Forensic Taphonomy:
processes associated with cadaver decomposition in soil

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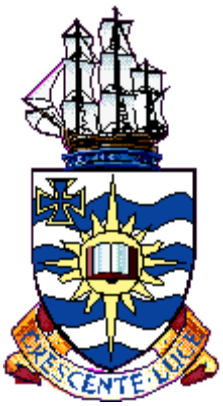
January 2005



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ABSTRACT

A series of laboratory and field incubations were carried out where juvenile rat (*Rattus rattus*) cadavers were buried in three soils of contrasting texture from tropical savanna ecosystems in Queensland, Australia. This work was done in order to develop an understanding of the effect of environmental variables (temperature, moisture), the soil in which a cadaver is buried and the nature of the cadaver on the processes associated with cadaver decomposition in soil. A pattern of mass loss comprised of an "Early Phase" of slow mass loss, "Intermediate Phase" of rapid mass loss and a "Late Phase" of slow mass loss. Early Phase decomposition coincided with an initial increase in carbon dioxide (CO_2) evolution, microbial biomass carbon (C_{mic}), phosphodiesterase activity, protease activity and soil pH. Microbial activity was triggered within 24 hours of cadaver burial and this initial flush of activity was likely due to both soil-borne and cadaveric microbes. Intermediate Phase decomposition was typically associated with peak levels of CO_2 evolution, C_{mic}, phosphodiesterase activity and soil pH. Late Phase decomposition typically coincided with a slowing down of process rates. In some cases, however, peak levels of protease activity were observed during late phase decomposition.

The rate of cadaver decomposition increased with an increase in temperature and moisture. However, the rate of cadaver decomposition was slowed at a matric potential of –0.3 megapascals (MPa) in coarse-textured soil and a matric potential of –0.01 MPa in fine-textured soil. Temperature and moisture also had similar effects on CO_2 evolution, C_{mic}, protease activity, phosphodiesterase activity and soil pH. In addition, the soil matrix and the soil microbial biomass had a significant effect on cadaver decomposition. The rate of cadaver decomposition following

burial in soil was greater than when a cadaver was exposed to a sterile, soil-free environment. Furthermore, cadaver decomposition was greatest in sandy soil. These phenomena were likely due to a greater rate of gas diffusion associated with sandy soil and the activity of aerobic microorganisms. The activity of aerobic decomposers was reflected as a significant relationship between CO_2 evolution and cadaver mass loss.

The structure of the soil microbial community determined by analysis of phospholipid fatty acids (PLFAs) was affected by the presence of a cadaver. However, soil type and seasonal variation in temperature and moisture had a much greater effect on the soil microbial community. In addition, the current study provided more evidence to show that the structure of the soil microbial community can be related to the function (protease, phosphodiesterase activity) of the soil microbial community. Furthermore, temporal changes in the microbial community of gravesoils were also observed.

Te results from the laboratory incubations were used to interpret the results from the field incubations. However, some results differed between laboratory and field settings. The rate of cadaver mass loss was greater in a field setting. Also, cadaver decomposition was greatest in sandy soil in the laboratory while cadaver mass loss was greatest in clay soil in the field. Moreover, changes in soil pH and the concentration of ammonium were less in a field setting.

The current thesis has demonstrated that the introduction of a cadaver into the soil can have a significant effect on the biological and chemical characteristics of soils.

In turn, this phenomenon can be affected by environmental variables, the soil in which a cadaver is placed and the nature of the cadaver. Biological and chemical measurements conducted in the current study hold potential for forensic applications, including markers of clandestine graves and a basis for the estimation of postmortem and postburial intervals.

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ABBREVIATIONS

ACE	Aerobic catabolic efficiency
C_{mic}	Microbial biomass carbon
CI	Incised, stitched cadaver
CO_2	carbon dioxide
EC	Eviscerated cadaver
FA	Yabulu/FACE site
MPa	Megapascals
NH_4^+-N	Ammonium-nitrogen
NO_3^--N	Nitrate-nitrogen
PLFA	Phospholipid fatty acid
PR	Pallarenda site
qCO_2	Metabolic quotient
SIR	Substrate-induced respiration
WB	Wambiana site

CHAPTER 1

INTRODUCTION

Approximately 99% of the organic material that undergoes decomposition in a terrestrial ecosystem is plant-derived (e.g. leaf litter, seeds, stems) (Swift et al., 1979). However, the decomposition of vertebrate (e.g. human, porcine, bovine) cadavers and their components (e.g. bone, skeletal muscle tissue) has received increased attention in recent years (Towne, 2000b; DeVault et al., 2003; Tibbett et al., 2004). A cadaver has recently come to be viewed as a source of sequestered nutrients and energy that can only be returned to the wider ecosystem upon decomposition (Putman, 1983). However, very little is known about the processes associated with cadaver decomposition in terrestrial ecosystems, particularly following burial. As a result, it is unknown how the release of cadaveric nutrients and energy contributes to the stability and function of terrestrial ecosystems. Furthermore, belowground processes associated with cadaver decomposition or only beginning to be explored for their potential to contribute to crime scene investigation.

Most research into cadaver decomposition is done under the guise of taphonomy. Taphonomy, originally a branch of palaeontology, was developed in order to better understand the processes associated with the fossilisation of animal and plant materials (Efremov, 1940). It was proposed that this could be achieved through examination of the conditions in which fossils are preserved. Thus, the goals of taphonomy are to understand the ecology of the fossilisation site, how site ecology changes upon the introduction of organic material and, in turn, how site ecology

affects the preservation of organic material (Efremov, 1940). In recent years these goals have been incorporated into forensic science as a way to understand processes associated with cadaver decomposition. The primary goals of forensic taphonomy are to estimate postmortem interval, postburial interval as well as assist in the determination of cause and manner of death (Haglund and Sorg, 1997). In addition, taphonomic processes have been used to aid the search and location of clandestine graves (Morse et al., 1976; Sigler-Eisenberg, 1985; Hunter, 1994). This has been attempted through the study of the internal (autolysis, putrefaction) and external (decay) processes of cadaver decomposition and the factors that influence them, such as temperature and moisture.

In order to undertake a study in forensic taphonomy it is necessary to understand the fundamental decomposition processes associated with a cadaver: autolysis, putrefaction and decay. Internal, aerobic microorganisms deplete the tissues of oxygen following the cessation of the heart. This results in the termination of aerobic metabolism and the destruction of cells by enzymatic digestion (i.e. autolysis) (Evans, 1963; Dent et al., 2004). Autolysis can begin within minutes after death (Vass, 2001; Vass et al., 2002) and can be significantly affected by temperature and moisture (Gill-King, 1997). Concomitantly, optimal conditions are created for fungi and anaerobic microorganisms originating from the gastrointestinal tract and respiratory system (Evans 1963; Jawetz et al. 1982). Following the establishment of an anaerobic environment, these cadaveric microorganisms transform carbohydrates, lipids and proteins into acids and gases that result in colour change, odour and bloating of the cadaver. This process is known as putrefaction. Autolysis and putrefaction will dominate cadaver

decomposition until the skin loses integrity, which results in the reintroduction of oxygen to the system. This re-establishes aerobic metabolism and designates the beginning of the decay process (Micozzi, 1986; Micozzi, 1991). Decay typically represents the period of most rapid breakdown. This is due to the activity of decomposer organisms such as insects, vertebrate scavengers and the soil microbiota (e.g. bacteria, fungi, microarthropods). In addition, the loss of the integrity of the skin results in a significant loss of moisture and, thus, mass. This can have a substantial effect on cadaver breakdown since human and other mammalian cadavers comprise approximately 75% water (Widdowson, 1950; Reinoso et al., 1997; Tortora and Grabowski, 2000). Indeed, cadaver decomposition has been described as a "competition" between desiccation and decomposition (autolysis, putrefaction, decay) (Aufderheide, 1981; Micozzi, 1986). The relationship between desiccation and decomposition is important because rapid desiccation, while resulting in a substantial loss of mass, can inhibit decomposition of the remaining tissue. This can result in the natural preservation of a cadaver for thousands of years, such as those observed in Egypt (Ruffer, 1921) and Peru (Allison, 1979).

One focus of forensic taphonomy has been to understand the contribution that the non-cadaveric decomposer population makes to cadaver decomposition. The vast majority of these studies have focused on the activity of insects in association with cadaver decomposition (e.g. Bornemissza, 1957; Johnson, 1975; Rodriguez and Bass, 1983; Kocárek, 2003). This focus is well justified as insects can arrive at a cadaver within minutes of death (Nuorteva, 1977) and are often responsible for the breakdown of the skin and release of decomposition fluids into the soil (Payne, 1965). It is also known that the burial of a cadaver in soil can result in a decrease in

the rate of cadaver decomposition. This is due to a restriction on the activity of insects and vertebrate scavengers as well as a decrease in temperature upon burial.

The activity of the soil microbiota following cadaver burial has received relatively little attention (Putman, 1978; Sagara, 1995; Hopkins et al., 2000; Carter and Tibbett, 2003; Tibbett et al., 2004). As a result, the extent of current knowledge is limited to the understanding that cadaver decomposition can prompt the growth (Hopkins et al., 2000) and activity (Putman, 1978) of the soil microbial biomass. These phenomena can be associated with a significant increase in the concentration of inorganic nitrogen (Vass et al., 1992; Hopkins et al., 2000; Towne, 2000a) and phosphorus (Towne, 2000a) in the soil as well as an increase in soil pH (Rodriguez and Bass, 1985; Vass et al., 1992; Hopkins et al., 2000; Towne, 2000a). Furthermore, the effect of soil type and/or texture on cadaver decomposition has not undergone thorough examination although Mant (1950) reported an increased occurrence of desiccation in coarse-textured soils associated with war dead in Europe. Clearly very little is understood about the processes associated with cadaver decomposition in soil.

A number of decomposition ‘stages’ have been proposed in order to define cadaver breakdown (Fuller, 1934; Bornemissza, 1957; Reed, 1958; Payne and King, 1968; Payne et al., 1968; Rodriguez and Bass, 1983). Only Payne and King (1968) and Payne et al. (1968) propose decomposition stages for cadavers buried in soil (Table 1.1). These stages typically coincide with a decomposition curve that follows a sigmoidal pattern (Payne et al., 1968; VanLaerhoven and Anderson, 1999) (Figure 1.1). The ‘Fresh’ and ‘Inflated” stages of decomposition (Table 1.1) correspond to

the initial period of slow mass loss (Figure 1.1), probably due to the presence of skin. The ‘Deflation & Decomposition’ stage is related to the period of rapid mass loss observed in Figure 1.1 and is attributed to the loss of integrity of the skin, which can be related to the activity of arthropods and vertebrate scavengers. Thus, this relatively rapid rate of cadaver mass loss is related to the loss of cadaveric moisture. The final two stages, ‘Disintegration’ and ‘Skeletonisation’ correspond to a second period when the rate of cadaver mass loss slows (Figure 1.1), probably from the depletion of moisture and readily available nutrients.

Table 1.1. Decomposition stages of pig (*Sus scrofa*) cadavers buried in soil proposed by Payne and King (1968).

Stage number	Stage name	Stage characteristics
I	Fresh	Initial discolouration
II	Inflated	Visible bloating of abdomen and scrotum
III	Deflation & Decomposition	Escape of blood and decomposition fluids from mouth, nose, ears, anus and arthropod-induced skin ruptures. Strong odour
IV	Disintegration	Removal of soft tissue and appearance of bones
V	Skeletonisation	Removal of hair, skin and remaining tissue fragments

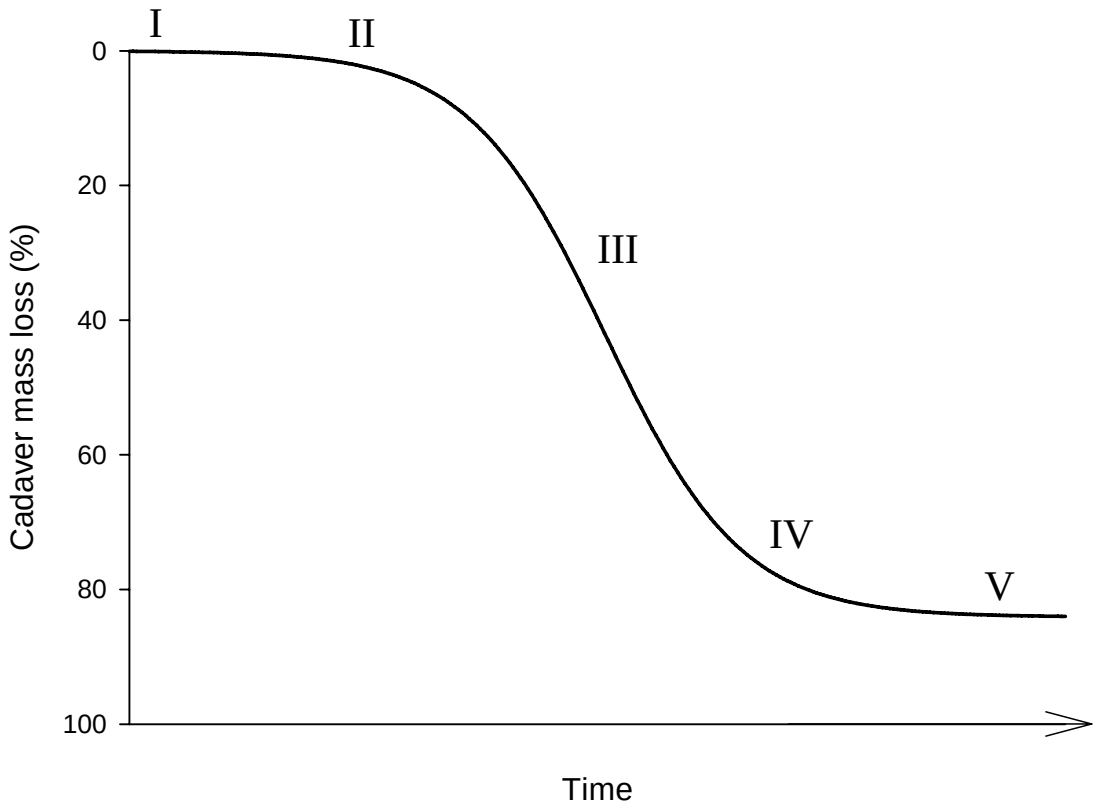

Figure 1.1. Sigmoidal pattern associated with cadaver decomposition following burial in soil and/or exclusion from arthropod and scavenger activity. Roman numerals correspond to the 'Stages' described in Table 1.1. Stage I (Fresh) and Stage II (Inflated) are associated with a slow rate of cadaver decomposition. Stage III (Deflation & Decomposition) is associated with a rapid rate of breakdown. Stage IV (Disintegration) and Stage V (Skeletonisation) represent a slowing down of the rate of cadaver mass loss.

Forensic taphonomy primarily relies on case studies, anecdotal evidence and unreplicated experimental work for data. In contrast, the decomposition of other organic resources such as plant leaves (Webster et al., 2000; Coûteaux et al., 2002), stems (Hopkins et al., 2001), seeds (Tibbett and Sanders, 2002) and sewage sludge (Clark and Gilmour, 1983; Ajwa and Tabatabai, 1994) has received a vast amount of contrived, replicated experimental attention. As a result, it is well understood that the decomposition of organic resources in soil is regulated by the physicochemical environment (e.g. climate, soil texture, pH), the quality of the resource (carbon:nitrogen ratio, phenol content) and abundance and activity of decomposer organisms (Swift et al., 1979).

The primary aim of this thesis is to better understand how the decomposition of a buried cadaver is affected by environmental variables, the soil in which it is buried and the nature of the cadaver itself. In addition, this thesis aims to identify processes that may be helpful to forensic investigators. The current study will examine how physicochemical characteristics (temperature, moisture, soil texture, season), resource quality (removal of internal organs) and decomposer organisms (removal of internal organisms, soil sterilisation) can affect cadaver decomposition in soil. Laboratory decomposition studies will be conducted to determine if an increase in temperature (Chapter 4) and soil moisture (Chapter 5) will result in an increase in cadaver decomposition. The dynamics of cadaver breakdown will be further explored in a laboratory decomposition study testing the hypothesis that the removal of cadaveric internal organs (evisceration) will result in a decrease in cadaver decomposition (Chapter 6). The relationship between the soil matrix, soil microbial biomass and cadaver decomposition will be further investigated via a laboratory decomposition study that utilises sterilised soils and sterilised microcosms without soil (Chapter 7). This study aims to determine if the absence of the soil microbial community will result in a decrease in cadaver decomposition. The results from each of these laboratory incubations will be used to interpret cadaver decomposition in a field setting in tropical savanna ecosystems during wet and dry seasons (Chapter 8). This field experiment will also allow for an investigation into the structural and functional dynamics of the soil microbial community associated with cadaver decomposition in soil (Chapter 9).

CHAPTER 2

MATERIALS & METHODS

A series of laboratory and field experiments were designed where cadaver mass loss was used as a direct measure of decomposition. In addition, a number of analyses were conducted on the soil in intimate association with a buried cadaver. Soils were analysed in order to assess the biological and chemical processes associated with cadaver decomposition, since these properties are recognised as having an effect on the rate of decomposition of an organic resource in soil (Swift et al., 1979).

2.2. Cadavers

Juvenile rat (*Rattus rattus*) cadavers (~18 g wet weight) aged eight to ten days were used as organic resource patches (Figure 2.1).

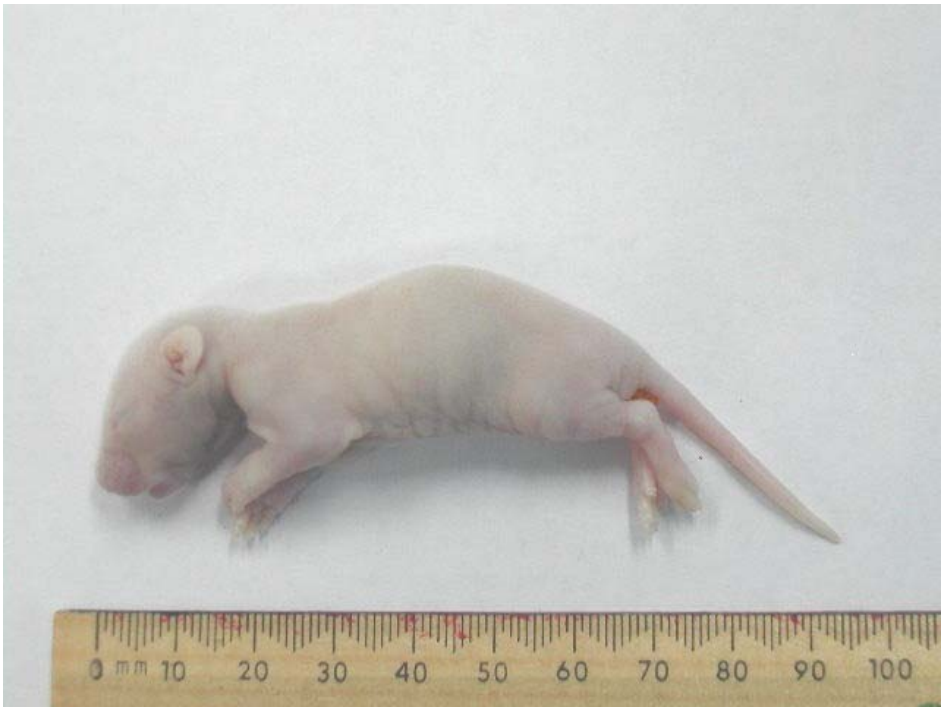

Figure 2.1. Juvenile rat (*Rattus rattus*) cadaver.

2.3. Soils

Soils, along with site ecology are described in Chapter 3.

2.4. Experimental design

2.4.1. Laboratory experiments

A sequential harvesting regime was implemented based on Tibbett et al. (2004) where cadaver and soil samples were destructively harvested following 7, 14, 21 or 28 days of incubation. Soil (500 g dry weight) was weighed into incubation chambers (2 l, high density polyethylene tubs: Crown Scientific, Newstead, Queensland, Australia; Product no. A80WTE+9530C) to a bulk density shown in Table 3.2 and amended with distilled water (see individual Chapters for moisture regimes). Soils were allowed to equilibrate for seven days following the calibration of moisture content. Following equilibration, rats were killed with carbon dioxide (CO_2), weighed and buried in soil on their right side at a depth of 2.5 cm (Figure 2.2). Soils with a cadaver will be referred to as test samples. Soils without a cadaver will be referred to as control samples. In control samples soil was disturbed in order to simulate cadaver burial and account for any effect of soil disturbance.

Figure 2.2. Cadaver (*Rattus rattus*) burial at a depth of 2.5 cm in a soil microcosm.

2.4.2. Field experiments

The experimental design implemented during the field trials is described in Chapter 8.

2.5. Cadaver & soil analyses

At each harvest event designated cadavers were exhumed along with soil directly surrounding the cadaver (detritisphere: (Tilston et al., 2004)) (approximately 50 g). The detritisphere represented the soil that adhered to the cadaver and was collected manually. In control samples the disturbed soil was collected at each harvest event.

2.5.1. Cadaver mass loss

The mass loss of an organic resource represents the simplest, most direct measure of decomposition (Swift et al., 1979). As such, cadavers were exhumed and immediately frozen (-20 °C). Once frozen the cadavers were rinsed with distilled water to remove any loose soil, dried with a paper towel and weighed. Freezing cadavers prior to the measurement of mass loss was done because it resulted in a reduced loss of loose cadaveric material (e.g. skin, bone) during the rinsing process. Harvested soil samples were weighed into sterile culture tubes for further analysis. Soil samples were immediately stored at -20 °C.

2.5.2. Soil physicochemical characteristics

Physicochemical properties of the soil such as texture and moisture can affect the decomposition of an organic resource (Jenkinson, 1977; Swift et al., 1979; Ilstedt et al., 2000). In turn, the breakdown of an organic resource can result in a change in the physicochemical environment (Swift, 1982; Carter, 2000). Therefore, it is

necessary to examine the physical and chemical properties and changes associated with the decomposition of an organic resource.

Soil physicochemical characteristics were determined using the methods commonly used for Australian soils. Bulk density and particle size distribution were determined after Coventry and Fett (1979). Soil moisture release curves (e.g. matric potential) were generated using soil cores (209 cm^3) placed on ceramic tension plates with hanging bottles of distilled water (-0.001 to –0.01 megapascals (MPa)) and pressure chambers with ceramic pressure plates (-0.03 to –1.5 MPa) (Klute, 1986; Ford, 1997). Moisture release curves were generated for cores containing undisturbed and sieved (2 mm) soils (see Chapter 3).

Soil pH was calculated at a 1:5 soil:water ratio (w:w) following shaking at 150 rpm for 30 minutes (Rayment and Higginson, 1992). Total carbon (C), nitrogen (N) and sulphur (S) were measured using a Leco dry combustion analyser. Organic C was determined after Heanes (1984). Total phosphorus (P), ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) were measured using a colorimetric autoanalyser following shaking (150 rpm for 30 minutes) and extraction with a 2 molar (M) potassium chloride (KCl) solution (1:10 soil:extract (w:v)) (Rayment and Higginson, 1992).

2.5.3. Microbial biomass

Decomposer organisms can play a significant role in the decomposition of an organic resource (Swift et al., 1979). In order to understand processes associated with the decomposition of an organic resource patch in soil it is necessary to assess the biomass and composition of the soil microbial population (Horwath and Paul,

1994). The soil microbial biomass is composed of many organisms (e.g. bacteria, fungi, yeasts, nematodes, protozoa) and, although each species plays a particular role, the biomass may be considered a single entity (Brookes et al., 1985). The microbial biomass component of the soil organic matter is responsible for the decomposition of organic materials as well as nutrient transformation and storage (Horwath and Paul, 1994). The soil microbial biomass typically represents 2-5% of total soil C and up to 5% of total soil N (Horwath and Paul, 1994).

Many methods can be used to estimate the size of the microbial biomass. Standard methods include the chloroform fumigation extraction (CFE) method (Vance et al., 1987), substrate-induced respiration (SIR) (Anderson and Domsch, 1978; Lin and Brookes, 1999) and adenosine triphosphate (ATP) analysis (Horwath and Paul, 1994). The CFE method estimates microbial biomass C based on the amount of extractable C following fumigation with chloroform. The concentration of ATP is used to estimate microbial biomass carbon because ATP is present in all viable cells and is readily degraded in soil. The current study employed SIR to estimate soil microbial biomass C. SIR was developed in order to provide an inexpensive, fast, accurate method to estimate microbial biomass C (Anderson and Domsch, 1978) and was chosen because of the large number of samples generated as a result of the experimental designs. In addition, SIR was chosen over CFE because of the potential for the extraction of cadaveric C instead of biomass C.

Microbial biomass C (C_{mic}) was estimated within one hour of harvest using the SIR technique (Anderson and Domsch, 1978) with some modifications (Lin and Brookes, 1999). Soil (5 g dry weight) was weighed into 30 ml McCartney bottles

and amended with glucose solution (6 mg g^{-1} soil) in order to calibrate them to 95% water-holding capacity. Following amendment with glucose solution a 6 ml vial containing 5 ml NaOH (0.1 M) (CO_2 trap) was placed on the soil surface and the McCartney bottle was immediately sealed. Samples were then incubated in the dark at 22 °C for 3 hours or 4 hours based on preliminary experiments (Appendix A). After the incubation period CO_2 traps were removed and sealed. The NaOH solution from the CO_2 traps was back-titrated with HCl (0.1 M) into 5 ml $BaCl_2$ (1.0 M) and 3 drops phenolphthalein as indicator (Rowell, 1994). Microbial biomass C was then calculated using the equation in Anderson and Domsch (1978):

$$x = 40.04y + 0.37 \qquad \text{Equation 1.}$$

Where x equals C_{mic} (mg C_{mic} $unit^{-1}$ soil) and y equals CO_2 evolution (ml CO_2 h^{-1} $unit^{-1}$ soil).

2.5.4. Microbial activity

Microbial activity includes all biochemical reactions catalysed by soil microorganisms (Alef and Nannipieri, 1995). Certain reactions, such as CO_2 evolution and heat output, can be carried out by the majority of soil organisms and therefore can be used as an assessment of microbial activity in general. Other reactions, such as nitrification and nitrogen fixation, can only be conducted by a specific suite of soil microbiota (Alef and Nannipieri 1995). The assessment of microbial activity is important in decomposition studies because decomposer organisms can play a significant role in the decomposition of organic materials whereas abiotic chemical oxidation will typically only account for approximately 5 to 20% of organic resource decomposition (Seastedt, 1984; Moorhead and Reynolds, 1989).

2.5.4.1. Carbon dioxide evolution

The aerobic heterotrophic microflora (which are credited with the majority of decomposition) derive energy via the oxidation of organic resources where oxygen (O_2) acts as the terminal electron acceptor. The products of this reaction are CO_2 and H_2O. Therefore, the metabolic activity of aerobic microflora may be quantified through the measurement of CO_2-C evolution and/or O_2 consumption. The measurement of CO_2-C evolution is a commonly used technique (e.g. Jenkinson, 1977; van Veen and van Elsas, 1986; Orchard et al., 1992; Hassink et al., 1993; Thomsen et al., 1999) and is based on the assumption that an increase in the rate of CO_2-C evolution equates to an increase in aerobic microbial activity. In the current study CO_2-C evolution was analysed following cadaver burial/soil disturbance using the alkali absorption/conductivity method (Rodella and Saboya, 1999). A 30 ml vial (Crown Scientific, Newstead, Queensland, Australia; Product no. 735) (CO_2 trap) was filled with 20 ml sodium hydroxide (NaOH) (0.465 M) and suspended inside each incubation chamber. The incubation chamber was immediately sealed. CO_2 traps and the air in the incubation chamber headspace were replaced every 24 hours. Upon removal from the incubation chamber the electrical conductivity of the NaOH solution inside the CO_2 trap was measured using a Metrohm 660 Conductometer (Herisau, Switzerland). Carbon dioxide evolution was calculated after Rodella and Saboya (1999):

$y = 316.69 – 3.75x$ Equation 2.

Where y equals the CO_2 absorbed (ml) in the NaOH solution and x equals the electrical conductivity (mS cm^{-1}) of the NaOH solution. The fraction of C in CO_2 was then determined by dividing y by 0.2727.

2.5.4.2. Metabolic efficiency

In the current study the metabolic quotient (qCO_2) was calculated in order to assess the efficiency with which C was utilised by the microbial biomass. The metabolic quotient equals the amount of CO_2-C evolved per unit of microbial biomass C and has been used to provide insight into the presence of zymogenous or autochthonous microorganisms (Dilly and Munch, 1998). Zymogenous microbes are typically associated with a high qCO_2 (inefficient C use) and tend to proliferate following the introduction of a readily available organic resource. Autochthonous microbes are associated with low qCO_2 (efficient C use) and represent the indigenous, steady-state microbial population that is primarily responsible for the decomposition of more recalcitrant organic resources such as organic matter. The qCO_2 was calculated by dividing the CO_2 evolution rate (μg CO_2-C g^{-1} soil h^{-1}) by the concentration of C_{mic} (μg C_{mic} g^{-1} soil) and was presented as μg CO_2-C μg^{-1} C_{mic} h^{-1} (Dilly and Munch, 1998). The CO_2 evolution rate from the 24 hour period prior to the estimation of C_{mic} was used in the calculation.

In addition to qCO_2, the aerobic catabolic efficiency (ACE) was used as an index of metabolic efficiency. The ACE was calculated as mg CO_2-C g^{-1} soil $\%^{-1}$ of cadaver mass loss. Thus, a decrease in the amount of CO_2-C evolved per unit cadaver mass lost equals an increase in ACE.

2.5.4.3. Enzyme activities

All biochemical activities in soil are subject to enzymatic processes (Tabatabai 1994). Enzymes act as mediators of the decomposition of organic resources (Tabatabai and Dick, 2002) and are present as endoenzymes (enzymes confined within the plasma membranes of viable cells), ectoenzymes (extracellular enzymes associated with viable cells) and abiontic enzymes (enzymes released into the environment by secretion or lysis, and active enzymes associated with dead cells and other non-living soil fractions) (Sinsabaugh et al., 2002). Enzyme activity is indicative of microbial activity because microbes supply most of the enzymes to the soil (Speir and Ross, 1978). This is due to their relatively large biomass and high metabolic activity, which allow them to secrete larger amounts of extracellular enzymes than plants and animals (Speir and Ross, 1978). In addition, the assay of enzyme activity can potentially anticipate changes in soils before they are detected by other soil properties (Brookes, 1995). The quantitation of enzyme activity can lead to inferences concerning the effort of microorganisms toward obtaining nutrients from specific resources and thus, provides functional information concerning the soil microbiota. However, these measurements represent maximum potential rather than actual enzyme activity because incubation conditions are manipulated to ensure optimum rates of catalysis.

