CSIRO PUBLISHING

Marine and Freshwater Research, 2008, **59**, 780–791 www.publish.csiro.au/journals/mfr

Photodegradation, interaction with iron oxides and bioavailability of dissolved organic matter from forested floodplain sources

Julia A. Howitt[A,B,C,D], *Darren S. Baldwin*[A], *Gavin N. Rees*[A] *and Barry T. Hart*[B]

[A]Murray-Darling Freshwater Research Centre, PO Box 991, Wodonga, Victoria 3689, Australia.
[B]Water Studies Centre, Monash University Clayton, Victoria 3800, Australia.
[C]School of Life and Environmental Science, Deakin University, PO Box 423, Warrnambool, Victoria 3280, Australia.
[D]Corresponding author. Email: jhowitt@csu.edu.au

Abstract. Photochemical degradation of dissolved organic matter (DOM) can influence food webs by altering the availability of carbon to microbial communities, and may be particularly important following periods of high DOM input (e.g. flooding of forested floodplains). Iron oxides can facilitate these reactions, but their influence on subsequent organic products is poorly understood. Degradation experiments with billabong (= oxbow lake) water and river red gum (*Eucalyptus camaldulensis*) leaf leachate were conducted to assess the importance of these reactions in floodplain systems. Photochemical degradation of DOM in sunlight-irradiated quartz tubes (with and without amorphous iron oxide) was studied using gas chromatography and UV-visible spectroscopy. Photochemical reactions generated gaseous products and small organic acids. Bioavailability of billabong DOM increased following irradiation, whereas that of leaf leachate was not significantly altered. Fluorescence excitation-emission spectra suggested that the humic component of billabong organic matter was particularly susceptible to degradation, and the source of DOM influenced the changes observed. The addition of amorphous iron oxide increased rates of photochemical degradation of leachate and billabong DOM. The importance of photochemical reactions to aquatic systems will depend on the source of the DOM and its starting bioavailability, whereas inputs of freshly formed iron oxides will accelerate the processes.

Additional keywords: aquatic carbon cycle, excitation-emission matrix (EEM), humic substances, Murray River.

Introduction

Photochemical modification of dissolved organic matter (DOM) has been observed through changes in both the properties of the DOM and the concentration of low molecular weight photoproducts (Kieber *et al*. 1990; Moran and Zepp 1997; Benner and Biddanda 1998; Howitt *et al*. 2004). These changes may be significant to aquatic food webs, through alterations in carbon availability for metabolism by aquatic organisms – a process that depends on factors such as the complexity of the components and their susceptibility to biotic and abiotic degradation (Geller 1986; Baldwin 1999; Robertson *et al*. 1999). Exposure of dissolved organic matter to sunlight has potential to influence microbial cycling of organic matter in the environment, and evidence exists to suggest that these effects also influence the metazooplankton (Daniel *et al*. 2006). However, the effects of photochemical modification of DOM vary widely with DOM from different sources. The follow-on effects on DOM bioavailability will always be complex as a result of production of labile compounds from refractory material (increasing bioavailability) (Kieber *et al*. 1989; Wetzel *et al*. 1995), but also mineralisation of labile material or its transformation into more refractory substances (potentially decreasing bioavailability) (Kieber *et al*. 1997).

It is well known that iron minerals can catalyse the photodegradation of dissolved organic carbon (Wells *et al*. 1991; Faust and Zepp 1993; Voelker *et al*. 1997) and they can be produced at the oxic/anoxic interface in floodplain wetlands. These reactions can also act as a sink for dissolved oxygen (Miles and Brezonik 1981; Howitt *et al*. 2004). However, the effect of photochemical interaction with iron on DOM end-products and subsequent carbon bioavailability is less well understood.

Techniques to examine changes in the remaining bulk DOM are very important for understanding how the reactions influence the cycling of carbon in aquatic food webs. In addition, these techniques have the potential to use organic matter to follow a pulse of water as it moves though a system and to characterise organic matter from different locations (Larsson *et al*. 2007). Decreasing fluorescence following irradiation has been widely used to follow photochemical degradation. Since the mid 1990s, 3-D fluorescence contouring (or excitation-emission matrix (EEM) spectroscopy) has been increasingly used to characterise and follow changes in the properties of DOM, and this technique has been the subject of recent reviews (Coble 2007; Hudson *et al*. 2007). EEMs have been used to characterise DOM from a variety of sources, including to differentiate samples from marine and terrestrial inputs (Coble 1996; Baker and

 10.1071/MF07225 1323-1650/08/090780

Spencer 2004; Stedmon and Markager 2005*a*), to characterise effluent-impacted waters (Baker 2001, 2002*a*, 2002*b*) and to differentiate microbially and terrestrially-derived DOM (McKnight *et al*. 2001).

In addition to the information provided by individual fluorescence scans, EEMs allow the fluorescence maximum (Ex_{max}/Em_{max}) – the combination of excitation and emission wavelengths, which results in maximum fluorescence – to be determined. A large proportion of the fluorescence of humic substances has been attributed to the presence of quinone functional groups (Klapper *et al*. 2002; Cory and McKnight 2005), and the effect of the substituents on the fluorescence has been used to investigate the origins of the humic substances. Fulvic acids derived from microbial material exhibit a sharper peak and at lower wavelengths than is found in terrestrially derived fulvic acids (McKnight *et al*. 2001) and a combination of peak location and the ratio of emission at 450–500 nm (excitation 370 nm) can be used to differentiate between the two classes of fulvic acid. Fluorescence from a protein- or amino acid-like component has also been found in marine surface waters and porewaters (Coble 1996) and studies of fluorescence peaks in this region indicate that they are linked with (but not necessarily completely explained by) products of bacterial metabolism (Cammack *et al*. 2004; Elliott *et al*. 2006).

Blue-shifting of fluorescence peaks (moving towards shorter wavelengths) has been associated with photochemical transformation of dissolved organic matter (Hayakawa *et al*. 2003; Ma and Green 2004; Kelton *et al*. 2007). The effect has also been observed with DOM modification associated with extraction of marine DOM, increasing salinity of the water (i.e. as the sample becomes more marine than riverine) and biological activity (Coble 1996). Blue-shifting of emission maxima is an indication of an increase in the energy difference between the ground and first excited states of the fluorophores, a reduction in the extent of the π-electron system, or the loss of functional groups such as carbonyl, hydroxyl or amine groups (Coble 1996).

In the present study, we examined the photodegradation and bioavailability of DOM derived from leachate from the leaves of the river red gum (*Eucalyptus camaldulensis*) and from a floodplain billabong (oxbow lake). Irradiation with sunlight was conducted in the presence and absence of amorphous iron oxide, and the reactions followed using a variety of techniques, including EEMs. These experiments aimed to establish: (i) whether amorphous iron oxide accelerated the rate of photodegradation of DOM from both organic matter sources; (ii) if the photodegradation of DOM from leaf leachate and billabong water sources increased the bioavailability of the carbon; and (iii) if photochemical degradation and any subsequent alteration to bioavailability differed between the leachate (a model for fresh organic matter inputs) and the billabong water (DOM that has been 'aged' on the floodplain).

Materials and methods

Two sets of outdoor experiments were conducted to examine the alteration of DOM by natural light, using similar methodology. The first was conducted using a leaf leachate and the second was conducted using water from a billabong on the River Murray (36°55′44″S, 146°55′56″E). *Eucalyptus camaldulensis* is the dominant tree in the remaining forested floodplains of the Murray River and is potentially a significant source of DOM to both floodplain wetlands and the river (Baldwin 1999). The billabong DOM is more representative of organic matter that has been subject to processing on the floodplain, and so these experiments are designed to cover the range of reactivities expected from fresh to aged DOM.

Leachate experiment

Five litres of river red gum (*E. camaldulensis*) leachate was prepared and sterilised using previously described methods (Howitt *et al*. 2004). Briefly, 0.5 g of ground, dried leaf was added to Milli-Q water, shaken in the dark at 20°C for 1 h, and then prefiltered through a Whatman GF/C filter (Whatman, Maidstone, Kent, UK). Leachate was filter-sterilised using 0.2-μm pore-size Millipore Stericap filters (Millipore, Billerica, MA, USA). This method produces a leachate with an average dissolved organic carbon content of 14 mg C L^{-1}, which is within the range of values found in freshwater ecosystems (Morel and Hering 1993) and similar to the concentrations observed during flooding of the Barmah-Millewa Forest on the Murray River (Howitt *et al*. 2007).

