Increased CO₂ fluxes from a sandy Cambisol under agricultural use in the Wendland region, Northern Germany, three years after biochar substrates application

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Abstract
In recent years, biochar has been discussed as an opportunity for carbon sequestration in arable soils. Field experiments under realistic conditions investigating the CO₂ emission from soil after biochar combined with fertilizer additions are scarce. Therefore, we investigated the CO₂ emission and its ¹³C signature after addition of compost, biogas digestate (originating from C₄ feedstock) and mineral fertilizer with and without biochar (0, 3, 10, 40 Mg biochar/ha) to a sandy Cambisol in Northern Germany. Biomass residues were pyrolized at ~650°C to obtain biochar with C₃ signature. Gas samples were taken biweekly during the growing season using static chambers three years after biochar substrate addition. The CO₂ concentration and its δ¹³C isotope signature were measured using a gas chromatograph coupled to an isotope ratio mass spectrometer. Results showed increased CO₂ emission (30%–60%) when high biochar amount (40 Mg/ha) was applied three years ago together with mineral fertilizer and biogas digestate. On average, 59% of the emitted CO₂ had a C₃ signature (thus, deriving from biochar and/or soil organic matter), independent of the amount of biochar added. In addition, our results clearly demonstrated that only a small amount of released CO₂ derived from biochar. The results of this field experiment suggest that biochar most likely stimulates microbial activity in soil leading to increased CO₂ emissions derived from soil organic matter and fertilizers mineralization rather than from biochar. Nevertheless, compared to the amount of carbon added by biochar, additional CO₂ emission is marginal corroborating the C sequestration potential of biochar.

KEYWORDS
biomass residues, carbon sequestration, CO₂ emission, field experiment, microbial biomass, organic fertilizer, stable isotope

1 | INTRODUCTION

It is estimated that 1550 billion tons (Pg) of the global carbon stock is stored in soils as soil organic carbon (Lal, 2004). Due to the conversion of natural ecosystems to arable land and intensive agricultural land use, soils lose about 1.2 Pg C annually emitted as CO₂ into the atmosphere, corresponding to about 10% of globally emitted annual CO₂ emission (Glaser & Stoknes, 2014). As a direct consequence, soil quality and biomass productivity decrease and atmospheric...
CO₂ concentration increases (Glaser & Stoknes, 2014; Lal, 2004). Aside from reduced soil C stocks caused by human activities, some soils have low soil C stocks due to natural limitations. Examples are sandy soils in the North German Plain, formed during the late Quaternary by glacial and periglacial deposits, being poor in soil organic matter and nutrients. Only high amounts of mineral fertilizer allow agricultural use of these disfavored regions although being ineffective.

In recent years, the idea of transferring atmospheric CO₂ into stable soil C pools was triggered by studies about charcoal-rich Anthrosols such as Terra Preta de Indio in the humid tropics of Brazil (Glaser, 2007; Glaser & Birk, 2012; Glaser, Haumaier, Guggenberger, & Zech, 2001). Soils, similar to Terra Preta are also described in other climate regions, which leave no doubt that charcoal may last in soil over millennia (Wiedner & Glaser, 2015; Wiedner, Schneeweiß, Dippold, & Glaser, 2015). However, stabilization mechanisms of pyrogenic carbon are still poorly understood but most likely a combination of chemical recalcitrance and physical protection takes place (Glaser, Balashov, Haumaier, Guggenberger, & Zech, 2000; Kuzyakov, Bogomolova, & Glaser, 2014; Marschner et al., 2008). Furthermore, type of feedstock and charring conditions (e.g., temperature and duration) heavily influence physical and chemical properties of charred particles (biochar) and thus their fate in soil environment (Schmelpfenning & Glaser, 2012; Wiedner et al., 2013). Nevertheless, the presence of oxygen-containing functional groups on surfaces of ancient biochar indicates degradation processes, which are probably affected by the complex interaction of abiotic and biotic factors such as soil moisture, soil temperature, soil microbial community structure, and land use practices (Wang, Xiong, & Kuzyakov, 2016; Wiedner, Fischer, et al., 2015).

