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Soil incubation methods lead to large differences in inferred methane production temperature sensitivity

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Abstract

Quantifying the temperature sensitivity of methane (CH_4) production is crucial for predicting how wetland ecosystems will respond to climate warming. Typically, the temperature sensitivity (often quantified as a Q_{10} value) is derived from laboratory incubation studies and then used in biogeochemical models. However, studies report wide variation in incubation-inferred Q_{10} values, with a large portion of this variation remaining unexplained. Here we applied observations in a thawing permafrost peatland (Stordalen Mire) and a well-tested process-rich model (*ecosys*) to interpret incubation observations and investigate controls on inferred CH_4 production temperature sensitivity. We developed a field-storage-incubation modeling approach to mimic the full incubation sequence, including field sampling at a particular time in the growing season, refrigerated storage, and laboratory incubation, followed by model evaluation. We found that CH_4 production rates during incubation are regulated by substrate availability and active microbial biomass of key microbial functional groups, which are affected by soil storage duration and temperature. Seasonal variation in substrate availability and active microbial biomass of key microbial functional groups led to strong time-of-sampling impacts on CH_4 production. CH_4 production is higher with less perturbation post-sampling, i.e. shorter storage duration and lower storage temperature. We found a wide range of inferred Q_{10} values (1.2–3.5), which we attribute to incubation temperatures, incubation duration, storage duration, and sampling time. We also show that Q_{10} values of CH_4 production are controlled by interacting biological, biochemical, and physical processes, which cause the inferred Q_{10} values to differ substantially from those of the component processes. Terrestrial ecosystem models that use a constant Q_{10} value to represent temperature responses may therefore predict biased soil carbon cycling under future climate scenarios.

1. Introduction

Understanding and quantifying methane (CH_4) production temperature sensitivity are important to improve predictions of how wetland ecosystems will respond to and feedback on climate warming (Davidson and Janssens 2006). The sensitivity of CH_4 production to temperature is often described by a Q_{10}

value, which is defined as the factor by which CH_4 production increases when temperature increases by 10 °C (van Hulzen *et al* 1999). Q_{10} is usually derived from laboratory incubation experiments where soil samples are placed in controlled conditions at different temperatures and CH_4 production is measured over time (Zheng *et al* 2018). Such incubation-inferred Q_{10} values are often incorporated into terrestrial ecosystem models. A constant Q_{10} value of 2 is often assumed (Walter and Heimann 2000, Riley *et al* 2011), although a large variation of Q_{10} values has been reported, and a large portion of that variability remains unexplained (Craine *et al* 2010, Hamdi *et al* 2013, Meyer *et al* 2018, Haaf *et al* 2021). The significant effects Q_{10} value can have on modeled CH_4 emissions implies the need to better understand and quantify variations in Q_{10} (Riley *et al* 2011). We note that many of these issues have also been shown to be important for evaluating the temperature sensitivity of soil carbon dioxide (CO_2) production (Gu *et al* 2004, Davidson *et al* 2006, Fierer *et al* 2006, Zhou *et al* 2009).

The limited understanding of CH_4 production temperature sensitivity arises from several factors (Segers 1998). First, CH_4 production processes are complex and involve various microbial activities, including syntrophic interactions and competition for key substrates (Le Mer and Roger 2001, Bridgham *et al* 2013). Heterotrophic microbes drive the breakdown of complex organic polymers to simple substrates. Fermentation of these substrates result in production of additional substrates including H_2, CO_2, and acetate. Acetate can be fermented to form CO_2 and CH_4 by acetoclastic methanogens (AM), and CO_2 can be reduced to CH_4 using H_2 as an electron donor by hydrogenotrophic methanogens (HM). The rates of these processes are also affected by environmental factors such as soil moisture, soil temperature, oxygen concentration, and substrate concentrations (Schlesinger and Bernhardt 2013).

Although laboratory experiments provide a controlled environment with comparatively stable soil moisture and temperature, uncertainties can arise from the time or season of soil collection, conditions under which the soil is stored (i.e. temperature, duration), and pre-treatment periods (Rhymes *et al* 2021, Schroeder *et al* 2021, Wilson *et al* 2021). Previous work assessing the impacts of sampling time and storage on microbial activities, CH_4 production, and inferences of Q_{10} values had inconsistent results (Rhymes *et al* 2021, Wilson *et al* 2021). For example, Lupascu *et al* (2012) found sampling time affects CH_4 production but not inferred Q_{10}. In contrast, Bergman *et al* (2000) found that Q_{10} values of CH_4 production varies with the time of collection due to substrate availability and seasonal variability in active microbial biomass. These conflicting results may stem from challenges in quantifying controlling factors (e.g. carbon quality, substrate concentrations and composition, and microbial biomass and activity) continuously and accurately (Blagodatskaya and Kuzyakov 2013), thereby hindering interpretation of incubation measurements.

Here we apply observations and a well-tested process-rich model, *ecosys*, to (1) interpret laboratory incubation observations; (2) investigate controls on inferred CH_4 production temperature sensitivity; and (3) inform incubation strategies. The *ecosys* model simulates the physical, hydrological, and biological processes that govern ecosystem responses to environmental conditions and has been applied in dozens of permafrost sites (Grant *et al* 2017, Mekonnen *et al* 2021, Riley *et al* 2021). *Ecosys* represents multiple microbial functional groups that affect complex biogeochemical transformations of carbon and nutrients. Our study site is Stordalen Mire, a permafrost site in northern Sweden, with an extensive research history, including modeling (Chang *et al* 2019a, 2019b, 2020) and a rich observational record (Bolduc *et al* 2020). We chose this site because permafrost regions contain a large amount of organic carbon which is vulnerable to decomposition by soil microbes, releasing greenhouse gases including CH_4 and CO_2 (Tarnocai *et al* 2009). We hypothesized that incubation methods (i.e. sampling time, storage temperature, and storage duration) would influence CH_4 production during incubation by altering the initial incubation conditions, consequently impacting inference of the temperature sensitivity. To address our hypothesis, we developed a field-storage-incubation (FSI) modeling approach that mimics the full incubation process, including the timing of field soil sample collection, soil sample storage, and incubation. Then we evaluated our model and FSI approach through comparison with field observations and laboratory incubation measurements. After model validation, we conducted FSI simulation experiments where we varied storage duration, storage temperature, sampling time, initial substrate and microbial biomass conditions, and incubation temperatures to investigate how these factors affect CH_4 production and inferred Q_{10} values.