Amorphous iron oxyhydroxide was prepared in a laminar flow cabinet, using autoclaved Milli-Q water and glassware. Iron oxide was prepared from a 0.4-M $FeCl_3$ solution via slow addition of 1 M NaOH solution to give a final pH of 6.9 (Lovley and Phillips 1986; Howitt *et al*. 2004). Oxide was collected by centrifugation at 8700*g* for 10 min and washed with 5 × 20 mL of sterile water (to give a final conductivity in the rinsing water of 100 μS cm^{-1}). Iron oxide was stored as slurry in 200 mL of water at 4°C. The mass of oxide per mL of slurry was calculated from six replicate samples of 700 μL dried at 80°C. Slurry equivalent to 8.6 mg L^{-1} of oxide was added to the DOM solutions, which gives iron concentrations within the range previously reported for the Murray River (Maier *et al*. 1998).

Quartz tubes (1 m long, 13 mm inner diameter) were acid washed (5% HCl), rinsed and air-dried. The tubes were sterilised by UV irradiation for 1 h in a laminar flow cabinet. Tubes were stoppered using acid-washed, autoclaved, ultrapure silicon stoppers (Cole-Parmer, Vernon Hills, IL, USA) and 70 mL of leachate was added to each. Dark control tubes were completely wrapped in aluminium foil.

Forty tubes were prepared: four for initial measurements (measured 30 min after sealing), and nine each of leachate (unwrapped), leachate (wrapped), leachate plus oxide (unwrapped) and leachate plus oxide (wrapped). The tubes were mounted in polystyrene racks and stored overnight at room temperature. The racks were immersed in a small wading pool filled with tap water (1.6 × 1.2 m, filled to a depth of 20 cm) with the tubes incubated just below the water surface. A small garden fountain was used to keep the water moving (providing a small amount of agitation to the tubes) and encourage some evaporative cooling.

An Odyssey photosynthetic irradiance sensor (Dataflow Systems, Christchurch, New Zealand) was used to record incident radiation at 5-min intervals. Three tubes from each treatment were removed after 2 days, 1 week and 2 weeks.

Billabong water experiment

Seven litres of water was collected from Normans Lagoon (36°55′44″S, 146°55′56″E), a billabong adjacent to the Murray River, near Albury, NSW, at 09.30 hours on 6 March 2002 and transported to the laboratory in a 10-L acid-washed carboy. The water was filtered through 35-μm gauze then Whatman GF/F filters (Whatman, Maidstone, Kent, UK) (nominal pore size 0.7 μm) before filter-sterilisation (as described above) and then stored overnight at 4°C. Fresh oxide was prepared for this experiment. Tube preparation, treatments and sample analyses were as for the leachate experiment, with samples removed after 2 days and 7 days. Tubes were placed in the pool at 21.00 hours on the day they were filled.

Gas analysis

Headspace gas samples (2 mL) were directly injected into a Varian Star 3400 gas chromatograph (Varian, Mulgrave, Australia) for CO_2 analysis and total production was calculated as described previously (Howitt *et al*. 2004). Liquid samples were then taken for organic acid analysis, fluorescence, absorbance and pH measurements and preparation of bacterial cultures.

Organic acid analysis

Organic acids were analysed using high performance liquid chromatography (HPLC): injection of 50 μL of sample into a Waters HPLC fitted with a U6K Injection port, a 450-variable wavelength detector set at 210 nm (Waters, MA, USA), and an Aminex HPX-87H (BioRad, Hercules, CA, USA) organic acid column. The mobile phase was 0.005 M H_2SO_4 at a flow rate of 0.6 mL min^{-1} and a column temperature of 40°C. Samples were centrifuged (15 000*g*) to remove particles.

Optical measurements

Samples for fluorescence measurements were acidified to pH 2.0 with HCl to encourage complexes between DOM and dissolved metals to dissociate and minimise fluorescence quenching (McKnight *et al*. 2001). Fluorescence measurements were made on an f-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan), scanning the excitation wavelength from 200 nm to 400 nm in 10-nm steps, and measuring the emission intensity every 2 nm between 200 nm and 550 nm for each excitation wavelength. Absorbances of both acidified and unmodified samples were measured in 2-nm steps between 200 nm and 550 nm using a Varian Carey 1E UV-Vis spectrophotometer (Varian, Mulgrave, Australia). Unmodified samples were used for photobleaching measurements (Whitehead *et al*. 2000), whereas the absorbances of acidified samples were used to correct the fluorescence results. Both absorbance and fluorescence measurements were made in a 1.0-cm quartz cuvette. The data were exported to Excel (Microsoft, Redmond, WA, USA) and corrected for primary and secondary inner-filtering to prevent concentration effects (Mobed *et al*. 1996). A correction factor is applied to each measured fluorescence result (Eqn 1):

$$I = \frac{I_m}{10^{-0.5(A_{ex}+A_{em})}} \quad (1)$$

where I is the corrected intensity, I_m is the measured intensity and A_{ex} and A_{em} are the measured absorbances at the excitation and emission wavelengths respectively.

Bioavailability experiments

Bacterial cultures were prepared from each of the sampled quartz tubes after 7 days of irradiation (and 14 days in the case of the leachate). Twenty-five millilitres of each sample was transferred into a sterile serum bottle. Nitrogen (9.5 mM NH_4^+) and phosphorus (3.5 mM PO_4^{3-}) were added to ensure that the cultures were neither N nor P limited. The pH was adjusted to 7.0 by addition of NaOH.

An inoculum was prepared in advance for use in all cultures to allow for consistent dosing with a natural assemblage. A concentrated leachate was prepared (1 g of powdered leaf in 1 L of ultrapure (Milli-Q) water for 1 h). The extract was filtered (Whatman GFF), 9.5 mM NH_4Cl was added (preliminary experiments had indicated the leachate was nitrogen limited) and the pH adjusted to 7.5. The extract was inoculated with 10 mL of GFF-filtered water from Normans Lagoon and incubated in the dark for 7 days. The culture was collected by centrifugation at 8700*g* and the bacterial pellet resuspended in 10 mL of 5 mM NaH_2PO_4 (pH of 7.0), and then divided between five 2-mL Eppendorf tubes. Tubes were centrifuged at 15 000*g* for 2 min, the liquid removed and the pellets frozen (−18°C) in their tubes until use (within 3 weeks). It was assumed that any negative effects of freezing would be consistent between tubes. Immediately before use the pellet was thawed and resuspended in 2 mL of a combined solution of 9.5 mM NH_4Cl, 3.5 mM NaH_2PO_4. A 2-mL syringe was used to add 0.1 mL of inoculum to each culture, which was then capped and incubated at 25°C for 9 days. Headspace gas samples were analysed as above.

Bacterial numbers were estimated from direct counts made using epifluorescence spectroscopy. One hundred microlitres of acridine orange solution (1 g L^{-1}) was added to 5 mL of bacterial sample in a 25-mm filter set (Sartorius, Goettingen, Germany) and left to stain for 10 min. The bacteria were then filtered onto a pre-blackened 0.2-μm polycarbonate filter under low vacuum and counted at a magnification of 1200× on a Zeiss Axioskop 2 (Carl Zeiss, Oberkochen, Germany). Seven fields of view (chosen at random) were counted from each sample to ensure that in excess of 500 bacteria were counted in most samples.

Statistical analyses

The effects of light treatment and oxide treatment on the bioavailability experiments were tested using two-way ANOVAs (SPSS, Chicago, IL, USA) with variation between containers as the residual term. For bacteria, replicate counts within containers were averaged for the ANOVAs. For leachate experiments, sampling time was included as an additional factor, although interactions between other factors and time meant separate two-way analyses were carried out for each time separately. Normality and homogeneity of variance were checked with plots of residuals but no transformations were necessary.

There were too few replicates to conduct a complete factorial ANOVA when testing the effects of light treatment and oxide on photochemical CO_2 production. A one-way ANOVA on the four light/oxide groups was done for each time separately, followed by a Tukey's test.

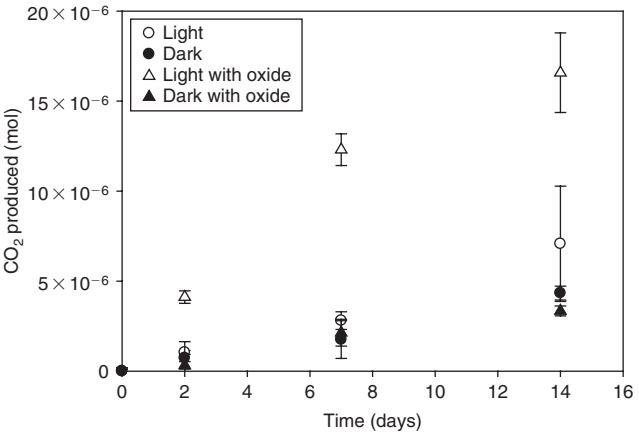

Fig. 1. Photochemical carbon dioxide production from sunlight irradiated leachate. t = time. $n = 2$ for $t = 0$, time = 2 (dark), time = 14 (light, dark, dark + oxide), $n = 3$ for all others. Error bars equal 1 s.d.

