Mechanisms for the suppression of methane production in peatland soils by a humic substance analog

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Abstract

Methane (CH₄) production is often impeded in many northern peatland soils, although inorganic terminal electron acceptors (TEAs) are usually present in low concentrations in these soils. Recent studies suggest that humic substances in wetland soils can be utilized as organic TEAs for anaerobic respiration and may directly inhibit CH₄ production. Here we utilize the humic analog anthraquinone-2, 6-disulfonate (AQDS) to explore the importance of humic substances, and their effects on the temperature sensitivity of anaerobic decomposition, in two peatland soils. In a bog peat, AQDS was not instantly utilized as a TEA, but greatly inhibited the fermentative production of acetate, carbon dioxide (CO₂), and hydrogen (H₂), as well as CH₄ production. When added together with glucose, AQDS was partially reduced after a lag period of 5 to 10 days. In contrast, no inhibitory effect of AQDS on fermentation was found in a fen peat and AQDS was readily reduced as an organic TEA. The addition of glucose and AQDS to both bog and fen peats caused complicated temporal dynamics in the temperature sensitivity of CH₄ production, reflecting temporal changes in the temperature responses of other carbon processes with effects on methanogenesis. Our results show that the humic analog AQDS can act both as an inhibitory agent and a TEA in peatland soils. The high concentrations of humic substances in northern peatlands may greatly influence the effect of climate change on soil carbon cycling in these ecosystems.

1 Introduction

Due to anaerobic soil conditions, wetlands store globally significant amounts of carbon (C) (Maltby and Immirzi, 1993), which may decompose to either carbon dioxide (CO₂) or methane (CH₄). Given that CH₄ has a global warming potential 25-times greater than CO₂ over 100 yr (Forster et al., 2007), the ratio of CO₂:CH₄ produced during anaerobic C decomposition may have substantial impacts on the Earth’s future cli-
mate. It is therefore essential to understand the fundamental controls over organic C mineralization to CO₂ and CH₄ in these systems.

Rates of anaerobic C mineralization and the ratio of its end products, CO₂ and CH₄, are the result of a suite of complicated interactions among multiple microbial functional groups (Bridgham et al., 2013; Megonigal et al., 2004). Under anaerobic conditions, organic polymers are ultimately converted to acetate, dihydrogen (H₂) and CO₂ by fermenting and syntrophic bacteria, and acetate and H₂ are further utilized as substrates for microbial respiration. In general, microbes will preferentially use a variety of thermodynamically favorable terminal electron acceptors (TEAs), such as nitrate (NO₃⁻), iron (Fe(III)), manganese (Mn(III, IV)), and sulfate (SO₄²⁻), for respiration before CH₄ production becomes important, which results in a higher ratio of CO₂ : CH₄ production. After these more favorable TEAs have been depleted, methanogens use either acetate (acetoclastic methanogenesis) or CO₂/H₂ (hydrogenotrophic methanogenesis) to produce CH₄ resulting in an approximately equal molar production of CO₂ and CH₄ (Conrad, 1999).

Despite northern peatlands generally having low concentrations of inorganic TEAs (Keller and Bridgham, 2007; Vile et al., 2003a), their ratio of CO₂ : CH₄ production is often much greater than 1 (Duddleston et al., 2002; Hines et al., 2001; Keller and Bridgham, 2007; Ye et al., 2012). Moreover, the production ratio of CO₂ : CH₄ can vary by several orders of magnitude among different types of peatlands suggesting distinct pathways and controls of anaerobic decomposition (Ye et al., 2012; Hines et al., 2008; Bridgham et al., 1998). To date it is not clear what ultimately limits CH₄ production and causes the large variations of CO₂ : CH₄ production in northern peatlands (Bridgham et al., 2013), but there is a growing consensus that these patterns cannot be explained by the respiration of inorganic TEAs.

Humic substances have been hypothesized to play multiple roles in anaerobic C cycling beyond their effect as organic substrates for decomposition. Humic substances are traditionally thought to be a unique, heterogeneous class of macromolecules, yet recent research suggests that they are collections of relatively small molecules derived from biological materials (Piccolo, 2002; Sutton and Sposito, 2005; Lehmann et al., 2008). Irrespective of the exact chemical nature of humic substances, aromatic substances have been shown to occur at high concentrations in peatlands (Collins and Kuehl, 2001; Tfaily et al., 2013). It is well recognized that humic substances can act as TEAs (Cervantes et al., 2000; Keller et al., 2009; Lovley et al., 1996). Galand et al. (2010) hypothesized that the unequal production of CO₂ and CH₄ in peat soils results from the reduction of humic substances as TEAs and that this process is more significant in bogs than in rich fens (Galand et al., 2010). Keller and Takagi (2013) verified in a bog soil that organic TEAs could explain a significant fraction of the CO₂ produced during anaerobic respiration and that CH₄ was not produced until the electron-accepting capacity of the organic TEAs was exhausted. Recent research has shown that humic substances are able to oxidize sulfur species, promoting sulfate reduction and contributing to high CO₂ : CH₄ production ratios (Heitmann and Blodau, 2006; Minderlein and Blodau, 2010). Humic substances can also promote iron reduction in wetland sediments by serving as electron shuttles (Rodén et al., 2010).

