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Biochar promotes methane production during anaerobic digestion of organic waste

Leilei Xiao¹,² · Eric Lichtfouse³,⁴ · P. Senthil Kumar⁵ · Quan Wang¹ · Fanghua Liu¹,²

Abstract
Climate change and energy demand are calling for more sustainable fuels such as biomethane produced by anaerobic digestion of organic waste. Biochar addition to waste is presumed to enhance the efficiency of methane production, yet individual reports disclose contradictory results. Therefore, we performed a meta-analysis of 27 selected publications containing 156 paired measurements of control and biochar-amended treatments to assess the impact of biochar on the methanogenic performance. Results show that biochar promotes biomethane production substantially with a high Hedge’s $d$ value of $5.7 \pm 1.04$, yet sporadic publications report a methane decline. Methanogenic performance is statistically controlled by feedstock type, pyrolysis temperature and biochar concentration, but not controlled by pH, size, surface area and methanogen species. These findings should help to tune the parameters of anaerobic digestion with biochar to optimize biomethane productions. Moreover, our results cast some doubt on the efficiency of adding biochar to soil to sequester carbon in soils because biochar promotes methane generation and, in turn, emissions of methane, a greenhouse gas, to the atmosphere.

Keywords Anaerobic digestion · Biochar · Methane · Meta-analysis · Wastewater treatment

Introduction
Global warming and the rising energy demand are calling for more circular processes where waste is recycled into materials and energy. Biomethane is a carbon–neutral, sustainable fuel produced by anaerobic fermentation of organic matter in natural and anthropic environments, yet the efficiency of actual processes is limited (Chen et al. 2018; Gao et al. 2020; Garcia-Mancha et al. 2017). Strategies have been recently developed to improve anaerobic fermentation by microbial immobilization, pH buffering and enzymatic induction (Gao et al. 2020; Xiao et al. 2020a). Anaerobic degradation and biomethane production are also promoted by electromethanogenesis using electroactive microorganisms and conductive materials such as biochar (Fig. 1; Li et al. 2018; Xiao et al. 2020b; Yuan et al. 2018). Recent research has also focused on the use of nanomaterials to favor methanogenesis (Ma et al. 2020; Xiao et al. 2018, 2019c).

Biochar is carbon negative and comprises a wide variety of complex materials produced by pyrolysis of biomass (Glaser et al. 2009; Gunarathne et al. 2019; Akhil et al. 2021; Fawzy et al. 2021). Biochar has been applied to reduce nutrient leaching from soils, to recover resources from water, to accelerate waste disposal and for biomethane production
Biochar properties and molecular composition vary widely with the nature of the feedstock and pyrolysis conditions (Keiluweit et al. 2010; Gao et al. 2020). Several biochar properties have been proposed to favor biomethane production, e.g., microbial immobilization, pH buffering, and controlling metal ion availability and enzymatic processes (Yuan et al. 2018; Xiao et al. 2019b; Gao et al. 2020; Huang et al. 2020b; He et al. 2020). Overall, mechanisms fostering methanation by biochar are better understood but individual studies report sometimes contradictory results, e.g., rising or declining biomethane production (Cheng et al. 2018; Luo et al. 2015; Shen et al. 2016). Therefore, we report here a meta-analysis to clarify the impact of biochar properties on methanogenesis during anaerobic digestion of environmental waste.

**Experimental**

**Biochar data**

We found 105 publications in the Web of Science and Bing search engine for documents on biochar application to methane production during anaerobic digestion (AD) for treating environmental waste and pollution, excluding soil-related research, from January 1 2010 to June 15 2020, using the keywords “biochar” AND “methane” OR “CH\textsubscript{4}.” We extracted the following variables: feedstock, pyrolysis temperature, pH, size, surface area, conductivity, methanogen species and methanogenic performance. Variable means, standard deviations and sample replicate number were extracted from publication tables and text. When data were only reported in image format in graphs, data points were extracted using Plot Digitizer 2.6.8 and Web Plot Digitizer. When relevant data were not present in publications, corresponding authors were contacted to get the data. We first considered the highest rate of methane production, but, if not available, we used the highest yield of methane. When accurate maximum rate of methane production or yield could not be obtained due to too much fluctuation of methane concentrations, publications were excluded from this study.

From this initial pool, we selected only documents reporting three or more replicates for each run, and we found 19 publications containing 105 data pairs of treatment data versus control data (Table S1). Control is defined as runs without biochar. We used the Hedges method, rather than the response ratio, because the Hedges method is adapted to data samples of relatively small size (Jeffery et al. 2016; Larry and Ingram 1985). Therefore, a minimum of two replicates is meeting the analysis standard. Consequently, data on biomethane production were collected from 27 articles containing 156 paired measurements of control and biochar-amended treatments for disposal of environmental waste and pollution.

Biochar variables were grouped to facilitate cross-comparisons, e.g., the nature of biochar feedstock was grouped in ‘wood and sawdust,’ ‘herbaceous and lignocellulosic waste,’ ‘manure,’ and ‘sludges’ (Table S1). Similarly, pyrolysis temperatures were grouped in ‘below 500 °C,’ ‘500–700 °C,’ and ‘above 700 °C.’ Conductivities were grouped in ‘below 450 μS/cm’ and ‘above 450 μS/cm.’ Biochar pH was grouped into ‘acidic below 7,’ ‘weakly alkaline from 7 to 9,’ and ‘alkaline above 9.’ Sizes were grouped in ‘below 1 mm’...
and ‘above 1 mm.’ Brunauer, Emmett and Teller (BET) surface areas were grouped in ‘below 100 m² g⁻¹’ and ‘above 100 m² g⁻¹.’ Biochar concentrations were grouped in ‘below 10 g dm⁻³,’ ‘equal to 10 g dm⁻³’ and ‘above 10 g dm⁻³.’ Two types of methanogenic archaea were distinguished: acetoclastic methanogens and hydrogenotrophic methanogens.