Results

Leachate experiments

The experiment was conducted over a fortnight of predominantly fine weather, although 2 days were considerably affected by cloud cover. Some early-morning and late-afternoon sunlight was excluded from the experiment as a result of shading by buildings, but on most days the incident light peaked between 2000 and 2500 μE m^{-2} s^{-1} (data not shown).

Photochemical carbon dioxide production was clearly evident in the light-exposed tubes where amorphous iron oxide was added (Fig. 1). There was a significant difference between treatments at all three sampling times (ANOVA: Time = 2 days, $F_{3,7} = 52.86$, $P < 0.001$; Time = 7 days, $F_{3,8} = 114.85$, $P < 0.001$; Time = 14 days, $F_{3,5} = 23.61$, $P = 0.002$). In each case the Tukey test indicated that the light with oxide treatment was different to the other three groups.

Photobleaching (measured as loss of absorbance) is evident in the light-exposed samples in both the presence and absence of iron oxide (Fig. 2). The figure illustrates data from a representative sample for each treatment. In both treatments with and without oxides, the rate of bleaching is slower in the second week of the experiment. Some loss of absorbance is associated with dark reactions between the DOM and iron oxides. The proportion of absorbance remaining at 250 nm and 340 nm after 7 days is summarised in Table 1. The rate of bleaching is accelerated at both wavelengths in the presence of iron oxide.

All samples had an initial pH of 5.2 (±0.1). For the first week, all treatments except light (no oxide) remained within error of the initial measurements. In the light-exposed samples, the pH decreased to 4.9 (±0.2) after 2 days and 4.70 (±0.03) after 7 days. After 14 days, samples from this treatment had an average pH of 4.66 (±0.06), whereas the dark-control had a pH of 5.06 (±0.01), the dark treatment with added oxide had a pH of 4.89 (±0.04) and the light-exposed with added oxide had a pH of 5.18 (±0.2).

HPLC chromatograms exhibited a large unresolved early peak with a shoulder and two small, broad peaks which had similar elution times to formic and acetic acid respectively.

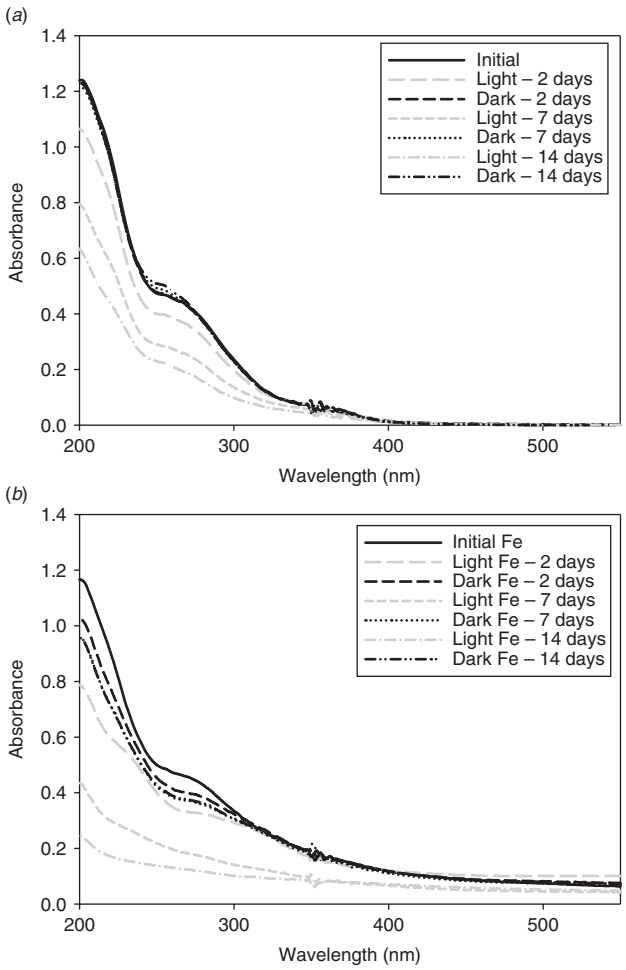

Fig. 2. Absorbance of the leachate (*a*) without iron oxide and (*b*) with iron oxide, over the period of the experiment.

Light-exposed treatments contained additional minor peaks with retention times coincident with succinic, formic, malic and citric acids (Fig. 3).

The initial EEM spectrum was characterised by the presence of two peaks (Em 305 nm, Ex 280 nm and Em 305 nm, Ex 230 nm), both covering relatively small regions of the spectrum (Fig. 4*a*,*b*). The spectrum of the leachate was concentrated at the high-energy end of the spectrum. After 14 days, the peaks in the light treatment (Fig. 4*c*) were less intense and less broad than in the initial sample (Fig. 4*a*) and the dark control (Fig. 4*d*). The light + oxide treatment (Fig. 4*e*) had more substantial changes – the two peaks merge into one peak of reduced intensity and substantially reduced area.

Billabong water

Sunlight exposure during this experiment was slightly lower than over the same time-frame in the previous experiment, with only one day exceeding 2000 μE m^{-2} s^{-1}. Considerable scatter between replicates existed in the carbon dioxide results and the CO_2 in each tube increased (Fig. 5). There was some suggestion of iron catalysis of the degradation reactions after 7 days. One-way ANOVA on the four light/oxide combinations indicated

Table 1. Remaining absorbance at 250 and 340 nm as a percentage of the initial after 7 days, and the ratio of the absorbances at each wavelength

Values in parentheses indicate standard deviations, $n = 3$. DOM, dissolved organic matter

DOM	Treatment	250 nm (%)	340 nm (%)	A_{250}/A_{340}
Leachate	Light	63 (±4)	84 (±5)	4.48 (±0.03)
Leachate	Dark	105 (±1)	102 (±1)	6.13 (±0.04)
Leachate	Light with oxide	48 (±8)	57 (±14)	2.02 (±0.15)
Leachate	Dark with oxide	87 (±3)	99 (±5)	2.09 (±0.04)
Billabong	Light	73 (±2)	57 (±3)	4.05 (±0.10)
Billabong	Dark	100 (±1)	98 (±1)	3.21 (±0.01)
Billabong	Light with oxide	68 (±2)	70 (±4)	1.89 (±0.03)
Billabong	Dark with oxide	88 (±1)	91 (±2)	1.88 (±0.03)

a significant difference ($F_{3,8} = 8.00$, $P = 0.009$) and the Tukey test indicated that the light with oxide treatment was significantly different to both the light (no oxide) and the dark with oxide treatments.

Photobleaching effects were evident in the light-exposed treatments, both with and without added iron oxide (Fig. 6). After 7 days the photobleaching was substantial across a range of wavelengths, and was clearly evident at both 250 and 340 nm (Table 1). Photobleaching was slightly increased in the iron oxide treatment compared with the treatment without oxide at 250 nm.

The initial HPLC chromatograms consisted of two peaks – an early large peak and a small peak coincident with acetate (Fig. 7). After 7 days, the early peak was reduced slightly in the dark + oxide treatment, but the reductions were greater in the light-exposed treatment and light + oxide treatment. The two light-exposed treatments contained new peaks with retention times coincident with citric, succinic, formic and acetic acid. These peaks were close to the limit of detection, and of similar size to a peak produced by 2×10^{-5} M formate. An additional peak in the light-exposed treatment with added iron oxide has a retention time coincident with oxalic acid. Traces of this peak occur as a shoulder in the light (no oxide) treatment.

The initial fluorescence spectrum of the billabong water (Fig. 8*a*) has broad peaks, at longer emission wavelengths than for the leachate. Slight differences exist between the initial samples with (Fig. 8*b*) and without (Fig. 8*a*) added iron oxide, possibly owing to some fluorescence quenching as a result of complexation with iron, despite attempts to minimise this with the low pH environment. Excitation also occurs over a wider range of wavelengths than in the leachate. After 2 days all samples had changed. Each graph represents one of three replicates, but replicate samples were quite similar. All four treatments contained an additional peak (although traces of the new peak at (Em 300 nm, Ex 240 nm) had been present in the initial sample to which iron oxide had been added). The intensity of the peak at (Em 410 nm, Ex 240 nm) also increased. In contrast, fluorescence induced by light in the 280–350 nm range was reduced in each light treatment relative to the corresponding dark control. After 7 days (Fig. 8*g–j*) the fluorescence spectra of the dark (no oxide) control had diminished slightly in intensity, but the overall characteristics remain unchanged. The dark (with oxide) control also had reduced intensity, especially in the (Em 410 nm, Ex 330 nm) region. This peak had gone from the light + oxide treatment and almost gone from the light (no oxide) treatment. In these two treatments the peak at (Em 410 nm, Ex 240 nm) had also diminished substantially relative to the corresponding dark controls.

