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Potential of biochar to mitigate methane production in paddy soils—application of a new incubation and modelling approach

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Abstract Paddy soils are a significant source of methane (CH_4) affecting the global climate. Therefore, it is important to investigate both emission mitigation strategies and the underlying biogeochemical processes. The application of biochar into paddy soils has emerged as a promising measure to mitigate CH_4 emissions. However, it has not yet been clarified why such effects are usually weaker in field studies than in laboratory incubations and which properties of biochar specifically decrease the production of CH_4. We conducted two incubation experiments, one with 1.5% addition of untreated biochars and one with same amounts, but pH-levelled, rinsed biochars. According to the common experimental design of existing incubation studies (experiment 1) biochar addition induced a mean soil pH increase of 0.28 after anaerobic incubation compared to the contro. In these treatments, biochar significantly extended the pre-methanogenic stage (mean 24.23%). However, this effect was weakened or even reversed when pH-levelled, rinsed biochars were amended in experiment 2, which was intended to mimic the persistent long-term effects in the field. This indicated that the provision of electron accepting capacities to suppress methanogenesis may be less important than previously thought. The addition of biochar significantly lowered CH_4 production rates *m* in both experiments with no significant influence of the pH (mean 25.89%), though. Our study demonstrated that incubation studies on CH_4 production in paddy soils can be improved by separating the pre-methanogenic and the methanogenic stage. This facilitates future research to compare characteristics of biochar, but also combinations of measures to optimise CH_4 mitigation strategies.

Keywords Electron accepting capacity (EAC) · Methanogenesis · pH effect · Respiration model · Feedstock · Redox chemistry

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Introduction

The widespread cultivation of paddy soils in the tropics and subtropics strongly affects the global climate: 20% of anthropogenic CH_4 emissions are due to rice production (Cai et al. 2018). To effectively mitigate climate change without jeopardising the important role of rice in human food security (Kögel-Knabner

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et al. 2010), scalable and easily applicable solution strategies to lower the CH_4 burden are needed. The application of biochar into paddy soils is seen as a promising measure in this regard (Qin et al. 2016; Ma et al. 2019; Mohammadi et al. 2020). An often discussed explanation for the mitigation of CH_4 emissions is that biochar contains redox-active functional groups, primarily quinone moieties (Klüpfel et al. 2014a, b; Zhang et al. 2019a, b; Pascual et al. 2020). Due to their reversible binding of electrons (Aeschbacher et al. 2011), biochar could delay the onset of CH_4 production (if it can act as an electron acceptor above – 200 mV) or slow production rates after the onset of methanogenesis when redox potentials (Eh) of redox-active functional groups is below – 200 mV (Klüpfel et al. 2014a, b). In a global meta-analysis, Ji et al. (2018a, b) calculated a cross-trial mitigation of 12% in CH_4 emissions after the application of biochar into paddy soils on the time scale of growing seasons from 61 peer-reviewed individual studies, including field, pot, and incubation studies. Laboratory experiments with in-vitro incubations showed with a mean mitigation potential by 23% an even greater effect on CH_4 emissions than in the field experiments with only 4% (Ji et al. 2018a, b). More recent research results also pointed to this difference, e.g. Lee et al. 2023, who similarly confirmed this discrepancy for their target region of Southeast Asia. In the case of their meta-analysis, the deviation was 9% points (Lee et al. 2023). An extensive number of individual studies therefore show in general a clear reduction of CH_4 emissions upon biochar addition, yet they strongly differ in terms of the effect size, which apparently depends on the experimental approach. The reasons for such clear effect-size differences between field and incubation studies remain elusive, complicating the challenge of transferring research results into practice.

Incubation experiments are a useful approach to isolate small-scale processes occurring within paddy soils (Schütz et al. 1990; Achtnich et al. 1995; Ji et al. 2018a, b). For example, in an incubation experiment on the biogeochemistry of paddy soils, the authors found that cumulative CH_4 production was strongly pH-dependent (Wang et al. 1993). They documented that for a pH increase from 6.25 to 7.00 CH_4 concentrations increased by factor 8 (Wang et al. 1993). It is important to note that biochar remains in soils for several centuries but may raise pH significantly shortly after addition and for only a few years thereafter (Nan et al. 2021), there is a risk that initial, temporary, pH-induced effects are extrapolated to long-term CH_4 balances. To date, to the best of the authors' knowledge, there is no published biochar incubation experiment with an experimental design that has excluded the influence of pH and initial leaching on CH_4 production. To explore the underlying processes in more detail, we want to outline our procedural understanding of anaerobic incubations (Fig. 1). While CH_4 production is largely suppressed by abundant alternative electron acceptors, and solely CO_2 production occurs, during the pre-methanogenic stage (Achtnich et al. 1995), CO_2 and CH_4 are theoretically produced at a 1:1 ratio in the methanogenic stage (Conrad 1999). The turning point between these two stages is described in redox chemistry as the time at which Eh falls below a certain threshold, e.g. – 200 mV as proposed elsewhere (Wang et al. 1993). At this time point, thermodynamically more favourable electron acceptors are depleted. After a short transition, given by soil heterogeneities, kinetics and accessibility of electron acceptors, methanogenesis sets in and becomes the dominant anaerobic mineralization pathway (Achtnich et al. 1995). Ultimately, end product accumulation of CO_2 and CH_4 leads to a slowing of mineralization rates again induced by thermodynamic constraints (Bonaiuti et al. 2017).

The pre-methanogenic stage is characterised by the highest respiratory activity of the soil microbiome, characterised by high rates of CO_2 production (Knoblauch et al. 2011). The CO_2 is derived from the degradation of organic matter, acting as an electron donor, and a concomitant reduction of an electron acceptor (Segers 1998). While remaining oxygen and alternative electron acceptors such as nitrate, ferric iron, sulphate, and redox-reactive organic matter (in dissolved and solid forms) are used in sequential redox reactions, the production of new CO_2 gradually decreases (Achtnich et al. 1995; Klüpfel et al. 2014a, b; Lau et al. 2014). As soon as the availability of alternative electron acceptors decreases, upon depletion of electron acceptors, the production of CH_4 becomes relevant and provides sufficient energy yields for microbial metabolism (start of methanogenic stage) (Cai et al. 2018). Each of the metabolic pathways leading to CH_4 production (mainly hydrogenotrophic or acetoclastic methanogenesis) results in an equal amount of carbon dioxide (CO_2) and CH_4

