Wetlands Ecology and Management **13:** 43–54, 2005.

Response of soil microbial activity to temperature, moisture, and litter leaching on a wetland transect during seasonal refilling

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Received 27 November 2002; accepted in revised form 20 August 2003

Key words: CO_2 efflux, Litter leaching, Microbial activity, Soil respiration, Wetland soil

Abstract

In nutrient impoverished landscapes in southwest Australia, terrestrial litter appears to be important in phosphorus (P) turnover and in the gradual accumulation of P in wetland systems. Little is known about the fate of P leached from litter during the wet season and the associated effects of soil microclimate on microbial activity. The effects of temperature, moisture, and litter leaching on soil microbial activity were studied on a transect across a seasonal wetland in southwestern Australia, after the onset of the wet season. Heterotrophic respiration (CO_2 efflux) was higher in the dried lakebed and riparian areas than in upland soils, and higher during the day than at night. There were significant variations in CO_2 efflux with time of sampling, largely caused by the effect of temperature. The addition of litter leachate significantly increased CO_2 efflux, more significantly in soils from upland sites, which had lower moisture and nutrient contents. There was a difference in response of microbial respiration between upland soils and wetland sediments to litter leachate and wetter, warmer conditions. In general, the litter leachate enhanced heterotrophic microbial respiration, and more significantly at warmer conditions (31 °C). The relative fungal to bacterial ratio was 2.9–3.2 for surface litter and 0.7–1.0 for soils, suggesting a fungal dominance in heterotrophic respiration of surface litter, but increased bacterial dominance in soils, especially in exposed sediments in the lakebed.

Introduction

Microbial biomass plays a key role in soil nutrient cycling (McLaughlin et al. 1988; Frossard et al. 2000). In agricultural soils, microbial activity in the form of phosphate-solubilizing bacteria improves P transfer from soil to plants; microbial biomass may comprise a significant pool of P, typically 10–50 kg P ha^{-1}, or 1–10% of the total P, and around 10–15% of soil organic P (Brookes et al. 1984; Richardson 2001) and is a major factor controlling organic and inorganic P concentrations in temperate soils (Seeling and Zasoski 1993). Compared with agricultural soils, much less is known about the role of microbial biomass in natural systems, especially in relation to P cycling between the catchment and adjacent wetland.

Generally, soils in southwestern Australia have been severely weathered since the Pleistocene, and thus contain very little total P, of which a significant proportion is associated with organic matter (McArthur 1991; McComb and Lukatelich 1995; Gilkes and Dimmock 1998). Wetlands in the region, and in particular those in interdunal depressions of the coastal sand dunes, are commonly surrounded by sedgeland and woodland (McArthur and Bettenay 1960). Because of low inorganic P in these severely weathered soils, it seemed likely that P availability would depend heavily on microbially mediated organic P turnover (Oberson et al. 2001). Qiu et al. (2002) reported that litterfall from

fringing areas of these wetlands had a high potential for P leaching during 'first storm' events, and microbial activity modified P concentrations in the leachate. A subsequent study suggested that the dynamics of P bioavailability in the catchment and wetland soils were associated with microbial activity, which differs between dry and wet conditions and varied significantly on spatial and temporal scales (Qiu et al. 2003).

It is therefore important to understand the response of soil microbial activity to litter-sourced P flux, and the effects of soil environment on microbial respiration. Climatic factors such as moisture and temperature are often confounded in field observations of microbial activity (Davidson et al. 1998; Xu and Qi 2001a). Severe drying may be lethal to bacteria, leading to the release of cell-bound P to soil and sediment (Sparling et al. 1995; Qiu and McComb 1995; Turner and Haygarth 2001). The influence of temperature on microbial respiration (Q_{10}) varies with soil and environment conditions (Xu and Qi 2001b). Specifying these effects is important not only for understanding microbial dynamics under the seasonal wet–dry conditions like that prevailing in southwestern Australia, but also for comparing data of carbon flux between different ecosystems and regions.

This paper reports the response of microbial activity on a transect across a seasonal wetland to changes in major environmental conditions, especially temperature and moisture. Heterotrophic microbial activity was measured either in the field as CO_2 efflux or in the laboratory as substrate-induced respiration (SIR). The microbial response to litter leaching during the wet season was of particular concern and was examined using litter leachate derived from catchment litter under rainfall. The eukaryote (fungal) and prokaryote (bacterial) contributions to microbial activity were determined using selective inhibition techniques applied to soils, wetland sediments, and surface litter at early stages of degradation.

Materials and methods

Study area

Thomsons Lake is a sub-coastal freshwater wetland, and part of the 'East Beeliar' chain of wetlands, located in interbarrier depressions between the Bassendean and Spearwood Sand Dunes of the Swan Coastal Plain (Arnold 1990). The lake is about 20 km from Perth and 5 km from the ocean (Figure 1). It is oval-shaped and flat bottomed, with a total area of 250 ha. Water levels are highly seasonal with the highest water level at 15.51 m Australian Height Datum (AHD) and a minimum of 10.75 m (Arnold 1990).