The current study assayed the activity of casein-hydrolising protease and phosphodiesterase (orthophosphodiester phosphohydrolase: EC 3.1.4.1). Proteases catalyse the breakdown of proteins to peptides and, thus, the proteolytic hydrolysis of casein was assayed due to the relatively large proportion of protein (10% cadaver

mass) associated with a newly born rat (Widdowson, 1950). Phosphodiesterase was assayed because of its recognised role in the degradation of nucleic acids (Razzell and Khorana, 1959).

In the current study proteolytic hydrolysis of casein was measured according to Ladd and Butler (1972) following modification in order to be carried out at a smaller scale. Soil (0.5 g dry weight) was amended with 2.5 ml 50 mM tris(hydroxymethyl)aminomethane buffer (pH 8.1) and 2.5 ml 2% (w/v) sodium caseinate. Samples were incubated in a water bath at 50 °C for 2 hours. Following incubation sodium caseinate was added to controls and standards (0, 200, 400, 600, 800, 1000 μg tyrosine solution (500 μg ml^{-1}) made up to 10 ml with tris buffer). The reaction was stopped with 2.5 ml 918 mM trichloroacetic acid and the samples were immediately centrifuged (5000 rpm for 6 minutes). The supernatant (1 ml) was amended with 1.5 ml alkaline reagent [472 mM sodium bicarbonate (Na_2CO_3) in 6 mM NaOH, 20 mM cupric sulphate ($CuSO4•5H_2O$), 35 mM potassium sodium tartrate in the ratio of 50:1:1 (v/v/v)] and 1 ml 33% Folin-Ciocalteu phenol reagent. After 60 minutes, the absorbance was read at 700 nm.

Soil phosphodiesterase (EC 3.1.4.1) activity was assayed following Tabatabai (1994). Soil (1 g dry weight) was amended with 400 μl toluene, 4 ml 50 mM tris(hydroxymethyl)aminomethane-sulphuric acid (H_2SO_4) buffer (pH 8.0) and 1 ml 50 mM sodium bis-*p*-nitrophenyl phosphate (Sigma N3002). Samples were incubated in a water bath at 37 °C for 1 hour. Following incubation bis-*p*-nitrophenyl phosphate was added to control samples and all samples were amended with 1 ml 0.5 M calcium chloride ($CaCl_2$) and 4 ml 50 mM

tris(hydroxymethyl)aminomethane-NaOH extractant solution (pH 12). The suspension was then filtered through Whatman No. 2 filter paper and the absorbance of the filtrate was read at 400 nm. Phosphodiesterase activity was calculated with reference to a *p*-nitrophenol standard containing 0, 10, 20, 30, 40 and 50 μg *p*-nitrophenol. To prepare standards 1 g *p*-nitrophenol was dissolved in 100 ml distilled water. This was then diluted (1 ml of *p*-nitrophenol solution to 100 ml water) in a volumetric flask. Aliquots (0, 1, 2, 3, 4, and 5 ml) of the diluted standard was then pipetted into a 50 ml Ehrlenmeyer flask, the volume was adjusted with 5 ml distilled water, and 1 ml $CaCl_2$ (0.5 M) and 4 ml NaOH (0.5 M) were added.

2.6. Statistical analyses

Descriptive and inferential statistics were generated using Microsoft Excel 2000 and SPSS 11.0.1 (Chicago, USA). Cadaver mass loss, microbial biomass C, qCO_2, enzyme, and soil pH data were analysed using univariate ANOVA when the assumptions set forth for the generation of parametric statistics (normality, homogenous variance) were met. When these assumptions were not met nonparametric statistics were generated using Mann-Whitney U and Kruskall-Wallis H tests. Carbon dioxide evolution data was analysed using a Repeated Measures ANOVA following rank transformation. Regression relationships between cadaver mass loss and carbon dioxide evolution were analysed using Pearson and Spearman correlation coefficients.

CHAPTER 3

SITE & SOIL CHARACTERISTICS

3.1. Site location

Three contrasting soil types from three sites in the tropical savannas of Queensland, Australia were used in the current study (Figure 3.1). The sites were: Yabulu/FACE (FA: 19°12'S, 146°36'E), Pallarenda (PR: 19°11'S, 146°46'E) and Wambiana (WB: 20°33'S, 146°08'E) (Figure 3.1).

3.2. Climate

The research sites are located in tropical savanna ecosystems (Gillison, 1983). A tropical savanna can be defined as an ecosystem that (1) has alternating wet and dry seasons, (2) a plant community structure primarily determined by competition between woody and grass plants for available moisture and (3) is principally modified by fire, herbivores and soil nutrients (Bourlière and Hadley, 1983). The majority of annual rainfall occurs during the summer months (November to April) while the rest of the year comprises the dry season. This can result in a water deficit and tends to produce landscapes dominated by grasses with scattered trees. Temperature and rainfall data for each site is presented in Table 3.1.

Table 3.1. Climate information at the FACE/Yabulu (FA), Pallarenda (PR) and Wambiana (WB) sites in the seasonally dry tropics of Queensland, Australia.

Site	Season	Mean maximum temp. (°C)	Mean minimum temp. (°C)	Mean rainfall (mm)
FA	Dry	22.9	16.7	140
	Wet	30.5	27	995
PR	Dry	26.9	16.4	120.3
	Wet	30.7	23.1	1005.1
WB	Dry	20.5	13.7	117.1
	Wet	32.7	27.5	542.5

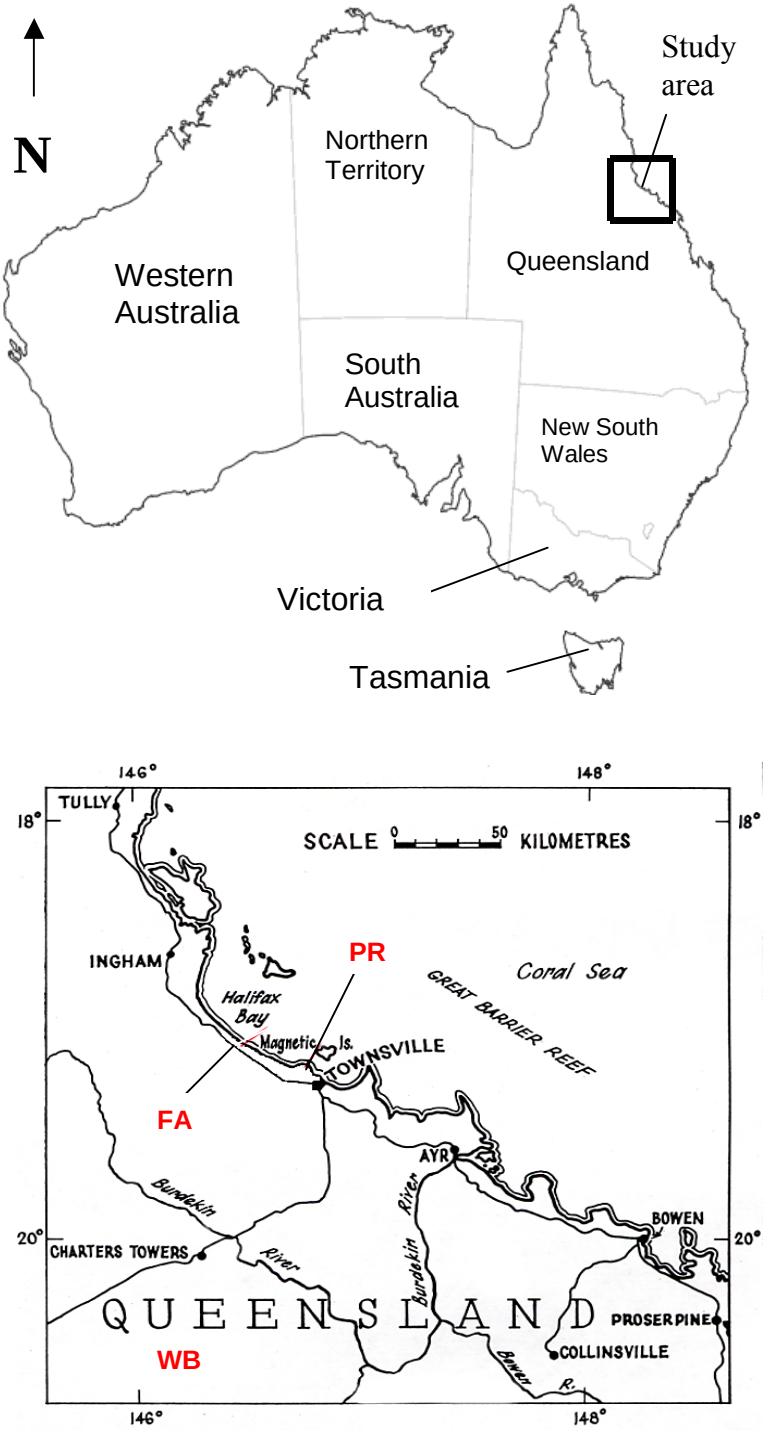

Figure 3.1. Map of Australia (above) showing study area. Enlarged study area (below) identifying the location of the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) field sites. Enlarged study area map adapted from Murtha (1975).

Figure 3.2. The Yabulu/FACE site during the dry (above) and wet (below) seasons.

3.3. Soil type and vegetation

The soil from the FA site (Figure 3.2) (19°12’S, 146°36’E) was a Brown Sodosol (Isbell, 2002) and had a loamy sand texture (Table 3.2). Sodosols roughly approximate to Planosols in the World Reference Base for Soil Resources (Deckers et al., 1998) and Albaqualfs, in the USA Soil Taxonomy (Soil Survey Staff, 2003). The soil at the FA site was typical of Sodosols in that it was located in a seasonally wet area and comprised a bleached, light-coloured A horizon overlying a dense clay

B horizon. Sodosols may form from Vertosols (see below) exposed to periods of increased wetness which result in clay removal or clay destruction in the A horizon. Sodosols also occur in Latin America, South America (from Argentina to southern Brazil) and southern and eastern Africa. The soil at the FA site (and WB site: see below) can be classified as a hydromorphic soil characterised by alternating dry and excessively wet seasons (Montgomery and Askew, 1983). These soils experience water deficiency during the dry season and are subject to excess water and intermittent flooding during the wet season. This can result in standing water for several months of the year. This promotes reducing conditions with subsequent gleying of the soils, the most common of which are the dark grey or black cracking clays. The typical vegetation at the FA site was Kangaroo Grass (*Themeda australis*), Black Spear Grass (*Heteropogon contortus*) and *Melaleuca* spp.

Table 3.2. Physical characteristics of sieved (2 mm) soils at the Yabulua/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in the seasonally dry tropics of Queensland, Australia.

	Soil		
Determinant	FA	PR	WB
Bulk density (ρ_b) (mg cm^{-3})	1.5	1.4	1.05
Particle density (ρ_s) (mg cm^{-3})	2.48	2.37	2.23
Total porosity (θ) (cm^3 cm^{-3})	0.395	0.410	0.552
% coarse sand (2.0-0.2 mm)	35.2	69.6	8.2
% fine sand (0.2-0.02 mm)	49	28.1	22.7
% silt (0.02-0.002 mm)	11	1.3	20.8
% clay (< 0.002 mm)	4.8	1	48.3
Soil texture	Loamy sand	Sand	Medium clay

The soil at the PR site (19°11’S, 146°46’E) was a Rudosol (Isbell, 2002) and had a sand texture (Table 3.2). Rudosols roughly approximate to Psamments in the USA Soil Taxonomy (Soil Survey Staff, 2003) and Arenosols in the World Reference Base for Soil Resources (Deckers et al., 1998). Rudosols are relatively young soils and are weakly developed. Rudosols can occur on aeolian, marine, lacustrine and alluvial sands. The total estimated extent of Rudosols and analogous soils types is 900 million hectares, mainly in the Kalahari and Sahel regions of Africa, Western Australia and South America.

Figure 3.3. The Pallarenda (PR) site during the dry (above) and wet (below) seasons.

The soil at the PR site can be described as a well-drained moist seasonal savanna soil (Montgomery and Askew, 1983). This soil never reaches saturation during the wet season. During the dry season the upper parts of the profile can reach wilting point while the soil will remain moist below a depth of approximately one metre (Montgomery and Askew, 1983). The typical vegetation of the PR site included Moreton Bay ash (*Eucalyptus tessellaris*), blue gum (*Eucalyptus tereticornis*), poplar gum (*Eucalyptus alba*) and mahogany (*Tristania suaveolens*). The PR site included Mango (*Mangifera indica*) trees and the dominant grass was Couch Grass (*Cynodon dactylon*) with *Stylosanthes* spp. (Figure 3.3).

The soil from the WB site (20°33'S, 146°08'E) was a Grey Vertosol (Isbell, 2002) with a medium clay texture (Table 3.2). Vertosols roughly approximate to Vertisols in the World Reference Base of Soil Resources (Deckers et al., 1998) and the USA Soil Taxonomy (Soil Survey Staff, 2003). Vertosols are deep clayish soils (> 30% clay) that swell upon wetting and shrink upon drying. Upon drying Vertosols form wide cracks that extend up to a depth of 50 cm below the surface. The Vertosols in the Townsville area contain high concentrations of smectite minerals and are mainly derived from fine-grained, base-rich parent material such as basalt and limestone. This soil type mainly occurs in tropical and sub-tropical regions with a seasonally wet climate. Major occurrences of Vertosols are in Australia, India, Sudan, Ethiopia, Texas, Uruguay, Paraguay and Argentina. The dominant plant species at the WB site were Brigalow (*Acacia harpophylla*), Yellow wood (*Terminalia oblongata*), Bauhinia (*Lysiphyllum carronii*) and currant bush (*Carissa ovata*). The dominant grass was forest bluegrass (*Bothriochloa ewartiana*) with Katoora (*Sporobolus actinocladus*) and Brigalow (*Paspalidium caespitosum*) Grass.

Figure 3.4. The Wambiana (WB) site during the dry (above) and wet (below) seasons.

Soil moisture retention characteristics were analysed using the method described in Chapter 2. Moisture release curves were generated for cores containing undisturbed (Figure 3.5a) and sieved (2 mm) (Figure 3.5b) soils.

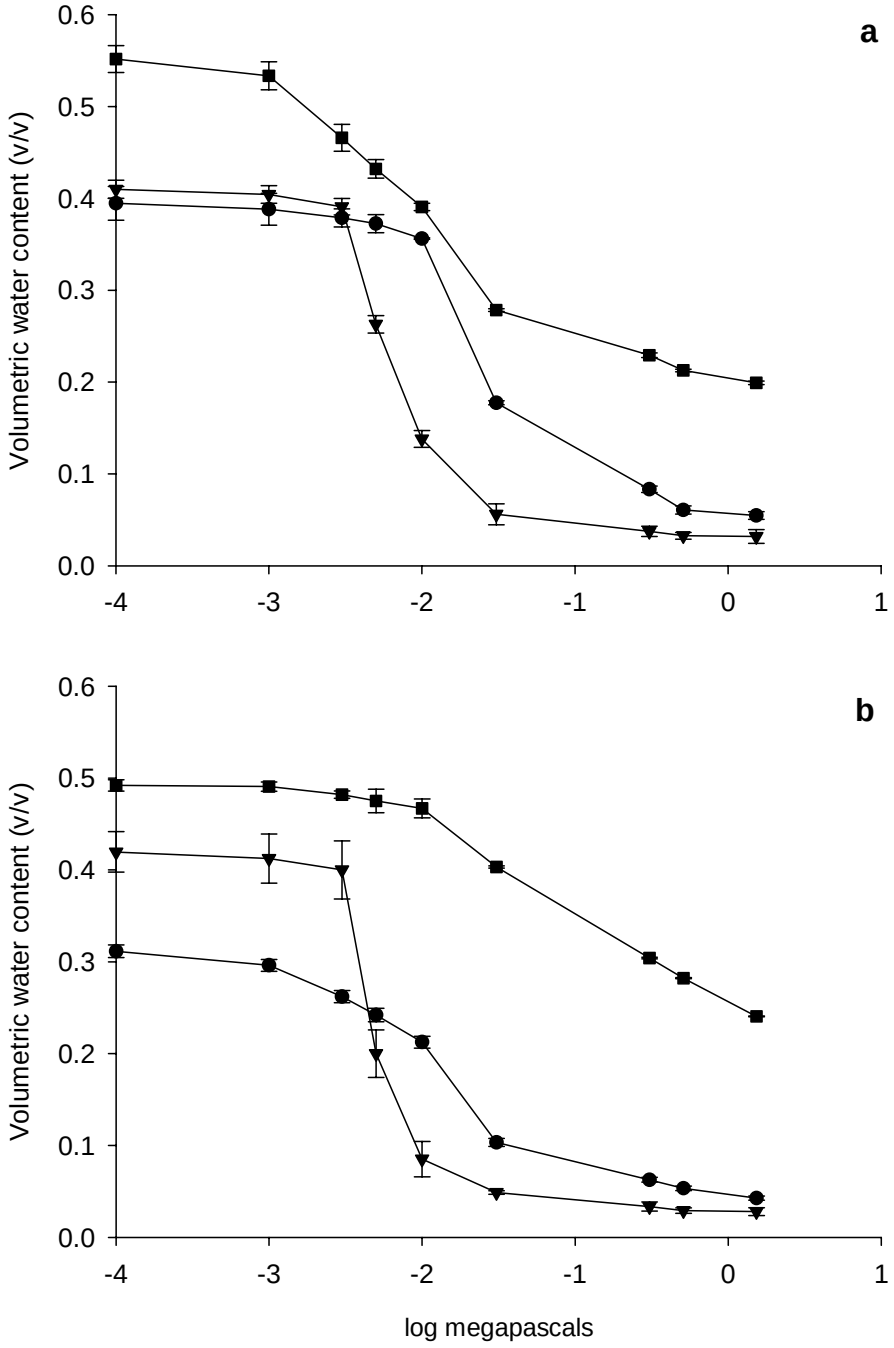

Figure 3.5. Moisture retention curves of undisturbed cores (a) and sieved (2 mm) (b) soil from the Yabulu/FACE (●), Pallarenda (▼) and Wambiana (■) sites located in the seasonally dry tropics of Queensland, Australia. Bars represent standard errors.

3.4. Soil chemical and biological characteristics

Soil biophysicochemical characteristics (Table 3.3) were determined on soils sampled from the A horizon (0-5 cm) during the dry season (October 2002) and wet

season (March 2003). Soil samples were sieved (2 mm) field fresh and analysed using the methods described in Chapter 2.

Table 3.3. Chemical and biological characteristics of soils at the Yabulu/FACE (FA), Pallarenda (PR), and Wambiana (WB) sites in tropical savanna ecosystems in Queensland, Australia during the dry (October 2002) and wet (March 2003) season. Numbers in brackets represent standard errors where n = 6. * indicates significant differences within soils between seasons: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

	Soil					
	FA		PR		WB	
Determinant	Dry	Wet	Dry	Wet	Dry	Wet
pH (H_2O)	3.4 (0.2)	3.2 (0.1)	4.9 (0.1)	5.1 (0.1)	6.1 (0.1)	6.1 (0.1)
Total C (%)	1.09	1.21	1.35	1.42	1.18	1.33
Organic C (%)	0.84	0.76	1.30	1.64	0.99	1.13
NH_4^+-N (mg kg^{-1})	11.5* (2.5)	5.5 (1.0)	8.8 (2.3)	5.3 (0.9)	2.5 (0.2)	2.6 (0.3)
NO_3^--N (mg kg^{-1})	3.2 (0.9)	3.8 (0.6)	17.5** (2.7)	4.7 (0.8)	2.5 (0.8)	3.0 (0.7)
Total P (%)	0.01	0.01	0.02	0.03	0.01	0.01
Total S (%)	<0.01	<0.01	0.01	0.01	0.01	0.01
Cation exchange capacity (cmol kg^{-1})	2.3	2.3	6.4	6.4	31.7	31.7
Electrical conductivity (mS cm^{-1})	0.02	0.02	0.05	0.05	0.10	0.10
Microbial biomass C (μg g^{-1} soil)	810* (0)	1091 (133)	839* (0)	925 (132)	766 (50)	793 (42)
Protease activity (μg tyrosine g^{-1} soil h^{-1})	418 (46)	395 (103)	714* (45)	447 (90)	134** (63)	843 (174)
Phosphodiesterase activity (μg *p*-nitrophenol g^{-1} soil h^{-1})	7.1** (0.7)	14.3 (2.0)	13.2** (1.3)	50.4 (5.8)	25.0** (2.4)	59.3 (3.3)

There was a clear difference in pH between the soils and the values are slightly lower than those observed in other tropical savanna soils in Australia (Mott et al., 1985; Chen et al., 2003). The soils used in the current study are typical of tropical savanna soils in that they are relatively low in C, N, P and S. A concentration of organic C of approximately 1% to 2% (Table 3.3) is common in tropical savanna soils (Montgomery and Askew, 1983). The onset of the wet season resulted in an

increase in organic C (except in FA soil), along with Total C (Table 3.3). All of the soils were relatively low in Total N, which is also common for tropical savanna soils, as N tends to be limiting in these ecosystems (Singh et al., 1989). However, the concentration of NH_4^+-N and NO_3^--N was higher than observed in tropical savanna soils in India (Singh et al., 1989). The cation exchange capacity (CEC) of FA and WB soils was similar to CEC values common to similarly textured soils. However, the CEC of PR (6.4 cmol kg^{-1}) was higher than expected for sandy textured soils (e.g. 2-4 cmol kg^{-1}). Electrical conductivity of each soil was low and was not affected by a change in season.

Microbial biomass C was slightly higher than observed in other tropical savanna soils (Singh et al., 1989; Srivastava, 1992) and an increase following the onset of the wet season was observed in FA and PR soil. This is in contrast to studies on Indian tropical savanna soils that have reported elevated microbial biomass C during the dry season (Singh et al., 1989; Srivastava, 1992). Phosphodiesterase activity was greater during the wet season and the activity of this enzyme was low in FA soil relative to PR and WB soil ($P < 0.05$). In contrast, protease activity was greater during the dry season in PR soil ($P < 0.05$). Little work has been done with these enzymes in tropical savanna soils so it is difficult to ascertain how the enzyme activities in the current soils relate to other soils in similar ecosystems.

CHAPTER 4

THE EFFECT OF TEMPERATURE ON PROCESSES ASSOCIATED WITH CADAVER DECOMPOSITION IN SOIL

Temperature can have a significant effect on decomposition of organic resources in soil (Swift et al., 1979). This is due to the effect of temperature on the metabolism of decomposer microorganisms (Clark and Gilmour, 1983; Carter, 2001; Coûteaux et al., 2002) and abiotic chemical reactions (van't Hoff, 1898). The metabolism of a mesotrophic microbial population typically increases with temperature up to 30 °C/35 °C and begins to decline at approximately 40 °C (Paul and Clark, 1996; Vinolas et al., 2001). Thus, an increase in temperature is typically associated with an increase in the decomposition of an organic resource patch (Kirschbaum, 1995; Carter, 2001; Coûteaux et al., 2002).

It is well known that temperature has a significant influence on cadaver decomposition. Field observations have repeatedly demonstrated that cadaver decomposition increases as temperature increases (Mant, 1950; Morovic-Budak, 1965; Vass et al., 1992). More recently, a controlled laboratory experiment demonstrated that an increase of 10 °C can significantly increase the decomposition of skeletal muscle tissue (*Ovis aries*) and microbial activity in a sandy loam soil (Carter and Tibbett, 2005). This phenomenon was associated with an increase in CO_2 evolution, microbial biomass C, qCO_2 and soil pH. These results show that temperature can regulate microbial activity and growth in the presence of one cadaver component (skeletal muscle tissue).

To determine if temperature affects cadaver decomposition soils were collected (August 2002) from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites located in tropical savanna ecosystems of Queensland, Australia (Chapter 3). Soils and cadavers were prepared as described in Chapter 2 and incubated at 29 °C, 22 °C or 15 °C following calibration to a matric potential of –0.05 megapascals (MPa). This moisture regime was chosen as an approximation to 50% water-holding capacity, which is generally regarded as resulting in peak microbial activity (Linn and Doran, 1984). Incubation temperatures were based on mean temperature at the research sites (Chapter 3) and since the current experiment was conducted in a laboratory setting, air temperature and soil temperature were identical. The experiment was replicated four times and set-up with sufficient replicates for four sequential harvest events over a period of 28 days resulting in a total of 288 microcosms. This experiment tested the hypothesis that an increase in temperature will result in an increase in cadaver decomposition.

4.2. Results

4.2.1. Cadaver mass loss

An increase in temperature resulted in an increase in cadaver mass loss at each harvest (Figure 4.1) except on day 7 in FA soil incubated at 22 °C or 29 °C (Figure 4.1). Cadaver mass loss was greatest in PR soil ($P < 0.001$) while burial in FA and WB soil resulted in similar levels of mass loss. The majority of mass loss was probably due to the loss of water as it is unlikely that ~80% cadaver mass was lost within 28 days. This resulted in a thin, flaccid cadaver by day 28 (see Figure 7.3)

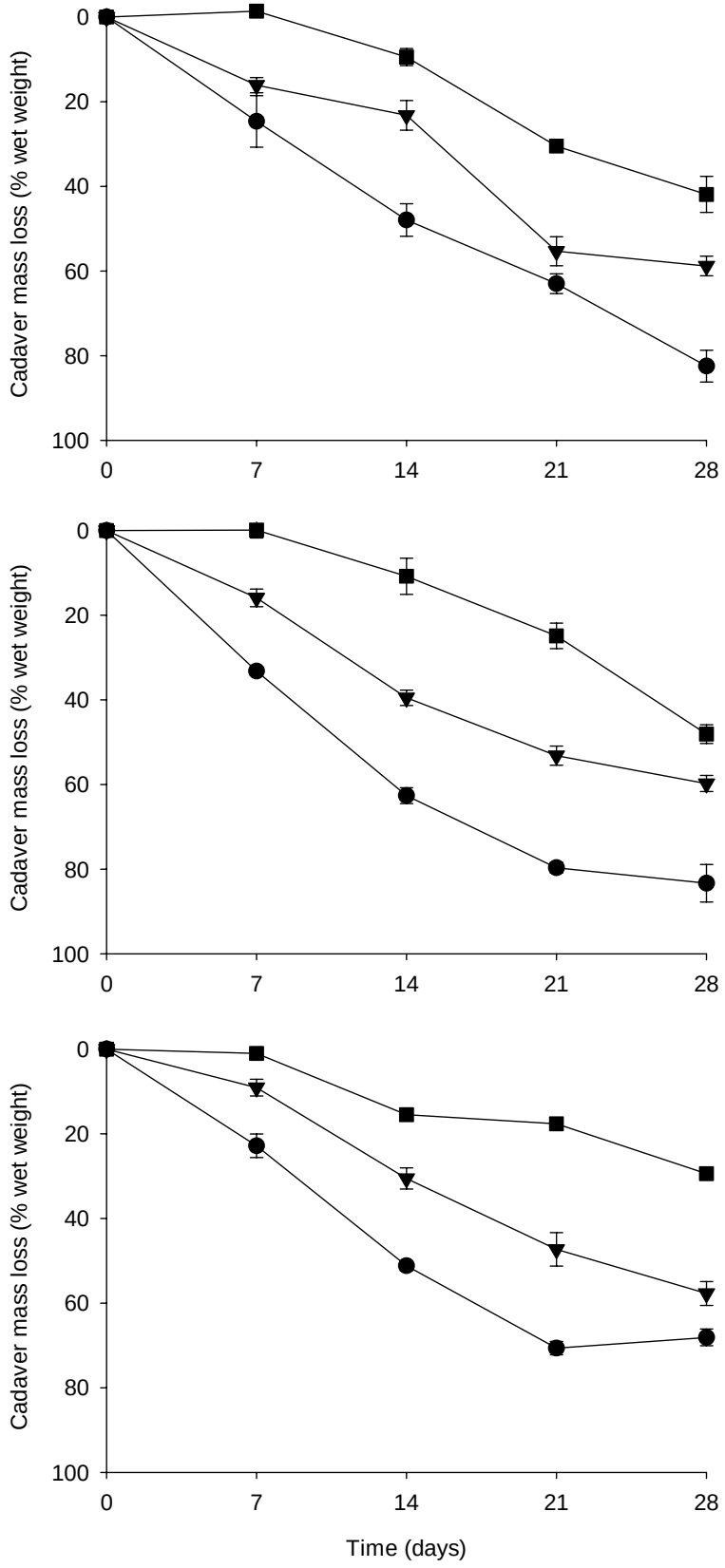

Figure 4.1. Mass loss (% wet weight) of a juvenile rat (*Rattus rattus*) cadaver following burial (2.5 cm) in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia incubated at 29 °C (●), 22 °C (▼) and 15 °C (■). Bars represent standard errors where n = 4.

4.2.2. Carbon dioxide (CO_2-C) evolution

Cadaver burial resulted in an increase in the rate of CO_2-C evolution (Figure 4.2). The rate of CO_2-C evolution in test samples was characterised by the presence of an initial lag phase followed by a period of peak of CO_2-C evolution, which was followed by a gradual decline in CO_2-C evolution. The duration of the lag phase and length of time before peak CO_2-C evolution was apparently regulated by temperature with an increase in temperature resulting in a decrease in the duration of the lag phase. The rate of CO_2-C evolution in all test samples was greater than in control samples ($P < 0.05$). An anomalous peak was observed in WB soil on day 15. This might be related to the significant loss of mass between day 7 and day 14 (Figure 4.1) or the result of incubator malfunction.

4.2.3. Cumulative CO_2-C evolution

Cumulative CO_2-C evolution in test samples was greater than in control samples ($P < 0.05$) and higher temperature resulted in higher CO_2-C evolution (Figure 4.2). Temperature also affected CO_2-C evolution in control samples (Appendix C). Carbon dioxide evolution in FA control samples at 15 °C was greater than at 29 °C, which was greater than at 22 °C ($P < 0.05$). Carbon dioxide evolution in PR control samples incubated at 29 °C was greater than at 15 °C ($P < 0.05$) while CO_2-C evolution in WB soil incubated at 22 °C was less than at 29 °C and 15 °C ($P < 0.05$). Soil type did not have an effect on CO_2-C evolution in test samples. In contrast, CO_2-C evolution in FA control samples was less than in PR and WB control samples ($P < 0.05$). Cumulative CO_2-C evolution in test samples was described using sigmoidal and quadratic polynomial equations. A significant

correlation was demonstrated between cadaver mass loss and cumulative CO_2-C evolution (Figure 4.3) (Appendix B: Table B.2).