2. Methods and data

2.1. Study site description

Stordalen Mire is a peatland situated in northern Sweden (68.35°N, 19.05°E). The climate in this area is subarctic with annual mean temperature of 0.07 °C and mean precipitation of 308 mm y^{-1} (1986–2006) (Bäckstrand *et al* 2010). The fen, one of the three sub-habitats in the study site, is fully thawed and inundated, with large reported CH_4 emissions (McCalley *et al* 2014, Holmes *et al* 2022). On this site, sedges (*Eriophorum angustifolium*) are the dominant plant species. Due to recent permafrost collapse and

increasing inundation, the fen area has increased by 100% from 1970 to 2014, increasingly offsetting the CO_2 sink at the Mire (Varner *et al* 2022). The Mire has been closely monitored since the 1970s and a comprehensive dataset of the site has been generated (Bolduc *et al* 2020). Peat cores were collected and transported for laboratory analysis including microbial analysis (Woodcroft *et al* 2018), biogeochemistry, and incubation experiments (Hodgkins *et al* 2014).

2.2. Measurements

Climate forcing data that is used to drive the *ecosys* model includes air temperature, precipitation, radiation, wind speed, and relative humidity. Forcing data prior to year 2014 are based on the GSWP3 reanalysis dataset and bias-corrected using long-term Abisko research station measurements at the Stordalen Mire (Chang *et al* 2019b). From 2014–2017, forcing data were obtained from the European center for medium-range weather forecasts atmospheric reanalyses (ERA5) (supplemental material). Terrestrial gas flux data, including CH_4 and CO_2 fluxes from 2011–2017, were recorded from auto-chamber systems on-site (McCalley *et al* 2014, Mondav *et al* 2014, Holmes *et al* 2022). Water table depth and thaw depth were also recorded and published (Crill *et al* 2023). Soil organic matter measurements are available at different depths at the autochamber sites (Hodgkins *et al* 2014). The incubation experimental data (i.e. CH_4 production) used for model testing in this study were also from Hodgkins *et al* (2014), which provides details on the site, storage, and incubation protocols.

2.3. Ecosys model

The *ecosys* model is a mechanistically-based terrestrial ecosystem model that couples hydrological, thermal, plant, and microbial dynamics and their exchanges with the atmosphere. *Ecosys* has been tested on multiple ecosystems including the Stordalen Mire site in this study (Chang *et al* 2019a, 2019b). *Ecosys* has also been successfully tested against scenarios of environmental change, including warming experiments (Bouskill *et al* 2020) and elevated atmospheric CO_2 conditions (Grant 2013). Eleven microbial functional groups that regulate carbon, nitrogen, and phosphorus dynamics are explicitly represented in *ecosys*. Decomposition rates of different soil organic matter pools are a function of decomposer biomass and substrate concentration, and are also affected by soil moisture. The effect of temperature on decomposition rate is represented with a modified Arrhenius function which considers the inactivation of enzymes under high and low temperatures (Sharpe and DeMichele 1977, Grant 2014). This temperature sensitivity function does not consider other environmental constraints (e.g. soil moisture, substrate levels) beyond temperature, so we term it the intrinsic temperature sensitivity (Davidson and Janssens 2006, Wu *et al* 2021). We then calculate Q_{10} values based on that intrinsic temperature sensitivity and compare them with the Q_{10} values inferred from the simulated incubation experiments. Soil moisture and temperature are solved based on heat and water transfer schemes though canopy-snow-litter-soil profiles. Microbial respiration rates are represented based on Michaelis-Menten kinetics with influences from soil water potential, oxygen concentration, nutrient availability, and temperature. *Ecosys* represents CH_4 production (acetoclastic methanogenesis and hydrogenotrophic methanogenesis), and CH_4 oxidation (table S1 and figure S1). A detailed description of model structure, inputs, and outputs of *ecosys* can be found in the data availability statement.

2.4. The simulation experiment

Here we developed and applied an FSI simulation approach (figure 1). In this approach, we first use *ecosys* to model a soil profile under long-term field conditions to create a modeled soil core consistent with the field soil core. A soil layer is then extracted numerically from that modeled field soil core and used in the model incubation protocol, which mimics laboratory sampling, storage, and incubation procedures. This modeling approach creates reasonable initial conditions for the incubation simulations and allows us to evaluate the model against field observations and laboratory incubation measurements.

We apply this FSI approach in Stordalen Mire, using field observations and incubation measurements from the fen site. For the field simulations, we run *ecosys* for the field site from 1980 until 2017 using the climate forcing described above. Simulation results are then compared against field observations to determine the appropriate field simulation scenario. From this baseline field simulation a soil layer is numerically extracted on the modeled date, 15 June 2011, mimicking the field sampling. Then the model is run with that soil layer through the storage and incubation periods. The storage and incubation conditions of that soil layer follow the incubation experiment procedure in Hodgkins *et al* (2014). However, a preincubation was not performed in the simulation because we forced the modeled soil and headspace to have no oxygen at the beginning of the incubation (baseline scenario). In the storage and incubation phases, modeled plants are removed and therefore no fresh litter inputs occur, and temperature and humidity are held constant (figure 1). Anaerobic conditions in the model are maintained by setting oxygen concentrations in the soil and headspace to zero at the start of storage. High N_2 concentrations in the headspace are set to mimic the N_2 flushing in the initial incubation experiment. Simulated CH_4 production is then compared against incubation experimental results for model evaluation.