The lake perimeter is vegetated, with the riparian zone being covered by bands of native sedges of various densities (mainly *Baumea articulata*, with a few *Typha*). The lakebed is usually dominated by ephemeral *Myriophyllum*. Upslope from the riparian zone the catchment is sedgeland and poorly developed woodland. There is a weed-infested low-lying area between the wetland and dryland, connecting the sedge fringe to the terrestrial woodland (*Banksia* and *Eucalyptus)*. 'Dieback', caused by the fungal pathogen *Phytophthora cinnamomi*, has been recorded on the eastern-side of the catchment for some years, and in part of the catchment area (dryland) in this study. Dieback was manifested as many decayed trees and branches lying between healthy trees on the eastern-side of the dryland catchment.

The lake was dry at the beginning of this study (March 2002), with most of the lakebed exposed. A few square meters of open water remained in the deepest area. Most aquatic plants in the lake and fringing areas were desiccated.

Study design

As a part of the study of the role of catchment litter in wetland P cycling, a transect was established in 2001 to examine dynamics of litter-sourced P flux during the winter refilling (Figure 1). The transect passed through the lakebed, the sedge area and riparian zone, ending in woodland on the eastern catchment of the lake, and had a slope of about 2% in the direction of the lakebed. The transect cut through vegetation bands of macrophytes, sedges, and woodland parallel to the lake margin on the east catchment. Seven sites (including a control site) were selected along the transect, characterized by their distinctive vegetation types, plant litter, and general morphological features (Table 1). The distance between each site was about 50 m, approximately the width of various vegetation

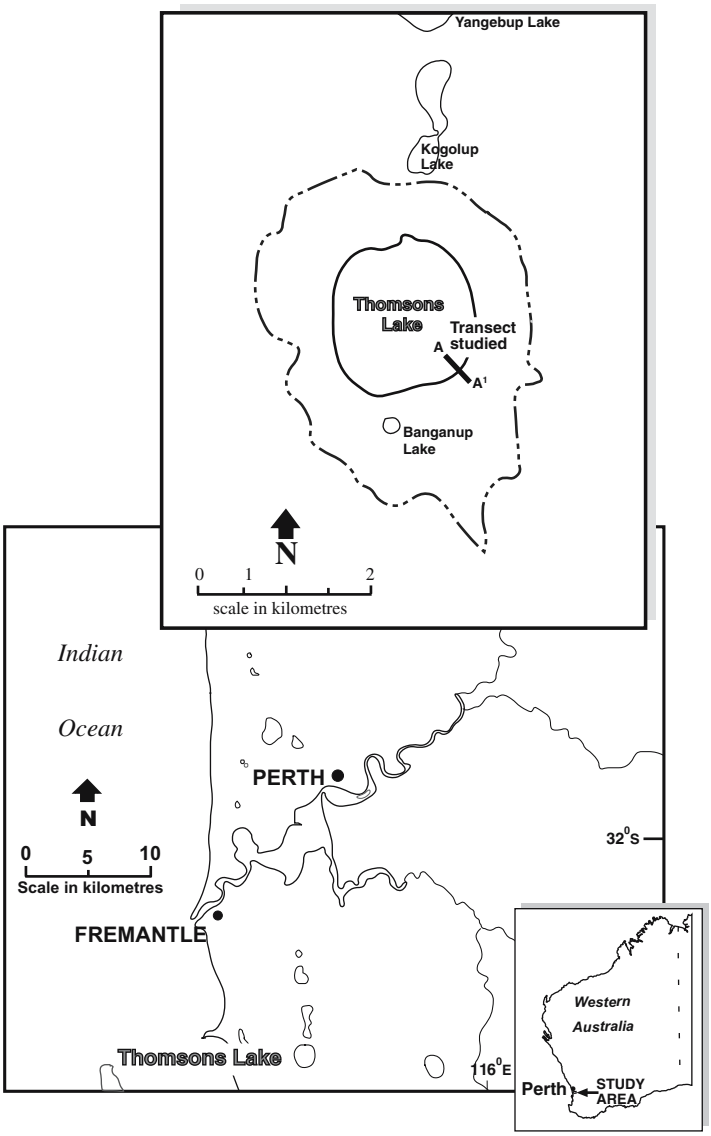

Figure 1. Study area and location of transect.

bands. A control site (Th7) was selected at the far end of the upland near the boundary of Thomsons Lake Reserve, in an elevated area with no vegetation, and the soil was bare with no obvious organic debris. Soils on the transect generally showed a pattern with higher nutrients in the low-lying and wetland area (Table 2).

It is generally assumed that the litter-sourced P flux during winter rains is related to site litter and hydrological properties, and is reflected in the level of soil microbial activity. Soil respiration was measured directly from these sites using soil chambers, and in the laboratory as SIR. Because of the high variations of microbial respiration between days and sites, which appeared to be confounded by microclimate factors (Qiu et al. 2003), soil cores were taken after the field CO_2 efflux measurement and incubated under various conditions to examine the effects of temperature, soil moisture, and litter leachate on heterotrophic microbial respiration. Fungal and bacterial activity were partitioned from litter and soil samples from the transect to

Table 1. Selected sites on a transect from the lakebed to the dry upland catchment.

