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Short communication

# Increased methane emissions from an invasive wetland plant under elevated carbon dioxide levels

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ABSTRACT

Wetlands function as important climate regulators by providing conditions for the large-scale production and release of methane from vegetation. Several studies have suggested an apparent link between two global warming gases that result in higher emissions of methane from rice paddies and wetlands subjected to elevated levels of atmospheric $CO_2$. We show that an increase in the relative abundance of methane-producing archaea isolated from the rhizosphere of a highly invasive plant was associated with enhanced plant biomass from $CO_2$-stimulated roots. Methane emissions from a cattail invasive to North America *Typha angustifolia* (narrow leaf cattail) increased 148% under an enriched atmosphere of $CO_2$ (700 ppm). Root biomass also increased under elevated $CO_2$ for *T. angustifolia* and was correlated with methane flux, suggesting that future $CO_2$ stimulation may lead to higher methane emissions from *Typha*-dominated wetlands.



## 1. Introduction

Many wetlands in North America are increasingly dominated by only a few noxious plant species. Among these highly invasive wetland plants is *Typha angustifolia*. Once formerly restricted to the eastern coast of North America, *T. angustifolia* has expanded much of its range west and southward since the mid- to late-20th century (Shih and Finkelstein, 2008). By the mid-20th century, the expansion of *T. angustifolia* progressed from the eastern coast to include Quebec, Ontario, and 19 states from Maine to North Dakota and Missouri to Pennsylvania (Hotchkiss and Dozier, 1949). The range of *T. angustifolia* continues to expand across North America encompassing 41 U.S. states and 8 Canadian provinces (USDA and NRCS, 2011). The geographic expansion of the species coincides with the distribution of *Typha latifolia*, to produce their F1 hybrid *T* × *glauca* (Kuehn et al., 1999). All three forms of *Typha* dominate large expanses of wetlands throughout eastern North America.

The displacement of diverse wetland plant communities with near monocultures of *Typha* vegetation has the potential to alter regional carbon (C) cycling. Future predictions of atmospheric $CO_2$ could further impact the biogeochemistry of wetlands, as plant traits are closely linked with microbial activity in anoxic sediments. Results from elevated $CO_2$ studies in wetlands and rice paddies indicate that methane emissions rise in response to higher levels of atmospheric $CO_2$ (Dacey et al., 1994; Hutchin et al., 1995; Megonigal and Schlesinger, 1997; Ziska et al., 1998; Inubushi et al., 2003). The apparent link between the two greenhouse gases may be due to increased exudation of plant-derived C in the presence of elevated $CO_2$, or it may be a result of other plant traits that influence net methane production. Plants stimulated by $CO_2$ can increase primary production, resulting in greater exudation of organic C (Dacey et al., 1994; Hutchin et al., 1995; Inubushi et al., 2003; Freeman et al., 2004). The increase in root-derived C, in turn, directly stimulates the activity and growth of acetoclastic methanogens that produce methane (Lu et al., 2000; Marschner, 1995). Microbial community analysis of rice rhizospheres indicates high enrichment of a diverse pool of methanogenic archaeal populations (Wantanabe et al., 2010). Rhizophere acetate levels can differ greatly among plant species and is considered an important precursor to methane formation (Strom et al., 2003).

Ultimately, net methane release depends on movement through the sediment–plant–atmosphere continuum, with several control points determining net methane flux. Plants in anoxic soil environments (such as wetlands) significantly alter net methane emissions by increasing methane diffusion or flow from flooded sediments via physical traits (Van der Nat and Middelburg, 2000). Many of the plant physical traits that are affected by elevated $CO_2$ also impact methane flux. Therefore, changes in the parameters that allow for greater diffusion and flow of methane from soil, through the plant, and into the atmosphere, should increase net

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methane emissions. Increased biomass under elevated $CO_2$ may raise emissions of methane by increasing the rate or volume of methane ventilated through the plant, while greater root biomass could allow more diffusion of dissolved methane into the roots and aerenchyma where methane ventilation proceeds (Inubushi et al., 2003).

