Aquatic Botany 93 (2010) 107–113

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Methane dynamics across wetland plant species

Jenny Kao-Kniffin[a,*], Dominique S. Freyre[b], Teri C. Balser[a]

[a] Department of Soil Science, University of Wisconsin, 1525 Observatory Drive, 263 Soils Bldg, Madison, WI 53706-1299, United States
[b] Department of Biology, University of Nebraska, Omaha, NE 68182, United States

ARTICLE INFO

Article history:
Received 22 July 2009
Received in revised form 11 March 2010
Accepted 16 March 2010
Available online 24 March 2010

Keywords:
Functional group
Invasive plant
Methane
Methanogen
Microbial community
T-RFLP

ABSTRACT

We examined patterns of methane flux, plant biomass, and microbial methanogenic populations in nine wetland plant species. Methane dynamics varied across plant functional groupings, with patterns distinctive among forbs, clonal dominants, and tussock/clump-forming graminoids. *Carex stricta* and *Scirpus atrovirens* showed the highest emissions (31.7 and 20.6 mg CH_4-C m^{-2} h^{-1}), followed by other tussock- or clump-forming graminoids that averaged 11.0 mg CH_4-C m^{-2} h^{-1} (*Scirpus cyperinus*, *Glyceria striata*, and *Juncus effusus*). The clonal dominants (*Phalaris arundinacea* and *Typha angustifolia*) had the lowest methane emissions (1.3 and 3.4 mg CH_4-C m^{-2} h^{-1}) of all seven graminoid species, and the forbs (*Mimulus ringens* and *Verbena hastata*) emitted no detectable methane flux from their leaves. In general, methane emissions decreased with greater plant biomass. Terminal restriction fragment analysis (T-RFLP) of archaeal 16S rRNA revealed that the structure of the soil methanogen communities isolated from plant rhizospheres had no effect on methane flux. The relative proportions of the different terminal fragments were not correlated with either methane emissions or plant biomass. Methanogen populations from *J. effusus* soils were dominated by acetoclastic archaea of the Methanosarcinaceae and Methanosaetaceae families, while all other graminoid soils were colonized primarily by hydrogenotrophic archaea of the Methanobacteriaceae family. The results indicate that plant functional groups and plant biomass are useful in predicting methane flux differences across plant species, while soil methanogen community structure showed no distinguishable patterns.

Published by Elsevier B.V.

1. Introduction

Wetland ecosystems are the single largest natural source for methane emissions into the atmosphere, contributing one-third of global emissions (Solomon et al., 2007). Methane (CH_4) is among the most potent greenhouse gases, trapping as much as 24 times more heat per molecule than carbon dioxide (Lashof and Ahuja, 1990). Studies that examine controls over methane dynamics in wetlands are therefore crucial in our understanding of how disturbance, land use, and climate change alter methane emissions.

Among the important drivers of methane dynamics in wetland systems is vegetation. The mere presence or absence of vegetation as a gas-channel heavily impacts the magnitude of methane flux from sediments to the atmosphere (Schimel, 1995; King et al., 1998; Bellisario et al., 1999). In an arctic wet meadow, the removal of sedges led to reduced CH_4 emissions and increased accumulation of CH_4 in flooded soils (Torn and Chapin, 1993; Verville et al., 1998). Other sedge removal sites in Sweden showed reductions of methane emissions from 30% at a *Carex rostrata* site to 55–85% at an *Eriophorum vaginatum* site (Waddington et al., 1996). For each location, the sedges provided the dominant transport pathway for CH_4 release into the atmosphere. Similarly, Van der Nat and Middelburg (2000) found that over 85% of net CH_4 emissions from the *Scirpus* and *Phragmites* wetland was a result of CH_4 venting to the atmosphere through the root–shoot system.

Although the physical structure of vegetation provides an effective pathway for the transport of gases between the sediment and atmosphere, plant composition can influence the differences in methane flux across plant communities. Among sedges in the same habitat, species differences better explained methane flux rates than plant biomass or water table depth (Schimel, 1995). A comparison of three species derived from a common habitat also indicated that plant productivity was not a predictor of methane flux (Strom et al., 2005). Instead, differences in methane consumption among the three species better explained methane flux.

The role of plant composition in influencing methane emissions is governed by the numerous biogeochemical mechanisms controlling methane production, consumption, and transport. As mentioned, wetland vegetation serves as an important conduit for methane release from anoxic sediments into the atmosphere. Net methane release depends on movement through the root–shoot system, with several control points determining net methane flux. Plants in anoxic soil environments (such as wetlands) significantly

* Corresponding author. Tel.: +1 608 335 0289; fax: +1 608 265 2595.
E-mail address: jtkao@wisc.edu (J. Kao-Kniffin).