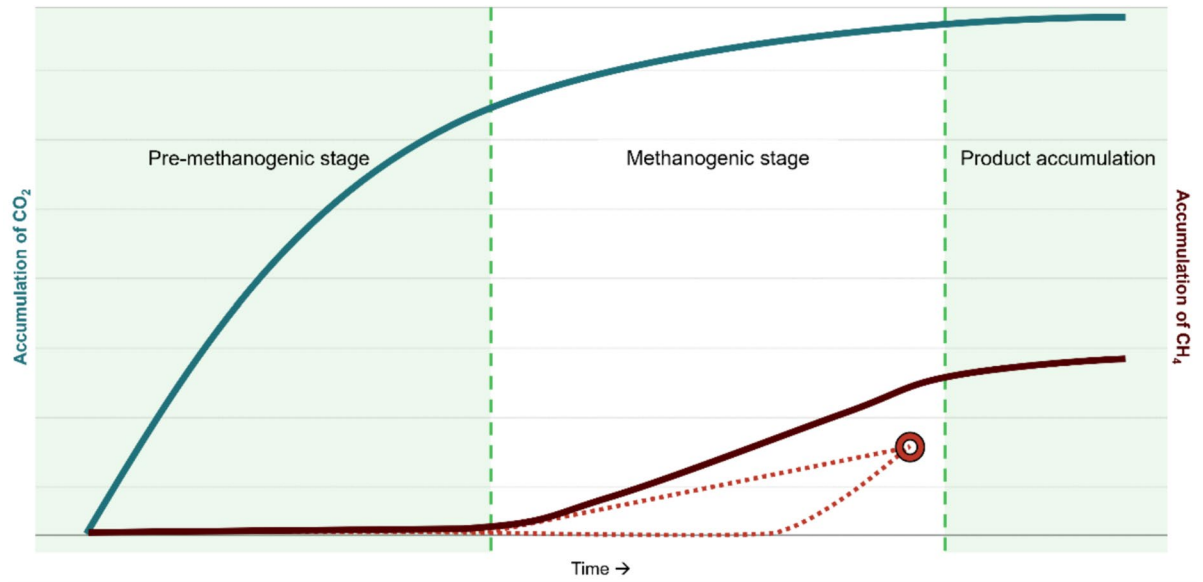


Fig. 1 Schemeof the three temporal stages of anaerobic incubations of paddy soils. Gas accumulation is described by the blue (CO_2) and brown (CH_4) lines. The dotted red lines show that focussing on a single parameter, namely CH_4 accumulation at an arbitrary point in time, does not explain whether CH_4 production rates were reduced or the onset of methanogenesis was delayed

being produced, assuming a zerovalent carbon source (Liu and Whitman 2008; Yu et al. 2016). This means that in strictly methanogenic respiration, the ratio of production rates must be 1 mol CO_2 to 1 mol CH_4 (Conrad 1999; Yavitt and Seidman-Zager 2006). In the third stage of the incubation, CH_4 and CO_2 production typically decrease. This phenomenon can be explained by high CH_4 partial pressures and concentrations, i.e., an end-product accumulation: The metabolic pathways of methanogenesis thereby yield less energy and are no longer thermodynamically feasible for the microbes (Beer and Blodau 2007). This pattern has been well documented in incubation studies (Liu et al. 2020; Sriphirom et al. 2020) and can, in some cases, correspond well to the processes occurring under water-logged field conditions of certain wetland ecosystems, such as peatlands (Beer and Blodau 2007). However, this effect might not directly apply to paddy soils, as paddy cultivation is an intensively managed cropping system that drains at the latest before harvest and is affected by regular soil disturbance, with CH_4 bubbles easily escaping (Guo and Zhou 2007). Moreover, the aerenchyma of rice plants provide a CH_4 transport pathway that significantly exceeds the emission rates of diffusion and ebullition (Groot et al. 2003). Finally, aerobic microsites exist in the rhizosphere as well as in the upper centimetres of the paddy soil where methanotrophic bacteria colonise to oxidise CH_4, providing a CH_4 sink (Conrad and Rothfuss 1991; Kögel-Knabner et al. 2010). Thus, the inhibition of CH_4 production through end-product accumulation is probably not important in managed paddy soils. Instead, we argue that of major importance, besides labile organic substrate supply, is the availability of electron acceptors, which control whether methanogenesis is a competitive process or suppressed at all.

Figure 1 also illustrates that a separation between the two stages described enables the effects of reduced CH_4 production to be clearly differentiated. CH_4 production in paddy soils can therefore be mainly affected through two different mechanisms: firstly, the amended biochar may provide electron accepting capacity (EAC) and can therefore suppress methanogenesis and extend the pre-methanogenic stage. This mechanism is e.g. supported by Klüpfel et al. (2014a, b), Li et al. (2020) and Zhang et al. (2019a) who demonstrated the role of biochar as a potent electron acceptor. Secondly, the effect of biochar may be in affecting the soil microbiome via

inhibitory effects, which in general slow anaerobic respiration rates and rates of methanogenesis. Only by clearly differentiating between the pre-methanogenic from the methanogenic stage in incubations, these two potential effects may be separated.

To summarise, although previous research has documented biochar application as a promising tool for CH_4-emission management in paddy soils, the mechanisms of biochar effects on CH_4 dynamics have not been systematically investigated under consideration of factors such as microbial activity, electron acceptors, pH, biochar feedstock and texture. To ensure more controlled conditions, and to exclude initial leaching and pH effects, pH-levelled, rinsed biochar was applied in this study and compared to the commonly applied experimental setup with untreated biochar. Corresponding with available studies, we further hypothesised that biochar can act as an electron acceptor, thus delaying the onset of methanogenesis. Of course, effects of short-term pH changes and leaching of readily soluble, alkaline elements on anaerobic carbon mineralization cannot be neglected and may obscure the effects of surplus electron acceptor capacity provided by biochar. Therefore, comparing the contrasting effects of untreated versus leached and pH-levelled biochar, and by separation of the pre-methanogenic and the methanogenic stage in the incubations, our study offers new insight into apparently contrary biochar effects on anaerobic carbon mineralization in paddy soils.

Materials and methods

Concepts for incubation and modelling approach

To address the above-described knowledge gap, our approach is based on a mathematical modelling of the amounts of CO_2 and CH_4 (µmol g^{-1} soil) produced over time t (d). At first-order kinetics was assumed for CO_2 production over time:

$$CO_2(t) = -\frac{a}{k} * e^{-k*t} + b \quad (1)$$

Here k (d^{-1}) is the decomposition constant of the labile and microbially accessible soil organic matter, which is assumed to be approximately homogeneous in the incubation process according to a one-pool model. The initial CO_2 production rate a (µmol CO_2 g^{-1} soil d^{-1}) and the maximum theoretical CO_2 accumulation b (µmol CO_2 g^{-1} soil) approximate the observed, thermodynamically justified effect of the accumulation of end-products (here CO_2) over time. Note that this concept does not imply that such limitation indeed occurs within our time scale of incubation, yet it shapes the evolution of CO_2 over time. Assuming no inhibition by product accumulation also for CH_4 (see above), the production rates of methanogenesis after its onset can be defined as a very rapid approach towards the constant CH_4 production rate m (µmol CH_4 g^{-1} d^{-1}). We justify a constant production, assuming a continuous substrate availability and resupply in paddy soils and no substrate limitation or end-product inhibition within the time scale of incubation due to the regular management of paddy soils. Moreover, in the field, the effective transport pathways of CH_4 in paddy soils would provide a steady gas removal and thus further prevent a product accumulation. The accumulation of CH_4 in the incubations can therefore be interpreted as the integral of the constant CH_4 production over time. As methanogenesis is initially suppressed, we introduce a term to account for this effect caused by abundant electron acceptors. Thus, the result for the accumulation of CH_4 is:

$$CH_4(t) = \frac{m * (t - d)}{1 + e^{-1000*(t-d)}} \quad (2)$$

Here, d (d) describes the delay of the onset of methanogenesis in the incubation process. The construction of $e^{-1000*(t-d)}$ creates a shift term that suppresses the influence of the numerator in the equation until t equals d. For our system, this means that the maximum CH_4 production rate has no influence on the onset of CH_4 production. However, as soon as CH_4 production starts, the rapidly reached maximum methanogenesis production rate m shapes the course of gas accumulation. Since CH_4 production very soon stabilises at a constant level until it gradually converges to product accumulation (which is not to be considered due to the shorter time scale of incubation, in accordance with regular disturbance in the field), a simplified linear approach can be used for this limited interval of the experimental setup (Fig. S1, SI). The determination of the onset of methanosgenesis can be derived from the

