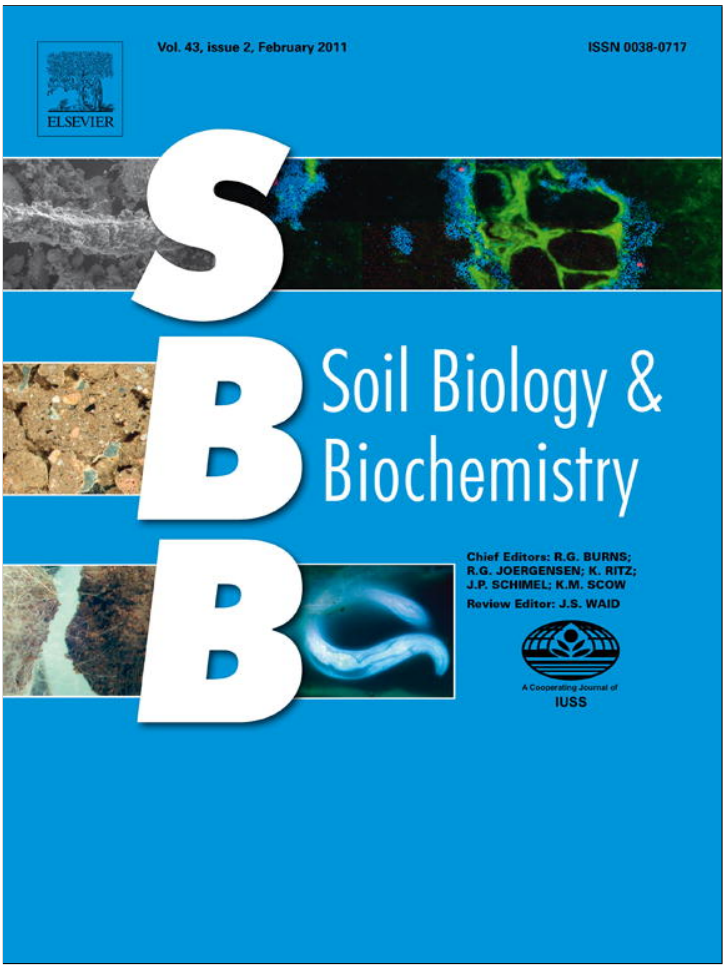

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Plant species traits regulate methane production in freshwater wetland soils

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ABSTRACT

Ecosystem and biogeochemical responses to anthropogenic stressors are the result of complex interactions between plants and microbes. A mechanistic understanding of how plant traits influence microbial processes is needed in order to predict the ecosystem-level effects of natural or anthropogenic change. This is particularly true in wetland ecosystems, where plants alter the availability of both electron donors (e.g., organic carbon) and electron acceptors (e.g., oxygen and ferric iron), thereby regulating the total amount of anaerobic respiration and the production of methane, a highly potent greenhouse gas. In this study, we examined how plant traits associated with plant inputs of carbon (photosynthesis and biomass) and oxygen (root porosity and ferric iron on roots) to mineral soils relate to microbial competition for organic carbon and, ultimately, methane production. Plant productivity was positively correlated with microbial respiration and negatively correlated to methane production. Root porosity was relatively constant across plant species, but belowground biomass, total biomass, and the concentration of oxidized (ferric) iron on roots varied significantly between species. As a result the size of the total root oxidized iron pool varied considerably across plant species, scaling with plant productivity. Large pools of oxidized iron were related to high CO_2:CH_4 ratios during microbial respiration, indicating that as plant productivity and biomass increased, microbes used non-methanogenic respiration pathways, most likely including the reduction of iron oxides. Taken together these results suggest that increased oxygen input from plants with greater biomass can offset any potential stimulation of methanogenic microbes from additional carbon inputs. Because the species composition of plant communities influences both electron donor and acceptor availability in wetland soils, changes in plant species as a consequence of anthropogenic disturbance have the potential to trigger profound effects on microbial processes, including changes in anaerobic decomposition rates and the proportion of mineralized carbon emitted as the greenhouse gas methane.

1. Introduction

Complex interactions between plants and microorganisms dominate biogeochemical cycling and the response of ecosystems to anthropogenic stresses such as climate change, eutrophication, and invasive species (Vitousek et al., 1997; Mack et al., 2000; Houghton et al., 2001). The nature of these interactions is defined by the distribution of physiological and morphological attributes among the organisms that compose ecosystems (Hobbie, 1992; Hooper and Vitousek, 1997; Hooper et al., 2005). For example, it is well known that variation in plant traits influences plant community persistence (Tilman, 1988), and there is a growing understanding that plant traits and plant community composition influence ecosystem function (Hooper and Vitousek, 1998; Wardle et al., 2004). The effects of plants on ecosystem processes are often mediated through their interactions with microbial communities, yet it remains quite difficult to predict how shifts in plant species composition affect belowground processes and vice versa (Wardle et al., 2004). We need a better understanding of how specific plant traits influence ecosystem processes via their effects on microbial communities. Here we consider the influence of a key plant trait – primary production – on the generation of methane (CH_4), a product of microbial respiration that is highly sensitive to microbial competition for organic carbon between methanogens and several other microbial functional groups.