**Meta-analysis**

We used the standardized mean difference metric Hedge’s $d$ in Eq. 1, which induces less biases that the Hedge’s $g$ factor in Eq. 2 (Larry and Ingram 1985):

\[ d = \left(1 - \frac{3}{4(n - 2) - 1}\right)g \]  
\[ (1) \]

\[ g = \frac{X_1 - X_2}{S_p} \]  
\[ (2) \]

\[ S_p = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 - 1 + n_2 - 1}} \]  
\[ (3) \]

where $n$ denotes the total sample size, and $\bar{x}_1$ and $\bar{x}_2$ depict the means of experimental and control treatments. Experimental data refer to the treatment with biochar, whereas control data refer to the treatment without biochar. A categorical random effect model was applied to $d$, with means weighted by the inverse of the variance. Here, $S_p$ is the pooled standard deviation in Eq. 3, where $n_1$ and $n_2$ are the number of repetitions in the control and experimental groups, and $s_1$ and $s_2$ depict the standard deviations of control and experimental groups.

Contrary to the response ratio commonly used in ecological research, the standardized mean effect sizes are probabilistic (Hedges et al. 1999; Larry and Ingram 1985). That is, the mean effect sizes describe the probability that a sample would fall between the experimental mean and the control mean, assuming a normal distribution (Hedges et al. 1999). Consequently, confidence intervals of 95% were generated based on a normal distribution. When the 95% confidence interval of the parameter does not overlap with Hedge’s $d$ of 0, this implies that the variable promotes biomethane production, which suggests the promotion of anaerobic digestion of environmental waste. When the 95% confidence interval of a biochar parameter does not overlap with that of another variable, there is a statistically significant difference.

By convention, for variables that do not overlap with the Hedge’s $g$ of 0, a $d$ value higher than 0.8 indicates a large effect, $d$ of 0.2–0.8 shows a moderate effect, and $d$ of 0.0–0.2 displays a small effect (Hedges et al. 1999; Jeffery et al. 2016). A key point is that, using the Hedge’s $d$ metric, an effect size of a variable analysis does not equate to an effect size of others in independent analyses presented in this study. As a consequence, only categories within individual analyses, e.g., feedstocks, as differentiated by the horizontal dotted bars, can be compared. The effect sizes do not mean that the extent of the actual biomethane production increase or decrease. Small effect sizes may indicate significant value in biomethane production in absolute terms. For instance, in small effect sizes, the actual biomethane parameter may be several times larger than that of the large effect sizes.

**Results and discussion**

**Overall effect of biochar addition**

We assessed the global effect of biochar addition on anaerobic methanogenesis by calculating the grand mean of the Hedge’s $d$ for 156 published data pairs of treatment versus control without biochar (Fig. 2). Results show a $d$ value of 5.70 ± 1.04, which evidences a large effect size and implies that the presence of biochar statistically induces an increase in biomethane in most investigations. Yet sporadic studies have also shown the inhibitory effect of biochar or no effect (Cheng et al. 2018; Shen et al. 2016). This discrepancy is probably due to the high heterogeneous nature of biochar (Diao et al. 2020; Gao and Goldfarb 2019), suggesting that biomethane production may be enhanced by specific biochar properties, as discussed below.

**Effect of biochar feedstock**

We calculated $d$ values of feedstock including sludges, manure, herbaceous and lignocellulosic waste, and wood and sawdust (Fig. 2). All feedstock types show high $d$ values from 4.71 ± 1.72 to 7.99 ± 1.51, implying that biochar addition improves biomethane generation whatever the type of feedstock. Furthermore, there is no statistical difference within feedstock types, sludges displaying the highest $d$ of 7.99. Manure, plant waste and woody materials appear equally competitive with Hedge’s $d$ values around 5.0. High $d$ values for sludges are supported by the fact that sludge biochar provides more nutrients for fermentative bacteria and methanogens (Wang et al. 2020). Moreover, biochar from sludge has also induced better pollutant removal and heavy metal adsorption (Diao et al. 2020; Regkouzas and Diampopoulos 2019; Singh et al. 2020), which may be explained by a more favorable living environment for microbial organisms. Overall, the slight advantage of sludge biochar in terms of methanogenesis is likely due to its ability to adsorb and store nutrients for activating methanogens. We conclude that biochar improves methanogenesis for all biochar feedstocks, but there is no statistical advantage of the feedstock type.
Effect of pyrolysis temperature

We calculated $d$ values of biochar produced by pyrolysis below 500 °C, of $6.72 \pm 1.86$, between 500 and 700 °C, of $6.40 \pm 1.11$, and above 700 °C, of $0.840 \pm 1.50$ (Fig. 2). Results imply that biochar favors biomethane generation below 700 °C. There is no significant difference between 500 and 700 °C-produced biochar and biochar produced below 500 °C. On the other hand, pyrolysis above 700 °C induces a drastic decline of biomethane promotion. These findings may be explained by changes of the biochar molecular structure with temperature (Hao et al. 2018). Indeed, Keiluweit et al. (2010) observed a gradual change in the molecular structure of plant biomass-derived biochar with temperature.

