



# REPORT ON THE EFFECT OF BBFs ON SOIL QUALITY AND CARBON SEQUESTRATION

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## OPTIMISING BIO-BASED FERTILISERS IN AGRICULTURE – PROVIDING A KNOWLEDGE BASIS FOR NEW POLICIES

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LEX4BIO aims to reduce the dependence upon mineral/fossil fertilisers, benefiting the environment and the EU's economy. The project will focus on collecting and processing regional nutrient stock, flow, surplus and deficiency data, and reviewing and assessing the required technological solutions. Furthermore, socioeconomic benefits and limitations to increase substitution of mineral fertiliser for BBFs will be analysed. A key result of LEX4BIO will be a universal, science-based toolkit for optimising the use of BBFs in agriculture and to assess their environmental impact in terms of non-renewable energy use, greenhouse gas emissions and other LCA impact categories. LEX4BIO provides for the first-time connection between production technologies of BBFs and regional requirements for the safe use of BBFs.

The project runs from June 2019 to May 2024. It involves 20 partners and is coordinated by Luke (Luonnonvarakeskus - Natural Resources Institute Finland).

More information on the project can be found at <http://www.lex4bio.eu>



## TABLE OF CONTENTS

Acknowledgement.....	3
List of figures .....	5
List of tables .....	5
1. INTRODUCTION .....	6
2. MATERIALS AND METHODS .....	7
2.1. Data collection.....	7
2.2. Data treatment and analysis .....	8
3. RESULTS.....	9
3.1. Characterisation of observations .....	9
3.2. Effects of BBFs on SOC concentrations .....	10
3.3. Effects of soil characteristics on BBF effects .....	10
3.4. Effects of site characteristics on BBF effects.....	11
4. DISCUSSION.....	12
4.1. Solid and carbon-rich BBFs are most efficient in increasing SOC concentrations.....	12
4.2. Less developed soils and loamy soils in dry climates show the highest potential for carbon sequestration through BBF application.....	14
5. CONCLUSION AND RECOMMENDATIONS .....	16
6. REFERENCES .....	16
Literature considered in meta-analysis.....	19



## LIST OF FIGURES

Figure 1. Geographic distribution of the considered studies in Europe and North America.

## LIST OF TABLES

Table 1: Effects of BBF quality and amount on SOC concentration.

Table 2: Effects of soil characteristics on the efficiency of BBFs to affect SOC concentration.

Table 3: Effects of environmental characteristics on the efficiency of BBFs to affect SOC concentration.



## D2.2: REPORT ON THE EFFECT OF BBFs ON SOIL QUALITY AND CARBON SEQUESTRATION

### 1. INTRODUCTION

Soil organic matter (SOM) is the nexus of soil quality as well as soil functionality because it holds a large share of nutrients and virtually all energy, and with this mediates and regulates the dynamics of biological, chemical and physical soil processes (Wiesmeier et al., 2019). SOM is an important soil component that can be directly managed by humans in order to improve soil fertility for food production. Humans directly benefit from this resource since agricultural land use began, continuously depleting SOM stocks (Sanderman et al., 2017). Together with this often not recognised depletion, other soil functions and in consequence, ecosystem services aggravated. Soils and especially SOM play vital roles in the global carbon and water cycles, and SOM depletion now has critical effects on the livelihood of humans. The fading productivity of soils was not perceived earlier because mineral fertilisers partly compensated the nutritional effects of SOM in food production since the 19<sup>th</sup> century. With the awareness of the planetary boundaries and global limitation of natural resources, especially phosphorus (P) and nitrogen (N), the concept of closed nutrient cycles and circular economy emerged. Today, imported mineral P and fossil energy-intensive N fertilisers cause major detrimental impacts on the environment, while a wide variety of nutrient-rich side-streams and organic wastes remain under-used. In the last 20 years, many new bio-based fertilisers (BBF) emerged and are currently considered for commercial application in agriculture. Ideally, the application of these BBFs in agriculture will also improve soil quality and contribute to climate mitigation. Freibauer et al. (2004) quantified the potential of agricultural soils to sequester organic carbon (OC) and identified organic inputs on arable land as the most efficient measure. Riggers et al. (2021) and Wiesmeier et al. (2016) modelled the development of soil organic carbon (SOC) until 2050/2100 under different climate scenarios. Both publications point out that organic inputs need to be increased significantly even just to maintain current SOC stocks.

Beside positive effects, application of BBFs may involve the risk of spreading organic and inorganic pollutants and pathogens. Among the potentially toxic elements (PTE, also known as heavy metals), copper, zinc and cadmium are of concern and enter agricultural soils via manure, sewage sludge, mineral P fertilizers and copper-containing fungicides (Bünemann et al., in prep). Various organic and emerging contaminants (Rigby et al., 2021), microplastics (Braun et al., 2021), and pathogens likewise occur in organic wastes. In the case of sewage sludge in particular, concerns about potential disease transmission has led to banning of land application in some countries, fuelling the development of a range of technologies to recycle nutrients such as P after ignition of sewage sludge (Harder et al., 2019). The obvious trade-off of such an approach is the loss of OC that would be valuable as an input in order to maintain SOC stocks.