modelled function for CH_4 accumulation. The shift term *t-d* describes the time *d* by which the onset of methanogenesis in anaerobic respiration is delayed by abundant electron acceptors. Even though methanogenesis only reaches maximum production rates with a short delay from this point, this time is relevant, because up to this moment, virtually all soil respiration can be ascribed to non-methanogenic pathways. Besides the delay of methanogenesis *d* and the subsequently evolving production rate *m,* another parameters can also be calculated for this point in time for each individual sample: $pmCO_2$ (µmol CO_2 g^{-1} soil) is the corresponding value of CO_2 accumulation at timepoint *d* and describes the pre-methanogenic respiration, that serves as an indicator of the EAC present in the sample. In the common incubation setup, as also reproduced in this study, the last day of measurement is an arbitrarily chosen moment. The snapshot of amounts of substances measured on that occasion should therefore not be decisive for the results of an experiment or the evaluation of an effect. According to the model assumptions described above, each state of CH_4 accumulation is composed of the parameters *d* and *m* (cf. Eq. 2). For this reason, the approach of this study is to consider these two parameters, delay of methanogenesis *d* and rate of methanogenesis *m*, in order to be able to draw targeted conclusions from their fitted values.

Soils and biochars

Starting in August 2020, two parallel incubation experiments were carried out, each consisting of two parallel redox cycles. For this purpose, soil samples of a paddy field of the Italian rice research institute *Ente Nazionale Risi* in Mortara (Lombardy, 8° 41′ 59″ E, 45° 14′ 47″ N) were taken and analysed at the *Institute of Landscape Ecology* of the *University of Münster*. For chemical characterisation (Table 1), triplicate samples were suspended in 0.01 M $CaCl_2$ solution (pH measurement) and in H_2O (measurement of electrical conductivity) and finely ground for C/N analysis (Eurovector EA3000, Pavia, IT). Plant-available phosphate (PO_4-P) and potassium (K) were determined using the CAL method (Schüller 1969). The soil (5 g) was extracted with 100 ml calcium lactate acetic acid (90 min shaking). In the extracts, K was determined with a flame photometer (JENWAY, Stoffordshire, UK) and PO_4-P with a UV-VIS spectrophotometer (Agilent Cary 100 E, Santa Clara, USA) after molybdate blue staining at 578 nm.

Table 1 Soil chemical characterisation of the paddy soil (n = 3; soil type is Hydragric Anthrosol, originally Luvisol)

	Unit	Mean	Std.dev
Ash content	m%	**95.9**	0.1
pH	–	**4.6**	0.1
Electrical conductivity	mS cm^{-1}	**2.4**	0.0
Organic carbon content (C_{org})	mg C g^{-1}	**13.6**	0.3
Nitrogen content (N)	mg N g^{-1}	**1.2**	0.0
C/N ratio	G g^{-1}	**11.1**	0.1
Phosphorus content (P; CAL-method)	mg P kg^{-1}	**14.8**	0.5
Potassium content (K; CAL-method)	mg K kg^{-1}	**104.4**	4.0

From the air-dried soil, 12.0 g of each sample were transferred to incubation flasks with a volume of 116 ml. All soil samples except those of the control group were mixed with biochar amendments at this point, resulting in a biochar content of 0.015 g g^{-1} dry soil. This content would be reached after about 15 years of continuous cultivation, depending on the feedstock and usual amount of annual incorporations (Nan et al. 2020) and corresponds to biochar contents of other incubation studies (Liu et al. 2011, 2020; Fu et al. 2021). Sorghum straw (SOR), urban tree cuttings (WO), wheat straw (WHE) were selected as feedstocks and comparable pyrolysis temperatures were chosen (Table 2): The biochars were divided into subdivisions with finer ground and coarser grain sizes (for experiment 2: SORc, WOc and WHEc). C/N analysis (Eurovector EA3000, Pavia, IT) was carried out for all biochars, after drying at 40 °C (and after rinsing the biochars of experiment 2, see description below).

Two experimental set-ups: untreated biochar versus rinsed, pH-levelled biochar

For all incubations the headspace volume was set to 86 ml gas space in the incubation bottle so that the solid and liquid phases made up 30 ml volume (water about 25.2 ml). Following a commonly chosen aspect of the experimental design of other studies (cf. Table 3 in Discussion section), the selected biochars were finely ground but not rinsed or otherwise treated

Table 2 Properties of tested biochars

	SOR			WO			WHE		
Feedstock	Sorghum straw			Urban tree cuttings			Wheat straw		
Pyrolysis temp. (°C)	600–700			500–750			600–700		
Experiment	1	2		1	2		1	2	
Treatment	n	c	f	n	c	f	n	c	f
Texture	fine		c	fine		c	fine		c
pH ($CaCl_2$)	10.2	5.5		9.1	5.5		8.8	5.5	
Carbon content (C; m%)	45.3	54.6	69.9	85.8	86.5	91.0	55.2	76.6	58.1
Nitrogen content (N; mg g^{-1})	7.9	8.4	3.9	4.3	4.2	4.7	8.2	8.4	8.1
C/N ratio (g g^{-1})	57	65	179	200	206	194	67	91	72

Biochar texture has defined value ranges (fine < 20 µm; coarse = 63…2000 µm). The last three rows represent the measurement results of C/N analysis of the prepared biochars (n = 2). Information on pyrolysis temperature and pH of the untreated biochars are taken from: SOR & WHE = Wiedner et al. 2013; WO = https://pyreg.com/de/unsere-technologie-alt/ (last accessed in April 2024). In the temperature range between 600 and 700 °C, biochars often have a high degree of aromatisation and quinone content (Zhang et al. 2019b)

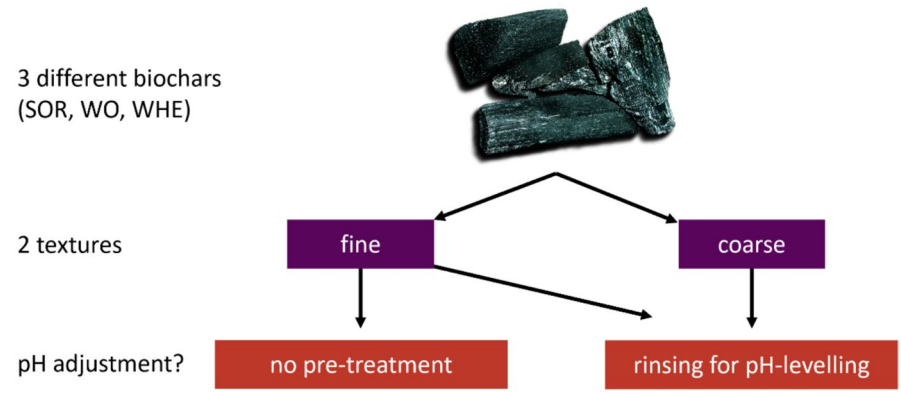
Table 3 Key findings of the present study compared with the outcomes of other incubation studies on biochar effects on methanogenesis

Publication	Fig. of interest	Feedstock	pH levelling?	Onset of methanogenesis	CH_4 production rate
Knoblauch et al. (2011)	4	Rice husks	No	Unrecognisable	Little lower
Liu et al. (2011)	4	Rice straw, bamboo chips	No	Equal	Lower
Xiao et al. (2018)	S1.a	Rice straw	No	Later	Lower
Liu et al. (2020)	3.b	Wheat straw	No	Little later	Little lower
Sriphirom et al. (2020)	1.c + d	Mangrove	No	Equal	Lower
Fu et al. (2021)	1.a	Rice straw, orange peel, bamboo powder	No	Later	little lower
Nguyen et al. (2023)	1.c + d	Corn stalks	No	Later	Lower
Wu et al. (2023)	2.a	Wheat straw	No	Later	Lower
Experiment 1: Untreated biochar	6 + 7	Sorghum straw, tree cuttings, wheat straw	No	Later (+ 24.24%)	Lower (− 38.96%)
Experiment 2: pH-levelled biochar	6 + 7	Sorghum straw, tree cuttings, wheat straw	Yes	Little earlier (− 7.00%)	Lower (− 20.10%)