Anaerobic environments present a unique opportunity to study plant trait effects on competition among heterotrophic microorganisms because the least competitive microbial functional group produces a distinct respiratory end product – CH_4. In anaerobic environments, a consortium of organisms is required to break down organic carbon to the inorganic end products carbon dioxide (CO_2) and CH_4, a potent greenhouse gas with 21 times the radiative

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forcing effect of CO_2 (Houghton et al., 2001). The terminal step of anaerobic decomposition is performed by microbial functional groups that tend to specialize in using one of several alternate terminal electron acceptors for respiration, particularly nitrate (NO_3), ferric iron (Fe(III)), sulfate (SO_4), or CO_2 (Megonigal et al., 2004). These respiration processes compete for organic carbon, or its derivative H_2, as an electron donor, and are known, respectively, as denitrification, iron reduction, sulfate reduction, and methanogenesis (listed in order of decreasing competitive ability based on free energy yield). Methanogenesis yields approximately 80% less free energy than denitrification and is the only pathway that produces CH_4 as an end product. In order to quantify the influence of wetland plant traits on the outcome of methanogen competition for organic carbon, we capitalize on the fact that competitively superior respiration pathways (e.g., iron reduction and sulfate reduction) produce CO_2 exclusively. In particular, we use the ratio of CO_2 to CH_4 as a measure of competitive suppression of methanogenic respiration (Keller et al., 2009); a ratio of approximately one indicates that methanogens dominate anaerobic microbial respiration and competition for electron donors is relatively weak, while ratios >1 suggest that methanogens are being outcompeted by other microbial functional groups (Conrad, 1999).

Wetland plants have the ability to determine the outcome of microbial competition for resources by influencing both the availability of electron donors (i.e., organic carbon) and electron acceptors (e.g., O_2 or Fe(III)). The availability of electron donors is largely controlled by plants because they are the source of nearly all organic carbon in soils (Megonigal et al., 2004). Plant carbon is made available to microbial communities through root exudation or biomass turnover, and several studies have found that wetland CH_4 emissions increase with primary productivity (Whiting and Chanton, 1993; Updegraff et al., 2001; Vann and Megonigal, 2003; Kankaala et al., 2005), suggesting that anaerobic respiration is carbon limited and coupled to plants as a labile carbon source (Megonigal et al., 1999). Because plants with high productivity tend to have more root growth and belowground biomass, and therefore more carbon inputs to the rhizosphere (Farrar et al., 2003), traits such as photosynthetic rate and biomass can be expected to be predictors of electron donor availability for CH_4 production.

In addition to controlling the supply of electron donors to microbes, plants also influence the supply of electron acceptors by supplying oxygen to the rhizosphere. There are two primary mechanisms by which oxygen in the rhizosphere can influence anaerobic respiration and decrease CH_4 production. Oxygen can be used directly as an electron acceptor in aerobic respiration and/or regenerate oxidized forms of nitrogen, iron, and sulfur that serve as electron acceptors in anaerobic respiration (Frenzel et al., 1999; Neubauer et al., 2005; Laanbroek, 2010). Thus, plants can indirectly suppress methanogenesis by supplying electron acceptor substrates to microbial functional groups with more energetically favorable respiration pathways. Second, oxygen can directly inhibit CH_4 production due to oxygen toxicity (Fridovich, 1998; Megonigal et al., 2004). Regardless of the mechanisms, plant traits that affect oxygen flux into a wetland soil, such as root porosity (the amount of conductive air space in plant roots) and plant biomass, have the potential to influence microbial competition for resources and CH_4 production.

Plants also influence net CH_4 emissions through several post-production processes. Plant oxygen inputs to soils can lower net CH_4 emissions by supporting aerobic methane-oxidizing bacteria that consume CH_4 (Gerard and Chanton, 1993; Calhoun and King, 1997; Bouchard et al., 2007). Conversely, the ability of wetland plants to vent CH_4 from soils directly to the atmosphere through aerenchymal transport allows CH_4 to bypass the soil surface where aerobic CH_4 oxidation also occurs, increasing the fraction of CH_4 production that escapes oxidation (Sebacher et al., 1985; Schimel, 1995; Van der Nat and Middelburg, 2000; Ding et al., 2005). These post-productive plant effects on CH_4 emissions are well studied and were not addressed in the current investigation; rather we focused on the less studied issue of plant-mediated controls on anaerobic respiration and CH_4 production.

Because plant traits that influence carbon and oxygen inputs to soils can operate independently and in opposite directions, the net effect of plants on anaerobic decomposition leading to CH_4 production has the potential to vary strongly across plant species. Previous studies have reported both increases and decreases in CH_4 production in response to an increase in plant biomass or productivity (Van der Nat and Middelburg, 1998b; Neubauer et al., 2005; Laanbroek, 2010). In order to understand the influence of plant species on microbial competition impacting CH_4 production, we measured differences in anaerobic microbial respiration and CH_4 production for four diverse plant species grown in common wetland soil. We hypothesized that differences in traits such as belowground biomass and root porosity would regulate the outcome of competition in microbial communities, resulting in differential rates of CH_4 and CO_2 production. Specifically, we predicted that more productive plants would deliver more carbon and oxygen to soils, stimulating overall respiration activity while decreasing CH_4 production.