0304-3770/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.aquabot.2010.03.009

alter net methane emissions by increasing methane diffusion or flow from flooded sediments via physical traits (Chanton et al., 1989; Van der Nat and Middelburg, 2000).

Plant traits also control methane flux by influencing the microbial mechanisms involving methane production and consumption. Factors directly influencing the growth and physiology of the methane-producing microorganisms are the central determinants of methane production in wetlands. Nonetheless, it is the difference between methane produced by methanogens and that consumed by methanotrophs that determines *net* methane emission into the atmosphere (Segers, 1998). Plants influence microbially mediated methane production and consumption processes through altering substrate availability (such as providing root exudates) (King and Reeburgh, 2002; Saarnio et al., 2004), competing for nutrients (Lipson et al., 1999; Bardgett et al., 2003) and creating microenvironments of aerobic conditions (Wigand et al., 1997; Jackson and Armstrong, 1999).

Little is known of plant species effects on methanogen and methanotroph community composition and activity (Van der Nat and Middelburg, 1998; Wang et al., 2008). Likewise, we do not know the extent to which microbial community composition impacts net methane emissions (Lueders and Friedrich, 2000; Ramakrishnan et al., 2001). Given that microorganisms are responsible for methane production and consumption, understanding the effects of plant species on the structure and activity of soil microorganisms is essential in predicting methane emissions under global change.

The objective of this study was to identify the plant and microbial mechanisms underlying net ecosystem methane flux in wetlands. Much of the body of research conducted on plant species effects on methane flux includes typically three or fewer plant species (Chanton et al., 1993; Schimel, 1995; King et al., 1998; Strom et al., 2005). The work by Bouchard et al. (2007) examines the effects of multiple plant species and functional groups on methane flux, but does not assess microbial community composition.

We examined nine different wetland plant species to determine if and how methane flux is influenced by plant and microbial composition. In this study, we refer to plant composition as either individual plant species or a collection of plant species grouped by function (functional group). Microbial composition in this study indicates phylogenetic groups of microorganisms clustered together by sequence similarity. We hypothesized that plant species or functional groups of species exert control over methane flux through their influence on plant biomass and microbial community composition.

2. Materials and methods

2.1. Experimental design

The plants used in this experiment were grown for 4 months in a greenhouse facility located on the University of Wisconsin-Madison campus. For each plant species, a total of nine replicates of plants were grown in individual pots located within three replicated greenhouse rooms (9 plant species × 9 replicate plants = 81 individual pots total). Each of the three greenhouse rooms contained three replicate pots of each plant species. The plants were grown in pots measuring 38.1 cm × 15.2 cm, containing leak-proof plastic lining bags. The plants received a daily watering of dilute (10%) liquid nutrients of Hoagland's solution that consisted of Ca, Cl, Fe, Mg, N, P, S, B, Cu, Mn, Mo, and Zn. We used a drip system for continuous flooding to maintain a water level 2.5 cm above the soil surface. Daily room temperatures ranged from 24 °C for 14 h to 18 °C for 10 h. Photosynthetic photon flux density in the rooms measured 490 μmol m^{-2} s^{-1}, with the photoperiod set at 14 h light and 10 h dark. Carbon dioxide (CO_2) concentrations in the rooms averaged 370 ppm (ambient level).

The soil used in this experiment was derived from a wet meadow community located near Dickeyville, Wisconsin, USA (42°39′21″N, 90°34′39″W) and from various wetlands located in the University of Wisconsin-Madison Arboretum (43°1′40″N, 89°26′15″E). To minimize excavating large amounts of wetland sediment, we added equal parts of topsoil (Liesener Soils Inc., Jackson, WI) and homogenized the medium in a large plastic swimming pool using shovels. We surmised that the sediment derived from a variety of natural wetlands would provide microbial inoculum to promote growth of methanogenic and methanotrophic microorganisms. Moreover, we minimized variability across plant species and replicates by homogenizing sediments to create standard soil mixes in all pots. The homogenized soil was placed into the pots, on top of 5 cm of washed sand.

