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Wetland methane emissions altered by vegetation disturbance: An interaction between stem clipping and nutrient enrichment

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ABSTRACT

Plant-mediated transport is the dominant means of methane release from vegetated wetlands. Whether aboveground plant disturbances affect the emission of methane is largely unknown. We tested the effects of stem clipping on methane emissions from freshwater wetland mesocosms vegetated with *Sagittaria lancifolia*, *Panicum hemitomon*, *Echinochloa walteri*, or *Eleocharis macrostachya* under different nutrient regimes. Mesocosms vegetated with *E. macrostachya* showed an effect of treatments on CH_4 emission which was elevated in high nutrient level treatment and suppressed in mid nutrient level treatment as compared to controls when the plant was clipped to 3 cm above water line. *S. lancifolia* and *P. hemitomon* showed a similar pattern, and *E. walteri* showed no response to treatments. Mean CH_4 emission significantly differed between species, being up to 56% higher in mesocosms vegetated with *P. hemitomon*. Treatments where plants were clipped below the water line had low initial emission rates, but these increased after three days. Sediment samples were analyzed for patterns in the microbial functional genes *mcrA* (methanogens) and *pmoA* (methanotrophs), but no changes in microbial communities in response to treatments were observed. Changes in methane emissions due to plant disturbances in wetlands warrant further investigation, but this study demonstrates that the available nutrient pool must also be considered. Additionally, initial responses to treatments were observed, but these responses tended to dissipate after three days, emphasizing the importance of monitoring CH_4 emissions over time rather than only observing initial responses. Our results suggest that nutrient enrichment alone can suppress CH_4 emission, however interactions with plant disturbances can be of equal importance.

1. Introduction

Wetland ecosystems are the largest natural source of methane (CH_4) emissions, representing 20–40% of global emissions (Kirschke et al., 2013), even as wetlands constitute only 5–8% of worldwide land area (Lehner and Döll, 2004). CH_4 concentrations in the atmosphere have exhibited an increasing trend since 1750 (Ciais et al., 2013), but there is a large amount of interannual variability in global emissions with an estimated 50–70% of the global interannual variability attributed to wetland CH_4 emissions (Bousquet et al., 2006; Chen and Prinn, 2006). Wetland vegetation is known to influence the production and consumption of CH_4 (Carmichael et al., 2014), modulating CH_4 fluxes to the atmosphere directly and indirectly (Schimel, 1995; Chanton et al., 2008). Given the influence of vegetation over CH_4 consumption, production, and emission, it is likely that disturbances to wetland vegetation can affect CH_4 processes (King et al., 1998; Ding et al., 2005; Schultz et al., 2011), and may play a role in the observed variability in wetland CH_4 emissions.

Transmission of sediment produced CH_4 through vegetation to the atmosphere is particularly important in wetlands; up to 90% of total CH_4 emissions from vegetated wetlands may result from emergent aquatic vegetative transport (Sebacher et al., 1985; Van der Nat and Middelburg, 1998; Bergstrom et al., 2007). Gas transport through wetland vegetation could potentially be altered by herbivores feeding on the stems of these plants (Bodelier et al., 2006; Dingemans et al., 2011; Petruzzella et al., 2015). The wetlands in southeastern Louisiana support many species of waterfowl and mammals (Nyman et al., 2013), but the effects of aboveground herbivory on the release of CH_4 from these wetlands are unknown. Extrapolations from previous herbivory or clipping studies provide

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little guidance due to the lack of studies utilizing plant species common to the region. Previous studies examining CH_4 emission in relation to herbivory or stem clipping have also maintained constant nutrient conditions (Kelker and Chanton, 1997; Greenup et al., 2000; Ding et al., 2005), whereas it is quite common for southeastern Louisiana wetlands to vary widely in nutrient content due to surrounding land use, river diversion control structures, wastewater treatment, and management (Brantley et al., 2008; Hyfield et al., 2008). In this study, we examined the effects of plant stem clipping on CH_4 emission via three levels of clipping on four different aquatic plants common to freshwater marshes of southeastern Louisiana (*Sagittaria lancifolia*, *Panicum hemitomon*, *Echinochloa walteri*, and *Eleocharis macrostachya*) and under three levels of nutrient enrichment. Sediment samples from each treatment were analyzed for methanogen and methanotroph diversity using denaturing gradient gel electrophoresis (DGGE) of genes linked to methane cycling (*mcrA*, *pmoA*) to determine if treatments also led to structural changes in the methane-active microbial community. By measuring CH_4 emission and methane-related microbial diversity, we sought to determine if plant disturbances similar to herbivory altered plant mediated CH_4 emission, and whether any observed effects could be related to the community of methanogenic and methanotrophic microorganisms.