2. Methods

2.1. Experimental set-up

We selected four common freshwater wetland species for this experiment, the forbs *Peltandra virginica* (arrow arum, herein *Peltandra*) and *Typha latifolia* (broadleaf cattail, herein *Typha*), and the grasses *Juncus effusus* (common rush, herein *Juncus*) and *Phragmites australis* (common reed, herein *Phragmites*). These four species have distinct morphological traits, such as rhizomatous (*Typha* and *Phragmites*) versus non-rhizomatous root systems and diffusive (*Peltandra*) versus pressurized flow internal gas flow mechanisms (Brix et al., 1992; Frye et al., 1994; Tornbjerg et al., 1994). Species with internal pressurization can transport more oxygen belowground (Laanbroek, 2010).

Peltandra and *Phragmites* were grown from seed obtained from local sources. *Phragmites* was collected from a patch that was identified genetically to be the introduced genotype from Europe (McCormick et al., 2010). In January, 2009, *Peltandra* and *Phragmites* seeds were germinated in moist sand and subsequently transplanted to seedling trays filled with a wetted potting soil mix. Plugs of *Juncus* and *Typha* were obtained from Environmental Concern (St. Michaels, MD) in early February 2009 and allowed to break dormancy before beginning the experiment.

2.2. Soil collection and planting

We collected approximately the top 20 cm of sediment from unvegetated mudflats in the Jug Bay wetland, a freshwater tidal wetland on the Patuxent River in Maryland, USA. The sediment was consolidated, mixed well in a large plastic container, then transferred to PVC pots that were 10 cm internal diameter and 45 cm tall with a plastic cap for the bottom. Nine pots per species were planted with one individual per pot; three pots were left unplanted. We carefully removed as much of the potting soil as possible from the roots of each species prior to transplanting them. The 39 pots were then randomly placed in one of three large plastic tubs filled with distilled water and maintained in a growth chamber. Water levels were maintained above the surface of the soil throughout the length of the experiment in order to ensure that changes in the

redox status of the soils were influenced primarily by plant roots. Plants were grown under typical spring growing conditions for four months: a minimum temperature of 14 °C at night, a maximum temperature of 20 °C during the day, with full light (approximately 800 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) between 0900 h and 1730 h every day), and relative humidity >60%. The light source was a combination of 50% tungsten and 50% fluorescent lights.

2.3. Plant trait selection

To understand plant contributions to the electron donor pool, we measured plant traits that related to plant productivity: aboveground biomass, total productivity (i.e., aboveground biomass + belowground biomass) and photosynthetic rate. To understand how plants influence electron acceptor pools we selected traits related to potential radial oxygen loss from roots: belowground biomass, root porosity, and iron oxide coatings (i.e., Fe(III) plaque) on roots.

2.4. Photosynthesis measurements

Shortly before we harvested the plants, in late June 2009, we measured photosynthetic rates on all species using an LI-6400 IRGA (LI-COR, Inc., Lincoln, NE). Ambient photosynthetic rates were measured using the internal red + blue LED light source set to 800 μmol m^{-2} s^{-1} PAR (measured ambient light conditions in the chamber), the CO_2 concentration within the IRGA chamber was maintained at 400 μL L^{-1} using the CO_2 mixer, and relative humidity was maintained >60% during all measurements. Due to morphological differences, there were differences in the placement of the IRGA chamber on the plant. Measurements were also corrected for leaf area if the leaf did not fill the entire chamber. For *Juncus*, photosynthetic rates were measured on several stems simultaneously, approximately half way up the stem while for *Peltandra* we measured photosynthesis on a mature leaf. For *Typha* we measured a healthy leaf approximately half way up the plant and for *Phragmites* we measured photosynthetic rates on the third fully expanded leaf from the top of the plant.

2.5. Plant harvest and microbial activity measurements

In July 2009, over a period of two weeks we harvested each plant and measured several plant traits. Total leaf area was measured immediately upon harvest using an LI-3100 Leaf Area Meter (LI-COR, Inc., Lincoln, NE). To provide estimates of whole plant photosynthesis, we multiplied our mean rate of photosynthesis by total leaf area for each individual. Leaves and stems were dried at 60 °C to constant mass and weighed to calculate aboveground biomass for each mesocosm. Once the aboveground biomass was removed, we transferred the upper 15 cm of soil, which contained the majority of plant root biomass, to an anaerobic chamber (Coy Laboratory Products, Inc., Grass Lake, MI). Visible roots were removed from the chamber and gently rinsed with DI water. A representative 0.2 g sample was excised to measure root porosity using the pycnometer method (Jensen et al., 1969) and root-surface iron by dithionite–citrate–bicarbonate (Taylor and Crowder, 1983; Weiss et al., 2003). The remaining roots were dried at 60 °C to constant mass, then they were combusted at 450 °C for 4 h to yield ash-free weight and this weight is reported as belowground biomass. Belowground biomass multiplied by either root-surface iron concentration or root porosity provided Root System Iron (RSFe) or Root System Porosity, respectively.