In addition to our baseline simulation, we simulated the following incubation scenarios: sampling

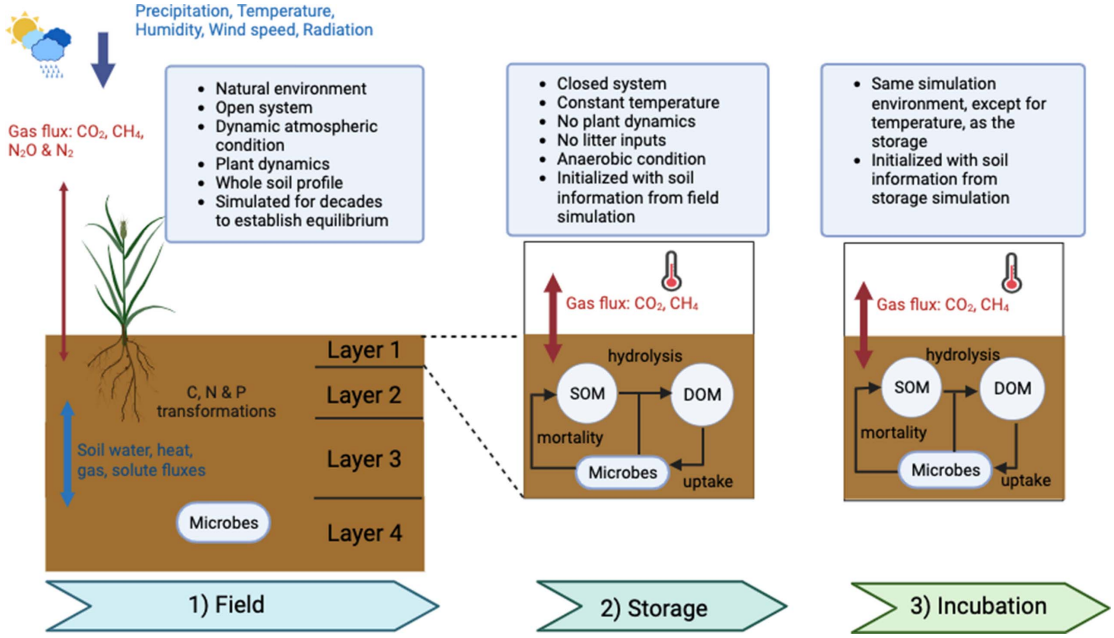

Figure 1. A schematic representation of the three-phase FSI modeling approach: (1) field, (2) storage, and (3) incubation. The baseline scenario includes simulation under field conditions until sampling on 15 June 2011, sample storage for 7 months at 4 °C, and sample incubation at 22 °C for 90 d.

times during the growing season (15 July, 15 August, 15 September), storage conditions (temperature: −20 °C; storage duration: 2 and 12 months), and incubation temperatures (4, 11, 33 °C). The cumulative CH_4 production under each combination of factors was used to infer Q_{10} values following the 'equal-time' approach (Hamdi *et al* 2013), wherein cumulative CH_4 production is evaluated at the same time from two cores incubated at different temperatures. The inferred Q_{10} value is calculated as:

$$Q_{10} = \left(\frac{C_2}{C_1}\right)^{10/(T_2-T_1)} \quad (1)$$

where C_2 and C_1 are cumulative CH_4 production at incubation temperatures T_2 and T_1, respectively.

3. Results and discussion

We next describe our model validation, which involved two steps: (i) comparison with time series of field measurements, and (ii) comparison with laboratory incubation experiments, for which both measured datasets have been previously published as described in methods and data.

3.1. Model validation

In addition to *ecosys* validation at the same site using field observational data from earlier years (i.e. 2003–2014 (Chang *et al* 2019a, 2019b)), we compared simulation results with the newest site observations through 2017 (Holmes *et al* 2022). Our modeled CH_4 emissions agree well with measurements across the simulation period (RMSE = 68 mgC m^{-2} d^{-1}, R = 0.76, figure 2(a)). Measured and modeled thaw depth, water table depth, and net ecosystem carbon exchange (NEE) also agree well (figure S2). Finally, modeled soil organic carbon content (440–460 g kg^{-1}) also compares well with observations (466 g kg^{-1}). These comparisons give confidence that *ecosys* reasonably captures the thermal-hydrological state and carbon cycling dynamics at the site. Also, these comparisons give us confidence that the numerically extracted soil for the FSI simulation experiments broadly matches the actual soil samples used in the laboratory incubation experiments.

CH_4 production during the modeled baseline incubation experiments broadly matched the observations from Hodgkins *et al* (2014) (figure 2(b); RMSE = 21 umol (g dry)$^{-1}$), with modeled and observed gas production both accumulating more slowly with time, in line with many published incubations studies, which show that respiration rates decline after the first few days as fast-cycling carbon is depleted and slow-cycling carbon becomes the main contributor to microbial activity (Fang *et al* 2005, Schädel *et al* 2020).

3.2. Time-dependent CH_4 production, biogeochemistry, and microbial biomass during incubations

Modeled CH_4 production by acetoclastic methanogenesis and hydrogenotrophic methanogenesis both decrease with time during the baseline incubation simulation (figure 3(a)). To disaggregate the