High-temperature biochar is characterized by fewer labile compounds at the surface of biochar particles, and therefore less microbial substrates for fermentative bacteria and methanogenic archaea (Bruun et al. 2011). This explanation is strengthened by the declining CH$_4$ and N$_2$O emissions from soils amended with high-temperature biochar, which are thus better suited for mitigation of greenhouse gas emissions (Cayuela et al. 2015). This scenario is also supported
by the biochar release of less degradable organic compounds when the pyrolysis temperature increases (Ji et al. 2020). By contrast, slow pyrolysis at low temperature yields more biochar with diverse chemical groups (Chen et al. 2019; Sohi et al. 2010), which are likely to promote methane production in anaerobic digesters, or methane emissions from soils (Jeffery et al. 2016). Overall, our findings show that biochar produced below 700 °C improves methanogenesis. Pyrolysis at lower temperature is also saving energy.

Effect of biochar conductivity

Biochars having low conductivity, below 450 μS/cm, show a much higher $d$ value, of 7.58 ± 1.79, than high-conductivity biochar, displaying a $d$ value of 2.06 ± 1.29 (Fig. 2). Low conductivity biochar is therefore statistically more effective at accelerating biomethane production. This finding is unexpected because recent research suggests that biochar acts as an electron shuttle, which should favor microbial activity (Viggi et al. 2017; Xiao et al. 2019b; Yuan et al. 2018). Nonetheless, a recent report explains that electrical conductivity of biochar is controlling only the rate of anaerobic degradation, not the yield of biogas (Rasapoor et al. 2020). Moreover, conductivity does not appear as a relevant factor for choosing which biochar should be used for degrading environmental waste (Lu et al. 2020a), and some studies suggest that attributing rising biomethane production to high material conductivity requires caution (Martins et al. 2018; Van Steendam et al. 2019; Wang et al. 2021). Overall, our findings show that low conductivity biochar favors methanogenesis, yet underlying mechanisms are unclear.

Effect of biochar pH

Figure 2 displays the effect of biochar of different pH on biomethane production. Results show that varying the biochar pH induces no statistical difference in biomethane production, despite the fact that pH is known to modify fermentation rates (Begum et al. 2018; Feng et al. 2020; Mao et al. 2017). Yet, most investigations included in this meta-analysis did not report the pH of the system before and after biochar application, though pH is expected to vary widely because some biochar contains oxygen-containing organic anions and carbonates that increase alkalinity (Fidel et al. 2017; Yuan et al. 2011; Meng et al. 2020). Overall, varying the pH of biochar does not statistically improve methanogenesis.

Effect of surface area and biochar size

Values of $d$ for biochar with BET surface area above 100 m$^2$/g, of 5.06 ± 1.83, are not statistically different from those of biochar with surface area below 100 m$^2$/g, of 4.45 ± 1.06 (Fig. 2). Similarly, the size to biochar particles does not appear to modify biomethane generation, yet a trend for higher $d$ value is observed for particle size below 1 mm. This implies that smaller particles of biochar may be beneficial to the degradation of environmental waste. For instance, the addition of powdered biochar to a pig manure/wheat straw aerobic compost increased biomethane emissions by 57%, whereas granular biochar decreased biomethane emissions by 22% (He et al. 2018). On the contrary, other investigations have shown that large biochar particles promote methanogenesis (Cheng et al. 2018; Viggi et al. 2017). Overall, there is no clear global effect of surface area and size on anaerobic degradation of waste and pollutant and on biomethane production.

Effect of biochar concentration

Biochar concentration caused a strong and statistically significant difference in the strength of biomethane production, with a maximal impact for concentrations exceeding 10 g/L and a $d$ value of 7.87 ± 0.35 (Fig. 2). Increasing biochar concentration is therefore an efficient means to improve methanogenesis, which may further result in a promotion of waste degradation. This finding is supported by biochar properties that are likely to stabilize anaerobic digestion and rise biomethane yield (Gao et al. 2020; Lim et al. 2020; Ma et al. 2021). For instance, providing immobilization sites for microorganisms could explain the higher anaerobic degradation and methanogenic performance (Zhang et al. 2018).

Moreover, even though biochar itself is not a substantial source of labile carbon, biochar is a sponge-like material able to adsorb and store organo-mineral nutrients for further microbial feeding (Cross and Sohi 2011; Demisie et al. 2014). In this line, elevated biochar concentrations have been shown to increase the availability of organic carbon for fermentation bacteria and methanogenic archaea (Lu et al. 2020b; Jiang et al. 2020; Zhang et al. 2020). Based on this, environmental waste and pollution can be degraded more easily, which in turn is more conducive to biological activities (Xiao et al. 2021a; b). Overall, high biochar concentrations foster methanogenesis, yet underlying mechanisms remain undeciphered.

Methanogenic species

Values of $d$ for acetoclastic methanogens, of 5.19 ± 2.06, and hydrogenotrophic methanogens, of 3.08 ± 1.4, are not statistically different, implying a similar contribution of these species to biomethane production (Fig. 3). These high $d$ values also reveal that both acetoclastic and hydrogenotrophic methanogens produce more biomethane following biochar addition. This finding is strengthened by an investigation revealing that Methanosarcina, Methanosaeta and
Methanobacterium methanogens predominate in paddy soil-amended biochar during the anaerobic decomposition of rice straw (Huang et al. 2020a). Trophic methanogens, hydrogenotrophic and acetoclastic methanogens may actively participate in the methane production process. Indeed, reports have shown that methanogens that use acetate and hydrogen as substrates coexist in the anaerobic fermentation system (Madigou et al. 2019; Zhang et al. 2019). Compared to hydrogenotrophic methanogens, acetoclastic methanogens should contribute more to methane production with sufficient organic substrates (Garcia-Mancha et al. 2017; Lim et al. 2020; Xiao et al. 2019a). Overall, biochar addition improves biomethane production by methanogens, yet acetoclastic and hydrogenotrophic methanogens display similar performances.

