogeneity (τ^2) of the model was estimated by the method of Sidik and Jonkman (2005).

The 95% confidence interval (CI) was calculated to determine the statistical significance of RR_+. Thus, the effect of applying biochar to the soil was significant when the CI did not include zero. The CI was corrected according to the method of Knapp and Hartung (2003). Additionally, to facilitate understanding the RR_+ values were converted into percentage change (Pc) using equation (2).

$$P_c = (e^{RR_+} - 1) \times 100 \tag{2}$$

For the fractions in which a robust dataset (TC, OC, MBC and DOC) was obtained, subgroup analysis was performed. Within a group (for example, raw material), the subgroups were subjected to multiple comparisons using Tukey contrasts, and the p-value correction was performed according to Holm (1979).

The relative influence of predictor variables on the percent change of C fractions was determined through “boosted regression trees” using machine learning (Greenwell et al., 2020). To find the best hyperparameters, 500 combinations were tested: learning rates of 0.05, 0.1, 0.2, 0.3 and 0.4; tree complexities of 1, 2, 3, 5 and 7; minimum observations in terminal node tree of 2, 3, 5, 7, and 10; and bag fractions of 0.5, 0.65, 0.8 and 0.9. The combination of hyperparameters with the smallest root mean square error for each C fraction is shown in Table 1. Normal distribution was used to adjust the model. All statistical analyses were performed using the R software version 4.0.2 (R Core Team, 2020).

Table 1. Optimal hyperparameters used in the construction of decision trees for C fractions

Hyperparameter	TC	OC	MBC
Learning rate	0.4	0.4	0.3
Tree complexity	3	3	2
Minimum observations in node	5	2	2
Bag fraction	0.8	0.8	0.9

5.3. Results and Discussion

5.3.1. Descriptive analysis of the results

The 169 articles included in this global meta-analysis contain results from 184 distinct study sites. Approximately 43% of the studies were carried out in China, 10% in the European Union, 9% in the United States, 9% in Australia and 29% in other countries around the world (Figure 1). The studies are concentrated in regions with a subtropical or temperate climate, with little participation of intertropical regions. Only 11% of the results were obtained in tropical climate regions, specifically in Latin America and Africa, where there are high rates of soil C emissions from agricultural activities (Lal, 2006; Palmer et al., 2019).

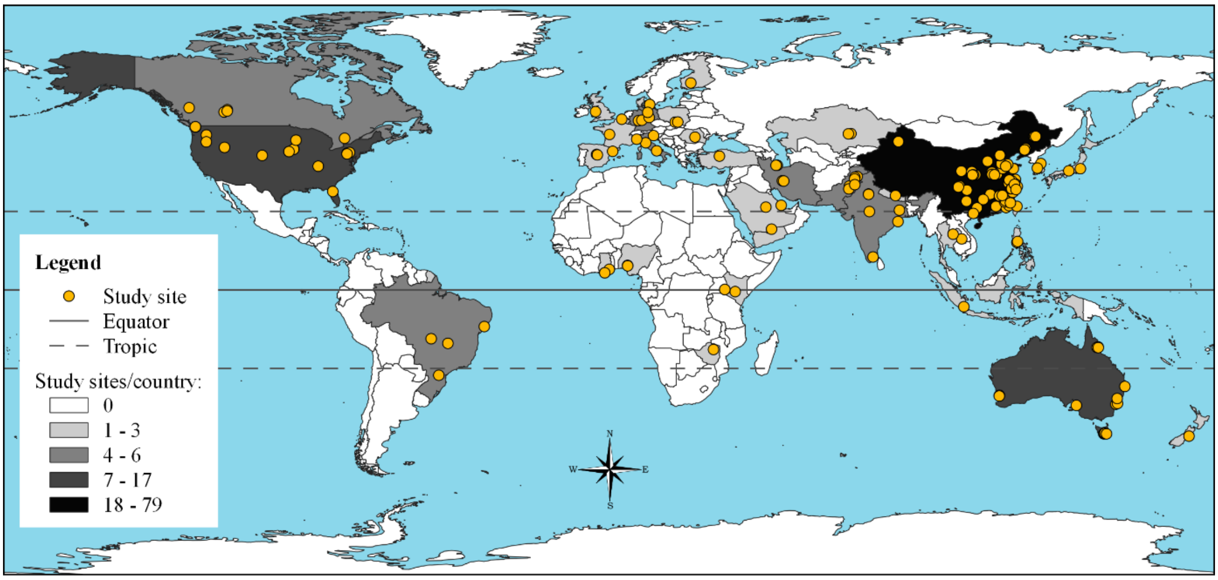
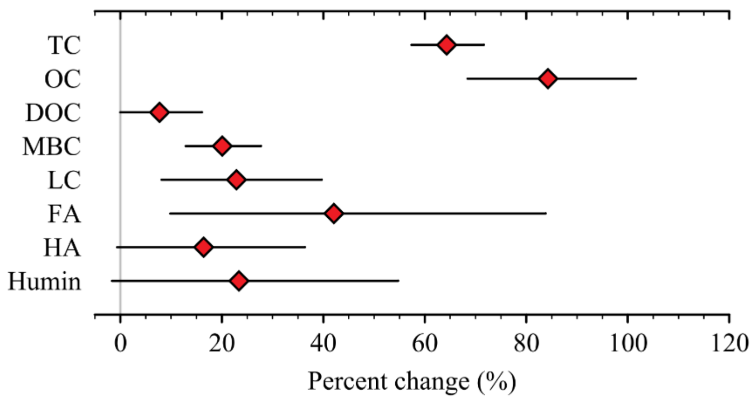


Figure 1. Location of the studies included in the meta-analysis. Each study site can represent more than one paired comparison

In the selected studies, the biochar pyrolysis temperature ranged from 200 to 1200 °C, rates of up to 50% biochar were applied in soils with C content ranging from 0.04 to 17.19% and the maximum study duration was 10 years. In general, the set of paired comparisons (586) satisfactorily covered the different types of soils, biochars and experimental conditions. Despite this, there is a predominance of results for biochars from crop residues (51%) pyrolyzed between 350 and 600 °C (67%) and applied at rates lower than 1% (40%) to acidic soils (59%), with total C content less than 1% (41%), in trials lasting between 3 months and one year (37%), conducted in temperate regions (45%).

5.3.2. General effect of biochar on the soil C fractions

The application of biochar significantly increased the contents of TC (64.3%), OC (84.3%), MBC (20.1%), LC (22.9%) and FA (42.1%) of the soil for the set of experimental conditions, types of biochar and soil properties (Figure 2). Other meta-analyses, evaluating up to 56 articles, found overall increases of 39% (Bai et al., 2019) and 52% (Liu et al., 2016) in organic C for soils with biochar. In these studies, it was not possible to identify which analytical methods were used for the determination of organic C in the soil. Additionally, different names for organic C were used in these previous studies, which makes comparisons between results difficult, especially in soils with biochar. Despite the difficulty compared with other reviews, the C increments promoted by biochar in the present study, both for TC and OC, were greater than those observed in previous studies (Bai et al., 2019; Liu et al., 2016). This result demonstrates an improvement in the effect size previously verified since 154 studies that evaluated TC or OC were included in the present study, approximately 175% higher than the 56 studies previously evaluated.



	Pc	**[-CI$_{95}$; +CI$_{95}$]**	**(N/n)**
TC	64.3***	[57.3; 71.6]	(368/103)
OC	84.3***	[68.4; 101.6]	(166/54)
DOC	7.7^{ns}	[0.0; 16.1]	(131/44)
MBC	20.1***	[12.9; 27.7]	(151/53)
LC	22.9**	[8.1; 39.7]	(34/16)
FA	42.1*	[9.8; 83.8]	(14/6)
HA	16.4^{ns}	[-0.6; 36.4]	(16/7)
Humin	23.4^{ns}	[-1.7; 54.7]	(10/5)

Figure 2. Percent change (Pc) of total C (TC), organic C (OC), dissolved organic C (DOC), microbial biomass C (MBC), labile C (LC), fulvic acid (FA), humic acid (HA) and humin in the soil under biochar application. CI95: 95% confidence interval; N: number of paired

comparisons; n: number of studies. Pc significant at 5% (*), 1% (**) and 0.1% (***) or not significant (ns)

The C fractions most responsive to biochar were OC and TC. The increase in TC is due to the contribution of C stabilized by the thermochemical conversion of raw materials via pyrolysis (Wei et al., 2019). In addition to increasing C, biochar also contributes to increasing the soil nutrient content (Ding et al., 2016; Hossain et al., 2020). Besides the direct increase in pyrogenic C, by increasing crop productivity biochar indirectly contributes to the increase of SOM fractions via cycling of crop residues or via rhizodeposition, and stimulates the development of the living SOM fraction, normally expressed by MBC (Ali et al., 2020; Wu et al., 2021). The abundance of biochar pores serves as a shelter for soil microorganisms, which can favor the growth of microbial biomass (Quilliam et al., 2013). A recent meta-analysis showed that the portion of recalcitrant C in biochar represents 97% of TC and that its average residence time in the soil is 556 years (Wang et al., 2016). These findings support the potential of biochar to increase soil organic C by 6x more than other alternatives for increasing soil C, such as the use of cover crops and the adoption of conservation tillage systems (Bai et al., 2019). Given these results, the role of biochar in accumulating C in the soil is reaffirmed, and thus its potential to mitigate climate change.

In addition to the potential to accumulate TC in soil, the present study confirms that biochar also increases labile forms of soil C, notably OC, MBC and LC (Figure 2). The C present in these labile fractions of SOM responds quickly to environmental and management changes. Therefore, it plays a fundamental role in cycling this element (Zhang et al., 2020). However, only 3% of biochar TC is present in labile forms, with an average decomposition time of 108 days (Wang et al., 2016). Thus, the decomposition of labile forms should not pose a problem in these soils compared to the amount of recalcitrant C that may be added via biochar.

On the contrary, when applied to cultivated soils biochar promotes an increase in labile C forms that have the potential to improve the chemical, physical and biological properties of the soil, even promoting the release of nutrients (Zhang et al., 2020). In addition, methods that employ $KMnO_4$ only account for labile C forms used by heterotrophic soil microorganisms as a source of energy (Stott, 2019). Therefore, increases in LC are positively correlated with increases in MBC (Nunes et al., 2020), except under limiting conditions to soil microorganisms. The stimulation of LC for soil microbiota growth can contribute to the humification of crop residues, and therefore increase in the soil C stock. Biochar is also capable of favoring the assimilation of C derived from crop residues through changes in composition of the soil microbial community (Liao et al., 2019). The leading site of microbial biomass increase is at the interface between the soil and biochar particles, called the "charsphere" (Luo et al., 2013). At this interface, the efficiency of C use is greater than in soils without biochar or with the application of residues, which also favors the accumulation of C (Liu et al., 2020). Finally, the microbiota increases soil aggregation (Cooper et al., 2020) and can contribute to preserving native organic matter and even reducing erosion losses.

Although positive, changes in the DOC, HA and humin fractions were not significant (7.7, 16.4 and 23.4%, respectively) (Figure 2). The group of humic substances includes compounds that are relatively stable in soil. Thus, it was expected that the application of biochar would increase the content of C in these pools, especially humin, a fraction of humic substances considered more stable (Hayes et al., 2017). However, the increase was significant only for FA, 42.1% (Figure 2), which is associated with the higher content of nutrients in the soil and consequent stimulus of soil microbiota development, which is essential for humification of plant remains (Leal et al., 2019). Among the other humic substance fractions, FA has greater functionalization (Klučáková, 2018) and, therefore, can contribute to nutrient retention and contaminant immobilization (Gerke, 2018), in addition to its hormonal effect on plant growth (Shah et al., 2018). The reasons for no change in the DOC will be discussed in section 5.3.6.

Because few studies evaluated humic substances and soil LC, subgroup analyses were not performed for these fractions. These analyses were expected to confirm the influence of different raw materials and pyrolysis temperatures on the C content in these fractions. However, subgroup analyses that included few studies may indicate unrealistic relationships (Higgins and Thompson, 2004). Therefore, further studies are needed to confirm the meta-analysis results for these soil C fractions.

5.3.3. Driving factors of the total C content (TC) in soils with biochar

Overall, the factor that most affected soil TC was the biochar rate (Figure 4a). The higher the biochar rate, the higher the soil TC, and on average, the application of biochar to the soil increased the TC by 64.3% (Figure 3). All analyzed factors resulted in significant positive percent changes to TC. As discussed previously, this stems from applying a recalcitrant C source.

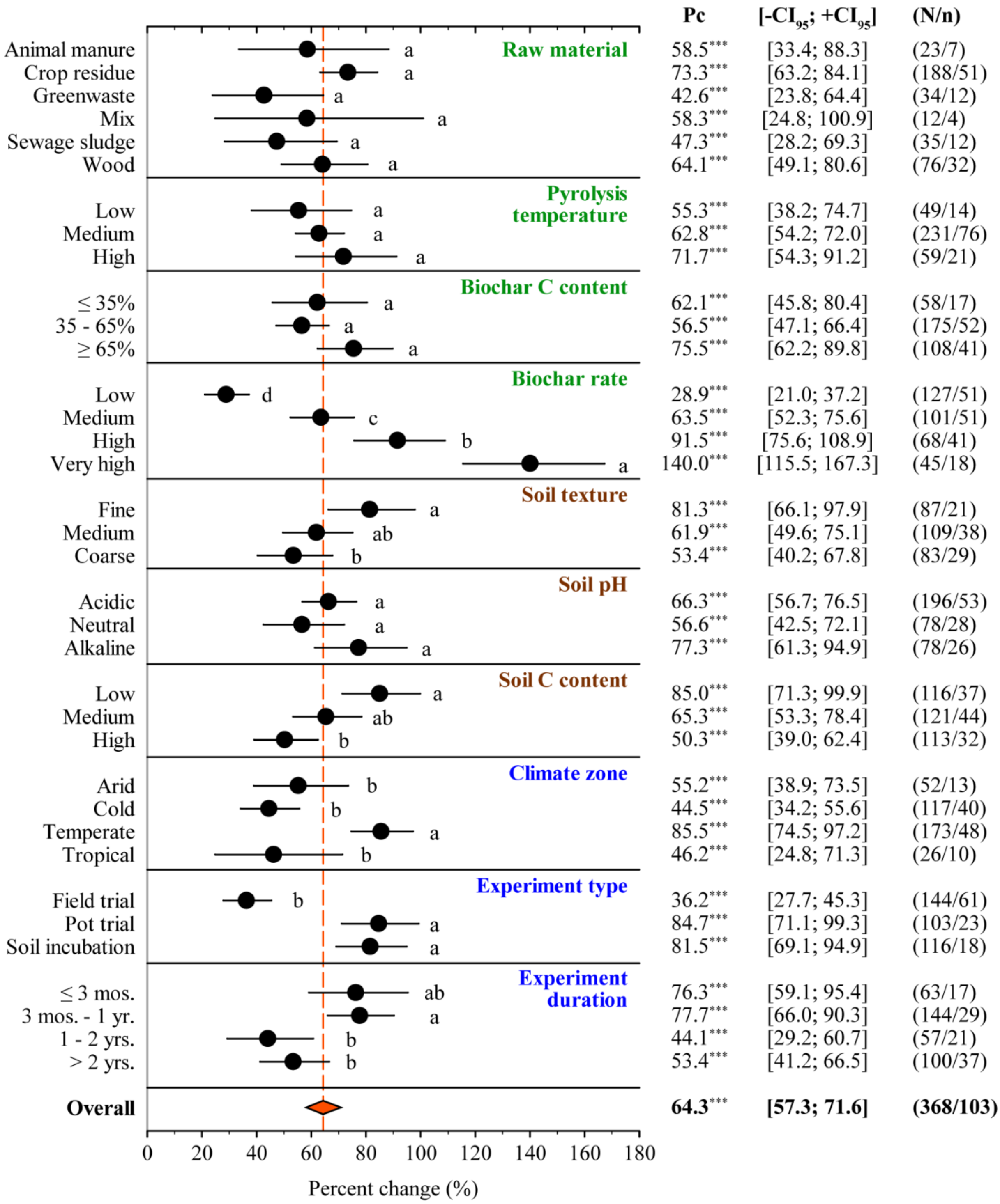


Figure 3. Percent change (Pc) of the total C content (TC) in the soil as a function of the characteristics of the biochar, the soil and the experimental conditions. CI_{95}: 95% confidence interval; N: number of paired comparisons; n: number of studies. Pc significant at 5% (*), 1% (**) and 0.1% (***) or not significant (ns). For the same factor, different letters indicate that the subgroups differed significantly by Tukey contrasts (p<0.05)

Among the factors directly related to characteristics of the biochars evaluated in this study, only the rate applied to the soil resulted in different responses between the categories. The higher the biochar rate applied to the soil, the greater the increase in TC, ranging from

28.9% for rates ≤1%–140.0% for rates >5%. Moreover, the results showed no differences between the raw materials, nor did the pyrolysis temperature or the C content of the biochar on the percent change in soil TC. In a previous meta-analysis, the response of soil C to the addition of biochar was reported to be more dependent on biochar properties and land use properties than on the application rate (Liu et al., 2016). The influence of raw material and the pyrolysis temperature on the C content of biochar is widely discussed in the literature (Ippolito et al., 2020; Tomczyk et al., 2020; Wei et al., 2019). However, in general, biochar has a much higher TC content than soil (on average 56.2 and 1.9%, respectively). Therefore, regardless of temperature and raw material, due to the mass balance of C in the soil after the addition of biochar, it is evident that the amount of this material applied exerts a greater influence on the TC content of the soil than the biochar C content. This perception was confirmed by the influence analysis (Figure 4a). Thus, soils with less than 1% TC before biochar application showed higher percent increases than soils with high initial content (>2%).

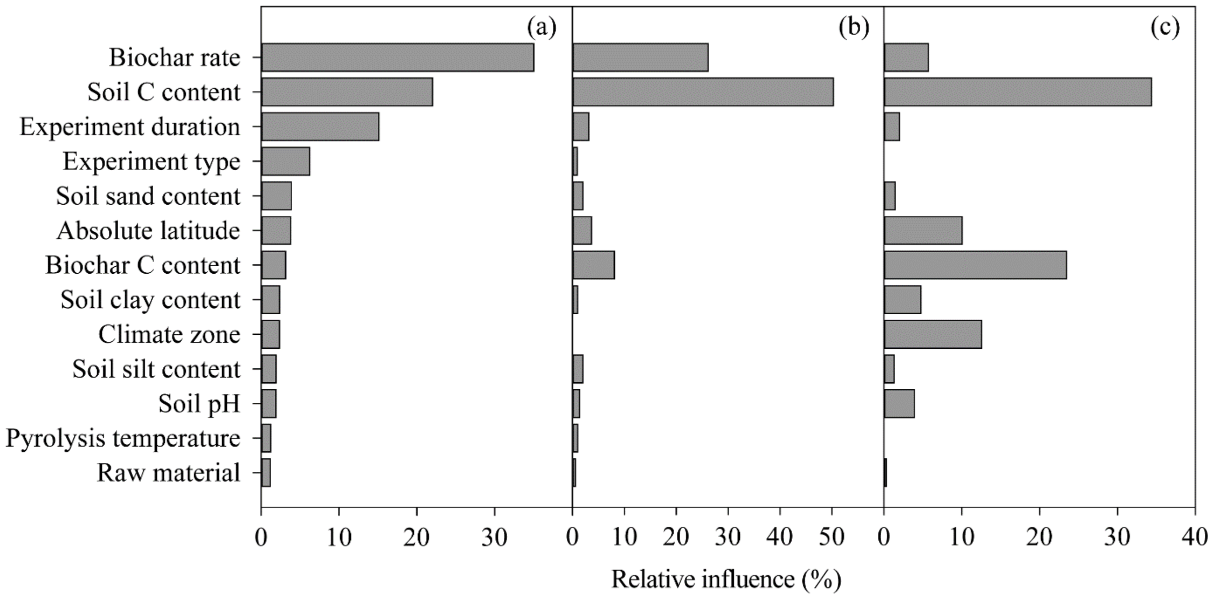


Figure 4. Relative influence of predictor variables on the percent changes in TC (a), OC (b) and MBC (c) contents in soils with biochar application

Texture affected the biochar’s ability to increase TC in relation to other soil properties. In fine-textured soils the increase was greater (81.3%) than in coarse-textured soils (53.4%). In addition to enabling strong chemical interactions, clay minerals provide physical protection to SOM in different ways, either by increasing the stability of aggregates (Omondi et al., 2016; Zong et al., 2018) or by adsorption and blocking of enzymes in the C cycle (Zhang et al., 2019). Furthermore, in sandy soils biochar C is more subject to losses from runoff and leaching (Yang et al., 2019) due to the high susceptibility to erosion and low ion exchange capacity of these soils, respectively. The soil pH did not interfere with the effectiveness of the biochar to increase the TC contents. Despite this, there is a tendency for alkaline soils (pH > 7.5) amended with biochar to present the greatest increases in C, as verified by Liu et al. (2016).

Regarding the experimental conditions, responses of the soil TC content to biochar application were different ($p < 0.05$) between the climatic zones, type and duration of the experiments. In temperate climate regions, the variation in TC was greater (85.5%) than in the others. Furthermore, experiments conducted in pots (84.7%) or soil incubations (81.5%) showed greater increases compared to field experiments (36.2%). First, it is important to consider the influence of other factors on these results. In studies carried out in temperate climate regions, an average biochar rate applied was 3.9%, 60% higher than that applied in other climate zones (1.6–2.3%). Higher biochar rates were also applied in soil incubations (on average 3.0%) and pot trials (4.1%) than in field experiments (1.7%). In general, lower biochar rates are applied in field experiments due to economic and operational issues. Therefore, as discussed above, due to the considerable influence of biochar rate on soil TC (Fig. 4a), it is likely that in temperate climates and controlled experiments the actual increase in TC is smaller than that quantified in this meta-analysis for such categories. Furthermore, biochar particles are subject to a much greater number of disturbances in the field than in controlled experiments (Leng et al., 2019; Yi et al., 2020). Agricultural practices of soil preparation (eg, ploughing and harrowing) (Naisse et al., 2015), climatic seasonality (Yi et al., 2020), interaction with soil meso- and macrofauna (Domene, 2016), intense nutrient cycling and susceptibility to leaching (Liu et al., 2019a; Yang et al., 2019) and erosion (Abney and Berhe, 2018) contribute to the lower levels of soil TC under field conditions.

Finally, experiments lasting longer than one year showed smaller increases in soil TC (44.1–53.4%). However recalcitrant it may be, there is inevitably a loss of C from the biochar over time, which negatively contributes to the remaining TC content in the soil1 (Bai et al., 2019; Wang et al., 2016). Over time, the probability of losing C in the ways mentioned above increases.

5.3.4. Driving factors of the organic C content (OC) in soils with biochar

The effects of different categories on the OC content of the soil after biochar application are shown in Figure 5. OC was generally more sensitive to the different factors analyzed than TC. Animal manure and sewage sludge biochars were responsible for the largest increases in OC, 289 and 242%, respectively. Other raw materials of plant origin had lower responses, ranging from 42 to 72%. Biochars produced from manure and sludge have a high concentration of volatile compounds (El-Naggar et al., 2019; Xing et al., 2021) which are more easily oxidized in soil than pyrogenic C. Because they are also rich in nutrients, these biochars contribute to improving soil fertility (Frišták et al., 2018; Xing et al., 2021), thus increasing plant development. The greater production of crop residues and roots (Faria et al., 2018; Gonzaga et al., 2020) adds organic matter to the soil and increases its OC content (Figueiredo et al., 2019; Luo et al., 2018). Among the few studies that determined OC after application of sewage sludge or animal manure biochars, all were conducted under controlled conditions (i.e., pot experiment or soil incubation). Therefore, the effect reported in this meta-analysis for biochars produced from these raw materials may be overestimated. The influence of experiment type on soil OC will be discussed below.

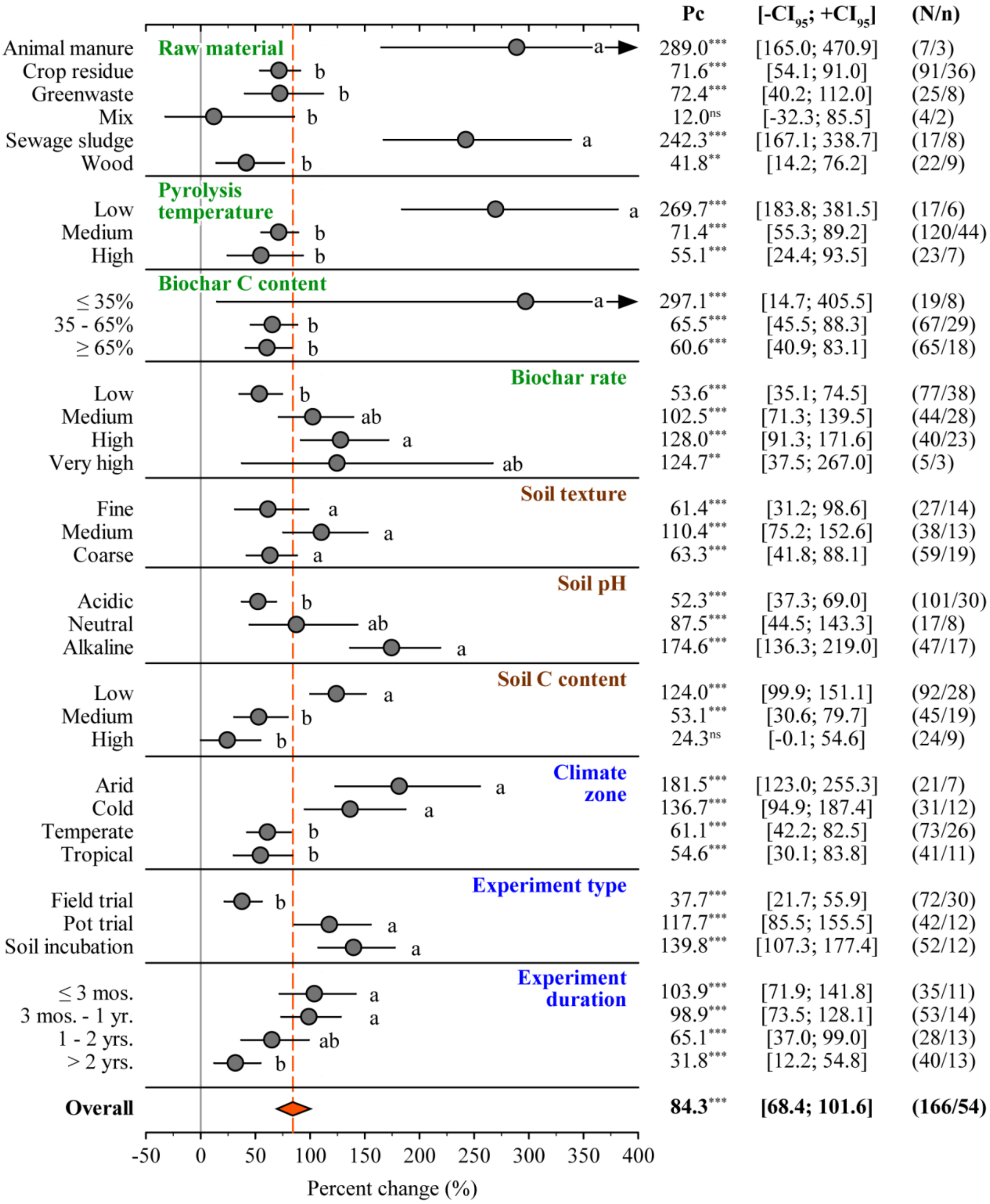


Figure 5. Percent change (Pc) of the organic C content (OC) in the soil as a function of biochar, soil and experimental conditions. CI_{95}: 95% confidence interval; N: number of paired comparisons; n: number of studies. Pc significant at 5% (*), 1% (**) and 0.1% (***) or not significant (ns). For the same factor, different letters indicate that the subgroups differed significantly by Tukey contrasts (p<0.05)

The application of biochars pyrolyzed at temperatures of up to 350 °C or with low C content (≤35%) also resulted in the most significant increases in soil OC. Although the increase in pyrolysis temperature concentrates C in the biochar (Ippolito et al., 2020; Tomczyk et al., 2020), there is also a conversion from labile forms of C to stable forms (Wei et al., 2019). Thus, as confirmed in this study, biochars pyrolyzed at low temperatures have greater potential to increase the OC content of the soil. Despite the significant difference of the group of biochars with up to 35% C compared to the others, due to the variability of the results, further studies using biochars with this characteristic should be performed to improve the accuracy of the estimate obtained here.

Like with the TC, the OC was also influenced by the biochar rate applied to the soil (Figure 4b). Higher rates resulted in a greater increment ($p < 0.05$) than lower rates. The absence of significant difference between the very high rate and the others can be explained by the small number of studies (3) that applied more than 5% of biochar to the soil. All three studies were conducted in pots, because applying very high rates under field conditions is still unfeasible. For example, considering a layer of 0–20 cm, it would be necessary to apply rates of 100 t ha^{-1} of biochar or greater for a concentration of 5%.

Regarding the soil properties, the largest relative increases in OC resulting from biochar application occurred in alkaline soils (174.6%) or soils with low initial C content (124%). Thus, with the application of biochar in acidic soils there are fewer limitations for the growth of the microbiota (Ali et al., 2020), which favors OC mineralization (Ding et al., 2018). On the other hand, biochar application in alkaline soil can further increase the pH and thus limit the growth of the microbiota, preserving soil OC for longer (Liu et al., 2016). However, alkaline soils had low initial C content (<1%) in 77% of the paired comparisons. This demonstrates that the influence of alkaline pH on soil OC content may be overestimated since soil C content exerted a greater influence on the results than soil pH (Figure 4b).

Considering the C content, soils with a large amount of OC are close to saturation, and therefore, they tend to present lower responses to the application of organic residues (Paustian et al., 2019). Similar behavior was verified in soils with manure application, and a greater relative increase in OC (%) in soils with low C content did not imply a greater absolute increase (Mg ha^{-1}) (Gross and Glaser, 2021). Soils with a high C concentration (e.g., from peat) should not be used for agricultural production because interventions in these soils impact all components of the peat ecosystem in addition to releasing large amounts of C into the atmosphere (Leifeld and Menichetti, 2018; Zomer et al., 2017). Furthermore, the soil texture was not determinant for the OC content. A similar result was obtained by Liu et al. (2016) and Bai et al. (2019).

In arid soils the potential for OC accumulation via cultivation and soil management strategies is limited by the low water availability and the small plant biomass production (Bai et al., 2019; Sultan et al., 2019). However, because biochar represents an external source of C, its application enhances the increase of OC in soils with this climatic characteristic. Additionally, soils from arid regions have low C contents (Lal, 2009), which can be confirmed by 76% of the results in soils from arid regions presenting an initial C content of less than 1%. About 65% of the paired comparisons in continental climate regions also occurred in soils with low C content. Therefore, the results in these climatic zones were indirectly influenced by this characteristic, with greater increases ($p < 0.05$) than in soils from tropical or temperate regions.

As observed for TC, increases in OC in the field and long-term experiments were smaller than those conducted under controlled conditions and lasting up to one year. The smaller long-term effect size is associated with the decomposition of labile C forms (Wang et al., 2016) and a reduction in the growth stimulus of plants, which contribute OC to the soil through their roots and decomposition of biomass. This reduction is mainly related to removing nutrients (Griffin et al., 2017) and loss of the alkalizing effect of biochar (Jin et al., 2019).

5.3.5. Driving factors of the microbial biomass carbon content (MBC) in soils with biochar

The set of results showed an average soil MBC increase of 20.1% due to biochar application (Figure 6). This increase corroborates the results obtained in previous meta-analyses, in which the average increase in MBC in soils with biochar was ~17% (Liu et al., 2016) and 26% (Zhou et al., 2017). Thus, the percent change estimated in the present study is included in the confidence interval of these previous estimates.

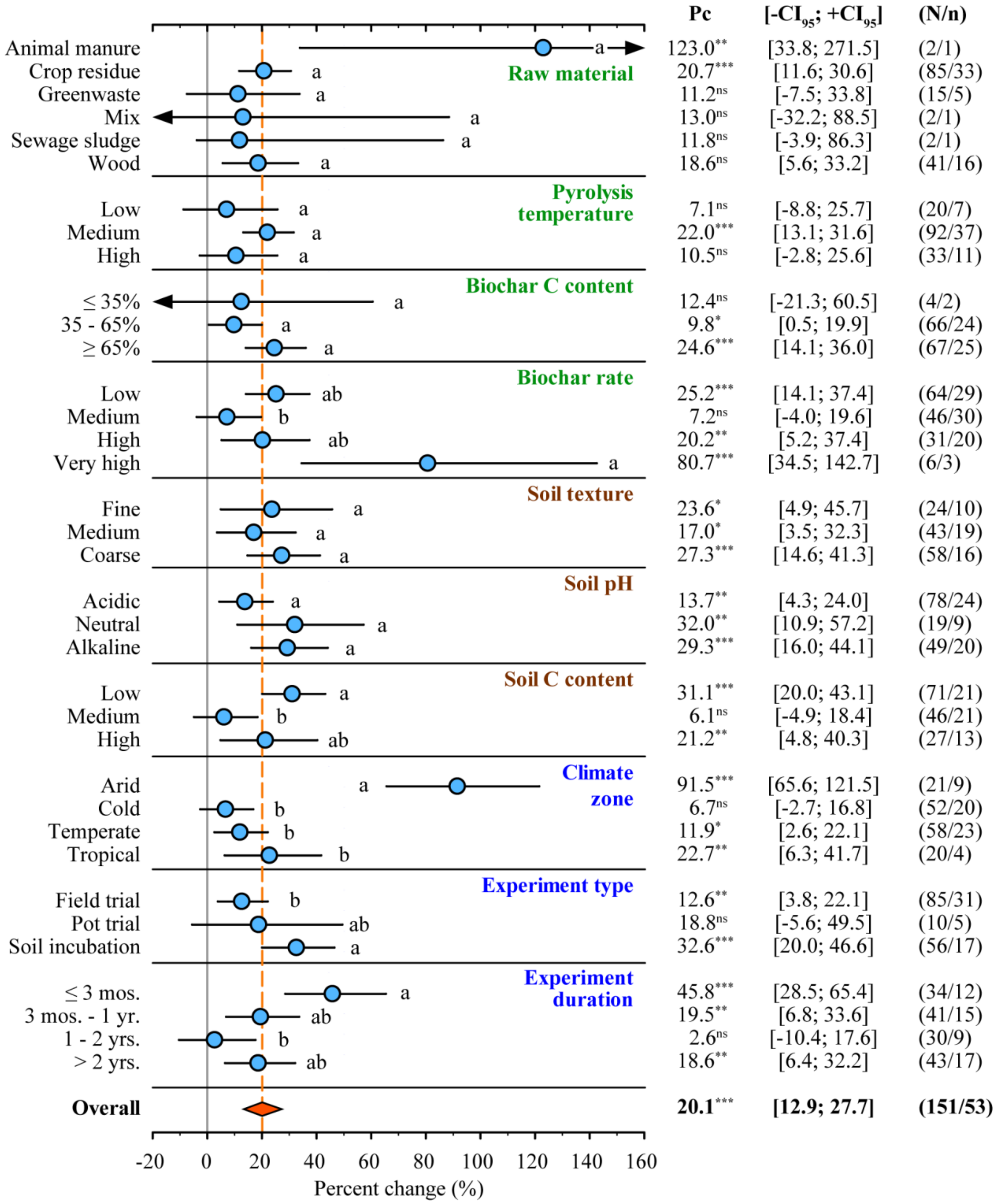


Figure 6. Percent change (Pc) of the soil microbial biomass C content (MBC) as a function of biochar and soil characteristics and experimental conditions. CI_{95}: 95% confidence interval; N: number of paired comparisons; n: number of studies. Pc significant at 5% (*), 1% (**) and 0.1% (***) or not significant (ns). For the same factor, different letters indicate that the subgroups differed significantly by Tukey contrasts (p<0.05)

Nutrient richness, labile C compounds and the absence of microbial inhibitors in biochar, among other factors, stimulate microbial biomass growth. Biochars from crop residues or wood, which are nutrient-poor residues, accounted for 83% of the paired comparisons for MBC. Then, this fact may explain why MBC has the smallest significant percent change (Figure 2). Biochar produced from animal manure, usually rich in nutrients and LC, showed the greatest increase in MBC (123%) compared to the control. However, due to the small number of published studies, it was impossible to statistically confirm biochars' potential from raw materials rich in nutrients and LC to increase the soil MBC. Sultan et al. (2019) reported the greatest increase in MBC in soil with animal manure biochar after 30 days of incubation compared to wheat straw and sugarcane bagasse biochars. However, this difference has changed over time due to the different speeds of LC mineralization between biochars of different raw materials.

