Potential and constraints of organic soil amendments for long-term carbon storage and soil improvement

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Abbreviations

OSA	Organic soil amendments (organischer Bodenhilfsstoff)
SOC	Soil organic carbon
CDR	Carbon dioxide removal (Kohlenstoffdioxid-Entfernung)
TC	Total carbon
C_{org}	Organischer Bodenkohlenstoff
C	Carbon
SOM	Soil organic matter
CO_2	Carbon dioxide
N	Nitrogen
P	Phosphorus
K	Potassium
GHG	Greenhouse gas
MRT	Mean residence time
C/N ratio	Carbon-to-nitrogen ratio
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
O	Oxygen
NO_3^-	Nitrate
N_2O	Nitrous oxide
CH_4	Methane
SSA	Specific surface area
BPCA	Benzene polycarboxylic acids
EC	Electrical conductivity
WHC	Water holding capacity

TN	Total nitrogen
TP	Total phosphorus
NH_4^+-N	Ammonium-nitrogen
NO_3^--N	Nitrate-nitrogen
PCA	Principal component analysis
DOC	Dissolved organic carbon
ADE	Anthropogenic dark earth
Ca^{2+}	Calcium ion
O/C ratio	Oxygen-to-carbon ratio
EU	European Union
ANOVA	Analysis of variance
H/C ratio	Hydrogen-to-carbon ratio
CCS	Carbon capture and storage
CCU	Carbon capture and use
NPK	Nitrogen, phosphorus and potassium
TOC	Total organic carbon
R	Response ratio
ΔSOC	Soil organic carbon stock mean difference
X_E	Mean soil organic carbon stock with application of manure
X_C	Mean soil organic carbon stock without application of manure
CI	Confidence interval
CEC	Cation exchange capacity
RR	Response ratio
dSOC	Soil organic carbon stock mean difference
REM	Random effects model

NMR	Nuclear magnetic resonance
BPCA	Benzene pentacarboxylic acid
B3CA	Sum of hemimellitic, trimellitic and trimesic acid
B4CA	Sum of pyromellitic, melophanic and prehnitic acid
B5CA	Benzene pentacarboxylic acid
B6CA	Mellitic acid
BD	Bulk density
REML	Residual maximum likelihood
numDf	Degrees of freedom in the numerator
denDf	Degrees of freedom in the denominator
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
N_2	Elemental nitrogen
BET	Brunauer-Emmett-Teller method
BJH	Barrett-Joyner-Halenda model
HSD	Tukey's honestly significant difference

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Study 3: Long-term biochar and soil organic carbon stability – evidence from field experiments in Germany

Study 4: Impact of biochar aging on soil health

Summary

This Ph.D. thesis is composed of four published studies on the environmental behavior of organic soil amendments (OSA), their persistence and influence on soil organic carbon (SOC) dynamics, and their long-term impact on soil properties. A special focus lies on biochar, which is considered as a promising measure for soil improvement and as a strategy for long-term carbon dioxide removal (CDR) from the atmosphere. Compared to conventional OSA such as compost, digestate, or manure, biochar consists of condensed aromatics C compounds and, therefore, has much higher stability. Consequently, biochar possesses greater potential for long-term and stable SOC sequestration than conventional OSA.

To further investigate this comparison, two meta-analyses were conducted. Meta-analyses quantitatively compile existing data from peer-review literature on a specific research question and evaluate it statistically. The first meta-analysis aimed to describe the impact of manure applications on SOC stocks, based on 592 individual observations from 101 studies. The second meta-analysis examined the impact of biochar applications on SOC stocks. This study was conducted based on 736 individual observations from 64 studies. On average, repeated manure applications led to an SOC stock increase of 10.7 Mg ha^{-1} (35%), while biochar amendments, mainly added as single application, increased SOC stocks on average by 13.0 Mg ha^{-1} (29%). However, the repeated manure applications, such as at the beginning of each growing season, did not result in significantly higher SOC stocks in long-term observations compared to short-term studies, indicating lacking stability of manure-derived carbon. In contrast, single applications of biochar led to a continuous increase of SOC stocks with longer observation periods of up to 10 years, highlighting biochar's higher carbon sequestration potential compared to manure applications. Acidic soils demonstrated a higher SOC stock increase after the application of manure than neutral and alkaline soils. This can be explained by reduced microbial activity in acidic soils, leading to lower SOC decomposition. On the other hand, biochar application resulted in larger SOC stocks in neutral or alkaline soils. This is likely due to the liming effect of biochar, which increases the pH of acidic soil, and thus, enhances microbial activity. Another explanation for the higher SOC stock increases in biochar-applied neutral and alkaline soils is the greater amount and availability of Ca^{2+} ions, which may favor mineral-organic complex formation. Within different manure types, animal manure showed the highest SOC increases, while green manure and straw had only minor effects. This observation is unsurprising, considering that manure of animal origin contains a higher amount of nutrients. Higher nutrient availability generally enhances both above- and below-ground plant production, which favors rhizodeposition and contributes to SOC storage. Biochar made from wood and plant-based sources led, on average, to larger SOC stock increases than biochars made from animal sources. Larger SOC stock increases were observed under conventional tillage rather than reduced tillage for both manure (+2.2 Mg ha^{-1}) and biochar applications (+7.0 Mg ha^{-1}). This may be due to the deeper incorporation of the amendment into soil, into regions with lower microbial activity and turnover, while simultaneously the formation of larger and more aromatic humic substances can be promoted. At clay-rich sites, manure applications resulted in +3.1 Mg ha^{-1}, and biochar applications resulted in +11.6 Mg ha^{-1} more SOC stock buildup compared to sandy sites. Both OSA also showed stronger increases in SOC in temperate climates compared to subtropical climates (+2.7 Mg ha^{-1} for manure applications and +8.5 Mg ha^{-1} for biochar applications), while observations in tropical climates were overall underrepresented in both datasets.

One main outcome of Study 2, the meta-analysis on the effects of biochar application on SOC stocks in agricultural soils, was the lack of long-term observations across various locations. To fill this knowledge gap, in the third study, two biochar field experiments were re-sampled after nine and eleven years, respectively, and stocks of SOC and black carbon were analyzed. The two locations differed in their site characteristics, the type and amounts of biochar, and other OSA. The application of 31.5 Mg ha^{-1} biochar (pristine, co-amended with compost, or co-composted) on the loamy soil at the Bayreuth experimental site increased the SOC stock by 38 Mg ha^{-1}. This increased stock was still detectable eleven years later, although the black carbon stocks decreased over time. The high application rate of 40 Mg ha^{-1} biochar in combination with digestate, compost, or mineral fertilizer on the sandy soil at the Gartow experimental site led to a short-term increase of SOC stocks by 61 Mg ha^{-1}. In the following four years, the stock decreased by 38 Mg ha^{-1}, and nine years later, at the time of re-sampling, the biochar plots showed only slightly elevated SOC stocks compared to the untreated control plots (+7 Mg ha^{-1}). A similar trend was observed for black carbon stocks. At both sites, the long-term effects were mainly dependent on the amount of applied biochar, and the effects of other OSA, whether applied alone or combined with biochar, were negligible. This study demonstrated the SOC sequestration potential of biochar when used as a OSA at a loamy site, despite the long-term decrease of detectable biochar in the experimental plots. This suggests stabilization or even buildup of native SOC. Conversely, the losses of SOC and black carbon at the Gartow site highlights the significant impact of lateral and vertical transport processes in sandy soils due to the lack of physical stabilization because biochar mineralization was very low.

As biochar resides in soil, it experiences alterations of its original properties due to chemical, physical, and biological processes. These changes affect the agronomic benefits of biochar application and its CDR potential. Long-term observations of biochar aging effects in soil are limited but highly relevant, as they provide a more realistic picture of the agronomic and societal benefits of biochar than short-term studies with relatively “fresh” biochar. Study 4 of this dissertation aimed to describe the aging effects of biochar and their impact on a range of soil properties at the Bayreuth site. For this purpose, soil and biochar samples were taken 13 years after application (two variants: 1. co-composted and 2. pristine biochar) and compared with a “fresh” variant in which the same unaged biochar was freshly mixed with the control soil. The freshly mixed variant showed the largest soil pH and electrical conductivity, and had the largest total carbon (TC) content. Due to biochar aging, these properties decreased in both the pristine aged and co-composted aged biochar variant. The co-composted aged variant showed increased nitrogen retention compared to the pristine aged variant, along with a higher TC content. This study identified numerous aging effects of biochar after 13 years in a loamy soil. It was demonstrated that co-composting does not negatively affect biochar's effect on TC, and is recommended to fully realize the potential agronomic benefits.

Zusammenfassung

Diese Disserationsschrift umfasst die Inhalte von vier in peer-review Zeitschriften publizierten Studien zum Verhalten von organischen Bodenhilffstoffen (engl. organic soil amendments, OSA) in der Umwelt, ihrer Persistenz, dem Einfluss auf den Gehalt an organischem Bodenkohlenstoff (C_{org}) und der langfristigen Wirkung auf Bodeneigenschaften. Der Fokus liegt hierbei auf Pflanzenkohle, die als vielversprechende Maßnahme zur Bodenverbesserung und als Strategie zur Kohlenstoffdioxid-Entfernung aus der Atmosphäre (engl. carbon dioxide removal, CDR) angesehen wird. Verglichen zu konventionellen OSA wie Komposten, Gärresten oder Mist, besteht Pflanzenkohle aus kondensierten Aromaten und weißt deshalb eine höhere Stabilität auf. Deshalb wird der Pflanzenkohle auch ein höheres Potential zum langfristigen und stabilen Aufbau von C_{org} (Kohlenstoffsequestrierung) zugeschrieben, als den konventionellen OSA.

Um diesen Vergleich zu untersuchen, wurden zwei Meta-Analysen durchgeführt. In Meta-Analysen werden vorhandene Daten aus Peer-Review-Literatur zu einer bestimmten Forschungsfrage objektiv zusammengefasst und sowohl quantitativ als auch statistisch ausgewertet. Die erste Meta-Analyse hatte zum Ziel, die Auswirkung von Mist-Applikationen auf die C_{org}-Vorräte auf der Basis von 592 Einzelbeobachtungen aus 101 Studien zu beschreiben. In der zweiten Meta-Analyse wurde die Auswirkung von Pflanzenkohle-Applikationen auf die C_{org}-Vorräte untersucht. Diese Studie wurde auf der Basis von 736 Einzelbeobachtungen aus 64 Studien durchgeführt. Durchschnittlich führten kontinuierliche Mist-Applikationen zu einer Steigerung der C_{org}-Vorräte von 10.7 Mg ha^{-1} (35%). Pflanzenkohle-Anwendungen, in den allermeisten Fällen als einzelne Gabe appliziert, erhöhten die C_{org}-Vorräte um durchschnittlich 13.0 Mg ha^{-1} (29%). Die kontinuierliche Applikation von Mist, z. B. zu Beginn jeder Vegetationsperiode, führte in längerfristigen Beobachtungen nicht zu signifikant höheren C_{org}-Vorräten als in Kurzzeit-Studien. Daher eignet sich Mist nicht zur C-Sequestrierung aufgrund der fehlenden Langzeitstabilität. Einmalige Anwendungen von Pflanzenkohle hingegen führten zu einer kontinuierlichen Erhöhung des C_{org}-Vorrats mit zunehmender Beobachtungszeit von bis zu 10 Jahren, was eine deutlich bessere Eignung von Pflanzenkohle zur Kohlenstoffsequestrierung im Vergleich zu Mist-Applikationen unterstreicht. Gegensätzliche Beobachtungen wurden an sauren Standorten gemacht. Nach der Anwendung von Mist konnten hier die C_{org}-Vorräte stärker erhöht werden, während Pflanzenkohle-Applikationen im Durchschnitt zu größeren C_{org}-Vorräten führte, wenn der Boden-pH-Wert neutral oder alkalisch war, was durch den Kalkungseffekt der Pflanzenkohle erklärt werden kann, da dieser zum pH-Anstieg saurer Standorte führt und damit zu erhöhter mikrobieller Aktivität. Ein weiterer Grund für die stärkere C_{org}-Zunahme an neutralen und alkalischen Standorten könnte durch die bessere Verfügbarkeit von Ca^{2+} Ionen bedingt sein, die die Bildung von mineral-organischen Komplexen begünstigt. Die Anwendung von Mist zeigte die größte Steigerung der C_{org}-Vorräte, während Gründünger und Stroh nur geringe Effekte hatten. Diese Beobachtung ist wenig überraschend, da Mist eine größere Menge an Nährstoffen enthält. Eine höhere Nährstoffverfügbarkeit fördert in der Regel sowohl das oberirdische als auch das unterirdische Pflanzenwachstum, was auch Rhizodeposition begünstigt und zur Speicherung von C_{org} beiträgt. Holz- und pflanzenbasierte Pflanzenkohle führte im Durchschnitt zu größeren C_{org}-Zuwächsen als Pflanzenkohle tierischen Ursprungs. Sowohl bei Mist (+2.2 Mg ha^{-1}) als auch bei Pflanzenkohle (+7.0 Mg ha^{-1}) wurde unter konventioneller Bodenbearbeitung größere C_{org}-Zuwächse beobachtet als bei reduzierter Bodenbearbeitung. Dies könnte daran liegen, dass der Eintrag des OSA bei konventioneller Bearbeitung tiefer in den Boden erfolgt, in Regionen mit geringerer mikrobieller Aktivität und Umsatz, während gleichzeitig die Bildung größerer und aromatischerer Huminstoffe gefördert wird. An tonreichen Standorten führten Mistanwendungen zu einem C_{org}-Zuwachs von +3.1 Mg ha^{-1} und die Anwendung von Pflanzenkohle zu +11.6 Mg ha^{-1}, verglichen mit sandigen Standorten. Beide OSA zeigten zudem stärkere C_{org}-Zunahmen in gemäßigten Klimazonen im Vergleich zu subtropischen Klimazonen (+2.7 Mg ha^{-1} bei Mistanwendungen und +8.5 Mg ha^{-1} bei Pflanzenkohleanwendungen), während Beobachtungen in tropischen Klimazonen in beiden Datensätzen insgesamt unterrepräsentiert waren.

Eine der Haupterkenntnisse aus Studie 2, der Meta-Analyse zu den Effekten der Pflanzenkohleapplikation auf die C_{org}-Vorräte landwirtschaftlich genutzter Böden war der Mangel an Langzeitobservationen über viele Jahre hinweg, an verschiedenen Standorten. Im Rahmen der dritten Studie, wurden daher zwei Pflanzenkohle-Feldversuche nach neun bzw. elf Jahren neu beprobt und hinsichtlich des Vorrats an C_{org} aber und Black Carbon, einem Biomarker für Pflanzenkohle untersucht. Beide Standorte unterschieden sich in ihren Standorteigenschaften, sowie der Art und Menge an Pflanzenkohle, aber auch anderen Bodenhilfsstoffen. Die Applikation von 31.5 Mg ha^{-1} Pflanzenkohle (unbehandelt, co-appliziert mit Kompost oder co-kompostiert) auf den lehmigen Boden am Experimentstandort Bayreuth erhöhte den C_{org}-Vorrat um 38 Mg ha^{-1}. Der erhöhte Vorrat war auch elf Jahre später noch vorzufinden, wenngleich die Vorräte an Black Carbon im Laufe der Jahre abnahmen. Die hohe Applikationsmenge von 40 Mg ha^{-1} Pflanzenkohle in Kombination mit Gärresten, Kompost oder Mineraldünger am sandigen Experimentstandort Gartow führte zu einer kurzfristen Erhöhung der C_{org}-Vorräte um 61 Mg ha^{-1}. In den folgenden vier Jahren nahm der Vorrat um 38 Mg ha^{-1} ab und neun Jahre später, zum Zeitpunkt der Neubeprobung, wiesen die Pflanzenkohleplots im Schnitt nur noch leicht erhöhte C_{org}-Vorräte verglichen zu den unbehandelten Kontrollplots auf (+7 Mg ha^{-1}). Einen ähnlichen Verlauf wiesen auch die Vorräte an Black Carbon auf. An beiden Standorten waren die Langzeiteffekte vor allem von der Menge an applizierter Pflanzenkohle abhängig und die Effekte anderer Bodenhilfsstoffe, separat oder co-appliziert konnten vernachlässigt werden. In dieser Studie konnte das Kohlenstoffsequestrierungspotenzial von Pflanzenkohle in der Anwendung als Bodenhilfsstoff an einem lehmigen Standort bewiesen werden, trotz langfristig schwindender Mengen an Pflanzenkohle in den Experiment-Plots. Dies deutet auf eine Stabilisierung bzw auf einen Aufbau des natürlichen Kohlenstoffs hin. Gleichzeitig unterstreichen die Verluste an C_{org} und Black Carbon am Standort Gartow die hohe Bedeutung von lateralen und vertikalen Transportprozessen in sandigen

Böden aufgrund fehlender physikalischer Stabilisierung. Signifikante Mineralisierung konnte in einer früheren Studie durch CO_2-Messungen ausgeschlossen werden.

Während Pflanzenkohle im Boden verweilt, ändert sie aufgrund chemischer, physikalischer und biologischer Prozesse viele ihrer ursprünglichen Eigenschaften. Diese Änderungen beeinflussen unter anderem auch die agronomischen Vorteile, die durch die Anwendung von Pflanzenkohle im Boden erzielt werden, sowie das CDR-Potenzial. Langzeitbeobachtungen der Alterungseffekte von Pflanzenkohle im Boden sind bisher kaum bekannt, aber von hoher Relevanz, da diese ein realistischeres Bild des agronomischen und gesellschaftlichen Nutzens von Pflanzenkohle zeichnet. Studie 4 hatte daher zum Ziel, die Alterungseffekte der Pflanzenkohle sowie der Auswirkungen auf eine Reihe von Bodeneigenschaften am Standort Bayreuth zu beschreiben. Hierfür wurden Boden- und Pflanzenkohleproben 13 Jahre nach der Applikation entnommen (zwei Varianten: 1. vorher co-kompostierte und 2. unbehandelte Pflanzenkohle) und mit einer „frischen“ Variante verglichen, in der die gleiche ungealterte Pflanzenkohle dem Kontrollboden beigemengt wurde. Der pH-Wert und die elektrische Leitfähigkeit war in der frisch gemischten Variante am höchsten und nahm durch die Pflanzenkohlealterung ab, sowohl in der gealterten unbehandelten als auch co-kompostierten Variante. Auch der Gehalt an Bodenkohlenstoff war in der frisch gemischten Variante deutlich höher als in beiden gealterten Varianten. Die gealterte co-kompostierte Variante wies jedoch einen erhöhten Stickstoffgehalt gegenüber der unbehandelten gealterten auf, ebenso wie einen höheren Gehalt an Bodenkohlenstoff.

In dieser Studie konnten eine Vielzahl von Alterungseffekte von Pflanzenkohle nach 13 Jahren in einem lehmigen Boden festgestellt werden. Gleichzeitig konnte gezeigt werden, dass eine Co-Kompostierung positive Effekte auf die Stickstoffverfügbarkeit hat und keinen negativen Effekt auf die Menge an Bodenkohlenstoff besitzt. Hinsichtlich dieser aufgezeigten agronomischen Vorteile sollte eine Co-Kompostierung vor der Anwendung von Pflanzenkohle im Boden durchgeführt werden.

I. Extended Summary

1. Introduction

1.1 Background

Soils are the basis of a functioning food system and source of income for billions of humans worldwide. Besides providing economic value and nutrition, soils fulfill several ecosystem functions, such as biomass and fiber production, regulation of water, and nutrient cycles (Lal, 2016). Moreover, soils serve as habitat for 25% of global biodiversity (FAO, 2020) and is thereby the foundation of the food chains nourishing above-ground species, thus humanity (European Commission, 2021).

In addition, soils are the largest terrestrial pool of carbon (C) on the planet, containing 3200 Gt of C, which significantly exceeds the amount of C in the atmosphere (850 Gt) and the amount held in terrestrial vegetation (450-700 Gt) (EEA, 2022), making them a critical source and sink for atmospheric C (Balesdent et al., 2018). Therefore, it is of great importance not just considering soil as an economic resource and means for our global food system but also being a key ally in mitigating climate change. Climate change poses several threats to soil health. Increasingly occurring weather extremes such as droughts and heavy rainfalls accelerate soil erosion rates (Borrelli et al., 2020), leading to loss of soil organic matter (SOM) and soil nutrients, and a net release of carbon dioxide (CO_2) into the atmosphere (Gerke, 2022). Moreover, soil erosion alone comes with enormous consequences and costs for European farmers ~1.25 billion € per year (European Commission, 2021). Simultaneously, the growing world population increases pressure on soils, enhancing the demand for crop yields and, therefore, productive soils. Enhancing soil's productivity is typically done via fertilization. By returning nutrients back to the soil which were removed through harvesting (e.g., nitrogen (N), phosphorus (P) and potassium (K)), soil fertility, and thus, productivity could be sustained. Since the invention of the synthetic N fertilizer via the Haber Bosch process and significant advancements in plant breeding during the early 20th century, soil and plant productivity was dramatically enhanced - the following decades of worldwide agricultural growth are widely known as the "green revolution". However, the increased use of synthetic fertilizers worldwide led to economic dependencies of farmers and several ecological problems, such as decreasing groundwater quality and loss of soil biodiversity by suppressing the role of N-fixing bacteria (Tripathi et al., 2020), and enhanced greenhouse gas (GHG) emissions from soil and as a consequence of the energy intensive production of synthetic fertilizer products. Another, much older approach to sustain agricultural land productive and fertile is the use of organic soil amendments (OSA).

1.2 Organic soil amendments

Since ancient times, humans have used different forms of OSA (Foxhall, 1998), the most common forms being straw, slurries, manures and compost. More modern forms are biogas digestates, sewage sludges, biosolids and biochar. These amendments not just contain nutrients, but also varying amounts of C-rich organic compounds. Therefore, the application of OSA leads to an increase of soil organic carbon (SOC) stocks (Alvarenga et al., 2020). The process of removing atmospheric CO_2 and storing it in SOC stocks for long periods of time (over 100 years) is known as “C sequestration”. Removing and storing atmospheric CO_2 in a stable form over long periods makes C sequestration a viable carbon dioxide removal (CDR) strategy. However, not all types of OSA increase SOC stocks in the long term, i.e. longer than 100 years, as they contain different types of C compounds with varying stability and mean residence times (MRT) in soil.

The carbon-to-nitrogen (C/N) ratio can be used as a proxy for organic matter and OSA stability. Liquid slurries and sludges usually possess high contents of N and water, and low amounts of C-rich organic material, making them prone to fast microbial decomposition, turnover and mineralization. Solid manures and composts usually show higher dry matter contents and a higher C/N ratio and, therefore, have higher persistence in soil, need to be applied regularly (same as for slurries and sludges), e.g., at the beginning of a new growing season to contribute to soil fertility and productivity, and an SOC stock increase in longer terms. Biochar amendments, in contrast, contain highly aromatic C compounds and only little amounts of N, most of them being polycyclic and not available for plants or microorganisms (Singh et al., 2014), and are, therefore, highly stable against microbial decomposition. Even if applied once, biochar amendments significantly increase SOC stocks over long periods of time (Gross et al., 2023).

1.3 Added value of meta-analysis

The magnitude of SOC stock increase was found to vary strongly between the different OSA types, as a range of different global meta-analysis suggested (Table 1). In contrast to a review, which provides a subjective overview of the state of research on a specific topic, the meta-analysis method enables a quantitative statistical analysis of a specific research question. This involves literature analysis methods such as Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) to establish objective and transparent criteria for deciding whether or not a study should be included into the quantitative analysis (Page et al., 2021). The “PRISMA 2020 statement paper” has the objective to guide authors conducting their meta-analysis thoroughly, ensuring objectiveness and transparency, and to provide meta-analysis readers with a plausible and traceable flow diagram of how many studies were included or excluded, and why (Page et al., 2021). PRISMA was originally designed for meta-analysis to synthesize health-related studies. However, it has since become an established and widely adopted standard in ecology and agronomy research as well.

Given the many factors influencing the impact of OSA on SOC stocks and the large number of available research articles on this topic, meta-analyses are of critical importance in synthesizing findings and identifying general trends and conclusions. There are several factors, making the comparison between the OSA types using previously published meta-analysis difficult. Firstly, the authors included studies into their dataset, which used fertilized controls and compared them with OSA additions, or considered mixed treatments of OSA and synthetic fertilizer (Han et al., 2016; Maillard and Angers, 2014). As a result, the observed effect sizes cannot be attributed to the respective OSA addition alone. Secondly, published meta-analyses often focused on specific geographic regions rather than using global datasets, which limited drawing general conclusions for a wider range of regions, climatic setting and different soil types. Global datasets with a feasible number of data entries from a wide set of regions can be used for sub-analysis in specific regional settings. Thirdly, previously published meta-analyses only considered certain OSA as a collective term, such as manure. Manure is a collective term for excreta of different animal species, urine, plant materials and straw but also livestock feed residues and organic household residues. Therefore, one must consider these different sub-types of OSA separately, each of them possessing individual properties and thus effects on SOC stocks. Fourthly, depending on the year of publication, previously published meta-analyses could only consider studies up to a certain date. Last but not least, some novel forms of OSA, such as biosolids have not been thoroughly investigated yet in their ability to increase SOC stocks using meta-analysis, involving the analysis of a wide set of potential explanatory variables. Only one master thesis on the SOC stock increase potential of biosolids could be identified (Snyder, 2021).

Table 1: Compilation of recent meta-analysis results of organic soil amendments effects on soil organic carbon stocks. CI = confidence interval, n = number of observations. n.s. = not significant.

	SOC stock increase [95% CI]	Long-term SOC stock increase	Note	Reference
Organic soil amendment				
Compost	29% [25-33%]	30%	Long-term effect estimated across all application frequencies over 25 years	Bai et al., 2023
Slurry	15% [10-15%]	16%		
Sewage sludge	80% [41-119%]	n.s.		
Manure	26% [24-28%]	36%		
Biosolids	72% [57-87%]	38%	Long-term effect estimated across all application frequencies over 11-30 years; single application: n.s	Snyder, 2021
Biochar	29% [26-33%]	49%	Single application effect after 10 years	Gross et al., 2021

1.4 History of biochar

Biochar is the product of thermochemical conversion of organic biomass under limited oxygen (O) supply, also known as pyrolysis. One must distinguish biochar from other carbonaceous material produced from pyrolysis. In contrast to char or charcoal, biochar is specifically produced for the purpose to be applied to and improve soil (Regkouzas et al., 2024). First uses of biochar, intended or unintended, date back to over 2000 years ago, when ancient people of Amazonia created fertile and SOM-rich Anthrosols called Terra Preta. Terra Preta soils were formed by incorporation and microbial transformation of agricultural and household residues, leaves, bones, excrements, and combustion residues such as chars and ashes (Glaser and Birk, 2012; Lombardo et al., 2022). These charred residues, today known as biochar, was found to be one key factor for the high fertility of Terra Preta, compared to the surrounding nutrient-poor tropical soils (Glaser and Birk, 2012). Terra Preta soils nowadays contain five times more SOC than adjacent tropical soil, 20% of which being biochar (Glaser, 2021), which underlines the longevity of biochar in soil and its large SOC sequestration potential.

1.5 Integration of biochar into modern agricultural systems

In modern times, biochar is increasingly seen as a system approach because the benefits can often only be realized if biochar is perceived as such (Lehmann and Joseph, 2024). Within a biochar system, the goal is to realize as many advantages of biochar as possible while maximizing the CDR potential at the same time (Gross et al., 2023). Biochar systems have the potential to address multiple problems of the agricultural sector even beyond CDR. In a series of meta-analyses (Schmidt et al., 2021), biochar use was found to increase crop yield by 10-15% (Jeffery et al., 2017), reduce nitrate (NO_3^-) leaching by 11% (Borchard et al., 2019), and increase the availability of P by 45% (Gao et al., 2019). Moreover, soil compaction can be reduced (Blanco-Canqui, 2021), and several soil hydraulic properties were found to improve after biochar usage (Edeh et al., 2020). Climate-relevant impacts include the SOC sequestration potential, the ability to reduce nitrous oxide (N_2O) from soil (Borchard et al., 2019), and the reduction of methane (CH_4) and N_2O from ruminant digestion if the biochar is used as fodder additive. Even beyond that, biochar has proven its ability to improve water quality (Blanco-Canqui, 2019), to serve as a resource for renewable energy (Kant Bhatia et al., 2021), and to support waste management and remediation of contaminated land (Zheng et al., 2022). Once applied to soil, biochar does not remain rigidly in place but reacts with the environment including soil flora, fauna and minerals as well as plants and water. This involves various dissipation processes including mineralization, metabolization and vertical and lateral translocation of biochar. Despite the fact that there are few individual studies describing one of these mentioned biochar dissipation pathways, describing biochar dissipation dynamics holistically with respect to all relevant dissipation processes, have been proven challenging. Moreover, the question of how these biochar dissipation dynamics influence the ability to sequester SOC remains largely unknown, since there is a general lack of experimental data on biochar SOC sequestration effects in the long-term and under different real field conditions.

1.6 Role of biochar aging

While biochar resides in soil, several reactions with its immediate surrounding environment are taking place. These processes are not just affecting biochar dissipation, but are leading to physical, chemical and biological alterations of biochar particles. Physical alterations include changes in particle size, porosity and surface area, and chemical alterations affecting mostly surface properties, such as the incorporation of O-containing functional groups, enhanced cation retention and aromaticity (Pignatello et al., 2024). Surface alterations due to biochar aging are also often linked to the sorption of organic compounds, leading to increased surface polarity, decreasing specific surface area (SSA) and increasing surface charge. The process of SOM sorption depends on pH, with lower pH facilitating associations of positive surface charge of metal (hydr-)oxides and negatively charges organic compounds (Wang and Kuzyakov, 2023). SOM sorption can, moreover, block pores and thereby prevent microbes and minerals from penetrating into the particle and interacting with the particle`s inside (Hagemann et al., 2017). Changes of the oxidation state of aged biochar are mostly biologically driven because aging leads to a colonization of soil microorganisms, which oxidize the altered surface (Lehmann et al., 2024). This then leads to incorporation of O into functional groups at the biochar surface, which makes the surface more hydrophilic and due to more negative charges, there is high potential for cation retention.

Field aging of biochar leads to multiple processes occurring simultaneously and sequentially. Aging and experiments aiming to imitate these processes artificially are less time-consuming than field aging, but do not represent the multiple facets of aging of biochar and their agronomic implications. Field aging experiments are often carried out over several years (Dong et al., 2017; Haider et al., 2020; Martin et al., 2012; Rechberger et al., 2019; Sorrenti et al., 2016), but none of them observed field aging processes on a decadal scale. However, it is critical to understand how these aging dynamics impact biochar effects in the environment and its agronomic benefits. Since aged biochars better reflect what biochar effects on soil properties would look like in hundreds and thousands of years (Lehmann et al., 2024), these processes are particularly interesting to understand biochars' persistence under field aging conditions and thus realistic CDR potential.

1.7 Dissertation objectives

The state of knowledge on the effectiveness of OSA, particularly biochar as a CDR agent and long-term soil conditioner described so far, revealed a number of research gaps, which are to be closed in the framework of this dissertation. The goal hereby is to find answers to the following three research questions:

1. ***To which extent are the two different OSA manure and biochar able to increase SOC stocks, and what are global explanatory variables controlling their SOC sequestration potential?***

2. ***How do different OSA, including biochar, influence the SOC stocks dynamics of two different soils in Germany in the long-term and how persistent is the added biochar under real field conditions?***

3. ***How does the long-term aging of biochar affect its efficacy as a CDR agent and measure to improve soil properties?***

2. Material and Methods

2.1 Meta-analysis

To answer the first research question, two meta-analyses were conducted. The first one had the scope to analyze how different OSA (summarized under the common term "manure") influence SOC stocks. The second meta-analysis was specifically focused on biochar amendments. In both meta-analyses, the goal was to analyze the SOC sequestration and CDR potential of both amendment types, and moreover, to identify important variables, influencing the SOC response.

Both meta-analyses were based on a systematic literature review performed using "ISI Web of Science". The literature analysis for the manure and biochar studies were conducted in late 2020 and early 2021, respectively, which was either before or shortly after the PRISMA guidelines were published in January 2021. However, both studies fulfill the PRISMA requirements, and the PRISMA flow diagrams which were filled out subsequently for each study are presented in Fig. 1. In total, 101 manure-related and 64 biochar-related studies, providing 592 and 736 pairwise observations, respectively, were considered usable for further analysis.

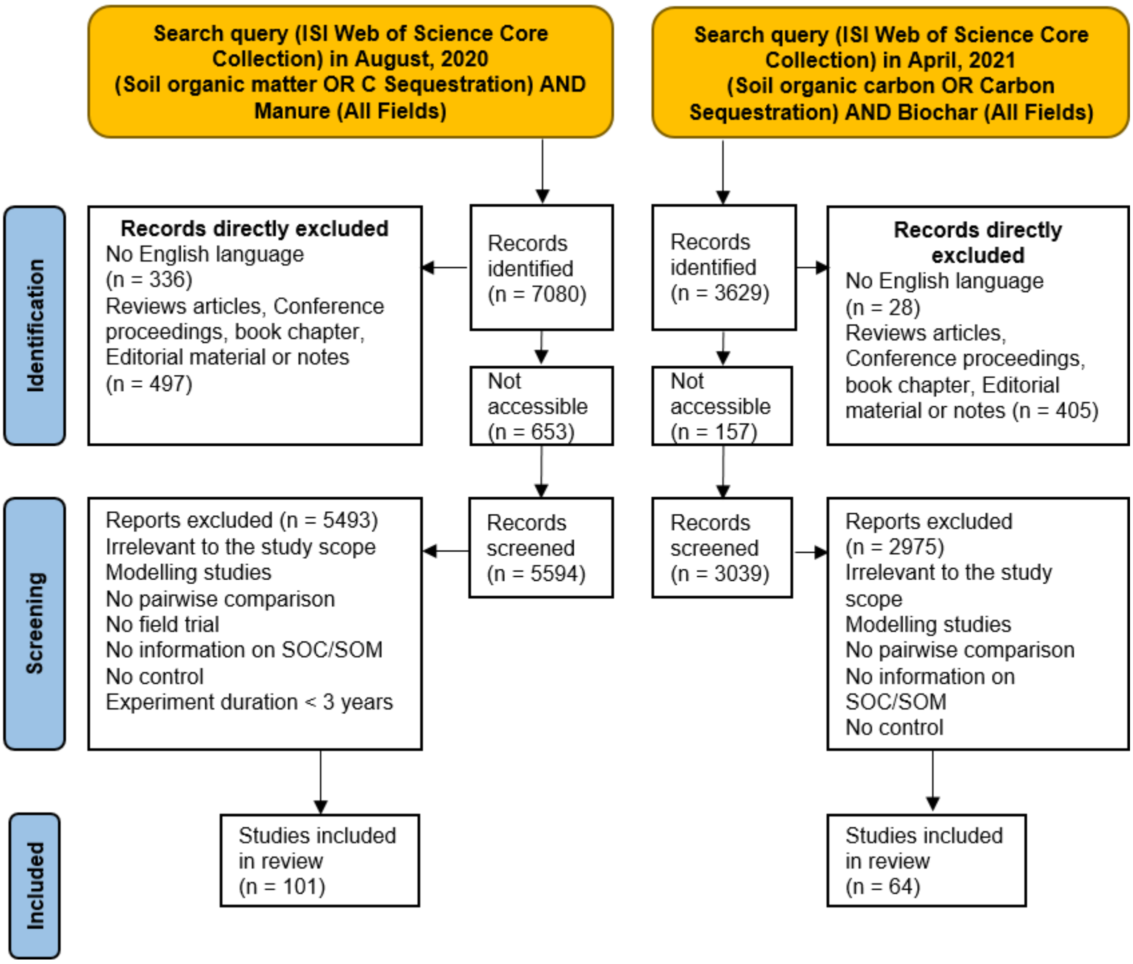

Figure 1: PRISMA flow chart of the two meta-analyses (manure on the left and biochar on the right) within the Ph.D. project.

2.2 Field experiments

Two biochar field experiments were sampled and analyzed to answer the second research question. In these field experiments, biochar and other OSA were added to soil nine and eleven years before sampling. Both locations provided insights into long-term dynamics of OSA induced SOC stock dynamics and long-term biochar stability.

Both study areas are located in Germany and differ in their location characteristics and amounts of biochar added (Fig. 2). One is located in northern Bavaria, near the town of Bayreuth. This field experiment was established in 2010. The other experiment is located in northern Germany, near the town of Gartow, and was established in 2012. Both experiments are arranged in a Latin rectangle design, to avoid disturbance between individual treatments and from surrounding influencing factors (Fig. 2). At the Bayreuth site, soil sampling was conducted in 2010 (prior to and after the soil was amended), in 2011, 2013, 2016 and 2021. At Gartow, soil sampling was conducted in 2012, in 2013 (spring and fall), in 2014, in 2016 and 2021.

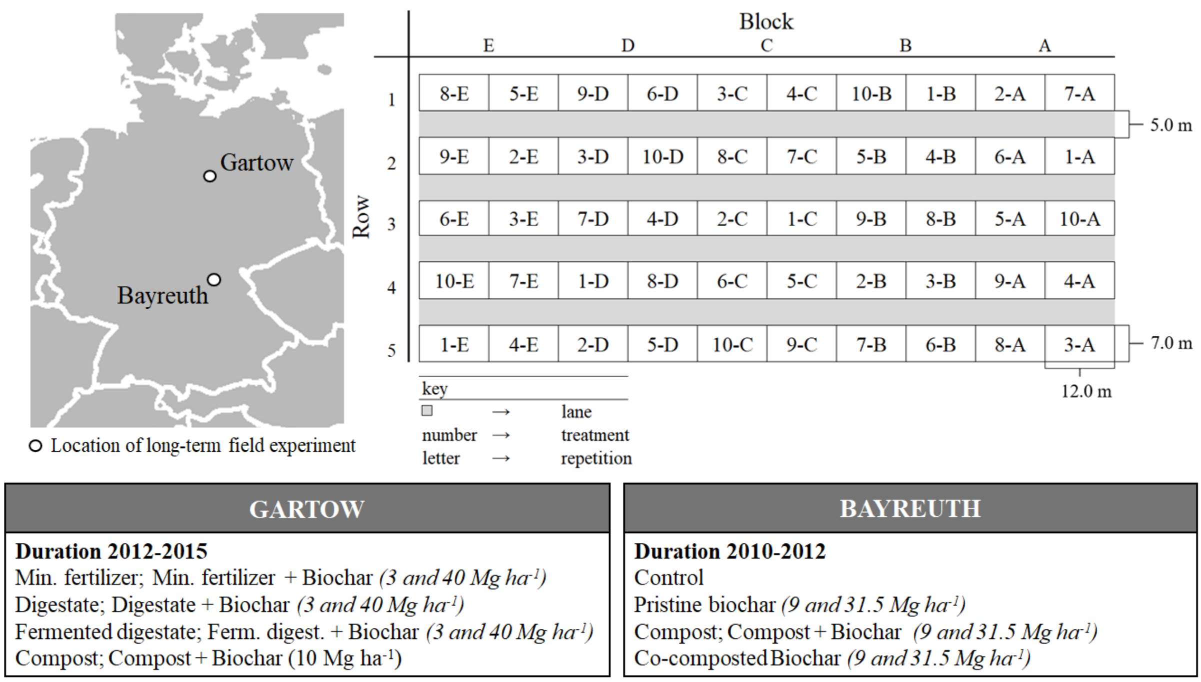

Figure 2: Location and experiment layout and treatment overview of the investigated biochar field experiments in Germany.

SOC was determined by dry combustion using a CN elemental analyzer (Elementar Vario El, Heraeus, Hanau, Germany). Black carbon contents were analyzed using the benzene polycarboxylic acids (BPCA) method of Glaser et al. (1998), modified by Brodowski et al. (2005). SOC and black carbon were expressed as stocks in the first 30 cm.

The goal of this study was to analyze temporal dynamics of SOC and black carbon stocks among different sampling dates at both locations. Temporal differences were analyzed using a linear mixed-effects model (random-intercept model). Each time series started with the first sampling date after the addition of biochar, thus the initial SOC and black carbon stock was excluded, since we wanted to analyze the differences after the application of biochar.

2.3 Biochar aging analysis

To answer the third and last research question of this dissertation, the biochar field experiment located in Bayreuth was revisited a second time in 2023. However, the scope of this additional approach was to analyze aging effects of the biochar, which has been added thirteen years ago in 2010.

For this study, three out of the ten treatments from the original Latin rectangle field experiment structure were used: pristine biochar applied at a rate of 31.5 Mg ha^{-1}, co-composted biochar mixed at a rate of 31.5 Mg ha^{-1} biochar and 70 Mg ha^{-1} compost, and an untreated control (Fig. 2). Soil sampling was conducted in March 2023. Soils were taken from each of the five field replicate plots treated with pristine biochar, co-composted biochar or the control soil from a soil depth of 0-30 cm, and mixed as composites for each respective treatment. For a better elucidation of the impact of biochar aging on soil properties, we prepared a reference soil by mixing 6 kg of the material from five control plots with 472.5 g fresh biochar used as amendment at the beginning of the field experiment in 2010. This mixture was calculated to correspond with the conditions in the first 30 cm of the field soils at the beginning of the experiment. For clarity, the soil treated with aged pristine biochar will from now on be referenced as "A_BC_S", the soil treated with aged co-composted biochar as "CC_BC_S", the reference soil which received fresh biochar as "F_BC_S", and the untreated soil as the "control".

Various soil physicochemical properties were analyzed on the four treatments. This included determination of pH, electrical conductivity (EC), water holding capacity (WHC), SOM, total carbon (TC), total nitrogen (TN), total phosphorus (TP), soluble P, available inorganic N in the forms of ammonium-nitrogen (NH_4^+-N) and nitrate-nitrogen (NO_3^--N), SSA, total pore volume and pore radius. In addition, the biochar (F_BC) used to prepare the fresh biochar amended soil for this study was also analyzed for pH, EC and WHC, as well as SSA, total pore volume and pore radius. Furthermore, SSA, pore volume and pore radius were also determined in two additional biochar samples, A_BC and CC_BC; these biochar samples were separated from a small amount of aged biochar treated soils, A_BC_S and CC_BC_S, respectively.

Principal component analysis (PCA) was conducted across the entire dataset to evaluate the influences of treatments on soil property variation.

3. Results and Discussion

3.1 Comparison of manure and biochar application effects on soil organic carbon storage

The application of manure on agricultural soils increased SOC stocks by 10.7 Mg ha^{-1}, corresponding to 35% (Fig. 3). Biochar applications to agriculturally used soils showed a mean increase in SOC stocks by 13.0 Mg ha^{-1} on average, corresponding to 29% (Fig. 3).

Both the application of manure and of biochar to soil are a direct input of organic C compounds to soil. Therefore, elevated SOC stocks after the application of these amendments were expected. However, to understand if these amendments lead to SOC sequestration and, therefore, to enhanced SOC storage on a longer-term scale (>100 years), one has to distinguish the types and properties, especially the stability of organic C compounds introduced to the soil. In Fig. 3 it can be seen that the increase of total SOC stocks following the application of both types of amendments depends on many influencing factors and partly show large differences.

Larger SOC stock increases were observed under conventional tillage rather than reduced tillage for both manure (+2.2 Mg ha^{-1}) and biochar applications (+7.0 Mg ha^{-1}). Biochar application under conventional tillage also led to enhanced SOC stocks (+27%) compared to reduced tilled systems. Conventional tillage mostly covers depths of up to 30 cm and thus leads to a deeper incorporation of the amendment into soil, into regions with lower microbial activity and SOC turnover compared to reduced tillage with incorporation depths limited to the first few cm (Mihelič et al., 2024). At the same time, more intensive tillage promotes soil mixing, aeration, decay and decomposition of both the amendment and native SOC (Al-Kaisi et al., 2014), while simultaneously the formation of larger and more aromatic humic substances can be promoted (De Mastro et al., 2019). The positive surplus observed in both our results indicated that deeper incorporation seemingly outweighs losses through increased activity. Additionally, our results show that the combination of manure applications and reduced tillage initiated a small but significant enrichment of SOC in regions deeper than 30 cm (see Gross and Glaser (2021)), which contradicts the previous statement that reduced tillage only lead to shallow soil SOC enrichment (Baker et al., 2007). Krauss et al. (2022) found increasing SOC stocks under reduced tillage even in 70-100 cm soil depth. Nonetheless, irrespective of the applied tillage, the topsoil SOC increase (0-15 cm) following the application of both manure and biochar was larger compared to deeper layers, which makes sense, since OSA are mostly incorporated into the first few cm.

The SOC stock increase following the application of both OSA types was higher in soils under temperate climate conditions compared to soils in the sub-tropics and tropics. Soils under temperate and cool climate conditions typically have lower decomposition rates and a larger potential to accumulate SOC (Lal, 2004). Subtropical soils generally have low native SOC, explaining the larger relative increases compared to observations under temperate climate conditions, and underlining their potential for additional C storage. Manure and biochar observations under tropical conditions are generally underrepresented (Fig. 3), which makes it difficult to draw meaningful conclusions.