Long-term trials are needed to evaluate the risks of contaminants when applied to soil, as well as to detect bulk SOC stock changes, which occur slowly despite highly dynamic SOC fractions, climate fluctuations and management effects. It is generally accepted that SOM, regardless of its great importance, is highly variable – in quantity, quality, turnover dynamics and spatial distribution from the molecular to the global scale. Nevertheless, the concentration of SOC is an easy to determine but



robust and meaningful indicator for SOM quantity and soil quality (Bünemann et al., 2018). Since the total N content can be determined in the same analysis, the C/N ratio offers a simple qualitative indicator of SOM. Hence, many publications use the SOC concentration as comprehensive soil quality indicator and prove that most soil functions are affected by SOM.

Several reviews and meta-analyses compiled the current knowledge on the effects of different organic amendments on agronomic and environmental soil properties. Diacono and Montemurro (2010) assessed the long-term effects of organic amendments on soil fertility and found positive effects on yield quality, soil biological, chemical and physical properties, carbon sequestration and no evidence for negative effects from heavy metals. Chen et al. (2018) reviewed studies considering a wide variety of organic amendments and concluded that this measure generally provides some additional benefits besides the pure nutritional effect on yield. They demand the careful selection of organic amendment type and application rate in order to maximise nutrient use efficiency and minimise any undesirable effects to the environment.

Despite these comprehensive compilations, we are still lacking information on the effects of various BBFs (especially digestates) on soil quality. Moreover, most studies focus on yields and neglect important soil and site characteristics, which are decisive for the expression of the BBF effects as well as the identification of the optimal BBF type and application rate. Their focus on productivity also puts long-term effects especially on SOC stocks on the side and therefore rules out the evaluation of BBF effects on the sequestration rates. We conducted a literature search and a meta-analyses to consider these points and thus, aimed to 1.) evaluate the effects of BBF type on SOC concentrations in long-term experiments; 2.) quantify the effects of soil and site characteristics; and 3.) derive application scenarios for different BBFs.

## 2. MATERIALS AND METHODS

### 2.1. Data collection

Chen et al. (2018) kindly provided their data set as the basis of this review. They published a meta-analysis including 132 long-term studies, with their definition of long-term being 10 years and more. We filtered the studies based on two criteria: 1. to include typical climatic zones of Europe (continental, mediterranean, subtropical and temperate); and 2. to include studies that report analytically determined SOC concentrations (using an elemental analyser) for each observation. This filtering reduced the number of studies to 42. In a second step, we contacted researchers running long-term trials that include BBFs and asked them for overlooked or new publications. The third step consisted of an additional literature review on ISI Web of Science (<http://apps.isiknowledge.com>) covering the period 1997–2021. We used key words from the following four groups in different combinations using the boolean operators AND and OR: 1. (field) trial/(field) experiment; 2. Long-term; 3. Fertilization/Fertilisation (organic waste, organic amendment/organic fertilizer, digestate, and biochar); and 4. soil organic carbon/SOC/organic carbon/carbon/soil organic matter/SOM.

In contrast to Chen et al. (2018), we set a minimum duration of trials of five years. Only studies that reported data for at least one control treatment besides the BBF treatment were added to the existing dataset. We defined two different types of control treatments: 1. In a negative control, no fertilizer is used, neither organic nor mineral; 2. A positive control includes the application of mineral fertilizer.



We extracted mean, standard deviation and sample size for all observations. If the data of interest was only shown in graphs or figures, we used the online tool [webplotdigitizer](https://automeris.io/WebPlotDigitizer/) (https://automeris.io/WebPlotDigitizer/, last access 24.04.2022) to extract it. If one paper reported various independent experiments (e.g., two experiments at separate locations), each of them was considered as an individual study and incorporated as an independent observation into our dataset (Garcia-Palacios et al., 2015; Luo et al., 2018). If one paper contained results from various sampling dates and soil depths, we used the data from the latest sampling time-point and from the sample collected from the uppermost layer of soil. If the study did not report the standard deviation, we reassigned the mean standard deviation of the existing data (Luo et al., 2006). We extracted metadata from each of the 60 studies in order to evaluate effects of BBF quality and quantity, soil and site characteristics.

## 2.2. Data treatment and analysis

We reclassified and harmonised the data to evaluate the effects of different BBFs on the SOC concentration under various soil and site characteristics. We reclassified all BBFs in the following seven categories: 1. biosolids (sewage sludge, sludge compost), 2. biowaste (compost from domestic organic waste, organic household waste), 3. Digestate (co-product of biogas production), 4. green manure (green manure crops such as rape crop, lantana and some leguminous plants), 5. Lignocellulose (lignin-containing waste or compost, e.g. sawdust, pruning waste), 6. manure (farmyard manure, livestock manure and manure-based materials), and 7. straw (crop straw, straw husk, straw compost). The C/N ratio was used as a qualitative characteristic of the BBFs. We grouped the observations into four classes: C/N <10, 10-30, >30, and N/A for observations that did not report the C/N ratio. We used the applied BBF amount as a quantitative characteristic. All observations were clustered in the following four classes: <5 Mg\*ha<sup>-1</sup>\*yr<sup>-1</sup>, 5-10 Mg\*ha<sup>-1</sup>\*yr<sup>-1</sup>, >10 Mg\*ha<sup>-1</sup>\*yr<sup>-1</sup> and N/A if the respective information was not given.

