Effects of Nutrient Addition and Two Invasive Plants on Wetland Methane Production

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Abstract
Wetlands provide a number of important ecosystem services, but their anoxic conditions also favor the production of several greenhouse gases. Wetlands are an ideal environment for methanogenesis, the process by which carbon dioxide is reduced to methane by wetland microbes (methanogens). As wetlands are the largest natural contributor to the atmospheric methane pool, it is important to understand variables that control wetland methane production. Nitrogen availability is one variable that likely affects methanogen communities. Nitrate drains from agricultural areas, where wetlands act as a nitrogen sink, preventing nitrate from contaminating aquatic systems. Another factor that may affect wetland methanogenesis is vegetation type. In recent years, invasives such as exotic cattail (Typha angustifolia and Typha x glauca) and Phragmites have spread through wetlands, negatively impacting ecosystem processes. To determine the influence of nitrate availability and vegetation type on wetland methanogenesis, we incubated sediment from Phragmites and exotic cattail dominated stands, added increasing concentrations of nitrate, and measured methane production. We hypothesized that if nitrate allows methanogens to be outcompeted by another group of wetland microbes, denitrifiers, then we would observe decreased methane production with high nitrate concentrations.

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EFFECTS OF NUTRIENT ADDITION AND TWO INVASIVE PLANTS ON WETLAND METHANE PRODUCTION

By

Jaynes Dempsey

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ABSTRACT

Wetlands provide a number of important ecosystem services, but their anoxic conditions also favor the production of several greenhouse gases. Wetlands are an ideal environment for methanogenesis, the process by which carbon dioxide is reduced to methane by wetland microbes (methanogens). As wetlands are the largest natural contributor to the atmospheric methane pool, it is important to understand variables that control wetland methane production. Nitrogen availability is one variable that likely affects methanogen communities. Nitrate drains from agricultural areas, where wetlands act as a nitrogen sink, preventing nitrate from contaminating aquatic systems. Another factor that may affect wetland methanogenesis is vegetation type. In recent years, invasives such as exotic cattail (Typha angustifolia and Typha x glauca) and Phragmites have spread through wetlands, negatively impacting ecosystem processes. To determine the influence of nitrate availability and vegetation type on wetland methanogenesis, we incubated sediment from Phragmites and exotic cattail dominated stands, added increasing concentrations of nitrate, and measured methane production. We hypothesized that if nitrate allows methanogens to be outcompeted by another group of wetland microbes, denitrifiers, then we would observe decreased methane production with high nitrate concentrations. Increased nitrate lowered methane production (p=0.03) and heightened denitrification potential (p<0.005). Exotic cattail increased methane production (p<0.01). Our findings suggest that nitrate bolsters denitrifiers, causing methanogens to be outcompeted. Our findings also suggest that exotic cattail facilitates methane production. Nitrate availability and vegetation type likely influence sediment methane production. It is important to integrate these conclusions into our understanding of invasive vegetation, water contaminants, and methane production, to consider the best possible methods of mitigation and prevention.
INTRODUCTION

Wetlands provide myriad ecosystem services. They supply habitat for organisms and offer recreational value (Silvius 2000, Millennium Ecosystem Assessment 2005). As a primary contributor to flood mitigation, millions of people depend upon wetlands (Silvius 2000, Millennium Ecosystem Assessment 2005). Additionally, their unique biogeochemical processes situate wetlands at the center of several significant environmental issues. Wetlands are nitrogen sinks, decreasing agricultural run-off from contaminating downstream waterways (Huang and Pant 2009). However, wetlands also contribute to global warming (Segers 1998). Wetlands emit methane, a potent greenhouse gas, which is produced by sediment microbes (methanogens) (Segers 1998, Huang and Pant 2009). Methanogen communities are affected by soil properties such as nitrate availability (Kim et al. 2015), and by direct interactions with vegetation (Wolfe and Klironomos 2005). Different plant species offer root exudates, leachates, and leaf litter that shapes soil microbial communities (Wolfe and Klironomos 2005, Weidenhamer and Callaway 2010). The invasion of exotic cattail to wetlands in the Great Lakes region, followed by the more recent invasion of Phragmites genotypes, has resulted in a need to understand the effects these plants have on wetland ecosystems.