For the results of the present study (last two rows), the average effects of all tested biochars compared to the control group of the respective experiment were calculated and shown in brackets

(experiment 1). Four replicates of the control without biochar and four replicates from each of the biochar treatments (SORn, WOn and WHEn, "n" stands for "no rinsing") resulted in a total sample size of 16 incubation flasks. Experiment 2 was carried out to investigate the extent to which findings based on this conventional approach (experiment 1) change when short-termed effects such as pH or a relatively high fraction of labile particulate carbon are excluded. The set-up of experiment 2 also corresponded to the descriptions above, but the difference was an increased sample size of 30 incubation flasks. In this case, six of them served as controls without the addition of biochar. The other 24 samples represented four replicates of the six above mentioned biochars (three feedstocks × two textures, cf. Figure 2).

Both the coarse and the fine biochars were pH-levelled prior to the mixing with homogenised and untreated soils. To this end, 8 g of biochar of each experimental group were thoroughly rinsed in 0.02 M



Experiment	Exp. 1: untreated biochar	Exp. 1: pH-levelled biochar
Number of incubation flasks	4 controls + 3 feedstocks x 4 replicates = **16**	6 controls + 3 feedstocks x 2 textures x 4 replicates = **30**
Objective	Follow common biochar incubation assessment as established in other studies	Improve the transferability of laboratory results by excluding pH effects

Fig. 2 Schematic methodological summary of the two experiments carried out

KCl solution and titrated with 0.1 M HCl until a stable equilibrium at pH = 5.5 was reached. The attainment of pH equilibrium was determined after 2 days of continuous shaking and post-titration at least three times. In the following step, the suspensions containing the ground biochar were filtered off using a 700 nm filter (Whatman GD/X 13). In parallel, the coarse biochar samples were separated from the solution using a 0.63 mm sieve. The described process was carried out a second time to ensure the constancy of the pH adjustment. Following drying, biochar was mixed with otherwise untreated soil (as in experiment 1). The mixtures of biochar and soil (or without biochar in the control) were first moistened but not yet saturated with water to slowly reactivate the microbial metabolism. After one week, the flasks were filled with deionised water up to a mark at 30 ml. After sealing the incubation flasks airtight, they were flushed with molecular nitrogen (99.99% N_2) for ten minutes.

Analytical methods

For incubation, the flasks were stored at 23 °C in darkness for the incubation period of several months. At intervals of 3 to a maximum of 22 days, 2 ml gas samples were taken from the headspace of the flasks and analysed by gas chromatography (SRI 8610C, California, USA) for the partial pressure of CH_4 and CO_2. The headspace air pressure was controlled to compensate for sampling volumes by injecting corresponding amount of dinitrogen. Each experiment was terminated as soon as methanogenesis was the dominant respiration process in all treatments (ratio of new production rates in at least half of the replicates below 2 mol CO_2 (mol CH_4)$^{-1}$). Then the flasks were opened to immediately determine the pH of the anoxic soil solution (Multi 9310 IDS, Weilheim, GER). The individual calculation steps used to convert the gas concentration data into amount of substance concentrations in the incubations (µmol g^{-1} soil) are shown in the supporting information (SI, chapter 1).

Data analysis and modelling

The resulting data from each sample (in total 96 samples with 10 to 31 measurements of CO_2 and CH_4 during the incubation period) were analysed using the „non-linear least squares “-algorithm in R (version 4.2.3; R Core Team 2023). Gas accumulation was modelled for each sample by fitting the CO_2 and CH_4 functions (Eqs. 1 and 2) to the observed totals of each measurement. Using the resulting model functions, the parameters d, k, $pmCO_2$ and m were calculated for each treated sample (i.e. each pair of CO_2 and CH_4 accumulation over time). In addition, affiliation to

experiment 1 or 2 (i.e. belonging to the conventional or alternative experimental setup), biochar feedstock (SOR, WO, WHE and C), biochar texture (coarse or finely ground) and pH value during methanogenesis (i.e. immediately after opening the incubation flasks) were integrated as possible explanatory variables in linear models (see Tables S2–S4, SI). All statistical tests were carried out in R (version 4.3.1; R Core Team 2023). First, the measurement results were evaluated separately for each experiment. The significance ($p < 0.05$) of the difference between the various experimental groups was tested using ANOVA and a subsequent Tukey test for pairwise comparisons. If the assumptions for ANOVA were not met, the non-parametric alternative in form of a Kruskal–Wallis test with a post-hoc Dunn's test was used. The influence of possible explanatory variables on the measurement results was tested by ordinary least squares regression. Homoscedasticity and normal distribution of the data were determined with Levene's and Shapiro–Wilk test, respectively.

Results

Experimental results: general patterns

The accumulation of CO_2 and CH_4 over time are shown in Fig. 3 as mean values of the diverse biochar treatments of the two experiments. The incubation curves of the two experiments clearly show that the CH_4 concentrations start to increase abruptly at