We extracted three different soil characteristics from the studies to evaluate their effect on the efficiency of BBF amendment on SOC concentrations: soil texture, soil pH, and the initial SOC content. Texture information was classified into sandy, silty, loamy and clayey (WRB, 2015). We combined the texture classes loam and clay (further denominated as loam) because only three studies with seven observations reported results from clayey soils. Information on soil pH – if measured in CaCl<sub>2</sub> – was allocated into the groups <5.5, 5.5-6.5, 6.5-7.4, >7.4 and N/A. Similarly, all observations of initial SOC content before start of the trial were grouped in the following five classes: <10 g\*kg<sup>-1</sup>, 10-15 g\*kg<sup>-1</sup>, 15-20 g\*kg<sup>-1</sup>, >20 g\*kg<sup>-1</sup> and N/A.

Soils were classified according to the World Reference Base for Soil Resources 2014 (WRB, 2015) at the level of the 32 reference soil groups without any qualifier. The whole data set contained seven soil types, while 31 observations did not report any soil type. We grouped and simplified these soils in four groups to enable the evaluation of BBF effects for all observations. We report the results for Cambisols, Luvisols, Mollisols, Regosols and Other. Cambisol, Luvisol and Mollisol contained solely these soil types, while we combined six Calcisols, four Aridisols together with 84 Regosols in the group Regosol, and all observations with unknown soil types and two Ultisols in the group Other. We reclassified the information on climate zone according to the Köppen climate classification into the four groups subtropical, continental, mediterranean and temperate. All observations were classified in the following four groups based on the duration of the trial: 5-10 years, 11-15 years, 16-25 years and >25 years.



The effect sizes (responses) of SOC concentrations were calculated using a categorical random effects model, where the effect size is weighted by the inverse of the variance (Adams et al. 1997). A random effects meta-analysis was performed using the restricted maximum likelihood estimator and using the Knapp-Hartung adjustment to account for the uncertainty in the estimate of (residual) heterogeneity. Datasets were analysed with SPSS 28 (IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp) to calculate the effect sizes and their significance levels.

### 3. RESULTS

#### 3.1. Characterisation of observations

We collected 18 new studies and combined them with 42 from the Chen et al. (2018) dataset, resulting in 260 observations from 60 field trials. 75% of the observations were located in humid-temperate and continental climates, while only 12% were in subtropical and 13% in mediterranean climates (Figure 1). Almost all studies (98%) reported the soil texture, with 39% on loamy to clayey, 31% on silty and 25% on sandy soils. Of the 80% of studies that mentioned the initial SOC content of the soil, 25% indicated a SOC content  $< 10 \text{ g} \cdot \text{kg}^{-1}$  and 39% between 10 and  $15 \text{ g} \cdot \text{kg}^{-1}$ . In the remaining 36% of studies SOC content was  $15 \text{ g} \cdot \text{kg}^{-1}$  or higher. The duration of the trials spanned between 5 and 110 years, with a median of 17 years and a mean of 27 years. 38% of the studies lasted longer than 25 years. The remaining 62% were distributed relatively evenly across the other three classes (5-10, 11-15 and 16-25 years).

In most studies (90%), the BBFs were applied multiple times. Of all 260 observations, the BBF was in 84% classified as solid whereas the remaining 16% were in liquid form, of which in 18 observations as digestates and in 22 observations as slurry. The most common BBF categories were manure, biosolids and biowaste, followed by straw, digestates, green manure and lignocellulosic waste. For most observations (78%), the C/N ratio of the BBF was given, ranging mostly (54%) between 10 and 30. 35% of the observations indicated a C/N ratio  $\leq 10$  and 11% a ratio  $\geq 30$ . The application rate of the BBFs