It is generally believed that quinone moieties contained in humic substances are important electron-accepting groups (Scott et al., 1998), and humic respiration has been frequently investigated with a functional analog, anthraquinone-2,6-disulfonate (AQDS) in many systems (Lovley et al., 1996; Keller et al., 2009; Cervantes et al., 2000). AQDS reduction (i.e., quinone respiration) to anthrahydroquinone-2,6-disulfonate (AHQDS) is thermodynamically more favorable than methanogenesis, which should lead to an increase in CO₂ : CH₄ production ratio in soils where AQDS-like humics are being utilized for microbial respiration (Cervantes et al., 2000). Keller et al. (2009) demonstrated that additions of AQDS to wetland soils resulted in decreased CH₄ production and increased ratios of CO₂ : CH₄, although this pattern was confounded by changes in pH. Amendment of AQDS to Arctic peat soils also stimulated iron reduction and resulted in higher production ratios of CO₂ : CH₄ (Lipson et al., 2010).

The large variety of aromatic compounds in peatlands (Tfaily et al., 2013) may also have direct inhibitory effects on various microbial groups due to their high concentra-
tions of polyphenolic and quinone moieties. For example, the addition of a “humic”-rich peat extract was found to be inhibitory to CO$_2$ production, sulfate reduction, and methanogenesis, but not to acetogenesis in a bog soil (Minderlein and Blodau, 2010). Polyphenols inhibit carbon mineralization by inhibiting microorganisms, binding proteins and polysaccharides, and inactivating enzymes (Harborne, 1997; Freeman et al., 2012). These compounds are degraded by phenol oxidase and peroxidase exoenzymes, but the activity of these enzymes is constrained by the low oxygen availability, low pH, and low temperature common to many peatlands (Freeman et al., 2012; Limpenes et al., 2008). These factors along with vegetation with high foliar phenolics concentrations (Bragazza et al., 2013) often cause very high soluble polyphenol concentrations in the porewaters of many peatlands. Moreover, Sphagnum mosses, a dominant component of the plant community in many peatlands, contain high concentrations of unique polyphenolic compounds that have long been known to have antibiotic properties (McClymont et al., 2011; Verhoeven and Toth, 1995; van Breemen, 1995). Quinone compounds are also well known to have strong antibiotic effects (Shyu et al., 2002; O’Brien, 1991), in addition to their roles as TEAs, although their toxicity role in natural soils is much less studied than polyphenolics. For example, Cervantes et al. (2000) suggested that AQDS may have a direct toxic effect on methanogens in some sediments.

Thus, it is apparent that humic substances can potentially influence anaerobic C mineralization in multiple ways, but untangling these multiple roles in peatland decomposition remains a challenge. We have recently observed different rates of CO$_2$ and CH$_4$ production in soils from six peatland types across a hydrogeomorphic landscape gradient even when incubated at common pHs (Ye et al., 2012). All of the peats contained minimal concentrations of inorganic TEAs, yet none of them exhibited methanogenic conditions during a 43-days incubation, with particularly high CO$_2$:CH$_4$ in bog peats. We hypothesized that humic or phenolic-like substances in these peats were particularly inhibitory to methanogens. In the present study, we used the humic analog AQDS to examine (1) whether humic substances are important in organic decomposition in peatland soils, (2) if the effect of humic substances is primarily as an organic electron acceptor or through direct inhibition, and (3) how humic substances influence the temperature responses of anaerobic decomposition, including methanogenesis.

## 2 Methods and materials

### 2.1 Site description

We collected soil samples from a bog and a rich fen in the Upper Peninsula of Michigan, USA in June 2011. These sites were previously described as “Bog 1” and “Rich Fen” in Ye et al. (2012). The bog (46°6′6″ N, 88°16′25″ W) had a pH of 3.7 and a peat depth of ~3.8 m with an average water table of ~27 cm during the growing season (water tables were measured in hollows). The bog is dominated by > 90% of cover of Sphagnum spp. mosses with stunted (< 1 m height) ericaceous shrubs such as leatherleaf (Chamaedaphne calyculata (L) Moench), small cranberry (Vaccinium oxycoccos L.), and bog Labrador tea (Rhododendron groenlandicum Oeder), and scattered low stature black spruce (Picea mariana (Mill.) Britton, Sterns & Poggen). The rich fen (46°13′27″ N, 89°29′53″ W) had a pH of 5.9, a peat depth of ~ 6.4 m with consistent standing water, and it is dominated by upright sedge (Carex stricta Lam.) tussocks with leatherleaf also present on the tussocks.

### 2.2 Sample preparation

On June 2011, 4 soil cores were randomly extracted from hollows in each site with PVC pipes (10 cm diameter) to a depth of 15 cm below the water table (~14 cm) at the bog site or 15 cm below the soil surface at the rich fen site (water table + 15 cm). Upon extraction, cores were intermittently capped after filling with porewater to prevent oxidation of the peat and transported on ice to our laboratory at the University of Oregon. The cores were stored at 4 °C and used within a week after collection.
Peat was processed in a glove box (Coy Laboratory Products Inc., Grass Lake, MI, USA) filled with 98 % of N₂ and ~ 2 % H₂ gas. Following the removal of green vegetation, large roots, and woody material, cores from the same site were combined and homogenized in a food processor with degassed deionized water at a peat : water ratio of 5 : 19 by mass. A subsample was collected and dried at 60 °C for 3 days to determine the moisture content. Subsamples of ~ 24 mL of the homogenized peat slurries were transferred to 125 mL serum bottles, which were capped with butyl septa and incubated at room temperature (22 ± 1 °C) in the dark for 15 days to reduce any electron acceptors that were initially present.