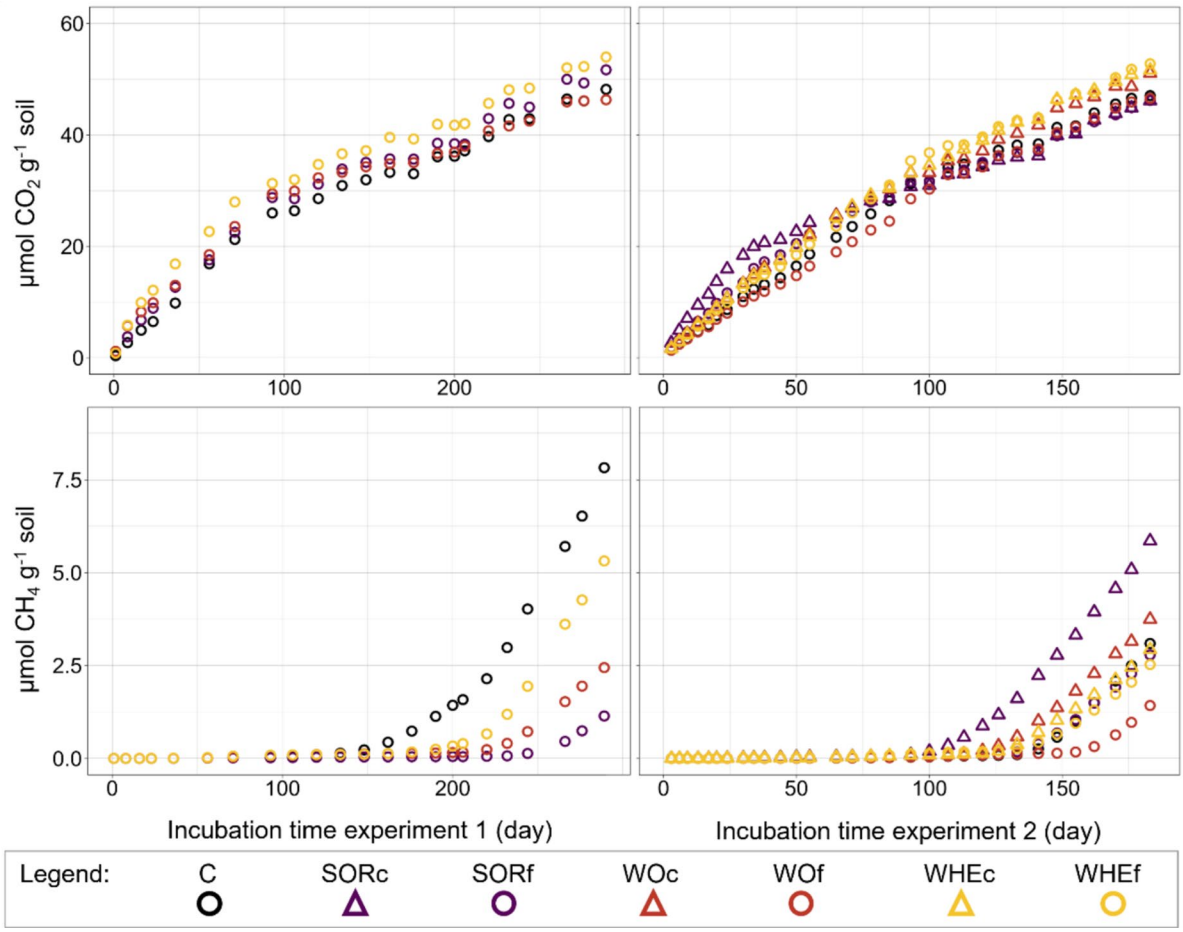


Fig. 3 Gas accumulation over the incubation periods of the two different experiments. Symbols represent mean values of each experimental group (n = 4, except for the control group in experiment 2, where n = 6). Groups contain (C) no biochar, (SOR) sorghum biochar, (WO) wood biochar and (WHE) wheat biochar. (c) is coarse biochar and (f) is fine biochar

different times, followed by a linear increase in both experiments.Short-term fluctuations in the accumulation curves are also recognisable, which deviate from the general trend of the time series on one or two measurement days. Here, we won't go more into detail describing the incubation patterns but underline that the data of each individual replicate of each treatment provided the data basis for the modelling approach and the calculation of the three target parameters: delay of methanogenesis, amount of alternative electron acceptors and subsequent CH_4 production rate. Our modelling approach and the underlying equation outlined above served to determine unambiguous parameters from each incubation in a standardised, systematic way beyond manual selection of time points.

For the total of 46 incubation runs of the two experiments, reasonable parameters of the two defined model functions were calculated. 23 (experiment 1; untreated set-up) and 31 (experiment 2; pH-levelled) measurements for CO_2 and CH_4 were carried out for each replicate. To assess the quality of the modelling approach, the relative standard errors of the CH_4 model parameters d and m were calculated and depicted for both experimental set-ups (Fig. 4). The standard errors of both parameters relative to the respective estimated value were rather low (median of $d = 0.77\%$ and $m = 3.51\%$), but the estimated values for m were less accurate than the estimation of d. It can also be observed that for both parameters in experiment 1, higher relative standard errors compared to experiment 2 show that the modelling of CH_4 accumulation was subject to slightly larger uncertainties.

The pH measurements after the onset of methanogenesis revealed differences between the two experiments, but pH of the control groups in both experiments were very similar at the end of the incubations, which is why these are shown in a single box (Fig. 5). pH of the biochar treatments in experiment 1 (mean 6.92) was remarkably higher than that of the control groups (mean 6.64), while the mean pH of the biochar amended soils of experiment 2 was 6.66.

Pre-methanogenic stage

In experiment 1, the untreated biochar significantly delayed the initiation of methanogenesis (Fig. 6): The biochar treatments increased d by an average of 24.24% in relation to the control group. In contrast,

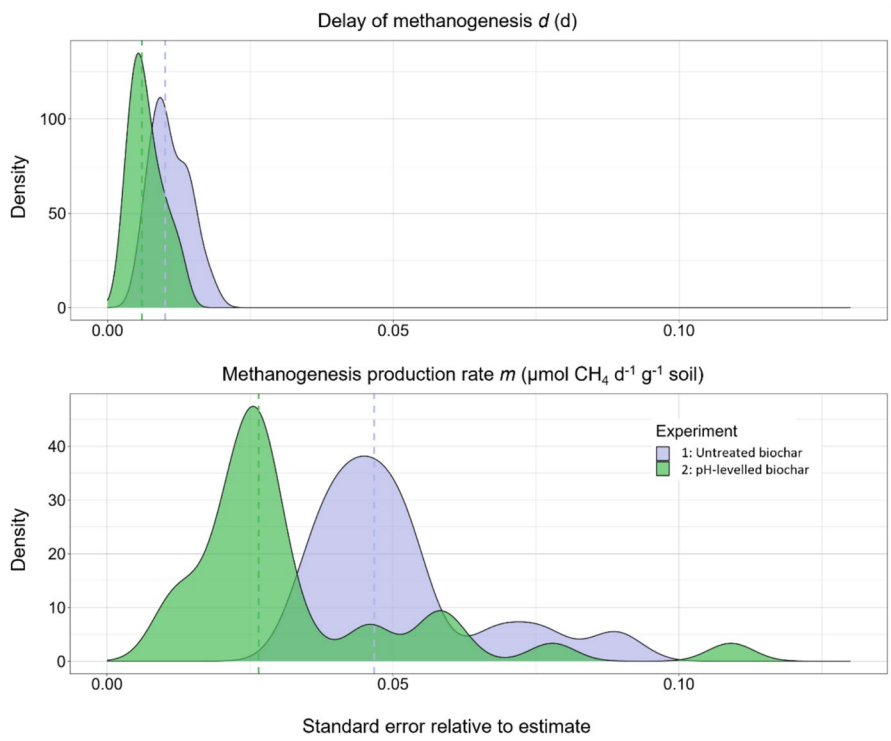


Fig. 4 Probability density function of relative standard errors of the model parameters d and m for modelling CH_4 accumulation. Dashed lines show median relative standard error of each parameter per experiment. Data basis are the calculated models of all replicates from experiment 1, "untreated biochar", n = 16 and experiment 2, "pH-levelled biochar", n = 30