Wetlands and Methane

Wetlands negatively impact the environment by releasing methane and other greenhouse gases into the atmosphere. Wetlands are the single largest natural methane source, contributing approximately 78% of methane produced in natural systems (Figure 1; Bosquet et al. 2006). Wetlands also account for 32-47% of total methane emissions (Denman et al. 2007).
Natural Sources of Methane Emissions

Figure 1 Wetlands are the largest natural contributors to the global methane pool, followed by termites and oceans (based on figure from Bosquet et al. 2006).

This is due to the anaerobic conditions in water-logged wetland sediments, which are ideal for methane-producing microbial processes called methanogenesis (Huang and Pant 2009). As methane is a potent greenhouse gas (28 times stronger than CO₂) (Segers 1998), the release of methane from ecosystems can substantially increase global warming and alter natural systems. Wetland methanogenesis is part of the natural decomposition process. During methanogenesis, the breakdown of organic matter under anaerobic conditions results in accumulation of carbon dioxide, before it is reduced by microbes to methane (Figure 2). These carbon dioxide reducing microbes are members of the domain Archaea, known as methanogens (Segers 1998, Denman et al. 2007). While the atmospheric methane pool has been rising for decades, in recent years it has experienced significant unexplained growth (Kirschke et al. 2013). Possible explanations include increased anthropogenic combustion of fossil fuels, and increased wetland methanogenesis (Kirschke et al. 2013; Figure 3).
Methane oxidation:

\[ \text{CH}_4 + 2 \text{O}_2 \rightarrow \text{CO}_2 + 2 \text{H}_2\text{O} \]

Methanogenesis:

\[ \text{CO}_2 + 4 \text{H}_2 \rightarrow 2 \text{H}_2\text{O} + \text{CH}_4 \]

\[ \text{CH}_3\text{COOH} \rightarrow \text{CO}_2 + \text{CH}_4 \]

**Figure 2** Methanogens take up \( \text{CO}_2 \), and convert it to \( \text{CH}_4 \). This methane may then be released into the atmosphere directly from the sediment; alternatively, it may escape through the leaves of wetland plants (Diagram modified from Brevik 2012).
Figure 3 Atmospheric methane levels since 1980 (Modified from Kirschke et al. 2013). Methane concentrations leveled off from 2000-2005, then began increasing in 2006. Dotted lines correspond to methane mole fractions, and solid lines correspond to growth rate.
Nutrient Enrichment and Methane

Nutrient enrichment (eutrophication) negatively impacts ecosystems (Smith et al. 1999). Excess nitrogen and phosphorus in aquatic systems cause increased algae growth, decreased biodiversity, and reduced ecosystem services (Smith et al. 1999). Nitrate, as a mobile form of nitrogen, often drains from agricultural areas into waterways (Randall and Mulla 2001, Mitsch et al. 2005). However, wetlands are often geographically situated to intercept this nitrate runoff before it can contaminate aquatic systems (Mitsch et al. 2005). Plant uptake and denitrification then remove this excess nitrogen from the wetland ecosystem (Mitsch et al. 2005). Additionally, increased nitrate availability has been implicated in the spread of exotic Typha spp. (a US invasive) (Morton 1975), and Phalaris arundinacea, another aggressive wetland species (Craft et al. 2006). Eutrophication also results in fish kills and contaminates water sources (Carpenter et al. 1998). These have become especially significant problem in recent years, due to increasing nitrogen and phosphorus inputs from agriculture and industry (Carpenter et al. 1998).