Remaining soil was homogenized and sub-sampled for microbial respiration analysis. Microbial CO_2 respiration and methanogenesis were determined over a period of four days by measuring CH_4 and CO_2 production rates from a 15 g (wet weight) soil sample, slurried with 5 mL of degassed DI water in a 70 mL serum vial with a nitrogen atmosphere. Once a day during a four-day incubation, CH_4 was measured using a Shimadzu GC-14A gas chromatograph (Kyoto, Japan) equipped with a flame ionization detector and CO_2 was measured using an LI-7000 CO_2/H_2O Analyzer (LI-COR, Inc., Lincoln, NE). Rates of CH_4 and CO_2 production were calculated from the linear ($r^2 > 0.90$) accumulation of these gases in the headspace of the serum bottle. Moisture content was determined from a 20 g subsample dried at 100 °C for 24 h. Because roots were removed prior to these incubations, accumulated CO_2 was assumed to come from heterotrophic respiration.

2.6. Statistical analysis

We used linear regression, correlation, and ANOVA to make comparisons between species and examine the relationships between species traits and microbial community activity. The assumptions of constant variance of the errors and normality of the error distribution were examined using plots of residuals versus predicted values and QQ plots of residuals. In cases where we identified outliers, we tested the sensitivity of our results with and without the outliers. Because our results were generally robust to the few outliers identified, we did not remove outliers from our analyses. The one exception to this was the relationship between belowground biomass and microbial CH_4 production where removing one observation changed the significance of this relationship, so we removed the outlier. All analyses were performed in the statistical software R 2.7.2 (R Development Core Team, 2008).

3. Results

Some plant traits varied more strongly than others across species. Mean belowground biomass and total biomass varied from 17 g and 23 g per plant, respectively, for *Peltandra*, to 39 g and 66 g, respectively, per plant for *Juncus* (Fig. 1a). Across all the plants in the study, belowground biomass and total biomass were tightly correlated ($\rho = 0.96$, $p < 0.0001$). Rates of photosynthesis were lowest for *Juncus* and *Peltandra* (0.30 and 0.33 μmol CO_2 s^{-1}, respectively) and highest for *Typha* (0.79 μmol CO_2 s^{-1}) (Fig. 1b). The root-surface iron oxides varied from 70 to 134 μmol g^{-1} root and showed significantly higher concentrations on the roots of *Juncus* and *Typha* than the other species (Fig. 1c). By contrast, root porosity varied between 35 and 45% of root volume and did not differ across the species (Fig. 1d).

From a soil biogeochemistry perspective, the concentration of iron oxides per mass root and the volume-percent root porosity are less important than the total quantities of these variables in a given mesocosm. Because the species with high belowground biomass (and therefore high total biomass) tended to also have higher concentrations of iron oxides (Fig. 1), there was a significant positive correlation between total plant biomass and total RSFe (Fig. 2a). Similarly, total root porosity was significantly, positively related to belowground biomass because percent root porosity was constant across species, while belowground biomass varied strongly ($\rho = 0.83$, $p < 0.0001$, data not shown). The net result of these relationships was that plants with more belowground biomass had more potential O_2-conducting root tissue and a larger pool of root-surface iron oxides. In terms of the individual species relationships, *Typha* total biomass and belowground biomass were significantly, positively related to RSFe (data not shown), but there were no other significant relationships between plant traits and microbial processes within individual species.