Both manure and biochar application to clay soils showed on average larger SOC increases than sandy soils. Sorption processes of SOC and SOM on soil mineral surfaces and physical stabilization within soil aggregates are both enhanced in clay-rich soils. Sandy soils tend to have higher leaching losses of dissolved organic carbon (DOC) than finer soil material and are usually more aerated, which favors decomposition rates. Additionally, sandy soils are more prone to erosion losses of both SOC and biochar particles (Yang et al., 2019), which could be another explanation for the lower SOC responses. However, the discovery of black carbon-rich Anthropogenic Dark Earth (ADE) soils in northern Germany, with a soil texture dominated by sand showed that it is in principle possible to stabilize SOM with biochar, even under sandy soil conditions and thus lack of fine material and soil aggregates (Glaser et al., 2024).

Soils with acidic conditions demonstrated a higher stock increase after the application of manure than neutral and alkaline soils, which can be explained by reduced microbial activity in acidic soils and thus lower decompositions rates. Biochar applications, however, led on average to larger SOC stock increases if the soil pH was neutral or alkaline. Biochar additions to acidic soils can lead to an increased priming effect (Sheng et al., 2016), meaning that the fresh biochar biomass input stimulates microbes to decompose native SOC. By enhancing the low soil pH through biochar’s liming effect, microbes would have more favorable conditions for priming, which could explain SOC degradation in short-term studies (Whitman et al., 2024). In turn, biochar added to neutral and alkaline soil could shift the soil pH to regions less favorable for microbial activity, which could decrease mineralization (Fernández-Calviño et al., 2011). Another explanation for higher SOC stock increases of biochar added to neutral and alkaline soils is the higher amount and availability of calcium ions (Ca^{2+}) in the soil, which could favor mineral-organic complex formation.

Animal manure showed the highest SOC increases (41% for farmyard-manure, 32% for cattle manure and 41% for pig manure), while green manure and straw showed only minor effects. This observation is not surprising considering the fact that manure of animal origin contains a higher amount of nutrients. Higher nutrient availability generally enhances plants’ above- and below-ground production, which favors rhizodeposition, which both contributes to the SOC storage.

Biochar made of wood and plant-based sources led on average to larger SOC stock increases than biochars made from animal sources. This is because biochar made of more lignin material generally contains of higher amounts of C, and possess higher stability due to their higher C/N and lower oxygen-to-carbon (O/C) ratios compared to biochars from animal sources, such as manure or sludges (Figueredo et al., 2017). Thus, their persistence is longer and the duration of positive effects on soil systems is elevated. These positive effects include improved crop productivity through enhanced soil nutrient availability, soil physical properties and WHC, and plant-microbe interaction. All of these effects, at least to some degree, can lead to increased native SOC stocks.

Nutrient-poor biochar additions do not deliver nutrients to soil, meaning that biochar should not be added instead of fertilizers but combined with them. The combined addition of biochar with synthetic fertilizer led to lower SOC increases than if the biochar was added combined with organic fertilizer, which is not surprising since organic fertilizers contain C and thus contribute additionally to SOC build-up. If manure applications were combined with additional synthetic fertilizer, the SOC increases were higher (+1.7 Mg ha^{-1}) compared to manure alone, because more nutrients entered the system and increased the fertilizing effect of plants and thereby the indirect increase of ‚native‘ SOC.

While both OSA seemingly have a large potential to increase SOC stocks, the most significant difference becomes obvious when looking at the duration effect. Continuous seasonal or annual applications of manure did not lead to significantly higher SOC stock between different duration classes. This is because the SOC stock increase is mainly controlled by the input rate and does not change much if the input rate remains constant, even after several decades. Most of the organic matter entering the soil is easily degradable and will not persist and accumulate in long-terms. Leuther et al. (2022) found that very high manure input rates of 100 and 200 Mg ha^{-1} yr^{-1} are necessary to significantly increase subsoil SOC stocks and thus soil regions with lower turnover rates and higher potential for C accumulation. However, only 2 to 3% of the total manure-C applied at such high rates for 36 consecutive years was detected in the subsoil, which highly questions the potential of manure for long-term SOC storage.

Biochar in contrast, even applied once, continuously increase SOC stock with increasing time up to 10 years. This observation underlines biochar’s persistence on the one hand and its potential for SOC stabilization on the other. Whether this trend continues over time frames longer than 10 years still remains unknown due to missing observations on decadal scales.

In sum, it could be shown that both types of OSA may have a large effect on enhancing SOC stocks but C sequestration effects (by definition >100 years) can only be achieved using biochar, mainly because of its higher long-term stability. The potential to use manure for CDR is also limited because periodic manure input is necessary to enhance SOC stocks in long terms. Available manure should first and foremost be used for fertilization and thus for synthetic fertilizer substitution. While selecting suitable areas for manure application, the potential to enhance food production should be prioritized over CDR, as available area is limited. Additionally, practitioners should be aware of nutrient thresholds in soil while selecting areas for application of manure. Co-composting manure together with biochar could serve as a solution. Thereby, N leaching can be avoided (Kammann et al., 2015), without compromising biochar's SOC sequestration potential (Apostolović et al., 2024). Finally, it should be noted that in order to move towards more sustainable and climate friendly food systems, a profound transformation of livestock practices is needed, including reduced dependencies on intensive livestock systems (Herrero et al., 2023; United Nations, 2023). Thus, it can be assumed that less manure should be available in the future which underlines the necessity to select carefully, for which purpose available manure should be used. Similarly, available biomass for biochar production should be prioritized with care. Generally, only biomass that do not fulfill another important purpose should be considered for pyrolysis. Current estimates quantify the available biomass to be ~152 Mio. t in Germany (DBFZ, 2020) and ~580 Mio. t in the European Union (EU) (Tripathi et al., 2019). Using the assumption of a pyrolysis yield of 30% at 600 °C (Altıkat et al., 2024), this would be equal to a biochar mass of 174 Mio. t, and each t saves ~3 t CO_2-eq., which would be the total equivalent of 522 Mio. t CO_2-eq. Even with less optimistic estimates and achieving only a portion of that, this number highlights the large potential to save emissions using biochar, which we urgently need to meet the target of the EU 'Green Deal' of saving 310 Mio. t of CO_2-eq. With pyrolysis production capacities exponentially growing in the EU (IBI, 2024), the potential for widespread implementation of biochar systems and thus saving these emissions exists.

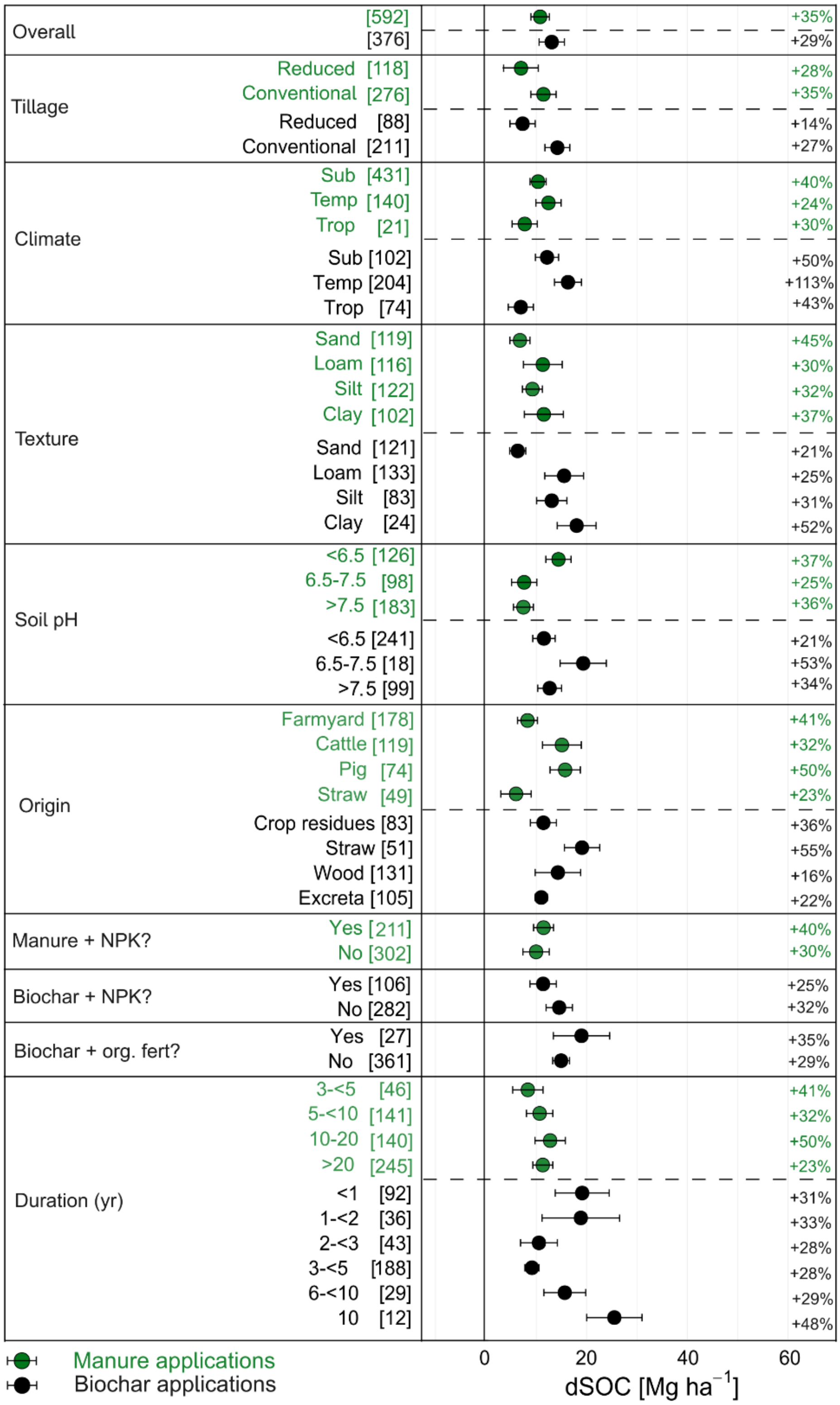

Figure 3: Meta-analysis results of manure and biochar application on SOC level changes, given as a forest plot. Presented is the mean difference and relative change of soil organic carbon stocks (SOC) after manure and biochar application (dSOC) differentiated by different variables. Number in brackets represent the number of included treatments. Points within the range represent the mean dSOC and the line within the 95% confidence interval represents the range of the effect size. Green points represent application of manure and black points the addition of biochar. If the effect size range crosses the "zero-effect-line", given as a solid vertical line at 0 Mg ha^{-1}, the result can be interpreted as statistically insignificant. The effect sizes of each group were considered to be significantly different at $p < 0.05$ from each other if the 95% confidence intervals did not overlap. Data obtained from Gross et al. (2021) and Gross and Glaser (2021).

3.2 Long-term biochar and soil organic carbon stability

Analysis of variance (ANOVA) revealed that the non-biochar OSA compost in Bayreuth and digestates, compost and synthetic fertilizer in Gartow, added alone or as biochar co-amendment did not exhibit significant effects after eleven and nine years on SOC and black carbon stocks, respectively. Only the factors ‘biochar’ (yes/no) and ‘biochar amount’ produced significant differences. Therefore, all individual treatments were aggregated according to the amount of biochar added and thus, three new amount-related treatments were generated per location: no biochar (control, compost, digestates, synthetic fertilizer), low biochar (3 Mg ha^{-1} in Gartow, 9 Mg ha^{-1} in Bayreuth), and high biochar (40 Mg ha^{-1} in Gartow, 31.5 Mg ha^{-1} in Bayreuth). The focus in this section of the extended summary lies on the high biochar containg time series since low biochar additions at both locations did not significantly affect the long-term dynamics (more details see Study 3: Gross et al., 2024).

Following the addition of OSA containing 31.5 Mg biochar ha^{-1}, the SOC stock increased by 38 Mg ha^{-1} (from 54 to 92 Mg ha^{-1}) and remained at the same level until 2021 (Fig. 4). The black carbon stock increased by 8 Mg ha^{-1} to 12 Mg ha^{-1}, increased again by 3 Mg ha^{-1} in the following year, and significantly dropped back to 12 Mg ha^{-1} in 2013. Between 2013 and 2021, the black carbon stock remained stable. In general, the black carbon stock data showed high variation, which limited the ability to draw definitive conclusions. However, since the black carbon stock median after eleven years remained at a level comparable to that immediately following biochar addition, this suggests biochar stability rather than dissipation.

OSA additions containing 40 Mg ha^{-1} biochar combined with fertilizers to the sandy and nutrient-poor soil in Gartow led to a short-term increase of SOC stocks of 60 Mg ha^{-1}, 30 Mg ha^{-1} dissipated in the following four years, and after nine years the biochar-amended soils showed only slightly higher SOC stocks (+7 Mg ha^{-1}) than the non-biochar-amended soil (Fig. 4). The black carbon stocks increased in the short- and mid-term and decreased almost to the original stock levels after nine years (Fig. 4).

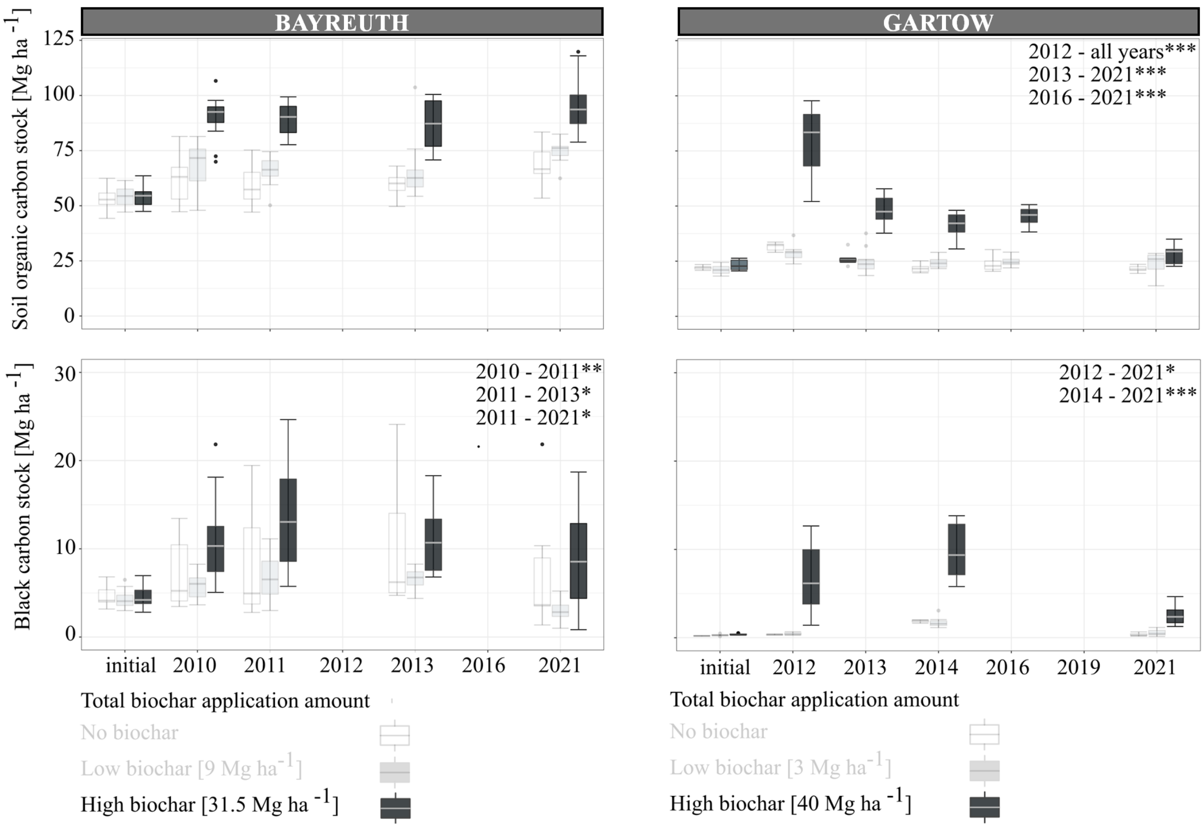

Figure 4: Box plots of soil organic carbon stock and black carbon stock time series of the Bayreuth and Gartow field experiments (soil organic carbon stock data from fall 2013 and 2014 at Gartow are not shown). The median of the data is shown as a horizontal solid line within the box. Each box contains the middle 50% of the data of a category. The whiskers indicate the lower and upper quartile of the data, respectively, and are limited to 1.5 times the interquartile range. Black dots outside the whiskers represent outliers. Significant differences were tested using estimation of least-squares means and are indicated by asterisks in the top right corner of each plot. One, two or three asterisks indicate the level of significance ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***). Data obtained from Gross et al. (2024).

At the Bayreuth site, the initial SOC stock increases due to the high amounts of biochar addition were stabilized over time. Within the same time period the black carbon stocks slightly declined, which indicates that additional SOC must not have originated from the biochar itself but could be related to management factors such as reduced tillage or other OSA. Additional long-term SOC-build up following the addition of biochar has previously been observed (Blanco-Canqui et al., 2020; Guo et al., 2024), and has been described as “negative priming” (Maestrini et al., 2015). Negative priming was found to be positively correlated with soil clay content and the pyrolysis temperature of the biochar (Ding et al., 2018), which matches with the clay-rich soil texture of the experiment soil (26% clay content) and high pyrolysis temperature of the biochar (up to 800 °C) that was used. Blanco-Canqui et al. (2020) observed such negative priming on loamy soil and under reduced tillage. If the SOC stabilization observed in Bayreuth is due to negative priming, the co-addition of OSA at the beginning of the experiment, reduced tillage, the periodic organic fertilization or a combination of these factors, cannot be certainly disentangled. What can, however, be concluded is that the initial SOC build-up after the application of the high biochar amounts reached a new and high plateau, which has been stabilized over a period of eleven years, thus underlining biochar’s SOC sequestration potential.

In contrast, the Gartow time series indicated pronounced SOC dissipation over time. The contrasting dynamics between both sites might therefore be related to the different soil texture of the two field experiments. While the soil at the Bayreuth site contains 12% silt and 26% clay, the soil texture at the Gartow site is dominated by 95% sand. Soils with a finer texture have higher amounts of clay minerals and iron oxides. These fine components associate with SOC and protect it from enzymatic breakdown and turnover - a process known as mineral-organic stabilization - while also increasing SOC stability through physical protection within soil aggregates, according to Lal (2018). Conversely, sandy soils offer less physical protection, making SOC more prone to oxidation and decomposition (Gross et al., 2021), and losses of SOC through leaching and runoff (Yang et al., 2019).

Other dominant factors influencing amendment-induced C sequestration are the OSA properties. The biochar used at the Bayreuth site was made of wood and showed a higher C/N ratio than the biochar used at the Gartow site (239 vs. 75), which was made of green cuts. The C and N content in biochar, and their ratio, are critical factors determining the stability of biochar and its ability to contribute to SOC build-up (Liu et al., 2016). Another decisive property to describe biochar stability is the hydrogen-to-carbon (H/C) ratio (Budai et al., 2013; Schimmelpfennig and Glaser, 2012), which is an indicator of the aromaticity of biochar. Compared to uncharred biomass, which typically possesses higher H/C ratios, biochar with low ratios is expected to be more stable in the long-term (Budai et al. 2013). Both of the biochars used at either location possessed very low H/C ratios, and should still contain >95% of its original mass after 100 years. Thus, the dissipation of black carbon stocks observed at both location and SOC stocks in Gartow cannot be explained by decreasing biochar stability.

More likely, lateral and vertical transport of biochar particles might have occurred at both locations to different degrees. Vertical transport, which is mainly driven by tillage, rainfall, bioturbation and soil macro pores (Major, 2010; Obia et al., 2017), occurs in rates up to 30 mm per year and can account for up to 40-70% of the applied biochar, as observed in agricultural experiment plots (Rumpel, 2024). Downward movement of biochar with time could be observed in both experiments (Table 2). Black carbon stocks significantly increased in the 10-30 cm layer while biochar dissipated in the 0-10 cm layer. When biochar moves deeper into soil, it continues to stabilize SOC stocks and contributes to sequestration effects (Wang et al., 2023). SOC stocks in 10-30 cm depth increased with progressing time (Table 2), which corroborates this theory.

Lateral transport is mainly driven by erosion, tillage and soil water flow and was found to be responsible for 20-53% of total dissipation (Rumpel, 2024). Lateral particle movement could not be systematically analyzed, as neither of the field experiments was specifically designed for this purpose. However, in Bayreuth, nearly all biochar-free plots adjacent to plots receiving high biochar applications (31.5 Mg ha^{-1}) exhibited increases in SOC and black carbon stocks over time (for more details see Study 3: Gross et al., 2024), suggesting lateral transport. In Gartow, however, a similar lateral movement was not observed, as SOC and black carbon stocks showed no specific trends between biochar-free plots adjacent to plots with high biochar applications (40 Mg ha^{-1}). This may indicate that vertical biochar transport is more prevalent in a highly sandy soil matrix.

To systematically, quantitatively, and statistically investigate lateral transport effects, an experimental design focused on transport dynamics or systematic sampling outside biochar plots—at increasing distances with sufficient replication—would be necessary. Given that biochar particle migration occurs over time, we would expect, on one hand, to detect biochar beyond the experimental plots, and on the other hand, an increase in SOC stocks in deeper soil layers over the long term, which should be explored in future studies.

Table 2: Median soil organic carbon (SOC) stocks and black carbon (BC) stocks of the organic soil amendments containing high biochar amounts (31.5 Mg ha^{-1} and 40 Mg ha^{-1}) in two soil depths 0-10 cm and 10-30 cm. SE = standard error. Significant differences between the years were tested using estimation of least-squares means and are indicated by different letters.

	Soil depth			
	0-10 cm		10-30 cm	
	SOC stocks ± SE	BC stocks ± SE	SOC stocks ± SE	BC stocks ± SE
	Mg ha^{-1}			
Bayreuth				
Year				
2010	52.80 ± 1.92a	8.54 ± 0.35a	39.63 ± 1.69a	3.25 ± 0.80a
2011	56.24 ± 2.08a	12.39 ± 1.37b	38.08 ± 3.35a	2.80 ± 0.57a
2013	30.45 ± 1.17b	2.79 ± 0.67c	51.58 ± 2.08b	7.36 ± 0.65b
Gartow				
Year				
2012	34.27 ± 4.31a	7.27 ± 1.63a	15.99 ± 0.45a	0.28 ± 0.03a
2014	24.73 ± 2.41a	1.06 ± 0.69b	15.03 ± 0.86a	1.65 ± 0.86b
2016	17.08 ± 1.39b	2.33 ± 0.27b	25.31 ± 1.90b	3.11 ± 0.47b

3.3 Impact of biochar aging on physicochemical properties

The soil pH of the variant which received fresh biochar (F_BC_S) was significantly higher (p<0.05) than the control, and the two variants containing aged biochar (A_BC_S and CC_BC_S) (Fig. 6a). Increasing soil pH following the addition of biochar is due to the release of alkaline cations known as "liming effect". During aging of biochar in soil, the acid-neutralizing effect diminishes, eventually leading to a decrease in soil pH. Dissolving basic compounds such as carbonates and hydroxides in biochar may contribute to the observed decrease in soil pH (Mukherjee et al., 2014). In addition, surface oxidation of biochar could have led to formation of carboxylic groups, which affect the soil pH (Mukherjee et al., 2014; Yao et al., 2010; Sorrenti et al., 2016). Decreasing soil pH during biochar aging is well documented (De La Rosa et al., 2018; Joseph et al., 2010; Spokas, 2013; Yao et al., 2010). Fresh addition of biochar increased soil EC in the F_BC_S variant, suggesting a higher concentration of soluble salts (Fig. 5a) than in the aged biochar treatments, which showed significantly lower EC levels ($p<0.05$), likely due to a reduction in soluble salts and ions, which may result from leaching (Joseph et al., 2010; Wu et al., 2014). The development of O-functional groups during aging could have contributed to the decreasing EC additionally (Kane et al., 2021).

Fresh biochar addition to soil (F_BC_S) significantly increased TC ($p < 0.05$) (Fig. 5b) compared to the control soil. After 13 years of aging, the TC content in both aged variants declined significantly compared to the freshly mixed soil variant. However, the decline in the co-composted biochar added to soil (CC_BC_S) was significantly lower ($p < 0.05$) than that of the pristine aged biochar added to soil (A_BC_S). The difference of SOM between the variants followed a similar pattern but the decline during aging was not as drastic as TC, and both aged variants showed no significant difference. During aging, biochar particles tend to sorb organic matter components from their surrounding soil material, resulting in organic coatings on the particle surface and reduced SOM loss or increased SOM stabilization. This SOM coating strongly affects biochar physicochemical properties and influences the stability of the aromatic "backbone" (Hagemann et al., 2017). Co-composting of biochar particles prior to application leverages this natural process of SOM sorption and coating formation, and could therefore explain higher TC and SOM levels than the pristine biochar treatment. However, the declining TC content of the aged variants compared to the fresh biochar variant cannot be explained by aging alone, but might be connected to vertical and lateral transport, which has already been observed eleven years after biochar addition (see Chapter 3.2).

In aged biochar treatments especially the co-composted biochar, TN levels were higher than in the treatment which received fresh biochar (Fig. 5b). Fresh addition of biochar decreased plant-available N (Fig. 5c), indicating immobilization effects, which had already been observed before (Clough et al., 2013; DeLuca et al., 2015). Immobilization of N is more likely if the biochar was added without additional fertilizer as co-amendment or without a pre-treatment with nutrients, such as co-composting. The co-composted treatment showed the highest NH_4^+-N levels. Contrarily, the presence of biochar led to significantly lower NO_3^--N levels compared to the control soil, likely due to a more negative charge on the biochar surface, resulting in reduced NO_3^--N retention. Kammann et al. (2015) however, demonstrated that co-composting biochar can enhance NO_3^--N retention, attributing this effect to the formation of acidic and basic functional groups, as well as organo-mineral complexes on the biochar surface. No significant differences were observed between treatments for TP and the soluble P fractions (Fig. 5d).

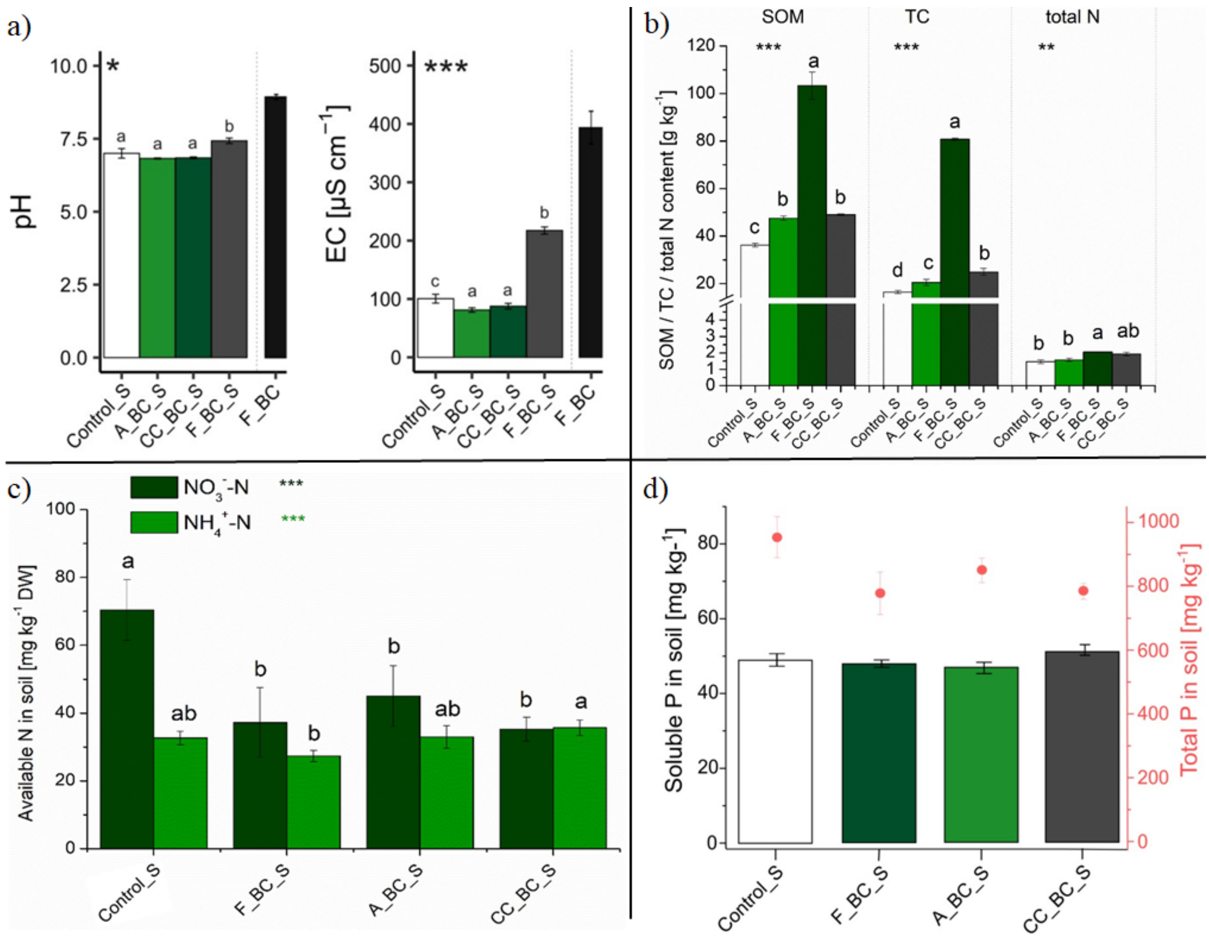

Figure 5: Soil and biochar pH (left) and electric conductivity (EC) values (right) of the different treatments (a). Soil organic carbon and total N (b), nitrate-nitrogen (NO_3^--N) and ammonium-nitrogen (NH_4^+-N) content (c), and soluble P and total P content of the four different soil treatments (d). Each bar represents the mean of three replicates. Error bars indicates the standard deviation. ANOVA and Tukey's honestly significant test were employed to analyze significant differences between means. Different letters indicate significant differences between the treatments. Asterisks indicate the level of significance (ns: not significant, $p < 0.05$*; $p < 0.01$**; $p < 0.001$***). Obtained from Apostolovic et al. (2024).

By applying PCA, the dimensionality of the entire soil property data set was aligned to two major axes: the first principal component, PC1, accounts for 59.4%, and the second principal component, PC2, accounts for 24.6% of the total variance in the data set (Fig. 6). In sum, both axes captured 84% of the total variance, thus, most of the variability in the data.

Four cluster could be identified. The one representing fresh biochar-treated soil (F_BC_S) was separated from the other three clusters, meaning that the changing soil properties upon fresh biochar additions make F_BC_S distinguishable from the aged biochar soil treatments and the control. The aged biochar soil treatments and the control formed individual clusters at approximately similar positions on the PC1, suggesting that 13 years of aging diminish those effects made these treatments become more similar. However, different positions along the PC2 axis suggest that the addition of biochar itself (compared to the control), and co-composting modified the impacts on soil properties.

The vectors representing NH_4^+-N (8) and pore radius (12) point towards the two aged biochar soils, underlining the significantly higher NH_4^+-N availability in both variants compared to the freshly amended soil (Fig. 5c), and suggesting higher mobility of these NH_4^+-N ions in larger pores. The vectors representing total P (9) and NO_3^--N point towards the control soil, and showed no relation to any of the biochar treatments. The vectors representing SSA (11), pH (1) and EC (2) point towards the freshly amended soil, underlining their immediate effects on these properties. Their magnitude eventually decreases during progressing aging time.

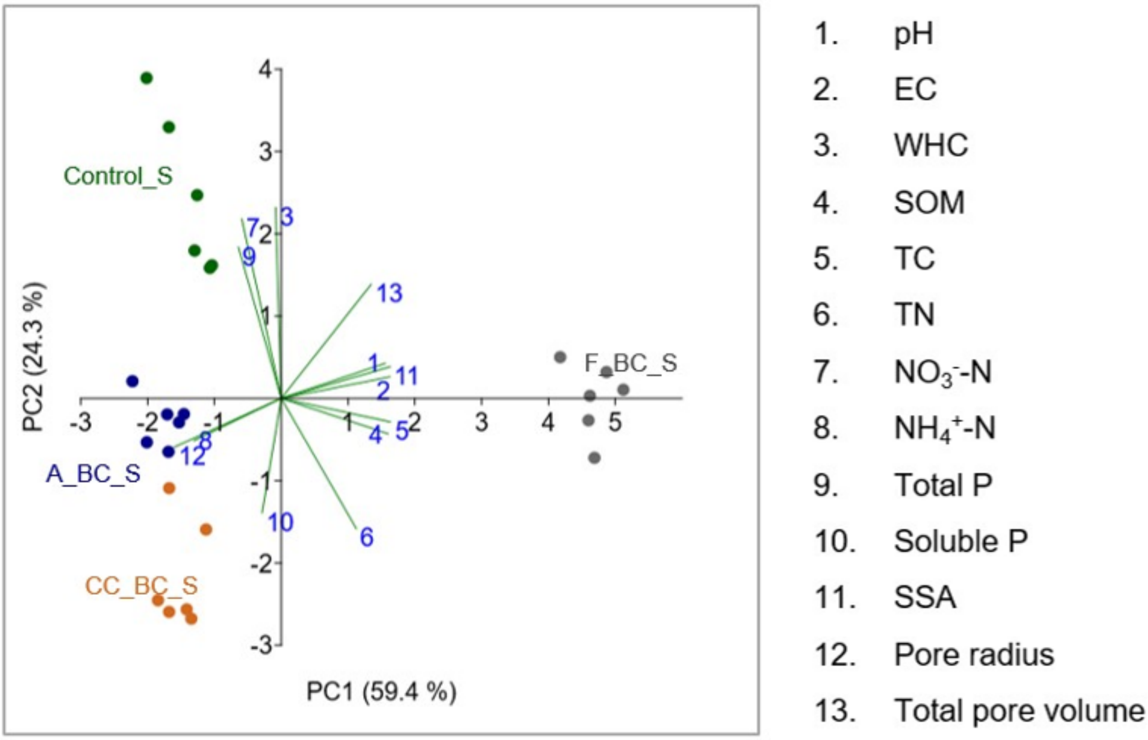

Figure 6: Principal component analysis (PCA) biplot. Control_S is the control soil, A_BC_S is aged biochar treated soil, F_BC_S is fresh biochar treated soil, CC_BC_S is co-composted biochar treated soil. Obtained from Apostolovic et al. (2024).

4. Conclusions

This Ph.D. project had the objective to fill apparent research gaps regarding effectiveness of OSA, particularly biochar as a CDR agent and long-term soil conditioner. The goal was to answer three essential research questions.

1. *To which extent are the two different OSA "manure" and "biochar" able to increase SOC stocks, and what are global explanatory variables controlling their SOC sequestration potential?*

Two meta-analyses showed that both types of OSA have a large potential to increase SOC stocks. However, the size of this potential depends on a series of regional factors, which have to be taken into account before using OSA for CDR measures. Most of these factors influenced the potential to increase SOC stocks upon application of both OSA in a similar way. However, achieving similar effects requires periodic manure application, whereas a single biochar application is sufficient. Another very notable difference could be identified when looking at long-term observations. Periodic applications of manure did not lead to significantly higher SOC stock in longer-term observations. Single applications of biochar in contrast to that, continuously increased SOC stock with increasing observation time up to 10 years. Due to missing observations at the time this analysis has been conducted, it remains unknown if this trend will continue on decadal scales. What this finding however underlines is the high persistence of biochar and its higher SOC stabilization and sequestration potential in contrast to manure. Longer term observations >10 years are urgently needed to confirm this trend.

2. *How do different OSA, including biochar, influence the SOC stocks dynamics of two different soils in Germany in the long-term and how persistent is the added biochar under real field conditions?*

By re-sampling and analyzing two abandoned biochar field experiments, it could be shown that the SOC sequestration potential after biochar-containing OSA applications and biochar stability in soils mainly depends on regional factors rather than the properties of the amendments used. The addition of biochar-containing OSA to a loamy soil in northern Bavaria led to SOC sequestration effects, while additions to a sandy soil in eastern Lower Saxony increased the SOC stocks only slightly, mainly due to missing physical protection within the soil matrix. Black carbon stock results indicated considerable biochar dissipation at both locations. This may be related to multiple dissipation processes occurring at the same time, such as oxidation, co-metabolic decomposition, or vertical and lateral particle transport. However, due to the inert stability of the biochars used in both field experiments with H/C ratios < 0.2, mineralization processes are less likely than particle translocation. Disentangling these dissipation pathways under field conditions and in the long term should be prioritized in future research. This paper was able to demonstrate that re-sampling old, abandoned field experiments can still be useful to understand the long-term behavior of SOC stocks and biochar. The observed dynamics should be further validated in future sampling.

3. *How does the long-term aging of biochar affect its efficacy as a CDR agent and measure to improve soil properties?*

Our findings demonstrate that the application of biochar, even after 13 years of aging, still have significant positive effects on soil physicochemical properties, although the magnitude decreases with the time in soil. While some soil quality increasing effects such as soil pH and EC diminished with aging time, the ability to retain N increased, especially if the biochar was co-composted before being applied. Moreover, the soil treated with co-composted biochar could preserve more TC during aging than the soil treated with pristine biochar. This confirmed previous findings that co-composting does not influence biochar's stability and persistence in soil and, considering its benefits over untreated pristine biochar, should be conducted before applying biochar to soil.

While the three research questions could be solved successfully, many more became apparent while delving into the objectives of this dissertation. There is an urgent need for research to identify unused biomasses suitable for pyrolysis. Biomass selection should preferably be based on holistic assessment methods to avoid potential negative trade-offs that could impede the CDR potential, e.g., long transport distances or material loss. Additionally, future studies should try to disentangle the different dissipation pathways of biochar and their impact on SOC sequestration. Further experimental evidence is necessary on how biochar-induced increases in SOC stock are influenced by biochar properties and the respective agroecosystem with its unique soil properties as well as agricultural management decisions. Without a broad empirical basis, these findings are not transferable into agronomic practice and will not find wide acceptance.

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Authors' contributions to the included manuscripts

This cumulative Ph.D. dissertation includes four manuscripts. All manuscripts were prepared by me as the first author, in collaboration with shared-first or co-authors. The estimated contributions of the authors for each of the four manuscripts are detailed as follows.

Study 1: Meta-analysis on how manure application changes soil organic carbon storage

Published in *Scientific Reports*, Impact factor: 3.8 (source: Clarivate)

DOI: 10.1038/s41598-021-82739-7

Arthur Groß	80%	Data collection, data preparation, data analysis, visualization, preparation of the manuscript, manuscript review and editing
Bruno Glaser	20%	Discussion of results, manuscript review and editing

Study 2: Soil organic carbon sequestration after biochar application: A global meta-analysis

Published in *Agronomy*, Impact factor: 3.3 (source: Clarivate)

DOI: 10.3390/agronomy11122474

Arthur Groß	80%	Data collection, data preparation, data analysis, visualization, preparation of the manuscript, manuscript review and editing
Tobias Bromm	10%	Manuscript review and editing
Bruno Glaser	10%	Manuscript review and editing

Study 3: Long-term biochar and soil organic carbon stability – evidence from field experiments in Germany

Published in *Science of The Total Environment*, Impact factor: 8.2 (source: Clarivate)

DOI: 10.1016/j.scitotenv.2024.176340

Arthur Groß	45%	Conceptualization, field work, laboratory analysis, data analysis, visualization, preparation of the manuscript, manuscript review and editing
Tobias Bromm	15%	Laboratory analysis, field work, data validation and discussion, manuscript review and editing
Steven Polifka	10%	Data validation and discussion, manuscript review and editing
Daniel Fischer	10%	Data validation and discussion, manuscript review and editing
Bruno Glaser	20%	Conceptualization, field work, data validation and discussion, manuscript review and editing

Study 4: Impact of biochar aging on soil physicochemical properties

Accepted for publication in *Agronomy,* Impact factor: 3.3 (source: Clarivate)

Tamara Apostolović	32.5%	Laboratory analysis, data analysis, visualization, manuscript review and editing
Arthur Groß	32.5%	Data analysis, visualization, preparation of the manuscript, manuscript review and editing
Álvaro Fernando García Rodríguez	2%	Laboratory analysis
José María de la Rosa	3%	Data validation and discussion, manuscript review and editing
Bruno Glaser	10%	Conceptualization, data validation and discussion, manuscript review and editing
Heike Knicker	10%	Conceptualization, data validation and discussion, manuscript review and editing
Snežana Maletić	10%	Conceptualization, data validation and discussion, manuscript review and editing

II. Publications and Manuscripts

Study 1: Meta-analysis on how manure application changes soil organic carbon storage

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Abstract

Manure application to agricultural soils is widely considered as a source of nutrients and a method of maintaining levels of soil organic carbon (SOC) to mitigate climate change. At present, it is still unclear which factors are responsible for the SOC stock dynamics. Therefore, we analyzed the relationship between SOC stock changes and site characteristics, soil properties, experiment characteristics and manure characteristics. Overall, we included 101 studies with a total of 592 treatments. On average, the application of manure on agricultural soils increased SOC stocks by 35.4%, corresponding to 10.7 Mg ha^{-1}. Manure applications in conventional tillage systems led to higher SOC stocks (+ 2.2 Mg ha^{-1}) than applications under reduced tillage. SOC increase upon manure application was higher in soils under non-tropical climate conditions (+ 2.7 Mg ha^{-1}) compared to soils under sub-tropical climate. Larger SOC increases after manure application were achieved in intermediate and shallow topsoils (in 0–15 cm by 9.5 Mg ha^{-1} and in 16–20 cm by 13.6 Mg ha^{-1}), but SOC stocks were also increased in deeper soils (> 20 cm 4.6 Mg ha^{-1}), regardless of the tillage intensity. The highest relative SOC increase (+ 48%) was achieved if the initial SOC was below 1% but the absolute SOC increased with increasing initial SOC. Clay soils showed higher SOC increase rates compared to sandy soils (+ 3.1 Mg ha^{-1}). Acidic soils showed comparable relative effects but a higher stock difference than neutral (+ 5.1 Mg ha^{-1}) and alkaline soils (+ 5.1 Mg ha^{-1}). The application of farmyard-, cattle- and pig manure showed the highest SOC increases (50%, 32% and 41%, respectively), while green manure and straw showed only minor effects. If manure applications were combined with additional mineral fertilizer, the SOC increases were higher (+ 1.7 Mg ha^{-1}) compared to manure alone. Higher applied amounts generally led to higher SOC stocks. However, the annually applied amount is only important under conventional tillage, non-tropical climate conditions, and pH-neutral as well as SOC-rich or SOC-depleted soils and if no additional mineral fertilization is applied. Further studies should focus on the SOC dynamics under tropical climate conditions and factors influencing a potential carbon saturation. In both cases, the number of data was too small. For this reason, additional field studies should be conducted primarily in the tropics. On the other hand, long-term field trials should be re-assessed or newly established to specifically investigate potential saturation effects and long-term (> 20 years) fertilizer effects and carbon sequestration.

1. Introduction

The continuously rising concentrations of atmospheric greenhouse gases (GHG) due to human and natural emissions are the main drivers of climate change (Schimel, 1996). This necessitates approaches for mitigating GHG emissions. Strengthening renewable energies or mitigating emissions using carbon capture and storage (CCS) or carbon capture and use (CCU), are possible GHG mitigation options (IPCC, 2007). However, since the switch to renewable energy supplies is still limited by political and market barriers, and geological storage technologies such as CCS are associated with certain risks

(Mazzoldi et al., 2011), they have less acceptance (Wallquist et al., 2012). Nonetheless, there remains a need for sustainable and safe carbon removal from the atmosphere.

Soils are an important carbon sink, as they contain more carbon than stored in terrestrial vegetation and the atmosphere combined (Lehmann and Kleber, 2015). Several regional studies showed that there is still potential to store even more carbon in soils, if certain management practices are applied (Glaser and Birk, 2012; Minasny et al., 2017). This process of storing organic carbon in soils, better known as carbon sequestration, describes how organic carbon is put into soils and converted into a stabilized form in the long-term (> 100 years). Besides its beneficial climatic effects, higher soil organic carbon (SOC) content promotes several important soil functions, such as nutrient transformation and supply, soil–water balance control or buffering of pollutants (Baldock and Skjemstad, 1999). In short, soil organic matter (SOM) is important for adapting to climate change as well as mitigating it.