2.3 Laboratory experiment

After pre-incubation, each sample was amended with one of the following treatments: (1) control (as water), (2) 1.4 mM glucose, (3) 10.2 mM AQDS or (4) 1.4 mM glucose plus 10.2 mM AQDS. All treatments were added as 11 mL of degassed solutions in the anaerobic glove box and were mixed well with peat slurries by gently shaking, followed by bubbling the slurries with oxygen-free N₂ gas for 10 min. Parallel samples were incubated at 7 °C, 15 °C, and 25 °C in the dark. Four replicates of each treatment incubated at each temperature were destructively sampled (see below) on the 2nd, 5th, 10th, 15th, 30th, and 45th day of incubation.

2.4 CO₂, CH₄, and H₂ measurement

Samples were shaken gently to release trapped gas bubbles. Headspace gases were analyzed for CO₂ and CH₄ by gas chromatography using a flame ionization detector equipped with a methanizer (SRI Instruments, Torrance, CA, USA). An aliquot of the headspace gases was used to determine H₂ concentration with a Peak Performer gas chromatograph with a reducing compound photometer (Peak Laboratories, Mountain View, CA, USA). Total CO₂ and CH₄ production were calculated from both gas and liquid phases, adjusting for solubility, temperature, and pH (Stumm and Morgan, 1995).

2.5 Water chemistry

Following gas measurement, 10 mL of water was collected from each sample and centrifuged at 5000 rpm for 5 min in the glove box. Aliquots of the water sample were used for quantifications of reduced AQDS (AHQDS) and glucose, while the remaining sample was frozen at −20 °C for acetate analysis. Reduced AQDS was determined as described by Cervantes et al. (2000) with slight modifications. In brief, 1 mL of water from the incubation was mixed well with 2 mL of degassed 60 mM bicarbonate buffer, pH 6.7, in a 5 mL cuvette, followed by measurements of the absorbance at 450 nm with a spectrophotometer (Genesys S, Thermo Scientific, Waltham, MA, USA). Stock AHQDS standards were obtained by chemically reducing AQDS with dithionite, while working standards were prepared by serially diluting the stocks with degassed water. All procedures were performed anaerobically in the glove box. Glucose concentration was determined colorimetrically (Fournier, 2001), and acetate was analyzed with a Dionex DX500 ion chromatography system equipped with a HC-75 (H⁺) column (Hamilton Company USA, Reno, NV, USA) and a Dionex AD20 absorbance detector (Dionex Corporation, Bannockburn, Illinois, USA). pH was measured at each sampling point, and did not differ between treatments within a peat type (data not shown).

2.6 Temperature sensitivity of CO₂ and CH₄ production and AQDS reduction

Temperature sensitivity was described by the Q₁₀, calculated as:

\[
Q_{10} = \left( \frac{k_2}{k_1} \right)^{(T_2-T_1)/10}
\]

where \( k_1 \) and \( k_2 \) are rates of production at temperatures \( T_1 \) and \( T_2 \), respectively (Fissore et al., 2009; Inglett et al., 2012). The production rates were calculated from cumulative production of CO₂ and CH₄ as well as the cumulative reduction of AQDS (measured as the production of AHQDS) during the incubation. Both \( k_1 \) and \( k_2 \) were the average of four replicates within each temperature. As such, no standard errors for \( Q_{10} \)s are provided.
2.7 Statistical analyses

Results were analyzed with the MIXED procedure of SAS 9.1 (SAS Institute). Tukey’s test was conducted to determine significant differences at $\alpha = 0.05$. The data were tested for normality and log-transformed if the transform resulted in significant improvements in the overall distribution.

3 Results

3.1 AQDS reduction

Background AHQDS concentrations in treatments without AQDS amendment were consistently < 0.1 mM (Fig. 1), suggesting that measured increases in AHQDS accurately approximated AQDS reduction. AHQDS concentrations were only slightly greater in the bog peat in the AQDS treatment at all temperatures, and in the AQDS + glucose treatment at 7 °C (0.15 to 0.21 mM), and did not increase through time (Fig. 1a–c). AHQDS production increased in the glucose + AQDS treatment after a lag period of 10 days at 15 °C and of 5 days at 25 °C (Fig. 1b and c). In the rich fen peat, AHQDS concentrations in peats amended with AQDS, with or without glucose, increased from day 2 to the last day of the experiment and were highest at 25 °C (Fig. 1d–f). Regardless of temperature and time, AHQDS concentrations were generally higher in the rich fen peat when both glucose and AQDS were added than when AQDS was added alone.