Nitrate availability also affects microbial communities. However, the influence of nitrate on methanogens is unclear. While some studies have found that nitrate increases methane production (Liu and Greaver 2009), others have suggested that nitrate suppresses methanogen growth (Kim et al. 2015). Because both methanogens and denitrifiers (another group of wetland microbes) require organic carbon (Vymazal 2013), competition may occur between the two groups. Nitrate addition potentially favors denitrifier growth, bolstering the ability of denitrifiers to compete with methanogens. This competition may result in the inhibition of methanogen activity (Figure 4 and 5), causing reduced methane production (Kim et al. 2015).

Denitrifiers are a group of microbes that are also active in anaerobic sediments, where they carry out an ecologically valuable process: denitrification (Knowles 1982). Denitrifying
bacteria reduce nitrate (NO₃⁻) to nitrogen gas (N₂), removing nitrate contaminants from the ecosystem (Knowles 1982). Therefore, denitrification is a vital process, mitigating the threat that nitrate poses to the environment. Like methanogenesis, denitrification must take place under anaerobic conditions (Chen and Lin 1993). However, because denitrification is carried out at a higher redox potential than methanogenesis, the addition of nitrate, which elevates redox potential (Chen and Lin 1993), results in conditions more favorable to denitrifiers. Thus, increased nitrate availability likely bolsters denitrifier growth. Over the last century, use of nitrogen in fertilizers has led to increased nitrate levels in lakes, rivers, and oceans (Galloway and Cowling 2002). These denitrifier-methanogen interactions may lead to an environmental trade-off concerning nitrogen: nitrate potentially suppresses methane production, but is also linked with many environmental problems (Galloway and Cowling 2002, McIsaac et al. 2001, Mitsch and Reeder 1991).
**Figure 4** Interactions between denitrifiers and methanogens in low nitrate conditions.

Methanogens successfully obtain sediment carbon, and produce higher methane quantities.

Arrows indicate size of interaction.

**Figure 5** Interactions between denitrifiers and methanogens in high nitrate conditions. If nitrate increases denitrifier populations, denitrifiers would potentially compete with methanogens for sediment carbon, reducing methanogen growth, and thus reducing methane production. Arrows indicate size of interaction.
**Vegetation Type and Methane**

Vegetation type is another factor that influences many microbial processes, including methanogen activity (Bubier 1995, Wolfe and Klironomos 2005, Audet *et al.* 2013). Specific wetland plant species (e.g., *Scheuchzeria palustris*) have been linked to high methane output (Bubier 1995), potentially due to ways in which these wetland plants alter soil conditions. Plant species affect soil hydrology, soil structure, and soil biogeochemistry (Weidenhamer and Callaway 2010). This, in turn, shapes soil microbial communities (Wolfe and Klironomos 2005). For instance, various plant species differentially influence soil nutrient availability by taking up nutrients and by producing leaf litter (Weidenhamer and Callaway 2010). In addition, some plants release root exudates into the sediment (Caillo-Quiroz *et al.* 2009, Zhai *et al.* 2013). Exudates can be conducive to microbial growth, especially when rich in nutrients (Caillo-Quiroz *et al.* 2009). These exudates differ in quantity and quality depending on plant species and environmental conditions (Wolfe and Klironomos 2005, Zhai *et al.* 2013). By offering exudates, leachates, and leaf litter of differing quantity and quality, certain species have the potential to facilitate the growth of certain microbes (Wolfe and Klironomos 2005): belowground microbial communities differ depending on aboveground vegetation (Callaway *et al.* 2004). Furthermore, increased microbial growth is linked with the production of substrates by plants (e.g., acetate) that can be used by methanogens (Jespersen *et al.* 1998). Plants also release oxygen from roots, further modifying the surrounding environment in a way that can potentially favor the growth of one microbial species over another (Jespersen *et al.* 1998).

