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Elevated CO_2 and water depth regulation of methane emissions: Comparison of woody and non-woody wetland plant species

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Abstract. Elevated CO_2 has been shown to increase methane emissions in herbaceous wetlands, but it is not clear that this will occur in wetlands dominated by woody plants or in wetlands that are not inundated. We determined the effects of elevated CO_2 and water table position on methane emission and oxidation rates from plant-soil microcosms planted with a woody tree, *Taxodium distichum*, or an emergent aquatic macrophyte, *Orontium aquaticum*. Experiments were conducted in replicate glasshouses (n = 2) at CO_2 concentrations of either 350 or 700 ppmv. Plants were grown from seed and subjected to two water level depths, flooded (+5 cm above the soil surface) and non-flooded (−10 cm for *T. distichum* and −6 cm for *O. aquaticum*). Elevated CO_2 increased whole-plant photosynthetic rates in both water table treatments. Methane emission rates increased by 62 to 69% in the *T. distichum* treatment and 27 to 29% in the *O. aquaticum* treatment. Whole-plant photosynthesis and biomass were strongly correlated with methane emissions ($r^2 \geqslant 0.75$, $P \leqslant 0.01$). This relationship provides evidence of a tight coupling between plant and microbial activity and suggests that similar relationships from other wetland studies measured at ambient CO_2 can be extrapolated into the future. In the *O. aquaticum*, non-flooded treatment, methanotrophy consumed 14 and 22% (replicate glasshouses) of the methane produced in the ambient treatment compared to 29 and 36% in the elevated CO_2 treatment. However, there was no significant methane oxidation detected in the flooded treatment. We concluded that woody and non-woody wetland ecosystems growing in a future CO_2-enriched atmosphere will emit more methane regardless of water table position, but the degree of stimulation will be sensitive to changes in water table position, particularly in forested wetlands.

Introduction

The effects of elevated CO_2 on methane emissions have been investigated in natural and artificial ecosystems representing peatlands, freshwater marshes, salt marshes and rice paddies, all of which were dominated by non-woody plants. These studies indicate that CH_4 emissions will increase substantially as a direct result of elevated CO_2, particularly in temperate and tropical zones, and regardless of the effect of elevated CO_2 on climate. Given that forested wetlands account for $\sim 60\%$ of the total wetland area globally (Matthews and Fung 1987), the response of

woody plant-soil systems to elevated CO_2 is key to predicting the impacts on future atmospheric methane concentrations. This is the first study to investigate methane emissions from a woody plant-soil system in a CO_2-enriched atmosphere.

The reason that elevated CO_2 can increase CH_4 emissions in a period of several weeks has not been established. One possibility is a tight coupling between plant and microbial metabolism whereby recently-assimilated photosynthates become available for fermentation through root exudation or rapid root turnover (Huang et al. 1998). Indeed, the stimulation of CH_4 emissions found in most studies of herbaceous species was accompanied by an increase in photosynthesis (Dacey et al. 1994; Hutchin et al. 1995; Megonigal and Schlesinger 1997; Ziska et al. 1998). A related possibility is that elevated CO_2 increases plant biomass, which in turn increases ventilation of CH_4 from soils. Regardless of the mechanisms, the same effects should occur in wetland trees provided they respond to elevated CO_2 with increased rates of photosynthesis and growth, as do most upland trees (Curtis (1996) and Delucia et al. (1999); but see Oren et al. (2001)).

Previous studies of elevated CO_2 on wetland herbaceous plant-soil systems may not be applicable to woody plant-soil systems. Although wetland trees develop root and stem aerenchyma tissue in response to flooding (Pezeshki 1991; Megonigal and Day 1992), limited data suggests they transport gases less effectively than herbaceous species (Hook and Brown 1971; Grosse et al. 1992). Because woody roots have more lignin and suberin than herbaceous roots, they may also have lower rates of radial oxygen loss and exudation. For example, lignification of the root cell wall and suberization coincided with reduced radial oxygen loss in the apical roots of two herbaceous wetland species (Armstrong and Armstrong 2001). Carbon allocation to wood reduces carbon allocated to relatively labile pools such as fine roots and root exudates. Finally, wood decomposition is relatively slow, which could limit the supply of acetate and H_2 to methanogenic bacteria.

Elevated CO_2 and flooding may interact to influence methane emissions from wetlands dominated by woody plants. For example, an increase in leaf-level photosynthesis due to elevated CO_2 may be offset by a decrease in leaf area due to flooding. Elevated CO_2 may influence water table depth directly by decreasing transpiration rates (Megonigal and Schlesinger 1997). Because methane production and oxidation are regulated to a large extent by water table position, this would constitute an indirect positive feedback on methane emissions.

The objectives of our study were to determine the influence of elevated CO_2 and water table position on CH_4 emissions from model woody and non-woody wetland plant-soil systems. We hypothesized that: (1) elevated CO_2 would increase methane emissions more in continuously flooded soils than partially saturated soils that have high methane oxidation rates, and (2) CO_2 stimulation of CH_4 emissions would be less from a woody plant-soil system than a non-woody system because woody plants have less aerenchyma and highly suberized roots that should inhibit root exudation and CH_4 transport.

