

Oxygen depletion and carbon dioxide and methane production in waters of the Pantanal wetland of Brazil

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Abstract. This study examines dissolved O₂, CO₂ and CH₄ in waters of the Pantanal, a vast savanna floodplain in Brazil. Measurements are presented for 540 samples from throughout the region, ranging from areas of sheet flooding to sluggish marsh streams to the major rivers of the region. Dissolved O₂ is often strongly depleted, particularly in waters filled with emergent vascular plants, which are the most extensive aquatic environment of the region. Median O₂ concentrations were 35 μM for vegetated waters, 116 μM for the Paraguay River, 95 μM for tributary rivers, and 165 μM for open lakes (atmospheric equilibrium, 230–290 μM). Air-water diffusive fluxes were calculated from dissolved gas concentrations for representative vegetated floodplain waters, based on data collected over the course of an annual cycle. These fluxes reveal about twice as much CO₂ evasion as can be accounted for by invasion of O₂ (overall means in nmol cm⁻² s⁻¹: O₂ 0.18, CO₂ 0.34, and CH₄ 0.017). Methanogenesis is estimated to account for ca. 20% of the total heterotrophic metabolism in the water column and sediments, with the remainder likely due mostly to aerobic respiration. Anaerobic respiration is limited by the low concentrations of alternate electron acceptors. We hypothesize that O₂ transported through the stems of emergent plants is consumed in aerobic respiration by plant tissues or microorganisms, producing CO₂ that preferentially dissolves into the water, and thus explaining most of the excess CO₂ evasion. This hypothesis is supported by measurements of gases in submersed stems of emergent plants.

Key words: aquatic plants, decomposition, dissolved gases, floodplains, methanogenesis, wetlands

Introduction

Concentrations of dissolved O₂, CO₂, and CH₄ in wetland waters deviate from atmospheric equilibrium under the influence of biotic activity, including photosynthesis, aerobic and anaerobic respiration, and methanogenesis. The direction and magnitude of these deviations reflect the relative importance of the various biotic processes that consume or produce gases, and provide an indication of the overall nature of the aquatic metabolism, which may be autotrophic or heterotrophic. Many surface waters and especially wetland waters tend to contain dissolved O₂ at concentrations well below atmospheric equilibrium, indicating net heterotrophy beneath the water surface (Beadle 1981; Welcomme 1985; Richey et al. 1988). Although commonly assumed

to be the result of microbial decomposition of organic matter, the specific processes responsible for net heterotrophy in wetland waters are not well understood. Depletion of dissolved O_2 is of fundamental importance to the ecosystem because it changes the nature and rate of microbial metabolism, influences the redox states and mobilities of elements such as Fe and S (Ponnamperuma 1972), and presents a strong limiting factor for many higher plants and animals (Armstrong 1979; Kramer et al. 1978).

The objective of this study was to investigate the spatial and temporal variations in dissolved gas concentrations in waters of the Pantanal, a vast savanna floodplain that represents a widespread but little studied type of tropical ecosystem. We analyze a large set of measurements of dissolved O_2 , CO_2 , and CH_4 from throughout the region to gain insight into the processes that control the concentrations of these gases. Additional data on water chemistry and on gases in plant stems are presented to aid in interpreting the patterns in the dissolved gases. We focus on understanding the major controls of dissolved gases in the seasonally flooded, often densely vegetated floodplain areas that comprise most of the inundable area in the region. These waters are shown to be characteristically O_2 -depleted. Comparison of diffusive fluxes of O_2 , CO_2 and CH_4 provides evidence that, on the scale of the ecosystem, a large fraction of the O_2 consumed beneath the water surface enters through the stems of emergent plants as well as by diffusive exchange with the atmosphere, and that the respiratory demands of the plant tissues can account for much of the O_2 consumption.

Study area

One of the world's largest tropical wetlands, the Pantanal occupies an area of approximately 140,000 km² in the upper Paraguay River basin, mostly within Brazil (Fig. 1). The Paraguay River runs from north to south along the western side of the Pantanal, collecting water from the various tributaries and non-channelized floodplain inflows. Flooding in the region is distinctly seasonal, although the flooding period may be delayed for months after the summer rains due to slow passage of floodwaters through the Pantanal. Many areas are flooded by riverine overflow, while other areas normally flood with local rainfall or mixtures of rain and river water.

Savanna and mixtures of grassland with patches of semideciduous forest are the most common vegetation formations in inundable areas (Prance & Schaller 1982). Trees and shrubs are often present but usually have sparse canopies in inundable areas, allowing herbaceous plants to coexist. Aquatic or semi-aquatic vascular plants occur almost everywhere surface water persists for more than a few weeks, except in the deepest basins that remain

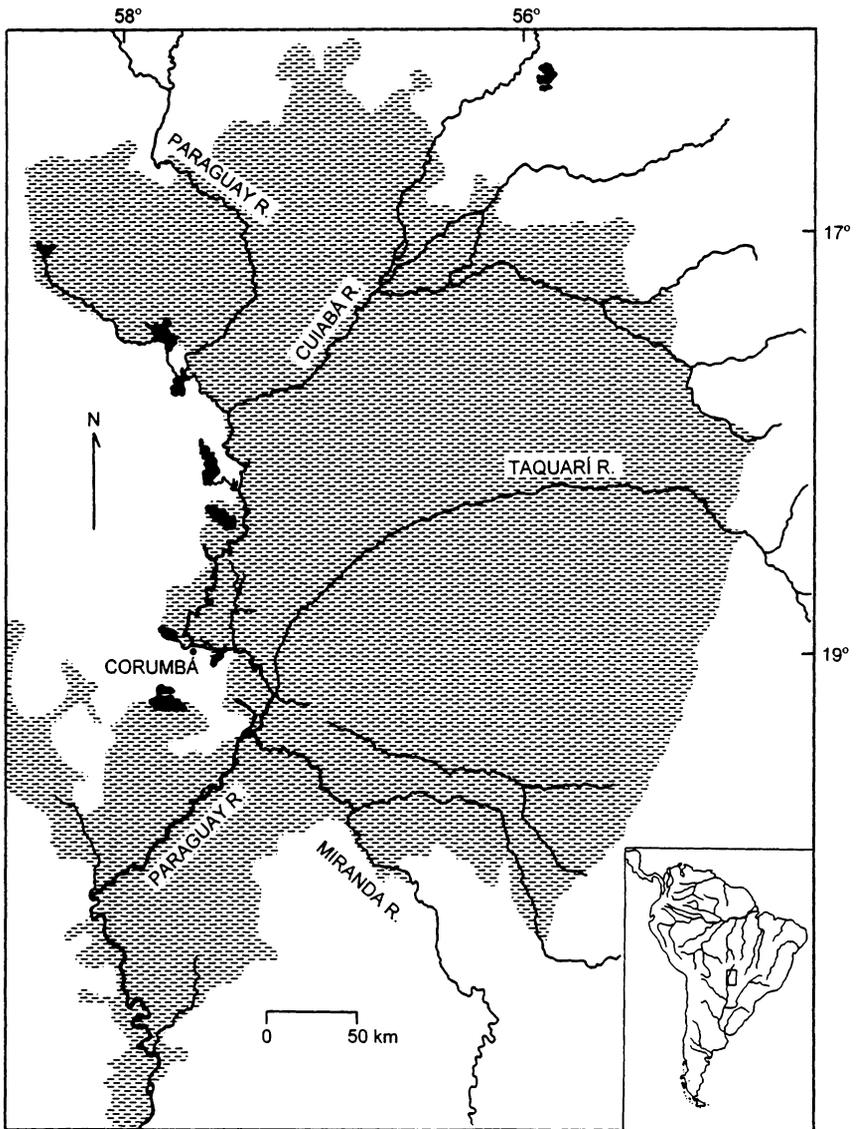


Fig. 1. Location of the Pantanal wetland. Most of the inundable territory is within Brazil, with lesser areas to the west of the Paraguay River in Bolivia and Paraguay. Area subject to seasonal inundation is shaded and large permanent lakes are black.

inundated throughout the year. Floating and rooted emergent forms are most abundant (Prance & Schaller 1982; Pott et al. 1989; Pott et al. 1992). Floating emergent plants such as *Eichhornea azurea*, *E. crassipes*, *Scirpus cubensis*, and *Salvinia* spp. tend to dominate in more deeply flooded environments. The

Table 1. Summary of water depth and flow at the sampling sites. Data are means \pm s.d. Mean flow for the vegetated waters does not include the 99 of 277 vegetated-water samples in which no flow was visible.