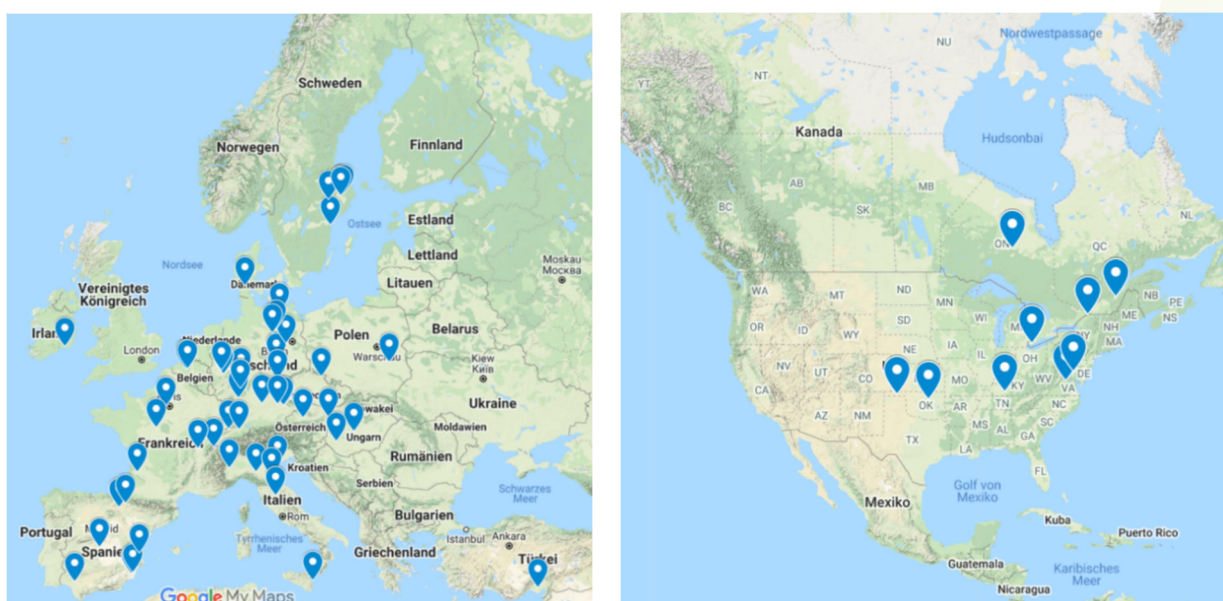


Figure 1: Geographic distribution of the considered studies in Europe and North America (both maps were created using Google Maps).



was frequently (42%) less than  $5 \text{ t*ha}^{-1}\text{*year}^{-1}$ , but also the groups  $5\text{-}10 \text{ t*ha}^{-1}\text{*year}^{-1}$  and  $> 10 \text{ t*ha}^{-1}\text{*year}^{-1}$  were well represented, with 32% and 26%, respectively.

### 3.2. Effects of BBFs on SOC concentrations

We found significant positive effects of BBFs on SOC concentrations when compared either to unfertilised or to minerally fertilised plots (Table 1). The effect was generally stronger when compared to the unfertilised plots. Of the different types of BBFs, lignocellulose had by far the strongest effect of  $7.6 \text{ g*kg}^{-1}$  higher SOC concentrations compared to the unfertilised control. These nine observations report the effects after application of woody materials with high contents of lignin (sawdust, green waste compost, vegetal pruning waste, conifer compost, crushed pruned vine-wood and urban tree and shrub leaves, grass cuttings and chopped brush). Stronger than average effects were also found for biosolids and biowaste. The effects of manure and straw were close to the overall average, while digestates and green manure showed effect sizes clearly below the mean. Green manure was the only BBF that showed no significant effect on SOC concentration. The meta-analysis showed the strongest effects for BBFs with a C/N ratio between 10 and 30 and still greater than average effects for BBFs with a C/N ratio  $>30$ . Less than average but still significant effects were reported for BBFs with C/N ratios  $<10$ . Obviously, SOC increased more, the greater the amount of BBF applied.

Table 1: Effects of BBF type, C/N ratio and applied amount on SOC concentration

	Treat vs. negative control				Treat vs. positive control				
	N	Effect size	Std. error	Sig. (2-sided)	N	Effect size	Std. error	Sig. (2-sided)	
Overall	200	2.94	0.17	0.0E+00	186	1.80	0.14	0.0E+00	
BBF type	Biosolid	45	3.84	0.46	0.0E+00	35	2.74	0.46	2.1E-09
	Biowaste	25	3.23	0.39	0.0E+00	25	2.30	0.41	1.8E-08
	Digestate	8	0.94	0.27	4.9E-04	19	0.55	0.17	1.1E-03
	Green manure	11	1.59	0.96	9.8E-02	13	1.99	0.93	3.2E-02
	Lignocellulose	9	7.61	2.13	3.5E-04	4	6.50	1.96	9.0E-04
	Manure	79	2.73	0.24	0.0E+00	64	1.89	0.18	0.0E+00
	Straw	23	2.88	0.42	5.3E-12	26	0.99	0.29	7.4E-04
BBF C/N	<10	52	1.70	0.21	2.2E-16	49	1.20	0.18	3.6E-11
	10-30	92	4.00	0.27	0.0E+00	71	2.94	0.27	0.0E+00
	>30	19	3.21	0.50	9.5E-11	14	2.27	0.38	3.5E-09
	N/A	37	2.01	0.33	1.4E-09	52	0.83	0.19	1.7E-05
BBF amount [ $\text{Mg*ha}^{-1}\text{*yr}^{-1}$ ]	<5	75	2.49	0.23	0.0E+00	77	1.31	0.15	0.0E+00
	5-10	60	3.22	0.31	0.0E+00	50	2.45	0.35	1.6E-12
	>10	51	3.53	0.38	0.0E+00	35	2.54	0.40	1.5E-10
	N/A	14	1.39	0.31	5.6E-06	24	1.11	0.29	1.1E-04