**Conclusion**

Our findings show that, on the average, biochar addition is favoring biomethane generation, whereas this was not clear in previous individual reports. Our identification of biochar properties that favor or do not favor methanogenesis will be helpful for basic research to decipher underlying mechanisms, and for applied research to improve biomethane production as a sustainable fuel and benefit perfection of environmental waste and pollution control measures. Last, the fact that biochar globally promotes biomethane generation in anaerobic media is casting some doubt on the use of biochar to sequester carbon in soils. Indeed, our findings suggest that soils amended with biochar may accelerate methane emissions in the atmosphere, notably in anaerobic soils where fermentation of organic matter and pollution takes place, thus counteracting the sequestrating effect of biochar.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10311-021-01251-6.

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**Author contributions** LX and EL designed the research. LX and QW collected the data. LX, EL, SK, FL analyzed the data. LX and EL wrote the article.

**Declarations**

**Conflict of interest** Authors declare no competing financial interest.

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Supplementary Material

Biochar promotes methane production during anaerobic digestion of organic waste

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Number of Tables: 1

Table SI 1 Literature data and references of the effect of biochar addition on biomethane production during anaerobic disposal of solid waste and wastewater.
Table S1 Literature data and references of the effect of biochar addition on biomethane production during anaerobic disposal of solid waste and wastewater.

| Feedstock | Pyrolysis temperature (°C) | pH       | Conductivity (mS/m) | Size (mm) | BET (m²/g) | Doze       | Methanogens | Methanogens | Max rate or yield (SD) | Number | Max rate or yield (SD) | Number | Hedge's References |
|------------|----------------------------|----------|---------------------|-----------|------------|------------|-------------|-------------|------------------------|---------|------------------------|---------|---------------------|
| Pine wood  | 980                        | NA       | 2.2 ± 0.46          | 2.0-2.4   | 451 ± 11   | NA         | NA          | NA          | 0.87 0.018              | 2       | 1.06 0.0792           | 2       | (Cheng et al., 2018)  |
| Pine wood  | 980                        | NA       | 2.2 ± 0.46          | 2.0-2.4   | 451 ± 11   | NA         | NA          | NA          | 0.935 0.015             | 2       | 1.3 0.0532            | 2       | (Cheng et al., 2018)  |
| Pine wood  | 980                        | NA       | 2.2 ± 0.46          | 2.0-2.4   | 451 ± 11   | NA         | NA          | NA          | 1.16 0.145              | 2       | 1.11 0.0166           | 2       | -0.277               |
| Pine wood  | 980                        | NA       | 2.2 ± 0.46          | 2.0-2.4   | 451 ± 11   | NA         | NA          | NA          | 1.08 0.037              | 2       | 0.741 0.0748          | 2       | -3.28                |
| Pine wood  | 980                        | NA       | 2.2 ± 0.46          | 2.0-2.4   | 451 ± 11   | NA         | NA          | NA          | 1.11 0.014              | 2       | 0.748 0.184           | 2       | -1.59                |
| Pine wood  | 980                        | NA       | 2.2 ± 0.46          | 2.0-2.4   | 451 ± 11   | NA         | NA          | NA          | 1.08 0.014              | 2       | 0.413 0.0317          | 2       | -15.54               |
| Fruitwoods | 800                        | 8.63 ± 0.13 | NA NA NA | 2        | NA         | NA          | NA          | NA          | 2.81 0.624              | 3       | 2.25 0.32909          | 3       | -0.899               |
| Fruitwoods | 800                        | NA       | 0.5-1 Methanobacterium m | 6 | Methanobacterium | 1.26 0.104 | 3 | 1.53 | 0.10392 | 3 | 3 | (Luo et al., 2015) |
| Fruitwoods | 800                        | 8.63 ± 0.13 | NA NA NA | 8        | Methanobacterium | 0.97 0.104 | 3 | 1.02 | 0.10392 | 3 | 3 | (Luo et al., 2015) |
| Sawdust    | 500                        | 0.12 ± 0.05 | NA NA NA | 1        | 34 10     | NA         | NA          | NA          | 1.349 0.095            | 2       | 1.351 0.0843          | 2       | 0.0127               |
| Biomass         | pH   | COD | VFA | Methanogenic Bacteria | Methanosarcina | Methanobacterium |
|-----------------|------|-----|-----|-----------------------|----------------|------------------|
| Sawdust 500     | 9.15 | 0.12| 248 | Methanosaeta          | 5.696          | 0.025            |
| Sawdust 500     | 9.15 | 0.12| 248 | Methanosaeta          | 5.696          | 0.025            |
| Sawdust 500     | 9.15 | 0.12| 248 | Methanosaeta          | 5.696          | 0.025            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Hardwood        | 7.9  | 0.3 | 14  | NA                    | 1.813          | 0.142            |
| Rice straw      | 9.6  | 0.6 | 20  | Methanosarcina        | 0.025          | 0.025            |
| Wood chips cow  | 9.6  | 0.6 | 20  | Methanosarcina        | 0.025          | 0.025            |
| Manure biomasses| 9.6  | 0.6 | 20  | Methanobacterium      | 0.025          | 0.025            |