Most of the studies dealing with biochar stability in soil environment are performed in laboratory experiments or extrapolations based on investigations of fire-affected soils (He et al., 2017). For instance, half-life of biochar exposed in temperate rainforests are estimated up to 6623 years (Preston & Schmidt, 2006). Long-term mean residence time (MRT) of biochar in two different savanna regions were estimated up to 1300 and 2600 years (Lehmann et al., 2008). In contrast, Hammes, Torn, Lapenas, and Schmidt (2008) calculated a turnover time of biochar in a Russian steppe soil of only 293 years and Bird, Moyo, Veenendaal, Lloyd, & Frost, (1999) report a half-life of charcoal in savannah soil of <100 years. A meta-analysis by Wang et al., (2016) revealed that the decomposed amount of biochar increased with experimental duration, but the decomposition rate decreased with time and MRT of biochar was estimated to 556 ± 483 years. The examples listed show clearly that biochar decomposition differs between different climates, soil types, experiment duration, feedstock, and biochar production conditions (Lorenz & Lal, 2014; Wang et al., 2016).

A further complication is the big knowledge gap on biochar interactions and stability combined with other fertilizers under realistic field conditions. It is mandatory to understand and characterize the behavior of biochar in arable soils to estimate its potential of long-term carbon sequestration. Thus, we performed a large-scale field experiment under both practical agronomic conditions and scientific requirements to investigate long-term C sequestration potential of complex biochar fertilizers in a sandy soil under temperate climate conditions. For this purpose, different amounts of biochar (0, 3, 10, 40 Mg/ha) were co-applied with compost, biogas digestate and mineral fertilizer to determine the release of CO₂ after three years of realistic agronomic land use. Furthermore, the emission source will be identified using stable carbon isotope signature (δ¹³C). The study addresses the following three research questions:

1. Does biochar increase CO₂ emissions when combined with mineral fertilizer, biogas digestate, or compost?
2. Are CO₂ emissions different when low or high amounts of biochar (3 or 40 Mg/ha) were applied to mineral fertilizer and biogas digestate?
3. Does the emitted CO₂ derive from biochar, soil organic matter, and/or applied fertilizers?

2 | MATERIALS AND METHODS

2.1 | Study site and agricultural management

The large-scale field experiment is located near Gartow at 53°1.154’N and 11°29.834’E, 19 m above sea level in the eastern part of Lower Saxony, Germany. The experiment was established and run under agronomic practice since the end of May 2012 (Glaser, Wiedner, Seelig, Schmidt, & Gerber, 2015).

Gartow has a mean annual temperature and precipitation of 8.8°C and 575 mm, respectively (Glaser et al., 2015). During the growing season 2014 (April to August 2014), mean temperature and precipitation were 16.2°C and 271 mm, respectively (DWD Climate Data Center, 2015). The sandy soil was classified as Stagnic Cambisol resulting from Quaternary dynamics (Glaser et al., 2015) with a δ¹³C isotope signature of −26.7 mUr and a soil organic carbon content of 0.6%.

The field experiment was designed as a Latin rectangle consisting of 50 plots in total, divided into ten different treatments with five replicates (Glaser et al., 2015). Treatments comprised mineral fertilizer, biogas digestate, fermented biogas digestate (inoculated with indigenous microorganisms, which were extracted from neighboring forest soils), and compost produced from local biomass.
residues. In addition, biochar was added to these pure fertilizers either annually in low amount (1 Mg biochar/ha) or once in high amount (10 Mg biochar/ha for compost and 40 Mg biochar/ha for the other treatments). Before application, all fertilizers were adjusted to common practice nitrogen levels depending on the cultivated crop. All field management activities were carried out according to agronomic practice.

Biochar was made out of nutrient-poor biomass residues produced by pyrolysis in a PYREG reactor at ~650°C (PYREG GmbH, Dörth, Germany) with the following properties: pH$_{\text{CaCl}_2}$ 8.6, electrical conductivity 1000 µS/cm, ash content 12.6%, total carbon content 71.3%, total nitrogen content 1.0% (Wiedner, Fischer, et al., 2015). Further information about the preparation and properties of the fertilizers are published by Glaser et al. (2015).