The relative increase in MBC was also significantly influenced by the biochar rate, soil C content, climatic zone, experiment type and duration (Figure 6). When soils of arid regions received biochar, the average increase in MBC content reached 91.5%. In general, the stimulation of soil microbiota occurs primarily in soils with limitations (Wang et al., 2016), such as those of coarse texture. Therefore, this effect is associated with increased water retention capacity in soils with biochar (Omondi et al., 2016), which is one of the main factors affecting the soil microbiota (Tomar and Baishya, 2020).

Furthermore, the difference ($p<0.05$) between very high and medium biochar rates was masked by the coincidence that two-thirds of the results included in that group were obtained in arid regions. Thus, the influence of climatic zone on MBC variation was 1.2 times greater than the influence of biochar rate (Figure 4c). This result needs to be confirmed with a larger number of studies. Other factors not addressed in this study are also very important for MBC, such as the C:N ratio of biochar. Higher N contents (low C:N ratio) in biochars stimulate soil microbiota, while those with high C:N ratio can reduce MBC due to soil N immobilization (Liu et al., 2016). For example, in the study by Liu et al. (2016), the application of rates greater than 20 t ha^{-1} reduced the MBC due to the high C:N ratio of biochar.

Initially, biochar's oxidation and physical fragmentation facilitated microbial colonization (Wang et al., 2016). However, as discussed in section 3.4, labile forms of soil C such as OC and LC decrease over time. This reduction starts to limit the growth and maintenance of the soil microbial community (Wang et al., 2018).

As in the other C fractions discussed above, for MBC the increase was greater in soil incubations (32.6%) than in field experiments (12.6%). Under field conditions, the soil microbiota is subjected to continuous or extreme variations in soil water content and changes in the composition of the microbial community occur (Wang et al., 2020a). On the other hand, in soil incubations the moisture content is maintained close to the field capacity the entire time. This fact may explain the difference between the effect size as a function of the experiment type.

The average variation of MBC was slightly higher in coarse-textured soils (27.3%), despite the absence of statistical differences. Biochar particles have a large surface area with a porous structure (Tomczyk et al., 2020), and over time an organic layer forms on its surface (Yi et al., 2020). Thus, biochar is a favorable habitat for microbial communities in sandy soils with larger mean particle diameters and smaller surface areas. The data variability did not identify differences between the soil pH ranges. An increase in MBC was expected in acidic soils due to the alkalizing power of biochar. Liu et al. (2016) estimated an average 49% increase in MBC in initially acidic soils which were amended with biochar. There was also no difference between the pyrolysis temperature groups, and only the biochar pyrolyzed at medium temperatures resulted in a significant variation (22%; $p < 0.001$).

5.3.6. Driving factors of the dissolved organic carbon content (DOC) in soils with biochar

Overall, there was no change ($p<0.05$) to the DOC content in soil with the application of biochar (Figure 7). It can also be noted that for most factors evaluated, the percent change in DOC was not significant ($p>0.05$). Furthermore, the relatively large number of studies that evaluated DOC shows that this fraction is not as sensitive to biochar application as the others. Biochars have a small quantity of compounds in their composition that will join the DOC in the soil. However, the small DOC increase is temporary, and with the ageing of the biochar, this effect quickly disappears (Liu et al., 2019b), as confirmed in the present study. This finding implies that the risk of biochar application altering soil water quality via DOC leaching is minimal (Yang et al., 2019). DOC has been reported to decrease in soils with biochar through adsorption (DeCiucies et al., 2018).

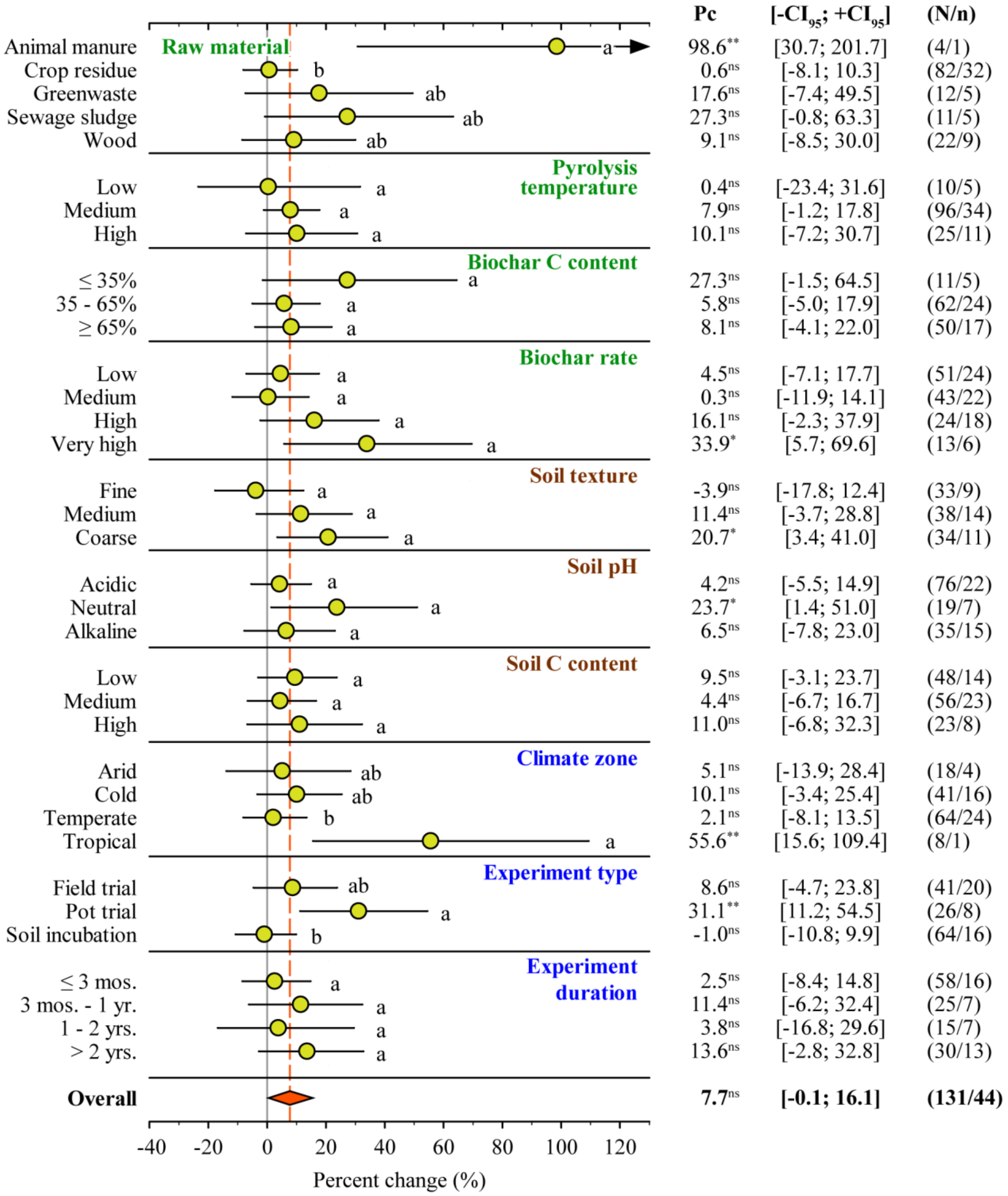


Figure 7. Percent change (Pc) of the dissolved organic C content (DOC) as a function of biochar and soil characteristics and experimental conditions. CI_{95}: 95% confidence interval; N: number of paired comparisons; n: number of studies. Pc significant at 5% (*), 1% (**) and 0.1% (***) or not significant (ns). For the same factor, different letters indicate that the subgroups differed significantly by Tukey contrasts ($p<0.05$)

In some conditions where significant increases were identified (animal manure feedstock and tropical climate zone) only one study was included in the subgroup analysis. Thus, these results may be inconsistent and require confirmation from a larger number of studies. On the other hand, the number of studies conducted in pots was higher (8) and allows for greater certainty in stating that there was an increase in soil DOC compared to soil incubations in these studies. In contrast to the results obtained in the present study, differences in soil DOC content as a function of the experiment type were reported by Liu et al. (2019a, b). The authors attributed the discrepancy between laboratory and field results to natural soil wetting and drying cycles.

5.4. Conclusion

This study presents results from different climatic regions of the globe and confirms the potential of biochar to accumulate C in the soil. Biochar application resulted in an increase in both labile and stable C fractions, which confirms this technology's dual environmental and agronomic potential. On average, biochar increased the TC content by 64%, confirming its importance in sequestering C in the soil. Biochar stimulates nutrient cycling and agricultural production by contributing to the soil labile C fractions (LC, MBC and OC). In addition, the preferential use of local waste as a raw material also reinforces the sustainability of this technology. The results indicated that multiple factors are involved in the response of C fractions to biochar application, such as biochar rate, soil C content, experiment duration, experiment type and climatic zone. Furthermore, quantifying the addition of C to the soil in relation to the initial content (rather than in absolute values) and detailing this estimate for different types of biochar, soil properties, and experimental conditions can help improve the estimate of potential soil C accumulation via biochar application. This improvement is critical to assist in decision making and in the definition of strategies and policies to encourage the use of biochar as a tool to achieve the goals established in global climate agreements.

5.5. References

Abney, R.B., Berhe, A.A., 2018. Pyrogenic carbon erosion: implications for stock and persistence of pyrogenic carbon in soil. Front. Earth Sci. 6, 1–16. https://doi.org/10.3389/feart.2018.00026.

Ali, I., Ullah, S., He, L., Zhao, Q., Iqbal, A., Wei, S., Shah, T., Ali, N., Bo, Y., Adnan, M., Amanullah, Jiang L., 2020. Combined application of biochar and nitrogen fertilizer improves rice yield, microbial activity and N-metabolism in a pot experiment. PeerJ 8, e10311. https://doi.org/10.7717/peerj.10311.

Amoakwah, E., Arthur, E., Frimpong, K.A., Parikh, S.J., Islam, R., 2020. Soil organic carbon storage and quality are impacted by corn cob biochar application on a tropical sandy loam. J. Soils Sediments 20, 1960–1969. https://doi.org/10.1007/s11368-019-02547-5.

Bai, X., Huang, Y., Ren, W., Coyne, M., Jacinthe, P., Tao, B., Hui, D., Yang, J., Matocha, C., 2019. Responses of soil carbon sequestration to climate-smart agriculture practices: a meta-analysis. Global Change Biol. 25, 2591–2606. https://doi.org/10.1111/gcb.14658.

Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A., Wood, E.F., 2018. Present and future Köppen-Geiger climate classification maps at 1-km resolution. Sci. Data 5, 180214. https://doi.org/10.1038/sdata.2018.214.

Chan, K.Y., Heenan, D.P., Oates, A., 2002. Soil carbon fractions and relationship to soil quality under different tillage and stubble management. Soil Tillage Res. 63, 133–139. https://doi.org/10.1016/S0167-1987(01)00239-2.

Cooper, J., Greenberg, I., Ludwig, B., Hippich, L., Fischer, D., Glaser, B., Kaiser, M., 2020. Effect of biochar and compost on soil properties and organic matter in aggregate size fractions under field conditions. Agric. Ecosyst. Environ. 295, 106882. https://doi.org/10.1016/j.agee.2020.106882.

DeCiucies, S., Whitman, T., Woolf, D., Enders, A., Lehmann, J., 2018. Priming mechanisms with additions of pyrogenic organic matter to soil. Geochem. Cosmochim. Acta 238, 329–342.

Ding, F., Van Zwieten, L., Zhang, W., Weng, Z., Shi, S., Wang, J., Meng, J., 2018. A meta-analysis and critical evaluation of influencing factors on soil carbon priming following biochar amendment. J. Soils Sediments 18, 1507–1517. https://doi.org/10.1007/s11368-017-1899-6.

Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L., Zheng, B., 2016. Biochar to improve soil fertility. Rev. Argon. Sustain. Dev. 36, 36. https://doi.org/10.1007/s13593-016-0372-z.

Domene, X., 2016. A critical analysis of meso- and macrofauna effects following biochar supplementation. In: Ralebitso-Senior, T.K., Orr, C.H. (Eds.), Biochar Application. Elsevier, pp. 268–292. https://doi.org/10.1016/B978-0-12-803433-0.00011-4.

Duddigan, S., Shaw, L.J., Alexander, P.D., Collins, C.D., 2019. A comparison of physical soil organic matter fractionation methods for amended soils. Appl. Environ. Soil Sci. 1–12. https://doi.org/10.1155/2019/3831241.

El-Naggar, A., El-Naggar, A.H., Shaheen, S.M., Sarkar, B., Chang, S.X., Tsang, D.C.W., Rinklebe, J., Ok, Y.S., 2019. Biochar composition-dependent impacts on soil nutrient release, carbon mineralization, and potential environmental risk: a review. J. Environ. Manag. 241, 458–467. https://doi.org/10.1016/j.jenvman.2019.02.044.

Faria, W.M., Figueiredo, C.C. de, Coser, T.R., Vale, A.T., Schneider, B.G., 2018. Is sewage sludge biochar capable of replacing inorganic fertilizers for corn production? Evidence from a two-year field experiment. Arch. Agron Soil Sci. 64, 505–519. https://doi.org/10.1080/03650340.2017.1360488.

Figueiredo, C.C. de, Farias, W.M., Melo, B.A. de, Chagas, J.K.M., Vale, A.T., Coser, T.R., 2019. Labile and stable pools of organic matter in soil amended with sewage sludge biochar. Arch. Agron Soil Sci. 65, 770–781. https://doi.org/10.1080/03650340.2018.1524577.

Frišták, V., Pipíška, M., Soja, G., 2018. Pyrolysis treatment of sewage sludge: a promising way to produce phosphorus fertilizer. J. Clean. Prod. 172, 1772–1778. https://doi.org/10.1016/j.jclepro.2017.12.015.

Gerke, J., 2018. Concepts and misconceptions of humic substances as the stable part of soil organic matter: a review. Agronomy 8, 76. https://doi.org/10.3390/agronomy8050076.

Ghosh, D., Maiti, S.K., 2021. Eco-Restoration of coal mine spoil: biochar application and carbon sequestration for achieving UN sustainable development goals 13 and 15. Land 10, 1112. https://doi.org/10.3390/land10111112.

Ghosh, D., Maiti, S.K., 2020. Can biochar reclaim coal mine spoil? J. Environ. Manag. 272, 111097. https://doi.org/10.1016/j.jenvman.2020.111097.

Ghosh, D., Masto, R.E., Maiti, S.K., 2020. Ameliorative effect of *Lantana camara* biochar on coal mine spoil and growth of maize (*Zea mays*). Soil Use Manag. 36, 726–739. https://doi.org/10.1111/sum.12626.

Gonzaga, M.I.S., Matias, M.I. de A.S., Andrade, K.R., Jesus, A.N. de, Cunha, G. da C., Andrade, R.S. de, Santos, J.C. de J., 2020. Aged biochar changed copper availability and distribution among soil fractions and influenced corn seed germination in a copper-contaminated soil. Chemosphere 240, 124828. https://doi.org/10.1016/j.chemosphere.2019.124828.

Greenwell, B., Boehmke, B., Cunningham, J., 2020. GBM Developers. Generalized Boosted Regression Models, gbm.

Griffin, D.E., Wang, D., Parikh, S.J., Scow, K.M., 2017. Short-lived effects of walnut shell biochar on soils and crop yields in a long-term field experiment. Agric. Ecosyst. Environ. 236, 21–29. https://doi.org/10.1016/j.agee.2016.11.002.

Gross, A., Glaser, B., 2021. Meta-analysis on how manure application changes soil organic carbon storage. Sci. Rep. 11, 5516. https://doi.org/10.1038/s41598-021-82739-7.

Hayes, M.H.B., Mylotte, R., Swift, R.S., 2017. Humin: its composition and importance in soil organic matter. In: Advances in Agronomy. Elsevier Inc., pp. 47–138. https://doi.org/10.1016/bs.agron.2017.01.001.

Hedges, L.V., Gurevitch, J., Curtis, P.S., 1999. The meta-analysis of response ratios in experimental ecology. Ecology 80, 1150. https://doi.org/10.2307/177062.

Higgins, J.P.T., Thompson, S.G., 2004. Controlling the risk of spurious findings from meta-regression. Stat. Med. 23, 1663–1682. https://doi.org/10.1002/sim.1752.

Holm, S., 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6, 65–70.

Hossain, M.Z., Bahar, M.M., Sarkar, B., Donne, S.W., Ok, Y.S., Palansooriya, K.N., Kirkham, M.B., Chowdhury, S., Bolan, N., 2020. Biochar and its importance on nutrient dynamics in soil and plant. Biochar 2, 379–420. https://doi.org/10.1007/s42773-020-00065-z.

Ippolito, J.A., Cui, L., Kammann, C., Wrage-Mönnig, N., Estavillo, J.M., Fuertes-Mendizabal, T., Cayuela, M.L., Sigua, G., Novak, J., Spokas, K., Borchard, N., 2020. Feedstock choice, pyrolysis temperature and type influence biochar characteristics: a comprehensive meta-data analysis review. Biochar 2, 421–438. https://doi.org/10.1007/s42773-020-00067-x.

Jeffery, S., Abalos, D., Prodana, M., Bastos, A.C., van Groenigen, J.W., Hungate, B.A., Verheijen, F., 2017. Biochar boosts tropical but not temperate crop yields. Environ. Res. Lett. 12, 053001 https://doi.org/10.1088/1748-9326/aa67bd.

Jin, Z., Chen, C., Chen, X., Hopkins, I., Zhang, X., Han, Z., Jiang, F., Billy, G., 2019. The crucial factors of soil fertility and rapeseed yield - a five year field trial with biochar addition in upland red soil, China. Sci. Total Environ. 649, 1467–1480. https://doi.org/10.1016/j.scitotenv.2018.08.412.

Klučáková, M., 2018. Size and charge evaluation of standard humic and fulvic acids as crucial factors to determine their environmental behavior and impact. Front. Chem. 6, 1–8. https://doi.org/10.3389/fchem.2018.00235.

Knapp, G., Hartung, J., 2003. Improved tests for a random effects meta-regression with a single covariate. Stat. Med. 22, 2693–2710. https://doi.org/10.1002/sim.1482.

Kuzyakov, Y., Bogomolova, I., Glaser, B., 2014. Biochar stability in soil: decomposition during eight years and transformation as assessed by compound-specific 14C analysis. Soil Biol. Biochem. 70, 229–236. https://doi.org/10.1016/j.soilbio.2013.12.021.

Laird, D.A., Brown, R.C., Amonette, J.E., Lehmann, J., 2009. Review of the pyrolysis platform for coproducing bio-oil and biochar. Biofuels, Bioprod. Biorefining 3, 547–562. https://doi.org/10.1002/bbb.169.

Lal, R., 2010. Managing soils and ecosystems for mitigating anthropogenic carbon emissions and advancing global food security. Bioscience 60, 708–721. https://doi.org/10.1525/bio.2010.60.9.8.

Lal, R., 2009. Sequestering carbon in soils of arid ecosystems. Land Degrad. Dev. 20, 441–454. https://doi.org/10.1002/ldr.934.

Lal, R., 2006. Soil carbon sequestration in Latin America. In: Lal, R., Cerri, C.C., Bernoux, M., Etchevers, J., Cerri, E. (Eds.), Carbon Sequestration in Soils of Latin America. CRC Press, pp. 77–92. https://doi.org/10.1201/9781482298031-11.

Leal, O., dos, A., Dick, D.P., De La Rosa, J.M., Leal, D.P.B., Gonzalez-Pérez, J.A., Campos, G.S., Knicker, H., 2019. Charcoal fine residues effects on soil organic matter humic substances, composition, and biodegradability. Agronomy 9, 1–16. https://doi.org/10.3390/agronomy9070384.

Leifeld, J., Menichetti, L., 2018. The underappreciated potential of peatlands in global climate change mitigation strategies. Nat. Commun. 9, 1071. https://doi.org/10.1038/s41467-018-03406-6.

Leng, L., Xu, X., Wei, L., Fan, L., Huang, H., Li, Jianan, Lu, Q., Li, Jun, Zhou, W., 2019. Biochar stability assessment by incubation and modelling: methods, drawbacks and recommendations. Sci. Total Environ. 664, 11–23. https://doi.org/10.1016/j.scitotenv.2019.01.298.

Liao, H., Li, Y., Yao, H., 2019. Biochar amendment stimulates utilization of plant-derived carbon by soil bacteria in an intercropping system. Front. Microbiol. 10, 1–13. https://doi.org/10.3389/fmicb.2019.01361.

Liu, C.-H., Chu, W., Li, H., Boyd, S.A., Teppen, B.J., Mao, J., Lehmann, J., Zhang, W., 2019a. Quantification and characterization of dissolved organic carbon from biochars. Geoderma 335, 161–169. https://doi.org/10.1016/j.geoderma.2018.08.019.

Liu, C., Wang, H., Li, P., Xian, Q., Tang, X., 2019b. Biochar's impact on dissolved organic matter (DOM) export from a cropland soil during natural rainfalls. Sci. Total Environ. 650, 1988. https://doi.org/10.1016/j.scitotenv.2018.09.356.

Liu, S., Zhang, Y., Zong, Y., Hu, Z., Wu, S., Zhou, J., Jin, Y., Zou, J., 2016. Response of soil carbon dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar amendment: a meta-analysis. GCB Bioenergy 8, 392–406. https://doi.org/10.1111/gcbb.12265.

Liu, X., Zhang, A., Ji, C., Joseph, S., Bian, R., Li, L., Pan, G., Paz-Ferreiro, J., 2013. Biochar's effect on crop productivity and the dependence on experimental conditions - a meta-analysis of literature data. Plant Soil 373, 583–594. https://doi.org/10.1007/s11104-013-1806-x.

Liu, Z., Wu, X., Liu, W., Bian, R., Ge, T., Zhang, W., Zheng, J., Drosos, M., Liu, X., Zhang, X., Cheng, K., Li, L., Pan, G., 2020. Greater microbial carbon use efficiency and carbon sequestration in soils: amendment of biochar versus crop straws. GCB Bioenergy 12, 1092–1103. https://doi.org/10.1111/gcbb.12763.

Luo, C., Deng, Y., Inubushi, K., Liang, J., Zhu, S., Wei, Z., Guo, X., Luo, X., 2018. Sludge biochar amendment and alfalfa revegetation improve soil physicochemical properties and increase diversity of soil microbes in soils from a rare earth element mining wasteland. Int. J. Environ. Res. Publ. Health 15, 965. https://doi.org/10.3390/ijerph15050965.

Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Devonshire, B.J., Brookes, P.C., 2013. Microbial biomass growth, following incorporation of biochars produced at 350 °C or 700 °C, in a silty-clay loam soil of high and low pH. Soil Biol. Biochem. 57, 513–523. https://doi.org/10.1016/j.soilbio.2012.10.033.

Naisse, C., Girardin, C., Lefevre, R., Pozzi, A., Maas, R., Stark, A., Rumpel, C., 2015. Effect of physical weathering on the carbon sequestration potential of biochars and hydrochars in soil. GCB Bioenergy 7, 488–496. https://doi.org/10.1111/gcbb.12158.

Nunes, M.R., Karlen, D.L., Veum, K.S., Moorman, T.B., Cambardella, C.A., 2020. Biological soil health indicators respond to tillage intensity: a US meta-analysis. Geoderma 369, 114335. https://doi.org/10.1016/j.geoderma.2020.114335.

Omondi, M.O., Xia, X., Nahayo, A., Liu, X., Korai, P.K., Pan, G., 2016. Quantification of biochar effects on soil hydrological properties using meta-analysis of literature data. Geoderma 274, 28–34. https://doi.org/10.1016/j.geoderma.2016.03.029.

Palmer, P.I., Feng, L., Baker, D., Chevallier, F., Bösch, H., Somkuti, P., 2019. Net carbon emissions from African biosphere dominate pan-tropical atmospheric CO2 signal. Nat. Commun. 10, 3344. https://doi.org/10.1038/s41467-019-11097-w.

Paustian, K., Larson, E., Kent, J., Marx, E., Swan, A., 2019. Soil C sequestration as a biological negative emission strategy. Front. Clim. 1, 8. https://doi.org/10.3389/fclim.2019.00008.

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G.P., Smith, P., 2016. Climate-smart soils. Nature 532, 49–57. https://doi.org/10.1038/nature17174.

Quilliam, R.S., Glanville, H.C., Wade, S.C., Jones, D.L., 2013. Life in the 'charosphere' – does biochar in agricultural soil provide a significant habitat for microorganisms? Soil Biol. Biochem. 65, 287–293. https://doi.org/10.1016/j.soilbio.2013.06.004.

R Core Team, 2020. R: a Language and Environment for Statistical Computing.

Shah, Z.H., Rehman, H.M., Akhtar, T., Alsamadany, H., Hamooh, B.T., Mujtaba, T., Daur, I., Al Zahrani, Y., Alzahrani, H.A.S., Ali, S., Yang, S.H., Chung, G., 2018. Humic substances: determining potential molecular regulatory processes in plants. Front. Plant Sci. 9, 1–12. https://doi.org/10.3389/fpls.2018.00263.

Sidik, K., Jonkman, J.N., 2005. Simple heterogeneity variance estimation for meta-analysis. J. R. Stat. Soc. Ser. C (Applied Statistics) 54, 367–384.

Smith, P., 2012. Soils and climate change. Curr. Opin. Environ. Sustain. 4, 539–544. https://doi.org/10.1016/j.cosust.2012.06.005.

Stockmann, U., Adams, M.A., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., Minasny, B., McBratney, A.B., Courcelles, V.de R.de, Singh, K., Wheeler, I., Abbott, L., Angers, D.A., Baldock, J., Bird, M., Brookes, P.C., Chenu, C., Jastrow, J.D., Lal, R., Lehmann, J., O'Donnell, A.G., Parton, W.J., Whitehead, D., Zimmermann, M., 2013. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agric. Ecosyst. Environ. 164, 80–99. https://doi.org/10.1016/j.agee.2012.10.001.

Stott, D.E., 2019. Recommended Soil Health Indicators and Associated Laboratory Procedures, Soil Health Technical Note No. 450-03. U.S. Department of Agriculture, Natural Resources Conservation Service.

Sultan, S., Khan, K.S., Akmal, M., Ahmed, Z.I., Hussain, Q., Khosa, S.A., 2019. Carbon mineralization in subtropical dryland soil amended with different biochar sources. Arabian J. Geosci. 12 https://doi.org/10.1007/s12517-019-4618-3.

Tomar, U., Baishya, R., 2020. Seasonality and moisture regime control soil respiration, enzyme activities, and soil microbial biomass carbon in a semi-arid forest of Delhi, India. Ecol. Process. 9, 50. https://doi.org/10.1186/s13717-020-00252-7.

Tomczyk, A., Sokołowska, Z., Boguta, P., 2020. Biochar physicochemical properties: pyrolysis temperature and feedstock kind effects. Rev. Environ. Sci. Bio/Technology 19, 191–215. https://doi.org/10.1007/s11157-020-09523-3.

United Nations Framework Convention on Climate Change (UNFCCC), 2021. "4 Per 1000" Initiative (4p1000) [WWW Document]. URL https://www.4p1000.org/ 4.19.21.

Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. J. Stat. Software 36, 1–48. https://doi.org/10.18637/jss.v036.i03.

Ventura, M., Alberti, G., Panzacchi, P., Delle Vedove, G., Miglietta, F., Tonon, G., 2019. Biochar mineralization and priming effect in a poplar short rotation coppice from a 3-year field experiment. Biol. Fertil. Soils 55, 67–78. https://doi.org/10.1007/s00374-018-1329-y.

von Lützow, M., Kogel-Knabner, ̈ I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biol. Biochem. 39, 2183–2207. https://doi.org/10.1016/j.soilbio.2007.03.007.

Wang, C., Liu, J., Shen, J., Chen, D., Li, Y., Jiang, B., Wu, J., 2018. Effects of biochar amendment on net greenhouse gas emissions and soil fertility in a double rice cropping system: a 4-year field experiment. Agric. Ecosyst. Environ. 262, 83–96. https://doi.org/10.1016/j.agee.2018.04.017.

Wang, D., Felice, M.L., Scow, K.M., 2020a. Impacts and interactions of biochar and biosolids on agricultural soil microbial communities during dry and wet-dry cycles. Appl. Soil Ecol. 152, 103570. https://doi.org/10.1016/j.apsoil.2020.103570.

Wang, D., Jiang, P., Zhang, H., Yuan, W., 2020b. Biochar production and applications in agro and forestry systems: a review. Sci. Total Environ. 723, 137775. https://doi.org/10.1016/j.scitotenv.2020.137775.

Wang, J., Xiong, Z., Kuzyakov, Y., 2016. Biochar stability in soil: meta-analysis of decomposition and priming effects. GCB Bioenergy 8, 512–523. https://doi.org/10.1111/gcbb.12266.

Wei, S., Zhu, M., Fan, X., Song, J., Peng, P., Li, K., Jia, W., Song, H., 2019. Influence of pyrolysis temperature and feedstock on carbon fractions of biochar produced from pyrolysis of rice straw, pine wood, pig manure and sewage sludge. Chemosphere 218, 624–631. https://doi.org/10.1016/j.chemosphere.2018.11.177.

Woolf, D., Amonette, J.E., Street-Perrott, F.A., Lehmann, J., Joseph, S., 2010. Sustainable biochar to mitigate global climate change. Nat. Commun. 1, 56. https://doi.org/10.1038/ncomms1053.

Wu, L., Zhang, S., Ma, R., Chen, M., Wei, W., Ding, X., 2021. Carbon sequestration under different organic amendments in saline-alkaline soils. Catena 196, 104882. https://doi.org/10.1016/j.catena.2020.104882.

Xing, J., Xu, G., Li, G., 2021. Comparison of pyrolysis process, various fractions and potential soil applications between sewage sludge-based biochars and lignocellulose-based biochars. Ecotoxicol. Environ. Saf. 208, 111756. https://doi.org/10.1016/j.ecoenv.2020.111756.

Yang, X.Y., Chang, K.H., Kim, Y.J., Zhang, J., Yoo, G., 2019. Effects of different biochar amendments on carbon loss and leachate characterization from an agricultural soil. Chemosphere 226, 625–635. https://doi.org/10.1016/j.chemosphere.2019.03.085.

Yang, Y., Guo, J., Chen, G., Yin, Y., Gao, R., Lin, C., 2009. Effects of forest conversion on soil labile organic carbon fractions and aggregate stability in subtropical China. Plant Soil 323, 153–162.

Yi, Q., Liang, B., Nan, Q., Wang, H., Zhang, W., Wu, W., 2020. Temporal physicochemical changes and transformation of biochar in a rice paddy: insights from a 9-year field experiment. Sci. Total Environ. 721, 137670. https://doi.org/10.1016/j.scitotenv.2020.137670.

Zhang, L., Chen, X., Xu, Y., Jin, M., Ye, X., Gao, H., Chu, W., Mao, J., Thompson, M.L., 2020. Soil labile organic carbon fractions and soil enzyme activities after 10 years of continuous fertilization and wheat residue incorporation. Sci. Rep. 10, 11318. https://doi.org/10.1038/s41598-020-68163-3.

Zhang, L., Xiang, Y., Jing, Y., Zhang, R., 2019. Biochar amendment effects on the activities of soil carbon, nitrogen, and phosphorus hydrolytic enzymes: a meta-analysis. Environ. Sci. Pollut. Res. 26, 22990–23001. https://doi.org/10.1007/s11356-019-05604-1.

Zhou, H., Zhang, D., Wang, P., Liu, X., Cheng, K., Li, L., Zheng, Jinwei, Zhang, X., Zheng, Jufeng, Crowley, D., van Zwieten, L., Pan, G., 2017. Changes in microbial biomass and the metabolic quotient with biochar addition to agricultural soils: a Meta-analysis. Agric. Ecosyst. Environ. 239, 80–89. https://doi.org/10.1016/j.agee.2017.01.006.

Zomer, R.J., Bossio, D.A., Sommer, R., Verchot, L.V., 2017. Global sequestration potential of increased organic carbon in cropland soils. Sci. Rep. 7, 1–8. https://doi.org/10.1038/s41598-017-15794-8.

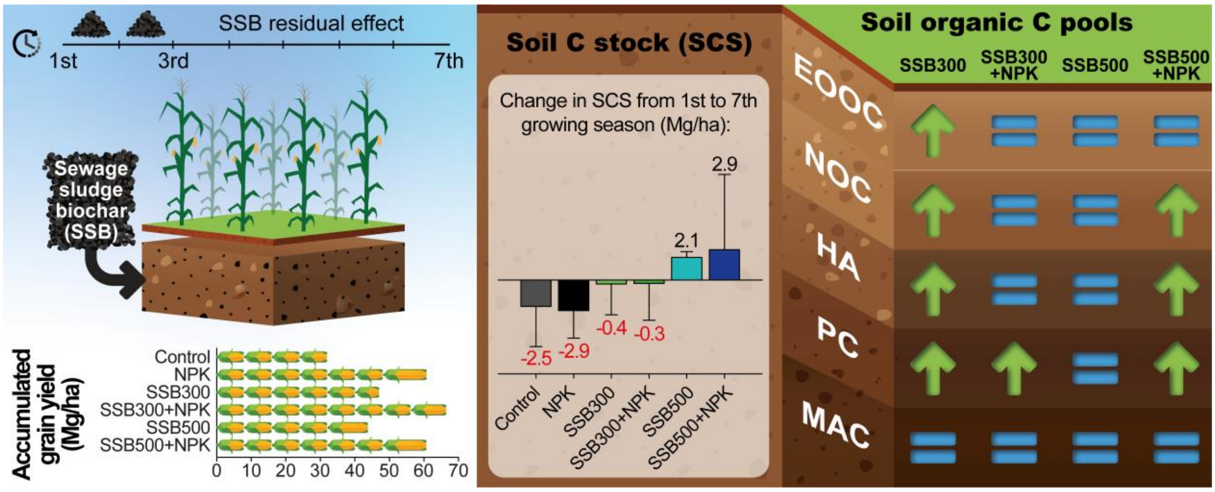
Zong, Y., Wang, Y., Sheng, Y., Wu, C., Lu, S., 2018. Ameliorating soil acidity and physical properties of two contrasting texture Ultisols with wastewater sludge biochar. Environ. Sci. Pollut. Res. 25, 25726–25733. https://doi.org/10.1007/s11356-017-9509-0.

CHAPTER II

SEVEN-YEAR EFFECTS OF SEWAGE SLUDGE BIOCHAR ON SOIL ORGANIC CARBON POOLS AND YIELD: UNDERSTANDING THE ROLE OF BIOCHAR ON CARBON SEQUESTRATION AND PRODUCTIVITY

6. SEVEN-YEAR EFFECTS OF SEWAGE SLUDGE BIOCHAR ON SOIL ORGANIC CARBON POOLS AND YIELD: UNDERSTANDING THE ROLE OF BIOCHAR ON CARBON SEQUESTRATION AND PRODUCTIVITY[3]

Graphical abstract



SSB300: sewage sludge biochar pyrolyzed at 300°C; SSB500: sewage sludge biochar pyrolyzed at 500°C; NPK: nitrogen, phosphorus, and potassium (mineral fertilization); EOOC: easily oxidizable organic C; NOC: non-oxidizable carbon; HA: humic acid; PC: particulate carbon; MAC: mineral-associated carbon.

