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Sarah E. Andrews
University of New Hampshire - Main Campus, Sarah.Andrews@unh.edu

R Schultz
Ohio State University - Main Campus

Serita D. Frey
University of New Hampshire - Main Campus, serita.frey@unh.edu

V Bouchard
Ohio State University - Main Campus

R Varner
University of New Hampshire - Main Campus

See next page for additional authors

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Plant community structure mediates potential methane production and potential iron reduction in wetland mesocosms

S. E. ANDREWS,[1,†] R. SCHULTZ,[2,4] S. D. FREY,[1] V. BOUCHARD,[2] R. VARNER,[3] AND M. J. DUCEY[1]

[1]*Department of Natural Resources and the Environment, University of New Hampshire, Durham, New Hampshire 03824 USA*
[2]*School of Environment and Natural Resources, Ohio State University, Columbus, Ohio 43210 USA*
[3]*Earth Systems Research Center, Institute for the Study of Earth Oceans and Space, University of New Hampshire, Durham, New Hampshire 03824 USA*

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Abstract. Wetlands are the largest natural source of methane to the atmosphere, but factors controlling methane emissions from wetlands are a major source of uncertainty in greenhouse gas budgets and projections of future climate change. We conducted a controlled outdoor mesocosm experiment to assess the effects of plant community structure (functional group richness and composition) on potential methane production and potential iron reduction in freshwater emergent marshes. Four plant functional groups (facultative annuals, obligate annuals, reeds, and tussocks) were arranged in a full-factorial design and additional mesocosms were assigned as no-plant controls. Soil samples from the top 10 cm were collected three times during the growing season to determine potential methane production and potential iron reduction (in unamended soils and in soils amended with 200 mM formate). These data were compared to soil organic matter, soil pH, and previously published data on above and belowground plant biomass. We found that functional group richness was less important than the presence of specific functional groups (reeds or tussocks) in mediating potential iron reduction. In our mesocosms, where oxidized iron was abundant and electron donors were limiting, iron reducing bacteria outcompeted methanogens, keeping methane production barely detectable in unamended lab incubations. When the possibility of re-oxidizing iron was eliminated via anaerobic incubations and the electron donor limitation was removed by adding formate, potential methane production increased and followed the same patterns as potential iron reduction. Our findings suggest that in the absence of abundant oxidized iron and/or the presence of abundant electron donors, wetlands dominated by either reeds or tussocks may have increased methane production compared to wetlands dominated by annuals. Depending on functional traits such as plant transport and rhizospheric oxygenation capacities, this could potentially lead to increased methane emissions in some wetlands. Additional research examining the role these plant functional groups play in other aspects of methane dynamics will be useful given the importance of methane as a greenhouse gas.

Key words: freshwater emergent marsh; functional group composition; functional group richness; iron reduction; methanogenesis.

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[4] Present address: Center for Earth and Environmental Science, State University of New York, Plattsburgh, New York 12901 USA.

† **E-mail:** sarah.andrews@wildcats.unh.edu

Introduction

The structure and function of ecosystems are being greatly altered by human activities (Vitousek et al. 1997), and the resulting loss of species worldwide has led to increasing concern for the consequences of reduced biodiversity across a wide range of ecosystems (Naeem et al. 1994, Chapin et al. 2000, Loreau et al. 2001, Zedler et al. 2001, Hooper et al. 2005, Lovett et al. 2009, Geyer et al. 2011). Freshwater wetland ecosystems provide a number of valuable services, including biodiversity support, water quality maintenance and improvement, flood control, and carbon storage (Zedler and Kercher 2005). While there are a number of studies documenting changes in wetland biodiversity as a consequence of human activities (Findlay and Houlahan 1997, Findlay and Bourdages 2000, Houlahan and Findlay 2004, Rosas et al. 2006, Schooler et al. 2006), there have been fewer studies on links between changes in biodiversity and functions that support wetland services (Engelhardt and Ritchie 2001, Mahaney et al. 2006, Bouchard et al. 2007, Schultz et al. 2011).

Alterations in plant community structure can affect ecosystem functioning because plants differ in their rates and mechanisms of resource utilization and in how they influence other plants and their physical environment (Chapin et al. 2000). Growing research indicates that restored and created wetlands do not exhibit or maintain the same functional or physical characteristics, including plant biomass, plant species richness, and biogeochemical functions, as natural wetlands (Zedler et al. 2001, Hossler et al. 2011, Moreno-Mateos et al. 2012). Thus, it is especially important that we understand the role that plant community structure plays in mediating wetland properties such as methane production and oxidation, organic matter dynamics, and plant biomass (Joabsson et al. 1999, Engelhardt and Richie 2001, Vann and Megonigal 2003, Bouchard et al. 2007).

Of the long-lived greenhouse gases, methane is second only to CO_2 in radiative forcing (Forster et al. 2007); because methane is such a potent greenhouse gas, understanding factors that affect methane dynamics is especially important. Wetlands are the single largest natural source of methane to the atmosphere, contributing an estimated 100–231 Tg CH_4 yr^{-1} (Christensen et al. 2003, Shindell 2004, Denman et al. 2007). Freshwater wetlands may account for 20–39% of the total global methane emissions and as much as 90% of natural emissions (Denman et al. 2007). Previous studies have measured highly variable rates of methane production in wetland sediments (Schimel 1995, Whalen and Reeburgh 2000, Krüger et al. 2001, Freeman et al. 2002, Megonigal and Schlesinger 2002, Keller et al. 2005, Welsch and Yavitt 2007, Sutton-Grier and Megonigal 2011) but the controlling factors contributing to this variability are still not well understood. Despite the significance of freshwater wetlands as a source of methane, this continued uncertainty about controls on sediment methane cycling stresses the need for further research of belowground processes. This task is particularly complex in vegetated wetlands due to the number of feedbacks between plants and microbes, the number of transport pathways plants provide, and competition with other microbes for electron donors (see Laanbroek 2010 for a review).

Methane production is performed by methanogenic archaea in the anaerobic zones of wetland sediments, but methanogens face competition for methanogenic substrates (i.e., organic acids for acetoclastic methanogens and hydrogen for hydrogenotrophic methanogens) from bacteria that can utilize more energetically favorable electron acceptors such as sulfate and oxidized iron. Competition with sulfate reducers is more likely to dominate in marine or other sulfate rich wetlands, while in freshwater wetlands competition with iron reducers is more likely (Laanbroek 2010). This competition has been shown to suppress methane production and reduce methane emissions (Roden and Wetzel 1996, van der Nat and Middelburg 1998, Frenzel et al. 1999, Neubauer et al. 2005). Plants contribute to these processes by providing carbon substrates (electron donors) to methanogens and their competitors through root exudation and root turnover and by creating an oxygenated rhizosphere where reduced iron can be re-oxidized and methane can be consumed (Laanbroek 2010).