*Typha* is a genus of wetland plants that has been negatively impacting Great Lakes ecosystems (Tuchman *et al.* 2009). While *Typha latifolia* (broad leaf cattail) is native to North America, *Typha angustifolia* is a non-native species (Grace and Harrison 1986). *Typha latifolia*
and *Typha angustifolia* hybridize, resulting in *Typha x glauca*, a second invasive *Typha* genotype (Grace and Harrison 1986; Figure 6). *Typha angustifolia* and *Typha x glauca* (hereafter referred to as "exotic cattail") have colonized much of the United States, including the Great Lakes area (Morton 1975, Grace and Harrison 1986). Exotic cattail decreases wetland biodiversity (Tuchman et al. 2009), in part due to the large amounts of leaf litter that it produces (Mitchell et al. 2011), which reduces seed growth of native plants (Vaccaro et al. 2009). Exotic cattail has also been observed to decrease wetland function; for example, *Typha x glauca* may reduce the capacity of wetlands to remove nutrients from waterways (Angeloni et al. 2006).

*Phragmites australis* is a wetland plant that is native to N. America, but a non-native genotype has recently spread extensively thought wetlands in the United States, often replacing exotic cattail in freshwater wetlands (Chambers et al. 1999; Figure 7). The Eurasian variety of *Phragmites australis* (hereafter referred to as *Phragmites*) that has recently invaded the Great Lakes region (Saltonstall 2001, Tulbure et al. 2007) has proven to be especially aggressive (Saltonstall 2002). The spread of *Phragmites* is thought to have been facilitated by many factors, including wetland habitat alteration and wetland eutrophication (Chambers et al. 1999, Tulbure et al. 2007). Concerns center around the loss of biodiversity resulting from wetland invasion by *Phragmites*, as well as the potential for *Phragmites* to modify trophic structures or disturbance regimes (Chambers et al. 1999).
Figure 6 Typha x glauca has invaded Great Lakes wetlands, with detrimental effects.

Image Credit: Wisconsin State Herbarium—University of Wisconsin, Madison.
Figure 7 *Phragmites australis* is a Great Lakes invasive that has been causing much concern in recent years. Image credit: http://swbiodiversity.org.
Experimental Approach

To understand factors affecting methane release from wetlands (Kirschke et al. 2013), in addition to the effects of increased nitrate draining from agricultural systems (Galloway and Cowling 2002), we focused on two broad questions. First, what are the effects of elevated nitrate concentrations on methanogen activity? Second, what are the effects of vegetation type (i.e., exotic cattail and Phragmites) on methanogen activity? We hypothesized that if nitrate bolstered the growth of competitors of methanogens, higher nitrate concentrations would result in reduced methane production. We also hypothesized that if exotic cattail facilitated methanogen growth, sediment from exotic cattail dominated stands would exhibit higher methane production. We sampled wetland sediment from stands dominated by exotic cattail or Phragmites, and incubated the sediment in jars. We added various levels of nitrate, before measuring methane production. To further examine whether competition occurs between methanogens and denitrifiers, we also measured sediment denitrification potential. We predicted that if nitrate bolsters denitrifier growth, then the sediment with greater nitrate availability would exhibit increased denitrification potential.
MATERIALS AND METHODS

Study Site

Sampling was conducted at Paint Creek Wetland, Ypsilanti, Michigan (42°12’N, 83°37W) in both fall of 2015 and fall of 2016. Paint Creek’s waters come from the Paint Creek watershed. Paint Creek is a mitigated wetland, with vegetation of two main types: *Phragmites australis*, exotic cattail (*Typha angustifolia* and *Typha x glauca*). Paint Creek has swathes of land dominated by each species, with little overlap.

Sediment sampling for the first experiment was conducted in November 2015; sediment sampling for the second experiment was conducted in November 2016. A soil corer was used to obtain sediment from exotic cattail dominated stands (referred to as exotic cattail sediment) and *Phragmites* dominated stands (referred to as *Phragmites* sediment) from the topmost 15 cm of sediment. In 2015, the sediment was frozen until early December, when it was thawed, homogenized, and placed in jars. In 2016, homogenization and jar set-up occurred the day after sediment sampling.