Abstract

The increasing need for sustainable agricultural practices and climate change mitigation has driven research into biochar's role in enhancing soil carbon (C) sequestration and fertility. This study investigates the long-term effects of sewage sludge biochar (SSB) on soil C stocks and organic matter fractions, addressing whether SSB can improve soil C sequestration. Over seven years, soil samples were collected post-harvest each season to analyze total C (TC), total nitrogen (TN), and various organic matter fractions, including easily oxidizable organic C, permanganate-oxidizable C, non-oxidizable C, humic substances, particulate C, and mineral-associated C. Thermogravimetric analysis, soil magnetic susceptibility, and $\delta^{13}C$ and $\delta^{15}N$ measurements were also performed. Results demonstrate that SSB application enhances soil TC and TN levels, indicating improved soil fertility and C sequestration potential. Notably, SSB amendments increased the non-oxidizable organic C pool, contributing to soil organic matter stabilization. While the easily oxidizable organic C pool was increased under SSB at 300°C, the permanganate oxidizable C pool was not affected by treatments, suggesting that SSB primarily affects more recalcitrant C fractions, essential for long-term C sequestration. Additionally, SSB application substantially increased crop yield, with higher grain yield and shoot biomass observed over multiple growing seasons. However, a decline in corn yield from the fourth season onwards in SSB-only treatments highlights a limited capacity of biochar to sustain long-term productivity. These findings underscore the effectiveness of SSB in

[3] Manuscript submitted to the journal *Soil Use and Management*.

enhancing soil C pools and its potential role in sustainable agricultural practices. Future research should focus on long-term field studies under various environmental conditions and explore the potential of co-pyrolysis of sewage sludge with other feedstocks to enhance C stability. The broader adoption of biochar technology could play a vital role in mitigating climate change and promoting sustainable agricultural development.

Keywords: soil carbon sequestration, organic matter fractions, biosolids biochar, sustainable agriculture, long-term field study

Resumo

A crescente necessidade de práticas agrícolas sustentáveis e a mitigação das mudanças climáticas têm impulsionado pesquisas sobre o papel do biochar em melhorar a sequestro de carbono (C) no solo e a fertilidade. Este estudo investiga os efeitos de longo prazo do biochar de lodo de esgoto (SSB) nos estoques de C no solo e nas frações da matéria orgânica, abordando se o SSB pode melhorar a sequestro de C no solo. Ao longo de sete anos, amostras de solo foram coletadas após a colheita de cada safra para analisar o carbono total (TC), o nitrogênio total (TN) e várias frações da matéria orgânica, incluindo carbono orgânico facilmente oxidável, carbono oxidável por permanganato, carbono não oxidável, substâncias húmicas, carbono particulado e carbono associado a minerais. Análises termogravimétricas, susceptibilidade magnética do solo e medições de $\delta^{13}C$ e $\delta^{15}N$ também foram realizadas. Os resultados demonstram que a aplicação de SSB aumenta os níveis de TC e TN no solo, indicando uma melhoria na fertilidade do solo e no potencial de sequestro de C. Notavelmente, os tratamentos com SSB aumentaram o compartimento de carbono orgânico não oxidável, contribuindo para a estabilização da matéria orgânica do solo. Embora o carbono orgânico facilmente oxidável tenha aumentado sob SSB a 300°C, o compartimento de carbono oxidável por permanganato não foi afetado pelos tratamentos, sugerindo que o SSB afeta principalmente frações de C mais recalcitrantes, essenciais para o sequestro de C a longo prazo. Além disso, a aplicação de SSB aumentou substancialmente a produtividade das culturas, com maior produtividade de grãos e biomassa da parte aérea observados ao longo de várias safras. No entanto, a queda na produtividade de milho a partir da quarta safra em tratamentos apenas com SSB destaca uma capacidade limitada do biochar em sustentar a produtividade a longo prazo. Esses achados ressaltam a eficácia do SSB em melhorar os compartimentos de C no solo e seu potencial papel em práticas agrícolas sustentáveis. Pesquisas futuras devem se concentrar em estudos de campo de longo prazo em várias condições ambientais e explorar o potencial da co-pirólise do lodo de esgoto com outros materiais para aumentar a estabilidade do C. A adoção mais ampla da tecnologia de biochar pode desempenhar um papel vital na mitigação das mudanças climáticas e na promoção do desenvolvimento agrícola sustentável.

Palavras-chave: sequestro de carbono no solo, frações da matéria orgânica, biochar de biossólidos, agricultura sustentável, estudo de campo de longo prazo

6.1. Introduction

Soil is the largest terrestrial carbon (C) reservoir, storing more C than the atmosphere and vegetation combined (Lal, 2010). Enhancing soil C sequestration is a key strategy for mitigating climate change and improving soil quality (Das et al., 2021). Biochar, a solid byproduct of pyrolysis, is a promising amendment for increasing soil C stocks due to its high C content and stability (Chagas et al., 2022; Gross et al., 2021). Pyrolysis is a versatile process that converts biomass into gaseous, liquid and solid products, the proportions of which are strongly influenced by the pyrolysis conditions (Vuppaladadiyam et al., 2023). Among the resulting products, the non-solid fractions are valuable for bioenergy and emission reduction purposes (Werner et al., 2018), while biochar has significant potential for climate change mitigation due to its C-rich nature (Lehmann et al., 2021). Biochar is formed through the thermochemical transformation of feedstocks, resulting in a stable C structure characterized by low oxygen and hydrogen content (Leng et al., 2019). Its C stability increases with longer pyrolysis time and lower ash content in the feedstock (McBeath et al., 2015).

The world produces approximately 359.4 billion m^3 of wastewater annually, with Brazil contributing 18.5 billion m^3, or ~5% of the global total. Of this total, 52% undergoes treatment (Jones et al., 2021), resulting in the generation of sewage sludge (SS) (Wu et al., 2020). As urbanization intensifies, converting SS to sewage sludge biochar (SSB) has emerged as a sustainable solution, presenting an agricultural opportunity with positive environmental impacts. Pyrolysis has emerged over the past two decades as an alternative method to convert SS into a safe and nutrient-rich amendment (Liu et al., 2018).

The chemical composition and properties of SSB differ from those of biochars derived from lignocellulosic feedstocks. SSB has a lower total C (29.8%) and higher total N (4.8%) than lignocellulose-based biochars (57.3% and 1.5%, respectively), resulting in a lower C:N ratio (6.2 vs. 54.7) (Gonzaga et al., 2018; Jafari Tarf et al., 2022; Khan et al., 2015; Shao et al., 2020). Furthermore, among 21 different biochar samples with contrasting feedstocks, SSB at different temperatures had lower C stability (Adhikari et al., 2024). Because of its nutrient richness, SSB is considered preferable for plant nutrition purposes (Faria et al., 2018), increasing crop yields by 10-42%, especially in acidic tropical soils (Joseph et al., 2021). Consequently, unlike other C-rich biochars, SSB was not primarily intended for soil C sequestration. However, it is crucial to assess whether it can directly or indirectly promote C stock gains and improve soil organic matter (SOM) quality when used for nutritional purposes.

SOM is essential for soil health, fertility, and C sequestration. However, SOM is heterogeneous and consists of different fractions with distinct turnover rates and functions in soil (Lehmann and Kleber, 2015). To evaluate how SOM quality changes over time, it is important to measure and compare these fractions and their interactions (Chan et al., 2002). Biochar can affect SOM fractions in several ways. However, not all SOM fractionation methods are suitable for biochar-amended soils, as some may interfere with the separation process or be misclassified as native SOM (Paetsch et al., 2017). Therefore, selecting an appropriate fractionation method is crucial for studying the effects of biochar on SOM (Cooper et al., 2020).

In general, SOM can be fractionated based on physical or chemical characteristics/reactivity (von Lützow et al., 2007). In the former method, SOM is separated into light and heavy fractions, where biochar can affect these fractions differently based on its properties and interactions with soil particles (Dong et al., 2016). Biochar can increase the particulate C fraction, which consists of large organic particles that are typically easily decomposed (Yang et al., 2018). However, being resistant to decomposition, biochar can introduce recalcitrant C into this labile fraction (Paetsch et al., 2017), requiring caution when measuring SOM fractions in biochar-amended soils. In the chemical fractionation method, SOM is separated into components like humic substances, labile C, easily oxidizable C, and inert C (von Lützow et al., 2007). Based on its properties and soil environment interactions, biochar can influence these components differently. It can increase humic substances retention by adsorbing them onto its porous structure (Pignatello et al., 2017), affect microbial decomposition of labile organic matter through priming or protective effects, especially in low-fertility soils (El-Naggar et al., 2019; Figueiredo et al., 2019c), and alter the amount and composition of easily oxidizable organic C, reflecting microbial activity and biochar-soil interactions (Abbas et al., 2019; Yang et al., 2018). Biochar can also contribute to non-labile pools like inert C and humin, with long-term effects on soil C sequestration (Hayes et al., 2017; Wander, 2004).

SSB typically contains high volatile content, especially at low temperatures (Zhang et al., 2015). As a result, C supplied via SSB may have a shorter half-life compared to biochar from other feedstocks (Leng and Huang, 2018). This highlights the importance of considering the distinct properties of SSB when assessing its impact on SOM dynamics. When used in agriculture, SSB can fully or partially replace chemical fertilizers, with residual effects lasting up to three years (Faria et al., 2018). Therefore, elucidating the indirect effects of agricultural SSB use requires assessing soil C dynamics after ceasing SSB application (residual effect); interactions with mineral fertilizers, particularly N; and comparisons with conventional fertilization.

However, several knowledge gaps hinder a comprehensive understanding of the potential of SSB for soil C sequestration and its interactions with native SOM. These gaps include limited long-term field studies on SSB-amended soils, a small number of studies focused on SSB, uncertainty about residual effects after SSB application ceases, unclear interactions between SSB and mineral fertilizers (particularly N), and lack of comprehensive field assessments of SSB effects on total soil C stocks and SOM pools. To address these gaps and unlock the potential of SSB to provide C sequestration benefits, it is imperative to conduct long-term field studies that cover different aspects of SSB application (Gross et al., 2021). The results of this study provide evidence-based guidance for optimizing the use of SSB as a soil amendment with unique properties.

Despite the relatively low C content of SSB compared to lignocellulose-derived biochar, even modest soil C gains over large areas could contribute significantly to climate change mitigation. Therefore, assessing the C sequestration potential of SSB under different real-world conditions is essential for a holistic sustainability assessment. Using waste streams for nutrient cycling and soil C enhancement has multiple global sustainability benefits.

To address these issues, the objectives of this study were i) to assess the dynamics of C in labile and stable SOM pools over seven years following SSB application, and ii) to evaluate the direct and indirect contribution of SSB to soil C stocks.

6.2. Material and Methods

6.2.1. Sewage sludge biochars: production and characterization

The feedstock for the biochar was SS from tertiary treatment plants dried in solar drying beds at 20% humidity. SS was collected from the sewage treatment plants of the Environmental Sanitation Company of the Federal District (CAESB) located in Gama and Samambaia (Brasília, Brazil).

Biochars were produced at 300°C (SSB300) and 500°C (SSB500). For this purpose, 8 mm sieved SS was pyrolyzed in a muffle furnace (Linn Elektro Therm, Eschenfelden, Germany) at an average heating rate of 2.5°C min^{-1} and a residence time of 30 min. After pyrolysis, the SSB were stored in plastic bags until their application to the soil.

The physicochemical characterization of SS and SSB was performed (Table 2). The pH was determined in $CaCl_2$ 0.01 mol L^{-1} (1:5 m/v) (Brazil, 2014). The total contents of C, N, H and O were determined in an elemental analyzer (PE 2400, series II CHNS/O, PerkinElmer, Norwalk, USA). Nitrate (NO_3^-–N) and ammonium (NH_4^+–N) were determined by the Kjeldhal method (Bremner and Keeney, 1965). Macronutrients were determined after nitroperchloric acid digestion (Silva, 2009) and quantified by the following methods: P was determined by molybdovanadophosphoric acid method; K by flame photometry; Ca, Mg and S were determined by ICP-OES (ICPE-9000, Shimadzu, Japan). Pore volume (PV) and specific surface area (SSA) were measured by N_2 adsorption isotherms at −196.2 °C in a surface area analyzer

(NOVA 2200, Quantachrome). The proximate analysis of SSB was conducted using simultaneous thermal analyzer (details in the Appendix). Yield was calculated as the ratio of the masses before and after pyrolysis.

Table 2. Characteristics of sewage sludge feedstock and biochars pyrolyzed at 300°C (SSB300) and 500°C (SSB500)

Characteristic[1]	Sewage sludge	SSB300	SSB500
pH ($CaCl_2$)	4.8±0.4	5.8±0.2	6.5±0.3
C (%)	21.0±0.4	23.4±0.4	19.0±0.2
H (%)	4.2±0.1	3.6±0.1	1.7±0.1
N (%)	3.0±0.1	3.3±0.1	2.3±0.1
O (%)	72.0±0.5	69.7±0.5	77.0±0.2
H/C	2.4±0.1	1.8±0.1	1.1±0.1
O/C	2.6±0.1	2.2±0.1	3.0±0.1
C/N	7.0±0.1	7.0±0.1	8.3±0.1
NO_3^-–N (mg kg^{-1})	23.3±3.4	17.5±2.8	5.8±0.9
NH_4^+–N (mg kg^{-1})	461.2±36.0	431.9±31.0	169.3±19.8
P (g kg^{-1})	35.7±2.8	41.1±3.2	61.3±5.6
K (g kg^{-1})	0.8±0.1	1.6±0.1	1.25±0.1
Ca (g kg^{-1})	6.6±0.1	6.7±0.2	8.2±0.3
Mg (g kg^{-1})	0.8±0.1	1.8±0.1	1.7±0.1
S (g kg^{-1})	6.7±0.2	15.1±1.0	7.4±0.4
PV (mL g^{-1})	0.022±0.001	0.027±0.001	0.053±0.002
SSA (m^2 g^{-1})	18.2±1.2	20.2±1.8	52.5±4.3
Moisture (%)	-	6.86	9.22
Volatile materials (% db)	-	43.90	31.83
Fixed carbon (% db)	-	3.03	5.47
Ash (% db)	-	53.07	62.71
Yield (%)	-	86±8	65±4

[1] mean ± standard deviation (n=3); PV: pore volume; SSA: specific surface area; db: on dry basis. Adapted from (Figueiredo et al., 2019a).

6.2.2. Field trial: location and experimental design

The field trial was set up at Fazenda Água Limpa (FAL/UnB), Brasília-DF, Brazil (latitude 15°56’45”S, longitude 47°55’43”W and altitude 1095 m). The area previously covered by native vegetation, Cerrado, was converted to pasture in 2005. Subsequently, the experiment was established in November 2014 when the area presented characteristics of degraded pasture due to lack of management (Figure 8).

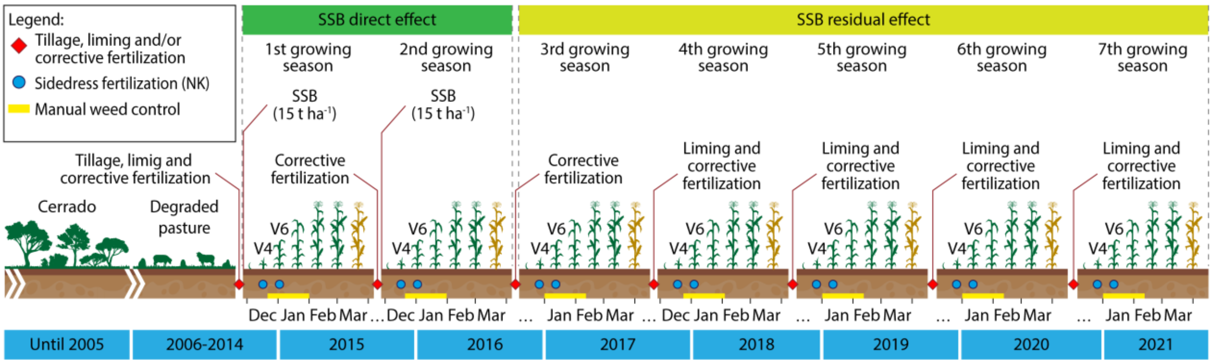


Figure 8. History of the field trial, including sewage sludge biochar (SSB) amendments, fertilization, and other management practices used during the evaluated period

The region has a tropical savanna climate (Aw, Köppen) with a dry winter from April to September. The average annual precipitation is 1400 mm (2001-2018) and the average annual temperature ranges from 14.7 to 25.4 °C. The soil was classified as *Latossolo Vermelho-Amarelo* according to the Brazilian Soil Classification (Santos et al., 2018), clayey Oxisol (Typic Haplustox) (Soil Survey Staff, 2014), Gibbsic Ferralsol (IUSS Working Group WRB, 2015). The physical and chemical properties of the soil (0-0.2 m) prior to the experimental setup are presented in Table 3 and were determined according to Teixeira et al. (2017).

Table 3. Physical and chemical properties of the soil prior to experimental setup

Soil property	Value	Soil property	Value
pH (H_2O)	4.9	Al^{3+} ($cmol_c\ kg^{-1}$)	0.04
OC ($g\ kg^{-1}$)	19.8	H+Al ($cmol_c\ kg^{-1}$)	5.9
P ($mg\ kg^{-1}$)	2.3	CEC ($cmol_c\ kg^{-1}$)	9.3
K ($cmol_c\ kg^{-1}$)	0.1	BS (%)	36.6
Ca^{2+} ($cmol_c\ kg^{-1}$)	2.4	Sand ($g\ kg^{-1}$)	45.0
Mg^{2+} ($cmol_c\ kg^{-1}$)	0.9	Silt ($g\ kg^{-1}$)	360.0
SB ($cmol_c\ kg^{-1}$)	3.4	Clay ($g\ kg^{-1}$)	595.0

OC: organic carbon; SB: sum of bases; CEC: cation exchange capacity; BS: base saturation.

A randomized block design with four replications and six treatments was used: 1) control - no biochar and no mineral fertilization; 2) NPK - mineral fertilization with N, P and K; 3) SSB300 - application of pyrolyzed biochar at 300°C; 4) SSB500 - application of pyrolyzed biochar at 500°C; 5) SSB300+NPK; 6) SSB500+NPK. The experimental plots were 20 m² (5x4 m).

6.2.3. History and crop management practices

Seven crop seasons were evaluated, according to the harvest dates as follows: 1st - 2015, 2nd - 2016, 3rd - 2017, 4th - 2018, 5th - 2019, 6th - 2020, and 7th - 2021. Details of the crop management practices applied in each season are provided in Table 4 and Figure 8. Prior to the first growing season, the soil was plowed, harrowed, and limed to raise the BS to 55%. In subsequent seasons, limestone was applied as needed without tillage to maintain 55% BS. Due to naturally low soil P and K levels, corrective fertilization was applied before planting the first two crops. Thereafter, corrective fertilization was applied as needed based on post-harvest soil analysis for each plot.

Table 4. Description of seasonal agricultural practices and fertilizer inputs during the sewage sludge biochar (SSB) field experiment

Season	Treatment	Prior to corn planting (Oct/Nov)				Planting fertilization (Nov/Dez)	Side-dressing fertilization
		Tillage*	Lime[a]*	Corrective fertilization[b]*	SSB (t ha^{-1})		
(1st) 2015	Control	Soil was plowed and harrowed	1.24 t ha^{-1}	87.3 kg ha^{-1} P (1110 kg ha^{-1} single superphosphate) and 42.3 kg ha^{-1} K (85 kg ha^{-1} potassium chloride)	-	-	-
	NPK				-	NPK[c]	NK[d]
	SSB300				15	-	-
	SSB300+NPK				15	NPK	NK
	SSB500				15	-	-
	SSB500+NPK				15	NPK	NK
(2nd) 2016	Control	No-till	-	43.6 kg ha^{-1} P (555 kg ha^{-1} single superphosphate) and 50 kg ha^{-1} K (100 kg ha^{-1} potassium chloride)	-	-	-
	NPK				-	NPK	NK
	SSB300				15	-	-
	SSB300+NPK				15	NPK	NK
	SSB500				15	-	-
	SSB500+NPK				15	NPK	NK
(3rd) 2017	Control	No-till	-	According to the necessity of each plot	-	-	-
	NPK				-	NPK	NK
	SSB300				-	-	-
	SSB300+NPK				-	NPK	NK
	SSB500				-	-	-
	SSB500+NPK				-	NPK	NK
(4th) 2018	Control	No-till	It was applied to increase the base saturation to 55%	According to the necessity of each plot	-	-	-
	NPK				-	NPK	NK
	SSB300				-	-	-
	SSB300+NPK				-	NPK	NK
	SSB500				-	-	-
	SSB500+NPK				-	NPK	NK
(5th) 2019	Control	No-till	It was applied to increase the base saturation to 55%	According to the necessity of each plot	-	-	-
	NPK				-	NPK	NK
	SSB300				-	-	-
	SSB300+NPK				-	NPK	NK
	SSB500				-	-	-
	SSB500+NPK				-	NPK	NK
(6th) 2020	Control	No-till	It was applied to increase the base saturation to 55%	According to the necessity of each plot	-	-	-
	NPK				-	NPK	NK
	SSB300				-	-	-
	SSB300+NPK				-	NPK	NK
	SSB500				-	-	-
	SSB500+NPK				-	NPK	NK
(7th) 2021	Control	No-till	It was applied to increase the base saturation to 55%	According to the necessity of each plot	-	-	-
	NPK				-	NPK	NK
	SSB300				-	-	-
	SSB300+NPK				-	NPK	NK
	SSB500				-	-	-
	SSB500+NPK				-	NPK	NK

*: in all treatments; a: dolomite lime with 100% acid-neutralizing potential; b: according to Sousa and Lobato (2004); c: 30 kg ha^{-1} N + 45 kg ha^{-1} P + 48 kg ha^{-1} K (714 kg ha^{-1} NPK [4-14-8]); d: 75 kg ha^{-1} N (urea) + 48 kg ha^{-1} K (KCl) at V4 stage + 75 kg ha^{-1} N (urea) at V6 stage.

In the first two growing seasons (2015-2016), SSB was applied at 15 t ha^{-1} (dry weight) per crop and incorporated into the top 0.2 m of soil using a rotary hoe before planting. No SSB was applied in the following five growing seasons (2017-2021) in order to evaluate residual effects. The biochar rate was selected based on a previous study showing optimal yields with 10-20 t ha^{-1} (Sousa and Figueiredo, 2016).

Planting was carried out at a density of 66666 plants ha^{-1}, with 5 furrows per plot at a distance of 0.9 m and 6 plants m^{-1}. In the first four seasons, corn hybrid LG 6030 was grown, while hybrid RB9789 VIP3 was sown in the remaining seasons. Regardless the cessation of biochar application, mineral fertilizer was applied in the sowing furrow of the respective plots (NPK, SSB300+NPK and SSB500+NPK) for all years (Table 4). Side dressing fertilization was applied at the stages of V4 (four developed leaves) and V6 (six developed leaves). These fertilizations were based on the post-harvest soil analysis of the previous crop and local recommendations for producing 10 t ha^{-1} of corn grain (Sousa and Lobato, 2004).

Weeds were controlled manually by hoeing whenever necessary to prevent interference to the crop. No pesticides were applied. In the period between harvest and subsequent seeding the weeds were not managed. Harvesting was done manually between May and June. Immediately after harvesting, the soil was sampled in the 0-0.2 m layer with a Dutch auger, taking 5 subsamples in the central furrows of each plot. Soil samples were passed through a 2 mm sieve, air-dried, and stored in plastic bags.

6.2.4. Soil analysis

Despite the uncertainties surrounding physical fractionation methods (Paetsch et al., 2017), the organic C in biochar-amended soils from the experimental site was subjected to both physical fractionation and chemical procedures, with soil samples collected from the 0-0.2 m depth at post-harvest of each growing season. Additionally, soil samples from an area of native Brazilian Cerrado near the experimental site were collected and analyzed from the same depth for comparison, with the Cerrado sampling occurring specifically at post-harvest of the 2018 growing season.

6.2.4.1. Chemical characterization

6.2.4.1.1 Total carbon (TC) and nitrogen (TN) analysis

Soil samples were passed through a 150 µm sieve. Approximately 2 to 4 mg of ground soil was weighed into tin capsules and the TC and TN content were determined in a CHN elemental analyzer (Eurovector EA3000, Milan, Italy) at 980°C. The C:N ratio was also calculated.

6.2.4.1.2 Organic carbon pools by oxidation methods

The easily oxidizable organic carbon (EOOC) in soil was determined by wet oxidation with potassium dichromate ($K_2Cr_2O_7$) without an external heat source (Walkley and Black, 1934). After drying and sieving through 0.5 mm, 0.5 g of soil sample was used for the EOOC determination. Then 20 mL of sulfuric acid (H_2SO_4) was added, stirred, and allowed to settle for 30 minutes. After resting, it was titrated with ferrous ammoniacal sulfate ($Fe(NH_4)_2(SO_4)_2.6H_2O$). Non-oxidizable C (NOC) content was calculated as the difference between TC and EOOC (Chan et al., 2001).

Permanganate-oxidizable carbon (POXC) was evaluated by oxidation with potassium permanganate ($KMnO_4$) (Blair et al., 1995). To 1 g of soil passed through a 500 µm sieve, 25 mL of $KMnO_4$ 333 mmol L^{-1} was added. This mixture was stirred for 1 h and then centrifuged. An aliquot of the supernatant was collected for measurement in a spectrophotometer (Espec-UV-5100, Metash, China) at 565 nm. The difference between EOOC and POXC was calculated on the assumption that there is an overlap between the C extracted by these methods.

6.2.4.1.3 Humic substances fractionation

The soil humic substances were fractionated into fulvic acid (FA), humic acid (HA) and humin (HU) by means of the difference in solubility in acidic and alkaline media (Swift, 1996) with adaptations reported by Benites and Machado (2003). A 1 g soil (≤2 mm) was shaken with 20 mL sodium hydroxide (NaOH) 0.1 mol L^{-1}. This mixture was centrifuged, and the extract was retained. Another 20 mL of NaOH was added to the solid material, and the extract was again retained after centrifugation. The solid material was the HU fraction, which was oven-dried and stored. The alkaline extract was acidified with H_2SO_4 and centrifuged to separate the HA. The precipitated HA was solubilized in NaOH. The C content of the HA, FA and HU fractions was quantified by oxidation with $K_2Cr_2O_7$.

6.2.4.2. Physical fractionation

Particulate carbon (PC) was extracted by dispersing 20 g of air-dried soil with 80 mL of 5 g L^{-1} sodium hexametaphosphate ($(NaPO_3)_6$) and shaking for 16 h on an orbital shaker at 150 rpm (Cambardella and Elliott, 1992). The soil suspension was sieved through 53 µm to retain particulate organic matter and sand. This fraction was washed with deionized water, oven-dried at 60°C, ground through 150 µm, and analyzed for PC content using an elemental analyzer (Eurovector EA3000, Milan, Italy) at 980°C, correcting for soil sand content. The mineral-associated carbon (MAC) was calculated as the difference between TC and PC.

6.2.4.3. Thermogravimetric analysis (TGA)

The thermal stability of SOM was assessed using a thermogravimetric analyzer (Shimadzu DTG-60H, Kyoto, Japan). Approximately 10 mg of soil (150 µm) was placed in a platinum crucible and heated from room temperature to 1000°C at 20°C min^{-1} under synthetic air (80% N_2, 20% O_2), with a flow rate of 50 mL min^{-1}. This analysis was conducted exclusively on samples from the 2018 growing season.

Weight loss (WL) was calculated across different temperature ranges, reflecting the stability of different SOM pools or fractions. Thermostability indices were also determined based on the WL. These temperature ranges and indices were derived from a comprehensive literature review.

Further TGA data analysis involved the calculation of mean thermal mass losses (TML) within 10°C intervals across the 30–1000°C temperature range. The coefficient of determination (R^2) between TML and the C fractions or pools described in sections 6.2.4.1 and 6.2.4.2 was calculated, providing a quantitative measure of their relationship (Tokarski et al., 2020).

6.2.4.4. Magnetic susceptibility

Magnetic susceptibility was measured at a low frequency (0.47 kHz) using 10 g of air-dried soil in a Bartington MS2 instrument coupled to a Bartington MS2B sensor (Dearing, 1999). The reported results are the average of six measurements per sample, obtained from three replicates with two measurements each.

6.2.4.5. $\delta^{13}C$ and $\delta^{15}N$

Approximately 20 to 30 mg of soil (150 µm) was weighed in tin capsules and the C and N isotope ratio (δ^{13}C and δ^{15}N) was determined in an elemental analyzer (Carlo Erba, CHN-1100) coupled to a Finnigan DELTAplus mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) at the Laboratory of Isotope Ecology, Center for Nuclear Energy in Agriculture (CENA/University of São Paulo), Piracicaba, SP, Brazil. The isotope ratios were calculated according to equation (3). This analysis was conducted exclusively on samples from the 2018 growing season.

$$\delta X(‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000 \quad (3)$$

Where δX is the isotopic ratio; R_{sample} and $R_{standard}$ are the isotopic ratios (^{13}C:^{12}C or ^{15}N:^{14}N) of the samples and standard, respectively.

The $\delta^{13}C$ and $\delta^{15}N$ were expressed relative to Vienna Pee Dee Belemnite (VPDB; ^{13}C:^{12}C=0.0112372) and to atmospheric N. The long-term analytical error for the internal standard is ±0.2‰ for $\delta^{13}C$ and ±0.4‰ for $\delta^{15}N$.

6.2.5. Soil carbon stock

Soil C stock (SCS) was estimated according to the following equation (4):

$$SCS = TC \times D \times H \quad (4)$$

where TC represents the total C content in the soil, D is the soil density, and H is the thickness of the evaluated soil layer. To determine the density, undeformed soil samples were collected from the 0.075–0.125 m layer using a metal cylinder of known volume, with one cylinder per plot. They were oven-dried and weighed, and the density was calculated using the ratio of mass to volume.

Since the soil density did not show significant variation among the treatments, the average density was used to calculate the equivalent layer in the Cerrado (Ellert and Bettany, 1995). Changes in SCS were calculated relative to the control treatment, and the results were expressed as the difference between the control and the treatment, in Mg ha^{-1}.

6.2.6. Crop yield

Post-harvest, the grain yield and shoot biomass dry matter were determined. Fifteen plants from the three central rows of each plot were sampled. These plants were dried in a circulating oven at 65°C until a constant weight was achieved, which allowed calculating the shoot biomass dry matter. The ears from these plants were manually shelled, and the grain yield was adjusted to account for 13% moisture. The yield accumulated over the growing seasons was also calculated.

6.2.7. Biochar carbon stability indices

Distinct indices were calculated to estimate the biochar C stability, including the H:C ratio, organic C content on a dry weight basis for biochar type t in year y ($CC_{y,t}$), thermostable fraction (TSF), and Enders' index.

$CC_{y,t}$ was determined as the product of the mass ($M_{y,t}$), a permanence factor (PR_{de}), and the elemental C content (F_{cp}) (Verra, 2023) according to equation (5):

$$\mathrm{CC_{y,t}} = \mathrm{M_{y,t}} \times \mathrm{F_{cp}} \times \mathrm{PR_{de}} \tag{5}$$

where $M_{y,t}$ was assumed to be 1 ton; and PR_{de} was applied based on its H:C ratio: for H:C < 0.4, PR_{de} was 0.74, and for H:C > 0.4, PR_{de} was 0.56 (Adhikari et al., 2024).

TSF was defined as the ratio of fixed C to the sum of volatile matter and fixed C from proximate analysis (Cely et al., 2014). The index proposed by Enders et al. (2012) classifies biochar according to the combination of its volatile matter and O:C ratio. The elemental ratios H:C and O:C, and the volatile matter content, were determined according to section 6.2.1. The H:C values were applied according to criteria established by Budai et al. (2013).

These indices were then compared with the assumed values for C stability, providing insights into the C stability of these biochars.

6.2.8. Statistical analysis

To compare treatments and cropping seasons (fixed effects), along the years the data were analyzed considering an experiment with repeated measures in time using a linear mixed model with the help of PROC MIXED of SAS (SAS Inc., USA). This type of analysis was selected based on previous long-term studies with biochar application (Cornelissen et al., 2018; Griffin et al., 2017; Kätterer et al., 2019). In addition, the model parameters were estimated using the restricted maximum likelihood (REML) method, and the Satterthwaite method was used to calculate the approximate number of degrees of freedom. The variance component structure was selected according to the Akaike's information criterion (AIC) or the Bayesian information criterion (BIC) by the minimum value criterion. The normality of the residuals was checked by the Anderson-Darling test and the non-normal data were transformed by Box-Cox (Box and Cox, 1964) using the convenient lambda value. The means of fixed factors with $p<0.05$ were compared by Tukey or Tukey-Kramer test ($\alpha = 0.05$) for balanced and unbalanced data, respectively.

The sensitivity index was calculated by determining the percentage change in the concentration of the TC and C pool/fraction in soil resulting from a specific treatment, relative to the concentration in the control (Yang et al., 2017).

Differences in WL and thermostability indices between treatments were statistically compared using analysis of variance (ANOVA) in R 4.3.1 (R Core Team, 2023). The curves of R^2 between TML and the C pools/fractions were fitted using generalized additive models with a 95% confidence interval.

6.3. Results

6.3.1. Biochar carbon stability

Table 5 presents a comparative analysis of C stability indices for SSB pyrolyzed at different temperatures, using biochars of distinct feedstocks as reference. The indices evaluated include the H:C ratio, $Cc_{y,t}$, TSF, and Enders' index. All indices consistently classify both SSB300 and SSB500 as having low C stability. In particular, C stability of SSB was inferior to biochar derived from other feedstocks such as hardwood, rice husk and rapeseed, which are classified as moderately to highly stable.

Table 5. Comparative analysis of carbon stability (CS) indices for SSB300 and SSB500, and biochars of other feedstocks

Biochar characteristics/ index	SSB300	SSB500	Assumed values for CS indices	Conclusion	Biochars from other feedstocks[a]
H:C	1.8	1.1	< 0.4 = high CS > 0.4 = low CS	Both SSB: low CS	Hardwood: high CS Rice husk: high CS Rapeseed: high CS
$CC_{y,t}$	13.1	10.6	< 25 = low CS 25 – 50 = moderate CS > 50 = high CS	Both SSB: low CS	Hardwood: high CS Rice husk: moderate CS Rapeseed: moderate CS
TSF	6.5	14.7	< 20 = low CS 21 – 59= medium CS > 60 = high CS	Both SSB: low CS	Hardwood: medium CS Rice husk: high CS Rapeseed: high CS
Enders' index	VM: 43.9 O:C: 2.2	VM: 31.8 O:C: 3.0	VM > 80% = negligible CS VM < 80% and (O:C > 0.2 or H:C > 0.4) = low CS VM < 80% and (O:C < 0.2 or H:C < 0.4) = high CS	Both SSB: low CS	Hardwood: high CS Rice husk: high CS Rapeseed: high CS

SSB300 and SSB500: sewage sludge biochar pyrolyzed at 300°C and 500°C; $CC_{y,t}$: organic C content on a dry weight basis for biochar type t in year y (%); TSF: thermostable fraction (%); VM: volatile matter; a: results from Adhikari et al. (2024) – biochars of hardwood pyrolyzed at 600°C, rice husk at 550°C, and rapeseed at 550°C; Green color represents current accepted analysis methods according to VCS for C credits certification (Verra, 2023).

6.3.2. TC and TN

The application of SSB affected soil TC, TN, and the C:N ratio in this seven-year field experiment ($p<0.05$). Soils amended with SSB300, SSB300+NPK, and SSB500+NPK, regardless of additional mineral fertilization, exhibited consistently significantly higher TC content (28.96, 28.36, and 28.54 g kg^{-1}, respectively) compared to control (26.44 g kg^{-1}) and NPK (26.70 g kg^{-1}) treatments (Figure 9A). SSB treatments had similar TC contents. On average, SSB treatments increased TC by 2.3-9.5% relative to the control, reaching levels comparable to the native Cerrado soil. The temporal dynamics of TC showed that the SSB-amended soils maintained higher TC levels than the control and NPK soils in all seasons, except for SSB500+NPK in the first season.