Group	Number of samples	Depth (m)	Flow (m/s)
Paraguay River	110	8.6 \pm 3.1	0.62 \pm 0.16
Tributary Rivers	123	4.1 \pm 2.0	0.35 \pm 0.22
Open Lakes	30	3.5 \pm 1.8	no flow
Vegetated Waters	277	1.9 \pm 1.6	0.15 \pm 0.19

most abundant rooted emergent species include *Cyperus giganteus*, *Thalia geniculata*, *Typha domingensis*, *Pontederia cordata* var. *lancifolia*, *Ipomoea* sp., *Polygonum* spp., *Oryza* sp., and *Paspalum* spp. The first four of these taxa have more vertical, erect stems and tend to colonize areas of shallower water (< ca. 1-m depth). The latter four taxa commonly develop long, floating stems but retain roots in the sediments, and can survive deeper inundation. Floating species typically produce large quantities of adventitious roots in the water during inundation, and these water roots commonly occur through most of the water column. Submersed vascular plants are less conspicuous in most environments, but *Utricularia* spp. and *Cabomba piauhyensis* are often present.

Vegetated waters, defined here as waters containing emergent herbaceous plants, are by far the most extensive aquatic environment of the Pantanal. Most of these environments dry seasonally, although some have remained flooded continuously during recent series of relatively wet years. During inundation, the amount of vegetation that remains above the water is variable, but dense canopies are common. The water in most vegetated floodplain areas tends to flow slowly in a characteristic direction, at least during high water.

Lakes in the Pantanal comprise a smaller proportion of the overall floodplain area than do lakes in the fringing floodplains of the Amazon and Orinoco rivers (Sippel et al. 1992; Hamilton & Lewis 1990a). Most lakes of the Pantanal can be grouped into three major types: 1) the large lakes in the vicinity of the Paraguay River, 2) the small lakes and ponds of the Taquari River alluvial fan, and 3) the small lateral levee lakes of the riverine floodplains. We define lakes here as basins that contain open water throughout the year and that do not exhibit substantial flow (e.g., not >ca. 5 cm/s).

Previous limnological research in the region has focused on floodplain lakes along the Cuiabá River in the northern Pantanal (Da Silva 1990; Pinto-Silva 1991), on the small lakes of the Taquari alluvial fan (Mourão 1988;

Mourão et al. 1988), and on the larger rivers (Alexandre 1982; EMBRAPA 1990). Da Silva and Pinto-Silva (1989) sampled floodplain waters along the road between Poconé and Porto Jofre throughout the year, measuring several limnological variables including dissolved O₂.

Methods

Sampling schedule and sites

Samples were collected between March 1992 and November 1993 from a wide range of aquatic environments throughout the southern Pantanal. Sampling included synoptic surveys as well as regular sampling at fixed points throughout the annual cycle (Hamilton 1994). The majority of the samples were collected in the vicinities of the Paraguay, Paraguai-mirim, Taquari, Negro, and Miranda rivers and their associated floodplains, and fall within a 100-km radius of Corumbá (Fig. 1).

The 540 samples included in this study were segregated into groups for data analysis based on the environmental characteristics of the sampling sites (Table 1). The Paraguay River was sampled mostly near Corumbá and at Porto Esperança (below the Miranda River). Tributary rivers are mainly those listed above, all of which were sampled within the Pantanal, usually near their confluences with the Paraguay River. The outflow from Lake Cáceres (Bolivia), which enters the river near Corumbá, was sampled regularly to represent the large open lakes associated with the Paraguay River. Synoptic samples were collected from other lakes.

Vegetated waters comprise about half of the total samples (Table 1). The sampling sites were chosen to represent the hydrologic and vegetational diversity of floodplain environments in the region. These samples can be further subdivided into those in which flow was visible at the time of sampling (“flowing vegetated waters”) and those which appeared stagnant. Many of the sampling sites were points of outflow of water from densely vegetated floodplain areas. Outflows were selected because the vegetated areas are generally inaccessible by boat or foot during inundation, and because water at outflows represents an integrated sample of water from a much wider area upstream.

Sampling and field measurements

Sampling was performed during daylight hours. Regular sampling sites were usually visited at the same time of day throughout the year because of their location in the sampling trip route. To check for diel variation, samples were

occasionally collected both in the late afternoon and early morning from representative sites. Water samples were collected with a vertical Van Dorn sampler from mid-depth if the water column was not stratified (most cases), or from the thickest homogeneous stratum, as indicated by the thermal profile (usually close to mid depth). In some vegetation-choked waters, we used a large-capacity hand-operated peristaltic pump fitted with low-permeability tubing to obtain water without disturbing the submersed plant surfaces.

After June 1992, thermal and dissolved O₂ profiles were measured with a YSI meter equipped with a polarographic oxygen sensor and a submersible stirrer (401 of the 540 samplings). The meter and probe were carried in a Styrofoam box to minimize heating and the system was air-calibrated before each use. For the earlier survey samples, we measured dissolved O₂ with the Winkler analysis (unmodified; APHA et al.1989), adding the reagents in the field upon collection.

Water samples were partitioned immediately upon collection as follows. A subsample for analysis of dissolved inorganic carbon and CH₄ was collected by filling a glass 120-mL serum bottle through a section of tubing attached to the sampler, maintaining the tubing at the bottom of the bottle and allowing at least three bottle volumes to overflow. After adding 400 μ L of a 50 g/L solution of HgCl₂ to arrest microbial activity, the bottle was sealed without air bubbles using a screw cap and heavy-duty TFE-lined silicone septum. A 250-mL polyethylene bottle was filled in a similar manner for later analysis of pH and acid neutralizing capacity. Details on sample filtration, preservation, and analysis for Fe, Mn, and major ions are given by Hamilton (1994).

A closed-system technique was used for pH measurement to avoid losses of dissolved CO₂. Measurement of pH and sample filtration and preservation were performed in the field. A pH meter equipped with an Orion Ross combination electrode (Model 8155 or 8102) was first calibrated using freshly prepared buffer solutions of pH 7.00 and 4.00. The electrode was subsequently rinsed and allowed to soak in river water for at least 15 min before beginning the measurements. The precision of the pH measurements is better than 0.02 pH unit.

Laboratory analyses

Samples collected for analysis of dissolved inorganic carbon (DIC) and CH₄ were analyzed by gas chromatography at the field lab, using thermal conductivity and flame ionization detectors. We calculated *p*CO₂ from measured DIC, pH, and acid neutralizing capacity, as discussed later. The precision of these measurements, as determined from analyses of sets of triplicate subsamples poured in the field, was 2.4% for DIC and 3.1% for dissolved CH₄.

Dissolved gases were extracted into a gaseous headspace using a static headspace equilibration technique (Ioffe & Vitenberg 1984). Hydrogen from a H₂ generator served as the headspace gas for equilibrations as well as for the carrier gas in the gas chromatograph. A 40-mL H₂ headspace was created in the 120-mL serum bottle by insertion of needles through the septum, leaving approximately 80 mL of water. Care was taken during headspace creation to maintain the pressure at 1 atm in the equilibration vessel. The 40 mL of water withdrawn for headspace creation was immediately analyzed for DIC by a method similar to that of Stainton (1973). The bottle was then rolled for several hours to equilibrate gases between the gas and liquid phases, and the headspace was subsampled and analyzed for CH₄ by gas chromatography. Certified standards of comparable concentrations were used for calibration of gas chromatograph (Scott Specialty Gases, Inc.). Methodological details on sample processing and calculations are given in Hamilton (1994).