While there have been studies examining the effects of specific plant species on methane dynamics (Chanton et al. 1993, Calhoun and King 1997, Ström et al. 2005, Smialek et al. 2006,

Welsch and Yavitt 2007), including studies looking at suppression by iron reducers (Roden and Wetzel 1996, Frenzel et al. 1999, Neubauer et al. 2005, Sutton-Grier and Megonigal 2011), there has been very little work looking at the effects of plant community structure on methane or iron cycling. As part of a larger project, our overall objective was to explore the interactions between plant community structure and belowground processes in the context of experimental freshwater wetland mesocosms. Here we focus specifically on the effects of plant community structure (functional group richness and composition) on potential methane production and potential iron reduction. We chose to utilize functional groups (facultative annuals, obligate annuals, reeds, and tussocks) rather than individual species in order to account for redundancy among species with similar functional traits.

Previous findings suggest that belowground biomass can increase with functional group richness (the number of functional groups present) as plants from different functional groups penetrate different niches (Bouchard et al. 2007). Increases in belowground biomass should lead to greater root turnover and root exudates, which contribute to the pool of available carbon substrates for methanogens and iron reducing bacteria. With that in mind, we chose functional groups that differ in their belowground biomass and morphology: facultative and obligate annuals tend to have shallow roots and lower root biomass than the reeds and tussocks, which typically have increased root biomass, penetrate more deeply and exhibit lateral spreading (Boutin and Keddy 1993). We hypothesized that potential methane production and potential iron reduction would be positively correlated with (1) increases in belowground plant biomass, (2) increases in plant functional group richness, and (3) the presence of reeds and/or tussocks.

Methods

Site description and experimental design

We tested our hypotheses using outdoor experimental wetland mesocosms that allowed us to manipulate the number and composition of functional groups present in the plant community. The mesocosms (130 cm length × 86 cm width × 40 cm depth) were located at the Waterman Agricultural and Natural Resources Laboratory on the main campus of Ohio State University. All mesocosms were filled with low organic matter soil (50/50 silt and sand mix, 3% C, 0.03% N, 2.6% Fe) to minimize the effects of existing organic matter stocks on carbon and nutrient cycling. Four plant functional groups (facultative annuals, obligate annuals, reeds, and tussocks) were chosen to represent a range of plants known to associate closely in freshwater emergent marshes, and were defined based on physiological, morphological, and life history traits (Boutin and Keddy 1993). The functional groups were arranged in a full-factorial design, giving 16 levels of functional group composition (one with no plants, four with single functional groups, six with two functional groups, four with three functional groups, and one with all four functional groups; FGC); each level was randomly assigned to five mesocosms. Throughout the paper we refer to these by capital letter designations: C (no-plant control), F (facultative annuals), O (obligate annuals), R (reeds), and T (tussocks). When more than one functional group is present, they are designated by the combination of letters. For example, FT indicates a community with both facultative annuals and tussocks. These treatments also gave us the ability to look at five levels (0–4) of functional group richness (number of functional groups present; FGR) and the presence/absence of each functional group (denoted by italicized capital letters). Plantings (18–20 plants per mesocosm) occurred in June 2006, with each functional group represented by four species (Appendix: Table A1). A drip irrigation system was installed to keep the mesocosms flooded during the growing season and the mesocosms were irrigated every three days to keep the water level at 10 cm above the soil surface. Further details on the site and experimental design are reported in Schultz et al. (2012).

Plant sampling and analysis

Details on plant sampling are reported in Schultz et al. (2012). Briefly, destructive sampling for root and shoot biomass was conducted at peak biomass (1 September 2008) from one half of each mesocosm. All stems were clipped down to the soil surface and plants were sorted for each plot by species and placed in paper bags and

dried. Immediately after aboveground samples were collected, two soil cores (7 cm diameter, 10 cm depth) were collected from each mesocosm and bulked for analysis of root biomass. Soil cores for root biomass were washed with a Delta-T Root Washer (500 μm mesh filter; Delta-T Devices Ltd, Cambridge, UK) followed by a 1 mm sieve and stored at −10°C until analysis. Live roots were then manually sorted from detritus. Root and shoot samples were oven dried for 72 hours at 55°C to constant mass. Subsamples of dried roots and shoots were then combusted in a muffle oven at 450°C for eight hours to determine the ash-free dry weight.

Soil sampling and analysis

Soil samples were collected in June, August, and November 2008. Because these were young systems (only two years old) we expected to see stronger effects of plants close to the soil surface (top 10 cm) where most of the root growth was occurring (root biomass increased by 219% in the top 10 cm from 2007 to 2008 but only by 33% below 10 cm; Schultz 2010). Therefore, because logistical constraints limited our ability to look at multiple depths, we chose to use the top 10 cm of soil. It should be noted however that due to this approach we may have missed treatment effects of root distribution on potential methane production and potential iron reduction. Surface water was temporarily drained and three soil cores (2 cm diameter) were collected from each mesocosm and bulked for analysis. Soil samples were immediately double bagged in re-sealable plastic zipper bags (to minimize oxygen infiltration), homogenized, and shipped overnight to the University of New Hampshire, where they were stored at 4°C until analysis.

Subsamples were used to estimate potential methane production and potential iron reduction and to measure soil moisture, soil organic matter (SOM), and pH. Soil moisture was determined by drying subsamples at 105°C to constant mass. The dried soils were then combusted in a muffle oven at 450°C for 6 hours to determine organic matter by loss-on-ignition. The compact nature of the soils and the high concentration of fine roots made it difficult to remove all roots from the samples; as a consequence the SOM results may include some fine root biomass. Soil pH was determined on 1:2 soil:deionized water slurries.

Potential methane production and potential iron reduction

Early trial incubations in unamended soils (only water added) yielded very low (often undetectable) rates of potential methane production (PMP) and so we chose to test a variety of carbon substrate amendments utilized by both methanogens and iron reducers (acetate, formate, and an H_2-CO_2 gas mix). In these test runs, potential methane production and potential iron reduction did not differ significantly between acetate amended soils and unamended soils, nor between formate amended soils and H_2-CO_2 amended soils (S. E. Andrews, *unpublished data*). Therefore, due to time and space constraints, we chose to use formate as the carbon substrate amendment for all subsequent incubations. For potential methane production, soils (1 g wet weight ± 0.1 g) were loaded into 20 mL clear serum vials inside an anaerobic N_2 filled chamber. Vials were capped with red rubber septa and sealed prior to removal from the chamber. Vials were then flushed with ultrapure N_2 for two minutes to ensure anaerobic conditions and were incubated overnight at 25°C. The following day, 2 mL of either deionized water (unamended) or 200 mM formate (amended) were injected through the septa, and vials were vortexed for 30 seconds and incubated for 10 days at 25°C. Vials were flushed with N_2 gas for two minutes twice within the first three days of the incubation to ensure that anaerobic conditions were being maintained. Four headspace samples were taken starting at 72 hours after the last flush (day six): vials were vortexed for 30 seconds before the entire headspace was evacuated into 60 mL syringes (used to create a strong enough vacuum to pull the bulk of the headspace from the vials). Immediately after sampling, vials were re-flushed with ultrapure N_2 for two minutes to maintain pressure and anaerobic conditions. Headspace samples were stored in evacuated 20 mL clear serum vials at room temperature until the contents could be analyzed for methane concentration (within 24 hours of headspace sample collection). Triplicate blanks (3 mL deionized water) were treated and sampled identically to the soil samples. Methane samples were analyzed using a gas chromatograph equipped with a flame ionization detector and a 1 mL sample loop (Shimadzu GC-8A, Shimad-