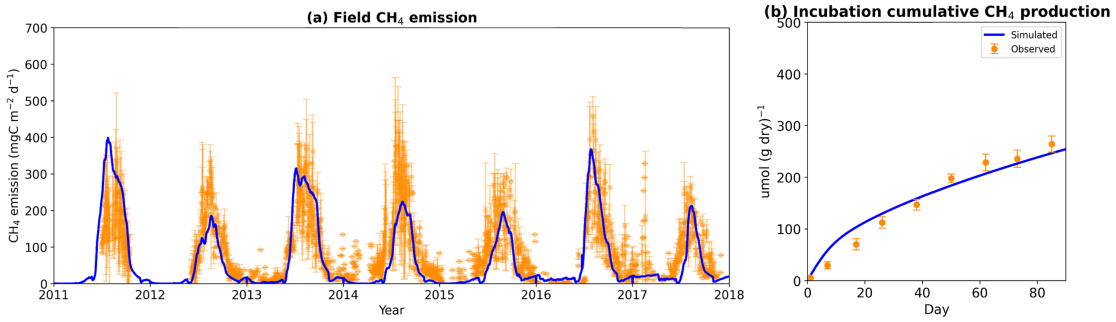

Figure 2. (a) Modeled CH_4 emissions closely align with field observations at the fen site for the period 2011–2017. Solid blue lines represent simulation results, while markers denote measurements. Error bars indicate the standard deviation of daily CH_4 emission measurements obtained from two autochamber systems. (b) Modeled cumulative CH_4 production is consistent with incubation measurements reported by Hodgkins *et al* (2014). The solid blue line represents simulation results and the markers represent measurements. Error bars in (b) depict the standard deviation of CH_4 production measurements in incubation experiments across three soil samples from the same site as the field observations.

factors regulating modeled CH_4 production, we analyzed the modeled substrate concentrations (i.e. dissolved organic carbon (DOC), acetate, and hydrogen (H_2)) and active microbial biomass (i.e. AM, HM, fermenter, and methanotroph) most closely related to CH_4 emissions (figure S1). These substrates and active microbial biomass change dynamically during incubations. H_2, the product of fermentation and a substrate for HM, shows a similar trend as the decreasing CH_4 production rate. Microbial biomass shows an initial slight increase followed by a steady decrease, in contrast to DOC and acetate concentration trends. Microbial growth, accompanied by DOC and acetate consumption, result in increased need for microbial maintenance respiration. When total respiration falls short of maintenance respiration needs, microbes senesce, producing microbial residue (Grant *et al* 1993). Decomposition of microbial residue contributes to the slight accumulation of DOC and acetate after 20 d (figure 3(b)).

We hypothesized that DOC and microbial biomass concentrations at the beginning of the incubation experiment were important controllers for subsequent CH_4 production. To explore this idea, we performed two simulation scenarios with increased levels of initial DOC (factor of ten) and microbial biomass (factor of ten) of all microbial functional groups (figures S3 and S4). Both simulations have similar patterns in the relationship between CH_4 production rates, substrates, and active microbial biomass. Increased DOC leads to an initial increase in CH_4 production, driven by sustained high H_2 concentrations from fermentation and growth of fermenters and methanogens. This modeled increase in soil respiration rate from adding initial substrates has also been reported in incubation experiments (Bergman *et al* 2000, Pegoraro *et al* 2019). Notably, the effect on CH_4 production of increasing initial microbial biomass is about twice that of increasing initial DOC, averaged over 3 months. A tenfold increase in microbial biomass resulted in sustained high DOC concentrations due to increased hydrolysis of soil organic matter (SOM) to soluble DOC. In this scenario, we did not see an increase in microbial biomass, possibly due to high maintenance respiration rates (caused by the high microbial biomass) that lead to the senescence of microbes. Our results demonstrate that DOC concentration and active microbial biomass are limiting factors of CH_4 production in incubations.

The simulated biomass of active AM, HM, and fermenters all decreased during baseline incubations with the relative proportions of these functional groups remaining relatively constant (figure 3(c)). The methanotroph biomass remains low due to anaerobic conditions. We confirmed that modeled methanotrophs will grow when oxygen is added to the modeling system, as expected. Our results of microbial biomass composition align with results in Wilson *et al* (2021) that microbial relative abundances were not changed significantly during incubations of fen samples at Stordalen Mire. However, accurately measuring active microbial biomass in experimental incubations has been challenging (Blagodatskaya and Kuzyakov 2013), and debates persist about microbial changes during storage and incubation periods (Stenberg *et al* 1998). Stenberg *et al* (1998) found (a) microbial biomass decreased significantly at 2 °C, as estimated by the chloroform fumigation-extraction method and (b) small changes in biomass estimated by the substrate induced respiration method. Microbiome 16 S rRNA copy number, an indicator of microbial biomass, has been reported not changing significantly during incubation (Wilson *et al* 2019, Fofana *et al* 2022).

3.3. Factors regulating incubation results

3.3.1. Storage duration

To test the effect of storage duration (at 4 °C) on CH_4 production, we conducted simulations for two additional storage duration scenarios (2 and

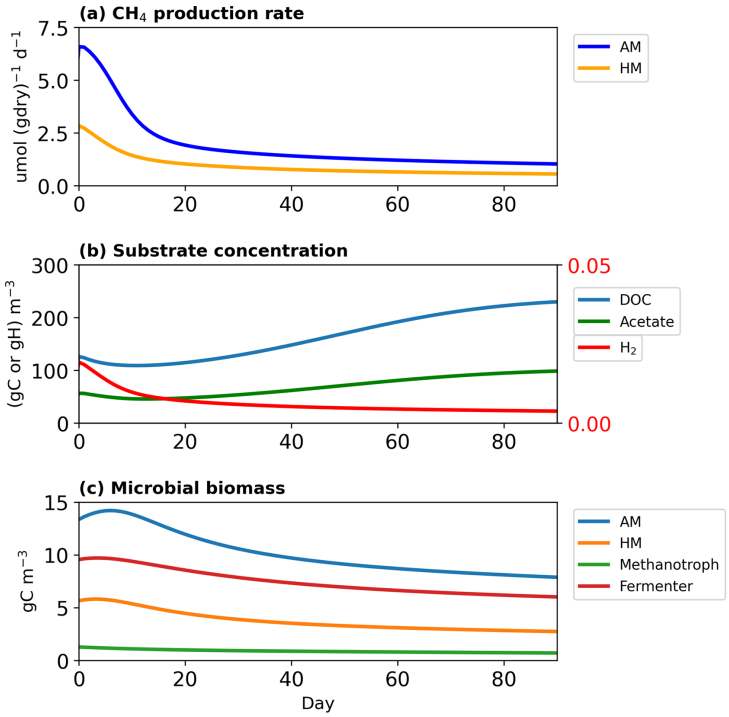

Figure 3. Modeled substrate concentration and active microbial biomass drive decreases in CH_4 production during baseline scenario incubations. (a) CH_4 production rates by AM (acetoclastic methanogens) and HM (hydrogenotrophic methanogens); (b) concentrations of DOC, acetate, and H_2; and (c) active AM, HM, methanotroph, and fermenter biomass.

