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Abstract

We have examined sediments from a fringing salt marsh in Maine to further understand marine CO metabolism, about which relatively little is known. Intact cores from the marsh emitted CO during dark oxic incubations, but emission rates were significantly higher during anoxic incubations, which provided evidence for simultaneous production and aerobic consumption in surface sediments. CO emission rates were also elevated when cores were exposed to light, which indicated that photochemical reactions play a role in CO production. A kinetic analysis of marsh surface sediments yielded an apparent K_m of about 82 ppm, which exceeded values reported for well-aerated soils that consume atmospheric CO (65 nM). Surface (0–0.2 cm depth interval) sediment slurries incubated under oxic conditions rapidly consumed CO, and methyl fluoride did not inhibit uptake, which indicated that neither ammonia nor methane oxidizers contributed to the observed activity. In contrast, aerobic CO uptake was inhibited by additions of readily available organic substrates (pyruvate, glucose and glycine), but not by cellulose. CO was also consumed by surface and sub-surface sediment slurries incubated under anaerobic conditions, but rates were less than during aerobic incubations. Molybdate and nitrate or nitrite, but not 2-bromoethanesulfonic acid, partially inhibited anaerobic uptake. These results suggest that sulfidogens and acetogens, but not dissimilatory nitrate reducers or methanogens, actively consume CO. Sediment-free plant roots also oxidized CO aerobically; rates for *Spartina patens* and *Limonium carolinianum* roots were significantly higher than rates for *Spartina alterniflora* roots. Thus plants may also impact CO cycling in estuarine environments.

Introduction

Carbon monoxide occurs in the troposphere at concentrations of about 60–350 parts per billion (ppb; Khalil & Rasmussen, 1990; Khalil, 1999). In spite of these low concentrations, it plays major roles in atmospheric chemistry (Crutzen & Gidel, 1983; Daniel & Solomon, 1998). Although atmospheric CO sources and sinks have been defined relatively well, the role of marine systems in global CO dynamics remains somewhat conjectural, largely due to the small number of studies to date. Several reports have documented super-saturated CO concentrations in surface waters, which support small net emissions (Conrad & Seiler, 1982; Jones, 1991; Johnson & Bates, 1996). Estimated emission rates represent just a fraction of total water column CO production, however, with the difference between production and emission attributed to microbial oxidation (Zafiriou *et al.*, 2003).

Although substantial CO oxidation may occur in the oceans (Zafiriou *et al.*, 2003; Tolli & Taylor, 2005), the organisms involved and the process itself have received relatively little attention. Initial studies of the water column (Conrad & Seiler, 1982) suggested a role for as yet unknown 'high affinity' carboxydotrophic bacteria, whereas other studies reported results consistent with activity by ammonia oxidizers (Jones & Morita, 1983). More recently, the first marine CO-oxidizing bacterial isolates have been described. Heterotrophic CO oxidizers, such as *Silicibacter pomeroyi* (King, 2003; Moran *et al.*, 2004) and members of the cosmopolitan genus *Stappia* (King, 2003), may prove to contribute significantly to CO uptake. Recent studies by Tolli & Taylor (2005) and Tolli *et al.* (2006) have also suggested a role for heterotrophic or facultatively lithotroph CO oxidizers, especially members of the *Alphaproteobacteria*, and have documented inhibition of activity by light. Nonetheless, much remains unknown about the

distribution, diversity and activity of marine CO-oxidizing bacteria in the water column.

Benthic CO dynamics are even less well understood. Results from terrestrial and water column studies suggest that several processes may be involved. CO can be produced by benthic algae at the surface of illuminated sediments, or by photochemical organic matter degradation (Schade *et al.*, 1999; King, 2001). In the absence of light, CO may be produced by abiological decomposition of organics (Conrad & Seiler, 1985), and by a variety of biological reactions (Engel *et al.*, 1972; Troxler & Dokos, 1973; Yoshida *et al.*, 1982; Hino & Tauchi, 1987; Migita *et al.*, 1998). CO may be consumed by multiple functional groups of bacteria, including aerobic CO oxidizers, methane- and ammonia-oxidizers, and anaerobes, such as acetogens, methanogens, and sulfate reducers (Meyer & Schlegel, 1983; Frunzke & Meyer, 1990; Svetlichny *et al.*, 1991; Mörsdorf *et al.*, 1992; Kerby *et al.*, 1995; Ragsdale, 2004; King, 2006). It is not evident, though, how CO production and consumption vary spatially or temporally, and whether sediments consume or emit CO.