Acid neutralizing capacity (ANC) was measured by Gran titration of unfiltered samples following the method of Gran (1952) with the modifications for low ionic-strength waters proposed by Cantrell et al. (1990). The normality of the titrant stock solution of HCl was determined using Na₂CO₃, and titrant concentrations were adjusted accordingly in the calculations (Drouse et al. 1986). The precision of this analysis is 0.8%.

Specific conductivity was measured on unfiltered water within 1–2 days of collection; measurements are standardized to 25 °C. Subsamples of filtered water were analyzed for major cations by flame atomic absorption spectrophotometry and for major anions by ion chromatography. In 58 representative samples, the sum of NO₃⁻ and NO₂⁻ was measured 1–2 days after sample collection by colorimetry after Cd-Cu reduction to nitrite (Wood et al. 1967). Total Fe and Mn were measured by atomic absorption spectrophotometry (flame or graphite furnace), employing standard protocols for aqueous samples.

The concentration of dissolved free CO₂ was calculated from measurements of pH, ANC, DIC, temperature, and major ions. For most samples we determined dissolved CO₂ as the difference between total inorganic carbon (DIC) and dissolved carbonates (assuming that Gran ANC is equivalent to carbonate alkalinity)(Skirrow 1975). This method works best when the difference is much larger than the analytical error of the two measurements, as is usually the case for waters of the Pantanal. We calculated dissolved CO₂ from pH, ANC, and major ions for about 25% of the samples, using the methods of Kempe (1982).

Table 2. Physicochemical characteristics of the waters of the Pantanal, including several alternative electron acceptors for anaerobic respiration.

Variable	Median	Interquartile range	Range	N
Temperature (°C)	27.5	24.0–30.0	16.0–36.8	540
Conductance ($\mu\text{S}/\text{cm}$; 25 °C)	50.0	41–78	5–479	540
pH	6.69	6.45–7.05	5.56–8.13	540
Gran ANC ($\mu\text{eq}/\text{L}$)	446	320–470	34–4835	540
$\text{NO}_3^- + \text{NO}_2^-$ (μM)	1.1	0.8–2.4	0.0–9.3	58
Total Mn (μM)	0.3	0.1–0.9	1.0–569	170
Total Fe (μM)	9.1	5–21	63–5277	170
SO_4^{2-} (μM)	0.5	0.3–1.5	0.04–113	540

Calculation of diffusive gas exchange rates

Diffusive gas exchange between the water and atmosphere was estimated from dissolved gas concentrations using the stagnant film model (Liss & Slater 1974; Broecker & Peng 1982). Gas exchange was estimated only for certain vegetated-water sites. These sites had very slow flow (<20 cm/s) or none at all, and the water surface was protected from wind by dense emergent plant canopies. Waves were never observed in these waters. A boundary layer of 300 μm was therefore used in the equation. This boundary layer corresponds to estimates for quiet waters in which gas exchange has been measured by several techniques (data compiled by Barber et al. 1988), and is similar to the lowest gas-exchange rates that have been measured by the SF_6 tracer technique in lakes (equivalent $z = 210 \mu\text{m}$: Upstill-Goddard et al. 1990).

Sampling and analysis of plant stem gases

Gases in the lacunae of submerged plant stems were sampled from a variety of the common plant species with emergent leaves. Stems were sampled in the upper 20 cm of the water column. The stems were first cut off at about 5 cm below the water surface, then 15-cm segments were cut and the gases were squeezed out and collected underwater (Hamilton 1994). Extractions were performed in triplicate for each species, with each replicate composed of stems from different plants in the same vicinity. Stem gas samples were analyzed within 24 hours for CO_2 , $\text{O}_2 + \text{Ar}$, N_2 and CH_4 by gas chromatography. Separation of O_2 and Ar was not possible with our chromatographic system, so Ar partial pressures were estimated from N_2 assuming an atmospheric

N₂:Ar molar ratio. The estimated Ar was subtracted from the measured O₂ + Ar to yield an estimate of O₂. Calibration was based on Scott Gas standards (CO₂ and CH₄) or ambient air (for O₂ + Ar and N₂).

Results

Physicochemical characteristics

The waters of the Pantanal are generally shallow and frequently flowing (Table 1). The Paraguay River is the deepest water in the region. Tributary rivers tend to be shallower, even close to their confluences with the Paraguay River. The large lakes along the course of the Paraguay River are not as deep as the river, although in most years they retain water throughout the dry season. Vegetated waters, most of which are flooded only seasonally, are the shallowest environments. Flow was visible in 178 of the 278 samplings in vegetated waters. These flow observations include those made at points of outflow from floodplain areas, such as natural channels or bridges along raised roads; the flow upstream of such outflows was generally lower (Hamilton 1994).

The water temperature varied seasonally in a regular fashion, with most samples ranging between 24–30 °C (Table 2). Many sites did not have detectable thermal gradients because of flow, which usually prevented significant stratification; thermal gradients were generally weak or absent when flow exceeded about 5 cm/s. Strong thermal stratification was sometimes observed in more stagnant waters, even in very shallow water columns (<0.5 m deep). Although it was usually only a diurnal phenomenon, significant vertical O₂ gradients sometimes developed by the afternoon (Hamilton 1994). Thermal gradients of ≥ 0.1 °C between the surface and bottom waters were recorded in 79 of 540 samplings. The means \pm s.d. of the thermal and O₂ gradients in the 79 observations were 1.2 ± 1.5 °C and 36 ± 49 μ M, respectively. No waters were observed in which thermal stratification persisted continuously for longer than a few days. Based on the shallow water depths and on observations of other shallow waters in similar tropical climates (e.g., Carter & Beadle 1932; MacIntyre & Melack 1984), it is likely that the sampling sites with thermal stratification during the day became isothermal at night, at least with respect to the 0.1 °C resolution of our measurements. Thermal stratification was generally not observed in early morning samplings.

Samples were occasionally collected in the late afternoon and early morning from representative sites to show diel variation in dissolved gas concentrations. Maximum differences would be expected between dusk and morning if aquatic photosynthesis is important in affecting O₂ and CO₂ concentrations

(Odum 1957). Overall, the observed diel changes were not large. Dissolved O_2 changed the most as a proportion of the total concentration, dropping $20 \pm 26 \mu\text{M}$ overnight (mean \pm s.d.; $N = 19$). Changes in concentrations of CO_2 and CH_4 were sometimes substantial but the direction was variable; the CO_2 change averaged $+26 \pm 46 \mu\text{M}$ ($N = 14$) and the CH_4 change averaged $+3.3 \pm 8.5 \mu\text{M}$ ($N = 15$). Rivers showed the least change. Relatively large changes at several of the stagnant, vegetated sites can be attributed to nocturnal mixing of the water column after diurnal thermal stratification during the previous day had allowed distinct strata to diverge in gas concentrations.

The major solute composition varied among sites due to the variable chemical composition of tributary rivers as well as the greater importance of local rainfall at some floodplain sites. In general, waters of the Pantanal are dilute and slightly acidic (Table 2), with low concentrations of suspended matter and moderate concentrations of dissolved organic carbon (Hamilton 1994). More alkaline waters occur in the southernmost areas of the Pantanal, particularly south of the Miranda River, where limestones occur in the upland watersheds. However, within the Pantanal, even the most alkaline waters do not carry detectable concentrations of carbonate solids in suspension, and virtually all of the waters that were sampled were undersaturated with respect to calcium carbonate (Hamilton 1994).