### 3.3. Effects of soil characteristics on BBF effects

We considered the effects of three soil characteristics on the efficiency of BBFs to increase the SOC concentration in long-term trials, namely soil texture, soil pH and the initial SOC content before BBF application (Table 2). All categories exhibit high numbers of observations, allowing a sound statistical evaluation. All three parameters significantly affected changes in SOC concentrations. Soil texture



showed a clear sequence of increasing effect size with finer particle sizes. Sandy soils showed a significant but weak positive effect of  $1.88 \text{ g*kg}^{-1}$  higher SOC concentrations after BBF application. Silty and especially loamy soils exhibited significant and strong positive effects on SOC concentration through BBF application. The same pattern appeared when compared to minerally fertilised soils. The initial SOC content was also decisive for the effect size of the BBFs. The effect size continuously increased from soils with low initial SOC concentrations of  $<10 \text{ g*kg}^{-1}$ , that showed weak but still significant responses to BBF applications, to soils with intermediate SOC concentrations of  $10\text{-}15 \text{ g*kg}^{-1}$  and showed strongest effects for soils with high initial SOC concentrations of  $15\text{-}20 \text{ g*kg}^{-1}$ . Surprisingly, the effect size decreased in soils with highest initial SOC concentrations of  $>20 \text{ g*kg}^{-1}$ .

*Table 2: Effects of soil characteristics on the efficiency of BBFs to affect SOC concentration*

	Treat vs. negative control				Treat vs. positive control				
	N	Effect size	Std. error	Sig. (2-sided)	N	Effect size	Std. error	Sig. (2-sided)	
Overall	200	2.94	0.17	0.0E+00	186	1.80	0.14	0.0E+00	
Soil texture	Sand	64	1.88	0.15	0.0E+00	56	0.86	0.13	7.7E-12
	Silt	42	3.03	0.38	3.6E-15	61	1.95	0.24	2.2E-16
	Loam	94	4.47	0.43	0.0E+00	69	2.82	0.35	1.8E-15
Soil pH	<5.5	30	3.39	0.51	3.3E-11	21	2.18	0.46	1.8E-06
	5.5-6.5	96	2.65	0.22	0.0E+00	79	1.97	0.22	0.0E+00
	6.5-7.4	23	2.40	0.33	6.4E-13	49	1.46	0.17	0.0E+00
	>7.4	46	3.90	0.46	0.0E+00	26	1.32	0.48	6.4E-03
	N/A	5	3.06	1.18	9.5E-03	11	2.53	0.86	3.1E-03
Initial SOC [ $\text{g*kg}^{-1}$ ]	<10	64	1.95	0.17	0.0E+00	54	1.09	0.14	6.5E-14
	10-15	71	3.76	0.36	0.0E+00	54	2.08	0.30	3.1E-12
	15-20	24	5.67	0.77	1.4E-13	23	3.37	0.57	3.5E-09
	>20	28	2.48	0.75	8.8E-04	13	1.02	0.55	6.4E-02
	N/A	13	2.99	0.33	0.0E+00	42	2.11	0.34	5.4E-10

### 3.4. Effects of site characteristics on BBF effects

We evaluated the effects of the site characteristics climate, soil type and duration of the long-term trials on the efficiency of BBFs to increase the SOC concentration. All three site characteristics and their respective expressions had sufficient numbers of observations in each group (except Mollisols when comparing with a positive control) and showed significant effects when compared to unfertilised and minerally fertilised controls. The pattern of the effect size between the different expressions of each characteristic was the same when compared to the positive and the negative control, with the latter always having the stronger effect size. We found the strongest effects of BBFs on the SOC concentration in Mediterranean and Continental climates. Long-term trials in subtropical and temperate climates showed significant but weaker effects after BBF application. All soil types showed significant effects of BBFs on their SOC concentrations when BBF were applied in comparison to minerally and unfertilised controls, with strongest effects in less-developed Regosols, while the more developed Luvisols and especially Cambisols showed weaker effects than on average. The duration of the long-term trials and with this, the duration of BBF amendment had a significant effect on the SOC concentration. The effect size increased proportionately with the duration of the long-term trial. We found effect sizes that were higher than average for long-term trials running since 15 to 25 years and the highest effects for trials running for more than 25 years. Trials running for less than 15 years



showed effect sizes below the average, and trials running between 5 and 10 years had the lowest values.

Table 3: Effects of environmental characteristics and trial duration on the efficiency of BBFs on SOC concentration