zu, Kyoto, Japan). The carrier gas was N_2 with a flow of 30 mL/min. Standardization was done using a calibrated breathing air cylinder based on NOAA ESRL standards. Linear regression analysis (PROC REG, SAS 9.3) was used to calculate the rate of methane produced over time. Rates were accepted for lines with $r^2 \geq 0.75$ and $P \leq 0.05$ (95% of rates met these criteria); rates that did not meet these criteria were not accepted and were treated as missing data.

Incubations for potential iron reduction (PIR) were conducted on soils collected in August and November of 2008. To determine potential iron reduction, Fe(II) concentrations were measured twice: once on unamended soil samples that were destructively sampled prior to the incubation (treated exactly as those for potential methane production except all vials received only 2 mL of water, Fe(II) was determined immediately after vials were vortexed on the first day, and soils were subsequently discarded) and once on the soil samples that were used to measure potential methane production (both the amended and unamended soils were destructively sampled after the last methane headspace sample was taken). Potential iron reduction rates were then determined by subtracting the initial Fe(II) concentration from the final Fe(II) concentration and dividing by the length of the incubation (the Fe(II) determined on unamended soils destructively sampled prior to incubation was used as the initial Fe(II) concentration for both the amended and unamended incubated soils). While we were unable to measure Fe(II) at more than two sampling times, others have found linear increases in iron reduction within the first three to twenty days of incubation (Roden and Wetzel 1996, Frenzel et al. 1999, and Roden and Wetzel 2003). We cannot rule out the possibility that our rates might not be linear but our rates do reflect the total accumulated iron reduced during our incubation and thus do not limit our ability to make comparisons across the treatments.

The method used to determine reduced iron content was modified from methods used by others (Sørensen 1982, Lovley and Phillips 1987, Achtnich et al. 1995) so that samples could be analyzed using a microplate reader. Briefly, vials were shaken and 0.3 mL of slurry was incubated with 5 mL of 0.5 M HCl for one hour at room temperature to dissolve poorly crystalline iron. After incubation, the slurry-HCl mix was shaken and 0.1 mL was added to 1 mL of Ferrozine reagent (1 g Ferrozine in 1000 mL of 50 mM HEPES buffer) in 2 mL amber microcentrifuge tubes. Tubes were centrifuged at 10,000 g for two minutes (Microfuge Centrifuge, Beckman Coulter, Brea, California, USA) and then samples were pipetted into microplates (clear, flat-bottomed, 96 well, 350 μl well volume). Absorbance at 562 nm was measured immediately on a Synergy HT microplate reader (BioTek Instruments, Winooski, Vermont, USA) using Gen5 Data Analysis software (2005). Triplicate blanks (deionized water) were treated identically to the soil samples. Initial and final Fe(II) concentrations were determined using standards made from ferrous ammonium sulfate.

Statistical analyses

To test for the effects of functional group richness, functional group composition, and presence/absence of functional groups, separate linear mixed effects models (LME) were done with either potential methane production or potential iron reduction as the dependent variable (PROC MIXED, SAS 9.3). All data were log transformed to adjust for heteroscedasticity and non-normality. We used LME for several reasons. First, LME are less sensitive to missing observations than ANOVA (SAS Institute 2008) and the 5% of our potential methane production rates that did not satisfy our criteria for acceptance ($r^2 \geq 0.75$, $P \leq 0.05$) were treated as missing observations. Second, our design includes a repeated measure (month) on each mesocosm (a random effect), which gives rise to correlated errors. By using LME we could model an appropriate covariance structure to account for correlated errors (Littell et al. 2006). Finally, we also have a split-plot factor (amended versus unamended) that gives rise to multiple sources of random error. In SAS, ANOVAs (proc GLM) incorrectly compute standard errors for interactions in split-plot experiments and cannot complete a correct analysis; therefore LME are recommended (Littell et al. 2006).

For all models a factorial analysis of the fixed effects was performed, where the whole-plot factor was functional group richness (0–4), functional group composition (16 levels), or presence/absence of functional groups (factorial

of *F*, *O*, *R*, and *T*; coded as present or absent), the repeated measure was month (June, August, and November for methane and August and November for iron), the split-plot factor was substrate (amended or unamended), and mesocosm was specified as a random effect (nested in the whole-plot factor). For the presence/absence models the no-plant controls were removed from analysis and only two-way interactions amongst *F*, *O*, *R*, and *T* were included. Including the higher-order interactions in the models resulted in parameter instability that in some cases led to least square means estimates outside of the data range. While there were a few statistically significant three-way interactions in earlier models, these were not biologically meaningful nor did they explain anything that could not already be seen from the two-way interactions. Similar presence/absence models were run with root biomass or shoot biomass as the dependent variable (see Schultz et al. 2012 for the effects of functional group richness and composition on plant biomass) but neither substrate nor month was included in those models as they were not applicable. Models were also run using pH or soil organic matter (SOM) as dependent variables; substrate was not included in these models as pH and SOM were measured prior to amendment.

Models were left in their full form because we were more interested in exploring significant effects than parsing down to predictive models at this stage. Where main effects were significant, differences in least squares means were assessed using Tukey's test of multiple comparisons. For significant interactions, differences in means of one effect were examined while holding the other effect(s) constant. Relationships amongst response variables (potential methane production, potential iron reduction, root biomass, shoot biomass, soil pH, and SOM) were analyzed separately by Pearson correlations for all combinations of response variables (proc CORR, SAS 9.3).

Results

Potential methane production and potential iron reduction

Potential methane production and potential iron reduction were significantly higher in amended soils than unamended for all sampling months (Fig. 1). For potential methane production there was a significant interaction between month and substrate (Table 1): for unamended soils there were no significant differences in potential methane production amongst months ($<$0.07 ng CH_4-C·g^{-1}·dry soil·h^{-1}), but soils amended with formate had significantly higher potential methane production in June (0.43 ng CH_4-C·g^{-1} dry soil·h^{-1}) than in August or November (0.29 and 0.25 ng CH_4-C·g^{-1} dry soil·h^{-1}, respectively; Fig. 1A). There was no month $\times$ substrate interaction for potential iron reduction (Table 1): regardless of substrate addition, potential iron reduction was significantly higher in August than in November (13.16 and 8.17 μg Fe(II)·g^{-1} dry soil·h^{-1}, respectively; note that data in Fig. 1B is shown by month and substrate so that differences can be more easily visualized).