Materials and methods

Plant material

We studied the elevated CO_2 responses of a woody conifer, *Taxodium distichum* and an emergent aquatic macrophyte, *Orontium aquaticum*. *O. aquaticum* has well developed stem and root aerenchyma tissue, and a root system that can reach depths of nearly 2 m. It grows from Massachusetts to Florida and west to Kentucky (Tiner 1988) and is predominately an understory plant in freshwater wetlands. *T. distichum* is a tree that can tolerate hydrologic regimes ranging from well drained to flooding depths of < 3 m (Mattoon 1915). Over 13 million ha of the southeastern United States are dominated by *T. distichum* and *Nyssa aquatica* (Hefner and Brown 1985).

Experimental procedure

T. distichum seeds were obtained commercially (F.W. Schumacher Co., Inc., Sandwich, MA); the original seed source was in the Gulf States region. *O. aquaticum* seeds were collected from a tidal freshwater wetland on the White Oak River in North Carolina (Megonigal 1996). Seeds of *O. aquaticum* were surface sterilized with a 1:10 bleach solution to prevent fungal growth and stored in plastic bags at 5 °C until planting. *T. distichum* seeds were also stored at 5 °C, but under dry conditions. *T. distichum* seeds were soaked for 48 hours in a 0.01% nitric acid bath to break internal dormancy. Both species were planted on 17 May 1998 into flats filled with Canadian *Sphagnum* peat moss, amended with dolomitic lime to achieve a pH of approximately 6.7. Germination flats were placed into two separate environmentally-controlled growth chambers with CO_2 concentrations maintained near 350 or 700 ppmv. Temperatures were 25 °C/20 °C (day/night) and photon flux density was 1000 μmol m^{-2} s^{-1} for a 14 hour day-length period. Relative humidity in both chambers was < 70% during the germination period. Seedlings of *O. aquaticum* were watered until the soil was saturated, while *T. distichum* seeds were watered less frequently to maintain a moist, but non-flooded soil. Plants of approximately equal size with two true leaves (*O. aquaticum*) or six true leaves (*T. distichum*) were selected for the study. *O. aquaticum* seedlings were transplanted on 9 June 1998 and *T. distichum* seedlings were transplanted on 23 June 1998 into 43 cm deep × 10 cm-diameter polyvinyl chloride (PVC) containers. Two transplanted seedlings that died within one week were replaced with another seedling of similar size.

Each container had four 1 cm-diameter holes at 28 cm above the base of the container and four holes at 6 cm above the base. A 40 × 5-cm strip of aluminum screen was secured over each set of holes to prevent excessive soil loss. The bases were sealed with a 10 cm-diameter PVC end cap and 250 ml of pea gravel was added to prevent the pot from floating. Each container was filled to a depth of 39 cm with a peat-based soil that had been mixed three weeks prior to transplanting. The soil mixture consisted of 95% Canadian *Sphagnum* peat, 3.5% wetland soil

collected from the White Oak River, and 1.5% dolomitic lime, which was added to raise the soil pH to 6.7. A slow release all-purpose fertilizer (Scott's Master Collection 15-13-13 NPK) was added to the soil mixture at a rate of 3.2 L m^{-3} of soil. This was supplemented by the addition of a water-soluble fertilizer, Miracle-Gro 15-30-15 NPK, applied every three weeks at half strength beginning on 20 June.

The transplanted seedlings were grown in four environmentally controlled glasshouses at the Duke University Phytotron (Durham, NC) from 8 June to 16 September 1998. CO_2 concentrations were maintained near 350 and 700 ppmv for each replicate (n = 2) glasshouse. The glasshouse temperatures were controlled near 28 °C from 0600 to 2000 hours and 23 °C overnight. Daily relative humidity levels were between 60 and 70%. Filtered sunlight provided a photon flux density of $\sim$1500 μmol m^{-2} s^{-1} at 1300 hours.

Individual seedlings were randomly placed within the glasshouses and water treatments. *O. aquaticum* containers were placed into 133-L tubs (Tucker Housewares, Leominster, MA) and *T. distichum* were placed into 83-L tubs (Rubbermaid Inc., Wooster, OH). There were two tubs per species in each glasshouse and each tub was a different water treatment, either flooded or non-flooded. There were 8 no-plant controls per treatment. Styrofoam disks (0.6 cm thick) on the surface of the water in each tub minimized algal growth and evaporation. Water levels for *O. aquaticum* were initially set at −3 cm (below the soil surface) in each tub on 10 June. As the seedlings grew, water levels were slowly lowered to −6 cm for the non-flooded treatment and +5 cm (above the soil surface) for the flooded treatment over a 16-day period. *T. distichum* water levels were initially set at −6 cm for the non-flooded and −2 cm for the flooded water treatments, then changed to the target levels of −10 cm for the non-flooded treatment and +5 cm for the flooded treatment over a 10 day period. Water levels were maintained at the target levels by replacing evapotranspired water daily with tap water. Tubs were drained every three weeks to prevent salt accumulation. The influence of water table manipulations on redox potential was measured at the end of the study using an Orion Model 250A redox meter inserted 6.5 cm below the soil surface.

Methane

Methane emissions were measured between 10 July and 22 August on eight plants per treatment using a closed chamber technique. Polyvinyl chloride flux chambers enclosed both the plant and soil surface. The chambers were capped with a 10 cm plastic lid with a rubber septum for headspace sampling. Twenty-four hours prior to sampling, the water infiltration holes in the PVC containers were plugged with closed-cell foam to prevent methane emissions. Five consecutive methane samples were taken from the headspace of the flux chambers and analyzed on a Varian Model 3700 Gas Chromatograph equipped with a flame ionization detector.