Bioavailability of leachate

The production of CO_2 in cultures growing on leachate (Fig. 9*a*) after sunlight irradiation for 7 and 14 days did not lead to a substantial difference in the amount of organic carbon lost via bacterial respiration. After 7 days of irradiation, ANOVA indicated a significant effect of light ($F_{1,8} = 5.643$, $P = 0.045$) and oxide ($F_{1,8} = 11.448$, $P = 0.010$) but no interactive effect ($F_{1,8} = 4.049$, $P = 0.079$). However, after 14 days of irradiation no significant trends remained ($F_{1,5} < 0.96$, $P > 0.37$ for all treatments). Light exposure did not significantly alter the abundance of bacteria in the cultures (Fig. 9*b*). ANOVA revealed no significant effect of oxide ($F_{1,8} = 1.671$, $P = 0.232$), light ($F_{1,8} = 0.012$, $P = 0.914$) or light in combination with oxide ($F_{1,8} = 0.928$, $P = 0.364$) on the size of the bacterial population after 7 days, and no significant effect was present after a further 7 days ($F_{1,5} < 0.85$, $P > 0.39$ for all three).

Bioavailability of billabong DOM

Both carbon dioxide production (Fig. 9*c*) and bacterial abundance (Fig. 9*d*) indicate an increase in the bioavailability of billabong dissolved organic matter after irradiation, and that the bioavailability of the DOM was influenced by the presence of iron oxide. ANOVA on respiration data indicates significant influences of light ($F_{1,8} = 13.266$, $P = 0.007$) and oxide ($F_{1,8} = 13.458$, $P = 0.006$), but no significant interactive effect ($F_{1,8} = 0.130$, $P = 0.727$).

The bacterial abundance followed the same pattern as the CO_2 respiration; however, these results were accompanied by much greater variability. ANOVA indicates a non-significant effect of light ($F_{1,8} = 3.813$, $P = 0.087$) but a significant effect of oxide ($F_{1,8} = 8.250$, $P = 0.021$), with no interactive effect ($F_{1,8} = 0.350$, $P = 0.570$).

Discussion

The present study clearly shows that amorphous iron oxide accelerated the photodegradation of DOM from both leachate and billabong sources, but that the effect of the photodegradation reactions on bioavailability was variable.

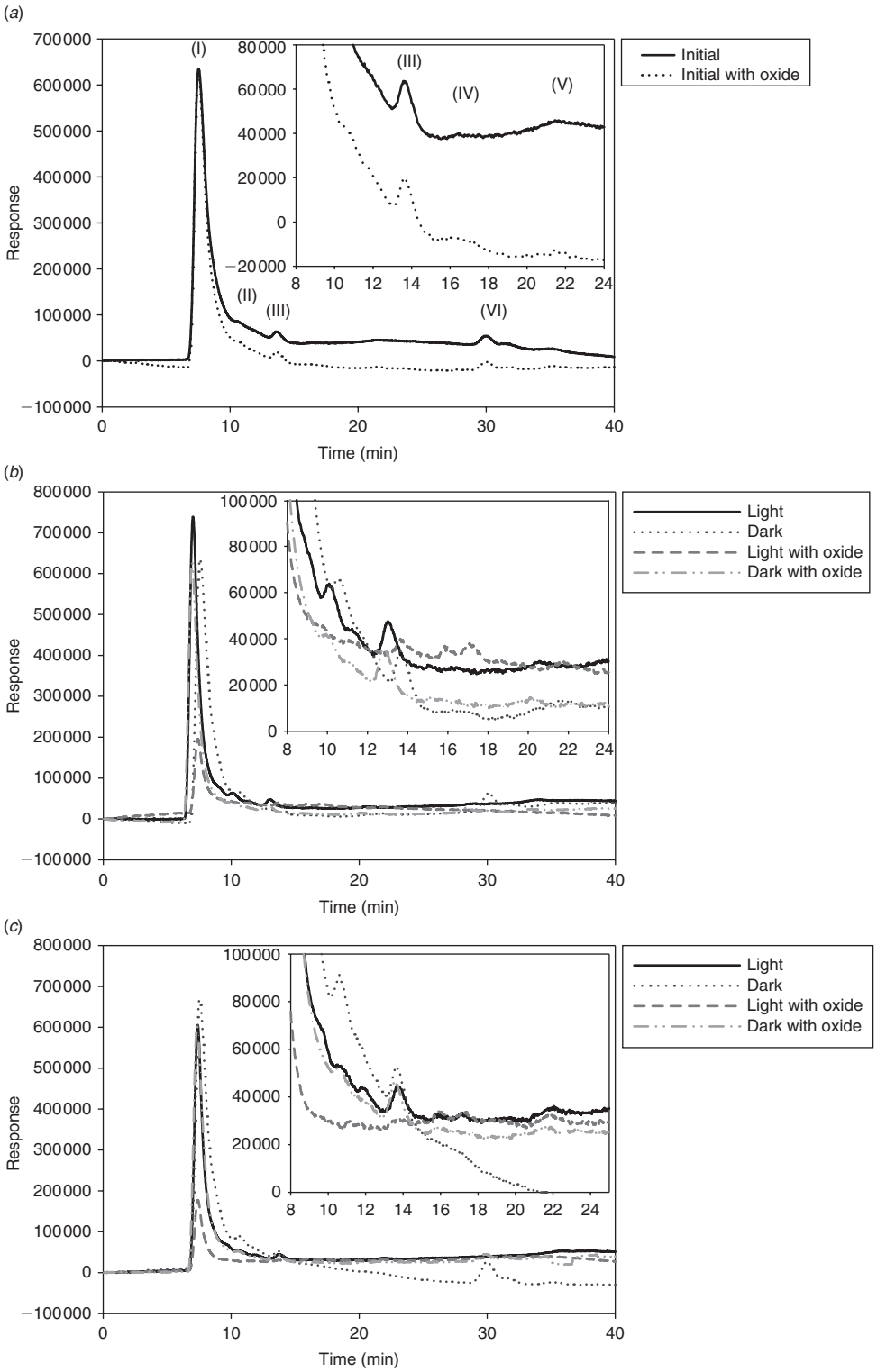

Fig. 3. High performance liquid chromatography (HPLC) analysis for the photochemical production of organic acids from leachate. Chromatograms are example results from initial (*a*), 1 week (*b*) and 2 week (*c*) samples.