There were no significant interactions between month and functional group richness or month and functional group composition for either potential methane production or potential iron reduction (data not shown). The lack of significant interactions indicates that even though rates decreased with month (Fig. 1), the patterns remained the same (data not shown). Therefore, for the remaining results, data from all sampling months were combined.

Functional group richness and composition

Potential methane production in unamended soils did not vary with functional group richness, however, potential methane production in amended soils was lowest (0.08 ng CH_4-C·g^{-1} dry soil·h^{-1}) in the no-plant controls and highest (0.62 ng CH_4-C·g^{-1} dry soil·h^{-1}) when all four functional groups were present (Fig. 2A; FGR $\times$ substrate interaction in Table 1). For unamended and amended soils potential iron reduction was also lowest (2.35 and 6.74 μg Fe(II)·g^{-1} dry soil·h^{-1}, respectively) in the no-plant controls and highest (7.74 and 22.00 μg Fe(II)·g^{-1} dry soil·h^{-1}, respectively) when all four functional groups were present (Fig. 2C). While functional group richness was a significant effect for both potential methane production (amended only) and potential iron reduction (amended and unamended; Table 1), the only significant differences amongst treatment levels were between the

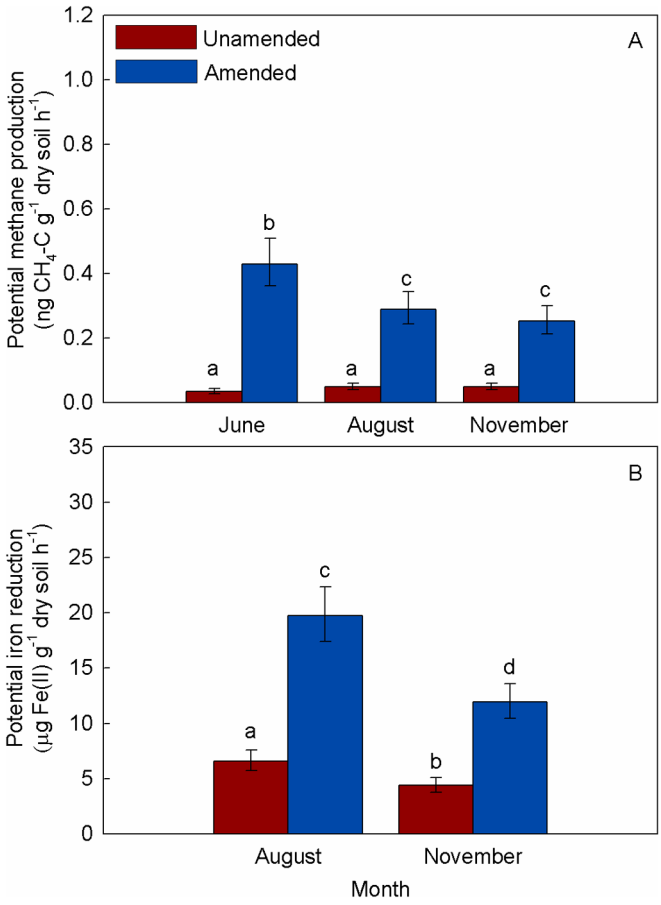

Fig. 1. Potential methane production (A) and potential iron reduction (B) by month and substrate. Unamended (red bars) = soils incubated with water alone; amended (blue bars) = soils amended with 200 mM formate. All 16 treatments (including no-plant controls) included. Bars are geometric means with 95% CI. Within a panel, bars with different letters are significantly different ($P < 0.05$).

no-plant controls and the vegetated treatments (0 and 1–4 respectively, Fig. 2A, C). Soil pH and SOM showed similar patterns (though pH in the no-plant controls was higher than other levels of richness rather than lower; Table 1 and Fig. 2B, D); however, the models for pH and SOM were not significantly better than the null models (i.e., models without fixed effects) and so we cannot say that there were truly differences in pH or SOM with the treatments. This is most likely due to the very narrow ranges over which pH and SOM varied (Fig. 2A, B; Appendix: Table A2).

While potential methane production (amended only) and potential iron reduction (unamended and amended) varied amongst the 16 levels of plant community composition (Appendix: Table A3), and functional group composition was a significant effect (Table 1), the only significant findings for vegetated treatments were that potential methane production in amended soils was significantly lower in the O treatment (0.14 ng CH_4-C·g^{-1} dry soil·h^{-1}) than in the T, FOR, and FORT treatments (0.49, 0.52, and 0.62 ng CH_4-C·g^{-1} dry soil·h^{-1}, respectively), while potential iron reduction in the amended soils was significantly lower in the O and F treatments (8.88 and 9.23 μg Fe(II)·g^{-1} dry soil·h^{-1}, respectively) than in the OR treatment (26.94 μg Fe(II)·g^{-1} dry soil·h^{-1}; Appendix: Table A3). Potential iron reduction in unamended soils did not vary significantly with functional group

Table 1. Linear mixed effect model F values (with df in parentheses) for main effects and significant interactions from functional group richness (FGR), functional group composition (FGC), and presence/absence models for soil response variables.

Main effects and interactions	PMP	PIR	pH	SOM
FGR model				
FGR	4.88 (4, 71)**	6.25 (4, 74)***	5.80 (4, 75)***	4.98 (4, 74)***
Month	0.95 (2, 276)	20.93 (1, 147)***	62.34 (2, 149)***	1.43 (2, 147)
Substrate	256.10 (1, 69)***	336.49 1, 70)***	n.a.	n.a.
FGR × substrate	4.29 (4, 70)**	1.98 (4, 71)	n.a.	n.a.
Month × substrate	11.59 (2, 276)***	1.16 (1, 147)	n.a.	n.a.
FGC model				
FGC	3.64 (15, 61)***	3.97 (15, 64)***	3.39 (15, 65)***	2.81 (15, 62)**
Month	1.33 (2, 237)	44.63 (1, 128)***	95.26 (2, 128)***	6.42 (2, 124)**
Substrate	470.77 (1, 61)***	573.85 (1, 61)***	n.a.	n.a.
FGC × substrate	2.09 (15, 61)*	1.17 (15, 61)	n.a.	n.a.
Month × substrate	13.66 (2, 237)***	1.16 (1, 128)	n.a.	n.a.
Pres/abs model				
F	0.16 (1, 62)	0.68 (1, 64)	0.02 (1, 65)	1.99 (1, 62)
O	1.31 (1, 63)	1.05 (1, 64)	0.27 (1, 65)	0.08 (1, 62)
R	4.81 (1, 63)*	13.42 (1, 64)***	8.16 (1, 64)**	9.98 (1, 61)**
T	1.12 (1, 63)	4.09 (1, 64)*	0.74 (1, 65)	0.00 (1, 62)
R × *T*	9.55 (1, 61)**	5.19 (1, 64)*	3.72 (1, 65)	3.19 (1, 61)
Month	1.12 (2, 237)	31.45 (1, 127)***	85.19 (2, 127)***	4.11 (2, 124)*
Substrate	442.57 (1, 61)***	454.43 (1, 61)***	n.a.	n.a.
R × *T* × substrate	4.47 (1, 60)*	4.59 (1, 61)*	n.a.	n.a.
Month × substrate	11.22 (2, 237)***	0.90 (1, 127)	n.a.	n.a.