Photosynthesis

Measurements of whole-plant photosynthetic rates for this study are reported in Vann (2000), but are used here for regression analyses. Briefly, individual leaf age class was determined at the time of harvest, and whole-plant photosynthetic rate was calculated as age-specific photosynthesis multiplied by the leaf area of the age class. Photosynthetic rates were determined on 6 replicate plants using a Licor 6200 (Lincoln, NB).

Methane oxidation

Methane oxidation rates were measured for the *O. aquaticum* system between 4 and 16 September using the CH_3F inhibition technique. Pre-treatment methane flux measurements were made on 12 replicate *O. aquaticum* plants per treatment by enclosing both the plant and soil in a 46-cm high PVC flux chamber for 20 minutes. Seven hours later, all 12 replicate plants were enclosed in flux chambers and CH_3F was injected into six of them to achieve a final concentration of 1.7%. CH_3F was permitted to diffuse into the soil and rhizosphere for 14 to 16 hours before post-treatment measurements were taken. After the CH_3F treatment, the flux chambers were vented for five minutes and CH_4 emissions were re-measured. In the flooded treatment, the floodwater was drawn off and replaced with deionized water prior to each CH_4 flux measurement in order to reduce oxidation at the soil surface and maximize the contribution of rhizospheric oxidation (Epp and Chanton 1993). Methane concentrations were analyzed using a Gas Chromatograph, Varian Model 3700, with a flame ionization detector.

Statistical analysis

The SAS univariate procedure was used to assess normality and Levene's test for equality of variance was used for homogeneity of variance (SAS Institute 1987). An ANOVA model was used to determine differences between treatments for the soil redox and whole-plant photosynthetic measurements. A linear regression (SAS Institute 1987) of a 5-point curve of methane concentration versus time was used to calculate methane fluxes. Fluxes were analyzed by a two-way ANOVA with CO_2 and H_2O as main effects. CO_2 effects were calculated using a Type III mean square error term. Data were log transformed when necessary. Methane oxidation rates were analyzed with paired t-tests (SAS Institute 1987) applied to fluxes measured in the presence of ambient air or CH_3F on the same pots. An adjustment for the increased probability of a significant difference due to a fixed number of repeated t-tests (n = 8) was made with Bonferroni's correction (Day and Quinn 1989). For a one-tailed t-test of the null hypothesis that CH_3F had no significant effect, the corrected α-value for a 0.05 significance level was 0.0125.

Results

Methane

Elevated CO_2 significantly increased CH_4 emissions from soils planted with the tree (*T. distichum*) after 72 days of treatment, and from soils planted with the herbaceous species (*O. aquaticum*) after 33 days ($P < 0.05$; Figures 1 and 2). Flooding did not reduce the magnitude of the CO_2 stimulation in the *T. distichum* microcosms (62% flooded vs. 69% non-flooded), but not the *O. aquaticum* microcosms (29% vs. 27%). The relative increase in emissions was larger for *T. distichum* than *O. aquaticum*.

After 72 days, methane emissions were similar in the *T. distichum* and *O. aquaticum* microcosms of the flooded treatment (ambient: 1167 vs. 1302 mg m^{-2} d^{-1}, respectively; elevated: 1953 vs. 1685 mg m^{-2} d^{-1}), but far lower for *T. distichum* than *O. aquaticum* in the non-flooded treatment (ambient: 909 vs. 436 mg m^{-2} d^{-1}; elevated: 1165 vs. 805 mg m^{-2} d^{-1}). Flooding increased CH_4 emissions by up to 141% in the *T. distichum* microcosms and up to 72% in the *O. aquaticum* microcosms ($P < 0.0001$; Figures 1 and 2). There were no significant $CO_2 \times H_2O$ interactions for CH_4 emissions.

When plants in the ambient and elevated CO_2 treatments were pooled within water table treatments, methane emissions were highly correlated with whole-plant photosynthesis for *T. distichum* (flooded $r^2 = 0.92$; non-flooded $r^2 = 0.87$) and *O. aquaticum* (flooded $r^2 = 0.97$; non-flooded $r^2 = 0.89$) (Figure 3). In both species, methane flux was also correlated with shoot productivity and total biomass, although they explained slightly less of the variation than photosynthesis (Table 1). Methane emissions were also strongly correlated to *T. distichum* root biomass in the flooded treatment and *O. aquaticum* root biomass in both treatments. Methane flux was not correlated with whole-plant transpiration in any case (Table 1).

Methane oxidation

Blocking methane oxidation with CH_3F significantly increased methane emissions in the *O. aquaticum* microcosms in the non-flooded treatment at both ambient and elevated CO_2 concentrations (Table 2). Emissions in the control group also increased for both water treatments in just one glasshouse (#4), suggesting enhanced CH_4 diffusion or ebullition. This could have been caused by a decrease in atmospheric pressure because air circulation is controlled separately in each glasshouse. Methane oxidation consumed 14 and 22% (replicate glasshouses) of gross CH_4 emissions at ambient CO_2, compared to 29 and 36% at elevated CO_2. Adjusting for the increase in control emissions in glasshouse #4 would reduce methane oxidation from 36% to 19% (Table 2). Methane emissions from the flooded water table treatment did not significantly increase in response to the CH_3F block. There were no significant CO_2 effects on methane oxidation, but there was a trend for an increase in methane oxidation rates in the elevated $CO_2 \times$ non-flooded treatment ($P = 0.07$). Methane oxidation in the *T. distichum* treatment was not determined.