The experimental design included nine species of wetland plants typically found in wet meadows and other freshwater wetlands in the northern United States. The plant species were chosen to represent major functional types: (1) forbs (monkey flower [*Mimulus ringens* L.] and blue vervain [*Verbena hastata* L.]; (2) clonal dominant graminoids (reed canary grass [*Phalaris arundinacea* L.] and narrowleaf cattail [*Typha angustifolia* L.]); (3) tussock- or clump-forming graminoids (woolgrass [*Scirpus cyperinus* L. Kunth], fowl mannagrass [*Glyceria striata* Lam.], common rush [*Juncus effusus* L.]; green bulrush [*Scirpus atrovirens* Willd.], and tussock sedge [*Carex stricta* Lam.]. Most species were germinated in either sand or potting soil before being transferred to the pots as seedlings. We added rhizome clippings of *T. angustifolia* and seedling plugs of *C. stricta* to the pots. The original experimental design included 14 different plant species, but six species failed to germinate under the growth conditions: *Phragmites australis* (Cav.) Trin. ex Steud., *Asclepias incarnata* L., *Calamagrostis canadensis* (Michx.) Beauv., *Typha* × *glauca* Godr., and *Carex vulpinoidea* Michx.

2.2. Gas sampling and analysis

Methane flux via the plant pathway was measured by placing PVC chambers over the plant leaves and stems. To minimize methane diffusion and ebullition from the flooded soil, stakes were used to hold the chambers in place on top of the sediment surface. The high water level at the sediment surface served as a barrier to gases emanating from the chamber base. Although this method does not provide complete exclusion of sediment-derived methane, we optimized collection of plant-derived methane by measuring gas flux when the plants reached full canopy maturity. Additionally, the top of the chambers were left open for approximately 45 min for gas equilibration 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. As a result of coordinating gas collections with the timing of canopy peak maturity, the flux measurements represent one sampling per plant during the period of this study. We used 30 mL polyurethane syringes to collect methane samples from the chambers at times 0, 15, and 30 min. Samples were injected into the Shimadzu 14B (Shimadzu Corporation, Kyoto, Japan) with a flame ionization detector. Fluxes were calculated using linear regression of the three time samples (0, 15, 30 min). Methane flux is reported as the ground surface area covered by the chambers.

2.3. Soil and plant samples

After 4 months of growth from germination in the greenhouse, we harvested plant shoots and roots from the pots. Soil adhering to the roots was removed using spatulas and considered rhizosphere soil in our microbial community analysis. The rhizosphere soil was homogenized and frozen at −20 °C prior to analysis. Aboveground

plant biomass was determined as the sections of the plant containing green leaves or stems, and was separated from belowground plant biomass. The plants were washed, dried at 60 °C until constant weight, and weighed to determine plant dry weight.

2.4. Microbial analysis

2.4.1. Molecular fingerprinting (T-RFLP)

In order to assess general methanogenic community structure, we used terminal restriction fragment length polymorphism (T-RFLP). This molecular fingerprinting method allows for rapid identification of major microbial populations based on PCR amplification of target sequences. Restriction enzyme digestion of the amplicons and subsequent measurement of the fluorescent-labeled terminal restriction fragments (T-RFs) provide a snapshot of microbial diversity and community structure derived from environmental samples. To characterize community structure using T-RFLP data, microbial ecologists calculate the signal intensity of a single T-RF in proportion to all T-RFs in a sample. The resulting ratio characterizes the relative gene frequency of different microbial populations (or T-RFs).

2.4.2. DNA extraction and amplification

To determine methanogen relative gene frequencies, we extracted total community DNA from 1.0 g soil in each plant species using the UltraSoil DNA extraction kit (MoBio Laboratories, Solana Beach, CA). Methanogen 16S rRNA genes were amplified from the purified extracts using the following primers: Ar109f (5′-ACG/TGCTCAGTAACACGT-3′) and Ar912r (5′-CTCCCCCGCCAATTCCTTTA-3′) (Lueders and Friedrich, 2000). The 50 μL PCR mixtures contained 50 μM each of the four deoxynucleotide triphosphates (dNTP—Promega, Madison, WI), 0.5 μM each primer, 1.5 U BlueTaq DNA polymerase (Denville Scientific, South Plainfield, NJ), 1× polymerase buffer (Denville Scientific, South Plainfield, NJ), 3.0 mM $MgCl_2$, and 5 μL of a 1:10 dilution of the purified DNA extracts. The reaction tubes were placed in the Eppendorf Mastercycler Gradient thermocycler for 5 min at a preheated 94 °C for denaturation, 28 cycles of further denaturation at 94 °C (1 min), annealing at 52 °C (1 min), and elongation at 72 °C (1.5 min). The final elongation was set at 72 °C for 6 min. *Taq* polymerase was added during the preheat stage (Hotstart method). Aliquots (5 μL) of the PCR products were analyzed by electrophoresis on 1% agarose gels stained with ethidium bromide and visualized using the Alpha Imager 2200 gel imaging system.