(Li et al., 2018) (Paritosh and Vivekanand, 2019) (Yuan et al., 2018) (Paritosh and Vivekanand, 2019) (Paritosh and Vivekanand, 2019) (Paritosh and Vivekanand, 2019)
| Material      | Density | Density Deviation | Moisture | Moisture Deviation | Ash | Ash Deviation | Fixed Carbon | Fixed Carbon Deviation | Ultimate Energy | Ultimate Energy Deviation | (Sunyoto et al., 2016) |
|--------------|---------|-------------------|----------|-------------------|-----|-------------|--------------|------------------------|----------------|--------------------------|----------------------|
| Pine sawdust | 650     | ±0.025            | 9.6      | ±0.003            | 130 | ±0.025      | 16.6        | ±9                     | 113            | ±8.66                    | 3 160 5.19615 2 3 5.27  |
| Pine sawdust | 650     | ±0.025            | 9.6      | ±0.003            | 130 | ±0.025      | 25.1        | ±9                     | 113            | ±8.66                    | 3 145 5.19615 2 3 3.58  |
| Pine sawdust | 650     | ±0.025            | 9.6      | ±0.003            | 130 | ±0.025      | 33.3        | ±9                     | 113            | ±8.66                    | 3 128 5.19615 2 3 6.10  |
| Switchgrasses| 500     | ±0.05 ± 0.3       | 7.3      | ±0.669            | ±5.7 ± 2.0 | NA  NA | 190.8        | ±328.7 4.458 3 7.40 | (Shanmugam et al., 2018) |
| Ashe juniper | 400     | ±0.2 ± 0.53       | 35 ± 14  | ±0.838            | ±8.0 ± 3.0 | NA  NA | 190.8        | ±327.2 14.7 3 6.10 | (Shanmugam et al., 2018) |
| Fruitwoods   | 800–900 | ±0.13 ± 0.63      | NA       | ±NA               | ±NA  | ±NA | ±4          | ±NA  NA 5.6 3 0.691 | (Cai et al., 2016)    |
| Fruitwoods   | 800–900 | ±0.13 ± 0.63      | NA       | ±NA               | ±NA  | ±NA | ±10         | ±NA  NA 4.8 3 0.230 | (Cai et al., 2016)    |
| Fruitwoods   | 800–900 | ±0.13 ± 0.63      | NA       | ±NA               | ±NA  | ±NA | ±20         | ±NA  NA 4.6 3 1.30  | (Cai et al., 2016)    |
| Fruitwoods   | 800–900 | ±0.13 ± 0.63      | NA       | ±NA               | ±NA  | ±NA | ±2          | ±NA  NA 3.5 3 0.586 | (Cai et al., 2016)    |
| Fruitwoods   | 800–900 | ±0.13 ± 0.63      | NA       | ±NA               | ±NA  | ±NA | ±5          | ±NA  NA 4.2 3 2.60  | (Cai et al., 2016)    |
| Fruitwoods   | 800–900 | ±0.13 ± 0.63      | NA       | ±NA               | ±NA  | ±NA | ±10         | ±NA  NA 1.05 3 0.105 | (Cai et al., 2016)    |
| Fruitwoods   | 800–900 | ±0.13 ± 0.63      | NA       | ±NA               | ±NA  | ±NA | ±1.6        | ±NA  NA 5.23 3 4.90 | (Cai et al., 2016)    |
| Fruitwoods   | 800–900 | ±0.13 ± 0.63      | NA       | ±NA               | ±NA  | ±NA | ±4          | ±NA  NA 4.6 3 4.69  | (Cai et al., 2016)    |
| Sewage sludge | 350 | 6.4 ± 0.1 | 44.22 ± 0.02 | NA | NA | 0.5 | NA | NA | 156.5 | 3.226 | 3 | 187.9 | 4.839 | 3 | 6.11 (Ambaye et al., 2020) |
| Sewage sludge | 350 | 6.4 ± 0.1 | 44.22 ± 0.02 | NA | NA | 1 | NA | NA | 156.5 | 3.226 | 3 | 208.9 | 4.032 | 3 | 11.5 (Ambaye et al., 2020) |
| Sewage sludge | 350 | 6.4 ± 0.1 | 44.22 ± 0.02 | NA | NA | 1.5 | NA | NA | 156.5 | 3.226 | 3 | 190.3 | 5.645 | 3 | 5.88 (Ambaye et al., 2020) |
| Sewage sludge | 350 | 6.4 ± 0.1 | 44.22 ± 0.02 | NA | NA | 2 | NA | NA | 156.5 | 3.226 | 3 | 182.3 | 4.839 | 3 | 5.02 (Ambaye et al., 2020) |
| Sewage sludge | 550 | 9.5 ± 0.1 | 21.07 ± 0.02 | NA | NA | 0.5 | NA | NA | 156.5 | 4.839 | 3 | 166.9 | 2.42 | 3 | 2.17 (Ambaye et al., 2020) |
| Sewage sludge | 550 | 9.5 ± 0.1 | 21.07 ± 0.02 | NA | NA | 1 | NA | NA | 156.5 | 4.839 | 3 | 188.7 | 3.225 | 3 | 6.26 (Ambaye et al., 2020) |
| Sewage sludge | 550 | 9.5 ± 0.1 | 21.07 ± 0.02 | NA | NA | 1.5 | NA | NA | 156.5 | 4.839 | 3 | 208.9 | 4.032 | 3 | 9.41 (Ambaye et al., 2020) |
| Sewage sludge | 550 | 9.5 ± 0.1 | 21.07 ± 0.02 | NA | NA | 2 | NA | NA | 156.5 | 4.839 | 3 | 196.8 | 4.032 | 3 | 7.25 (Ambaye et al., 2020) |
| Sewage sludge | 350 | 6.4 ± 0.1 | 44.22 ± 0.02 | NA | NA | 0.5 | NA | NA | 156.5 | 6.14 | 3 | 233.3 | 4.386 | 3 | 11.5 (Ambaye et al., 2020) |
| Sewage sludge | 350 | 6.4 ± 0.1 | 44.22 ± 0.02 | NA | NA | 1 | NA | NA | 156.5 | 6.14 | 3 | 227.2 | 4.386 | 3 | 10.6 (Ambaye et al., 2020) |
| Sewage sludge | 350 | 6.4 ± 0.1 | 44.22 ± 0.02 | NA | NA | 1.5 | NA | NA | 156.5 | 6.14 | 3 | 214.9 | 3.509 | 3 | 9.34 (Ambaye et al., 2020) |
| Sewage sludge | 350 | 6.4 ± 0.1 | 44.22 ± 0.02 | NA | NA | 2 | NA | NA | 156.5 | 6.14 | 3 | 207 | 4.384 | 3 | 7.57 (Ambaye et al., 2020) |
| Sewage sludge | 550 | 9.5 ± 0.1 | 21.07 ± 0.02 | NA | NA | 0.5 | NA | NA | 146.5 | 4.386 | 3 | 166.7 | 4.386 | 3 | 3.68 (Ambaye et al., 2020) |
| Sewage sludge | 550 | 9.5 ± 0.1 | 21.07 ± 0.02 | NA | NA | 1 | NA | NA | 146.5 | 4.386 | 3 | 194.7 | 3.509 | 3 | 9.71 (Ambaye et al., 2020) |