Theoretically, there are many ways of increasing the SOC pool (Lal, 2004). However, most of their practical potential is limited. For instance, while no tillage did not significantly increase SOC stocks (Poeplau and Don, 2015), cover crops increased SOC stock by 9–10%, based on a review of global meta-analysis data (Bolinder, 2020). Another option is to use different organic materials with high carbon content as soil amendments. Manure is a collective term for excrements of different animal species, urine, plant materials and straw but also livestock feed residues and human household waste. Manure nitrogen production increased from 21.4 Tg N year^{-1} in 1860 to 131.0 Tg N year^{-1} in 2014 with an annual increasing trend of 0.7 Tg N year^{-1} (Zhang et al., 2019). Cattle dominated the manure nitrogen production and contributed ~44% of the total manure nitrogen production in 2014, followed by goats, sheep, swine, and chicken (Zhang et al., 2019). The manure nitrogen application to cropland accounts for less than one-fifth of the total manure nitrogen production (Zhang et al., 2019). Manure might also increase carbon in soils as these materials have high carbon content. However, organic matter in manure might also be easily degraded due to its high nitrogen content or its low carbon-to-nitrogen (C/N) ratio. Many individual studies measured the impact of manure application on SOC stocks, with few studies showing increases in SOC stocks, but also studies showing only small or even negative impacts. Due to this wide variation in results, there is a need for studies clarifying factors that control the magnitude of change in SOC stocks due to manure application. Up to now, only two quantitative reviews have tried to find global relationships between the magnitude in SOC stock changes and different explanatory factors. Han et al. (2016) focused on combined treatments of manure and mineral fertilizer and Maillard and Angers (2014) included studies with mineral fertilizer as reference to manure treatments. Furthermore, Maillard and Angers (2014) only considered articles published up to 2011.

Due to this current lack of clear evidence and statistically significant relationships between SOC stock changes upon manure application and global explanatory factors, we conducted a meta-analysis. The aim of this study was to calculate the response ratio (RR) of carbon stocks to manure application and the SOC stock difference under consideration from data available from peer-reviewed studies (ISI Web of Science). Furthermore, our target was to identify clear evidence of influencing factors. For this purpose, we grouped and analyzed the results according to the following criteria: site characteristics (climate zone), soil properties (initial SOC content, pH value, soil texture), experiment characteristics (tillage intensity, experiment duration, sampling depth) and manure characteristics (manure type, added manure amount, additional mineral nitrogen, phosphorus, and potassium (NPK) fertilizer). In addition to the analysis within individual categories, we also examined intercategorical effects to investigate possible interactions between the investigated factors.

2. Material and Methods

2.1 Data sources, collection and categorization

In order to analyze SOC stock changes following manure application, a meta-analysis was conducted. Within this framework, we performed a systematic literature review using “ISI Web of Science (Core Database)”. The search term was “(Soil organic matter OR C Sequestration) AND Manure”. Studies were included if they were performed under field conditions and if the effect and control size was expressed as content of total organic carbon (TOC) or quantified as SOC or TOC stocks. If SOM rather than SOC information were given in a study, we calculated SOC as SOM multiplied by 0.58. All treatments with a duration of <= 3 years were removed to exclude short-term effects and the influence of the cultivated crops. Overall, 101 studies with a total of 592 treatments were included.

Besides information on SOC content, we also extracted information on soil properties (initial SOC content, texture, bulk density, soil pH class), experiment characteristics (tillage intensity, duration, sampling depth), manure characteristics (type, added amount, additional mineral fertilizer use) and site characteristics (longitude, latitude, altitude, climate zone). To limit the variety of different soil texture classes, we decided to group them into their respective dominant particle size class (sand, silt or clay). Exceptions are the middle classes “clay loam and loam”. These have been added to “loam”. If data were only presented in figures, WebPlotDigitizer Version 4.2 was used for the extraction of data. In order to analyze the total amount of manure added, annual amounts were accumulated.

If no information on SOC stocks was provided, we quantified them using the following equation (1) (FAO, 2018),

$$SOC\ stock = SOC * Bulk\ density * Depth * 0.1 \tag{1}$$

where SOC stock is expressed as Mg ha^{-1}, bulk density as g cm^{-3}, depth as cm and SOC as g kg^{-1}. In a few studies, no soil bulk density was given. In these cases, we used different pedotransfer functions. If studies included information on the initial SOC, silt and clay content, we used the pedotransfer function given in Men et al. (2008) *(Equation 2).* If studies included information on the initial SOC and the clay content, we used an equation given in Bernoux et al. (1998) *(Equation 3).* If studies only provided information on initial SOC, we used a pedotransfer function given in La Manrique and Jones (1991) (Equation 4).

$$Bulk\ density = 1.386 - 0.078 \times SOC + 0.001 \times Silt + 0.001 \times Clay \quad (2)$$

$$Bulk\ density = 1.398 - 0.0047 \times Clay - 0.042 \times SOC \quad (3)$$

$$Bulk\ density = 1.660 - 0.318 \times SOC^{0.5} \quad (4)$$

where bulk density is expressed as g cm^{-3} and the SOC, silt and clay content as %. To better understand the factors influencing SOC stock changes, we grouped the study results as follows: tillage intensity type, climate zone, initial SOC, soil texture, sampling depth, soil pH class, added annual manure amount, cumulative manure amount, manure type, additional mineral fertilizer and experiment duration.

2.2 Data analysis

To estimate the effects of manure applications on SOC stock changes, we used two different indices. We calculated the response ratio (RR), which is the mean of the manure treatment divided by the mean of the control group (all the same but without manure application) and we calculated the SOC stock mean difference (ΔSOC). To measure experimental effect sizes, RR and ΔSOC are both very common and wide-spread in meta-analyses (Gattinger et al., 2012; Han et al., 2016; Hedges et al., 1999; Liu and Greaver, 2010; Maillard and Angers, 2014). It is essential to calculate both indices as RR only gives information on relative changes whereas ΔSOC considers the absolute impact. The consideration of only one of those indices can be misleading. Two similar absolute SOC changes can be the result of either a low or a high relative SOC change, depending on the initial SOC content.

RR was calculated using the following equation:

$$RR = \left(\frac{X_E}{X_C}\right) - 1 \quad (5)$$

where X_E is the mean SOC stock with manure application and X_C is the mean SOC stock without application of manure (control group) for each treatment. In order to better interpret the result, 1 was subtracted from each RR value

More precise meta-analysis are using a weighting according to the number of repetitions, the standard deviation or the standard error. Considering the fact that only a few of the analyzed studies provided sufficient information on statistical measures and replicates, we decided to use un-weighted meta-analysis, to include as many treatments as possible. “Un-weighted” meta-analysis is a commonly used approach, which gives all included studies the same weight, e.g. a weight of 1 (Guo and Gifford, 2002;

Han et al., 2016; Johnson and Curtis, 2001; Qin et al., 2021). SOC stock differences were calculated by using equation 6:

$$\Delta SOC = X_E - X_C \tag{6}$$

where X_E represents the mean SOC stock in Mg ha^{-1} of the experimental group and X_C the mean SOC stock in Mg ha^{-1} of the control group.

For reasons of better interpretation, 95% confidence intervals (CI) were calculated as follows:

$$CI\ upper = R\ or\ \Delta SOC\ \frac{+1.96*\sigma}{\sqrt{n}} \tag{7}$$

$$CI\ lower = R\ or\ \Delta SOC\ \frac{-1.96*\sigma}{\sqrt{n}} \tag{8}$$

with the mean response ratio RR or the SOC stock mean difference ΔSOC in Mg ha^{-1}, 1.96 the confidence coefficient, σ the standard deviation and n the number of individual treatments.

All of these statistical measures are presented as forest plots. Visualization was conducted with R Version 3.5.2 (R Core Team, 2021). The overall grand mean of all individual treatments is presented in the first row. The grey solid line represents an RR, or a mean difference equal to 0, thus no effect. An effect size larger than 0 indicates a positive effect (i.e. an increase of SOC upon manure application), and lower than 0 a negative effect (i.e. a decrease of SOC upon manure application). Each effect size is presented as the range between the upper and lower 95% confidence interval. The line inside of both confidence intervals represents the range of the effect size. The range between both confidence intervals of the grand mean is shown by the extent of the rectangle. If the effect size range crosses the "zero-effect-line", the result can be interpreted as statistically insignificant. The mean effect sizes of each group were considered to be significantly different at $p < 0.05$ from each other if the 95% confidence intervals were not-overlapping. N represents the number of included treatments.

In the inter-categorial evaluation, all influencing factors were compared with each other. Due to the resulting large number of data, we decided to examine only those intermediate category treatments that occurred at n >= 10. Furthermore, we eliminated all treatments in the intercategorical evaluation, which applied combinations of manure types due to too many different combinations and, therefore, too few repetitions per manure treatment class.

To analyze the connection of ΔSOC and added manure amounts under the influence of various factors, a linear regression analysis was conducted using R Version 3.5.2 (R Core Team, 2021). We calculated the coefficient of determination R^2 and the statistical connection was determined by using the Pearson correlation coefficient RR. Normal distribution was checked using the Shapiro-Wilk test.

3. Results and Discussion

3.1 General effect

Overall, 101 studies with a total of 592 treatments were analyzed in this study (Appendix S1-1). All of them were conducted under field conditions. No laboratory experiments were included. Locations in North America (n = 8), South America (n = 2), Sub-Saharan Africa (n =5), Europe (n =11), West Africa (n = 2), South Asia (n = 20) and East Asia (n = 53) were included. The results of all subcategories, including their standard deviation can be found in the Appendix S1-2. The results of the intercategorical grouping is located in the Appendix S1-3 and their corresponding forest plots can be found in the Supplementary Material.

As expected, the results obtained from 592 pairwise comparisons showed a significant increase of SOC stocks of 35% (95% CI 32% - 39%) and a ΔSOC of 10.7 Mg ha^{-1} (95% CI 9.8 – 11.6 Mg ha^{-1}) on average, after manure was applied despite high variation among different groups. This positive effect can mainly be explained by the fact that manure applications are direct inputs of carbon into soil and a source of nutrients (especially nitrogen), which results in an increased net primary production of plants and increased yields (Cai et al., 2018; Du et al., 2020; Obour et al., 2017). Increasing plant primary production leads to an increase of crop residue inputs and rhizodeposition, which both enhance SOC sequestration (Stewart et al., 2007).

3.2 Tillage intensity effect

Out of 592 treatments that we analyzed, 394 treatments provided information on tillage intensity. 276 treatments were conducted on soils under conventional tillage and 118 treatments on reduced tillage soils. The relative SOC change of the tillage intensity group is presented in Fig. S1-1. Both treatments showed positive magnitudes with a mean increase of SOC stocks of 35% for conventional tillage and 28% for reduced tillage systems. ΔSOC was 10.7 Mg ha^{-1} for conventional tillage and 8.5 Mg ha^{-1} in reduced tillage systems (Fig. S1-2). It is known that reduced tillage has beneficial effects on soil quality, e.g. physical, biological and chemical properties (Liebig et al., 2004; Rasmussen, 1999; Willekens et al., 2014), but the effects of tillage on carbon accumulation are controversially discussed. While many studies showed higher SOC accumulation in reduced tillage systems after manure application (Bogužas et al., 2015; Mando et al., 2005; Yaduvanshi and Sharma, 2008), Baker et al. (2007) argued that SOC accumulation caused by reduced tillage are biased, as most of the studies conducted only involved shallow sampling. Studies which involved deeper sampling often show no positive or insignificant sequestration effects (Baker et al., 2007). Our results point to different dynamics. The intercategorical evaluation of tillage intensity and sampling depth shows that manure applications even under reduced tillage led to the smallest but still a significant enrichment of SOC in depths > 30 cm (Appendix Fig.

S1-1 and S1-2). ΔSOC increased by 3.7 Mg ha^{-1} corresponding to an RR of 19%. Conventional tillage in depths > 30 cm led to a SOC increase of 23% corresponding to 5.6 Mg ha^{-1}. Shallow sampling depths <= 15 cm led to a SOC increase of 21% under reduced tillage and a ΔSOC of 7.2 Mg ha^{-1}. Under conventional tillage, shallow sampling depths showed a larger SOC increase of 40% and also a larger ΔSOC of 9.0 Mg ha^{-1}. Overall, sampling depth-wise both tillage intensities showed the same SOC increase with large relative and absolute SOC increases in shallow soil depth and smaller responses in deeper regions. This seems logical, as manure applications under conventional tillage are usually only ploughed into the soil up to a depth of 20-30 cm, or in the case of reduced tillage only very shallowly or not at all.

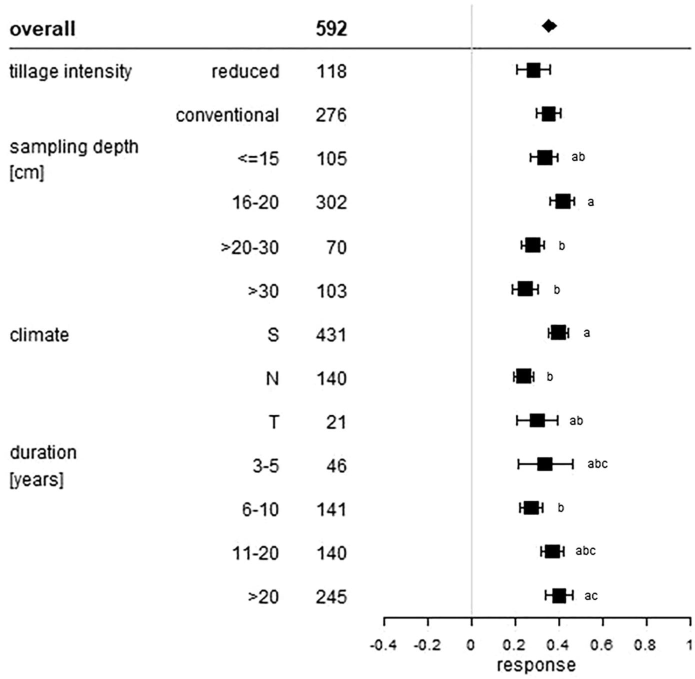

Figure S1-1: Relative response of manure applications on soil organic carbon stocks influenced by tillage intensity, sampling depth (cm), climate and duration (years) of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at response ratio = 0. Different letters in each subcategory indicate statistically significant differences.

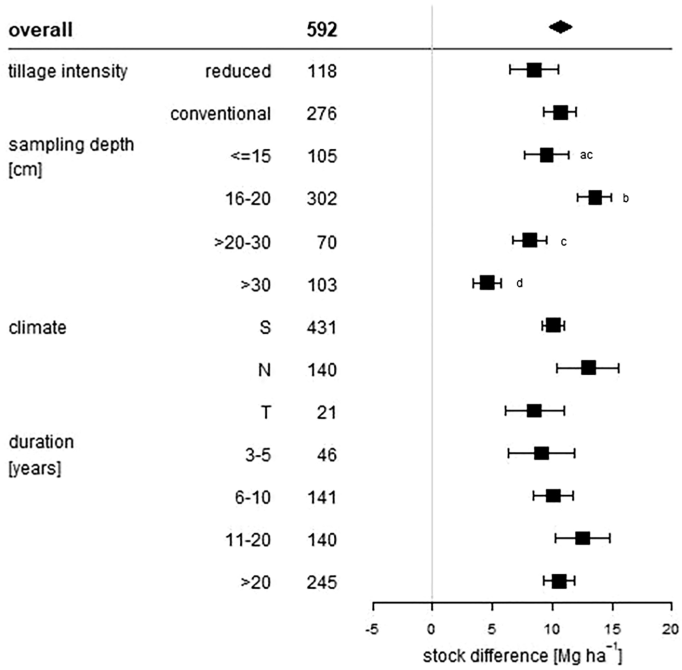

Figure S1-2: Absolute response (Mg ha^{-1}) of manure applications on soil organic carbon stocks influenced by tillage intensity, sampling depth (cm), climate and duration (years) of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response is presented as range between the upper and lower 95% confidence intervals. Points within the range represent the mean response. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at stock difference = 0 Mg ha^{-1}. Different letters in each subcategory indicate statistically significant differences.

Regarding sampling depth, a 12% lower SOC stock response was observed at near-surface sampling depth equal to or less than 15 cm, compared to 16 – 20 cm soil depth (Fig. S1-1). If sampling depth was > 20 - 30 cm, SOC stock increased by 28%. In > 30 cm soil depth, SOC increased by 24%. ΔSOC showed higher results in near-surface regions than in greater soil depth, with 9.5 Mg ha^{-1} in the first 15 cm and 4.6 Mg ha^{-1} in depths > 30 cm (Fig. S1-2). The largest ΔSOC was achieved in 16 – 20 cm soil depth with 13.6 Mg ha^{-1}. The vertical distribution of SOC in agriculturally used soils can differ largely, depending on the applied tillage practice (Angers and Eriksen-Hamel, 2008). Where SOC accumulates in the soil surface in reduced- or no tillage systems, ploughing in conventional systems can lead to a shift of SOC in deeper soil regions (Piccoli et al., 2016). However, our results showed no significant differences in ΔSOC in shallow regions of conventional tillage soils compared to reduced tillage soils. Conventionally tilled soils showed a larger relative response, but again this difference was not significant. This result corresponds to findings of a meta-analysis of cover crop induced SOC effects, where also no significant differences between SOC stocks of conventional and reduced tillage soils could be identified (Poeplau and Don, 2015).

3.3 Climate effect

Fig. S1-1 presents the relative SOC stock change induced by climatic conditions. The lowest response ratio was observed in non-tropical climates, with an average SOC stock increase of 24%. SOC stock changes in tropical climates had a positive mean value of 30%. The highest positive response, with an average of 40%, was accounted for in sub-tropical climates. Tropical climate responses showed a large range and were not significantly different from the other climatic categories due to the low number of only 21 treatments. ΔSOC showed different dynamics. The highest difference of 12.8 Mg ha^{-1} was reached under non-tropical climatic conditions (Fig. S1-2). Subtropical and tropical conditions led to lower ΔSOC of 10.1 Mg ha^{-1} and 8.5 Mg ha^{-1}, respectively. The relative and absolute SOC changes confirms common paradigm. Generally, soils in cool and humid climates have a larger potential to store SOC than soils in dry and warm regions, due to lower decomposition rates and, therefore, higher carbon accumulation (Lal, 2004). Also Maillard and Angers (2014) showed that the absolute difference in SOC stocks after manure application is lower in tropical and warm regions than in cool regions (Maillard and Angers, 2014). The response ratio results can be explained by initial SOC content and stocks in tropical and sub-tropical soils, which are generally lower than in soils of cooler regions (Lal, 2004). SOC-poor soils have a larger potential to store additional SOC than SOC-rich soils and, therefore, have higher initial SOC accumulation rates (West and Six, 2007). This leads to a larger relative SOC increase in sub-tropical and tropical soils, compared to SOC-richer soils in non-tropical regions. Our results of the intercategorical grouping of the climate categories and initial SOC partly confirm this understanding (Appendix Fig. S1-3 and S1-4). Out of 263 treatments under subtropical conditions, which reported initial SOC values, 160 reported an initial SOC content < 1%. These treatments showed a large response ratio of 48% but low ΔSOC results of 10.3 Mg ha^{-1}. In turn, most of the treatments under non-tropical conditions which reported SOC, showed an initial SOC content > 2%. These treatments were characterized by a low mean response ratio of 23% but a high SOC stock difference of 28 Mg ha^{-1}. Due to low number of samples (n = 23), the error bar is wide and limits a conclusive statement. The analysis under tropical conditions was only possible for intermediate initial SOC contents due to the small number of samples.

3.4 Temporal effect

The relative SOC stock response connected with the durations of the experiments are shown in Fig. S1-1. Four different durations were analyzed. The relative mean stock increase of treatments with durations between 3 and 5 years was 34%. If experiments had durations between 6 and 10 years, SOC stocks increased by 27%. Between 11 and 20 years, mean response ratio was 36%. For durations higher than 20 years, SOC stocks changed by 40%. The highest stock difference was gained between 11 and 20 years and > 20 years, with a ΔSOC of 12.5 and 10.6 Mg ha^{-1} (Fig. S1-2). If the duration was between 3 and 5 years, stocks only changed by 9.1 Mg ha^{-1}, but showed a high relative gain. This is not surprising, as initial SOC accumulation rates are generally high if the area is feasible and management practices are of good choice (Minasny et al., 2017). As there is no large difference in the effect sizes (relatively and absolutely) between durations between 3 and 5 years and more than 20 years, SOC stocks do not change systematically with time, if applied manure amounts did not differ interannually. This finding indicates the potential of manure to store carbon in the long term. But, as SOC stocks do not increase with duration, carbon saturation is indicated. The timing of saturation not only depends on soil and input material properties, but also on the initial SOC content. Soils with high initial SOC content reach carbon saturation within a short period of time, whereas soils with low initial SOC need more time (Liu et al., 2014). West and Six (2007) showed that carbon saturation might occur over a period of 26 years under conventional rotation and 21 years under no till. Moreover, the saturation equilibrium seem to depend on the soil texture, sandy soils being more prone to C saturation (Angers et al., 2011). According to Wiesmeier et al. (2014), finer textured soils showed a depletion of SOC. Due to the variety of different factors influencing SOC saturation, we further analyzed the intercategorical effect of the initial SOC content, soil texture and tillage intensity on the temporal SOC storage dynamics.

Our findings regarding the influence of initial SOC on carbon saturation supports the common paradigm. Treatments with low initial SOC show large relative responses in all durations and no depletion over time in absolute terms (Appendix Fig. S1-5). Treatments with intermediate initial SOC showed lower relative responses and a depletion of ΔSOC between durations between 11 and 20 years and durations > 20 years (Appendix Fig. S1-6).

With regard to the tillage intensity, the experiment duration does not seem to play a major role. All response ratios show wide ranges and quite similar mean values but slight increases regarding long experimental duration > 20 years with 32% under reduced and 40% under conventional tillage (Appendix Fig. S1-7). The ΔSOC results also do not allow a meaningful conclusion (Appendix Fig. S1-8). Error bars are too wide and mean values are too similar. However, a slight depletion is indicated under conventional tillage at durations > 20 years. Here, the ΔSOC showed a lower response of 9.3 Mg ha^{-1}.

Texture-wise the situation is different. Mean response ratios were high in sandy soils with experimental duration between 11 and 20 years and > 20 years with 56% and 40% respectively (Appendix Fig. S1-9). However, with respect to SOC stocks, both analyzed durations did not differ largely and showed a low level (Appendix Fig. S1-10). Fine textured clay soils however, showed both high relative and absolute mean responses in large durations with 74% and 21.3 Mg ha^{-1} in durations between 11 and 20 years. Our results therefore seem to contradict the statement that clay soils show SOC depletions over time. But a more conclusive statement requires more long-term experiments with durations > 20 years.

3.5 Soil properties effect

The influence of initial SOC content on the relative effect size within our analysis is presented in Fig. S1-3. Treatments with low initial SOC content < 1% (46%) showed higher stock increases than treatments with initial SOC content between 1 and 2%, with stock increases of 25%. Treatments with initial SOC content > 2% resulted in a mean response of 37%. ΔSOC results were highest in treatments with initial SOC > 2% (21.5 Mg ha^{-1}) and were lower with decreasing initial SOC content with 12.4 Mg ha^{-1} in treatments with 1 – 2% initial SOC and 9.8 Mg ha^{-1} in treatments with initial SOC < 1% (Fig. S1-4). This finding is not unexpected as even small relative SOC stock changes in soils with high SOC content are leading to large absolute stock differences. In turn, large relative changes in soils with a low initial SOC are leading to a low absolute difference. Soils with an intermediate initial SOC showed an intermediate absolute stock difference. The relative effect of intermediate initial SOC content was the lowest of all responses but the difference was not significant compared to high initial SOC content.

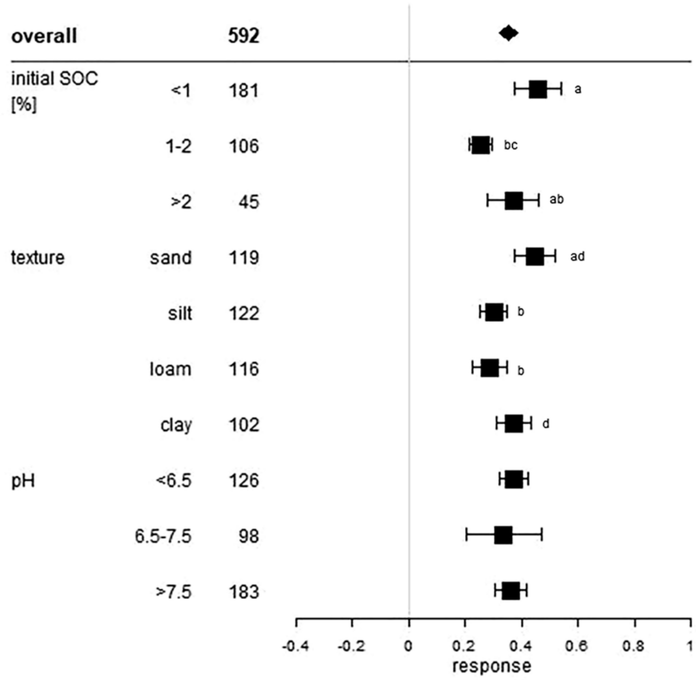

Figure S1-3: Relative response of manure applications on soil organic carbon stocks influenced by the initial soil organic carbon content (%), soil texture and soil pH value of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at response ratio = 0. Different letters in each subcategory indicate statistically significant differences.

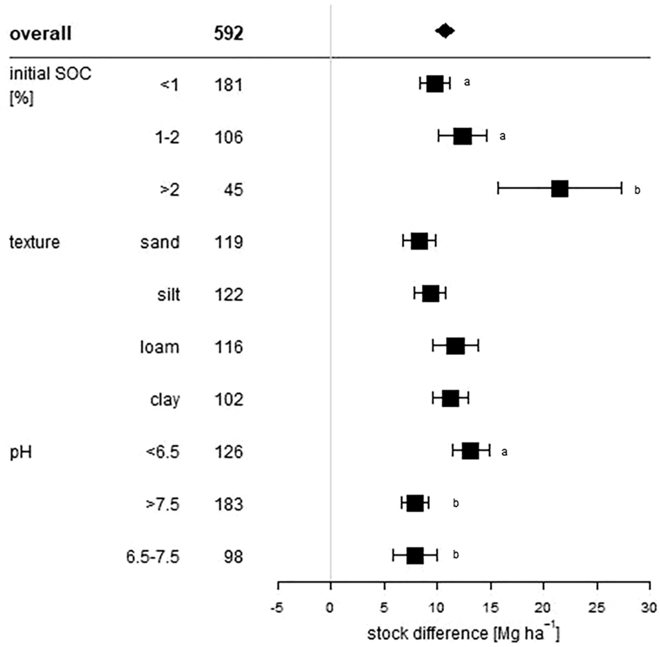

Figure S1-4: Absolute response (Mg ha^{-1}) of manure applications on soil organic carbon stocks influenced by the initial soil organic carbon content (%), the soil texture and the soil pH value of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at stock difference = 0 Mg ha^{-1}. Different letters in each subcategory indicate statistically significant differences.

All soil texture classes had positive significant responses on SOC stocks after manure was applied, but responses differed largely between the texture classes (Fig. S1-3). There are different processes related to soil C stabilization as a function of soil texture. These processes often depend on the soil clay content. Sorption processes of SOC on soil mineral surfaces and SOC incorporation within soil aggregates can both be enhanced by higher content of clay-sized particles, as clay-sized particles have a higher reactive surface area than coarser particles (Dungait et al., 2018). This supports our findings that texture classes with higher clay content showed significantly increased ΔSOC in loam and clay soils with mean differences of 11.7 and 11.3 Mg ha^{-1} (Fig. S1-4). Furthermore, clay soils also showed high relative SOC increases (37%), suggesting that soils with small particle sizes are best suited for SOC storage. Due to the lower specific surface area, sandy soils tend to have higher leaching losses of dissolved organic carbon than finer soil material and are usually more aerated, which favors SOC decomposition. Both processes underpin our finding concerning manure application on sandy soils. Results showed a high relative increase of 45% but the lowest of all analyzed absolute SOC responses with 8.2 Mg ha^{-1}. The high relative increase seems to be related to low initial SOC values, which in our evaluation often occurred in sandy soils (n = 51) (Appendix Fig. S1-11 and S1-12). Out of all initial SOC value classes, only the < 1% class could be evaluated because higher initial values occurred too rarely (n < 10).

Soils with pH < 6.5 showed a mean response of 37% and a mean SOC increase of 13.1 Mg ha^{-1}. Neutral soils (6.5 – 7.5) had a mean SOC increase of 7.9 Mg ha^{-1} corresponding to 25%, while alkaline soils (> 7.5) showed a SOC increase of 7.9 Mg ha^{-1} corresponding to 36% (Fig. S1-3, Fig. S1-4). The supply of protons to soils, from atmospheric or organic sources, influences several biological and chemical processes e.g. soil microbial activity, which affects decomposition of organic matter and carbon sequestration (Paul, 2015). Generally, increasing soil pH stimulates microbial activity and decomposition rates of fresh organic matter and, therefore, favors SOC mineralization (Andersson and Nilsson, 2001). However, it is still unknown whether a higher net primary production as a consequence of raising soil pH (e.g. through lime application) and, therefore, higher plant residue and root biomass inputs could possibly offset higher soil respiration and promote carbon sequestration in the long term (Holland et al., 2018; Paradelo et al., 2015). Furthermore, a higher amount of Ca^{2+} ions could favor formation of mineral-organic complexes in soils with higher pH. Decreasing pH values, in turn, can reduce decomposition rates of SOC (Motavalli et al., 1995). Therefore, acidity could possibly promote SOC accumulation which our results confirmed as manure application showed the highest absolute and relative SOC stock responses in acid soils.

3.6 Fertilizer properties and amount effect

In total, 16 different manure types from different origins (including farmyard manure, i.e. various excretions originating from agricultural activity) were categorized of which seven were a combination of single manure types, which occurred in a low number and therefore were difficult to evaluate individually. The application of each manure type had a significantly positive response on SOC stocks (Fig. S1-5, Fig. S1-6). The lowest effects came from the application of green manure, straw, and combined applications of both with 17% and 5.1 Mg ha^{-1}, 23% and 6.4 Mg ha^{-1} and 11% corresponding to 4.5 Mg ha^{-1} respectively. In contrast, pig manure, cattle manure and farmyard manure led to the highest responses with 50% and 15.8 Mg ha^{-1}, 32% and 15 Mg ha^{-1} and 41% corresponding to 9.7 Mg ha^{-1}, respectively. Other livestock excretions, namely poultry manure, sheep manure and horse manure responded with 39% and 8.9 Mg ha^{-1}, 35% and 7 Mg ha^{-1} and 23% corresponding to 8.3 Mg ha^{-1}, respectively, and thus also showed good SOC storage performances. Maillard and Angers (2014) found a comparable result with cattle manure, inducing high SOC stock differences, but they only considered three livestock species (cattle, pig, poultry) and they included only a small number of treatments, which led to a high variability. Liu et al. (2014) found an SOC response ratio of 12.8% after straw application on paddy and upland soils. This result corroborates our findings. Out of all manure types, pig manure showed the highest C accumulation potential. However, all manure types showed positive responses, especially those of livestock.

The effect of SOC stock changes influenced by additional added mineral fertilizer are presented in Fig. S1-5 and S1-6. If mineral fertilizer was added, the SOC stocks increased significantly by 40% and absolutely by 11.9 Mg ha^{-1}. Treatments with no additional mineral fertilizer raised SOC stocks by 30%. Here, the absolute difference was slightly lower, with 10.2 Mg ha^{-1} (Fig. S1-6). As already explained in section 3.1, additional mineral fertilizer input provides a delivery of nutrients. Plant growth is promoted, aboveground and belowground. This enhanced net primary production with higher biomass inputs explains higher SOC stocks, as rising biomass yields generally correlate with rising SOC values. Although aboveground biomass is removed after harvest, increased root growth and higher crop residue amounts have a positive effect on SOC content, compared to unfertilized treatments, especially if manure and mineral fertilizer application is combined (Li et al., 2010; Manna et al., 2007; Yu et al., 2012). Further, also the relative SOC gain was higher if additional mineral fertilizer was used. Initial SOC values could be the explanation. The intercategorical evaluation of NPK and initial SOC values identified the most NPK treatments with low initial SOC values and high response ratios of 54% (Appendix Fig. S1-13). Treatments with low initial SOC were also the majority in the non-NPK grouping, but many treatments with high SOC levels were also found. Here, response ratios only hardly differed from each other. ΔSOC showed the same dynamics for both NPK and non-NPK treatments with higher stock differences in soils (Appendix Fig. S1-14).

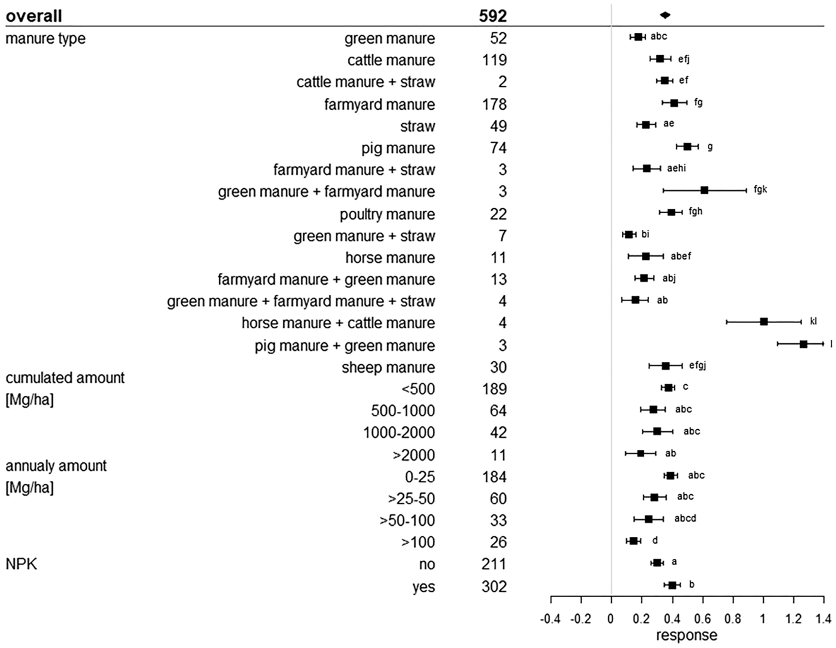

Figure S1-5: Relative response of manure applications on SOC stocks influenced by manure type, annual manure amount (Mg ha^{-1}), the accumulated manure amount (Mg ha^{-1}), and additionally added chemical fertilizer (NPK) of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at response ratio = 0. Different letters in each subcategory indicate statistically significant differences.

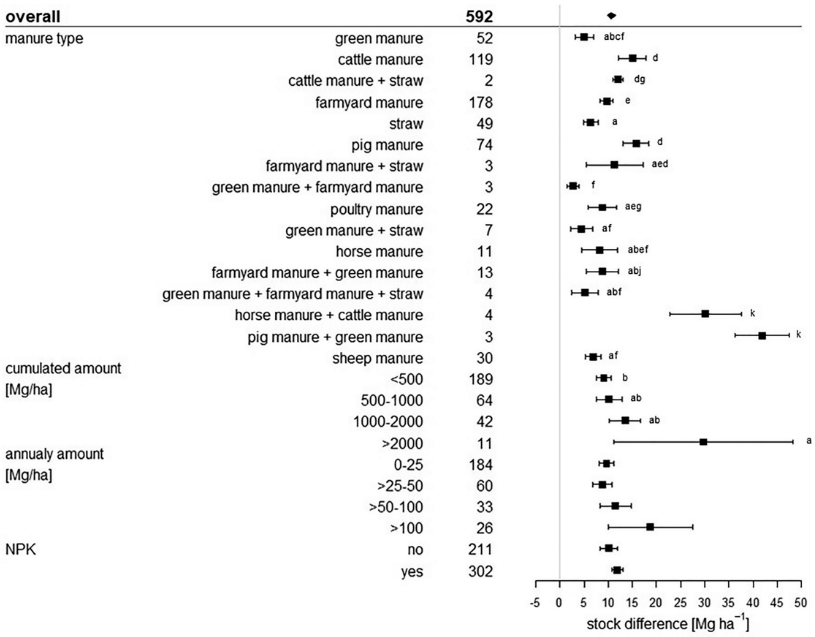

Figure S1-6: Absolute response (Mg ha^{-1}) of manure applications on soil organic carbon stocks influenced by the manure type, the annual manure amount (Mg ha^{-1}) the accumulated manure amount (Mg ha^{-1}) and additional added chemical fertilizer (NPK) of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response is presented as the range between the upper and lower 95% confidence interval. Points within the range represent the mean response. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at stock difference = 0 Mg ha^{-1}. Different letters in each subcategory indicate statistically significant differences.

SOC stock responses induced by different amounts of manure application are presented in Fig. S1-5 and S1-6. Cumulative and annual manure amounts, each in four different quantities were analyzed. Amounts <500 Mg ha^{-1}, the lowest cumulative quantity, showed the highest relative SOC stock response, which was 38%, but had a low absolute SOC gain, with 9.2 Mg ha^{-1} (Fig. S1-5 and S1-6). The amounts ranging between 500 and 1000 Mg ha^{-1} showed a response ratio of 27% and a stock difference of 10.1 Mg ha^{-1}. Between 1000 – 2000 Mg ha^{-1} the response was 30% relatively and 13.5 Mg ha^{-1} absolutely. The last and highest amount range classified was >2000 Mg ha^{-1} with a response ratio of 19% and a 29.7 Mg ha^{-1} SOC stock change, which is the highest absolute value. Annual amount results showed a similar dynamic. Low annual manure amounts of 0 – 25 Mg ha^{-1} a^{-1} resulted in a low ΔSOC of 9.6 Mg ha^{-1} but a high relative change of 39%. High annual amounts >100 Mg ha^{-1} a^{-1}, however showed a high ΔSOC of 18.8 Mg ha^{-1} but a lower response ratio of 14%. The relative change in SOC stocks does not increase with higher annual input amounts. Rather, the response ratio reached the highest relative change at the lowest annual and cumulative input amount. However, our results indicate that high input amounts seem to be connected with high SOC stock differences. A regression analysis of the connection between the input amount and ΔSOC indicated a significant linear, but weak relationship for both annual ($p = 4.1e^{-11}$; $R^2 = 0.13$) (Fig. S1-7a) and cumulative quantities ($p = 1.6e\text{-}07$; $R^2 = 0.087$) (Fig. 7b). Maillard and Angers (2014) also found a linear relationship between cumulative carbon input and SOC stock difference up to very high levels of carbon inputs which support our finding. To further evaluate this relationship, we analyzed the link between ΔSOC and annual manure inputs as a function of the subcategories we investigated. The regression plots are located in the Appendix Fig. S1-15a-t. A Shapiro-Wilk test, which was carried out in advance, showed a non-normal distribution of the data. Regarding tillage intensity effects, no significant relationship could be found for reduced tillage treatments (Supplementary Material, Fig. S1-15a), whereas conventional tillage treatments showed a linear relationship between annual amounts and ΔSOC ($p < 2.2e^{-16}$; $R^2 = 0.5$) (Appendix Fig. S1-15b). Differences between soil texture groups could not be identified. All texture classes showed no significant relationships (Appendix Fig. S1-15c - f). In the climate subcategories (Appendix Fig. S1-15g – h), a significant relationship was only identified under non-tropical conditions ($R^2 = 0.24$; $p = 0.0019$) (Appendix Fig. 15g). Applications under tropical conditions were not included in the regression analysis due to the low number of treatments. Considering the various sampling depths (Appendix Fig. S1-15i) only depths between 16 and 20 cm showed significance ($R^2 = 0.34$; $p =3.1e^{-13}$) (Appendix Fig. S1-15j). Regarding soil pH conditions (Appendix Fig. S1-15m – o), a significant linear increase was identified in pH neutral soils but ($R^2 = 0.2$; $p = 9.9e^{-5}$) (Appendix Fig. S1-15o), whereas acidic soils showed a significant linear decrease ($R^2 = 0.1$; $p = 0.044$) (Appendix Fig. S1-15m). Low initial SOC < 1% ($R^2 = 0.037$; $p = 0.049$) (Appendix Fig. S1-15p) and high initial SOC > 2% ($R^2 = 0.57$; $p = 1.6e^{-6}$) (Appendix Fig. S1-15r) showed significant positive relationships. The application of additional mineral fertilizers led to an insignificant relation

(Appendix Fig. S1-15s) whereas non-NPK treatments showed a significant positive link (Supplementary Dataset Fig. S1-15t) (R^2 = 0.41; p = 7.8e^{-15}). To summarize, the annual amount of application seems to be important only under conventional tillage, non-tropical climate conditions and pH-neutral as well as SOC-rich or SOC-depleted soils and only if no additional mineral fertilization is applied. Under other conditions, there seems to be no statistically significant relation between ΔSOC and annual manure amounts.

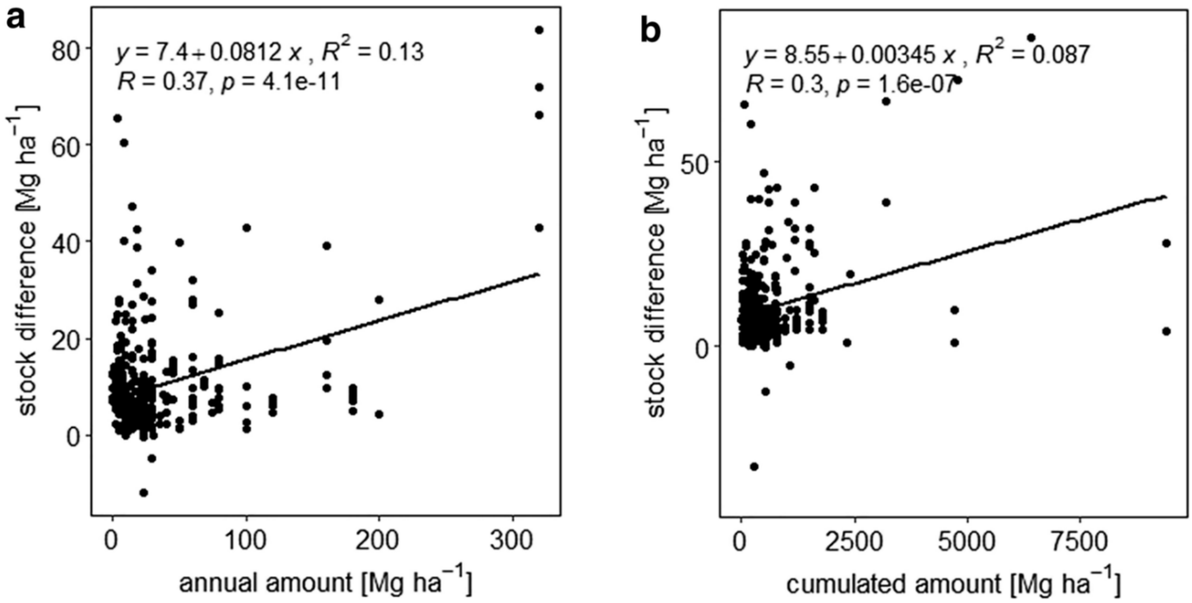

Figure S1-7: Relationship between the SOC stock difference (Mg ha^{-1}) and cumulative manure-C input (Mg ha^{-1}) (**a**) and the SOC stock difference (Mg ha^{-1}) and annual manure input (Mg ha^{-1}) (**b**). R^2 represents the coefficient of determination.

4. Conclusions

Globally, manure applications induced a raise of SOC stocks. However, our results indicate that the increase effect is linked to many factors and can show large differences. These factors included management decisions (tillage intensity, manure amount, duration of application), site properties (climate, initial SOC content, soil texture) and manure characteristics (manure origin and the combined application with synthetic fertilizer). To better understand carbon dynamics, more long-term SOC field data are required, especially the factors influencing carbon saturation need to be further investigated. Moreover, many measurements under tropical conditions need to be conducted because the small number of treatments found made it impossible to draw definitive conclusions. Additional to that, more holistic approaches within carbon dynamics assessment methods need to be established. For example, although, conventional tillage and synthetic fertilization have high effects in terms of SOC enrichment, positive aspects through reduced tillage and external effects (e.g. through the production of synthetic fertilizers) should play a role in the development of sustainable management strategies. Expanding the scope will help to avoid misleading conclusions.