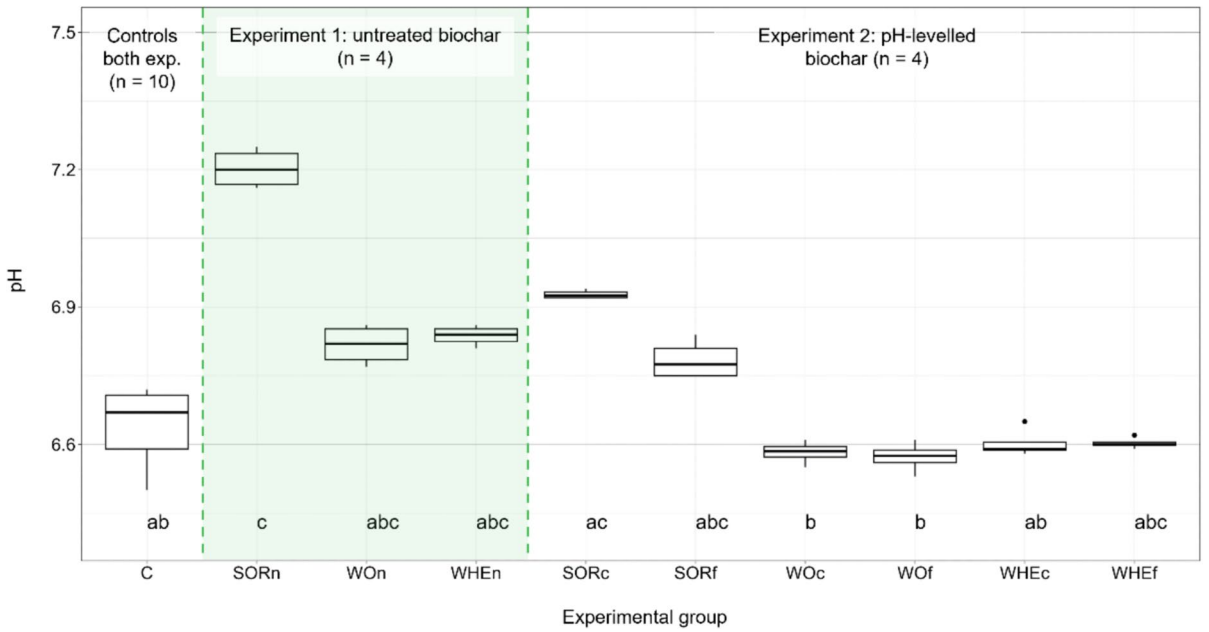


Fig. 5 pH values of soil solutions at the final measurement of incubations. The groups contain (C) no biochar, (SOR) sorghum biochar, (WO) wood biochar and (WHE) wheat biochar. (n) is no rinsing, (c) is coarse biochar and (f) is fine biochar. Letters below indicate the results of Dunn's test following Kruskal–Wallis analysis of variance. Groups that don't share any letter are significantly different ($p < 0.05$)

in experiment 2 with alternative experimental set-up for controlled pH, there was a mean decrease of *d* by 7.00% of the mean of control group. The mean values of all treatments in experiment 2were below the average of the control group, in two of six cases with significant difference ($p < 0.05$). The only exception of the described pattern is fine-wood biochar showing an increase with time until onset of methanogenesis. The ordinary least squares regression with the whole data set confirmed that biochar had no consistent effect on *d*, but the effect of the pH value on d was highly significant ($p < 0.001$; Table S2, SI).

In experiment 2, the texture of biochar also had an effect: coarse biochar (indicated by "c"; SORc, WOc, WHEc) lowered *d* with an effect of 22.18 days (− 15.57%) compared to fine biochar ($p < 0.001$). Of highest significance for the variation of *d* was the measured pH ($p < 0.001$): Raising the pH value by 1 increases *d* by 139.77 days, which means that the time to the start of methanogenesis almost doubled (Table S2, SI, last column). Analogous to the delay of methanogenesis, there was also an increase in pre-methanogenic respiration ($pmCO_2$) by 21.20% relative to the control group in experiment 1, where pH was not controlled (Fig. S2, SI). The pH-levelling pre-treatment of experiment 2 blurred this tendency and led to a slight decrease of mean $pmCO_2$ by 0.50% in the biochar containing soils. Not only was the effect size variable and small, but also the direction heterogenous, as in the case of a significant decrease in $pmCO_2$ ($p < 0.05$) for coarse sorghum biochar and a significant increase ($p < 0.05$) in the case of fine wheat biochar.

The overall data set shows a significant ($p < 0.05$) but relatively small increase of 7.06% additional electron acceptor capacity due to biochar addition (Table S3, SI). Only the results of wheat biochar showed a remarkable increase in electron accepting capacity with an effect of 5.1 µmol CO_2, i.e. 20.4 µmol electrons per g soil ($p < 0.001$), which equals an increase of 13.22%.

Methanogenic stage

During the ongoing methanogenesis in experiment 1 the wood (WOn) and sorghum (SORn) biochar groups indicated a significant mitigation in CH_4 production rates ($p < 0.05$; Fig. 7), WOn by an average of

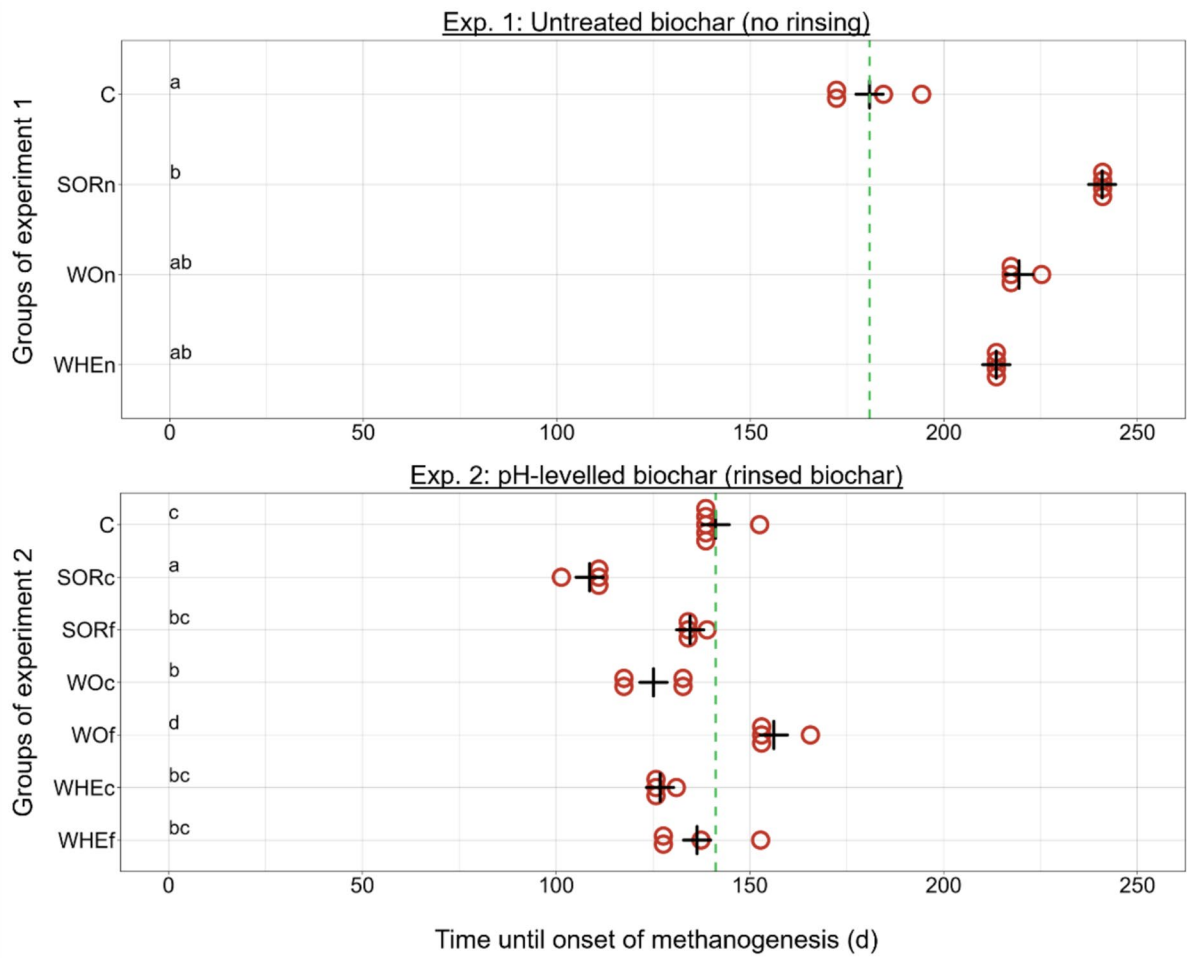


Fig. 6 Distribution of the delay of methanogenesis *d* among the experimental groups. Rings show the modelled results of each replicate. Groups contain (C) no biochar, (SOR) sorghum biochar (WO) wood biochar and (WHE) wheat biochar. (n) is no rinsing, (c) is coarse biochar and (f) is fine biochar. Black crosses show mean values and green lines mean values of control groups. Letters (at x = 0) indicate significant differences between groups ($p < 0.05$), separated by experiments (Exp. 1: Dunn's test; Exp. 2: Tukey's test)

50.2%, SORn even by 67.5%. In the same experiment control group and wheat biochar treatment showed no significant difference with average CH_4 production rates near to 0.07 µmol CH_4 d^{-1} g^{-1} soil. This tendency to lower the amount of CH_4, *m,* was apparent also in experiment 2, although this effect was not significant here. Nevertheless, the CH_4 production rate of the biochar treatments was decreased on average by 20.10% compared to the control group.