12 months), and compared with the default scenario (i.e. 7 month storage duration). While the results of the 12 month storage scenario resemble those of the 7 month scenario, distinct differences emerged for the 2 month storage scenario, suggesting that storage duration significantly affects cumulative CH_4 production rates during incubation (figures 4(a) and (b)). The 2 month scenario is similar to the scenario with added initial DOC described above (figure S3). The initial DOC concentration is much larger (about 2 times greater) when storage duration is 2 versus 7 months. The additional initial resource in the 2 month storage scenario leads to a rapid increase in CH_4 production rates, and a >40 d period to decline to rates comparable to the other storage duration scenarios (figure 4(b)). The rapid increase and subsequent decrease of CH_4 production rates are regulated by the interplay of substrates and microbial biomass (figures 4(c)–(h)). The rapid decrease in DOC between about days 3 and 20 is consistent with increased fermentation biomass and products including acetate and H_2 (figures 4(d), (e), and (h), $C_6H_{12}O_6 + 2H_2O \text{—>} 2CH_3COOH + 2CO_2 + 4H_2$). This quick decrease in DOC concentration also aligns with the rapid growth of AM and HM (figures 4(f) and (g)). The slow accumulation of DOC and acetate after 20 d is similar to patterns observed in other storage scenarios, as described above.

Recognizing the significance of initial incubation conditions, we extended the analysis to include the storage phase in both the 7 month and 2 month storage scenarios (figures S5 and S6) to investigate the reason for their distinct initial conditions. Although the initial storage conditions are identical for the 7 month and 2 month scenarios, the continuous depletion of DOC from prolonged storage results in a higher DOC concentration in the shorter 2 month storage scenario compared to the 7 month storage scenario. Additionally, the close correspondence between substrates and microbial biomass during storage mirrors the relationship shown in the incubation phase. This close correspondence might be attributed to microbial decomposition being the sole source of substrates, given the absence of growing plants and root exudates during both phases.

There is ongoing debate regarding whether storage duration affects incubation data analysis. Stenberg *et al* (1998) found that microbial biomass and activity in soil are influenced by storage conditions, with storage duration being less important when soils are stored at freezing (−20 °C) compared to refrigeration (2 °C) temperatures. However, other studies found that microbial biomass carbon and enzyme activities were not substantially affected when stored at 4 or −20 °C (Lee *et al* 2007, Wilson *et al* 2021).

Our results indicate that 4 °C storage duration significantly affects CH_4 production through its effects on initial incubation substrate concentrations, which then affect microbial activity and biomass. The simulated storage temperature of −20 °C led to negligible microbial activity during storage