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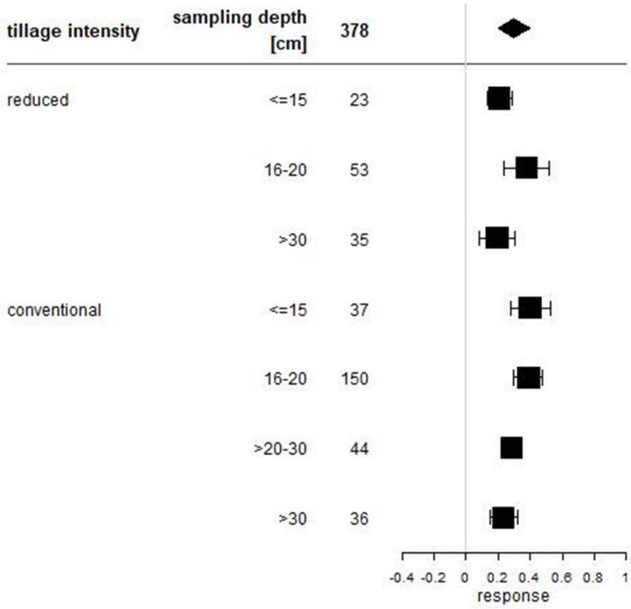
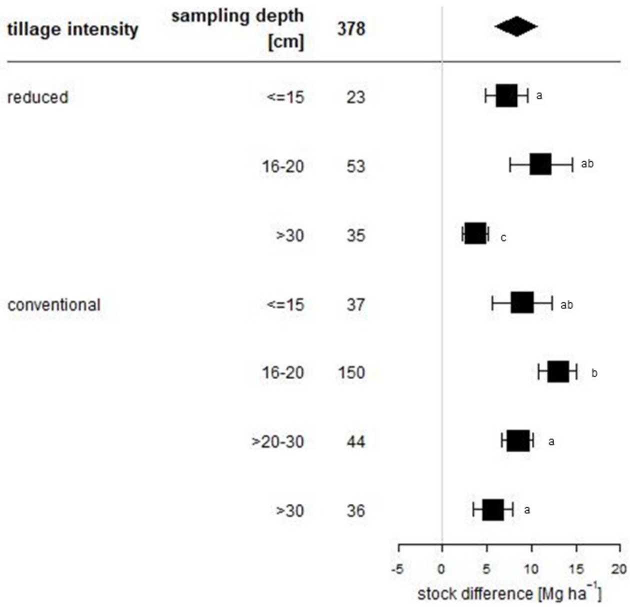
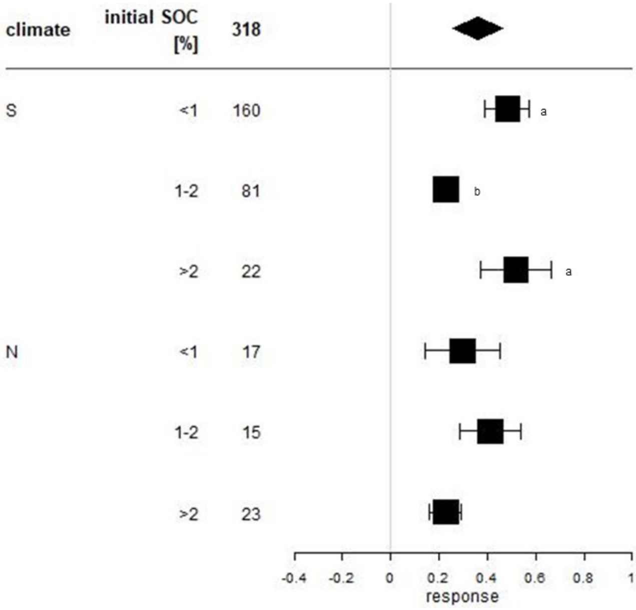
Appendix



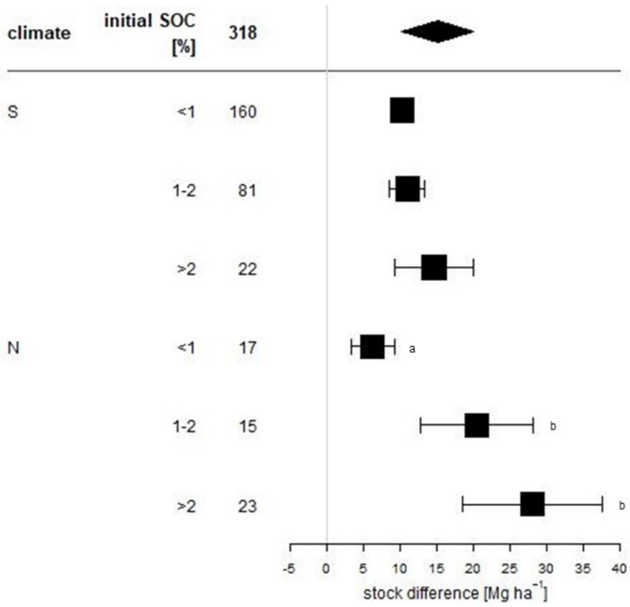
Appendix Figure S1-1: Relative response of manure applications on soil organic carbon stocks influenced by the tillage intensity in different sampling depths of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The greyline was drawn at response ratio = 0. Different letters in each subcategory indicate statistically significant differences.



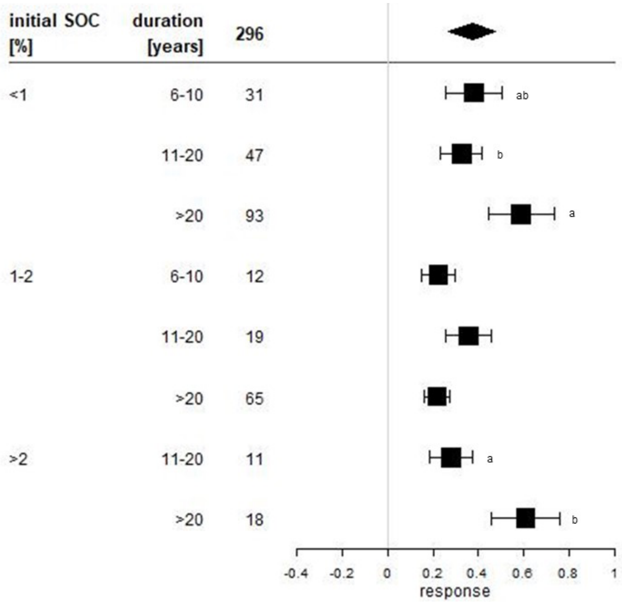
Appendix Figure S1-2: Absolute response (Mg ha^{-1}) of manure applications on soil organic carbon stocks influenced by the tillage intensity in different sampling depths of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at stock difference = 0 Mg ha^{-1}. Different letters in each subcategory indicate statistically significant differences.



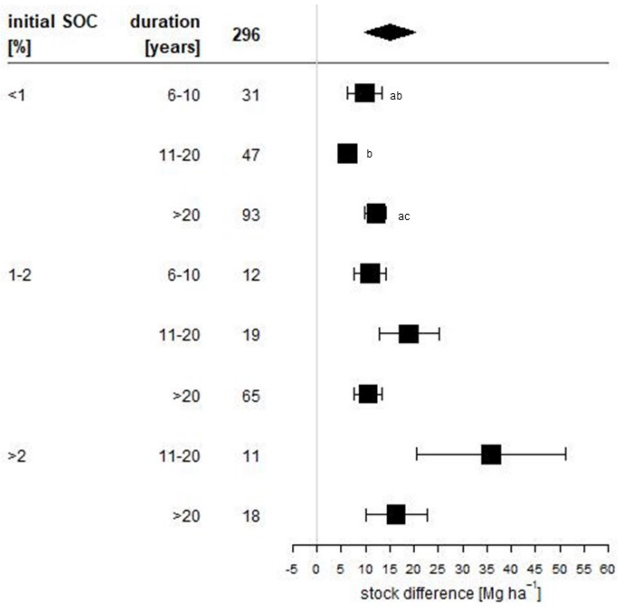
Appendix Figure S1-3: Relative response of manure applications on soil organic carbon stocks influenced by the combined effect of the climate and different initial soil organic carbon contents (%) of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at response ratio = 0. Different letters in each subcategory indicate statistically significant differences.



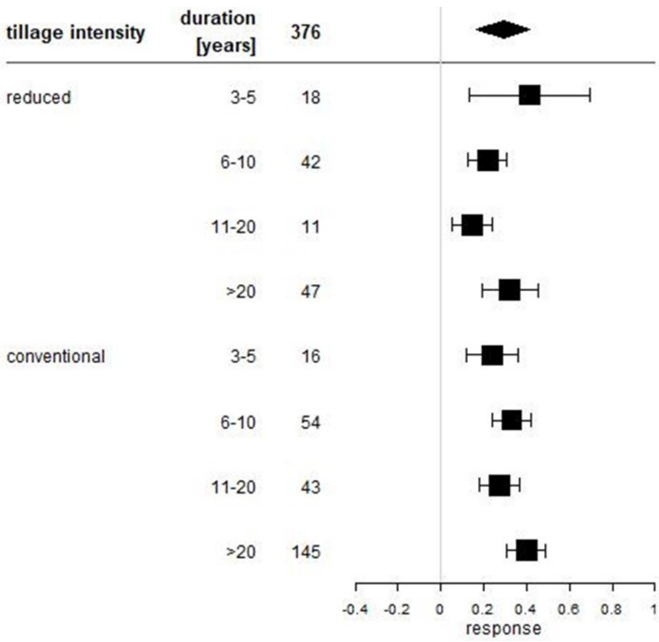
Appendix Figure S1-4: Absolute response (Mg ha^{-1}) of manure applications on soil organic carbon stocks influenced by the combined effect of climate and different initial soil organic carbon contents (%) of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at stock difference = 0 Mg ha^{-1}. Different letters in each subcategory indicate statistically significant differences.



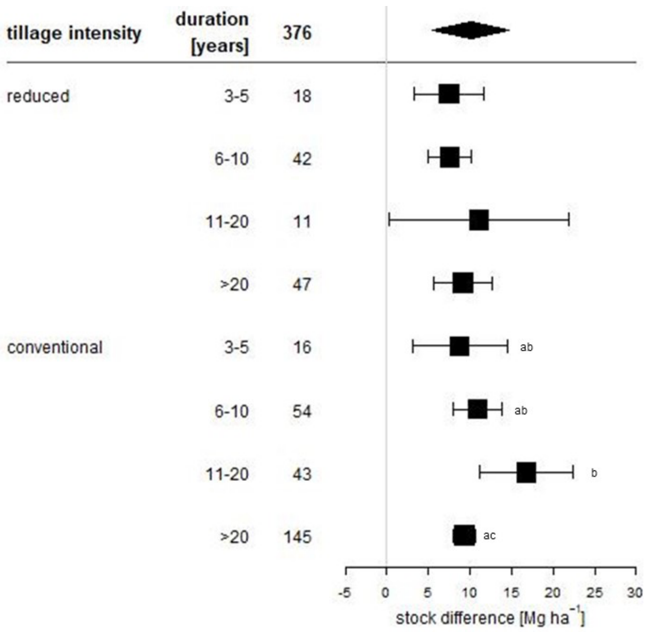
Appendix Figure S1-5: Relative response of manure applications on soil organic carbon stocks influenced by the combined effect of the initial soil organic carbon content (%) and different experiment durations of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at response ratio = 0. Different letters in each subcategory indicate statistically significant differences.



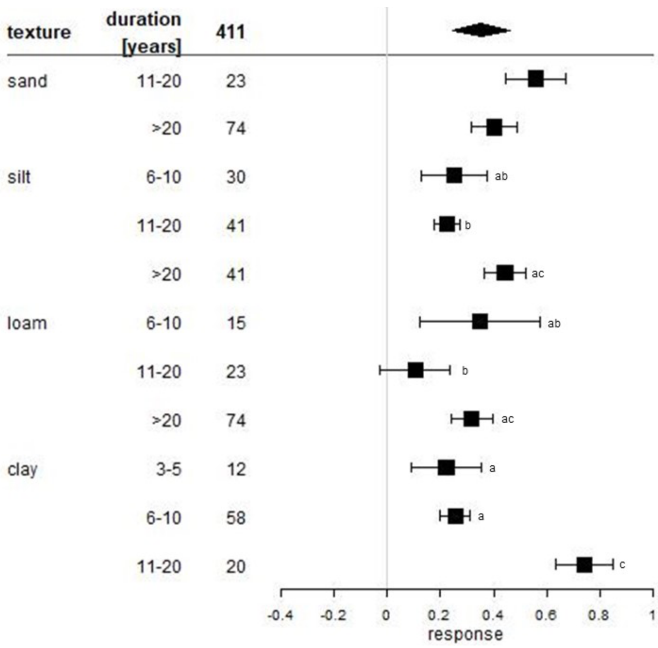
Appendix Figure S1-6: Absolute response (Mg ha^{-1}) of manure applications on soil organic carbon stocks influenced by the combined effect of the initial soil organic carbon content (%) and different experiment durations of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at stock difference = 0 Mg ha^{-1}. Different letters in each subcategory indicate statistically significant differences.



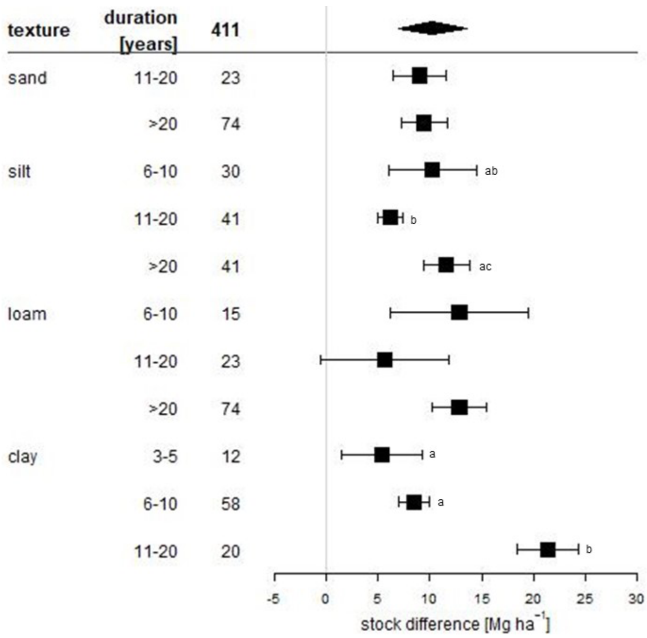
Appendix Figure S1-7: Relative response of manure applications on soil organic carbon stocks influenced by the combined effect of the tillage intensity and the experiment duration of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at response ratio = 0. Different letters in each subcategory indicate statistically significant differences.



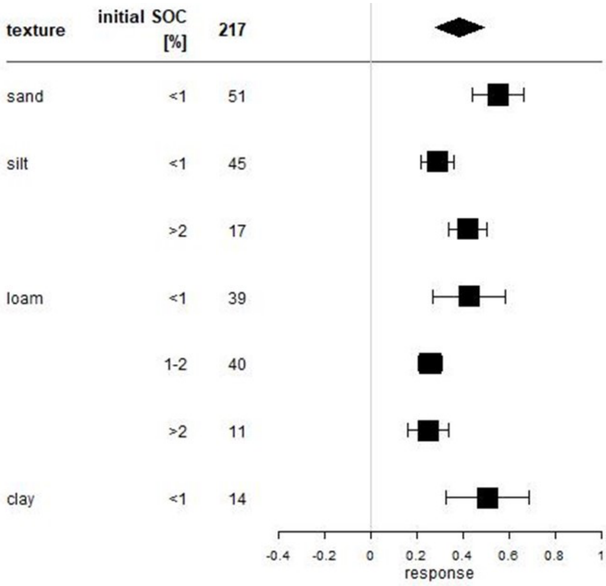
Appendix Figure S1- 8: Absolute response (Mg ha^{-1}) of manure applications on soil organic carbon stocks influenced by the combined effect of the tillage intensity and the experiment duration of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at stock difference = 0 Mg ha^{-1}. Different letters in each subcategory indicate statistically significant differences.



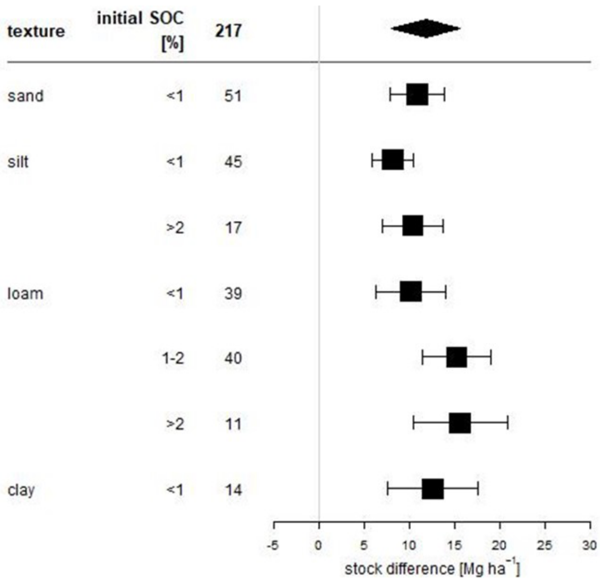
Appendix Figure S1-9: Relative response of manure applications on soil organic carbon stocks influenced by the combined effect of different soil textures and the experiment duration of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at response ratio = 0. Different letters in each subcategory indicate statistically significant differences.



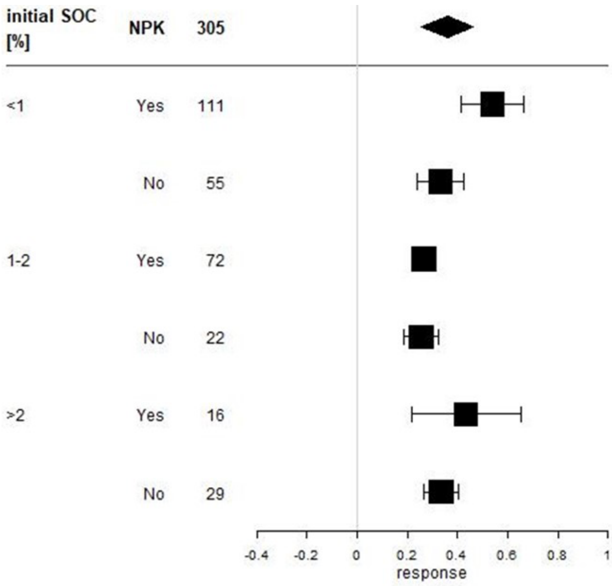
Appendix Figure S1-10: Absolute response (Mg ha^{-1}) of manure applications on soil organic carbon stocks influenced by the combined effect of different soil textures and the experiment duration of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at stock difference = 0 Mg ha^{-1}. Different letters in each subcategory indicate statistically significant differences.



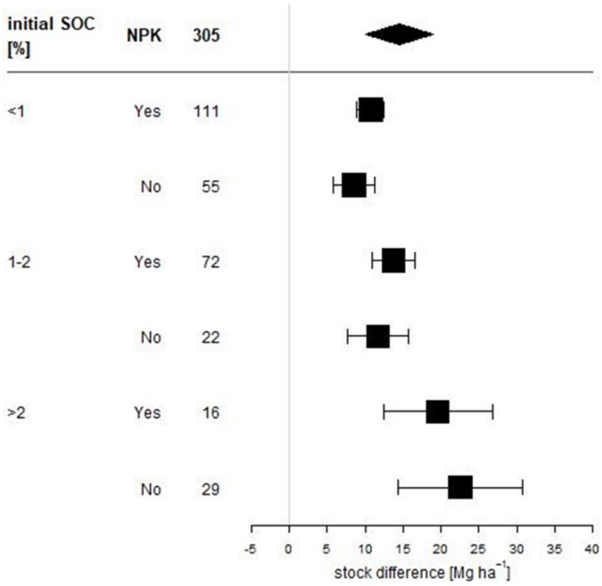
Appendix Figure S1-11: Relative response of manure applications on soil organic carbon stocks influenced by the combined effect of different soil textures and the initial soil organic carbon content (%) of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at response ratio = 0. Different letters in each subcategory indicate statistically significant differences.



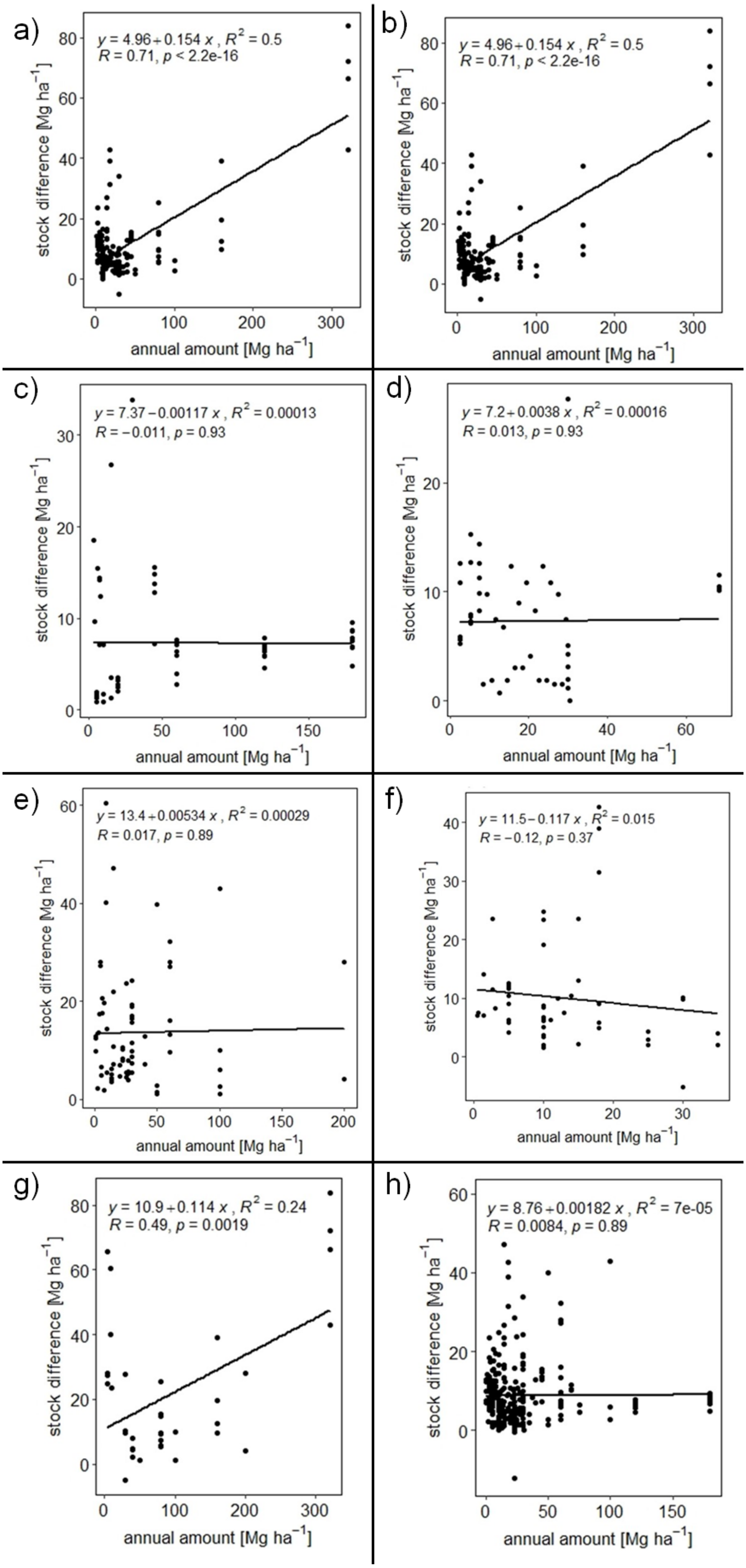
Appendix Figure S1-12: Absolute response (Mg ha^{-1}) of manure applications on soil organic carbon stocks influenced by the combined effect of different soil textures and the initial soil organic carbon content (%) of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at stock difference = 0 Mg ha^{-1}. Different letters in each subcategory indicate statistically significant differences.

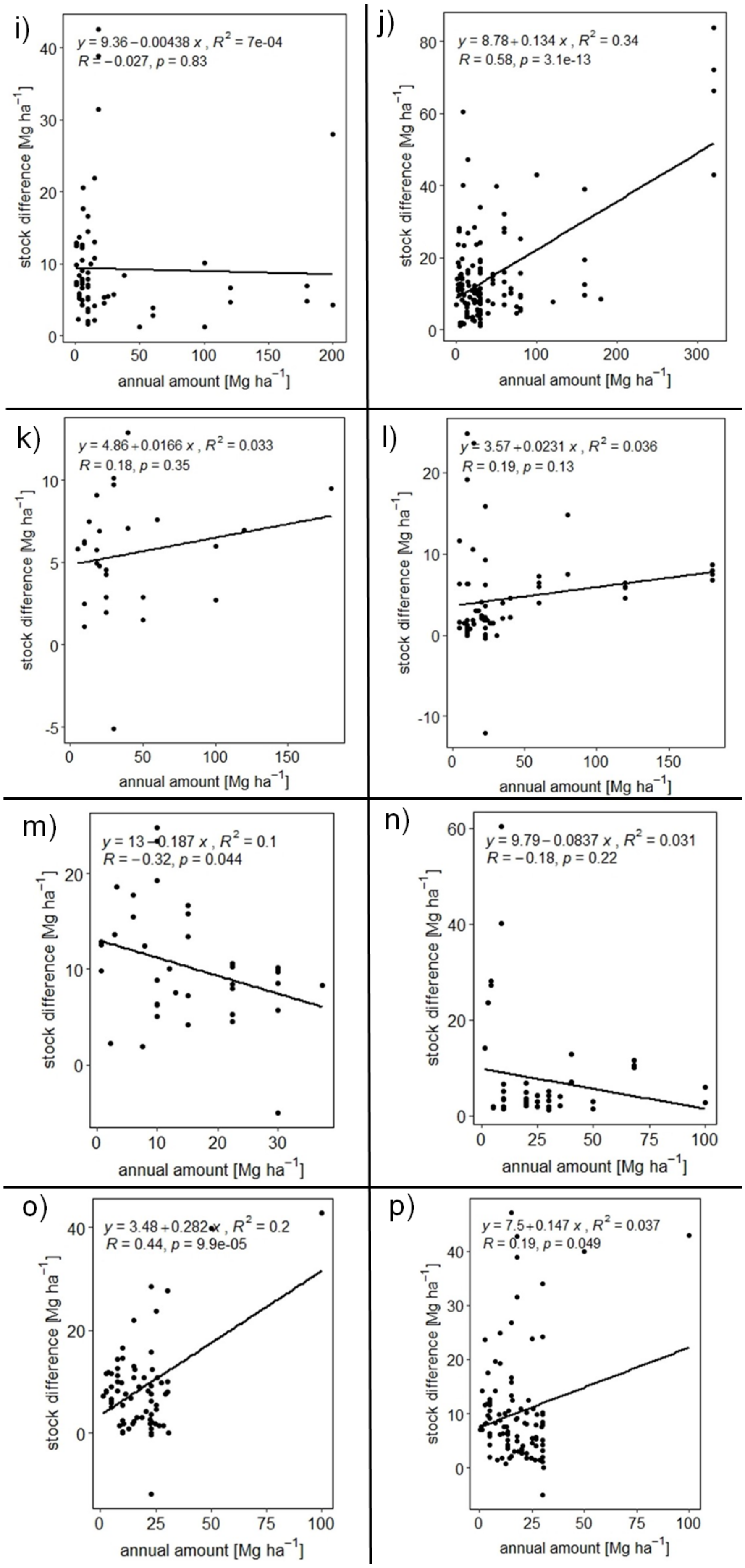


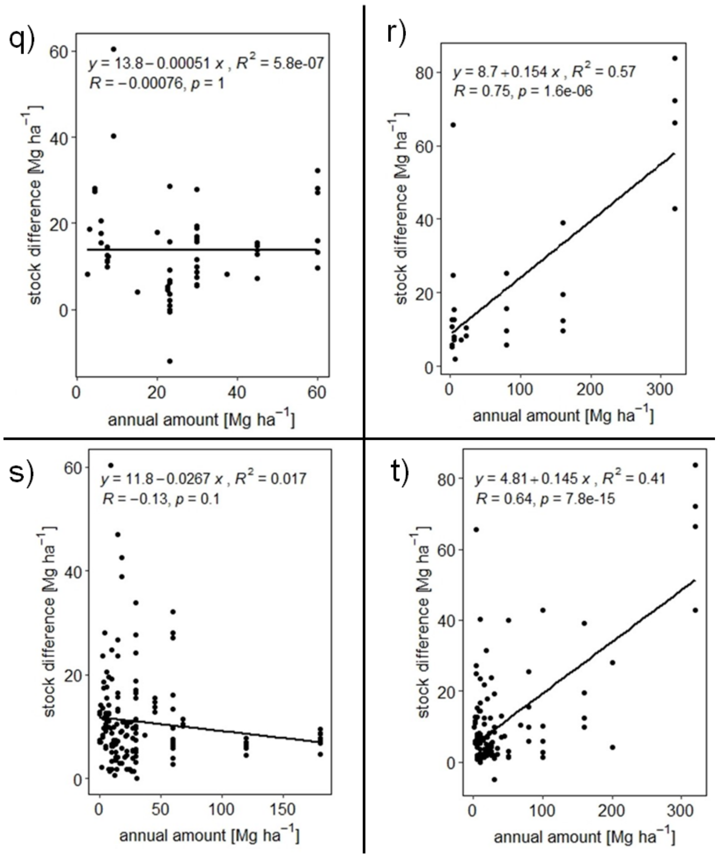
Appendix Figure S1-13: Relative response of manure applications on soil organic carbon stocks influenced by the combined effect of the initial soil organic carbon content (%) and additional added chemical fertilizer (NPK) of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at response ratio = 0. Different letters in each subcategory indicate statistically significant differences.



Appendix Figure S1-14: Absolute response (Mg ha^{-1}) of manure applications on soil organic carbon stocks influenced by the combined effect of the initial soil organic carbon content (%) and additional added chemical fertilizer (NPK) of the considered treatments. The overall grand mean of all individual treatments is presented in the first row followed by the considered subcategories below. Each response ratio is presented as the range between the upper and lower 95% confidence intervals. Points within the range represent the mean response ratio. The range between both 95% confidence intervals of the grand mean is shown by the extent of the rectangle. The number in each treatment row represents the number of pairwise comparisons on which the statistic is based. The grey line was drawn at stock difference = 0 Mg ha^{-1}. Different letters in each subcategory indicate statistically significant differences.


a)
y = 4.96 + 0.154 x , R² = 0.5
R = 0.71, p < 2.2e-16
stock difference [Mg ha⁻¹]
annual amount [Mg ha⁻¹]
b)
y = 4.96 + 0.154 x , R² = 0.5
R = 0.71, p < 2.2e-16
stock difference [Mg ha⁻¹]
annual amount [Mg ha⁻¹]
c)
y = 7.37 − 0.00117 x , R² = 0.00013
R = −0.011, p = 0.93
stock difference [Mg ha⁻¹]
annual amount [Mg ha⁻¹]
d)
y = 7.2 + 0.0038 x , R² = 0.00016
R = 0.013, p = 0.93
stock difference [Mg ha⁻¹]
annual amount [Mg ha⁻¹]
e)
y = 13.4 + 0.00534 x , R² = 0.00029
R = 0.017, p = 0.89
stock difference [Mg ha⁻¹]
annual amount [Mg ha⁻¹]
f)
y = 11.5 − 0.117 x , R² = 0.015
R = −0.12, p = 0.37
stock difference [Mg ha⁻¹]
annual amount [Mg ha⁻¹]
g)
y = 10.9 + 0.114 x , R² = 0.24
R = 0.49, p = 0.0019
stock difference [Mg ha⁻¹]
annual amount [Mg ha⁻¹]
h)
y = 8.76 + 0.00182 x , R² = 7e-05
R = 0.0084, p = 0.89
stock difference [Mg ha⁻¹]
annual amount [Mg ha⁻¹]


i)
y = 9.36 − 0.00438 x , R² = 7e-04
R = −0.027, p = 0.83
j)
y = 8.78 + 0.134 x , R² = 0.34
R = 0.58, p = 3.1e-13
k)
y = 4.86 + 0.0166 x , R² = 0.033
R = 0.18, p = 0.35
l)
y = 3.57 + 0.0231 x , R² = 0.036
R = 0.19, p = 0.13
m)
y = 13 − 0.187 x , R² = 0.1
R = −0.32, p = 0.044
n)
y = 9.79 − 0.0837 x , R² = 0.031
R = −0.18, p = 0.22
o)
y = 3.48 + 0.282 x , R² = 0.2
R = 0.44, p = 9.9e-05
p)
y = 7.5 + 0.147 x , R² = 0.037
R = 0.19, p = 0.049
stock difference [Mg ha⁻¹]
annual amount [Mg ha⁻¹]



Appendix Figure S1-15: Relationship between SOC stock difference (Mg ha^{-1}) and the annual manure input (Mg ha^{-1}) under reduced tillage (a) and conventional tillage (b), the annual manure input in clay soils (c), silt soils (d), loam soils (e) and sand soils (f), the annual manure input under non-tropical (g) and sub-tropical (h) climate conditions, the annual manure input in sampling depths <=15 cm (i), 16-20 cm (j), 21-30 cm (k) and >30 cm (l), the annual manure input in acidic soils (m), alkaline (n) and pH neutral soils (o), the annual manure input in soils with low initial SOC (p), intermediate initial SOC (q) and high initial SOC (r) and the annual manure input in treatments with additional added chemical fertilizer (NPK) (s) and without additional NPK fertilizer (t). R^2 represents the coefficient of determination.

Supplementary datasets

Three supplementary datasets can be found online via:

https://www.nature.com/articles/s41598-021-82739-7#Sec13

The datasets contain of the following:

The results of all 592 treatments from 101 studies are located in Dataset 1.

The results of all subcategories, including their standard deviation can be found in Dataset 2.

The results of the intercategorical grouping is located in Dataset 3.

Study 2: Soil organic carbon sequestration after biochar application: A global meta-analysis

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Abstract

Biochar application to soil has high potential to sequester carbon in the long term because of high stability and large scale production potential. However, biochar technologies are still relatively new, and the global factors affecting long-term fate of biochar in the environment are still poorly understood. To fill this important research gap, a global meta-analysis was conducted including 64 studies with 736 individual treatments. Field experiments covered experimental durations between 1–10 years with biochar application amounts between 1-100 Mg ha^{-1}. They showed a mean increase in soil organic carbon (SOC) stocks by 13.0 Mg ha^{-1} on average, corresponding to 29%. Pot and incubation experiments ranged between 1–1278 days and biochar amounts between 5 g kg^{-1} and 200 g kg^{-1}. They raised SOC by 6.3 g kg^{-1} on average, corresponding to 75%. More SOC was accumulated in long experimental durations of >500 days in pot and incubation experiments and 6-10 years in field experiments than in shorter experimental durations. Organic fertilizer co-applications significantly further increased SOC. Biochar from plant material showed higher carbon (C) sequestration potential than biochar from fecal matter, due to higher carbon-to-nitrogen (C/N) ratio. SOC increases after biochar application were higher in medium to fine grain textured soils than in soils with coarse grain sizes. Our study clearly demonstrated the high C sequestration potential of biochar application to agricultural soils of varying site and soil characteristics.

Keywords Organic soil amendments; Climate change mitigation; C sequestration; Charcoal; Pyrogenic C

1. Introduction

Many international governmental efforts aim to reduce large amounts of greenhouse gases (GHG), to stay in line with the “Paris Agreement” by mitigating the global mean air temperature below 2 °C compared to the pre-industrial level, with efforts to even reach the 1.5 °C mark.

Being part of the “Paris agreement”, the European Union (EU) is pursuing carbon dioxide (CO_2) neutrality by 2050 within their “Green Deal”. To achieve this ambitious goal, the EU Commission proposed a net reduction in emission of GHG of 55% from 1990 emission levels as a target milestone by 2030. To reach this challenging objective, efficient CO_2 removal (CDR) technologies are needed.

Compared to suggested technical solutions such as carbon capture and storage (CCS), natural soils are an important carbon sink, as they contain more carbon than stored in terrestrial vegetation and the atmosphere combined (Lehmann and Kleber, 2015). Different studies demonstrated the potential to store even more carbon in soils, by applying certain management practices, such as afforestation, conservational tillage practices, or the use of soil amendments, with the latter showing high potentials (Glaser and Birk, 2012; Minasny et al., 2017).

This process of storing organic carbon in soils (SOC), better known as SOC sequestration, describes how organic carbon is incorporated into soils and converted into a long living C pool, that would otherwise be emitted as CO_2 (Lal, 2008). SOC sequestration cannot only be considered a CDR, but also enhances the soil quality and thus improves ecosystem functions and services, food security and resilience to climate change (Lal et al., 2015, 2007; Rumpel et al., 2020). Increases of SOC stocks can be achieved using different strategies such as reduced tillage (Bernacchi et al., 2005), organic farming (Gattinger et al., 2012; Leifeld and Fuhrer, 2010), agroforestry (De Stefano and Jacobson, 2017), and soil amendments such as straw (Berhane et al., 2020; Liu et al., 2014) sewage sludge (Pitombo et al., 2015; Soriano-Disla et al., 2010) or manure (Gross and Glaser, 2021; Han et al., 2016; Maillard and Angers, 2014). However, the strategies differ greatly in the amount of carbon stored and the long-term nature (Donigian et al., 1994; Gattinger et al., 2012; Gross and Glaser, 2021; Kopittke et al., 2017), with agroforestry showing the greatest potential (Feliciano et al., 2018; Shi et al., 2018). Soil amendments need to be applied regularly (e.g. at the beginning of a growing season), to store C in the long-term (Donigian et al., 1994; Gross and Glaser, 2021).

Another option to sequester SOC is the use of carbon-rich soil amendments with long mean residence times and low decay rates. Especially biochar application has a high SOC sequestration potential in the long term because of high stability (Wang et al., 2016), and large scale production potential (Azzi et al., 2021; Coomes and Miltner, 2017; Duku et al., 2011; Koçer et al., 2020), which is only limited by available biomass. In addition, biochar has the potential to increase nutrient availability (Prendergast-Miller et al., 2014), crop yields (Glaser et al., 2015; Jeffery et al., 2017; Rogovska et al., 2014; Vaccari et al., 2011), soil water availability (Rogovska et al., 2014), microbial biomass (Liu et al., 2016), and soil microbial diversity (Xu et al., 2014). By increasing biomass yields, biochar could substitute mineral fertilizers and thus reduce the carbon footprint of crops by avoiding energy intensive fertilizer production processes (Glaser et al., 2015).

In sum, biochar as a soil amendment provides solutions to the most recent threats to soil health and the mitigation of climate change. However, as biochar technologies are relatively new, there is a lack of data regarding short and especially long-term stabilization of SOC stocks and their fate after biochar amendment. Many individual studies aimed to fill these research gaps, but, to our best knowledge, there is no study available which analyzed global explanatory factors influencing the SOC dynamics after biochar amendments.

To fill this important research gap, a global meta-analysis was conducted. The first objective of this study was to collect available data from peer-reviewed studies using ISI Web of Science as reliable database. The second objective was to analyze this data collection for explanatory factors, which may affect SOC differences after biochar soil amendments. That is why we further analyzed the relationship between SOC sequestration and experiment characteristics (field, laboratory, or greenhouse study, single vs. continuous application, duration, applied amounts, sampling depth), site characteristics (climate zone, tillage intensity, crop type, additional fertilization), soil properties (texture, soil pH class, initial SOC content), biochar characteristics (feedstock, C content, C/N ratio, specific surface area (SSA), cation exchange capacity (CEC)), and interactions between explanatory factors. The third objective was to identify similarities and differences between studies, which were performed on real field scale and studies conducted in the greenhouse or laboratory, as a comprehensive understanding of both settings can lead to an even better understanding of the whole research question (Calisi and Bentley, 2009).

2. Material and Methods

2.1 Data sources, collection, and categorization

To quantify the response of SOC stocks following biochar applications, a meta-analysis was conducted. The systematic literature review was performed using "ISI Web of Science" by using the search term "Soil organic carbon OR Carbon Sequestration AND Biochar". Studies were included, if the effect and control size was expressed as content of total organic carbon (TOC) or quantified as SOC or TOC stocks. In total, 64 studies were considered usable within our approach. In five studies soil organic matter (SOM) rather than SOC or TOC information was given (Jin et al., 2020; Khan et al., 2020; Lebrun et al., 2021; Mohan et al., 2018; Tan et al., 2021). We calculated SOC or TOC as SOM *0.58 in these cases (NSW Government, 2021). Outdoor field studies, greenhouse studies and studies with laboratory treatments were all included. Studies were excluded, if total carbon (TC) rather than TOC or SOC was given, or SOC was given in its fractions, such as the light or heavy fraction. Moreover, studies were excluded if they did not present a "clear" control. A control was considered "clear" if they were treated in the exact same way and the only difference to the treatment was the absence of biochar.

Field studies included biochar treatments on natural soils (n = 376), and on lysimeters and columns (n = 36). Non-field experiments included incubation experiments (n = 182), all of which were carried out in laboratories. Furthermore, non-field experiments included pot experiments (n = 141). These pots were either placed in the open air (n = 96) or indoor e.g., in greenhouses (n = 45).

We divided all treatments into two separate datasets. All field treatments were allocated to the "field dataset" (n = 412) (Supplementary dataset, Table S2-1) and the pot and incubation treatments were allocated to the "non-field dataset" (n = 324) (Table S2-2).

Besides information on SOC content, we also extracted information on experiment characteristics (field, laboratory, or greenhouse study, single vs. continuous application, duration, applied amounts, sampling depth), site characteristics (climate zone, tillage intensity, crop type, additional fertilization), soil properties (texture, soil pH class, initial SOC content), and biochar characteristics (feedstock, C content, C/N, surface area, CEC). To limit the variety of different soil texture classes, we decided to group them according to their dominant particle size class (sand, silt, or clay). Exceptions are the classes “clay loam and loam”. These have been added to the fourth category “loam”. In cases data was only presented in figures, WebPlotDigitizer Version 4.4 was used for the extraction of data (Rohatgi, 2020). In case of annual biochar applications, annual amounts were accumulated in order to analyze total amounts.

In the field dataset, SOC stocks given as Mg ha^{-1} were used to quantify SOC dynamics after biochar application. To enable the consideration of absolute SOC stock differences among studies with different layer thicknesses, we computed them to a common layer thickness of 30 cm using weighted average. A total of twelve treatments could not be computed and were eliminated from the field dataset, as studies did not report a clear layer thickness. If no information on SOC stocks was provided at all, we quantified them using the following equation (FAO, 2018),

$$SOC\ stock = SOC * Bulk\ density * Depth * 0.1 \quad (1)$$

where the SOC stock is expressed as Mg ha^{-1}, bulk density as g cm^{-3}, layer thickness as cm and SOC as g kg^{-1}.

In a few studies, no soil bulk density was given. In these cases, we used different pedotransfer functions and followed an approach, already applied in meta-analysis (Gross and Glaser, 2021), If studies included information on the initial SOC, silt and clay content, we used the pedotransfer function given in Men et al. (2008) (*Equation 2*). If studies included information on the initial SOC and the clay content, we used an equation given in Bernoux et al. (1998) (*Equation 3*). If studies only provided information on initial SOC, we used a pedotransfer function given in La Manrique and Jones (1991) (*Equation 4*).

$$Bulk\ density = 1.386 - 0.078 \times SOC + 0.001 \times Silt + 0.001 \times Clay \quad (2)$$

$$Bulk\ density = 1.398 - 0.0047 \times Clay - 0.042 \times SOC \quad (3)$$

$$Bulk\ density = 1.660 - 0.318 \times SOC^{0.5} \quad (4)$$

where bulk density is expressed as g cm^{-3} and the SOC, silt and clay contents as %. To better understand the factors influencing SOC stock changes, we grouped the study results as follows: tillage intensity type, climate zone, initial SOC, soil texture, sampling depth, soil pH class, added biochar amount, biochar type, additional fertilizer, and experiment duration.

The non-field dataset contained solely pot or incubation experiments, thus the quantification of SOC stocks was not practicable. Here, the relative or absolute difference of SOC content after biochar application given as g kg^{-1} was used.

2.2 Data analysis

We used two ratios to describe the SOC dynamics following biochar applications. To describe the relative effect, we calculated the response ratio (RR) (*Equation 5*) according to Hedges et al. (1999), and transformed it into $RR_{[\%]}$ (*Equation 6*), in order to interpret results more effectively. The absolute effect was described by dSOC (*Equation 7*).

$$RR = \ln\left(\frac{X_E}{X_C}\right) \quad (5)$$

$$RR_{[\%]} = 100\ \times (-1 + e^{RR}) \quad (6)$$

$$dSOC = X_E - X_C \quad (7)$$

where X_E is the mean SOC stock with biochar application and X_C is the mean SOC stock without application of biochar (control group) for each treatment. Both effect sizes were estimated using a random-effects model (REM), as heterogeneity was assumed among the individual studies. REM's rely on the inverse-variance method (*Equation 8*), to estimate the weighting factor w_k of each individual effect size k.

$$w_k = \frac{1}{s_k^2 + \tau^2} \quad (8)$$

with s_k^2 being the variance of each individual effect size k and τ^2 being the variance of the distribution of effect sizes within their population. The Restricted Maximum Likelihood method was used to account for τ^2, being the variance of the distribution of effect sizes within their population.

$$\Theta = \frac{\sum_{k=1}^{K} \Theta_k w_k}{\sum_{k=1}^{K} w_k} \quad (9)$$

The weighting factor w_k was then used to calculate the pooled REM effect size Θ for each respective category using *Equation 9*.

To explore interactions between variables, we conducted a subgroup analysis of variables. We assumed that the studies within each subgroup was drawn from a universe of populations and therefore used *Equation 8* and *Equation 9* like we did in the REM. However, as we have assumed both random effects (within the subgroups) and fixed effects (the subgroups themselves were assumed to be fixed) in this subgroup analysis, this is a mixed-effects model approach (Harrer et al., 2022).

We used R Version 4.0.3 (RStudio Team, 2020), and the "meta" package for calculation (Balduzzi et al., 2019). Considering the fact that ~20% of the included studies provided insufficient information on statistical measures, we decided to assume a standard deviation of 10% in those cases, as already performed in a recent meta studies to include as many treatments as possible (Gattinger et al., 2012; Han et al., 2016; Luo et al., 2006; Tian et al., 2015).