2.4.3. Cloning and sequencing

The methanogen 16S rRNA PCR products were cloned in *Escherichia coli* competent cells, JM109, using the pGEM-T Easy Vector Kit (Promega, Madison, WI). We made the following modifications to the manufacturer's instructions: for the ligation step, we halved the amount of reagents in the reactions and controls to a total volume of 5 μL. The standard reaction contained 2.5 μL ligation buffer, 0.5 μL vector, 0.5 μL DNA ligase, 1.5 μL PCR product, and no water. The positive and background controls also contained half the amount of reagents, with water added for a final volume of 5 μL. For the transformation step, we used 20 μL of JM109 cells and half the amount of bacterial nutrient broth (SOC medium). Colony PCR was performed on randomly selected clones using 1× Go *Taq* Green Master Mix (Promega, Madison, WI) and 0.5 μM each primer (M13F-40 and M13R) to a total volume of 40 μL. Aliquots (2 μL) of the colony PCR products, along with 5.2 μL of water, were cleaned with 0.2 μL of ExoSAP-IT reagent (USB, Cleveland, OH). The purified products (7.43 μL) were used in Big Dye sequencing reactions using 0.75 μL BigDye Terminator v. 3.1 mix (Applied Biosystems), 1.5 μL of dilution buffer (Applied Biosystems), 0.32 μL primer (M13F-20 or M13R) to a total volume of 10 μL. The thermal profile was set at 95 °C for 3 min, followed by 36 cycles at 95 °C for 20 s, 45 °C for 30 s, and 60 °C for 4 min. The final elongation was set at 72 °C for 7 min. Excess dye terminators were removed using MagDTR Dye Terminator Removal Resin (Edge Biosystems, Gaithersburg, MD) prior to sequencing. 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.

2.4.4. Fragment analysis

For T-RFLP analysis of archaeal 16S rRNA, we used the primer Ar109f with a 6-carboxy-flourescein (FAM) labeled 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).

2.5. Statistical analysis

We analyzed our data using JMP version 5.0 (SAS Institute Inc., Cary, NC). Plant species were treated as the fixed factor and greenhouse rooms as the random factor in the ANOVA analysis. We determined the effects of plant species on methane flux, plant biomass, and microbial relative gene frequencies using Fisher's LSD post hoc analysis. When necessary, data were log- or square root-transformed to meet the assumptions of normality and homogeneity of variance. Microbial relative gene frequencies (also known as the relative signal intensity of each T-RF) were calculated based on the signal intensity of each T-RF, in relation to the total signal intensity of all T-RFs.

3. Results

3.1. Net methane flux

Net methane emissions from the nine wetland plant species showed patterns separated across functional groupings. The two forbs, *V. hastata* and *M. ringens*, showed no fluxes of methane from the leaves, while the seven graminoids differed significantly by species ($F = 24.5$, $p < 0.0001$) (Fig. 1). The two clonal dominants, *P. arundinacea* and *T. angustifolia*, showed the lowest rates of methane flux of all the graminoids. In contrast, the two graminoids with the highest methane flux rates were tussock- or clump-forming species (*C. stricta* and *S. atrovirens*). The plant species showing intermediate methane flux rates consisted of other clump-forming species, including a bulrush (*S. cyperinus*), a reed (*J. effusus*), and a grass (*G. striata*).

3.2. Plant biomass

Biomass differed significantly among species and showed patterns of functional groupings. Plant biomass was generally highest among the graminoids compared to the forb species (Fig. 2). The two clonal dominants (*T. angustifolia* and *P. arundinacea*), along with *G. striata*, had the highest total plant biomass, while the forbs and *C. stricta* showed the lowest (aboveground biomass: 49.09, $p < 0.0001$, belowground biomass: $F = 28.5$, $p < 0.0001$). Three of the clump-forming reed and bulrushes (*J. effusus*, *S. atrovirens*, and *S. cyperinus*) showed intermediate values in biomass. When we regressed plant biomass and methane flux across all graminoids, we found an overall negative correlation ($r = -0.6$, $p < 0.0001$) (Fig. 3).