| Sewage sludge | 550 | 9.5 ± 0.1 | 21.07 ± 0.02 | NA | NA | 1.5 | NA | NA | 146.5 | 4.386 | 3 | 211.4 | 4.385 | 3 | 11.8 (Ambaye et al., 2020) |
| Sewage sludge | 550 | 9.5 ± 0.1 | 21.07 ± 0.02 | NA | NA | 2 | NA | NA | 146.5 | 4.386 | 3 | 185.1 | 5.263 | 3 | 6.37 (Ambaye et al., 2020) |
| Material          | 1st Value | 2nd Value | 3rd Value | 4th Value | 5th Value | 6th Value | 7th Value | 8th Value | 9th Value | 10th Value | 11th Value | 12th Value | 13th Value | 14th Value | 15th Value | 16th Value | 17th Value | 18th Value |
|-------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Sewage sludge     | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.4 ± 0.1 |
| Vineyard prunings | 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1| 0.60 ± 0.1|
|                        |          |          |          | Methanosetaeaceae |          |          |          |          |          |          |          |          |
|------------------------|----------|----------|----------|-------------------|----------|----------|----------|----------|----------|----------|----------|----------|
| Wheat straw             | NA       | NA       | NA       | Methanosetaeaceae | NA       | 1.4      | 0.1      | 2        | 1.2      | 0.1      | 2        | -1.14    | (Indren et al., 2020) |
| Sheep manure            | NA       | NA       | NA       | Methanosetaeaceae | NA       | 1.4      | 0.1      | 2        | 1.2      | 0.1      | 2        | -1.14    | (Indren et al., 2020) |
| Dry dairy manure        | 350      | NA       | NA       | 0.6               | 6.3      | 1        | NA       | 2.0      | 19.2     | 0.9      | 2        | 1.09     | 2.673     | (Jang et al., 2019)   |
| Dry dairy manure        | 350      | NA       | NA       | 0.6               | 6.3      | 1        | NA       | 2.0      | 19.2     | 0.9      | 2        | 1.09     | 2.673     | (Jang et al., 2019)   |
| Dry dairy manure        | 350      | NA       | NA       | 0.6               | 6.3      | 1        | NA       | 2.0      | 19.2     | 0.9      | 2        | 1.09     | 2.673     | (Jang et al., 2019)   |
| Dry dairy manure        | 350      | NA       | NA       | 0.6               | 6.3      | 1        | NA       | 2.0      | 19.2     | 0.9      | 2        | 1.09     | 2.673     | (Jang et al., 2019)   |
| Dry dairy manure        | 350      | NA       | NA       | 0.6               | 6.3      | 1        | NA       | 2.0      | 19.2     | 0.9      | 2        | 1.09     | 2.673     | (Jang et al., 2019)   |
| Dry dairy manure        | 350      | NA       | NA       | 0.6               | 6.3      | 1        | NA       | 2.0      | 19.2     | 0.9      | 2        | 1.09     | 2.673     | (Jang et al., 2019)   |
| Corn stover             | 500      | NA       | NA       | 841 to 0.42       | 6.3      | 10       | NA       | 243.2    | 21.4     | 2        | 1.65     | 2        | 1.26     | (Jang et al., 2019)   |
| Corn stover             | 500      | NA       | NA       | 841 to 0.42       | 6.3      | 10       | NA       | 243.2    | 21.4     | 2        | 1.65     | 2        | 1.26     | (Jang et al., 2019)   |
| Corn stover             | 500      | NA       | NA       | 841 to 0.42       | 6.3      | 10       | NA       | 243.2    | 21.4     | 2        | 1.65     | 2        | 1.26     | (Jang et al., 2019)   |
| Douglas fir wood        | 400      | 5.62     | 0.01     | 46.8±1.9          | 16.99    | 0.58     | 10       | Methanobacteractae | Methanosaeae | 19.3 | 1.4 | 2 | 31 | 1.9 | 2 | 4.01 | (Wang et al., 2020) |
| Douglas fir wood        | 500      | 5.62     | 0.00     | 49.9±1.2          | 13.17    | 4.05     | 10       | Methanobacteractae | Methanosaeae | 19.3 | 1.4 | 2 | 38 | 2.2 | 2 | 5.80 | (Wang et al., 2020) |
| Douglas fir wood        | 600      | 5.62     | 0.00     | 51.2±5.7          | 18.36    | 2.61     | 10       | Methanobacteractae | Methanosaeae | 19.3 | 1.4 | 2 | 33.5 | 1.6 | 2 | 5.40 | (Wang et al., 2020) |
| Douglas fir wood        | 730      | 5.62     | 0.00     | 63.6±3.4          | 18.39    | 0.29     | 10       | Methanobacteractae | Methanosaeae | 19.3 | 1.4 | 2 | 25.6 | 1.8 | 2 | 2.23 | (Wang et al., 2020) |
| Douglas fir wood        | 400      | 5.62     | 0.00     | 46.8±1.9          | 16.99    | 0.58     | 10       | Methanobacteractae | Methanobacteria | 3.9 | 1.8 | 2 | 7.4 | 0.5 | 2 | 1.51 | (Wang et al., 2020) |
| Douglas fir wood        | 500      | 5.62     | 0.00     | 49.9±1.2          | 13.17    | 4.05     | 10       | Methanobacteractae | Methanobacterium | 3.9 | 1.8 | 2 | 6.7 | 0.2 | 2 | 1.25 | (Wang et al., 2020) |
| Douglas fir wood        | 600      | 5.62     | 0.00     | 51.2±5.7          | 18.36    | 2.61     | 10       | Methanobacteractae | Methanobacterium | 3.9 | 1.8 | 2 | 6.8 | 1 | 2 | 1.14 | (Wang et al., 2020) |