From May 30, 2012 to September 24, 2012, silage hybrid maize (Zea mays, variety KALVIN, Syngenta Agro GmbH, Maintal, Germany) was cultivated. Hybrid winter rye (Secale cereale, variety BRASETTO, KWS SAAT SE, Einbeck, Germany) was sown after maize harvest at the mid of October 2012. On April 11, 2013, a treatment-specific fertilization on a fixed nitrogen level of 120 kg/ha was performed. Three weeks after the rye harvest on July 24, 2013, a mixture of 13 catch crops was sown. Before the third fertilization on March 23, 2014, the catch crops were chopped by a rotary hoe and subsequently incorpo rated by a shallow plow. Due to cultivation of the legume narrow-leaved lupine (Lupinus angustifolius, variety BOREGINE, Saatzucht Steinach GmbH & Co. KG, Steinach, Germany) in 2014, nitrogen application was reduced to 36 kg/ha for each treatment. For this purpose, each plot was fertilized uniformly with 252 L biogas digestate (Raiffeisen Warenengenossenschaft Jameln eG, Jameln, Germany) mixed with 0.37 kg elemental sulfur (90% S) except the plots treated with mineral fertilizer. The mineral fertilizer mixture for each of the conventional farming treatments consisted of 2.0 kg KALISOP (50% K$_2$O and 45% SO$_3$) from K+S Kali GmbH, 0.35 kg MgSO$_4$, 0.40 kg quick-lime, and 14.6 L Organic Plant Feed (9% N, 2% P$_2$O$_5$ and 2% K$_2$O) from Plant Health Cure BV. As in the previous two years of the field experiment, the treatments with annual biochar application received 77 kg biochar (47% DM), corresponding to 1 Mg biochar/ha (Biochar (3) digestate and biochar (3) mineral fertilizer). Immediately after the application of fertilizers, a shallow plow was used for fertilizer incorporation and narrow-leaved lupine was sown with a spring-tooth harrow. In 2014, no irrigation or application of herbicide or fungicide was performed during the whole growing season. A tined weeder was used once to control weeds mechanically during the growing season. The lupine harvest was on August 4, 2014.

### 2.2 Gas sampling

CO$_2$ released from the sandy soil was sampled biweekly from April 1, 2014 to August 5, 2014, using static PVC-U chambers (Gebr. Ostendorf Kunststoffe GmbH, Vechta, Germany). To avoid boundary effects, for example, of neighboring plots, each chamber was placed in the plot center (Figure 1a). The chambers were 0.55 m long with 0.11 m inner diameter (Figure 1b). Each chamber was inserted 0.15 m deep into the soil (Figure 1b). Therefore, the chamber covering an area of 0.01 m$^2$. Together with the remaining 0.40 m length above the soil surface, each chamber had an aboveground cylinder volume of 3.8 dm$^3$.

The gas samples were transferred through multisample needles (Ø 0.8 × 38 mm) (Vacutest Kima S.r.l., Piove di Sacco, Italy) to sealed and pre-evacuated 0.2 dm$^3$ headspace glass vials, which were closed by aluminum crimp caps combined with butyl hollow stopper (IVA Analysentechnik GmbH & Co. KG, Meerbusch, Germany). Gas samples were taken immediately after chamber closure as well as after 10, 20 and 30 min. To avoid measurement of root respiration, chambers were kept free of vegetation. According to Parkin and Kaspar (2003), gas sampling was carried out during 11 am to 1 pm to get representative results reflecting the daily mean emissions.

### 2.3 Instrumentation and calculations

CO$_2$ measurements were performed using a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) additionally equipped with a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland). Carbon dioxide concentration and stable C isotope composition was detected by a Finnigan Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, Bremen, Germany), coupled via a ConFlo IV universal interface (Thermo Fisher Scientific, Bremen, Germany) to the Trace GC Ultra gas chromatograph.

Injections were carried out using a 2.5 cm$^3$ gas-tight headspace syringe SYRC HS2.5-23-5 (CTC Analytics, Zwingen, Switzerland) in split mode (split ratio of 5 and split flow of 13 cm$^3$/min) through a glass inlet liner (TQ CE 5 mm inner diameter, SGE Europe Ltd., Milton Keynes, UK). The injector block was heated at 100°C. To remove potential isobaric interferences (e.g., N$_2$O), a chromatographic separation of CO$_2$ was carried out using a Carboxen 1010 PLOT column (30 m, 0.32 mm internal diameter, Supelco, Bellefonte, PA, USA) with a constant helium flow (99.9997%, pure) of 2.5 cm$^3$/min. The temperature program started at 40°C (1.00 min holding time), heated up to 100°C at 30°C/min (holding time 2.50 min) ended with 230°C at 100°C/min (holding time 2.00 min).
ISODAT 3.0 (Thermo Fisher Scientific) and EXCEL 2013 (Microsoft, Redmond, WA, USA) were used for data processing. An external standard (IAEA-CO-8, -5.764 mUr, Vienna Pee Dee Belemnite (VPDB), International Atomic Energy Agency (IAEA), Vienna, Austria) was analyzed with each measurement and used for carbon concentration and isotope calibration. To produce CO2 from these inorganic solid materials, orthophosphoric acid (85%, Baker analyzed, Mallinckrodt Baker, Deventer, the Netherlands) was added to the argon (99.999%, pure) purged vial, which contained weighted portion of the reference standard. This procedure was performed for three different amounts of the standard (30, 60, and 90 µg).