We report here the first analyses of benthic marine CO metabolism. We have observed net CO emission to the atmosphere from *Spartina alterniflora* salt marsh sediments. Emission patterns obtained under oxic and anoxic conditions suggested that CO production and oxidation occur simultaneously, with the latter exceeding the former. Sediment slurry analyses revealed active aerobic CO oxidation that was not inhibited by methyl fluoride, which suggests that facultatively lithotrophic or heterotrophic CO oxidizers dominate activity rather than ammonia- or methane-oxidizing bacteria. Slurry results have also suggested that CO oxidation rates may be sensitive to organic substrate availability, and that sulfate reducers and acetogens oxidize CO anaerobically. Incubations of washed, sediment-free excised roots from three common marine macrophytes suggest that plants may represent CO sources in vegetated sediments, and suggest that plants affect the distribution and activity of CO-oxidizing bacteria.

Materials and methods

Site description

CO uptake and production analyses used sediment and macrophyte roots collected from an *S. alterniflora*-dominated fringing salt marsh in Lowes Cove, Maine. Salinities of the Damariscotta River, which floods the salt marsh, varied between about 30–32 ppt. Other aspects of the Lowes Cove system have been described previously (King *et al.*, 1983, 1986, 1990b; Findlay *et al.*, 1989; Sawyer & King, 1993; Hansen *et al.*, 1996; Therkildsen *et al.*, 1996).

Intact core CO fluxes

To determine the magnitude of CO exchange rates between marsh sediments and the atmosphere, triplicate intact cores were collected using aluminum tubes [7.2 cm inner diameter (ID) × 30 cm], the tops of which were sealed after returning to the laboratory. Cores were first incubated with an ambient air headspace to determine fluxes under oxic conditions. Subsequently, tube headspaces were flushed for 30 min with oxygen-free nitrogen. CO fluxes were then measured under anoxic conditions. Headspace samples were removed by needle and syringe and analyzed as described previously (King, 2000).

A second set of triplicate intact cores was collected during low tide using stainless steel collars (9.2 cm ID × 10 cm; King, 2000). Cores were returned to the laboratory, fitted with a quartz chamber and incubated at ambient temperature in a greenhouse either in the absence of light, or under a 1000 W sodium vapor lamp with about 290 μE incident radiation for 400–700 nm wavelengths (PAR, photosynthetically active radiation; measured with a Li-Cor quantum sensor), and about 2.8 watt m^{-2} for total UV radiation wavelengths between 295 and 385 nm (UV-A+B measured with an Eppley TUVR radiometer). Cores were incubated without illumination first followed by incubation with illumination. CO accumulation in the chambers was monitored by periodically sampling the headspaces with a needle and syringe for analysis by gas chromatography as described previously (King, 2000).

Slurry preparation and analyses

Intact cores were collected during June-September using acrylic tubes (6.5 cm ID × 30 cm). Sediment was extruded after returning to the laboratory, and the 0–0.2 cm (surface) and 7–10 cm (sub-surface) depth intervals were pooled to prepare slurries. These intervals were selected to represent zones of primarily oxic and anoxic metabolism, respectively, as micro-oxygen electrode profiles reveal 1–2 mm oxygen penetration depths (G.M King, unpublished data). Slurries consisted of sediment and sterile artificial seawater (ASW) in a 1 : 4 ratio (gram fresh weight (gfw) : vol). Surface sediment was slurried in the presence of ambient air and sub-surface sediment was slurried in a glovebag containing a headspace of oxygen-free nitrogen. Slurries (10 cm^3) were dispensed into 160-cm^3 serum bottles that were subsequently sealed with neoprene stoppers. After adding CO to serum bottle headspaces, uptake was monitored by sampling with syringes and needles, and analyzing concentrations by gas chromatography as previously described (King, 2000). All slurries were incubated in triplicate for each treatment with rotary shaking (150 r.p.m.) at ambient temperature.