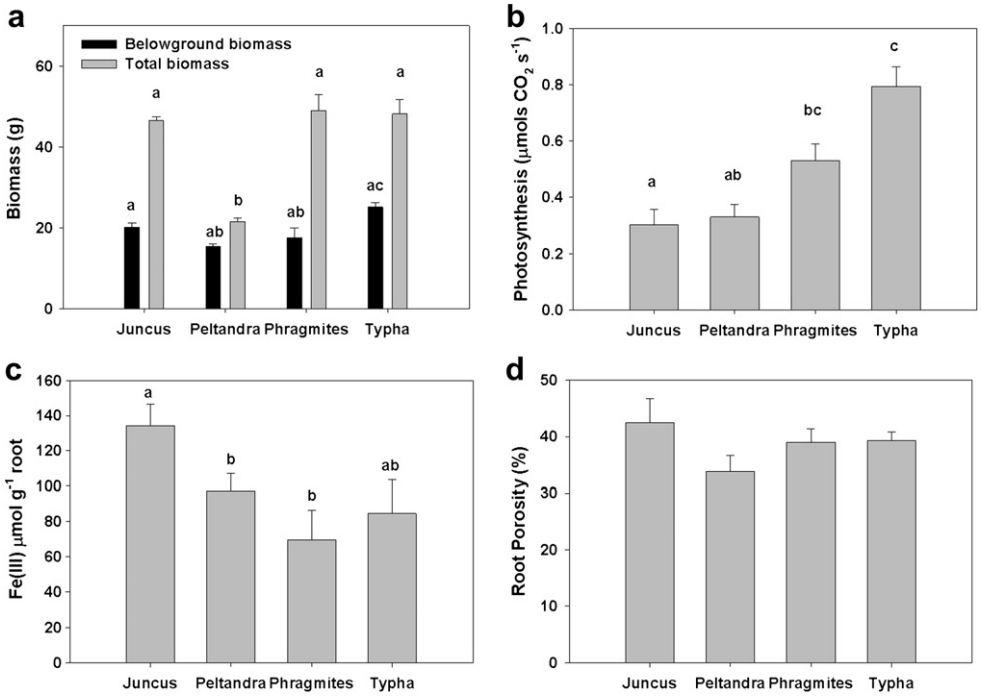

Fig. 1. Comparison by species of (a) belowground (black) and total (gray) biomass, (b) photosynthesis, (c) root Fe(III) concentration, and (d) percent root porosity. Error bars are one standard error. Significant differences of at least $p = 0.05$ are indicated by different letters. For (a) the letters denote differences between species for either belowground biomass or total biomass, but not differences between belowground and total biomass within a species. Statistics were performed on log transformed data for photosynthesis and Fe(III) to conform to the assumptions of normality, but untransformed data are presented in the figure. Belowground biomass is ash-free dry weight.

There was a positive relationship between microbial respiration ($CO_2 + CH_4$) and total plant biomass (Fig. 2b and Table 1), as well as with plant above and belowground biomass (Table 1) indicating that plant carbon inputs increased with plant productivity and stimulated overall microbial activity; however, biomass explained only 31% of the experiment-wide variation in microbial respiration. Total plant biomass had a significant, positive relationship with CO_2:CH_4 ratios (Fig. 2c and Table 1), explaining 68% of the experiment-wide variation

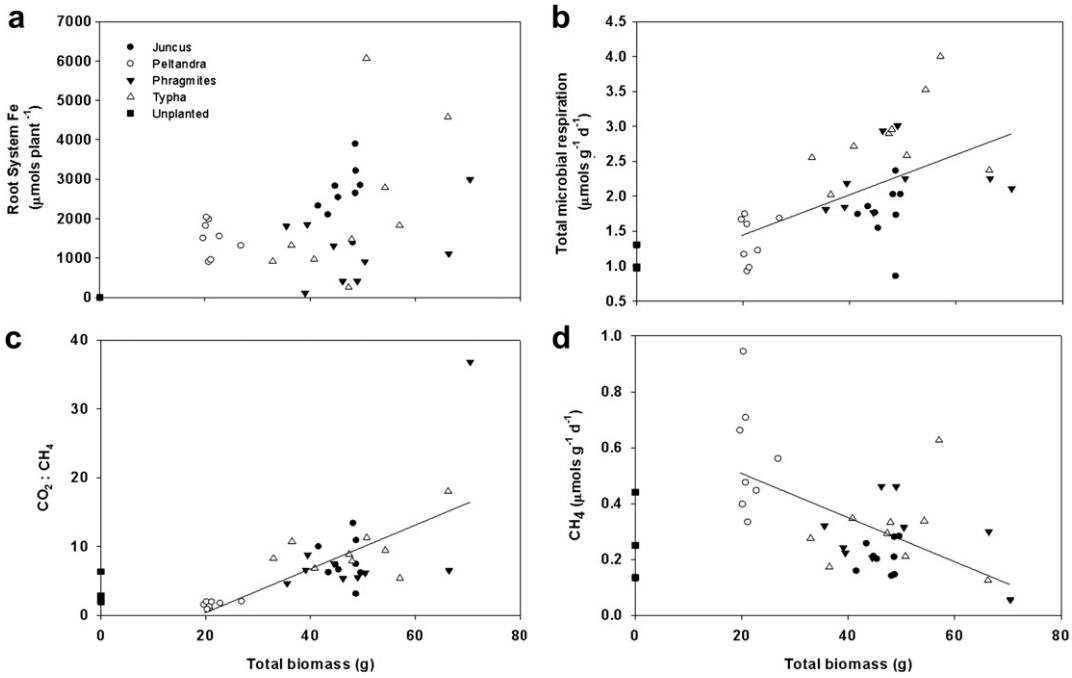

Fig. 2. Comparisons of total plant biomass versus (a) root system Fe for all species ($\rho = 0.335$, $p = 0.05$), (b) total microbial respiration ($CO_2 + CH_4$) for all species, (c) CO_2:CH_4 ratios for all species, (d) CH_4 production for all species. A correlation was calculated for (a) because these two variables were not independent given belowground biomass was used to calculate root system Fe. Regression coefficients for (b), (c), and (d) are listed in Table 1. Note that for (c) and (d), statistics were performed on log CO_2:CH_4 and CH_4 production data but the untransformed data are presented in the figure.

Table 1
Regression results including r^2 and p-values (slope, intercept) for the plant trait predictors of microbial processes including total microbial respiration ($CO_2 + CH_4$), CH_4 production, and CO_2:CH_4 production ratio. CH_4 production, CO_2:CH_4 ratio, belowground biomass, and Root System Fe(III) were log transformed to conform to the assumptions of normality. Significant relationships are highlighted in bold. Belowground biomass is ash-free dry weight.