	Treat vs. negative control				Treat vs. positive control				
	N	Effect size	Std. error	Sig. (2-sided)	N	Effect size	Std. error	Sig. (2-sided)	
Overall	200	2.94	0.17	0.0E+00	186	1.80	0.14	0.0E+00	
Climate	Continental	48	4.80	0.52	0.0E+00	38	3.62	0.51	2.2E-12
	Mediterranean	26	6.85	0.91	5.0E-14	17	2.73	1.00	6.1E-03
	Subtropical	26	1.98	0.39	3.1E-07	15	2.21	0.44	5.9E-07
	Temperate	100	2.11	0.17	0.0E+00	116	1.20	0.11	0.0E+00
Soil type WRB	Cambisol	32	1.98	0.26	1.7E-14	33	0.59	0.13	1.0E-05
	Luvisol	38	2.49	0.26	0.0E+00	48	1.50	0.17	0.0E+00
	Mollisol	31	2.91	0.49	3.6E-09	9	3.38	1.22	5.6E-03
	Regosol	79	4.53	0.47	0.0E+00	65	2.37	0.34	1.9E-12
	Other	20	2.89	0.40	4.6E-13	31	2.28	0.37	7.3E-10
LTE duration [yr]	5-10	33	1.80	0.22	2.2E-16	52	0.84	0.14	6.0E-09
	10-15	45	2.57	0.34	2.3E-14	25	2.04	0.36	1.3E-08
	15-25	38	3.46	0.51	1.2E-11	25	1.36	0.43	1.5E-03
	>25	84	3.52	0.28	0.0E+00	84	2.69	0.25	0.0E+00

## 4. DISCUSSION

### 4.1. Solid and carbon-rich BBFs are most efficient in increasing SOC concentrations

Our meta-analysis showed that the application of BBFs has a significant positive effect on the SOC concentration, even when compared to minerally fertilised control plots. The mean effect size of  $2.9 \text{ g} \cdot \text{kg}^{-1}$  is not much from an ecological perspective, but it represents a significant carbon sequestration of  $7 \text{ Mg C} \cdot \text{ha}^{-1}$ , if we assume a horizon thickness of 20 cm and a bulk density of  $1.2 \text{ g} \cdot \text{cm}^{-3}$ . We propose that the increase in SOC concentration following BBF application results from the combination of a direct effect of the BBF applied and an indirect effect via its fertilisation of the following crop and thus higher OM inputs (Bunemann et al., 2006).

The meta-analysis shows that the effect size depends mainly on the BBF type, its quality and quantity. It is not surprising that the SOC concentration increases with increasing BBF quantity applied and that we found the strongest effects for application rates of  $>10 \text{ Mg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ . Diacono and Montemurro (2010) concluded in their review article that the best agronomic performance, higher organic carbon contents and improved soil biological functions are achieved in long-term trials with repeated application of organic amendments at highest rates and frequencies. Gross and Glaser (2021) also conclude that higher applied amounts of manure generally lead to higher SOC stocks.

However, we found that BBF type and quality are even more decisive for the increase in SOC concentration. Now the question is if BBFs that support crop production more efficiently (higher nutrient content, narrow C/N) have a stronger effect on the SOC concentration than BBFs that directly increase SOC concentrations and have less fertilisation effect on the crop (lower nutrient content, wide



C/N ratio). The C/N ratio is a robust and easy to determine but not very precise indicator for the BBF quality. Johnson et al. (2007) and Zhang et al. (2008) conclude that the chemical constituents and the C/N ratio of organic matter are important characteristics controlling their decomposition dynamics. Our meta-analysis shows that BBFs with C/N ratios  $>10$  increase SOC concentrations more than BBFs with more narrow C/N ratios, and that solid BBFs were generally more efficient than liquid BBFs. Taking this into account, the considered BBFs in this study can be sorted by their quality from stable and being more efficient in sequestering SOC to labile and having less effect on SOC concentrations. BBFs in the category lignocellulose were always solid, had C/N ratios  $>10$  (50 % of the observations  $>30$ ) and showed the strongest effect of increasing SOC stocks by  $18 \text{ Mg C}^* \text{ha}^{-1}$  if compared to a negative control, and by  $16 \text{ Mg C}^* \text{ha}^{-1}$  if compared to a positive control. On the lower end, digestates were mostly liquid (90 % of the observations), had in 85 % of the observations a C/N ratio  $<10$  and showed a significant but very low SOC increase of just  $2 \text{ Mg C}^* \text{ha}^{-1}$ . Biosolids and biowastes also showed effects above the mean effect size, but not as strong as Lignocellulose. All BBFs in both groups were solid and had more narrow C/N ratios. Biosolids were in 70 % of the observations characterised by narrow C/N ratios  $<10$  (70 % of the observations). Both BBF types have in common that they already underwent a decomposing process (waste water treatment, typically with an anaerobic digestion phase, or composting) that removed labile carbon and enriched more stable C forms (Bernal et al., 2009). Confirmation for this can be drawn from two recent studies on a 42-year old long-term trial that show higher SOC concentrations in plots fertilised with composted manure in comparison to plots with staple manure (Krause et al., 2022; Mayer et al., 2022). In our dataset, straw had C/N ratios  $>10$  and showed effect sizes slightly below the mean. The BBF type manure is considered in two forms: 23% of the observations used liquid manure of which 64% showed narrow C/N ratios of  $<10$ , while 77% of the observations used solid manure with 97% C/N ratios  $>10$ . We found significant but slightly less effect for the BBF type manure compared to the mean across all observations. Green manure was always solid, with wide C/N ratios  $>10$ , but showed the second lowest effects. Gross and Glaser (2021) describe similar results in a meta-analysis on the effects of manure application on SOC stocks. Farmyard-, cattle- and pig manure showed the highest SOC increases, while green manure and straw showed only minor effects. We assume that this low efficiency of green manure despite its theoretically high resistance towards decomposition is because the crops often grow under sub-optimal conditions and thus the OM input cannot be standardised or controlled as well as the precise application of BBFs. Blanco-Canqui (2022) reviewed the literature on the effects of cover crops on carbon sequestration. He concludes that in only 22% of the studies a significant sequestration took place. He found many potential explanations for green manure not being as efficient as expected because of environmental and management reasons. This fits to our findings of green manure not significantly affecting SOC stocks in European long-term trials. Similar results and reasons for no strong effects of cover crops are reported for German croplands (Seitz et al., 2022).