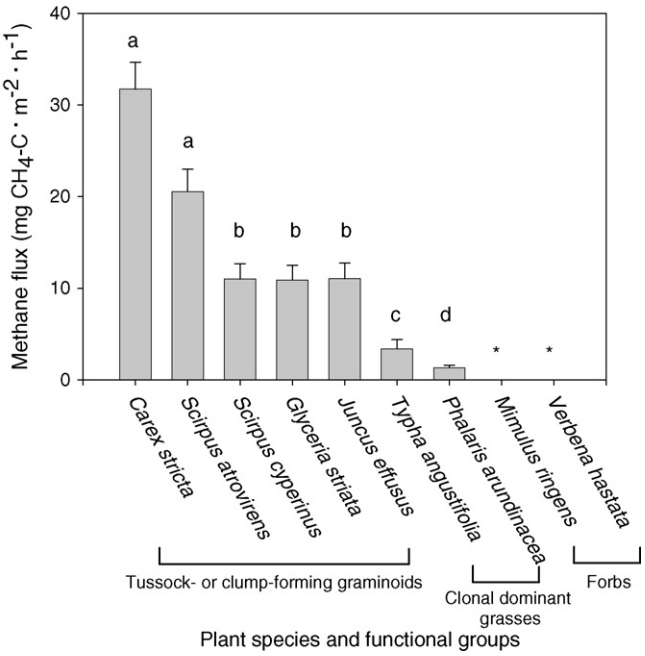

Fig. 1. Methane flux measurements from the shoots of seven graminoid species reported on a ground surface area basis (means ± 1 SE). The bases of the chambers were sealed with water to restrict gas emissions from the sediment into the chamber air space. Bars showing different letters indicate significantly different means at $p < 0.05$ (Fisher's LSD).

When regressed by individual plant species, no significant relationships with methane flux were detected.

3.3. Methanogen population structure

Terminal restriction fragments (T-RFs) are terminal fragments of amplified genetic sequences that represent microbial assemblages. In this study, the different size fragments correspond to phylogenetic groups of archaea. The soil samples resulted in five different terminal fragments: (82 bp) Methanomicrobiaceae, (91 bp) Methanobacteriaceae, (184 bp) Methanosarcinaceae, (283 bp) Methanosaetaceae, and (393 bp) Rice Cluster I. Library clones generated from DNA extracts of the same soil samples showed similar phylogenetic clusters indicated by the T-RFLP groupings.

We found that the profile of the soil methanogens differed among plant species, but did not indicate patterns distinguished by plant functional groupings. With the exception of *J. effusus*, the profile of the methanogenic community from the soil of the graminoid plants was dominated largely by archaea from the Methanobacteriaceae (as distinguished by the 91 bp terminal fragment) (Fig. 4, Table 1). In general, the relative proportions of methanogens isolated from rhizospheric soil showed that between 65 and 85% of the community was comprised of hydrogenotrophic methanogens (archaea that utilize H_2 and CO_2 as energy sources). *J. effusus* was the only plant species that was dominated by acetoclastic methanogens (archaea that utilize acetate as the energy source). The relative proportions of acetoclastic and hydrogenotrophic methanogens were not correlated with methane flux rates.

4. Discussion

Our primary objective in this study was to assess the role of plant composition in influencing net methane emissions. Methane flux exhibited a general pattern across plant species: tussock- and clump-forming graminoids released the highest rates of methane,

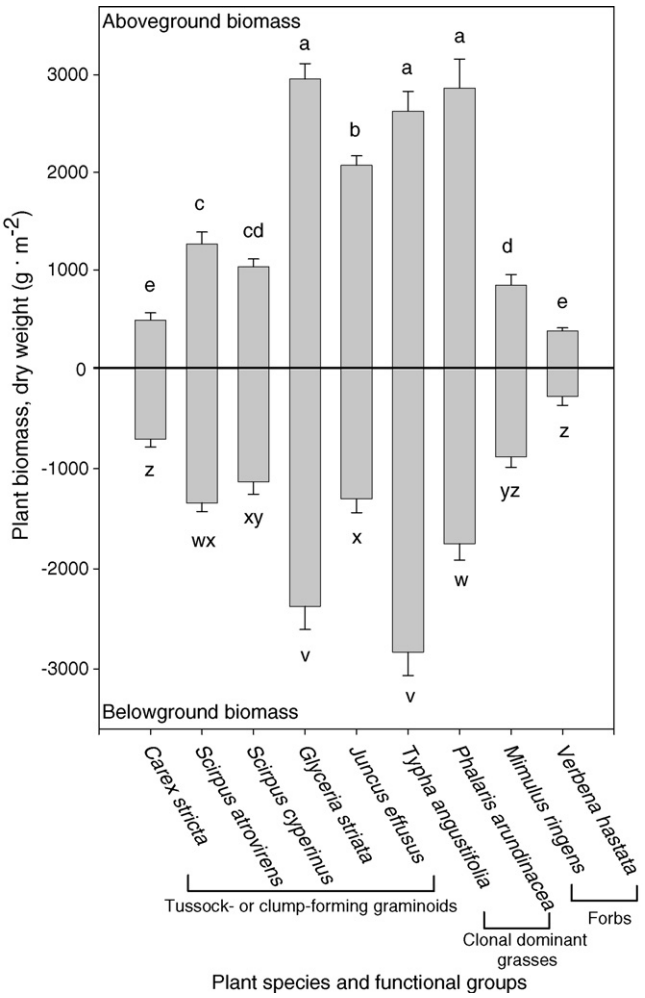

Fig. 2. Plant aboveground and belowground biomass for nine wetland species (means ± 1 SE). Bars showing different letters indicate significantly different means at $p < 0.05$ (Fisher's LSD). Letters a–e correspond to means differences among aboveground biomass, and letters v–z denote means differences among belowground biomass.