3.2 Glucose concentration

Glucose concentrations were generally higher in the control than in the AQDS treatment in both the bog and fen peat, although the difference was not always significant (Fig. 2). In bog peat, adding only glucose increased its concentration in the early stages of the experiment relative to the control, but the difference diminished as the experiment continued and disappeared on day 30 at 7 °C, on day 10 at 15 °C, and on day 5 at 25 °C (Fig. 2a–c). However in the glucose + AQDS treatment, the difference persisted through the entire incubation at 7 °C and disappeared only at day 30 at 15 °C and 25 °C (Fig. 2a–c). In contrast, added glucose (with or without AQDS) was rapidly consumed in the rich fen peat such that its concentration was not different from the control by day 2 at 25 °C and by day 5 at 7 °C and 15 °C (Fig. 2d–f). Glucose concentrations in rich fen peat in the glucose + AQDS treatment were less than the control after day 5 regardless of the temperature, although the difference was not always significant (Fig. 2d–f).

3.3 Acetate concentration

In the bog peat, acetate concentrations in the control increased constantly from days 2 to 45 and were generally greater at higher temperatures (Fig. 3a–c). Adding glucose greatly promoted acetate accumulation, but adding glucose in combination with AQDS caused a lag period in acetate accumulation of 30 days at 7 °C, 10 days at 15 °C, and 5 days at 25 °C. On days 30 and 45, acetate concentrations were similar in the glucose and glucose + AQDS treatments at 15 °C and 25 °C. In both the bog and fen peats, acetate was not detected at all temperatures during the entire course of the experiment when AQDS was added alone (Fig. 3). In the rich fen peat, acetate concentrations were initially lower in the glucose + AQDS treatment than in the glucose treatment, but the difference was not significant by day 45 at 7 °C, day 30 at 15 °C, and day 10 at 25 °C.

3.4 H₂ partial pressure

In the bog peat, higher partial pressures of H₂ were generally observed at higher temperatures in the control (Fig. 4a–c). Addition of glucose increased H₂ partial pressures at all temperatures, with a maximum on day 30 at 7 °C, on day 15 at 15 °C, and on day 10 at 25 °C. In contrast, addition of glucose with AQDS did not cause significant in-
creases in H$_2$ at any temperature, except at 15°C after a 10-days lag period. H$_2$ partial pressures in both the bog and rich fen peats amended with AQDS alone were consistently < 2 Pa regardless of the temperature (Fig. 4). In the rich fen peat, addition of glucose increased H$_2$ production at all temperatures, with a maximal value on day 10 at 7°C and on day 5 at 15°C and 25°C (Fig. 4d–f). The H$_2$ partial pressures decreased at all temperatures after their peak and were all < 2 Pa at end of the experiment. H$_2$ partial pressures in the glucose + AQDS treatment were consistently very low.

### 3.5 CO$_2$ production rates

CO$_2$ production in both the bog and rich fen controls increased with temperature (Fig. 5). In the bog peat, addition of glucose caused a substantial increase in CO$_2$ production at all temperatures. In contrast, addition of AQDS generally caused lower CO$_2$ production relative to the control after day 2 at all temperatures, though the difference was not always significant. The glucose + AQDS treatment occasionally caused a small increase in CO$_2$ production at 15°C and 25°C. In rich fen peat, addition of glucose increased CO$_2$ production at all temperatures (Fig. 5d–f). Addition of glucose and AQDS caused even a larger increase in CO$_2$ production initially, though this stimulatory effect decreased through time. Addition of AQDS alone had no effect on CO$_2$ production, except for a small stimulatory effect on day 2 at 25°C.

### 3.6 CH$_4$ production rates

CH$_4$ production increased with temperature in both the bog and rich fen peats (Fig. 6). In the bog peat, addition of glucose did not result in significantly higher CH$_4$ production, except on day 45 at 7°C, day 5 at 15°C, and days 5 and 30 at 25°C (Fig. 6a–c). Amendment with AQDS, with or without glucose, decreased the rates of CH$_4$ production after day 5 at 7°C and after day 2 at both 15°C and 25°C. In contrast to the bog peat, addition of glucose in the rich fen peat increased CH$_4$ production rates at all temperatures during the entire course of the experiment (Fig. 6d–f). Addition of AQDS decreased CH$_4$ production after day 2 at all temperatures. However, there was no difference between the glucose + AQDS treatment and the control.

### 3.7 $Q_{10}$ for CO$_2$, CH$_4$, and AQDS reduction

The $Q_{10}$ for cumulative CO$_2$ production in bog peat was similar in the control and AQDS + glucose treatments (2.1 to 2.2), slightly lower in the AQDS treatment (1.9), and lowest in the glucose treatment (1.3, Table 1). However, these cumulative $Q_{10}$ values mask complicated temporal dynamics in temperature sensitivity for CO$_2$ production (Fig. 7a), with the $Q_{10}$ peaking at day 5 in the glucose treatment and decreasing to close to one by day 30. In contrast, the glucose + AQDS treatment had an increasing $Q_{10}$ through the first 15 days of the incubation and it then decreased somewhat on day 45. The $Q_{10}$ in the rich fen peat was similar in the control and AQDS treatment (from 2.0 to 2.1), slightly lower in the glucose treatment (1.9), and lowest in the glucose + AQDS treatments (1.6, Table 1).