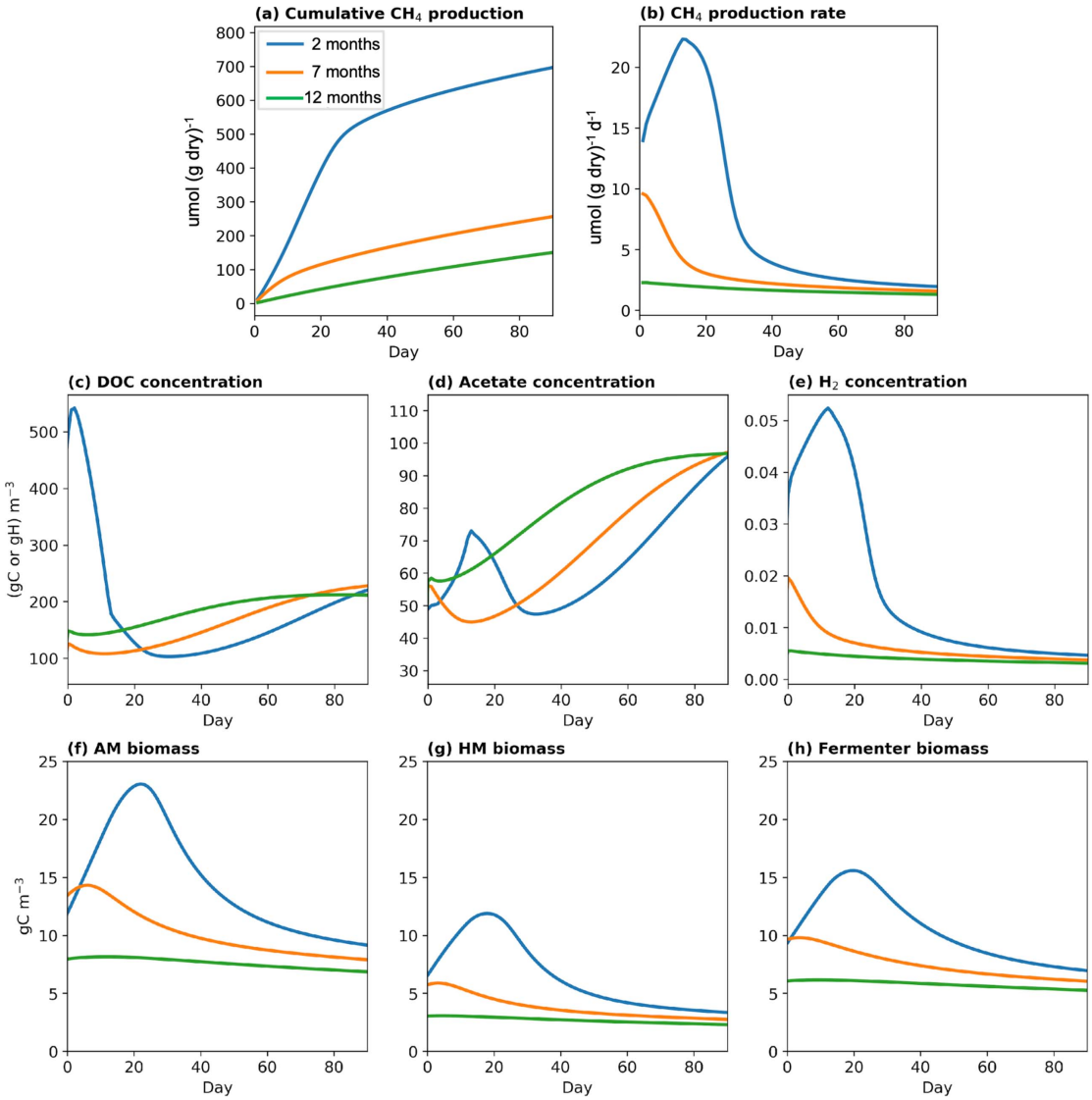

Figure 4. Storage duration affects CH_4 production during incubation through regulating substrate concentrations and microbial biomass. Legend labels refer to storage duration. Comparisons are made for storage durations of 2, 7, and 12 months for: (a) cumulative CH_4 production, (b) CH_4 production rate, (c) DOC concentration, (d) acetate concentration, (e) H_2 concentration, (f) AM biomass, (g) HM biomass, and (h) fermenter biomass. Initial conditions of the storage phase, storage temperature (4 °C), and incubation conditions are the same for all the storage duration scenarios. Except for storage duration, all the simulation conditions are the same as the default scenario.

and thereby high initial incubation DOC concentration (figure S7). Subsequently, the CH_4 production is much higher during incubation compared to storage at 4 °C under the same storage duration (figure S5). Based on these results we suggest either limiting storage duration or using a freezing storage temperature to minimize consumption of substrates during storage. Note that we focus on permafrost soils in this study where microbes are accustomed to freezing conditions. Introducing freezing temperature to warmer soil might lead to death of microbes and distorted interpretation of the incubation (Weiser Russell and Osterud Clarice 1945).

3.3.2. Sampling time

Sampling time (i.e. the time in the growing season when incubation material is collected from the field) influences CH_4 production during soil incubations and the effects on CH_4 production rate diminish over time (figures 5(a) and (b)). Notably, CH_4 production is larger during the incubation period when samples are taken in June, with production declining each sampling month until September. This effect is most prominent in the initial ∼20 d of the incubation period. The higher modeled CH_4 production rates for June samples are attributed to the higher availability of H_2 (i.e. substrate for HM, figure 5(e)) and active biomass of AM, HM, and fermenters (figures 5(f)–(h)) during this period. Tracing back to the storage phase, we found higher initial storage microbial biomass for all functional groups when sampling in September (figure S8) compared with sampling in June (figure S5), leading to more rapid DOC consumption and earlier mortality of microbes. As a