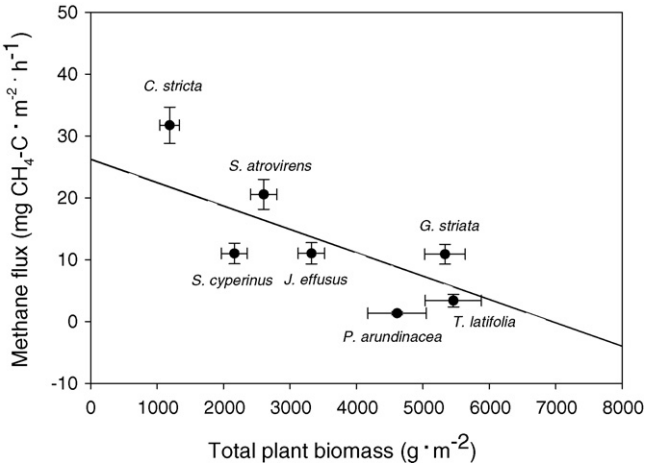

Fig. 3. Regression analysis of methane flux measurements with total plant biomass for all seven graminoid species. The regression ($r = -0.6$, $p < 0.0001$) was generated using all data points ($n = 62$) without separation by species. For ease of visualizing data clustering by plant composition, the graph depicts means with standard error bars (means ± 1 SE) for each species.

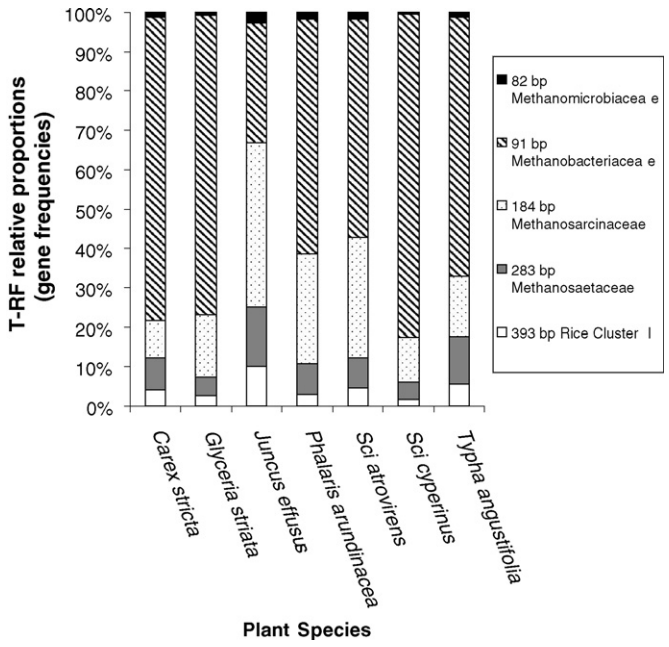

Fig. 4. Relative proportions of terminal restriction fragments (T-RFs) derived from soil DNA extracts under seven different graminoid species. The standard errors are listed under Table 1. T-RFs include 82 bp-Methanomicrobiaceae (solid black), 91 bp-Methanobacteriaceae (diagonal lines), 184 bp-Methanosarcinaceae (dots), 283 bp-Methanosaetaceae (solid gray), and 393 bp-Rice Cluster I (solid white).

followed by clonal dominant grasses, and no release by forbs. The lack of a methane response from the two forb species suggests that the forbs in this study did not serve as conduits for methane emission from the sediment to the atmosphere. Surprisingly, the invasive plants in our study (clonal dominants) had the lowest methane emissions of all nine species. We expected *P. arundinacea* (reed canary grass) and *T. angustifolia* (narrowleaf cattail) to have the greatest methane flux measurements, because both species exhibit high plant productivity and have a physical structure conducive to gas flux. Instead, the large aboveground and belowground biomass of the two invasive plants may have aided in greater oxygen transport to anoxic sediments, which could stimulate methane consumption by rhizospheric bacteria (Brune et al., 2000). Many of the most invasive wetland plants are matrix clonal dominants that form dense and deeply rooted belowground structures with well-developed aerenchyma (continuous system of air spaces in rhizomes and roots). Reed canary grass (*P. arundinacea*), narrowleaf cattail (*T. angustifolia*), and common reed (*P. australis*) are examples of invasive clonal dominants that invade many types of wetlands across the U.S.