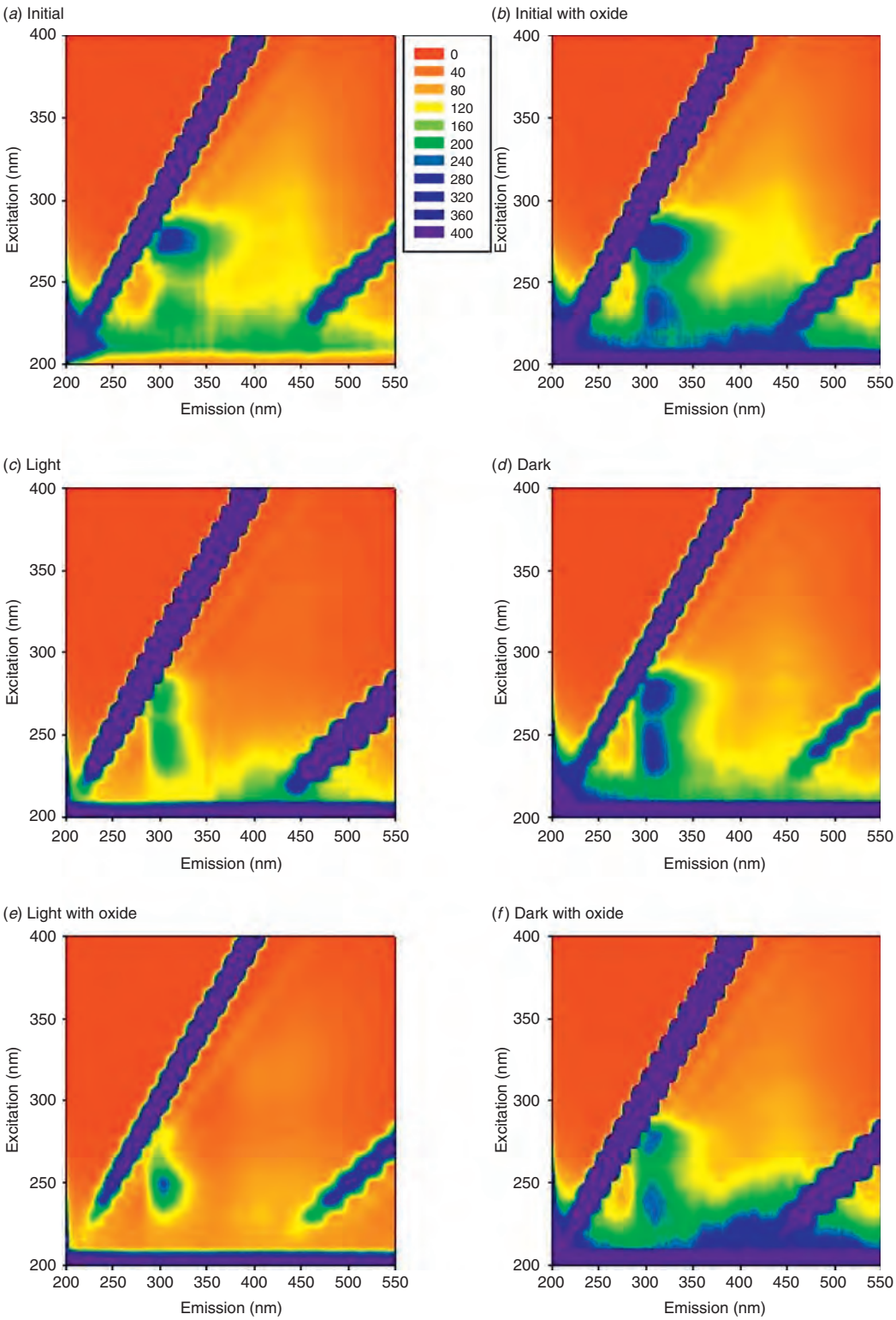

Fig. 4. Excitation-emission matrices (EEMs) for leachate from initial (*a*, *b*) and 14 day (*c*–*f*) samples.

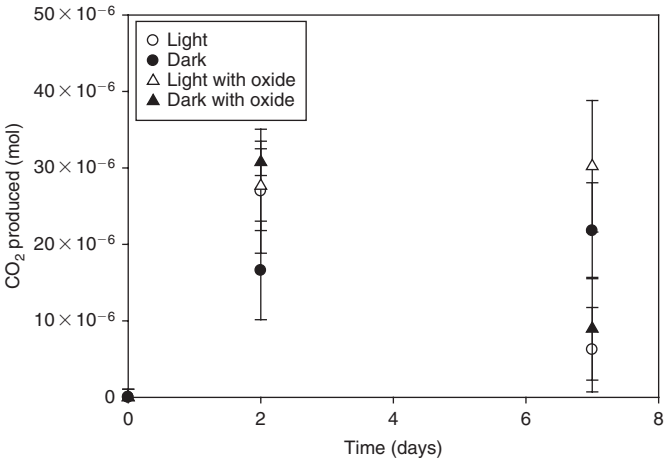

Fig. 5. Photochemical carbon dioxide production from sunlight irradiated billabong water. $n = 3$, except for $t = 0$: light and dark, $n = 2$, light and dark with oxide, $n = 1$. Error bars indicate one standard deviation.

Photodegradation experiments

Photodegradation of DOM was evident in the absorbance data for both the leachate and billabong water experiments. Photobleaching of leachate was fairly uniform across the spectrum (spectral shape was largely retained), although after 7 days bleaching was greater at 250 nm than at 340 nm (Table 1). Photobleaching increased in the presence of iron oxide. Similar effects were observed for billabong water but with preferential bleaching at longer wavelengths, which has previously been linked to changes in the molecular size distribution (Schmitt-Kopplin *et al*. 1998; Bertilsson and Bergh 1999) and suggests that destruction of large conjugated bond structures was occurring preferentially in the light (no oxide) billabong samples.

Addition of iron oxide increased the photoproduction of carbon dioxide in both experiments, although the effect was much more pronounced in the leachate experiment. The pH in this treatment did not drop to the same extent as in the light (no oxide) treatment, suggesting that carboxylate groups were being formed in the absence of oxide, but the oxide catalysed their destruction to CO_2 (Howitt *et al*. 2004).

Some evidence was found of the formation of succinic, formic, malic and citric acids in the light-exposed leachate treatments, and these appeared more rapidly in the treatment with added oxide. Citrate, acetate, formate and other fatty acids have previously been observed following photodegradation of leachate from macrophytes (Wetzel *et al*. 1995) most likely from the oxidation of the side-chains of lignin-based macromolecules. Succinic acid has also been observed in irradiated lake water (Bertilsson and Allard 1996). Initial chromatograms for billabong water were simpler than for leachate. As simple organic acids are likely to be rapidly consumed in aquatic environments (Moran and Zepp 1997), the relatively featureless chromatograms are not surprising. Production of several small organic acids was observed in both light-exposed treatments.

Initial EEM spectra of leachate suggested that the DOM was dominated by molecules in the region previously identified as being protein-like (Coble 1996). Loss of fluorescence was evident in light-exposed treatments, relative to the corresponding

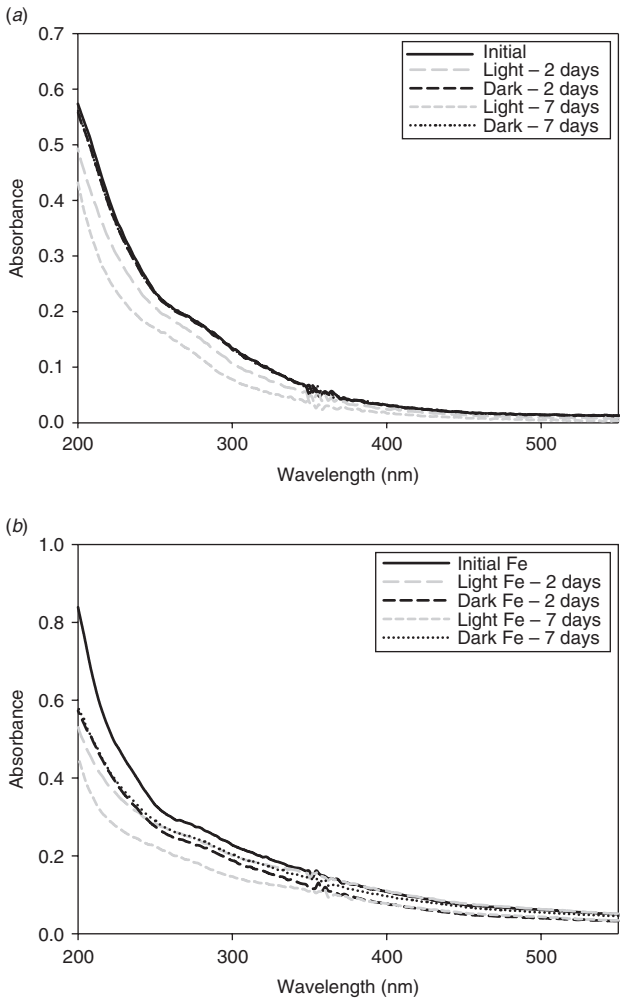

Fig. 6. Absorbance of billabong water samples (*a*) without iron oxide and (*b*) with iron oxide, during sunlight irradiation.

dark controls. Some dark reactions also occurred. Dark reactions between iron and DOM are reported to influence light absorption and fluorescence of solutions, and the addition of either iron (III) or iron (II) results in organic matter inducing changes in the redox state of the iron (Pullin *et al*. 2007). The influence of iron has been shown here to be greatly enhanced in the presence of sunlight.

Alterations in billabong water fluorescence suggested much greater changes to the bulk properties of the organic matter, and the initial fluorescence covered a much greater region of the spectrum. Slight differences exist between the initial samples with and without added iron oxide, most likely as a result of some fluorescence quenching associated with the iron addition. Although acidification of samples is intended to reduce complexation of metals by organic matter (McKnight *et al*. 2001), at higher pH values, blue-shifting and fluorescence quenching can result from the addition of iron to un-irradiated DOC (owing to complexation at phenolic and carboxylic groups) (Kelton *et al*. 2007; Ohno *et al*. 2008). Formation of the peak at (Em 300 nm, Ex 240 nm) and increased intensity of the peak at (Em 410 nm,

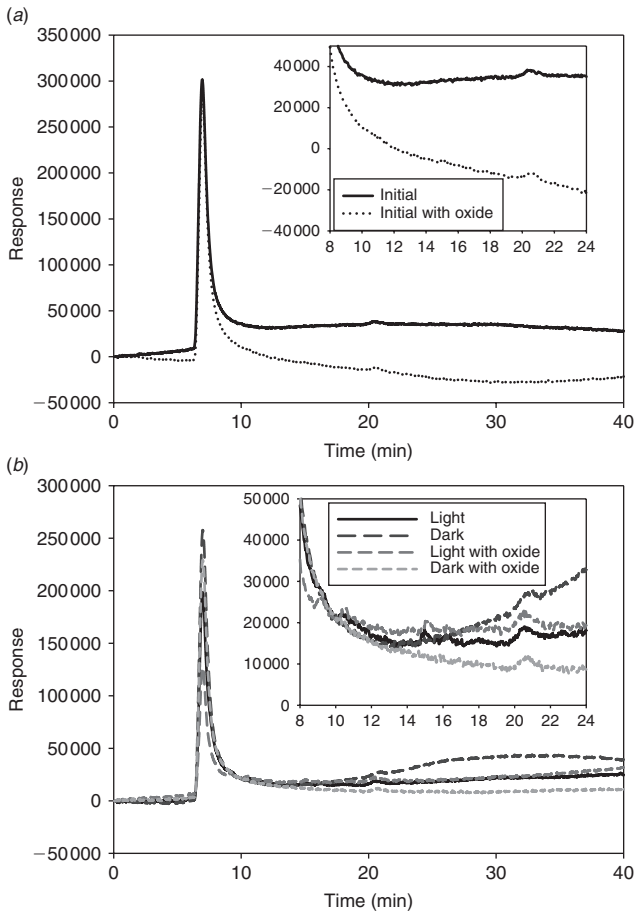

Fig. 7. Example of high performance liquid chromatography (HPLC) results for the analysis of organic acids in billabong water initially (*a*) and after 1 week (*b*).