Site	Location	Morphology	Vegetation	Inundation	Topsoil
Th1	Dried lakebed	Dried bed, 20 m from remaining water body; cracked sediment surface	Scattered dried rooted aquatic weeds	In the middle of rainy season	Oozy, black sediment, fine textured with some plant debris
Th2	Lake margin	Damp area surrounding the lake, gently sloping to lake, soft surface	Sparse macrophytes (*B. articulata*)	In the middle of rainy season	Oozy, black soil, plant root debris
Th3	Fringing area	Elevated area beyond the damp area, solid surface supporting walking	Terrestrial weeds, bushes, and young eucalyptus trees	In the middle of rainy season	Sandy soil with degraded plant debris
Th4	Fringing area	Transition to a dryland area of catchment, in a damp depression area	*B. articulata* interspersed with young eucalyptus trees	During excessive rainfall	Damp, sandy soil mingled with degraded plant debris
Th5	Terrestrial catchment	Marginal area of the woodland, under canopy of eucalyptus trees	Eucalyptus woodland	Never inundated	Sandy soil with leaf and woody debris on surface
Th6	Dryland, dieback area	Elevated dryland on the high side of the internal drive	Collapsed eucalyptus, banksia, poorly developed understory plants	Never	Greyish sands with degraded leaf and woody debris

Table 2. Properties of surface soils (0–5 cm) from transect sites in Thomsons Lake in February 2001 (before wet season).

	Th1	Th2	Th3	Th4	Th5	Th6	Th7
Color[a]	BRBK	BRBK	BRBK	BRBK	BRBK	BRBK	BR
Organic matter (LOI%)	38.0	31.7	21.9	6.9	3.5	1.4	0.7
Extractable S (mg kg^{-1})	2126	1563	1268	651	221	27	5.7
Colwell K (mg kg^{-1})	579	959	525	209	159	27	13
NO_3–N (mg kg^{-1})	99	165	85	1	1	3	1
NH_4–N (mg kg^{-1})	13	21	34	41	11	24	2
Total P (mg kg^{-1})	617	700	1007	555	196	154	50

[a] BR – brown; BK – black.

understand microbial community structure. Catchment litter production was recorded from April 2001 to May 2002 using 34 litter traps (0.25 m^2) at 17 sites in Thomsons Lake Reserve to estimate litter-sourced P budget during winter rains.

Field respiration

Soil chambers (plastic cylinders 10 cm diameter, 10 cm effective height above the soil surface) with an air space of 785 ml and a screw-on cap (sealed with rubber rings), and with air inlet and outlet ports were deployed in sites Th1, Th3, Th5, and Th6 along the transect from 14–17 May and 26–28 June 2002. Three chambers were installed at each site, 10 m apart and aligned with the lake margin. The chambers were closed from 10:00 a.m. to 4:00 p.m. to record respiration during the day and from 10:00 a.m. to 10:00 a.m. the next day to record daily respiration. After each reading the chamber was opened for 30 min to equilibrate with the ambient air. CO_2 in the chamber was measured by a portable gas analyser (GA 2000, Geotechnical Instrument, UK) equipped with an infrared CO_2 sensor. Q_{10}, a multiplier to the respiration rate for a 10-degree increase in temperature, was calculated using the following models:

$$F = b_0 \, e^{b_1 T}$$

$$Q_{10} = e^{10 b_1}$$

where F is CO_2 efflux rate (μmol m^{-2} s^{-1}) and b_1 and b_0 are constants fitted by the least-square method. Soil temperature (T) was taken at 10 cm depth when measuring CO_2 efflux.

Soil core incubation

To explore factors controlling field respiration, 10 soils cores (10 cm depth) were taken from the transect on 17 May: two from each of the four locations used for *in situ* CO_2 measurement and two from the control site, Th7. The bottom of each soil core/chamber was sealed by plastic bags bound with tape. The 'intact' soil cores were transferred to the laboratory, incubated under constant temperature (20 °C) beginning the day after sampling, and respiration rates recorded daily and hourly. The cores were moved outdoors to record diurnal fluctuations in CO_2 flux. After each test, the cores were re-opened to equilibrate with ambient air, and subsequent tests always started with ambient air conditions.

There was a gradual decrease in soil moisture due to evaporation between experiments and when cores were open for air exchange. Since it may simulate natural drying during no rainfall period, no attempt was taken to remoisten soil cores until the leachate treatment. After 30 days, the rates of CO_2 efflux and soil properties were again recorded. This was followed by an addition of litter leachate produced in parallel rain-leaching tests, with a litter density of 0.5 kg m^{-2} yr^{-1}. The leachate generated by rainfall was collected in a large container for six weeks: it contained 156 μg l^{-1} of bioavailable P measured by anion-exchange membrane (AEM) extraction. The litter leachate was expected to stimulate microbial activity and CO_2 efflux, as P loading is a major control of microbial activity in aerobic and wetland soils (DeBusk and Reddy 1998).

The amount of leachate added was equivalent to a 250 mm rainfall, and respiration recorded after 24 h. The leachate treatment was repeated by adding a volume of DI water equivalent to 250 mm of rain to the cores, simulating excessive rainfall conditions after the 'first flush' of nutrients from catchment litter.