\[ \delta^{13} \text{C} \text{ signature was calculated according to Craig (1957) and all isotope signatures are expressed by the SI-compliant unit Urey (Ur) as recommended by Brand and Coplen (2012) (Equation 1).} \]

\[
\delta \text{ [mUr]} = \left( \frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1 \right) \times 1000 \tag{1}
\]

where \( R \) is the \(^{13}\text{C}/^{12}\text{C} \) ratio of the standard or the sample and \( \delta \) describes the relative isotope ratio of the sample relative to the standard IAEA-CO-8 in mUr.

\( \delta^{13} \text{C} \) measurements were drift- and amount dependence-corrected according to Zech & Glaser, 2008.

Calculation of the CO2-C fluxes was performed using Equation (2) (Flessa, Wild, Klemisch, & Pfadenhauer, 1998; modify by Beetz et al. 2013):

\[
F_{\text{CO2-C}} = k_{\text{CO2}} \times \frac{273.15}{T} \times \frac{V}{A} \times \frac{\Delta C}{\Delta t} \tag{2}
\]

where \( F_{\text{CO2-C}} \) is the flux rate of CO2-C (mg CO2-C m\(^{-2}\) hr\(^{-1}\)), \( k_{\text{CO2}} \) (0.536 kg C/m\(^3\)) serve as conversion factor for the ideal gas at 273.15 K, \( T \) is the daily mean air temperature (K), \( V \) is the volume of the chamber (m\(^3\)), \( A \) is the basal area within the chamber and \( \Delta C/\Delta t \) is the concentration change of CO2-C over time (ppm, per hr) in the headspace of the chamber obtained by linear regression. For calculating the cumulative CO2-C fluxes, no linear interpolation between the sampling days was used, because of the strong variations under field conditions and unavailable data of needed parameters (e.g., soil temperature, soil moisture). Therefore, an interpolation would overestimate the actually emitted carbon dioxide.

In general, C3 and C4 vegetation shows average values of -27.7 mUr or -13.5 mUr (Troughton, Card, & Hendry, 1974). The isotope composition of CO2-C makes it possible to determine different sources of CO2-C emissions using a two source-mixing model (Fry, 2006) as shown in Equation (3):

\[
\text{Fraction}_{\text{C3 source}}[\%] = \frac{\left( \delta_{\text{sample}} - \delta_{\text{C4 source}} \right)}{\left( \delta_{\text{C3 source}} - \delta_{\text{C4 source}} \right)} \times 100 \tag{3}
\]

where the C3-derived fraction (including soil organic matter and biochar) or the C4-derived one (containing biogas digestate) in percent is calculated by isotope signatures of the sample and the two emission sources. We are aware that isotope fractionation during CO2 production through soil organic matter decomposition occurs. Isotope fractionation is highly complex and strongly influenced by several variables such as soil organic matter quality, water content, or the carbon-nitrogen ratio of soils (Wang, Jia, & Li, 2015). In our study, we decided against a correction factor, because the fractionation intensities were not determinable. For removing the atmospheric isotope background, Miller-Tans plots were used (Miller & Tans, 2003). For this purpose, the products of the individual CO2-C concentrations and the \( \delta^{13} \text{C} \) values of sampled CO2 were plotted against

**FIGURE 1** (a) Experimental plots equipped with static gas sampling chambers during the growing season 2014. (b) Outline and dimensions of static gas sampling chamber.
their corresponding CO₂-C concentrations. The resulting slope of the linear regression shows the isotope signature of the source without background. This technique allows to identify the isotope signature of the soil respired CO₂, especially if the air background concentrations vary over time and are not stable as required for the Keeling plot (Miller & Tans, 2003).

Feedstock and bulk soil samples were air-dried and ground before δ¹³C analysis. Total carbon and nitrogen content and the isotope signature of the amendments were detected by an Euro EA Elemental Analyser (Eurovector, Milan, Italy), which was coupled via a Finnigan ConFlo III (Thermo Fisher Scientific) universal interface to a Finnigan Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific).