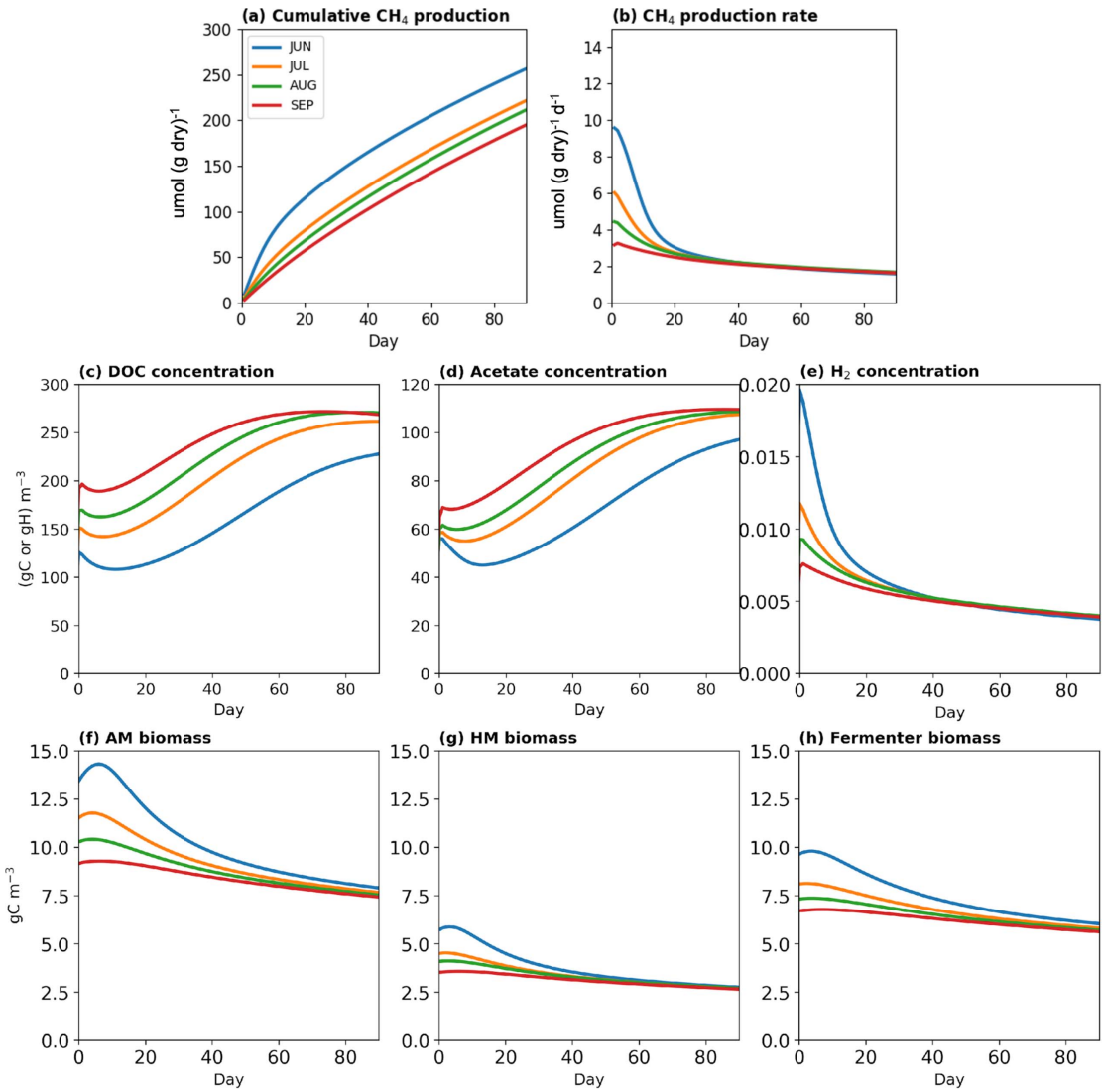

Figure 5. Sampling time affects CH_4 production during incubations through regulating the initial substrate concentrations and microbial biomass. Comparisons are made for sampling times of June 15, July 15, August 15, and September 15, denoted as JUN, JUL, AUG, and SEP, respectively. Results show (a) cumulative CH_4 production, (b) CH_4 production rate, (c) DOC concentration, (d) acetate concentration, (e) H_2 concentration, (f) AM biomass, (g) HM biomass, and (h) fermenter biomass.

result, in the initial phase of incubation, substrate concentrations in the September sampling scenario (compared to June sampling) are higher (from dead microbes) but active microbial biomass is lower, resulting in lower CH_4 production.

The conclusion above that earlier sampling time (i.e, June vs. September) leads to a higher cumulative CH_4 production during incubations is also valid for long-term incubations with −20 °C storage temperature (figure S9). However, for short-term incubations less than 10 d, CH_4 production rates are slightly higher when sampling in September. Experimental results from Lupascu *et al* (2012) show a progressive increase in CH_4 production rates when sampled from June to September for top 20 cm soil incubated at 4 °C. Bergman *et al* (2000) highlighted the significant impact of sampling time on incubation results, and showed that whether early season sampling time led to larger incubation CH_4 production varied across field sites.

Overall, our results demonstrate that sampling time can significantly affect incubation CH_4 production. However, the intricate interplay between substrates and microbial biomass, coupled with the initial incubation conditions influenced by storage temperature and duration, adds complexity to understanding how sampling time affects CH_4 production during incubation.

3.4. Sampling time, storage duration, and incubation duration affect inferred temperature sensitivity

As described in methods, we inferred Q_{10} values using cumulative CH_4 production in the same manner as is typically done from laboratory incubations (i.e. the equal-time method). We hypothesized that sampling

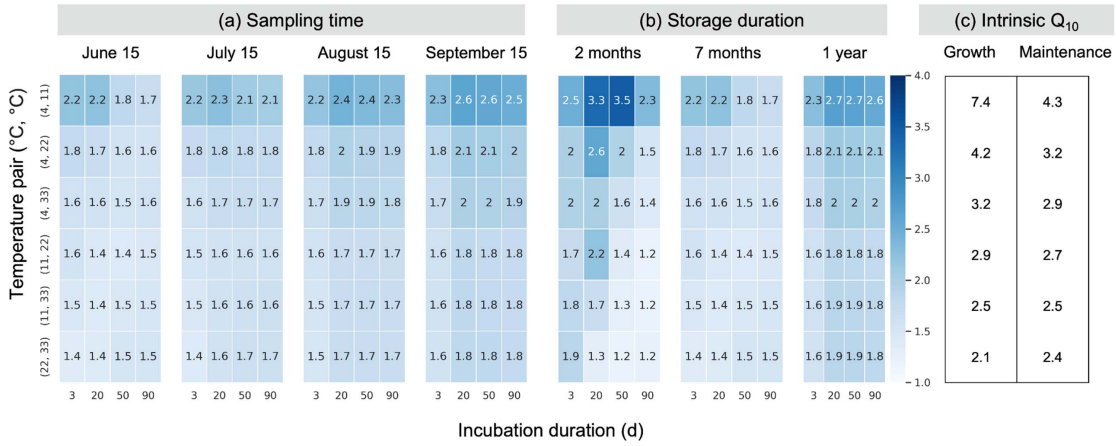

Figure 6. Q_{10} values vary with incubation duration (*x*-axis), temperature pairs (*y*-axis), (a) sampling time, and (b) storage duration. Temperature pairs represent the two temperatures chosen for calculating Q_{10} values using the equal-time method (equation (1)). Note Q_{10} values in the first (June 15) and sixth (7 months) groups of columns are identical because those Q_{10} values are inferred from the same default simulation with sampling time of June 15 and storage duration of 7 months. (c) Microbial growth and maintenance Q_{10} values calculated based on the modified Arrhenius function used in *ecosys*.

time (15 June, 15 July, 15 August, 15 September), storage duration (2, 7, 12 months), incubation duration (3, 20, 50, 90 d), and incubation temperature (4, 11, 22, 33 °C; figure S10) would all affect inferred Q_{10} values. Across all these cases, we inferred Q_{10} values between 1.2 and 3.5 (figure 6). These results align well with published Q_{10} values from laboratory incubation experiments on permafrost soils. For example, Lupascu *et al* (2012) reported values of 1.9–3.5 at a sedge site in Stordalen Mire based on laboratory incubation experiments. Dutta *et al* (2006) reported a mean Q_{10} value of 1.9 ± 0.3 for permafrost soil samples across tundra and boreal forest sites in Siberia, incubating at controlled temperatures of 5, 10, and 15 °C.

