1. ABSTRACT
This paper addresses litter decomposition in the terrestrial catchment of a wetland, and the consequent release of phosphorus (P). Microbial activity and P dynamics were monitored before and after the onset of winter rain along a transect from the bed of a small lake in Western Australia into its vegetated catchment. Microbial activity was measured in the field as CO$_2$ production from surface soils, and in the laboratory as substrate-induced respiration (SIR). Before rain, SIR was positively correlated with soil litter and organic content, and P extracted by anion exchange membrane ($P_{AEM}$) was strongly correlated with site litter. With the onset of winter rain, soil moisture favoured microbial activity in the presence of nutrient flux from decomposing litter, and much of the bioavailable P inherited from the dry season was immobilized in microbial biomass. It is concluded that microbial activity plays an important role in regulating P flux from catchment litter.

KEYWORDS: phosphorus, catchment, litter decomposition, soil respiration, soil phosphorus.

2. INTRODUCTION
Because of the focus of interest on processing organic waste produced by human activities, we can overlook the important role played by organic matter in the functioning of natural ecosystems. This paper addresses the degradation of leaf litter in the terrestrial catchment of a wetland, and the consequent release of phosphorus (P) from this source.

Some of the techniques and concepts are relevant to nutrient loss during the management of organic waste, including field and laboratory measurements of respiration, the use of anion-exchange membranes to trap and measure readily-available phosphorus, and of chloroform fumigation to disentangle the contribution of microorganisms to P dynamics. The full details of this work are being published elsewhere Qiu et al. (in review), but outlines of key methods and results are presented here.

Wetlands in Australia, like those in other countries, have been impacted by human activity, their catchments cleared for agriculture or urban development, and their waters showing consequences of nutrient enrichment (e.g. McComb and Lake 1988). Wetlands with less disturbed catchments provide an opportunity to infer what might take place under pristine conditions.

Topsoil in the region of our study is severely leached, infertile, and when not impacted typically contains low concentrations of P and organic matter (McArthur 1991; McComb and Lukatelich 1995; Gilkes and Dimmock 1998). There are exceptions: organic deposits occur in a chain of wetlands in an interdunal depression between two Pleistocene dune systems. Their sediments contain 23-48% organic matter, and organic P may account for 35%-73% of total P (Qiu and McComb 2000). In the absence of anthropogenic influence, the source of this organic matter and phosphorus is of particular interest, and we have suggested this is to be found in catchment litter; we already knew that this releases considerable P during decomposition and leaching, and could infer that microbial activity plays a key role in mediating this nutrient release (Qiu et al. 2002).
The present study examined heterotrophic microbial activity, soil type and litter along a transect running from a wetland into its catchment, in relation to the onset of winter rains.

3. MATERIALS AND METHODS

Thomsons Lake has an area (open water and emergent macrophytes) of some 250 ha, and the water level fluctuates some 5 m annually, leaving an exposed lakebed at the end of the dry season, and inundated fringing vegetation after refilling (Arnold 1990). Seven study sites were selected 50 m apart along a transect starting in the lake bed, passing through a riparian zone occupied by sedges, and ending in a woodland dominated by Banksia and Eucalyptus (Figure 1).

Soil respiration was measured in the field by analysing, with a portable gas analyser, CO₂ accumulating in chambers pressed into the soil surface. For measurement under controlled conditions, 'intact' soil cores were transferred to the laboratory.

Substrate induced respiration (SIR) was measured under conditions such that respiratory substrate was not limiting. Soil samples (5 g dw equivalent) were placed in volume-calibrated Erlenmeyer flasks, distilled water added to 20% water content, wrapped with plastic film, and incubated at 20°C for 7 days. Each then received 1.5 ml glucose (30 mg ml⁻¹) to a soil water content of about 50%, sufficient to thoroughly mix soil and glucose solution, without over-saturating. The glucose concentration added was 9 mg g⁻¹ (Anderson and Domsh 1978; Chen and Coleman 1988). Three litter types were also investigated: 'Fresh ground litter' was collected from the soil surface near the end of dry season. 'Moulded leaf litter' was from an area with a dense litter accumulation, and litter buried in soils and degraded to various stages was recovered manually from surface soils.