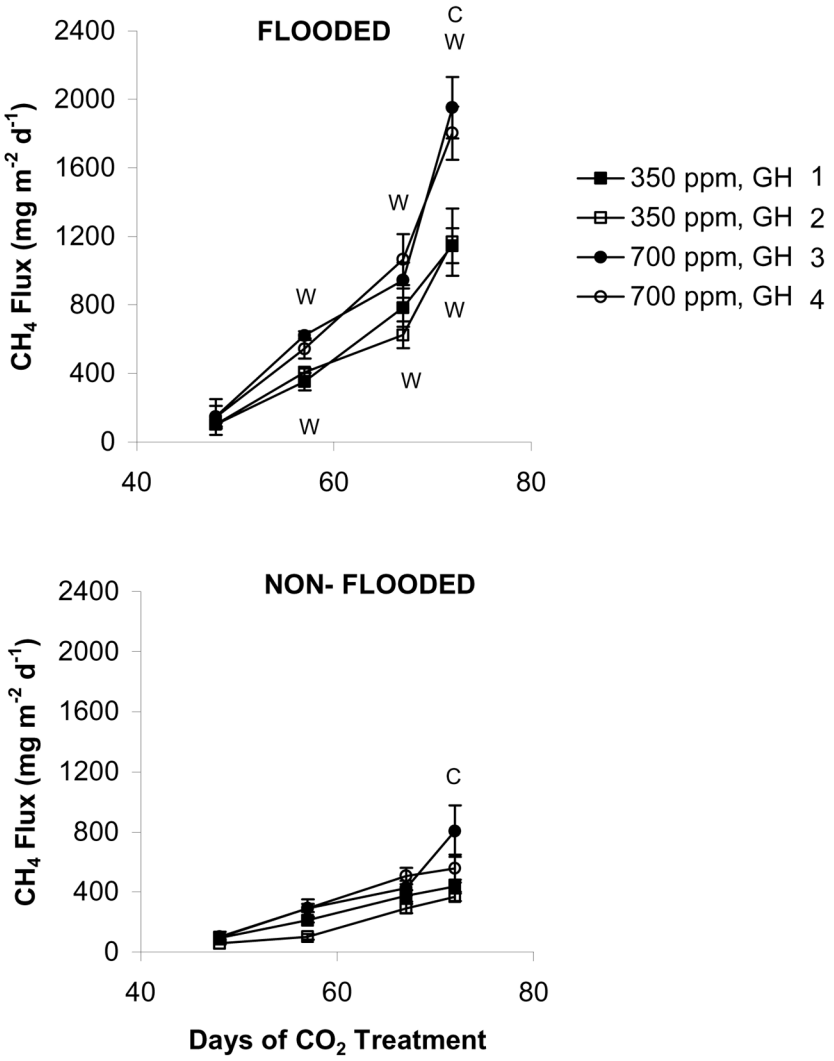

Figure 1. Methane flux from model *Taxodium distichum* soil microcosms. Values are means ± 1 standard error. Significant differences are indicated by the letters C and W located on the treatment with the highest mean. The letter C is a significant CO_2 effect and W is a significant water treatment effect. GH = Glasshouse.

Soil redox potential

Flooding significantly decreased the soil redox potential in all cases ($P < 0.0001$, Table 3). Due to the absence of radial oxygen loss from the roots, the no-plant control microcosms had more reduced soils in both water table treatments. Soil redox potential was not significantly affected by CO_2.