Experimental Set-Up

To examine the influence of nitrate on methane production from wetland sediments, nitrate was added to jar incubations of exotic cattail and *Phragmites* sediment, and sediment methane production was measured over the course of one week. Three nutrient concentrations and two sediment types were used, for a total of six treatments, with five replicates per treatment.

In the 2015 experiment, 15 g of sediment was placed in each jar (either *Phragmites* sediment or exotic cattail sediment), and 13 mL of nutrient solution was added. Three nutrient
concentrations were used: distilled water, 2.5 mM of KNO₃, and 5.0 mM KNO₃. To create anoxic conditions, the jars were bubbled with nitrogen gas for two minutes. The lids were secured, then the jar headspace was flushed out for four minutes with nitrogen gas, by way of rubber septa embedded in the lids. The jars were incubated in darkness at 25°C for five days.

The same experimental set-up was used in 2016, except with 30 g of sediment, 40 mL of nutrient solution (same concentrations), and bubbling and flushing with N2 gas for three and five minutes, respectively. We also modified the technique to ensure sediments were at atmospheric pressure following flushing by inserting a water-filled syringe in the jar septum and allowing bubbling to occur. The jars were incubated in darkness at 18°C for six days.

Methane Measurements:

To measure methane production, we used a Gas Chromatograph (Shimadzu 2014, equipped with a flame ionizing detector). A syringe and needle were used to sample the jar headspace through the septa in their lids; immediately after sampling, the gas sample was injected into a Gas Chromatograph to measure the methane concentration.

Sediment Carbon Content

To measure organic carbon content in sediment samples, _Phragmites_ and exotic cattail sediment was dried in a drying oven at 65°C. The dry weights were recorded. To remove all organic carbon content, the samples were placed in a combustion oven. The final (mineral) weights were recorded, and the difference between the dry weights and mineral weights was used to calculate exotic cattail and _Phragmites_ sediment carbon content.
Denitrification Measurements:

To further assess the effects of nitrate on microbial processes, we measured sediment
denitrification potential using the acetylene-inhibition method (as described by Groffman et al.
2006). This experiment was conducted only in 2016, using the sediment from the methane
experiment.

Sediment was removed from the jars, and approximately 7 g of each replicate was added
into an Erlenmeyer flask. Flasks were capped with rubber septa. A needle was used to inject 6
mL of acetylene through the septa to prevent the conversion of N₂O to N₂. The flasks were
shaken on a shaker-table for 30 minutes, before an additional 3 mL of acetylene was injected.
The flasks were shaken for an additional 60 minutes, then the flask headspace was sampled with
a syringe. A set of vials was flushed with nitrogen to ensure anoxic conditions, and the samples
were injected into the vials.

Approximately one month after gas sampling, the samples were run on a gas
chromatograph (equipped with an electron capture detector) to measure N₂O accumulation. The
standards for the GC machine were collected on the same day as the samples, and stored in
evacuated vials.

Data Analysis

Methane standards of known concentration were used to produce a standard curve and
calculate jar methane concentration. To better compare data between years, methane
measurements were converted to micrograms of carbon per day per dry gram of sediment using
the following equation:
A two-way ANOVA was run on both the methane and denitrification data, and a two-tailed t-test on the exotic cattail *Phragmites* denitrification control. A two-tailed t-test was run on the sediment carbon content data, and on the methane production data for 2015 and 2016.
RESULTS

Methane Emissions

To test the hypothesis that nitrate additions inhibit methanogen activity, we measured methane production of *Phragmites* and exotic cattail sediments supplied with varying nitrate additions. In fall 2015, methane production in sediment with nitrate addition was many times lower than methane production sediment with no nitrate addition (p<0.01; Figure 8). The same experiment examined whether exotic cattail facilitated methane production. In fall 2015, methane production in exotic cattail sediments was over twice that of *Phragmites* sediments (p<0.01; Figure 8).