For aerobic CO uptake assays, surface sediment slurries were incubated with an air headspace containing about

100 ppm CO initially, with or without 1% methyl fluoride (MF). MF was used to inhibit CO oxidation by methane- and ammonia-oxidizing bacteria (King, 1999). A second set of slurries was amended with pyruvate, glycine, or glucose (25 mM final concentrations), or cellulose at a final concentration of 4.5 mg cm^{-3} slurry (equivalent to 25 mM glucose carbon). Unamended slurries served as controls.

Surface sediment slurries were used to estimate CO uptake kinetic parameters (V_{max}, maximum uptake velocity; $_{app}K_m$, apparent K_m). CO was added at a final concentration of about 230 ppm to the headspaces of slurries incubated in 160-cm^3 serum bottles. Uptake was monitored at intervals to obtain 'progress curves', which were analyzed by the non-linear curve-fitting algorithm of Kaleidagraph software (Synergy Software, Inc.) to obtain V_{max} and $_{app}K_m$ values.

To determine the potential anaerobic fate of CO, surface sediment slurries were incubated with oxygen-free nitrogen headspaces containing initial CO concentrations of about 100 ppm with or without sodium nitrate added at a final concentration of 10 mM. A similar set of slurries was prepared using sub-surface (5–7 cm depth interval) sediment. These slurries were incubated with: no addition, sodium nitrate (10 mM), sodium nitrite (10 mM), sodium molybdate (20 mM), or 2-bromoethanesulfonic acid (BES, 20 mM). Molybdate and BES have been well documented as sulfate reduction and methanogenesis inhibitors, respectively (Oremland & Capone, 1988; Scholten *et al.*, 2000). Sodium nitrate and nitrite were added to stimulate denitrification and dissimilatory nitrate respiration.

An additional set of surface and sub-surface slurries was prepared to compare the effects of molybdate, tungstate and vanadate, all of which inhibit sulfate reduction (Oremland & Capone, 1988; Lie *et al.*, 1999). Oxic and anoxic slurries were prepared as before; oxic slurries were incubated with or without addition of 20 mM sodium vanadate and an air headspace containing about 100 ppm CO. Anoxic slurries were incubated with oxygen-free nitrogen headspaces containing about 100 ppm CO and 20 mM sodium molybdate, tungstate or vanadate; unamended slurries served as controls.

CO uptake by sediment-free plant root

Live, excised roots were obtained from three halophytes, *S. alterniflora*, *S. patens* and *L. carolinianum*. Roots were rinsed thoroughly with seawater from the Damariscotta River to remove all loosely adhering sediment. The fine root fraction (< 2 mm diameter) was separated from coarse roots, blotted gently to remove excess water, and transferred to 110-cm^3 jars that were subsequently sealed with neoprene stoppers. CO was added to final concentrations of approximately 150 ppm and uptake was monitored as above.

Results

Intact core CO fluxes

CO was emitted from two different sets of salt marsh cores incubated aerobically in darkness at rates from 50 ± 26 to 1380 ± 170 ng CO m^{-2} day^{-1}. No obvious differences other than core diameter were noted between the two core sets during collection or processing. The headspaces of the core set with the lowest emission rates were flushed with nitrogen to create an anoxic atmosphere. Subsequent emission rates rose to 320 ± 20 ng CO m^{-2} day^{-1} (Fig. 1). Based on the difference between oxic and anoxic emission rates, aerobic CO oxidation consumed up to $85 \pm 7\%$ of the potential CO flux. The core set with the higher dark emission rates was incubated with illumination (PAR = 290 μE; UV = 2.8 W m^{-2}). Emission rates during illumination rose to 2840 ± 650 ng CO m^{-2} day^{-1}, an increase of $110 \pm 40\%$.