In the bog peat, addition of glucose increased the $Q_{10}$ of cumulative CH$_4$ production relative to the control (from 2.3 to 2.6), but addition of AQDS with or without glucose eliminated any temperature response ($Q_{10}$ 1.0 to 1.1, Table 1). In the fen peat, addition of AQDS and glucose both caused a small increase in the $Q_{10}$ relative to the control (from 2.1 to 2.3), and adding them together increased the $Q_{10}$ further to 2.4. Similar to CO$_2$ production, cumulative CH$_4$ production masked complicated temporal changes in the temperature sensitivity of methanogenesis (Fig. 7b and e). The $Q_{10}$ increased through time in the bog peat in a parallel manner in the control and glucose treatments, but there was a flat response except for one anomalous value on day 10 when AQDS was added with or without glucose (Fig. 7b). In this anomalous case, a small increase was observed at higher temperature in a flux that was close to the limit of detection. In the rich fen peat, the $Q_{10}$ of CH$_4$ production steadily decreased in the control and glucose treatments in a parallel manner (Fig. 7e). During the first week, addition of AQDS decreased the $Q_{10}$, but this effect was ameliorated by the addition of glucose with AQDS by day 10, and by day 30 in the AQDS only treatment.
When AQDS was added alone to bog peat, there was almost no temperature response in cumulative AQDS reduction because of low reduction at all temperatures \( (Q_{10} = 1.1, \text{ Table 1}) \). However, cumulative AQDS reduction showed a moderate temperature response in the AQDS + glucose treatment \( (Q_{10} = 1.9) \). The lag period in this treatment before substantial AQDS reduction (Fig. 1) was evident also in the temporal dynamics of the \( Q_{10} \) for AQDS reduction, with the \( Q_{10} \) increasing steady after day 10 to a \( Q_{10} \) of 3.5 by day 45 (Fig. 7c). In contrast in the fen peat, the temperature response of AQDS reduction was higher in the AQDS only treatment \( (Q_{10} = 1.9) \) than in the AQDS + glucose treatment \( (Q_{10} = 1.3) \). These two treatments also had very different temporal responses with the \( Q_{10} \) increasing in the AQDS only treatment and decreasing in the AQDS + glucose treatment to day 10 and remaining relatively steady after that (Fig. 7f).

### 4 Discussion

Northern peatlands contain low concentrations of inorganic TEAs, and their reduction is generally not the major pathway for carbon mineralization (Ye et al., 2012; Keller and Bridgham, 2007; Vile et al., 2003b). Humic substances can be utilized as organic TEAs by humic-reducing microbes in many systems (Cervantes et al., 2000; Keller et al., 2009; Lovley et al., 1996), and it has recently been shown that the reduction of both liquid and solid phase humic substances can account for a significant fraction of \( \text{CO}_2 \) production in a bog soil (Keller and Takagi, 2013). In the present study, all peat samples were collected below the water table and processed anaerobically and we assume that after the 15 day pre-incubation period, reduction of endogenous inorganic and organic TEAs was likely minimal. Thus, in our experimental protocol, added AQDS was likely the most abundant TEA which allowed us to explore the role of this humic analog as both a TEA and a potentially inhibitory compound.

#### 4.1 Anaerobic decomposition

There was no evidence of substantial AQDS reduction in the bog peat when it was added alone (Fig. 1a–c). Instead AQDS had a severe inhibitory effect on fermentation and respiration reactions, with acetate concentration below detection (Fig. 3a–c), very low \( \text{H}_2 \) partial pressures (Fig. 4a–c), and suppressed rates of \( \text{CO}_2 \) production (Fig. 5a–c). As expected, we observed increased fermentative production of acetate, \( \text{H}_2 \), and \( \text{CO}_2 \) when glucose was added alone to bog peat. However, the stimulatory effect of added glucose on fermentation had a lag period that was temperature-dependent when added in combination with AQDS. Major decreases in glucose were seen on day 45 at 7°C and day 10 at 15°C and 25°C (Fig. 2a–c). At the two warmer temperatures, this was coincident with increases in acetate concentration and \( \text{H}_2 \) partial pressures, and generally increased \( \text{CO}_2 \) production. This enhanced anaerobic activity was accompanied by the reduction of AQDS (Fig. 1b and c), indicating that it was acting as a TEA. Thus, it was only with a substantial input of labile carbon and a lag period that the microbial community in the bog peat was able to use AQDS as a TEA in respiration, and the dominant effect of AQDS was inhibitory.

In contrast, AQDS stimulated both fermentation and respiratory activity in the fen peat through its role as a TEA, as demonstrated in both the AQDS and AQDS + glucose treatments by sustained production of AHQDS (Fig. 1d–f), rapid initial production of acetate (Fig. 3d–f), and an increase in \( \text{CO}_2 \) production (Fig. 5d–f) at all temperatures. Glucose was rapidly consumed in the fen peat with or without AQDS addition (Fig. 2d–f), indicating high fermentation potential compared to the bog peat.