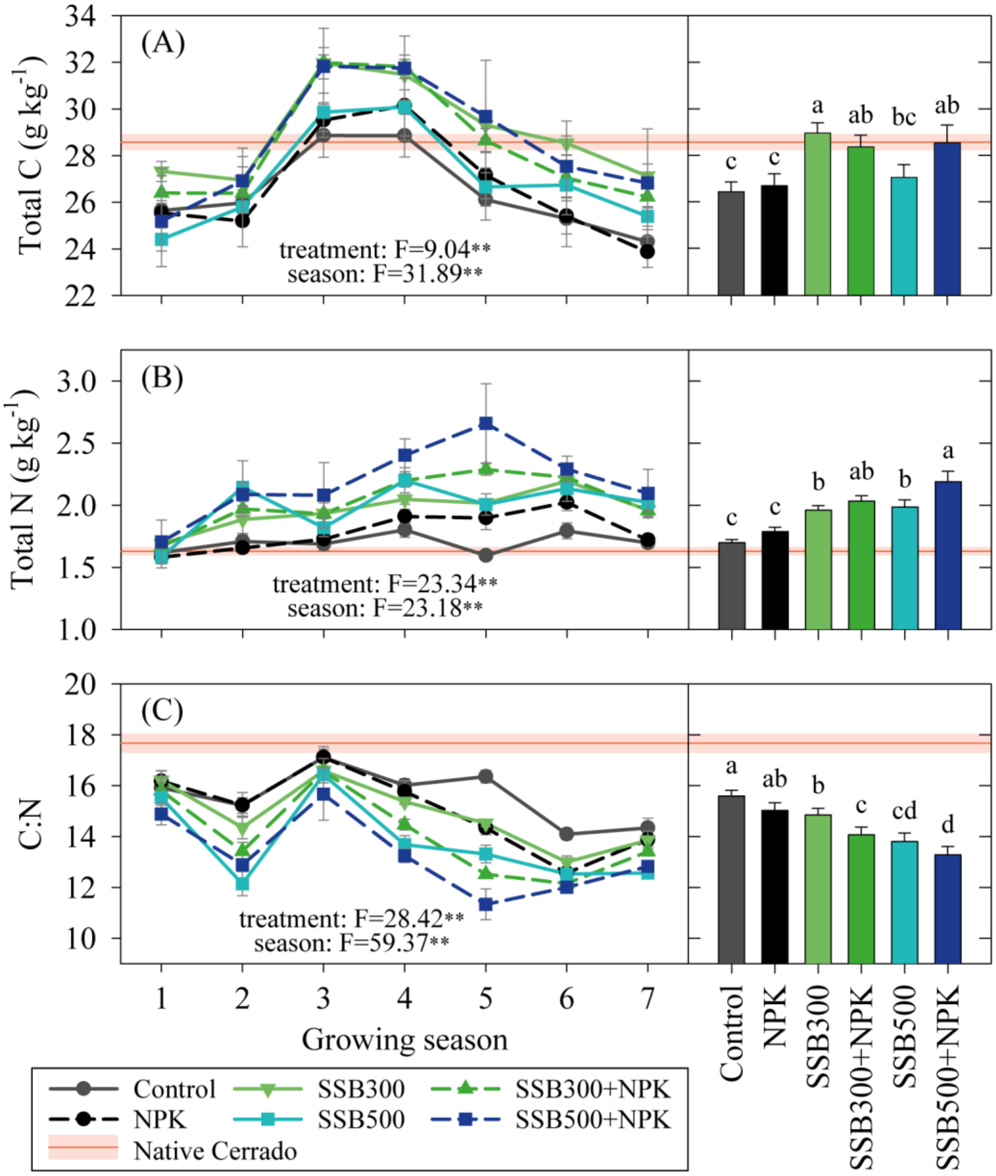


Figure 9. Soil total C content (A), total N content (B) and C:N ratio (C). NPK: mineral fertilizer; SSB300 and SSB500: sewage sludge biochar pyrolyzed at 300°C and 500°C. The bar plot represents the mean of growing seasons. Means followed by a common letter are not significantly different by the Tukey-test ($p < 0.05$). Error bars represent standard error. $^{**}p \leq 0.01$.

Similar trends were observed for TN, with all soils amended with SSB having significantly higher values than control and NPK (Figure 9B). Notably, the TN contents of SSB-amended soils exceeded 1.96 g kg^{-1}, while those of control and NPK soils were only 1.70 and 1.79 g kg^{-1}, respectively. This translates to average increases of TN ranging from 15.3% to 28.8% in SSB-amended soils relative to control. Importantly, no significant difference was found between control and NPK soils in terms of TN content. The SSB application alone resulted in higher TN levels than the exclusive mineral fertilization over the seven years. Furthermore, TN in SSB-amended soils even surpassed the native Cerrado content.

The C:N ratio of the soil was significantly reduced by SSB application, due to the greater proportional increases in TN content than TC (Figure 9C). The lowest average C:N ratio was observed in the soil with SSB500+NPK (13.27). In contrast, the control and NPK soils had similar and higher C:N ratios of 15.58 and 15.02, respectively. Therefore, SSB application led to average C:N reductions ranging from –4.7% to –14.8%, compared to the control. Moreover, the C:N ratio of the control, NPK, and SSB-amended soils was lower than that of the native Cerrado soil.

6.3.2.1. Soil C stock

The SCS under SSB500+NPK treatment was 49.47 Mg ha^{-1}, significantly higher than the control and NPK treatments (Figure 10). This meant a 6.53% increase in SCS compared to the average of these treatments, reaching the same level as the native Cerrado soil. However, applying SSB300, with or without NPK, did not result in significantly higher SCS, despite the significant increases in TC levels.

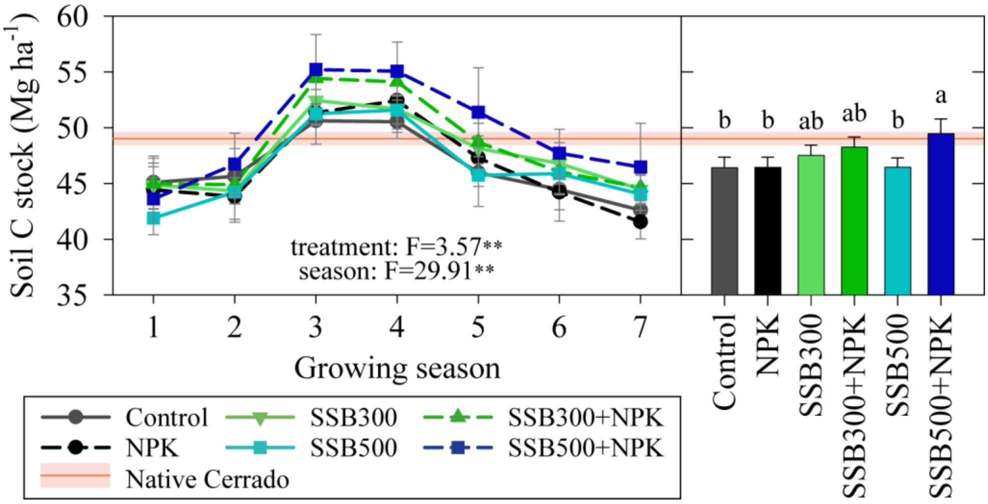


Figure 10. Soil C stock in 0-0.2 m layer. NPK: mineral fertilizer; SSB300 and SSB500: sewage sludge biochar pyrolyzed at 300°C and 500°C. The bar plot represents the mean of growing seasons. Means followed by a common letter are not significantly different by the Tukey-test ($p < 0.05$). Error bars represent standard error. **$p \leq 0.01$.

Figure S2 illustrates the temporal dynamics of SCS by showing the difference in SCS relative to the control over seven seasons. The SCS under NPK varied by up to 1.91 Mg ha^{-1}, but it was unstable over time. It decreased, increased, and then decreased again compared to the control. In contrast, starting from the second season, the SSB500+NPK amendment consistently increased the SCS, from 1.09 to 5.43 Mg ha^{-1}. The only exception was the first season, with an absolute reduction in SCS under SSB500+NPK. Notably, the other biochar treatments, SSB300, SSB500, and SSB300+NPK, only enhanced the carbon accumulation from the third season onwards.

To elucidate how the SCS was affected by time over seven seasons, the results were split into two overlapping periods: the first four seasons, when SSB was applied and had more noticeable effects, and the last four seasons, when only the SSB residual effect was present (Figure S3). The figure also shows the SCS difference across the whole study period. For this, the net SCS balance for each treatment was calculated as the difference between the SCS at the end and the beginning of each period. Over the entire experiment, only SSB500, with or without NPK, had a positive net SCS balance, 2.87 and 2.13 Mg ha^{-1}, respectively (Figure S3A). During the first four seasons, all treatments showed positive net SCS balance, ranging from 5.44 to 11.45 Mg ha^{-1}, with the highest value by SSB500+NPK (Figure S3B). This represents an average increase rate of 2.86 Mg ha^{-1} year^{-1}. However, from the fourth season onwards, there was a general decrease in SCS for all treatments, but more severe for those with NPK, regardless of SSB presence (Figure S3C). The SCS in plots without NPK dropped by –7.20 to –7.93 Mg ha^{-1}, while the plots with NPK showed an even more substantial decline of –8.58 to –10.89 Mg ha^{-1}.

6.3.3. Chemical pools of soil organic matter

6.3.3.1. Oxidizable and non-oxidizable organic carbon

The SSB300 addition increased the average EOOC pool to 23.24 g kg^{-1}, compared to 21.71, 21.99, and 21.92 g kg^{-1} in the control, NPK, and SSB500 treatments, respectively (Figure 11A). This increase even surpassed the EOOC content of native Cerrado soil. The EOOC pool accounted for a substantial portion of the TC, ranging from 78.1% to 82.4% across treatments (Figure 12).

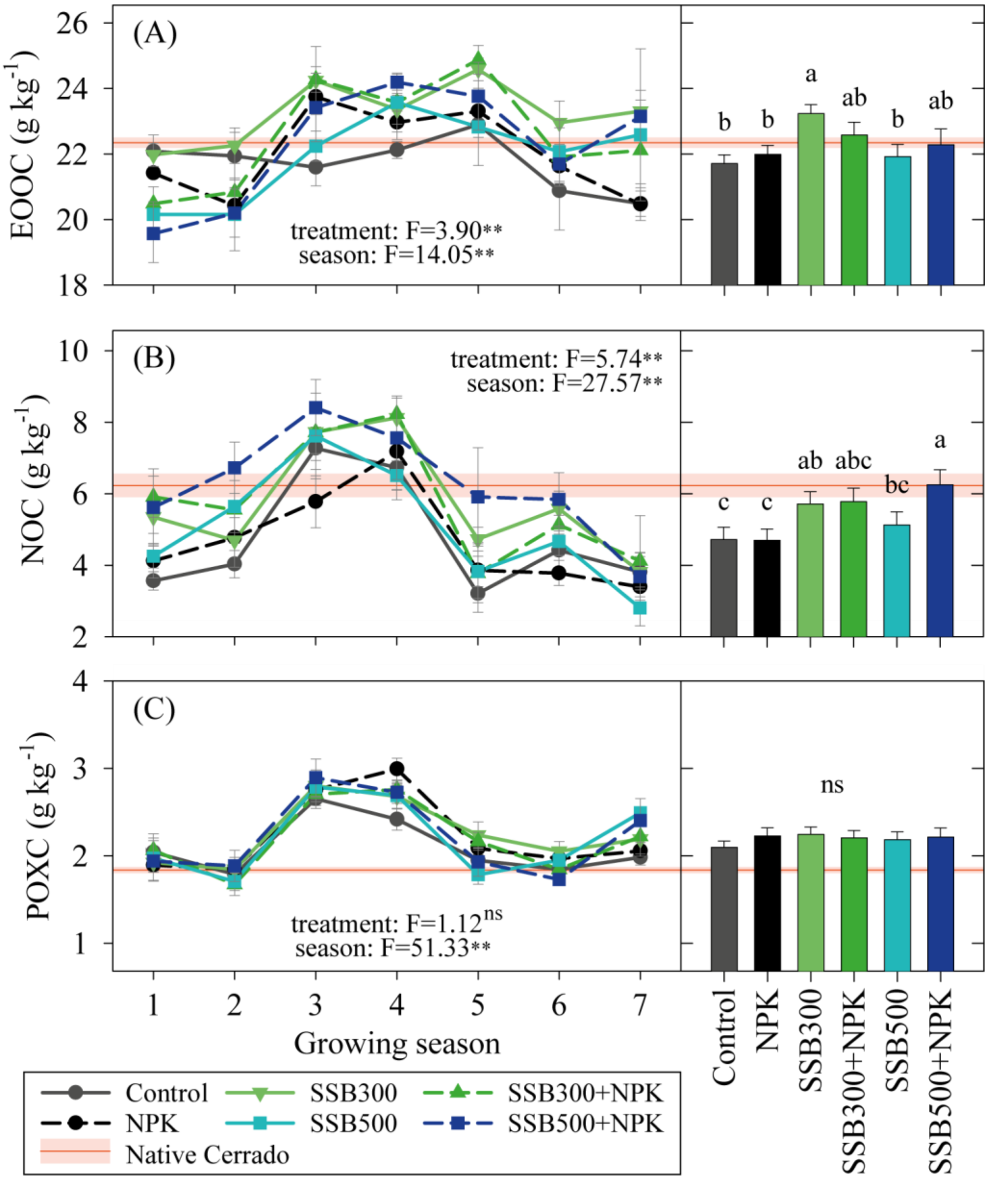


Figure 11. Easily oxidizable organic carbon – EOOC – (A), non-oxidizable carbon – NOC (B) and permanganate-oxidizable carbon – POXC (C). NPK: mineral fertilizer; SSB300 and SSB500: sewage sludge biochar pyrolyzed at 300°C and 500°C. The bar plot represents the mean of growing seasons. Means followed by a common letter are not significantly different (ns) by the Tukey-test ($p < 0.05$). Error bars represent standard error. **$p \leq 0.01$.

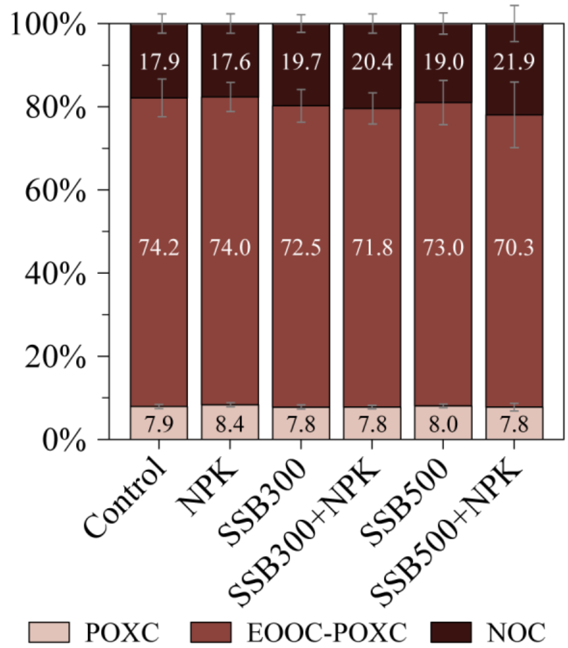


Figure 12. Average permanganate-oxidizable carbon (POXC), easily oxidizable organic carbon minus POXC (EOOC–POXC), and non-oxidizable carbon (NOC) in soil under different treatments. Error bars represent standard error.

Similarly, the SSB amendment positively affected the NOC pool. Both SSB500+NPK (6.25 g kg^{-1}) and SSB300 (5.72 g kg^{-1}) exhibited higher NOC contents than the control (4.72 g kg^{-1}) and NPK (4.70 g kg^{-1}) treatments (Figure 11B). This translated to increases of 32.4% and 21.1%, respectively, compared to the control. Notably, the NOC pool accounted for 19.0–21.9% of TC in the SSB-amended soil, which was higher than the control (17.9%; Figure 12). Additionally, the mean levels of NOC in the SSB300, SSB300+NPK, and SSB500+NPK treatments were comparable to native Cerrado soil, while the control and NPK treatments had lower NOC contents.

In contrast to the EOOC and NOC pools, the POXC pool remained relatively stable across treatments, averaging between 2.10 and 2.25 g kg^{-1} (Figure 11C). The only notable effect was a seasonal variation, consistent with other fractions/pools observations. On average, the POXC pool ranged 7.8–8.4% of TC. Interestingly, the control and NPK treatments had the highest EOOC-POXC percentage (74.2 and 74.0%, respectively), while the SSB-amended soil had a lower ratio (70.3–73.0%).

6.3.3.2. Humic substances

Analysis of humic substances revealed distinct responses to the different treatments. FA content remained unchanged across treatments, averaging around 6 g kg^{-1}, similar to the control or NPK (Figure 13A). The proportion of FA within the total humic substances remained consistent across treatments, ranging from 27.4 to 28.7% (Figure 14). However, compared to the native Cerrado soil, FA content was lower in all treatments.

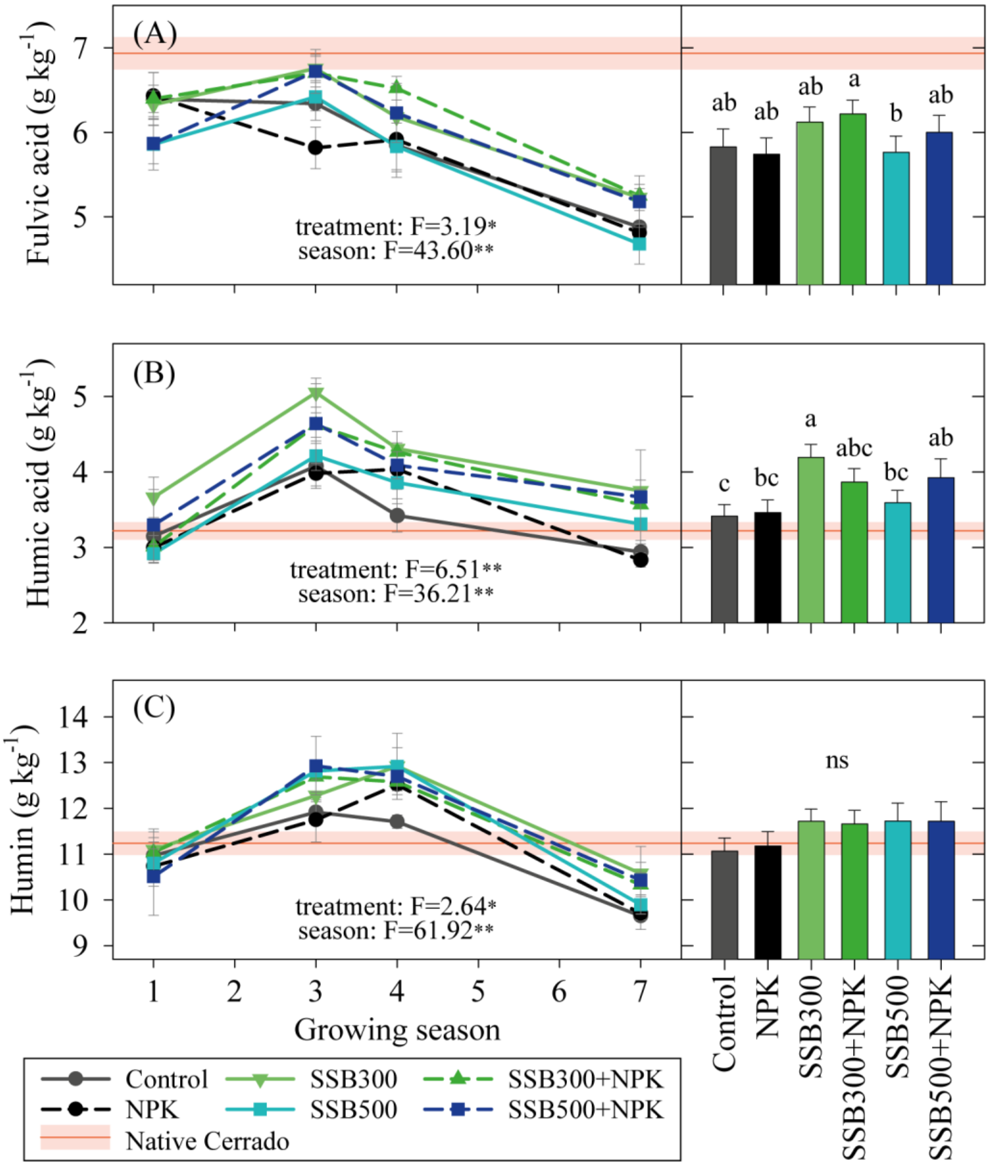


Figure 13. Soil fulvic acid (A), humic acid (B), and humin (C) contents. NPK: mineral fertilizer; SSB300 and SSB500: sewage sludge biochar pyrolyzed at 300°C and 500°C. The bar plot represents the mean of growing seasons. Means followed by a common letter are not significantly different (ns) by the Tukey-test ($p < 0.05$). Error bars represent standard error. *$p \leq 0.05$, **$p \leq 0.01$.

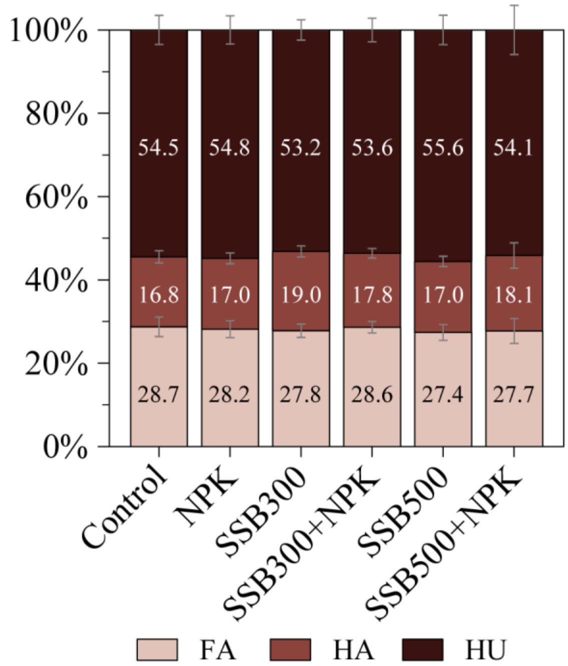


Figure 14. Average percentage of fulvic acid (FA), humic acid (HA), and humin (HU) in soil under different treatments. Error bars represent standard error.

Conversely, HA content displayed contrasting behavior. Both SSB300 (4.19 g kg^{-1}) and SSB500+NPK (3.93 g kg^{-1}) amendments significantly increased HA content compared to the control (3.41 g kg^{-1}; Figure 13B). These increases corresponded to 22.8% and 14.9%, respectively, resulting in HA values exceeding those of the native Cerrado soil. However, the proportion of HA within the total humic substances remained relatively constant across treatments, ranging from 16.8% to 19.0% (Figure 14).

Humin content was not affected by treatments (11.07–11.72 g kg^{-1}), but a slight upward trend was observed in SSB-amended soils, with an average increase of 5.8% compared to the control and NPK (Figure 13C). Despite this tendency, humin content across all treatments remained comparable to the native Cerrado soil. Notably, the proportion of humin within the total humic substances again showed minimal variation, ranging from 53.2% to 55.6% (Figure 14).

6.3.4. Physical fractions of soil organic matter

On average, SSB300, SSB300+NPK, and SSB500+NPK application increased the soil PC content to 6.16, 5.76 and 6.28 g kg^{-1}, respectively, which was significantly higher than the control (5.10 g kg^{-1}; Figure 15A). These treatments had 20.8%, 13.1%, and 23.1% higher PC, respectively. PC comprised a small proportion of soil TC (18.9–21.7%), while MAC constituted the largest (78.3–81.1%; Figure 16). Unlike PC, MAC content was not affected by SSB treatments (Figure 15B). The PC and MAC contents in SSB300, SSB300+NPK, and SSB500+NPK-amended soils had similar values to those of the native Cerrado.

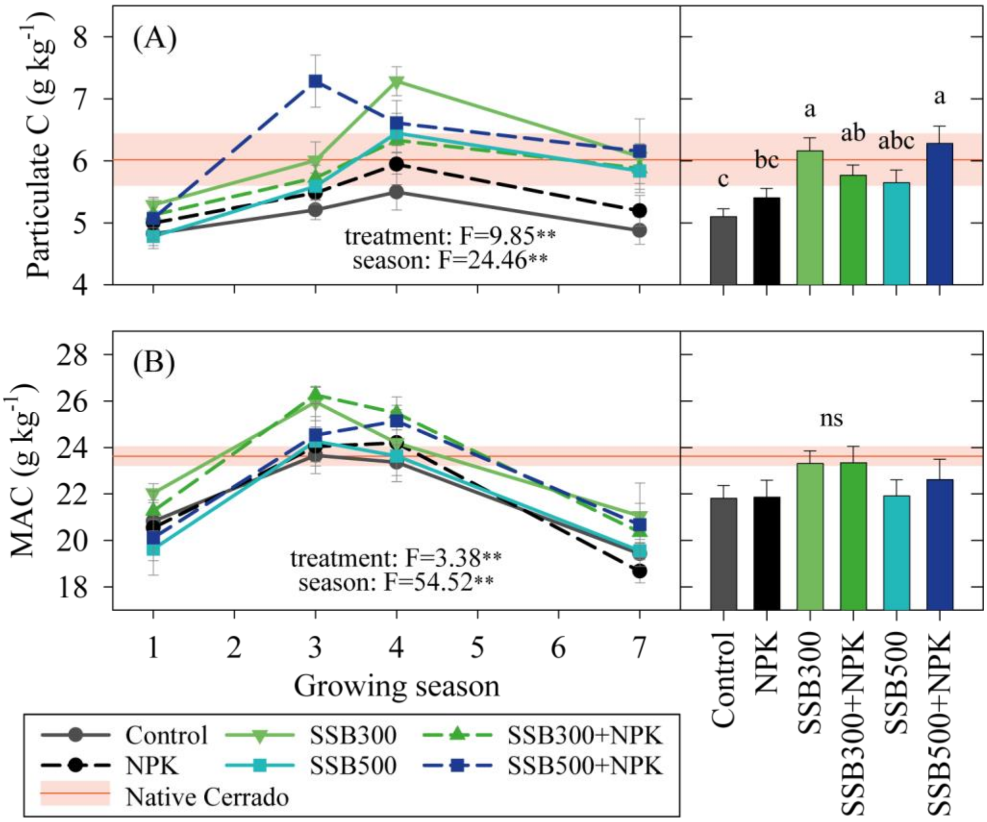


Figure 15. Particulate carbon (A) and mineral-associated carbon – MAC (B). NPK: mineral fertilizer; SSB300 and SSB500: sewage sludge biochar pyrolyzed at 300°C and 500°C. The bar plot represents the mean of growing seasons. Means followed by a common letter are not significantly different (ns) by the Tukey-test ($p < 0.05$). Error bars represent standard error. **$p \leq 0.01$.

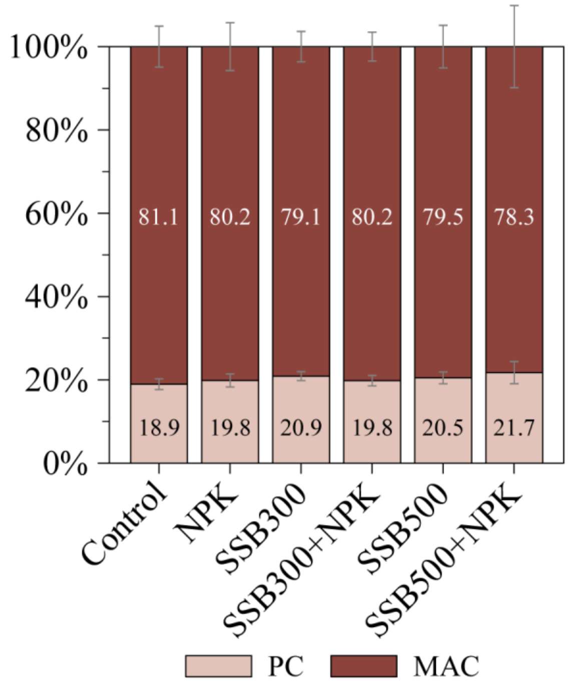


Figure 16. Average percentage of particulate carbon (PC), and mineral-associated carbon (MAC) in soil under different treatments. Error bars represent standard error.

6.3.5. Thermal stability of soil organic matter

None of the assessed WL or index, regardless of whether they were associated with labile or recalcitrant compounds, was affected by the soil amendments (Table 6).

Table 6. Comparison of TGA-derived weight loss (WL) and indices across treatments using ANOVA

WL or index	p-value	Reference	WL or index	p-value	Reference
$WL_{250-350}$	0.143	[1]	WL_{V}	0.379	[9]
$WL_{400-550}$	0.843	[1, 2]	$WL_{301-388}$	0.254	[10]
WL_{I}	0.594	[1]	$WL_{424-448}$	0.527	[10]
$WL_{200-400}$	0.169	[2, 3]	$WL_{507-570}$	0.768	[10]
$WL_{400-600}$	0.838	[3]	$WL_{200-350}$	0.170	[11]
WL_{II}	0.586	[3]	$WL_{350-500}$	0.537	[11]
$WL_{180-600}$	0.330	[4]	$WL_{180-325}$	0.216	[11]
$WL_{180-410}$	0.184	[4]	$WL_{325-540}$	0.626	[11]
$WL_{130-180}$	0.421	[4]	$WL_{295-344}$	0.221	[12]
$WL_{410-600}$	0.861	[4]	$WL_{414-473}$	0.630	[12]
WL_{III}	0.556	[4]	WL_{VI}	0.295	[12]
$WL_{180-310}$	0.267	[5]	$WL_{230-330}$	0.190	[13]
$WL_{310-450}$	0.334	[5]	$WL_{330-430}$	0.352	[13]
WL_{IV}	0.506	[5]	$WL_{430-530}$	0.899	[13]
$WL_{200-380}$	0.155	[6, 7]	$WL_{550-600}$	0.221	[2]
$WL_{380-475}$	0.565	[6, 7]	$WL_{338-341}$	0.506	[14]
$WL_{475-650}$	0.854	[6, 7]	$WL_{439-456}$	0.609	[14]
$WL_{130-280}$	0.117	[8]	$WL_{539-560}$	0.951	[14]
$WL_{280-520}$	0.257	[8]	$WL_{200-280}$	0.177	-
$WL_{295-307}$	0.263	[9]	$WL_{280-360}$	0.163	-
$WL_{436-469}$	0.670	[9]	$WL_{360-550}$	0.721	-

WL_{X-Y} refers to weight loss difference between the temperature range of X and Y°C. List of references in Appendix.

Examining of R^2 values between TML and different soil C pools and fractions showed a maximum value within the temperature range of 360 to 390°C (Figure 17). Notably, the peaks for different fractions overlapped surprisingly. However, this trend was not observed for POXC, which exhibited a peak at a higher temperature of 430°C. Therefore, no clear relationship was found between TML and the measured labile and recalcitrant soil C pools/fractions across the entire temperature spectrum. Despite having a peak at a higher temperature, POXC also exhibited the lowest maximum R^2 (0.32) compared to the other fractions. In contrast, TC had the highest R^2 value (0.74), with all other fractions/pools showing lower R^2 values. Notably, no significant peaks were observed for any of the fractions above 600°C.

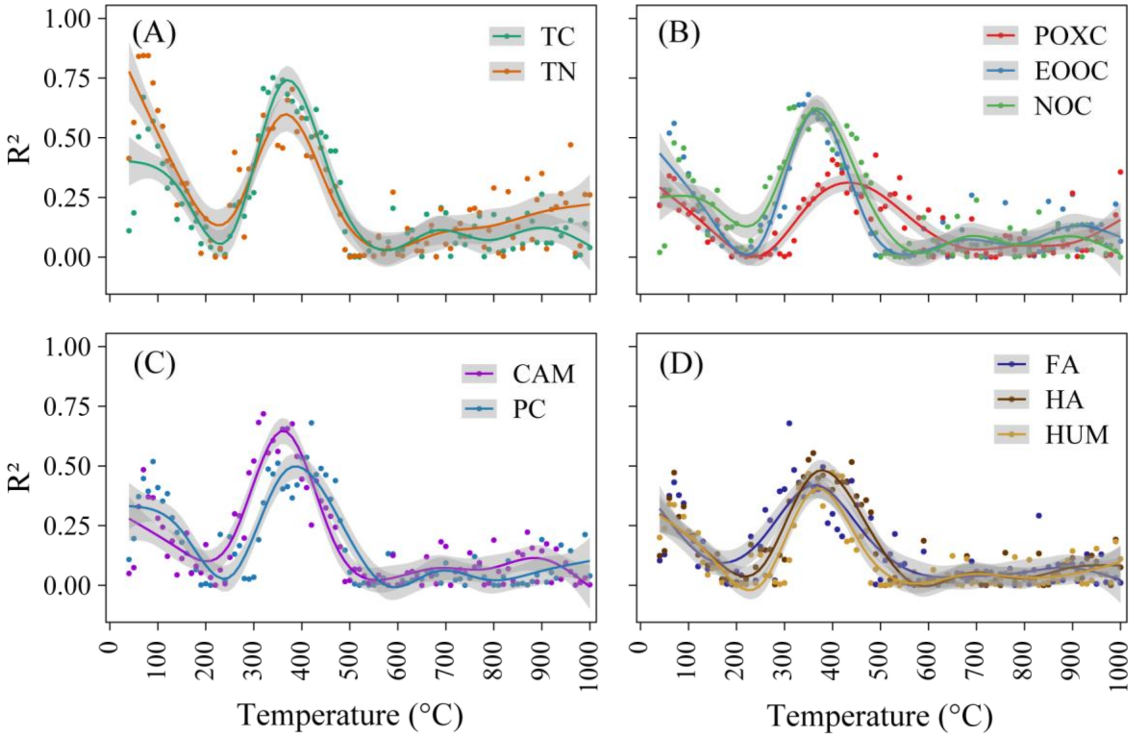


Figure 17. Coefficient of determination (R^2) between TML and the C pools/fractions. Gray band represents the 95% confidence interval.

6.3.6. Magnetic susceptibility of biochar-amended soils

The application of SSB300+NPK yielded the lowest magnetic susceptibility (118.18 $\times 10^{-8}$ m^3 kg^{-1}), followed closely by SSB500 (118.59 $\times 10^{-8}$ m^3 kg^{-1}; Figure 18). These values were significantly lower than the control treatment (122.04 $\times$ 10^{-8} m^3 kg^{-1}). The other treatments, including SSB300 and SSB500+NPK, displayed intermediate susceptibility values, similar to both the control and NPK treatments ($p > 0.05$).

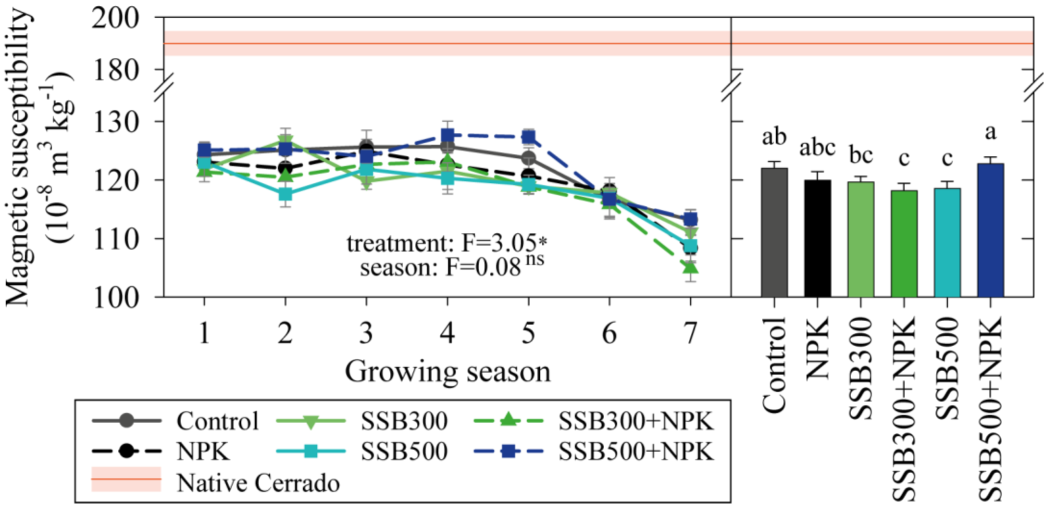


Figure 18. Magnetic susceptibility. NPK: mineral fertilizer; SSB300 and SSB500: sewage sludge biochar pyrolyzed at 300°C and 500°C. The bar plot represents the mean of growing seasons. Means followed by a common letter are not significantly (ns) different by the Tukey- ($p < 0.05$). Error bars represent standard error. *$p \leq 0.05$.