2. Methods

This experiment was conducted in wetland mesocosms (18 total) that were constructed from galvanized steel farm watering troughs (1.8 × 0.6 × 0.3 m, 280 l; Nasco, Fort Atkinson, WI), and were located outdoors on the Louisiana State University campus, Baton Rouge, LA. Each mesocosm was filled with high clay content fill dirt to approximately one-half of total depth. The upper sediment material consisted of surface sediment collected from a freshwater marsh at the confluence of Bayou Verret and Lake Cataouatche in southeastern Louisiana, and was added to a depth of approximately 20 cm. Each mesocosm was then subdivided into four 0.6 × 0.3 m sections using flat sheets of PVC plastic (Professional Plastics Inc., Fullerton, CA) pushed into the sediment to limit belowground competition between plants. Each subdivision within each trough was then vegetated with one of four plant species: *Sagittaria lancifolia*, *Panicum hemitomon*, *Eleocharis macrostachya*, or *Echinochloa walteri*, and two small remaining sections on each end of the trough were left unplanted. *S. lancifolia* was collected from the lower Atchafalaya delta (29°24′49.49 N, 91°19′37.45 W) and Bayou Verret, LA (29°52′32.54 N, 90°14′42.68 W). *P. hemitomon* was collected from a site just south of Raceland, LA, along Highway 182 West (29°40′37.76 N, 90°39′26.99 W). *E. macrostachya* was collected from the Farr Park Equestrian Center, Baton Rouge, LA (30°22′57.50 N, 91°12′39.35 W). All field-collected plants were transplanted into mesocosms on the day of collection. *E. walteri* was not collected, but was grown from seed outdoors at the Louisiana State University greenhouse facility, in a mixture of high clay fill dirt, potting soil, and vermiculite. Plants were randomly assigned to a subdivision within each trough and each subdivision received enough plant material to fill the area. Each trough was filled with water to the top of the trough and water levels were monitored daily.

The experimental design consisted of three levels of each of two treatments: nutrient enrichment (at zero, mid, and high nutrient addition), and clipping (at no clipping, clipping to 3 cm above the water line, and clipping to below the water line). Nutrient enrichment consisted of powdered 20-10-5 planting tablets (20% total N consisting of 4% ammoniacal N, 4.65% water soluble organic N, and 11.35% water insoluble N, 10% available phosphate, 5% soluble potash; Forestry Suppliers Inc., Jackson, MS) that were wrapped in a filter and enclosed in a 30 ml narrow mouthed sample bottle (Fisher Scientific Inc., Pittsburgh, PA), which contained 20 holes of approximately 0.15 cm diameter. Bottles were pushed into the sediment of each tank receiving nutrient enrichment. This method of nutrient enrichment was used to promote slow release over the course of the experiment (Williams and Ruckelshaus, 1993; Silliman and Zieman, 2001). The high nutrient addition was set to 20 g N m^{-2}, just above the yearly loading rate recommended for rice fertilization in Louisiana (Saichuck et al., 2011). The mid-level nutrient treatment was set at half of this amount for a total concentration of 10 g N m^{-2}, which is near the N-levels in marshes receiving diverted Mississippi river water (Hyfield et al., 2008). For troughs receiving no nutrient enrichment, sand was used in place of the powdered planting tablet to control for any disturbance effects. Nutrient enrichment began six weeks before sampling. Clipping was carried out just prior to sampling and consisted of clipping all of the plants in a particular mesocosm down to either 3 cm above the water, or to below the water line. Controls were not clipped. Each combination of nutrient amendment (zero, mid, high) and clipping (no, clipped to 3 cm above the water, clipped below water line) was carried out in two replicate mesocosms with 4 differing plant species (4 × 3 × 3 × 2 design).

Methane emissions were measured immediately following clipping of the stem. Sampling took place over three separate sampling events, each lasting three days, with six mesocosms sampled during each event. For each event and after methane measurements, the clipped plant material was collected, dried (70 °C, 48 h), and weighed to determine biomass. Plants clipped to 3 cm above the water were fully harvested to the top of the sediment after gas sampling on day 3, dried, and weighed. The roots of each plant within the confines of the base chamber were subsequently removed and washed clean of sediment and debris. Roots were then dried to determine belowground biomass. Sediment samples for mass measurements were weighed, dried (70 °C, 48 h), and reweighed to determine moisture content (Carter and Gregorich, 2007). Dry sediment was then ashed (500 °C, 2 h) and reweighed to determine organic matter content as ash free dry mass (% C) (Carter and Gregorich, 2007). Other environmental variables measured included stem number, water temperature and depth, pH, and percent cover and dieback were assessed.

2.1. Methane sampling

Methane sampling followed the common protocol of Lindau et al. (1991) for closed chamber measurements. Methane emissions were measured immediately after applying clipping treatments and again after three days, in order to detect responses other than the immediate effect of clipping. Clear Plexiglas chambers (30 × 30 × 30 cm), as described by Lindau et al. (1991), were installed into each subdivision of six randomly selected mesocosms three days prior to sampling, and this process was repeated 3 times, once per week for three weeks, until all 18 mesocoms were sampled. Chambers were installed to 10 cm of sediment depth. At the beginning of each sampling event, plants were clipped to the appropriate treatment level, and gas chamber tops added and sealed to the base units. A time-zero gas sample was taken and internal chamber temperature recorded. Each chamber top consisted of a sampling port (rubber septum), battery operated 12 v fan, pressure control tube, and a thermometer. The pressure control consisted of 1 m of plastic tubing (1.5 mm I.D.) that maintained equilibrium gas pressure between the outside and inside of chambers. Prior to sampling, Vacutainers were placed on a high-vacuum preparation line to remove any residual gases and then re-sealed with silicone rubber. For each headspace sample, 15 ml was removed from the flux chambers with a syringe and injected into a 10 ml evacuated gas Vacutainer. A slight over pressure of headspace gas