The range in pH of Pantanal waters (Table 2) indicates that virtually all of the dissolved carbonate would exist as the bicarbonate ion (HCO_3^-), with very little ($<0.3\%$) as carbonate (CO_3^{2-}). Given the typical major ion composition and the absence of significant amounts of carbonate solids in the suspended matter, dissolved CO_2 produced by the biota will accumulate in these waters and have little effect on dissolved carbonates.

Alternate electron acceptors that could support significant anaerobic respiration in freshwaters include NO_3^- , NO_2^- , Mn(IV), Fe(III), and SO_4^{2-} (Zehnder & Stumm 1988). Measurements of the concentrations of these species provide an indication of their availability for anaerobic respiration. Table 2 shows that the concentrations of these potential alternate electron acceptors were low in waters of the Pantanal. The highest concentrations of $NO_3^- + NO_2^-$ and of SO_4^{2-} tended to occur in waters of unusually high ionic strength, such as hydrologically isolated basins, whereas the highest concentrations of total Fe and Mn tended to be associated with O_2 -depleted waters. Additional evidence that little SO_4^{2-} reduction occurs in most Pantanal waters is provided by the observation that H_2S was undetectable by the methylene blue method in 65 representative samples that were analyzed (Hamilton 1994).

In addition to the more sensitive colorimetric analysis of $NO_3^- + NO_2^-$ in 58 selected samples, we measured NO_3^- in all 540 samples by ion chromatography, which revealed that all had concentrations $<20 \mu\text{M}$ and only 14

samples had concentrations above $5 \mu\text{M}$. Rivers in the uplands surrounding the Pantanal tend to carry slightly higher concentrations than rivers within the Pantanal, but concentrations of $\text{NO}_3^- + \text{NO}_2^-$ in 8 river samples of the upper Taquari River basin were below $10 \mu\text{M}$.

Differences in dissolved gases among environments

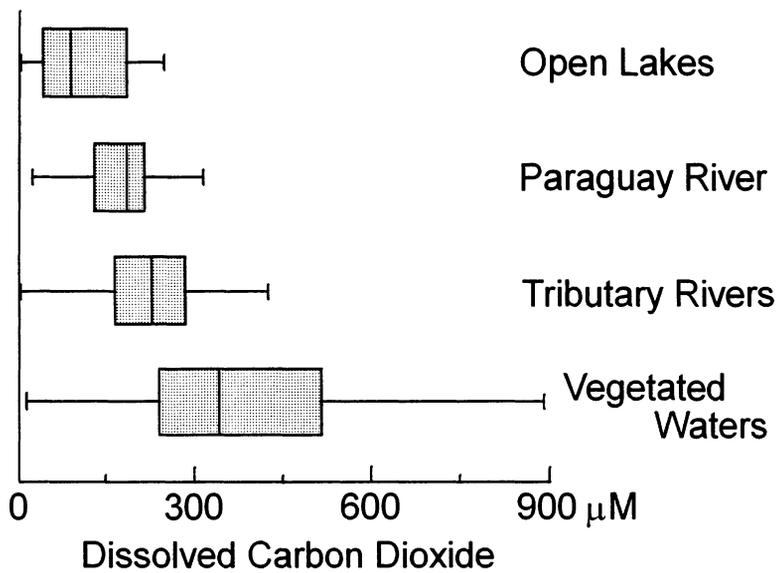
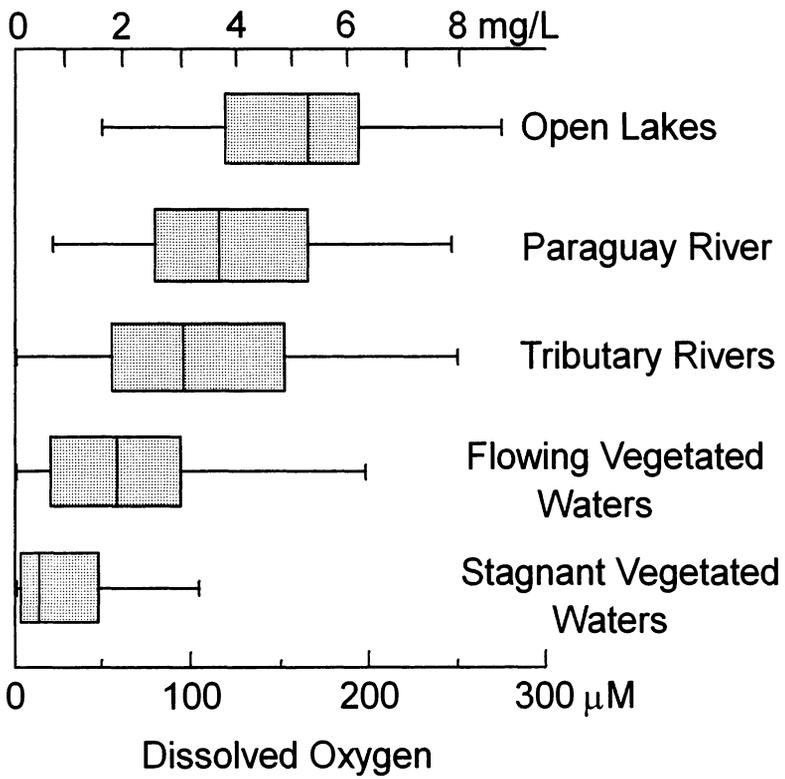
Dissolved O_2 was nearly always well below atmospheric equilibrium in waters of the Pantanal (Fig. 2). The rivers were usually at $<50\%$ O_2 saturation, and some tributary rivers became nearly anoxic at times (Hamilton 1994). Open lakes tended to have higher dissolved O_2 concentrations than the other environments, but supersaturation was recorded only once. Vegetated waters were the most O_2 -depleted environments, with the majority of measurements falling below $100 \mu\text{M}$ and a large number approaching anoxia (O_2 concentrations in the range $0\text{--}3 \mu\text{M}$ are difficult to quantify accurately by the methods employed). Vegetated waters in which no flow was visible were the most depleted in O_2 (Fig. 2).

Dissolved CO_2 showed the greatest deviations from atmospheric equilibrium in the more O_2 -depleted environments, as expected (Fig. 2). Supersaturation was nearly always observed. Vegetated waters generally had the highest CO_2 concentrations, and as with O_2 , the most extreme values were recorded in the stagnant vegetated waters. Dissolved CH_4 was consistently highly supersaturated in waters of the Pantanal (Fig. 2). The pattern for CH_4 resembles that for CO_2 , although open lakes were typically higher in CH_4 than the Paraguay River.

Time series of dissolved gases in representative environments indicate that the seasonal changes in gas concentrations in the Paraguay River and in tributary rivers are related to the degree of contact of river waters with the surrounding floodplain areas (Hamilton 1994). The greatest deviations of gas concentrations from atmospheric equilibrium occur at high water, particularly during the rising water phase. The vegetated floodplain environments appear to be responsible for much of the observed deviations in dissolved gas concentrations in the rivers and open lakes, whose waters tend to be much closer to atmospheric equilibrium when surrounding floodplain areas are dry.