![Figure 8: Effects of nitrate concentration on methane production in sediments from exotic cattail and Phragmites sediment with standard error. Fall, 2015. N=5.](image)
Figure 9: Effects of nitrate concentration on methane production in sediments from exotic cattail and *Phragmites* sediment with standard error. Winter 2016. N=5.

In fall 2016 samples, methane concentration in control jars after six days was notably higher than methane concentrations in the low and high nutrient treatments, especially in the exotic cattail sediment (*p*=0.15; Figure 9). Samples from exotic cattail sediment produced approximately double the methane than those from *Phragmites* sediment after six days (*p*<0.01; Figure 9). The data suggest that nitrate does inhibit methanogen activity, and that exotic cattail bolsters methanogen growth.

Difference between 2015 and 2016 methane production was significant, with 2015 methane production nearly an order of magnitude higher than that of 2016 methane (*t*=2.51; *p*=0.01). This suggests that there are other important factors that affect methane production in wetlands.
Sediment Carbon Content

To examine sediment carbon content, we combusted exotic cattail and *Phragmites* sediment in a muffle furnace, and calculated the percent carbon present in the sediment. In 2015, exotic cattail sediment had significantly higher carbon content as compared to *Phragmites* (t=3.72; p<0.005; Table 1). This trend was even more notable in 2016, when exotic cattail sediment had nearly 50% higher carbon content as compared to *Phragmites* sediment (t=8.74; p<0.005; Table 1). The data suggest that exotic cattail alters soil properties by increasing sediment carbon content.

Table 1: Relationship between vegetation (Exotic cattail and *Phragmites*) and percent carbon content ± standard error. N=15 and N=5 for 2015 and 2016, respectively.

<table>
<thead>
<tr>
<th>% Carbon</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exotic Cattail</td>
<td>31.7±0.01</td>
<td>34.9±0.01</td>
</tr>
<tr>
<td><em>Phragmites</em></td>
<td>26.7±0.008</td>
<td>21.5±0.007</td>
</tr>
</tbody>
</table>

Dentritfication Potential

To further examine whether nitrate additions increase competition between methanogens and denitrifiers, denitrification potential of 2016 sediments was measured. Nitrate addition nearly doubled denitrification potential (p<0.005; Figure 10). In the control, exotic cattail sediments had significantly lower denitrification potential as compared to *Phragmites* (t=7.35; p<0.01; Figure 10), though the effect of vegetation type on denitrification was not significant
overall (p=0.202; Figure 10). This suggests that nitrate addition does bolster denitrifier communities, increasing denitrifier-methanogen competition. This also suggests that *Phragmites* may facilitate denitrification.

![Figure 10: Effects of nitrate concentration and vegetation on denitrification potential with standard error. Winter 2016. N=5.](image)
DISCUSSION

To examine the relationship between nitrate, vegetation type, and wetland methane production, we incubated sediment in jars with different levels of nitrate availability and measured methane production. We hypothesized that if nitrate bolstered the growth of methanogen competitors, higher nitrate concentrations would result in reduced methane production. We also hypothesized that if exotic cattail facilitated methanogen growth, exotic cattail sediment would have higher methane production. Our results support these hypotheses. Nitrate availability was negatively correlated with decreased methane production and positively correlated with denitrification potential. These findings suggest that denitrifiers compete with methanogens, inhibiting methane production. Sediments from exotic cattail stands produced more methane than *Phragmites* sediments.