As hypothesized, plant biomass was an important measure in this study for predicting methane flux. A negative correlation between biomass and methane flux was also found by Strom et al. (2005), in which methane emissions decreased when all three plant species increased in biomass. In contrast, other studies have shown positive relationships between biomass, CO_2 fixation, or net ecosystem productivity (NEP) and methane flux (Chanton et al., 1993; Whiting and Chanton, 1993; Waddington et al., 1996; Christensen et al., 2000; Joabsson and Christensen, 2001). When examined at the plant species level, the relationship between biomass and methane flux becomes less predictable. A wetland in NE Greenland dominated by *Eriophorum scheuchzeri* and *Carex subspathacea* showed higher rates of methane with greater plant biomass, whereas no correlation was found with the biomass of another dominating sedge, *Dupontia psilosantha* (Joabsson and Christensen, 2001). Furthermore, in an Alaskan wet meadow tundra, plant biomass did not influence methane emissions from two different sedge species (Schimel, 1995). In this study, the relationship between biomass and methane emissions was inconsistent at the species level and showed no significant trends. Only when plant biomass was examined across the full collection of species, did we find the negative relationship with methane emissions.

Although we show that biomass at the plant species level is a poor predictor of methane emissions, we found that plant functional groupings were useful indicators. While several studies report species-specific differences in methane emissions (Schimel, 1995; Joabsson and Christensen, 2001; Garnet et al., 2005; Strom et al., 2005), little information is known of how methane flux differs across plant functional groups. A wetland mesocosm experiment that explicitly measured the effects of functional groups on methane emissions revealed no statistically significant differences in flux rates among four functional groups (Bouchard et al., 2007). However, the tussock-forming plants did show a trend towards higher emission amounts than the other functional groups, as was found in our study here. The major driver of methane emissions in the mesocosm experiment was the number of functional groups, not the composition. An increase in functional group richness led to a decrease in methane flux, most likely as a result of enhanced sediment oxidation and methane consumption in mesocosms showing greater plant structural and functional diversity. The study illustrates the important concept that plant physical traits not only impact methane emissions via effects on the gaseous transport pathway, but also by influencing belowground oxidation levels and microbial metabolism.

Table 1
Fixed effect factors (*F*- and *p*-values) and standard errors (±1 SE) for the relative proportions of each terminal restriction fragment (T-RF) within bp length. See Fig. 4 for accompanying means. For each T-RF, values not connected by the same letters indicate significantly different means among plant species ($p < 0.05$): 91 bp (lowercase a–g), 184 bp (lowercase u–z), 284 bp (uppercase A–F), and 393 bp (uppercase T–Z).

Plant species	82 bp	91 bp	184 bp	283 bp	393 bp
F- and *p*-values	$F = 0.83$ p = n.s.	$F = 11.5$ $p < 0.001$	$F = 11.5$ $p < 0.001$	$F = 7.0$ $p < 0.001$	$F = 11.7$ $p < 0.001$
Carex stricta	0.00	0.05 ab	0.02 y	0.02 BCD	0.01 TUVWX
Glyceria striata	0.00	0.04 abc	0.03 wxy	0.01 CDEF	0.01 VWXYZ
Juncus effusus	0.00	0.05 g	0.03 u	0.02 A	0.01 S
Phalaris arundinacea	0.01	0.12 def	0.11 v	0.03 BCDE	0.01 VWXYZ
Scirpus atrovirens	0.00	0.04 f	0.03 v	0.01 CDE	0.01 TUV
Scirpus cyperinus	0.00	0.03 a	0.02 y	0.01 DEF	0.01 YZ
Typha angustifolia	0.00	0.03 bcdef	0.01 wxy	0.02 AB	0.01 TU

In some environments, plant roots also provide substantial levels of carbon and nitrogen substrates for microbial metabolism. Certain groups of methanogens inhabit the surface and rhizosphere of plant roots, presumably specializing on plant-derived compounds (Grosskopf et al., 1998). Acetoclastic and hydrogenotrophic metabolism are the two dominant pathways of methanogenesis. Acetoclastic methanogens belonging to Methanosarcinaceae and Methanosaetaceae utilize certain forms of organic carbons, and are often limited by the availability of these substrates (Jetten et al., 1992).

In natural systems acetoclastic metabolism is dominant over hydrogenotrophic (Jones, 1991), but the dominant populations of methanogens in this study were represented overwhelmingly by the hydrogenotrophs. Acetoclastic archaea represented only a fraction of the population of total methanogens found in our rhizosphere samples. It may be that the acetate levels in our sediments were too low to influence the population dynamics of acetoclastic methanogens. The Methanosarcinaceae can utilize a wide variety of carbon sources, while those belonging to the Methanosaetaceae utilize acetate as a sole carbon source. In the case of the Methanosarcinaceae, a minimum threshold concentration of ~1 mM acetate is required for growth, but only a fraction of this amount (5–20 μM) is needed for the Methanosaetaceae (Jetten et al., 1992).