Gas concentrations in the vegetated waters

We have selected 8 vegetated-water sites to further investigate the patterns in dissolved gas concentrations (Table 3). They were selected because they represent the environments that cover most of the inundable area of the region, and include areas that are seasonally inundated by waters of several rivers, areas flooded by local rainfall, and a hydrologically isolated marsh that



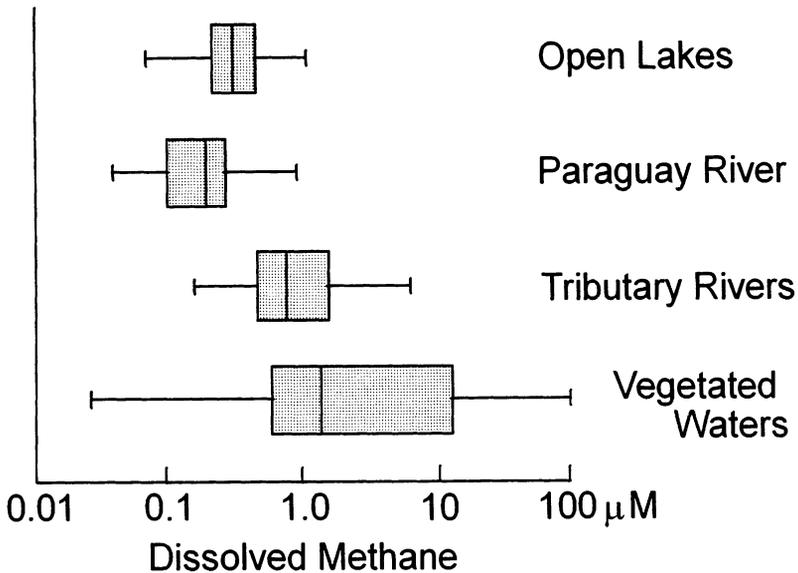


Fig. 2. Concentrations of dissolved O_2 , CO_2 , and CH_4 in the different aquatic environments of the Pantanal. In these box-and-whisker plots, the vertical line inside the rectangular box is the median, and the box spans the interquartile range (25th to 75th percentiles). Horizontal lines ("whiskers") show the range of values that fall within the interquartile range times 1.5; outliers are not included in the plots. At the commonly observed water temperatures of 18–32 °C, the concentration of dissolved O_2 at atmospheric equilibrium ranges from 290–230 μM (9.3–7.4 mg/L). The equilibrium concentration of CO_2 ranges from 13–9 μM (for atmospheric $P_{\text{CO}_2} = 350$ ppmv), and the equilibrium concentration of dissolved CH_4 is approximately 0.002 μM (for atmospheric $P_{\text{CH}_4} = 2$ ppmv).

remains wet throughout the year ("Pirizal") (Hamilton 1994). We sampled them at 4–6 week intervals over the course of an annual cycle (December 1992–November 1993); means and ranges of the gas concentrations during that period are given in Table 3. These sites were consistently depleted in dissolved O_2 despite considerable seasonal variation in water depth and flow. Concentrations of dissolved CO_2 and CH_4 were consistently very high.

The rates of diffusive gas exchange between the air and the water were calculated for each of the 8 sites on each sampling date, based on our measurements of dissolved gas concentrations and water temperature (Fig. 3). As discussed above, these sites presented favorable characteristics for the estimation of diffusive gas exchange. Diffusive transport of O_2 into these waters (invasion) is clearly exceeded by diffusive emission of CO_2 (evasion). Evasion of CH_4 is a small but variable fraction of the total carbon evasion ($\text{CO}_2 + \text{CH}_4$), ranging from 0.15–8.5% of the CO_2 evasion (mean, 5.0%). Note that these observations of relative differences in flux rates would not be

Table 3. Dominant aquatic plants, number of sampling dates, and concentrations of dissolved gases for 8 vegetated-water sites during the inundation phase. These sites were sampled at 4–6 week intervals beginning in December 1992 and continuing until the sites began to dry (most often in September or October 1993). Gas concentrations (μM) are means with ranges in parentheses.

Site (UTM Coordinates) ¹	Dominant plants ²	Sampling dates	Dissolved O ₂	Dissolved CO ₂	Dissolved CH ₄
Miranda floodplain (482, 7835)	1	5	24 (10–43)	495 (377–598)	18 (5–53)
Miranda floodplain (480, 7833)	1, 6	9	27 (12–43)	426 (297–564)	2.8 (0.2–18)
Miranda floodplain (493, 7819)	6	9	28 (9–94)	504 (375–602)	0.8 (0.3–1.7)
Taquarf floodplain (480, 7878)	2, 3	9	64 (0.6–108)	301 (219–466)	23 (2–62)
Paraguay floodplain (441, 7903)	2, 3, 4, 6	10	46 (0.9–108)	367 (235–613)	19 (0.4–78)
Negro floodplain (481, 7874)	2, 3, 4	8	34 (0.9–183)	410 (129–538)	37 (2–85)
Negro floodplain (491, 7871)	4, 6	10	12 (2–84)	561 (426–767)	36 (3–67)
Jacadigo (“Pirizal”) (436, 7866)	5	9	5 (3–8)	1084 (889–1300)	76 (31–95)

¹ Coordinates (x, y in km) are in the Universal Transverse Mercator (UTM) system to conform with Brazilian maps.

² Key to dominant plants: 1. *Paspalum pontanalis*; 2. *Paspalum hydrophyllum*; 3. *Oryza* sp.; 4. *Scirpus cubensis*; 5. *Cyperus giganteus*; 6. *Eichhornia azurea*.

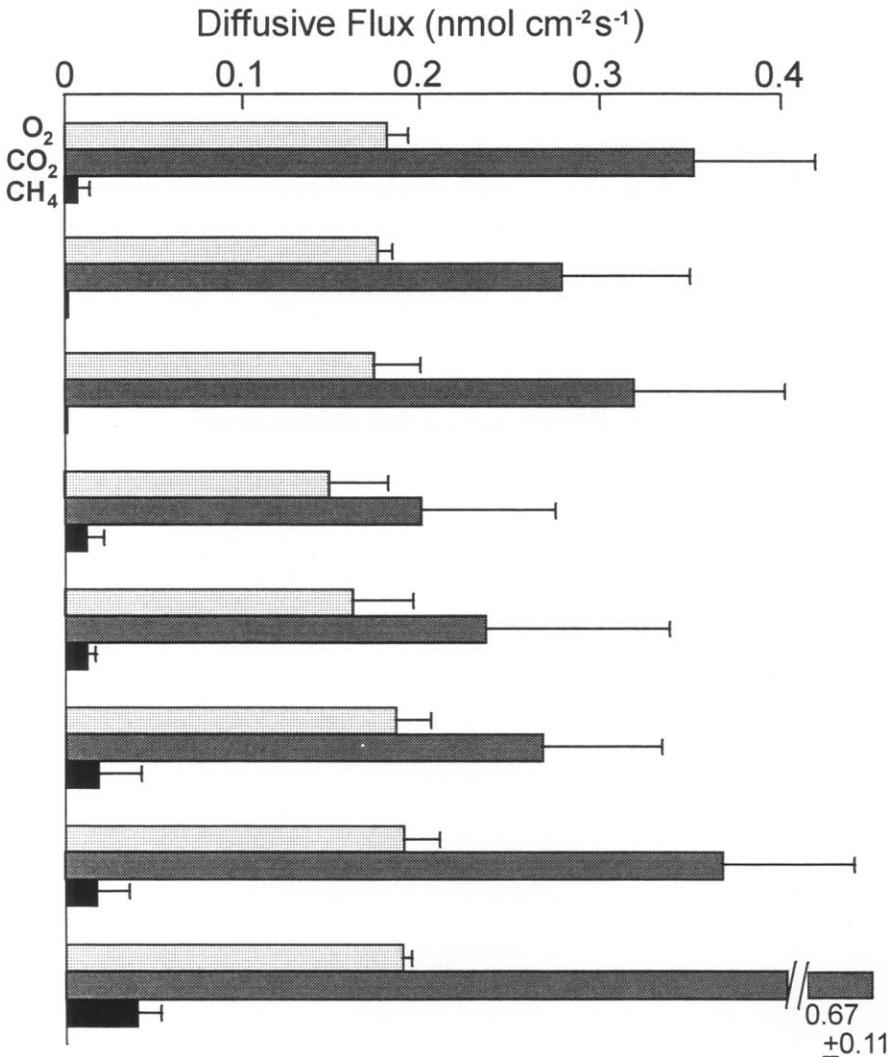


Fig. 3. Diffusive fluxes of gases between the air and water at selected vegetated-water sites, calculated using the stagnant film model. Each cluster of three bars represents a particular site, and each bar is the mean \pm s.d. for 5–10 dates during inundation (see Table 3). The sites are presented in the same order as in Table 3.

affected by the use of a different boundary-layer thickness or a different gas exchange model.