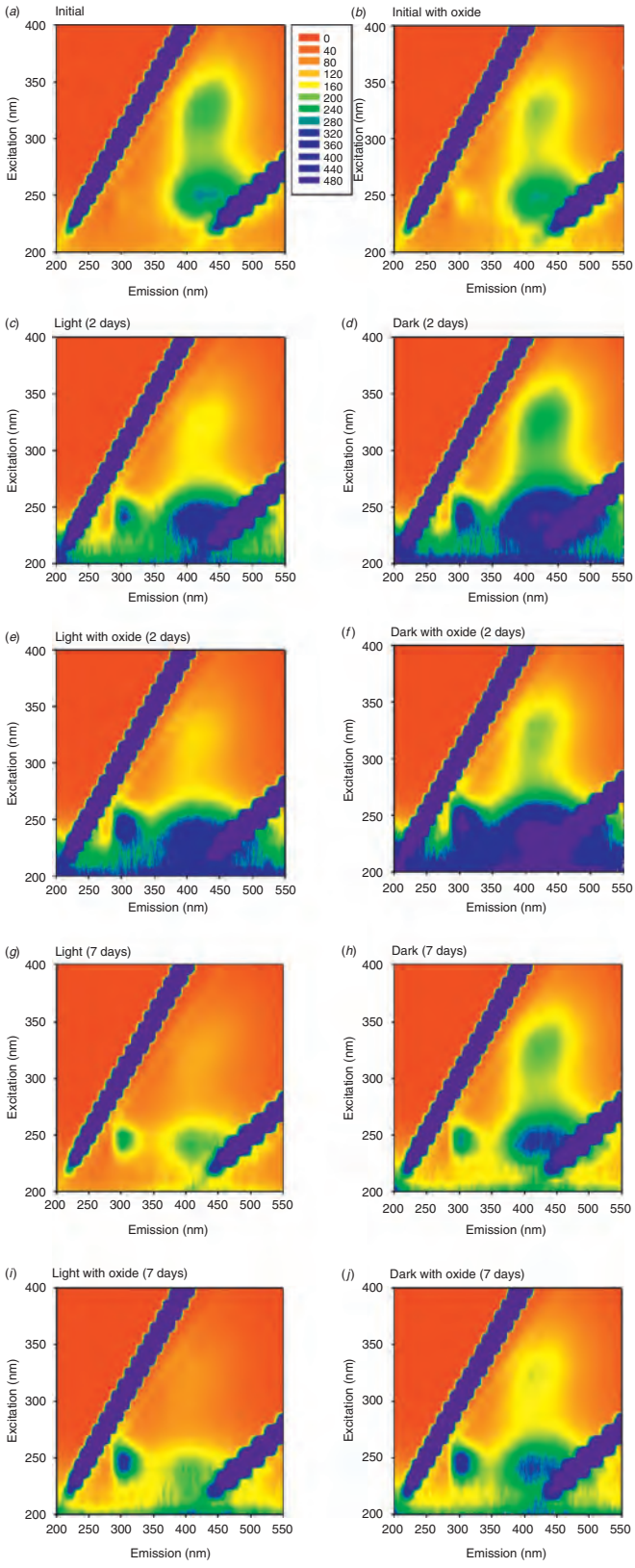

Fig. 8. Excitation-emission matrices (EEMs) for billabong water. Scans include the initial spectra (*a*, *b*), spectra 2 days into the experiment (*c*–*f*) and after 7 days (*g*–*j*).

Ex 240 nm) in all treatments indicate that some organic matter changes were occurring through non-photochemical processes. Fluorescence induced by light in the 280–350 nm range became less important in all but the dark (no oxide) control. The reactions responsible for this change appear to include a photochemical mechanism and are enhanced in the presence of both light and iron oxide. Fluorescence in this region is associated with humic substances (Coble 1996) and the reduction in both peak intensity and area suggests diminished humification of the samples. Blue shifting of peaks associated with photochemical degradation of organic matter has been reported in the literature, but not to the extent observed in these experiments (Kelton *et al*. 2007).

A shift from emission maxima in the 420–450 nm region to the 300–350 nm region, observed in the billabong water samples, is indicative of a shift from dominance by humic-like materials to aromatic protein-like materials (Coble 1996). Leachate fluorescence is also in the protein-like region. Increased fluorescence in the protein-like region is consistent with reports that this fluorescence is associated with two classes of compounds – proteinaceous materials and phenolic moieties of humic compounds (with a minor contribution from smaller amino acids and peptides) (Maie *et al*. 2007). Protein-like fluorescence signatures

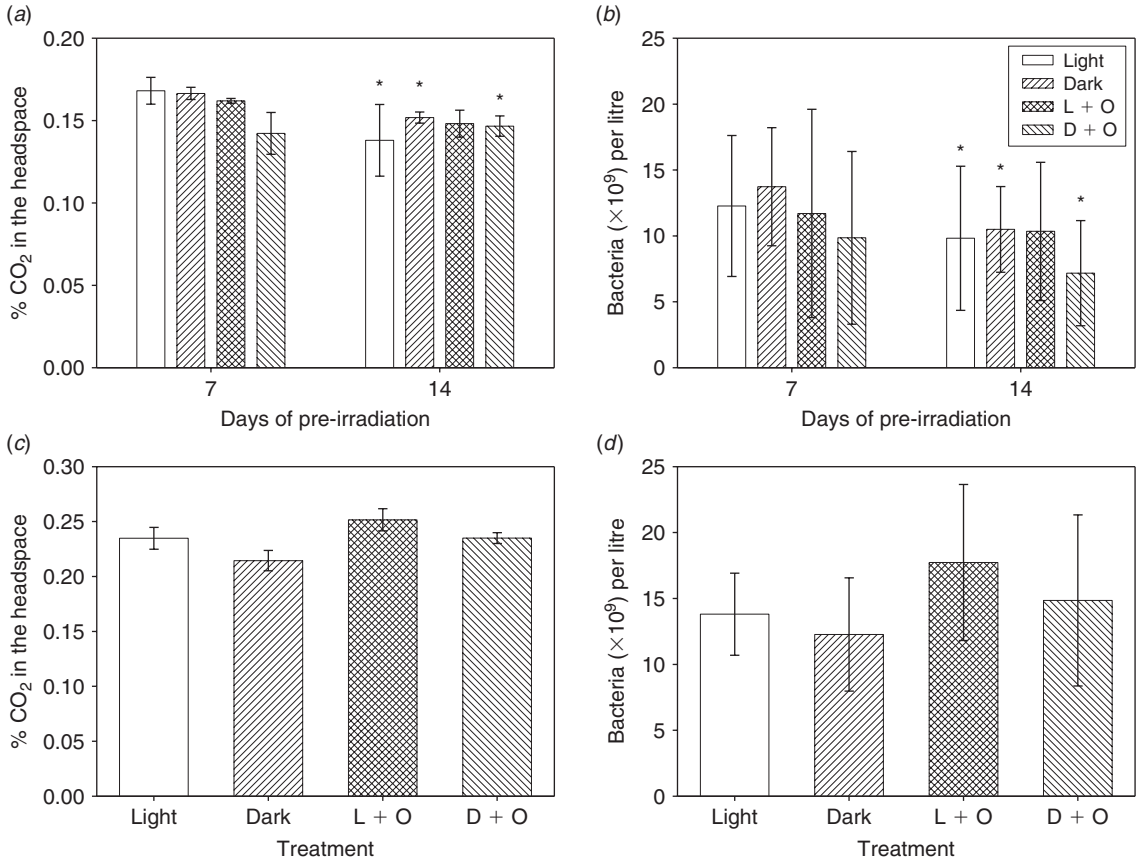

Fig. 9. Bioavailability experiments. Bacterial CO_2 production (*a*) and bacterial abundance (*b*) in cultures prepared on leachate after 7 and 14 days, and CO_2 production (*c*) and bacterial abundance (*d*) in cultures prepared on billabong water after 7 days. Error bars indicate one standard deviation ($n = 3$, except where * indicates $n = 2$). L + O = light plus oxide, D + O = dark plus oxide.

have been found to co-vary with terrestrially derived material in streams, and it has been suggested that they are associated with the degradation of the terrestrial inputs (Stedmon and Markager 2005*a*), but are also associated with bacterial metabolism (Cammack *et al*. 2004; Elliott *et al*. 2006). These experiments show they may be the product of photochemical degradation of humic materials and also be present in the fresh leachates of some plants. Bleaching of both leachate and billabong water suggests that photochemical reactions are destroying conjugated and aromatic molecules and producing low molecular weight compounds (likely to be organic acids) and this process has been suggested as a dominant driver of the DOM cycling in some systems (Waiser and Robarts 2000).