Sediment slurry CO consumption

Surface sediment slurries consumed CO added at about 100-ppm concentrations with initial rates of 5.5 ± 0.4 and 5.8 ± 0.3 nmol cm^{-3} slurry h^{-1} for air only and air plus 1% MF treatments, respectively (Fig. 2). These rates did not differ significantly ($P = 0.513$). A kinetic analysis revealed a maximum potential CO uptake rate (V_{max}) of 7.5 ± 1.3 nmol cm^{-3} slurry h^{-1} and an apparent K_m of 81.8 ± 20.4 ppm (equivalent to a dissolved concentration of about

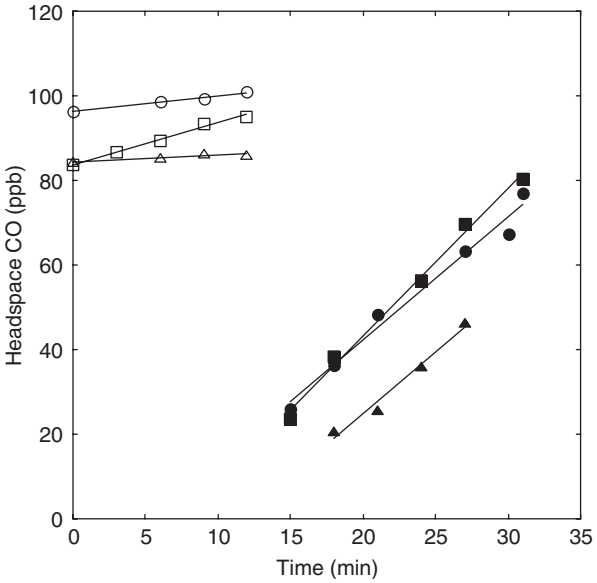

Fig. 1. Time course of CO accumulation in intact core headspaces. Different symbols represent individual replicates; open symbols represent cores incubated with an air headspace, closed symbols represent anoxic incubations.

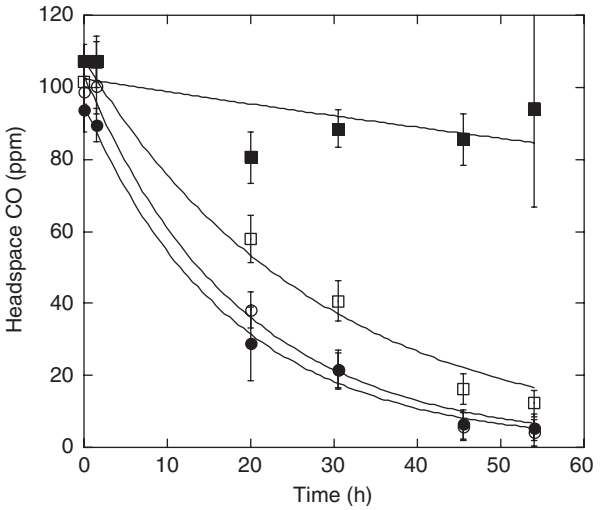

Fig. 2. Time course of headspace CO concentrations (means ± 1 SE, $n = 3$) for surface sediment slurries incubated with air only (oxic; ○), air plus 1% methyl fluoride (●), nitrogen only (anoxic, □), nitrogen plus nitrate (■).