| Douglas fir wood        | 730      | 5.62     | 0.00     | 63.6±3.4          | 18.39    | 0.29     | 10       | Methanobacteractae | Methanobacterium | 3.9 | 1.8 | 2 | 3.4 | 0.7 | 2 | 0.0418 | (Wang et al., 2020) |
| Douglas fir wood        | 400      | 5.62     | 0.00     | 46.8±1.9          | 16.99    | 0.58     | 10       | Methanobrevibacter | Methanobrevibacter | 30.7 | 1.4 | 2 | 35.8 | 1.9 | 2 | 1.75 | (Wang et al., 2020) |
| Material        | Value   | Standard Deviation | Count | Methanothermobacter | Methanomassiliicoccus | (Sun et al., 2020b) |
|-----------------|---------|--------------------|-------|---------------------|-----------------------|---------------------|
| Douglas fir wood| 500     | 5.77 ± 0.00        | 6.08  | 49.9 ± 1.2          | NA                    | 10                  |
|                 | 600     | 5.12 ± 0.04        | 6.13  | 18.36 ± 2.61        | NA                    | 10                  |
|                 | 730     | 63.6 ± 0.08        | 5.62  | 18.39 ± 0.29        | NA                    | 10                  |
|                 | 400     | 46.8 ± 1.9         | 5.77  | 16.99 ± 0.58        | NA                    | 10                  |
|                 | 500     | 49.9 ± 0.00        | 6.08  | 18.39 ± 4.05        | NA                    | 10                  |
|                 | 600     | 51.2 ± 0.04        | 6.13  | 18.36 ± 2.61        | NA                    | 10                  |
|                 | 730     | 63.6 ± 0.08        | 5.62  | 18.39 ± 0.29        | NA                    | 10                  |
| Sewage sludge  | 300     | 6.78 ± 6.41        | 6.13  | 21.319 ± 3.19       | NA                    | 10                  |
| Cow manure     | 500     | 7.5 ± 27.4         | 6.13  | 3 ± 31.74           | NA                    | 10                  |
|                 | 700     | 24.85 ± 6.13       | 6.13  | 3 ± 31.74           | NA                    | 10                  |
| Cow manure     | 500     | 8.5 ± 112.6        | 6.13  | NA                  | Methanospirillum       | 10                  |
| Cow manure     | 500     | 8.5 ± 112.6        | 6.13  | NA                  | Methanospirillum       | 10                  |
| Cow manure     | 500     | 8.5 ± 112.6        | 6.13  | NA                  | Methanospirillum       | 10                  |
| Cow manure     | 500     | 8.5 ± 112.6        | 6.13  | NA                  | Methanospirillum       | 10                  |
| Cow manure     | 500     | 8.5 ± 112.6        | 6.13  | NA                  | Methanospirillum       | 10                  |
| Cow manure     | 500     | 8.5 ± 112.6        | 6.13  | NA                  | Methanospirillum       | 10                  |
| Cow manure     | 500     | 8.5 ± 112.6        | 6.13  | NA                  | Methanospirillum       | 10                  |
| Cow manure     | 500     | 8.5 ± 112.6        | 6.13  | NA                  | Methanospirillum       | 10                  |
| Corn straw     | 400     | 8.2 ± 29.8         | 6.13  | NA                  | NA                    | 8                   |
| Corn straw     | 500     | 8.3 ± 32.8         | 6.13  | NA                  | NA                    | 8                   |
| Material       | NO. | Consistency | 
|----------------|-----|-------------|
| Corn straw     | 600 | 8.3         | NA          |
| Sewage sludge  | 400 | 9.3         | NA          |
| Sewage sludge  | 500 | 9.5         | NA          |
| Sewage sludge  | 600 | 9.7         | NA          |
| Coconut shell  | 400 | 8.7         | NA          |
| Coconut shell  | 500 | 9.5         | NA          |
| Coconut shell  | 600 | 11.1        | NA          |
| Corn straw     | 600 | 8.3         | NA          |
| Corn straw     | 600 | 8.3         | NA          |
| Sewage sludge  | 600 | 9.7         | NA          |
| Sewage sludge  | 600 | 9.7         | NA          |
| Sewage sludge  | 600 | 9.7         | NA          |
| Coconut shell  | 600 | 11.1        | NA          |
| Coconut shell  | 600 | 11.1        | NA          |
| Bamboo biochar | ±   | 0.024 ± 0.002 | 2-3         |
| Pine           | 800 | 0.5–1 < 0.00 | 5 | 210.78 |
| Pine           | 800 | 0.5–1 < 0.00 | 5 | 210.78 |
| Sample Type          | Sulfate (mg/L) | Phosphorus (mg/L) | Potassium (mg/L) | Methanomicrobia | Methanomicrobia (mg/L) | Methanosetaeae (mg/L) | Methanobacteriacea (mg/L) | Source |
|----------------------|----------------|-------------------|------------------|-----------------|------------------------|------------------------|--------------------------|--------|
| Pine                 | 800            | NA                | 7.3              | NA              | NA                     | NA                     | NA                       | (Lu et al., 2019)        |