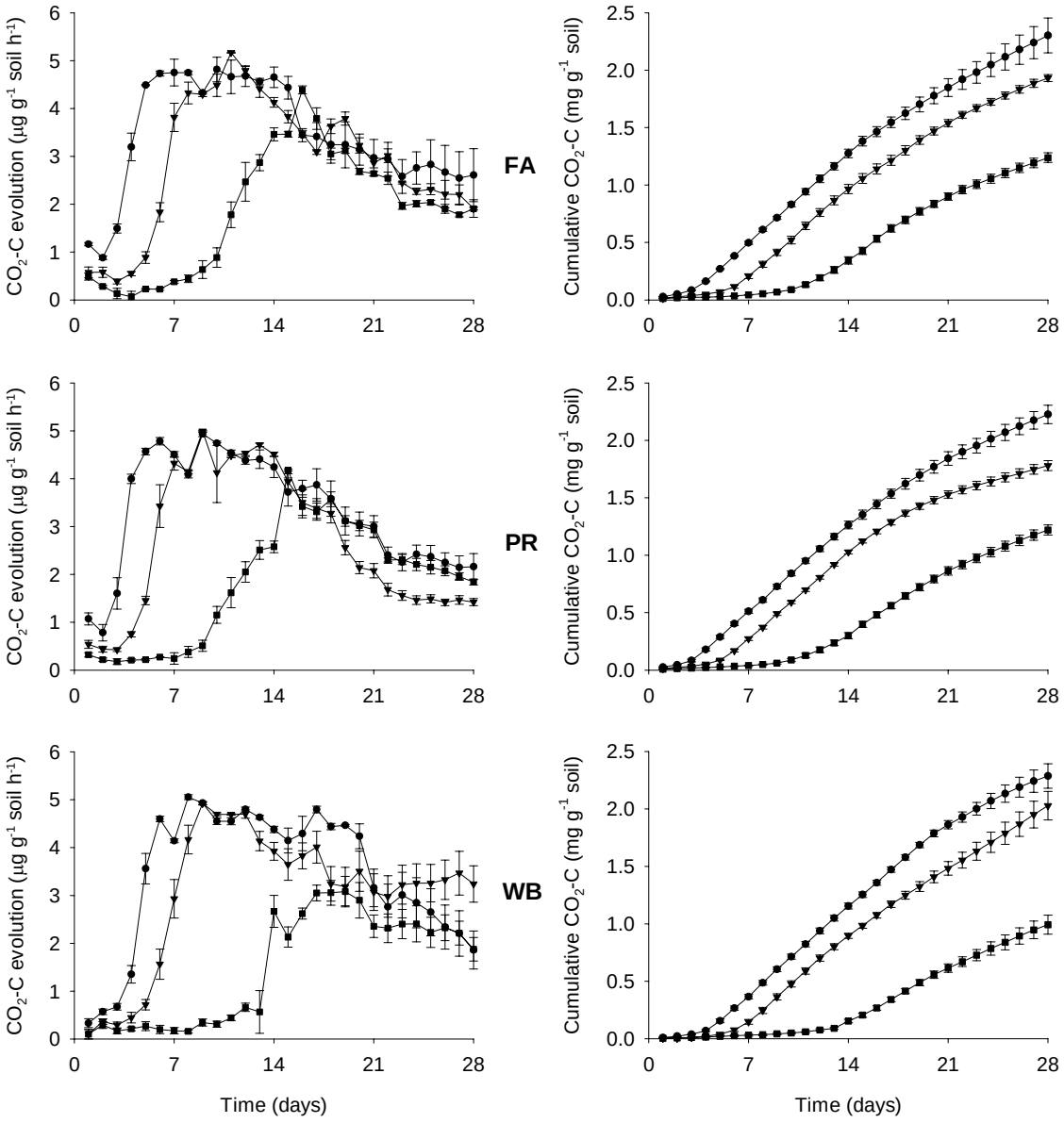

Figure 4.2. Daily (left column) and cumulative (right column) CO_2-C evolution (minus control data) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver in 500 g (dry weight) sieved (2 mm) soils from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia incubated at 29 °C (●), 22 °C (▼) and 15 °C (■). Bars represent standard errors where n = 4. Graphs displaying control data are presented in Appendix C: Figure C.1, Figure C.2.

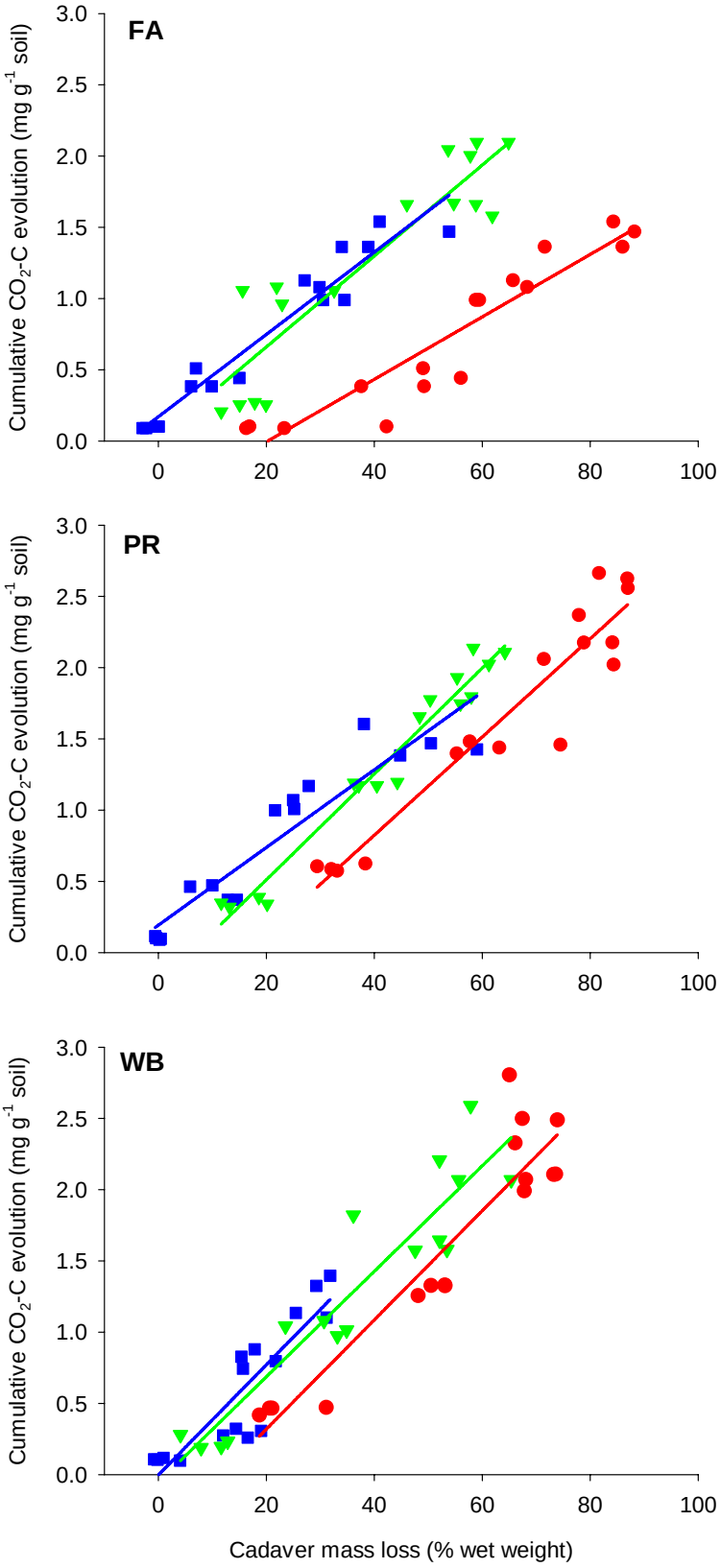

Figure 4.3. Correlation between cumulative CO_2-C evolution (mg g^{-1} soil) and mass loss (% wet weight) of a juvenile rat (*Rattus rattus*) cadaver following burial (2.5 cm) in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia incubated at 29 °C (●), 22 °C (▼) and 15 °C (■). Equations are presented in Appendix B: Table B.2.

There was no difference in aerobic catabolic efficiency (ACE) between soils. ACE tended to decline over time but no change was observed in some FA (15 °C) and PR (22 °C, 15 °C) samples (Table 4.1). A temperature effect was only observed in PR soil, as ACE at 29 °C was greater than at 15 °C. This effect can also be seen in Figure 4.3.

Table 4.1. Aerobic catabolic efficiency (ACE: mg CO_2-C % wet weight^{-1} cadaver mass loss) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia incubated at 29 °C, 22 °C and 15 °C. Numbers in brackets represent standard error of the mean. a, b, c represent a significant difference ($P < 0.05$) between time within soil and temperature. y, z, represent a significant difference ($P < 0.05$) .

		Day				
Soil	Temperature (°C)	7	14	21	28	Mean ACE
FA	29	0.02^{a} (0.00)	0.03^{a} (0.00)	0.03^{a} (0.00)	0.03^{a} (0.00)	0.03^{z} (0.00)
	22	0.01^{a} (0.00)	0.04^{c} (0.01)	0.03^{b} (0.00)	0.03^{bc} (0.00)	0.03^{z} (0.00)
	15	0.03^{a} (0.00)	0.04^{a} (0.01)	0.03^{a} (0.00)	0.03^{a} (0.00)	0.03^{z} (0.00)
PR	29	0.02^{a} (0.00)	0.02^{ab} (0.00)	0.02^{ab} (0.00)	0.03^{b} (0.00)	0.02^{z} (0.00)
	22	0.02^{a} (0.00)	0.03^{b} (0.00)	0.03^{b} (0.00)	0.03^{b} (0.00)	0.03^{yz} (0.00)
	15	0.02^{a} (0.00)	0.03^{a} (0.01)	0.03^{a} (0.00)	0.03^{a} (0.00)	0.03^{y} (0.00)
WB	29	0.02^{a} (0.00)	0.02^{a} (0.00)	0.03^{b} (0.00)	0.03^{b} (0.00)	0.03^{z} (0.00)
	22	0.02^{a} (0.01)	0.03^{a} (0.00)	0.03^{a} (0.00)	0.04^{a} (0.00)	0.03^{z} (0.00)
	15	0.02^{ab} (0.01)	0.01^{a} (0.00)	0.04^{b} (0.00)	0.03^{b} (0.00)	0.02^{z} (0.00)

4.2.4. Microbial biomass C (C_{mic})

Cadaver burial resulted in an increase in C_{mic} (Figure 4.4). This increase was affected by temperature as higher temperature resulted in an earlier increase. Thus, peak C_{mic} at 29 °C was typically observed from day 7 to day 14 while incubation at 22 °C or 15 °C resulted in peak C_{mic} from day 14 to day 21. One exception was PR

test samples incubated at 22 °C, which may not have reached peak levels by day 28. In most cases a significant increase in microbial biomass was associated with a loss of ~20% mass. The two exceptions were FA (15 °C: increase at 0% mass loss) and WB (22 °C: increase at 9% mass loss). FA test and control samples contained the smallest concentrations of C_{mic} ($P < 0.05$).

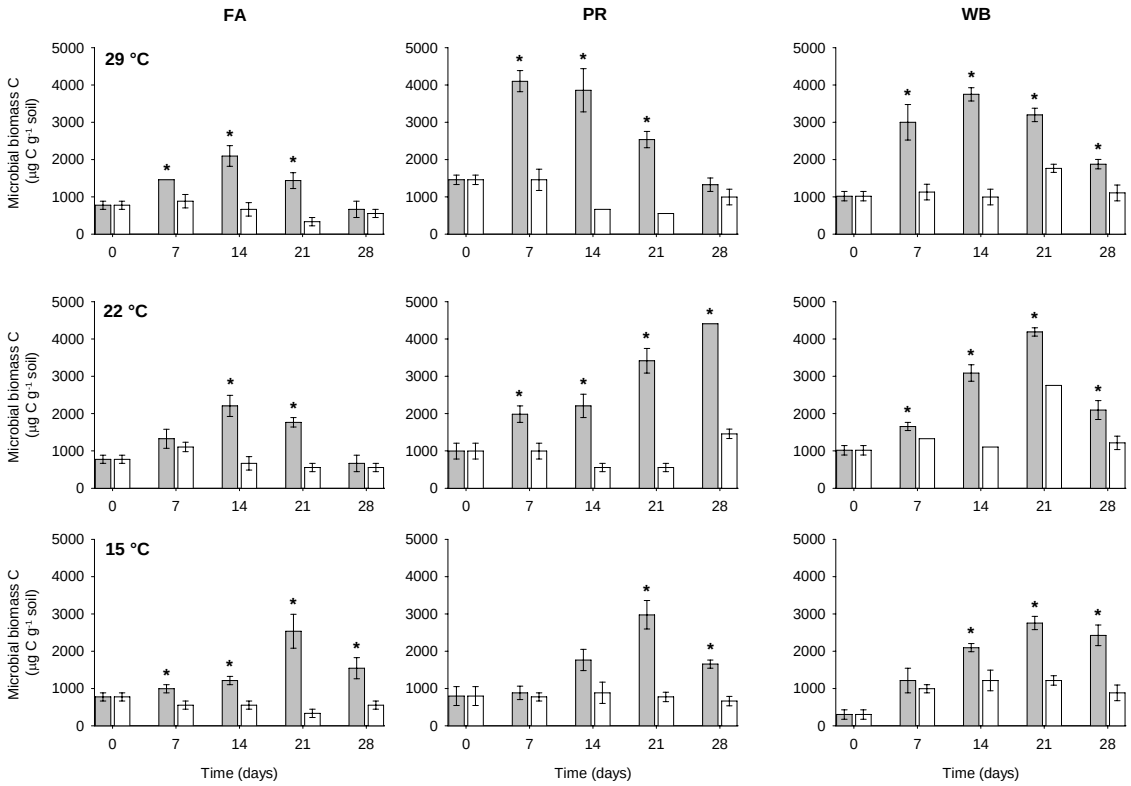

Figure 4.4. Microbial biomass C (µg g^{-1} soil) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (■) and in control (no cadaver: □) samples in 500 g (dry weight) sieved (2 mm) soils from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia incubated at 29 °C, 22 °C and 15 °C. Bars represent standard errors where n = 4. * denotes significant difference ($P < 0.05$) between cadaver treatments within time.

Fungal hyphae were seen on the soil surface following cadaver burial (Figure 4.5). The presence of fungal hyphae was most common in WB soil but first appeared on day 7 in FA test samples incubated at 29 °C (Table 4.1). There was an apparent relationship between incubation temperature and the appearance of fungal hyphae:

hyphae tended to appear earliest at 29 °C and latest at 15 °C. No fungal hyphae were seen on the surface of control soils.

Figure 4.5. Fungal hyphae on the surface of 500 g (dry weight) sieved (2 mm) soil from the Wambiana site located in a tropical savanna ecosystem of Queensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver.

Table 4.2. Macroscopic detection (+) of fungal hyphae on the surface of 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia following cadaver (*Rattus rattus*) burial and incubation at 29 °C, 22 °C or 15 °C over a period of 28 days. + represent the number of replicates in which fungal hyphae were observed (e.g. ++ = fungal hyphae present in two replicates).

	FA			PR			WB		
Time (days)	29 °C	22 ° C	15 °C	29 °C	22 °C	15 °C	29 °C	22 °C	15 °C
0									
7	++								
14	++++				+		++	++++	
21	+				+		++++	++++	
28		+++			++			++++	++++

4.2.5. Metabolic quotient (qCO_2)

Cadaver burial resulted in an increase in qCO_2 (Figure 4.6). This was apparently affected by temperature as incubation at 15 °C resulted in a relatively lower qCO_2 in FA ($P < 0.05$) and WB ($P < 0.05$) test samples. Temperature did not affect qCO_2 in

PR and WB control samples. However, qCO_2 in FA control samples incubated at 15 °C was greater than at 22 °C ($P < 0.05$). Soil had an affect on qCO_2 in test and control samples. The qCO_2 of FA test samples was greater than PR and WB test samples at each temperature ($P < 0.01$). Among control samples qCO_2 in PR soil was greatest at 22 °C ($P < 0.01$) and 29 ° C ($P < 0.001$). No soil effect was observed in control samples incubated at 15 °C.

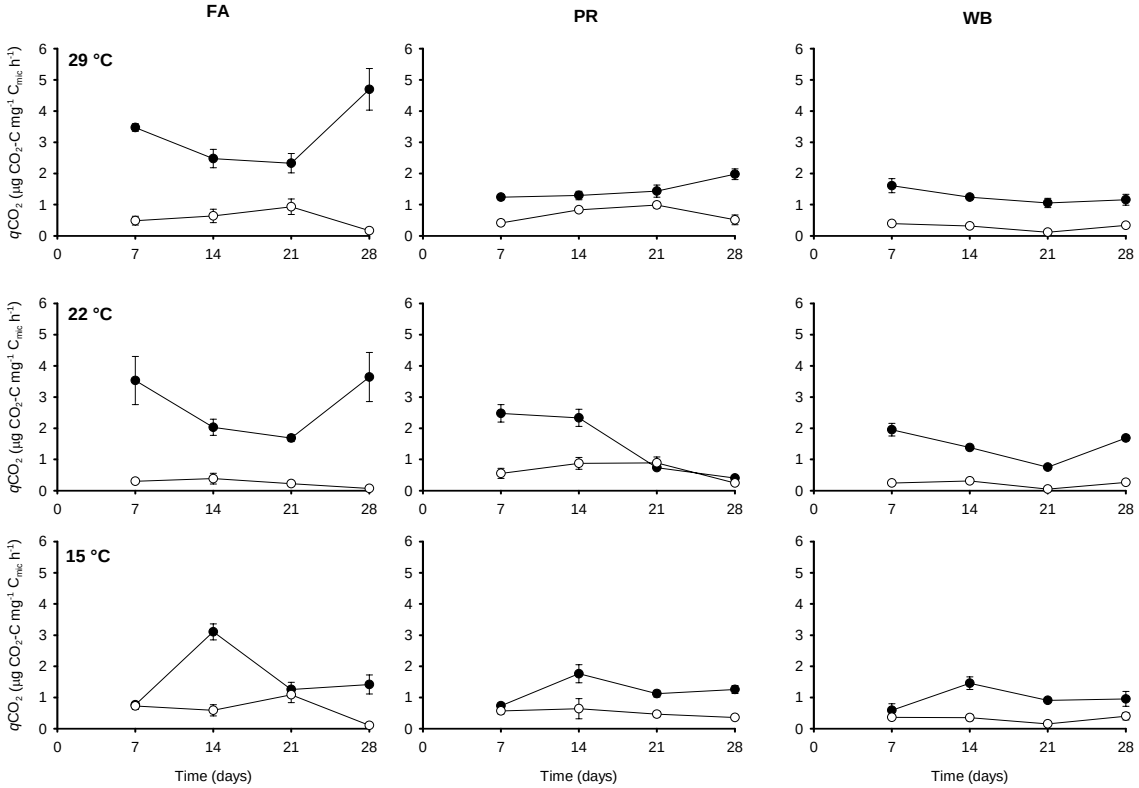

Figure 4.6. Metabolic quotient (qCO_2: µg CO_2-C mg^{-1} C_{mic} h^{-1}) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and in control (no cadaver: ○) samples in 500 g (dry weight) sieved (2 mm) soils from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems Queensland, Australia incubated at 29 °C, 22 °C and 15 °C. Bars represent standard errors where n = 4.

4.2.6. Protease activity

Cadaver burial resulted in an increase in protease activity, particularly during the latter part of the incubation (Figure 4.7). The observed increase in protease activity was apparently affected by temperature as incubation at 29 °C generally resulted in an increase in protease activity earlier than observed at 22 °C and 15 °C. There was

no difference between temperatures within FA and PR test samples. However, protease activity in WB test samples incubated at 22 °C was greater than at 15 °C ($P < 0.05$). Protease activity in control samples was constant over time but was affected by temperature. Protease activity in FA and WB control samples incubated at 29 °C was greater than at 15 °C ($P < 0.05$). Temperature did not affect PR control samples. WB soil always contained the greatest level of protease activity ($P < 0.05$).

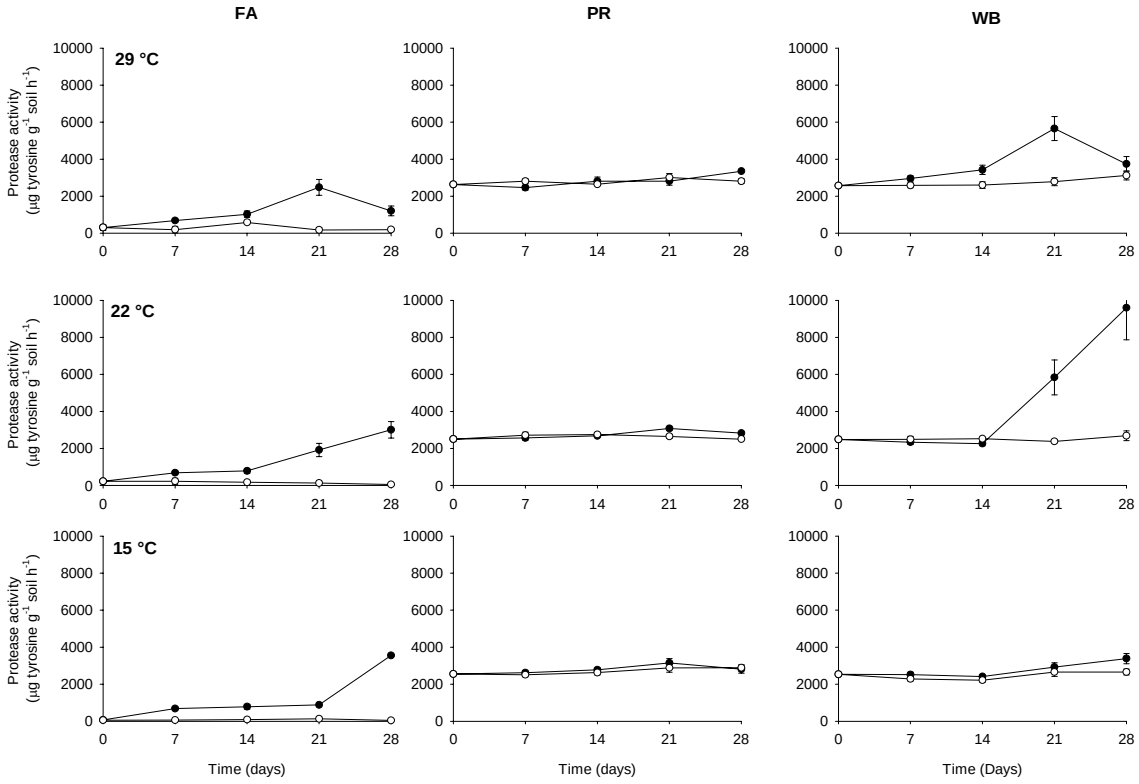

Figure 4.7. Protease activity (µg tyrosine g^{-1} soil h^{-1}) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and in control (no cadaver: ○) samples in 500 g (dry weight) sieved (2 mm) soils from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia incubated at 29 °C, 22 °C and 15 °C. Bars represent standard errors where n = 4.

4.2.7. Phosphodiesterase activity

Cadaver burial resulted in an increase in phosphodiesterase activity ($P < 0.05$) (Figure 4.8). This phenomenon was apparently regulated by temperature as an increase in phosphodiesterase activity in test samples incubated at 29 °C took place earlier than at 22 °C and 15 °C. Phosphodiesterase activity among test samples was

greatest in WB soil ($P < 0.05$). Among control samples phosphodiesterase activity in WB was greater than in PR, which was greater than in FA ($P < 0.05$).

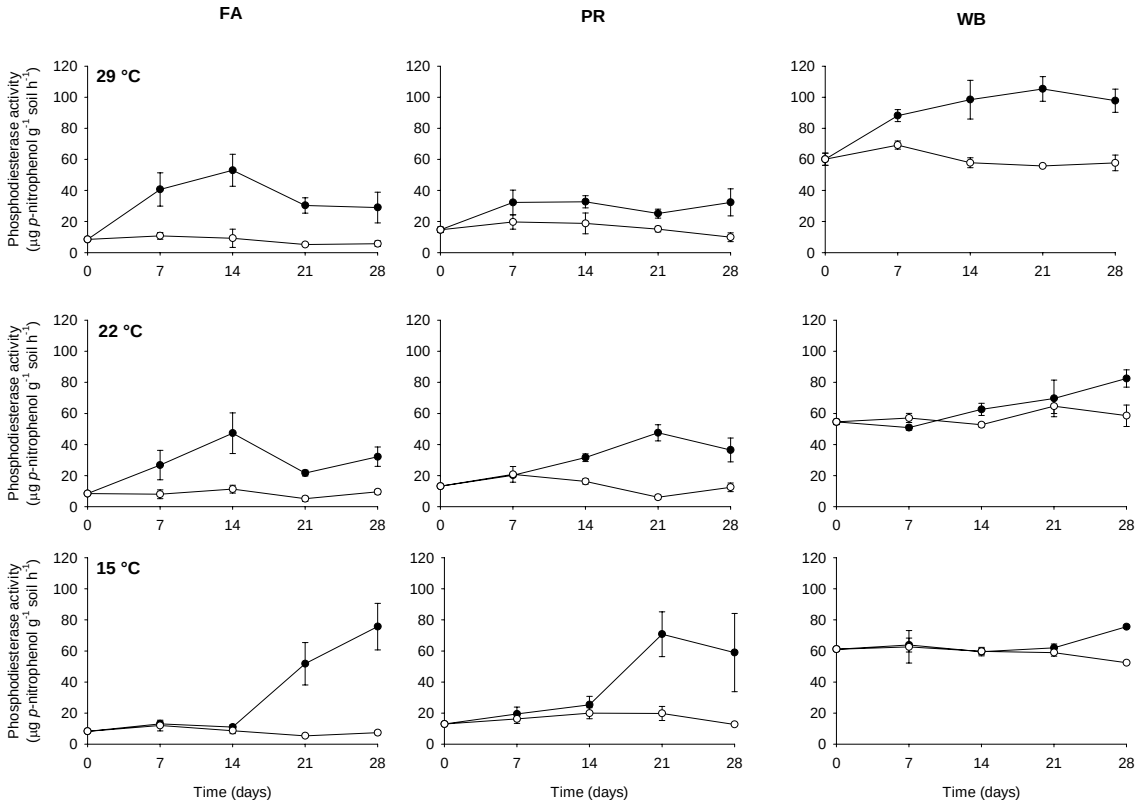

Figure 4.8. Phosphodiesterase activity (µg *p*-nitrophenol g^{-1} soil h^{-1}) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and in control (no cadaver: ○) samples in 500 g (dry weight) sieved (2 mm) soils from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia incubated at 29 °C, 22 °C and 15 °C. Bars represent standard errors where n = 4.

4.2.8. Soil pH

Cadaver burial consistently resulted in an increase of pH to a peak between 8 and 8.1 (Figure 4.9). This increase was apparently affected by temperature with an increase in temperature resulting in an earlier peak. In control samples the pH of WB was greater than PR, which was greater than FA ($P < 0.05$).

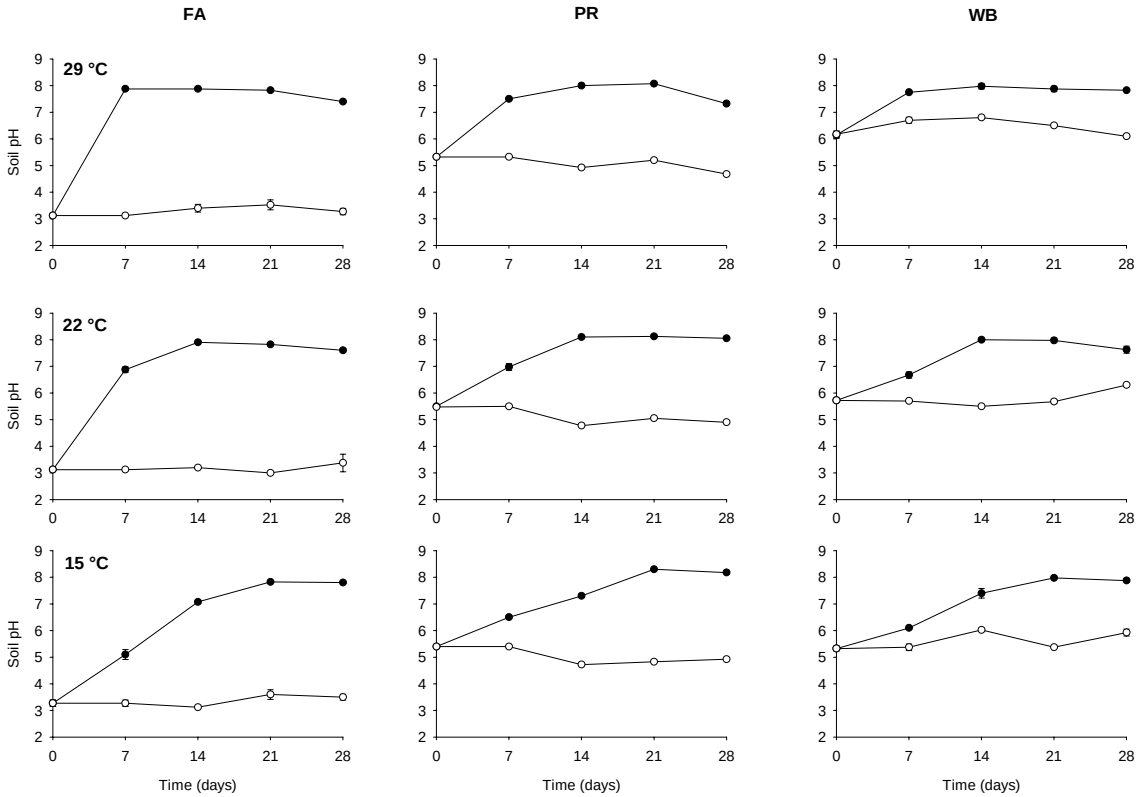

Figure 4.9. pH of sieved (2 mm) soils from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and in control (no cadaver: ○) samples incubated at 29 °C, 22 °C and 15 °C. Bars represent standard errors where n = 4.