Our results are intriguing when compared to those of Keller and Takagi (2013), which conclusively demonstrated that fully oxidized humic substances from the same bog as used in the present study acted as TEAs and accounted for a significant fraction of anaerobic \( \text{CO}_2 \) production until the humic substances were fully reduced. In their study, the solid phase peat was responsible for the vast majority of the reductive capacity. However, AQDS applied in our study is a surrogate for dissolved-phase quinone sub-
stances that may be taken up across cell membranes to provide a toxic effect (O’Brien, 1991; Shyu et al., 2002). The amount of our AQDS addition was similar to other studies that have examined its electron-accepting capabilities (e.g. Lovley et al., 1996; Keller et al., 2009; Cervantes et al., 2000) and was biologically reasonable considering the observed reductive capacity of the fen peats (up to 100% reduction of the added AQDS). Since AQDS addition caused no change in pH, its inhibitory effect in the bog peat in the present study was not due to an increase in acidity. Furthermore, addition of a “humic”-rich peat extract was found to be inhibitory to CO₂ production, sulfate reduction, and methanogenesis, but not to acetogenesis in a bog soil (Minderlein and Blodau, 2010). Thus, it appears that both AQDS and humic substances can act as TEAs or be inhibitory for anaerobic microbial metabolism under different circumstances. We hypothesize that solid phase humics act primarily as TEAs and solution-phase humics can act as inhibitory substances in certain environments (e.g., bogs).

4.2 Methanogenesis

As expected, CH₄ production was stimulated by glucose amendment regardless of the peat type and temperature (Fig. 6). It is apparent that the fermentation of glucose provided extra substrates, acetate (Fig. 3) and H₂ (Fig. 4), to methanogens resulting in the increase in CH₄ production. In contrast, AQDS amendment significantly decreased CH₄ production in both peats (Fig. 6) but because of different mechanisms. In the fen peat, AQDS acted as a TEA, and addition of glucose fully compensated for the reduction in CH₄ production by AQDS (Fig. 6d–f), providing strong evidence that the effect of AQDS on CH₄ production was primarily through substrate competition. In contrast, in the bog peat CH₄ production was not recovered in the glucose + AQDS treatment (Fig. 6a–c), despite high concentrations of acetate as a result of glucose fermentation after the lag period discussed above (Fig. 3a–c). We have previously shown that acetoclastic methanogenesis dominates in this bog soil (Ye et al., 2012), suggesting that AQDS suppressed CH₄ production in this bog soil through a direct inhibitory effect even in the presence of high concentrations of the dominant methanogenic substrate.

Our results are in agreement with other studies that have found that AQDS can have an inhibitory effect on methanogenesis (Keller et al., 2009; Cervantes et al., 2000). CH₄ production in the controls was up to 47-times lower in the bog peat compared to the rich fen peat (Fig. 6). Lower CH₄ production in bog soils compared to soils of other peatland types has been widely observed, indicating that methanogenesis in bog peats may be intrinsically inhibited (Bridgham et al., 2013). The low soil pH of these systems has often been implicated as a reason for this inhibition (Brauer et al., 2004; Valentine et al., 1994; Dunfield et al., 1993). However, in a pH manipulation experiment with soils from six peatlands across the ombrotrophic-minerotrophic gradient, we found that while pH was an important control over CH₄ production, this rate remained low in bog peat even after prolonged incubation at circumneutral pH and with substantial acetate accumulation (Ye et al., 2012).

In a review on this subject, Bridgham et al. (2013) hypothesized that bogs contain high concentrations of aromatic compounds that are particularly inhibitory to methanogens. Bog porewater has very high concentrations of aromatic compounds compared to fen porewater (Tfaily et al., 2013). Sphagnum mosses have high concentrations of phenolic substances that are inhibitory to microbial activity (Williams et al., 1998; McClymont et al., 2011), and Hines et al. (2008) found that CH₄ production in peat soils was highly negatively correlated with the proportional cover of Sphagnum mosses. Our results are in agreement with Hines et al. (2008) in that plant cover was dominated by Sphagnum spp. in the bog and by vascular plants in the rich fen. Our experimental results with the quinone analog AQDS support the hypothesis that the inherently low CH₄ production in bogs is attributable to the toxic effect of dissolved aromatic substances. However, we only examined the quinone component of these substances with the AQDS analog, and polyphenolics almost certainly play an additional important inhibitory role (Bragazza et al., 2013; Freeman et al., 2012). Our study provides an important additional perspective to the “enzyme-latch” hypothesis that peatlands accumulate carbon because of the low activity of phenol oxidase and the resultant accumulation of phenolic compounds, which would include the phenolic moieties of humic substances that may be taken up across cell membranes to provide a toxic effect (O’Brien, 1991; Shyu et al., 2002).
substances (Freeman et al., 2001, 2012; Limpens et al., 2008; Bragazza et al., 2013). This important body of research has to date not addressed the effects of aromatic substances on methanogenesis to our knowledge. While we are unaware of any studies that have examined phenol oxidase activity across the ombrotrophic–minerotrophic gradient, it is clear from our study that quinone-like humic substances have a broad inhibitory effect on anaerobic carbon mineralization in an ombrotrophic bog, with a particularly strong inhibitory effect on methanogenesis, whereas these substances largely act as organic TEAs in a minerotrophic fen. Additional research is needed to verify our findings in other peatlands, to identify the source of the inhibitory substances in bogs (e.g., are they derived primarily from Sphagnum spp. mosses?), and to relate these findings to the enzyme-latch hypothesis.