Specific plant responses to elevated $CO_2$ vary across species (Poorter, 1993; Poorter and Navas, 2003) and cultivars (Lou et al., 2008), which complicate efforts to predict changes in methane emissions from wetlands exposed to an enriched $CO_2$ atmosphere. The ecological consequences of replacing large tracts of wetlands with monocultures of an invasive plant could alter regional methane emissions, particularly if higher levels of methane are released under an enriched $CO_2$ atmosphere. In this study, we hypothesized that higher atmospheric $CO_2$ concentrations would increase methane flux from *T. angustifolia*, with an associated increase in plant biomass and methanogen activity.

## 2. Materials and methods

### 2.1. Experimental design

Rhizome clippings of narrow leaf cattail, *Typha angustifolia* L., were grown for four months under enriched $CO_2$ in a controlled greenhouse facility located on the University of Wisconsin–Madison campus. The $CO_2$ treatments consisted of plants grown in six separate rooms, with three replicate rooms receiving 700 $\mu l\,l^{-1}$ (elevated) $CO_2$ and the other three held at 380 $\mu l\,l^{-1}$ (ambient) $CO_2$. The rhizomes of *Typha* were obtained from a wetland restoration nursery (Southern Tier Consulting, Inc.) based in West Clarkville, NY. The plants were grown in circular pots measuring 38.1 cm height and 15.2 cm diameter, containing leak-proof plastic lining bags. The plants received a dilute (10%) concentration of Hoagland's solution with daily watering through a drip system. The pots were flooded continuously throughout the experiment at a water level of 2.5 cm above the soil surface. The room temperatures were set at 24 °C for 14 h to 18 °C for 10 h. The sediment used in this study was a mixture of 1:1 parts of wetland sediment and topsoil. The homogenized sediment was placed into the pots above a base of 5 cm washed sand. The pots were filled with sediment up to 2.5 cm below the pot surface.

### 2.2. Gas sampling and plant biomass

Methane flux was collected from plants during mid-day after three months by placing PVC chambers over the plant leaves and stems. The chambers were held in place on the pots using stakes. The high water level of the pots served as a seal for the base of the chamber, which minimized methane gas release into the chambers. The gases in the chambers were allowed to equilibrate with the atmosphere for 45 min before the chamber tops were closed and sealed with teflon tape. A 12-V fan circulated gases within the chamber, and a 20-gauge needle inserted into a gray butyl septa maintained atmospheric pressure within the chamber. Gases were collected with 30 mL polyurethane syringes at 0, 15, and 30 min, and immediately transferred to glass Wheaton serum bottles with rubber butyl caps. The samples were analyzed with a Shimadzu 14B gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with a flame ionization detector.

Rhizosphere soil was collected by scraping soil adhering to roots with spatulas. The soil was homogenized and frozen at −20 °C prior to analysis. Aboveground and belowground plant materials were separated to determine biomass. The plants were washed, dried at 60 °C until constant weight, and weighed to calculate plant dry weight.

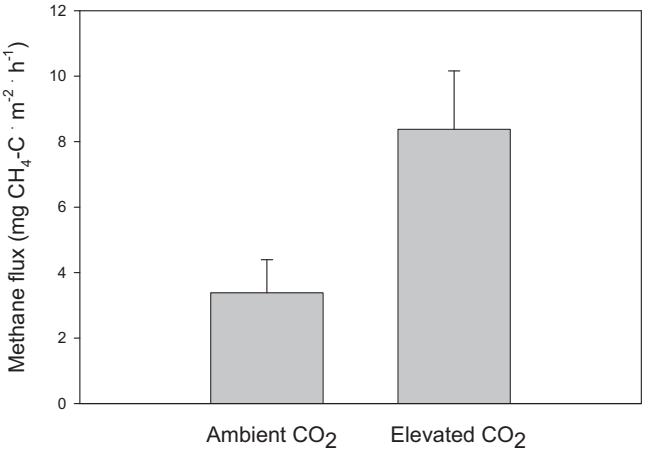


**Fig. 1.** Methane flux measurements from the shoots of *T. angustifolia* (means ± 1 SE). Bars represent data from ambient and elevated $CO_2$ rooms. Means are significantly different between ambient and elevated $CO_2$ at $p < 0.05$ (Fisher's LSD).

