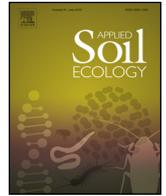




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Short communication

Spatial variation related to hydrologic patterns and vegetation in greenhouse gas fluxes from the Mississippi Delta agricultural region



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ABSTRACT

Analysis of microbial gas emissions collected with field chambers were combined with soil genetic and chemical analyses to determine hotspots of greenhouse gas emissions from vegetated cropland, drainage ditch, riparian zone, and wetland habitats in the Tunica and Sunflower Counties of the north Mississippi Delta agricultural region. Methane (CH₄) emissions were highest in sites with highest soil moisture (wetlands) and low water flow rates, whereas carbon dioxide (CO₂) emission peaked in sites with intermediate soil moisture (riparian zone). Nitrous oxide (N₂O) emissions were highest in the cropland and riparian zones, although soil genetic and chemical data suggest a difference in N₂O sources between these two sites. Denitrification gene (*nosZ*) abundances indicate denitrification-derived N₂O products in intermittently-flooded sites, while soil [NO₃⁻ + NO₂⁻] concentrations suggest nitrification-derived N₂O products in dry cultivated sites. Vegetation reduced gas emissions in riparian zone habitat, but had no effect in wetland habitat. Substantial and consistent differences in gas emission rates between two neighboring wetlands corresponded to differences in flow regime. Wetland hydrologic flow regime is the factor regulating the distinct gas emission differences observed between two neighboring wetlands. This study demonstrates the importance of hydrologic factors and location-specific vegetation on gas emission hotspots in this intensively-managed agricultural landscape.

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Agriculture comprises about 58% of the Mississippi River Basin land area (Goolsby and Battaglin, 2000) and is the dominant land-use in the alluvial floodplain of the Lower Mississippi River. Although this nearly flat, regularly tilled, agricultural landscape may appear homogeneous in form, it can be quite variable in habitat, including irrigated croplands, riparian zones, drainage ditches, and wetlands surrounded by cultivated fields. Stimulation of microbial activity by excess nutrients applied as plant fertilizer, combined with temporal and spatial variation in hydrological and redox conditions, can create hotspots of microbial metabolism and microbially-mediated nutrient transformations (McClain et al., 2003). Hotspots in microbial activities may occur at various spatial scales, from across a particular habitat type (Koh et al., 2009), or at smaller scale interfaces such as along a soil moisture gradient

(Hedin et al., 1998; McClain et al., 2003) or at plant root-sediment interfaces (Reddy et al., 1989).

The purpose of this study was to examine if within this intense agricultural landscape there was a spatial pattern related to soil moisture, hydrology, or the presence of vegetation, affecting microbial production of the most common greenhouse gases, CH₄, N₂O, and CO₂. Our objectives were to: (1) determine rates of greenhouse gas emissions from these contiguous, but hydrologically-distinct, habitats; (2) examine the influence of hydrophytic vegetation in channeling gas emissions from agriculturally-impacted wetland and riparian habitats; (3) assess the role of soil saturation in determining gas emission variability; and (4) use soil genetics and chemical analyses to identify biotic sources of gas emissions.

The study was carried out in the north portion of the Mississippi-Yazoo alluvial valley (commonly referred to as the Mississippi Delta). Field gas flux measurements were combined with soil genetic and chemical analyses to explore spatial and temporal variation in microbial gas production in four habitats

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typical of this region—row croplands, drainage ditches, riparian zones, and wetlands. Study sites representing these habitat types, and a gradient of water saturation and flow regime, were selected in the Cow Oak Bayou, Ark Bayou and Beasley Lake drainage systems, located in Tunica and Sunflower Counties, Mississippi, U. S.A. (Fig. 1). Cow Oak Bayou and Ark Bayou study sites were predominantly Sharkey clay and Tensas silty clay loam soils, while the Beasley Lake sites were primarily Dundee silty clay loam soils (Web Soil Survey, 2015).