The REM results for both $RR_{[\%]}$ and dSOC are presented as forest plots. Visualization was conducted with R Version 4.0.3. The vertical black solid line represents an $RR_{[\%]}$, or a dSOC equal to 0, thus no effect. An effect size larger than 0 indicates a positive effect (i.e., an increase of SOC upon biochar application), and lower than 0 a negative effect (i.e., a decrease of SOC upon biochar application). Each effect size is presented as the range between the upper and lower 95% confidence interval (CI). The line inside of both confidence intervals represents the range of the effect size. If the effect size range crosses the "zero-effect-line", the result can be interpreted as statistically insignificant. The effect sizes of each group were considered to be significantly different at $p < 0.05$ from each other if the 95% CI were not-overlapping. The vertical black dotted line represents the grand overall mean. Group category names are presented on the y-axis in bold black letters, sub-categories are given in grey letters. The number of included treatments is given in grey brackets.

3. Results and Discussion

3.1 General effect

Overall, 64 studies with a total of 736 treatments were analyzed within this meta-analysis. The treatments were located in North America (n = 28), South America (n = 40), Sub-Saharan Africa (n = 43), North Africa (n = 4), Europe (n = 180), Australia (n = 13), South Asia (n = 90), and East Asia (n = 338). The results of all subcategories, including their REM statistics are given in Supplementary dataset Table S2-3 - Table S2-6. Results of the subgroup analysis obtained from the mixed-effects model are given in Supplementary dataset, Table S2-7 - Table S2-8.

3.2 Experiment setup effect

As expected, both the results of the field dataset and the non-field dataset showed a significant increase of SOC, although, among both datasets, field treatments showed a 46% lower $RR_{[\%]}$ than non-field treatments, on average. Treatments retrieved from field studies showed an absolute SOC increase of 13.0 Mg ha^{-1} (95% CI 11.5 – 14.6 Mg ha^{-1}) corresponding to a relative SOC increase of 29% (95% CI 26% - 33%) (Fig. S2-1 and S2-2). Greenhouse and laboratory studies showed an absolute SOC increase of 6.1 g kg^{-1} (95% CI 5.5 – 7.2 g kg^{-1}) corresponding to a relative SOC increase of 75% (95% CI 67% - 85%) after biochar application (Fig. S2-4 and S2-5).

Differences between field and greenhouse studies were already observed in previous studies (Fidel et al., 2019; Wang et al., 2019) and are mainly due to non-existing environmental factors such as temperature and moisture fluctuations, and "near ideal" conditions in the laboratory or the greenhouse with minimal disturbance, which is almost impossible to achieve under field conditions. Factors such as crop growth and soil tillage affect soil structure, which is strongly connected to SOC stabilization (Guo et al., 2020), and are therefore influencing factors for the C sequestration potential of soil amendments (Xu et al., 2019). In addition, under field conditions, also other dissipation pathways such as wind and/or water erosion, leaching, and bioturbation occur.

Despite this difference among the experimental setups, different groups in both datasets showed high variability, but almost all comparisons revealed increases in SOC content or stocks, mainly due to the fact that biochar mainly adds stable carbon to the soil.

The molecular structure and stability of carbon compounds added to soil by various soil amendments is, among many other biotic and abiotic influences, a controlling factor in soil carbon persistence and SOC sequestration potential (Kimble et al., 2000; Lorenz and Lal, 2014; Wang et al., 2016). Labile C fractions such as the microbial biomass have short turnover rates and short soil persistence compared to humified, physically protected, and chemically recalcitrant C fractions (Silveira et al., 2008). Biochar consists mainly of highly stable aromatic C compounds, making up about 97% of the total biochar C, and therefore has a high mean residence time of 556 years and a low decay rate (Wang et al., 2016)

Field treatments were distinguished into lysimeter or column setups, and "classic" field experiments showing significantly higher SOC sequestration than classic field setups, with a higher absolute SOC increase of 22.6 and 12.6 Mg ha^{-1}, respectively, and a higher relative SOC increase of 98 and 26%, respectively (Fig. S2-1 and S2-2). Non-field treatments were subdivided into pot and incubation studies (Fig. S2-4 and S2-5). Pot experiments showed an absolute SOC increase of 5.6 g kg^{-1} corresponding to a relative increase of 81%. Incubation studies showed on average comparable SOC increases (absolute increase of 6.9 g kg^{-1} and relative increase of 70%). Pot studies conducted indoor showed a higher absolute increase of SOC (8.6 vs 4.5 g kg^{-1}) but a lower relative increase (55% vs. 92%) than those conducted outdoor. Comparable to the disparities between the field and laboratory scale, these differences can be explained by a lack of disturbance and environmental conditions, which are better controlled and limited in lysimeter and column trials.

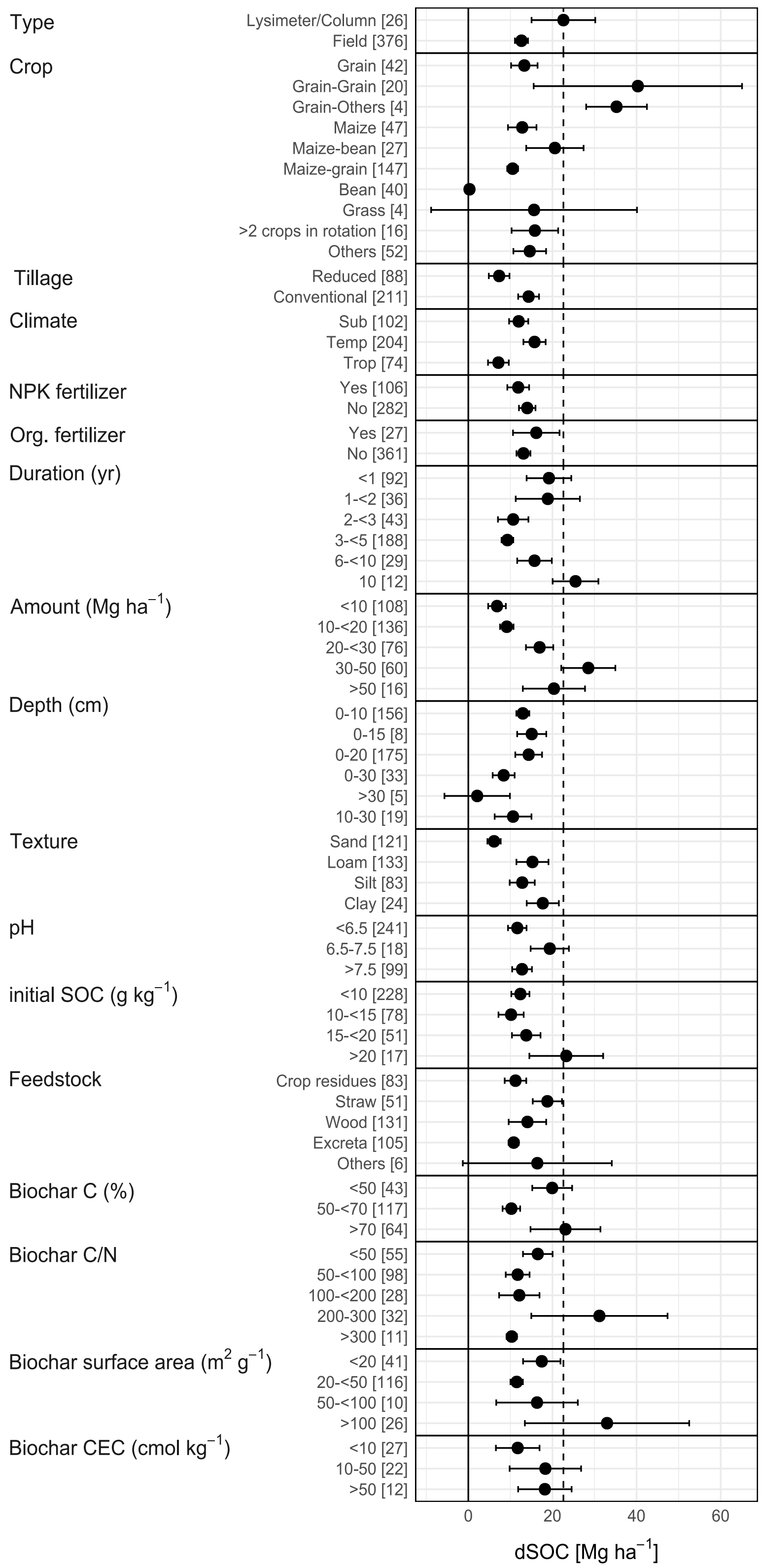

Figure S2-1: Meta-analysis results of the "field-dataset", given as a forest plot. Presented is the mean difference of soil organic carbon stocks after biochar application (dSOC) influenced by different data groups. Number in brackets represent the number of included treatments. Points within the range represent the mean dSOC and the line within the 95% confidence interval represents the range of the effect size. If the effect size range crosses the "zero-effect-line", given as a solid vertical line at 0%, the result can be interpreted as statistically insignificant. The effect sizes of each group were considered to be significantly different at $p < 0.05$ from each other if the 95% confidence intervals were not overlapping. The vertical dotted line represents the grand overall mean.

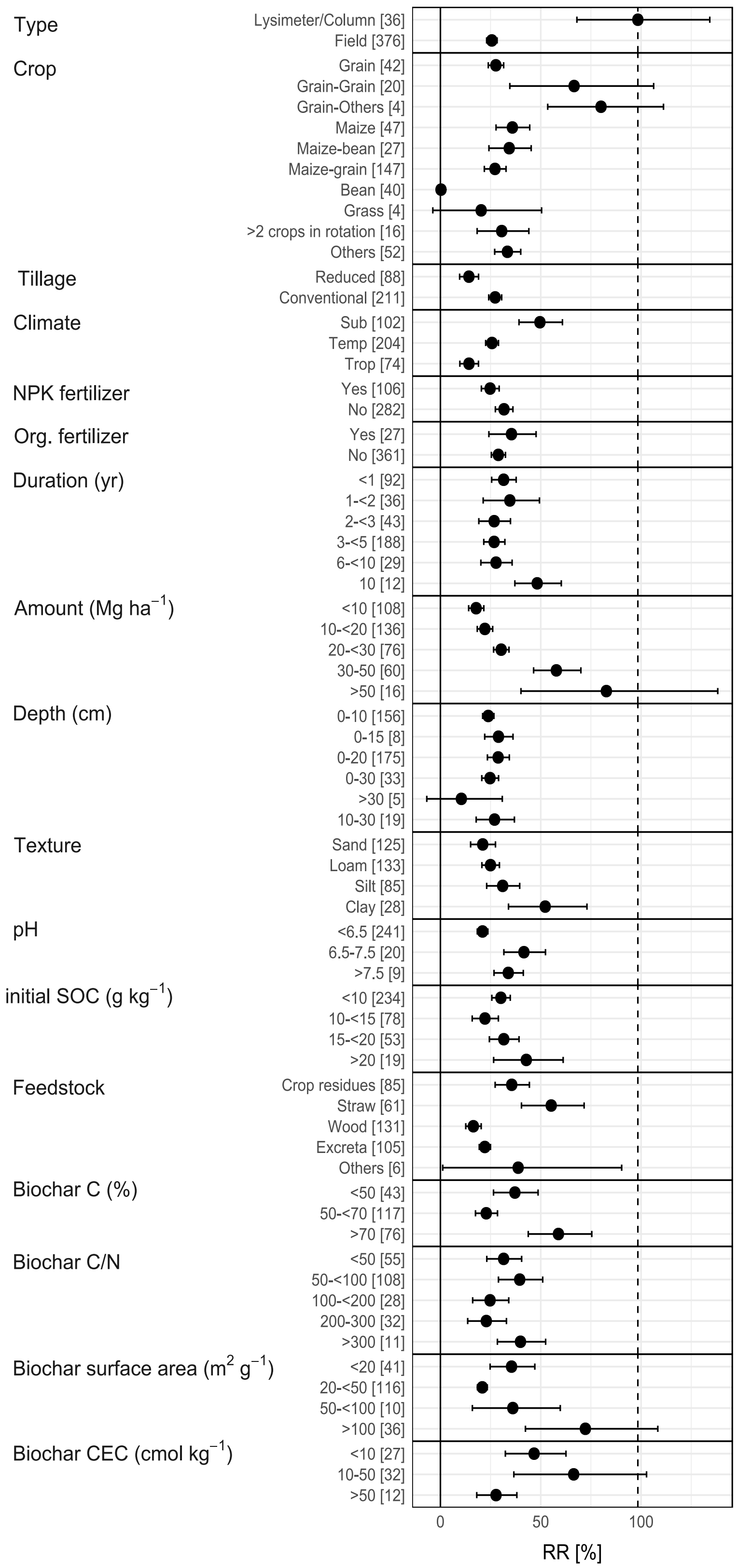

Figure S2-2: Meta-analysis results of the "field-dataset", given as forest plot. Presented is the relative change of soil organic carbon stocks ($RR_{[\%]}$) after biochar application influenced by different data groups. Numbers in brackets represent the number of included treatments. Points within the range represent the mean dSOC and the line within the 95% confidence interval represents the range of the effect size. If the effect size range crosses the "zero-effect-line", given as a solid vertical line at 0%, the result can be interpreted as statistically insignificant. The effect sizes of each group were considered to be significantly different at $p < 0.05$ from each other if the 95% confidence intervals were not overlapping. The vertical dotted line represents the grand overall mean.

3.3 Single versus continuous biochar application

Whether biochar was applied only once at the beginning of a field experiment or repeatedly (e.g., each year or at the beginning of a growing season) significantly influenced the relative increase in SOC stock. Single applications raised SOC stocks by 26% while continuous applications led to a mean of 55% SOC stock increase at the end of the experiments (Fig. S2-6). This difference in the relative SOC stock magnitude can be explained by different transport mechanisms and dissipation processes that determine biochar loss (Dong et al., 2017). Microbial decomposition reduces biochar C by 0.5% in one year (Maestrini et al., 2014), and total biochar amount in soils by 2.2% after two years (Major et al., 2010). Other biochar losses are due to leaching of dissolved organic carbon (~2% over two years) (Major et al., 2010), vertical transport (9-19%) (Obia et al., 2017), and lateral transport (20-53%) (Major et al., 2010). By re-applying biochar annually, those biochar losses are mitigated and SOC stocks are restored.

3.4 Duration effect

The experimental duration was grouped into six different classes, of which two can be described as short-term (<1 yr and 1 – <2 yr), two as mid-term (2 – <3 yr and 3 – <5 yr) and two as long-term (6 – <10 yr and 10 yr). The results of these duration classes show a highly interesting pattern, in which short and long-term treatments led to higher absolute and relative SOC increases. This effect was irrespective of whether biochar was applied once or repeatedly (Fig. S2-6 and S2-7). The same principle was observed in the non-field dataset. At experiment durations shorter than 10 days, SOC increased absolutely and relatively from 5.3 g kg^{-1} and 60%, respectively. In the case of long-term experiments with durations longer than 500 days, the SOC increase was up to 9.6 g kg^{-1} and 163%.

A high SOC increase in the short term is due to the application and incorporation of fresh and C-rich biochar into soil. This initial exposition of fresh biochar leads to a high microbial response and the turnover of the labile C fractions, often referred to as a positive priming effect (Wang et al. 2016). Positive and negative biochar-induced priming can co-exist but negative priming is more important in the long-term (Maestrini et al., 2014). Microbes prefer the utilization of easily degradable C pools, but they deplete with time (Wang et al., 2016; Maestrini et al., 2014). Additionally, biochar-C losses through microbial turnover are marginal, compared to losses through erosion and vertical/lateral transport (Maestrini et al., 2014; Major et al., 2010). In the short-term, the availability of labile biochar-C enhances microbial turnover and a loss of oxygen. With increasing time, the stable residue biochar-C is only slightly degraded. However, the O content increases here, due to the formation of O-containing functional groups (Wiedner et al., 2015).

Overall, durations of ten years led to the highest absolute (25.5 Mg ha^{-1}) and relative (48%) SOC increase, and surprisingly, these results were significantly higher than the SOC stock magnitudes of all shorter duration classes. Long-term SOC enrichments are consistent with previous research on Terra

Preta soils, where over a time span of ~2000a, SOC stocks were 3 times higher and biochar stocks were 70 times enriched compared to neighboring soils (Glaser, 2014). This is an even larger increase of SOC stock than observed in our dataset. Therefore, biochar seems to stimulate also non-biochar SOC sequestration with increasing time, up to very long durations. But at the same time, no biochar application experiments with an experimental duration longer than ten years could be identified, thus the long-term description of SOC stocks is still limited. It also means that biochar plays one of many key roles in Terra Pretas' SOM formation, and it is more likely that a combination of nutrient-rich household wastes, excrements, bones, ash and charred material caused Terra Pretas' SOC and nutrient enrichment (Glaser, 2014). In addition, this assumption is supported by our results on the combined application of biochar and organic fertilizers, as described earlier.

3.5 Amount effect

Increasing biochar application amounts showed a positive relationship with absolute SOC increases, both in "field" and "non-field" treatments (Fig. 3). Low amounts <10 Mg ha^{-1} led to significantly lower absolute and relative SOC increases than higher amounts of >20 Mg ha^{-1}. This relationship is irrespective of whether the biochar was applied once or repeatedly (Fig. S2-5 and Fig. S2-6). Similar amount responses were observed in the non-field dataset. An exception was observed at amounts >10-30 g kg^{-1}, where an increase at low amount levels appeared, but this increase was not significant. Different meta-analysis have shown that C-rich soil amendments such as straw (Berhane et al., 2020), and manure (Maillard and Angers., 2014; Gross and Glaser, 2021; Han et al., 2016) increase SOC with increasing C input amounts. Maillard and Angers (2014) additionally found the manure C input as the most decisive global influencing factor for increasing SOC stocks. Similar dynamics were therefore expected within our approach. Possible saturation effects, such as observed with straw returns (Liu et al., 2014), are not to be expected in the case of biochar. The observations of Anthropogenic Dark Earths, in which the SOC content could be increased three times over adjacent soils by the use of pyrogenic C in the course of hundreds to thousands of years (Glaser and Birk, 2012; Solomon et al. 2016), contradict the saturation effects. However, in order to disprove this irrevocably, long-term field tests are required, since saturation effects need experiment durations of about 26 years to occur (Liu et al. 2014; West and Six, 2007).

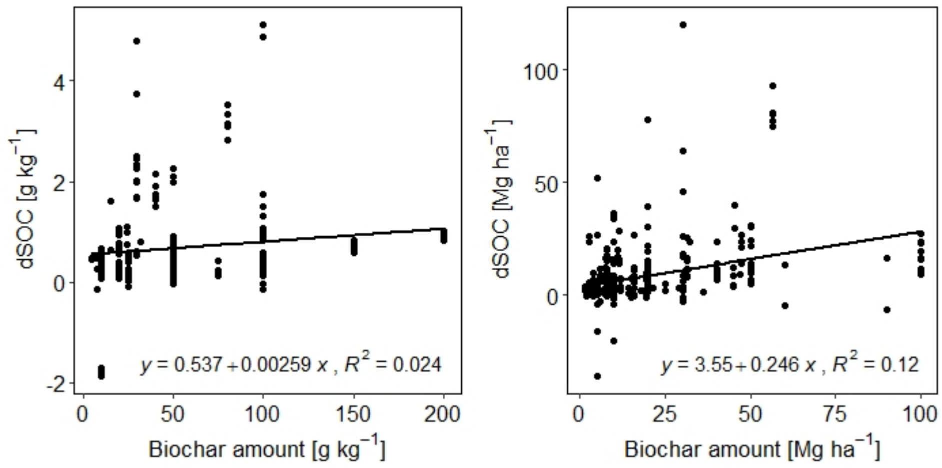

Figure S2-3: Relationship between the biochar amount and absolute SOC difference (dSOC) and, in laboratory and greenhouse treatments (left) and field treatments (right). In laboratory and greenhouse treatments both variables are given as g kg^{-1}, and field treatments both variables are given as Mg ha^{-1}. R^2 represents the coefficient of determination.

3.6 Soil depth effect

SOC increases after the application of biochar were higher in shallow compared to deep soil regions. The highest magnitudes were achieved if treatments sampled between 0-15 (15.1 Mg ha^{-1} and 29%) and 0-20 cm (14.4 Mg ha^{-1} and 29%). Both depth classes showed significantly higher SOC enrichment compared to 0-30 cm (8.4 Mg ha^{-1}) or higher than 30 cm soil thickness (2.1 Mg ha^{-1}), where the increase was not significant. But this finding is ambiguous as it might be related to the low number of five repetitions. A depth-related decline in SOC responses after incorporation of soil amendments was already observed in recent research (Gross and Glaser, 2021), and it can be expected due to the corresponding dilution effect. There is still an ongoing debate, whether the SOC increases after the application of certain management decisions, e.g. reduced tillage, agroforestry or soil amendments, are accountable across the whole soil profile (Gross and Glaser, 2021; Mando et al., 2005; Yaduvanshi and Sharma, 2008; Boguzas et al., 2015; Baker et al., 2007). The depth-wise decline is connected to the fact that biochar is generally incorporated into the topsoil or only into shallow soil depth. According to our subgroup analysis, SOC stock increases in shallow soil regions were higher under reduced tillage, whereas in intermediate soil depths (0-20 cm and 0-30 cm) conventional tillage showed stronger increases (Supplementary dataset, Table S2-7). In soil depths 10-30 cm, reduced tillage soils showed a higher absolute SOC stock increase than conventionally tilled soils. However, only four observations under conventional tillage could be observed, leading to large error bars and nonsignificant results. These results demonstrate that SOC stock increases in different soil depths are connected to tillage, which confirms previous findings (Silveira et al., 2008). The downward migration of biochar and SOC depend on tillage (Obia et al., 2017), soil texture and particle size distribution (Obia et al., 2017), rainfall amounts and hydraulic conductivity (Major et al., 2010; Obia et al., 2017), the activity of soil fauna and thus bioturbation (Major et al., 2010). As vertical biochar migration takes time, we expect the SOC stocks to increase in deeper soil regions only in the long-term. However, up to now, there is a lack of long-term field experiments that could deliver proof of concept.

SOC sequestration processes are particularly important in deeper soil regions because subsoils are generally far from being saturated (Lal et al., 2007; Gross et al., 2019). During downward migration, SOC is subject to preferential sorption to minerals, especially as dissolved organic carbon (Gross et al., 2019), which often have large surface areas due to high clay content and greater abundance of Fe and Al hydrous oxides in deep soil regions (Deb and Shukla, 2011; McCarthy, 2005; Rasse et al., 2005). Moreover, the translocation of SOC in deeper soil regions can increase its persistence. Globally, more than 50% of SOC is stored below ~20 cm (Batjes, 1996; Jobbágy and Jackson, 2000), with ages ranging from about 1,000 to 10,000 years (Fontaine et al., 2007; Jobbágy and Jackson, 2000; Rumpel et al., 2002; Schmidt et al., 2011).

3.7 Climate effect

In the field dataset (Fig. S2-1 and S2-2), the highest absolute SOC increase was observed in temperate regions (15.7 Mg ha^{-1}) and the highest relative SOC increase after biochar application was achieved in subtropical climates (50%). Non-field treatment results were comparable. Temperate (11.3 g kg^{-1} and 113%) and subtropical climates (6.5 g kg^{-1} and 94%) achieved higher SOC content increases than soil under tropical climate (4.6 g kg^{-1} and 43%). These findings are consistent with previous results obtained from different soil amendments (Maillard and Angers, 2014; Gross and Glaser, 2021; Tian et al., 2015), where absolute SOC increases under subtropical and temperate climate were generally higher. In our dataset, biochar applications under tropical conditions led both to significantly lower absolute (7.2 Mg ha^{-1}) and relative SOC increase (14%) than in any other climate region. In tropical soils with higher initial SOC, both absolute and relative SOC stock increases were at a high level (Supplementary dataset, Table S2-8). Research on “Terra Preta” genesis has demonstrated that with the use of soil amendments, especially biochar, high amounts of SOC could be sequestered over hundreds of years in tropical soils and could generate highly fertile soils (Glaser and Birk, 2012). Similar dynamics were observed in West Africa, also a region characterized by generally low initial SOC, where the historic application of soil amendments led to formation of carbon-rich and highly fertile African Dark Earths Frausin et al., 2014). Biochar application studies nowadays, generally restricted to a duration of 3-5 years, cannot display such long-term effects. That is why we suggest to either perform field application studies over a longer time frame, or to revisit or reanalyze locations where biochar has been applied longer ago.

3.8 Tillage intensity effect

Tillage intensity data were provided for 299 treatments, of which 88 were performed under reduced tillage, resulting in a lower relative SOC increase of 14% than conventional tilled soils with 27%. A similar pattern is observed for absolute SOC change, where reduced tillage soils showed a lower absolute SOC stock increase of 7.3 Mg ha^{-1} than conventionally tilled soils with 14.3 Mg ha^{-1}. Similar results were observed after the application of manure (Gross and Glaser, 2021). More intensive tillage promotes soil aeration and thus decay and decomposition. These oxidative conditions could promote the formation of humic substances (Mia et al., 2017). Additionally, tillage could also favor the biochar incorporation into the soil, as opposed to soil surface or shallow application. This enables biochar C to reach deeper soil region more easily. This effect has already been observed with manure application, which led to greater increases in deeper soil regions with conventional tillage, but also caused a significant increase in SOC content at depths below 30 cm with reduced tillage (Gross and Glaser, 2021). In any case, biochar application should be combined with tillage in order to maximize SOC sequestration.

3.9 Crop effect

Out of 412 field treatments, 410 provided information on crop type. The largest significant SOC gain was achieved by the combination of grains with “others”, a variety of crops appearing with a low number of replicates in the dataset. This combination led to a high absolute SOC increase of 35.2 Mg ha^{-1} and a relative SOC increase of 80%, and shows a large error bar, due to the small group size (n = 4). The second highest combination was grain followed by grain, which led to both a high absolute SOC increase of 40.3 Mg ha^{-1} and a high relative SOC increase of 66%. Grain treatments conducted only for one season led to an absolute SOC increase of 13.3 Mg ha^{-1} and a relative SOC increase of 28%. In contrast, maize treatments showed the highest SOC sequestration, if the experiment was only conducted for one growing season (dSOC = 12.8 Mg ha^{-1}, $RR_{[\%]}$ = 36%). Maize-bean combinations led to a SOC increase of 20.6 Mg ha^{-1} corresponding to 34%, whereas maize-grain combinations showed an absolute SOC stock increase of 10.5 Mg ha^{-1} and a relative SOC increase of 27%. Cultivation of beans and grass did not significantly increase SOC stocks. The implementation of a diverse crop rotation with more than two crops led to a SOC increase of 15.8 Mg ha^{-1} corresponding to 31%. Our data suggests that double-cropping systems show higher SOC accumulation after biochar application than single cropping systems. But these results show high variation and partly large error bars. Generally, more diverse crop rotations improve various ecosystem services and additionally offer the potential to increase SOC content (King and Blesh, 2018). In addition to ecological benefits, multiple-cropping systems also offer economic advantages and possibilities for farmers to adapt to climate change (Kawasaki, 2019).

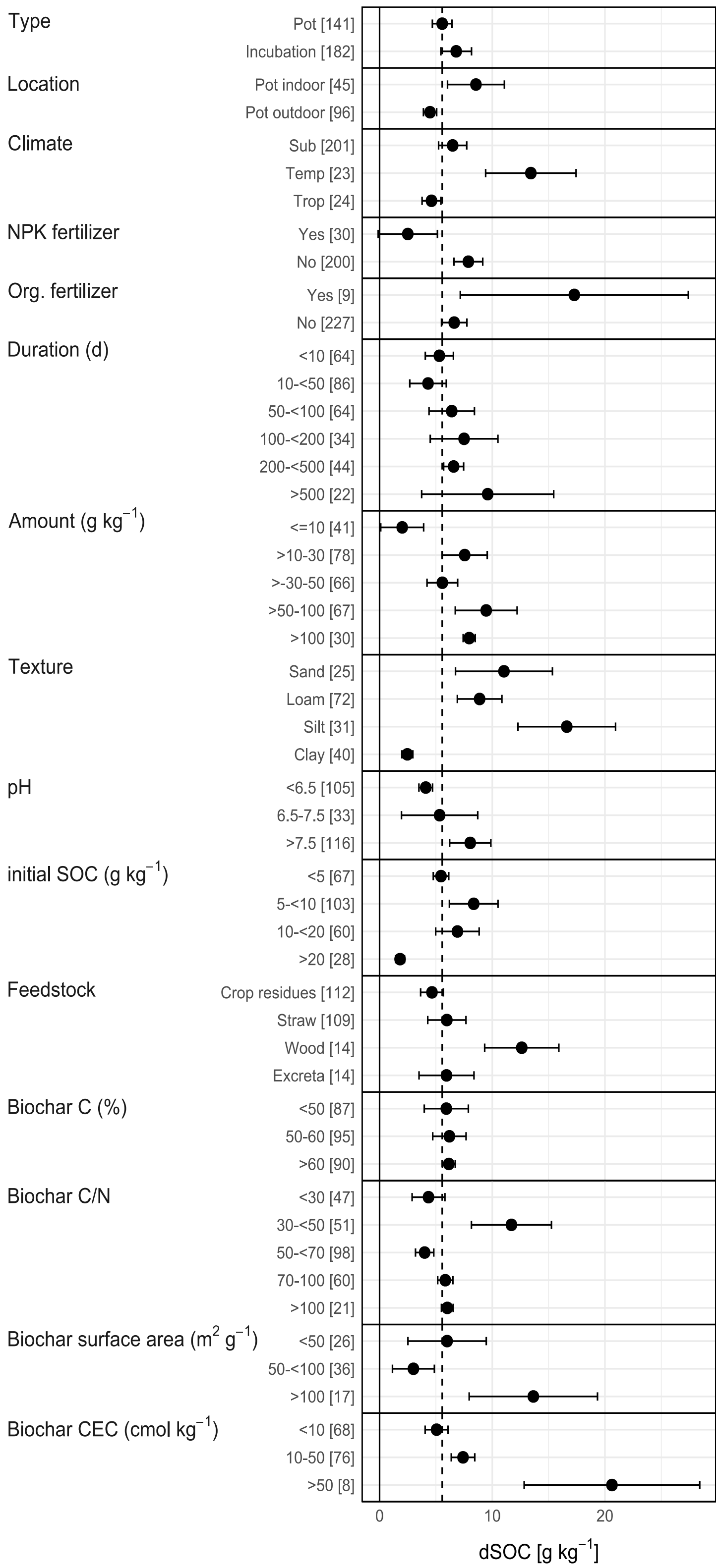

Figure S2-4: Meta-analysis results of the "non-field dataset", given as a forest plot. Presented is the mean difference of soil organic carbon content after biochar application (dSOC) influenced by different data groups. Number in brackets represent the number of included treatments. Points within the range represent the mean dSOC and the line within the 95% confidence interval represents the range of the effect size. If the effect size range crosses the "zero-effect-line", given as a solid vertical line at 0%, the result can be interpreted as statistically insignificant. The effect sizes of each group were considered to be significantly different at $p < 0.05$ from each other if the 95% confidence intervals were not overlapping. The vertical dotted line represents the grand overall mean.

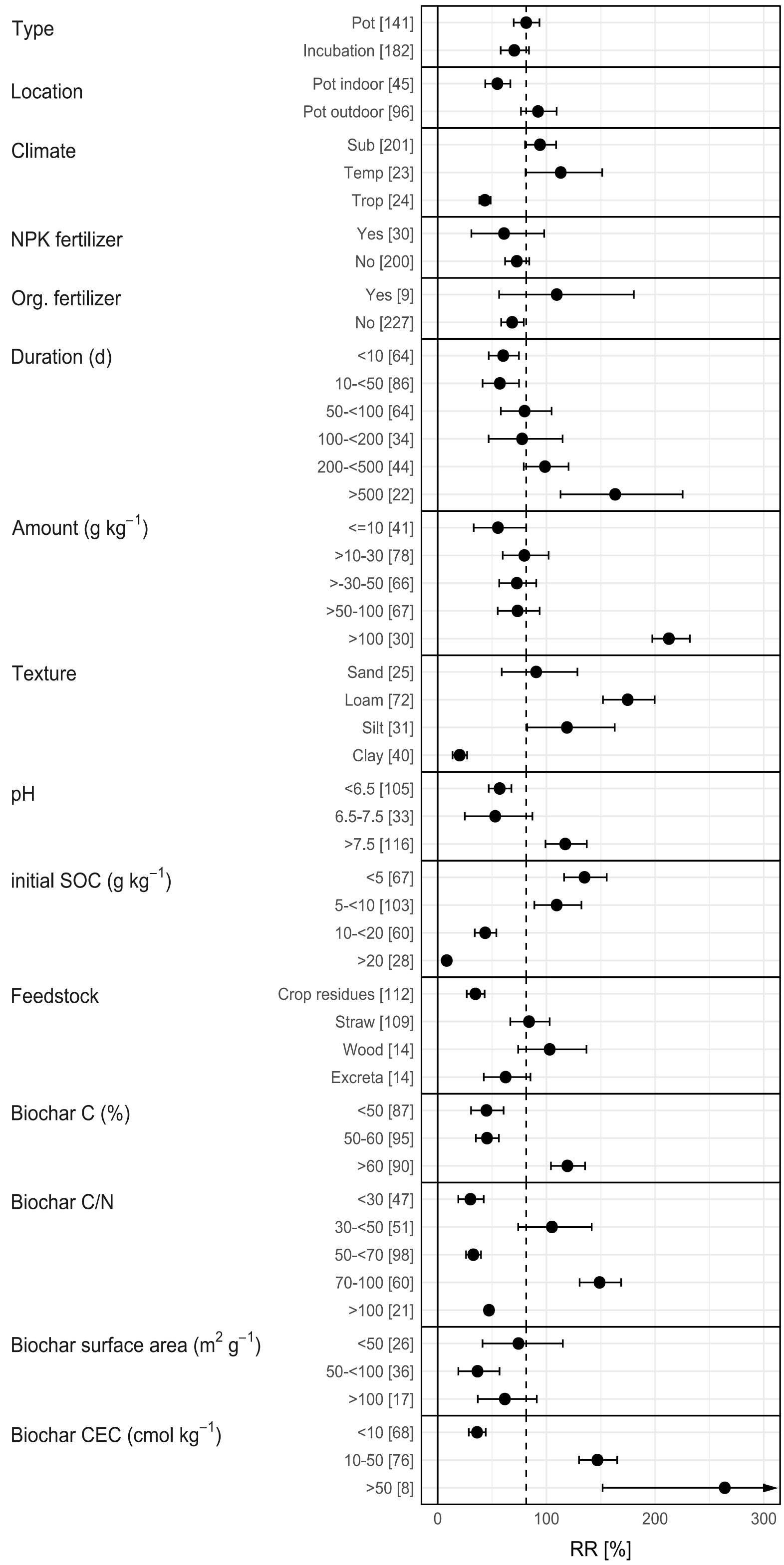

Figure S2-5: Meta-analysis results of the "non-field dataset", given as a forest plot. Presented is the relative response ratio $RR_{[\%]}$ of soil organic carbon content after the application of biochar influenced by different data groups. Numbers in brackets represent the number of included treatments. Points within the range represent the mean dSOC and the line within the 95% confidence interval represents the range of the effect size. If the effect size range crosses the "zero-effect-line", given as a solid vertical line at 0%, the result can be interpreted as statistically insignificant. The effect sizes of each group were considered to be significantly different at $p < 0.05$ from each other if the 95% confidence intervals were not overlapping. The vertical dotted line represents the grand overall mean.

3.10 Fertilizer effect

Additional organic fertilizer application led to higher SOC stock increases compared to biochar alone (Fig. S2-1 and S2-2), which was already shown elsewhere (Liu et al., 2016). The opposite was observed with additional synthetic fertilizer use compared to biochar alone. In non-field treatments (Fig. S2-4 and S2-5), additional synthetic fertilizer also led to a lower SOC increase than without synthetic fertilizers (7.9 vs 2.5 g kg^{-1} and 61 vs 73%). Organic fertilizer input led to a vice versa effect with higher SOC increases than in the unfertilized group (17.3 vs 6.6 g kg^{-1} and 109 vs 68%).

Synthetic fertilizers deliver nutrients, mainly NPK, enhance the net primary production, stimulates microbial activity and thus C and N turnover, resulting in increased biomass output Du et al., 2020; Cai et al., 2018; Obour et al., 2017). Organic fertilizers, however, not only provide nutrients, but also serve as a C source. Depending on the C content and C/N ratio, organic fertilizer amendments such as manure, straw, or slurry can increase and stabilize SOC stocks in the long term (Berhane et al., 2020; Liu et al., 2014; Gross and Glaser, 2021; Tian et al., 2015). Research on Terra Preta concluded that not only biochar itself, but the combination of stable pyrogenic C, labile C fractions, and other source of nutrients such as composts and manures formed the highly fertile and C-rich tropical soils, as biochar comprises just about 20% of the SOM present in Terra Preta (Glaser, 2014).

3.11 Soil texture effect

Soil texture differences in the dataset showed a clear relationship between an increasing SOC stock response with increasing clay content, both in relative and absolute terms. Biochar applications to clay soils resulted in the highest SOC stock increase (17.7 Mg ha^{-1} and 52%), followed by silty soils (12.8 Mg ha^{-1} and 31%) and loamy soils (15.3 Mg ha^{-1} and 25%). Sandy soils showed the lowest overall increases (6.1 Mg ha^{-1} and 21%), which was significantly lower than applications to other differently textured soil. In general, higher clay mineral content in finer textured soils not only provides physical protection of SOC to enzymatic activity and thus turnover (Nannipieri et al., 2018; Zhang et al., 2019), but also increases SOC stability in the form of aggregates (Lal, 2018; Zong, 2018). With decreasing grain size, the physical protection becomes less important and SOC is exposed to oxidation and thus decomposition (Gross and Glaser, 2021), as well as SOC losses due to leaching and runoff (Yang, 2019). Different SOC increases related to different soil texture classes are due to the various processes, that are driven by soil grain size.

Unexpectedly, the non-field treatments identified clay soils with the lowest results out of the four observed texture classes (2.5 g kg^{-1} and 20%). Sand (11.0 g kg^{-1} and 90%) and especially silt (16.6 g kg^{-1} and 119%) and loam (8.9 g kg^{-1} and 175%) showed significantly higher SOC sequestration potential. This difference to the field dataset is due to the importance of initial SOC for SOC increase dynamics (West and Six, 2007). Sandy soils generally show lower initial SOC contents, which can be seen in the non-field dataset (23 out of 25 observations with initial SOC content <10 g kg^{-1}) and in the field dataset (99 out of 119 observations with initial SOC <15 g kg^{-1}). In both datasets low absolute increases of the SOC content or the SOC stock corresponded with high relative SOC magnitudes (Supplementary dataset, Table S2-9). Similar effects were already observed in soils after manure application (Gross and Glaser, 2021). Under field conditions, sandy soils are subject to higher biochar and SOC losses than under controlled greenhouse or laboratory conditions. For this reason, relative SOC increases were higher in the field dataset, especially if the initial SOC content was between 5 and 10 g kg^{-1} (SOC content increase of 131.52%) (Supplementary dataset, Table S2-9). The low relative and absolute SOC increases of clay soils in the non-field dataset, however, was due to the fact that clay treatments could only be found in two studies, with one study using biochar with a comparably low C content and low C/N ratios, and the soil used was acidic (Nyambo et al., 2018), and the second using NPK fertilizer in most treatments (Khan et al., 2020).

3.12 Soil pH effect

Neutral soils (pH 6.5–7.5) significantly accumulated more SOC (19.4 Mg ha^{-1} and 42%) than acidic soils (pH <6.5; 11.6 Mg ha^{-1} and 21%) after the application of biochar. Alkaline soils (pH >7.5) showed a SOC increase of 12.8 Mg ha^{-1} corresponding to 34%, and thus showed a good potential to increase SOC stocks. SOC increases in the non-field dataset followed similar trends as seen in the field dataset with neutral (53% and 5.3 g kg^{-1}) and alkaline soils (8.1 g kg^{-1} and 117%) showing larger increases than acidic soils 4.1 g kg^{-1} and (57%). Our findings of field and non-field treatments are consistent with previous research on biochar applications to soils with different pH (Liu et al., 2016). Biochar application to acidic soils leads to an enhanced biochar and SOC degradation (Liu et al., 2014; Sheng et al., 2016). Liu et al. (2016) explained this finding with higher positive priming effect and higher native SOC mineralization after biochar use in acidic soils, than following the addition to neutral or alkaline soils. Therefore, biochar has higher stability in neutral or alkaline soils. Additionally, higher amounts of Ca^{2+} ions in neutral and alkaline soils could favor mineral-organic complex formation (Gross and Glaser 2021).

3.13 Initial SOC effect

Low initial SOC content <10 g kg^{-1} led to high relative SOC increase both in the field and in greenhouse/laboratory treatments (Fig. S2-1, S2-2, S2-4, and S2-5), as has already been shown for manure amendments (Gross and Glaser 2021). However, the fact that the highest relative and absolute SOC increase was observed in SOC-rich soils >20 g kg^{-1} (Fig. S2-4 and S2-5) was surprising, as this contradicts previous findings with manure amendments (Gross and Glaser 2021) and highlights the potential to increase SOC stocks by biochar application irrespective of the current or initial SOC content. With respect to the differences in the C content between manure and biochar, it becomes clear why both amendments respond differently to initial SOC content. Biochar has a much higher C content than manure and therefore generally exerts a greater influence on the soil carbon balance, even if the soil already contains of a relatively high SOC content. In addition, biochar-C is much more stable than any other SOM component.

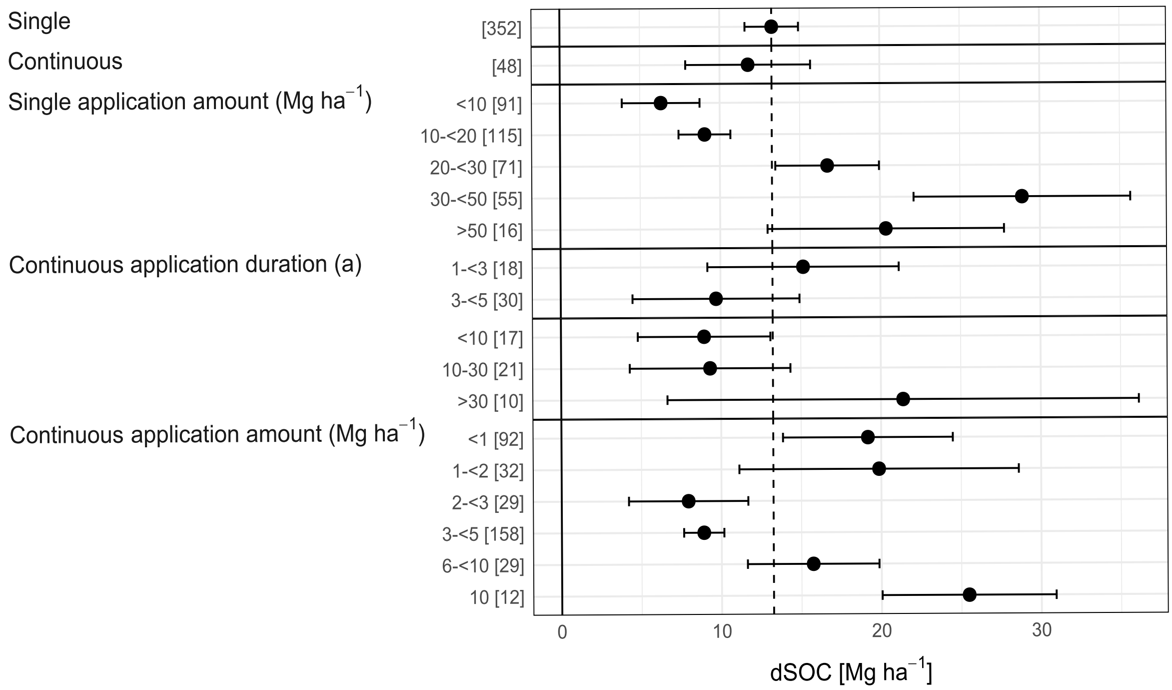

Figure S2-6: Meta-analysis results of the "field dataset", given as a forest plot. Presented is the absolute mean difference of soil organic carbon stocks (dSOC) after the application of biochar influenced by whether the application was conducted once (single application) or repeatedly (continuous application). Points within the range represent the mean dSOC and the line within the 95% confidence interval represents the range of the effect size. If the effect size range crosses the "zero-effect-line", given as a solid vertical line at 0%, the result can be interpreted as statistically insignificant. The effect sizes of each group were considered to be significantly different at $p < 0.05$ from each other if the 95% confidence intervals were not-overlapping. A vertical black dotted line represents the grand overall mean.