### 2.3. Microbial analysis

Terminal restriction fragment length polymorphism (T-RFLP) was used to identify major microbial populations based on PCR amplification of target sequences (Liu et al., 1997; Tiedje et al., 1999). The relative abundance (or gene frequency) of different microbial populations is calculated as the signal intensity of a single terminal restriction fragment (T-RF) in proportion to all T-RFs in a sample. Total community DNA was extracted from 1 g of soil for each $CO_2$ treatment using the UltraSoil DNA extraction kit (MoBio Laboratories, Solana Beach, CA). The following primers were used to amplify methanogen 16S rRNA: Ar109f (5′-ACG/TGCTCAGTAACACGT-3′) and Ar912r (5′-CTCCCCCGCCAATTCCTTTA-3′) (Lueders and Friedrich, 2000). Details of the PCR protocol are described in Kao-Kniffin et al. (2010). Samples were electrophoresed on the Applied Biosystems 3730xl automated DNA sequencing instrument, using 50 cm capillary arrays and POP-7 polymer. Data were analyzed using PE-Biosystems version 3.7 of Sequencing Analysis.

Primer Ar109f was labeled with a 6-carboxy-flourescein (FAM) at the 5′ end. The fluorescent-labeled PCR products (16.8 μl) were digested with 5 U *Taq*I (Promega, Madison, WI), 1× buffer, and 1 μg BSA for 2 h at 65 °C. A 1 μl aliquot of the digest was added to a 10 μl standard mixture containing formamide and a 625 bp ROX-labeled internal size standard (CHIMERx, Madison, WI). The diluted samples were analyzed through denaturing capillary electrophoresis on an ABI 3700 genetic analyzer (Applied Biosystems). The T-RFLP patterns were analyzed by peak area integration of the different T-RFs using GeneMarker v1.50 (SoftGenetics LLC, State College, PA).

### 2.4. Statistical analysis

Data were analyzed using JMP version 5.0 (SAS Institute Inc., Cary, NC). The $CO_2$ level (365 and 700 $\mu l\,l^{-1}$) was the fixed factor, while greenhouse rooms were the random factor. We determined the effects of $CO_2$ on methane flux, plant biomass, and microbial relative abundance using ANOVA with Fisher's LSD post hoc analysis. Methanogen relative abundance represented the signal intensity of each T-RF in relation to the total signal intensity of all T-RFs.

## 3. Results

Methane flux from *T. angustifolia* increased 148% under an enriched atmosphere of $CO_2$ at 700 ppm ($F = 3.99$, $p < 0.05$) (Fig. 1). We used the average $CO_2$ level predicted by 2100, with all IPCC

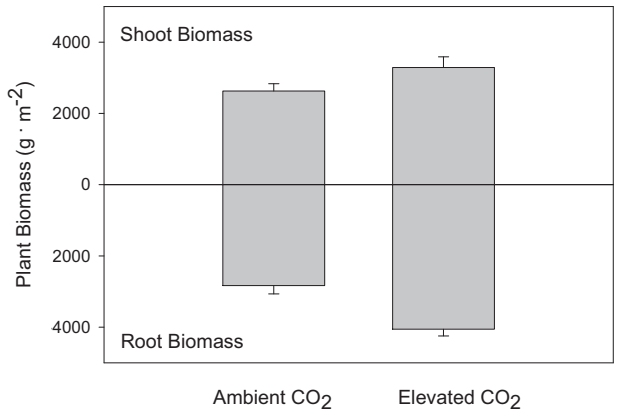


**Fig. 2.** Shoot and root biomass of *T. angustifolia* (means ± 1 SE) grown under ambient and elevated $CO_2$ levels. Bars represent significantly different means between ambient and elevated $CO_2$ for shoot, root, and total biomass at $p < 0.05$ (Fisher's LSD).