Notes: PMP = potential methane production, PIR = potential iron reduction, SOM = soil organic matter, *F* = presence/absence of facultative annuals, *O* = presence/absence of obligate annuals, *R* = presence/absence of reeds, and *T* = presence/absence of tussocks. Month = June, August, and November 2008 for PMP, SOM, and pH and August and November 2008 for PIR. Substrate = unamended (water only) versus amended (200 mM formate). SOM and pH were determined prior to amendment. For SOM and pH the fitted models are not better than the null models. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n.a. = not applicable,

composition; however, the trends were the same as for the amended soils (Appendix: Table A3).

When looking at the presence of each of the four functional groups compared to their absence, a more significant pattern emerged: potential methane production (amended only) and potential iron reduction (amended and unamended) were enhanced when either reeds or tussocks were present compared to when they were both absent (Fig. 3). In the absence of reeds potential methane production (amended only) and potential iron reduction were higher when tussocks were present compared to absent (though not significant for unamended potential iron reduction). Similarly, when tussocks were absent, potential methane production (amended only) and potential iron reduction (amended and unamended) were higher when reeds were present compared to absent. However, when reeds and tussocks were present together, neither potential methane production nor potential iron reduction was greater than when reeds were present without tussocks or when tussocks were present without reeds. The presence of reeds and tussocks together did result in significantly greater potential iron reduction (amended and unamended) than the absence of both (Fig. 3C, D), but potential methane production was not significantly greater when both reeds and tussocks were present than when both were absent (Fig. 3B). Root and shoot biomass were also significantly greater in the presence of reeds and/or tussocks compared to the absence of both (Fig. 4 and Appendix: Table A4).

Relationships amongst soil and plant properties

Potential methane production was positively correlated with potential iron reduction in amended and unamended soils (Table 2). Potential methane production (amended only) and potential iron reduction (amended and unamended) were positively correlated with root and shoot biomass (Table 2). Soil organic matter (SOM) remained low (1.6–2.4%) and soil pH remained slightly alkaline (7.7–8.0) throughout the experiment (Appendix: Table A2). Despite the narrow range over which SOM and pH varied,

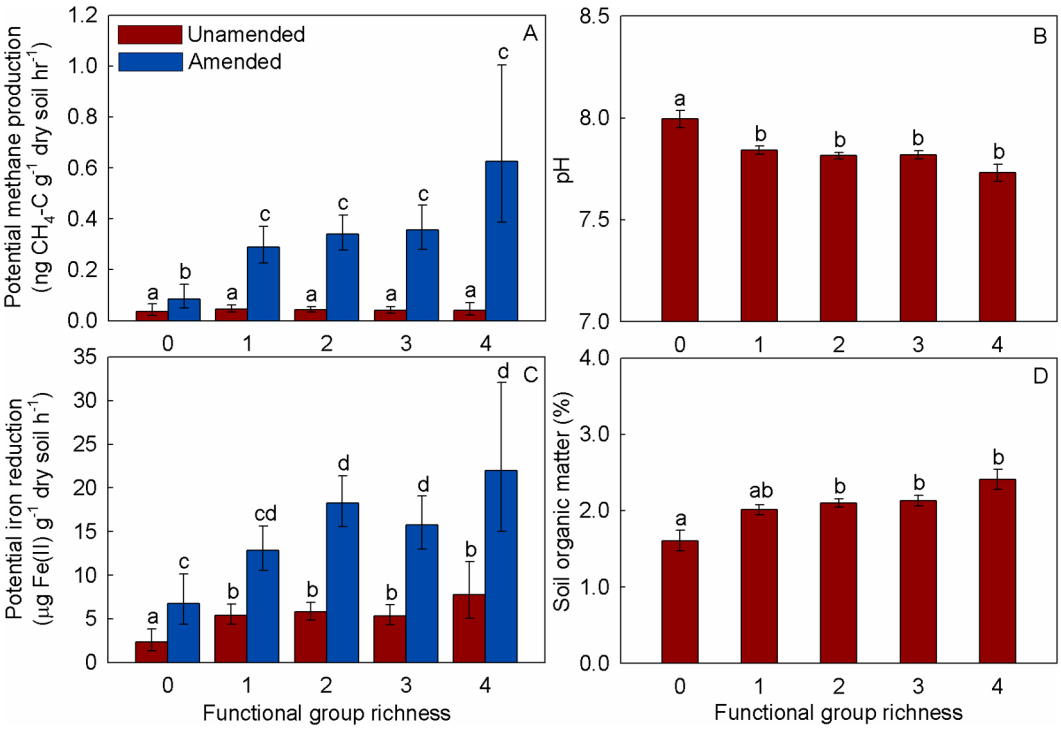

Fig. 2. The effect of functional group richness on potential methane production (A), soil pH (B), potential iron reduction (C), and soil organic matter (D). Unamended (red bars) = soils incubated with water alone; amended (blue bars) = soils amended with 200 mM formate. All sampling months are included. For (A) and (C) bars are geometric means with 95% CI; for (B) and (D) bars are arithmetic means ± 1 SE. Within a panel, bars with different letters are significantly different ($P < 0.05$). Note that the y-axis in (B) does not start at zero. Note also that the models for pH (B) and SOM (D) were not significantly better than the null models.

potential methane production (amended only) was significantly correlated with pH (negatively) and SOM (positively). Soil organic matter was also positively correlated with both root and shoot biomass, and pH was negatively correlated with SOM and root biomass but not shoot biomass. There were no significant correlations between potential iron reduction and pH or SOM (Table 2).

Discussion

We manipulated freshwater wetland plant functional groups in controlled outdoor mesocosms to study the effects of plant community structure on potential methane production and potential iron reduction. We hypothesized that potential methane production and potential iron reduction would increase with belowground biomass and that factors we expected would lead to increased belowground biomass (functional group richness and presence of reeds and/or tussocks) would also be correlated with increases in potential methane production and potential iron reduction.