Four ml glucose (30 mg ml⁻¹) were added to each flask containing litter (2 g dwt equivalent, cut into 0.5-1 cm pieces) after 'pre-wetting', and thoroughly mixed. Glucose addition was 60 mg g⁻¹, similar to Imberger and Chiu (2001) and Neely et al. (1991). SIR was measured with the CO₂ analyser 6 and 24 hr after incubation at 20 °C.

Measurements were made of properties likely to affect soil respiration, including moisture content, pH, plant debris and organic matter.

Bioavailable P was measured as anion exchange membrane (AEM) extractable P (P_AEM), which includes soluble P and a pool of desorbable P (Bentley et al. 1999; Saggar et al. 1999; Nuemberg et al. 1998). It was determined by shaking 1 g soil with an AEM strip (2 x 2 cm) in 20 ml distilled water (16 hours); retained P was eluted in 0.1 N H₂SO₄ (Kouno et al.1995).

Microbial biomass P (P_MB) was determined by adding liquid chloroform to soil suspensions, and P released measured as the difference in P_AEM between 'fumigated' and 'non-fumigated' samples (McLaughlin et al. 1986; Kouno et al.1995). To each 'fumigated' sample (after extraction of P_AEM, 20 ml) was added 1 ml of alcohol-free chloroform, shaken 16 hr to lyse microbial cells, and placed under vacuum to remove chloroform. Lysed cell-P was extracted by AEM strips. Non-fumigated samples received only AEM-strips. The difference in P_AEM between 'fumigated' and 'non-fumigated' samples was taken as P_MB. The total biomass P in the sample was estimated using a recovery factor (K_P), analogous to Brookes et al. (1982).
4. RESULTS

At the end of the dry season, after 6 months of high evaporation and almost no rain, surface
soils were desiccated and the lake bed exposed, although there was high moisture content
beyond 20 cm below the sediment surface.

'Background P' was determined for an upland site without vegetation, and was 50 mg kg$^{-1}$ of
total P. In contrast, surface soil on the lakebed had a total P content of 600-700 mg kg$^{-1}$.

Field rates of CO$_2$ efflux from soils (g m$^{-2}$ h$^{-1}$) were well correlated ($R^2 = 0.93$, $p < 0.0001$)
with SIR rates measured in the laboratory (g CO$_2$ kg h$^{-1}$), and were higher in surface soils (0-5
cm) than 20 cm below the surface (Figure 2).

CO$_2$ efflux from the 'background soil' without vegetation was only 5.3 μg g$^{-1}$ h$^{-1}$ before rain,
an order of magnitude lower than in surface soils from vegetated sites.

Compared with soils, there was significantly higher microbial activity (SIR) for all litter
types, and especially for degraded leaf litter buried in surface soil. Activity was lower from
'fresh ground litter', collected before the onset of the wet season (Table 1).

Before the wet season, SIR was closely correlated with litter and organic content, and plant
debris (> 1 mm) was correlated with anion-exchange-membrane extractable P ($P_{AEM}$).

Microbial activity was concentrated in the 10 – 20 cm depth zone, and depleted below 40 cm
(Figure 2)
Table 1. Substrate-induced respiration of leaf litter and surface soil from selected sites. Assumed stoichiometric ratio of aerobic respiration CO₂/O₂ = 1.38.

<table>
<thead>
<tr>
<th>Litter type</th>
<th>CO₂ consumption ( \mu g , g^{-1} , h^{-1} )</th>
<th>SE</th>
<th>( \mu g , g^{-1} , h^{-1} )</th>
<th>O₂ consumption</th>
<th>SE</th>
<th>Ratio of CO₂/O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moulded leaf litter</td>
<td>614</td>
<td>30</td>
<td>484</td>
<td>27</td>
<td>1.27</td>
<td></td>
</tr>
<tr>
<td>Ground litter</td>
<td>281</td>
<td>18</td>
<td>220</td>
<td>14</td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>Soil litter</td>
<td>1036</td>
<td>34</td>
<td>709</td>
<td>37</td>
<td>1.46</td>
<td></td>
</tr>
<tr>
<td>Upland soils</td>
<td>25</td>
<td>8</td>
<td>22</td>
<td>6</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td>Soils from Low-lying area</td>
<td>83</td>
<td>5</td>
<td>58</td>
<td>4</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td>Exposed sediment</td>
<td>123</td>
<td>12</td>
<td>78</td>
<td>7</td>
<td>1.57</td>
<td></td>
</tr>
</tbody>
</table>

\[ P_{AEM} \text{ and } P_{MB} \, \text{mg kg}^{-1} \]