The effect of raised temperature on CO_2 efflux was subsequently tested at day 33, following treatment with leachate and 'rain water'. All soil cores were incubated at 31 °C for 24 h and CO_2 recorded.

Substrate induced respiration (SIR)

Soil samples were collected from the transect in early April 2002, before the first heavy rain and after the onset of the wet season on 14 May and 26 June, by coring randomly at each site. Three samples (10 m apart) were taken from each site at depths of 0–5 cm and 5–20 cm, large pieces of litter were removed manually (referred to as litter %), and samples were then sieved (1 mm) to remove small plant debris (referred to as debris %). Sieved soil was stored in plastic bags at room temperature at field moisture, and semi-sealed to reduce evaporation whilst maintaining air exchange.

Before measuring SIR, triplicates of soil samples (5 g dry weight equivalent) were pre-wet in Erlenmeyer flasks (volume calibrated) by adding DI water to 20% water content (w/w), wrapped in thin plastic film, and left at room temperature for 7 days. Soil samples were then treated with 1.5 ml glucose solution (30 mg ml^{-1}) to raise soil water content to about 50%. The glucose concentration added was 9 mg g^{-1} soil, similar to those described by Anderson and Domsch (1978) and Chen and Coleman (1988). Soils were then incubated at 21 °C, and CO_2 concentrations were recorded at 6 and 24 h using the infrared gas analyzer.

Partition of fungal and bacterial activity

The selective inhibition method originally developed by Anderson and Domsch (1975, 1978) partitioned prokaryotic and eukaryotic respiration based on physiological response of microbial biomass to antibiotics. The method may potentially encounter non-target effects, antibiotic inactivation, antibiotic resistant populations and microbial competition. These undesired effects can be managed by optimising inhibitor application and time (e.g., 2–8 h) of incubation (Anderson and Domsch 1975; Beare et al. 1990; Scheu and Parkinson 1994). Selective inhibition has often been used to discriminate between fungal and bacterial activity in plant residue and soils (Beare et al. 1990; Scheu and Parkinson 1994; Lin and Brooks 1999).

The concentrations and volumes of antibiotics used to partition eukaryote (fungal) and

prokaryote (bacterial) respiration were 32 mg ml^{-1} of streptomycin and 64 mg ml^{-1} of cycloheximide, and a combined solution of streptomycin (32 mg ml^{-1}) and cycloheximide (64 mg ml^{-1}), analogous to Imberger and Chiu (2001). The treatment was carefully adjusted to achieve effective concentration as well effective surface contact with soil particles. To litter samples (about 2 mm pieces), which had higher water sorption capacity, 2.0 ml g^{-1} of antibiotics solution 32 mg ml^{-1} streptomycin and 64 mg g^{-1} glucose (30 mg ml^{-1}) were added. For soils (4.0 g dwt) with low water holding capacity and significantly lower SIR, 32 mg g^{-1} (dwt) of streptomycin/ cycloheximide or their combination was added, but the concentration adjusted to allow for the field soil water content. For example, to wet sediments from Th1 with ~50% water content, 1.0 ml g^{-1} dwt (64 mg ml^{-1} of streptomycin/ cycloheximide containing glucose 60 mg ml^{-1}) was added. For sandy soils from Th6, which were dry (water content <10 %) and had little water holding capacity, 1.0 ml g^{-1} dwt of antibiotics (32 mg ml^{-1} streptomycin/ cycloheximide containing glucose 30 mg ml^{-1}) was added. Considering the original water content in these soils, the effective concentrations were 32 mg g^{-1} streptomycin/cycloheximide containing 30 mg ml^{-1} glucose for both soils.

The Erlenmeyer flasks were then stoppered and incubated (21 °C, 6 h) and CO_2 concentration measured using an infrared gas analyzer.

Fungal and bacterial respiration and their ratios were estimated as:

1. Prokaryote (bacterial) respiration (B) = total (non-inhibited, control) respiration (A) – respiration after addition of streptomycin.
2. Eukaryote (fungal) respiration (C) = A – respiration after addition of cycloheximide.
3. Residual respiration (D) = Respiration remaining after addition of combined antibiotics.
4. Eukaryote to prokaryote ratio = C/B.
5. Evaluation ratio = $(B + C)/(A-D)$.

Statistical analysis for microbial respiration was conducted using the statistical package of Microsoft Excel 2000, including modules of descriptive statistics and t-test for means (at 95% confidence level or otherwise specified). Correlation analysis was conducted using the bivariate regression module.

Results

Response of microbial activity to temperature

Field CO_2 efflux

Field CO_2 efflux measured from soil chambers was higher in the lakebed (Th1) and the low-lying area (Th3) than in upland sites Th5 and Th6 (Figure 2). There was significantly higher CO_2 efflux (mean 0.22 ± 0.031 g CO_2 m^{-2} h^{-1}) during daytime (10:00 a.m.–4:00 p.m.) compared with the 24-h means (0.10 ± 0.017 g CO_2 m^{-2} h^{-1}, $P < 0.001$), and this corresponded with higher temperatures. Rates were also significantly higher on the first day (14–15 May) than on the following day (0.060 ± 0.015 g CO_2 m^{-2} h^{-1}, $P < 0.001$). Accompanying changes in field respiration rates, there was a wide range of soil temperatures between sites and days (Figure 2).