Gas measurements were made and soil samples collected in June–August 2013 at multiple sites across each of the four habitats (Fig. 1). For clarification, we define a site as a distinct sample location within each of the four habitat types. There were two different cropland habitat types: corn or soybeans. Corn was fertilized (approximately 225 kg N fertilizer ha⁻¹); soybeans were not fertilized (A. Myers, personal communication). At Cow Oak Bayou and Ark Bayou, soil samples were taken from two cornfield sites and three soybean field sites, and gas measurements were made at one cornfield site and one soybean field site (Fig. 1A). At Beasley Lake, soil samples were taken from two soybean field sites and three cornfield sites, and no gas measurements were made (Fig. 1B). Within a single vegetated drainage ditch in the Beasley Lake watershed, comprising a two-cell sediment retention pond, we sampled at 7 sites along a gradient extending from croplands to wetland (Fig. 1B). One riparian zone habitat having moist, unsaturated soils was selected at Cow Oak Bayou, adjacent to the wetland, where soil samples and gas measurements were collected from 3 sites (Fig. 1A). There were 3 different wetland habitat types that were sampled, in Cow Oak Bayou, Ark Bayou, and in Beasley Lake. From these wetland habitats we took soil samples at 17 sites (5 at Cow Oak, 5 at Ark Bayou, 7 at Beasley Lake) and made gas measurements at all the wetland sites in Cow Oak Bayou and Ark Bayou.

Soil samples were collected at all 41 cropland, riparian zone, drainage ditch, and wetland sites in Ark Bayou, Cow Oak Bayou, and Beasley Lake on dry, summer afternoons. Soil analyses determined soil [NO₃⁻ + NO₂⁻] (Fawcett et al., 2014), soil *nosZ* gene abundance (Henry et al., 2006), and soil moisture content (DeAngelis, 2007) in all sites across all habitats. Gas flux measurements could not be performed at all sample sites but were made within both cropland fields (corn: *n* = 12 incubations, soybean: *n* = 9), one riparian zone habitat (*n* = 14), and two of the wetland habitats (Ark Bayou and Cow Oak Bayou; *n* = 34).

Wetland habitats were delineated from drainage ditches and the riparian zones by having continuously submerged soils. Sites in the Beasley Lake watershed (33°23'52.50"N, 90°40'38.13"W) were not sampled for gas emissions, but were sampled for soil and water chemistry and *nosZ* gene abundances. Dominant vegetation in the

Beasley wetland was *Alternanthera philoxeroides* and bottomland hardwood forest, while the Beasley drainage ditches also contained *A. philoxeroides*. The two wetland habitats sampled for gas emissions, Cow Oak and Ark Bayou, were separated from each other by about 400 m. The Cow Oak Bayou wetland (34°30'21.04"N, 90°25'33.88"W) was vegetated primarily by *Limnium spongia* and *Sagittaria latifolia*, had flowing water (0.15–0.43 m³ s⁻¹), and was subject to pumping for agricultural irrigation. The Ark Bayou wetland (34°30'08.48"N, 90°25'26.82"W) was vegetated primarily with *L. spongia* and *Polygonum persicaria*, and had stagnant hydrological conditions due to upstream beaver impoundments. The riparian zone habitat was adjacent to the Cow Oak Bayou wetland (34°30'21.16"N, 90°25'33.38"W) and vegetated primarily with *Phanopyrum gymnocarpon*.

Gas measurements were made from field chambers at approximately one-week intervals on 15 days through the summer 2013 growing period. For each gas measurement on each of the 15 sample days there generally were 3 replicate chambers enclosing vegetation and 3 replicate chambers from which vegetation had been removed. Ten wetland sites were sampled on 10 days, 3 riparian zone sites on 3 days, and 2 cropland sites on 2 days. Within a habitat location, sample sites varied with each incubation so that disturbance from prior incubations would not carry over.

Chambers were 40 × 30 × 30 cm in size, and constructed of opaque plastic (c.f. Waletzko and Mitsch, 2013). They were protected from over-heating by a layer of neutral density screening, which also reduced photosynthetically-active radiation at the chamber interior by 65%. The chambers were tightly fitted over soils, either enclosing vegetation (V chambers), or from which above-ground vegetation had been removed by clipping immediately prior to chamber installation (VR chambers) (Frenzel and Karofield, 2000; Ding et al., 2005). From each chamber, gas samples were collected via a stopcock once per hour over a three-hour period (1:00–4:00 pm). Samples were immediately sealed in glass vials and analyzed within three weeks for CH₄, N₂O, and CO₂ concentrations on a Shimadzu Gas Chromatograph (Shimadzu, Kyoto, Japan) coupled with an Electron Capture Detector and Flame Ionization Detector. Hydrologic measurements at Cow Oak Bayou and Ark Bayou wetland sites were made on site (water depth) on sample days, and occasionally through the summer by the USDA-ARS National Sedimentation Laboratory, Oxford, MS (water flow rates).