Although several studies suggest that acetoclastic methanogens are widely distributed in natural systems and anaerobic digesters, and may prevail as the dominant methanogens compared with the hydrogenotrophs (Lueders and Friedrich, 2000; Smith and Ingram-Smith, 2007), we found the opposite trend. The methanogen populations represented in our study were more similar to those found on rice roots, where archaea of the acetoclastic Methanosarcinaceae and Methanosaetaceae were in lower proportions than the hydrogenotrophs (Grosskopf et al., 1998; Ramakrishnan et al., 2001; Chin et al., 2004). This finding may be a product of our study design, though we were careful to utilize soil from a variety of local wetlands as a mesocosm starter. However, using a wetland-topsoil mixture that was homogenized prior to being placed in pots may have inhibited the development of methanogen populations requiring high microsite concentrations of organic carbon. A 4-month long experiment may not have allowed enough time to simulate certain environmental conditions that influence methane production, such as organic matter decomposition, stand age variability, and sediment nutrient heterogeneity. Nevertheless, the assumption that acetoclastic populations are dominant in natural systems merits further investigation.

Although methanogen relative proportions (T-RFLP fragments) differed across particular plant species, no patterns emerged when examined with plant biomass, plant functional groupings, or methane flux. The lack of a relationship between methane emissions and the frequencies of T-RFLP fragments might suggest that amounts of methane emitted from plants are independent of microbial community structure. Other studies have shown limited correlations between microbial function and community structure in methanogenic populations (Lueders and Friedrich, 2000; Chin et al., 2004; Hoj et al., 2005).The standard techniques used by researchers to characterize microbial community structure may not accurately depict the actual population structure of microorganisms from environmental samples. For example, our sampling procedure may have influenced the relative proportions of different T-RFs represented in our samples. A single gram of fresh soil is typically used to characterize microbial community composition. Using small amounts of soil to determine microbial composition can result in highly variable communities that characterize soil aggregates or microsites, instead of the experimental variables tested. Although we aimed to capture representative samples by selecting well-homogenized composites of sediments scraped from randomly chosen roots, microbial community analysis is still constrained by sample size and replication. Other researchers have noted that the proportions of the T-RF (283 bp) for archaea belonging to the Methanosaetaceae are heavily influenced by low acetate concentrations and proximity to plant roots (Lehmann-Richter et al., 1999; Kruger et al., 2005). The high proportion of non-acetoclastic methanogens in the overall archaeal profile (T-RF 92 bp, Methanobacteriaceae) indicate that hydrogenotrophic, rather than acetoclastic methanogenesis, may be the dominant form of methane production from soil collected the majority of the plant species. Therefore, overall methane emissions from plant shoots are likely not to be significantly affected by plant-derived carbon compounds when the dominant rhizospheric methanogens are hydrogenotrophs.

Also, the use of T-RFLP as a semi-quantitative PCR-based method may not capture the true population structure of the soil community. The limitations of any PCR-based method (low soil sample size, PCR amplification bias, and PCR inhibitors) can impede accurate comparisons of relative proportions and diversity between samples (Suzuki and Giovannoni, 1996). Despite these potential barriers, we found that T-RFLP revealed a number of compelling relationships regarding the role of plant and microbial composition in methane dynamics. Most notably, we found that methane emissions are not determined by the proportions of acetoclastic or hydrogenotrophic methanogens in the rhizosphere. However, we examined only a portion of microorganisms in our study that regulate methane emissions from plants. This study does not include the population dynamics of methanotrophs, which are represented mainly by methane-oxidizing bacteria. Advancements in molecular methods that improve rapid identification of microorganisms across a diverse range of species will be critical in extending plant functional group studies beyond environmental and plant productivity measurements.

Acknowledgments

We thank Katherine Faust, Marlo Dobrient, and Vivian Chiang for their help with gas sampling, plant harvesting, soil collection, and gas analysis. Katherine Faust also helped with microbial analysis. Dirk Krueger provided assistance and training in cloning and sequencing analysis. Randy Jackson and Gary Oates provided access to and training on the Shimadzu 14B. A grant from the Department of Energy's National Institute for Climatic Change Research (NICCR) and assistance from the University of Wisconsin-Madison Graduate School funded components of the research. An NSF Doctoral Dissertation Improvement Grant, awarded to Jenny Kao-Kniffin, provided additional funding for the project.

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