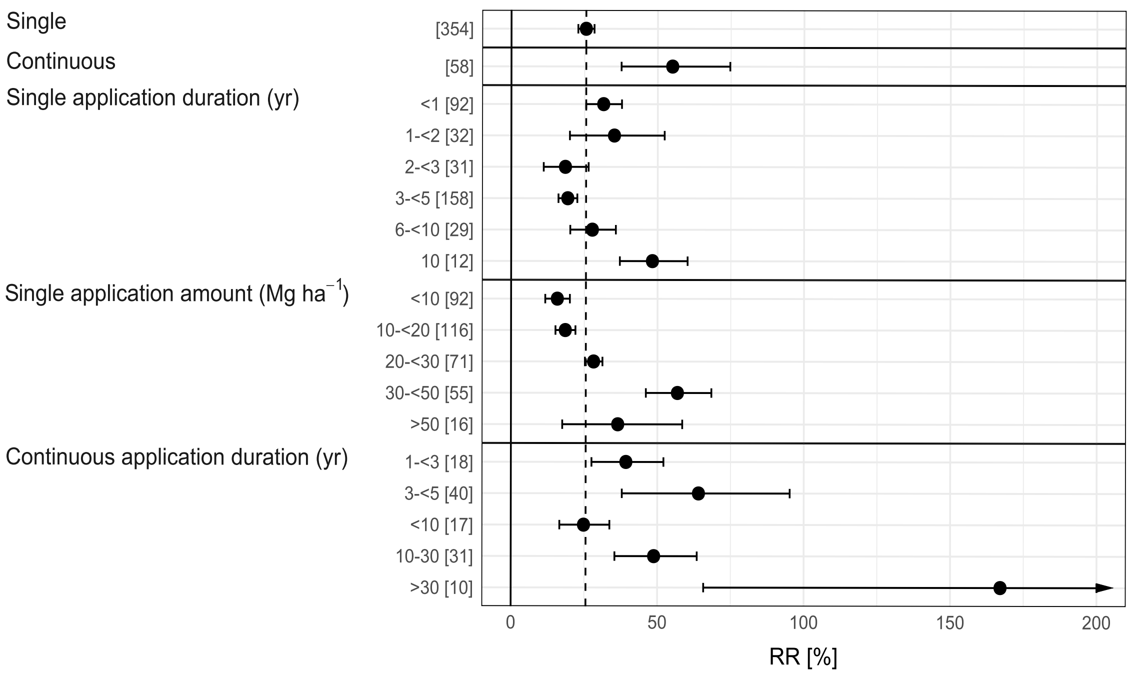

Figure S2-7: Meta-analysis results of the "field dataset", given as a forest plot. Presented is the relative response ratio (RR [%]) of soil organic carbon stocks (dSOC) after the application of biochar influenced by whether the application was conducted once (single application) or repeatedly (continuous application). Points within the range represent the mean dSOC and the line within the 95% confidence interval represents the range of the effect size. If the effect size range crosses the "zero-effect-line", given as a solid vertical line at 0%, the result can be interpreted as statistically insignificant. The effect sizes of each group were considered to be significantly different at $p < 0.05$ from each other if the 95% confidence intervals were not-overlapping. A vertical black dotted line represents the grand overall mean.

3.14 Biochar C and C/N effect

In the "field" dataset, the highest absolute and relative SOC increases were observed when biochar contained more than 70% C. A high magnitude of 20.0 Mg ha^{-1} and 37% could also be found if the C content of biochar was below 50% (Fig. S2-1 and S2-2). In the "non-field" dataset, the C content of biochar positively influenced the relative SOC increase. When the biochar C content was 60% or higher, the relative increase was 119% and thus higher than at lower C content (Fig. S2-4 and S2-5). However, higher absolute SOC increases could not be observed. The findings in both datasets indicate that the biochar C content has a secondary role in relation to SOC increase dynamics.

Additionally, in both datasets there was no clear statistical evidence as to whether a relatively low C/N ratio or a relatively high C/N ratio indicates a higher SOC increasement. A high relative gain, however, was observed in the "field" dataset, if C/N was >300 (40%) and the highest absolute increase was found at a relatively high C/N of 200-300 (31.2 Mg ha^{-1}). The C and N content of biochar and their ratio are generally very decisive values for biochar stability and the formation of SOC (Liu et al., 2016). Higher biochar C contents logically lead to larger C inputs, with positive effects on SOC. Low biochar C/N ratios have shown to increase soil respiration and CO_2 flux (Liu et al., 2016), due to higher N availability and thus higher microbial C mineralization rates (Zou et al., 2004; Huang et al., 2004). Consequently, high biochar C/N ratios in turn, led to increasing SOC (Liu et al., 2016). However, these effects could not be statistically substantiated in either of the two datasets.

3.15 Biochar feedstock effect

Different biochars vary in their ability to alter soil properties (Liu et al., 2016), due to varying structural components in their parent material (Raveendran et al., 1995; Aller, 2016), referred to as their feedstock. Therefore, it was not surprising that there were large differences in the SOC stock magnitudes after the application of biochar retrieved from different feedstocks. The highest magnitude overall was found with straw as the feedstock (55% and 18 Mg ha^{-1}), followed by crop residues (11.0 Mg ha^{-1} and 36%). Woods showed a comparatively high dSOC value of 16.4 Mg ha^{-1} but a low relative gain (16%). All in all, plant and wood-based sources showed a higher performance than biochars retrieved from animal excreta (10.8 Mg ha^{-1} and 22%). These findings are consistent with previous research (Liu et al., 2016), and are connected to higher C/N ratios in plant and wood based biochar (mean of 65.7 in our datasets) and, contrarily low C/N ratios of manure and excreta based biochar (mean of 18.1 in our datasets), which generally show enhanced C mineralization rates due to higher microbial N availability, as described in the previous chapter. In the contrary, high C/N ratios have positive effects on SOC, described above. Straw and especially wood as biochar feedstock led to the highest SOC responses in non-Field studies, with a dSOC of 6.0 and 12.6 g kg^{-1} and RR% of 84% and 103%, respectively. Crop residues, however, did not show as large increases (4.7 g kg^{-1} and 35%) as in the field-dataset. They were even lower than SOC increases of biochars retrieved from excreta feedstock (6.0 g kg^{-1} and 62%). This difference in the impact of crop residue biochar between both datasets (field vs. non field) is due to way higher C/N ratios of crop residue-based biochar used in field treatments (mean of 202.0) compared to biochar in non-field treatments (mean of 63.0).

3.16 Biochar cation exchange capacity and specific surface area effect

Biochars' CEC did not show significant differences among groups. However, biochars with a medium CEC of 10-50 cmol kg^{-1} raised SOC stocks the largest (18.3 Mg ha^{-1} and 66%). Regarding biochars' surface area, high SOC stock increases (33.0 Mg ha^{-1} and 72%) were observed if the surface area was high (>100 m^2 g^{-1}) (33.0 Mg ha^{-1} and 72%). However, this observation showed a large error bar and thus, the difference was not significant. Below 100 m^2 g^{-1}, the different surface area classes did not vary significantly. In non-field treatments, the highest SOC increases were achieved if the CEC was higher than 50 cmol kg^{-1}. However, only eight treatments were analyzed and thus they showed a large range. Regarding the SOC increase as a result of a high biochars' surface area, results were also quite ambiguous at least in their relative increase. Here, low surface areas <10 m^2 g^{-1} as well as higher areas >100 m^2 g^{-1} led to large increases with 74% and 62%, respectively. The absolute increase, however, showed a clear tendency regarding high areas with a dSOC of 13.7 g kg^{-1}. But still, this result did not significantly differ from the lower biochars' surface area subgroups.

The surface area is an important indicator of the adsorption rate and porosity of biochar when biochar is added to soil (Cabrera and Spokas, 2011; Shackley and Sohi, 2010), whereas the CEC determines the biochars' ability to exchange cations with the soil solution (Shackley and Sohi, 2010; Lee et al., 2010; Lehmann, 2007). Both biochar properties have influences on the soil quality after application, such as water retention (Suliman et al., 2017), nutrient availability and biomass production. High aboveground and root biomass, and therefore additional C inputs into soil, favor SOC sequestration. Increases of both properties in our datasets seem to have positive effects on SOC. However, it is not possible to draw definitive conclusions from our datasets.

4. Conclusions

We present a quantitative and systematic global evaluation of the C sequestration potential of biochar as a soil amendment with respect to a wide range of site and soil characteristics and differences between laboratory and field studies. Based on a meta-analysis approach, we found that biochar has a huge ability to increase and stabilize SOC.

SOC sequestration potential differed significantly between field treatments and treatments conducted in greenhouses and laboratories, with lower responses observed on field scale. Our study indicated that SOC sequestration upon biochar application was highest under alkaline soil pH, additional organic fertilizer, plant residues as biochar feedstock, and finer soil texture. As the longest reported study was 10 years, it is very difficult to extrapolate SOC sequestration potential beyond this time scale. Therefore, longer term biochar field experiments longer than 10 years are urgently needed to evaluate the climate change mitigation potential of biochar.

Further research should therefore conduct field application studies over a longer time frame, or re-visit and re-analyze locations where biochar has been applied longer ago and respect subsoil processes, in order to achieve a holistic understanding of SOC turnover and stabilization dynamics across the soil profile.

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Supplementary datasets

A supplementary dataset can be found online via:

https://www.mdpi.com/article/10.3390/agronomy11122474/s1

The dataset contains the following content:

Table S2-1: Overview of the studies in the "field" dataset and their treatments and results.

Table S2-2: Overview of the studies in the "non-field" dataset and their treatments and results.

Table S2-2: Overview of the results of the random effects model as well as their statistics in the "field dataset". Shown is the RR according to Hedges et al. 1999 and converted into percent. From column L onwards, measures of heterogeneity of the random effects model are presented, once for the entire data set and once for within groups.

Table S2-4: Overview of the results of the random effects model as well as their statistics in the "field dataset". Shown is the absolute mean difference dSOC. From column L onwards, measures of heterogeneity of the random effects model are presented, once for the entire data set and once for within groups.

Table S2-5: Overview of the results of the random effects model as well as their statistics in the "non-field dataset". Shown is the RR according to Hedges et al. 1999 and converted into percent. From column L onwards, measures of heterogeneity of the random effects model are presented, once for the entire data set and once for within groups.

Table S2-6: Overview of the results of the random effects model as well as their statistics in the "non-field dataset". Shown is the absolute mean difference dSOC. From column L onwards, measures of heterogeneity of the random effects model are presented, once for the entire data set and once for within groups.

Table S2-7: Results of the subgroup analysis Tillage x Soil depth obtained from a mixed-effects model. Results from the field dataset are presented. T

Table S2-8: Results of the subgroup analysis Climate x Initial SOC obtained from a mixed-effects model. Results from the field dataset are presented.

Table S2-9: Results of the subgroup analysis Soil texture x Initial SOC obtained from a mixed-effects model. Results from the non-field and the field dataset are presented. Table S10: Reference list of the literature used in this meta-analysis.

Study 3: Long-term biochar and soil organic carbon stability – evidence from field experiments in Germany

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Abstract

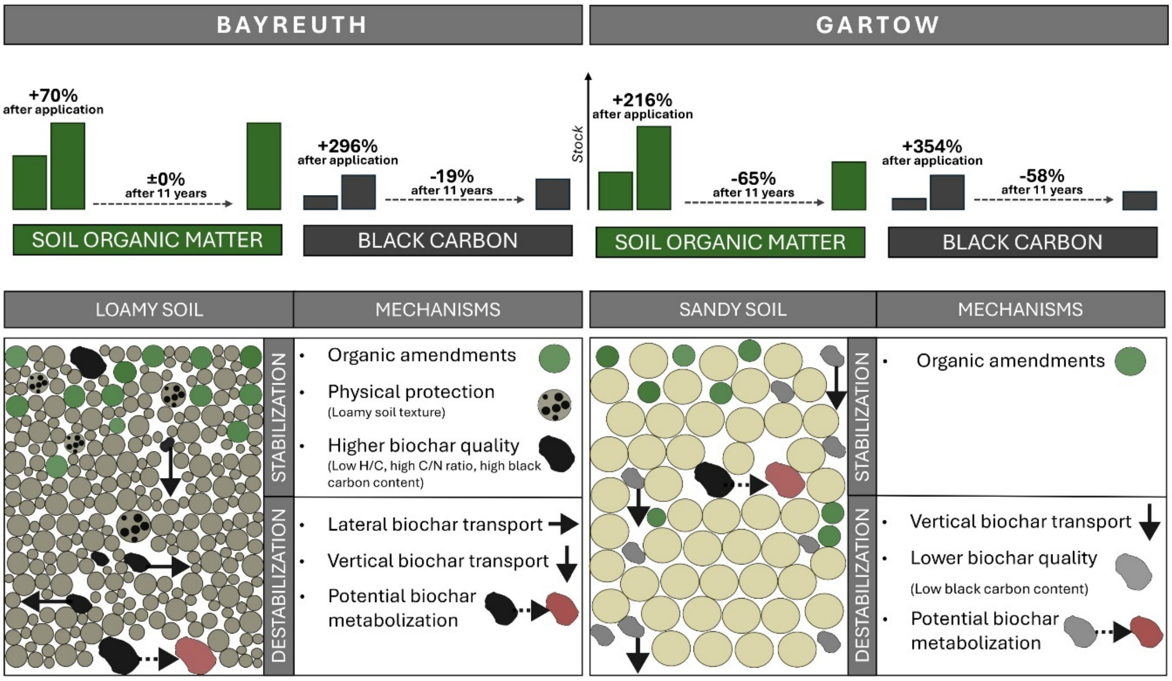
Organic soil amendments (OSA) with long residence times, such as biochar, have a high potential for soil organic carbon (SOC) sequestration. The highly aromatic structure of biochar reduces microbial decomposition and explains the slow turnover of biochar, indicating long persistence in soils and thus potential SOC sequestration. However, there is a lack of data on biochar-induced SOC sequestration in the long-term and under field conditions. We sampled two long-term field experiments in Germany, where biochar was applied 12 and 14 years ago. Both locations differ in soil characteristics and in the types and amounts of biochar and other OSA. Amendments containing compost and 31.5 Mg ha^{-1} of biochar on a loamy soil led to a SOC stock increase of 38 Mg ha^{-1} after OSA addition. The additional increase is due to non-biochar co-amendments such as compost or biogas digestate. After eleven years, this SOC stock increase was still stable. High biochar amount additions of 40 Mg ha^{-1} combined with biogas digestate, compost or synthetic fertilizer on a sandy soil led to an increase of SOC stocks of 61 Mg ha^{-1}; 38 Mg ha^{-1} dissipated in the following four years most likely due to lacking physical protection of the coarse soil material, and after nine years the biochar-amended soils showed only slightly higher SOC stocks (+7 Mg ha^{-1}) than the control. Black carbon stocks on the same soil increased in the short- and mid-term and decreased almost to the original stock levels after nine years. Our results indicate that in most cases the long-term effect on SOC and black carbon stocks is controlled by biochar quality and amount, while non-biochar co-amendments can be neglected. This study proves that SOC sequestration through the use of biochar is possible, especially in loamy soils, while non-biochar OSA cannot sequester SOC in the long term.

Highlights:

- SOC after eleven years of biochar application on a loamy soil in northeastern Bavaria could be demonstrated
- Biochar application to a sandy soil led to large SOC dissipation, most likely due to lacking physical stabilization
- Aged biochar particles mainly lost labile black carbon compounds while stable compounds persisted

Keywords: Carbon sequestration, Biochar dissipation, Molecular marker, Carbon dioxide removal, Long-term biochar field experiment, Organic soil amendments.

Graphical abstract:



1. Introduction

The growing world population (UN 2015) and climate change are increasing pressures on soil resources and destabilize the world's food security (Tumwesigye et al. 2021). Sustainable agriculture must mitigate the consequences of current and future soil threats while adapting to future climate conditions and maintaining food production. Increasing pressure on soils already jeopardizes soil quality and several ecosystem functions, e.g., the role of soils as a carbon (C) sink (FAO and ITPS 2015). Soils are the most important terrestrial C sink, storing 3500–4800 Pg of C (Lehmann and Kleber 2015), while terrestrial vegetation and the atmosphere store only around 800 Pg C each.

The centuries-long storage of organic carbon in the soil, better known as soil organic carbon (SOC) sequestration (Lal 2008), is not only crucial for carbon dioxide removal (CDR) from the atmosphere, but also has a positive effect on soil health and promotes the functionality of ecosystems (Lal et al. 2007; Lal et al. 2015; Rumpel et al. 2020). Increasing the SOC storage can be achieved by using different organic soil amendments (OSA), the most common forms being straw, slurries, manures, compost, biogas digestates, sewage sludges biosolids. Often, these OSA are used for fertilization due to delivery of nutrients. However, since they contain varying amounts of C-rich organic compounds, the application of OSA leads to an increase of SOC stocks (Alvarenga et al. 2020). The aforementioned OSA types contain very different forms of C-compounds, the majority of them possessing low stability and short mean residence times (MRT) in soil. Thus, these amendments need to be applied regularly, e.g. at the beginning of a new growing season to contribute to soil fertility and SOC stock increases in longer term (Alvarenga et al. 2020; Gross and Glaser 2021). Biochar amendments, in contrast, contain highly aromatic C compounds and only little amounts of nitrogen (N) (most of them being polycyclic and not available to microbes), and are, therefore, highly stable against microbial decomposition. When applied to soil, biochar's MRT is estimated to be 556±483 years (Kuzyakov et al. 2014, Wang et al. 2016). However, adding pure biochar alone to soil does not necessarily improve the soil quality. Pure biochar added to soil can lead to immobilization of nitrogen and reduced plant growth (Kammann et al. 2015). Immobilization of N is more likely if the biochar's carbon-to-nitrogen (C/N) ratio is very high (Mukome and Parikh, 2016) and the biochar was added without additional fertilizer as co-amendment or without a pre-treatment with nutrients, such as co-composting (Fischer and Glaser 2012). Mixing biochar with compost has been shown to prevent N immobilization. Co-composting of biochar, moreover, leads to the formation of a coating on the surface of biochar that on the one hand serve as a slow releasing reservoir for nutrients and on the other hand protects the aromatic C structure of biochar from further oxidation while enhancing biochar's stability in soil (Hagemann et al. 2017).

However, there is a lack of data on agricultural and environmental benefits of biochar in the long-term, specifically on a decadal time scale. In particular, biochar aging and the long-term fate of SOC stocks after biochar amendment remain poorly understood, as short-term studies and studies performed under laboratory conditions are not useful for predicting the long-term fate of biochar (Kuzyakov et al. 2014; Gross et al. 2021). Once applied to soil, biochar does not remain rigidly in place but reacts with the environment, with consequences for biochar's persistence, stability and traceability in soil. Biochar's persistence refers to the presence of biochar as opposed to its mineralization to carbon dioxide (CO_2) through biotic and abiotic processes (Lehmann et al. 2024), and thus persistence cannot be used as a synonym for stability. However, factors that influence the stability of biochar, can affect mineralization rates and thus persistence. This includes biotic processes such as the presence of microorganisms, but also larger soil fauna like earthworms. This includes chemical processes, mainly abiotic oxidation of biochar surfaces such as the reaction with water (Spokas and Reicosky, 2009) and desorption of CO_2 (Bruun et al. 2014). Last but not least, this includes the physical disintegration of large biochar particles into smaller particles (Spokas et al. 2014), due to frost, changing temperature and moisture, salt weathering, solubilization, roots or mechanical stress through e.g. soil tillage (Lehmann et al. 2024). Since biotic, chemical and physical processes typically occur simultaneously, and often sequentially while biochar resides in soil, experiments conducted under field conditions and with a feasible observation time are needed to achieve a more realistic idea about biochar's soil persistence and stability. Many approaches exist to trace biochar in soil and to assess its stability. To distinguish biochar from other C compounds in soil, the aromaticity and degree of aromatic condensation of organic C compounds in soil can be used since they are a key feature of biochar. They can be measured using solid-state ^{13}C Nuclear magnetic resonance spectroscopy (NMR) or by using molecular markers, e.g. benzene polycarboxylic acids (BPCA) (De la Rosa et al. 2018, Glaser et al. 1999). The advantage of using molecular markers over e.g. quantification of labile and stable C pools in soils via thermochemical oxidation resistance methods is the unambiguity in distinguishing biochar quantity and quality/stability.

Short-term studies have shown ambiguous results on biochar stability (Knicker 2011; Wang et al. 2016). This is due to the fact that mainly the labile biochar-C is decomposed in the short-term (Wang et al. 2016). These labile fractions decompose rapidly, leading to a positive priming effect. However, it has been observed that in the long-term, pyrogenic organic matter may promote physical protection through sorption, leading to negative priming at a later stage (Maestrini et al. 2015). So far, only a few studies have observed comparable negative priming effects in field experiments following the application of biochar (Blanco-Canqui et al. 2020; Guo et al. 2024). Thus, studies that solely focus on short-term effects underestimate biochar's true MRT considerably. A recent meta-analysis on the potential of biochar to increase the soil C stock in agricultural soils indicates the high persistence of biochar contributing to an additional build-up of SOC with increasing observation time (Gross et al. 2021). The organic C stocks increased significantly over a period of up to ten years. However, this observation was very limited in the number of included studies, and studies with a duration longer than ten years were missing completely.

This research gap is unfortunate, because efficient CDR technologies are urgently needed, and the European Union (EU) is pursuing CO_2 neutrality by 2050 as part of the "Green-Deal". To achieve this ambitious goal, the EU Commission proposed a net reduction in emission of greenhouse gases of 55% by 2030 compared to 1990 emission levels as a milestone for the new EU climate law.

The aim of this study is to provide insights into long-term SOC and biochar stock dynamics on a decadal scale and under field experiment conditions. The first objective was to analyze the SOC stock dynamics over time after the application of varying biochar amounts at two different long-term biochar field experiments in Germany. In addition to SOC, as a second objective, we analyzed the black carbon stocks, which we used as a molecular marker for biochar, to verify whether the biochar applied long ago is still traceable and stable, and to quantify its remaining amount. The third objective was to analyze the influence on the SOC stocks induced by different organic and mineral fertilizers used as co-amendment to biochar application and to determine whether they still had a co-effect on SOC sequestration and biochar stability at the two different sites, eleven and nine years after application.

2. Material and Methods

2.1 Study area and experiment characteristics

To achieve the objectives of this study, two long-term field experiments located in Germany were examined (Fig. S3-1).

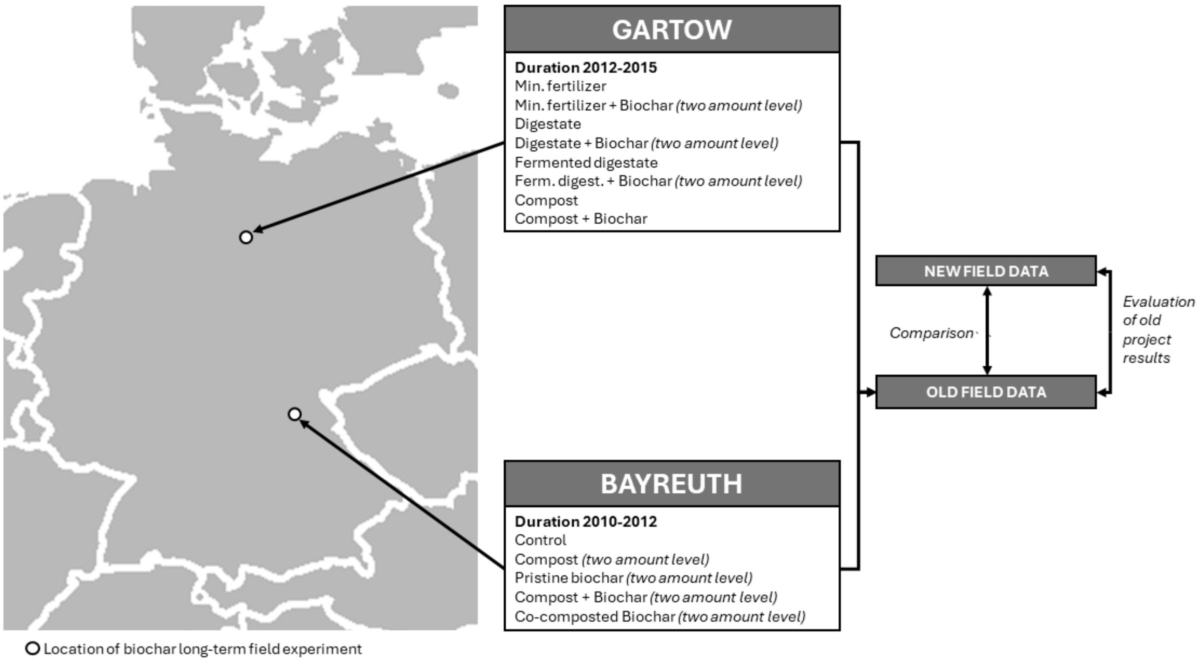


Figure S3-1: Location of the investigated biochar field experiments and the scope of this study.

Both field experiments used biochar-treated fertilizers and cover different soil conditions. Table S3-1 summarizes the main characteristics and properties of the two experimental sites.

Table S3-1: Main site characteristics, soil properties and biochar properties of the two biochar field experiments in Bayreuth and Gartow.

	Bayreuth (Northeastern Bavaria)	Gartow (Eastern Lower Saxony)
Site characteristics		
Latitude	49°56′01.7″	53°01′09.26″
Longitude	11°31′17.1″	11°29′50.04″
Precipitation [mm]	507	575
Temperature [°C]	8.2	8.8
Current use	Cropland	Cropland
Tillage depth [cm]	0–10	0–20
Soil properties [0–30 cm]		
Soil type	Cambisol	Cambisol
Soil texture	Sandy loam	Sand
Sand [%]	62	94
Silt [%]	12	4
Clay [%]	26	2
Initial SOC [%]	1.6	0.6
pH	5.4	5.7
Biochar properties		
Feedstock	Pine wood	Green cuts
Pyrolysis temperature [°C]	550	650
Total carbon [g kg^{-1}]	843	667

Total nitrogen [g kg^{-1}]	4	30
Black carbon [g kg^{-1} C]	795	259
C/N	239	75
H/C [atomic ratio]	0.11	0.1–0.2
pH [H_2O]	9.8	10.3
Ash [g kg^{-1}]	90	230

One of the two field experiments is located near Bayreuth (Donndorf) in northeastern Bavaria and was established in 2010 to analyze the effects of different organic soil amendments (OSA), consisting of biochar mixed with compost in different amounts, on soil properties and crop yields under organic farming conditions (Fig. S3-1). The experimental site covered a total area of 3600 m² (30 m x 120 m) and consists of 50 individual plots of 72 m² (6 m x 12 m), each of them arranged according to a Latin rectangle in a row-column design so that each of the ten treatments was present in each row and each pair of columns in a grid across the field (Appendix Fig. S3-1; Meyer et al. 2012). Each of the ten treatments was replicated five times and each experiment plot received a OSA treatment in July, 2010. The organic material was applied and distributed on the plots manually. Afterwards, it was incorporated to a depth of 10 cm using a rotary tiller. Biochar containing treatments were applied once, while compost was applied every experiment year until 2012. Total C input by each of the treatment is shown in Appendix Fig. S3-3. From 2012 on, all plots have been treated equally with organic fertilizers every year since then, either with maize biogas digestate using a liquid manure spreader or by broadcasting cow or horse slurry at amounts of about 15–30 Mg ha^{-1}. More details on the Bayreuth field experiment can be found in Meyer et al. (2012) and Cooper et al. (2020).

The second experimental site is located in the Wendland region, near Gartow in northern Germany (Fig. S3-1). The experiment was established in 2012 with the objective to compare different OSA from regional agricultural residues, including biochar, with synthetic fertilization in their effects on soil properties, nutrient dynamics, and crop yields. Similar to the Bayreuth site, the experiment field consisted of 50 individual plots of 72 m² (6 m x 12 m), arranged as a Latin rectangle (Appendix Fig. S3-1b; Glaser et al. 2015). Due to the space between plots which were used as driving lanes, the total area was 7200 m² (60 m x 120 m). Ten different treatments were tested in five-fold replication (Appendix Fig. S3-2). Total C input by each of the treatment is shown in Appendix Fig. S3. All biochar treatments except the ones containing of 1 Mg ha^{-1} were applied once at the beginning of the experiment in May 2012. All other treatments received annual applications for three years in spring (including 3 times 1 Mg biochar per hectare summing up to 3 Mg biochar per hectare in total after application). The organic material was applied and distributed on the plots by hand, and incorporated into the first 15 cm using a disc harrow. The experiment discontinued in fall 2014 after the last harvest and all plots have been treated with biogas digestate every year in spring since then. In 2020 and 2021, the plots were amended with compost in spring. More details on the Gartow field experiment can be found in Glaser et al. (2015).

2.2 Soil sampling and preparation

At the Bayreuth site, soil sampling was conducted twice in 2010 (immediately prior to and one month after the soil was amended in July). In 2011, 2013, 2016 and 2021 soil samplings were conducted after harvesting in fall. Between 2009 and 2016, samples were taken at two depths (0–10 cm and 10–30 cm), and in 2021 in 0–30 cm. At the center of each plot, three to five samples were taken using an auger and were then mixed into one composite sample.

At Gartow, soil sampling was conducted in 2012 (immediately before and after amendment application in May), twice in 2013 (May and September), twice in 2014 (May and September), once in 2016 and 2021. From 2012 to 2016, sampling was conducted at two soil depths (0–10 cm and 10–30 cm), whereas in 2021, samples were gathered from a unified depth of 0–30 cm. At the center of each plot, two to five samples were taken using an auger and then mixed into one composite sample.

The soil samples from both locations were dried in an oven at 40 °C for a duration of 48 hours. In preparation for further analysis, the samples were ground using a vibratory disc mill.

2.3 Soil analysis

2.3.1 Soil organic carbon and black carbon

SOC was determined by dry combustion using a CN elemental analyzer (Elementar Vario El, Heraeus, Hanau, Germany). Every sample was treated with diluted hydrochloric acid to eliminate inorganic C. Samples were measured as complete time series of each treatment to avoid a systematic offset over time.

To analyze black carbon contents, we used the BPCA method of Glaser et al. (1998), modified by Brodowski et al. (2005). Individual BPCA were isolated and measured using a Shimadzu GC 2010 gas chromatograph, equipped with a flame ionization detector and an HP5 column (30 m × 0.25 mm × 0.25 µm). The total black carbon content was determined by calculating the sum of BPCA, which was then converted into biochar equivalents using the factor 2.27 (Glaser et al. 1998). To assess the aromaticity of the samples, the relative contribution of the sum of hemimellitic, trimellitic and trimesic acid (B3CA), the sum of pyromellitic, melophanic and prehnitic acid (B4CA), benzene pentacarboxylic acid (B5CA) and mellitic acid (B6CA) was used.

2.3.2 Soil texture, bulk density, and carbon stocks

Soil texture was estimated based on the particle size distribution that was analyzed using laser diffractometry. Contents of silt, clay and SOC were then used to estimate bulk density (BD) using the pedotransfer function given in Men et al. (2008) (Equation 1).

$$Bulk\ density = 1.386 - 0.078 \times SOC + 0.001 \times Silt + 0.001 \times Clay \quad (1)$$

where BD is expressed in g cm^{-3} and the SOC, silt, and clay content in %.

The use of a pedotransfer function to estimate BD was necessary since BD was not measured in the field consecutively at each sampling date. The estimated BD, however, was compared with that measured in the field, when available, to verify its plausibility.

In this study, SOC and black carbon are expressed as stocks. Carbon stocks were quantified using an equation, provided by FAO (2019) (Equation 2).

$$Carbon\ stock = Carbon\ content \times Bulk\ density \times Depth \times 0.1 \qquad (2)$$

where the carbon stock (SOC or black carbon) is expressed in Mg ha^{-1}, bulk density in g cm^{-3}, layer thickness in cm and the carbon content (SOC or black carbon) in g kg^{-1}.

The calculation of SOC and black carbon stocks was essential for comparing data obtained from soil depths of 0–10 cm and 10–30 cm prior to 2021 with data from 2021, which was collected from a unified soil depth of 0–30 cm. In addition, with this approach it was possible to calculate the recovery of applied biochar and other OSA.

2.4 Statistical analysis

Statistical analysis was carried out using R 4.1.2 (R Core Team 2021). The differences of SOC and black carbon stocks among different sampling dates in time were analyzed using a linear mixed-effects model (random-intercept model), as the observed variable (SOC or black carbon) eventually becomes dependent through repeated measuring the same plots. In such cases, mixed-effects models should be used since we assume fixed effects (e.g., treatments and application amounts) and multiple random effects (rows, columns, and time) to influence our model results (Piepho et al. 2003). Separate mixed-effects models were carried out to analyze temporal differences between the different sampling dates in time within a certain amount level (low and high added biochar amounts). All individual treatments per field experiment were therefore aggregated according to the amount of biochar added (Appendix Fig. S3-3). In our mixed-effects model, we focused on the amount levels because according to analysis of variance (ANOVA), the individual biochar treatment structure (except for one single treatment in Gartow, see Appendix Table S3-2) showed no significant effects on SOC and black carbon stocks in Bayreuth and Gartow in 2021, respectively. What did, however, matter was the biochar amount added. Details on the conducted ANOVA and the obtained results can be found in the Supplementary Material file.

Only the time series containing biochar additions were statistically evaluated since our focus was the long-term effect of biochar, but non-biochar treatments are included in the box-plots of the results section for a visual impression of their effects on the C stocks. Each of the four time series started with the first sampling date after the addition of biochar, thus the initial SOC and black carbon stock was excluded, since we wanted to analyze the differences after the application of biochar. Additionally, the SOC and black carbon stock time series in 0-10 cm and 10-30 cm soil depth between 2010- 2013 in Bayreuth and 2012-2016 in Gartow was analyzed, to investigate vertical biochar transport with time. Only the variants with a high biochar content were used for the evaluation, as they showed pronounced effects. In addition, the black carbon data of the high biochar variants from the 2016 sampling in Gartow showed many data gaps, while the variants with a high biochar content were complete. Therefore, the 2016 black carbon data from Gartow was only used to evaluate vertical transport. Mixed-effects modelling was conducted using the R package lme4 (Bates et al. 2015). Variance components were estimated with the residual maximum likelihood (REML) method (Kenward and Roger 1997). In order to meet parametric model conditions, a Box-Cox transformation was performed in the case of non-normal model residuals. Significant effects were observed along the time series of each biochar amount level at each of the two field experiments using the estimation of least-squares means with the R package emmeans (Lenth et al. 2023). Results of this post-hoc test are provided in Table S3-3 and S3-5 of the Appendix. Significant effects are marked with one, two or three asterisks in the results tables, depending on the level of significance ($p < 0.05$*; $p < 0.01$**; $p < 0.001$***).

3. Results

3.1 Effects of different biochar amounts on temporal soil organic carbon dynamics

The addition of OSA containing low biochar amounts (9 Mg ha^{-1}; total C inputs are shown in Appendix Fig. S3-3) led to an initial median SOC stock increase of 17 Mg ha^{-1} at the Bayreuth site (Fig. S3-2; from 54 to 71 Mg ha^{-1}, +31%). In the following three years, the median SOC stock slightly decreased (−9 Mg ha^{-1}, -13%) and increased again between 2013 and 2021 (from 63 to 76 Mg ha^{-1}, +21%), but both changes were not significant (Table S3-2). A comparable temporal dynamic was observed when high biochar levels (31.5 Mg ha^{-1}) were added. Initially, the median SOC stock increased by 38 Mg ha^{-1} (from 54 to 92 Mg ha^{-1}, +70%) and remained at the same level until 2021 (Fig. S3-2). At the Gartow site, OSA additions containing 3 Mg ha^{-1} of biochar initially led to an SOC stock increase of 8 Mg ha^{-1} (from 21 to 29 Mg ha^{-1}, +38%). The SOC stock significantly decreased by 17% between 2012 and 2014 to 24 Mg ha^{-1} (Fig. S3-2, Table S3-2, and Appendix Table S3-4). Between 2014 and 2016, the SOC stock remained at almost the same level and increased slightly but not significantly between 2016 and 2021 (from 24 to 28 Mg ha^{-1}, +17%). The addition of OSA mixed with 40 Mg ha^{-1} of biochar led to an overall SOC stock increase of 61 Mg ha^{-1} (from 22 to 83 Mg ha^{-1}, +277%). One year later, in 2013, the SOC stock dropped by 31 Mg ha^{-1} and continued to significantly decrease by 37% between 2013 and 2014 to 41 Mg ha^{-1} (Fig. S3-2). Between 2014 and 2016, the median SOC stock slightly increased to 46 Mg ha^{-1} (+12%), but this change was not significant. In the following five years, the median SOC stock dropped significantly by another 17 Mg ha^{-1} (-63%).

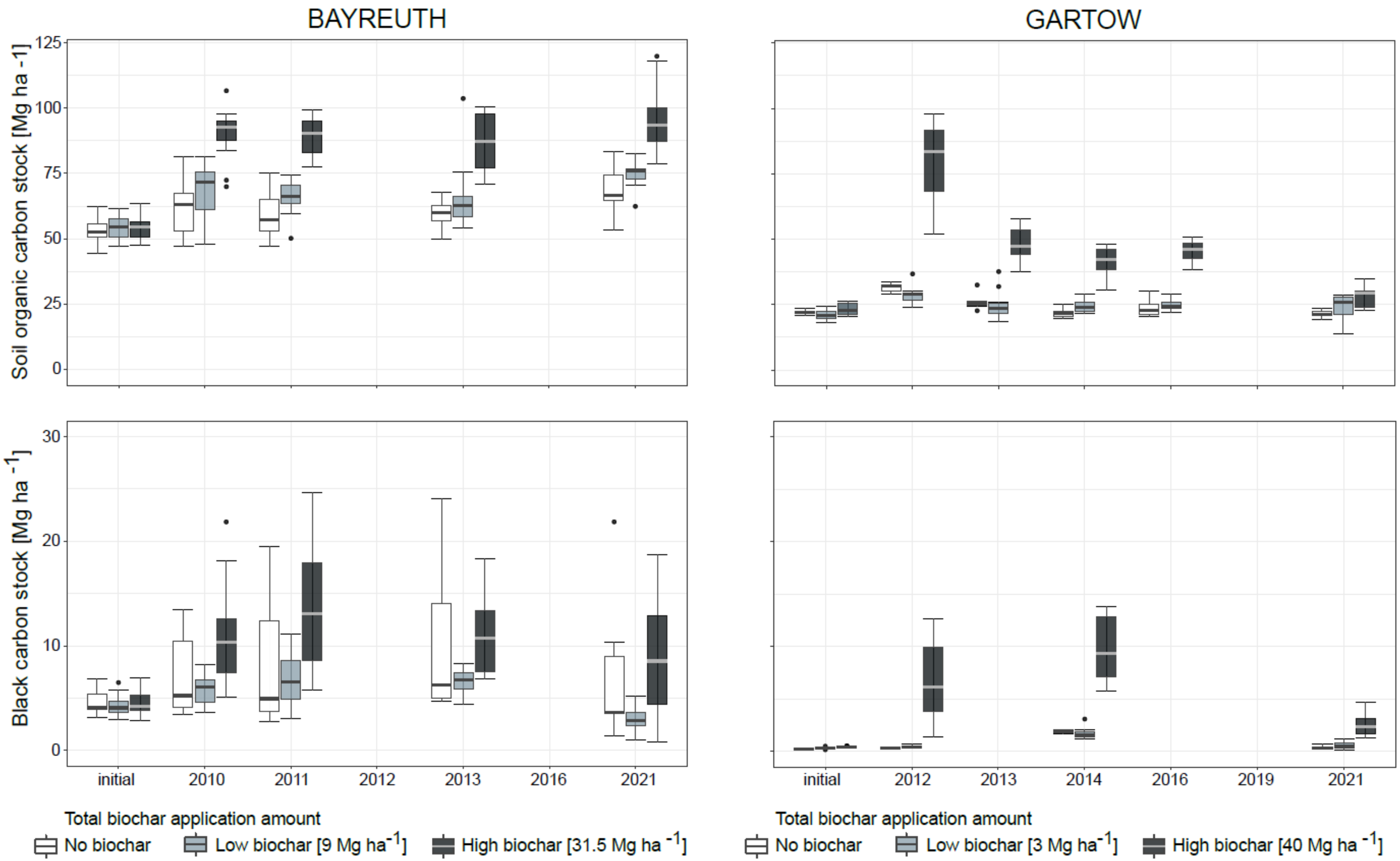


Figure S3-2: Box plots of soil organic carbon stock and black carbon stock time series in the Bayreuth and Gartow field experiment (soil organic carbon stock data from fall 2013 and 2014 in Gartow are not shown). The median of the data is shown as a horizontal solid line within the box. Each box contains the middle 50% of the data of a category. The whiskers indicate the lower and upper quartile of the data, respectively, and are limited to 1.5 times the interquartile range. Black dots outside the whiskers represent outliers.

Table S3-2: Results of linear mixed-effects model analysis of the soil organic carbon stocks time series at both locations. Separate mixed-effects models were carried out for each amount (low and high) to analyze the effect of the different sampling dates in time (year). numDf = degrees of freedom in the numerator; denDf = degrees of freedom in the denominator.

	numDf	denDf	F value	p value
Bayreuth				
Low biochar (9 Mg ha^{-1})				
Row	4	6	3.59	0.08
Column	4	6	9.79	<0.01**
Year	3	42	0.35	0.79
High biochar (31.5 Mg ha^{-1})				
Row	4	6	4.20	0.06
Column	4	6	2.09	0.20
Year	3	42	1.62	0.20
Gartow				
Low biochar (3 Mg ha^{-1})				
Row	4	5	0.91	0.52
Column	4	50	0.41	0.80
Year	6	50	3.43	<0.05*
High biochar (40 Mg ha^{-1}) - Box-Cox transformed				
Row	4	6	1.45	0.33
Column	4	6	1.29	0.37
Year	6	84	37.99	<0.001***

3.2 Effects of different biochar amounts on temporal black carbon dynamics

At the Bayreuth field experiment, the addition of OSA containing low biochar amounts increased the black carbon stocks initially by 200% (2 Mg ha^{-1} to 6 Mg ha^{-1}) (Fig. S3-2). In the following three years, the black carbon stock remained stable (Appendix Table S3-5). Initially, the addition of 31.5 Mg ha^{-1} of biochar combined with co-amendments significantly increased the black carbon stock by 50% (8 Mg ha^{-1} to 12 Mg ha^{-1}), increased the stock again by 3 Mg ha^{-1} in the following year (+25%), and led to a significant drop back to 12 Mg ha^{-1} (-20%) in 2013 (Table S3-3 and Appendix Table S3-5). Between 2013 and 2021, the black carbon stock remained stable. The addition of 3 Mg ha^{-1} mixed with co-amendments on the sandy soil in Gartow did not change black carbon stocks significantly (Fig. S3-2). The addition of OSA containing 40 Mg ha^{-1} of biochar increased the black carbon stock by 28% (7 Mg ha^{-1} to 9 Mg ha^{-1}) and did not change significantly over the next two years. In the long-term, however, the median black carbon stock dropped by 56% (5 Mg ha^{-1} to 4 Mg ha^{-1}) and was therefore just slightly higher than initially before the application (2 Mg ha^{-1}).

Table S3-3: Results of linear mixed-effects model analysis of the black carbon stocks time series at both locations. Separate mixed-effects models were carried out for each amount (low and high) to analyze the effect of the different sampling dates in time (year). numDf = degrees of freedom in the numerator; denDf = degrees of freedom in the denominator.

	numDf	denDf	F value	p value
Bayreuth				
Low biochar (9 Mg ha^{-1})				
(Intercept)	1	42.00	4175.17	<0.001***
Row	4	6.00	0.41	0.80
Column	4	6.00	7.25	<0.05*
Year	3	42.00	77.94	<0.001***
High biochar (31.5 Mg ha^{-1}) - Box-Cox transformed				
(Intercept)	1	42.00	7405.61	<0.001***
Row	4	6.00	3.69	0.08
Column	4	6.00	1.14	0.45
Year	3	42.00	5.37	<0.001***
Gartow				
Low biochar (3 Mg ha^{-1})				
(Intercept)	1	23.00	1057.14	<0.001***
Row	4	5.00	0.71	0.62
Column	4	23.00	0.26	0.90
Year	3	23.00	11.45	<0.001***
High biochar (40 Mg ha^{-1})				
(Intercept)	1	42.00	235.22	<0.001***
Row	4	6.00	2.23	0.18
Column	4	6.00	0.46	0.76
Year	3	42.00	7.75	<0.001***

3.3 Effects of biochar additions on temporal soil organic carbon and black carbon dynamics in different soil depth

After the addition of OSA containing high biochar amounts (31.5 Mg ha^{-1} in Bayreuth and 40 Mg ha^{-1} in Gartow), SOC and BC stocks in 0-10 cm significantly decreased with time. In Bayreuth, the SOC stock of 52.8 Mg ha^{-1} in 2010 after the addition of biochar decreased by 42% to 30.45 Mg ha^{-1} in 2013. Black carbon stocks decreased by 67% from 8.54 to 2.79 Mg ha^{-1} in the same time period. In Gartow, the SOC stocks in 0-10 cm decreased from 34.27 to 17.08 Mg ha^{-1}, corresponding with 50% between 2012 and 2016. Black carbon stocks decreased by 68% from 7.27 to 2.33 Mg ha^{-1}. In 10-30 cm soil depth, SOC and black carbon stocks significantly increased with time at both locations. In Bayreuth, SOC stocks increased by 30% from 39.63 to 51.58 Mg ha^{-1}, while black carbon stocks increased by 135% from 3.25 to 7.36 Mg ha^{-1}. In Gartow, SOC stocks in 10-30 cm increased by 58% between 2012 and 2016 while black carbon stocks increased by 1010% from 0.28 to 3.11 Mg ha^{-1}.