4.3. Discussion

The current results show that an increase in temperature can result in an increase in cadaver mass loss and microbial activity. The mass loss of the cadavers in the current study progressed through an "early phase", "intermediate phase" and "late phase" (only observed at 29 °C and 22 °C) that were characterised by cadaver mass loss and microbial activity (Figure 4.1). Early phase decomposition roughly approximated to the "Fresh" and "Inflated" stages in Payne et al. (1968). Intermediate phase decomposition approximated to the "Deflation &

decomposition" stage while late phase decomposition approximated to the "Disintegration" and "Skeletonisation" stages (Payne et al. 1968).

Early phase decomposition at 29 °C and 22 °C took place between day 0 and day 7 and was characterised by initial increases in CO_2-C evolution (Figure 4.2), C_{mic} (Figure 4.4), qCO_2 (Figure 4.6), protease activity (Figure 4.7), phosphodiesterase activity (Figure 4.8) and soil pH (Figure 4.9). At 15 °C early phase decomposition took place from day 0 to day 14 and included many of the same patterns as the samples incubated at 29 °C and 22 °C. However, incubation at 15 °C typically resulted in the delay of an increase in process rates by approximately seven days.

Intermediate phase decomposition was characterised by the maintenance of an elevated rate of CO_2-C evolution (Figure 4.2) C_{mic} (Figure 4.4), phosphodiesterase activity (Figure 4.8) and soil pH (Figure 4.9). At 29 °C and 22 °C intermediate phase decomposition took place between day 7 and day 21 but there were minor differences between these two temperatures. Peak C_{mic} took place on day 14 at 29 °C and day 21 at 22 °C (Figure 4.4). At 15 °C intermediate phase decomposition took place between day 14 and day 28.

Late phase decomposition was only observed at 29 °C and 22 °C and coincided with a general slowing down of cadaver mass loss (Figure 4.1) and a gradual decrease in CO_2-C evolution (Figure 4.2). Late phase decomposition likely reflects the depletion of readily-available resources (Ajwa and Tabatabai, 1994). Interestingly, peak protease activity was associated with late phase decomposition and coincided

with a decrease in C_{mic} (Figure 4.4), an increase in qCO_2 (Figure 4.6) and a rate of CO_2-C evolution that was significantly greater than in control soils (Figure 4.2). This pattern has been observed during the decomposition of leaf litter (Nannipieri et al., 1983; Dilly and Munch, 1996) and might be due to the release of labile organic materials upon the death of the microbial biomass (Ladd and Paul, 1973; Nannipieri et al., 1979; Nunan et al., 2000), which might signal a successional change in community structure. This may explain the increase in qCO_2 values in FA (29 °C, 22 °C), PR (29 °C) and WB (22 °C) test samples (Figure 4.6): the proliferation of zymogenous microorganisms in response to the introduction of dead microbial cells.

Greater cadaver mass loss in PR soil leads shows that cadaver burial in a coarse-textured soil can result in greater cadaver decomposition than in a fine-textured soil. Because cadaver mass loss was determined on a wet weight basis the increased mass loss increased desiccation associated with a greater rate of gas diffusion in a coarse-textured sandy soil (see Chapter 5). While the current results are in accordance with research using non-cadaveric organic resources (e.g. Sörensen, 1975; Jenkinson, 1977; Sørensen, 2001), it is in contrast to research using cadaveric resources. Soil type is traditionally thought to result in minor differences in the short term processes associated with cadaver decomposition (Mant, 1950; Morovic-Budak, 1965; Mant, 1987) although Mant (1950) observed an increased occurrence of tissue desiccation in sandy soil during the exhumation of war dead in Europe. Turner and Wiltshire (1999) suggested that clayey soil may slow cadaver decomposition by acting as a seal that prevents decomposer organisms (e.g. arthropods, vertebrate scavengers) from participating in decomposition but they did not conduct a comparative study using a soil of contrasting texture.

In the current study qCO_2 was used to assess the efficiency with which the microbial population utilises organic C (Anderson and Domsch, 1990; Dilly and Munch, 1998). Cadaver burial clearly resulted in an increase in qCO_2, which is common following the introduction of an organic resource (e.g. Mamilov and Dilly, 2002). This phenomenon reflects active microorganisms responding to an increase in resource availability, which previously limited metabolism. Interestingly, the ACE conflicted with the qCO_2 except for the detection of a loss in efficiency over time (Figure 4.6; Table 4.1). The ACE is likely to be the more accurate assessment of metabolic efficiency since it is uses a direct measure of cadaver mass. Also, qCO_2 can be subject to a number of variables including temperature (Anderson and Domsch, 1986), moisture stress (Killham, 1985) and a change in pH (Anderson and Domsch, 1993).

An increase in CO_2-C evolution is associated with the decomposition of many organic resources such as skeletal muscle tissue (*Ovis aries*) (Carter, 2001), sewage sludge (Albiach et al., 2000), glucose (Nicolardot et al., 1994), holocellulose (Nicolardot et al., 1994) and pine (*Pinus halepensis*) needle litter (Coûteaux et al., 2002). The rates of CO_2-C evolution in the current study are similar to previous studies focusing on the decomposition of exposed (i.e. on the soil surface) mice in field settings (Putman, 1978a; Putman, 1978b): CO_2–C evolved from soils associated with cadaver leachate was greater than control soils (Putman, 1978a). In addition, a rapid increase in soil-derived CO_2-C occurred two to three days after the increase of enteric CO_2-C evolution (Putman, 1978a). This may explain some of the multiple peaks in CO_2-C evolution in the current study (Figure 4.2). The initial peak

may be due to the enteric community while the second peak might be the result of soil microbial activity, possibly resulting from bacterial and fungal utilisation of decomposition fluids that escape from the cadaver during intermediate phase decomposition (Payne et al., 1968). The relative contribution of cadaver-borne and soil-borne microbes to decomposition has been addressed in more detail in Chapters 6 and 7.

A common indicator of the microbial utilisation of an organic nutrient source is the estimation of microbial biomass, which can be significantly affected by temperature and nutrient status (Wardle, 1992). Hopkins et al. (2000) reported a significant increase in C_{mic} in clay soil associated with buried pig cadavers (*Sus scrofa*). It is important to note that the burial of a cadaver under the current experimental conditions can result in a concentration of C_{mic} greater than 4000 $\mu g\ g^{-1}$ soil (Figure 4.4). This concentration of C_{mic} has not been found in the literature and illustrates the high level of activity associated with a nutrient patch such as a cadaver.

The proliferation of fungal hyphae on the surface of test soils (Table 4.2, Figure 4.5) supports the suggestion that the fungal portion of the microbial biomass might play a significant role in processes associated with cadaver decomposition in soil (Tibbett and Carter, 2003). The macroscopic presence of fungal hyphae has been observed on the surface of a sandy loam soil associated with skeletal muscle tissue (*Ovis aries*) (Carter, 2001) and on decomposition fluids associated with buried pig (*Sus scrofa*) cadavers (Payne et al., 1968). These hyphae might be representative of the postputrefaction fungi, a group of terrestrial fungi that produce fruiting structures in association with decomposing cadavers (Sagara, 1995) and/or

keratinophilic fungi (Pugh and Evans, 1970; Kaul and Sumbali, 2000; Deshmukh and Agrawal, 2003). It is unknown whether these fungi actively participate in cadaver decomposition or are utilising decomposition by products as a source of nutrients and energy.

The increase of soil pH has been attributed to the accumulation of NH_4^+ (Hopkins et al., 2000) and has been reported in association with human (Rodriguez and Bass, 1985; Vass et al., 1992), dog (*Canis familiaris*) (Reed, 1958) and pig (*Sus scrofa* L.) (Hopkins et al., 2000) cadavers as well as skeletal muscle tissue (*Ovis aries*) (Carter, 2001). Interestingly, cadaver burial resulted in a pH of approximately 8 regardless of temperature and soil type (Figure 4.10). A similar trend was observed in association with human cadavers (Rodriguez and Bass, 1985) and skeletal muscle tissue (*Ovis aries*) (Carter, 2001). The decrease in pH observed in some of the test samples incubated at 29 °C and 22 °C (Figure 4.10) could be the result of the soil returning to its basal pH because of the microbial utilisation of base cations. A decrease in pH during late phase decomposition is in accordance with more complete decomposition and is similar to the findings from human decomposition studies (Vass et al., 1992). To date, the effect of basal soil pH on cadaver decomposition is unknown.

The current findings show that the introduction of a complex organic resource patch, in the form of a cadaver, into the soil results in a significant increase of mass loss, CO_2-C evolution, C_{mic}, enzyme activities, pH as well as the presence of fungal hyphae on the soil surface. Furthermore, these effects can be significantly affected by temperature whereby an increase in temperature can result in an increase in

cadaver decomposition. This temperature effect resulted in a “temporal wave” throughout the current data where peaks or differences that occurred at 29 °C at one sample period would occur at later sample periods at lower temperatures.

CHAPTER 5

THE EFFECT OF MOISTURE ON PROCESSES ASSOCIATED WITH CADAVER DECOMPOSITION IN SOIL

Soil moisture can have a significant effect on decomposition (Swift et al., 1979). This is due, in part, to the fact that soil moisture can effect the metabolism of the decomposer microorganisms. This effect can be modified by soil texture because bioavailable moisture is determined, in part, by the suction with which water is held between soil particles (i.e. matric potential). Thus, the calibration of soils to a known matric potential can lead to the assessment of the effect of bioavailability of moisture in soil (Hillel, 1982) and allow for the comparison of process rates between soils at the same matric potential (Orchard and Cook, 1983). Microbial metabolism has been observed to be greatest at a matric potential of –0.01 megapascals (MPa) (Linn and Doran, 1984; Paul and Clark, 1996) and can decline as suction increases or decreases.

The decline in metabolic activity associated with a matric potential of less than –0.01 MPa has been attributed to a decrease in the rate of gas diffusion through the liquid phase (Papendick and Runkles, 1965; Moldrup et al., 1997). This is because the rate of gas diffusion in water is less than in air. The diffusion of gases (e.g. oxygen (O_2), carbon dioxide (CO_2)) in soil can have a profound effect on the decomposition of organic nutrient patches because aerobic microorganisms are typically more effective at decomposing organic resources than anaerobic microorganisms (Swift et al., 1979). Microbial activity tends to increase in the presence of an organic resource (e.g. Hodge et al., 2000) and, therefore, a low matric potential may result in a rate of gas diffusion that is insufficient to support an

aerobic microbial population. This effect may be enhanced by a change in the shape and formation of air-filled pores upon the introduction water (due to the swelling of some clays), which can cause increased tortuosity for gas diffusion (Moldrup et al., 2000).

An assessment of the rate of gas diffusion can be made via the calculation of gas diffusivity (the ratio of gas diffusion coefficients in soil and in air). In addition to matric potential, gas diffusivity is dependent upon soil type and structure (Schjønning et al., 1999) but this effect is apparently less significant in sieved soil (Moldrup et al., 1997). Gas diffusivity for sieved, wetted soil can be calculated by introducing a water-induced linear reduction (WLR) to the model for gas diffusivity (Equation 5.1) in dry porous media (Marshall, 1959; Moldrup et al., 2000; Rolston and Moldrup, 2002).

$$\frac{D_P}{D_0} = \frac{\varepsilon^{2.5}}{\Phi} \qquad \text{Equation 5.1.}$$

Where D_P is the gas diffusion coefficient in soil (cm^3 soil air cm^{-1} soil s^{-1}), D_0 is the gas diffusion coefficient in free air (cm^2 air s^{-1}), ε is the soil air-filled porosity (cm^3 soil air cm^{-3} soil), Φ is the soil total porosity (cm^3 cm^{-3}).

A decline in microbial metabolism at a matric potential greater than –0.01 MPa has been attributed to a decrease in microbial mobility (Griffin, 1981; Killham et al., 1993) as well as intracellular water potential, which can alter enzyme conformation and activity (Skujins and McLaren, 1967b; Csonka, 1989). Microbial mobility in soil is regulated by pore size and the diameter of water-filled pores (Elliot et al., 1980; Griffin, 1981). Typically, nematodes require a water-filled pore diameter of

15 μm for mobility while the movement of protozoa requires a diameter of at least 6 μm (Elliot et al., 1980). Bacteria can move freely in water-filled pores with a diameter of less than 6 μm (Elliot et al., 1980) although bacterial mobility can decline sharply as matric potential increases from –0.05 MPa to –0.3 MPa (Wong and Griffin, 1976a; Wong and Griffin, 1976b). One possible exception to matric potential induced restrictions on microbial mobility is the growth of fungal hyphae, which can bridge air-filled pores (Griffin, 1981; Savin et al., 2001).

Maximum water-filled pore diameters can be calculated from matric potential using the capillary rise equation (Equation 5.2) (Hillel, 1982):

$$d = 300/\psi_m \qquad \text{Equation 5.2.}$$

where d is pore size (μm) and ψ_m is the matric potential in kilopascals (kPa). As water becomes available in increasingly smaller pores it becomes more difficult for microbes to take up. A decrease in available water can affect the catalysis of organic nutrient patches because, under dry conditions, the concentration of ions in soil solution can increase. This can trigger microbes to intracellularly accumulate ions in order to maintain osmotic balance (Sparling and Ross, 1988; Csonka, 1989), which can retard enzyme activity (Skujins and McLaren, 1967a; Csonka, 1989).

Moisture can have a significant effect on the decomposition of cadavers in soil. It is generally understood that a dry environment promotes cadaver desiccation (Galloway et al., 1989; Galloway, 1997; Aturaliya and Lukasewycz, 1999) while wet environments can retard decomposition via the formation of adipocere

(hydrolysed fat) when sufficient fatty tissues or lipids are present (Mant, 1987; Fiedler and Graw, 2003; Forbes et al., 2004a; Forbes et al., 2004b). The burial of a cadaver in a wet clay soil can lead to conditions indicative of an anaerobic environment (Hopkins et al., 2000) which can result in a rate of cadaver decomposition less than under aerobic conditions (Turner and Wiltshire, 1999; Hopkins et al., 2000). The contribution of the soil microbial biomass to these processes is unknown.

To assess the effect of soil moisture on cadaver (*Rattus rattus*) decomposition soils were collected from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia (see Chapter 3). Soils and cadavers were prepared as described in Chapter 2 and soil matric potential was calibrated to one of three matric potentials (-0.01 MPa, -0.05 MPa and –0.3 MPa) based on the data from the moisture retention curves presented in Chapter 3. Following the addition of water, soils were allowed to equilibrate in the dark at 22 °C for seven days. Relative gas diffusivity for each soil and matric potential was calculated using the WLR (Marshall) model (Equation 5.1) (Table 5.1). Using the capillary rise equation (Equation 5.2) it was determined that matric potentials of –0.01 MPa, -0.05 MPa and –0.3 MPa corresponded to water-filled pore diameters of 30 μm, 6 μm and 1 μm, respectively. A matric potential of –0.01 MPa will allow for the movement of all soil microbes. A matric potential of –0.05 MPa will restrict the movement of nematodes and protozoa while –0.3 MPa will restrict the mobility of all microbes including, possibly, bacteria.

Table 5.1. Gas diffusivity (D_P/D_0) of sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites located in tropical savanna ecosystems Queensland, Australia calibrated to a matric potential of –0.01 megapascals (MPa), -0.05 MPa or –0.3 MPa and incubated at 22 °C where D_P is the gas diffusion coefficient in soil (cm^3 soil air cm^{-1} soil s^{-1}) and D_0 is the gas diffusion coefficient in free air (0.0207 cm^3 soil air cm^{-3} soil).

	Matric potential		
Soil	-0.01 MPa	-0.05 MPa	-0.3 MPa
FA	0.001	0.056	0.138
PR	0.093	0.181	0.206
WB	0.013	0.057	0.089

The current experiment was replicated three times resulting in a total of 216 microcosms. The current experiment tested the hypothesis that a decrease in soil matric potential (i.e. increase in bioavailable moisture) will result in an increase in cadaver decomposition.

5.2. Results

5.2.1. Cadaver mass loss

Matric potential had a significant effect on cadaver mass loss in all soils. In FA and WB soil cadaver mass loss was greatest at –0.05 MPa ($P < 0.01$) (Figure 5.1). In contrast, cadaver mass loss in PR soil was greatest at –0.05 MPa and –0.01 MPa ($P < 0.01$) (Figure 5.1). A soil effect was also observed as cadaver mass loss at –0.05 MPa and –0.01 MPa was greatest in PR soil ($P < 0.05$). However, there was no difference in cadaver mass loss between soils at –0.3 MPa.

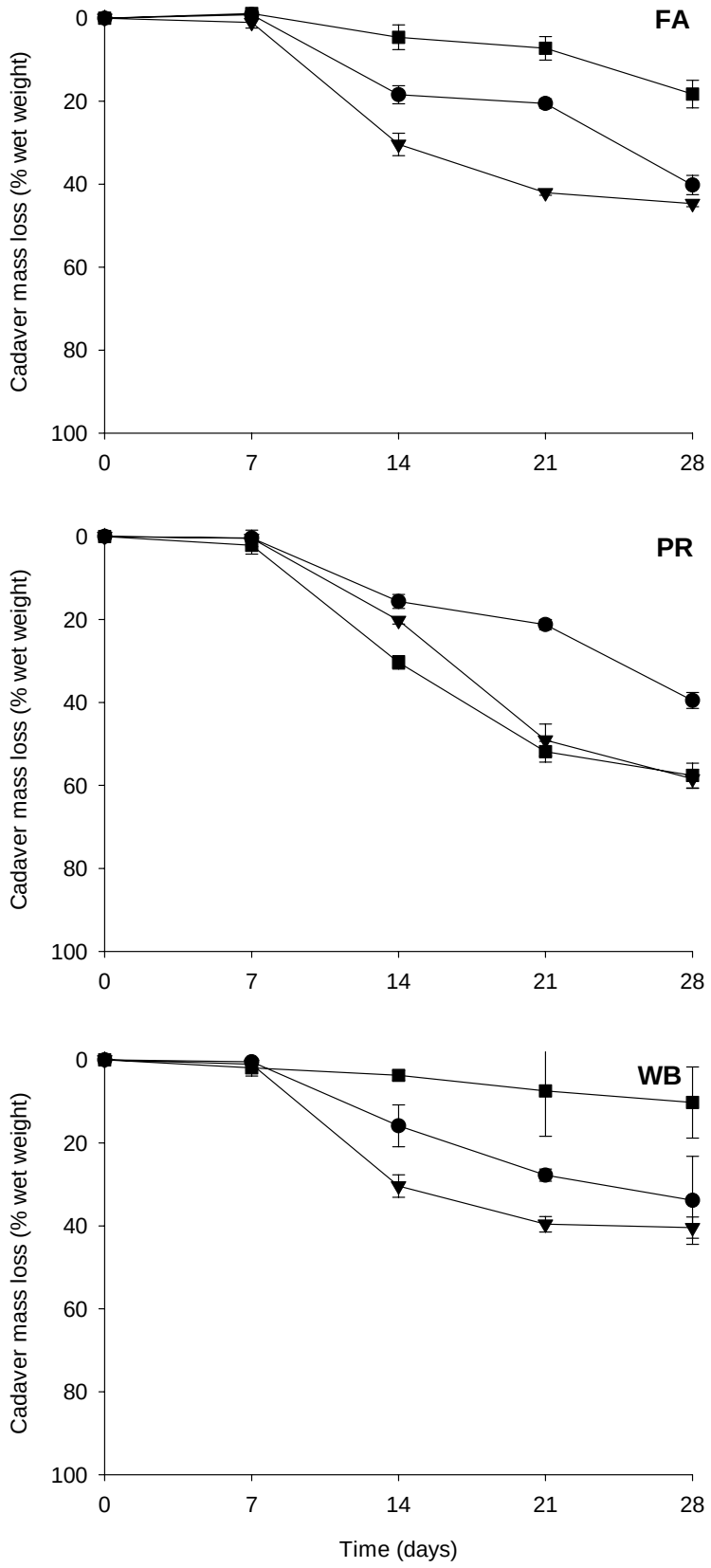

Figure 5.1. Mass loss (% wet weight) of a juvenile rat (*Rattus rattus*) cadaver buried (2.5 cm) in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR), and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia calibrated to a matric potential of –0.3 megapascals (MPa) (●), -0.05 MPa (▼) and –0.01 MPa (■) and incubated at 22 °C. Bars represent standard errors where n = 3.

5.2.2. Daily carbon dioxide (CO_2-C) evolution

Cadaver burial resulted in an increase in CO_2-C evolution in all soils at each matric potential (Figure 5.2). However, CO_2-C evolution in FA and WB test samples calibrated to –0.01 MPa was significantly less ($P < 0.001$) than at –0.3 MPa and –0.05 MPa. Conversely, CO_2-C evolution in PR test samples calibrated to –0.3 MPa was less than at –0.05 MPa and –0.01 MPa, but only during the latter 14 days of incubation ($P < 0.05$). The rate of CO_2-C evolution in control soils was not affected by matric potential, however, differences were observed between control soils, as CO_2-C evolution was always greatest in WB soil ($P < 0.01$) (Appendix C: Figure C.3).

5.2.3. Cumulative CO_2-C evolution

Cadaver burial resulted in an increase in cumulative CO_2-C evolution in all soils at each matric potential (Figure 5.2). Cumulative CO_2-C evolution in FA and WB test samples calibrated to –0.01 MPa was significantly less ($P < 0.001$) than at –0.05 MPa and –0.3 MPa. In contrast, cumulative CO_2-C evolution in PR test samples calibrated to –0.3 MPa was less than at –0.05 MPa and –0.01 MPa ($P < 0.05$). A soil effect was observed at –0.3 MPa and –0.05 MPa as WB test samples generated more CO_2-C than FA and PR test samples ($P < 0.01$). However, in test samples calibrated to –0.01 MPa, CO_2-C evolution in PR soil was greater than in WB soil which was greater than in FA soil ($P < 0.01$). Soil matric potential did not affect cumulative CO_2-C evolution in control samples. Carbon dioxide evolution among control samples was greatest in WB samples ($P < 0.05$) (Appendix C: Figure C.3). Significant correlation relationships were observed between cadaver mass loss and

cumulative CO_2-C evolution in all test samples except WB calibrated to –0.01 MPa (Figure 5.3; Table B.2).

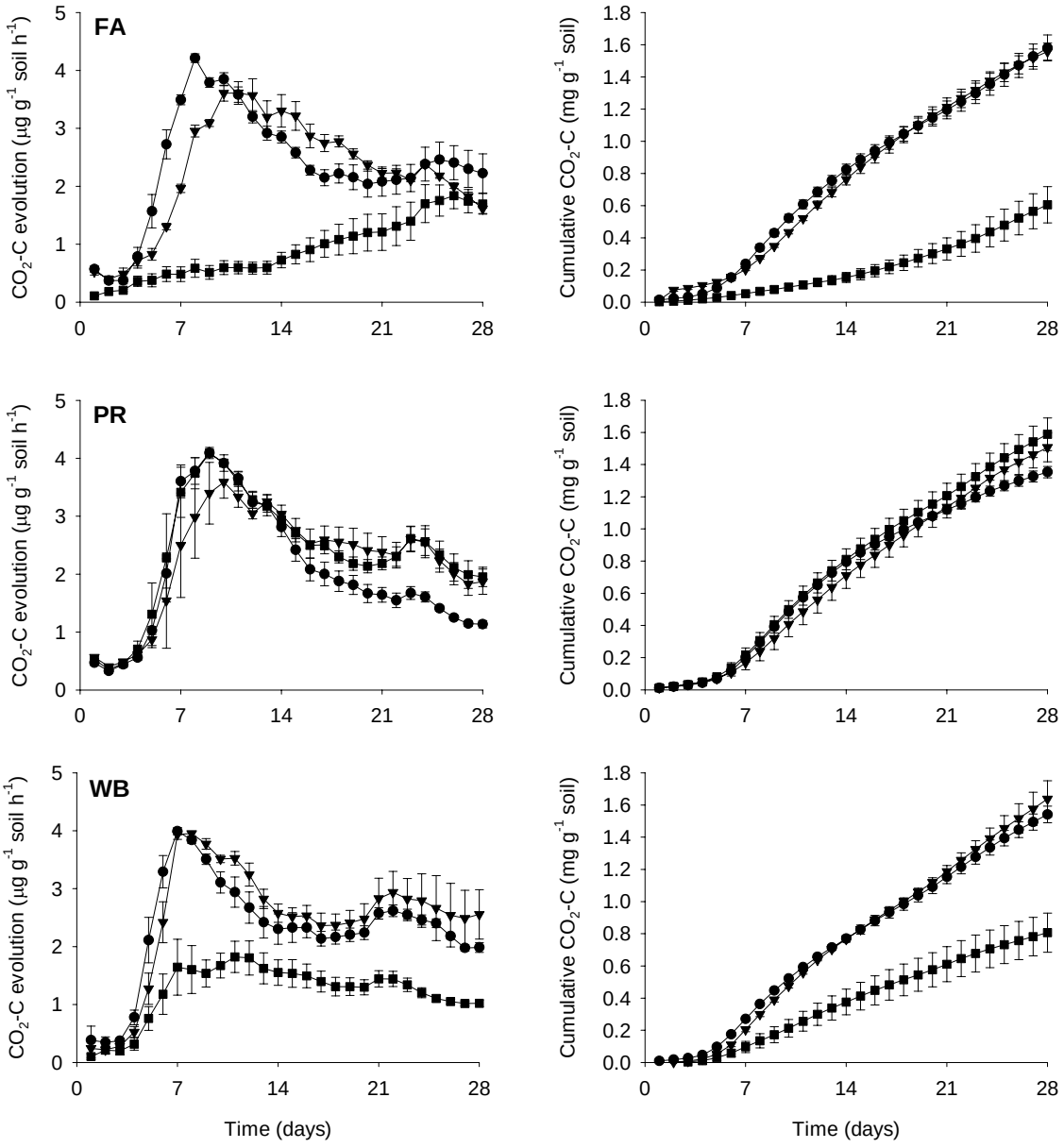

Figure 5.2. Daily (left column) CO_2-C (mg CO_2-C g^{-1} soil h^{-1}) and cumulative (right column) CO_2-C evolution (mg CO_2-C g^{-1} soil) (minus control samples) in 500 g (dry weight) sieved (2 mm) soil following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (filled symbols) and in control samples from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queeensland, Australia calibrated to a matric potential of –0.3 megapascals (MPa) (●), -0.05 MPa (▼) or –0.01 MPa (■) and incubated 22 °C. Bars represent standard errors where n = 3.

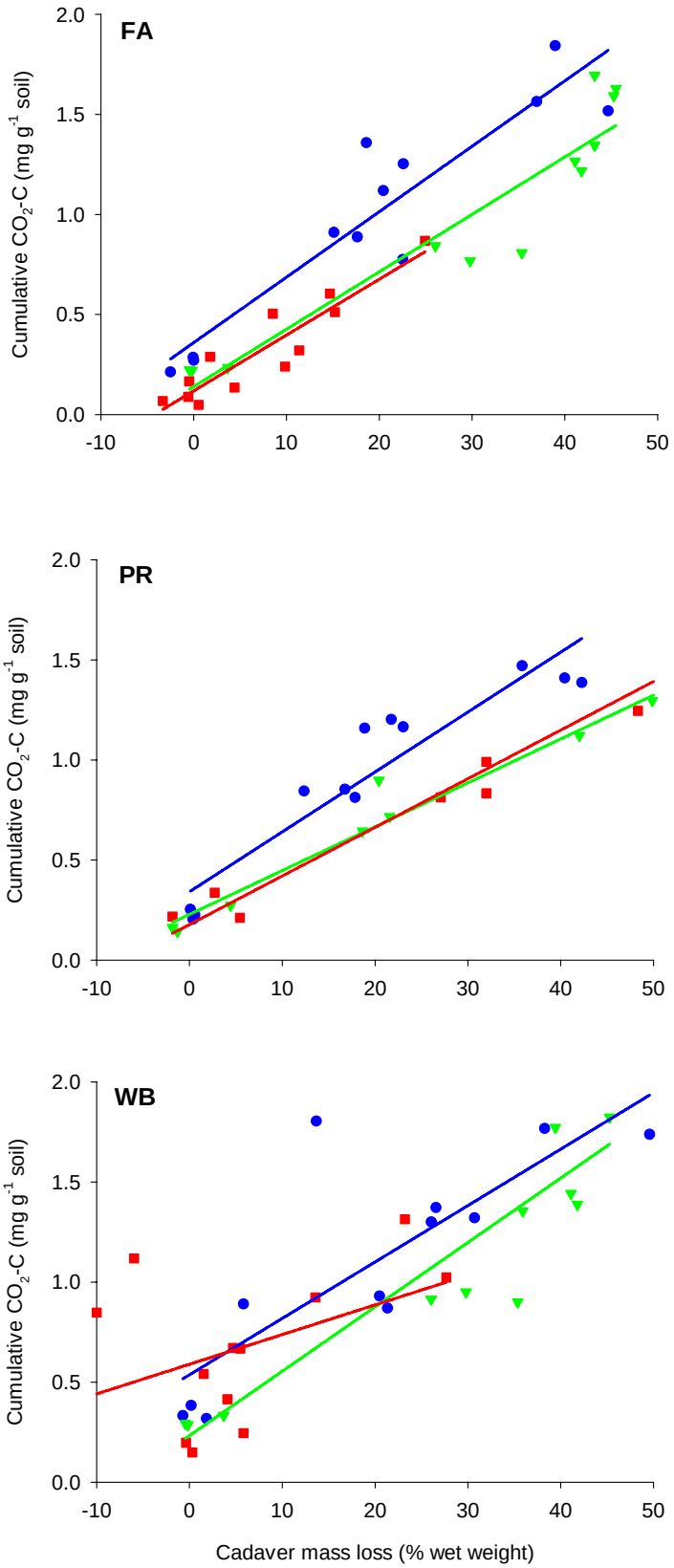

Figure 5.3. Correlation between cumulative CO_2-C evolution (mg g^{-1} soil) and mass loss (% wet weight) of a juvenile rat (*Rattus rattus*) cadaver buried (2.5 cm) in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savannas of Queeensland, Australia calibrated to a matric potential of –0.3 megapascals (MPa) (●), -0.05 MPa (▼) or –0.01 MPa (■) and incubated 22 °C. Regression equations are presented in Appendix B: Table B.2.