Overall, our findings corroborate the theory of recalcitrance as one mechanism stabilising SOM against decomposition. This theory states that specific organic components like lignin retard the decomposition of organic matter, but only during the initial phase of one or a few years (von Lützow et al., 2006). In the last two decades, this concept was continuously developed and more and more replaced by the concept of accessibility (Dungait et al., 2012; Schmidt et al., 2011). This concept relates the decomposition dynamics of organic matter with the microbial community in the respective soil and how well these microbes can access the organic matter to be decomposed (Lehmann and Kleber, 2015). Other authors go even further and give evidence that the so called low-quality litter with low N concentrations, high C/N ratios, and high phenol/lignin concentrations contribute less to the stable SOM pool (Cotrufo et al., 2013) than high-quality litter because these labile plant constituents are utilized more efficiently by microbes. The resulting microbial products would thus become the main



precursors of stable SOM by promoting aggregation and through strong chemical bonding to the mineral soil matrix. This model contrasts with our findings of higher SOC concentrations in plots fertilised with low-nutrient BBFs like lignocellulose. However, Castellano et al. (2015) advanced the Microbial Efficiency-Matrix Stabilization (MEMS) framework (Cotrufo et al., 2013) by including the carbon saturation concept. They proposed that litter quality affects stable SOC storage only when there is a saturation deficit, e.g. in sandy soils where non-protected SOM can account for a significant share of total SOM due to enhanced biochemical recalcitrance (Carrington et al., 2012; Creamer et al., 2013; Filley et al., 2008; Gaiser et al., 2012). Lehmann et al. (2020) developed the stabilisation mechanisms and proposed that molecular diversity of the OM input and temporal variability of microbial activity together with the spatial heterogeneity control the persistence of SOM in soils. Our findings corroborate this theory as the BBFs showing the strongest effects on SOC concentrations are the ones that are most different to the inherent SOM. Lignocellulosic BBFs, biowaste and biosolids are chemically much more different from the typical SOM of agriculturally managed soils than crop residues, manure, straw or green manure.

In summary, solid and C-rich BBFs showed significant and strong positive effects on SOC concentrations. BBFs with higher fertilisation qualities like digestates and manure showed less or no effects. We assume that the slow decomposition of the solid and C-rich BBFs in combination with their fertilising effect that induces higher crop yields and in turn organic matter input explain their stronger effect.

#### 4.2. Less developed soils and loamy soils in dry climates show the highest potential for carbon sequestration through BBF application

Our meta-analysis shows strong effects of soil and site properties on the efficiency of BBFs to increase SOC concentrations. The initial SOC concentration and the texture showed stronger effects than the pH value. Site characteristics were decisive, too, especially the soil type and the climate, while the duration of the trial was not as important. These findings corroborate the general understanding of SOM dynamics in agroecosystems, but also identify important drivers and the most promising sites for BBF application.

We found the highest effect size in loamy soils compared to sandy soils, indicating that soils with finer texture are more efficient in sequestering SOC. This underlines the generally accepted stabilisation mechanism of physical protection, either as intermediate process within aggregates or long-term stabilisation in organo-mineral complexes, being mostly active in the fine silt and clay fraction (Dungait et al., 2012; von Lützow et al., 2006). Wiesmeier et al. (2019) proposed an indicator system to predict actual and potential SOC stocks for most soil types worldwide and defined the fine mineral fraction as the key element. It is generally accepted that this size fraction has a limited potential to stabilise SOC because firstly the surface area for organo-mineral associations is limited (Hassink, 1997; Stewart et al., 2008; Wiesmeier et al., 2014), and secondly the potential of a given soil material to form aggregates is limited (Brown et al., 2014; Gulde et al., 2008; Kölbl and Kögel-Knabner, 2004). We found evidence for this limitation, because the effect of BBF application was influenced by the initial OC content of the soils. The effect size continuously increased from soils with low initial SOC concentrations to those with medium SOC and then dropped sharply for soils with high initial SOC concentrations. We assume that this finding is the consequence of a SOC sequestration limit. It is clear that a meta-analysis does not replace mechanistic studies elaborating the exact sequestration potential of a soil, but its statistical approach can point towards these mechanistic relations. We assume that the increasing effect size



from low to intermediate initial SOC concentrations is the result of a higher fertility with increasing SOC concentrations and therefore better growing conditions, leading to more biomass input from crops. Thus, soils with a loamy texture and not too high initial SOC concentrations have a larger potential to sequester and stabilise SOC from BBFs.