Table S3-4: Median soil organic carbon (SOC) stocks and black carbon (BC) stocks of the organic soil amendments containing of high biochar amounts (31.5 Mg ha^{-1} and 40 Mg ha^{-1}) in two soil depths 0-10 cm and 10-30 cm. SE = standard error. Different letters indicate significant differences between the years.

	Soil depth			
	0-10 cm		10-30 cm	
	SOC stocks±SE	BC stocks±SE	SOC stocks±SE	BC stocks±SE
	Mg ha^{-1}			
Bayreuth				
Year				
2010	52.80 ± 1.92a	8.54 ± 0.35a	39.63 ± 1.69a	3.25 ± 0.80a
2011	56.24 ± 2.08a	12.39 ± 1.37b	38.08 ± 3.35a	2.80 ± 0.57a
2013	30.45 ± 1.17b	2.79 ± 0.67c	51.58 ± 2.08b	7.36 ± 0.65b
Gartow				
Year				
2012	34.27 ± 4.31a	7.27 ± 1.63a	15.99 ± 0.45a	0.28 ± 0.03a
2014	24.73 ± 2.41a	1.06 ± 0.69b	15.03 ± 0.86a	1.65 ± 0.86b
2016	17.08 ± 1.39b	2.33 ± 0.27b	25.31 ± 1.90b	3.11 ± 0.47b

3.4 Long-term effects of different biochar treatments on biochar quality

Biochar of the Bayreuth field experiment contained three time as much black carbon compared to the biochar used for the Gartow field experiment (Table S3-1), indicating a higher biochar stability of the former. In addition, the relative contribution of higher aromatic BPCA (B5CA and B6CA) increased between 2010 and 2021 in all high biochar treatments at Bayreuth (Fig. S3-3). Apart from the biochar (40) digestate, the relative contribution of higher aromatic BPCA slightly decreased over time between 2012 and 2021 in all treatments containing high biochar amounts of the Gartow field experiment (Fig. S3-3).

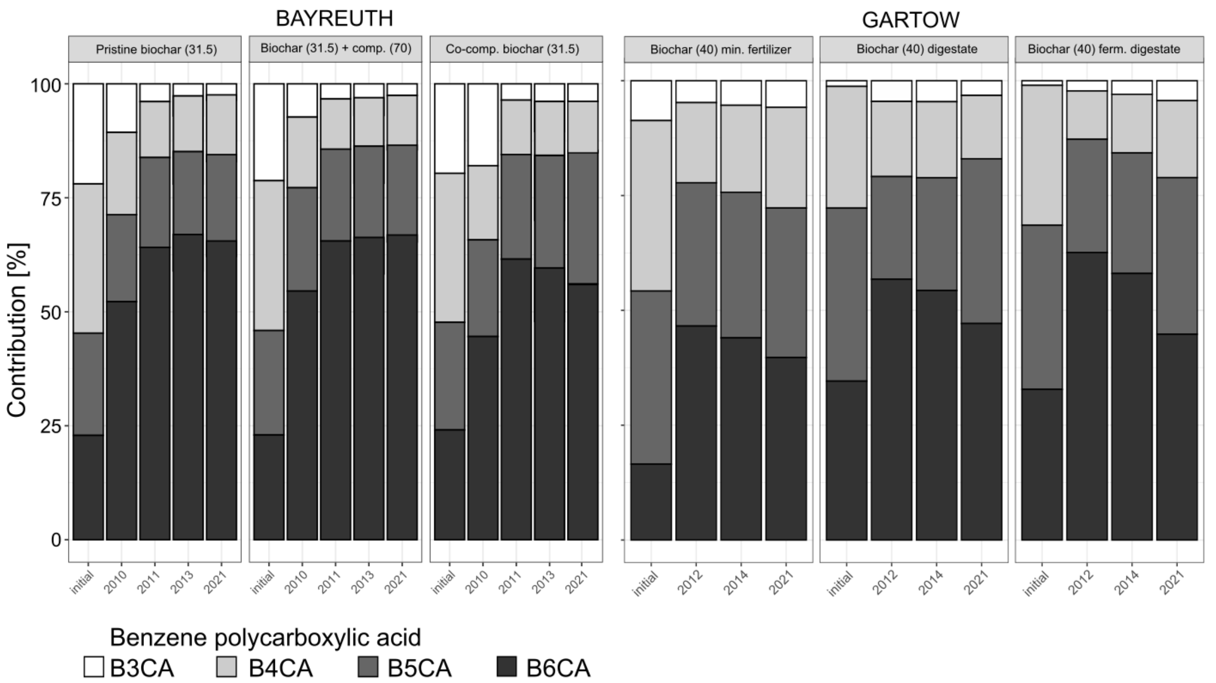


Figure S3-3: Relative contribution of the sum of hemimellitic, trimellitic and trimesic acid (B3CA), the sum of pyromellitic, melophanic and prehnitic acid (B4CA), benzene pentacarboxylic acid (B5CA) and mellitic acid (B6CA) to the sum of all BPCA of each of the treatments containing high biochar amounts of both field experiments.

4. Discussion

4.1 Long-term effects of biochar treatments on SOC stock changes

It is known that the addition of biochar increases SOC stocks (Gross et al. 2021; Huang et al. 2023), however, little is known about the long-term effects under real agronomic field conditions. In a recently published meta-analysis, the longest observation duration under field experiment conditions was ten years (Gross et al. 2021). According to this study, SOC stocks of biochar-amended soils were significantly higher than the control soil after nine and eleven years, but only if high amounts of 31.5 Mg ha^{-1} of biochar at the Bayreuth site and 40 Mg ha^{-1} of biochar at the Gartow site were added (Appendix Table S3-1). However, both sites investigated showed contrasting SOC stock dynamics over time (Fig. S3-2). At the Bayreuth site, the initial SOC stock increases induced by high amounts of biochar addition could be stabilized over time (Fig. S3-2). Black carbon stocks slightly decreased within the same time period, indicating that the additional SOC must not have originated from the biochar itself. This finding corroborates with results of Blanco-Canqui et al. (2020), who made similar observations on a loamy soil under reduced tillage in southwestern Iowa. Negative priming has previously been found to be positively correlated with soil clay content and the pyrolysis temperature of the biochar (Ding et al., 2018). Maestrini et al. (2015) found that with increasing time, biochar could further enhance SOC accumulation through adsorption and physical protection of dissolved organic C. Whether the SOC buildup observed in our study was due to negative priming effects, the initial high compost application (70 Mg ha^{-1}) or the co-composting of the biochar, or due to the organic farming practices including reduced tillage and annual manure input after discontinuation of the experimental treatment, could not be determined certainly, since the SOC stocks of the high biochar- containing OSA showed a large dispersion (Appendix Table S3-3). What could be concluded however, was that the initial SOC build-up by the biochar reached a new and high plateau, which has been stabilized over a period of eleven years. In contrast, the Gartow experiment showed pronounced SOC dissipation over time at both amounts of added biochar (3 and 40 Mg ha^{-1}). Polifka et al. (2018) showed that the more biochar was added, the more CO_2 was released, up to 60%. However, most of this CO_2 was C4-derived and therefore, not due to biochar or SOC. More likely, the elevated CO_2 stems from mineralization of the maize-based biogas digestates and C4 plant residues, since maize was cultivated in the years before the gas measurement. Due to the same C3-isotope composition of soil organic matter and the applied biochar, it is not possible to differentiate between biochar-derived and soil organic matter-derived emitted CO_2. However, compared to the high amount of biochar added (40 Mg ha^{-1}), additional CO_2 release from biochar and SOC was negligible (about 0.1 Mg ha^{-1}). There is still no consensus about how the added biochar affects the priming of native SOC due to the very complex interplay between various biochar treatments, soil properties and the soil microbial community (Rasul et al. 2022). These contrasting SOC stock dynamics observed in our data might, therefore, be related to the different soil properties, and the different biochar properties of the two field experiments, especially the higher polycondensed aromatic carbon content of the biochar used at the Bayreuth experiment.

4.1.1 Influence of location properties on SOC stock changes

Most obviously, both locations show a different soil texture. While the soil at the Bayreuth site contains 12% silt and 26% clay, the soil texture at the Gartow site is dominated by 95% sand. Texture is one of the most important influencing factors of amendment-induced SOC stabilization (Han et al. 2016; Berhane et al. 2020; Gross and Glaser 2021; Gross et al. 2021). Soils with a finer texture have higher amounts of clay minerals and iron oxides. These components not only protect SOC from enzymatic breakdown and turnover as noted by Nannipieri et al. (2018) and Zhang et al. (2019), but also increase SOC stability in the form of physical protection within soil aggregates, according to Lal (2018) and Zong et al. (2018). Conversely, sandy soils offer less of this physical protection, making SOC more susceptible to oxidation and decomposition (Gross et al. 2021), which leads to positive SOC priming (Rasul et al. 2022), along with losses of SOC through leaching and runoff (Yang et al. 2019). On average, the increases in SOC stock post-biochar application were higher in silty (13 Mg ha$^{−1}$ and 31%), loamy (15 Mg ha$^{−1}$ and 25%), and clayey soils (18 Mg ha$^{−1}$ and 52%) compared to sandy soils (6 Mg ha$^{−1}$ and 21%) (Gross et al. 2021).

In principle, our results support the findings of the mid-term SOC effects that were found at the Bayreuth site (Cooper et al. 2020) and the Gartow site (Greenberg et al. 2016b). The latter study found that the co-amendment of fertilizers had no effect on SOC after four years, which we now can confirm after a duration of nine years (Appendix Table S3-1). Greenberg et al. (2016b) additionally analyzed SOC in different aggregate size fractions and found slight SOC increases after the application of 40 Mg ha^{-1} of biochar in the 0.25–0.053 mm and < 0.053 mm fractions, the most stable aggregate size fractions. Since they did not differentiate between unpyrolized and pyrolized organic C, they argued that increased content of SOC in the fine fractions might be related to higher crop yields and thus higher plant-derived inputs to SOC. Based on our results, we can support this theory, as not only bulk SOC but also bulk black carbon disappears in the long-term, seemingly contradicting a stabilization of black carbon in the fine fraction of the Gartow soil, which is reasonable, as it is dominated by sand. In contrast to that, Cooper et al. (2020) demonstrated that only high biochar application amounts of 31.5 Mg ha^{-1} could stabilize SOC in the stable < 0.053 mm fraction, both in 0–10 cm and 10–30 cm soil depth, six years after application. This corroborates our findings that only high amounts of biochar showed significant effects on SOC sequestration after eleven years (Appendix Table S3-1).

4.1.2 Influence of biochar properties on SOC stock changes

Other dominant factors influencing amendment-induced SOC sequestration are the biochar properties. The biochar used at the Bayreuth site was made of wood and showed a higher content of C, black carbon, and a higher C/N ratio than the biochar used at the Gartow site, which was made of green cuts. The C and N content in biochar, along with C/N ratio, are critical factors determining the stability of biochar and its ability to contribute to SOC build-up (Liu et al. 2016), with higher biochar C contents leading to more C input and therefore enhanced SOC sequestration potential. Conversely, OSA with higher N contents, resulting in lower C/N ratios, may lead to an increase in soil respiration and CO_2 emissions due to the greater availability of N and enhanced rates of microbial C mineralization (Huang et al. 2004; Zou et al. 2004). Another decisive property to describe biochar stability is the H/C ratio (Schimmelpfennig and Glaser 2012; Budai et al. 2013). The H/C ratio is an indicator for the aromaticity of biochar. Compared to uncharred biomass, which typically possesses higher H/C ratios, biochar with low ratios is expected to be more stable in the long-term (Budai et al. 2013). The fact that the biochar used at both sites showed a high stability based on the low H/C ratio results indicated that the stability of biochar must also be assessed in the context of its intended use and location.

4.2 Long-term effects of different biochar treatments on black carbon stocks

Little is known about the long-term fate of black carbon in agricultural soils after the addition of biochar. We used black carbon as a molecular marker for the amount and quality of biochar in both soils. However, the amount of black carbon and biochar is not identical due to a conversion factor of 2.27 that we used to multiply the sum of BPCA into biochar equivalents (Glaser et al. 1998). For higher accuracy, individual conversion factors for individual fresh and aged biochar should be determined in the future, which was beyond the scope of this study. Nevertheless, differing black carbon stocks can be used as a relative indicator for altering biochar stability because it specifically traces the stable polyaromatic backbone of biochar (Glaser et al. 1998). Significant effects of biochar addition on the black carbon stocks in 2021 were only observable, when at least 31.5 Mg ha^{-1} of biochar was added (Appendix Table S3-2). By looking at the black carbon time series at the Bayreuth and Gartow sites (Fig. S3-2), it could be seen that both the low amount and high amount of biochar were affected by dissipation; in Bayreuth, however, much weaker and with large data variability.

Not just the black carbon stock itself can be used as an indicator for altering biochar stability, but also the relative contribution of individual BPCA. Larger shares of B5CA and B6CA reflect higher degrees of aromatic condensation (Glaser et al., 1998), stronger oxidation resistance and are often related to higher pyrolysis temperature (Chang et al. 2019), and thus higher stability. The relative contribution of B5CA and B6CA increased between 2010 and 2021 in all treatments containing high biochar amounts at the Bayreuth site, while, apart from the biochar + digestate treatment, all treatments containing high biochar amounts of the Gartow field experiment slightly lost higher aromatic BPCA (Fig. S3-3). In the case of the Bayreuth field experiment, this suggests that black carbon stocks may be heading towards a long-term steady state, as the more stable compounds may prevail in the long-term.

At the Gartow site, however, the slight loss of higher aromatic compounds was probably not due to biochar decay, since the H/C ratio indicated a high stability but was more likely to be related to the sandy soil texture in Gartow and the associated lack of physical stabilization and protection against biochar movement (Polifka et al. 2018), e.g., through vertical transport. This finding agrees with Wang et al. (2023), who found subsoil accumulation of B5CA and B6CA compounds after the use of biochar.

4.2.1 Influence of biochar oxidation and metabolization on black carbon stocks

During biochar aging, oxidation processes typically decline biochar C compounds (Li et al. 2019). These oxidation processes may lead to biochar degradation, but the adsorption of organic materials and inorganic materials increase the protection of biochar and thus the stability of biochar's aromatic backbone (Nguyen et al. 2008; Hagemann et al. 2017). Such an organic coating is typical for aged co-composted biochar particles (Hagemann et al. 2017).

At the Bayreuth site, the co-composted biochar treatment affected black carbon stocks similarly like the pristine biochar and biochar mixed with compost did (Appendix Table S3-2) and showed a comparable BPCA pattern over time (Fig. S3-3). Therefore, there was no evidence for enhanced biochar oxidation due to compost-induced increase of microbial activity. Higher overall black carbon stocks of the co-composted biochar treatment suggests that the organic coating associated with co-composted biochar particles might have led to biochar stabilization (Appendix Table S3-3), which could, however, not be statistically corroborated, since there was no significant difference between the biochar-containing OSA treatments (Appendix Table S3-2).

It could not be ruled out that biochar metabolization led to molecules not visible by our analytical procedure, such as free BPCA as biochar metabolites, which may be stabilized into soil organic matter (Di Rauso Simeone et al. 2018). Evidence of such stabilization was observed in the diminishing quantities of biochar over time, as suggested by black carbon analysis, alongside an increase in the relative presence of more highly aromatic black carbon compounds (Fig. S3-3), with SOC levels remaining relatively stable (Fig. 4-2). Co-composting with biochar significantly improves the accessibility of nutrients on the biochar's surface (Hagemann et al. 2017), potentially facilitating co-metabolism. Once these surface nutrients are depleted, microorganisms begin to "mine" for new sources of N and P, while releasing enzymes that break down organic matter under stress (Whitman et al. 2015). This co-metabolic degradation becomes crucial when there is no mineral-organic interaction to stabilize the soil, which can be the case in very sandy soil environments (Polifka et al. 2018). In soils with low clay content, like in Gartow, the loss of C following the addition of large amounts of biochar can reach up to 20%, a significantly higher rate than in soils with high clay content (Wang et al. 2016). In the Gartow experiment, fermented digestate combined with 40 Mg ha^{-1} of biochar led to significantly higher black carbon stocks (Appendix Table S3-3). Additional fermentation of digestate reduces the amount of easily degradable organic matter and thus the availability of nutrients, which could have diminished co-metabolic degradation of biochar.

4.2.2 Influence of lateral and/or vertical biochar particle transport on black carbon stocks

Biochar particles are usually more susceptible to vertical and/or lateral transport than mineral soil particles. This movement is largely influenced by water flow, wind, and soil macrofauna activity. The downward movement of biochar is particularly influenced by tillage and the physical structure of the soil (Obia et al. 2017), the amount of rainfall and hydraulic conductivity (Major et al. 2010; Obia et al. 2017), as well as bioturbation (Major et al. 2010). Obia et al. (2017) found that between 9 and 19% of the total loss of biochar in general is due to vertical transport. Downward movement of biochar with time could be observed in both experiments (Table S3-4). Black carbon stocks significantly increased in the 10-30 cm layer while biochar dissipated in the 0-10 cm layer. As biochar moves into deeper soil layers, it continues to participate in carbon sequestration. We found that SOC stocks in the subsoil layer 10-30 cm increased with progressing time, confirming this theory. Biochar particles can enhance C sequestration even additionally through the formation of inorganic carbon in the subsoil (Wang et al. 2023).

Lateral biochar movement, which is predominantly due to wind erosion and surface water runoff, is also an important transport pathway and can account for 20 to 53% of total dissipation (Major et al. 2010). To quantify the impact of lateral transport of biochar on the experimental results, we looked at potential pathways (Supplementary Dataset). We compared SOC and black carbon stocks between plots that received no biochar at all and plots that received high amounts of biochar in both experiments. At the Bayreuth site, nearly all biochar-free-plots adjacent to a plot which received high amounts of biochar (31.5 Mg ha^{-1}) exhibited increases in SOC and black carbon stocks over time (Supplementary Dataset), indicating lateral transport. In Gartow, similar lateral movement could not be observed. SOC and black carbon stocks showed no specific trend between biochar-free-plots adjacent to a plot which received high amounts of biochar (40 Mg ha^{-1}). This could indicate that vertical biochar particle transport is more important in a very sandy soil matrix.

In order to investigate this effect systematically, quantitatively, and statistically, an experimental design focusing on transport dynamics is needed, or systematic sampling outside the biochar plots, at increasing distances with sufficient repetition. As such biochar particle migration takes time, we expect on the one hand to find significant amounts of biochar outside the experiment plots and on the other hand, the SOC stocks to increase in deeper soil regions in the long-term, which should be studied in the future. To date, there is a lack of long-term field experiments that could deliver proof of concept.

4. Conclusions

So far, there is lack of evidence on the fate of SOC and black carbon stocks under field experiment conditions, on a decadal time scale under realistic field conditions. Moreover, previous approaches trying to quantify SOC stock differences and biochar loss and migration rates are characterized by high uncertainties, have not been tested in more than one agroecosystem and are not based on long-term observations. The bottom line is, therefore, that it is difficult to generalize SOC and biochar dynamics. In this study, we present results of long-term biochar field experiments, conducted in two contrasting agroecosystems in Germany.

Our study indicates that it depends on soil and biochar properties such as soil texture and the black carbon content of the biochar, whether SOC stocks are stable in the long-term and biochar dissipation can be mitigated. Under loamy soil conditions and with the usage of C-rich wood-based biochar, the initial SOC stock increases were stable over a time frame of eleven years and thus SOC sequestration was confirmed. In contrast, the observations on the sandy soil made over nine years and under the use of biochar with a lower C/N ratio and a lower content of stable poly-condensed aromatic moieties were characterized by large SOC and black carbon losses, seemingly related to lacking physical protection and vertical biochar particle transport. According to black carbon stock results, considerable biochar loss was observed in both soils, which may be related to multiple dissipation processes occurring at the same time, such as oxidation and/or co-metabolic decomposition, or vertical and lateral particle transport. However, persisting high SOC levels at the Bayreuth site despite decreasing black carbon levels indicate biochar stabilization even if black carbon detection was not always possible. This study was able to demonstrate that the re-sampling of long-term biochar field experiments provided insights into the long-term behavior of SOC stocks and biochar. The observed dynamics should be further validated in future sampling.

Additionally, future studies should disentangle the different dissipation pathways and their impact on SOC sequestration. More experimental proof is necessary on how the long-term fate of biochar induced SOC stock increases are influenced by biochar properties and the respective agroecosystem, with unique soil properties and agricultural management decisions. Without a broad empirical basis, these findings are not transferable into agronomic practice.

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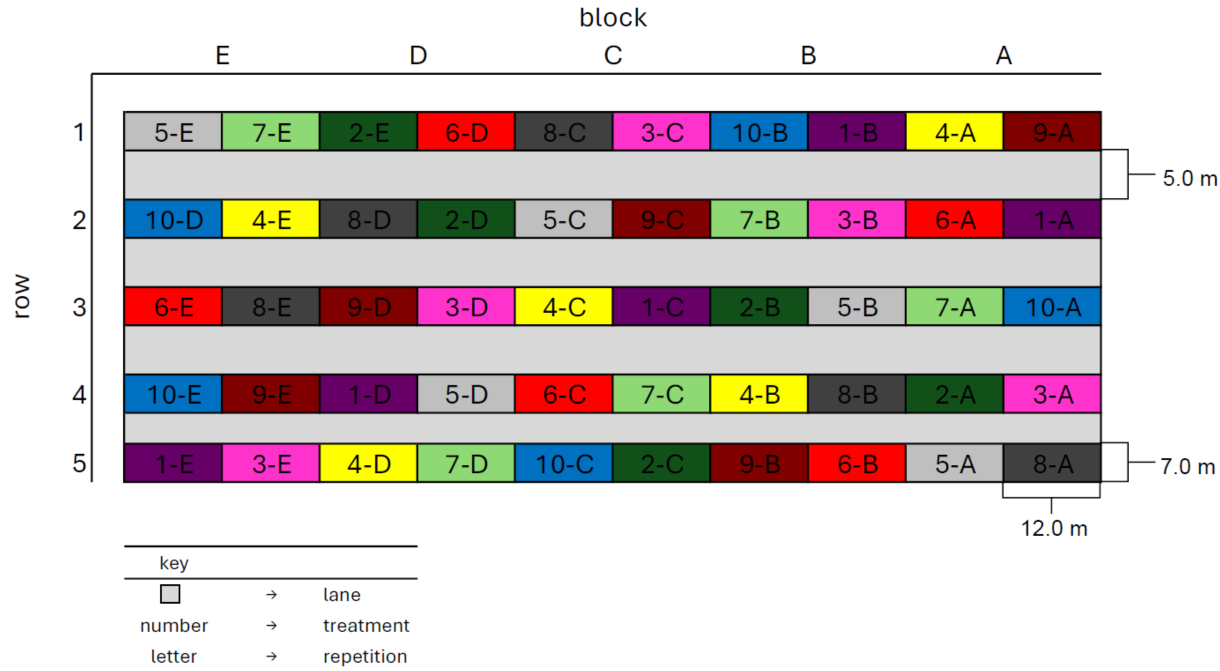
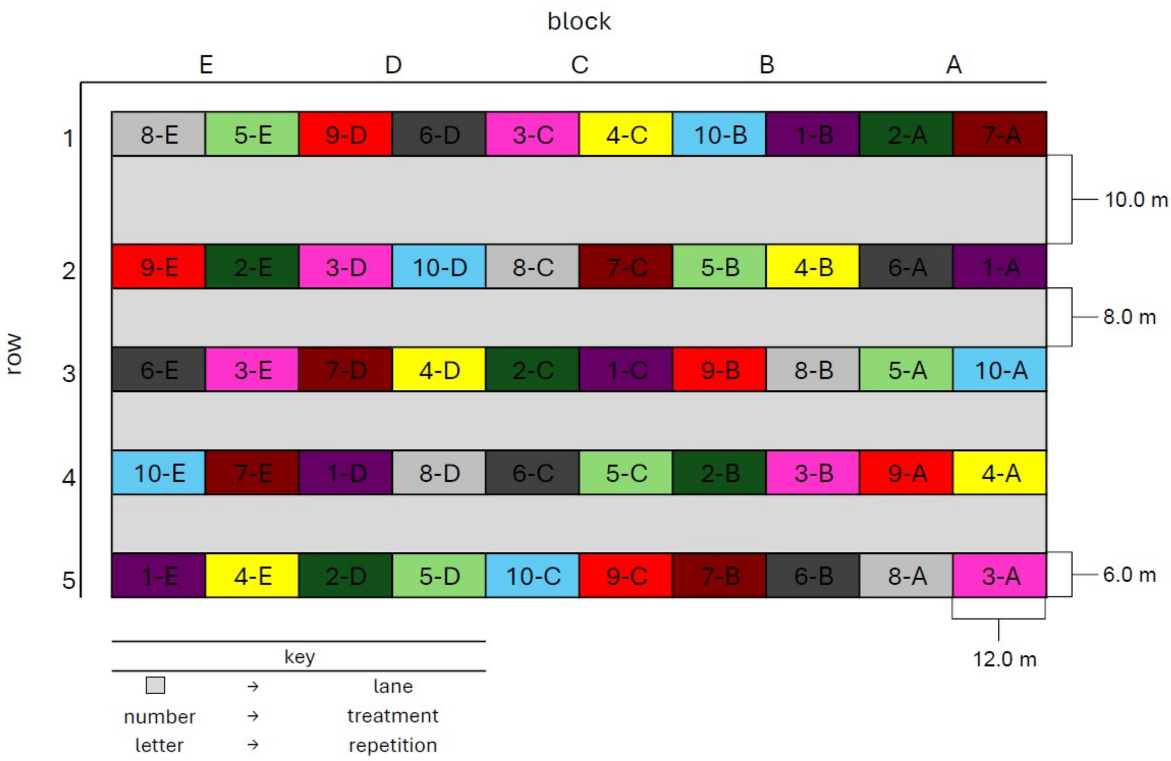
Appendix



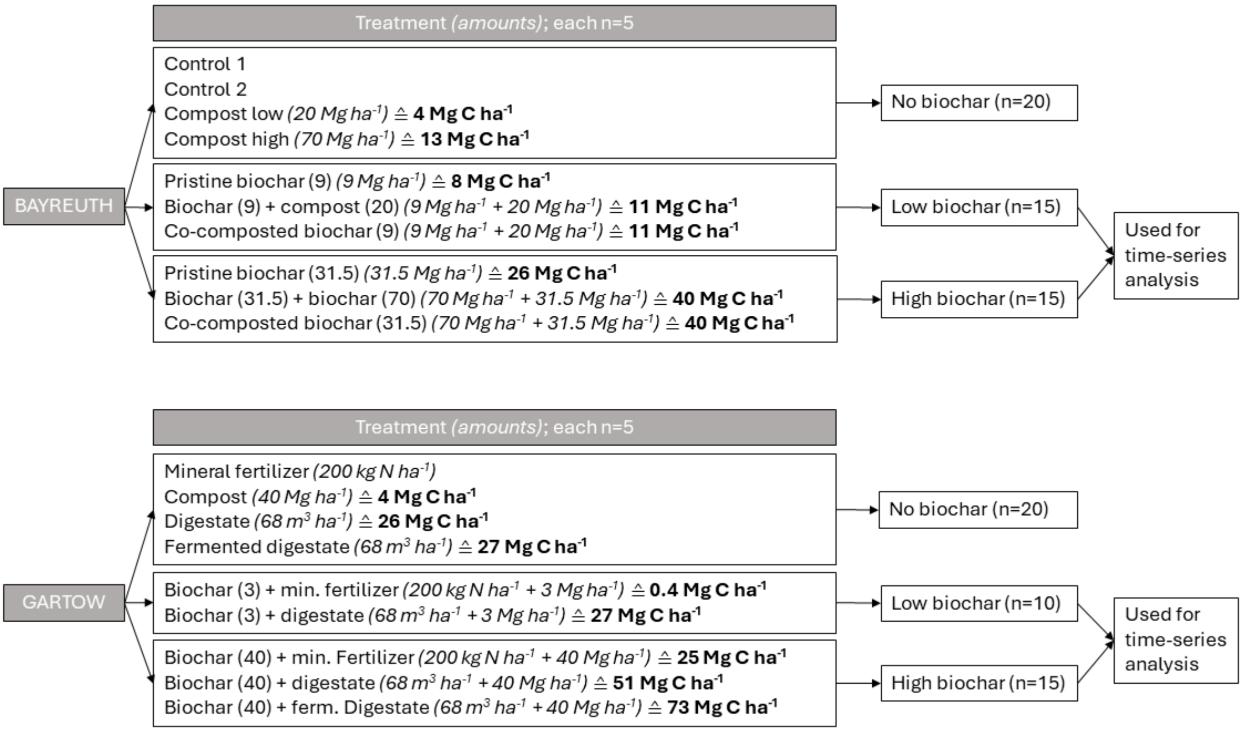
Treatment number	Treatment	Fertilizer	Biochar Mg ha^{-1}
1	Control 1	-	-
9	Pristine biochar (9)	-	9
5	Compost (20)	Compost	-
2	Biochar (9) + Compost (20)	Compost	9
10	Co-composted biochar (9)	Compost	9
6	Control 2	-	-
4	Pristine biochar (31.5)	-	31.5
8	Compost (70)	Compost	-
3	Biochar (31.5) + Compost (70)	Compost	31.5
7	Co-composted biochar (31.5)	Compost	31.5

Appendix Figure S3-1: Design and treatments of the Bayreuth biochar field experiment. The plot number in the experiment rectangle refers to the treatment type, which can be identified in the table. The plot letter refers to the number of repetitions.



Treatment number	Treatment	Fertilizer	Biochar
			Mg ha^{-1}
1	Mineral fertilizer	Min. fertilizer	-
9	Biochar (3) + min. Fertilizer	Min. fertilizer	3
5	Biochar (40) + min. Fertilizer	Min. fertilizer	40
2	Digestate	Maize digestate	-
10	Biochar (3) + digestate	Maize digestate	3
6	Biochar (40) + digestate	Maize digestate	40
4	Fermented digestate	Maize digestate	-
8	Biochar (40) + ferm. digestate	Maize digestate	40
3	Compost	Compost	-
7	Biochar (10) + compost	Compost	10

Appendix Figure S3-2: Design and treatments of the Gartow biochar field experiment. The plot number in the experiment rectangle refers to the treatment type, which can be identified in the table. The plot letter refers to the number of repetitions.



Appendix Figure S3-3: Aggregation of individual treatments and their input amounts of both field experiments for usage in time series analysis. Total C contents of the two co-composted biochar treatments of the biochar field experiment were not measured and thus, total C inputs can not be calculated.

Analysis of variance of the 2021 dataset

We conducted analysis of variance (ANOVA) to find out whether the combination of biochar with co-amendments still have an effect, nine and eleven years after the application at both locations. After visual inspection of boxplots of the results of the latest sampling date (2021), ANOVA was performed. As a precondition, homogeneity of variance was checked using Levene`s test. After each ANOVA, normality of residuals was checked using the Shapiro-Wilk test. To maintain a balanced design in the ANOVA model, outlier values were imputed using the mean of the remaining four replicate values of the same treatment. Since that affected only three SOC and two black carbon values, this was conducted instead of using unbalanced ANOVA models. Separate ANOVAs were carried out to test the biochar addition effect and the fertilizer effect, their interaction, as well as the influence of rows and columns in the field, according to the Latin rectangle design of both field experiments. Stepwise model reduction was performed, in accordance with Greenberg et al. (2019a), Greenberg et al. (2019b) and Cooper et al. (2020), including first, the elimination of non-significant interactions and second, non-significant main effects irrespective of the row and column design (Crawley, 2012). Significant effects are marked with one, two or three asterisks in the results tables, depending on the level of significance ($p<0.05$ =*; $p<0.01$**; $p<0.001$***).

Long-term effects of different biochar treatments on SOC stocks

The addition of 31.5 Mg ha^{-1} biochar at the Bayreuth site significantly increased SOC stocks in the long-term (11 years). The initial co-addition of compost or biochar co-composting did not have an additional effect (Appendix Table S3-1), probably because since 2012 all plots have been treated equally with organic fertilizers in each of the following years. The addition of 9 Mg ha^{-1} biochar on the same site did not have an increasing effect on SOC stock after eleven years.

Nine years after application, organic amendments containing of 40 Mg ha^{-1} biochar experiment resulted in significant SOC stock increase at the Gartow site (Appendix Table S3-1) while low amendment amounts (3 Mg ha^{-1}) showed no significant differences (Table S3-3, Fig. S3-2).

Appendix Table S3-1: Results of analysis of variance for the effect of row and column in the field, treatment type, co-amendment type, biochar application, and their interaction on the soil organic carbon stocks in 0-30 cm soil depth of both field experiments. Model simplification was carried out with stepwise elimination of non-significant factors, independent of field experiment design factors row and column. Df = degrees of freedom.

	Df	Sum of Squares	Mean Square	F ratio	p value
Bayreuth					
No biochar					
Row	3	442.95	147.65	5.82	<0.05*
Column	4	127.22	31.81	1.25	0.3967
Compost	2	31.88	15.94	0.63	0.5710

Residuals	5	126.89	25.38		
Low biochar (9 Mg ha^{-1})					
Row	4	2868.90	717.22	5.81	<0.05*
Column	4	857.26	214.31	1.74	0.07
Biochar	1	1012.52	1012.52	8.20	<0.05*
Residuals	10	1234.59	123.46		
High biochar (31.5 Mg ha^{-1})					
Row	4	2868.90	717.22	5.81	<0.05*
Column	4	857.26	214.31	1.74	0.07
Biochar	1	1012.52	1012.52	8.20	<0.05*
Residuals	10	1234.59	123.46		
Gartow					
No biochar					
Row	4	6.79	1.70	0.04	1.00
Column	4	301.96	75.49	1.76	0.30
Treatment	2	239.39	119.70	2.79	0.17
Residuals	4	171.67	42.92		
Low biochar (3 Mg ha^{-1})					
Row	4	73.43	18.36	0.46	0.77
Column	4	91.06	22.77	0.57	0.69
Biochar	1	145.56	145.56	3.61	0.09
Residuals	10	402.90	40.29		
High biochar (40 Mg ha^{-1})					
Row	4	192.82	48.21	0.98	0.44
Column	4	100.85	25.21	0.51	0.73
Biochar	1	314.57	314.57	6.41	<0.05*
Residuals	20	981.06	49.05		

Long-term effects of different biochar treatments on black carbon stocks

The addition of 9 Mg biochar ha^{-1} at the Bayreuth site did not significantly influence black carbon stocks in the long term (Appendix Table S3-2). High biochar additions of 31.5 Mg ha^{-1} at the Bayreuth site led to a significant increase of black carbon stocks, eleven years after biochar addition.

The addition of 3 Mg biochar ha^{-1} at the Gartow site did not significantly influence black carbon stocks in the long term (Appendix Table S3-2). High biochar additions of 40 Mg ha^{-1} at the Gartow site led to a significant increase of black carbon stocks, nine years after biochar addition. The combined application of high biochar additions fermented digestate led to significantly higher black carbon stocks than combined with mineral fertilizer or digestate.

Appendix Table S3-2: Results of analysis of variance for the effect of row and column in the field, treatment type, co-amendment type, biochar application, and their interaction on the black carbon stocks in the 0-30 cm layer of both field experiments. Model simplification was carried out with stepwise elimination of non-significant factors, independent of field experiment design factors row and column. Df = degrees of freedom.

	Df	Sum of Squares	Mean Square	F ratio	p value
Bayreuth					
Low biochar (9 Mg ha^{-1})					
Row	4	1.83	0.46	0.26	0.90
Column	4	10.23	2.56	1.45	0.29
Biochar	1	3.06	3.06	1.73	0.22
Residuals	10	17.65	1.76		
High biochar (31.5 Mg ha^{-1})					
Row	4	80.21	20.05	0.82	0.54
Column	4	147.87	36.97	1.51	0.27
Biochar	1	242.80	242.80	9.92	<0.05*
Residuals	10	244.78	24.48		
Gartow					
Row	4	3.71	0.93	0.53	0.72
Column	4	3.04	0.76	0.43	0.78
Biochar	1	5.39	5.39	3.06	0.11
Residuals	10	17.60	1.76		
High biochar (40 Mg ha^{-1})					
Row	4	9.04	2.26	1.21	0.34
Column	4	2.33	0.58	0.31	0.87
Biochar	1	32.40	32.40	17.31	<0.001***
Fermented digestate	1	3.92	3.92	2.10	0.16
Biochar x Fermented digestate	1	10.88	10.88	5.81	<0.05*
Residuals	18	33.70	1.87		

Appendix Table S3-3: Median soil organic carbon stocks and black carbon stocks of the organic soil amendments containing of high biochar amounts (31.5 Mg ha^{-1} and 40 Mg ha^{-1}) of both field experiments. SE = standard error.

	Soil organic carbon stock	±	SE	Black carbon stock	±	SE
	Mg ha^{-1}					
Bayreuth						
Treatment						
Control	63.75	±	15.35	4.61	±	0.74
Pristine biochar (31.5)	98.41	±	17.63	7.85	±	1.81
Biochar (31.5) + Compost (70)	91.45	±	7.57	10.78	±	3.49
Co-composted biochar (31.5)	89.02	±	4.14	12.87	±	2.32
Gartow						
Treatment						
Mineral fertilizer	19.49	±	3.74	0.24	±	0.07
Biochar (40) + syn. Fertilizer	23.97	±	2.93	2.15	±	0.35
Biochar (40) + digestate	23.50	±	2.66	2.18	±	0.50
Biochar (40) + ferm. digestate	30.61	±	2.75	4.66	±	0.74

Mixed-effect model post-hoc results

Appendix Table S3-4: Post-hoc test results of each of the four analyzed soil organic carbon stock time-series (two field experiments, two amount levels each). Post-hoc results were estimated using least-squares means. Df = degrees of freedom.

	Df	T ratio	p value
Bayreuth			
Low biochar (9 Mg ha^{-1})			
2010 - 2011	42	0.79	0.86
2010 - 2013	42	0.56	0.94
2010 - 2021	42	0.72	0.89
2011 - 2013	42	0.10	1.00
2011 - 2021	42	0.29	0.99
2013 - 2021	42	0.20	1.00
High biochar (31.5 Mg ha^{-1})			
2010 - 2011	42	-1.31	0.56
2010 - 2013	42	1.26	0.59
2010 - 2021	42	-0.27	0.99
2011 - 2013	42	2.03	0.20
2011 - 2021	42	0.91	0.80
2013 - 2021	42	-1.03	0.73
Gartow			
Low biochar (3 Mg ha^{-1})			
2012 - 2013 (spring)	50	1.46	0.77
2012 - 2013 (fall)	50	3.82	<0.05*
2012 - 2014 (spring)	50	3.36	<0.05*
2012 - 2014 (fall)	50	3.15	<0.05*
2012 - 2016	50	2.70	0.12
2012 - 2021	50	2.64	0.13
2013 (spring) - 2013 (fall)	50	2.36	0.24
2013 (spring) - 2014 (spring)	50	1.90	0.49
2013 (spring) - 2014 (fall)	50	1.69	0.63
2013 (spring) - 2016	50	1.24	0.87
2013 (spring) - 2021	50	1.20	0.89
2013 (fall) - 2014 (spring)	50	-0.45	1.00
2013 (fall) - 2014 (fall)	50	-0.67	0.99
2013 (fall) - 2016	50	-1.11	0.92
2013 (fall) - 2021	50	-1.12	0.92
2014 (spring) - 2014 (fall)	50	-0.22	1.00
2014 (spring) - 2016	50	-0.66	0.99
2014 (spring) - 2021	50	-0.67	0.99
2014 (fall) - 2016	50	-0.45	1.00
2014 (fall) - 2021	50	-0.46	1.00
2016 - 2021	50	-0.02	1.00
High biochar (40 Mg ha^{-1})			
2012 - 2013 (spring)	84	7.09	<0.001***
2012 - 2013 (fall)	84	8.75	<0.001***

2012 - 2014 (spring)	84	10.33	<0.001***
2012 - 2014 (fall)	84	10.27	<0.001***
2012 - 2016	84	9.29	<0.001***
2012 - 2021	84	14.21	<0.001***
2013 (spring) - 2013 (fall)	84	1.66	0.65
2013 (spring) - 2014 (spring)	84	3.24	<0.05*
2013 (spring) - 2014 (fall)	84	3.18	<0.05*
2013 (spring) - 2016	84	2.20	0.31
2013 (spring) - 2021	84	7.12	<0.001***
2013 (fall) - 2014 (spring)	84	1.59	0.69
2013 (fall) - 2014 (fall)	84	1.52	0.73
2013 (fall) - 2016	84	0.54	1.00
2013 (fall) - 2021	84	5.46	<0.001***
2014 (spring) - 2014 (fall)	84	-0.07	1.00
2014 (spring) - 2016	84	-1.05	0.94
2014 (spring) - 2021	84	3.88	<0.01**
2014 (fall) - 2016	84	-0.98	0.96
2014 (fall) - 2021	84	3.94	<0.01**
2016 - 2021	84	4.92	<0.001***

Appendix Table S3-5: Post-hoc test results of each of the four analyzed black carbon stock time-series (two field experiments, two amount levels each). Post-hoc results were estimated using least-squares means. Df = degrees of freedom.

	Df	T ratio	p value
Bayreuth			
Low biochar (9 Mg ha^{-1})			
2010 - 2011	42	-2.69	<0.05*
2010 - 2013	42	7.05	<0.001***
2010 - 2021	42	8.94	<0.001***
2011 - 2013	42	7.55	<0.001***
2011 - 2021	42	9.09	<0.001***
2013 - 2021	42	2.43	0.09
High biochar (31.5 Mg ha^{-1}) - Box-Cox transformed			
2010 - 2011	42	-3.43	<0.01**
2010 - 2013	42	1.00	0.75
2010 - 2021	42	0.65	0.92
2011 - 2013	42	2.98	<0.05*
2011 - 2021	42	3.10	<0.05*
2013 - 2021	42	-0.31	0.99
Gartow			
Low biochar (3 Mg ha^{-1}) - Box-Cox transformed			
2012 - 2014	12	-7.41	<0.001***
2012 - 2021	12	-0.66	0.79
2014 - 2021	12	6.58	<0.001***
High biochar (40 Mg ha^{-1})			
2012 - 2014	12	-2.01	0.15
2012 - 2021	12	3.05	<0.05*
2014 - 2021	12	4.89	<0.001***

Appendix Table S3-6: Post-hoc test results of the soil organic carbon stock time series of high biochar containing OSA variants in two soil depths. Post-hoc results were estimated using least-squares means. Df = degrees of freedom.

	Df	T ratio	p value
Bayreuth			
0-10 cm			
2010 - 2011	42	-0.56	0.84
2010 - 2013	42	9.77	<0.001***
2011 - 2013	42	10.33	<0.001***
10-30 cm – Box-Cox transformed			
2010 - 2021	42	-0.35	0.94
2011 - 2013	42	-3.93	<0.01**
2011 - 2021	42	-3.58	<0.01**
Gartow			
0-10 cm			
2012 – 2014	12	1.53	0.29
2012 – 2016	12	4.16	<0.001***
2014 - 2016	12	2.71	<0.05*
10-30 cm			
2012 – 2014	12	1.14	0.50
2012 – 2016	12	-5.35	<0.001***
2014 - 2016	12	-6.07	<0.001***

Appendix Table S3-7: Post-hoc test results of the black carbon stock time series of high biochar containing OSA variants in two soil depths. Post-hoc results were estimated using least-squares means. Df = degrees of freedom.

	Df	T ratio	p value
Bayreuth			
0-10 cm - Box-Cox transformed			
2010 - 2011	42	-3.67	<0.01**
2010 - 2013	42	5.67	<0.001***
2011 - 2013	42	9.35	<0.001***
10-30 cm - Box-Cox transformed			
2010 - 2011	42	-0.01	0.99
2010 - 2013	42	-5.61	<0.001***
2011 - 2013	42	-5.60	<0.001***
Gartow			
0-10 cm - Box-Cox transformed			
2012 – 2014	12	4.28	<0.001***
2012 – 2016	12	2.89	<0.05*
2014 - 2016	12	-2.45	0.05
10-30 cm - Box-Cox transformed			
2012 – 2014	12	-6.43	<0.001***
2012 – 2016	12	-13.80	<0.001***
2014 - 2016	12	-1.63	0.25

Supplementary dataset

A supplementary dataset can be found online via:

https://www.sciencedirect.com/science/article/pii/S0048969724064969?via%3Dihub#s0120

The datset contains the following content:

Figure S3-3a: Comparison of soil organic carbon stocks over time in plots that have received high amounts of biochar (31.5 Mg ha^{-1}) and non-biochar plots that are directly adjacent in the Bayreuth field experiment. We used this comparison as an indicator for lateral transport. Plot number corresponds with the type of treatment and the letter represents the repetition (see Fig. S1a).