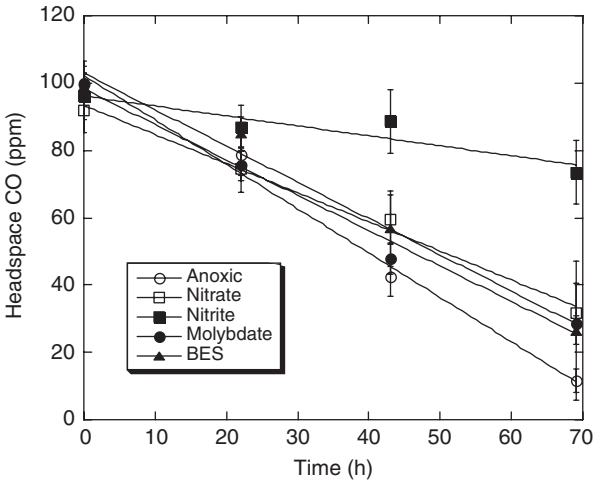

Fig. 3. Time course of headspace CO concentrations (means ± 1 SE, $n = 3$) for sub-surface sediment slurries incubated with nitrogen only (anoxic; ○), nitrogen plus nitrate (□), nitrogen plus nitrite (■), nitrogen plus molybdate (●) and nitrogen plus BES (▲).

65 ± 16 nM). The decrease in headspace CO concentrations during this assay was modeled well ($r^2 = 0.99$) using a non-linear algorithm for the integrated form of the Michaelis–Menten expression: $V_{max}t = C_0 - C_t - K_m \times \ln(C_t/C_0)$.

CO was also consumed at rates of 3.6 ± 0.3 nmol cm^{-3} slurry h^{-1} by surface sediment slurries incubated anaerobically (Fig. 2). These rates were about 33% less than and significantly different from aerobic rates ($P < 0.0001$). Surface sediment slurries incubated anaerobically in the presence of 10 mM sodium nitrate consumed CO at initial rates of about 1.5 nmol cm^{-3} slurry h^{-1}, but uptake was completely inhibited after 20 h.

Sub-surface sediment slurries consumed CO during anaerobic incubations at rates of 0.8 ± 0.1 nmol cm^{-3} slurry h^{-1} that were about fourfold lower than rates for surface sediment slurries (Fig. 3). Both nitrate and nitrite (10 mM) significantly inhibited uptake (35% and 78%, respectively) based on ANOVA. Molybdate and BES additions resulted in slight inhibition, about 20%, which was not statistically significant.

Results from additional analyses of surface sediment slurries incubated aerobically revealed CO uptake rates (3.0 ± 0.1 nmol cm^{-3} slurry h^{-1}) comparable to those previously observed, and also showed a small, statistically significant inhibition (about 32%) by 20 mM sodium vanadate (Fig. 4). Likewise, rates for anaerobically incubated sub-surface sediment slurries (0.5 ± 0.02 nmol cm^{-3} slurry h^{-1}) were comparable to values obtained initially. However, 20 mM molybdate and tungstate each inhibited uptake significantly (about 50%), while 20 mM vanadate resulted in complete inhibition (Fig. 4).

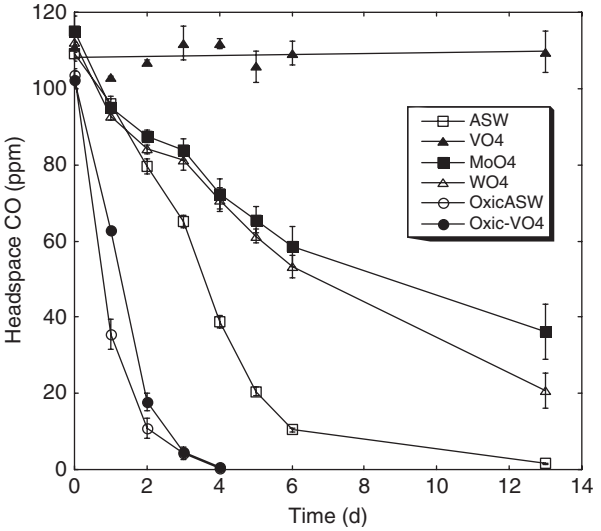

Fig. 4. Time course of headspace CO concentrations (means ± 1 SE, $n = 3$) for surface sediment slurries incubated with air only (oxic; ○) or air plus vanadate (●) and sub-surface sediment slurries incubated with nitrogen only (□), nitrogen plus molybdate (■), nitrogen plus tungstate (Δ) and nitrogen plus vanadate (▲).