The magnetic susceptibility was not affected by the growing seasons ($p > 0.05$). Interestingly, all treatments, including the control and NPK, exhibited lower susceptibility (189.97×10^{-8} m^3 kg^{-1}) compared to the native Cerrado soil used as a reference.

6.3.7. $\delta^{13}C$ and $\delta^{15}N$

No significant change was observed on δ^{13}C between treatments, ranging from –18.43 to –19.34‰ (Figure 19A). However, δ^{15}N showed a distinct response to biochar application, particularly with SSB500 (Figure 19B). Soils amended with SSB500, alone (8.24‰) or combined with NPK (7.96‰), exhibited significantly higher δ^{15}N values compared to the control (6.68‰).

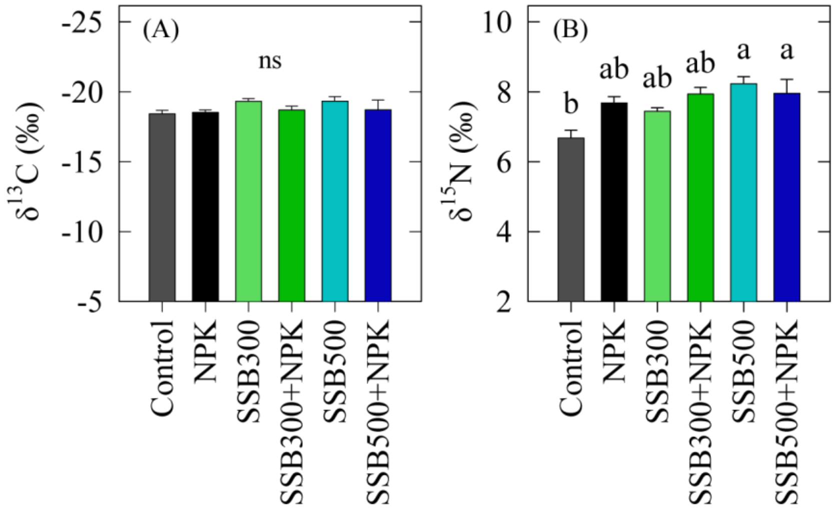


Figure 19. $\delta^{13}C$ (A) and $\delta^{15}N$ (B). Means followed by the same letter are not significantly (ns) different by the Tukey-test ($p < 0.05$). Error bars represent standard error.

6.3.8. Crop yield

Across the seven growing seasons, applying SSB, with or without NPK, increased both grain yield and shoot biomass compared to the control treatment ($p < 0.05$; Figure 20). This positive effect was consistent from the second to the sixth growing season. On average, plots amended with SSB300 and SSB500 achieved annual grain yields of 6.68 and 6.32 Mg ha^{-1}, respectively, representing increases of 49.8% and 41.7% compared to the control (4.46 Mg ha^{-1}). Similar trends were observed for shoot biomass, with SSB300 and SSB500 treatments yielding 4.99 and 5.01 Mg ha^{-1} annually, translating to increases of 32.7% and 33.3% over the control (3.76 Mg ha^{-1}).

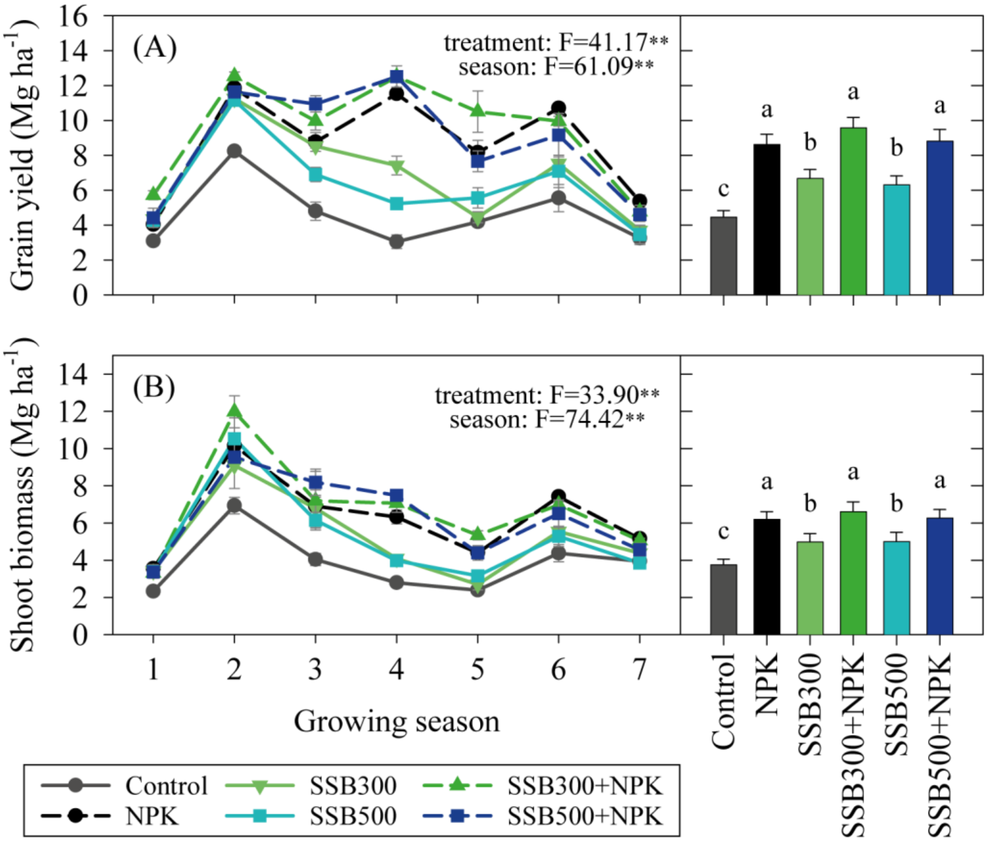


Figure 20. Grain (A) and shoot biomass (B) yields. NPK: mineral fertilizer; SSB300 and SSB500: sewage sludge biochar pyrolyzed at 300°C and 500°C. The bar plot represents the mean of growing seasons. Means followed by a common letter are not significantly different by the Tukey-test ($p < 0.05$). Error bars represent standard error.

However, even higher grain yields were observed in plots receiving NPK fertilizer alone or combined with SSB. The average grain yield under NPK alone was 8.63 Mg ha^{-1}, with the addition of SSB300 and SSB500 it was 9.58 and 8.82 Mg ha^{-1}, respectively. These represent increases of 93.4%, 114.9%, and 97.8% compared to the control.

Interestingly, the grain yield of SSB-only treatments (without NPK) exhibited a sharp decline from the fourth season onwards, diverging from the yield of NPK-fertilized plots and eventually returning to control levels by the seventh season. This trend was further reflected in the cumulative grain yield, whereby SSB300+NPK outperformed both the control (31.88 Mg ha^{-1}) and NPK alone (60.66 Mg ha^{-1}), reaching 66.35 Mg ha^{-1} (Figure S4A). This translates to a 108.11% increase in grain yield compared to the control and a 9.37% advantage over NPK alone across the entire trial period.

While the cumulative shoot biomass yield in NPK+SSB-amended treatments did not differ significantly from NPK alone (43.69 Mg ha^{-1}), it remained significantly higher than all other treatments (Figure S4B). Notably, the addition of SSB300 and SSB500 increased the cumulative shoot biomass by 9.07 and 9.60 Mg ha^{-1}, respectively, compared to the control. This positive impact was further amplified in the SSB300+NPK and SSB500+NPK plots, whereby shoot biomass yield increased by 19.73 and 16.61 Mg ha^{-1} compared to the control.

6.4. Discussion

6.4.1. Unveiling the effects of SSB on TC and TN, and soil carbon stock (SCS)

This seven-year field study demonstrates the effectiveness of SSB as a soil amendment for enhancing soil C sequestration and overall soil quality. Soils amended with SSB, either alone or combined with mineral fertilizer (NPK), consistently exhibited higher TC and TN levels than the control and NPK-only treatments.

While SSB was primarily designed to address nutritional needs (Faria et al., 2018), the sustained rise in TC observed in most SSB-amended soils (except SSB500) points to the long-term stability and persistence of the C added through SSB and crop residues, potentially reducing GHG emissions (Ibrahim et al., 2017). Notably, the TC levels in these amended soils matched or surpassed those of the native Cerrado, highlighting the potential of SSB to reverse soil C losses from land-use changes. These findings align with previous research emphasizing the effectiveness of biochar in promoting soil C sequestration (Kätterer et al., 2019; Shi et al., 2021). Earlier studies with SSB found that the TC increased by 4.28% on average for each percent of C applied via SSB relative to the control (Mierzwa-Hersztek et al., 2018; You et al., 2019). However, the present study observed a lower increase of 0.58% to 2.96%.

The higher TC content in SSB-amended soils compared to NPK suggests additional benefits beyond mineral fertilization, particularly in enhancing soil C content. Fluctuations in SCS under NPK treatment suggest an inherent lack of stability in the C sequestration potential of conventionally fertilized fields (Figure S2). This highlights the potential limitations of relying solely on mineral fertilizers for long-term C sequestration strategies in agricultural soils.

The positive influence of SSB500+NPK on C sequestration highlights the synergistic effect of combining SSB and mineral fertilization in promoting C accumulation within the soil, overcoming the limitation of SSB in supplying potassium to plants (Chagas et al., 2021). While biomass yield was comparable between SSB+NPK and NPK-amended soils (Figure S4B), the SSB combination proved more effective in augmenting soil TC and SCS. This suggests that SSB was the primary factor responsible for the observed increase in SCS, rather than the comparable biomass input.

The analysis of C stability indices (Table 5) reveals that both SSB exhibited low C stability across all evaluated indices, which explains the relatively modest increase in TC and

SCS observed in this field trial. These low stability indices suggest that the C in SSB is more prone to microbial decomposition and less likely to persist in the soil over long periods, leading to reduced C sequestration. The high ash content and lower pyrolysis temperatures employed reduce the potential of SS for soil C sequestration (McBeath et al., 2015). Utilizing a lignocellulosic feedstock with lower ash content in a co-pyrolysis process could potentially enhance the SSB performance on increasing the soil C sequestration. Adhikari et al. (2024) reported feedstock as the primary factor influencing C structural stability, with SSB pyrolyzed at 550 and 700°C exhibiting lower C sequestration potential due to higher O:C ratio, lower recalcitrance index, higher ash, and lower C content. Additionally, Nair et al. (2023) observed that co-pyrolysis of SSB with plant materials increased carbonization and maximized C sequestration potential, requiring maximum temperature pyrolysis for over an hour, contrasting the 30-minute duration used in the present study, potentially limiting carbonization and C sequestration.

The reduction in the plant biomass yield in soil amended with biochar alone from the fourth growing season onwards probably occurred due to the nutritional limitations of SSB. This led to a decoupling from the TC level of NPK-treated soils and approached the control TC level. Consequently, the C contribution to the soil became limited, and the SCS remained relatively constant thereafter compared to the baseline level of the control.

An immediate increase in SCS was anticipated in the initial two growing seasons due to C contribution from SSB application. However, a net reduction in SCS was observed during the same period, indicating a positive priming effect, which later transitioned to negative priming (Figure S2). This aligns with a meta-analysis that identified experimental time and biochar C:N ratio as the key factors influencing the priming effect in biochar-amended soils (Ding et al., 2018).

Interestingly, soils receiving SSB effectively accumulated C only from the third growing season onwards. This delayed increase corroborates previous observations of a lag in the manifestation of biochar's effects on other soil properties (Chagas et al., 2021; Cornelissen et al., 2018). The initial delay in C accumulation may be attributed to the time required for recovering the degraded area, particularly in correcting soil acidity and re-establishing soil balance. Furthermore, the high shoot biomass yield in SSB-amended soils likely contributed indirectly to the observed effects after the third growing season, as the plant residues provided continuous organic matter inputs to the soil.

Seasonal variations in TC over multiple growing seasons are common (Wuest, 2014; Yang et al., 2018) and may be linked to changes in climate, collection time, collection depth, among other factors. Regarding this, the more pronounced decline in SCS between the 7th and 4th growing seasons in soils receiving NPK (Figure S3C) suggests a potential negative interaction between SSB and mineral fertilizer on long-term soil C stability. This interaction may be attributed to the readily available nutrients provided by mineral fertilization enhancing soil microbial biomass, consequently increasing the net mineralization of SOM. Moreover, the necessary change of the corn hybrid sown in the trial could have contributed to the observed decline in SCS due to differences in shoot biomass yield and subsequent soil C input. The interplay of these factors, including mineral fertilization, microbial activity, and plant residue inputs, may have collectively influenced the long-term soil C dynamics.

These findings have significant implications for farmers and policymakers. For farmers, SSB application presents an opportunity to increase soil C stock, potentially improving soil health, fertility, crop yields, and profitability. Additionally, the enhanced soil C sequestration potential through biochar application could enable farmers to generate C credits, providing an additional revenue stream (Salma et al., 2024). Concurrently, policymakers can promote the use of SSB as a sustainable soil amendment, to enhance soil health and C sequestration and contribute to climate change mitigation efforts and bolster the resilience of agricultural systems. Furthermore, developing C credit markets and incentives for biochar-based soil amendments could encourage their widespread adoption.

The elevated TN content in SSB-amended soils indicates the ability of SSB to enrich soil N reserves, surpassing native Cerrado levels. Despite 1260 kg ha^{-1} of N applied as mineral fertilizer, the exclusive NPK application did not significantly increase TN compared to the control. Remarkably, the average TN increase in SSB-amended soils (428–492 kg ha^{-1} N) was achieved with a lower actual N input from SSB (690 and 990 kg ha^{-1} N for SSB300 and SSB500, respectively). This highlights an enhanced long-term N-use efficiency in SSB-amended soils (Figueiredo et al., 2021). Therefore, SSB presents a viable alternative or complementary N source to synthetic fertilizers, potentially increasing crop yields and indirectly enhancing soil C stocks while reducing dependence on mineral fertilizers.

The decrease in the C:N ratio in SSB-amended soils contrasts previous findings of increased C:N ratio following SSB amendment (You et al., 2019). This discrepancy may be attributed to SSB characteristics, pyrolysis conditions, soil type, or environmental factors. Nevertheless, the observed decrease suggests that SSB application may promote a favorable microbial environment, facilitating organic matter breakdown and mineralization, ultimately enhancing nutrient availability for plant growth. Simultaneously, the low C:N ratio may also explain the lower long-term C sequestration capacity of SSB compared to biochars from other feedstocks with higher C:N ratios and more recalcitrant C forms.

6.4.2. Effects of SSB on soil C pools

Although there was a significant increase in SCS, it is important to determine if there has been a change in the quality of SOM by assessing the soil C pools. Multiple methods of fractionation and quantification of soil C pools were performed for this purpose.

6.4.2.1. Chemical pools of SOM

The long-term increase in TC after SSB amendment prompted an analysis of the changes in different soil C pools, which are relevant indicators of SOM quality and dynamics. To our knowledge, this is the first long-term study to assess the effects of SSB on soil C pools. Previous studies used biochars from other feedstocks, such as wood (Giannetta et al., 2024; Leal et al., 2019), greenwaste (Abbas et al., 2019; Paetsch et al., 2017), animal manure (Jarosz et al., 2020), and crop residues (Yang et al., 2018).

The chemical characterization of soil C pools revealed that SSB application affected the quality and dynamics of SOM in the long term. The analysis focused on three distinct pools: EOOC, POXC, and NOC, which represent different degrees of stability and reactivity of soil C. The results showed that the increase of TC content in SSB-amended soils mainly occurred due to the accumulation of NOC. This recalcitrant and non-labile pool (Chan et al., 2001) accounted for a larger proportion of TC under SSB treatments than under the control and NPK treatments (Figure 12), indicating a shift in the soil C stabilization mechanisms. The increase in NOC was more pronounced under SSB500+NPK and SSB300, which also had NOC contents similar to those of native Cerrado soil. This suggests that SSB application restored the soil C sequestration potential, which was reduced by soil degradation (Figure 8). The NOC pool consists mainly of biochar-derived C and native black-C, which can resist long-term microbial and chemical degradation (Kopecký et al., 2021). The NOC pool can also interact with soil minerals, especially clay, and form stable organo-mineral complexes, further protecting the C from decomposition (Dwivedi et al., 2019).

The EOOC pool, representing the C that can be naturally mineralized (Nelson and Sommers, 1996), also increased significantly under SSB300, but not SSB500+NPK. This agrees with previous studies that reported a EOOC increase after SSB application in short-term soil incubations of up to 24 months (Gonzaga et al., 2020; Jafari Tarf et al., 2022). The lower pyrolysis temperature of SSB300, which resulted in a higher content of volatile materials (43.90%; Table 2), may have contributed to this increase. In contrast, our initial evaluation of this field trial over two cropping seasons showed a transient EOOC increase only under SSB300+NPK (Figueiredo et al., 2019c). In a longer timeframe, the response changed: SSB300, not SSB300+NPK, enhanced EOOC. Therefore, the interaction between SSB and NPK fertilization influenced the EOOC pool. This was due to the lower C:N ratio in soil from mineral fertilization (Figure 9C), which potentially increased the turnover rate of this C pool.

The POXC pool, which reflects the active and labile pool that is associated with soil aggregation and nutrient cycling (Wander, 2004), did not differ significantly between treatments. This suggests that long-term POXC levels rely more on crop inputs and native SOM than SSB amendments. POXC may also fluctuate with climatic conditions and plant growth, affecting microbial activity and C fluxes (Lehmann et al., 2020; Yang et al., 2018). Since soil samples were collected after harvest, most of the POXC from SSB may have been mineralized early, not detected in the analysis. This agrees with Figueiredo et al. (2019b), who reported that the same SSB300 used in this study increased C-CO_2 emissions by 60% within 127 days. Our initial study (Figueiredo et al., 2019c) also observed a POXC increase under SSB300 in the first two growing seasons, but this effect did not persist over time. This indicates that the POXC increase under SSB300 came from some labile C fractions from SSB, such as carbohydrates and aliphatic compounds, which are susceptible to oxidation by permanganate (Weil et al., 2003). However, this pool may have been quickly depleted, returning POXC to control levels. While other field studies observed POXC increases with biochar (Wu et al., 2021; Yang et al., 2018), they used different feedstocks, pyrolysis temperatures, and doses, highlighting the influence of these properties on C stability and availability. Moreover, the soil type, climate, and cropping system can also modulate POXC by altering the soil moisture, temperature, pH, and microbial community (Culman et al., 2012).

The EOOC–POXC difference reflects the soil's capacity to store and release C. While higher values in control and NPK treatments might suggest increased C stabilization by SSB, it is important to note that Figure 12 shows relative values to TC, while both EOOC and NOC reserves increased in absolute terms (Figure 11). Therefore, no trade-off between labile and stable C pools was evident. As POXC remained constant across treatments, the EOOC-POXC pattern followed that of EOOC.

6.4.2.1.1 Humic substances

The findings show contrasting responses within the humic fraction, warranting further investigation into the mechanisms and implications for soil C stabilization. HA and FA are the most active players in soil humic substances, influencing soil structure and properties (Tan, 2014). While FA exhibited remarkable stability across treatments, mirroring previous findings (Jarosz et al., 2020; Leal et al., 2019), HA displayed a significant response to SSB amendments, particularly SSB300. This aligns with observations in other biochar studies (Amoakwah et al., 2020; Li et al., 2015).

Several factors explain the divergent behavior of HA compared to FA. Firstly, FA's inherently lower molecular weight and higher susceptibility to microbial degradation (Gramss et al., 1999) likely account for its long-term stability regardless of treatment. Secondly, biochar harbors pre-existing HA due to the thermochemical transformation during pyrolysis (Jin et al., 2018). Additionally, its porous structure and large surface area provide a favorable environment for HA formation but also actively stimulate and accelerate the humification process (Cybulak et al., 2021). Furthermore, recent study suggest that compared to FA, HA in the superficial soil layer demonstrates greater sensitivity to organic amendments (Feng et al., 2024). This may be because the HA has a higher affinity and binding capacity to form stable organo-mineral complexes with clay and metal oxides in the soil compared to the more soluble and mobile FA (Tan, 2014). SSB may enhance the HA-mineral interactions by providing more HA-like compounds and increasing soil pH, favoring organo-mineral complexes formation (Lehmann and Kleber, 2015), protecting HA from degradation and increasing the soil C sequestration potential. This explains why HA contributes more significantly to changes in SOM than FA in the current study. Interestingly, while FA quantity remained unaltered, applying organic amendments like SSB can still enhance the aromaticity and molecular weight of FA to a greater extent than HA (Feng et al., 2024), which contributes to the stability of the C in the FA fraction.

While the total content of humic substances increased with SSB amendments, their relative proportions within the soil remained stable across treatments. This indicates that SSB additions primarily influenced the total quantity of humic substances, potentially benefitting soil quality and crop performance (Rahim et al., 2024). This aligns with similar observations by Aydin et al. (2020), who reported no changes in FA and HA proportions under low biochar application rates. Notably, this stability in proportions does not guarantee maintained quality. For instance, González Pérez et al. (2004) found cultivated areas (with frequent crop residue inputs) had less aromatic, potentially less stable humic acid, despite higher carbon content, compared to uncultivated areas. This highlights the importance of examining both quantity and quality of humic substances for a comprehensive understanding of their long-term impacts on soil health.

Although SSB amendments did not alter humin content, this observation offers valuable insights, indicating that the SSB had a minimal impact on humin formation or stability. This aligns with expectations, as humin accounts for more than half of soil C and is resistant to changes, longer study periods or more sensitive techniques might be necessary to detect subtle changes. Furthermore, the predominance of humin in clay soils (Lima et al., 2010) such as the one used in this study (Table 3), suggests that the soil is relatively stable and has a high potential to store C. Additionally, the relatively low pyrolysis temperatures and low fixed C content of the SSB (Table 2) likely limited their potential contribution to soil humin.

6.4.2.2. Physical fractions of SOM as affected by SSB amendment

SOM carbon was also fractionated into PC and MAC based on particle size (von Lützow et al., 2007). PC, composed of plant residues, microbial and microfaunal debris, provides readily available C and nutrients for soil biota. Conversely, MAC, protected by association with soil minerals, contributes to a long-term C storage (Christensen, 2001).

The sensitivity of PC to the soil management reflects the dynamics of fresh organic inputs and decomposition processes (Luo et al., 2020). While constituting a small proportion of soil TC, PC plays a crucial role in soil fertility and C sequestration. The PC increase in SSB-amended soil can be attributed to several synergistic factors. Firstly, biochar can directly contribute to the PC fraction by adding organic material to the soil surface (Shi et al., 2021). Its inherent C content and the formation of new soil aggregates around biochar particles physically protect organic matter from decomposition (Sun et al., 2023), promoting its accumulation as PC. Secondly, SSB indirectly stimulates PC accumulation through its positive effects on plant growth (Chagas et al., 2021) and microbial activity (Chagas et al., 2022). Increased plant biomass, particularly root exudates, provides readily available C for soil microbes, enhancing their activity and necromass production (Kalu et al., 2024). This microbial-derived organic matter further contributes to the PC fraction. Additionally, biochar may adsorb dissolved organic C from the soil solution, including root exudates, into its porous structure (DeCiucies et al., 2018), effectively storing it within the PC fraction for long-term. These combined mechanisms explain the observed rise in PC content in SSB-amended soils.

The resilience of MAC to changes in soil management reflects the complex dynamics of soil C fractions (Lehmann et al., 2020). MAC dynamics are primarily controlled by climate and soil-properties (Luo et al., 2020), both of which are homogeneous along the experimental field. The inherent high clay content and surface area of the local soil (Table 3) facilitate strong physical and chemical interactions with SOM, contributing to MAC formation and stabilization. Consequently, MAC constitutes a large proportion of soil TC, requiring substantial SSB inputs or longer-term studies to detect significant changes. This pre-existing high MAC content might have limited the potential for further increases through SSB application (Luo et al., 2020). Additionally, the effect of biochar on MAC is also influenced by various factors, including feedstock type, pyrolysis temperature, soil properties, and study duration, as demonstrated by previous research (Giannetta et al., 2024; Shi et al., 2021).

The similarity in PC and MAC contents between SSB-amended soils and the native Cerrado suggests that SSB application did not alter the natural soil C dynamics. These results contrast with those of Schellekens et al. (2023), who found that vegetation composition, water availability and wildfires are key drivers of soil C fractions in Brazilian Cerrado. Notably, the comparable PC levels between SSB-amended soils and the native Cerrado indicate that SSB has the potential to restore soil C lost due to land-use change and intensive agricultural practices. This highlights the promising role of SSB as a sustainable amendment for improving soil health.

6.4.2.3. Soil carbon pools affected by SSB addition: advantages and limitations of different analytical methods

The investigation into the effects of SSB amendments on soil C pools and fractions unveiled an intricate interplay between various analytical approaches. While chemical characterization indicated an increase in the stable pool (NOC) under specific treatments (SSB300 and SSB500+NPK), this trend was not reflected in the non-labile pools obtained through humic substances (humin) and physical fractionation (MAC). This apparent contradiction can likely be attributed to the substantial differences in the magnitude of these fractions. The labile pools (POXC, FA) did not respond clearly to the treatments.

It is important to consider the influence of the chosen fractionation method when interpreting changes in soil C quality. Sherrod et al. (2019) reported a moderate correlation ($r = 0.49$) between HA and PC, as slow-cycling pools, in contrasting US soils. Interestingly, the present study found a stronger correlation ($r = 0.64$) between these fractions. Additionally, Sherrod et al. (2019) noted a potential overlap between FA and HA with POXC. It is important to acknowledge these methodological limitations to avoid misinterpretations.

The generally accepted notion is that labile soil C fractions are most affected by changes in SCS (Culman et al., 2012). However, our research indicates that NOC, which is theoretically a stable pool, is the most sensitive based on the sensitivity index (Figure S5). Although NOC exhibited a proportional increase in response to the amendments, its absolute increase may not have been as substantial. Further investigation is necessary to clarify the reasons behind this observation. This statement contradicts previous studies that emphasize the sensitivity of POXC to management practices (Weil et al., 2003).

TGA was used to evaluate the SOM continuum, but the results were inconclusive in capturing changes in either labile or stable pools. Previous studies have shown contrasting results with TGA for SSB-amended soils. For instance, Cely et al. (2014) found that soil amended with 8% SSB pyrolyzed at 600°C exhibited a thermal behavior similar to the unamended soil, indicating a similar organic matter composition. In contrast, Gascó et al. (2012) reported an increase in the thermostable portion of soils amended with SSB pyrolyzed at 500°C and applied at 4% and 8% rates compared to the control soil. However, our study did not show any response, which is consistent with the findings of Tokarski et al. (2020). They attributed the lack of response in recalcitrant pools (HU, NOC) to interference from soil clay minerals. In addition, the low fixed C content (3.03-5.47%) of the applied SSB (Table 2) limited the technique's ability to detect small changes. Adhikari et al. (2024) suggest that SSB may be less suitable for substantial C sequestration due to their low fixed C content, among other factors. These points collectively suggest that TGA might be less sensitive than other methods employed in this study for detecting alterations in soil quality.

6.4.3. Magnetic susceptibility

The study results did not demonstrate a significant relationship between soil C fractions and magnetic susceptibility. The anticipated positive correlation between SOM and magnetic susceptibility, as suggested by Mullins (1977), was not observed. This expectation was based on the premise that organic matter could enhance magnetic susceptibility by creating conditions conducive to microbial iron reduction.

The lack of significant changes in soil magnetic properties following SSB application can be attributed to several factors. First, the relatively low application rates of SSB in this study may have been insufficient to induce measurable changes in soil magnetic susceptibility. It is possible that higher biochar application rates might be necessary to observe significant alterations in soil magnetic properties.

Second, the inherent homogeneity of the soil type and origin within the experimental area likely masked any subtle changes induced by the SSB amendments. Uniform soil characteristics contribute to minimal variations across treatments, making it challenging to detect minor changes when the baseline variability is already low.

Additionally, previous studies on agricultural soils have reported mixed results, with some finding positive (Jakšík et al., 2016; Matias et al., 2014) and others negative (Siqueira et al., 2016) correlations between SOM and magnetic susceptibility. This inconsistency further suggests that magnetic susceptibility may not be a reliable parameter for predicting changes in soil properties related to organic matter content.

At a regional scale, variations in soil magnetic susceptibility are influenced by geology, soil processes, and anthropogenic activities (Fialová et al., 2006). In agricultural fields, soils irrigated with sewage have been found to have higher magnetic susceptibility compared to those irrigated with groundwater, yet no strong correlation between SOM and magnetic susceptibility was found in these studies (Yang et al., 2015). This regional variability highlights the complexity of factors influencing soil magnetic properties and the challenges in using magnetic susceptibility as a universal indicator of SOM changes.

6.4.4. $\delta^{13}C$ and $\delta^{15}N$

The observed δ^{13}C values, greater than –20‰, align with the historical vegetation of the area, previously dominated by Cerrado (woody savanna) vegetation (Martinez et al., 2022). Sagrilo et al. (2015) reported minimal isotopic differences between control and biochar-treated soils at low application rates (5 and 10 Mg ha^{-1}). Thus, the absence of significant changes in δ^{13}C values among treatments in this study is consistent with previous research, suggesting that the applied SSB amounts were insufficient to alter the δ^{13}C signature significantly.

In contrast, the δ^{15}N values exhibited a more pronounced response to biochar application, particularly with SSB500. This increase in δ^{15}N is consistent with previous studies, which reported that organic amendments tend to elevate soil δ^{15}N compared to control and mineral fertilization (Li et al., 2021; Mani et al., 2021). The δ^{15}N enrichment observed in this study can be attributed to increased nitrogen availability in SSB500-amended soils, as higher TN content was recorded in these treatments (Figure 9B).

The mechanism behind this δ^{15}N enrichment involves several factors. First, the addition of organic carbon sources, such as biochar, can enhance microbial nitrate immobilization, which retains nitrogen in the soil. This process can occur without significantly increasing denitrification losses, thereby promoting nitrogen retention and influencing δ^{15}N values (Wang et al., 2021). The higher $\delta^{15}N$ values in SSB500-amended soils suggest enhanced microbial activity and nitrogen cycling, which are consistent with the observed increases in TN content.

Moreover, the higher $\delta^{15}N$ values under SSB500 treatments might indicate a more substantial contribution of biochar-derived nitrogen to the soil nitrogen pool (Figueiredo et al., 2021). Biochar produced at higher pyrolysis temperatures, such as 500°C, typically has a more stable C structure (Wei et al., 2019), which can slow down nitrogen mineralization and increase nitrogen retention over time. This slow-release mechanism could lead to a gradual increase in soil $\delta^{15}N$ values as biochar-derived nitrogen becomes a more prominent component of the soil nitrogen pool.

6.5. Conclusion

This seven-year field study demonstrated that the application of SSB enhances SCS and organic matter fractions. SSB-amended soils consistently exhibited higher TC and TN levels, indicating improved soil fertility and C sequestration potential. The increase in the NOC pool further contributed to the stabilization of SOM, highlighting SSB's effectiveness in enhancing soil health and its potential role in sustainable agricultural practices. The positive effects of SSB on crop yield were evident, with increases in grain yield and shoot biomass of corn. This enhancement was consistent across multiple growing seasons, demonstrating the agronomic benefits of SSB application. However, the decline in yield from the fourth season onwards in SSB-only treatments underscores the importance of supplementing biochar with mineral fertilizers to sustain long-term yield. These findings have important implications for sustainable agriculture and climate change mitigation. SSB offers farmers an option to enhance soil fertility and crop yields while contributing to C sequestration. Policymakers can promote the use of SSB as a sustainable soil amendment to improve soil health, support waste management, and achieve climate goals. Future research should focus on long-term field studies under various environmental conditions to better understand the dynamics of SSB in different soil types. Additionally, exploring the potential of co-pyrolysis of sewage sludge with other feedstocks may enhance C stability and further improve the benefits of biochar application. In conclusion, SSB application enhances SCS and organic matter fractions, contributing to improved soil health and sustainability. These findings support the broader adoption of biochar technology as a viable practice for mitigating climate change and promoting sustainable agricultural development. Continued research and practical implementation are essential to fully realize the potential of biochar in diverse agricultural systems.

6.6. References

Abbas, M., Ijaz, S.S., Ansar, M., Hussain, Q., Hassan, A., Akmal, M., Tahir, M., Iqbal, M., Bashir, K., Sana-ur-Rehman, 2019. Impact of biochar with different organic materials on carbon fractions, aggregate size distribution, and associated polysaccharides and soil moisture retention in an arid soil. Arabian Journal of Geosciences 12. https://doi.org/10.1007/s12517-019-4792-3

Adhikari, S., Moon, E., Paz-Ferreiro, J., Timms, W., 2024. Comparative analysis of biochar carbon stability methods and implications for carbon credits. Science of The Total Environment 914, 169607. https://doi.org/10.1016/j.scitotenv.2023.169607

Amoakwah, E., Arthur, E., Frimpong, K.A., Parikh, S.J., Islam, R., 2020. Soil organic carbon storage and quality are impacted by corn cob biochar application on a tropical sandy loam. J Soils Sediments 20, 1960–1969. https://doi.org/10.1007/s11368-019-02547-5

Aydin, E., Šimanský, V., Horák, J., Igaz, D., 2020. Potential of Biochar to Alternate Soil Properties and Crop Yields 3 and 4 Years after the Application. Agronomy 10, 889. https://doi.org/10.3390/agronomy10060889

Benites, V.D.M., Machado, P., 2003. Extração e fracionamento quantitativo de substâncias húmicas do solo: um procedimento simplificado de baixo custo, Comunicado Técnico (16). Embrapa Solos, Rio de Janeiro.

Blair, G., Lefroy, R., Lisle, L., 1995. Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. Aust J Agric Res 46, 1459. https://doi.org/10.1071/AR9951459

Box, G.E.P., Cox, D.R., 1964. An analysis of transformations. Journal of the Royal Statistical Society: Series B (Methodological) 26, 211–252. https://doi.org/10.1111/j.2517-6161.1964.tb00553.x

Brazil. Ministry of Agriculture, Livestock and Supply, 2014. Manual of official analytical methods for mineral, organic, organic-mineral and corrective fertilizers [*Manual de métodos analíticos oficiais para fertilizantes minerais, orgânicos, orgaminerais e corretivos*]. MAPA/SDA/CGAL, Brasília. (in Portuguese)

Bremner, J.M., Keeney, D.R., 1965. Steam distillation methods for determination of ammonium, nitrate and nitrite. Anal Chim Acta 32, 485–495. https://doi.org/10.1016/S0003-2670(00)88973-4

Budai, A., Zimmerman, A.R., Cowie, A.L., Webber, J.B.W., Singh, B.P., Glaser, B., Masiello, C.A., Andersson, D., Shields, F., Lehmann, J., Camps Arbestain, M., Williams, M., Sohi, S., Joseph, S., 2013. Biochar Carbon Stability Test Method: An assessment of methods to determine biochar carbon stability, International Biochar Initiative.