Soil core incubation

When soil cores were incubated under a controlled temperature (21 °C), CO_2 efflux was stable over the three consecutive days (Table 3). Respiration rates were considerably higher than those measured in the field, possibly because of the raised temperature.

When incubated under field conditions, CO_2 efflux (hourly) showed marked diurnal variation in response to temperature, with highest rates in late afternoon, and lowest in early morning. Temperature dependence varied between cores. There was a stagnant phase recorded from several cores during the lower temperatures of early morning. There was for example, virtually no CO_2 efflux and O_2 consumption in the core from Th6 (water content 8.4%) when the temperature was below 20 °C at night (Figure 3a). For cores from the exposed lakebed, where the surface soil had a water content of over 40%, respiration continued despite the temperature being below 20 °C, though the rate fell markedly (Figure 3b). Nocturnal respiration became steady when the temperature was maintained at 21 °C (Figure 3c).

Response to raised temperature (31 °C)

Soil cores were incubated under a raised temperature (31 °C) after the treatment with leachate at 21 °C. The increased temperature further enhanced CO_2 efflux (Figure 4). On average, the rates of the

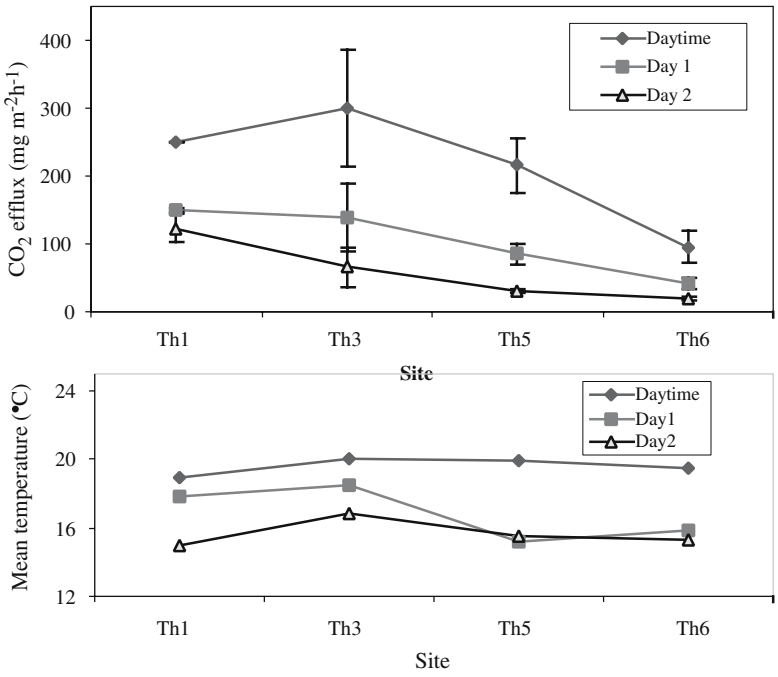

Figure 2. Averaged field CO_2 efflux during the daytime (10:00 a.m.–16:00 p.m.) and for 24 h (10:00 a.m.–10:00 a.m.) on the study transect on15 and 16 May, with corresponding soil temperatures shown below. Error bars indicate standard error of three chambers on each site, 10 m apart aligned to the lake margin.

Table 3. Respiration rates of soil cores incubated under controlled temperature (21 °C).

	Day 1		Day 2		Day 3	
Site	CO_2 flux	O_2 consumption	CO_2 flux	O_2 consumption	CO_2 flux	O_2 consumption
Th1	0.18 (0.02)	0.23 (0.02)	0.20 (0.01)	0.23 (0.01)	0.21 (0.02)	0.22 (0.02)
Th3	0.20 (0.04)	0.16 (0.04)	0.20 (0.05)	0.16 (0.05)	0.20 (0.06)	0.15 (0.05)
Th5	0.22 (0.02)	0.16 (0.02)	0.21 (0.02)	0.15 (0.02)	0.20 (0.03)	0.14 (0.02)
Th6	0.18 (0.05)	0.13 (0.03)	0.17 (0.04)	0.13 (0.04)	0.16 (0.04)	0.11 (0.03)
Mean	0.20	0.17	0.2	0.17	0.19	0.16

Values represent 24 h means ± 1SE (g m^{-2} h^{-1}).

efflux increased by 2 times, from 175 ± 28 mg m^{-2} h^{-1} at 21 °C to 365 ± 63 mg m^{-2} h^{-1} at 31 °C. Although there were high between-core variations in the lakebed and catchment sites of Th5 and Th6, average CO_2 rates were well above the original levels in the field, and in laboratory incubation (day 3, 21 °C) after the collection (Figure 4).

Soil moisture and microbial activity

On the transect, increased soil water content towards the wetland during the wet season (May–June) was correlated with increased CO_2 efflux. Both field (uncontrolled conditions) and laboratory CO_2 efflux (21 °C) was positively correlated with soil water content ($P = 0.04$ and 0.05, respectively).