**Effects of Nitrate Addition on Sediment Methane Production**

Our findings support the competition hypothesis, which suggests that the increased denitrification and inhibited methanogenesis is caused by denitrifiers (Kim *et al*. 2015), which are bolstered by higher nitrate availability (Huang and Pant 2009). Given the competition over carbon substrate between the microbes discussed, this denitrifier growth appears to be at the expense of the methanogen population (Fang and Zhou 1999). Previous studies have found that denitrifiers outcompete methanogens in certain decomposition processes (Fang and Zhou 1999). A second possibility is that nitrate increases denitrification, and that toxic intermediates, such as NO₂, are produced during this process, which inhibit methanogen activity (Banihani *et al*. 2009). Even small increases in nitrate have been observed to inhibit methanogenesis (Yuan and Lu 2009). These mechanisms are not mutually exclusive; methanogens and denitrifiers may be impacted by both. To determine the extent to which each of these factors affects methane
production, further studies could be conducted in which methane production is measured after
the sediment has been exposed to denitrification intermediates. If methane production decreases,
this would offer evidence that methanogens are inhibited by these gases. The extent to which
they are inhibited could then be investigated with a more careful quantitative analysis in which
methanogens are exposed to differing concentrations of denitrification intermediates.

Our results are generally consistent with those from other studies. Several studies found a
similar relationship between nitrate and methane production (Kim et al. 2015, Yuan and Lu
2009, Banihani et al. 2009). One study had results notably different from ours, indicating
increased methane production with increased nitrate (Liu and Greaver 2009). However, this
study did not specifically isolate production from sediment, but instead conducted a meta-
analysis that considered overall production from wetlands (Liu and Greaver 2009). Our results
account only for production directly from sediments, and do not include other potential
contributors to the atmospheric methane pool, such as methane transported through plant
aerenchyma systems (Ding et al. 2005). In addition, methane production from wetlands may be
context-dependent, depending on factors such as water level and climate. Future research could
consider various factors that influence the effects of nitrate addition on methane production by
conducting similar jar incubations with varying water levels or temperature.

Our findings indicate significant year-to-year variation in methane production. This may
have been caused, in part, by the higher incubation temperature in 2015 as compared to 2016.
Ideally, incubation would have been conducted at the same temperature both years. However, it
is unlikely that this can account completely for the observed variation. Another possible cause
could be water level differences at the sampling site. Lower water levels allow for greater
sediment oxygen availability, which would raise the redox potential and favor denitrifiers. This,
in turn, may suppress methanogen communities and alter methanogen community composition. Future studies should investigate annual and seasonal differences in methane production, and consider such additional variables that might be correlated with these differences. Another study on the relationship between nitrate and denitrification revealed a relationship parallel to our results, with increased nitrate correlated with increased denitrification (Hanson et al. 1994). There is also evidence that the relationship between nitrate, denitrification, and methanogenesis is not as straightforward as it appears, with other nutrients, such as carbon and phosphorus, altering the influence of nitrate (Kim et al. 2015). For example, phosphorus was observed to decrease methane production when added alongside nitrate; phosphorus was also observed to increase denitrification potential when added in conjunction with carbon and nitrate (Kim et al. 2015). This is another avenue for further study: methane production and denitrification potential could be measured in samples containing various combinations of nitrate, phosphorus, and carbon.

Nitrate is a waterway contaminant; over the last century, the use of nitrogen in fertilizers has led to increased nitrate levels in lakes, rivers, and oceans with detrimental effects (Galloway and Cowling 2002). Increases in nitrate availability have also been implicated in eutrophication (McIsaac et al. 2001), adverse health effects on both humans and animals, and water acidification (Camargo and Alonso 2006). Yet nitrate appears to provide some benefit: it decreases methane production. Therefore, the impacts of nitrate addition into waterways are not simple, but multifaceted. To what extent does this suppression of methane production counterbalance the negative effects caused by nitrate pollution? Further studies could attempt to quantify the extent to which nitrate influences methanogenesis by conducting jar incubations with more incremental differences in nitrate concentration.
Effects of Vegetation on Sediment Methane Production

Our results support the hypothesis that exotic cattail facilitate methanogen growth. In both years, exotic cattail sediments had higher methane production than Phragmites sediments. The results also suggest that *Phragmites australis* facilitates denitrifier growth, as denitrification in the *Phragmites* sediment control was significantly higher than denitrification in exotic cattail sediment control.