Cambardella, C.A., Elliott, E.T., 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Science Society of America Journal 56, 777–783. https://doi.org/10.2136/sssaj1992.03615995005600030017x

Cely, P., Tarquis, A.M., Paz-Ferreiro, J., Méndez, A., Gascó, G., 2014. Factors driving the carbon mineralization priming effect in a sandy loam soil amended with different types of biochar. Solid Earth 5, 585–594. https://doi.org/10.5194/se-5-585-2014

Chagas, J.K.M., Figueiredo, C.C. de, Paz-Ferreiro, J., 2021. Sewage sludge biochars effects on corn response and nutrition and on soil properties in a 5-yr field experiment. Geoderma 401, 115323. https://doi.org/10.1016/j.geoderma.2021.115323

Chagas, J.K.M., Figueiredo, C.C. de, Ramos, M.L.G., 2022. Biochar increases soil carbon pools: Evidence from a global meta-analysis. J Environ Manage 305, 114403. https://doi.org/10.1016/j.jenvman.2021.114403

Chan, K.Y., Bowman, A., Oates, A., 2001. Oxidizible organic carbon fractions and soil quality changes in an Oxic Paleustalf under different pasture leys. Soil Sci 166, 61–67. https://doi.org/10.1097/00010694-200101000-00009

Chan, K.Y., Heenan, D.P., Oates, A., 2002. Soil carbon fractions and relationship to soil quality under different tillage and stubble management. Soil Tillage Res 63, 133–139. https://doi.org/10.1016/S0167-1987(01)00239-2

Christensen, B.T., 2001. Physical fractionation of soil and structural and functional complexity in organic matter turnover. Eur J Soil Sci 52, 345–353. https://doi.org/10.1046/j.1365-2389.2001.00417.x

Cooper, J., Greenberg, I., Ludwig, B., Hippich, L., Fischer, D., Glaser, B., Kaiser, M., 2020. Effect of biochar and compost on soil properties and organic matter in aggregate size fractions under field conditions. Agric Ecosyst Environ 295, 106882. https://doi.org/10.1016/j.agee.2020.106882

Cornelissen, G., Jubaedah, Nurida, N.L., Hale, S.E., Martinsen, V., Silvani, L., Mulder, J., 2018. Fading positive effect of biochar on crop yield and soil acidity during five growth seasons in an Indonesian Ultisol. Science of The Total Environment 634, 561–568. https://doi.org/10.1016/j.scitotenv.2018.03.380

Culman, S.W., Snapp, S.S., Freeman, M.A., Schipanski, M.E., Beniston, J., Lal, R., Drinkwater, L.E., Franzluebbers, A.J., Glover, J.D., Grandy, A.S., Lee, J., Six, J., Maul, J.E., Mirksy, S.B., Spargo, J.T., Wander, M.M., 2012. Permanganate Oxidizable Carbon Reflects a Processed Soil Fraction that is Sensitive to Management. Soil Science Society of America Journal 76, 494–504. https://doi.org/10.2136/sssaj2011.0286

Cybulak, M., Sokołowska, Z., Boguta, P., 2021. The influence of biochar on the content of carbon and the chemical transformations of fallow and grassland humic acids. Sci Rep 11, 5698. https://doi.org/10.1038/s41598-021-85239-w

Das, R., Ghosh, A., Das, S., Basak, N., Singh, R., Priyanka, Datta, A., 2021. Soil Carbon Sequestration for Soil Quality Improvement and Climate Change Mitigation, in: Pant, D., Nadda, A.K., Pant, K.K., Agarwal, A.K. (Eds.), Advances in Carbon Capture and Utilization (Series: Energy, Environment, and Sustainability). Springer, Singapore, pp. 57–81. https://doi.org/10.1007/978-981-16-0638-0_4

Dearing, J.A., 1999. Environmental magnetic susceptibility: using the Bartington MS2 system, 2nd ed. Chi Publishing, Kenilworth.

DeCiucies, S., Whitman, T., Woolf, D., Enders, A., Lehmann, J., 2018. Priming mechanisms with additions of pyrogenic organic matter to soil. Geochim Cosmochim Acta 238, 329–342. https://doi.org/10.1016/j.gca.2018.07.004

Ding, F., Van Zwieten, L., Zhang, W., Weng, Z., Shi, S., Wang, J., Meng, J., 2018. A meta-analysis and critical evaluation of influencing factors on soil carbon priming following biochar amendment. J Soils Sediments 18, 1507–1517. https://doi.org/10.1007/s11368-017-1899-6

Dong, X., Guan, T., Li, G., Lin, Q., Zhao, X., 2016. Long-term effects of biochar amount on the content and composition of organic matter in soil aggregates under field conditions. J Soils Sediments 16, 1481–1497. https://doi.org/10.1007/s11368-015-1338-5

Dwivedi, D., Tang, J., Bouskill, N., Georgiou, K., Chacon, S.S., Riley, W.J., 2019. Abiotic and Biotic Controls on Soil Organo–Mineral Interactions: Developing Model Structures to Analyze Why Soil Organic Matter Persists. Rev Mineral Geochem 85, 329–348. https://doi.org/10.2138/rmg.2019.85.11

Ellert, B.H., Bettany, J.R., 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. Can J Soil Sci 75, 529–538. https://doi.org/10.4141/cjss95-075

El-Naggar, A., El-Naggar, A.H., Shaheen, S.M., Sarkar, B., Chang, S.X., Tsang, D.C.W., Rinklebe, J., Ok, Y.S., 2019. Biochar composition-dependent impacts on soil nutrient release, carbon mineralization, and potential environmental risk: a review. J Environ Manage 241, 458–467. https://doi.org/10.1016/j.jenvman.2019.02.044

Enders, A., Hanley, K., Whitman, T., Joseph, S., Lehmann, J., 2012. Characterization of biochars to evaluate recalcitrance and agronomic performance. Bioresour Technol 114, 644–653. https://doi.org/10.1016/j.biortech.2012.03.022

Faria, W.M., Figueiredo, C.C. de, Coser, T.R., Vale, A.T., Schneider, B.G., 2018. Is sewage sludge biochar capable of replacing inorganic fertilizers for corn production? Evidence from a two-year field experiment. Arch Agron Soil Sci 64, 505–519. https://doi.org/10.1080/03650340.2017.1360488

Feng, H., Han, X., Zhu, Y., Zhang, M., Ji, Y., Lu, X., Chen, X., Yan, J., Zou, W., 2024. Effects of long-term application of organic materials on soil water extractable organic matter, fulvic acid, humic acid structure and microbial driving mechanisms. Plant Soil. https://doi.org/10.1007/s11104-024-06522-z

Fialová, H., Maier, G., Petrovský, E., Kapička, A., Boyko, T., Scholger, R., 2006. Magnetic properties of soils from sites with different geological and environmental settings. J Appl Geophy 59, 273–283. https://doi.org/10.1016/j.jappgeo.2005.10.006

Figueiredo, C.C. de, Chagas, J.K.M., da Silva, J., Paz-Ferreiro, J., 2019a. Short-term effects of a sewage sludge biochar amendment on total and available heavy metal content of a tropical soil. Geoderma 344, 31–39. https://doi.org/10.1016/j.geoderma.2019.01.052

Figueiredo, C.C. de, Coser, T.R., Moreira, T.N., Leão, T.P., Vale, A.T., Paz-Ferreiro, J., 2019b. Carbon mineralization in a soil amended with sewage sludge-derived biochar. Applied Sciences 9, 4481. https://doi.org/10.3390/app9214481

Figueiredo, C.C. de, Farias, W.M., Melo, B.A. de, Chagas, J.K.M., Vale, A.T., Coser, T.R., 2019c. Labile and stable pools of organic matter in soil amended with sewage sludge biochar. Arch Agron Soil Sci 65, 770–781. https://doi.org/10.1080/03650340.2018.1524577

Figueiredo, C.C. de, Wickert, É.G., Vieira Neves, H.C., Coser, T.R., Paz-Ferreiro, J., 2021. Sewage sludge biochar increases nitrogen fertilizer recovery: Evidence from a 15N tracer field study. Soil Use Manag 37, 689–697. https://doi.org/10.1111/sum.12672

Gascó, G., Paz-Ferreiro, J., Méndez, A., 2012. Thermal analysis of soil amended with sewage sludge and biochar from sewage sludge pyrolysis. J Therm Anal Calorim 108, 769–775. https://doi.org/10.1007/s10973-011-2116-2

Giannetta, B., Plaza, C., Galluzzi, G., Benavente-Ferraces, I., García-Gil, J.C., Panettieri, M., Gascó, G., Zaccone, C., 2024. Distribution of soil organic carbon between particulate and mineral-associated fractions as affected by biochar and its co-application with other amendments. Agric Ecosyst Environ 360, 108777. https://doi.org/10.1016/j.agee.2023.108777

Gonzaga, M.I.S., Mackowiak, C., Quintão de Almeida, A., Wisniewski, A., Figueiredo de Souza, D., da Silva Lima, I., Nascimento de Jesus, A., 2018. Assessing biochar applications and repeated Brassica juncea L. production cycles to remediate Cu contaminated soil. Chemosphere 201, 278–285. https://doi.org/10.1016/j.chemosphere.2018.03.038

Gonzaga, M.I.S., Matias, M.I. de A.S., Andrade, K.R., Jesus, A.N. de, Cunha, G. da C., Andrade, R.S. de, Santos, J.C. de J., 2020. Aged biochar changed copper availability and distribution among soil fractions and influenced corn seed germination in a copper-contaminated soil. Chemosphere 240, 124828. https://doi.org/10.1016/j.chemosphere.2019.124828

González Pérez, M., Martin-Neto, L., Saab, S.C., Novotny, E.H., Milori, D.M.B.P., Bagnato, V.S., Colnago, L.A., Melo, W.J., Knicker, H., 2004. Characterization of humic acids from a Brazilian Oxisol under different tillage systems by EPR, 13C NMR, FTIR and fluorescence spectroscopy. Geoderma 118, 181–190. https://doi.org/10.1016/S0016-7061(03)00192-7

Gramss, G., Ziegenhagen, D., Sorge, S., 1999. Degradation of Soil Humic Extract by Wood- and Soil-Associated Fungi, Bacteria, and Commercial Enzymes. Microb Ecol 37, 140–151. https://doi.org/10.1007/s002489900138

Griffin, D.E., Wang, D., Parikh, S.J., Scow, K.M., 2017. Short-lived effects of walnut shell biochar on soils and crop yields in a long-term field experiment. Agric Ecosyst Environ 236, 21–29. https://doi.org/10.1016/j.agee.2016.11.002

Gross, A., Bromm, T., Glaser, B., 2021. Soil organic carbon sequestration after biochar application: a global meta-analysis. Agronomy 11, 2474. https://doi.org/10.3390/agronomy11122474

Hayes, M.H.B., Mylotte, R., Swift, R.S., 2017. Humin: its composition and importance in soil organic matter, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, Burlington, pp. 47–138. https://doi.org/10.1016/bs.agron.2017.01.001

Ibrahim, M., Li, G., Khan, S., Chi, Q., Xu, Y., 2017. Biochars mitigate greenhouse gas emissions and bioaccumulation of potentially toxic elements and arsenic speciation in

Phaseolus vulgaris L. Environmental Science and Pollution Research 24, 19524–19534. https://doi.org/10.1007/s11356-017-9605-1

IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, update 2015: international soil classification system for naming soils and creating legends for soil maps. Food and Agriculture Organization of United Nations, Rome.

Jafari Tarf, O., Akça, M.O., Donar, Y.O., Bilge, S., Turgay, O.C., Sınağ, A., 2022. The short-term effects of pyro-and hydrochars derived from different organic wastes on some soil properties. Biomass Convers Biorefin 12, 129–139. https://doi.org/10.1007/s13399-021-01282-7

Jakšík, O., Kodešová, R., Kapička, A., Klement, A., Fér, M., Nikodem, A., 2016. Using magnetic susceptibility mapping for assessing soil degradation due to water erosion. Soil and Water Research 11, 105–113. https://doi.org/10.17221/233/2015-SWR

Jarosz, R., Mierzwa-Hersztek, M., Gondek, K., Kopeć, M., Lošák, T., Marcińska-Mazur, L., 2020. Changes in quantity and quality of organic matter in soil after application of poultry litter and poultry litter biochar—5-year field experiment. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-020-01005-4

Jin, J., Sun, K., Yang, Y., Wang, Z., Han, L., Wang, X., Wu, F., Xing, B., 2018. Comparison between Soil- and Biochar-Derived Humic Acids: Composition, Conformation, and Phenanthrene Sorption. Environ Sci Technol 52, 1880–1888. https://doi.org/10.1021/acs.est.7b04999

Jones, E.R., van Vliet, M.T.H., Qadir, M., Bierkens, M.F.P., 2021. Country-level and gridded estimates of wastewater production, collection, treatment and reuse. Earth Syst Sci Data 13, 237–254. https://doi.org/10.5194/essd-13-237-2021

Joseph, S., Cowie, A.L., Van Zwieten, L., Bolan, N., Budai, A., Buss, W., Cayuela, M.L., Graber, E.R., Ippolito, J.A., Kuzyakov, Y., Luo, Y., Ok, Y.S., Palansooriya, K.N., Shepherd, J., Stephens, S., Weng, Z. (Han), Lehmann, J., 2021. How biochar works, and when it doesn’t: A review of mechanisms controlling soil and plant responses to biochar. GCB Bioenergy 13, 1731–1764. https://doi.org/10.1111/gcbb.12885

Kalu, S., Seppänen, A., Mganga, K.Z., Sietiö, O.-M., Glaser, B., Karhu, K., 2024. Biochar reduced the mineralization of native and added soil organic carbon: evidence of negative priming and enhanced microbial carbon use efficiency. Biochar 6, 7. https://doi.org/10.1007/s42773-023-00294-y

Kätterer, T., Roobroeck, D., Andrén, O., Kimutai, G., Karltun, E., Kirchmann, H., Nyberg, G., Vanlauwe, B., Röing de Nowina, K., 2019. Biochar addition persistently increased soil fertility and yields in maize-soybean rotations over 10 years in sub-humid regions of Kenya. Field Crops Res 235, 18–26. https://doi.org/10.1016/j.fcr.2019.02.015

Khan, S., Waqas, M., Ding, F., Shamshad, I., Arp, H.P.H., Li, G., 2015. The influence of various biochars on the bioaccessibility and bioaccumulation of PAHs and potentially toxic elements to turnips (Brassica rapa L.). J Hazard Mater 300, 243–253. https://doi.org/10.1016/j.jhazmat.2015.06.050

Kopecký, M., Kolář, L., Váchalová, R., Konvalina, P., Batt, J., Mráz, P., Menšík, L., Hoang, T.N., Dumbrovský, M., 2021. Black Carbon and Its Effect on Carbon Sequestration in Soil. Agronomy 11, 2261. https://doi.org/10.3390/agronomy11112261

Lal, R., 2010. Managing soils and ecosystems for mitigating anthropogenic carbon emissions and advancing global food security. Bioscience 60, 708–721. https://doi.org/10.1525/bio.2010.60.9.8

Leal, O. dos A., Dick, D.P., De La Rosa, J.M., Leal, D.P.B., González-Pérez, J.A., Campos, G.S., Knicker, H., 2019. Charcoal fine residues effects on soil organic matter humic substances, composition, and biodegradability. Agronomy 9, 1–16. https://doi.org/10.3390/agronomy9070384

Lehmann, J., Cowie, A., Masiello, C.A., Kammann, C., Woolf, D., Amonette, J.E., Cayuela, M.L., Camps-Arbestain, M., Whitman, T., 2021. Biochar in climate change mitigation. Nat Geosci 14, 883–892. https://doi.org/10.1038/s41561-021-00852-8

Lehmann, J., Hansel, C.M., Kaiser, C., Kleber, M., Maher, K., Manzoni, S., Nunan, N., Reichstein, M., Schimel, J.P., Torn, M.S., Wieder, W.R., Kögel-Knabner, I., 2020. Persistence of soil organic carbon caused by functional complexity. Nat Geosci 13, 529–534. https://doi.org/10.1038/s41561-020-0612-3

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60–68. https://doi.org/10.1038/nature16069

Leng, L., Huang, H., 2018. An overview of the effect of pyrolysis process parameters on biochar stability. Bioresour Technol 270, 627–642. https://doi.org/10.1016/j.biortech.2018.09.030

Leng, L., Huang, H., Li, H., Li, J., Zhou, W., 2019. Biochar stability assessment methods: A review. Science of The Total Environment 647, 210–222. https://doi.org/10.1016/j.scitotenv.2018.07.402

Li, C., Wang, Q., Shao, S., Chen, Z., Nie, J., Liu, Z., Rogers, K.M., Yuan, Y., 2021. Stable Isotope Effects of Biogas Slurry Applied as an Organic Fertilizer to Rice, Straw, and Soil. J Agric Food Chem 69, 8090–8097. https://doi.org/10.1021/acs.jafc.1c01740

Li, H., Yutong, W., Tianpei, W., Hongrui, M., 2015. Effect of biochar on organic matter conservation and metabolic quotient of soil. Environ Prog Sustain Energy 34, 1467–1472. https://doi.org/10.1002/ep.12122

Lima, C.C., Mendonça, E. de S., Roig, A., 2010. Contribution of humic substances from different composts to the synthesis of humin in a tropical soil. Rev Bras Cienc Solo 34, 1041–1048. https://doi.org/10.1590/S0100-06832010000400004

Liu, Z., Singer, S., Tong, Y., Kimbell, L., Anderson, E., Hughes, M., Zitomer, D., McNamara, P., 2018. Characteristics and applications of biochars derived from wastewater solids. Renewable and Sustainable Energy Reviews 90, 650–664. https://doi.org/10.1016/j.rser.2018.02.040

Luo, Z., Viscarra Rossel, R.A., Shi, Z., 2020. Distinct controls over the temporal dynamics of soil carbon fractions after land use change. Glob Chang Biol 26, 4614–4625. https://doi.org/10.1111/gcb.15157

Mani, S., S, A., K, B., N, S., 2021. Effect of Nutrient Management on δ15N, δ13C Isotopes and Enzyme Activities in Higher Altitude Agricultural Soils, India. Geomicrobiol J 38, 174–180. https://doi.org/10.1080/01490451.2020.1822469

Martinez, P., Silva, L.C.R., Calegari, M.R., de Camargo, P.B., Vidal-Torrado, P., Kleber, M., 2022. Carbon stocks in umbric ferralsols driven by plant productivity and geomorphic processes, not by mineral protection. Earth Surf Process Landf 47, 491–508. https://doi.org/10.1002/esp.5262

Matias, S.S.R., Marques Júnior, J., Siqueira, D.S., Pereira, G.T., 2014. Outlining precision boundaries among areas with different variability standards using magnetic susceptibility and geomorphic surfaces. Engenharia Agrícola 34, 695–706. https://doi.org/10.1590/S0100-69162014000400009

McBeath, A. V., Wurster, C.M., Bird, M.I., 2015. Influence of feedstock properties and pyrolysis conditions on biochar carbon stability as determined by hydrogen pyrolysis. Biomass Bioenergy 73, 155–173. https://doi.org/10.1016/j.biombioe.2014.12.022

Mierzwa-Hersztek, M., Gondek, K., Klimkowicz-Pawlas, A., Baran, A., Bajda, T., 2018. Sewage sludge biochars management-Ecotoxicity, mobility of heavy metals, and soil microbial biomass. Environ Toxicol Chem 37, 1197–1207. https://doi.org/10.1002/etc.4045

Mullins, C.E., 1977. Magnetic susceptibility of the soil and its significance in soil science - a review. Journal of Soil Science 28, 223–246. https://doi.org/10.1111/j.1365-2389.1977.tb02232.x

Nair, R.R., Kißling, P.A., Marchanka, A., Lecinski, J., Turcios, A.E., Shamsuyeva, M., Rajendiran, N., Ganesan, S., Srinivasan, S.V., Papenbrock, J., Weichgrebe, D., 2023. Biochar synthesis from mineral and ash-rich waste biomass, part 2: characterization of biochar and co-pyrolysis mechanism for carbon sequestration. Sustainable Environment Research 33. https://doi.org/10.1186/s42834-023-00176-9

Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter, in: Sparks, D.L., Pag, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (Eds.), Methods of Soil Analysis. Part 3. Chemical Methods. Soil Science of America and American Society of Agronomy, Madison, pp. 961–1010. https://doi.org/10.2136/sssabookser5.3.c34

Paetsch, L., Mueller, C.W., Rumpel, C., Angst, Š., Wiesheu, A.C., Girardin, C., Ivleva, N.P., Niessner, R., Kögel-Knabner, I., 2017. A multi-technique approach to assess the fate of biochar in soil and to quantify its effect on soil organic matter composition. Org Geochem 112, 177–186. https://doi.org/10.1016/j.orggeochem.2017.06.012

Pignatello, J.J., Mitch, W.A., Xu, W., 2017. Activity and reactivity of pyrogenic carbonaceous matter toward organic compounds. Environ Sci Technol 51, 8893–8908. https://doi.org/10.1021/acs.est.7b01088

R Core Team, 2023. R: a language and environment for statistical computing.

Rahim, H.U., Allevato, E., Vaccari, F.P., Stazi, S.R., 2024. Biochar aged or combined with humic substances: fabrication and implications for sustainable agriculture and environment-a review. J Soils Sediments 24, 139–162. https://doi.org/10.1007/s11368-023-03644-2

Sagrilo, E., Rittl, T.F., Hoffland, E., Alves, B.J.R., Mehl, H.U., Kuyper, T.W., 2015. Rapid decomposition of traditionally produced biochar in an Oxisol under savannah in Northeastern Brazil. Geoderma Regional 6, 1–6. https://doi.org/10.1016/j.geodrs.2015.08.006

Salma, A., Fryda, L., Djelal, H., 2024. Biochar: A Key Player in Carbon Credits and Climate Mitigation. Resources 13, 31. https://doi.org/10.3390/resources13020031

Santos, H.G. dos, Jacomine, P.K.T., Anjos, L.H.C. dos, Oliveira, V.Á. de, Lumbreras, J.F., Coelho, M.R., Almeida, J.A. de, Araújo Filho, J.C. de, Oliveira, J.B. de, Cunha, T.J.F., 2018. Sistema Brasileiro de Classificação de Solos [Brazilian system of soil classification], 5th ed. Embrapa, Brasília, DF.

Schellekens, J., Justi, M., Macedo, R., Calegari, M.R., Buurman, P., Kuyper, T.W., Barbosa de Camargo, P., Vidal-Torrado, P., 2023. Long-term carbon storage in Brazilian Cerrado soils

– a conjunction of wildfires, bioturbation, and local edaphic controls on vegetation. Plant Soil 484, 645–662. https://doi.org/10.1007/s11104-022-05824-4

Shao, H., Wei, Y., Zhang, F., Li, F., 2020. Effects of Biochars Produced from Coconut Shell and Sewage Sludge on Reducing the Uptake of Cesium by Plant from Contaminated Soil. Water Air Soil Pollut 231. https://doi.org/10.1007/s11270-020-04922-2

Sherrod, L.A.A., Vigil, M.F.F., Stewart, C.E.E., 2019. Do fulvic, humic, and humin carbon fractions represent meaningful biological, physical, and chemical carbon pools? J Environ Qual 48, 1587–1593. https://doi.org/10.2134/jeq2019.03.0104

Shi, S., Zhang, Q., Lou, Y., Du, Z., Wang, Q., Hu, N., Wang, Y., Gunina, A., Song, J., 2021. Soil organic and inorganic carbon sequestration by consecutive biochar application: Results from a decade field experiment. Soil Use Manag 37, 95–103. https://doi.org/10.1111/sum.12655

Silva, F.C. da (Ed.), 2009. Manual of chemical analysis of soils, plants and fertilizers [*Manual de análises químicas de solos, plantas e fertilizantes*], 2nd ed. Embrapa Informação Tecnológica, Brasília, DF. (in Portuguese)

Siqueira, D.S., Marques Júnior, J., Teixeira, D.D.B., Matias, S.S.R., Camargo, L.A., Pereira, G.T., 2016. Magnetic susceptibility for characterizing areas with different potentials for sugarcane production. Pesqui Agropecu Bras 51, 1349–1358. https://doi.org/10.1590/s0100-204x2016000900034

Soil Survey Staff, 2014. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, Washington, DC.

Sousa, A.A.T.C., Figueiredo, C.C., 2016. Sewage sludge biochar: effects on soil fertility and growth of radish. Biological Agriculture and Horticulture 32, 127–138. https://doi.org/10.1080/01448765.2015.1093545

Sousa, D.M.G. de, Lobato, E. (Eds.), 2004. Cerrado: correção do solo e adubação [Cerrado: soil amendment and fertilizing], 2nd ed. Embrapa Informação Tecnológica, Brasília, DF.

Sun, J., Lu, X., Chen, G., Luo, N., Zhang, Q., Li, X., 2023. Biochar promotes soil aggregate stability and associated organic carbon sequestration and regulates microbial community structures in Mollisols from northeast China. SOIL 9, 261–275. https://doi.org/10.5194/soil-9-261-2023

Swift, R.S., 1996. Organic Matter Characterization, in: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H. (Eds.), Methods of Soil Analysis Part 3 - Chemical Methods. Soil Science Society of America, American Society of Agronomy, Madison, WI, pp. 1011–1069. https://doi.org/10.2136/sssabookser5.3.c35

Tan, K.H., 2014. Humic Matter in Soil and the Environment, 2nd ed. CRC Press, Boca Raton. https://doi.org/10.1201/b17037

Teixeira, P.C., Donagemma, G.K., Fontana, A., Teixeira, W.G. (Eds.), 2017. Manual de métodos de análise de solo, 3rd ed. Embrapa, Brasília.

Tokarski, D., Wiesmeier, M., Doležalová Weissmannová, H., Kalbitz, K., Scott Demyan, M., Kučerík, J., Siewert, C., 2020. Linking thermogravimetric data with soil organic carbon fractions. Geoderma 362. https://doi.org/10.1016/j.geoderma.2019.114124

Verra, 2023. Verified Carbon Standard (VCS) Methodology VM0044 - Methodology for biochar utilization in soil and non-soil applications.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. Soil Biol Biochem 39, 2183–2207. https://doi.org/10.1016/j.soilbio.2007.03.007

Vuppaladadiyam, A.K., Vuppaladadiyam, S.S.V., Sahoo, A., Murugavelh, S., Anthony, E., Bhaskar, T., Zheng, Y., Zhao, M., Duan, H., Zhao, Y., Antunes, E., Sarmah, A.K., Leu, S.-Y., 2023. Bio-oil and biochar from the pyrolytic conversion of biomass: A current and future perspective on the trade-off between economic, environmental, and technical indicators. Science of The Total Environment 857, 159155. https://doi.org/10.1016/j.scitotenv.2022.159155

Walkley, A., Black, I.A., 1934. An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37, 29–38. https://doi.org/10.1097/00010694-193401000-00003

Wander, M., 2004. Soil Organic Matter Fractions and Their Relevance to Soil Function, in: Magdoff, F., Weil, R.R. (Eds.), Soil Organic Matter in Sustainable Agriculture. CRC Press, Boca Raton, p. 412. https://doi.org/10.1201/9780203496374.ch3

Wang, J., Chen, Z., Xu, C., Elrys, A.S., Shen, F., Cheng, Y., Chang, S.X., 2021. Organic amendment enhanced microbial nitrate immobilization with negligible denitrification nitrogen loss in an upland soil. Environmental Pollution 288, 117721. https://doi.org/10.1016/j.envpol.2021.117721

Wei, S., Zhu, M., Fan, X., Song, J., Peng, P., Li, K., Jia, W., Song, H., 2019. Influence of pyrolysis temperature and feedstock on carbon fractions of biochar produced from pyrolysis of rice straw, pine wood, pig manure and sewage sludge. Chemosphere 218, 624–631. https://doi.org/10.1016/j.chemosphere.2018.11.177

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. American Journal of Alternative Agriculture 18, 3–17. https://doi.org/10.1079/AJAA200228

Werner, C., Schmidt, H.-P., Gerten, D., Lucht, W., Kammann, C., 2018. Biogeochemical potential of biomass pyrolysis systems for limiting global warming to 1.5 °C. Environmental Research Letters 13, 044036. https://doi.org/10.1088/1748-9326/aabb0e

Wu, B., Dai, X., Chai, X., 2020. Critical review on dewatering of sewage sludge: Influential mechanism, conditioning technologies and implications to sludge re-utilizations. Water Res 180, 115912. https://doi.org/10.1016/j.watres.2020.115912

Wu, L., Zhang, S., Ma, R., Chen, M., Wei, W., Ding, X., 2021. Carbon sequestration under different organic amendments in saline-alkaline soils. Catena (Amst) 196, 104882. https://doi.org/10.1016/j.catena.2020.104882

Wuest, S., 2014. Seasonal Variation in Soil Organic Carbon. Soil Science Society of America Journal 78, 1442–1447. https://doi.org/10.2136/sssaj2013.10.0447

Yang, P.G., Yang, M., Mao, R.Z., Byrne, J.M., 2015. Impact of Long-Term Irrigation with Treated Sewage on Soil Magnetic Susceptibility and Organic Matter Content in North China. Bull Environ Contam Toxicol 95, 102–107. https://doi.org/10.1007/s00128-015-1562-0

Yang, X., Meng, J., Lan, Y., Chen, W., Yang, T., Yuan, J., Liu, S., Han, J., 2017. Effects of maize stover and its biochar on soil CO2 emissions and labile organic carbon fractions in Northeast China. Agric Ecosyst Environ 240, 24–31. https://doi.org/10.1016/j.agee.2017.02.001

Yang, X., Wang, D., Lan, Y., Meng, J., Jiang, L., Sun, Q., Cao, D., Sun, Y., Chen, W., 2018. Labile organic carbon fractions and carbon pool management index in a 3-year field study with biochar amendment. J Soils Sediments 18, 1569–1578. https://doi.org/10.1007/s11368-017-1874-2

You, J., Sun, L., Liu, X., Hu, X., Xu, Q., 2019. Effects of sewage sludge biochar on soil characteristics and crop yield in loamy sand soil. Pol J Environ Stud 28, 2973–2980. https://doi.org/10.15244/pjoes/93294

Zhang, J., Lü, F., Zhang, H., Shao, L., Chen, D., He, P., 2015. Multiscale visualization of the structural and characteristic changes of sewage sludge biochar oriented towards potential agronomic and environmental implication. Sci Rep 5, 9406. https://doi.org/10.1038/srep09406

6.7. Appendix

The proximate analysis of SSB was conducted using simultaneous thermal analyzers (TGA/DSC) (SDT 2960, TA Instruments, Delaware, USA). Initially, the sample was heated from room temperature to 140°C at a rate of 50°C min^{-1} and held isothermal for 3 min in a N_2 atmosphere (40 mL min^{-1}) to remove moisture. Subsequently, it was heated linearly (100°C min^{-1}, 40 mL min^{-1} with N_2 flow) and held isothermally at 950°C for 3 min to remove volatile matter. The sample was then cooled to 450°C at a rate of –50°C min^{-1} and the atmosphere was switched to synthetic air (80% N_2, 20% O_2). A new heating ramp of 100°C min^{-1} was initiated until the temperature reached 800°C, which was maintained isothermally for 3 min to combust the fixed carbon (García et al., 2013). The graphs are depicted in Figure S1.

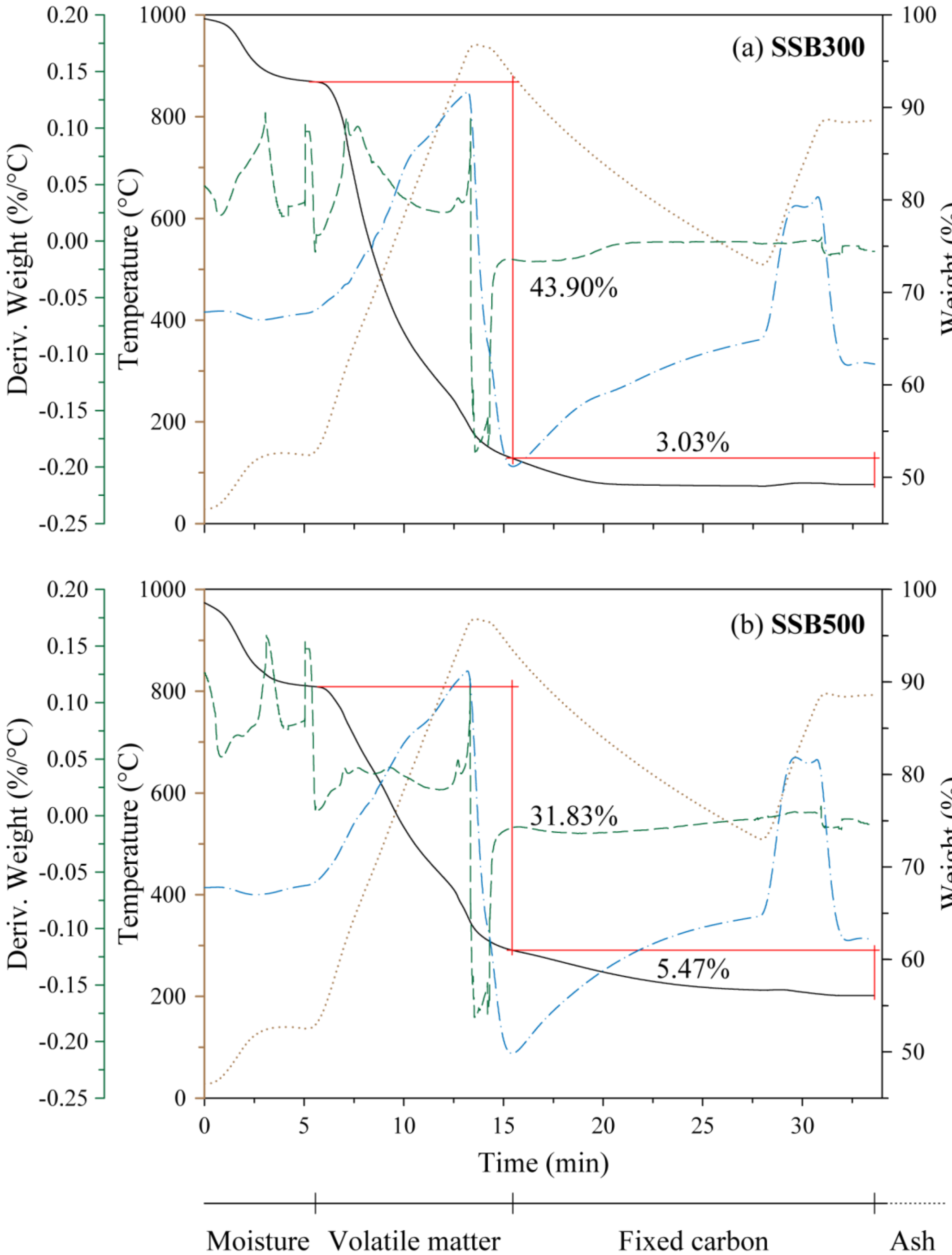


Figure S1. Proximate analysis of biochars pyrolyzed at 300°C – SSB300 – (a) and 500°C – SSB500 – (b) using TGA/DSC. Volatile matter and fixed carbon percentages are expressed on a dry basis.

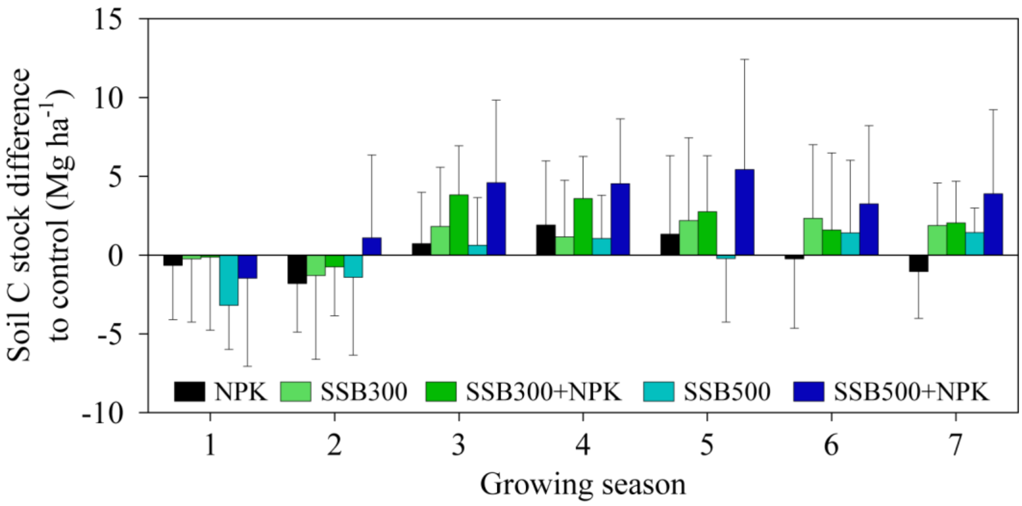


Figure S2. Difference in soil carbon stock to control. NPK: mineral fertilizer; SSB300 and SSB500: sewage sludge biochar pyrolyzed at 300°C and 500°C. Error bars represent standard error.