### 4.3 Temperature sensitivity of CH₄ production

Reported apparent Q₁₀'s for CH₄ production vary greatly in wetland soils, ranging from 1.3 to 28 (Segers, 1998). Better defining the temperature response of overall anaerobic C cycling and CH₄ production was recently identified as a major impediment to modeling CH₄ emissions from wetlands in response to climate change (Bridgham et al., 2013). Van Hulzen et al. (1999) suggested while the processes controlling CH₄ production (i.e., TEA reduction, fermentation reactions, and methanogenesis) all had intrinsic Q₁₀'s of ~ 2, typical for microbial processes, that their complex temporal dynamics could give very high apparent Q₁₀'s for CH₄ production.

Our anaerobic handling of samples and 15 day pre-incubation should have reduced any TEAs in the samples, and thus we isolated the temperature response of AQDS, as an aromatic substance analog, with and without a ready source of electrons caused by the addition of glucose in the two peats, and its effect on the Q₁₀ of methanogenesis. With the exception of the case when AQDS was added to bog peat and caused almost complete inhibition of CH₄ production and thus no temperature response, we observed a relatively narrow range of Q₁₀'s for cumulative CH₄ production ranging from an average of 2.3 to 2.6 in both peats (Table 1). Addition of glucose increased the Q₁₀ for CH₄ production in both peats, most likely through the stimulatory effect of higher temperatures on acetate and H₂ production (Figs. 3 and 4). The addition of AQDS to the fen peat, where it acted as a TEA, increased the Q₁₀ of cumulative CH₄ produced, although the effect was modest. Van Hulzen et al. (1999) suggested the presence of TEAs would increase the Q₁₀ for CH₄ production (van Hulzen et al., 1999). Thus the cumulative CH₄ results suggest a relatively straight forward temperature response of CH₄ based upon the factors identified previously by van Hulzen et al. (1999). However, the complex temporal dynamics of temperature effects on CH₄ production (Fig. 7b and e) suggests a more complicated set of factors controlling the apparent temperature sensitivity of that process. For example, the temporal dynamics of Q₁₀ in the control and glucose treatments in both peats generally mirrored each other but changed greatly through time, especially in the bog peat, for reasons that are not clear. Similarly quizzical, the negative effect of AQDS on the Q₁₀ in the fen peat was ameliorated by day 10 in the glucose + AQDS treatment and by day 30 in the AQDS treatment (Fig. 7e), although the majority of the 11.25 mM of added AQDS was not reduced by the end to the experiment (Fig. 1).

These complicated temporal dynamics in the temperature sensitivity of CH₄ production likely reflect temporal changes in the temperature responses of other microbial groups with effects on methanogens. For example, the initial decrease in the Q₁₀'s for AQDS reduction (Fig. 7f) and CO₂ production (Fig. 7d) in the AQDS + glucose treatment in the fen peat was likely due to rapid consumption of the added glucose in that treatment (Fig. 2d–f). In contrast, the increase in the Q₁₀ in AQDS reduction (Fig. 7f) and CO₂ production (Fig. 7d) in the AQDS treatment in the fen peat from day 2 to 5 was likely because of a small stimulatory effect of AQDS on mineralization of native peat on day 2 (Fig. 5d–f). The Q₁₀ in AQDS reduction and CO₂ production in the AQDS + glucose treatment in the bog peat (Fig. 7a and c) increased substantially as it began to be used as a TEA (Fig. 1a–c), but it likely continued to strongly direct inhibit methanogenesis also. The addition of glucose increased the Q₁₀ of CO₂ production by day 2 in the fen peat (Fig. 7d), and by day 5 in the bog peat (Fig. 7a), but in both cases the Q₁₀...
thereafter tended to be lower than the control, with the temperature effect almost disappearing from this treatment in the last half of the incubation in the bog peat (Fig. 7a). Part of the reason for this in the bog peat is the difference in the time it took to mineralize all of the added glucose at the various temperatures (Fig. 2), but the added glucose apparently had a priming effect that enhanced mineralization of the native peat in both the bog and fen, because the glucose treatment caused increased CO₂ production in these treatments long after it had been depleted (cf. Figs. 2 and 5).

These treatment effects are intriguing because kinetic theory suggests that more labile C compounds should have lower temperature sensitivity than more recalcitrant compounds (Davidson and Janssens, 2006). An addition of glucose may cause a large increase in the apparent $Q_{10}$, even if it has a low intrinsic $Q_{10}$, if it has a much higher absolute rate of mineralization. Additionally, Davidson and Janssens (2006) describe the difficulty in predicting temperature responses under conditions of substrate limitation or rapidly changing substrate conditions because of differential, and potentially offsetting, effects of temperature on the maximum rate of a reaction ($V_{max}$) and its half-saturation constant ($K_{m}$) if reactions are following Michaelis–Menten kinetics.