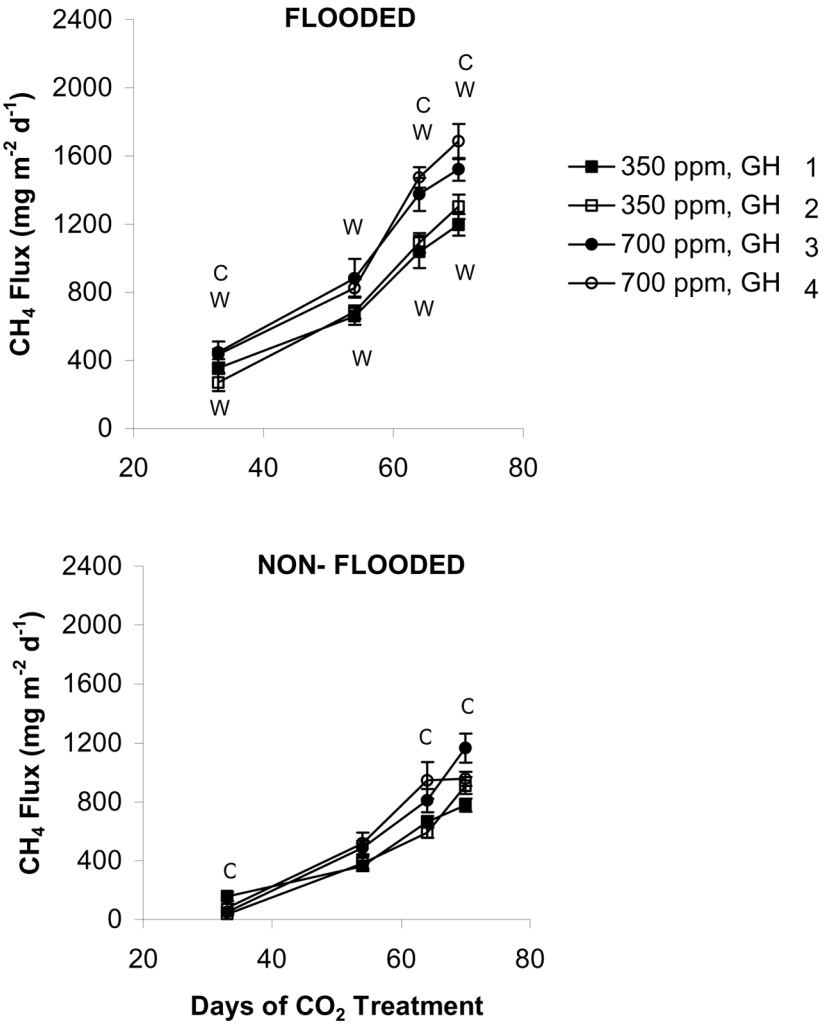

Figure 2. Methane flux from model *Orontium aquaticum* soil microcosms. Details as in Figure 1.

Root morphology

Water and CO_2 treatment did not visually affect the root morphology of *O. aquaticum*. However, coarse and fine roots of *T. distichum* in the non-flooded treatment were primarily woody with numerous branches. In contrast, flooded coarse and fine roots were primarily non-woody water roots with few branches.

Table 1. Regression equations and statistics relating CH_4 emissions to plant characteristics. All equations used data pooled from the ambient and elevated CO_2 treatments.

Species	Water Treatment	Independent Variable†	Regression Equation‡	R^2 value	P value
O. aquaticum	Flooded	Whole-Plant Photosynthesis	Y = 926.1X+504.3	0.966	< 0.0001
	Non-flooded	"	Y = 723.4X+324.8	0.886	0.0005
T. distichum	Flooded	"	Y = 3760.1X-74.6	0.917	0.0002
	Non-flooded	"	Y = 225.2X+116.3	0.872	0.0021
O. aquaticum	Flooded	Shoot Productivity	Y = 46.1X+637.9	0.931	0.0001
	Non-flooded	"	Y = 45.1X+379.2	0.867	0.0008
T. distichum	Flooded	"	Y = 214.6X-103.5	0.752	0.0053
	Non-flooded	"	Y = 24.2X+28.3	0.748	0.0120
O. aquaticum	Flooded	Total Biomass	Y = 48.0X+633.6	0.934	< 0.0001
	Non-flooded	"	Y = 46.5X+378.2	0.864	0.0008
T. distichum	Flooded	"	Y = 214.6X-103.5	0.751	0.0053
	Non-flooded	"	Y = 24.2X+28.3	0.747	0.0120
O. aquaticum	Flooded	Root Biomass	Y = 90.7X+685.6	0.926	0.0001
	Non-flooded	"	Y = 86.5X+418.2	0.839	0.0010
T. distichum	Flooded	"	Y = -638.6Ln(X)+159.8	0.794	0.0030
	Non-flooded	"	Y = -194.9Ln(X)+334.2	0.633	0.0180
O. aquaticum	Flooded	Whole-Plant Transpiration	Y = 0.82Ln(X)-4.82	0.301	0.1580
	Non-flooded	"	Y = 0.31Ln(X)-1.31	0.067	0.5360
T. distichum	Flooded	"	Y = 0.91Ln(X)-2.99	0.032	0.6740
	Non-flooded	"	Y = 0.05Ln(X)+0.15	0.444	0.0710

†Whole-plant photosynthesis in μmol s^{-1}; shoot productivity in biomass in g $plant^{-1}$; whole-plant transpiration in mg s^{-1}.
‡CH_4 emissions in mg m^{-2} d^{-1}.