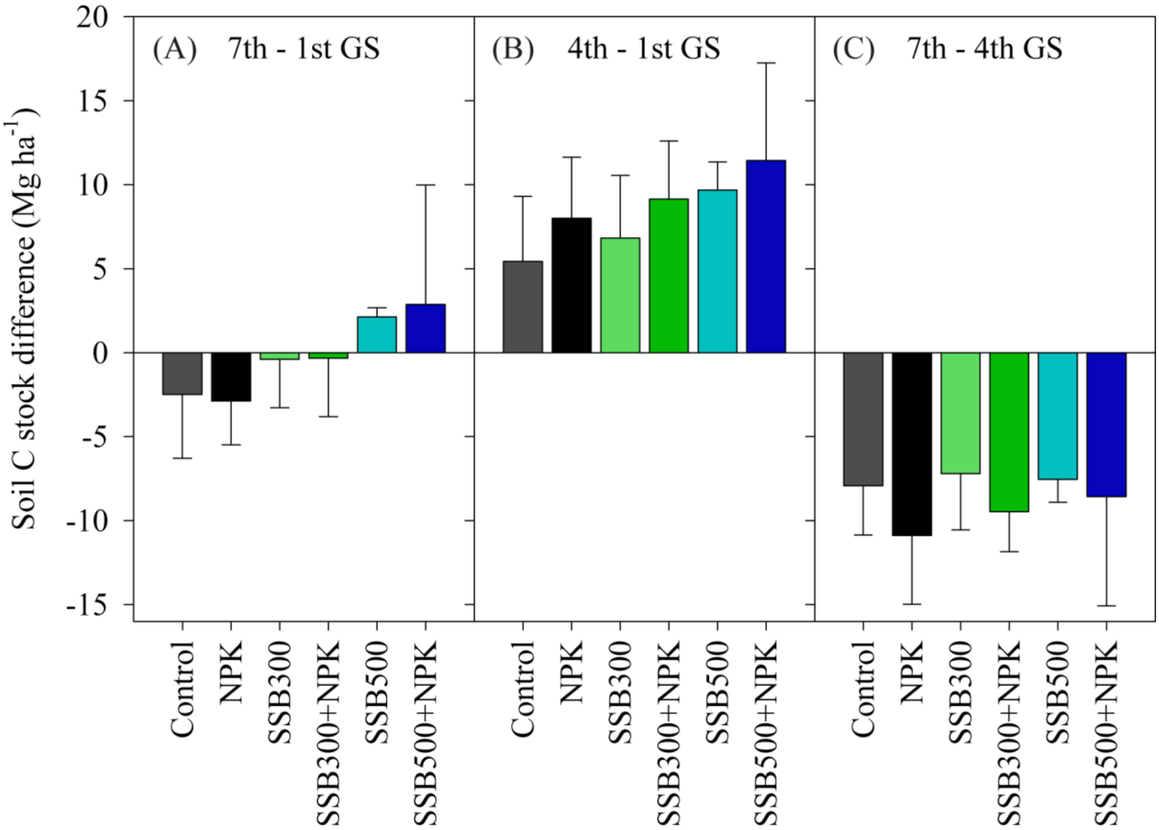


Figure S3. Differences in soil carbon stock between 7th and 1st (A), 4th and 1st (B), and 7th and 4th (C) growing seasons (GS). NPK: mineral fertilizer; SSB300 and SSB500: sewage sludge biochar pyrolyzed at 300°C and 500°C. Error bars represent standard error.

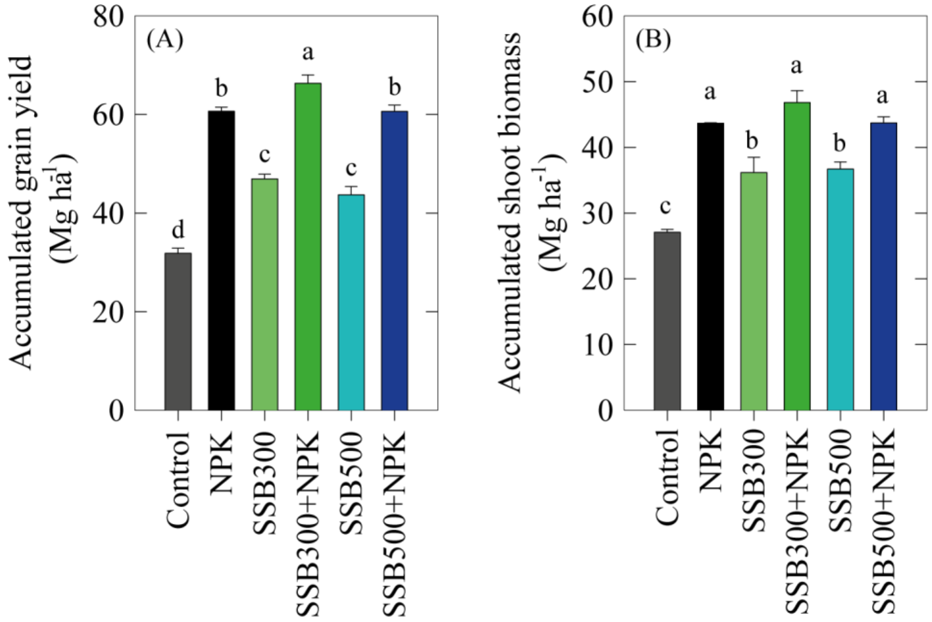


Figure S4. Accumulated grain yield (A) and accumulated shoot biomass (B) across growing seasons. Means followed by a common letter are not significantly different by the Tukey-test ($p < 0.05$). Error bars represent standard error.

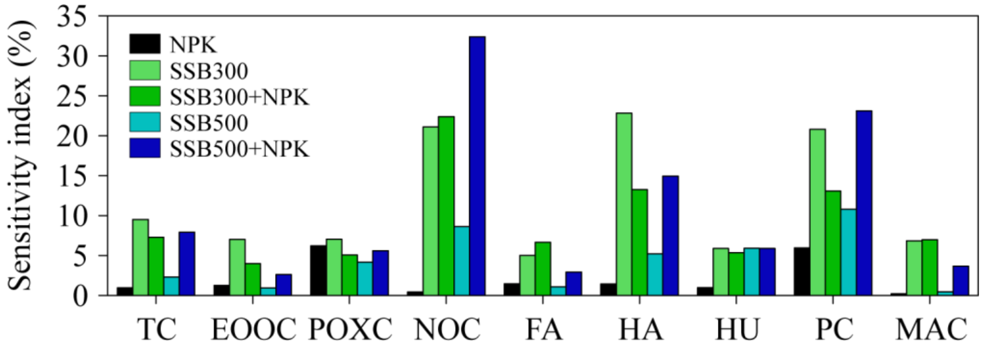


Figure S5. Comparison of the amendments on sensitivity indices of total carbon (TC), easily oxidizable organic carbon (EOOC), permanganate-oxidizable carbon (POXC), non-oxidizable carbon (NOC), fulvic acid (FA), humic acid (HA), humin (HU), particulate carbon (PC) and mineral-associated carbon (MAC) in the soil.

List of references cited in Table 6:

[1] Giannetta, B., Plaza, C., Cassetta, M., Mariotto, G., Benavente-Ferraces, I., García-Gil, J.C., Panettieri, M., Zaccone, C., 2023. The effects of biochar on soil organic matter pools are not influenced by climate change. J Environ Manage 341. https://doi.org/10.1016/j.jenvman.2023.118092

[2] Lopez-Capel, E., de la Rosa Arranz, J.M., González-Vila, F.J., González-Perez, J.A., Manning, D.A.C., 2006b. Elucidation of different forms of organic carbon in marine sediments from the Atlantic coast of Spain using thermal analysis coupled to isotope ratio

and quadrupole mass spectrometry. Org Geochem 37, 1983–1994. https://doi.org/10.1016/j.orggeochem.2006.07.025

[3] Gascó, G., Paz-Ferreiro, J., Méndez, A., 2012. Thermal analysis of soil amended with sewage sludge and biochar from sewage sludge pyrolysis. J Therm Anal Calorim 108, 769–775. https://doi.org/10.1007/s10973-011-2116-2

[4] Baffi, C., Dell'Abate, M.T., Nassisi, A., Silva, S., Benedetti, A., Genevini, P.L., Adani, F., 2007. Determination of biological stability in compost: A comparison of methodologies. Soil Biol Biochem 39, 1284–1293. https://doi.org/10.1016/j.soilbio.2006.12.004

[5] Plante, A.F., Pernes, M., Chenu, C., 2005. Changes in clay-associated organic matter quality in a C depletion sequence as measured by differential thermal analyses. Geoderma 129, 186–199. https://doi.org/10.1016/j.geoderma.2004.12.043

[6] De la Rosa, J.M., Knicker, H., López-Capel, E., Manning, D.A.C., González-Perez, J.A., González-Vila, F.J., 2008. Direct Detection of Black Carbon in Soils by Py-GC/MS, Carbon-13 NMR Spectroscopy and Thermogravimetric Techniques. Soil Science Society of America Journal 72, 258–267. https://doi.org/10.2136/sssaj2007.0031

[7] Yanardağ, İ.H., Zornoza, R., Cano, A.F., Yanardağ, A.B., Mermut, A.R., 2015. Evaluation of carbon and nitrogen dynamics in different soil types amended with pig slurry, pig manure and its biochar by chemical and thermogravimetric analysis. Biol Fertil Soils 51, 183–196. https://doi.org/10.1007/s00374-014-0962-3

[8] Kristensen, E., 1990. Characterization of biogenic organic matter by stepwise thermogravimetry (STG). Biogeochemistry 9, 135–159. https://doi.org/10.1007/BF00692169

[9] Dell'Abate, M.T., Benedetti, A., Sequi, P., 2000. Thermal Methods of Organic Matter Maturation Monitoring During a Composting Process. J Therm Anal Calorim 61, 389–396. https://doi.org/10.1023/A:1010157115211

[10] Dell'Abate, M.T., Benedetti, A., Trinchera, A., Dazzi, C., 2002. Humic substances along the profile of two Typic Haploxerert. Geoderma 107, 281–296. https://doi.org/10.1016/S0016-7061(01)00153-7

[11] Dell'Abate, M.T., Benedetti, A., Brookes, P.C., 2003. Hyphenated techniques of thermal analysis for characterisation of soil humic substances. J Sep Sci 26, 433–440. https://doi.org/10.1002/jssc.200390057

[12] Lopez-Capel, E., Sohi, S.P., Gaunt, J.L., Manning, D.A.C., 2005. Use of thermogravimetry–differential scanning calorimetry to characterize modelable soil

organic matter fractions. Soil Science Society of America Journal 69, 136–140. https://doi.org/10.2136/sssaj2005.0136a
[13] Lopez-Capel, E., Abbott, G.D., Thomas, K.M., Manning, D.A.C., 2006a. Coupling of thermal analysis with quadrupole mass spectrometry and isotope ratio mass spectrometry for simultaneous determination of evolved gases and their carbon isotopic composition. J Anal Appl Pyrolysis 75, 82–89. https://doi.org/10.1016/j.jaap.2005.04.004
[14] Fernández, J.M., Hockaday, W.C., Plaza, C., Polo, A., Hatcher, P.G., 2008. Effects of long-term soil amendment with sewage sludges on soil humic acid thermal and molecular properties. Chemosphere 73, 1838–1844. https://doi.org/10.1016/j.chemosphere.2008.08.001

References cited in the Appendix

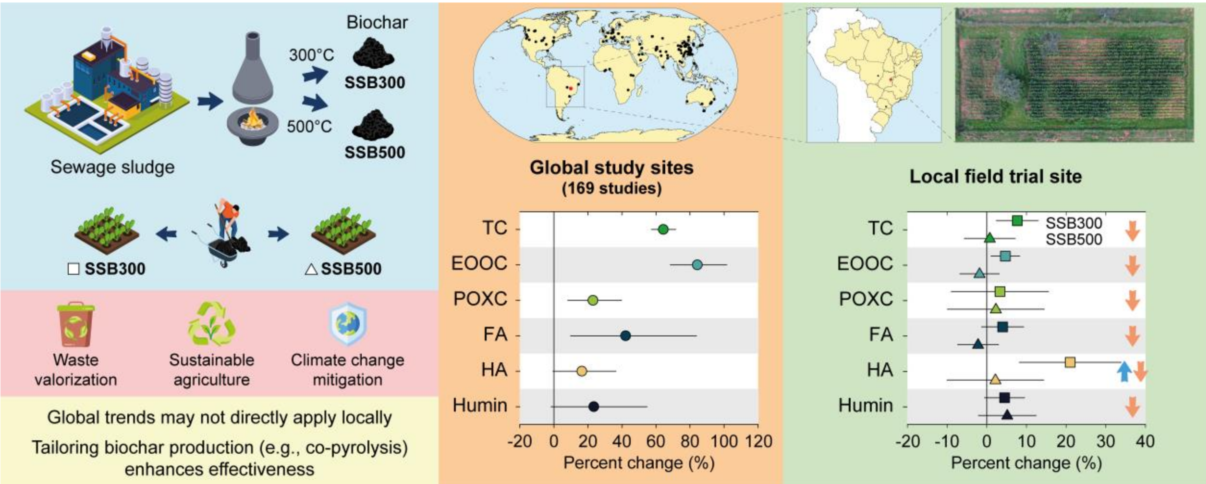
García, R., Pizarro, C., Lavín, A.G., Bueno, J.L., 2013. Biomass proximate analysis using thermogravimetry. Bioresource Technology 139, 1–4. https://doi.org/10.1016/j.biortech.2013.03.197

CHAPTER III

COMBINING META-ANALYSIS AND LOCAL ASSESSMENT: AN IN-DEPTH APPROACH ON BIOCHAR USE TOWARD SOIL CARBON SEQUESTRATION

7. COMBINING META-ANALYSIS AND LOCAL ASSESSMENT: AN IN-DEPTH APPROACH ON BIOCHAR USE TOWARD SOIL CARBON SEQUESTRATION

Graphical abstract



SSB300: sewage sludge biochar pyrolyzed at 300°C; SSB500: sewage sludge biochar pyrolyzed at 500°C; TC: total carbon; EOOC: easily oxidizable organic C; POXC: permanganate-oxidizable carbon; FA: fulvic acid; HA: humic acid.

Abstract

This study evaluates the impact of biochar, produced by valorizing waste sewage sludge, on soil carbon (C) sequestration, combining a global meta-analysis with a four-year tropical field trial. Biochar application can enhance soil C and mitigate climate change, contributing to sustainable resource management. The meta-analysis of 586 paired comparisons from 169 studies showed increases in total C (TC) and various soil C fractions post-biochar application. In contrast, the field trial using sewage sludge biochar pyrolyzed at 300°C (SSB300) and 500°C (SSB500) showed modest TC increases (7.7% with SSB300, 0.7% with SSB500) and minimal changes in other C fractions. Importantly, the absolute TC gain with SSB300 surpassed those from practices like no-till farming. These findings highlight the importance of considering local conditions when implementing biochar strategies. Adopting context-specific approaches can enhance waste recycling, promote sustainable agriculture, and aid in climate change mitigation.

Keywords: biochar, soil carbon sequestration, meta-analysis, sewage sludge biochar, tropical soils

Resumo

Este estudo avalia o impacto do biochar, produzido pela valorização do resíduo de lodo de esgoto, no sequestro de carbono do solo (C), combinando uma meta-análise global com um ensaio de campo de quatro anos em uma região tropical. A aplicação de biochar pode aumentar o C do solo e atenuar as mudanças climáticas, contribuindo para o gerenciamento sustentável dos recursos. A meta-análise de 586 comparações pareadas de 169 estudos mostrou aumentos no C total (TC) e em várias frações do C do solo após a aplicação do biochar. Por outro lado, o estudo de campo usando biochar de lodo de esgoto pirolisado a 300°C (SSB300) e 500°C (SSB500) mostrou aumentos modestos de TC (7,7% com SSB300, 0,7% com SSB500) e alterações mínimas em outras frações de C. É importante ressaltar que o ganho absoluto de TC com SSB300 superou o de práticas como o plantio direto. Essas constatações destacam a importância de considerar as condições locais ao implementar estratégias com biochar. A adoção de abordagens específicas ao contexto pode aprimorar a reciclagem de resíduos, promovendo a agricultura sustentável e ajudando na mitigação das mudanças climáticas.

Palavras-chave: biochar, sequestro de carbono no solo, meta-análise, biochar de lodo de esgoto, solos tropicais

7.1. Introduction

Managing soil carbon (C) pools is essential for improving soil health and mitigating climate change (Lal, 2016). Several agricultural practices contribute to increasing soil C pools and sequestration, including no-till farming (Corbeels et al., 2016), cover cropping (Poeplau and Don, 2015), integrated crop-livestock-forest systems (Coser et al., 2018), reforestation with planted forests (Silver et al., 2000), manure application (Gross and Glaser, 2021), and biochar use (Gross et al., 2021a).

Biochar, a C-rich material produced by pyrolysis of organic biomass under low-oxygen conditions, has gained attention for its potential to improve soil C fractions, enhance soil fertility, and reduce greenhouse gas emissions (Amoakwah et al., 2020; Li et al., 2024). Biochar can increase labile and stable soil organic C fractions, including total C (TC), easily oxidizable organic C (EOOC), permanganate oxidizable C (POXC), fulvic acids (FA), humic acids (HA), and humin (Jarosz et al., 2020; Wu et al., 2021). Understanding how biochar affects these different C fractions is crucial for optimizing its use in soil C management strategies.

Despite these benefits, the effects of biochar are highly variable, influenced by factors such as feedstock type, pyrolysis conditions, application rates, soil type, and climate (Gross et al., 2021b; X. Li et al., 2020). This variability makes it challenging to predict consistent outcomes of biochar application across different environments, emphasizing the need for approaches combining global meta-analytical insights and localized field data.

Global meta-analyses provide aggregated data on the effects of biochar on soil C pools, showing general trends like increases in various soil C fractions (Biederman and Harpole, 2013; Chagas et al., 2022; Liu et al., 2016). For instance, Chagas et al. (2022) reported significant increases in TC, EOOC, microbial biomass C, and FA following biochar application. However, these analyses often mask significant site-specific variations due to differing environmental conditions and management practices (Shackelford et al., 2021). The effects of biochar on other soil C fractions, such as dissolved organic C, humic acid, and humin, are less predictable, underscoring the complex interactions between biochar properties and environmental factors (Chagas et al., 2022).

Subgroup analyses within meta-analyses help to refine understanding by isolating effects under specific conditions, providing more targeted insights for guiding biochar application in diverse settings (Burford et al., 2013). For example, biochar produced from different feedstocks can have varying impacts on soil C fractions due to differences in their chemical composition and stability (Liu et al., 2016).

Local field trials are essential for understanding how biochar affects different soil C fractions and crop productivity under specific environmental conditions. Field studies have shown that biochar application can significantly increase stable soil C fractions like humic substances, while effects on labile fractions may vary (Ding et al., 2023; S. Li et al., 2020). A field study in Brazil using sewage sludge biochar (SSB) reported increases in stable soil C fractions, such as non-oxidizable organic C, but showed varied effects on more labile fractions and overall soil fertility, highlighting the influence of local soil properties and climate. These findings highlight the importance of localized data to validate or challenge global findings to ensure that biochar use is both effective and context-specific.

Comparing results from global meta-analyses with local studies is particularly relevant in countries like Brazil, which have a high potential for biochar use due to their large agricultural sector and diverse climatic conditions (Arias et al., 2023; Lefebvre et al., 2020). Despite this potential, there are few long-term studies on the effects of biochar in tropical regions, leading to a knowledge gap about its sustained impact under local conditions. Moreover, the accelerated expansion of the global C credit market (Yang and Luo, 2020) could benefit from reliable data on the efficacy of biochar, aiding the development of verified C standards and methodologies, such as those promoted by Verra (2023). Aligning local field trial results with global meta-analyses can enhance the accuracy of C credit metrics and promote sustainable agricultural practices.

This comparative analysis is crucial for providing evidence-based recommendations on biochar use, potentially influencing policy and market decisions regarding C credits. By aligning global evidence with local findings, this study aims to clarify the conditions under which biochar is most effective, contributing to the broader goal of enhancing soil health and mitigating climate change through optimized soil management practices.

This study aimed to integrate insights on the effects of biochar on soil C pools from global and local perspectives. By comparing global meta-analysis data with local field trials results, this research offers a comprehensive analysis of biochar use for soil C management, supporting more effective and sustainable agricultural practices.

7.2. Material and Methods

7.2.1. Data sources and selection process

The global meta-analysis results used in this study were derived from a comprehensive dataset that included 586 paired comparisons from 169 peer-reviewed articles reported in our previous study (Chagas et al., 2022). These articles were selected based on stringent criteria, such as randomized experimental designs, explicit replication numbers, control and treatment consistency, and clear evaluation of at least one soil C fraction using defined determination methods. Data extraction focused on key variables, including mean values, standard deviations, and the number of repetitions for various soil C fractions – total C (TC), easily oxidizable organic C (EOOC), permanganate oxidizable C (POXC), fulvic acid (FA), humic acid (HA), and humin. The data were categorized based on key factors such as biochar feedstock, pyrolysis temperature, application rate, soil C content, experiment type, and duration. A random-effects model was utilized to calculate the response ratios, expressed as log-transformed ratios of means, to quantify effect sizes across studies. Statistical significance was determined using 95% confidence intervals, and results were expressed as percent changes to evaluate the effectiveness of biochar in enhancing soil C pools globally.

The local field trial data were collected from an experiment conducted at Fazenda Água Limpa (FAL/UnB), Brasília-DF, Brazil, over seven growing seasons (2015-2021). This field trial specifically examined the effects of SSB produced at two pyrolysis temperatures, 300°C (SSB300) and 500°C (SSB500). Treatments included a non-fertilized control (no biochar, no mineral fertilization), SSB300 and SSB500 applications, with four repetitions. Biochars were applied at a rate of 15 t ha^{-1} during the initial two growing seasons and incorporated into the soil's top 0.2 m layer. The study focused on the first four seasons (2015-2018) to capture the direct effects of biochar amendments, as previous research indicates that the impact of biochar on soil properties often manifests one to two years post-application (Chagas et al., 2021a). The same soil C fractions assessed in the global meta-analysis were measured in this local study to ensure consistency and comparability between the datasets. Soil samples were collected from the top 20 cm at post-harvest. TC was determined using a CHN elemental analyzer (Eurovector EA3000, Milan, Italy) at 980°C. EOOC was measured by wet oxidation with potassium dichromate without external heating (Walkley and Black, 1934), and POXC by oxidation with potassium permanganate (Blair et al., 1995). Soil humic substances were fractionated into FA, HA, and humin based on solubility differences in acidic and alkaline media (Swift, 1996), adapted from Benites and Machado (2003). The C content of these fractions was quantified by oxidation with potassium permanganate.

7.2.2. Analytical approaches for data integration and comparison

The soil C fractions common to both studies (TC, EOOC, POXC, FA, HA, and humin) were identified and analyzed to integrate and compare the global meta-analysis with the local field trial data. However, subgroup analyses were only performed for TC and EOOC due to the availability of sufficient data in the meta-analysis. For the other soil C fractions (POXC, FA, HA, and humin) only overall effect sizes were calculated because the dataset did not support a more detailed subgroup analysis. The comparison focused on the overall effect sizes and specific subgroups matching the local field trial conditions. This methodology aligns with the concept of dynamic scoping as outlined by Shackelford et al. (2021), where subsets of the global dataset are filtered to match local conditions, thereby enhancing the relevance and applicability of the findings. This approach allows for a detailed examination of the effects of biochar on soil C pools observed globally compared to those found locally.

The local data were transformed into percent changes relative to the control treatment, based on mean values across the first four growing seasons, facilitating a direct comparison with the global dataset. The analysis involved calculating the mean, standard error of the mean (SEM), 95% confidence intervals (CI), and percent change relative to the control for each soil fraction. The 95% CI for each treatment was calculated using equation (6):

$$\mathrm{CI}_{95} = \mathrm{SEM} \times t_{(0.975,\mathrm{N-1})} \tag{6}$$

where $t_{(0.975,N-1)}$ is the critical value from the t-distribution for a 95% confidence level with N−1 degrees of freedom. The percent change (Pc) relative to the control was determined using equation (7).

$$\mathrm{P_C} = \left(\frac{\mathrm{Mean_{treatment}} - \mathrm{Mean_{control}}}{\mathrm{Mean_{control}}}\right) \times 100 \tag{7}$$

To estimate the variability of the Pc, the confidence interval for the percent change (CI_{PC}) was calculated using equation (8).

$$\mathrm{CI_{PC}} = \left(\frac{\mathrm{CI_{95}}}{\mathrm{Mean_{control}}}\right) \times 100 \tag{8}$$

The confidence intervals were also used to statistically compare the effect sizes between the field trial and the meta-analysis. If the confidence intervals of the percent change from the field trial and the meta-analysis overlapped, no significant difference was suggested. This statistical approach provided a standardized method for quantifying differences between the global and local datasets, enabling a comprehensive understanding of the effects of biochar on soil carbon sequestration in various contexts. The statistical analyses were conducted using Python with appropriate statistical libraries (Python Software Foundation, 2024).

7.3. Results and Discussion

7.3.1. A comparison of local field trial and global meta-analysis parameters

The parameters of the local field trial were categorized according to the global meta-analysis framework to ensure comparability, revealing several key differences (Table 7). The local study utilized SSB produced at a lower pyrolysis temperature (300°C), with low C content. The biochar was applied to fine-textured soil with a high initial soil C content in a tropical region. These characteristics were underrepresented in the meta-analysis, with fewer than one-third of the global studies investigating similar conditions. This discrepancy underscores the variability in biochar types and environmental conditions studied globally, which can affect the overall trends.

Table 7. Comparison of local field trial parameters with meta-analysis classifications and study distribution.

Parameter	Field trial value	Meta-analysis classification	% of studies in meta-analysis
Raw material	Sewage sludge	Sewage sludge	11%
Pyrolysis temperature (°C)	300	Low	14%
	500	Medium	76%
Biochar C content (%)	23.4 (SSB300)	≤ 35%	14%
	19.0 (SSB500)	≤ 35%	14%
Biochar rate (%)	0.75	Low	56%
Soil texture	Silty clay	Fine	23%
Soil pH	4.9	Acid	54%
Soil C content (%)	2.64	High	26%
Climate zone	-	Tropical	12%
Experiment type	-	Field trial	54%
Experiment duration	7 years	> 2 years	31%

The low representation of SSB in the meta-analysis dataset, comprising only 11% of the studies, reveals a significant gap in research. Despite its limited coverage, SSB remains a valuable input in sustainable agriculture, offering improvements in soil fertility (Tian et al., 2019), C sequestration (Yin et al., 2021), and reductions in greenhouse gas emissions (Ibrahim et al., 2017). Its distinct properties, including pathogen reduction, nutrient provision, and heavy metal stabilization, position it as a promising alternative to traditional waste disposal methods, especially in tropical regions (Chagas et al., 2021a, 2021b). Given the potential environmental and agricultural benefits of SSB, further research is necessary, particularly in underrepresented tropical environments, to optimize its use across different agroecological contexts (Ghorbani et al., 2022).

Tropical regions, which accounted for only 12% of the studies in the global meta-analysis, are particularly underrepresented (Figure 21). This is significant because tropical soils and climates, characterized by rapid organic matter turnover and unique microbial dynamics (Rasche and Cadisch, 2013), influence biochar's interaction with soil differently from temperate regions. These conditions present distinctive challenges and opportunities for C sequestration. Given that tropical regions occupy 40% of the Earth's surface and face substantial agricultural constraints, biochar – particularly SSB – could play a crucial role as a tool for C sequestration and soil enhancement. However, more extensive field trials and long-term studies are required to assess the potential impact of SSB in these regions.

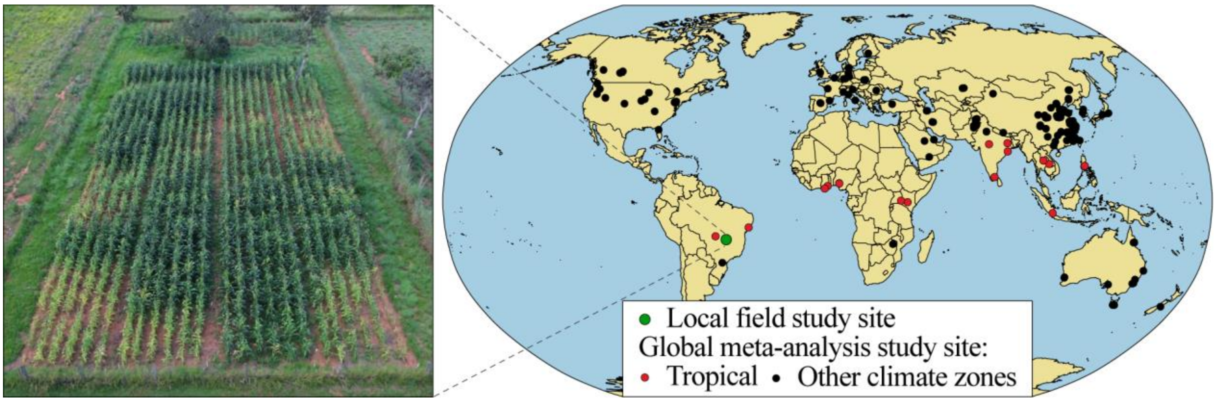


Figure 21. Global meta-analysis and local field trial sites for assessing soil carbon changes due to biochar amendment.

7.3.2. Comparative analysis for total carbon (TC)

Considerable differences in the TC values emerged between the global meta-analysis and the local field trial. The meta-analysis revealed a significant positive impact of biochar on TC across various contexts, with percent increases ranging from 28.9% to 64.3% (Figure 22). However, the local trial with SSB showed more modest gains, with TC increasing by 7.7% for SSB300 and only 0.7% for SSB500. Although the TC increase for SSB500 was not statistically significant, as its confidence interval overlapped zero, there was no significant difference in percent change between SSB300 and SSB500. This discrepancy highlights the importance of considering local soil and environmental conditions when interpreting global data, especially in tropical soils. The temporal dynamics of TC over the four years in the local trial are presented in Figure S6a.

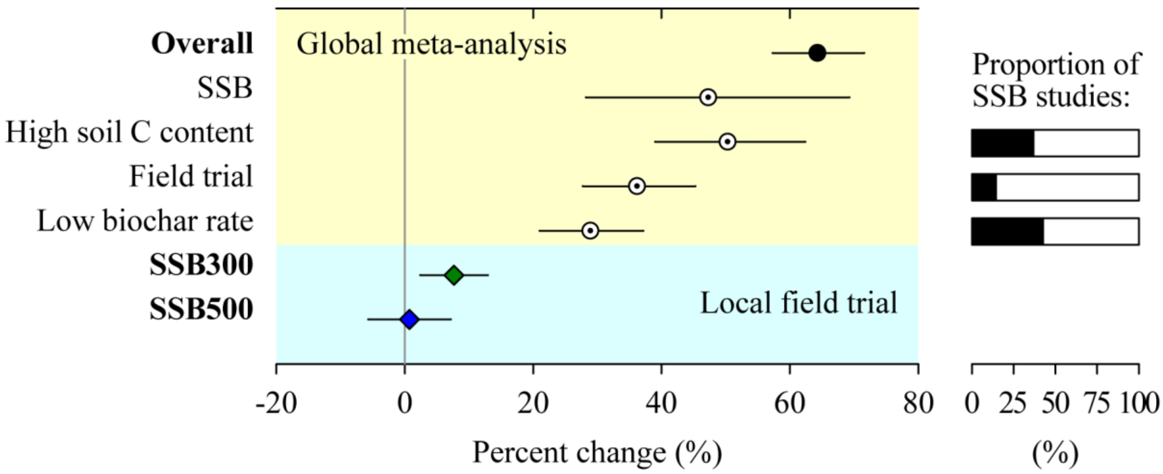


Figure 22. Percent change in soil total carbon (C) from global meta-analysis and local field trial and proportion of SSB studies for specific conditions. SSB: sewage sludge biochar pyrolyzed under 300°C (SSB300) and 500°C (SSB500).

Several factors contribute to the observed differences. First, SSB and soils with high initial C content are underrepresented in global datasets, as mentioned in section 7.3.1. These conditions reduced the overall effect size in TC increases. Field trials and studies using low biochar application rates are prevalent in the global dataset, accounting for over 50% of the cases (Table 7). However, among studies specifically using SSB, the proportion of field trials drops to 14.3%, and only 42.4% of studies employed low SSB application rates (Figure 22). This suggests that the global meta-analyses for SSB are predominantly based on controlled environments and higher application rates, which may overstate the TC increases compared to what is achievable under field conditions with lower application rates. Consequently, the global meta-analyses may not accurately reflect the performance of SSB under practical field conditions, potentially biasing the overall TC change.

High initial soil C content likely constrained the observed percent increases in the local trial. Soils with higher baseline C levels require more substantial C input to register noticeable percent changes (Paustian et al., 2019). Additionally, field trials tend to present lower TC increases due to the complexity of *in situ* conditions. Unlike controlled laboratory experiments, field trials expose biochar to various dynamic environmental disturbances, such as tillage, fluctuating temperature, water availability, redox cycles, and biotic activities (Yi et al., 2020). These factors contribute to reduced effect sizes in field settings, as biochar particles experience greater physical and chemical stress, limiting their stability and C sequestration potential over time.

According to the meta-analysis, the biochar application rate is the primary factor influencing TC increases (Chagas et al., 2022), with higher TC increases observed at higher biochar rates. The mean application rate in the meta-analysis studies assessing TC was 2.90%, which is almost three times higher than the 0.75% used in the local trial. This difference in biochar application rates likely contributes to the discrepancy between global and local results.

SSB, particularly when pyrolyzed at low to medium temperatures, is known for its nutrient supply capacity (Goldan et al., 2022). However, SSB typically has a lower C content and stability compared to other biochars, limiting its long-term C sequestration potential. This is attributed to its higher O/C ratios, lower recalcitrance indices, and higher ash content (Adhikari et al., 2024; McBeath et al., 2015). These factors reduce its effectiveness in maintaining soil C stocks over time, as observed in the local field trial.

Notably, the meta-analysis included no SSB results lasting more than two years, and only 5.7% of SSB results were obtained in tropical regions. Optimizing pyrolysis conditions to produce more recalcitrant biochar is crucial to enhance the C sequestration potential of SSB in the tropics. Co-pyrolysis of SSB with plant materials has shown a potential to increase the C content and improve stability (Nair et al., 2023). Co-pyrolysis with organic additives has been shown to reduce the H/C, N/C, and O/C ratios, thereby improving long-term SSB stability (Yin et al., 2021).

Despite the lower percent increases observed in the local trial, the absolute increases in TC were substantial when compared to other agricultural management practices. Over the 4-year period, the average increases in TC corresponded to 4.19 Mg C ha^{-1} for SSB300 and 0.40 Mg C ha^{-1} for SSB500. For instance, after 31 years of cultivation, reductions in C emissions under no-till corresponded to a linear rate of 0.35 Mg C ha^{-1} $year^{-1}$ compared to conventional tillage (Ferreira et al., 2016), amounting to an increase of 1.4 Mg C ha^{-1} over 4 years. Similarly, meta-analyses report positive effects of cover crops on SOC stocks with average SOC accrual rates of 0.21–0.56 Mg C ha^{-1} $year^{-1}$ (Qin et al., 2023), corresponding to an increase of 0.84–2.24 Mg C ha^{-1} over 4 years. Thus, the absolute increase in TC with SSB300 surpasses those achieved with no-till and cover crop adoption over a similar period. However, direct comparisons should be made cautiously due to differences in study durations and conditions.

Although the percent increase in TC with SSB300 (7.7%) was lower than the global average for biochar (64.3%), it is comparable to the percent increases observed for other widely recognized soil management practices that contribute to C sequestration and climate change mitigation, such as no/reduced tillage (5.0%) and cover crops (11.6%) (Beillouin et al., 2023). This indicates that, in absolute terms, the application of SSB can be an effective strategy for increasing soil C stocks, even if the percent increases appear modest compared to global averages for biochar.