For CH₄, for acceptance of the data, a threshold of $R^2 \geq 0.8$ was applied to emission values plotted at the four time points (Imer et al., 2013). For CO₂ and N₂O, concentration changes generally were more variable between time points, so a mean hourly emission rate was estimated from initial and final concentrations. This approach assumes that where the time-course data over short

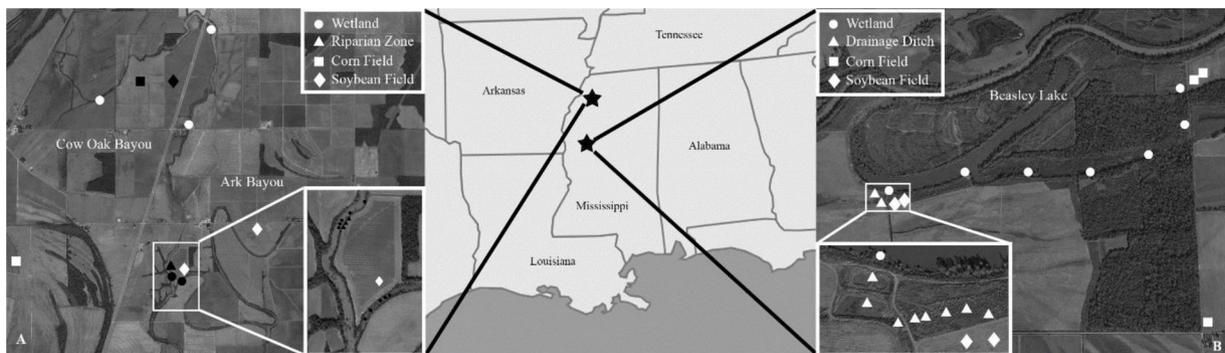


Fig. 1. Map of study sites at (A) Cow Oak Bayou and Ark Bayou (Tunica County, MS) and (B) Beasley Lake (Sunflower County, MS). Expanded boxes indicate in greater detail the spatial organization of sample sites. White symbol shapes indicate locations and habitat types where soil, water and the *nosZ* gene samples were collected. Black symbols represent locations where gas measurements were made in addition to soil and water sample collection.

intervals was variable, the best measure of the “signal” for rates of gas fluxes comes from changes over the maximum incubation time of 3 h. Standard errors in gas fluxes were computed from replicate chambers. Factorial and One-way Analysis of Variance (ANOVA; R, 2008) followed by Tukey's Highly Significant Differences (HSD) tests ($\alpha \leq 0.05$) were used to compare gas emission rates from V and VR chambers made on different dates. One-way ANOVA and Tukey's HSD was used to compare *nosZ* gene abundance means, soil $[\text{NO}_3^- + \text{NO}_2^-]$ means, and soil moisture means across land-use types; standard errors of the mean were calculated.

Cropland, riparian zone, and wetland habitats exhibited distinct patterns in rates and relative proportion of gas emissions, soil *nosZ* gene abundances, and soil chemistry (Table 1). Habitat differences were also strongly associated with soil moisture differences which followed a gradient from driest in cropland habitat to completely saturated in wetland habitat (Table 1). Methane and CO_2 emissions were highest in the wetlands ($p < 0.0001$) and riparian zones ($p < 0.0001$), respectively. Consistent with previous studies, CH_4 emission was at a maximum in flooded locations (e.g. Altor and Mitsch, 2006; Koh et al., 2009) with emission ranges ($0.6\text{--}112 \text{ mg m}^{-2} \text{ h}^{-1}$) comparable to other studies of freshwater riverine wetlands ($0.33\text{--}86 \text{ mg m}^{-2} \text{ h}^{-1}$; Sha et al., 2011). Mean $\text{CO}_2\text{-C}$ emissions were equivalent among wetland and cropland sites (VR chambers, $p=0.81$), but about 3 times higher in the riparian zone than in either wetland ($p < 0.0001$) or cropland ($p < 0.0001$), confirming that moderate soil moisture can spur high microbial CO_2 production (Xu and Qi, 2001; Koh et al., 2009). Emissions of N_2O were similar and highest in the cropland and riparian zones ($p=0.5$) compared to wetlands ($p < 0.0001$) with soil genetic and nutrient analyses (Table 1) suggesting both nitrification and denitrification as N_2O sources (see below).