Interestingly, the aerobic catabolic efficiency (ACE) in PR (-0.3 MPa, -0.05 MPa) and WB (-0.01 MPa) decreased over time, indicating an increase in catabolic efficiency (Table 5.2). Matric potential also had an effect on ACE in these soils. A decrease in catabolic efficiency was observed in PR soil calibrated to –0.3 MPa and WB soil calibrated to –0.01 MPa. All treatments were associated with similar ACE except WB calibrated to –0.01 MPa, which was associated with a significantly higher ACE value ($P < 0.05$).

Table 5.2. Aerobic catabolic efficiency (ACE: mg CO_2-C g^{-1} soil % wet $weight^{-1}$ cadaver mass loss) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia incubated at 22 °C and calibrated to a matric potential of –0.3 megapascals (MPa), -0.05 MPa or –0.01 MPa. Numbers in brackets represent standard error of the mean. a, b, c represent a significant difference ($P < 0.05$) between time within soil and temperature. y, z, represent a significant difference ($P < 0.05$) .

		Day				
Soil	Matric potential (MPa)	7	14	21	28	Mean ACE
FA	-0.3	0.04^{a} (0.01)	0.05^{a} (0.01)	0.06^{a} (0.01)	0.04^{a} (0.02)	0.04^{z} (0.01)
	-0.05	0.02^{a} (0.02)	0.03^{a} (0.00)	0.03^{a} (0.00)	0.04^{a} (0.00)	0.03^{z} (0.01)
	-0.01	0.03^{a} (0.03)	0.02^{a} (0.01)	0.08^{a} (0.04)	0.04^{a} (0.00)	0.04^{z} (0.02)
PR	-0.3	0.05^{ab} (0.00)	0.06^{a} (0.01)	0.06^{a} (0.00)	0.03^{b} (0.00)	0.05^{z} (0.00)
	-0.05	0.05^{a} (0.00)	0.03^{b} (0.00)	0.03^{b} (0.00)	0.03^{b} (0.00)	0.03^{y} (0.00)
	-0.01	0.03^{a} (0.01)	0.03^{a} (0.00)	0.03^{a} (0.00)	0.03^{a} (0.00)	0.03^{y} (0.00)
WB	-0.3	0.06^{a} (0.04)	0.08^{a} (0.04)	0.05^{a} (0.04)	0.07^{a} (0.03)	0.06^{y} (0.02)
	-0.05	0.03^{a} (0.02)	0.03^{a} (0.02)	0.04^{a} (0.02)	0.05^{a} (0.02)	0.04^{y} (0.01)
	-0.01	0.18^{a} (0.09)	0.19^{a} (0.09)	0.06^{b} (0.04)	0.04^{b} (0.02)	0.12^{z} (0.04)

5.2.4. Microbial biomass C (C_{mic})

Cadaver burial resulted in an increase in C_{mic} in all soils and this increase was modified by matric potential (Figure 5.4) and associated with a loss of cadaver mass as little as 0.5%. In FA soil an elevated level of C_{mic} took place over a period of two harvest events (day 14, 21), which was delayed by seven days at a matric potential of –0.01 MPa. In PR test samples elevated C_{mic} was observed on day 7 and elevated levels were prolonged with each decrease in matric potential. WB test samples calibrated to –0.3 MPa contained an elevated concentration of C_{mic} at each harvest while WB test samples showed a trend similar to that of FA test samples: a matric potential of–0.01 MPa resulted in a delay in the growth of the microbial biomass. Microbial biomass C was greatest in WB test and control samples ($P < 0.05$).

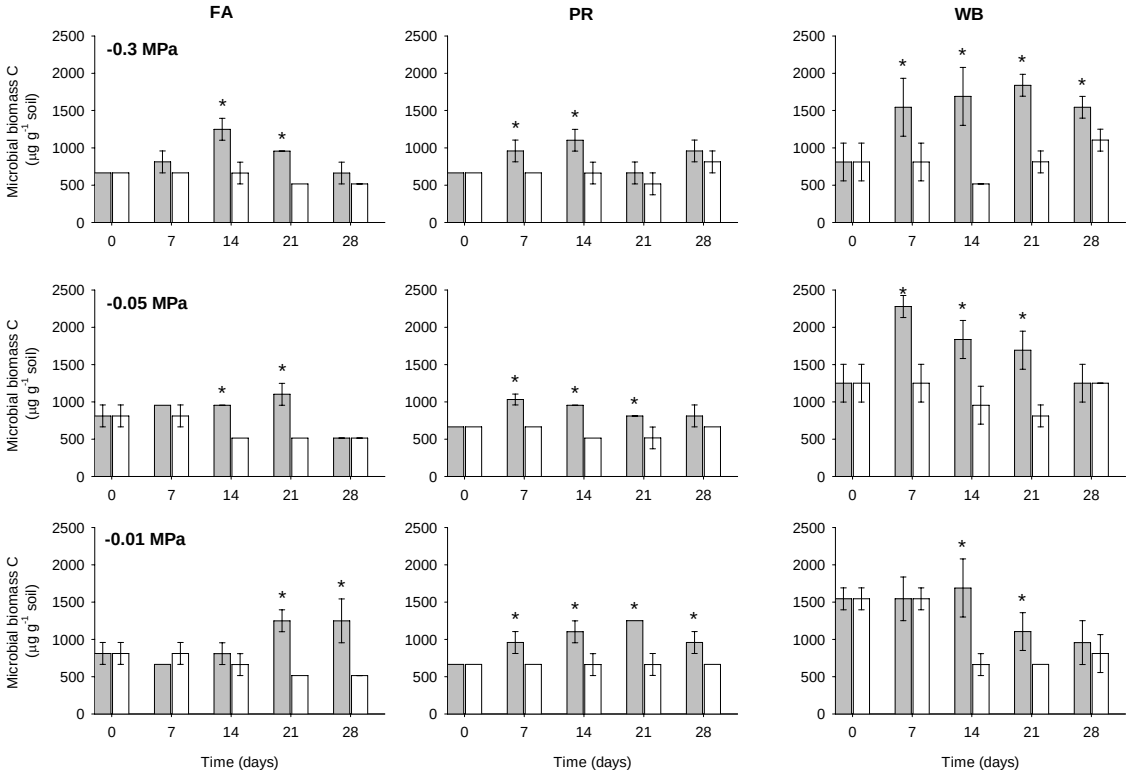

Figure 5.4. Microbial biomass C (µg g^{-1} soil) in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savannas of Queeensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (■) and in control samples (no cadaver: □) calibrated to a matric potential of –0.3 megapascals (MPa), -0.05 MPa or –0.01 MPa and incubated 22 °C. Bars represent standard errors where n = 3. * represents significant difference ($P < 0.05$) between test and control samples.

Fungal hyphae were seen on the soil surface of all test samples (Table 5.2; Figure 4.5). The hyphae were present on day 14 and day 21 in all test treatments but were first observed in PR (-0.3 MPa, -0.01 MPa) and WB (-0.3 MPa, -0.05 MPa) test samples on day 7 (Table 5.2). The hyphae were seen for the longest period of time in WB test samples calibrated to –0.3 MPa or –0.05 MPa (Table 5.2). No hyphae were seen on the surface of control soils.

Table 5.3. Macroscopic detection (+) of fungal hyphae on the surface of 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia following calibration to a matric potential of –0.3 megapascals (MPa), -0.05 MPa or –0.01 MPa and the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver. Samples were incubated at 22 °C. + represents the number of replicates in which fungal hyphae was observed (e.g. ++ = fungal hyphae seen in two replicates).

	FA			PR			WB		
Time (days)	-0.3 MPa	-0.05 MPa	-0.01 MPa	-0.3 MPa	-0.05 MPa	-0.01 MPa	-0.3 MPa	-0.05 MPa	-0.01 MPa
0									
7				+		+	+++	+++	
14	+++	+++	+++	+++	+++	+++	+++	+++	+++
21	+++	+++	+++	+++	+++	+++	+	++	++
28							+++	+++	

5.2.5. Metabolic quotient (qCO_2)

Cadaver burial resulted in an elevated qCO_2 (Figure 5.5). A modification of this increase was observed in FA test samples as a matric potential of –0.01 MPa was associated with a lower qCO_2 than at –0.3 MPa and –0.05 MPa ($P < 0.05$). The qCO_2 of WB control soil was greater than PR and FA control soil ($P < 0.05$).

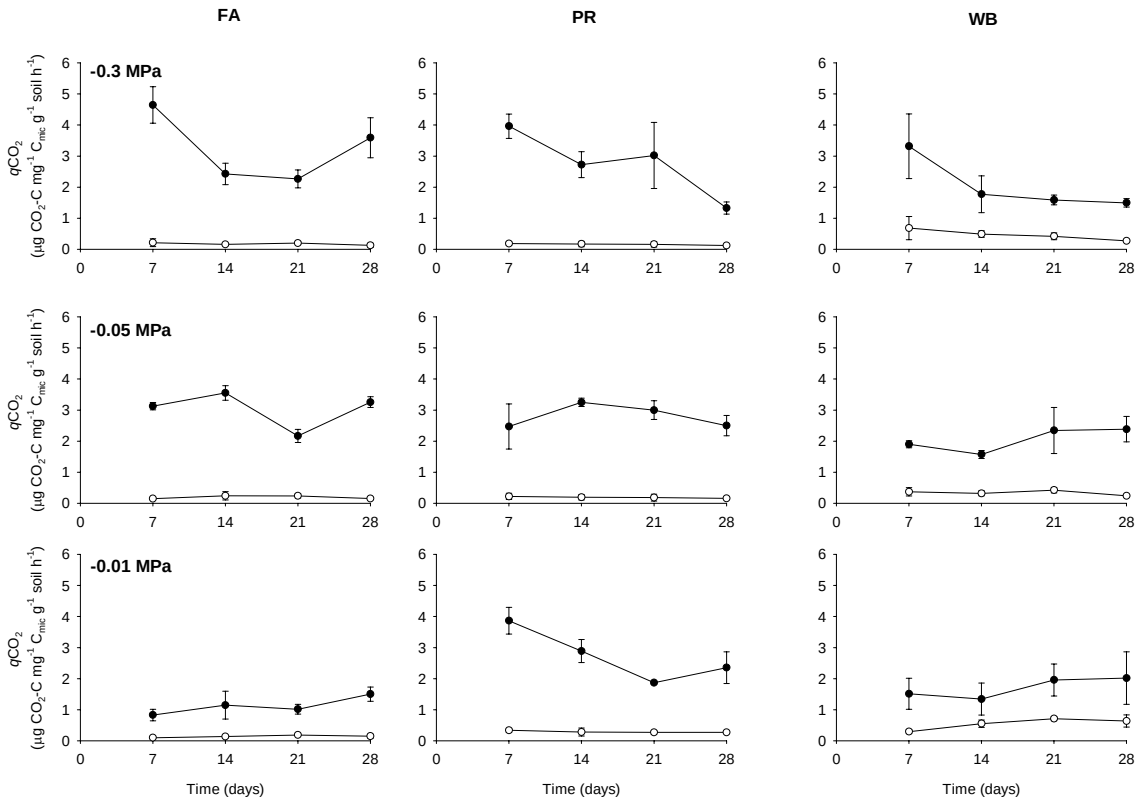

Figure 5.5. Metabolic quotient (qCO_2: µg CO_2-C mg^{-1} C_{mic} g^{-1} soil h^{-1}) in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites from tropical savanna ecosystems in Queeensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and in control (○) samples (no cadaver) calibrated to a matric potential of –0.3 megapascals (MPa), -0.05 MPa or –0.01 MPa and incubated 22 °C. Bars represent standard errors where n = 3.

5.2.6. Protease activity

Cadaver burial resulted in an increase in protease activity in all soils (Figure 5.6). Matric potential only had an effect on FA test samples, as protease activity was greatest at –0.05 MPa ($P < 0.05$). Protease activity among test samples was greatest in WB soil but this was only observed in soils calibrated to –0.05 MPa or –0.01 MPa ($P < 0.05$). Among control samples WB soil always contained the greatest levels of protease activity ($P < 0.05$).

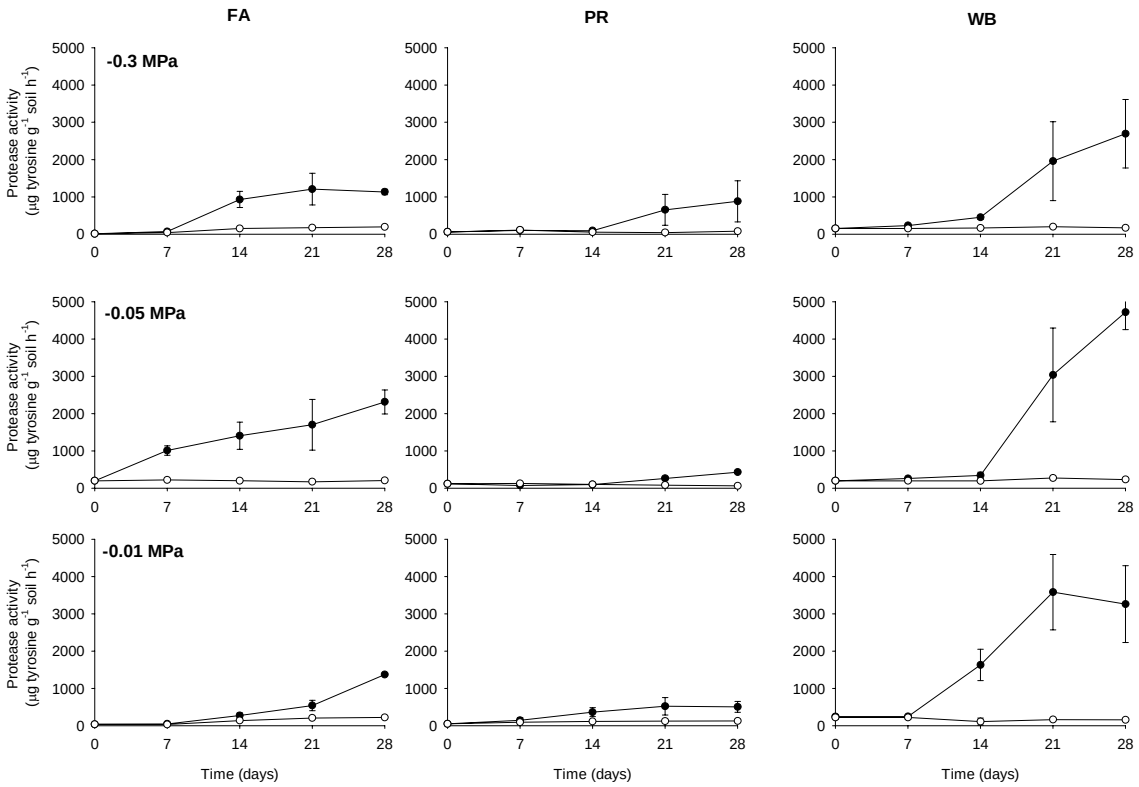

Figure 5.6. Protease activity (µg tyrosine g^{-1} soil h^{-1}) in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites from tropical savanna ecosystems in Queeensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and in control (○) samples (no cadaver) calibrated to a matric potential of –0.3 megapascals (MPa), -0.05 MPa or –0.01 MPa and incubated 22 °C. Bars represent standard errors where n = 3.

5.2.7. Phosphodiesterase activity

Cadaver burial resulted in an increase in phosphodiesterase activity in all soils (Figure 5.7). This increase was modified by matric potential in FA test samples as calibration to –0.05 MPa resulted in a greater rate of phosphodiesterase activity than at –0.3 MPa and –0.01 MPa ($P < 0.05$). A soil effect was observed in test samples at all matric potentials. At –0.3 MPa and –0.05 MPa phosphodiesterase activity was greatest in FA test samples ($P < 0.05$). Conversely, at –0.01 MPa the least phosphodiesterase activity was observed in FA test samples ($P < 0.05$). Matric potential did not affect phosphodiesterase activity in FA and PR control samples. However, the calibration of WB control soil to –0.01 MPa resulted in a lower rate of

phosphodiesterase activity than at –0.3 MPa ($P < 0.01$). Among control samples WB soil always contained the greatest level of phosphodiesterase activity ($P < 0.05$).

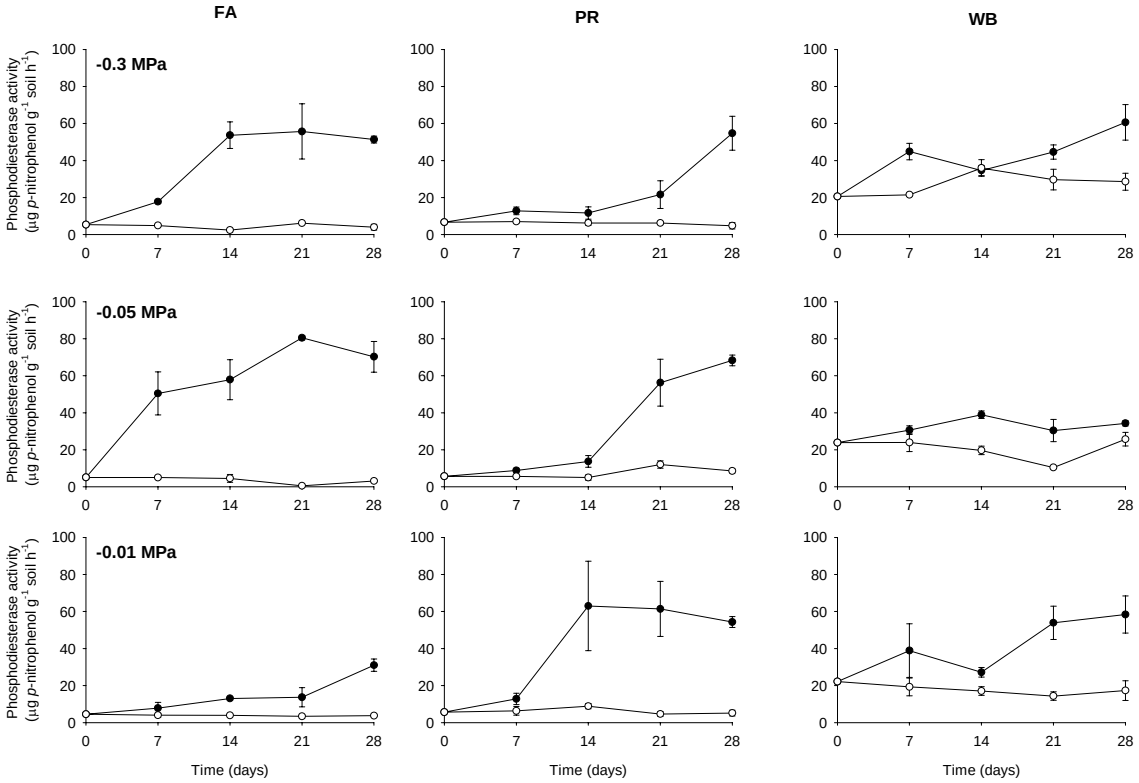

Figure 5.7. Phosphodiesterase activity (µg *p*-nitrophenol g^{-1} soil h^{-1}) in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites from tropical savanna ecosystems in Queeensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and in control (○) samples (no cadaver) calibrated to a matric potential of –0.3 megapascals (MPa), -0.05 MPa or –0.01 MPa and incubated 22 °C. Bars represent standard errors where n = 3.

5.2.8. Soil pH

Cadaver burial resulted in an increase in soil pH to approximately pH 8 and this was affected by matric potential (Figure 5.8). A matric potential of –0.01 MPa resulted in a slower rate of increase of soil pH in FA and WB test samples. Conversely, a matric potential of –0.3 MPa resulted in a slower pH increase in PR test samples. In addition, soil pH had begun to decrease by the end of the incubation in FA (-0.3 MPa, -0.05 MPa), PR (-0.05 MPa, -0.01 MPa) and WB (-0.3 MPa, -0.05 MPa) soils.

Among control samples the pH of WB was greater than PR, which was greater than FA ($P < 0.05$). A matric potential effect was observed in WB control samples as calibration to –0.3 MPa resulted in increase in soil pH ($P < 0.05$).

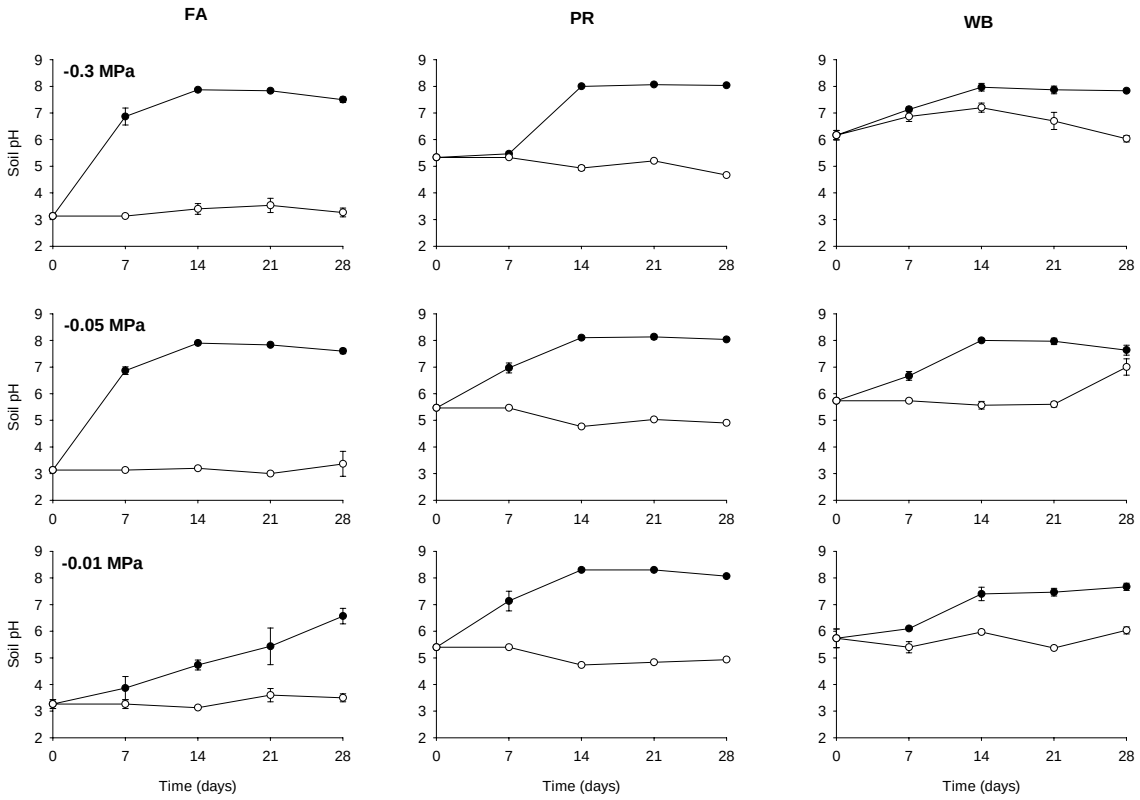

Figure 5.8. The pH of sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites from tropical savanna ecosystems in Queeensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and in control (○) samples (no cadaver) calibrated to a matric potential of –0.3 megapascals (MPa), -0.05 MPa or –0.01 MPa and incubated 22 °C. Bars represent standard errors where n = 3.

5.3. Discussion

The current results show that soil moisture can affect cadaver decomposition in soil. A decrease in matric potential (i.e. increase in bioavailable moisture) can result in greater cadaver decomposition but the optimal level of moisture can be exceeded, resulting in less cadaver decomposition. In FA and WB soil the greatest cadaver decomposition occurred at –0.05 MPa (Figure 5.1). In PR soil a matric potential of –0.05 MPa or –0.01 MPa resulted in the greatest cadaver decomposition (Figure

5.1). Cadaver decomposition at these matric potentials progressed through early phase, intermediate phase and late phase decomposition similar to that discussed in Chapter 4.

There was an interaction between matric potential and soil whereby cadavers buried in FA or WB soil calibrated to –0.01 MPa underwent slower cadaver decomposition than at higher matric potentials. Cadaver decomposition under these conditions was characterised by a decreased rate of cadaver mass loss (Figure 5.1) and CO_2-C evolution (Figure 5.2), as well as a delay in the increase of C_{mic} (Figure 5.3), phosphodiesterase activity (Figure 5.7) and soil pH (Figure 5.8). In WB soil calibrated to –0.01 MPa an uncoupling of the relationship between cadaver mass loss and cumulative CO_2-C evolution took place (Figure 5.3) and this was associated with a low aerobic catabolic efficiency (Table 5.2). These phenomena might be due to the decreased diffusion coefficient of gases (Table 5.1) and the consequent mobility to meet the high microbial demand for O_2 during the aerobic catabolism of an organic resource. This might result in the proliferation of anaerobic microorganisms, which are typically associated with a decreased rate of decomposition (Swift et al., 1979) and the evolution of incompletely oxidised C compounds (e.g. methane) (Sommers et al., 1981). Gases other than CO_2 (e.g. H_2S, NH_3, CH_4) have been associated with cadaver decomposition (Gill-King, 1997; Vass et al., 2004). This fact is the basis for the major criticism of the use of CO_2-C evolution as an index of microbial activity associated with cadaver decomposition: Any C evolved as a part of a compound other than CO_2 can result in an underestimation of microbial activity. Testing for other C containing compounds was not undertaken in the current study (due to technical reasons), however, it has

been shown (using gas chromatography) that CO_2 is the only respiratory gas detected during the decomposition of exposed mouse cadavers (Putman, 1976). However, this does not confirm that such was the case in the current study. The analysis of the gas in the incubation chamber headspace would have likely been able to confirm reducing conditions in the FA and WB test samples calibrated to –0.01 MPa. Unfortunately, this was unable to be carried out in the current study for technical reasons.

A higher matric potential (-0.3 MPa) in FA and PR soils also retarded the processes associated with cadaver decomposition. This was observed as a decreased rate of cadaver mass loss (Figure 5.1), a relatively short period of increased C_{mic} in PR soil (Figure 5.4) and a decreased rate of protease activity in FA soil (Figure 5.6). In PR soil the effect of high matric potential on mass loss was first observed on day 14 and continued until day 28 (Figure 5.1). This time period was associated with a lower rate of CO_2-C evolution (Figure 5.2), a decline in C_{mic}, which was similar to control samples on day 21 and day 28) (Figure 5.4) as well as increases in protease (Figure 5.6) and phosphodiesterase (Figure 5.7) activities. All of these findings are indicative of a lack of nutrient supply to the soil microbial biomass, which is probably due to the low water content. Bacterial movement tends to be restricted to water films as matric potential increases and these organisms can only remain active while nutrients are able to diffuse to them and waste products are able to diffuse away from them (Wong and Griffin, 1976a). The presence of cadaveric moisture, which might have been insufficient to sustain microbial activity throughout the incubation, could explain the lack of a decreased rate of CO_2-C evolution during the initial 7 days of burial (Figure 5.2). In addition, the rate of CO_2-C evolution may

have been augmented by an intensification of food web interactions in hydrologically isolated microsites (Görres et al., 1999). Decreased protease activity in FA soil supports the concept of altered hydrolytic enzyme activity with an increase in matric potential (Skujins and McLaren, 1967).

In the current study cadaver decomposition was observed to differ between soils. At –0.05 MPa and –0.01 MPa the greatest cadaver decomposition was observed in PR soil. The relatively high rate of gas diffusion (Table 5.1) in PR soil probably allowed for a sufficient exchange of gas to meet the microbial demand for O_2. This finding is in contrast to the belief that soil type does not have a significant influence on cadaver decomposition (Mant, 1950; Morovic-Budak, 1965; Mant, 1987). However, Mant (1950) did observe that cadaver burial in sandy soils tended to result in increased rates of cadaver desiccation. Interestingly, a soil effect was not observed at -0.3 MPa (Figure 5.1). This finding, along with the apparent interactions between soil and matric potential, might reflect the participation of the soil microbial biomass in cadaver decomposition. If the microbial population primarily responsible for cadaver decomposition is enteric (Evans, 1963) then it would seem that the burial environment would have little effect on cadaver decomposition until, possibly, the breakdown of the epidermis when the abdominal cavity would become exposed to the burial environment. If this were true then it would be logical to hypothesise that soil matric potential would have little effect on cadaver decomposition. This hypothesis would be rejected by the current results. The estimation of C_{mic} (Figure 5.4) shows a clear cadaver and matric potential effect although it is not possible to say with any certainty if the observed biomass has originated from the cadaver or the soil.

As discussed in Chapter 4 the measurement of qCO_2 does not provide a great deal of insight into the microbial metabolic efficiency associated with cadaver decomposition in soil. The qCO_2 does consistently provide a crude indication of the presence of a cadaver due its elevated value (Figure 5.5) although it is felt little else can be learned from this measure. The aerobic catabolic efficiency (ACE) (Table 5.2) showed that extreme matric potential (-0.3 MPa in PR; -0.01 in WB) can affect the efficiency with which a cadaver is broken down. The relative lack of soils effect on ACE is not surprising for soils from ecosystems defined, in part, by alternating wet season and dry season. The soil microbial biomass has probably adapted to exceedingly dry and wet conditions.

The measurement of soil pH (Figure 5.8) as well as the presence of fungal hyphae again proved to consistently indicate the presence of a cadaver regardless of soil and, thus, might have potential to become a forensic tool in the location of clandestine graves. Soil matric potential did, however, have an effect on the rate at which soil pH increased.

In conclusion, cadaver decomposition can increase with a decrease in matric potential. However, the optimal matric potential for cadaver decomposition can be exceeded, resulting in a retardation of process rates and less cadaver mass loss (probably due to the onset of anaerobiosis). In addition, a decrease in the rate of cadaver decomposition can take place at high matric potential (-0.3 MPa). This is likely due to a restriction on microbial activity high matric potential.

CHAPTER 6

CAN EVISCERATION RESULT IN A DECREASED RATE OF CADAVER DECOMPOSITION IN SOIL?