There was a close correlation between soil water content and SIR ($R^2 = 0.90$, $P = 0.004$). CO_2 efflux from incubated soil cores declined with time (days), attributable to loss of soil moisture and decreased substrate availability. At day 30, CO_2 efflux was significantly lower than the initial level recorded at day 3 (Figure 5), accompanied by a 26% loss of soil water during the period.

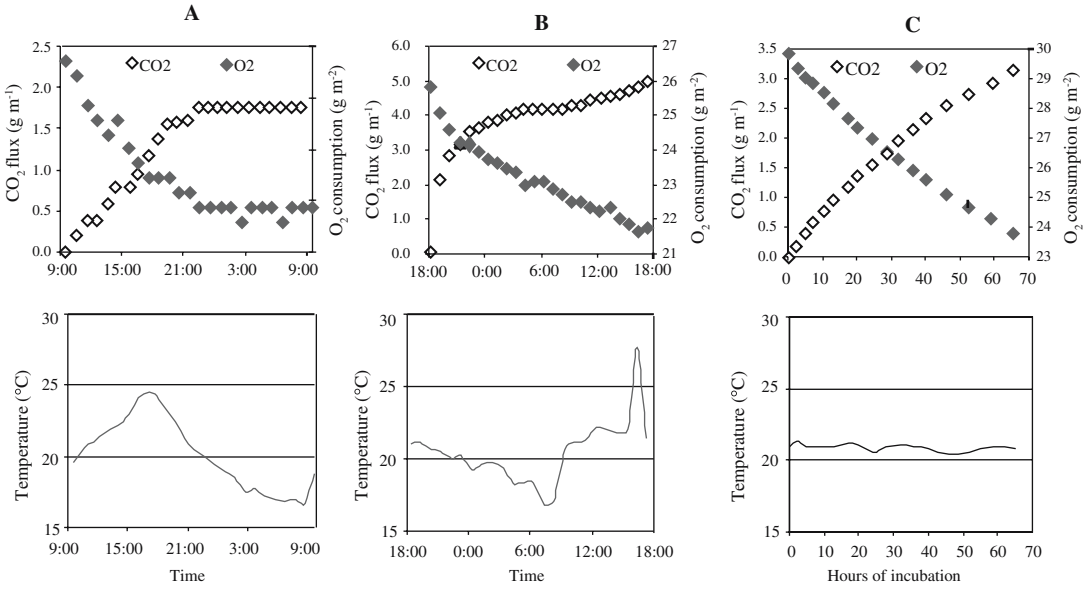

Figure 3. The effect of diurnal temperature on soil core respiration: (a) upland catchment (Th6) under field temperature; (b) lakebed (Th1) under field temperature; (c) subsequent re-incubation of lakebed (Th1) under controlled temperature (21 °C).

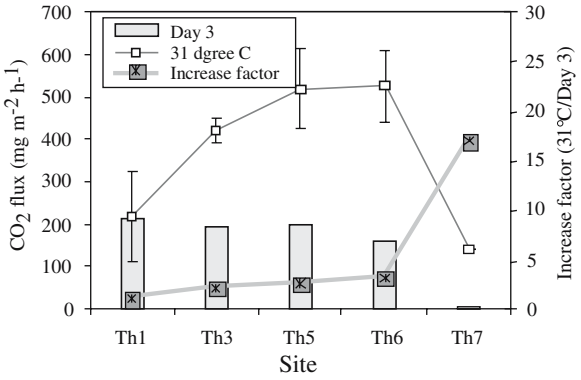

Figure 4. Effects of increased temperature (31 °C) on heterotrophic microbial activity in soil cores. Temperature increased from 21 to 31 °C after leachate and rainwater treatment. 'Day 3' represents the initial rates from three consecutive days of controlled incubation started from the next day of field coring (Table 3). Standard error of the measurements indicated in error bar.

Response to litter leachate

Based on the efflux of Day 30, representing drier conditions before the first storm, the addition of leachate increased CO_2 efflux in all soil cores ($P < 0.05$) except Th1 (Figure 5). The increase was significant in cores from upland sites, which had lower organic and P content, and were drier when collected. By contrast, the leachate failed to enhance CO_2 efflux in wetland soils (Th1). One core from Th1 was virtually submerged by the leachate and further 'rainwater', and showed significantly lower CO_2 efflux. Subsequent treatment with 'rainwater' following the leachate increased CO_2 rates in Th3, Th5 and Th6, but reduced CO_2 rates in wetland soils (Th1) and the controls (Th7).

Quotients of fungal and bacterial respiration

Average respiration rates before and after antibiotic treatment are shown in Table 4. Microbial activity was markedly higher in leaf litter (*Eucalyptus*) than in soils ($P < 0.05$). There were significantly higher fungal to bacterial ratios in litter than in soils ($P < 0.05$).