Nitrous oxide (N_2O) can be produced by either nitrification or as a product of denitrification depending primarily on soil O_2 levels (Morse and Bernhardt, 2013). A decline in soil O_2 availability causes aerobic nitrifiers to produce a higher ratio of $\text{N}_2\text{O}/\text{NO}_3^-$ (Goreau et al., 1980), whereas an increase in soil O_2 disrupts the final step of denitrification (Davidson et al., 2000). Both nitrification and denitrification are important sources of N_2O emissions in cropland habitat as irrigation practices can create heterogeneous soil O_2 conditions (Morse and Bernhardt, 2013). In addition to measuring N_2O production directly, we determined abundances of the *nosZ* gene through gene extraction from soil samples followed by quantitative polymerase chain reaction (qPCR). The *nosZ* gene facilitates the reduction of N_2O to N_2 in the final step of denitrification and therefore acts as an indicator of denitrification activity (Henry et al., 2006).

The *nosZ* gene abundances were measured in the cropland, drainage ditch, and wetland sites, but not the riparian sites. The lowest *nosZ* gene abundances combined with the highest

NO_x ($\text{NO}_3^- + \text{NO}_2^-$) concentrations in cropland ($p < 0.0001$) and low soil moisture suggests aerobic nitrification as the source of cropland N_2O emissions. Panek et al. (2000) used ^{15}N isotopic signals to show that N_2O emissions from dry agricultural land are due primarily to nitrifying bacteria though episodic irrigation can create soil conditions conducive for denitrification. In wetland sites, by contrast, greater *nosZ* gene abundances than in cropland ($p=0.07$), and the lowest NO_x concentrations ($p < 0.0001$) combined with water-saturated soils, suggest anaerobic denitrification as the source of N_2O emissions. Low wetland N_2O emissions suggest that complete denitrification is occurring which produces N_2 over N_2O . In the drainage ditch sites, *nosZ* gene abundances were two orders of magnitude higher than in cropland, and more than 10 times higher than in the wetlands ($p=0.0009$), soil saturation content was intermediate, and, despite being immediately adjacent to the croplands, soil NO_x concentrations were much more similar to wetlands than to cropland soils. These observations are consistent with a recent study that reported agricultural drainage ditches in the Lower Mississippi Delta as having the potential for significant N removal by denitrification (Kröger et al., 2014).

In the riparian zone, the presence of vegetation lowered the emission rates of all three gases (Table 1; CH_4 : $p=0.04$, N_2O : $p=0.03$, CO_2 : $p=0.0006$). Plants influence soil conditions by releasing O_2 and organic exudates from their roots into the soil (Reddy et al., 1989; Cheng et al., 2007), thereby affecting rhizosphere microbial activity. These results suggest that plant oxygenation of unsaturated, riparian zone soil creates aerobic conditions that limits the obligate anaerobic processes of methanogenesis and denitrification, thereby reducing both CH_4 and N_2O emissions. Similar results were obtained by Yan et al. (2000) who documented higher N_2O emissions from unsaturated soils in the absence, rather than in the presence, of rice plants. The *nosZ* gene data indicate denitrification potential of these soils, but gas emissions data indicate that plants reduce denitrification-derived N_2O products by approximately 2/3. In contrast to our results, Hernandez and Mitsch (2006) and Altor and Mitsch (2006) found no vegetative effects on N_2O or CH_4 emissions from soils manipulated to have alternating flooded and dry conditions; however, their studies were conducted in highly-managed experimental wetlands designed for nutrient processing (i.e. denitrification) as opposed to the natural, heterogeneous, agriculturally-impacted systems in this study. In contrast to the riparian zone, we found no effects of wetland plants on mean CH_4 and N_2O emissions (Tables 1 and 2), though, as expected, CO_2 emissions were significantly reduced ($p < 0.001$). These data suggest location-specific effects of the dominant vegetation on gas emissions, likely related to degree of soil oxygenation affected by plant respiration in riparian zone vs. wetland habitats.

Table 1
Locational differences of measured environmental parameters; V is vegetation; VR is vegetation removal (ND=not determined). Mean \pm standard error (SE) values are presented.