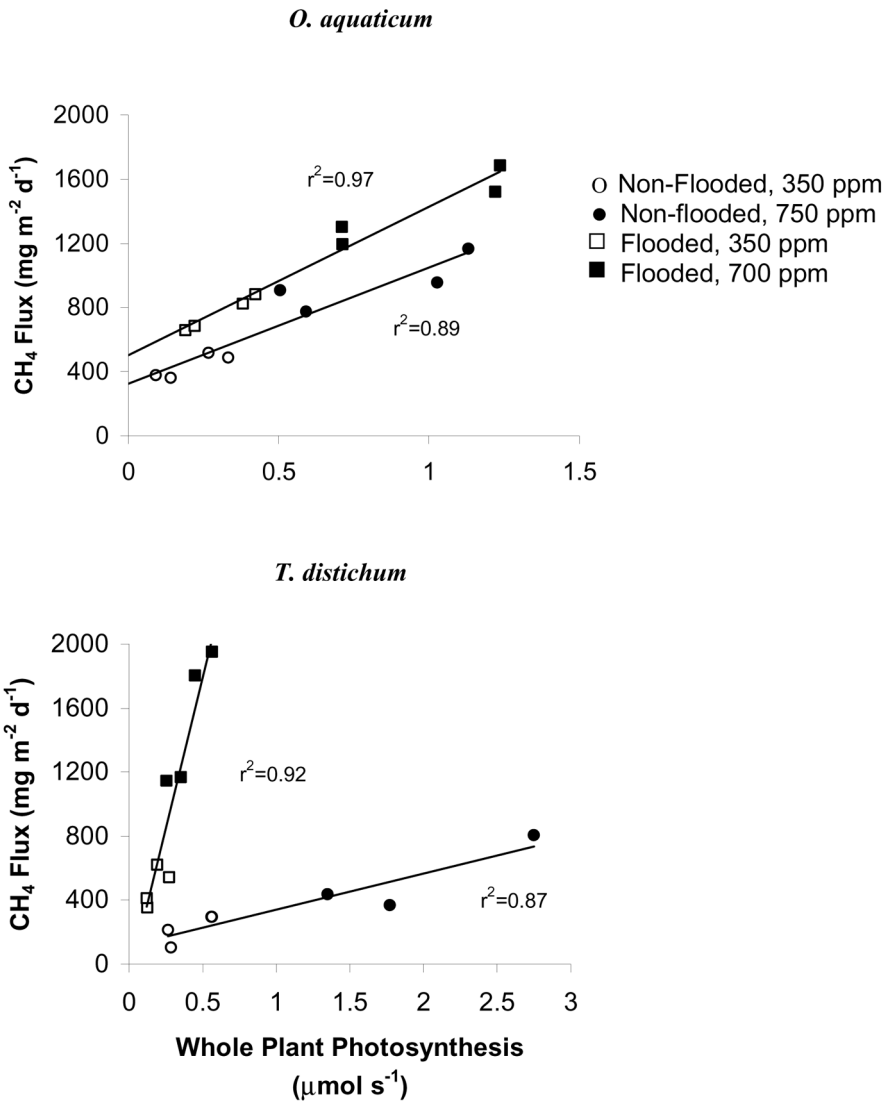

Figure 3. Regression of whole-plant photosynthesis and methane flux for *Orontium aquaticum* and *Taxodium distichum* plant-soil systems.

Discussion

Elevated CO_2 has been shown to increase CH_4 emissions in a wide variety of wetlands dominated by herbaceous plant species (Table 4). This is the first study to report an increase in CH_4 emissions from a soil in which a wetland tree was exposed to a CO_2-enriched atmosphere. The increase in emissions was substantial –

Table 2. Methane oxidation in replicate (n = 2) *Orontium aquaticum* microcosms estimated by application of CH_3F and compared to air (controls). Values are means ± 1 standard error.

Water Table Treatment	CO_2 Treatment	Inhibition Treatment	Pre-treatment flux	Post-treatment flux	Post – Pre Change	P-value†	Relative Oxidation
			mg CH_4 m^{-2} d^{-1}				%
Flooded	350	Air	1853 ± 149	1640 ± 91	−213 ± 92	0.0530	NS
		CH_3F	1760 ± 135	1913 ± 158	153 ± 100	0.2612	NS
	350	Air	1528 ± 87	1379 ± 84	−149 ± 56	0.0667	NS
		CH_3F	1751 ± 68	1582 ± 69	−169 ± 64	0.0529	NS
	700	Air	1438 ± 212	1642 ± 155	204 ± 91	0.0491	NS
		CH_3F	1609 ± 134	1793 ± 135	184 ± 54	0.0263	NS
	700	Air	2009 ± 131	2204 ± 160	195 ± 59	0.0110	10.0
		CH_3F	1731 ± 133	1950 ± 160	219 ± 143	0.2001	NS
Non-flooded	350	Air	1165 ± 55	1331 ± 95	166 ± 69	0.0568	NS
		CH_3F	1214 ± 69	1561 ± 78	347 ± 37	0.0002	22.2
	350	Air	1303 ± 83	1437 ± 71	134 ± 66	0.0667	NS
		CH_3F	1155 ± 84	1343 ± 62	188 ± 38	0.0088	14.4
	700	Air	1348 ± 174	1462 ± 175	114 ± 45	0.0491	NS
		CH_3F	1366 ± 151	1955 ± 249	589 ± 118	0.0002	29.0
	700	Air	1442 ± 303	1750 ± 483	308 ± 220	0.0110	17.6
		CH_3F	1437 ± 72	2296 ± 216	859 ± 148	0.0001	36.3

†Paired, one-sided t-tests on individual pots before and after treatment with CH_3F or air (control). Based on Bonferroni's correction for multiple t-tests, there was a significant increase in flux when P-values were $\leqslant$ 0.0125.

Table 3. Redox potential (mV) of no-plant controls, *Taxodium distichum*, and *Orontium aquaticum* microcosm soils. Values are means ± 1 standard deviation. Significant differences are indicated by the letter W where there was a water table treatment effect, placed on the larger mean.