2.4 | Statistical analyses

Statistical analysis and graphical design were carried out using R 3.3.1 (R Core Team, 2016) and EXCEL 2013 (Microsoft Corporation, Redmond, WA, USA). Box plots (not shown) were used to identify outliers. To do this, all values beyond the limit of 1.5 times of the interquartile range were eliminated from further analysis. Afterward, arithmetic means of the remaining replicates (n = 3–5) were calculated. Differences in hourly-emitted carbon dioxide (CO₂-C) during the growing season between treatments and corresponding pure fertilizer without biochar means were statistically evaluated using the two-sided unpaired two-sample t test. Prior test assumption of normally distributed data was examined using the Shapiro–Wilk test. In case of non-normal distributed data (treatment min-

3 | RESULTS

3.1 | CO₂ emissions

Temporal variation of the CO₂ fluxes and weather conditions for comparison are shown in Figure 2. From April to the beginning of May 2014, CO₂ fluxes within treatments were more or less stable but varied among treatments from approximately 50 mg CO₂-C m⁻² hr⁻¹ (pure fertilizer) to 150 mg CO₂-C m⁻² hr⁻¹ (high biochar addition). At the beginning of May 2014, CO₂ emissions increased continuously until June 10, 2014. On June 25, 2014, all treatments showed a reduced CO₂ emission. Thereafter, a strong increase in CO₂ flux was observed for all treatments.

The CO₂ flux generally increased in the order mineral fertilizer < digestate (pure and fermented) < compost. The yearly re-application of 1 Mg biochar/ha mixed with digestate did not increase the CO₂ fluxes substantially compared to the corresponding fertilizers. Furthermore, the differences between high biochar applications (40 Mg/ha) and the pure fertilizers were obviously higher using mineral fertilizer and digestate compared to compost and fermented digestate (Figure 2).

The mean CO₂ fluxes per hour (Table 1) varied from 86 to 151 mg CO₂-C m⁻² hr⁻¹ with significantly increased emissions when biochar was applied in high application amounts. For instance, CO₂ emission of mineral fertilizer or biogas digestate combined with 40 Mg/ha biochar increased up to 64% and 53% compared to the pure fertilizers, respectively (Table 1). Fermented digestate including 40 Mg biochar/ha emitted 30% more CO₂-C than the corresponding pure fermented digestate alone. Low amounts of biochar (3 Mg/ha) combined with biogas digestate did not increase the CO₂ flux, which is in contrast to mineral fertilizer where CO₂-C emission increased by 26% (Table 1). Pure compost showed higher CO₂-C fluxes than mineral fertilizer, fermented and nonfermented biogas digestate and biochar addition did not significantly increase CO₂-C emission of the compost treatment (Table 1).

3.2 | Carbon isotope signature (δ¹³C)

Table 2 shows the ¹³C isotope signature and total carbon content of the applied fertilizers within the three years of the field experiment. As expected, biochar addition to the fertilizers increased total carbon content multiple times. With exception of mineral fertilizer (2012), biochar addition increased ¹³C isotope signature of fertilizers. Furthermore, ¹³C isotope signature of pure fertilizers was more or less equal in each year with exception of the mineral fertilizer applied 2014, which was considerably higher compared to 2012 and 2013.

Maize biogas digestate showed a typical C4 plant ¹³C isotope signature of approximately −12.7 mUr. Due to the C3 signature of biomass residues biochar used in this study (−20.8 mUr), sources of emitted CO₂ could be determined. Yakir and Sternberg (2000) classified the ¹³C isotope composition of −25.0 mUr for soils containing C3 soil organic matter. During the growing season 2014, the CO₂ emissions showed mostly an intermediate ¹³C signature, indicating mixed C4 and C3 sources (Figure 3). However, short-term negative or positive offsets could be observed during the measurement (Figure 3). Especially digestate and mineral fertilizer treatments, including biochar-amended treatments, revealed a strong variation and a daily fluctuation of the emission source. In contrast, CO₂ emissions of compost and fermented digestate showed smaller variations during the growing season. Compared with the seasonal course of the CO₂ fluxes (Figure 2)
almost all treatments showed a rapid change to approximately \(-25\) mU/h on June 9, 2014. Only controls such as fermented digestate and compost as well as biochar (40) mineral fertilizer do not follow this pattern.