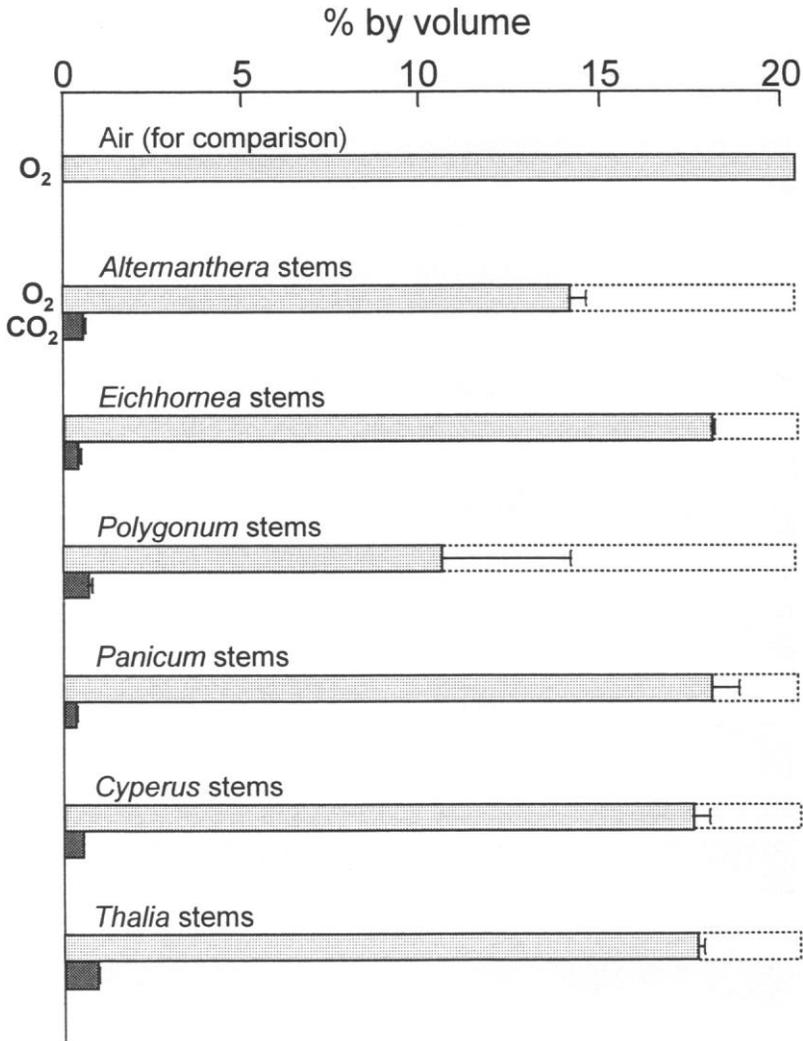


Fig. 4. Partial pressures of O₂ and CO₂ in the lacunae of submersed stems of aquatic vascular plants, sampled near the surface. The P_{O₂} in air is depicted at the top for comparison, and is shown by the dashed bar in the other plots. Each bar is the mean ± s.d. for triplicate samples.

Gases in submersed plant stems

Plant stems contained higher partial pressures of CO₂ and lower partial pressures of O₂ than the overlying air (Fig. 4). Compared with dissolved gas pressures in the surrounding water, the stem atmospheres had higher pO₂ but similar pCO₂ (Hamilton 1994). Methane was found in these plants at partial

pressures ranging from 56–14,100 ppmv (Hamilton 1994). The presence of CH₄ in submersed plant stems is attributed to entry of sediment gases through roots and rhizomes (Chanton & Dacey 1991; Schütz et al. 1991); the surficial sediments of the Pantanal contain CH₄ at high partial pressures (mean, 56%: Hamilton 1994).

Discussion

Processes controlling gas concentrations

The patterns that we have observed in dissolved gas concentrations indicate that the net ecosystem metabolism is strongly heterotrophic beneath the water surface. The net productivity of algae and submersed vascular plants must be low relative to the sum of processes that consume dissolved O₂. The lack of marked diel fluctuations in dissolved O₂ suggests that photosynthesis beneath the water surface does not strongly affect O₂ concentrations in most waters of the Pantanal.

The dissolved gas concentrations reflect the net balance of production and consumption rather than the absolute rates of these processes. The net heterotrophy of Pantanal waters could result from low rates of photosynthetic O₂ production. Alternatively, O₂ may be produced at high rates by aquatic autotrophs, but a strong O₂ demand may consume the photosynthetic O₂ as it is produced, maintaining a constant, low concentration throughout the day.

Casual examination of the vascular plants and algae that are typically present in the water column does not suggest that rates of aquatic photosynthesis would be particularly low in waters of the Pantanal. Phytoplankton abundance may frequently be limited by water movement, which removes suspended matter by straining through vegetation. However, attached algae are often abundant on submersed surfaces of vascular plants. Studies in the Amazon floodplain have demonstrated that epiphytic microalgae in floating grass mats can have high productivity (Engle and Melack 1990; Doyle 1991).

The shallow, seasonal flooding of savanna floodplains such as the Pantanal favors the luxuriant growth of aquatic vascular plants with rooted emergent or floating growth forms. These plants have high rates of primary production, but their metabolism is largely respiratory beneath the water surface, and many species produce roots in the water column. Their dense aerial canopies and underwater stems and roots reduce air-water gas exchange and turbulent mixing of the water column (Doyle 1991). Some fraction of their biomass ultimately decomposes underwater. The presence of these plants therefore results in both increased consumption of dissolved O₂ and decreased reaeration of

the water column. Although their submersed surfaces provide substrata for algal growth, dense stands of these plants reduce the penetration of light in the water column, and may thereby limit the potential for aquatic photosynthesis (Doyle 1991). Emergent vascular plants are thus capable of strongly influencing the nature and rates of aquatic metabolism.

Comparison of the concentrations and fluxes of O₂, CO₂ and CH₄ provides insight into the possible role of heterotrophic metabolism by emergent vascular plants. Although microbial decomposition of allochthonous organic matter is commonly assumed to be largely responsible for the heterotrophic nature of wetland waters, the principal processes that could be involved include: 1) aerobic respiration by microorganisms; 2) anaerobic respiration by bacteria; 3) fermentation and methanogenesis by bacteria; and 4) aerobic and anaerobic metabolism by higher plants.

The air-water diffusive fluxes of O₂, CO₂ and CH₄ for the vegetated-water sites can be considered to represent a quasi-steady state because of the lack of strong diel variations in dissolved gas concentrations and the short residence times of the dissolved gases, which are on the order of hours for O₂ and 1–3 days for CO₂ and CH₄ (Hamilton 1994). Thus O₂ invasion balances its consumption in the water column and sediments, and CO₂ evasion balances its production, assuming that diffusion is the main route of flux of these gases across the air-water interface (Devol et al. 1987; Denmead & Freney 1992). Diffusive evasion of CH₄ is probably substantially lower than the total emission because of the relative importance of ebullition and plant-mediated transport, which typically comprise about half of the total CH₄ emission from more deeply flooded wetlands (Devol et al. 1990; Chanton & Dacey 1991; Happell & Chanton 1993; Bartlett & Harriss 1993).

The air-water diffusive fluxes show that invasion of atmospheric O₂ was insufficient to balance the CO₂ evasion at the vegetated-water sites (Fig. 3). The sum of CO₂ and CH₄ evasion, which provides a minimum estimate of the overall rate of heterotrophic metabolism, ranged from 0.2–0.7 nmol cm⁻² s⁻¹, and on average the CO₂ evasion rate was about twice the O₂ invasion. Thus the waters produce twice as much CO₂ than can be accounted for by consumption of dissolved O₂.