scenarios ranging from 535 to 983 ppm by the end of this century (Solomon et al., 2007). Our measurements for methane flux (1–11 $m^2\ h^{-1}$) were comparable to published reports on *Typha*: 0–1259 $m^2\ d^{-1}$ (Johansson et al., 2004), 1–18 $m^2\ h^{-1}$ (Kaki et al., 2001), and 22–47 mg $m^2\ h^{-1}$ (Whiting and Chanton, 1996).

The root biomass of *T. angustifolia* increased significantly under elevated $CO_2$ ($F = 7.31$, $p < 0.01$) (Fig. 2), and was correlated with methane flux ($r = 0.5$, $p = 0.06$). As a measure of methanogen activity, we quantified the relative abundance of individual phylogenetic-based fragments in relation to the total fragment intensity using terminal restriction fragment length polymorphism (T-RFLP) (Fig. 3). The relative abundance of a restriction fragment representative of acetate-consuming methanogenic archaea (184 T-RF) was correlated with increases in the $CO_2$-stimulated root biomass of the invasive plant ($r = 0.4$, $p = 0.09$). The frequency values ranged between 0.08 and 0.2 for the T-RF 184.

## 4. Discussion

We expected that elevated $CO_2$ would increase methane flux from wetland plants by stimulating plant productivity (Ziska et al., 1998) and acetoclastic methanogen relative abundance (Yue et al., 2007). For *T. angustifolia*, two of the factors leading to higher methane emissions from $CO_2$ enrichment appeared to be root biomass gain and growth stimulation of archaea associated with the Methanosarcinaceae family. Increasing plant productivity results in changes to plant physical traits, such as increased leaf size, number of leaves, plant height, stem diameter, root length, and root diameter (Pritchard et al., 1999). Changes in one or several of these plant physical parameters can effectively alter methane flow rates, and consequently, net methane emissions (Sharkey et al., 1991). Plant species comparisons confirm positive associations between plant productivity and methane flux (Whiting and Chanton, 1993). Thus, elevated $CO_2$ could result in increased methane emissions by altering plant physical traits that impact methane flow, and by changing root exudation patterns that influence methane production. Feedback processes associated with elevated $CO_2$ could result in greater drought conditions in wetlands, which could reduce regional methane emissions (Freeman et al., 2002).

The same changes in plant productivity and physiology associated with elevated $CO_2$ growing conditions can alter the structure and function of methanotroph (methane-consuming) populations. A rice paddy receiving enriched $CO_2$ levels showed decreased abundance of methanogen populations that were linked to rice phenology (Yue et al., 2007). In this study, the increase in root and shoot biomass of *T. angustifolia* may have enhanced oxygen levels in actively growing sites of the rhizosphere, which could have resulted in localized increases in methanotroph populations and methane consumption. Although we did not measure methanotrophy or methanotroph abundance in this study, it is important to state that both methanogen and methanotroph populations are critical in influencing net methane flux from plants.

Similar to increased plant productivity, $CO_2$ stimulation of methanogen population activity or abundance could also explain higher methane flux under *T. angustifolia*. Methane producers utilize a variety of C sources and metabolic pathways (Ferry, 1993). Acetate splitting (acetoclastic) and $CO_2$-reduction are the two dominant pathways of methanogenesis. Methanogen populations associated with acetoclastic methanogenesis (Methanosarcinaceae and Methanosaetaceae) utilize specific forms of organic C, and are often limited by the availability of these substrates (Schlesinger, 1997). Therefore, the increased supply of organic C associated with $CO_2$-stimulated plants is thought to be the mechanism that induces higher net methane flux from wetland and rice plants (Hutchin et al., 1995; Inubushi et al., 2003; Freeman et al., 2004). On the other hand, methanogen populations that utilize the $CO_2$-reduction pathway (Methanomicrobiaceae and Methanobacteriaceae) are not likely to be stimulated by an increase in organic C substrates.