### 3.3 Cumulative emissions and emission sources

The cumulative CO\(_2\) emissions of all sampling dates confirm the results of increased CO\(_2\) releases after biochar addition (Figure 4). CO\(_2\) emissions of biochar-treated plots (40 Mg biochar/ha) increased up to 57\% compared to corresponding fertilizers without biochar. CO\(_2\)-C emissions of compost and biochar (10) compost were more or less equal. There is only a slight increase in the soil-derived CO\(_2\)-C by biochar addition to the mineral fertilizer treatment. On the other hand, the fertilizer-derived carbon dioxide output rises. Both digestate treatments (untreated and fermented) showed an increased level of soil-derived

| TABLE 1 | Hourly-emitted carbon dioxide-carbon (CO\(_2\)-C) of the sampling dates during the growing season of narrow-leafed lupine (Weighted mean \pm standard error of the weighted mean). Relative changes refer to the comparison of the treatment and pure fertilizers without biochar |
|---------------------------------|------------------|-----------------|--------------------------------------------------------------------|
| Treatment                        | CO\(_2\)-C (mg m\(^{-2}\) hr\(^{-1}\)) | Relative change (%) |
| Mineral fertilizer               | 86 \pm 3          | –                |
| Biochar (3) mineral fertilizer   | 108 \pm 4         | 26               |
| Biochar (40) mineral fertilizer  | 141 \pm 5         | 64*              |
| Digestate                       | 99 \pm 3          | –                |
| Biochar (3) digestate            | 95 \pm 3          | –4               |
| Biochar (40) digestate           | 151 \pm 4         | 53*              |
| Fermented digestate             | 105 \pm 3         | –                |
| Biochar (40) fermented digestate | 136 \pm 4         | 30               |
| Compost                         | 128 \pm 3         | –                |
| Biochar (10) compost             | 146 \pm 4         | 14               |

Significant differences between the treatment and the corresponding pure fertilizer without biochar are marked by *, which represent \(p < .05\).
carbon emissions after the addition of biochar. The mixture of 40 Mg biochar/ha and digestate increases also the emission from fertilizer.

Although the total emissions always increased after the addition of high biochar amounts (40 Mg/ha), the relative contribution of soil-derived CO$_2$-C mostly did not raise for all treatments (Figure 4). Carbon dioxide emissions with a C3 signature of biochar (40) mineral fertilizer and biochar (40) digestate showed a reduction of the relative contribution. On the other hand, fermented digestate mixed with 40 Mg biochar/ha did not follow this trend and showed minor enhancement C3-derived CO$_2$ emissions. It is conspicuous that the biochar (3) digestate does not follow this trend. For biochar (3) digestate, the cumulative emissions stagnate, but the relative contribution shifts to more C3-derived CO$_2$ emissions. In comparison with all other treatments, the addition of 10 Mg biochar/ha to compost effects only minor changes of the relative contribution of the CO$_2$ emission sources.

4 | DISCUSSION

4.1 | Heterotrophic respiration as function of fertilizer type

Against the observations of several laboratory experiments (e.g., Marstorp, 1996; Stumpe et al., 2012), no enhanced CO$_2$ production immediately after fertilizer application was observed (Figure 2). The reason could be the low soil temperature of approximately 11°C, dry soil conditions, which restrict the microbial activity, or the lagged gas sampling performed one week and a half after the fertilizer incorporation. Therefore, the initial microbial stimulation by the fertilizer ceased until we started gas sampling.

In general, it can be expected that the application of easily available carbon (e.g., in the form of liquid organic fertilizer or biogas digestate) enhances CO$_2$ emission of well-aerated soils such as sandy soils. The fast decomposition of easily degradable organic compounds is responsible for additional emissions (Bol, Moering, Kuzyakov, & Ame-lung, 2003; Joergensen, Meyer, Roden, & Wittke, 1996; Stumpe et al., 2012). In our case, CO$_2$ emission increased after organic fertilizers application compared to mineral fertilizer (all without biochar). Both average and total emissions of the whole growing season are increased by 13% for biogas digestate, 19% for inoculated biogas digestate, and 42% for compost (Table 1; Figure 4). The process of fermentation led to a reduced amount of easily degradable organic compounds. Therefore, the CO$_2$ emissions should be decreased on plots treated with fermented digestate. But our experiment shows contrary results. Additional fermentation of already fermented biogas digestate did not further reduce CO$_2$ emission. Another explanation of enhanced degradation of organic fertilizers is that nitrogen fertilization stimulates microbial activity (Lu et al., 2014). The use of organic fertilizers provides high nitrogen amounts and labile carbon structures, which stimulates heterotrophic respiration.