A cadaver is an extremely complex resource. This is due, in part, to the presence of the internal organs (viscera) that make up the gastrointestinal tract (oesophagus, stomach, small intestine, large intestine), lower respiratory system (larynx, trachea, bronchi, lungs) and accessory digestive organs (liver, gallbladder, pancreas) (see Tortora and Grabowski, 2000). These organs are associated with a substantial amount of water (Reinoso et al., 1997), a large microbial population (e.g. *Eschericia coli, Lactobacillus, Streptococcus, Bacteroidaceae*) (Yajima et al., 2001) and a rapid rate breakdown (Gill-King, 1997). Therefore, the internal organs play a major role in the physicochemistry, resource quantity/quality and population of decomposer organisms associated with a cadaver. Each of these factors can have an effect on the decomposition of an organic resource in a terrestrial ecosystem (Swift et al., 1979).

The gastrointestinal tract, lower respiratory system and accessory digestive organs are typically the first components of a cadaver to decompose (Gill-King, 1997). This is due to the large number and variety of hydrolytic enzymes (e.g. protease, phosphodiesterase, lipase) and microorganisms (e.g. *Clostridium, Streptococcus, Bacillus*) associated with these organs. It is well understood that these microorganisms are responsible for the anaerobic degradation of a cadaver (putrefaction) following the cessation of the heart (Clark et al., 1997; Gill-King, 1997) and they are credited with initiating and fuelling cadaver decomposition when the activity of arthropods and vertebrate scavengers is restricted, such as following

burial (Evans, 1963; Janaway, 1996). Thus, traditional dogma states that the internal organs play a major role cadaver decomposition (Evans, 1963; Micozzi, 1986; Janaway, 1996) and their removal will result in a slower rate of cadaver decomposition. However, this view has never been scientifically validated.

In order to examine the effect of evisceration on cadaver decomposition a controlled laboratory experiment was carried out to determine if the evisceration of a cadaver (*Rattus rattus*) would result in a decreased rate of decomposition. Soils from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites (see Chapter 3) were collected (September 2003) and prepared as described in Chapter 2. Soils were then calibrated to a matric potential of –0.05 megapascals (MPa) and equilibrated for seven days at 22 °C. The current experiment used four cadaver treatments including a complete cadaver (CC), an eviscerated cadaver (EC), a cadaver with a sown incision only (CI) and a control (soil without cadaver). In order to implement these treatments rats were euthanised with carbon dioxide (CO_2). For EC and CI treatments an incision was made from the anus to the sternum (Figure 6.1). The internal organs of the lower respiratory system (larynx, trachea, bronchi, lungs), gastrointestinal tract (oesophagus, stomach, small intestine, large intestine) and accessory digestive organs (liver, gallbladder, pancreas) were removed using a scalpel and forceps. Following evisceration the abdominal and thoracic cavities were rinsed with sterile distilled water and stitched using a 4/0 nylon suture (Braun Surgical, Emmenbrucke, Germany) (Figure 6.1). Cadavers designated for CI treatment were incised and stitched without evisceration. Complete cadavers did not receive an incision or stitching prior to burial. The experiment was replicated four times. Each treatment was set up with sufficient replicates for four sequential

harvest events resulting in a total of 192 samples. This experiment was conducted to test the hypothesis that the evisceration of a cadaver will result in a slower rate of decomposition relative to a complete cadaver.

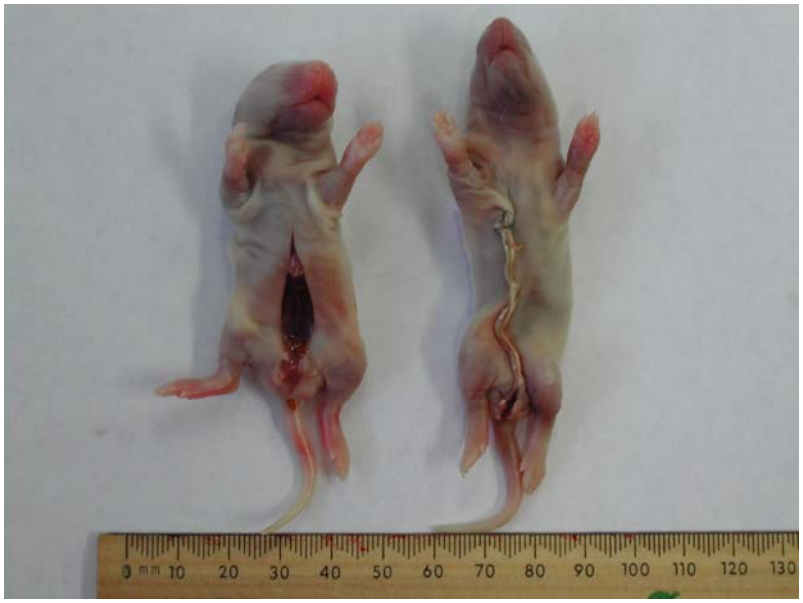

Figure 6.1. Eviscerated and stitched rat (*Rattus rattus*) cadavers. The cadaver on the left shows the incision from anus to sternum. The cadaver on the right shows the sown incision.

6.2. Results

6.2.1. Cadaver mass loss

Evisceration resulted in a decrease in cadaver mass loss in PR soil ($P < 0.05$) (Figure 6.2). Cadaver treatment did not affect mass loss in FA and WB soils (Figure 6.2). Among soil treatments the mass loss of CC and CI treatments was greatest in PR soil ($P < 0.05$) while mass loss of EC was greatest in FA soil ($P < 0.05$).

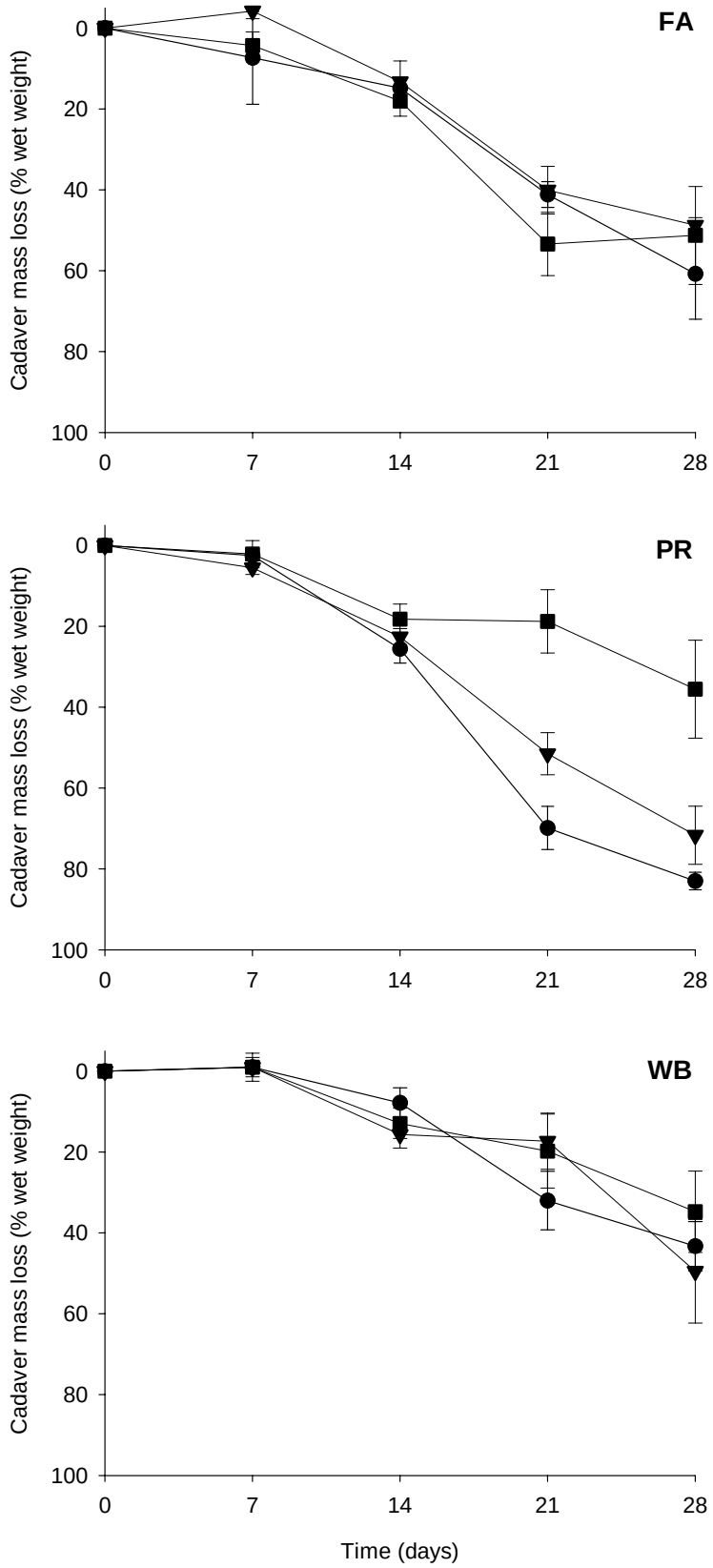

Figure 6.2. Mass loss (% wet weight) of a complete juvenile rat (*Rattus rattus*) cadaver (CC: ●), cadaver with sown incision (CI: ▼) or eviscerated cadaver (EC: ■) following burial (2.5 cm) in 500 g sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia. Soils were calibrated to a matric potential of –0.05 megapascals prior to burial. Bars represent standard errors where n = 4.

6.2.2. Daily carbon dioxide (CO_2-C) evolution

Cadaver burial resulted in an increase in CO_2-C evolution in all soils (Figure 6.3). However, the rate of CO_2-C evolution associated with EC was less than in other test samples at some point during the incubation (Figure 6.3). In FA soil this was observed from day 12 to day 19. In PR soil this was observed from day 13 to day 27. In WB soil this was observed from day 12 to day 14.

6.2.3. Cumulative CO_2-C evolution

Cadaver burial resulted in greater cumulative CO_2-C than observed in all control samples (Figure 6.3). In FA and WB soil cadaver treatment did not have an effect on cumulative CO_2-C evolution. However, the burial of EC in PR soil resulted in less cumulative CO_2-C ($P < 0.05$) relative to other PR test samples.

Significant correlation relationships existed between cadaver mass loss and cumulative CO_2-C in all samples (Figure 6.4) (Appendix B: Table B.3). The efficiency with which a cadaver was aerobically decomposed (aerobic catabolic efficiency) tended to increase over time. However, this was not observed with EC. A negative ACE value was observed on day 7 in FA (CI) and WB (CC, EC) samples.

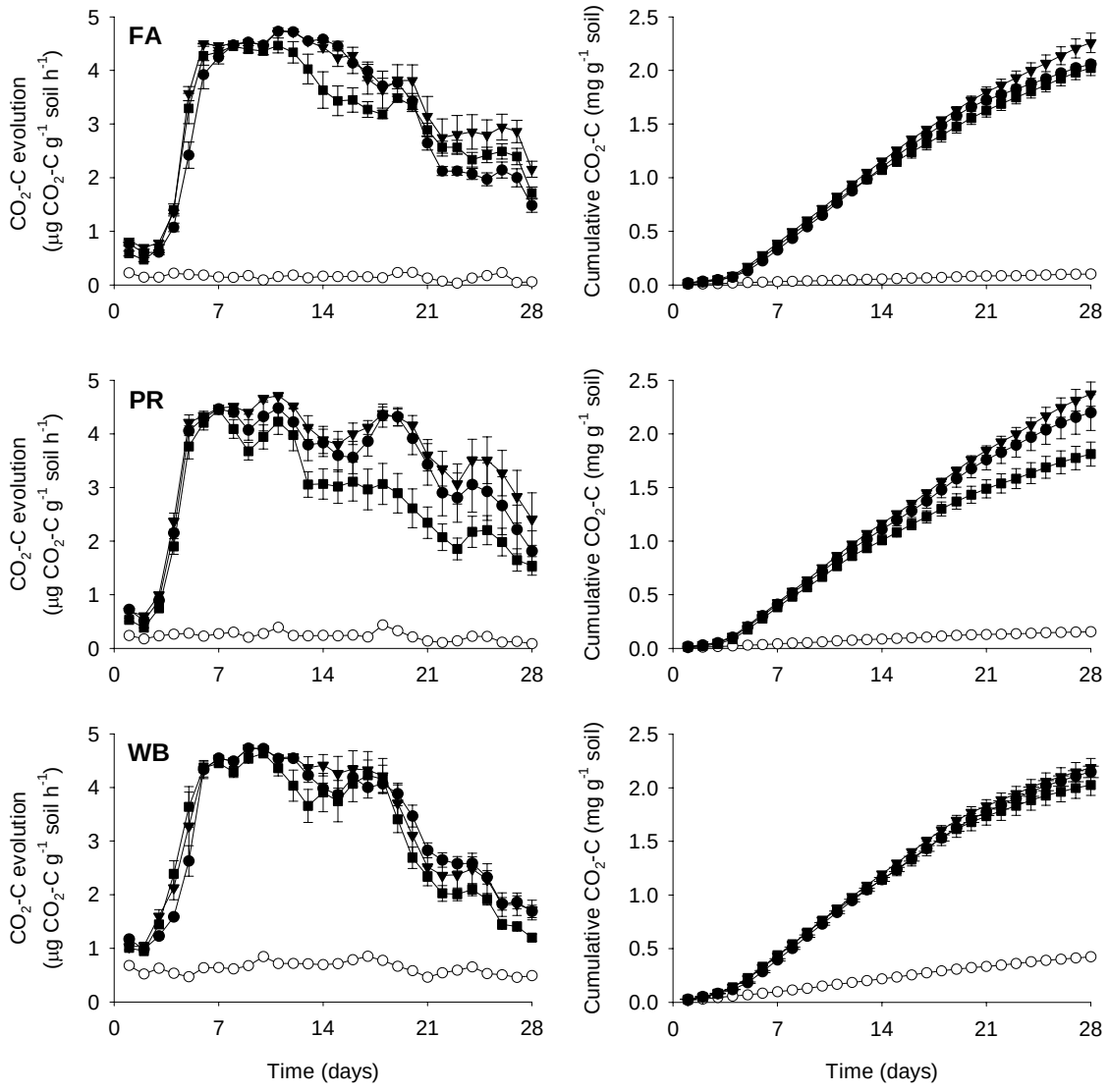

Figure 6.3. Daily (left column) and cumulative (right column) CO_2-C evolution in soil without a rat (*Rattus rattus*) cadaver (control: ○) and following burial (2.5 cm) of a complete cadaver (CC: ●), cadaver with a sown incision (CI: ▼) or eviscerated cadaver (EC: ■) in 500 g sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia. Soils were calibrated to a matric potential of –0.05 megapascals prior to burial. Bars represent standard errors where n = 4.

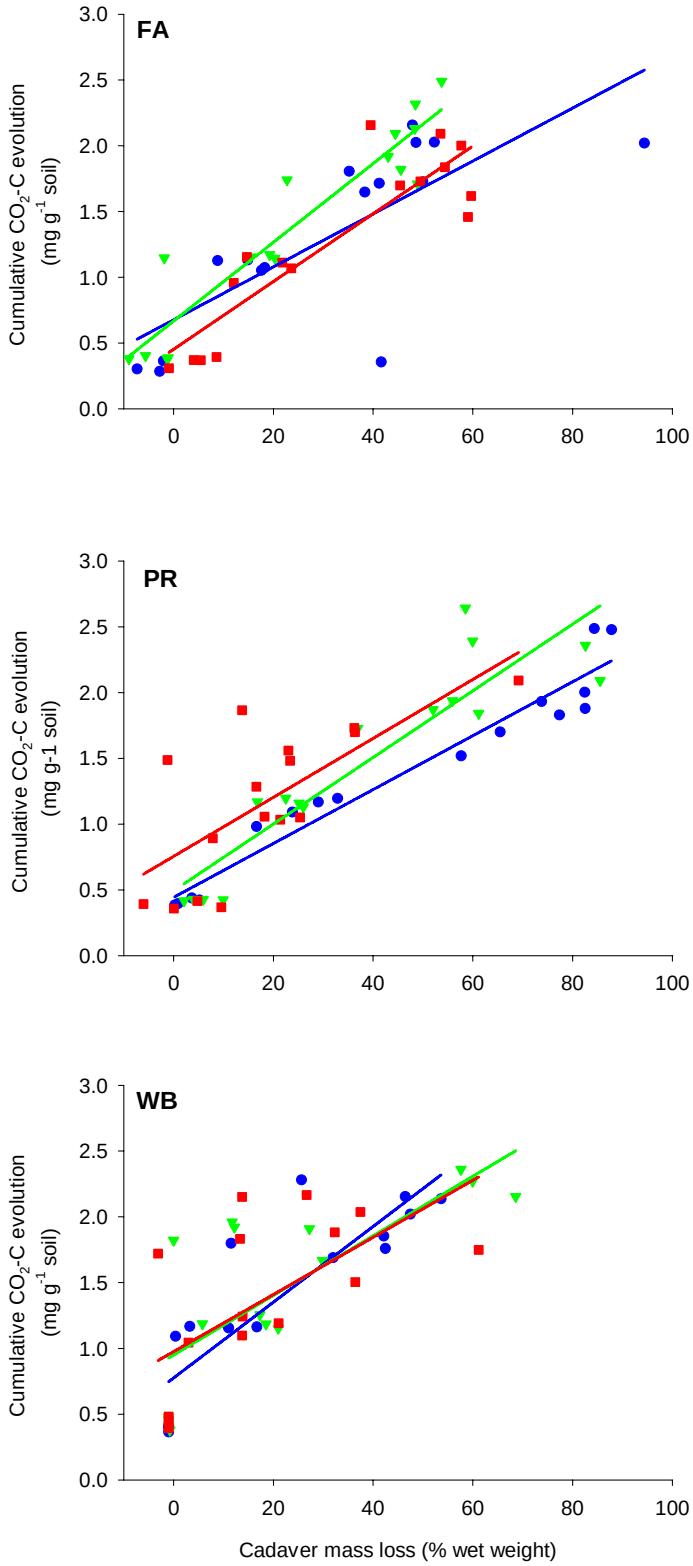

Figure 6.4. Correlation between cumulative CO_2-C evolution (g) and mass loss (% wet weight) following the burial (2.5 cm) of a complete cadaver (CC: ●), cadaver with sown incision (CI: ▼) or eviscerated cadaver (EC: ■) in 500 g sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia. Soils were calibrated to a matric potential of –0.05 megapascals prior to burial. Equations are presented in Appendix B: Table B.3.

Table 6.1. Aerobic catabolic efficiency (ACE: mg CO_2-C g^{-1} soil % wet $weight^{-1}$ cadaver mass loss) following the burial (2.5 cm) of a complete juvenile rat (*Rattus rattus*) cadaver (CC), cadaver with sown incision (CI) or eviscerated cadaver (EC) in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia incubated at 22 °C and calibrated to a matric potential of -0.05 megapascals. Numbers in brackets represent standard error of the mean. a, b, c represent a significant difference ($P < 0.05$) between time within soil and temperature. y, z, represent a significant difference ($P < 0.05$) .

Soil	Cadaver Treatment	Day 7	14	21	28	Mean ACE
FA	CC	0.08[a] (0.03)	0.08[a] (0.02)	0.04[a] (0.00)	0.04[a] (0.01)	0.06[z] (0.01)
	CI	-0.20[a] (0.08)	0.07[b] (0.00)	0.05[b] (0.01)	0.04[b] (0.00)	-0.01[z] (0.06)
	EC	0.07[a] (0.02)	0.07[a] (0.01)	0.03[a] (0.00)	0.04[a] (0.00)	0.05[z] (0.01)
PR	CC	0.09[a] (0.01)	0.05[b] (0.00)	0.03[b] (0.00)	0.03[b] (0.00)	0.05[z] (0.01)
	CI	0.11[a] (0.03)	0.05[b] (0.01)	0.04[b] (0.00)	0.03[b] (0.00)	0.06[z] (0.01)
	EC	0.07[a] (0.01)	0.07[a] (0.02)	0.07[a] (0.02)	0.07[a] (0.02)	0.07[z] (0.01)
WB	CC	-0.02[a] (0.17)	0.19[b] (0.07)	0.07[a] (0.03)	0.05[a] (0.01)	0.07[z] (0.05)
	CI	0.10[a] (0.14)	0.10[a] (0.14)	0.11[a] (0.04)	0.07[a] (0.03)	0.09[z] (0.03)
	EC	-0.01[a] (0.16)	0.14[a] (0.07)	0.10[a] (0.03)	0.08[a] (0.03)	0.08[z] (0.04)

6.2.4. Microbial biomass C (C_{mic})

Cadaver burial resulted in an increase in C_{mic} (Figure 6.5). In FA and PR soil this was associated with a 10%-20% loss of cadaver mass. In WB this occurred at ~1% loss of cadaver mass. An increase in C_{mic} in association with CC occurred by day 14. Increases in C_{mic} in association with CI were observed between day 7 and day 21. An increase in C_{mic} in soils containing EC tended to be restricted to the latter half of the incubation. An exception was WB soil, where an increase in C_{mic} with EC was observed on day 7. The greatest increase in C_{mic} was observed in WB soil.

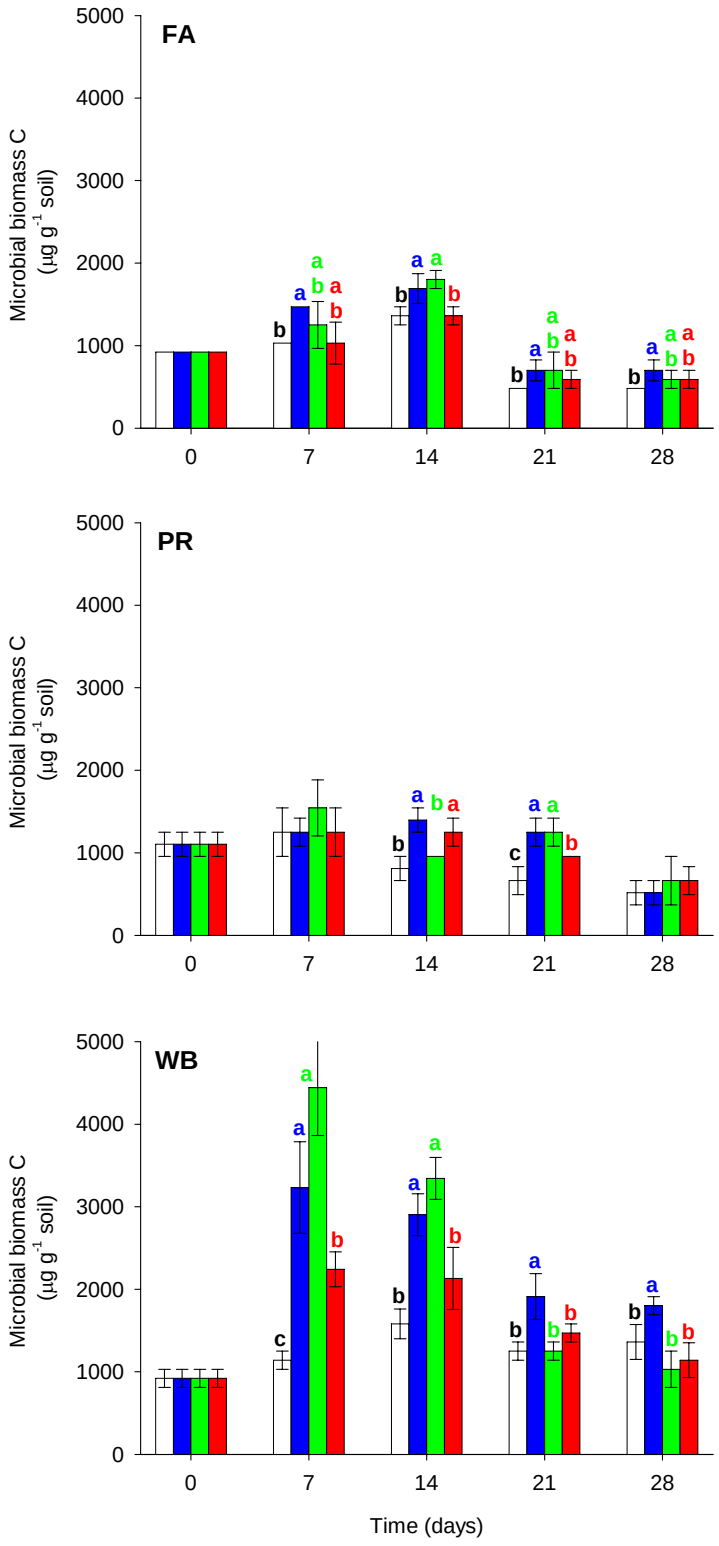

Figure 6.5. Microbial biomass C (µg g^{-1} soil) estimated via substrate-induced respiration associated with soil without a cadaver (*Rattus rattus*) (control: □) and following the burial (2.5 cm) of a complete cadaver (CC: ■), cadaver with a sown incision (CI: ■) or eviscerated cadaver (EC: ■) in 500 g sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia. Soils were calibrated to a matric potential of –0.05 megapascals prior to burial. Bars represent standard errors where n = 4. letters indicate an significant difference from the control ($P < 0.05$).

Fungal hyphae were macroscopically observed on the soil surface of test samples (Table 6.1). The presence of hyphae was most abundant in FA and PR soil. There was an apparent cadaver treatment effect in FA and PR soil as CI and EC resulted in an earlier presence of fungal hyphae. In addition, the fungal hyphae in association with EC in PR soil took on a different macroscopic appearance distinguished by the presence of conidia-like structures (Figure 6.6).

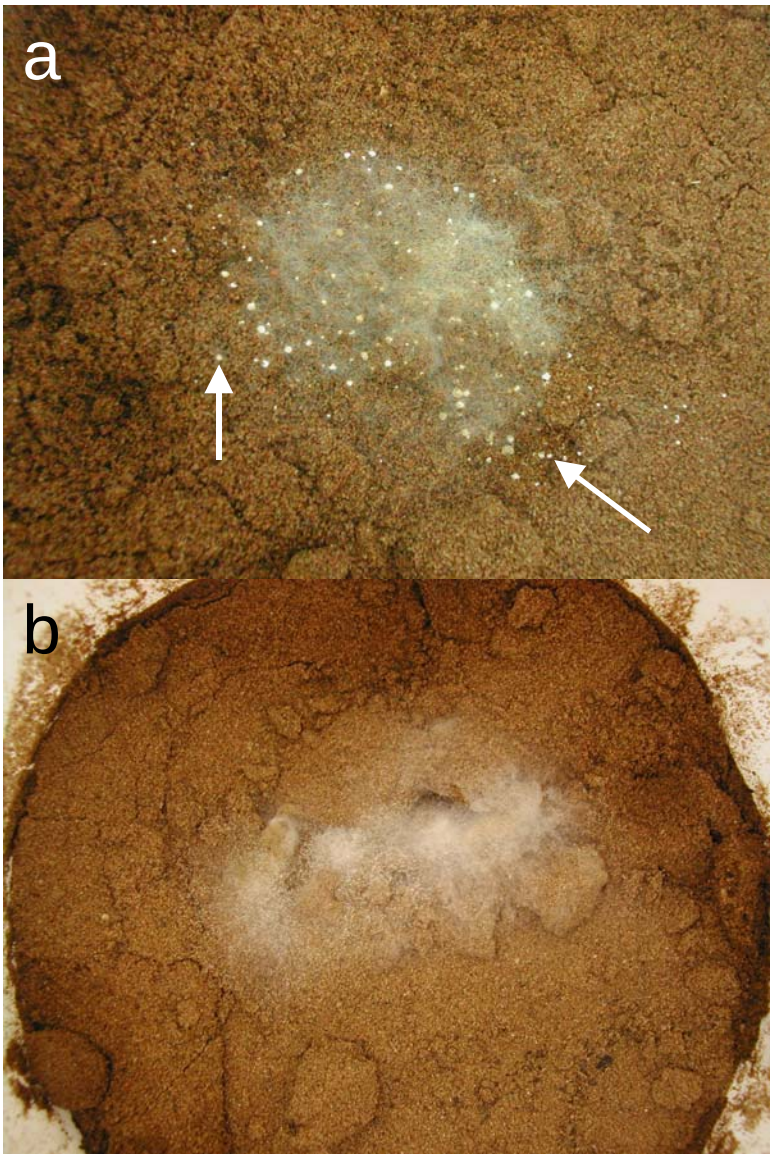

Figure 6.6. Presence of conidia-like structures (designated by arrows) on the soil surface above an eviscerated cadaver (*Rattus rattus*) (a) and presence of fungal hyphae on the soil surface above a complete cadaver (b) buried in soil from the Pallarenda site in a tropical savanna ecosystem in Queensland, Australia. Soils were calibrated to a matric potential of –0.05 megapascals.

Table 6.2. Macroscopic detection (+) of fungal hyphae on the surface of soil (500 g) from the Yabulu/FACE (FA), Pallarenda (PR) or Wambiana (WB) sites in the seasonally dry tropics of Queensland, Australia following the burial (2.5 cm) of a complete cadaver (*Rattus rattus*: CC), a cadaver with a sown incision (CI) or an eviscerated cadaver (EC) at 22 °C in soil calibrated to a matric potential of –0.05 megapascals over a period of 28 days. Number of + represents the number of replicates in which fungal hyphae was observed (e.g. ++ = fungal hyphae present in two replicates).

	FA			PR			WB		
Time (day)	CC	CI	EC	CC	CI	EC	CC	CI	EC
0									
7	++	++++	++++		++	+++			
14	++++	+	+	++++	++++	++++	++	++	+
21	++++	++++		+	++++	++++		+	
28						++++	+		+

6.2.5. Metabolic quotient (qCO_2)

Cadaver burial resulted in an increase in qCO_2 ($P < 0.01$) (Figure 6.7). Cadaver treatment did not have an effect on qCO_2.

6.2.6. Protease activity

Cadaver burial resulted in an increase in protease activity in all soils (Figure 6.8). Cadaver treatment only had an effect in PR soil whereby the burial of EC resulted in a decreased rate of protease activity ($P < 0.05$). Protease activity among test samples was least in WB soil ($P < 0.05$).

6.2.7. Phosphodiesterase activity

Cadaver burial resulted in an increase in phosphodiesterase activity in all soils (Figure 6.8). The burial of EC resulted in a decreased rate of phosphodiesterase activity in PR soil but an increased rate WB soil. Among test samples phosphodiesterase activity was greatest in FA soil ($P < 0.05$).