Plant predictors	Microbial processes		
	Total respiration ($CO_2 + CH_4$)	Log (CH_4)	Log (CO_2:CH_4 ratio)
Total biomass	**$r^2 = 0.31$, $p = 0.0005$ (0.03, 0.87)**	**$r^2 = 0.38$, $p < 0.001$ (−0.025, −0.19)**	**$r^2 = 0.68$, $p < 0.001$ (0.05, −0.44)**
Aboveground biomass	**$r^2 = 0.18$, $p = 0.01$ (0.03, 1.42)**	**$r^2 = 0.42$, $p < 0.0001$ (−0.036, −0.45)**	**$r^2 = 0.67$, $p < 0.0001$ (0.07, 0.18)**
Log (Belowground biomass)	**$r^2 = 0.25$, $p = 0.002$ (1.22, −1.51)**	**$r^2 = 0.15$, $p = 0.02$ (−0.20, 2.67)**	**$r^2 = 0.22$, $p = 0.004$ (1.37, −2.32)**
Root system porosity	**$r^2 = 0.79$, $p < 0.0001$ (0.17, 0.79)**	**$r^2 = 0.11$, $p = 0.05$ (−0.073, −0.68)**	**$r^2 = 0.36$, $p = 0.0001$ (0.20, 0.20)**
Log (Root System Fe(III))	$r^2 = 0.03$, $p = 0.33$ (−0.15, 3.18)	$r^2 = 0.09$, $p = 0.08$ (−0.21, 0.30)	$r^2 = 0.02$, $p = 0.46$ (0.14, 0.69)
Plant photosynthesis	**$r^2 = 0.26$, $p = 0.002$ (1.39, 1.39)**	$r^2 = 0.05$, $p = 0.19$ (−0.49, −1.00)	**$r^2 = 0.22$, $p = 0.005$ (1.50, 0.95)**

in CO_2-generating versus CH_4-generating anaerobic respiration pathways. This increase in CO_2:CH_4 ratio with increasing total biomass suggests that changes in total microbial respiration were accompanied by shifts in the underlying microbial respiration pathways. In fact, microbial respiration was significantly, positively related to total plant biomass while CH_4 respiration was negatively related (Fig. 2b, d, and Table 1). Photosynthesis was significantly, positively related to total microbial respiration (Fig. 3a), explaining 26% of the experiment-wide variation, an amount similar to total biomass; there was also a positive relationship between whole plant photosynthesis (scaled based on leaf area) and total plant biomass (Fig. 3b).

4. Discussion

Soil microbial respiration is generally stimulated by plant carbon inputs (e.g., Wardle, 2002; Hill and Cardaci, 2004; Wardle et al., 2004), suggesting that microbial activity is carbon limited in terrestrial ecosystems. Our results were broadly consistent with this generalization because microbial respiration increased with belowground biomass (Fig. 2b) and whole plant photosynthesis (Fig. 3a). Even though photosynthesis and biomass were positively related to total microbial respiration, the fact that neither variable explained more than 31% of the variation in microbial respiration may reflect the short (approximately one growing season) duration of the experiment. However, the experiment was long enough to observe immediate rhizosphere effects on redox potential and labile carbon pools as reflected by the 2- to 4-fold increase in microbial respiration over plant-free soils. The primary source of carbon inputs would have been via root exudation with a limited amount of carbon added to the soil through root turnover. Because we measured microbial respiration in strictly anaerobic serum bottle incubations with root-free soil, any plant-induced effects on soils or soil processes must have occurred before the incubations began. The additional 69% unexplained variance may be because of other unmeasured species-related factors such as substrate quality (e.g., C:N of root tissue or exudates) or root morphology (e.g., total root volume or surface area). Nonetheless, it appears that plant traits favoring faster growth and greater biomass stimulated carbon inputs to soils and subsequent microbial respiration, as reported in previous studies (Zak et al., 1994; Farrar et al., 2003).

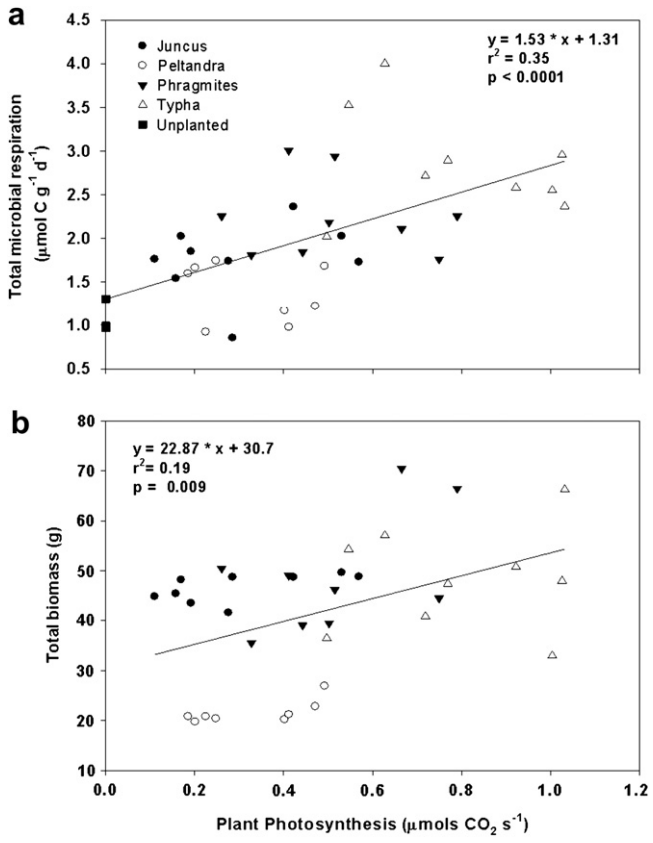

Fig. 3. Whole plant photosynthesis (scaled by leaf area) versus (a) total microbial respiration and (b) total plant biomass.