Richey et al. (1988) also observed this “excess CO₂” in waters of the Amazon floodplain, and they attribute it to anaerobic degradation of organic matter by microorganisms. Anaerobic processes of potential importance in waters of the Pantanal include 1) anaerobic respiration by bacteria, in which oxidation of organic matter to CO₂ is coupled with reduction of an inorganic electron acceptor; 2) methanogenesis by bacteria; and 3) anaerobic metabolism by the tissues of higher plants. In the following discussion, we propose that anaerobic degradation of organic matter is not necessarily the

principal process responsible for the excess CO_2 in waters dominated by emergent plants. Instead, we argue that the respiratory metabolism of the plants can explain much of the observed consumption of O_2 and production of CO_2 , and that aerobic respiration supported by O_2 influx through the emergent plant stems may account for much of the excess CO_2 .

Anaerobic respiration by bacteria is unlikely to be a major pathway of CO_2 production because of the scarcity of alternate electron acceptors (Table 2). A few μM of these alternate electron acceptors would support a relatively small amount of organic carbon respiration compared with dissolved O_2 , which is continually resupplied by invasion from the atmosphere in these shallow waters (Hamilton 1994).

Methanogenesis produces CH_4 and CO_2 in equimolar proportions, and thus could account for the large excess of CO_2 if most of the CH_4 that was produced were lost to the atmosphere by ebullition and plant stem transport, while the CO_2 dissolved into the water column. This could occur because CH_4 is much less soluble than CO_2 . However, the highest reported rates of total CH_4 emission (diffusive + ebullitive + plant transport) in wetlands are $<0.05 \text{ nmol cm}^{-2} \text{ s}^{-1}$ (Bartlett & Harriss 1993). This rate is well below the $\text{CO}_2 + \text{CH}_4$ evasion rates in Figure 3, suggesting that CO_2 produced directly by methanogenesis cannot account for most of the excess CO_2 that we have observed.

Oxidation of CH_4 could be a significant source of dissolved CO_2 . Studies in shallow rice fields (Conrad & Rothfuss 1991; Frenzel et al. 1992) and in shallow waters of the Everglades (King et al. 1990) show that as much as 80–90% of the total CH_4 produced in the sediments can be oxidized to CO_2 at the sediment surface and in the plant rhizospheres. Oxidation of CH_4 would consume 8 moles of O_2 for every 3 moles of CO_2 produced (Wetzel 1983), and thus it is not a potential source of the excess CO_2 unless the O_2 is supplied by transport through emergent plant stems. Oxidation of CH_4 supported by O_2 diffusion from plant rhizospheres is known to be important in shallow rice fields (Schütz et al. 1991). In many waters of the Pantanal, however, the diffusion paths are much longer than in the rice fields or in the experimental enclosures that are most often studied. In waters of ca. 1-m depth, we have observed that most of the floating aquatic plants have stem lengths of several meters between the water surface and the sediments because they tend to grow horizontally. It is therefore questionable whether such plants could deliver much O_2 to the sediments, where CH_4 oxidation is usually considered to be most important. Significant CH_4 oxidation supported by O_2 from plant stems could possibly occur in submersed plant parts nearer to the water surface, particularly in waters of $<10 \mu\text{M O}_2$.

Anaerobic metabolism by the submersed parts of vascular plants, in which fermentation reactions produce equimolar quantities of CO_2 and ethanol (Bertani et al. 1980; Mendelssohn & McKee 1987), has been demonstrated in some plants but its importance relative to aerobic metabolism for the plant species typical of tropical floodplains is unknown.

A process of more likely importance is the transport of O_2 into the water column through emergent plant stems. The lacunae of the plant stems provide a gas phase continuum from the overlying atmosphere into the water column (Armstrong 1979). The emergent stems of rooted or floating vascular plants could supply O_2 for heterotrophic metabolism by both the plant tissues and associated microorganisms, thereby producing the observed excess CO_2 . Respiratory demand for O_2 in the submersed stems and roots can be met largely by diffusion or mass flow of air from the aerial plant parts through the lacunae to the tissues, at least in species with well-developed aeration (Armstrong 1979; Grosse et al. 1991). Preferential dissolution of the CO_2 produced by aerobic respiration from the plant tissues into the surrounding water is likely because CO_2 is so much more soluble than O_2 (Beckett et al. 1988), and would yield excess dissolved CO_2 that cannot be accounted for by dissolved O_2 consumption. In addition, diffusion of O_2 from the gas phase of submerged plant tissues into the surrounding water could conceivably support microbial oxidation of organic carbon (including CH_4 oxidation), which would also produce excess dissolved CO_2 .

The consumption of O_2 from the lacunae and dissolution of the resultant CO_2 into the aqueous medium in plant roots has been noted by plant physiologists working on the scale of the individual plant (Armstrong 1979; Bertani et al. 1980; Raskin & Kende 1983), but until recently has not been recognized for its potential to control gas concentrations in wetland waters. Some recent studies of CO_2 emission from wetlands have concluded that root respiration may be the principal source of CO_2 emitted to the atmosphere (Morris & Whiting 1986; Pulliam 1993; Happell & Chanton 1993). Experiments with emergent plants grown in anoxic water in the laboratory confirm that roots release large amounts of CO_2 but little O_2 to the water (Bedford et al. 1991).

Our measurements of gases in the lacunae of submerged plant stems support the hypothesis that much of the CO_2 produced by aerobic respiration of the plant tissues diffuses into the surrounding waters (Fig. 4). The $p\text{O}_2$ and $p\text{CO}_2$ observed in the stems indicate that, relative to air, the stem gases had O_2 deficits that were not balanced by CO_2 surpluses. Hence O_2 had been consumed, but the CO_2 that must have been produced in roughly equimolar amounts by aerobic respiration had been lost from the gas phase. Photosynthetic fixation does not explain the missing CO_2 because it would

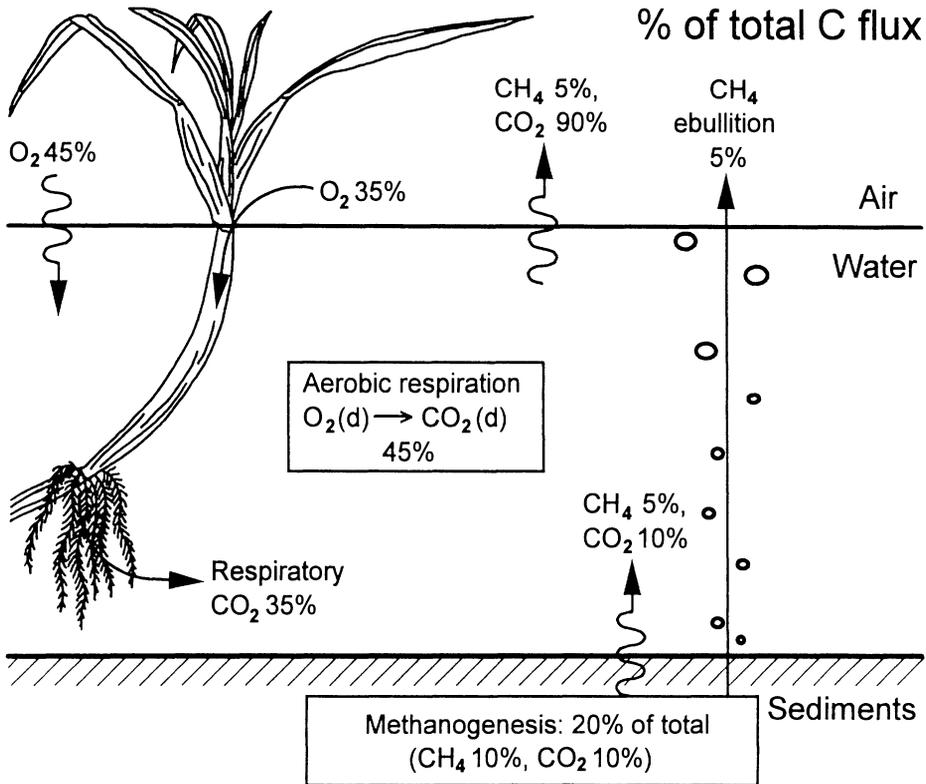


Fig. 5. Summary of the most important processes that control dissolved gas concentrations in the vegetated waters of the Pantanal. Fluxes are expressed as the % of the total C flux, which is the sum of CO_2 and CH_4 emission. See text for explanation.

have produced O_2 . Transport of CO_2 out of the stems by dissolution into the transpiration stream is a possible sink for CO_2 , but is probably less important than diffusion into the aqueous medium (Armstrong 1979).