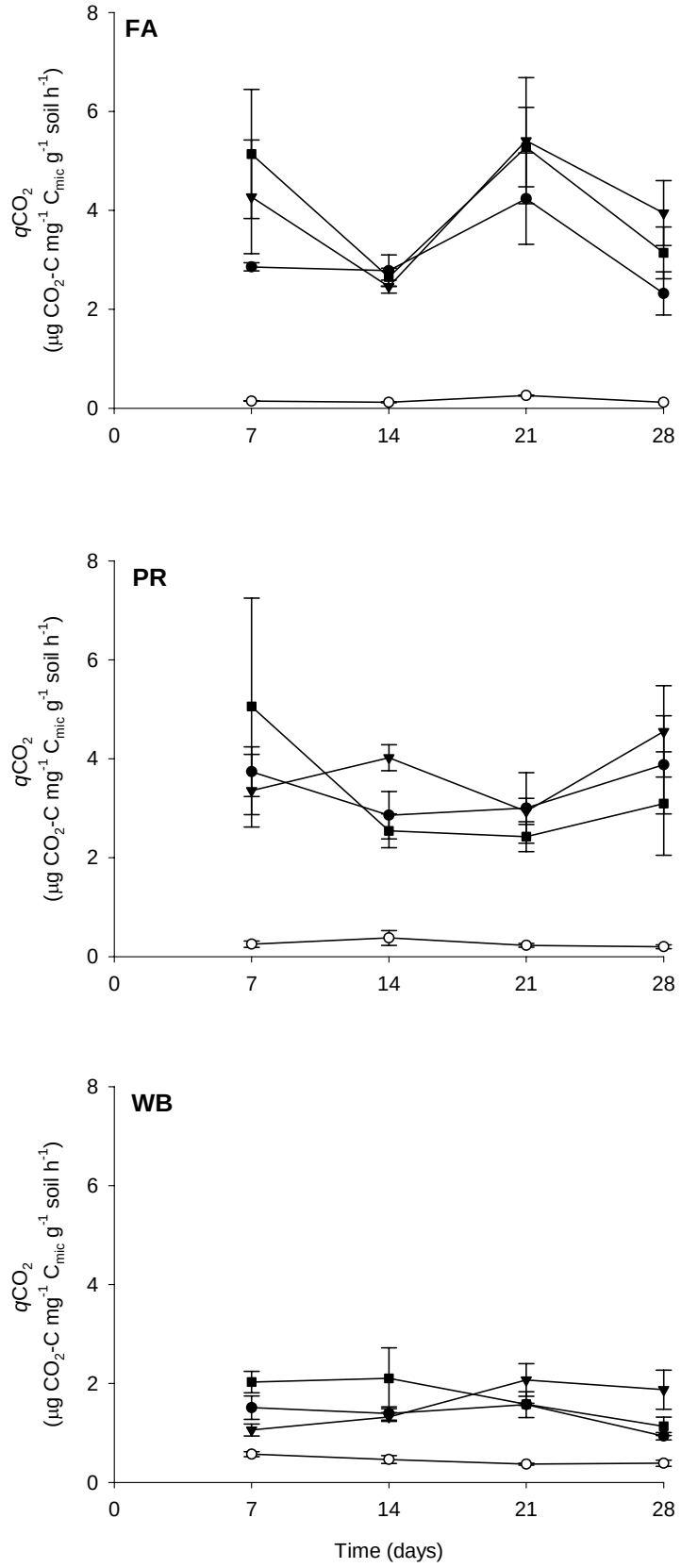

Figure 6.7. Metabolic quotient (qCO_2) (µg CO_2-C mg^{-1} C_{mic} g^{-1} soil h^{-1}) in soil without a cadaver (*Rattus rattus*) (control: ○) and following the burial (2.5 cm) of a complete cadaver (CC: ●), cadaver with a sown incision (CI: ▼) or eviscerated cadaver (EC: ■) in 500 g sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia. Soils were calibrated to a matric potential of –0.05 megapascals prior to burial. Bars represent standard errors where n = 4.

6.2.8. Soil pH

Cadaver burial resulted in an increase in pH to 8 in all soils (Figure 6.9). In PR soil a pH of 8 was reached by day 7. A pH of 8 was reached by day 14 in FA and WB soils. Cadaver treatment did not have an influence on soil pH in PR and WB soils, however, the burial of CI in FA soil resulted in a greater pH than other cadaver treatments on day 7.

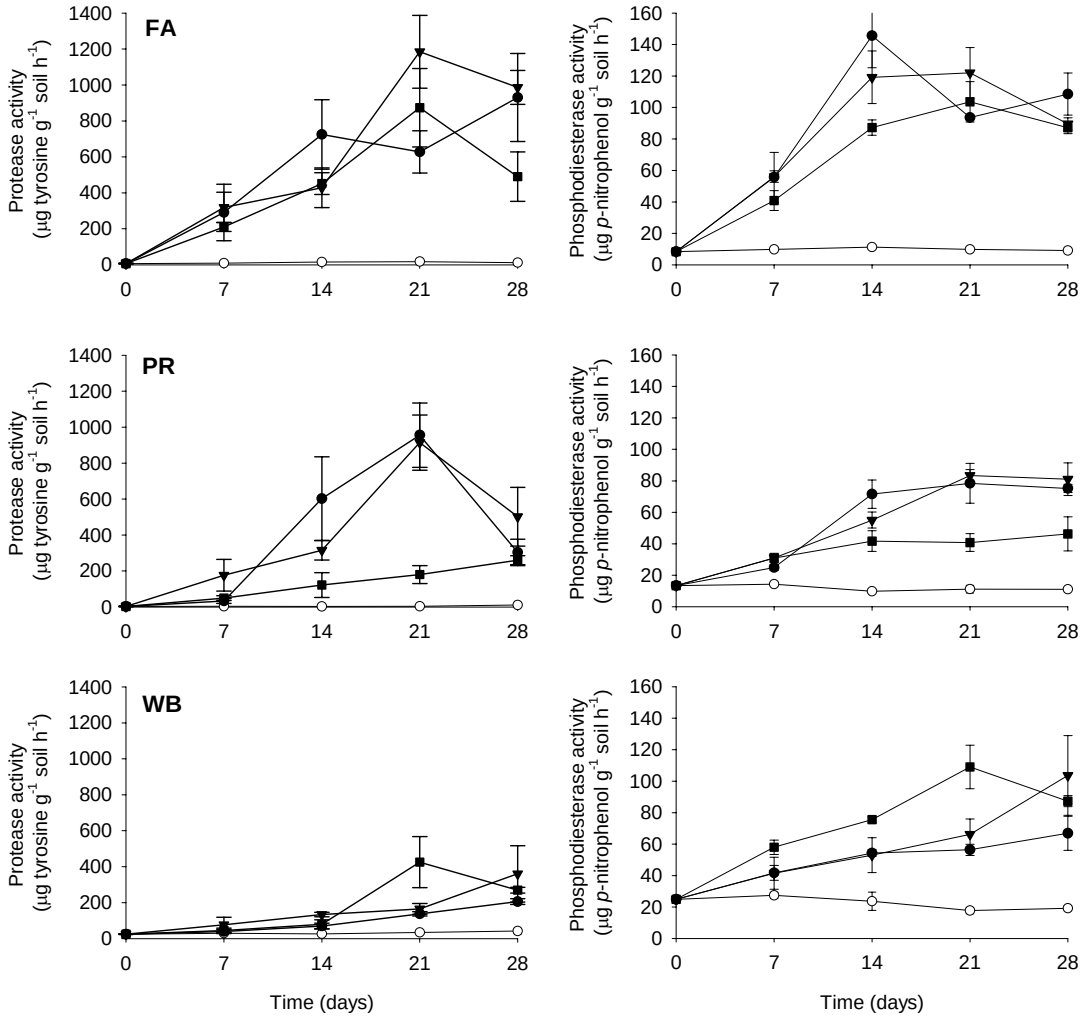

Figure 6.8. Protease activity (left column: µg tyrosine g^{-1} soil h^{-1}) and phosphodiesterase activity (right column: µg *p*-nitrophenol g^{-1} soil h^{-1}) in soil without a cadaver (*Rattus rattus*) (control: ○) and following the burial (2.5 cm) of a complete cadaver (CC: ●), cadaver with a sown incision (CI: ▼) or eviscerated cadaver (EC: ■) in 500 g sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia. Soils were calibrated to a matric potential of –0.05 megapascals prior to burial. Bars represent standard errors where n = 4.

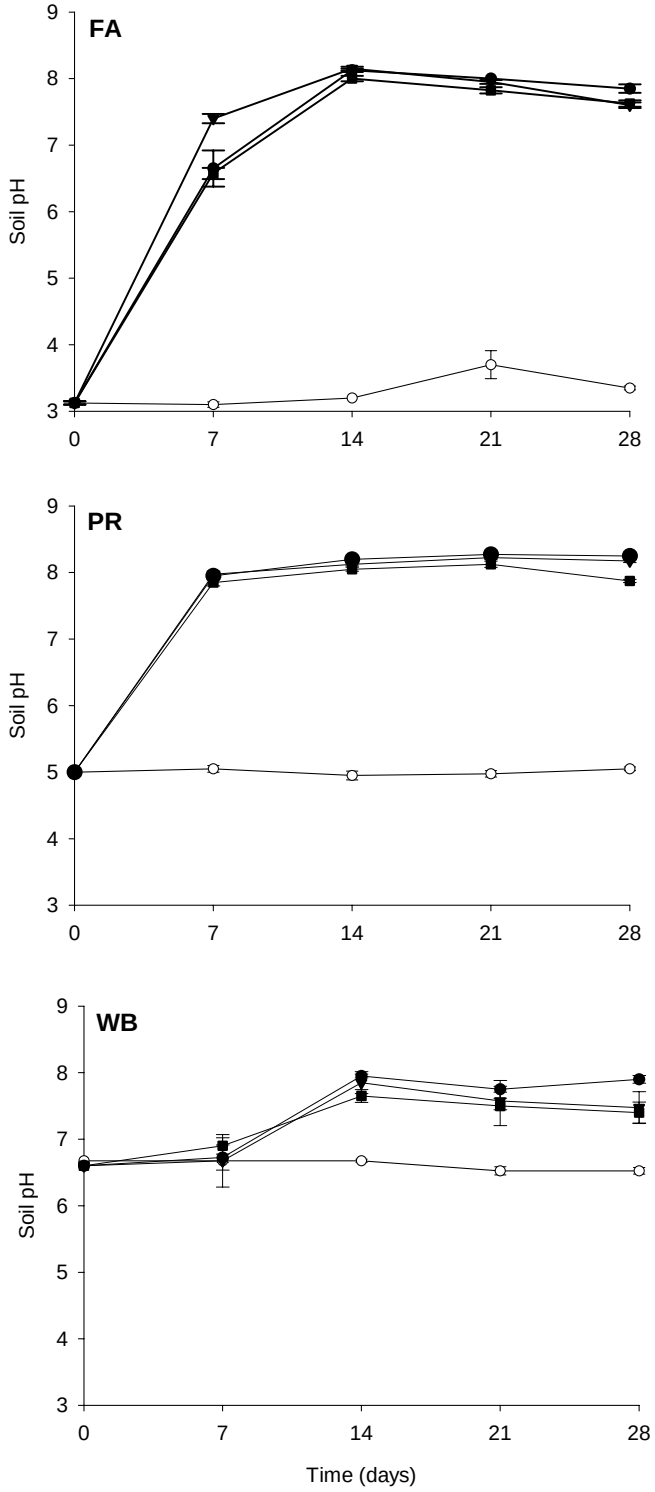

Figure 6.9. pH on soil without a cadaver (*Rattus rattus*) (control: ○) and following the burial (2.5 cm) of a complete cadaver (CC: ●),cadaver with a sown incision (CI: ▼) or eviscerated cadaver (EC: ■) in 500 g sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia. Soils were calibrated to a matric potential of –0.05 megapascals prior to burial. Bars represent standard errors where n = 4.

6.3. Discussion

Data presented in this chapter show that evisceration can result in decreased cadaver decomposition in a sandy soil. This was observed as decreased cadaver mass loss (Figure 6.2) and was associated with a decrease in cumulative CO_2-C evolution (Figure 6.3), protease and phosphodiesterase activity (Figure 6.8). This provides evidence that a significant amount of microbial activity is associated with the viscera. Interestingly, a difference in C_{mic} between CC and EC treatments was only observed once in PR soil. In contrast, C_{mic} in WB soil was consistently greater with CC than EC. This, along with the finding that evisceration did not affect cadaver mass loss in FA and WB soil (Figure 6.2), might indicate that the soil microbial biomass plays a more important role in cadaver decomposition in clayey soils while the enteric microbiota play a more prominent role in sandy soil. This reflects the importance of microbially mediated processes of cadaver decomposition in some soils and provides evidence that the soil microbiota and/or the microflora residing on the skin can effectively decompose a cadaver in the absence of the enteric microbial population. This efficacy is further supported by the consistent pH (Figure 6.9) values as well as an increase in phosphodiesterase activity associated with EC (in WB soil: Figure 6.9). An increase in pH has been observed in the presence of complete human (Vass et al., 1992) and porcine (Hopkins et al., 2000) cadavers and is credited to the accumulation of ammonium (Hopkins et al., 2000). However, further research is required in order to determine if the soil microbial biomass and/or the microbial population residing on the surface of a cadaver mediates these processes. This will be addressee in greater detail in Chapter 7.

The presence of fungal hyphae (Figure 6.6; Table 6.2) on the soil surface shows that microbes can proliferate relatively soon after burial although it is unknown if the hyphae are actively participating in decomposition or utilising by-products from other decomposition processes (e.g. organic C, ammonium, nitrate). Furthermore, it remains unclear whether the hyphae are cadaveric or soil-borne. The fungal hyphae may or may not belong to the postputrefaction (Sagara, 1995) or keratinophilic fungi, which can reside in the soil or on the skin (Pugh and Evans, 1970; Papini et al., 1998; Deshmukh et al., 2000; Kaul and Sumbali, 2000; Deshmukh and Agrawal, 2003). Unfortunately, microbial biomass estimates (Figure 6.5) do not provide evidence to resolve this matter.

Cadaver mass loss was similar to the results from Chapter 4 and Chapter 5 and was characterised by Early, Intermediate and Late Phase decomposition as described in Chapter 4. All phases were observed following the burial of CC. Interestingly, the burial of CI resulted in similar process rates as observed in association with CC, however, the mass loss of CI was more gradual than the mass loss of CC in PR soil (Figure 6.2). The difference between the mass loss of CI and CC might be due to a gradual release of internal fluids via the incision. Also, the incision might allow for the presence of oxygen inside the abdominal cavity, which may alter the succession of endogenous microorganisms that takes place following death (Gill-King, 1997; Vass, 2001).

It is extremely important to note that the endogenous cadaveric microbial population in the current study might not have developed into the complex community associated with adults. The foeti of monogastric animals, such as

humans and rats, are sterile (Inoue and Ushida, 2003a). The colonisation of the intestinal and respiratory tracts by microbes occurs immediately following birth and can take place vertically (e.g. suckling) and horizontally (e.g. exposure to environment) (Inoue and Ushida, 2003b). Vertical transmission of microbes is recognised as the dominant mechanism responsible for the colonisation of the neonatal intestinal tract (Inoue and Ushida, 2003b). While *Eschericia coli*, *Lactobacillus* and *Streptococcus* are the dominant microbes during the suckling stage, *Bacteroidaceae* and *Lactobacillus* become the dominant microbes during the weaning stage (Mitsuoka and Kaneuchi, 1977; Yajima et al., 2001). This is probably due to nutritional and physiological changes in the host animal (Umesaki and Setoyama, 2000). It is currently unknown if this shift in microbial community structure would have any effect on the microbially mediated processes associated with cadaver decomposition.

The measurement of qCO_2 was carried out in order to assess the microbial metabolic efficiency associated with cadaver decomposition. As observed in Chapter 4 and Chapter 5 this measure provides little more than a crude indication that a cadaver has been placed in the soil (Figure 6.7). Similarly, the ACE did not differ between cadaver treatments. The increase in efficiency over time is similar to the results from the other chapters. Interestingly, there was no cadaver treatment effect on ACE. The reason for this is unknown.

Soil pH again showed potential to be an effective marker of clandestine graves. The measurement of pH provided relatively consistent results regardless of soil type and cadaver treatment. One minor discrepancy was the delay in an increase of pH in FA

and WB soil. It is unknown what caused this and it could be due to a number of factors including an increase in clay content.

In conclusion, the evisceration of a cadaver can result in a decreased rate of cadaver decomposition in sandy soil. This reduced decomposition was associated with a decrease in the rate of CO_2-C evolution, protease activity and phosphodiesterase activity. It is possible that the enteric microbial biomass is more important t cadaver decomposition in sandy soil while the soil microbial biomass is more important in clayey soil. It is unknown if these phenomena are due to the dominance of desiccation, the removal of the viscera as a source of enteric microbes or as an organic resource for the soil microbial biomass. The lack of cadaver treatment effect on cadaver mass loss in FA and WB soil in addition to consistent soil pH provides evidence to show that the soil microbial biomass and/or the microbes residing on the skin are capable of decomposing a cadaver. In order to gain further insight into the role that the soil microbial biomass plays during cadaver decomposition, an experiment using sterilised soil was undertaken (Chapter 7).

CHAPTER 7

CADAVER DECOMPOSITION IN THE ABSENCE OF SOIL MICROORGANISMS

The breakdown of an organic resource patch in soil can be regulated by the activity of decomposer organisms (Swift et al., 1979). A cadaver, for example, can be subject to the activity of arthropods (Fuller, 1934; Kocárek, 2003), vertebrate scavengers (Haglund, 1997; DeVault et al., 2003) and microorganisms (Micozzi, 1986; Gill-King, 1997). The burial of a cadaver in soil can severely restrict the activity of arthropods and vertebrate scavengers (Rodriguez and Bass, 1985; Rodriguez, 1997) and, thus, select for a decomposer population comprised of cadaveric and soil-borne microorganisms. Consequently, burial in soil is widely recognised as resulting in a slower rate of cadaver decomposition than exposure on the soil surface (Rodriguez and Bass, 1985; Rodriguez, 1997). Interestingly, cadaver decomposition in soil has not been compared to cadaver decomposition in a sterile, soil-free environment where the decomposer population is comprised entirely of cadaveric microbes. It is therefore unknown how the soil microbial biomass can affect cadaver decomposition following burial, apart from the results in Chapter 6.

The participation of the soil microbial biomass in the decomposition of an organic resource patch, such as a cadaver, can be studied through removal of the microbial population by sterilisation of the soil. Sterilisation methods include fumigation with chloroform (Jenkinson and Powlson, 1976; Tiwari et al., 1988), autoclaving (Powlson and Jenkinson, 1976; Tiwari et al., 1988; Alphei and Scheu, 1993; Bennett et al., 2003), gamma irradiation (Powlson and Jenkinson, 1976; Alphei and Scheu, 1993; McNamara et al., 2003), microwaving (Alphei and Scheu, 1993) and

fumigation with methyl bromide (Powlson and Jenkinson, 1976). In the current study soils were sterilised via autoclaving and three soil treatments were used: autoclaved soil (sterile), autoclaved soil reinoculated with non-autoclaved soil (non-sterile) and a sterile, soil-free burial environment. Autoclaving was chosen as the method of sterilisation based on the equipment available.

Soils were collected from the Yabulua/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites (see Chapter 3), sieved (2 mm) and immediately placed in aluminium trays to a depth of 2.5 cm. The aluminium trays containing soil were covered with aluminium foil and autoclaved at 121 °C for 30 minutes on two consecutive days (Coûteaux, 1992; Bennett et al., 2003). Soils were incubated at 22 °C, while sealed, for 24 hours between autoclaving. Following autoclaving all work was conducted in a sterile laminar flow cabinet in order to keep the systems sterile throughout the incubation. Soils (500 g) were weighed into incubation chambers that had been sterilised with 70% ethanol and exposure (8 hours) to ultraviolet radiation. All carbon dioxide traps (see Chapter 2) were sterilised each day using these methods. In order to set up a non-sterile soil treatment, 50 g of autoclaved soil was removed from designated incubation chambers, replaced with 50 g non-autoclaved soil and mixed with a sterile spatula (Bennett et al., 2003). Soils were calibrated to a matric potential of –0.05 megapascals (see Chapter 3) with sterile distilled water, sealed and left to equilibrate at 22 °C for seven days. Distilled water was sterilised by autoclaving at 121 °C for 30 minutes. Following equilibration, rat (*Rattus rattus*) cadavers were killed and buried following the methods described in Chapter 2. Control samples (soil minus cadaver) were disturbed in order to simulate cadaver burial and, thus, account for any effects caused by soil disturbance. For the sterile,

soil-free treatment cadavers were placed on a sterile aluminium tray at the bottom of a sterile incubation chamber (Figure 7.1). The experiment was replicated four times. Each treatment was set up with sufficient replicates for four sequential harvest events over 28 days resulting in a total of 192 microcosms. The aim of the experiment was to test the hypothesis that cadaver decomposition in the presence of soil microorganisms is greater than in the absence of soil microorganisms.

Figure 7.1. Placement of juvenile rat (*Rattus rattus*) cadaver on a sterile aluminium tray in a sterile, soil-free environment.

7.2. Results

7.2.1. Effect of autoclaving

Autoclaving had an effect on the chemical and biological properties of the soils (Table 7.1). Some of these effects were relatively consistent across all soil types, such as a decrease in microbial biomass carbon (C_{mic}) and enzyme activity. Interestingly, phosphodiesterase activity in WB soil was not significantly affected by autoclaving. Other effects included a decrease in organic C and cation exchange

capacity of PR soil. Unfortunately, a technical error resulted in the loss of the cation exchange capacity and organic C replicates so it was unable to determine if this effect was significant.

Table 7.1. Biophysicochemical properties of field fresh and autoclaved soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of north Queensland, Australia. Soils were autoclaved at 121 °C for 30 minutes on consecutive days. In between autoclaving soil were sealed and incubated at 22 °C for 24 hours. Figures in brackets represent standard errors where n = 4. * denote significant differences where * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	FA		PR		WB	
	Field fresh	autoclaved	Field fresh	autoclaved	Field fresh	autoclaved
pH (H_2O)	5.3 (0.0)	5.5 (0.0)	5.8 (0.0)	6.0 (0.0)	7.1 (0.0)	7.0 (0.1)
Organic C (%)	0.76	0.72	1.64	0.88	1.13	1.10
Total N (%)	0.04	0.04	0.10	0.05	0.07	0.07
Total P (%)	0.01	0.01	0.03	0.02	0.01	0.01
Cation exchange capacity (cmol+ kg^{-1})	2.3	2.3	6.4	3.9	31.7	31.6
Electrical conductivity (mS cm^{-3})	0.02	0.04	0.05	0.05	0.10	0.12
Microbial biomass C (μg g^{-1} soil)	718*** (105)	0 (0)	682*** (55)	0 (0)	489*** (28)	0 (0)
Protease activity (μg tyrosine g^{-1} soil h^{-1})	395*** (103)	58 (5)	447*** (90)	51 (4)	843** (175)	118 (14)
Phosphodiesterase activity (μg *p*-nitrophenol g^{-1} soil h^{-1})	14.3*** (2)	1.2 (0.2)	50.4*** (5.8)	11.2 (0.3)	76.0 (2.8)	72.9 (1.4)

7.2.2. Cadaver mass loss

Cadaver mass loss in non-sterile soil was greater than in a soil-free environment but this difference was only observed during the latter 14 days of incubation (Figure 7.2). Cadaver mass loss in sterile PR soil was also greater than in soil-free samples during the latter half of incubation ($P < 0.05$) (Figure 7.2). In contrast, burial in sterile FA and WB soil resulted in cadaver mass loss similar to that observed in soil-

free samples (Figure 7.2). Furthermore, cadaver mass loss in non-sterile FA and WB soil was greater than in sterile soil from day 21 to 28 ($P < 0.05$).

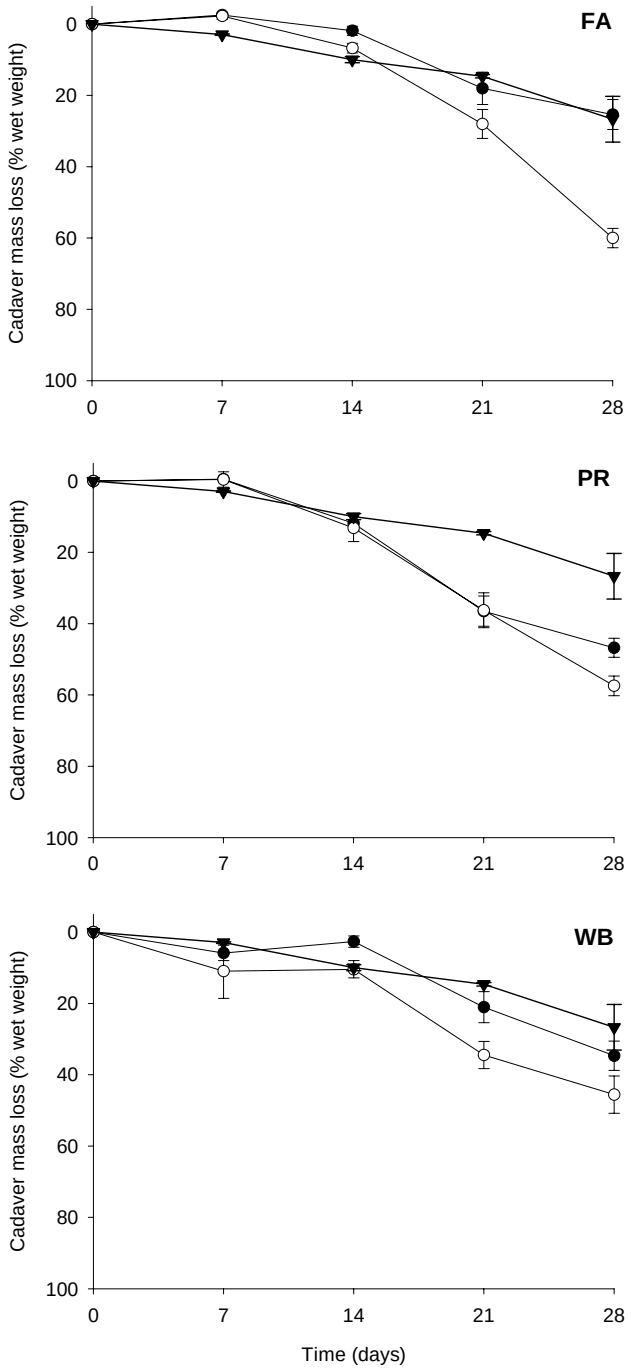

Figure 7.2. Mass loss (% wet weight) of a juvenile rat (*Rattus rattus*) cadaver following placement in a sterile, soil-free environment (▼) or burial (2.5 cm) in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia that was sterilised (●) or sterilised and reinoculated with unsterilised soil (○) and calibrated to a matric potential of –0.05 megapascals with sterile distilled water. Soils were sterilised by autoclaving at 121 °C for 30 minutes on two consecutive days. In between autoclaving soils were incubated at 22 °C for 24 hours. Bars represent standard error where n = 4.

Cadavers placed in a soil-free environment retained their basic antemortem size and shape throughout the incubation (Figure 7.3a). However, by day 7 cadavers began to darken in colour and become bloated. The darkening of the cadavers and degree of flaccidity increased over time. In addition, fungal hyphae were macroscopically observed on the mouth of these cadavers. In contrast, cadavers buried in soil (sterile and non-sterile) underwent significant morphological change (Figure 7.3b, c). By day 7 the cadavers had darkened and soil was observed to adhere to the cadaver. By day 28 the gross morphology of cadavers buried in non-sterile soil was different to those in the other treatments. Cadavers in non-sterile soil comprised little more than dark, flaccid specimens of skin, bone and soil where all internal organs appeared to be absent. This would account for the levels of mass loss observed in the previous chapters and show that the majority of cadaver decomposition following burial is due to the release of the contents of the thoracic cavity (viscera, water). In contrast, the cadavers in sterile soil and a sterile soil-free environment still appeared to contain some internal material.

7.2.3. Daily carbon dioxide (CO_2-C) evolution

Cadaver burial in soil resulted in a greater rate of CO_2-C evolution than observed in control samples ($P < 0.001$) (Figure 7.4). Furthermore, sterilisation had a significant effect on the rate of CO_2-C evolution following cadaver burial. In FA soil this effect was observed as a decrease in the rate of CO_2-C evolution during the final 10 days of incubation while in sterile PR and WB soils a delay in the flush of CO_2-C evolution following burial was observed (Figure 7.4).