Discussion

In general, heterotrophic microbial activity appears to depend heavily on litter turnover on this non-fertilized catchment. Our data suggest that microbial activity is predominantly associated with litter, in which the rates were several to

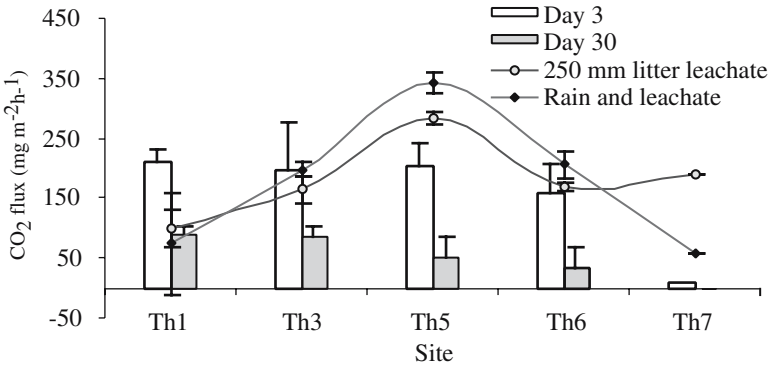

Figure 5. Effect of litter leachate and 'rainwater' on heterotrophic microbial activity in soil cores at 21 °C. Litter leachate applied on Day 30 from sampling, and 'rain water' was applied one day after the leachate treatment. Standard error of the measurements indicated in error bar.

Table 4. Fungal and bacterial contributions to substrate-induced respiration of litter and soils along transect.

	Total CO_2 ($\mu g\ g^{-1}\ h^{-1}$)	Bacterial CO_2 ($\mu g\ g^{-1}\ h^{-1}$)	Fungal CO_2 ($\mu g\ g^{-1}\ h^{-1}$)	Anti-biotic resistant CO_2 ($\mu g\ g^{-1}\ h^{-1}$)	Fungal to bacterial ratio	Additivity ratio
Old leaf litter[a]	451	106	305	80	2.9	1.11
Fresh leaf litter[b]	239	53	173	53	3.2	1.22
Th1 soil	80	40	27	27	0.7	1.27
Th6 soil	27	14	14	0	1.0	1.02

Respiration was measured using the selective inhibition technique.
[a] Decaying litterfall from Th3, mainly eucalyptus leaves.
[b] Eucalyptus leaves collected from upland catchment sites Th5 and Th6 at the end of the dry season.

more than a hundred fold higher than in soils (Qiu et al. 2003). This may be attributable to the role of litter in providing microbial organisms with a source of carbon and nutrients as well as habitat. Soil heterogeneity in terms of litter and organic matter thus appears to be the underlying factor determining the level of microbial activity on the study transect, and soil organic matter ($P = 0.03$) and P ($P = 0.05$) were higher in the wetland sites (Th1, Th2, and Th3) than in the upland sites (Table 2). However, litter-related soil heterogeneity in the field may often be confounded by environmental influence (moisture and temperature), resulting not only in high spatial and temporal variability in microbial activity but also a pattern not directly attributable to litter–microbial interactions. It is therefore necessary to partition the temperature and moisture effects from litter-related CO_2 dynamics, in order to understand surface microbial activity on an ecosystem level.

Effect of temperature on microbial activity

We incubated soils under a 'constant' temperature (21 °C, as recorded during the incubation period) to investigate temperature effects. Under such conditions, there were no differences in CO_2 efflux rates between days, confirming that the significant variation between days recorded in the field was largely a temperature effect (Table 3).

This region has a high diurnal fluctuation in temperature, with a 12 °C difference recorded during the period of field measurements. The soil temperature (10 cm depth) varied between sites and days, ranging from 14.2 to 20.7 °C in May and 12.0–20.4 °C in June. Such variation may well be expected to cause a significant change in field CO_2 efflux (Qi et al. 2002) and appears to be a major contributor to the short-term difference in CO_2 efflux between days and the diurnal average reported above (Figure 2). There were also significant differences in site temperature of the upland

soils (especially Th1 and Th2, $P = 0.01$–0.07). The level of soil water and the direct exposure to sun radiation appeared to be associated with these variations in the field. The Q_{10} value estimated from the exponential model was 6.6 for field efflux, a value higher than previously reported in soils, but similar to those reported for wetland and peaty soils (Trettin et al. 1996; Scanlon and Moore 2000). Warm weather, which increased nutrient loss from litter leaching, and the inclusion of wetland soils in this study may be the contributing factors to those higher values of Q_{10} (Trettin et al. 1996).

Chen et al. (2002), in a field study of a eucalypt forest in a wet–dry tropical savanna of northern Australia, found that daytime rates of CO_2 efflux were consistently higher than nocturnal values. Maximal rates occurred in late afternoon (16:00) when soil temperatures were maximal, minimum values during the early morning (4–8:00 a.m.). Our data are consistent with these observations.

Increase of temperature by 10 °C (21° vs. 31 °C) following leachate treatment further increased CO_2 efflux by a factor of 2.1, 1.5, and 2.5 fold in Th3, Th5, and Th6, respectively. Rates were well above initial levels when collected from the field (as in Day3) by a factor of 2.1, 2.6, and 3.3 for Th3, Th5, and Th6, respectively. The increment tended to increase from the lakebed towards the upland catchment, suggesting a lower temperature influence in wetter soils (Figure 4).