Figure S3-3b: Comparison of black carbon stocks over time in plots that have received high amounts of biochar (31.5 Mg ha^{-1}) and non-biochar plots that are directly adjacent in the Bayreuth field experiment. We used this comparison as an indicator for lateral transport. Plot number corresponds with the type of treatment and the letter represents the repetition (see Fig. S1a).

Figure S3-4a: Comparison of soil organic carbon stocks over time in plots that have received high amounts of biochar (40 Mg ha^{-1}) and non-biochar plots that are directly adjacent in the Gartow field experiment. We used this comparison as an indicator for lateral transport. Plot number corresponds with the type of treatment and the letter represents the repetition (see Fig. S1b).

Figure S3-4b: Comparison of black carbon stocks over time in plots that have received high amounts of biochar (40 Mg ha^{-1}) and non-biochar plots that are directly adjacent in the Gartow field experiment. We used this comparison as an indicator for lateral transport. Plot number corresponds with the type of treatment and the letter represents the repetition (see Fig. S1b).

Study 4: Impact of biochar aging on soil physicochemical properties

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Abstract

Biochar undergoes significant transformations in soil as a result of chemical, physical, and biological processes. These alterations can impact its initial properties, influencing both its agronomic effectiveness and its capacity for carbon sequestration. Long-term observations of biochar aging effects in soil are limited but highly relevant, as they provide a more realistic picture of the agronomic and societal benefits of biochar than short-term studies with relatively "fresh" biochar. This study aimed to describe the aging effects of biochar and their impact on a range of soil properties at a long-term biochar experiment in Bayreuth, Germany. For this purpose, soil and biochar samples were taken 13 years after application (two variants: 1. co-composted and 2. pristine biochar) and compared with a fresh variant in which the same unaged biochar was freshly mixed with the control soil.

The soil quality parameters, pH and electrical conductivity, decreased significantly ($p < 0.05$) during biochar aging. Specifically, the pH dropped from 7.4 in freshly biochar-amended soil to 6.8 in the pristine aged biochar variant and 6.9 in the co-composted aged biochar variant. Electrical conductivity decreased from 217.0 µS cm^{-1} in the freshly amended soil to 81.1 µS cm^{-1} in the pristine aged variant and 87.6 µS cm^{-1} in the co-composted aged variant. Nitrogen retention was enhanced in the soil amended with co-composted aged biochar compared to the pristine aged biochar soil. Total nitrogen (TN) was higher at 1.94 g kg^{-1} versus 1.57 g kg^{-1} ($p < 0.05$), and ammonium-nitrogen (NH_4^+-N) was slightly elevated at 35.7 mg kg^{-1} versus 33.0 mg kg^{-1}, although the difference was not statistically significant. The nitrate-nitrogen (NO_3^--N) content was significantly lower in all biochar-amended soil variants compared to the control soil. Total carbon (TC) levels decreased during biochar aging in all soil variants. However, the reduction was significantly lower in the co-composted aged biochar soil (25.0 g kg^{-1}) compared to the pristine aged biochar soil 20.5 g kg^{-1}, $p < 0.05$).

This study identified multiple aging effects on biochar following 13 years of exposure in loamy soil. Importantly, the results showed that compared to the amendment of pristine biochar, co-composting did not diminish TC of the treated soil, and more N could be retained, 13 years after amendment. In fact, co-composting prior to soil application is recommended to fully realize the potential agronomic benefits.

Keywords: Biochar aging, Soil health, Carbon Sequestration, Long-term field experiment

1. Introduction

Soils are the basis of a functioning food system and source of income for eight billion humans worldwide. Besides providing economic value and nutrition, soil fulfills several other services and ecosystem functions, such as biomass and fiber production, regulation of water, and nutrient cycles. Moreover, soils fulfill an enormous habitat function by hosting 25% of global biodiversity (FAO, 2020) and is thereby the foundation of the food chains nourishing above ground species, thus humanity (European Commission, 2021). In addition, soils are the most important terrestrial carbon (C) sink, storing 3500–4800 Pg of C (Lehmann and Kleber, 2015), whereas terrestrial vegetation and the atmosphere store only around 800 Pg C each. Therefore, it is of great importance to not just consider soil as an economic resource and means for our global food system but also being a key in climate change mitigation.

Since ancient times, humans use different forms of organic soil amendments (OSA) (Van Zwieten, 2018), the most common forms being straw, slurries, manures and compost. More modern forms are biogas digestates, sewage sludge biosolids and biochar. These amendments not just contain nutrients, but also varying amounts of C-rich organic compounds. Therefore, the application of OSA is often used as a way to increase soil organic carbon (SOC) (Bai et al., 2023; Chen et al., 2018). However, these different OSA have different mean residence times (MRT) in soil and usually (with the exception of biochar) have to be regularly reapplied to maintain the SOC stock increase in the soil.

Biochar is the product of thermochemical conversion of organic biomass under minimized oxygen supply, also known as pyrolysis. One must distinguish biochar from other carbonaceous material produced from pyrolysis. In contrast to char or charcoal, biochar is specifically produced for the purpose to be applied to soil (Lehmann and Joseph, 2024). Biochar contains highly aromatic C compounds and only small amounts of N (most of them being polycyclic and not available for plants), and are therefore, in contrast to other common types of OSA highly stable against microbial decomposition. Biochar's MRT is estimated to be 556 ± 483 years as a recent meta-analysis suggested (Wang et al., 2016), which underlines its high SOC sequestration potential. The wide variation of the mean MRT is mainly because of two factors. Firstly, the original studies included in the meta-analysis reported a broad range of MRTs, with estimates up to 891 years. And secondly, because both field and incubation studies were included in the literature assessment. The observation time of incubation studies being is shorter than the actual decomposition of biochar, thus, the MRT must be extrapolated with high uncertainty and often not reflect real field conditions. Long-term field observations of biochar stability are therefore urgently needed.

While biochar resides in soil, several reactions with its immediate surrounding environment are taking place. These processes are not just affecting biochar dissipation, but are leading to physical, chemical, and biological alterations of the biochar particles. Physical alterations include changes in particle size, porosity and surface area, and chemical alterations affecting mostly surface properties (Pignatello et al., 2024). Surface alterations due to biochar aging are often linked to the sorption of soil organic matter (SOM), leading to increased surface polarity, decreasing specific surface area (SSA), and increasing surface charge. The process of SOM sorption depends on pH, with lower pH generally leading to increased SOM sorption (Wang and Kuzyakov, 2024). SOM sorption can, moreover, block pores and thereby prevent microbes and minerals from penetrating into the particle and interacting with the particle`s inside (Hagemann et al., 2017). Changes of the oxidation state of aged biochar are mostly biologically driven because aging leads to a colonization of soil microbes which oxidize the altered surface (Lehmann et al., 2024). This then leads to the incorporation of oxygen into surface groups which makes the surface more hydrophilic and due to more negative charges, there is high potential for positive ion retention.

Field aging of biochar is leading to multiple processes occurring simultaneously and sequentially. Aging methods and experiments aiming to imitate these processes artificially are less time-intensive than field aging techniques, but do not represent the multiple facets of aging of biochar and their agronomic implications. Field aging experiments are often carried out with a limited duration (Dong et al., 2017; Haider et al., 2020; Pignatello et al., 2024), and none of them observed field aging processes on a decadal scale. However, it is critical to understand how aging dynamics impact biochar's environmental effects and its agronomic benefits on longer time scales. Since aged biochars better reflect what biochar mineralization would look like in hundreds and thousands of years (Lehmann et al., 2024), their process understanding is particularly interesting for long-term SOC sequestration and carbon dioxide removal.

Co-composting of biochar particles before application does not only "load" the biochar with nutrients and thereby making the soil more fertile, but it also mimics the natural aging process of SOM sorption and coating formation. This affects the particle stability against microbial decomposition, due to the microbial preference to utilize organic substrates on the particle surface that require less activation energy for metabolization (Pignatello et al., 2024). Co-composting also enhances the natural oxidation of biochar particles (Hagemann et al., 2017), which as already explained before, increasing the surface cation exchange capacity (CEC) and thereby nutrient availability for plants, consequentially leading to higher crop yields (Wang et al., 2019). Studies comparing co-composted and pure biochar have found that co-composting increases plant growth but has no negative effect on the long-term stability of biochar (Gross et al., 2024; Kammann et al., 2015). If the advantageous effects of co-composting on soil and biochar properties are limited to only few years after amendment or if they persist in the long-term is still unknown.

This study aimed to investigate the impact of long-term biochar aging on soil and biochar characteristics after long-term exposure to environmental conditions in a loamy soil under a temperate climate in Bayreuth, Germany. The first objective was to resample the biochar amended soil after 13 years of aging, and to create an un-aged reference by mixing unamended control soil with the original biochar, which was sealed for 13 years. The second objective was to analyze a variety of soil and biochar properties such as pH, electrical conductivity (EC), SOM, soil nutrients and soil C, in order to describe the soil health status as a function of biochar aging in soil. The third objective was to analyze if pristine aged biochar and co-composted aged biochar impact the soil differently in the long-term.

Our working hypotheses are:

1. Biochar aging leads to significant changes of soil chemical and physical properties;

2. Co-composted biochar increases soil fertility more than pristine biochar.

2. Material and Methods

2.1 Sampling, Site characteristics and Amendment Properties

To test the hypotheses of this study, a long-term biochar field experiment established in 2010 and located at Donndorf, a village close to Bayreuth, Germany, was sampled. Table 1 summarizes the main characteristics and properties of the experimental site and the used amendments. A more detailed description of its experimental design can be found in Cooper et al. (2020).

Table S4-1: Main site characteristics, soil, biochar and compost properties at the beginning of the field experiment in Bayreuth.

	Bayreuth field experiment
Site characteristics	
Latitude	49°56′01.7″
Longitude	11°31′17.1″
Mean annual precipitation [mm]	507
Mean annual temperature [°C]	8.2
Current use	Organic cropland
Tillage depth [cm]	0–10
Soil properties [0–30 cm]	
Soil type	Cambisol
Soil texture	Sandy loam
Sand [%]	62
Silt [%]	12
Clay [%]	26
Initial SOC [%]	1.6
pH	5.4
Biochar properties	
Feedstock	Pine wood
Pyrolysis temperature [°C]	550 + 800 (two stages)
Total carbon [g kg^{-1}]	843
Total nitrogen [g kg^{-1}]	4
Black carbon [g kg^{-1} C]	795
Carbon-to-nitrogen (C/N) ratio	239
Hydrogen-to-carbon (H/C) ratio [atomic ratio]	0.11
pH [$CaCl_2$]	8.77
Ash [g kg^{-1}]	90
CEC [$mmol_c$ kg^{-1}]	72.7
WHC	249.4
Compost properties	
Feedstock	Green litter
Total carbon [g kg^{-1}]	186
Total nitrogen [g kg^{-1}]	10
C/N	18.8

H/C [atomic ratio]	1.41
pH [$CaCl_2$]	6.99
Ash [g kg^{-1}]	66.1
CEC [$mmol_c$ kg^{-1}]	304.3
WHC [%]	203.8

In this experiment, three of the ten treatments from the original Latin rectangle field experiment design (Appendix Fig. S4-1) were selected. The Latin rectangle structure ensures treatment independence, as each treatment appears only once per row and column. The treatments applied were: pristine biochar at a rate of 31.5 Mg ha^{-1}, co-composted biochar combined with 70 Mg ha^{-1} compost at the same biochar rate, and an untreated control. Each treatment included five field replicates, with application occurring in May 2010. The commercial biochar (CarbonTerra, Wallerstein, Germany) was made from pine wood chips and produced in a gasification system via slow pyrolysis at 550 °C for 36 h followed by a second step of high temperature pyrolysis at 800 °C for 2h. The compost was produced by BKE Bio-Kompost and Disposal / GmbH & Co. and derived from green litter. The biochar-compost mixture was set up on 17.05.2010 at the “Bindlacher Berg” composting site, before being transported to the field experiment site on 21.05.2010 after four consecutive days of co-composting. The experiment site was under farming cultivation in each of the following years. More details of the farming activities can be found in Cooper et al. (2020) and Gross et al. (2024).

Soil sampling took place in March 2023 before the summer sowing. Prior to this, mustard was planted in fall 2022 as cover crop. Samples were collected from five field replicate plots. These plots had been treated with either pristine biochar, co-composted biochar, or left untreated as a control. Biochar amendments were incorporated to a depth of 10 cm using a rotary tiller in 2010. We assume vertical particle migration with time and therefore soil samples in 2023 were taken from a depth of 0–30 cm, and combined into composite samples for each treatment group. For clarity, we will refer to the soil treated with aged pristine biochar as “A_BC_S”, the soil treated with aged co-composted biochar as “CC_BC_S”, and the untreated soil as ”Control_S”.

For a better elucidation of the impact of biochar aging on soil properties, we prepared a reference soil by mixing 6 kg of the material from the control site with 472.5 g fresh biochar used as amendment at the beginning of the field experiment (F_BC_S) in 2010 (Table 1). This mixture was calculated to replicate the conditions found in the top 30 cm of the field soil at the start of the experiment. Given that biochar can undergo aging in the presence of oxygen, the used biochar was stored in sealed plastic buckets to prevent oxidation.

2.2 Soil and biochar analysis

The control soil (Control_S) and the biochar treated soils (F_BC_S, A_BC_S and CC_BC_S) were analyzed for the following parameters: pH, EC, water holding capacity (WHC), SOM, total carbon (TC), total nitrogen (TN), total phosphorus (TP), soluble phosphorus, available inorganic nitrogen (NH_4^+-N and NO_3^--N), SSA, total pore volume and pore radius. In addition, the biochar (F_BC) used to prepare the fresh biochar amended soil for this study was also analyzed for pH, EC and WHC, as well as SSA, total pore volume and pore radius. It is important to underline that this is the same biochar that was used in 2010 for treating the soil referred in this study as A_BC_S). Furthermore, SSA, pore volume and pore radius were also determined in two additional biochar samples, A_BC and CC_BC; these biochar samples were separated from a small amount of aged biochar treated soils, A_BC_S and CC_BC_S, respectively.

The pH of the soils and the biochar was measured in triplicates (CRISON pH Basic 20) in a 1:10 (w/v) soil:MiliQ water after being stirred for 30 min and left to rest for another 30 min, following the method described by Campos et al. (2020). After pH measurements, the supernatant was filtered and the EC was determined using a conductivity meter (CRISON ECmetro Basic 30) (de la Rosa et al., 2014).

The WHC of amended and un-amended soils, and biochar was determined in 6 replicates by weighing the water retained in 2 g of each material after saturation and subsequent settling for 2 h, in accordance with de la Rosa et al. (2014). Maximal WHC was calculated as the ratio of the weight of retained water to the dry weight of the sample expressed in percentage.

SOM content was determined according to the loss-of-ignition method based on gravimetric weight change associated with high temperature oxidation of organic matter. After initial oven drying at 105 °C overnight, the samples were ignited in a muffle furnace for 6 hours at 550 °C. The percent weight loss during the ignition step is reported SOM (% wt. loss).

Total C and TN contents were determined in duplicates by conducting dry combustion using an elemental analyser Flash 2000 elemental micro–analyser (Thermo Scientific, Bremen, Germany). Total P was determined in triplicates following controlled acidic digestion with ultrapure nitric and hydrochloric acid (DigiPREP Block Digestion Systems (SCP Science)) using and analysis by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Varian, Santa Clara, CA, USA).

Soluble phosphorus content was obtained in triplicates. Previously dried soil, passed through a 2 mm sieve, was mixed with activated carbon (about 6% w:w) in falcon tubes. Extraction was carried out according to the Olsen method (Olsen and United States. Department of Agriculture, 1954) with sodium bicarbonate extraction solution at solid to solution mass ratio 1:20, by shaking for 30 min in a bottle shaker. Supernatants were filtered twice, through folded filters (general filter) and Whatman No. 2 filters in succession, and measured by spectroscopy with Bran-Luebbe autoanalyzer.

Available inorganic nitrogen (NH_4^+-N and NO_3^--N), was quantified after extraction of the samples with 1 M potassium chloride (w/w 1:50) for 1 h at 180 rpm, centrifuged for 5 min at 4000 rpm and filtered through Whatman 2 filter paper (Jones, 2001). The ionic content was measured in the supernatant by colorimetric assays Omega SPECTROstar (BMG LABTECH GmbH, Germany). The NO_3^--N content of the extract was measured using the salicylic-sulfuric acid method (Cataldo et al., 1975) and NH_4^+-N was determined with an adapted protocol from the colorimetric method described by Greweling and Peech (1960).

SSA and pore volume were determined using adsorption-desorption analysis of elemental nitrogen (N_2) at 77 K using the Autosorb iQ Surface Area Analyzer (Quantochrome Instruments, USA). Prior to measurement, samples were degassed in vacuum at 378 K to remove surface adsorbates. SSA was calculated from the adsorption branches using the Brunauer-Emmett-Teller method (BET). The total pore volume was determined by applying the desorption isotherm of the Barrett-Joyner-Halenda (BJH) model. The average pore radius was estimated as a ratio of the total pore volume and SSA.

2.3 Statistical analysis

Statistical analyses were performed with Microsoft Excel and the software Past4.03. To determine significant differences of soil properties due to biochar treatments, one-way analysis of variance ANOVA was used. Differences were considered statistically significant at $p < 0.05$. After finding a significant result in ANOVA, a post-hoc Tukey's honestly significant difference (HSD) test was employed to compare all possible pairs of means. Pearson's correlation was used in order to assess the linear relationships between analyzed parameters (pH, EC, WHC, SOM, TC, TN, NO_3^--N, NH_4^+-N, total P, soluble P, SSA, total pore volume, and pore radius). The analysis was performed on three observations; where duplicates were analyzed, the missing values were replaced by mean imputation. For the significance testing, $p<0.05$ was set as a criterion. Principal component analysis (PCA) was conducted on the entire dataset to evaluate the influences of treatments on soil parameters variation. R (R Core Team, 2021) was used for visualization.

3. Results and Discussion

3.1 Aging effects on pH and EC

The freshly added biochar led to a significant increase ($p < 0.05$) of soil pH (Fig. S4-1). This initial pH increase is due to the "liming effect" of biochar and the release of calcium and other alkaline cations. However, as biochar ages, its acid-neutralizing effect diminishes, leading to a decrease in soil pH. Decreasing biochar pH after aging in the soil is well known and described (Pignatello et al., 2024). With increasing time and biochar aging basic species such as carbonates and hydroxides dissolute and could explain lower soil pH (Mukherjee et al., 2014). Another explanation could be surface oxidation of biochar (Mukherjee et al., 2014; Yao et al., 2010) and the increase of carboxylic groups during aging due to partial biochar oxidation (Sorrenti et al., 2016).

The EC of biochar in soil is responsible for the exchange of ions, and therefore a critical property for soil fertility. Fresh biochar increased soil EC, indicating a higher concentration of soluble salts in the soil (Fig. S4-1). The aged biochar treatments showed lower EC levels, indicating a reduction in soluble salts and ions, which might be due to leaching (Burrell et al., 2016; Wu et al., 2014), and microbial activity. Moreover, an increase of oxidation and O-functional groups on the surface of biochar, typical for biochar aging, is leading to decreasing EC (Kane et al., 2021).

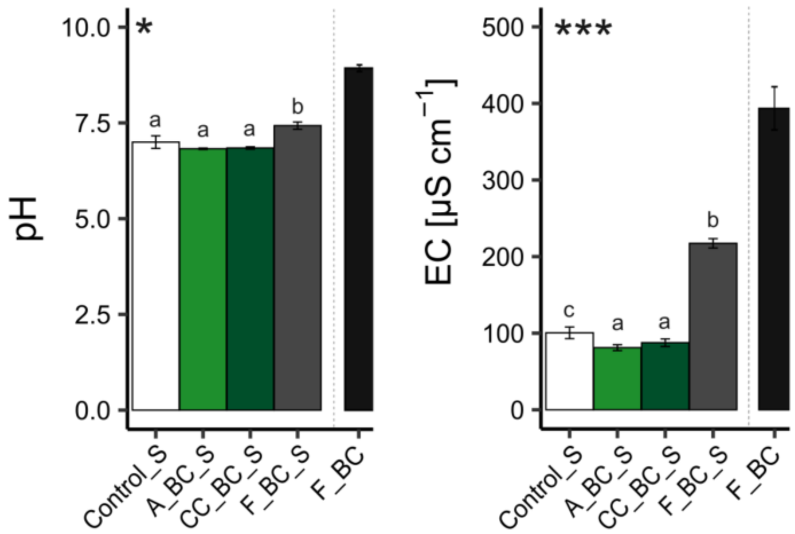


Figure S4-1: Soils and biochar pH (left) and electric conductivity (EC) values (right) of the different treatments (Control_S is the control soil, A_BC_S is aged biochar amended soil, F_BC_S is fresh biochar amended soil, CC_BC_S is co-composted biochar amended soil; F_BC is fresh biochar, stored and sealed for 13 years). Each bar represents the mean of three replicates. Error bar indicates the standard deviation. Asterisks indicate the level of significance (ns: not significant, $p < 0.05$*; $p < 0.01$**; $p < 0.001$***). Different letters indicate significant differences between the treatments.

3.2 Aging effects on TC, SOM, and soil nutrients

Fresh mixing of un-aged biochar with control soil after 13 years ("F_BC_S") increased TC significantly ($p < 0.05$) (Fig. S4-2). Aging resulted in a significant decline in TC levels when comparing fresh biochar amended soil, which showed ~three to fourfold more TC, with both aged biochar soil treatments A_BC_S and CC_BC_S. The CC_BC_S treatment had significantly more TC than the pristine A_BC_S treatment (Fig. S4-2). The SOM difference between the variants followed a similar pattern, with F_BC_S showing the highest content, and both aged variants showing significantly lower values ($p < 0.05$). However, the SOM decline during aging was not as drastic as TC, and both aged variants showed no significant difference. During aging, biochar particles tend to sorb organic matter components from their surrounding soil material, resulting in organic coatings on the particle surface and reduced SOM loss or increased SOM stabilization. This SOM coating strongly affects biochar physical-chemical properties and influences the stability of the aromatic "backbone" (Hagemann et al., 2017). Co-composting of biochar facilitates this natural process of coating formation and could therefore explain higher TC levels than the pristine biochar treatment. However, the threefold declining TC content of CC_BC_S and A_BC_S compared to the F_BC_S treatment cannot be explained by aging alone. Gross et al. (2024) found clear indication of vertical transport of biochar particles from topsoil to subsoil occurring in the Bayreuth experiment, and laterally between the experiment plots. Although these biochar particles disappear from the topsoil and lead to declining TC contents, they still have agronomic and ecological implications. Wang et al. (2023) found that vertically translocated biochar particles contribute to subsoil SOC sequestration, and higher subsoil pH. Biochar transport to different parts of the soil profile eventually depends strongly on soil and environmental properties (Rumpel, 2024) and needs further evidence from agronomic field trials with contrasting agricultural practices (Button et al., 2022).

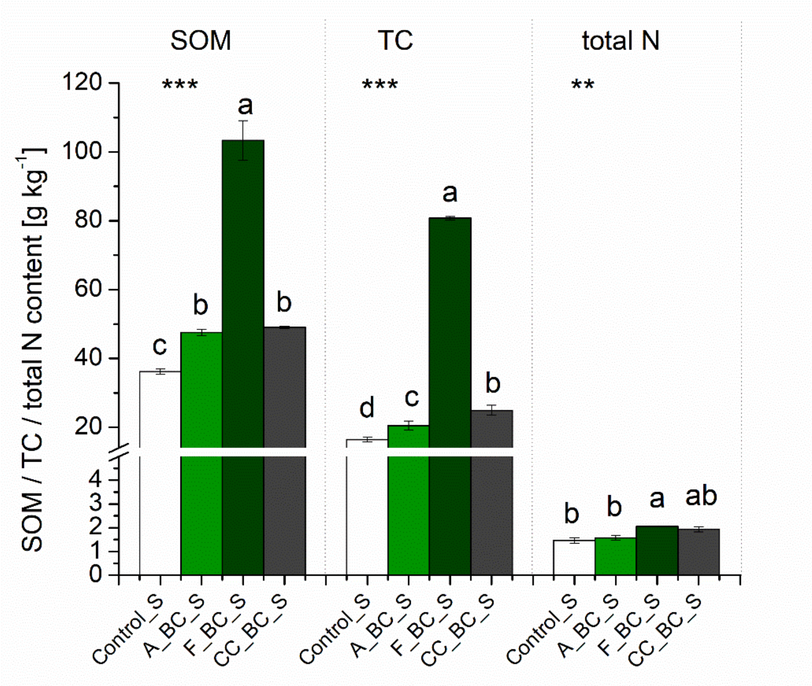


Figure S4-2: Soil organic matter (SOM), total carbon (TC) and total N content of the four different treatments (Control_S is the control soil, A_BC_S is aged biochar treated soil, F_BC_S is fresh biochar treated soil, CC_BC_S is co-composted biochar treated soil). Each bar represents the mean of three replicates. Error bar indicates the standard deviation. Asterisks indicate the level of significance (ns: not significant, $p < 0.05$*; $p < 0.01$**; $p < 0.001$***). Different letters indicate significant differences between the treatments.

In aged biochar treatments especially the co-composted treatment, TN levels were higher than in the treatment which received fresh biochar. Addition of fresh biochar decreased plant-available N (Fig. S4-2). Fresh biochar added to soil can lead to immobilization of N (DeLuca et al., 2015; Nguyen et al., 2017; Wang et al., 2019). Immobilization of N is more likely if the biochars C/N is very high (Mukome and Parikh, 2015) and the biochar was added without additional fertilizer as co-amendment or without a pre-treatment with nutrients, such as co-composting. Mixing biochar with compost has been shown to prevent N immobilization. According to our results, aging led to a significant increase of NH_4^+-N compared to the freshly added biochar (F_BC_S) (Fig. S4-3). The co-composted treatment thereby showed the highest NH_4^+-N. The presence of biochar led to significant lower NO_3^--N levels compared to the control soil (Fig. S4-3), which could be related to a more negative charge on the biochar surface and thus less NO_3^--N capture. Kammann et al. (2015) demonstrated that co-composting of biochar can enhance nitrate capture. They explained their surprising finding with the development of acid and basic functional groups, and organo-mineral complexes on the biochar surface. Total P and the soluble P fractions showed no significant differences between the treatments (Fig. S4-4).

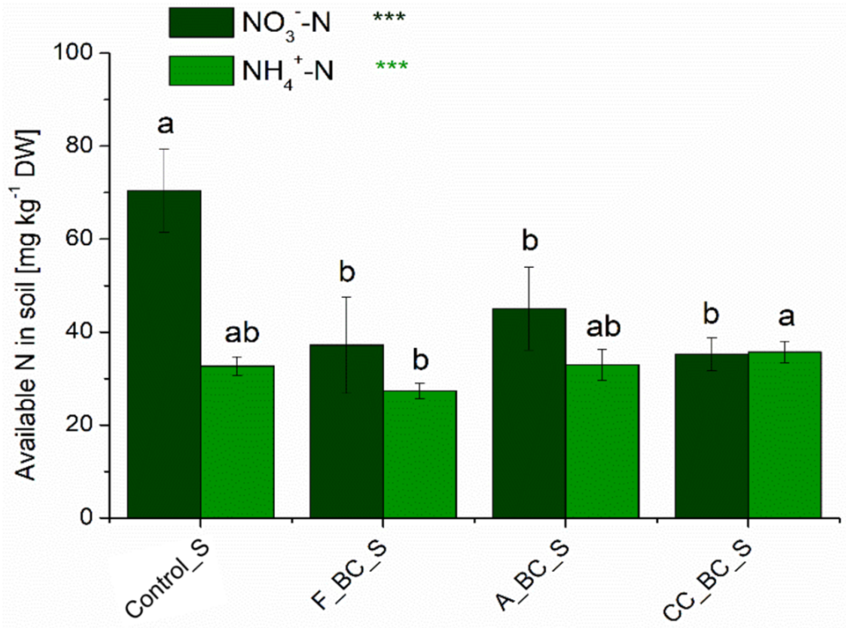


Figure S4-3: Nitrate-nitrogen (NO_3^--N) and ammonium-nitrogen (NH_4^+-N) content of the four different treatments (Control_S is the control soil, A_BC_S is aged biochar treated soil, F_BC_S is fresh biochar treated soil, CC_BC_S is co-composted biochar treated soil). Each bar represents the mean of three replicates. Error bar indicates the standard deviation. Error bar indicates the standard deviation. Asterisks indicate the level of significance (ns: not significant, $p < 0.05$*; $p < 0.01$**; $p < 0.001$***). Different letters indicate significant differences between the treatments.

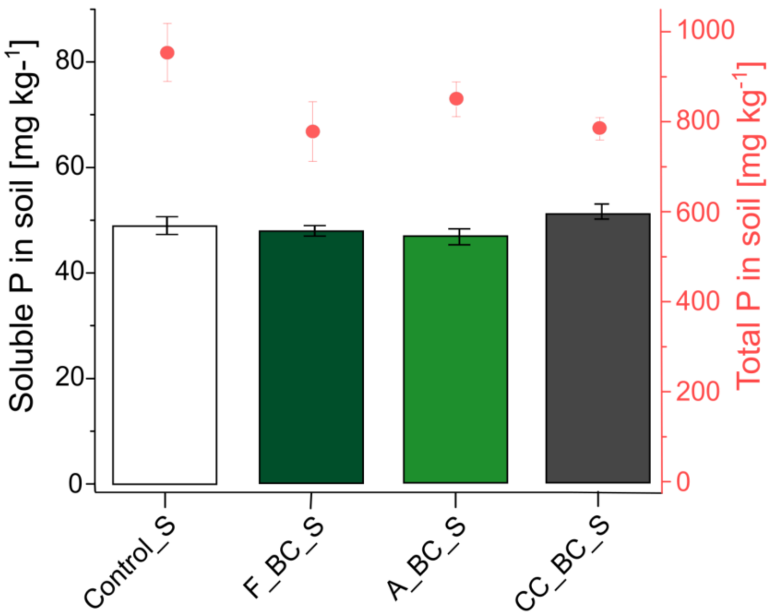


Figure S4-4: Soluble P and total P content of the four different treatments (Control_S is the control soil, A_BC_S is aged biochar treated soil, F_BC_S is fresh biochar treated soil, CC_BC_S is co-composted biochar treated soil). Each bar represents the mean of three replicates. Error bar indicates the standard deviation. Each bar represents the mean of three replicates. Error bar indicates the standard deviation.

3.3 Aging effects on SSA and WHC

Sorption of SOM to biochar surfaces during aging influences the SSA and eventually the hydraulic properties of the particle. N_2 BET SSA in both the soil and biochar of the co-composted variant was lower than in the other treatments (Table 2). This decrease in SSA reduces surface reactivity and sorption. However, the sorption of SOM could produce a more negative surface charge and thus higher potential for positively charged cations for sorption. Moreover, it intercepts compounds and organisms from penetrating into the biochar particle through pores and thereby prevents the aromatic core from potential degradation. According to our findings, biochar additions reduced the soil WHC. Limited pore access after the sorption of SOM to biochar surface can potentially negatively affect the WHC. However, this argumentation cannot explain lower WHC in the “F_BC_S” treatment, which was prepared by mixing the un-aged biochar with control soil sampled after 13 years. Considering that the biochar was added without milling, the pore size distribution is likely to be shifted to larger pores that allow preferential flow and decreases the water retention in the soil-biochar mixture. This is likely to be supported by the hydrophobicity of fresh biochar. Whereas these impact may be of minor importance in undisturbed soils, it can create artefacts during the determination of WHC in a laboratory.

Table S4-2: Specific surface area, pore size and volume, and water holding capacity ± standard deviation results of four different treatments measured of soil and biochar particles.

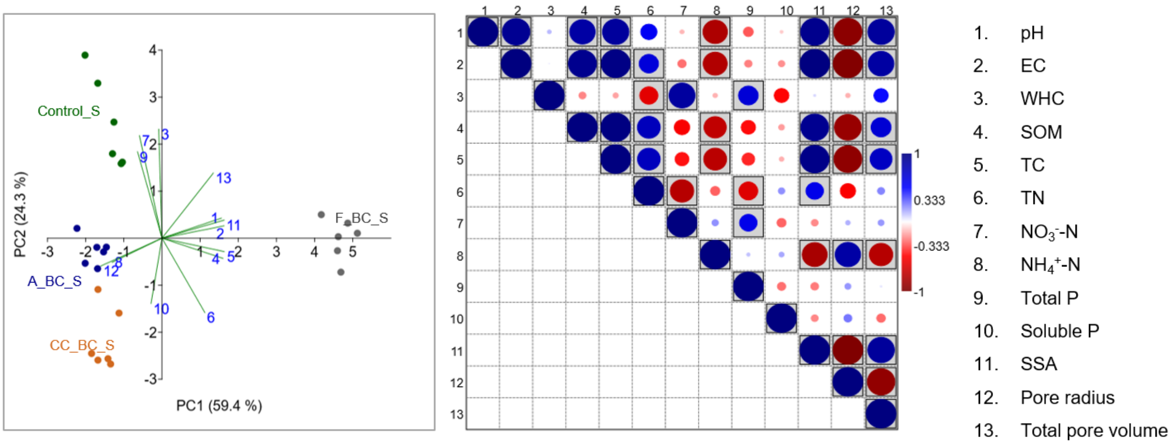
	Soil				Biochar		
	Control_S	F_BC_S	A_BC_S	CC_BC_S	F_BC	A_BC	CC_BC
Parameter							
SSA* [m^2 g^{-1}]	5.3	18.1	3.1	2.8	445.1	100.2	89.8
Total pore volume [cm^3 g^{-1}]	0.022	0.027	0.015	0.014	0.241	0.071	0.064
Pore radius [Å]	80.8	29.8	94.1	99.6	10.8	14.1	14.2
*WHC [%]	41.6 ± 4.1	31.5 ± 3.5	30.3 ± 2.8	25.9 ± 2.9	152.8 ± 7.2	n.a.**	n.a.**

*Specific surface area, **water holding capacity, ***not analyzed

3.4 Principal component analysis

Principal component analysis reduced the dimensionality of the data set which included 13 variables/soil properties determined in four soil variants, to two major axes (Fig. S4-5a), with the first principal component, PC1 accounting for 59.4%, and the second principal component, PC2 accounting for 24.6% of the variance in the data set. Together, they explain 84% of the total variance, suggesting that these two compounds capture most of the variability in the data. The analysis separated the soils by the applied soil treatments into distinct clusters. The cluster representing fresh biochar-treated soil (F_BC_S) was separated from the other clusters along the PC1 axis, suggesting that the fresh biochar significantly alters soil properties, distinguishing this soil from the other treatments and the control. The other three clusters can be found at approximately the same position on the PC1 axis but were spread out along the PC2 axis. This indicates that over time, the effect of pristine biochar and co-composted biochar on soil properties might diminish, and the biochar-amended soil eventually becomes more similar to the un-amended soil. Further, the separation of the two aged soils, treated with pristine biochar and co-composted biochar, could indicate that the interactions between compost and biochar during co-composting may have led to a modified impact of biochar on soil properties.

The vectors representing individual soil properties are tagged in Fig. S4-5 with numbers 1–13. The vectors for available nitrogen content in NH_4^+ form (8) and pore radius (12) point to the two aged soils, showing a positive long-term effect of biochar treatment on NH_4^+-N content in soil. This positive correlation between the pore radius and NH_4^+-N indicate higher mobility of these ions in larger pores. This could explain why in F_BC_S, which has significantly smaller average pore radius than the other soils (Table 2), we observed significantly lower NH_4^+-N content (Fig. S4-3). On the other hand, vectors representing total P (9) and available N in NO_3^- form point towards the control soil, showing no significant correlation with any of the treatments. Surface area (11), pH (1) and EC (2) vectors point to the fresh biochar treated soil, suggesting that biochar treatment has an immediate effect on these properties, the strength of which decreases over time. In addition, Pearson's correlation was performed to determine the strength and direction of the linear relationships between soil properties (Fig. S4-5b). Each dot in the Figure represents a correlation between two variables (soil properties), with blue and red representing positive and negative correlation, respectively. The intensity of the colors and the size of the dots indicate the strength of the correlation: larger dots with more intense color show a strong correlation, and smaller dots less intense in color indicate a weak correlation between the two variables. The boxed dots show statistically significant correlations ($p<0.05$). Results show a strong positive correlation between pH (1) and EC (2) indicating a common influence on these two parameters. Both of these parameters showed a significant positive correlation with TC (5), SSA (11) and pore volume (13), and a negative correlation with NH_4^+-N (8) and pore radius (12). The higher TC content stemming from biochar addition could possibly have a positive correlation with pH and EC as a result of the presence of ionizable functional groups as well as basic cations on the biochar. The negative correlation of pH and EC with some of the N species indicates that soil acidity and salinity need to be considered during nitrogen management in soil. Total P (9) and soluble P (10) showed no strong correlation with any other parameter, indicating soil treatments did not influence P content significantly. TC (5) and SOM (4) correlated positively with SSA (11) and negatively with pore radius (12). These correlations conformed well with correlations observed in the PCA biplot..



a) b)

Figure S4-5: Statistical analysis - a) principal component analysis (PCA) biplot, and b) Pearson's correlation of the soil properties (pH, electrical conductivity (EC), water hoding capacity (WHC), soil organic matter (SOM), total carbon (TC), total nitrogen (TN), available inorganic nitrogen in nitrate (NO_3^--N) and ammonium (NH_4^+-N) forms, total phosphorus (Total P), soluble phosphorus (Soluble P), specific surface area (SSA), pore radius and total pore volume) obtained for the four different treatments (Control_S is the control soil, A_BC_S is aged biochar treated soil, F_BC_S is fresh biochar treated soil, CC_BC_S is co-composted biochar treated soil). Correlations with $p < 0.05$ are boxed in Pearson's correlation graph.

4. Conclusion

Our findings demonstrate that the application of biochar, even after 13 years of aging, still have significant positive effects on physical and chemical soil properties, although the magnitude decreases with the time in the soil. Whereas some soil properties such as soil pH and EC showed decreasing effects with aging time, the ability to retain nitrogen increased, especially if the biochar was co-composted before being applied. Given the enhanced benefits of co-composted biochar compared to untreated biochar, co-composting should be strongly considered as a pre-treatment before biochar application to soils. The strongly declining TC cannot be explained by aging effects alone but is due to an interplay of biochar degradation and transport. Future studies will have to verify the proportion of biochar stability loss, vertical and lateral transport, and the impact of such transport dynamics on soil quality.

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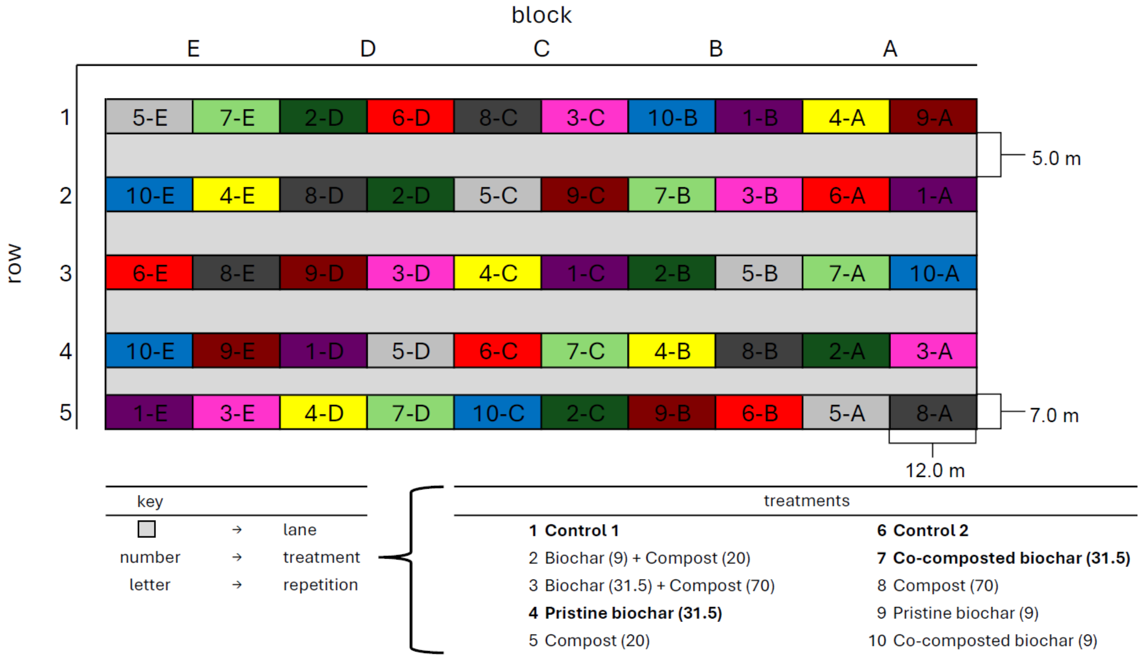
Appendix



Appendix Figure S4-1: Design and treatments of the Bayreuth biochar field experiment.

Curriculum Vitae

BERUFLICHE ERFAHRUNGEN

Wissenschaftlicher Mitarbeiter

Martin-Luther-Universität Halle-Wittenberg
Bodenbiogeochemie
Seit 01/2023

- Wissenschaftliche Bearbeitung des Projektes „TwinSubDyn"
- Koordination der Zusammenarbeit mit internationalen Kooperationspartnern
- Durchführung von Workshops und Summer Schools in Serbien
- Biomarkeranalysen
- Betreuung von ausländischen Gastwissenschaftlern
- Statistische Auswertung von Messergebnissen
- Wissenschaftliche Publikationen

Technischer Mitarbeiter

Martin-Luther-Universität Halle-Wittenberg
Bodenbiogeochemie
03/2021 bis 12/2022

- Bodenkundliche Laboranalytik
- Stabilisotopenanalysen (^{13}C und ^{15}N)
- Biomarker-Analysen (Black Carbon)
- Messungen am EA-IRMS, GC-FID und GC-MS
- Gerätewartung und Troubleshooting (Isotopenmassenspektrometrie)
- Statistische Auswertung von EA-IRMS Messergebnissen
- Durchführung von Lehrveranstaltungen

Wissenschaftliche Hilfskraft

Martin-Luther-Universität Halle-Wittenberg
Bodenbiogeochemie
10/2020 bis 02/2021

- Bodenkundliche Laboranalytik
- Biomarker-Analysen (Sterole, Stanole, Stanone)
- Messungen am EA-IRMS, GC-FID und GC-MS
- Statistische Auswertung von Gewächshaus- und Feldversuchen

Wissenschaftliche Hilfskraft

Leibniz-Institut für Agrarentwicklung in Transformationsökonomien
IAMO Land Systems Group
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- Übersetzungsarbeiten Deutsch-Englisch/Englisch-Deutsch
- Durchführung von Life Cycle Assessments

KOMPETENZEN

Labor-Analytik

Black Carbon

Gallensäuren

Sterole, Stanole, Stanone

Stabile Isotopen (^{13}C, ^{15}N)

Messung am GC-MS

Messung am GC-FID

Messung am EA-IRMS

PC-Kenntnisse

Microsoft Office

R

SPSS

GIS

OpenLCA

Fremdsprachen

Englisch

Sonstiges

Führerschein Klasse B

Akademischer Werdegang

Promotion

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Betreuer: Prof. Dr. Bruno Glaser

Promotionsthema: *„The potential of organic soil amendments for long-term carbon storage and soil improvement"*

Master of Science „Management natürlicher Ressourcen"

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Martin-Luther-Universität Halle-Wittenberg
Note: 1,2

Thema der Abschlussarbeit: *„The impacts on climate change when transitioning from conventional to organic milk production - a dynamic LCA case study"*

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Bachelor of Science „Management natürlicher Ressourcen"

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Martin-Luther-Universität Halle-Wittenberg
Note: 1,9

Thema der Abschlussarbeit: *„Beurteilung der Klimawirkung der Milchproduktion am Hof Pfaffendorf mit der Methode Ökobilanz"*

Note: 1,3

List of Publications

Publications

Handiso, M., Zebene A., Glaser, B., Bromm, T., Gross, A., Lemma, B. Effects of canopy management of umbrella tree (Terminalia brownii Fres.) on microclimate and maize (Zea mays L.) yield in agroforestry parkland of South Ari District, Southern Ethiopia. *Frontiers in Sustainable Food Systems* 8 (2024). https://doi.org/10.3389/fsufs.2024.1464609

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Conference presentations

Gross, A., Bromm, T., Polifka, S., Fischer, D., Glaser, B. Long-term biochar and soil organic carbon stability – evidence from long-term field experiments in Germany, *EGU General Assembly 2024*, Vienna, Austria, 14–19 Apr 2024, EGU24-9944 https://doi.org/10.5194/egusphere-egu24-9944

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Declaration under Oath

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.