We found that the plant community had a significant effect on microbial activity, but this effect was primarily on potential iron reduction. In unamended soils, where plant effects were not masked by the addition of a carbon substrate, we found a significant positive correlation between potential iron reduction and plant biomass (root and shoot), significant increases in potential iron reduction in vegetated treatments compared to the no-plant controls, and significantly higher potential iron reduction in the presence of reeds or both tussocks and reeds together compared to the absence of both, lending partial support to all of our hypotheses. We found these same patterns in amended soils (potential iron reduction and

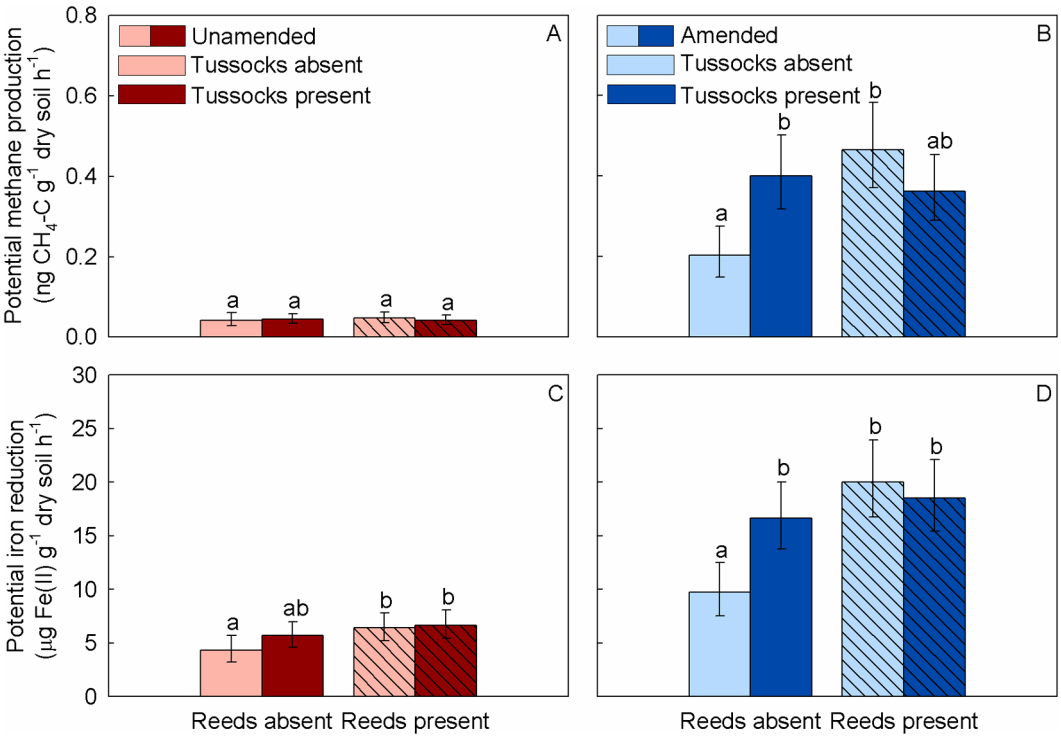

Fig. 3. The effect of functional group composition on potential methane production (A and B) and potential iron reduction (C and D) for unamended (water only, light and dark red bars) and amended (200 mM formate, light and dark blue bars) soils. In all four panels light bars = absence of tussocks, dark bars = presence of tussocks, unhatched bars = absence of reeds, and hatched bars = presence of reeds. All sampling months are included but the no-plant controls were excluded from analysis. Bars are geometric means with 95% CI. Within a panel, bars with different letters are significantly different ($P < 0.05$).

potential methane production), suggesting that the effects of plants on microbial activity were not masked entirely by the formate amendment.

Several studies have shown an inverse relationship between plant biomass and methane emissions from wetlands, which is often attributed to increased rhizospheric oxygenation and methane consumption with increasing biomass (Ström et al. 2005, Bouchard et al. 2007, Kao-Kniffin et al. 2010, Koelbener et al. 2010). Our finding that iron reduction was occurring in unamended soils while potential methane production remained barely detectable suggests that another possible explanation for this inverse relationship may be that competition from iron reducing bacteria can inhibit methane production. Iron reducing bacteria have been shown to have lower threshold concentrations for electron donors that are also utilized by methanogens (i.e., organic acids and hydrogen; Lovley 1985, Achtnich et al. 1995, Roden and Wetzel 2003). Therefore, in the presence of oxidized iron, iron reducing bacteria can outcompete methanogens by maintaining the concentration of electron donors at levels too low for methanogens to metabolize (Roden and Wetzel 1996, Frenzel et al. 1999, and Neubauer et al. 2005). If plant biomass positively affects iron reducing bacteria, as our positive correlation between plant biomass and potential iron reduction in unamended soils suggests, then in wetlands where oxidized iron is readily available an inverse relationship between plant biomass and methane emissions could be attributed to this competition from iron reducing bacteria. However, Achtnich et al. (1995) found that excess oxidized iron inhibited methanogenesis only if electron donors were limiting in the soil. Because SOM was low (~2%) in our soils (we started with low OM soil to minimize the effects of existing SOM stocks on carbon and

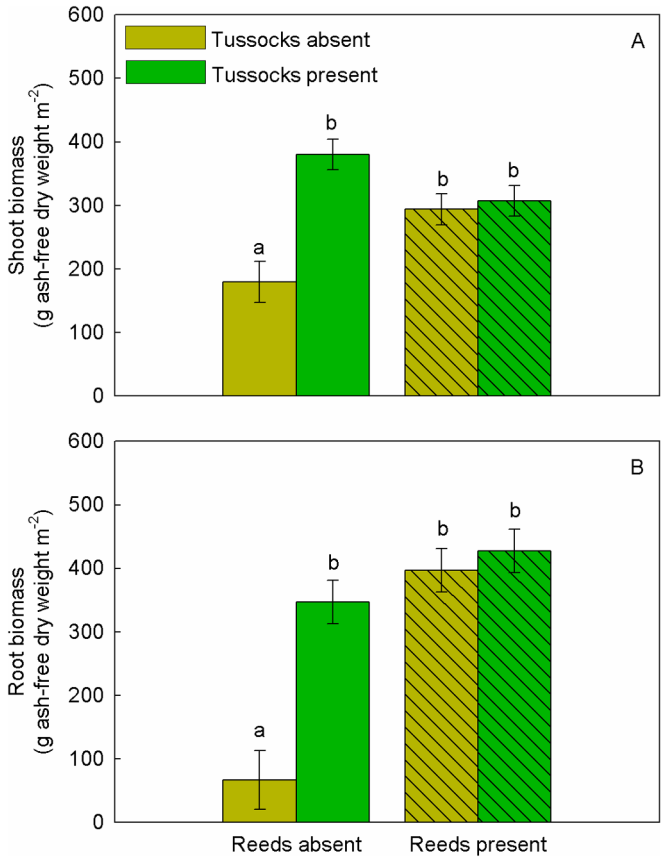

Fig. 4. The effect of functional group composition on shoot biomass (A) and root biomass (B) at peak biomass (early September). In both panels gold bars = absence of tussocks, green bars = presence of tussocks, unhatched bars = absence of reeds, and hatched bars = presence of reeds. The no-plant controls were excluded from analysis. Bars are arithmetic means ± 1 SE. Within a panel, bars with different letters are significantly different ($P < 0.05$).

nutrient cycling) and our systems were young (2 years), our soils were likely limited in electron donor availability. This is supported by our finding that potential methane production and potential iron reduction were both enhanced substantially in soils amended with formate compared to unamended soils.