Synthesizing both experiments a highly significant ($p < 0.01$) effect of biochar was found for the CH_4 production rate (Table S4, SI), which corresponds to a mean decrease of *m* by 25.89% ($p < 0.01$). Additionally, wood and sorghum biochar showed highly significant effects with a mitigation of CH_4 production rates by 0.023 and 0.020 µmol CH_4 g^{-1} soil d^{-1} ($p < 0.01$). The effect of wheat biochar was weaker (0.014 µmol CH_4 g^{-1} soil d^{-1}) and not significant. Comparing the effects of biochar application and alkalinisation in one regression model (Table S4, SI, last column) showed that biochar independently of its pH value had a significantly decreasing effect on *m* ($p < 0.05$), while pH was no significant explanatory variable for CH_4 production rates.

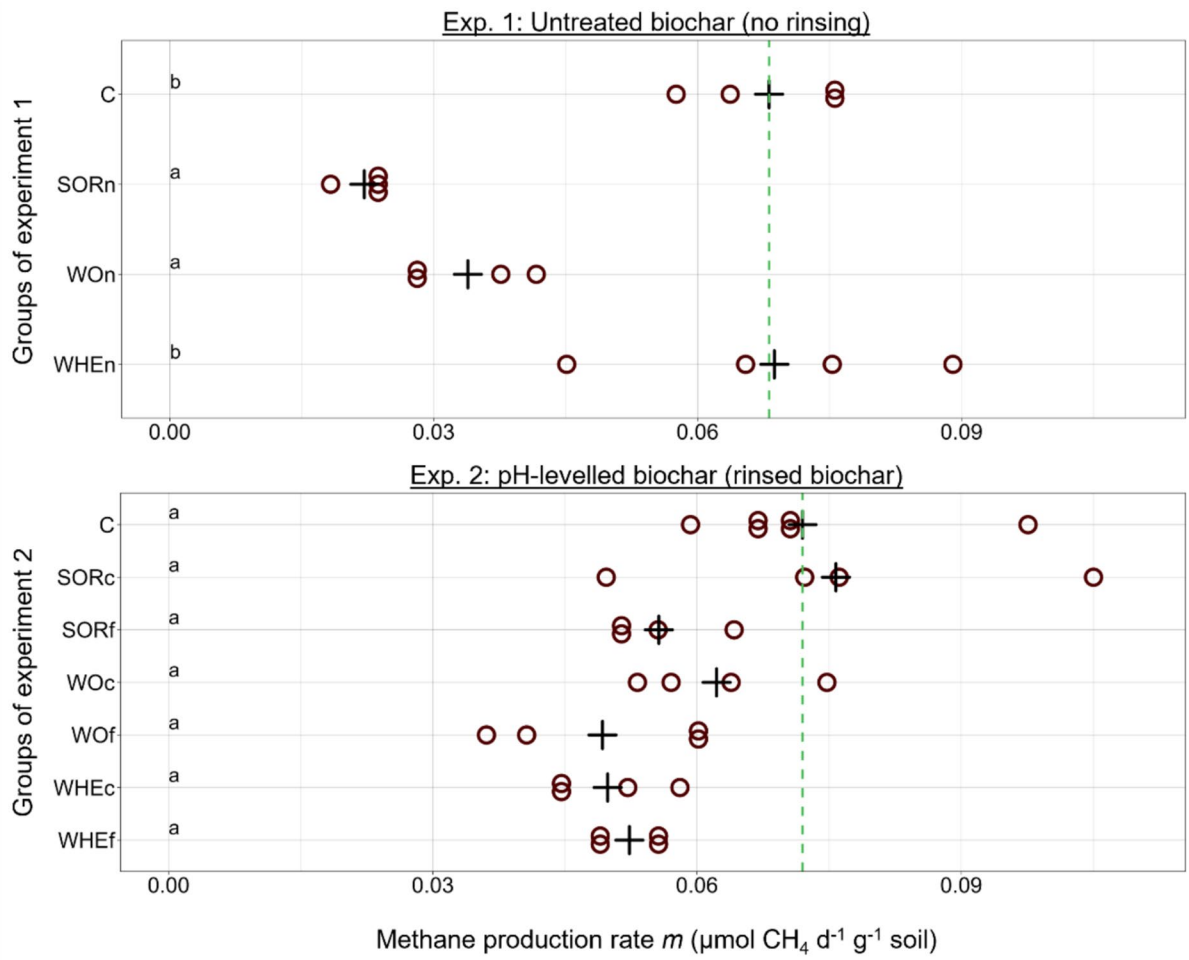


Fig. 7 Distribution of the CH_4 production rate *m* among the experimental groups. Rings show the modelled results of each replicate. Groups contain (C) no biochar, (SOR) sorghum biochar (WO) wood biochar and (WHE) wheat biochar. (n) is no rinsing, (c) is coarse biochar and (f) is fine biochar. Black crosses show mean values and green lines mean values of control groups. Letters (at x = 0) indicate significant differences between groups ($p < 0.05$), separated by experiments (Exp. 1: Tukey's test; Exp. 2: Dunn's test)

Discussion

Biochar-induced pH elevation

Our pH data (Fig. 5) clearly show that the conventional experimental approach (experiment 1) differs substantially in terms of soil chemistry from the proposed approach to exclude pH effects (experiment 2). As in our experiment 1, other studies also indicated a strong influence of biochar application on soil pH, especially in the first years after application (Wang et al. 2019; Jia et al. 2023). The increase in pH is due to the application of additional bases, which are present in the form of ash in the pores of the biochar (Nan et al. 2021). We assume that this property is dominated by pyrolysis conditions and above all by the chemical composition of the feedstock (e.g. sorghum straw probably had a lower C content but higher mineral content than green cuttings). This is also reflected by the alkaline biochar varieties used in this work (cf. Table 2) leading to high pH values of the incubated soils of experiment 1. Rinsing the biochar as a measure to level the pH of biochar treatments in experiment 2 achieved the desired effect of levelled pH values in the case of wood and wheat straw as feedstock. In contrast, the groups with

sorghum biochar still showed elevated pH values after rinsing, which means that the pH-levelling could still not be achieved in this case, despite of repeated treatment over 2 days. Nevertheless, for all rinsed biochars, the pH range of the soil solution was clearly narrowed compared to the untreated biochars.

Biochar-induced delay of methanogenesis

To categorise the key results of the two experiments, these were presented in Table 3 in line with comparable incubation studies. The last two columns represent the two possible pathways of decreasing CH_4 production by biochar application depicted in Fig. 1. It is important to emphasise that the delay of the onset of methanogenesis (in this study *d*) and CH_4 production rate (*m*) were not determined in previous studies. The figures in the respective publications were assessed and qualitative results were derived visually for this comparison.