There is an increasing acceptance in the terrestrial ecosystem literature that while the apparent temperature response of most microbially mediated reactions is $\sim 2$ that the apparent temperature response of soil respiration can be quite variable because of the effects of temperature on interacting processes (Schmidt et al., 2011; Conant et al., 2011). Our results strongly suggest that this is also the case in peatlands where we demonstrated complicated temperature sensitivity of anaerobic C cycling and CH₄ production under carefully controlled conditions of substrate and TEA availability. Our results also extend the work of van Hulzen et al. (1999) by demonstrating inhibitory quinone moiety effects on the temperature sensitivity of many anaerobic processes in bog soils.

5 Conclusions

We demonstrated that the quinone analog, AQDS, has broad-scale inhibitory effects on anaerobic C cycling in a bog soil, with methanogenesis being particularly sensitive, and it was only when glucose was added and a lag period that any reduction of AQDS was observed. In contrast, AQDS acted as an organic TEA in a rich fen soil. There is ample supportive evidence in the literature to suggest that quinone substances and aromatic compounds can both act as inhibitory substances and TEAs in peatlands, but the circumstances that determine when one or the other effect is dominant are unclear at this point. We suggest that the enzyme-latch hypothesis that has structured much current thinking about why peatlands accumulated C needs to be expanded to incorporate the effects of aromatic substances on anaerobic C cycling.

The addition of glucose and AQDS caused complicated temporal dynamics in terms of the apparent temperature sensitivity of various anaerobic C cycling processes. Under natural conditions the availability of TEAs and available substrates will vary dramatically in space and time in peatlands, and we suggest that these interactions will make modeling the temperature response of CH₄ production in peatlands particularly challenging. Our research adds to the growing body of literature in terrestrial soils that climate effects on soil C cycling will be mediated through complicated, interactive ecosystem responses.

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References


### Table 1. $Q_{10}$ values from 7–25°C for $\text{CH}_4$, $\text{CO}_2$, and AQDS reduction in peats from a bog and a rich fen with different treatments. Values were calculated as described in the method section, with the production rates being averaged of the cumulative production of $\text{CO}_2$ and $\text{CH}_4$ as well as the cumulative reduction of AQDS across time.

<table>
<thead>
<tr>
<th></th>
<th>$\text{CH}_4$</th>
<th>$\text{CO}_2$</th>
<th>AQDS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bog</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.30</td>
<td>2.13</td>
<td>N.A.</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.56</td>
<td>1.30</td>
<td>N.A.</td>
</tr>
<tr>
<td>AQDS</td>
<td>1.12</td>
<td>1.89</td>
<td>1.06</td>
</tr>
<tr>
<td>Glucose + AQDS</td>
<td>0.97</td>
<td>2.20</td>
<td>1.94</td>
</tr>
<tr>
<td><strong>Rich Fen</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
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<td>2.03</td>
<td>N.A.</td>
</tr>
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<td>2.26</td>
<td>1.87</td>
<td>N.A.</td>
</tr>
<tr>
<td>AQDS</td>
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<td>Glucose + AQDS</td>
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**Fig. 1.** AHQDS concentrations of peat slurries from a bog and a rich fen with different treatments. (a–c), bog peats incubated at 7°C, 15°C, and 25°C, respectively; (d–f), rich fen peats incubated at 7°C, 15°C, and 25°C, respectively. Bars indicate mean ±1 standard error. Note differences in scales between the bog and rich fen soils.

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**Fig. 2.** Glucose concentrations of peat slurries from a bog and a rich fen with different treatments. (a–c), bog peats incubated at 7°C, 15°C, and 25°C, respectively; (d–f), rich fen peats incubated at 7°C, 15°C, and 25°C, respectively. Bars indicate mean ±1 standard error.
Fig. 3. Acetate concentrations of peat slurries from a bog and a rich fen with different treatments. (a–c), bog peats incubated at 7°C, 15°C, and 25°C, respectively; (d–f), rich fen peats incubated at 7°C, 15°C, and 25°C, respectively. Bars indicate mean ±1 standard error.

Fig. 4. H₂ partial pressures in response to different amendments in peat slurries from a bog and a rich fen. (a–c), bog peats incubated at 7°C, 15°C, and 25°C, respectively; (d–f), rich fen peats incubated at 7°C, 15°C, and 25°C, respectively. Bars indicate mean ±1 standard error. Note differences in scales between the bog and rich fen soils.
**Fig. 5.** CO₂ production rates in response to different amendments in peat slurries from a bog and a rich fen. (a–c), bog peats incubated at 7 °C, 15 °C, and 25 °C, respectively; (d–f), rich fen peats incubated at 7 °C, 15 °C, and 25 °C, respectively. Bars indicate mean ±1 standard error.

**Fig. 6.** CH₄ production rates in response to different amendments in peat slurries from a bog and a rich fen. (a–c), bog peats incubated at 7 °C, 15 °C, and 25 °C, respectively; (d–f), rich fen peats incubated at 7 °C, 15 °C, and 25 °C, respectively. Bars indicate mean ±1 standard error. Note differences in scales between the bog and rich fen soils.
Fig. 7. Q_{10} for CO_{2} and CH_{4} production and AQDS reduction in peat slurries from a bog and a rich fen. (a–c), CO_{2}, CH_{4}, and AHQDS in bog peats, respectively; (d–f), CO_{2}, CH_{4}, and AHQDS in fen peats, respectively.