Bioavailability of dissolved organic matter

Photochemical modification of leachate samples had only a slight effect on the bioavailability of the leachate to bacteria. This DOM is known to be readily bioavailable before light exposure (O'Connell *et al*. 2000). Both carbon dioxide production and bacterial abundance in the billabong water experiment indicate that photochemical reactions increased the bioavailability of the DOM, and that the presence of iron oxide also influences the bioavailability of the DOM. It has previously been shown that billabong DOM may be less bioavailable than DOM in the adjacent river channel (McDonald *et al*. 2007) and that carbon quality (and therefore DOM history) may influence bacterial processing.

Increased bioavailability of billabong water appears to be associated with a decrease in the humic-like fluorescence of the samples. The fluorescence characteristics of the billabong water became more like the leachate as it degraded. The bulk characteristics of leachate were initially less complex than billabong water, and this may be the reason why the effects on the bioavailability of the DOM was less pronounced in this experiment, despite the observed photochemical degradation and production of trace quantities of organic acids. This supports the hypothesis that protein-like fluorescence signatures represent labile DOM (Stedmon and Markager 2005*b*; Hudson *et al*. 2008), and fluorescence in this region of the spectrum is correlated with rates of bacterial metabolism in lake water (Cammack *et al*. 2004) but may be a result of either labile substrate or the products of bacterial metabolism. Protein-like fluorescence from both billabong water and leachate suggests that fluorescence in this region is indicative of bioavailable material and bacterial metabolism, and may represent a balance of both production and consumption of these compounds by the bacterial population, input from terrestrial sources and photochemical generation from dissolved humic and fulvic acids.

The catalytic effect of amorphous iron oxide on the photodegradation of billabong water was not evident in the change to carbon bioavailability, but an effect of iron addition on the bioavailability was noted in both the light and dark treatments (indicating either carbon modification through dark reactions or iron limitation in the cultures). The data suggest that stimulation of bacterial growth and respiration, brought about by photochemical reactions, are the result of a reduction in the complexity and humic nature of the organic matter, and not exclusively from the production of low molecular weight organic acids. Increases in bioavailability have often been attributed to the production of low molecular weight photoproducts (Kieber *et al*. 1989; Moran and Zepp 1997; Goldstone *et al*. 2002). However, others have noted that production of low molecular weight photoproducts is insufficient to account for increased microbial activity and that modified higher molecular weight products contribute to increased bioavailability (Miller and Moran 1997; Obernosterer and Herndl 2000).

The present study showed that the addition of amorphous iron oxide can increase rates of degradation of both leachate and billabong water in sunlight. The billabong water samples are likely to contain a mixture of fresh and aged dissolved organic matter and showed considerable capacity to be modified. Modifications to the organic matter remaining in solution differed according to the source of the DOM. EEMs suggested that the humic component of billabong DOM was particularly susceptible to photochemical degradation. Both organic acid production and photodegradation of humic structures play a role in enhancing the biodegradability of organic matter derived from billabong water, but organic acids alone cannot account for enhanced bacterial activity (as bacterial metabolism did not increase in irradiated leachate). Changes to bioavailability were more closely correlated with the changes in fluorescence (and hence humic content) than to the production of organic acids. These results indicate photochemical degradation of billabong water can increase the subsequent bacterial metabolism of the dissolved organic matter, leading to increased bacterial biomass and CO_2 emitted from the aquatic ecosystem through bacterial respiration. Photochemical reactions in forested floodplain systems clearly contribute to the degradation of recalcitrant organic matter and the return of this carbon back into the food web.

Acknowledgements

We thank Dr Diane McKnight and Dr J. Robin Fulton from the University of Colorado, Boulder for advice on fluorescence corrections and Zygmunt Lorenz of the Murray-Darling Freshwater Research Centre (MDFRC) for assistance with the correction macro. We also thank Dr Patricia Bowen (MDFRC) for providing dried leaf material for use in the leachate experiments and Professor Gerry Quinn (Deakin University) for assistance with statistical analysis, and several anonymous reviewers for their suggestions on how the manuscript could be improved. This work was undertaken with the financial support of an Australian Postgraduate Award from Monash University and a top-up scholarship from the Cooperative Research Centre for Freshwater Ecology.

References

Baker, A. (2001). Fluorescence excitation-emission matrix characterization of some sewage-impacted rivers. *Environmental Science & Technology* **35**, 948–953. doi:10.1021/ES000177T

Baker, A. (2002*a*). Fluorescence excitation-emission matrix characterization of river waters impacted by a tissue mill effluent. *Environmental Science & Technology* **36**, 1377–1382. doi:10.1021/ES0101328

Baker, A. (2002*b*). Fluorescence properties of some farm wastes: implications for water quality monitoring. *Water Research* **36**, 189–195. doi:10.1016/S0043-1354(01)00210-X

Baker, A., and Spencer, R. G. M. (2004). Characterization of dissolved organic matter from source to sea using fluorescence and absorbance spectroscopy. *The Science of the Total Environment* **333**, 217–232. doi:10.1016/J.SCITOTENV.2004.04.013

Baldwin, D. S. (1999). Dissolved organic matter and phosphorus leached from fresh and 'terrestrially' aged river red gum leaves: implications for assessing river-floodplain interactions. *Freshwater Biology* **41**, 675–685. doi:10.1046/J.1365-2427.1999.00404.X

Benner, R., and Biddanda, B. (1998). Photochemical transformations of surface and deep marine dissolved organic matter: Effects on bacterial growth. *Limnology and Oceanography* **43**, 1373–1378.

Bertilsson, S., and Allard, B. (1996). Sequential photochemical and microbial degradation of refractory dissolved organic matter in a humic freshwater system. *Archiv für Hydrobiologie. Special Issues: Advances in Limnology* **48**, 133–141.

Bertilsson, S., and Bergh, S. (1999). Photochemical reactivity of XAD-4 and XAD-8 adsorbable dissolved organic compounds from humic waters. *Chemosphere* **39**, 2289–2300. doi:10.1016/S0045-6535(99)00152-6

Cammack, W. K. L., Kalff, J., Prairie, Y. T., and Smith, E. M. (2004). Fluorescent dissolved organic matter in lakes: Relationships with heterotrophic metabolism. *Limnology and Oceanography* **49**, 2034–2045.

Coble, P. G. (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry* **51**, 325–346. doi:10.1016/0304-4203(95)00062-3

Coble, P. G. (2007). Marine optical biogeochemistry: the chemistry of ocean color. *Chemical Reviews* **107**, 402–418. doi:10.1021/CR050350+

Cory, R. M., and McKnight, D. M. (2005). Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. *Environmental Science & Technology* **39**, 8142–8149. doi:10.1021/ES0506962

Daniel, C., Graneli, W., and Kritzberg, E. S. (2006). Stimulation of metazooplankton by photochemically modified dissolved organic matter. *Limnology and Oceanography* **51**, 101–108.

Elliott, S., Lead, J. R., and Baker, A. (2006). Characterisation of the fluorescence from freshwater, planktonic bacteria. *Water Research* **40**, 2075–2083. doi:10.1016/J.WATRES.2006.03.017

Faust, B. C., and Zepp, R. G. (1993). Photochemistry of aqueous iron(III)-polycarboxylate complexes: Roles in the chemistry of atmospheric and surface waters. *Environmental Science & Technology* **27**, 2517–2522. doi:10.1021/ES00048A032

Geller, A. (1986). Comparison of mechanisms enhancing biodegradability of refractory lake water constituents. *Limnology and Oceanography* **31**, 755–764.