Although the delay of methanogenesis has never been quantified in the existing literature, the results of experiment 1 are in agreement with observations of other studies that the incorporation of untreated biochar delays the onset of methanogenesis (incubation studies shown in Table 3 and pot studies as Pratiwi and Shinogi 2016; Qi et al. 2018). In contrast the results derived from experiment 2suggest that the experimental design (pH and biochar texture) has a decisive influence on the magnitude (eliminating the strong and significant delay of methanogenesis) and direction (even shortened duration until onset of methanogenesis) of the effect of biochar. A coarser texture of the biochar particles could minimise their interaction with the soil solution and thus their delaying effect as alternative electron acceptors for the microbiome (Sarfraz et al. 2020). The delaying effect on the onset of methanogenesis as documented in experiment 1 was eliminated by mimicking field conditions (experiment 2), where readily leachable elements would also be washed out and changes in pH would rather be minor compared to experiment 1. The thus reduced mitigation potential aligns with the discussion of several reports of a decrease in the CH_4 mitigating effect with advancing duration after the application of biochar (Lee et al. 2023). There are three possible factors that caused the weakening or partial reversal of the effect of biochar as observed in experiment 2. First of all, it can be assumed that pH has a strong influence on anaerobic respiration and subsequent methanogenesis, since the reduction of quinones implies a release of acidity (Scott et al. 1998). When the pH is raised by untreated biochars (experiment 1), the reduction of benzoquinone thermodynamically gains energy yield. Secondly, it could be that a soluble fraction of the quinone moieties was also lost by rinsing the biochars. However, it was reported that the soluble fraction often only contributes a small amount to the EAC, compared to the particulate fraction (Gao et al. 2019). Thirdly, it is possible that biochar selectively affects the microbiome through niche formation or toxicity, thereby shifting metabolic strategies (Smith et al. 2013).

Biochar effects on CH_4 production rate m

The results of both experiments agree with publications that address methanogenic activity after biochar applications (Table 3). In order to place this result in the context of pH dependence, pH was used as an additional explanatory variable in the last step of the regression models of *d*, *m* and $pmCO_2$ (Tables S2, S3 and S4, SI). This indicated that in contrast to the pre-methanogenic stage, the effect of biochar on CH_4 production is not dominated by a pH effect in the later, methanogenic stage. Thus, there may be a different mechanism by which biochar mitigates CH_4 production. Even though our data does not allow for a mechanistic explanation, we see two possible explanations how biochar could decrease the rate of CH_4 production *m*. One is based on the availability of alternative electron acceptors, which are available to the microorganisms at low redox potentials alongside methanogenesis. In this low redox range, the redox-active organic moieties of biochar could be of particular importance (Klüpfel et al. 2014a, b). Secondly, biochar can cause a general decline in microbial activity in paddy soils by lowering the dissolved organic matter content of the soil solution through adsorption (Nan et al. 2021).

Our finding that CH_4 production rate *m* was not dominated by a pH effect at first appears to contradict Wang et al. (1993), who demonstrated the importance of pH for CH_4 production in an incubation study. However, the authors of the latter study interpret the CH_4 accumulation at the end of their incubation as uniform CH_4 production rates, but do not differentiate between the delay of methanogenesis (high influence

of pH) and the later developing production rates (lower influence of pH). Thus, the mechanisms of the pH effect identified in the present study do not contradict but explain the results of fundamental research about pH dependence of methanogenic respiration in paddy soils through better temporal differentiation.

Effectiveness of modelling approach

The data quality of the modelled values strongly depends on the number of measurements, especially after the onset of methanogenesis. Therefore, the uncertainties in the estimation of the parameters delay of methanogenesis *d* and CH_4 production rate *m* can be explained by the fact that only a few measurements of the phase of constant CH_4 production were possible due to experimental conditions (cf. Chap. 2.1).In relation to the estimation of *d*, fewer measured values during ongoing methanogenesis underlie the modelling of *m*. Still, the overall effect on the results is comparably small. Instead of describing CH_4 accumulation along a time series of single measurements, the proposed model strictly separates the two stages, pre-methanogenic (Figs. 6 and S2, SI) and methanogenic respiration (Fig. 7). The measured amounts of CO_2 and CH_4 formed could be modelled with an accuracy that creates a contrast between the derived parameters for further analyses and the variability of individual measurements that are commonly used as results (compare Fig. 4 to Fig. 3). Considering the raw data, a remaining fitting error of the model can also be interpreted as an advantage: The residual uncertainty may be due to the operational measurement uncertainty or inaccuracies in sampling that confound the measured values and therefore prevent overly perfect fits.

Conclusions and outlook

In summary and based on the examination of the parameters 'delay of methanogenesis' *d* and the 'CH_4 production rate' *m*, our revised incubation and modelling approach was well suited to evaluate the effects of six different biochar additives on CH_4 production in paddy soils. This enabled us to develop a mechanistically differentiated understanding on the patterns of CH_4 production. By performing two different incubation experiments we were able to resolve the apparently contradicting results. The common, untreated laboratory set-up (experiment 1) led a clear delay of CH_4 production (mean delay by 24.24%) during the pre-methanogenic stage. In the proposed alternative experimental set-up (experiment 2) biochar did neither prolong the pre-methanogenic stage (*d*) (i.e., retard CH_4 production) nor did it provide additional electron acceptors ($pmCO_2$) (mean decrease by 7.00% and 0.50%, respectively. However, irrespective of the pre-treatment of the biochars during the methanogenic stage, their application led to a significant decrease of the production rates of CH_4 (mean effect of − 25.89%). The patterns of such effects can also be differentiated for different biochar treatments, showing the importance of feedstock selection and revealing lower interactions between microorganisms and coarse biochar compared to fine texture.

It has been reported that the addition of biochar raises the pH value in the short term (Jeffery et al. 2016), but this effect fades out over years due to leaching and changes in the surface chemistry of biochar (Chang et al. 2019). Under the assumption that the influence on the pH value of the soil solution is marginal in the long term and that various boundary effects buffer an alkalinisation of the soil even during incorporation into the field, the alternative experimental set-up (experiment 2) revealed biochar effects that could be isolated from the impact of pH. With the help of this study, we presume that the short-term elevation of pH may be the cause why laboratory studies so far report distinctly more positive effects on biochar in mitigating CH_4 production in paddy soils compared to large-scale field trials. We conclude that the mitigation under real field conditions is mostly achieved by decreased CH_4 production rates and not by a delayed onset of methanogenesis (cf. possible pathways depicted in Fig. 1).

We recommend further efforts in the research of CH_4 emissions from paddy cultivation to harmonise field, pot and laboratory experiments. The strong influence of pH observed here highlights the importance to identify and quantify short-term effects and to compare alternative study designs to isolate these effects. Further investigations with focus on the methanogenic stage is needed to get mechanistic insights into biochar effects on the availability of both electron acceptors and organic substrates for microbial communities. In addition, we also suspect a microbial black box in this field with regard to the

interactions between biochar and soil microorganisms beyond mere redox chemistry, e.g. toxicities or niche formation. Simplified evaluation strategies, such as the modelling approach presented here, can also be used to meet the challenge of investigating repeated redox cycles and the succession of the biochar-soil interactions.

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Author contributions K.-H. Knorr and T. Middelanis contributed to the study conception and design. Material preparation, data collection and analysis were performed by T. Middelanis with help in statistics by D. Looschelders. The first draft of the manuscript was written by Thomas Middelanis and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare that no funds, grants, or other support were received during the preparation of this manuscript. The authors have no relevant financial or non-financial interests to disclose.

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