In general, inferred Q_{10} values are largest for storage durations of 2 months, indicating that when substrate levels are high, the enhancement of CH_4 production due to warming is also higher. For the 2 month storage scenario, Q_{10} values initially increase and then decrease with incubation duration, corresponding to the phases of microbial growth and death. We also inferred much higher Q_{10} values under the simulation scenarios of higher initial DOC concentration and −20 °C storage temperature (figure S11). Except for the 2 month storage scenario, Q_{10} values stabilize after incubating for ∼50 d. This effect of incubation duration on inferred Q_{10} values has also been reported in incubation experiments (Reichstein *et al* 2005, Gudasz *et al* 2015). Additionally, our inferred Q_{10} values tend to be higher at lower temperature pairs (e.g. using 4 °C and 11 °C) compared to using higher temperature pairs, consistent with prior findings (Zhou *et al* 2009).

Using the modeled CH_4 production results to infer Q_{10} values allows a comparison to the intrinsic temperature sensitivity coded in the model. *Ecosys* uses a modified Arrhenius function (Sharpe and DeMichele 1977, Grant *et al* 1993) to infer the effect of temperature on microbial activity (termed the intrinsic temperature sensitivity). Because substrate concentrations, nutrient constraints, oxygen concentrations, and pH also affect activity rates in the model (and real world), the Q_{10} values inferred from emergent CH_4 production are expected to be different from the intrinsic value (Davidson *et al* 2006). This issue is important for model development, since it is common practice to use laboratory inferred Q_{10} values in biogeochemical models without accounting for all the other factors which affect CH_4 production rates already included in the model. The intrinsic Q_{10} values in *ecosys* range from 2.1 to 7.4 based on the prescribed temperature pairs (figure 6), decreasing as the incubation temperature pairs increase, and are a factor of about 1.4–2.5 higher than the inferred values (calculated using the mean for each temperature pair shown in figure 6). Thus, a model that used an inferred Q_{10} value from a laboratory incubation, and that also included other factors affecting respiration rates, could be substantially biasing low their predicted CH_4 production temperature sensitivities.

Further, the fact that the inferred Q_{10} values depend on substrate concentrations, microbial biomass, and temperature, as discussed above, and that those factors are often dynamic in real ecosystems, has significant implications for applications in terrestrial ecosystem models. Frequently, incubation experiments last days to months, with the resulting Q_{10} value calculated for the incubation duration. However, modelers often apply the inferred Q_{10} value to represent transient (often hourly) changes in respiration rates caused by temperature (Gu *et al* 2004). Misunderstanding of inferred Q_{10} values in terrestrial ecosystem models could lead to an underestimation of the response of soil respiration to warming, especially in cold regions (Zhou *et al* 2009).

Q_{10} values inferred from incubation studies have been widely adopted and have contributed to understanding of ecosystem responses to change. While it may be acceptable for empirical ecosystem models lacking representations of substrates and microbes to use a temperature sensitivity response that includes all those external effects, those models miss key mechanisms and are less favorable for simulating carbon-climate feedbacks with environmental change (Sulman *et al* 2018, Chang *et al* 2021, Tang *et al* 2023). Moving forward, particularly for process-based terrestrial ecosystem models that represent microbial activities and substrate dynamics, we recommend the use of mechanistic temperature sensitivity representations of component processes that contribute to the overall emission instead of a constant Q_{10} value for CH_4 production or emission (Davidson *et al* 2006, Tang and Riley 2020). The characterization of such temperature sensitivities can be informed by theoretical studies and incubation experiments. When characterizing temperature sensitivities based on incubation experiments, we suggest using short-term incubations with abundant substrates, avoiding storage (or above-freezing conditions), and measuring other factors known to affect CH_4 production rates (e.g. substrate levels, microbial biomass). The exact form of the temperature sensitivity representation is still under discussion. Some models, such as the modified Arrhenius function by Sharpe and DeMichele (1977), incorporate the high and low temperature inactivation of enzymes in organism activity. The macromolecular rate theory model, which accounts for the change in heat capacity associated with the transition between the enzyme–substrate complex and the enzyme–transition state, has recently been proposed (Alster *et al* 2016, 2020, Liang *et al* 2018), as have other approaches based on chemical kinetics (e.g. Tang and Riley 2024). Other studies argued that the emergent temperature response also depends on dynamic interactions between mineral surfaces and substrates, substrate lability, enzymes, and microbes (Tang and Riley 2014).

4. Conclusions

We applied observations and a well-tested process-rich model, *ecosys*, to interpret laboratory incubation observations and investigate controls on inferred temperature sensitivity (i.e. Q_{10}) of methane (CH_4) production. A field-storage-incubation (FSI) simulation approach was developed to mimic the incubation process. The *ecosys* model and the simulation approach were benchmarked using observations of field CH_4 emissions and incubation CH_4 production. Incubation simulation results show that dynamic CH_4 production rates are regulated by the interplay between substrates (DOC, acetate, and H_2) and activities of acetoclastic and hydrogenotrophic methanogens and fermenters. Using the model, we found that storage duration, storage temperature, and sampling time affect CH_4 production through interactions with substrates and microbial biomass.

Our findings explain how the inferred temperature sensitivity of CH_4 production is affected by incubation duration, incubation temperatures, storage duration, storage temperature, and sampling time. The inferred Q_{10} values were substantially lower than the intrinsic temperature sensitivity used in the model because other factors (e.g. substrates and microbial biomass) also affect CH_4 production. In other words, Q_{10} values of CH_4 production and emission are regulated by a complex interplay of biological, biochemical, and physical processes. This interaction leads to the aggregated Q_{10} being different than those of the component processes. Terrestrial ecosystem models relying on a constant Q_{10} value to characterize temperature responses may therefore predict biased soil carbon cycling under future climate scenarios. Our framework for simulating incubations and the associated findings provide valuable insights for interpreting incubation observations and lead us to propose hypotheses relating to the effects of storage duration and sampling time on Q_{10} that could be tested in incubation experiments. Further, our work emphasizes the need to accurately measure important auxiliary variables such as substrate availability and active microbial biomass during incubation experiments to improve mechanistic understanding and modeling of carbon cycling responses to warming.

Data availability statement

The *ecosys* model and documentation is available for download at https://github.com/jinyun1tang/ECOSYS

The data that support the findings of this study are openly available at the following URL/DOI: https://zenodo.org/records/10420649.

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