Goldstone, J. V., Pullin, M. J., Bertilsson, S., and Voelker, B. M. (2002). Reactions of hydroxyl radical with humic substances: bleaching, mineralization and production of bioavailable carbon substrates. *Environmental Science & Technology* **36**, 364–372. doi:10.1021/ES0109646

Hayakawa, K., Sekino, T., Yoshioka, T., Maruo, M., and Kumagai, M. (2003). Dissolved organic carbon and fluorescence in Lake Hovsgol: factors reducing humic content of lake water. *Limnology* **4**, 25–33. doi:10.1007/S10201-003-0092-3

Howitt, J. A., Baldwin, D. S., Rees, G. N., and Hart, B. T. (2004). Facilitated heterogeneous photodegradation of dissolved organic matter by particulate iron. *Environmental Chemistry* **1**, 197–205. doi:10.1071/EN04005

Howitt, J. A., Baldwin, D. S., Rees, G. N., and Williams, J. L. (2007). Modelling blackwater: Predicting water quality during flooding of lowland river forests. *Ecological Modelling* **203**, 229–242. doi:10.1016/J.ECOLMODEL.2006.11.017

Hudson, N., Baker, A., and Reynolds, D. (2007). Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters – A review. *River Research and Applications* **23**, 631–649. doi:10.1002/RRA.1005

Hudson, N., Baker, A., Ward, D., Reynolds, D. M., Brunsdon, C., and Carliell-Marquet, C. (2008). Can fluorescence spectrometry be used as a surrogate for the Biochemical Oxygen Demand (BOD) test in water quality assessment: An example from South West England. *The Science of the Total Environment* **391**, 149–158. doi:10.1016/J.SCITOTENV.2007.10.054

Kelton, N., Molot, L. A., and Dillon, P. J. (2007). Spectrofluorometric properties of dissolved organic matter from Central and Southern Ontario streams and the influence of iron and irradiation. *Water Research* **41**, 638–646. doi:10.1016/J.WATRES.2006.11.001

Kieber, D. J., McDaniel, J., and Mopper, K. (1989). Photochemical source of biological substrates in sea water: implications for carbon cycling. *Nature* **341**, 637–639. doi:10.1038/341637A0

Kieber, R. J., Zhou, X., and Mopper, K. (1990). Formation of carbonyl compounds from UV-induced photodegradation of humic substances in natural waters: Fate of riverine carbon in the sea. *Limnology and Oceanography* **35**, 1503–1515.

Kieber, R. J., Hydro, L. H., and Seaton, P. J. (1997). Photooxidation of triglycerides and fatty acids in seawater: Implication toward the formation of marine humic substances. *Limnology and Oceanography* **42**, 1454–1462.

Klapper, L., McKnight, D. M., Blunt-Harris, E. L., Nevin, K. P., Lovley, D. R., and Hatcher, P. G. (2002). Fulvic acid oxidation state detection using fluorescence spectroscopy. *Environmental Science & Technology* **36**, 3170–3175. doi:10.1021/ES0109702

Larsson, T., Wedborg, M., and Turner, D. (2007). Correction of inner-filter effect in fluorescence excitation-emission matrix spectrometry using Raman scatter. *Analytica Chimica Acta* **583**, 357–363. doi:10.1016/J.ACA.2006.09.067

Lovley, D. R., and Phillips, E. J. P. (1986). Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Applied and Environmental Microbiology* **51**, 683–689.

Ma, X., and Green, S. A. (2004). Photochemical transformation of dissolved organic carbon in Lake Superior – An *in-situ* experiment. *Journal of Great Lakes Research* **30**, 97–112.

Maie, N., Scully, N. M., Pisani, O., and Jaffe, R. (2007). Composition of a protein-like fluorophore of dissolved organic matter in coastal wetland and estuarine ecosystems. *Water Research* **41**, 563–570. doi:10.1016/J.WATRES.2006.11.006

Maier, H. R., Dandy, G. C., and Burch, M. D. (1998). Use of artificial neural networks for modelling cyanobacteria *Anabaena* spp. in the River Murray, South Australia. *Ecological Modelling* **105**, 257–272. doi:10.1016/S0304-3800(97)00161-0

McDonald, S., Pringle, J. M., Prenzler, P. D., Bishop, A. G., and Robards, K. (2007). Bioavailability of dissolved organic carbon and fulvic acid from an Australian floodplain river and billabong. *Marine and Freshwater Research* **58**, 222–231. doi:10.1071/MF06085

McKnight, D. M., Boyer, E. W., Westerhoff, P. K., Doran, P. T., Thomas, K., and Andersen, D. T. (2001). Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography* **46**, 38–48.

Miles, C. J., and Brezonik, P. L. (1981). Oxygen consumption in humic colored waters by a photochemical ferrous-ferric catalytic cycle. *Environmental Science & Technology* **15**, 1089–1095. doi:10.1021/ES00091A010

Miller, W. L., and Moran, M. A. (1997). Interaction of photochemical and microbial processes in the degradation of refractory dissolved organic matter from a coastal marine environment. *Limnology and Oceanography* **42**, 1317–1324.

Mobed, J. J., Hemmingsen, S. L., Autry, J. L., and McGown, L. B. (1996). Fluorescence characterization of IHSS humic substances: total luminescence spectra with absorbance correction. *Environmental Science & Technology* **30**, 3061–3065. doi:10.1021/ES960132L

Moran, M. A., and Zepp, R. G. (1997). Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. *Limnology and Oceanography* **42**, 1307–1316.

Morel, F. M. M., and Hering, J. G. (1993). 'Principles and Applications of Aquatic Chemistry.' (John Wiley and Sons, Inc.: New York.)

O'Connell, M., Baldwin, D. S., Robertson, A. I., and Rees, G. (2000). Release and bioavailability of dissolved organic matter from floodplain litter: influence of origin and oxygen levels. *Freshwater Biology* **45**, 333–342. doi:10.1111/J.1365-2427.2000.00627.X

Obernosterer, I., and Herndl, G. J. (2000). Differences in the optical and biological reactivity of the humic and nonhumic dissolved organic carbon component in two contrasting coastal marine environments. *Limnology and Oceanography* **45**, 1120–1129.

Ohno, T., Amirbahman, A., and Bro, R. (2008). Parallel factor analysis of excitation-emission matrix fluorescence spectra of water soluble soil organic matter as a basis for the determination of conditional metal binding parameters. *Environmental Science & Technology* **42**, 186–192. doi:10.1021/ES071855F

Pullin, M. J., Anthony, C., and Maurice, P. A. (2007). Effects of iron on the molecular weight distribution, light absorption, and fluorescence properties of natural organic matter. *Environmental Engineering Science* **24**, 987–997. doi:10.1089/EES.2006.0040

Robertson, A. I., Bunn, S. E., Boon, P. I., and Walker, K. F. (1999). Sources, sinks and transformations of organic carbon in Australian floodplain rivers. *Marine and Freshwater Research* **50**, 813–829. doi:10.1071/MF99112

Schmitt-Kopplin, P., Hertkorn, N., Schulten, H.-R., and Kettrup, A. (1998). Structural changes in a dissolved soil humic acid during photochemical degradation processes under O_2 and N_2 atmosphere. *Environmental Science & Technology* **32**, 2531–2541. doi:10.1021/ES970636Z

Stedmon, C. A., and Markager, S. (2005*a*). Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. *Limnology and Oceanography* **50**, 686–697.

Stedmon, C. A., and Markager, S. (2005*b*). Tracing the production and degradation of autochthonous fractions of dissolved organic matter by fluorescence analysis. *Limnology and Oceanography* **50**, 1415–1426.

Voelker, B. M., Morel, F. M. M., and Sulzberger, B. (1997). Iron redox cycling in surface waters: Effects of humic substances and light. *Environmental Science & Technology* **31**, 1004–1011. doi:10.1021/ES9604018

Waiser, M. J., and Robarts, R. D. (2000). Changes in composition and reactivity of allochthonous DOM in a prairie saline lake. *Limnology and Oceanography* **45**, 763–774.

Wells, M. L., Mayer, L. M., Donard, O. F. X., de Souza Sierra, M. M., and Ackelson, S. G. (1991). The photolysis of colloidal iron in the oceans. *Nature* **353**, 248–250. doi:10.1038/353248A0

Wetzel, R. G., Hatcher, P. G., and Bianchi, T. S. (1995). Natural photolysis by ultraviolet irradiance of recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. *Limnology and Oceanography* **40**, 1369–1380.

Whitehead, R. F., de Mora, S., Demers, S., Gosselin, M., Monfort, P., and Mostajir, B. (2000). Interactions of ultraviolet-B radiation, mixing, and biological activity on photobleaching of natural chromophoric dissolved organic matter: A mesocosm study. *Limnology and Oceanography* **45**, 278–291.

Manuscript received 28 November 2007, accepted 29 June 2008