Addition of exogenous carbon sources decreased surface sediment slurry CO uptake rates (Fig. 5). Unamended slurries consumed CO at a rate of 8.1 ± 1.5 nmol cm^{-3} slurry h^{-1}. Cellulose addition had no effect on activity (rate = 8.1 ± 1.6 nmol cm^{-3} slurry h^{-1}). Pyruvate inhibited uptake by 34% (rate = 5.3 ± 0.3 nmol cm^{-3} slurry h^{-1}); glycine inhibited uptake by 42% (rate = 4.7 ± 0.5 nmol cm^{-3} slurry h^{-1}), and glucose inhibited activity by 59% (rate-3.3 ± 0.2 nmol cm^{-3} slurry h^{-1}).

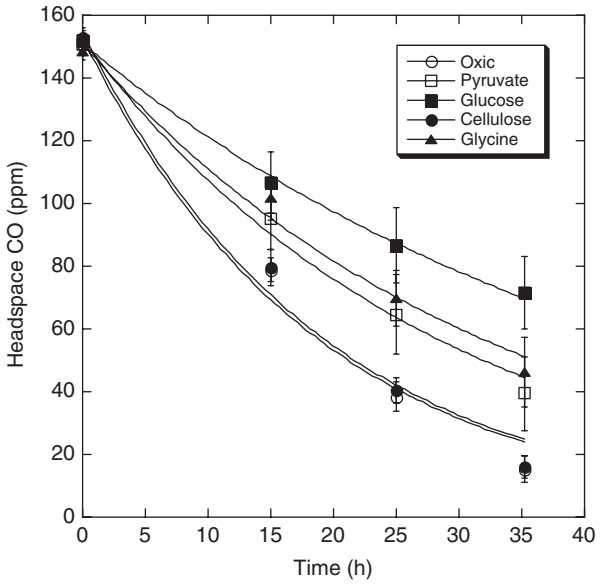

Fig. 5. Time course of headspace CO concentrations (means ± 1 SE, $n = 3$) for surface sediment slurries incubated with air only (○) or air plus glucose (■), air plus glycine (▲), air plus pyruvate (□) or air plus cellulose (●).

Root CO production and uptake

When incubated with ambient CO concentrations, washed excised roots produced CO at net rates ranging from 9 ± 1 to 39 ± 1 nmol gram dry weight $(gdw)^{-1}\,h^{-1}$, with the greatest activity observed for *S. alterniflora*. When incubated with air containing approximately 150 ppm initial CO concentrations, washed excised roots consumed CO at net rates of 107 ± 13 nmol $gdw^{-1}\,h^{-1}$ for *S. patens*, 100 ± 13 nmol $gdw^{-1}\,h^{-1}$ for *L. carolinianum* and 20 ± 7 nmol $gdw^{-1}\,h^{-1}$ for *S. alterniflora*. *Spartina alterniflora* rates were significantly lower than those for *S. patens* and *L. carolinianum* ($P < 0.1$), while the latter did not differ.

Discussion

CO was emitted at rates up to 1.4 mg CO $m^{-2}\,d^{-1}$ from intact salt marsh cores collected between plant culms and incubated without illumination. These rates were less than or comparable to values that have been reported for unilluminated Maine forest (1.4–3.6 mg CO $m^{-2}\,d^{-1}$) and agricultural (1.1 mg CO $m^{-2}\,d^{-1}$) soils, but net CO emission at these sites occurred only intermittently during periods when the soils were water saturated, and on an annual basis the soils were net consumers of atmospheric CO (King, 2000). In contrast, Lowes Cove marsh sediments may emit CO throughout the year as they remain water-saturated annually. This could mean that Maine and perhaps other salt marsh sediments represent small, net annual sources of atmospheric CO.