4.2 | Heterotrophic respiration as influenced by biochar application

Our experiment showed increased CO$_2$ release from the sandy Cambisol when high amount of biochar (40 Mg/ha)

| 2012 | 2013 | 2014 |
|------|------|------|
| Total C (%) | δ$^{13}$C (mUr, VPDB) | Total C (%) | δ$^{13}$C (mUr, VPDB) | Total C (%) | δ$^{13}$C (mUr, VPDB) |
| Mineral fertilizer | 5.6 | –40.3 | 6.5 | –43.3 | 27.5 | –17.4 |
| Biochar (3) mineral fertilizer | 21.4 | –29.4 | 50.8 | –28.1 | 63.3 | –24.6 |
| Biochar (40) mineral fertilizer | 62.7 | –27.5 | n.a. | n.a. | n.a. | n.a. |
| Digestate | 36.0 | –8.1 | 38.6 | –11.9 | 36.9 | –12.7 |
| Biochar (3) digestate | 44.8 | –18.7 | 53.5 | –20.9 | 62.4 | –23.7 |
| Biochar (40) digestate | 72.3 | –27.0 | n.a. | n.a. | n.a. | n.a. |
| Fermented digestate | 39.2 | –11.4 | 33.7 | –13.0 | 33.5 | –13.0 |
| Biochar (40) fermented digestate | 66.6 | –25.9 | n.a. | n.a. | n.a. | n.a. |
| Compost | 9.7 | –20.4 | n.a. | n.a. | n.a. | n.a. |
| Biochar (10) compost | 10.7 | –23.8 | n.a. | n.a. | n.a. | n.a. |
| Biochar, pure | 71.9 | –27.6 | 61.2 | –28.0 | 51.5 | –26.4 |

n.a., not available because of missing re-fertilization with biochar containing fertilizers.
was applied, independent from the fertilizer used in addition. These results are compliant with a meta-analysis by He et al. (2017), who found an increased CO₂ release from soils after biochar addition. Similar results were reported by Lanza, Wirth, Gessler, and Kern (2015) for a short-term dynamic incubation experiment, which showed higher CO₂ effluxes of amendments with fermented biochar. However, as we did not measure CO₂ release during fermentation, total carbon balance remains unclear. But it could be shown that total carbon balance does not differ between composting and fermentation of biochar (Fischer & Glaser, 2012).

Within this study, we could not clarify the responsible processes, which led to increased CO₂ emissions. However, in our opinion, the most probable explanation is that due to high porosity of biochar, microorganisms are protected against predators leading to higher microbial biomass (Lehmann et al., 2011; Thies & Rillig, 2009). In the presence of easily available carbon (e.g., in the form of liquid organic fertilizer or biogas digestate), microbial degradation

**FIGURE 3** Isotope composition ($\delta^{13}C$) of the CO₂ emissions obtained from the Miller-Tans mixing model (Miller & Tans, 2003).
(a) Mineral fertilizer. (b) Digestate. (c) Fermented digestate. (d) Compost (Missing data point on June 9 based on a deficient sampling).
of these products is increased, which leads to higher CO₂ emissions of well-aerated soils. As described in several studies (Kuzyakov et al., 2009, 2014; Wang et al., 2016), the decomposition of biochar mainly takes place in a co-metabolic way.

Furthermore, biochar significantly improved the plant-available water holding capacity of sandy soils (Glaser et al., 2015; Liu et al., 2012). Consequently, it also ensures water supply to soil microorganisms during dryer periods. On the other hand, a waterlogging situation on June 25, 2014, may explain the reduced CO₂ emission from all treatments.

Another explanation for increased CO₂ release upon high biochar application is a missing protection against co-metabolic degradation due to missing organic-mineral interactions between biochar and minerals caused by the sandy texture of our soil with a minor clay fraction (<5%). Also, Wang et al. (2016) showed in a meta-analysis that clay-poor soils (<10%) could have up to 20% higher carbon losses compared to clay-rich soils when high amounts of biochar were applied. Brodowski, Amelung, Haumaier, Abetz, and Zech (2005) and Glaser et al. (2000) demonstrated that oxidized zones on biochar surface are more susceptible to organic-mineral interactions, which are a main driver of long-term stability of biochar in addition to its intrinsic chemical recalcitrance. These interactions effect a physicochemical protection through aggregation, which prevent the ongoing microbial or chemical oxidation of the charcoal surface (Glaser et al., 2000). A sandy-textured soil is characterized by a bigger size of its primary particles and inert particle surfaces, compared to clay or silt, which reduces the formation of mineral aggregates containing biochar. Also, direct organic-mineral interactions between biochar and mineral surfaces, caused by electrostatic cation bridges, hydrophobic interactions or H-bondings, are inhibited because of the sandy texture (von Lützow et al., 2006). Therefore, physical protection against degradation can mostly be neglected. A further study by Brodowski, John, Flessa, and Amelung (2006) demonstrated that macroaggregates are not effective to enclose biochar in sandy soils. This missing physical protection could also enhance the vulnerability of biochar for (co-metabolic) degradation, which leads to higher carbon dioxide emissions.