In addition to providing carbon substrates to iron reducing bacteria, plants have been shown to positively influence iron reduction by creating

Table 2. Correlation coefficients for relationships amongst soil and plant response variables.

Response variable	Log(UPIR)	Log(APIR)	pH	SOM	Root biomass	Shoot biomass
Log(UPMP)	0.26**		0.18	0.15	0.13	0.10
Log(APMP)		0.47***	−0.32***	0.32***	0.41***	0.41***
Log(UPIR)			−0.11	0.15	0.23*	0.24*
Log(APIR)			−0.05	0.12	0.47***	0.36**
pH				−0.28***	−0.46***	−0.12
SOM					0.28*	0.24*

Notes: Log(UPMP) = log transformed unamended (water only) potential methane production, Log(APMP) = log transformed amended (200 mM formate) potential methane production, Log(UPIR) = log transformed unamended potential iron reduction, Log(APIR) = log transformed amended potential iron reduction, and SOM = soil organic matter. Correlations amongst PMP, pH, and SOM include June, August, and November 2008; correlations amongst PIR, pH, and SOM include August and November 2008; correlations with root and shoot biomass include only August 2008. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

an oxygenated rhizosphere where reduced iron can be re-oxidized (Roden and Wetzel 1996, Neubauer et al. 2005). Whether re-oxidation of iron in the rhizosphere was a factor limiting methane production in our soils is difficult to determine for two reasons. First, our starting soils contained high levels of total iron (25,000 mg Fe/kg soil) and the water used to flood our mesocosms likely brought in additional iron (total iron increased to an average of 27,000 mg Fe/kg soil after 18 months of flooding; Schultz 2010). This external source of iron may have masked any effects due to internal cycling of iron. Secondly, if re-oxidation was an important limitation on methane production in situ, we would expect to see an increase in methane production in the lab when re-oxidation of iron was prevented via anaerobic incubations. However, while we did see this in our amended soils (where electron donors were not limiting), this was not the case for our unamended soils. This is most likely due to low electron donor availability in conjunction with the high oxidized iron availability in our unamended soils. In our short incubations (10 days), and in situ where we were unable to detect methane emissions, iron reducing bacteria were likely unable to draw down the oxidized iron far enough for methanogens to compete successfully for the electron donors (though we cannot say for sure because we were only able to measure reduced iron at two sampling points). In the lab, our amended incubations removed the electron donor limitation, allowing methanogenic activity to increase to the point were patterns were detectable.

As reported in Schultz et al. (2012), the belowground biomass in these mesocosms increased significantly with functional group richness ($1 < 2 = 3 < 4$). However, while we did find significant positive relationships between functional group richness and potential methane production (amended only) and functional group richness and potential iron reduction (amended and unamended), the only significant differences were between the no-plant controls and the planted treatments, which does not fully support our second hypothesis. This suggests that the presence of vegetation was more important than plant functional group richness in mediating potential methane production and potential iron reduction. However, looking at richness masks differences among treatments in plant community composition within the same level of richness: knowing which functional groups are present is more important in this case than the number of functional groups present. When looking at the presence/absence of functional groups, we found that in vegetated mesocosms reeds and tussocks had the most influence on potential methane production and potential iron reduction: potential methane production (amended only) and potential iron reduction (amended and unamended) were significantly enhanced in the presence of reeds or tussocks (supporting our third hypothesis). Our findings suggest that this increased potential methane production and potential iron reduction is most likely due to higher root biomass and SOM, and therefore enhanced carbon substrate availability, in the treatments containing reeds or tussocks compared to treatments containing only annuals. However, we cannot rule out the possibility that litter chemistry might have played a role. For example, Williams and Yavitt (2010) also found reduced methane production in the presence of a facultative annual (*Lythrum salicaria*) compared to a reed (*Juncus effusus*) or a tussock (*Carex lacustris*), which they attributed to variation in biochemical composition of plant litter.

Studies have shown that increases in root exudates (Koelbener et al. 2010) and methane production (van der Nat and Middelburg 1998) are positively correlated with methane emissions. Therefore, if wetland systems dominated by reeds and/or tussocks have increased methane production (due to increased quantity and/or quality of root exudates and litter), this suggests that they might also have increased methane emissions compared to wetlands dominated by annuals. However, some research has shown that methane emissions are controlled more by differences in rhizospheric oxygenation or plant transport of methane than by root exudates or methane production. For example, Schimel (1995) found that total methane production was not a good predictor of actual emissions; instead, emissions were controlled primarily by the composition of the plant community and its ability to transport methane. Additionally, Ström et al. (2005) found that despite increased carbon substrate availability (acetate) under *Eriophorum vaginatum* or *Juncus effusus,* methane emissions

were reduced compared to areas dominated by *Carex rostrata*, which they attributed to the high rhizospheric oxygenation exhibited by *E. vaginatum* and *J. effusus*.

Because of this continued uncertainty surrounding the factors that contribute most toward methane emissions, we cannot infer whether wetlands dominated by reeds or tussocks would have greater emissions that those dominated by annuals based on potential methane production rates alone. However, a recent study looking at the influence of plant functional types on methane emissions (Kao-Kniffin et al. 2010) found no methane emissions from forb treatments (similar in species composition to our facultative annuals) and variable emissions (intermediate to high) from their tussock treatments (similar in species combination to our reeds and tussocks combined). They attributed these differences in emissions to differences in plant productivity, plant transport of methane to the atmosphere, and rhizospheric oxygenation. Their findings lend support to the hypothesis that methane emissions may be enhanced in wetlands dominated by reeds or tussocks compared to those dominated by annuals.

Finally, while we planted four different functional groups as defined by Boutin and Keddy (1993), with respect to potential methane production and potential iron reduction we didn't find any functional differences between the facultative and obligate annuals or between the reeds and tussocks. This suggests that for methane production and iron reduction we functionally only had two groups: annuals and perennials. However, the same may not be true for other aspects of methane and iron dynamics such as gas transport or rhizospheric oxygenation capacity. As methane emissions are controlled by the combination of such factors, future research examining the role of functional groups on methane and iron dynamics in wetlands should consider additional plant traits in determining which functional groups to use (for example, internal gas flow mechanisms, or other traits that mediate gas transport, and quantity/quality of plant litter and root exudates).