Response to litter leachate and raised temperature

Using litter traps, we recorded approximately 0.5 kg m^{-2} yr^{-1} of litterfall in the woodland catchment of this lake. Leaf litter (the predominant component of litterfall from the catchment) contained on average approximately 0.4 g P kg^{-1}. Based on these data, we calculate that the first heavy rain (assuming 25 mm in 24 h based on local records) could produce a P flux with a concentration of 2.4 mg l^{-1}, given the leaching rate of 30% reported in our previous study (Qiu et al. 2002). This value represents only the litter of the current year and does not include mineralization of soil organic matter, nor litter accumulated from past years.

We used a litter leachate produced by early winter rains during an extended period of six weeks, thus lowering P concentrations. The leachate significantly enhanced microbial activity in most soils, presumably due to increased moisture (equivalent to about 12% soil water), and to nutrients contained in the leachate. The average ratios of C : N (50) and C : P (845) of litterfall in the area were very high, compared with those of soil bacteria (typically C : N : P = 90 : 15 : 1). The scarce P availability, based on these ratios, is thus likely to be a limiting factor for soil microbial activity (Sylvia et al. 1998). However, the limiting effects would be more complicated and confounded by field conditions and the availability of other nutrients such as C and N. Further work is therefore necessary to ascertain limiting factors of the microbial activity.

Soils in upland sites had lower water content (average 4.2%) and nutrients than those in wetland areas. The lakebed, in which the addition of leachate failed to increase CO_2 efflux, was already wet (47.8% water content) before the treatment. The repression of CO_2 efflux in this core with 'follow-up rains' was probably due to water-saturation, which is known to depress microbial activity (Chen and Coleman 1988). In addition, some CO_2 may be lost in soil water due to dissolution and the formation of bicarbonates and carbonates in soil–water system (Hawkins and Freeman 1994).

The difference between dry and wet systems in response to increased moisture appears associated with the structure of the microbial community (D'Angelo and Reddy 1999). Rates of organic C mineralization could be 3 times faster using O_2 as electron acceptors (aerobic respiration) compared to those using alternative electron acceptors, such as denitrification and sulfate reduction (Wright and Reddy 2001). Thus, excessive rains could decrease measured heterotrophic microbial activity in the lakebed and low-lying areas, which are normally inundated in late wet season. A recent model of soil carbon decomposition shows that the decomposition rate of soil organic matter, under both dry and wet conditions, is sensitive to water content. Under wet conditions, decomposition rate decreases with increased water content (Yang et al. 2002).

Quotients of fungal and bacterial respiration

The range of fungal to bacterial ratios observed from litter samples here was 2.9–3.2, which is typical for plant residues in early stages of decomposition, such as 1.6–2.5 reported by Beare et al. (1990)

and 0.9–4.4 by Neely et al. (1991). The fungal to bacterial ratio in our soils was 0.7–1.0, comparable with ratios of 1.05–1.62 reported by Imberger and Chiu (2001), and 1.0–6.0 reported by Scheu and Parkinson (1994). Much higher ratios (29–15) have been reported for dry soils (Johnson et al. 1996). In general, our results suggest a fungal dominance in heterotrophic respiration of surface litter, but an increased bacterial dominance in soils, and especially in exposed sediments in Thomsons Lake.

Higher fungal to bacterial ratios may only be expected for upland soils, because fungi are more tolerant to dry soil conditions than bacteria due to their filamentous nature, allowing better translocation of nutrients between soil and surface residues for their growth activity (Holland and Coleman 1987; Johnson et al. 1996). The low ratio (0.7) found in the lakebed may suggest a bacterially dominated microbial structure in the exposed sediments. On the other hand, the estimation of fungal to bacterial ratio was based on microbial CO_2 production, without taking into account the rate repression in anoxic system discussed above. This anoxic repression has been suggested in the ratios of CO_2 efflux to O_2 consumption per unit soil, which was significantly higher in the lakebed (1.57) than in the upland soil (1.14). The low ratio in the lakebed should be, at least in part, attributable to lower capacity of CO_2 rates by anoxic bacterial activity.

Conclusions

Heterotrophic microbial activity in surface soils had high spatial and temporal variability on the study transect. This was fundamentally due to soil heterogeneity in terms of site litter and organic matter confounded by site microclimate factors, especially temperature and water content after the onset of the wet season. High diurnal fluctuations in ambient temperature, and contrasting site properties such as soil water content (depth to groundwater) appear to have caused spatial and temporal differences in soil temperature (10 cm) along the transect, contributing to high variability in heterotrophic respiration.

Incubating soil cores under controlled temperature eliminated between-day variations in CO_2 efflux, suggesting that temporal variation after onset of the wet season was largely a temperature effect. Simulated litter leachate significantly enhanced soil respiration, attributable to increased moisture in well-drained soils and increased substrate and nutrient availability.

In contrast to upland soils, the depressive response of CO_2 efflux to leachate and 'follow-up rains' in lakebed was probably related to water-saturation. A rise in temperature from 21 to 31 °C after leachate treatment further increased CO_2 efflux in most soils, but this did not occur when soils were flooded.

Heterotrophic microbial activity was dominated by the microbial community associated with surface litter. There was a fungal dominance in heterotrophic respiration from surface litter, but increased bacterial dominance in soils and exposed lake sediments.

Acknowledgements

This work forms part of on-going study supported by ARC Large Grant A00105241. The Department of Conservation and Land Management of Western Australia issued permits for field studies.

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