4.3 | Carbon sources of heterotrophic respiration

The shifting carbon isotope ratio of the mineral fertilizer represents the change from conventionally produced mineral fertilizer, which possess δ¹³C values of approximately −40 mUr (typical signature of fossil methane, which is a basic commodity of the urea production, Vitoria, Otero, Soler, & Canals, 2004), to an immediately available organic-based fertilizer, which was used in 2014. The carbon isotope signature of −17.4 mUr shows a mixture of C3 and C4 feedstocks. Both biogas digestates showed C4-type δ¹³C values well-reflecting maize used as feedstock (Table 2). Biochar exhibited a δ¹³C value indicating C3 origin of biomass used as feedstock, while compost showed a slightly more positive δ¹³C value still indicating C3 origin but also ¹³C enrichment due to intensive microbial degradation via composting process (Table 2).

Generally, δ¹³C value of heterotrophic respiration became more negative during the growing season due to the mineralization shift from fertilizer to soil organic matter, which is based on the short-term availability of the applied fertilizers (Figure 3). This effect was independent from the applied fertilizer (Figure 3). The shifting δ¹³C
values during the growing season suggest that directly after the fertilizer application, fertilizer-derived organic material was degraded by microorganisms. During the growing season 2014, the decrease of δ¹³C values indicates an increasing microbial degradation of soil- and biochar-derived organic matter. However, fluctuation of CO₂ δ¹³C values during the experimental period was rather high (Figure 3). Therefore, all δ¹³C values were integrated into an isotope mass balance (Figure 4).

Figure 4 clearly shows that the more biochar was added, the more CO₂ was released up to 60%. There was only a small increase of soil- and/or biochar-derived organic matter, indicated by a small increase of C₃-derived CO₂ (Figure 4). Due to the same 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 CO₂. Additional C₃-derived CO₂ of the biochar treatments compared to the corresponding pure fertilizers without biochar is either due to biochar degradation or caused by a positive priming of soil organic matter evoked by biochar. Most probably it is a mixture of both processes. Comparing isotope measurements of the treatments with and without biochar clearly indicates a minor contribution (positive priming or biochar degradation) to total CO₂ emissions when combined with mineral fertilizer or compost (Figure 4). On the other hand, more biochar or soil organic matter was decomposed, when biochar was combined with biogas digestate (fermented or nonfermented; Figure 4). However, compared to the high amount of biochar added (40 Mg/ha) additional CO₂ release was negligible (about 0.1 Mg/ha).

### 4.4 | Biochar as a passive influencer

Our study clearly shows increased CO₂ emissions at high biochar application amounts (40 Mg/ha) from a sandy Cambisol under practical agronomic conditions in Northern Germany, especially when combined with mineral fertilizer and digestate. Stable isotope (δ¹³C) measurements show the main source of enhanced CO₂ emissions being fertilizer-derived organic carbon when biochar was combined with mineral fertilizer (containing organic carbon) and compost, while the positive priming potential of biochar or the co-metabolic decomposition of biochar was a substantial source of enhanced CO₂ emissions, when combined with biogas digestate even in case this was fermented beforehand.

In view of the fact that biochar should be able to sequester large amounts of carbon due to long-term stability, the increased CO₂ emissions after the treatment with 40 Mg biochar/ha are negligible (about 0.1 Mg/ha). However, further long-term measurements of CO₂ emissions under field conditions are necessary to get a clear picture of the carbon sequestration potential of biochar.

In summary, increased carbon losses from biochar-treated agriculturally used soils under temperate conditions result from different factors, which are stimulated by the presence of biochar. The most important emission driver might be the enlargement of the microbial biomass (Figure 5) because of the suitable habitat and the protection against predators provided by the microporous surface of biochar. Another main driver of the microbial-derived soil emissions is the soil temperature and humidity, which are both modified by biochar. The decomposition of organic plant residues, soil organic matter, and biochar is strongly influenced by the amended fertilizers.

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