We also found a significant effect of the pH, with stronger effects for acid and alkaline soils than for neutral soils. We suppose that the limited biological activity in acid soils retards the decomposition of the added BBFs. In alkaline soils, two mechanisms can potentially stabilise SOM: 1. The presence of polyvalent cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) facilitate SOM stabilisation by binding OC to clay surfaces (O'Brien et al., 2015), and 2. Alkaline soils are abundant in dry and warm climatic regions and their water deficit retards the decomposition of BBFs. Further evidence for this theory can be drawn from the strong effect of BBFs on SOC concentrations in continental and especially mediterranean climates and the soils that are frequent in these climatic zones.

Our meta-analysis identified OM-rich Mollisols and less-developed Regosols as the soil types that showed stronger effects of BBF application on SOC concentrations, while the more developed soil types Cambisol and Luvisol showed lower effect sizes. This corroborates the results of Zhang et al. (2008) who compiled a data set on decomposition rates and concluded that geographical location and climate are most decisive for the variation in litter decomposition. Building on this concept, Prescott (2010) concluded that site characteristics play an important role in stabilising SOM against decomposition. Kramer and Chadwick (2018) showed that wet climates especially under forest have generally higher contributions of reactive mineral surfaces which stabilise a large share of the stored SOC in organo-mineral associations. We assume that the higher share of SOM stabilised at reactive mineral surfaces in combination with the saturated sequestration potential of the fine mineral fraction explains the lower effect size in the more developed soils of more wet temperate climates. In contrast, dry biomes contain only a small fraction of SOC retained by reactive minerals, while the larger part is stored in aggregates or in the labile free or particulate SOM pool. This is the result of less soil development because of the water limitation retarding the weathering of the parent material and the formation of pedogenic minerals. Concurrently the soil texture in dry climates is frequently coarser, thus having less surface area for sorption processes. The SOM in these climatic zones is not labile per se. It is sequestered as long as the climate remains dry and thus decomposition is retarded because of low water contents. In these regions inherently recalcitrant BBFs can accumulate and significantly affect SOC concentrations (Castellano et al., 2015) and soil quality by increasing water holding capacity and soil structure, partly compensating for the low reactive mineral surface.

Our meta-analysis underlines the classic stabilisation mechanisms of SOC in different climatic zones. In addition, we can show that the application of low nutrient BBFs in dry climates can result in significant increases of SOC concentrations and stocks. If this holds true, the application of high nutrient, liquid BBFs with narrow C/N ratios in dry climates will not help maintaining SOC stocks. Nevertheless, the sustainable food production is the main purpose of agricultural soil management. Thus, we recommend to closely monitor soil quality (especially SOC stocks) in agricultural soils and complement especially high nutrient BBFs with other materials (e.g. low nutrient BBFs) in order to maintain or even improve soil quality.



## 5. CONCLUSION AND RECOMMENDATIONS

We focussed our meta-analysis on the effects of different BBF types on the SOC concentration as an indicator for soil quality, while yield was not considered. This is important because soil quality and productivity do not always go hand in hand. We are aware that the findings presented in this deliverable may be of relevance for policy recommendations. Thus, they will be discussed in that context in further detail in Deliverable 7.6 “Policy brief with a clear concise summary of policy recommendations”. We found that low nutrient BBFs with wide C/N ratios (especially BBFs containing lignocellulose) significantly increase SOC concentrations, but we assume that their effect on yields will be low because of their low nutrient content and the retarded nutrient release as a consequence of their inherent recalcitrance. We found generally strong effects of BBFs that were depleted in labile and nutrient rich organic substances due to previous processing steps like anaerobic digestion and composting. The emissions and nutrient losses, as well as the potential accumulation of pollutants during these steps were not considered in this analysis. Complete life cycle analyses are needed to fully judge the sustainability of BBFs. This work is currently ongoing in WP5 and WP6 of LEX4BIO. While anaerobic digestion produces energy and thus returns less labile C to the soil, this trade-off is likely negligible, given that in biogas plants, often additional co-substrates are used and ultimately recycled to agriculture. However, we could not draw firm conclusions on C sequestration from digestates vs. other amendments since the number of observations for digestates was too small. Nevertheless, we have shown that BBFs have a significant potential to positively affect SOC concentrations and therefore soil quality. This underlines the common approach that the aim of applying BBFs has to be clear when deciding on the BBF type. Low nutrient BBFs can be an important additive for increasing SOC sequestration in less developed soils of dry climates, while high nutrient BBFs will not have similar effects. In wetter climates, it is quite likely that the SOC sequestration potential is already saturated and further application of BBFs will have no additional effect on soil quality. We recommend customized and site-adapted fertilisation strategies considering both aspects: plant nutrient and soil quality maintenance or improvement. We have a broad and growing spectrum of BBFs available ensuring that both requirements can be met. We conclude that the OC in BBFs can have strong positive effects on soil quality underlining the role of carbon as the ignored nutrient. Thus, C-depleting steps during BBF processing, e.g. incineration, should be applied with caution and only if needed to eliminate organic pollutants and pathogens.

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