		Redox Potential (mV)				
		Ambient CO_2 Treatment		Elevated CO_2 Treatment		Significant
Water Table Treatment	Species	350	350	700	700	Difference
Flooded	No-Plant Control	–297 ± 12	–297 ± 15	–294 ± 14	–299 ± 7	
	Taxodium distichum	–243 ± 16	–276 ± 21	–278 ± 20	–288 ± 15	
	Orontium aquaticum	–267 ± 12	–224 ± 11	–232 ± 12	–243 ± 9	
Non-flooded	No-Plant Control	95 ± 6	96 ± 6	93 ± 6	87 ± 4	W
	Taxodium distichum	147 ± 13	154 ± 12	143 ± 11	128 ± 8	W
	Orontium aquaticum	135 ± 14	151 ± 14	143 ± 19	145 ± 16	W

62 to 69%. A CO_2-induced stimulation of CH_4 emissions in a woody plant-soil system is significant because forested wetlands account for $\sim$60% of wetlands globally (Matthews and Fung 1987), and therefore have influence on atmospheric CH_4 concentrations.

Given the short duration of this study (18 weeks), there are two possible explanations for the increase in methane emissions in response to elevated CO_2. First, CH_4 production may have risen due to an increase in rhizodeposition (i.e. root exudation and/or rapid fine root turnover). Second, an increase in plant biomass may have increased the rate that soil CH_4 is ventilated through the plant. These explanations are not mutually exclusive and both processes may contribute to increased emissions. However, the first explanation represents an increase in gross CH_4 production with important implications for future emissions of CH_4, while the second represents a change in emissions pathway (i.e. plant transport vs. ebullition) that may be less important because it does not necessarily constitute a significant increase in methane flux to the atmosphere.

Evidence of a tight coupling between photosynthesis and methane production that would support the first mechanism (above) has been reported in carbon isotope tracer studies on *Oryza* spp. (rice) (Mindota and Kimura (1994, 1996)) and *O. aquaticum* (Megonigal et al. 1999). However, these studies could not determine whether carbon isotopes originating in the leaf were incorporated into CH_4 through acetate fermentation or CO_2-reduction. The later pathway does not necessarily indicate an energetic link between the plants and microbes (Megonigal et al. 1999). A positive correlation between methane emissions and net ecosystem exchange ex-

Table 4. Summary of studies on the effects elevated CO_2 on CH_4 emissions in wetlands.

System	Dominant Plant Species	Photosynthesis Stimulation †	Total Biomass Stimulation	CH_4 Stimulation	Citation
Sphagnum Bog	*Sphagnum* and Sedge Cores	ND	ND	0%	Saarino et al. (1998)
Oligotrophic Mire	*Eriophorum vaginatum*	100%	0%	250%	Hutchin et al. (1995)
Oligotrophic Mire	*Eriophorum vaginatum*	ND	ND	18%	Saarino and Silvola (1999)
Brackish Marsh	*Scirpus olneyi*	30–100%	19–76%	80%	Dacey et al. (1994) and Jacob et al. (1995)
Freshwater Marsh	*Orontium aquaticum*	54–71%	0%	136%	Megonigal and Schlesinger (1997)
Freshwater Marsh	*Orontium aquaticum*	50–67%	33–38%	27–29%	Vann (2000); this study
Freshwater Marsh	*Taxodium distichum*	50–107%	0%	62–69%	Vann (2000); this study
Rice Paddy	*Orzya sativa*	ND	35–83%	−86% ‡	Schrope et al. (1999)
Rice Paddy	*Oryza* spp.	ND	32%	55%	Ziska et al. (1998)

†Stimulation was calculated as (Elevated – Ambient)/Ambient. Statistically non-significant ($P < 0.05$) differences reported as zero.
‡Average of all treatments estimated from Figure 4 of Schrope et al. (1999).

ists for wetlands distributed from the subarctic to the subtropics (Whiting and Chanton 1993). Although net ecosystem exchange should be correlated with photosynthesis, a relationship between these parameters is confounded by microbial respiration in the soil. A study of bog and fen mecoscosms produced relationships between gross primary production and CH_4 emissions very similar to those reported here, with linear relationships that held across nutrient and heating treatments, but varied by water table depth treatment (Updegraff et al. 2001). The present study is unique in that photosynthetic rates were manipulated directly by elevating atmospheric CO_2. Because the relationship between photosynthesis and CH_4 emissions measured at ambient CO_2 levels held at elevated CO_2 levels (Figure 3), we consider these results the strongest evidence to date for a direct link between CO_2 assimilation and methanogenesis in wetlands. Furthermore, our work suggests that it may be possible to predict CH_4 emissions in a future, elevated CO_2 atmosphere by extrapolating published relationships between these variables that were determined at ambient CO_2.

Elevated CO_2 appears to always increase CO_2 assimilation and frequently increase CH_4 emissions from wetlands, even in the absence of an increase in plant biomass. Three of seven wetland studies that report both biomass and CH_4 emissions, found no increase in biomass but a 62 to 250% increase in CH_4 emissions (Table 4). In the present study, elevated CO_2 did not significantly increase total biomass of *T. distichum*, but CH_4 emissions increased by $\sim$ 66%. This is evidence that a substantial portion of the additional photosynthates produced in an elevated CO_2 atmosphere entered the soil as rhizodeposits and stimulated CH_4 production. In contrast to *T. distichum*, *O. aquaticum* exhibited a 36% increase in total biomass and a 28% increase in CH_4 emissions in response to elevated CO_2. In this case, an increase in CH_4 emissions may be due to both an increase in root exudation and CH_4 ventilation.