Microbial respiration is often carbon limited in anaerobic wetland soils because organic matter is recalcitrant to decomposition in the absence of oxygen. In fact, the recalcitrance of organic matter under anaerobic conditions explains why many wetland soils accumulate large pools of soil organic matter. Evidence of carbon-limited microbial respiration can be found in several studies that report a linear increase in CH_4 emissions with increasing primary production (Whiting and Chanton, 1993; Updegraff et al., 2001; Vann and Megonigal, 2003; Kankaala et al., 2005). There are at least two, non-exclusive, mechanisms by which an increase in plant carbon inputs to soils stimulates CH_4 production. First, carbon inputs can cause an increase in overall heterotrophic microbial respiration that stimulates both non-methanogenic and methanogenic respiration; second, carbon inputs can cause a shift toward methanogenic respiration because accelerated rates of respiration consume more of the competitive electron acceptor supply (Martens and Val Klump, 1984). If the dominant influence of plant traits on microbial community anaerobic respiration had been to regulate the supply of labile organic carbon (i.e., electron donors), we would have expected a positive relationship between primary production (both whole plant photosynthetic rates and biomass) and CH_4 production. In contrast to this expectation and several previous studies, we found a negative relationship between primary production and CH_4 production in our anaerobic incubations (Fig. 2d). Because this pattern was not matched by a decline in total microbial respiration (Fig. 2b), the most likely cause was an effect of plant traits on the supply of relatively high energy-yielding alternative electron acceptor compounds. Overall, our results suggest that plant inputs of carbon

were not the main driver of the plant species effects on CH_4 production.

Further evidence that plant traits influenced microbial competition for organic carbon resources is found in the ratio of CO_2 to CH_4, which approximates a value of one when methanogenesis is the dominant microbial metabolic pathway (Conrad, 1999). For three of the species, this ratio was much greater than one (*Juncus* = 7.9 ± 1.0 S.E., *Phragmites* = 9.8 ± 3.4, and *Typha* = 9.6 ± 1.2), reflecting significant microbial respiration by non-methanogenic metabolic pathways. Because increases in the CO_2:CH_4 ratio were driven both by increases in CO_2 production and decreases in CH_4 production, the most plausible explanation for these high ratios is a substantial pool of alternative electron acceptors at the time the soils were sampled.

It is noteworthy that the soils exposed to three of the species (excluding *Peltandra*) had absolute rates of CH_4 production that were similar to the controls (Fig. 2d), yet these plant-influenced soils supported much higher rates of total microbial respiration ($CO_2 + CH_4$) than the controls (Fig. 2b). This could occur if these three species simultaneously increased both the supply and consumption of non-methanogenic electron acceptors. In contrast, *Peltandra* had a fundamentally different influence on the dual processes of electron donor supply and electron acceptor supply. *Peltandra* had higher average CH_4 production than the unplanted control soils, total microbial respiration rates similar to the unplanted controls, and a low CO_2:CH_4 ratio (1.50 ± 0.16) that was much closer to one (Fig. 2d). Collectively, these data suggest that *Peltandra* was a weak source of both electron donor and acceptor compounds to the soil compared to the other species. This observation and the fact that CH_4 production was not correlated to plant production within any of the four species (only across species) suggests there are species-specific exceptions to the widely-held view that CH_4 emissions (and by implication CH_4 production) increase with plant production (Whiting and Chanton, 1993; Jespersen et al., 1998; Updegraff et al., 2001; Vann and Megonigal, 2003; Kankaala et al., 2005).

We cannot explain the morphological and physiological sources that caused *Peltandra* to influence microbial respiration differently than the other species. However, we noted that the *Peltandra* roots were comparatively lower in biomass, relatively thick and spongy, and had few fine roots. *Typha* and *Phragmites* had both relatively thick rhizomes and thin roots, *Juncus* had many thin roots, therefore all three species had overall greater surface area than the *Peltandra* roots. It is possible that these morphological differences between *Peltandra* and the other species are related to differences in oxygen (or carbon) inputs to soils, particularly given *Peltandra* has diffusive internal gas flow compared to pressurized gas flow in the other three species (Frye et al., 1994). A better understanding of the relationship between morphology, physiology and plant inputs (both carbon and oxygen) to soils will be useful in linking species diversity to microbial respiration and CH_4 emissions in wetland soils.