In conclusion, we found that the presence of vegetation (compared to no-plant controls), increases in plant biomass, and the presence of reeds or tussocks (compared to mesocosms containing only annuals) led to increased potential iron reduction in amended and unamended soils. In our mesocosms, where oxidized iron was abundant and electron donors were limiting, iron reducing bacteria outcompeted methanogens, keeping potential methane production barely detectable in unamended lab incubations and preventing in situ methane emissions. This inhibition of methanogenesis by iron reducing bacteria adds to a growing body of research highlighting the importance of considering the influence of microbes that utilize alternative electron acceptors when studying methane dynamics in wetlands. When the possibility of reoxidizing iron was eliminated (anaerobic incubations) and the electron donor limitation was removed (amending with formate), potential methane production increased and followed the same patterns as potential iron reduction. Taken together these findings suggest that in systems where oxidized iron availability is high (due to large pools of oxidized iron or rapid cycling of iron), particularly in wetlands where electron donors are limiting, competition with iron reducing bacteria may be an important control on methane emissions. In the absence of abundant oxidized iron and/or the presence of abundant electron donors wetlands dominated by reeds or tussocks may have increased methane production, and, depending on functional traits such as plant transport and rhizospheric oxygenation capacities, this may lead to increased methane emissions in certain wetlands. Additional research examining the role these plant functional groups play in other aspects of methane dynamics, particularly plant transport and rhizospheric oxygenation, will be useful given the importance of methane as a greenhouse gas.

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Supplemental Material

Appendix

Table A1. Representative plant species by functional group.

Functional group	Latin name	Common name
Facultative annuals	*Eupatorium perfoliatum* L.	Common boneset
	Lycopus americanus Muhl. ex W. Bart.	American water horehound
	Mimulus ringens L.	Allegheny monkeyflower
	Verbena hastata L.	Swamp verbena
Obligate annuals	*Bidens cernua* L.	Nodding beggartick
	Echinochloa muricata (Beauv.) Fern.	Rough barnyardgrass
	Panicum dichotomiflorum Michx.	Fall panicgrass
	Polygonum pensylvanicum L.	Pennsylvania smartweed
Reeds	*Eleocharis erythropoda* Steud.	Bald spikerush
	Eleocharis palustris L.	Common spikerush
	Juncus canadensis J. Gay ex Laharp	Canadian rush
	Juncus effusus L.	Common rush
Tussocks	*Acorus calamus* L.	Calamus or sweet flag
	Calamagrostis canadensis (Michx.) Beauv.	Bluejoint
	Carex crinita Lam.	Fringed sedge
	Scirpus cyperinus (L.) Kunth	Woolgrass

Table A2. Mean pH and soil organic matter (SOM) by plant functional group composition (FGC) treatment.

FGC	pH	SOM (%)
C	8.0 (0.04)	1.61 (0.13)
F	7.9 (0.04)	1.92 (0.12)
O	7.9 (0.04)	1.78 (0.12)
R	7.8 (0.04)	2.32 (0.13)
T	7.8 (0.04)	2.05 (0.13)
FO	7.9 (0.04)	1.99 (0.12)
FR	7.8 (0.04)	2.41 (0.12)
FT	7.8 (0.04)	2.06 (0.12)
OR	7.7 (0.04)	2.13 (0.12)
OT	7.9 (0.04)	2.06 (0.12)
RT	7.8 (0.04)	1.94 (0.12)
FOR	7.8 (0.04)	2.23 (0.13)
FOT	7.8 (0.04)	2.04 (0.13)
FRT	7.8 (0.04)	2.15 (0.12)
ORT	7.9 (0.04)	2.12 (0.13)
FORT	7.7 (0.04)	2.41 (0.12)

Notes: C = no-plant controls, F = facultative annuals, O = obligate annuals, R = reeds, T = tussocks. Means are arithmetic (SE).

Table A3. Mean potential methane production (PMP) and potential iron reduction (PIR) by plant functional group composition (FGC) treatment.

FGC	Unamended PMP (ng CH_4-C·g^{-1} dry soil·h^{-1})	Amended PMP (ng CH_4-C·g^{-1} dry soil·h^{-1})	Unamended PIR (μg Fe(II)·g^{-1} dry soil·h^{-1})	Amended PIR (μg Fe(II)·g^{-1} dry soil·h^{-1})
C	0.04 (0.02–0.06)	0.09 (0.05–0.14)	2.35 (1.40–3.67)	6.74 (4.54–9.81)
F	0.04 (0.02–0.07)	0.24 (0.15–0.37)	4.50 (2.94–6.69)	9.23 (6.32–13.28)
O	0.04 (0.02–0.07)	0.14 (0.08–0.22)	3.48 (2.16–5.37)	8.88 (6.07–12.80)
R	0.06 (0.04–0.10)	0.47 (0.29–0.75)	7.54 (5.11–10.93)	18.98 (13.30–26.91)
T	0.05 (0.03–0.08)	0.49 (0.31–0.77)	6.92 (4.67–10.06)	17.67 (12.15–25.50)
FO	0.04 (0.02–0.07)	0.16 (0.10–0.25)	3.49 (2.21–5.27)	11.05 (7.63–15.83)
FR	0.04 (0.02–0.07)	0.46 (0.30–0.73)	7.52 (5.10–10.91)	21.32 (14.97–30.17)
FT	0.04 (0.02–0.06)	0.43 (0.27–0.68)	5.95 (3.76–9.13)	16.04 (10.98–23.24)
OR	0.04 (0.02–0.08)	0.42 (0.26–0.67)	5.87 (3.91–8.59)	26.94 (19.00–38.04)
OT	0.06 (0.03–0.10)	0.35 (0.22–0.56)	5.69 (3.70–8.51)	17.12 (11.74–24.77)
RT	0.04 (0.02–0.07)	0.32 (0.20–0.50)	7.21 (4.88–10.48)	20.67 (14.51–29.27)
FOR	0.05 (0.02–0.08)	0.52 (0.33–0.81)	5.16 (3.34–7.74)	14.73 (10.26–20.98)
FOT	0.04 (0.02–0.06)	0.35 (0.22–0.55)	4.61 (3.02–6.84)	16.19 (11.31–23.02)
FRT	0.04 (0.02–0.07)	0.30 (0.19–0.48)	5.70 (3.79–8.36)	15.58 (10.87–22.17)
ORT	0.04 (0.02–0.08)	0.29 (0.18–0.45)	6.14 (4.11–8.97)	16.49 (11.52–23.44)
FORT	0.04 (0.02–0.07)	0.62 (0.40–0.97)	7.74 (5.25–11.20)	22.00 (15.46–31.13)

Notes: Abbreviations as in Table A2. Unamended = incubated in water only; Amended = amended with 200 mM formate. Means are geometric (95% CI).

Table A4. Linear mixed effect presence/absence model F values for main effects and significant interactions for root and shoot biomass.

Main effects and interactions	Root biomass	Shoot biomass
F	1.25	2.60
O	0.40	2.54
R	29.86***	0.58
T	17.02***	16.17***
$R \times T$	11.01***	12.41***

Notes: Abbreviations as in Table A2. Only significant interactions were included. Numerator df = 1 and denominator df = 64. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.