| Sawdust              | 300 ± 0.1      | 9.2 ± 1           | 5 ± 2            | 9.08            | 0.69                   | 3                      | 9.44                     | (Wang et al., 2020a)     |
| Sawdust              | 500 ± 0.1      | 10.0              | NA               | NA              | 115.9                  | 2.362                  | 3                       | (Wang et al., 2020a)     |
| Sawdust              | 700 ± 0.2      | 800               | NA               | NA              | 115.9                  | 2.362                  | 3                       | (Wang et al., 2020a)     |
| Straw                | 350–550        | 143               | NA               | NA              | 0.756                  | 0.092                  | 3                       | (Xiao et al., 2019)      |
| Sludge               | 500 ± 0.05     | 46.6 ± 2.8        | NA               | NA              | 20.14                  | 1.49                   | 3                       | (Yin et al., 2019)       |
| Sawdust              | 500            | 9.2 ± 1           | 248.6            | Methanomicrobia | Methanomicrobia        | NA                     | NA                       | (Wang et al., 2018)      |
| Sawdust              | 500            | 9.2 ± 1           | 248.6            | Methanomicrobia | Methanomicrobia        | NA                     | NA                       | (Wang et al., 2018)      |
| Sawdust              | 500            | 9.2 ± 1           | 248.6            | Methanomicrobia | Methanomicrobia        | NA                     | NA                       | (Wang et al., 2018)      |
| Sawdust              | 500            | 9.2 ± 1           | 248.6            | Methanomicrobia | Methanomicrobia        | NA                     | NA                       | (Wang et al., 2018)      |
| Red spruce woodchips | NA             | 8.4 ± 1           | 327              | Methanosetaeae  | Methanosetae           | NA                     | NA                       | (Mainardis et al., 2019) |
| Red spruce woodchips | NA             | 8.4 ± 1           | 327              | Methanosetaeae  | Methanosetae           | NA                     | NA                       | (Mainardis et al., 2019) |
| Pine biochar         | NA             | NA                | 310.19           | Methanosetaeae  | Methanosetae           | NA                     | NA                       | (Mainardis et al., 2019) |
| Pine biochar         | NA             | NA                | 310.19           | Methanosetaeae  | Methanosetae           | NA                     | NA                       | (Mainardis et al., 2019) |
| White oak biochar    | NA             | NA                | 296.81           | Methanosetaeae  | Methanosetae           | NA                     | NA                       | (Shen et al., 2016)      |
| White oak biochar    | NA             | NA                | 296.81           | Methanosetaeae  | Methanosetae           | NA                     | NA                       | (Shen et al., 2016)      |
| Pine biochar         | NA             | NA                | 310.19           | Methanosetaeae  | Methanosetae           | NA                     | NA                       | (Shen et al., 2016)      |
| Pine biochar         | NA             | NA                | 310.19           | Methanosetaeae  | Methanosetae           | NA                     | NA                       | (Shen et al., 2016)      |
| White oak biochar    | NA             | NA                | 296.81           | Methanosetaeae  | Methanosetae           | NA                     | NA                       | (Shen et al., 2016)      |
| White oak biochar    | NA             | NA                | 296.81           | Methanosetaeae  | Methanosetae           | NA                     | NA                       | (Shen et al., 2016)      |
| Wheat bran pellets   | 800 ± 0.05     | 1.7–2             | 55 ± 1           | Methanosarcina  | Methanosarcina         | 14.15                  | 4                       | 58.308                        |

(Wang et al., 2020a) (Mainardis et al., 2019) (Shen et al., 2016) (Viggi et al., 2017)
| Treatment           | Methanogens | Methanogens | 14.15 | 4.001 | 2   | 66.923 | 2.461 | 2 | 4.28 |
|---------------------|-------------|-------------|--------|--------|-----|--------|-------|---|------|
| Coppiced woodlands  | Methanosarcina | Methanosarcina | 4.15 | 4.001 | 2 | 66.923 | 2.461 | 2 | 4.28 |
| Orchard pruning     | Methanosarcina | Methanosarcina | 4.15 | 4.001 | 2 | 66.923 | 2.461 | 2 | 4.28 |

Notes: 1, NA, not available; 2, Not used; 3, The predominant methanogens; 4, Methanogens with the highest increase in abundance. These paired measurements of control and biochar-amended treatments was in duplicate with a gray background.
thermophilic anaerobic digestions of dairy manure.

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