Bacteria that consume CO aerobically in marine sediments are essentially unknown at present. Sediment slurry results, however, provide some insights. Neither methane- nor ammonia-oxidizing bacteria appear involved, since a specific inhibitor of these groups, methyl fluoride, did not affect activity. Instead, facultatively lithotrophic aerobic heterotrophs functionally similar to those in CO-oxidizing genera, such as *Stappia* and *Silicibacter*, are more likely candidates (King, 2003; Moran *et al.*, 2004). Though not yet isolated from coastal sediments specifically, these and other marine CO-oxidizing *Proteobacteria* occur ubiquitously, and have been isolated from salt marshes and other coastal environments (Buchan *et al.*, 2001; King, 2001).

As the sediments examined in this study did not consume atmospheric CO, it is perhaps not surprising that they exhibited a moderate ${}_{app}K_m$. The observed value, 82 ppm, is approximately an order of magnitude greater than values observed for oxic soils and the marine water column (Conrad & Seiler, 1980, 1982; King, 1999; Hardy & King, 2001; Tolli & Taylor, 2005), but is less than values reported for 'low affinity' carboxydotrophic isolates (Conrad *et al.*, 1981). Whether the ${}_{app}K_m$ for Lowes Cove sediment is an intrinsic property of the active microbes or a variable that responds to environmental conditions remains to be determined.

Multiple variables likely control the magnitude of salt marsh CO emission. Comparisons of CO fluxes under oxic and anoxic conditions (Fig. 1) suggest that oxygen availability constrains emission. A six-fold increase in emissions for anoxic versus oxic incubations likely reflects the impact of aerobic CO oxidizers at or near the sediment surface, and provides evidence supporting simultaneous production and consumption of CO as occurs in soils (Conrad, 1996; King, 1999). The apparent impact of aerobic CO oxidation, a reduction of 85% in CO emission rates, compares well with the reported impact of an analogous process, aerobic methane oxidation. Methane oxidation at the surface of wetlands typically reduces fluxes by > 50% (King, 1990a, 1992).

Relative to incubation in darkness, higher rates of CO emission were observed when sediments were illuminated. Emission rates doubled with a light source of 290 μE PAR and 2.8 W m^{-2} TUVR (total ultraviolet radiometer), which represent less than 20% of values for peak summer isolation in Maine. Emission rates for salt marsh sediments likely increase with increasing light intensity as has been reported for soils, plant litter, leaves and surface waters (Schade *et al.*, 1999), which suggests that changes reported here are minimal. Increased emission from sediments undoubtedly occurs due to photochemical CO production from organic matter (Schade *et al.*, 1999). Greater oxygen availability within the sediments resulting from increased photosynthetic activity (G.M King, unpublished results) does not compensate for photochemical CO production, though it is possible fluxes are attenuated to some extent by aerobic oxidation.

Anaerobic metabolism may also reduce CO fluxes. Both surface sediment and sub-surface sediment slurries consume CO anaerobically (Figs 2 and 3). Several functional groups may account for the observed activity, including denitrifiers, dissimilatory nitrate reducers, acetogens, sulfate reducers and methanogens, all of which are known to oxidize CO (Ragsdale, 2004; Jensen & Finster, 2005; King, 2006). Nitrate and nitrite additions inhibited slurry CO uptake (Figs 2 and 3), however, which suggests that denitrifiers and dissimilatory nitrate reducers play minor roles, if any, in anaerobic CO oxidation.

Nitrate inhibition implicates sulfate reducers, methanogens and acetogens, as these groups are nitrate-sensitive in general (Balderston & Payne, 1976; Achtnich *et al.*, 1995; Fröstl *et al.*, 1996). Of these, methanogens are likely unimportant as BES, a methanogen-specific inhibitor, did not affect CO uptake (Fig. 3). Molybdate and tungstate, both specific inhibitors of sulfate reducers, partially decreased CO uptake (20–50%; Figs 3 and 4), which suggests that anaerobic CO metabolism may be coupled to sulfate reduction. Nonetheless, significant activity remained in the presence of these inhibitors (50–80%), and this can be attributed to acetogens. Acetogens have previously been reported to consume CO in soils (Küsel & Drake, 1995; Wagner *et al.*, 1996), and likely do so in sediments as well. Although vanadate completely inhibited anaerobic CO uptake, it also partially inhibited aerobic uptake, likely due in part to the fact that vanadate can inhibit ATPase activity. Thus, vanadate appears to affect multiple functional groups, not only sulfate reducers, and cannot be used to infer which groups consume CO. In spite of these limitations, the results from anaerobic incubations support activity by both sulfate reducers and acetogens.