It is worth noting that biochar was only applied in the first two growing seasons of the local trial, yet the cumulative increase in TC over four years was substantial (Figure S6a). While the intention is not to propose replacing established practices like no-till farming or cover cropping with biochar application, these findings suggest that combined technologies could contribute synergistically to increasing soil C stocks. However, further long-term field studies are needed to confirm this hypothesis and to understand the interactions between biochar application and other soil management practices.

By adjusting biochar production and application strategies to match local environmental conditions, stakeholders can ensure that biochar projects contribute meaningfully to C sequestration efforts, particularly in regions like Brazil, where tropical soils present unique challenges and opportunities for C management. Contextualized biochar use can also support C credit markets, allowing for more accurate accounting of verifiable climate benefits.

7.3.3. Comparative analysis for easily oxidizable organic carbon (EOOC)

The global meta-analysis indicated a significant positive effect of biochar application on EOOC, with an overall percent increase of 84.3% (Figure 23). Notably, SSB exhibited an even larger effect, showing a percent increase of 242.3%. In contrast, the local field trial demonstrated a modest increase in EOOC for SSB300 (4.7%) and a non-significant decrease for SSB500 (–1.8%). No significant difference was observed between the SSB300 and SSB500 treatments, suggesting that pyrolysis temperature may not substantially impact EOOC under the conditions of the local field trial. The temporal dynamics of EOOC in the field trial is presented in Figure S6b.

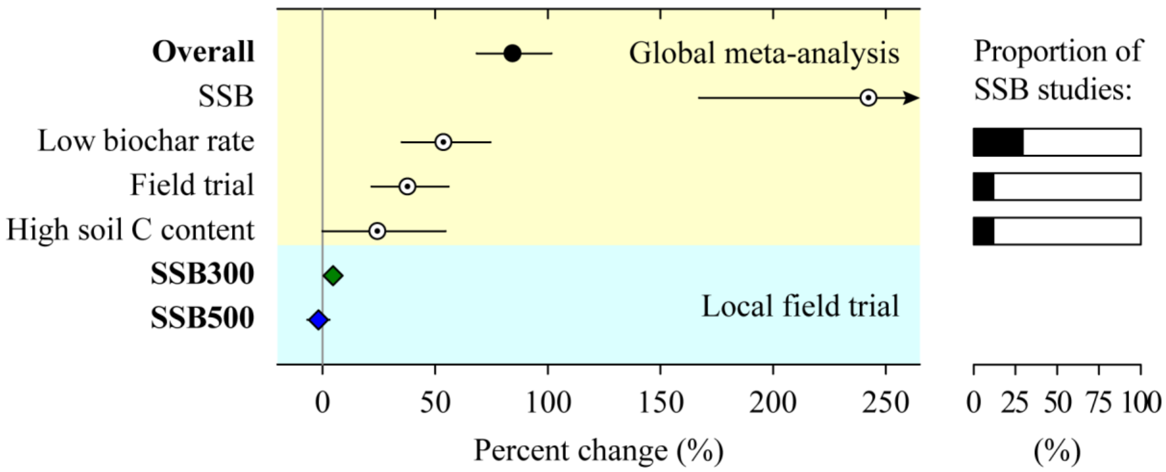


Figure 23. Percent change in soil easily oxidizable organic carbon (C) from global meta-analysis and local field trial and proportion of SSB studies for specific conditions. SSB: sewage sludge biochar pyrolyzed under 300°C (SSB300) and 500°C (SSB500).

The pronounced discrepancy between the global meta-analysis and the local field trial suggests that the large positive effects of SSB on EOOC observed globally may be overestimated due to the predominance of laboratory studies in the meta-analysis dataset. The meta-analysis primarily comprises laboratory studies, with only 11.8% of SSB studies conducted under field conditions and an equal proportion on soils with high initial C content (Figure 23). Although a higher proportion of SSB studies used low biochar application rates, this still represents less than one-third of the studies. This confirms the scarcity of SSB studies assessing not only TC but also EOOC under field conditions. Moreover, no experiments longer than two years were included in the SSB meta-analysis dataset, highlighting a gap in long-term field studies on the effects of SSB biochars on EOOC.

Underrepresentation of specific conditions or subgroups in meta-analyses can bias effect size estimates and limit the generalizability of the findings (Ding et al., 2022). The positive effects of SSB biochar on EOOC observed in laboratory settings may not directly translate to field conditions, especially in tropical soils.

Soil microbial dynamics influenced by biochar additions can also affect EOOC levels. Biochar can alter soil microbial community composition and activity, impacting the decomposition of organic matter and the turnover of labile C fractions (Ding et al., 2023). The specific microbial environment in the local trial's tropical soil may differ from those in temperate soils (Ngaba et al., 2024) commonly studied in the meta-analysis, leading to varying effects on EOOC.

From an agronomic perspective, the lack of a significant increase in EOOC in the local trial suggests that the immediate benefits of biochar application on soil fertility through increases in EOOC could be context-dependent. EOOC is crucial for nutrient cycling and availability, directly influencing plant growth and yield (Lal, 2020). These results affect soil organic matter dynamics, indicating that biochar may contribute more to stable C pools than labile ones in high-C tropical soils. This shift can influence the turnover rates of soil organic matter, potentially affecting long-term soil fertility and C sequestration strategies (Kuzyakov et al., 2014).

7.3.4. Comparative analysis for other soil carbon fractions

The effects of biochar on specific soil C fractions differed in terms of significance between the global meta-analysis and the local field trial (Figure 24), emphasizing the importance of biochar properties and site-specific conditions in soil C dynamics.

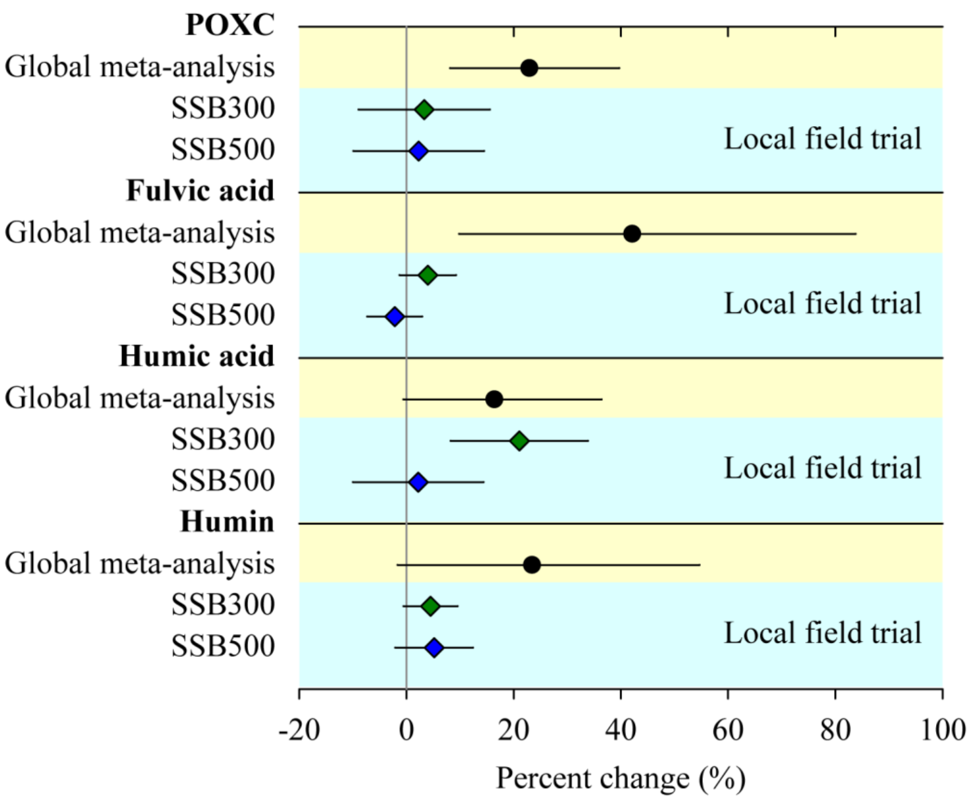


Figure 24. Percent change in soil permanganate-oxidizable carbon (POXC), fulvic acid, humic acid, and humin from global meta-analysis and local field trial. SSB300 and SSB500: sewage sludge biochar pyrolyzed under 300°C and 500°C, respectively.

The global meta-analysis indicated significant increases in labile C pools, with a 22.9% increase in POXC and a 42.1% increase in FA following biochar application. These fractions are crucial for microbial activity and nutrient cycling, serving as indicators of soil health and fertility (Bongiorno et al., 2019; Zhang et al., 2020). In contrast, the local field trial showed non-significant changes in both POXC and FA relative to the control, with percent increases of 3.30% and 3.97% for SSB300, and 2.27% and a slight decrease of 2.21% for SSB500, respectively. The temporal dynamics depicted in Figure S6c and Figure S6d illustrate that these fractions remained relatively stable over the four years. The coherence in results between these two labile pools suggests that the methods of C determination yielded consistent trends, regardless of whether POXC or FA was measured. This lack of significant change in the local trial suggests that the immediate benefits of biochar on microbial biomass and nutrient availability might not be evident under certain conditions. This finding aligns with observations by Qiu et al. (2023), who reported a decrease in POXC after 12 months of biochar application.

For HA, representing the intermediate soil C pool important for soil structure and nutrient retention (Ampong et al., 2022), the global meta-analysis suggested a moderate increase of 16.4%. The local trial demonstrated a significant increase of 21.03% with SSB300, while SSB500 showed a minor increase of 2.17%. Similarly, a previous study with corn cob biochar reported a stronger effect on HA compared to FA (Amoakwah et al., 2020), supporting the observation that certain biochars enhance HA more than FA.

This enhancement with SSB300 highlights that lower pyrolysis temperatures favor the retention of labile compounds, promoting humification. Lower temperatures (300–400°C) increase dissolved organic matter release, facilitating the transformation into humic substances due to higher oxygen-containing functional groups (Fan et al., 2023; Rajapaksha et al., 2019). In contrast, higher pyrolysis temperatures (500–600°C) lead to greater carbonization, forming more recalcitrant C structures with lower O/C and H/C ratios and reduced labile components like protein-like substances, limiting availability for humification (Fan et al., 2023). This underscores the importance of optimizing pyrolysis conditions to tailor biochar for specific soil management objectives.

Regarding humin, the most recalcitrant soil C fraction with turnover times from decades to centuries (Hayes et al., 2017), both the meta-analysis and local trial showed non-significant changes despite the meta-analysis reporting a 23.4% increase. The local trial observed modest increases of 4.47% for SSB300 and 5.15% for SSB500. These non-significant changes suggest that biochar's contribution to the most stable C pools may require higher application rates or longer periods to become significant. Additionally, the relatively low pyrolysis temperatures and low fixed C content of the SSB (3.03–5.47%) likely limited their potential contribution to soil humin.

These varying effects of biochar on different C fractions have essential implications for soil C dynamics and management strategies. As observed with HA in the local trial using

SSB300, the enhancement of intermediate C pools can improve soil structure and nutrient retention, contributing to soil fertility and resilience. Although the limited effect on labile C pools suggests that biochar may contribute more to stable C pools in tropical soils, this was not confirmed by the results for humin.

Therefore, tailoring biochar characteristics, such as feedstock selection and pyrolysis conditions, can optimize its effectiveness to match specific soil needs. Producing biochar at lower pyrolysis temperatures may enhance its ability to increase intermediate C fractions like HA. However, this must be balanced with the need for long-term stability, as lower temperature biochars may be less recalcitrant. These findings emphasize the necessity of considering biochar production parameters and site-specific factors to improve soil C sequestration and fertility through SSB application.

7.4. Conclusion

This study underscores the importance of integrating global meta-analytical insights with local field data to understand biochar's effects on soil C pools. While global meta-analysis showed positive impacts of biochar on TC and various soil C fractions, our local field trial using SSB in a tropical soil with high initial C content revealed more modest TC increases and minimal changes in other C fractions. These discrepancies highlight the necessity of considering local soil and environmental conditions when applying global findings. Despite lower percent increases, the absolute TC increase achieved with SSB300 was substantial compared to sustainable practices like no-till farming and cover cropping, indicating that SSB can still effectively enhance soil C stocks in tropical regions. Tailoring biochar production and application to local conditions, such as optimizing pyrolysis temperatures and exploring co-pyrolysis with plant materials, can enhance its effectiveness. Our findings have important implications for sustainable agriculture and climate change mitigation. Policymakers and stakeholders should consider local conditions and biochar properties to ensure meaningful contributions to soil health and climate goals. Future research should expand studies in tropical regions, conduct long-term field trials, and explore methods to improve biochar properties.

7.5. References

Adhikari, S., Moon, E., Paz-Ferreiro, J., Timms, W., 2024. Comparative analysis of biochar carbon stability methods and implications for carbon credits. Science of The Total Environment 914, 169607. https://doi.org/10.1016/j.scitotenv.2023.169607

Amoakwah, E., Arthur, E., Frimpong, K.A., Parikh, S.J., Islam, R., 2020. Soil organic carbon storage and quality are impacted by corn cob biochar application on a tropical sandy loam. J Soils Sediments 20, 1960–1969. https://doi.org/10.1007/s11368-019-02547-5

Ampong, K., Thilakaranthna, M.S., Gorim, L.Y., 2022. Understanding the Role of Humic Acids on Crop Performance and Soil Health. Frontiers in Agronomy 4. https://doi.org/10.3389/fagro.2022.848621

Arias, C.M., da Silva, L.F.S., Soares, M.R., Forti, V.A., 2023. A bibliometric analysis on the agricultural use of biochar in Brazil from 2003 to 2021: research status and promising raw materials. Renewable Agriculture and Food Systems 38, e19. https://doi.org/10.1017/S1742170522000412

Beillouin, D., Corbeels, M., Demenois, J., Berre, D., Boyer, A., Fallot, A., Feder, F., Cardinael, R., 2023. A global meta-analysis of soil organic carbon in the Anthropocene. Nat Commun 14, 3700. https://doi.org/10.1038/s41467-023-39338-z

Benites, V.D.M., Machado, P., 2003. Extraction and quantitative fractionation of humic substances from soil: a simplified, low-cost procedure [*Extração e fracionamento quantitativo de substâncias húmicas do solo: um procedimento simplificado de baixo custo*], Comunicado Técnico (16). Embrapa Solos, Rio de Janeiro. (in Portuguese)

Biederman, L.A., Harpole, W.S., 2013. Biochar and its effects on plant productivity and nutrient cycling: A meta-analysis. GCB Bioenergy 5, 202–214. https://doi.org/10.1111/gcbb.12037

Blair, G., Lefroy, R., Lisle, L., 1995. Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. Aust J Agric Res 46, 1459. https://doi.org/10.1071/AR9951459

Bongiorno, G., Bünemann, E.K., Oguejiofor, C.U., Meier, J., Gort, G., Comans, R., Mäder, P., Brussaard, L., de Goede, R., 2019. Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. Ecol Indic 99, 38–50. https://doi.org/10.1016/j.ecolind.2018.12.008

Burford, B., Lewin, S., Welch, V., Rehfuess, E., Waters, E., 2013. Assessing the applicability of findings in systematic reviews of complex interventions can enhance the utility of reviews for decision making. J Clin Epidemiol 66, 1251–1261. https://doi.org/10.1016/j.jclinepi.2013.06.017

Chagas, J.K.M., Figueiredo, C.C. de, Paz-Ferreiro, J., 2021a. Sewage sludge biochars effects on corn response and nutrition and on soil properties in a 5-yr field experiment. Geoderma 401, 115323. https://doi.org/10.1016/j.geoderma.2021.115323

Chagas, J.K.M., Figueiredo, C.C. de, Ramos, M.L.G., 2022. Biochar increases soil carbon pools: Evidence from a global meta-analysis. J Environ Manage 305, 114403. https://doi.org/10.1016/j.jenvman.2021.114403

Chagas, J.K.M., Figueiredo, C.C. de, Silva, J. da, Shah, K., Paz-Ferreiro, J., 2021b. Long-term effects of sewage sludge–derived biochar on the accumulation and availability of trace elements in a tropical soil. J Environ Qual 50, 264–277. https://doi.org/10.1002/jeq2.20183

Corbeels, M., Marchão, R.L., Neto, M.S., Ferreira, E.G., Madari, B.E., Scopel, E., Brito, O.R., 2016. Evidence of limited carbon sequestration in soils under no-tillage systems in the Cerrado of Brazil. Sci Rep 6, 21450. https://doi.org/10.1038/srep21450

Coser, T.R., de Figueiredo, C.C., Jovanovic, B., Moreira, T.N., Leite, G.G., Cabral Filho, S.L.S., Kato, E., Malaquias, J.V., Marchão, R.L., 2018. Short-term buildup of carbon from a low-productivity pastureland to an agrisilviculture system in the Brazilian savannah. Agric Syst 166, 184–195. https://doi.org/10.1016/j.agsy.2018.01.030

Ding, Q., Wan, S., Dowling, T.C., 2022. Research and scholarly methods: Subgroup analysis. Journal of the American College of Clinical Pharmacy 5, 551–558. https://doi.org/10.1002/jac5.1611

Ding, X., Li, G., Zhao, X., Lin, Q., Wang, X., 2023. Biochar application significantly increases soil organic carbon under conservation tillage: an 11-year field experiment. Biochar 5, 28. https://doi.org/10.1007/s42773-023-00226-w

Fan, J., Duan, T., Zou, L., Sun, J., 2023. Characteristics of dissolved organic matter composition in biochar: Effects of feedstocks and pyrolysis temperatures. Environmental Science and Pollution Research 30, 85139–85153. https://doi.org/10.1007/s11356-023-28431-x

Ferreira, E.A.B., Bustamante, M.M. da C., Resck, D.V.S., Figueiredo, C.C. de, Pinto, A. de S., Malaquias, J.V., 2016. Carbon Stocks in Compartments of Soil Organic Matter 31 Years after Substitution of Native Cerrado Vegetation by Agroecosystems. Rev Bras Cienc Solo 40, e0150059. https://doi.org/10.1590/18069657rbcs20150059

Ghorbani, M., Konvalina, P., Walkiewicz, A., Neugschwandtner, R.W., Kopecký, M., Zamanian, K., Chen, W.-H., Bucur, D., 2022. Feasibility of Biochar Derived from Sewage Sludge to Promote Sustainable Agriculture and Mitigate GHG Emissions—A Review. Int J Environ Res Public Health 19, 12983. https://doi.org/10.3390/ijerph191912983

Goldan, E., Nedeff, V., Barsan, N., Culea, M., Tomozei, C., Panainte-Lehadus, M., Mosnegutu, E., 2022. Evaluation of the use of sewage sludge biochar as a soil amendment - a review. Sustainability 14, 5309. https://doi.org/10.3390/su14095309

Gross, A., Bromm, T., Glaser, B., 2021a. Soil organic carbon sequestration after biochar application: a global meta-analysis. Agronomy 11, 2474. https://doi.org/10.3390/agronomy11122474

Gross, A., Bromm, T., Glaser, B., 2021b. Soil organic carbon sequestration after biochar application: a global meta-analysis. Agronomy 11, 2474. https://doi.org/10.3390/agronomy11122474

Gross, A., Glaser, B., 2021. Meta-analysis on how manure application changes soil organic carbon storage. Sci Rep 11, 5516. https://doi.org/10.1038/s41598-021-82739-7

Hayes, M.H.B., Mylotte, R., Swift, R.S., 2017. Humin: its composition and importance in soil organic matter, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, Burlington, pp. 47–138. https://doi.org/10.1016/bs.agron.2017.01.001

Jarosz, R., Mierzwa-Hersztek, M., Gondek, K., Kopeć, M., Lošák, T., Marcińska-Mazur, L., 2020. Changes in quantity and quality of organic matter in soil after application of poultry litter and poultry litter biochar—5-year field experiment. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-020-01005-4

Kuzyakov, Y., Bogomolova, I., Glaser, B., 2014. Biochar stability in soil: Decomposition during eight years and transformation as assessed by compound-specific 14C analysis. Soil Biol Biochem 70, 229–236. https://doi.org/10.1016/j.soilbio.2013.12.021

Lal, R., 2020. Soil organic matter content and crop yield. J Soil Water Conserv 75, 27–32. https://doi.org/10.2489/JSWC.75.2.27A

Lal, R., 2016. Beyond COP 21: Potential and challenges of the “4 per Thousand” initiative. J Soil Water Conserv 71, 20A-25A. https://doi.org/10.2489/jswc.71.1.20A

Lefebvre, D., Williams, A., Meersmans, J., Kirk, G.J.D., Sohi, S., Goglio, P., Smith, P., 2020. Modelling the potential for soil carbon sequestration using biochar from sugarcane residues in Brazil. Sci Rep 10, 1–11. https://doi.org/10.1038/s41598-020-76470-y

Li, B., Guo, Y., Liang, F., Liu, W., Wang, Y., Cao, W., Song, H., Chen, J., Guo, J., 2024. Global integrative meta-analysis of the responses in soil organic carbon stock to biochar amendment. J Environ Manage 351. https://doi.org/10.1016/j.jenvman.2023.119745

Li, S., Wang, S., Fan, M., Wu, Y., Shangguan, Z., 2020. Interactions between biochar and nitrogen impact soil carbon mineralization and the microbial community. Soil Tillage Res 196, 104437. https://doi.org/10.1016/j.still.2019.104437

Li, X., Wang, T., Chang, S.X., Jiang, X., Song, Y., 2020. Biochar increases soil microbial biomass but has variable effects on microbial diversity: A meta-analysis. Science of The Total Environment 749, 141593. https://doi.org/10.1016/j.scitotenv.2020.141593

Liu, S., Zhang, Y., Zong, Y., Hu, Z., Wu, S., Zhou, J., Jin, Y., Zou, J., 2016. Response of soil carbon dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar amendment: a meta-analysis. GCB Bioenergy 8, 392–406. https://doi.org/10.1111/gcbb.12265

McBeath, A. V., Wurster, C.M., Bird, M.I., 2015. Influence of feedstock properties and pyrolysis conditions on biochar carbon stability as determined by hydrogen pyrolysis. Biomass Bioenergy 73, 155–173. https://doi.org/10.1016/j.biombioe.2014.12.022

Nair, R.R., Kißling, P.A., Marchanka, A., Lecinski, J., Turcios, A.E., Shamsuyeva, M., Rajendiran, N., Ganesan, S., Srinivasan, S.V., Papenbrock, J., Weichgrebe, D., 2023. Biochar synthesis from mineral and ash-rich waste biomass, part 2: characterization of biochar and co-pyrolysis mechanism for carbon sequestration. Sustainable Environment Research 33. https://doi.org/10.1186/s42834-023-00176-9

Ngaba, M.J.Y., Mgelwa, A.S., Gurmesa, G.A., Uwiragiye, Y., Zhu, F., Qiu, Q., Fang, Y., Hu, B., Rennenberg, H., 2024. Meta-analysis unveils differential effects of agroforestry on soil properties in different zonobiomes. Plant Soil 496, 589–607. https://doi.org/10.1007/s11104-023-06385-w

Paustian, K., Larson, E., Kent, J., Marx, E., Swan, A., 2019. Soil C sequestration as a biological negative emission strategy. Frontiers in Climate 1, 8. https://doi.org/10.3389/fclim.2019.00008

Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops - A meta-analysis. Agric Ecosyst Environ 200, 33–41. https://doi.org/10.1016/j.agee.2014.10.024

Python Software Foundation, 2024. Python Language Reference [WWW Document]. URL https://docs.python.org/3/reference/index.html (accessed 7.16.24).

Qin, Z., Guan, K., Zhou, W., Peng, B., Tang, J., Jin, Z., Grant, R., Hu, T., Villamil, M.B., DeLucia, E., Margenot, A.J., Umakant, M., Chen, Z., Coppess, J., 2023. Assessing long-term impacts of cover crops on soil organic carbon in the central US Midwestern agroecosystems. Glob Chang Biol 29, 2572–2590. https://doi.org/10.1111/gcb.16632

Qiu, H., Liu, J., Boorboori, M.R., Li, D., Chen, S., Ma, X., Cheng, P., Zhang, H., 2023. Effect of biochar application rate on changes in soil labile organic carbon fractions and the association between bacterial community assembly and carbon metabolism with time.

Science of The Total Environment 855, 158876. https://doi.org/10.1016/j.scitotenv.2022.158876

Rajapaksha, A.U., Ok, Y.S., El-Naggar, A., Kim, H., Song, F., Kang, S., Tsang, Y.F., 2019. Dissolved organic matter characterization of biochars produced from different feedstock materials. J Environ Manage 233, 393–399. https://doi.org/10.1016/j.jenvman.2018.12.069

Rasche, F., Cadisch, G., 2013. The molecular microbial perspective of organic matter turnover and nutrient cycling in tropical agroecosystems - What do we know? Biol Fertil Soils. https://doi.org/10.1007/s00374-013-0775-9

Shackelford, G.E., Martin, P.A., Hood, A.S.C., Christie, A.P., Kulinskaya, E., Sutherland, W.J., 2021. Dynamic meta-analysis: a method of using global evidence for local decision making. BMC Biol 19, 33. https://doi.org/10.1186/s12915-021-00974-w

Silver, W.L., Ostertag, R., Lugo, A.E., 2000. The potential for carbon sequestration through reforestation of abandoned tropical agricultural and pasture lands. Restor Ecol 8, 394–407. https://doi.org/10.1046/j.1526-100x.2000.80054.x

Swift, R.S., 1996. Organic Matter Characterization, in: Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H. (Eds.), Methods of Soil Analysis Part 3 - Chemical Methods. Soil Science Society of America, American Society of Agronomy, Madison, WI, pp. 1011–1069. https://doi.org/10.2136/sssabookser5.3.c35

Tian, Y., Cui, L., Lin, Q., Li, G., Zhao, X., 2019. The sewage sludge biochar at low pyrolysis temperature had better improvement in urban soil and turf grass. Agronomy 9, 156. https://doi.org/10.3390/agronomy9030156

Verra, 2023. Verified Carbon Standard (VCS) Methodology VM0044 - Methodology for biochar utilization in soil and non-soil applications.

Walkley, A., Black, I.A., 1934. An examination of the degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37, 29–38. https://doi.org/10.1097/00010694-193401000-00003

Wu, L., Zhang, S., Ma, R., Chen, M., Wei, W., Ding, X., 2021. Carbon sequestration under different organic amendments in saline-alkaline soils. Catena (Amst) 196, 104882. https://doi.org/10.1016/j.catena.2020.104882

Yang, J., Luo, P., 2020. Review on international comparison of carbon financial market. Green Finance 2, 55–74. https://doi.org/10.3934/GF.2020004

Yi, Q., Liang, B., Nan, Q., Wang, H., Zhang, W., Wu, W., 2020. Temporal physicochemical changes and transformation of biochar in a rice paddy: Insights from a 9-year field experiment. Science of The Total Environment 721, 137670. https://doi.org/10.1016/j.scitotenv.2020.137670

Yin, X., Xi, M., Li, Y., Kong, F., Jiang, Z., 2021. Improvements in physicochemical and nutrient properties of sewage sludge biochar by the co-pyrolysis with organic additives. Science of The Total Environment 779, 146565. https://doi.org/10.1016/j.scitotenv.2021.146565

Zhang, X., Zhao, Y., Meng, H., Li, L., Cui, H., Wei, Z., Yang, T., Dang, Q., 2020. Revealing the Inner Dynamics of Fulvic Acid from Different Compost-Amended Soils through Microbial and Chemical Analyses. J Agric Food Chem 68, 3722–3728. https://doi.org/10.1021/acs.jafc.0c00658

7.6. Appendix

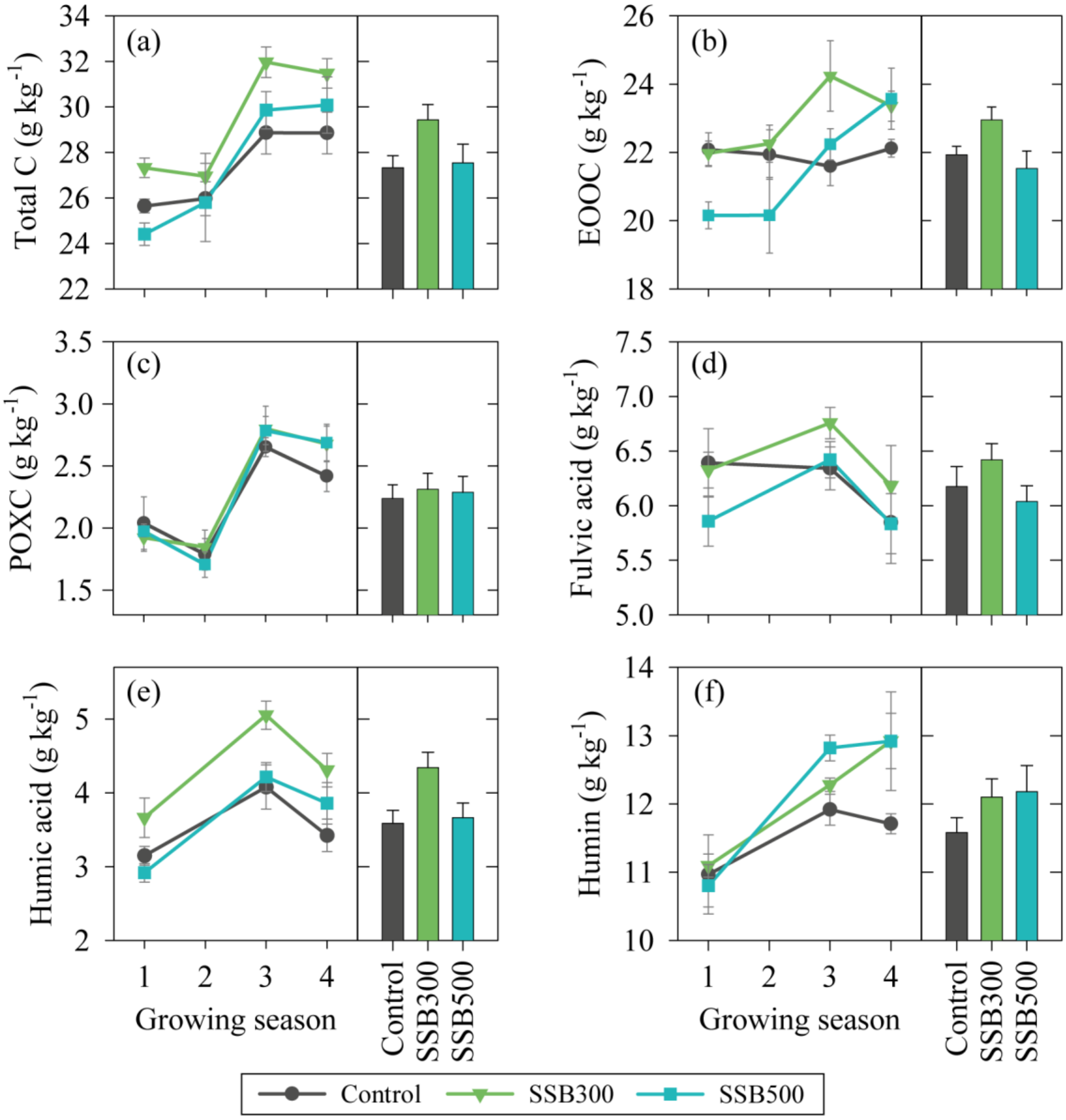


Figure S6. Soil total carbon content (a), easily oxidizable organic carbon – EOOC (b), permanganate oxidizable carbon – POXC (c), fulvic acid (d), humic acid (e), and humin (f). SSB300 and SSB500: sewage sludge biochar pyrolyzed at 300°C and 500°C. The bar plot represents the mean of growing seasons. Error bars represent standard error.

8. CONCLUDING REMARKS

This research comprehensively examines the effects of biochar on soil carbon (C) pools by integrating a global meta-analysis with a local field trial. The main objective was to assess how biochar application influences soil C sequestration and to identify factors that optimize its effectiveness under varying conditions.

Combining findings from all three chapters offers a nuanced understanding of biochar in soil C management. Chapter I presented a global meta-analysis of 586 paired comparisons from 169 studies, revealing that biochar enhances soil C pools: total C (TC) by 64.3%, organic C by 84.3%, microbial biomass C by 20.1%, labile C by 22.9%, and fulvic acid by 42.1%. These results highlight biochar's potential to improve soil health and mitigate climate change.

Chapter II examined a seven-year field trial using sewage sludge biochar (SSB) in a tropical Oxisol. SSB application increased soil TC and total nitrogen levels, enhancing soil fertility and C sequestration potential. Notably, SSB elevated the non-oxidizable organic C pool, indicating improved soil organic matter stabilization. However, while SSB initially boosted crop yields, productivity declined from the fourth season onward without supplemental mineral fertilization, emphasizing the need for integrated nutrient management to sustain long-term productivity with biochar amendments.

Chapter III integrated the global meta-analysis with local field trial findings, revealing discrepancies between global trends and local results. While the meta-analysis reported significant positive impacts on various soil C fractions, the local trial showed more modest TC increases and minimal changes in other fractions. These differences stem from variations in biochar types, soil properties, environmental conditions, and experimental durations. Specifically, the underrepresentation of tropical regions and SSB in global studies may lead to overestimations of the effectiveness of biochar in such contexts.

8.1. Achievement of objectives and confirmation of hypotheses

The research achieved its primary objectives: assessing biochar-induced changes in soil C sequestration across various organic matter pools and linking global results with a long-term SSB field trial.

In Chapter I, the global meta-analysis confirmed the hypothesis that biochar application enhances soil C pools, particularly TC and other fractions, and identified key influencing factors, validating most hypotheses.

Chapter II clarified the temporal dynamics of C in labile and stable pools during SSB application, elucidating the direct and indirect contributions of SSB to soil C. It confirmed the hypothesis that biochar stabilizes soil organic matter by increasing the non-oxidizable C pool. However, methodological limitations prevented confirming that SSB's direct C contribution to TC is negligible.

Chapter III integrated global and local findings, reinforcing that biochar effects vary with biochar type, soil properties, and environmental conditions. Thus, the research objectives and hypotheses were met, highlighting the need to consider specific conditions when applying biochar for soil C sequestration.

8.2. Implications and recommendations

These findings underscore the critical role of context in determining the effectiveness of biochar. Despite the lower percentage increases in soil C fractions observed in the local trial, the absolute TC gains with SSB300 were substantial, surpassing those achieved by practices like no-till farming and cover cropping over similar periods. This suggests that SSB can effectively enhance soil C stocks in tropical regions when properly managed.

To optimize the benefits of SSB, several factors warrant consideration: tailoring biochar production to achieve desired properties, recognizing the influence of soil type and climate, determining appropriate application rates, exploring co-pyrolysis with plant materials, and combining SSB with mineral fertilizers. Implementing integrated nutrient management and long-term monitoring is crucial for sustaining the benefits of SSB application.

These findings hold significant implications for policymakers. Biochar application can contribute to greenhouse gas mitigation efforts, support sustainable agriculture, and promote circular economy principles through waste material utilization. Reliable data on the effectiveness of biochar in various contexts can inform the development of C credit methodologies, encouraging the adoption of biochar technologies.

8.3. Future research directions

To fully realize the potential of biochar, future research should expand studies in underrepresented regions, focusing on tropical areas to understand its effects under diverse environmental conditions. Investigating methods to improve biochar properties, such as optimizing pyrolysis conditions and exploring co-pyrolysis with different feedstocks, is essential. Exploring the synergistic effects of combining biochar application with other sustainable practices like no-till farming and cover cropping could enhance soil C sequestration. Assessing the persistence of biochar's benefits over extended periods will inform sustainable land management strategies.

8.4. Final reflections

In conclusion, this research bridges global trends with local realities, emphasizing biochar's context-dependent effectiveness in enhancing soil C sequestration and promoting sustainable agriculture. By tailoring applications to local conditions and integrating comprehensive nutrient management strategies, we can unlock the full potential of biochar to mitigate climate change, improve soil health, and support sustainable agricultural development. This nuanced understanding, derived from integrating global meta-analytical insights with localized field data, underscores the importance of context in evaluating sustainable agricultural practices. Continued research and practical implementation are essential to advance biochar technology, contributing to global efforts in climate change mitigation and sustainable agriculture promotion.