Parameter	Cropland		Drainage ditch	Riparian zone		Wetland	
	Soybean (SB)	Corn (C)		V	VR	V	VR
CH_4 ($\text{mg C m}^{-2} \text{ h}^{-1}$)	0.2 ± 0.02 (1 site, $n=12$)	0.1 ± 0.04 (1 site, $n=9$)	ND	2.3 ± 1.7 (1 site, $n=14$)	6.5 ± 2.1 (1 site, $n=14$)	21.6 ± 5.7 (2 sites, $n=34$)	26.3 ± 6.9 (2 sites, $n=34$)
N_2O ($\mu\text{g N m}^{-2} \text{ h}^{-1}$)	64.2 ± 7.4 (1 site, $n=12$)	81.2 ± 7.3 (1 site, $n=9$)	ND	17.2 ± 5.4 (1 site, $n=14$)	56.7 ± 9.6 (1 site, $n=14$)	9.3 ± 6.7 (2 sites, $n=34$)	10.8 ± 6.1 (2 sites, $n=34$)
CO_2 ($\text{mg C m}^{-2} \text{ h}^{-1}$)	15.5 ± 1.0 (1 site, $n=12$)	14.8 ± 1.4 (1 site, $n=9$)	ND	14.2 ± 4.0 (1 site, $n=14$)	45.9 ± 7.4 (1 site, $n=14$)	-4.2 ± 1.2 (2 sites, $n=34$)	13.0 ± 1.3 (2 sites, $n=34$)
<i>nosZ</i> gene (mean no. copies/g soil $\times 10^7$)	2.3 ± 0.4 (10 sites, SB: $n=30$; C: $n=23$)		422.4 ± 300.6 (7 sites, $n=32$)	ND		36.2 ± 26.9 (9 sites, $n=71$)	
$\text{NO}_3^- + \text{NO}_2^-$ (μM)	25.1 ± 1.6 (10 sites, SB: $n=30$; C: $n=23$)		3.2 ± 1.6 (7 sites, $n=32$)	ND		1.3 ± 1.1 (9 sites, $n=71$)	
Mean soil moisture (%)	25.6 ± 1.5 (2 sites, $n=17$)		ND	46.7 ± 12.1 (1 site, $n=4$)		100	

Table 2

Gas emission differences between Ark Bayou (AB) and Cow Oak Bayou (COB); V is vegetation; VR is vegetation removal. Mean \pm standard error (SE) values are presented.

Parameter	V		VR	
	AB (n = 17)	COB (n = 17)	AB (n = 17)	COB (n = 17)
CH ₄ (mg C m ⁻² h ⁻¹)	40.8 \pm 10.1	3.4 \pm 0.6	50.1 \pm 13.2	7.8 \pm 4.0
N ₂ O (μ g N m ⁻² h ⁻¹)	-10.5 \pm 5.5	31.8 \pm 11.0	-8.0 \pm 5.0	30.7 \pm 12.6
CO ₂ (mg C m ⁻² h ⁻¹)	12.8 \pm 1.3	13.1 \pm 1.2	-5.4 \pm 0.9	-3.0 \pm 0.4

Distinct gas emission differences were found between Cow Oak Bayou wetland and Ark Bayou wetland with higher CH₄ emissions in Ark Bayou ($p < 0.0001$) and higher N₂O emissions in Cow Oak Bayou ($p < 0.0001$; Table 2), again regardless of the presence of vegetation. The Ark Bayou wetland water depth was relatively deep (mean = 50.5 cm) and non-flowing, compared to the Cow Bayou wetland (mean depth = 11.6 cm; flow rates = 0.15–0.43 m³ s⁻¹). Given the effect of O₂ on methanogenesis, nitrification, and denitrification (Reddy et al., 1989; Mitsch and Gosselink, 2007), we surmise that these hydrologic differences are the principal driver for patterns in wetland gas emissions, with stagnant wetland conditions favoring CH₄-production (Altor and Mitsch, 2006) in Ark Bayou, and steady flow conditions favoring N₂O-production (Hernandez and Mitsch, 2006) in Cow Oak Bayou (Table 2).

By combining several physical, chemical, and microbial genetic variables we detected high spatial variation, and the presence of hotspots, in greenhouse gas emissions due to soil microbial activity in the Mississippi Delta agricultural region. Soil water saturation appeared to be a critical driver of these hotspots, which varied in location for CH₄, N₂O, and CO₂. Even between neighboring wetlands, there was a distinct difference in gas production, probably associated with the hydrologic regime. After assessing the importance of vegetation on gas emissions, we conclude that vegetation reduced gas emissions in riparian zone soils, but had no effect in these wetland habitats. We do not know if the difference in plant effects in riparian vs. wetland sites was due to differences in soil conditions, plant species, or some combination of site and plant community. Agricultural ecosystems are widespread, often intensively fertilized, extensively irrigated, and on a global basis can be significant in greenhouse gas production (Smith et al., 2007). This study indicates the important role that landscape heterogeneity within these highly managed systems can play in greenhouse gas production.

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