There are a limited number of alternative electron acceptors that are regenerated by plant inputs of O_2 to saturated wetland soils, primarily nitrate, sulfate, and ferric iron. Nitrate and sulfate do not generally account for a large fraction of anaerobic respiration in the absence of an external source of these compounds, such as fertilizer (nitrate) or sea water (sulfate) (Capone and Kiene, 1988; Laanbroek, 2010). In the present study, nitrate and sulfate availability should have been negligible because the mesocosms were flooded with deionized water; ferric iron (Fe(III)) was the most likely non-methanogenic electron acceptor in our system. Like most mineral soils, our soils contained abundant iron, which is regenerated in the rhizosphere of wetlands plants through oxidation of ferrous iron (Fe(II)) (Weiss et al., 2003; Neubauer et al., 2005). Indeed, iron oxides accumulated on the root-surface of all four species (Fig. 1c). The strong positive relationships between total biomass, total RSFe (Fig. 2a), and total root porosity, suggest that plants with more belowground biomass transported more oxygen into the rhizosphere, oxidizing more Fe(II), and ultimately providing a larger supply of an alternate electron acceptor for microbial metabolism. The increased Fe(III) supply would have increased microbial competition between Fe(III)-reducing bacteria and methanogens for organic carbon resources. Because the variation in percent root porosity and root iron concentration was relatively low compared to variation in belowground biomass, these patterns were due primarily to inter-specific differences in primary production, and root production specifically. Similar findings have been reported in previous studies. Weißner et al. (2002) determined that plant production was strongly correlated with radial oxygen loss in *T. latifolia* and *J. effusus*. Laan et al. (1989) determined that differences in oxygen loss between three *Rumex* species were correlated with root growth, and found that the species with faster root growth rates had greater root oxygen losses and more soil Fe(III).

Our results suggest that plant inputs of oxygen to the soil can be as important as inputs of organic carbon for regulating CH_4 production. Several previous studies provided evidence that the influence of plants on methanogenesis is mediated by Fe(III) regeneration. Working in the same marsh complex where our soil was collected, Neubauer et al. (2005) reported a decline in iron reduction and a concomitant increase in CH_4 production across the growing season. They observed that peak rates of iron reduction (minimum rates of methanogenesis) coincided with peak plant biomass, and proposed that the increase in biomass had stimulated radial oxygen loss, rhizosphere Fe(III) regeneration, and ultimately suppression of methanogenesis by iron reduction respiration. Roden and Wetzel (1996) reported that soil cores vegetated with *Juncus* had larger Fe(III) pools and lower CH_4 production than unvegetated cores. Van der Nat and Middelburg (1998a) reported higher rates of CH_4 production in sediments either left unplanted or planted with *Phragmites* compared to sediments planted with *Scirpus*. These differences in CH_4 production were consistent with the fact that *Phragmites* had a relatively limited capacity to oxidize the sediment and generate Fe(III) oxides compared to *Scirpus*. In other cases, the effects of plant oxygen inputs are not likely to be mediated by Fe(III) regeneration. For example, Koelbener et al. (2010) reported a negative relationship between aboveground biomass and CH_4 *emissions* in a peatland where iron content is typically low; in this case, direct inhibition of methanogenesis through oxygen toxicity or aerobic CH_4 oxidation may explain the negative relationship (Bouchard et al., 2007).

In the present study, any influence of plant oxygen inputs on microbial activity, such as through accumulation of root iron oxides, had to be imparted to the soils before they were sampled because the soil incubations were anaerobic. We suggested this influence operated via rhizosphere regeneration of Fe(III) compounds and subsequent Fe(III) respiration effects on methanogenesis. However, it is possible that anaerobic CH_4 oxidation linked to Fe(III) occurred to some extent in our anaerobic incubations (Beal et al., 2009). Regardless of the mechanisms involved, our study indicates that species-specific differences in plant oxygen transport to the rhizosphere influence the strength of the CH_4 source apart from any effects that it may have on aerobic CH_4 oxidation (Calhoun and King, 1997; Bouchard et al., 2007).

In most cases significant relationships across all species did not emerge within a species. This suggests that intraspecies variation in traits may be less important than differences between species in the context of impacts on microbial processes. The exception to this was the relationship between RSFe and *Typha* biomass. It is possible that if we had had more individuals per species, we might have found that more relationships were significant within a species. Wetland plant communities in the field would likely experience

a much greater range of environmental conditions and could also include higher genotypic diversity than the individuals we used in this experiment, such that the dynamics of CH_4 production in the field would depend on trait variability both within and across species. Nevertheless, the strong relationships between plant traits and microbial processes across species that we found here suggest that wetland plant species composition plays an important role in influencing microbial dynamics.

5. Conclusions

In this experiment, we demonstrated that differences between wetland plant species in expressed plant traits related to productivity have consequences for microbial competition for resources and CH_4 production. Of particular interest, our results suggest that the consequence of differences in productivity among plant species was mainly to influence oxygen transport into the rhizosphere, regenerating non-methanogenic electron acceptors. This contrasts with the dominant view that the primary net influence of plants on CH_4 emissions is driven by differences in carbon inputs (Whiting and Chanton, 1993; Updegraff et al., 2001; Vann and Megonigal, 2003; Kankaala et al., 2005). We propose that plant-induced changes in electron acceptor abundance, most likely iron oxides, led to increased competition between microbes for carbon with the end result that the less favorable metabolic pathway – methanogenesis – was suppressed. These results suggest that the outcome of microbial competition for organic carbon, particularly CH_4 production, may be quite sensitive to plant-specific differences in oxygen transport to otherwise anaerobic soils. If so, anthropogenic perturbations, such as climate change or land use change, are likely to directly impact ecosystem processes through their influence on plant community composition.

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