One possible mechanism for increased methane production in exotic cattail sediment is that exotic cattail raises the carbon content of sediment, bolstering methanogen communities. Plants produce substrates that have been correlated with increased microbial growth (Jespersen *et al.* 1998), and carbon is required by methanogens (Vymazal 2013). If *Typha* spp. offer these carbon substrates, this is a potential source of methanogen growth. Furthermore, a hypothesis that focuses on carbon content is supported by our results, which found increased carbon content in exotic cattail as compared to *Phragmites* sediment (Table 1). Future studies could examine potential correlations between sediment carbon content and methane production, and at the effects of exotic cattail leaf litter on methane production by conducting jar incubations with leaf litter additions.

Another possible mechanism for increased methane production in exotic cattail sediments is that *Phragmites* may aerate sediments at an increased rate. Sediment aeration, in which oxygen is provided to sediment through plant roots, raises redox potentials (Jespersen *et al.* 1998). This, in turn, favors denitrifiers, as denitrification takes place under a higher redox potential than methanogenesis (Chen and Lin 1993). If exotic cattail aerates sediment at a greater rate than *Phragmites australis*, this could be the mechanism behind our observed facilitation of methanogenesis. This would also explain why *Phragmites* appeared to facilitate denitrification.
(Figure 10). However, there appears to be significant variation in terms of the effects that *Phragmites* spp. and exotic cattail spp. have on methane production, with one study observing no clear link between either *Phragmites* or exotic cattail stands and methane production (Kaki *et al.* 2001). Another study observed little difference between *Typha angustifolia* and *Phragmites australis* microbial biomass (Findlay *et al.* 2002). A third study, however, found that *Phragmites australis* reduced methane production (Grunfeld and Brix 1999). A useful next step would be to compare the microbial communities around the roots of these plants directly, by sampling for DNA to identify the microbes present. If vegetation type affects methanogen community size and community composition, then we expect significant differences in methanogen populations around exotic cattail roots as compared to roots of *Phragmites australis*.

Invasive plants, such as *Phragmites australis* and exotic cattail, have been observed to colonize wetlands, alter habitat structure, and reduce biodiversity (Chambers *et al.* 1999, Zedler and Kercher 2004). The ability of *Phragmites australis* to dominate ecosystems is of particular concern, which has led to attempts to remove these invaders from wetlands (Ailstock *et al.* 2001). However, it appears that *Phragmites australis* may offer an ecosystem service: the reduction of methane production from wetlands. Therefore, it is important to consider all factors before attempting to exterminate *Phragmites australis* and restore native wetland vegetation. Further studies could also examine the difference between methane production in native versus restored wetlands.

**Conclusion**

Given the increasing threat of global climate change as the result of rising levels of greenhouse gasses in the atmosphere, in part due to methane production (Seger 1998), and the current high levels of nitrate runoff (Naslas 1994), it is necessary to consider mitigation and
prevention of methane production and nitrate runoff. As wetlands play a significant role in the methane cycle and in nitrate cycling (Seger 1998, Denman 2007), it is essential that we consider the type and extent of their involvement. We must unravel the complex web of interacting factors in order to recommend avenues for change. Our results indicate that nitrate decreases methane production; this is likely due, at least in part, to the beneficial effects that nitrate has on denitrifier growth, bolstering the ability of these microbes to compete with methanogens. Our results also indicate that exotic cattail bolsters methane production, potentially due to effects of exotic cattail on sediment carbon content. Future studies should continue to look at the links between these variables, and especially assess the relationship between methanogenesis and nitrate, to better comprehend the intersection between these potentially harmful factors.