In addition to oxygen availability, soluble organic substrates affect aerobic CO uptake in surface sediment slurries (Fig. 5). Pyruvate, glycine and glucose additions each inhibit uptake. This response further supports a major role for facultatively lithotrophic CO oxidizers and a minimal role for methanotrophs and ammonia oxidizers. The former characteristically prefer organic substrates to CO (Meyer & Schlegel, 1983; Mörsdorf *et al.*, 1992), and, in at least some CO oxidizers, such substrates repress expression of the genes for CO oxidation and otherwise limit activity (Mörsdorf *et al.*, 1992). Inhibition of CO uptake is not consistent with a major role for methanotrophs and ammonia oxidizers, as neither typically uses heterotrophic substrates. An unusual methanotroph in the genus, *Methylocella*, grows with heterotrophic substrates (Dedysh *et al.*, 2005) but is not anticipated to contribute to benthic marine CO oxidation as it has only been isolated from acidic peat or soils thus far.

Although heterotrophic substrate regulation of short-term CO uptake has also been observed in studies of CO consumption by freshwater macrophyte roots (Rich & King, 1998), the role of organic substrates *in situ* is uncertain. Particulate organic matter, e.g. cellulose, appears to have little or no effect (Fig. 5) as it is not readily available. Further, concentrations of readily available soluble substrates are likely too low in most instances to have a major impact. Thus, heterotrophic substrate regulation may occur more as an indirect phenomenon, expressed through the impact of substrate on microbial biomass, as has been reported for soils (Moxley & Smith, 1998).

Salt marsh root activity may also affect CO dynamics. Results presented here document CO consumption and production by roots of three common halophytes. These observations are consistent with patterns for terrestrial plants, which emit CO produced from several biological and abiological reactions when incubated with an atmosphere containing low CO concentrations (King & Crosby, 2002). Roots consume CO when incubated with high concentrations due to the presence of CO-oxidizing bacteria in and on roots, which reduce the amount of total CO lost from root tissue (King & Crosby, 2002). Although *S. alterniflora* roots consumed CO at statistically lower rates than *S. patens* or *L. carolinianum*, factors that account for the differences are unclear. Similar variability exists in comparisons among terrestrial plants (King & Crosby, 2002).

Results from this study also reveal that salt marsh root CO consumption rates are similar to values obtained for freshwater plants when incubated with similar CO concentrations, but substantially higher than rates for terrestrial plant roots (Rich & King, 1998; King & Crosby, 2002). These comparisons suggest that aquatic plant roots generally support more abundant and active CO-oxidizer populations than terrestrial plants. Reasons for the observed trends are uncertain, but may include systematic differences in CO availability, water stress or other variables.

Regardless, salt marsh plant roots provide environments below the oxic sediment surface where CO-oxidizing populations may flourish. As CO oxidizers typically consume organic substrates and often fix nitrogen, denitrify or respire nitrate (Meyer & Schlegel, 1983; King, 2003), sub-surface populations may participate in salt marsh carbon and nitrogen cycling as well as CO biogeochemistry. In addition, plant roots likely increase the phylogenetic diversity of benthic CO oxidizers relative to unvegetated sediments by increasing the diversity of microhabitats available for colonization and by providing a sub-surface source of CO (i.e. produced by the roots themselves). The recent development of molecular approaches for analyzing carbon monoxide dehydrogenase genes (Dunfield & King, 2004, 2005) will facilitate assessments of marine CO oxidizer diversity in salt marshes and other systems.

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