As in similar studies, root exudation could not be separated from rapid root turnover, so we use the term 'rhizodeposition' to refer to the sum of the two processes. Several studies of upland plant species have observed enhanced rhizodeposition in response to elevated CO_2 (Dhillion et al. 1996; Paterson et al. 1997; Sadowsky and Schortemeyer 1997; Ball 1997). Because microorganisms are generally carbon limited, an increase in soil carbon through rhizodeposition often stimulates microbial activity. Soils treated with glucose typically exhibit higher methane emissions than untreated soils (Yavitt et al. 1987; Aerts and Caluwe 1999). Thus, it is reasonable to assert that elevated CO_2 is acting through rhizodeposition to increase methanogenic activity.

Approximately 90% of the methane emitted to the atmosphere travels through aerenchyma tissue in herbaceous wetland plants (Chanton and Dacey 1991) and aerenchyma is a pathway for methane transport in wetland tree species (Pulliam 1992; Rusch and Rennenberg 1998). Because elevated CO_2 often increases plant biomass, the quantity of methane ventilated to the atmosphere through plants should also increase, producing a correlation between diffusive CH_4 flux and biomass. The balance of the CH_4 produced would be episodically released to the atmosphere in ebullition. In this study, plant biomass was strongly correlated with methane flux in

both species, suggesting an increase in CH_4 ventilation. However, some elevated CO_2 studies have reported an increase in methane emissions in the absence of an increase in biomass (Hutchin et al. 1995; Megonigal and Schlesinger 1997). Further research is needed to determine whether elevated CO_2 increases CH_4 emissions by stimulating methanogenesis, by increasing ventilation through plants, or by a combination of these mechanisms.

The water table treatment did not change the slope of the relationship between photosynthesis and CH_4 emissions in the *O. aquaticum* microcosms, but had a dramatic affect on the *T. distichum* microcosms (Figure 3). This species-specific difference in the effect of flooding was most likely due to differences in root depth distribution. As is typical of wetland trees, *T. distichum* had a shallow root system, while the roots of *O. aquaticum* were distributed equally throughout the soil. Thus, in a flooded condition, rhizodeposition in both species occurred in the anaerobic zone. In the non-flooded treatment, the *O. aquaticum* root system remained largely in the anaerobic zone while the *T. distichum* root system was largely in the aerobic zone where high O_2 levels suppressed methanogenesis. This fundamental difference in root distribution will make CH_4 emissions from forested wetlands more sensitive than herbaceous wetlands to anticipated changes in precipitation (Kattenburg et al. 1995).

Elevated CO_2 tended to increase CH_4 oxidation in the non-flooded treatment of *O. aquaticum* ($P = 0.07$). This effect was most likely caused by an increase in plant biomass that facilitated O_2 transport into the soil. Additional radial O_2 loss should increase CH_4 oxidation in systems where oxidation rates are O_2 limited (Calhoun and King 1997; van der Nat and Middelburg 1998). Alternatively, additional O_2 may suppress methane production in soils with low O_2 demand. Schrope et al. (1999) reported that elevated CO_2 decreased CH_4 emissions from a sandy soil planted with rice (*Oryza sativa*, Table 4). Our data suggest that elevated CO_2 will stimulate CH_4 emissions more than CH_4 sinks in soils with moderate O_2 demand.

CH_4 oxidation consumed 14 to 36% of gross CH_4 emissions in the non-flooded treatment of *O. aquaticum*, but had no significant effect on methane flux in the flooded treatment. This suggests that much of the CH_4 oxidation occurred at the soil surface. The absence of rhizospheric methane oxidation in the flooded *O. aquaticum* plant-soil system may have been due to intense competition for O_2 between methanotrophic bacteria and other O_2 consuming processes such as root respiration and nitrification (van Bodegon et al. 2001). The absence of rhizospheric CH_4 oxidation has been reported previously for *Peltandra virginica* growing in a 20–40 cm inundated soil (Chanton et al. 1992).

Conclusions

Elevated CO_2 increased CH_4 emissions from both woody (*T. distichum*) and non-woody (*O. aquaticum*) plant-soil microcosms by 27 to 69%. This occurred in both flooded and non-flooded soils suggesting that emissions will increase even in drier-

end wetlands. Forested wetlands may respond more strongly than herbaceous wetlands to future changes in water table depth. Because photosynthesis and CH_4 emissions were strongly correlated, we suspect that the increase in CH_4 emissions was primarily due to an increase in the exudation of recently fixed photosynthates and to a lesser extent by an increase in root turnover. However, there was also evidence that an increase in root biomass may have facilitated an increase in CH_4 ventilation. For *O. aquaticum* there was a trend of increased CH_4 oxidation in response to elevated CO_2, but the net effect favored an increase in CH_4 emissions.

Determining the mechanisms by which elevated CO_2 increases CH_4 emissions will be useful for modeling past and future changes in CH_4 emissions from wetlands. We used a microcosm design to investigate processes such as rhizodeposition and plant ventilation that can respond to changes in atmospheric CO_2 in a matter of a few months. Field studies are required to address additional processes that respond more slowly, such as biomass turnover and decomposition. Caution should be exercised in extrapolating our results to mature forests because the present study was conducted on seedlings; it is important that future studies manipulate wetland systems in situ. It is clear that studies must also be done in both forested and non-forested wetland systems to adequately capture the range of possible responses to elevated CO_2.

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