Figure 2. Distribution of \( P_{AEM} \) and \( P_{MB} \) at various depths below the exposed lakebed of Thomsons Lake at the onset of the wet season.

With the onset of the wet season, SIR was positively correlated with organic matter (as loss on ignition), and AEM-extractable P (\( P_{AEM} \)) was low compared to P associated with microbial biomass (\( P_{MB} \)), suggesting that most bioavailable P had been taken up by microbial biomass, resulting in a low \( P_{AEM}/P_{MB} \) ratio before rain. Using chloroform to lyse microbial cells returned much of the P into solution, in which the concentration of P became about 100 times higher than non-cell \( P_{AEM} \) remaining in soils.

\( P_{AEM} \) was hardly detectable from surface sediment to a depth of more than 60 cm. \( P_{MB} \) was concentrated at 0-30 cm, then decreased sharply, in parallel with reduced substrate availability, loss on ignition decreasing from 40 to 50% at the surface 10-30 cm to 6.0% at 50-60 cm. With the onset of the wet season, surface microbial activity (SIR) was positively
correlated with organic matter (as loss on ignition). $P_{\text{AEM}}$ was low compared to $P$ associated with microbial biomass ($P_{\text{MB}}$).

$P_{\text{AEM}}$ was hardly detectable from surface sediment to more than 60 cm. $P_{\text{MB}}$ was concentrated at 0-30 cm, then decreased sharply, in parallel with reduced substrate availability; loss on ignition decreased from 40 to 50% at the surface 10-30 cm to 6.0% at 50-60 cm).

5. DISCUSSION
There was no significant nutrient source, other than catchment vegetation, for the relatively pristine wetland studied here, yet microbial processes and litterfall played major roles in $P$ bioavailability and fluxes. Microbial activity is largely associated with the mineralisation of woodland litter, consistent with the hypothesis that this is an important source of phosphorus for the wetland, and that microbial activity mediates in this phosphorus transfer. Using litter traps we found approximately 0.5 kg m$^{-2}$ yr$^{-1}$ of litterfall from the woodland catchment. Thus the first heavy rain (5 mm in 24 hr) could produce a $P$ flux with a concentration of 2.4 mg L$^{-1}$, given the leaching rate of 30% reported in our previous leaching tests. Thus a significant nutrient flux from catchment litter is likely to occur, and would affect microbial activity in surface soils on the catchment.

Surface litter appears important in providing organic carbon and nutrients during decomposition. The onset of the wet season favoured microbial activity, so that bioavailable $P$ was transferred into microbial biomass. The microbial $P$ ($P_{\text{MB}}$) measured here approximated the results of Grierson and Adams (2000) for jarrah forest in southwestern Australia, and our soil CO$_2$ efflux figures are similar to those of Raich et al. (1990) and Epron et al. (2001), but were substantially lower (about 1/3rd) compared with data from tropical rain forest in Australia (Holt et al. 1990; Kiese and Butterbach-Bahl 2002).

6. CONCLUSIONS
1. Measurement of substrate-induced respiration, and of available $P$ using anion-exchange membrane, are valuable tools for understanding heterotrophic microbial activity.

2. Litter provides a rich source of available $P$ and carbon for the microbial community in these $P$ deficient soils.

3. Microbial activity is a key regulator of bioavailable $P$ in surface soil during the wet season, the onset of which stimulated microbial activity, transferring most bioavailable $P$ into microbial biomass.

7. ACKNOWLEDGEMENT
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8. LIST OF REFERENCES