Data from the literature suggest that the magnitude of root respiration in the vegetated waters would be large enough to explain much of the observed heterotrophic metabolism, and could present a strong sink for dissolved O_2 as well as consuming atmospheric O_2 transported through the stems. Doyle (1991) used *in situ* chambers to measure a mean root respiration rate of $1.5 \text{ mg } O_2 \text{ g}^{-1} \text{ h}^{-1}$ for water roots of emergent grasses in an Amazon floodplain lake. Da Silva (1990) presents monthly measurements of root biomass of floating macrophytes in the Pantanal; means for the inundation phase range between $40\text{--}270 \text{ g/m}^2$ for three species. Combining these data yields areal root respiration rates that range from $0.05\text{--}0.35 \text{ nmol } O_2 \text{ cm}^{-2} \text{ s}^{-1}$, which encompasses the range of O_2 invasion rates that we observed in the vege-

tated waters (0.15–0.19; Fig. 3). Root respiration rates of this magnitude are consistent with estimates of the primary productivity of emergent species in tropical waters, considering that 30–60% of the gross primary production is typically consumed by respiration, mostly in the roots (Hamilton 1994).

The roots of emergent plants may rely on lacunar O₂ when they are closer to the water surface, but uptake of dissolved O₂ may be more important for roots further from the water surface. Plants with long, floating stems typically develop root clusters along several meters of stem, and live roots can be found along stems far from the water surface (Junk 1970; Doyle 1991). Thus the roots may present a strong sink for dissolved O₂ that could obscure the effect of aquatic photosynthesis by maintaining low O₂ concentrations throughout the day. This would explain the paradox of high productivity of epiphytic algae, as evidenced by direct measurements (Doyle 1991) and inferred from their importance to aquatic food webs (Hamilton et al. 1992), in an environment that does not show strong diel cycles in dissolved O₂.

Conclusions

The most important processes that control dissolved gas concentrations in vegetated waters of the Pantanal are hypothesized in Figure 5, which is based on the mean gas fluxes for the 8 sites over an annual cycle (Fig. 3). Each flux is expressed as the % of the total carbon flux in the aquatic phase, which is the sum of diffusive evasion of CO₂ and CH₄ plus the estimated emission of CH₄ by other routes. On average, diffusive evasion of CH₄ was 5% of CO₂ evasion from vegetated waters. For the present purposes, we assume that diffusive evasion comprises roughly half of the total emission, with the remainder occurring by ebullition or plant-mediated transport (see below), so the total CH₄ emission rate would comprise about 10% of the total C flux. Given that methanogenesis produces equimolar proportions of CO₂ and CH₄, an equivalent amount of the total C flux must be methanogenic CO₂ that diffuses into the water column, and methanogenesis therefore comprises about 20% of the total C flux.

Accounting for methanogenesis leaves 80% of the total C flux, of which 45% can be explained by some form of aerobic respiration in the water column that is supported by diffusive invasion of atmospheric O₂, which we found to be half of the CO₂ evasion rate or 45% of the total C flux (Fig. 3). The fluxes can then be balanced if the remainder of the total C flux (35%) is due to aerobic respiration supported by O₂ transported through the lacunae of emergent plant stems. This respiration could be due to the plant tissues or to associated microorganisms (including methanotrophs), although the former

is likely to be most important. In either case, it would be responsible for most of the excess CO_2 observed in the water column.

The proportion of total CH_4 emission due to diffusive evasion is the most uncertain of the assumptions in Figure 5. Several studies in the Amazon floodplain have shown that diffusive evasion ranges from 30–80% of the total CH_4 emission (reviewed by Bartlett & Harriss 1993). If diffusive evasion were 30% of the total CH_4 emission, then aerobic respiration supported by O_2 transported through the lacunae of emergent plant stems would comprise 29% of the total carbon flux. If evasion were 80% of the total, then stem O_2 transport would comprise 41% of the total carbon flux. Thus our hypothesis that stem O_2 transport is important in the overall aquatic metabolism remains plausible despite the uncertainty in the relative importance of diffusive evasion of CH_4 .

Comparison with other wetlands

Depletion of dissolved O_2 appears to be common in tropical freshwater wetlands (Beadle 1981; Welcomme 1985), although most of the available measurements have been made in support of ecological studies and detailed information on the processes that consume O_2 is lacking. Dissolved O_2 undersaturation has been observed in floodplain lakes of the Amazon River (Melack & Fisher 1983; Richey et al. 1988) and to a lesser extent in the shallower lakes of the Orinoco River floodplain (Hamilton & Lewis 1990b; Vásquez 1992). In contrast to the results reported in this study, however, the Amazon and Orinoco studies generally observed anoxic waters in the hypolimnia of floodplain lakes with persistent thermal stratification. In the Amazon floodplain, thermal stratification tends to persist when waters are deeper than about 5 m (MacIntyre & Melack 1988). Nearly all of the non-lotic environments in the Pantanal are <5 m in depth (Table 1). In addition, the studies cited above tended to sample open waters (lakes), although they were usually surrounded by abundant aquatic vegetation, whereas in this study we have sampled much more within the vegetated waters, which comprise the majority of the inundation area of most floodplains. In agreement with our results, Carter & Beadle (1932) also found strong depletion of dissolved O_2 in vegetated waters of the Paraguayan Chaco, south of the Pantanal.

In some respects the hydrological characteristics and the aquatic vegetation of the Pantanal resemble some subtropical wetlands of the southeastern U.S. more than the deeply flooded fringing floodplains of the Amazon and Orinoco. Floodplains of the Amazon and Orinoco tend to have very large fluctuations in water level and high rates of hydraulic through-flow, and the aquatic vegetation is largely limited to floating species. The forested swamps of the southeastern U.S. can show strong O_2 depletion and CO_2 and CH_4

accumulation (Pulliam 1993; Happell & Chanton 1993), but these ecosystems differ from the Pantanal because their dense forest canopies shade the water surface, thereby limiting the growth of herbaceous plants. Shallow sheet flooding, predominance of herbaceous plants, and lack of dense forest canopies are characteristic of marsh wetlands such as the Florida Everglades and the Okefenokee Swamp (Mitsch & Gosselink 1986). However, benthic and epiphytic algal mats are much more abundant in many waters of the Everglades, and their productivity substantially increases dissolved O_2 during the day (Browder et al. 1994). In waters of the Okefenokee Swamp, which are acidic and high in DOC, dissolved CH_4 concentrations may be similar to those in the Pantanal (Flebbe 1984), but dissolved O_2 may not be as low (Bosserman 1984).

It is unlikely that our findings are unique to the Pantanal because wetlands with similar hydrology and vegetation are widely distributed in the tropics and subtropics. The difficulty in citing comparable situations is probably due to the paucity of studies that have made simultaneous measurements of the major dissolved gases, and to the historical tendency for investigators to bias their sampling in favor of lakes and rivers rather than the more extensive vegetated waters typical of many wetlands.

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