The terminal fragment, 184 bp, was positively correlated with methane flux under *Typha* soil. In contrast, we found no relationship between measured methane flux and the relative abundance of another terminal fragment, 283 bp, representative of acetoclastic methanogens from the Methanosaetaceae family. For that reason, only the activity of soil methanogens belonging to the Methanosarcinaceae family appeared to be influenced by plant species. This may be because the archaea of the Methanosarcinaceae family can utilize a wide variety of C sources, while those belonging to the Methanosaetaceae family utilize acetate as a sole C source (Sharkey et al., 1991) In a mixed community, the range of C source use could be a competitive advantage.

In this study, methanogenic and methanotrophic activities (potential to produce or consume methane) were not directly measured. Therefore, the activities of the methane producers or consumers may have been altered under elevated $CO_2$, despite the lack of increase in the relative abundance of acetoclastic methanogens. Elevated $CO_2$ may alter the activity and structure of the methanogen and methanotroph communities by changing the availability of C sources (Mitchell et al., 2003) or modifying the concentration of $O_2$ in the root zone (Schrope et al., 1999; Ludemann et al., 2000) thus impacting methane production and consumption, respectively.

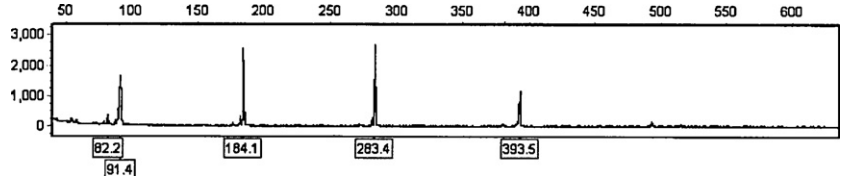


**Fig. 3.** Electropherogram of SSU rRNA fragments labeled with 6-FAM. The resulting T-RFs are 82 bp (Methanomicrobiaceae), 91 bp (Methanobacteriaceae), 184 bp (Methanosarcinaceae), 283 bp (Methanosaetaceae), and 393 bp (Rice Cluster I).

Finally, T-RFLP is a semi-quantitative PCR-based method that may not represent actual population dynamics of the soil microbial community. PCR-based methods to characterize soil microbial community composition and relative abundance are constrained by low soil sample volume (~0.5–1 g soil), PCR amplification bias, and PCR inhibitors (usually humic substances) (Suzuki and Giovannoni, 1996). Given the greater limitations in using phospholipid fatty acid analysis, microarray chips, and plate enumeration techniques to characterize soil microbial community structure, we found that T-RFLP provided a useful measure in elucidating potential relationships regarding the role of plant and microbial composition in methane dynamics.

Not all methanogenic archaeal species respond similarly to an enriched $CO_2$ atmosphere, as is the case with plant species. In this study, the invasive cattail (*T. angustifolia*), increased aboveground, belowground, and total biomass with higher $CO_2$ (Fig. 2), and this correlated with increased methane flux (Fig. 1). Other wetland species may not respond similarly as *T. angustifolia* to $CO_2$ enrichment. Additionally, methane flux levels can differ greatly among plant species (Strom et al., 2005). In a long-term $CO_2$ enrichment study, methane flux increased from specific plant species within the salt marsh instead of the whole plant community (Marsh et al., 2005). Given the large spatial distribution of *T. angustifolia* across many wetland habitats in North America and its ability to dominate the plant community, an enriched $CO_2$ atmosphere could stimulate regional levels of methane flux in cattail-dominated marshes. Further investigations of enhanced methane emissions by other dominant wetland plant species could reveal the extent to which $CO_2$ stimulation alters methane dynamics across a diversity of wetland plant species.

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