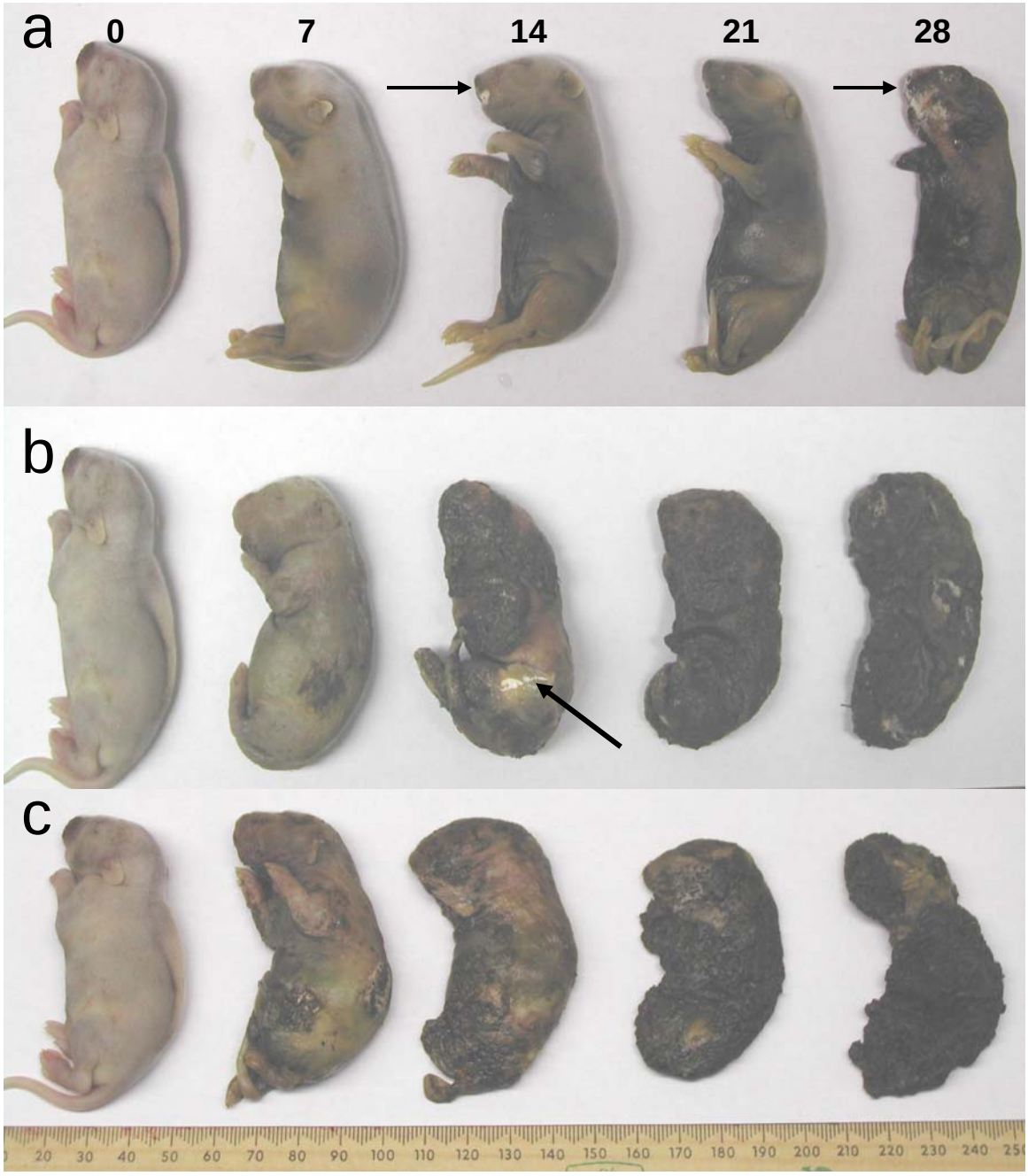

Figure 7.3. Gross morphology of cadavers exposed to a sterile, soil-free environment (a), sterilised soil (b) and non-sterilised soil (c) from the Yabulu/FACE site in a tropical savanna ecosystem in Queensland, Australia. Soils were sterilised by autoclaving at 121 °C for 30 minutes on two consecutive days. In between autoclaving soils were incubated at 22 °C for 24 hours. Numbers across the top indicate days of incubation. Arrows indicate presence of fungal hyphae.

7.2.4. Cumulative CO_2-C evolution

Cadaver burial in soil resulted in a greater accumulation of CO_2-C than observed in soil-free samples, which were greater than in control samples ($P < 0.001$) (Figure 7.4). Cumulative CO_2-C in soil-free samples was greater than in FA and PR control samples during the final 14 days of incubation in WB soil. However, cumulative CO_2-C evolution in a sterile, soil-free environment was only greater than control samples during the final two days of incubation. In addition, cadaver burial in non-sterile PR and WB soil resulted in greater CO_2-C evolution than in sterile test soil ($P < 0.05$). This effect was also observed in FA soils but only during the final 3 days of incubation.

A significant relationship was observed between cumulative CO_2-C evolution and cadaver mass loss. Correlation equations are presented in Appendix B: Table B.4. The aerobic catabolic efficiency (ACE) showed that the most efficient decomposition took place in soil-free and sterilised PR soil (Table 7.2). ACE was least in sterile FA soil. An increase in efficiency was observed over time in non-sterile PR and FA soil. I decrease in efficiency was observed on day 14 in sterile FA and non-sterile WB soil. A negative ACE was observed in sterile FA soil on day 7.

7.2.5. Microbial biomass C (C_{mic})

No C_{mic} was observed in sterile control samples (Figure 7.6). Cadaver burial in non-sterile soil resulted in an increase C_{mic} relative to sterile soil during the latter half of the incubation in FA (day 28) and WB soil (day 14, day 28). Sterilisation had no effect on C_{mic} following cadaver burial in PR soil. The concentration of C_{mic} in non-sterile WB control soil was consistently less than in WB test samples.

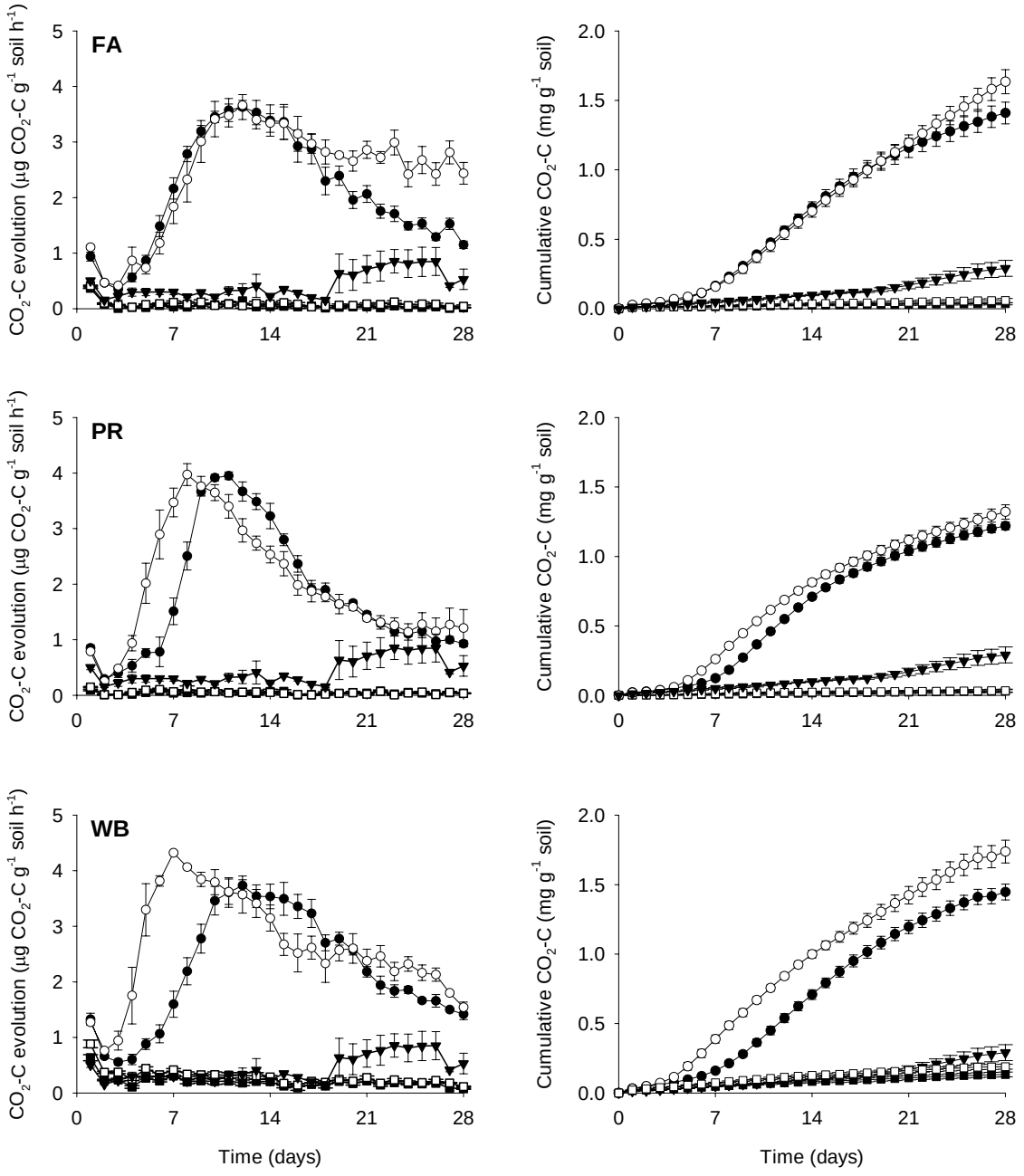

Figure 7.4. Daily (left column) and cumulative (right column) CO_2-C evolution following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia that was sterilised (●) or sterilised and reinoculated with non-sterile soil (○). CO_2-C evolution was also measured in control samples (no cadaver) of sterile (■) and sterile soil reinoculated with non-sterile soil (□). Cumulative CO_2 evolution was measured following the placement of a rat cadaver in a sterile, soil free environment (▼). Soils were sterilised by autoclaving at 121 °C for 30 minutes on two consecutive days. In between autoclave treatments soils were incubated at 22 °C for 24 hours. Following sterilisation soils were calibrated to a matric potential of –0.05 megapascals. Bars represent standard errors where n = 4.

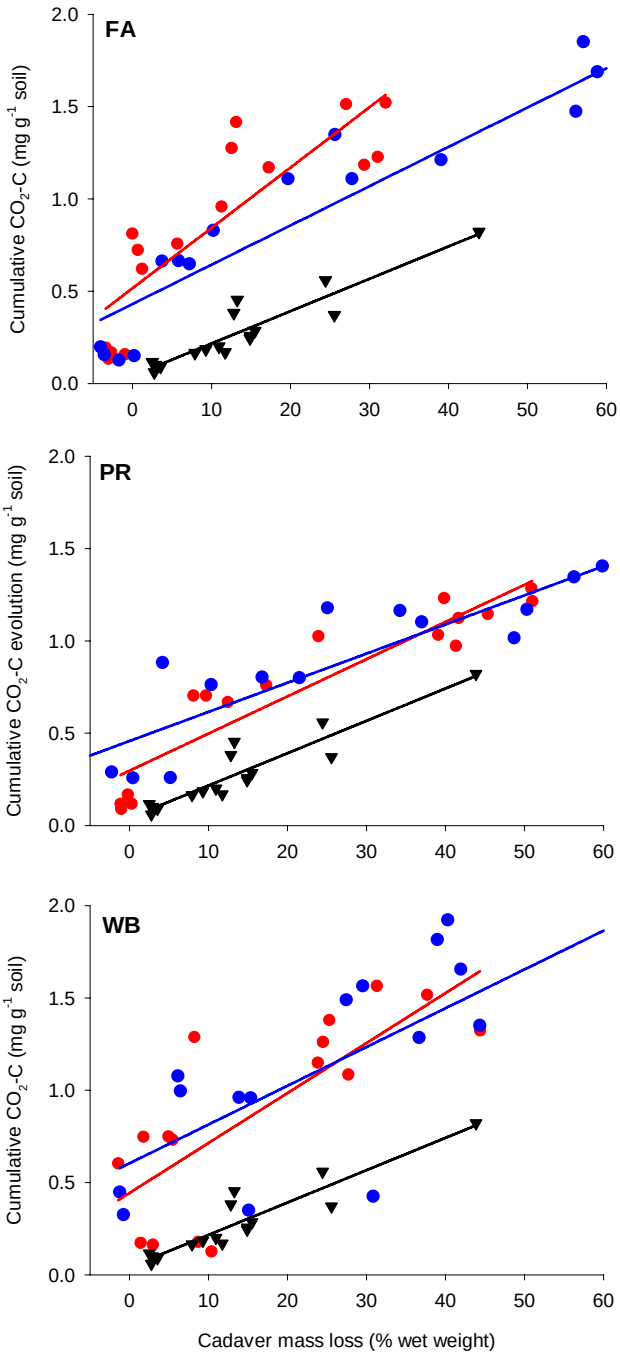

Figure 7.5. Correlation between cumulative CO_2-C evolution (mg g-1 soil) and mass loss (% wet weight) following the placement of a juvenile rat (*Rattus rattus*) cadaver in a sterile, soil-free environment (▼) or burial (2.5 cm) in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia that was sterilised (●) or sterilised and reinoculated with non-sterile soil (●). Soils were sterilised by autoclaving at 121 °C for 30 minutes on two consecutive days. In between autoclave treatments soils were incubated at 22 °C for 24 hours. Following sterilisation soils were calibrated to a matric potential of –0.05 megapascals.

Table 7.2. Aerobic catabolic efficiency (ACE: mg CO_2-C g^{-1} soil $\%^{-1}$ wet weight cadaver mass loss) following the placement of a juvenile rat (*Rattus rattus*) cadaver in a sterile, soil-free environment (SF) or burial (2.5 cm) in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia that was sterilised or sterilised and reinoculated with unsterilised soil, calibrated to a matric potential of -0.05 megapascals with sterile distilled water and incubated at 22 °C. Soils were sterilised by autoclaving at 121 °C for 30 minutes on tow consecutive days. In between autoclaving soils were incubated at 22 °C for 24 hours. Numbers in brackets represent standard error of the mean. a, b, c represent a significant difference ($P < 0.05$) between time within soil and temperature. y, z, represent a significant difference ($P < 0.05$) .

Soil	Soil Treatment	Day 7	14	21	28	Mean ACE
SF	Sterile, soil-free	0.02^{a} (0.00)	0.01^{a} (0.00)	0.01^{a} (0.00)	0.01^{a} (0.00)	0.01^{z} (0.00)
FA	Sterile	-0.08^{a} (0.03)	0.62^{b} (0.20)	0.07^{a} (0.01)	0.06^{a} (0.02)	0.17^{x} (0.08)
	Non-sterile	0.15^{ab} (0.21)	0.12^{a} (0.02)	0.05^{b} (0.01)	0.03^{b} (0.00)	0.09^{xy} (0.05)
PR	Sterile	-0.10^{a} (0.25)	0.06^{a} (0.01)	0.03^{a} (0.00)	0.03^{a} (0.00)	0.01^{z} (0.06)
	Non-sterile	0.08^{a} (0.03)	0.09^{a} (0.03)	0.03^{b} (0.01)	0.02^{b} (0.00)	0.06^{y} (0.01)
WB	Sterile	0.05^{a} (0.03)	0.08^{a} (0.18)	0.07^{a} (0.03)	0.04^{a} (0.01)	0.06^{y} (0.01)
	Non-sterile	0.05^{a} (0.02)	0.12^{b} (0.03)	0.04^{a} (0.01)	0.04^{a} (0.01)	0.06^{y} (0.01)

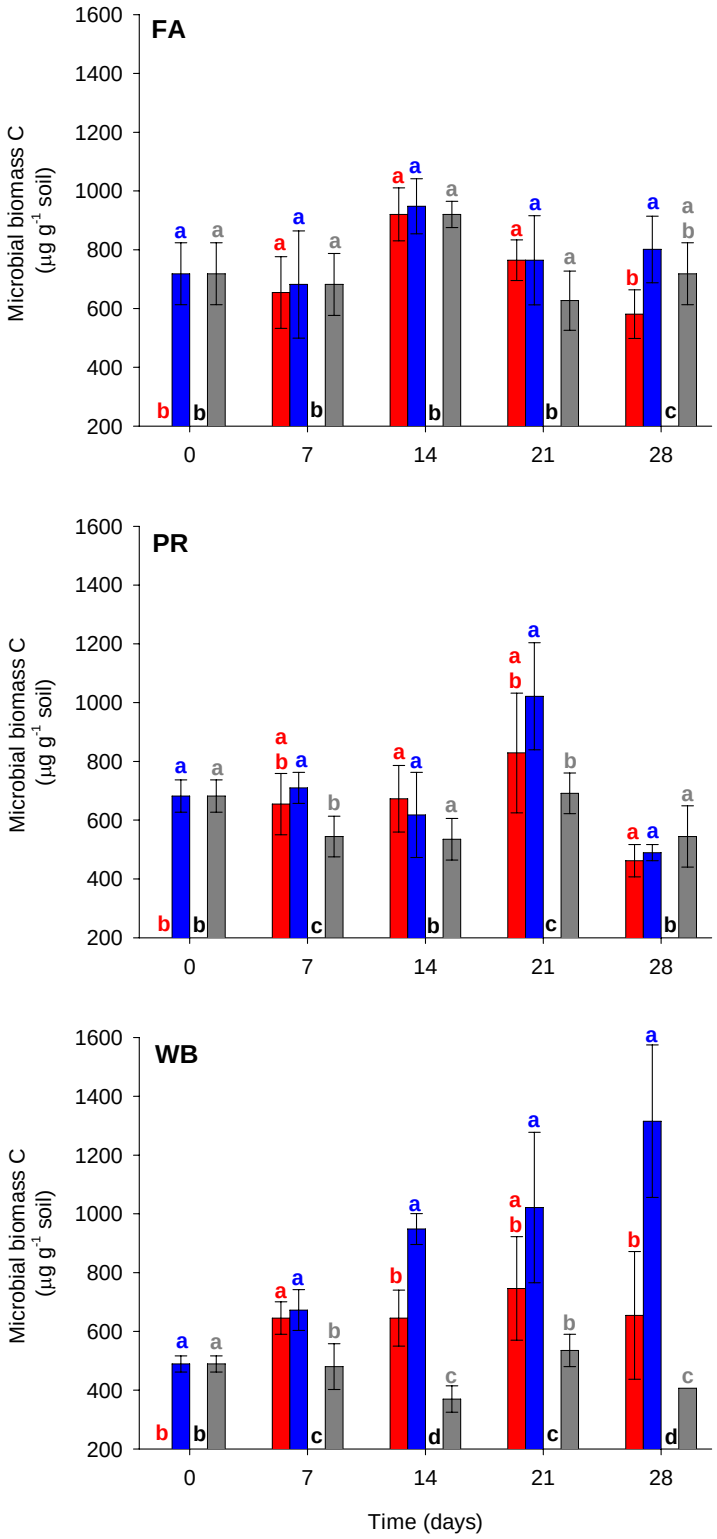

Figure 7.6. Microbial biomass C (µg C_{mic} g^{-1} soil) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia that was sterilised (■) or sterilised and reinoculated with non-sterile soil (■). Microbial biomass C was also measured in control samples (no cadaver) of sterile (■) and sterile soil reinoculated with non-sterile soil (■). Soils were sterilised by autoclaving at 121 °C for 30 minutes on two consecutive days. In between autoclave treatments, soils were incubated at 22 °C for 24 hours. Following sterilisation soils were calibrated to a matric potential of –0.05 megapascals. Bars represent standard errors where n = 4.

Fungal hyphae were seen on the soil surface above buried cadavers (Figure 7. 7; Table 7.2). In non-sterile soils the hyphae were able to colonise the soil surface directly above the entire cadaver and were observed 7 days earlier than on sterile soil in FA and WB samples. In contrast, the colonisation of fungal hyphae in association with cadavers buried in sterile soil was concentrated in the areas directly above the mouth and anus.

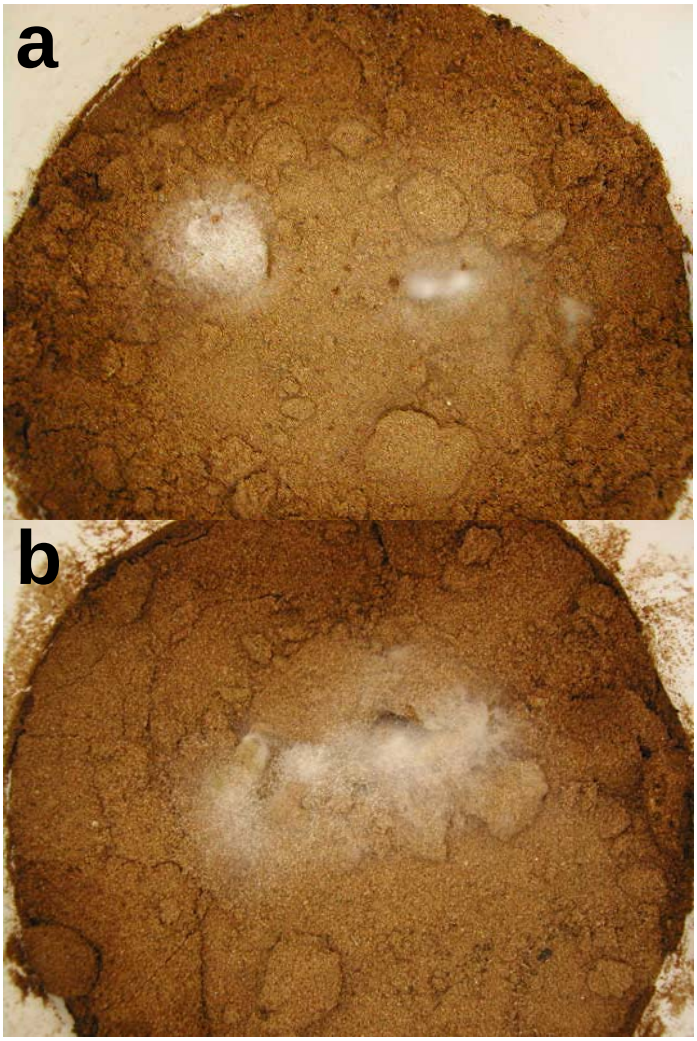

Figure 7.7. Fungal hyphae on the surface of 500 g (dry weight) sieved (2 mm) soil from the Pallarenda site in a tropical savanna ecosystem of Queensland, Australia that was sterilised (a) or sterilised and reinoculated with non-sterile soil (b) prior to the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver. Soils were sterilised by autoclaving at 121 °C for 30 minutes on two consecutive days. In between autoclave treatments soils were incubated at 22 °C for 24 hours. The areas of increased fungal hyphae concentration on the sterile soil are located above the mouth and anus.

Table 7.3. Macroscopic detection (+) of fungal hyphae on the surface of 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) or Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia that had been sterilised (sterile) or sterilised and reinoculated with non-sterile soil (non-sterile) prior to the burial of a juvenile rat (*Rattus rattus*) cadaver. Soils were calibrated to a matric potential of –0.05 megapascals and at 22 °C. Soils were sterilised by autoclaving at 121 °C for 30 minutes on consecutive days. Between autoclaving soils were incubated at 22 °C for 24 hours. Number of + represents the number of replicates in which fungal hyphae was observed (e.g. ++ = fungal hyphae present in two replicates).

	FA		PR		WB	
Time (day)	sterile	non-sterile	sterile	non-sterile	sterile	non-sterile
0						
7				++		++++
14	++++	++++	++++	++++	++++	++++
21	++++	++++	++++	++++	++++	+++
28	++++	++++	++++	++++		

7.2.6. Metabolic quotient (qCO_2)

Cadaver burial resulted in an increase in qCO_2 in all soils but little difference was detected between test samples: a greater qCO_2 was observed in FA non-sterile samples relative to sterile test samples on day 21 and day 28 ($P < 0.05$) (Figure 7.8). The qCO_2 was not measurable in sterile control soils because no C_{mic} was detected.

7.2.7. Protease activity

Protease activity was affected by cadaver burial and autoclaving. In FA and PR soil cadaver burial resulted in an increase in protease activity ($P < 0.05$). In FA soil this increase was enhanced by autoclaving (Figure 7.9). Sterilisation apparently had a greater effect than cadaver burial in WB soil as sterile samples were associated with a greater rate of protease activity than non-sterile samples. A similar trend was observed in FA control samples.

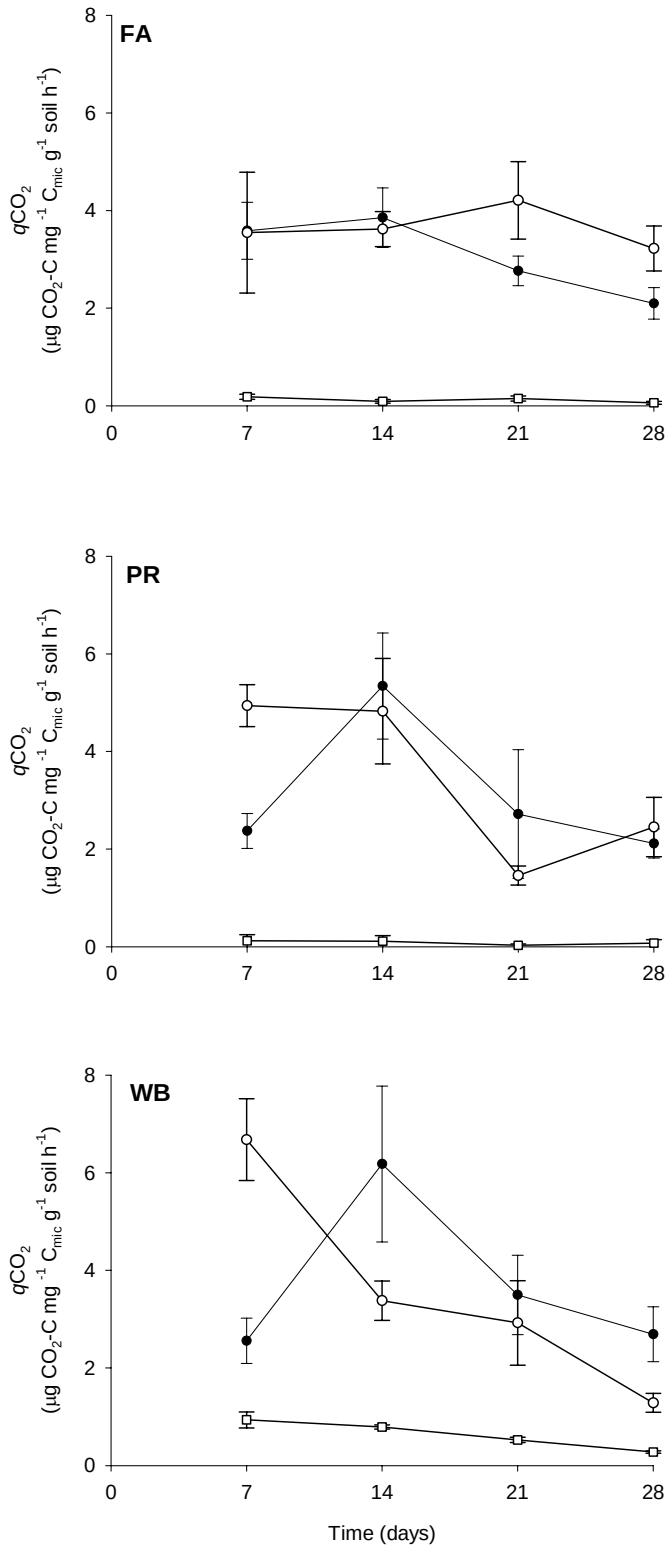

Figure 7.8. Metabolic quotient (qCO_2: µg CO_2-C mg^{-1} C_{mic} g^{-1} soil h^{-1}) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia that was sterilised (●) or sterilised and reinoculated with non-sterile soil (○). Metabolic quotient was also measured in control samples (no cadaver) of sterile soil reinoculated with non-sterile soil (□) but was not measured in sterile control soils because of the lack of C_{mic}. Soils were sterilised by autoclaving at 121 °C for 30 minutes on two consecutive days. In between autoclave treatments, soils were incubated at 22 °C for 24 hours. Following sterilisation soils were calibrated to a matric potential of –0.05 megapascals. Bars represent standard errors where n = 4.

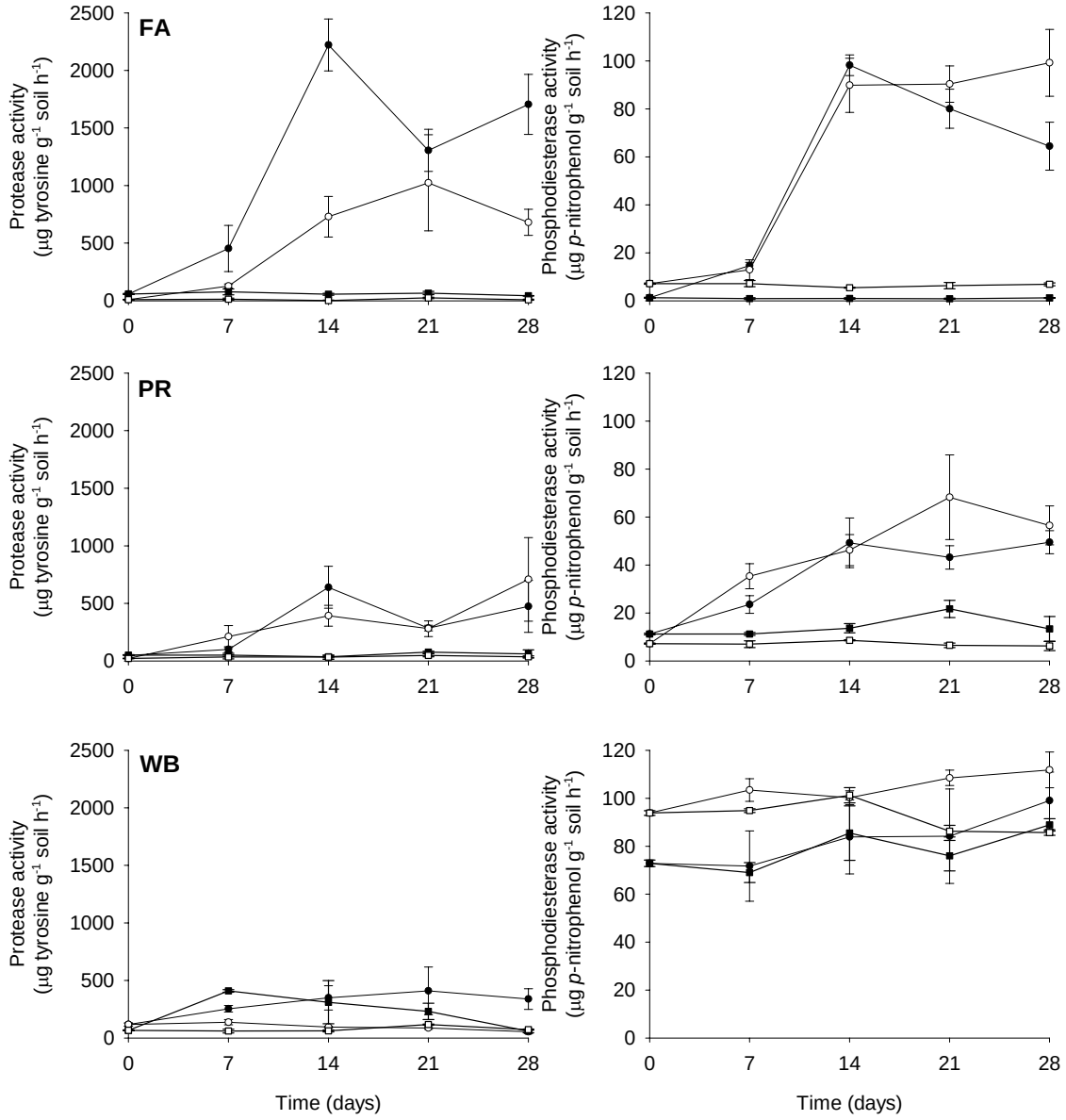

Figure 7.9. Protease (µg tyrosine g^{-1} soil h^{-1}: left column) and phosphodiesterase (µg *p*-nitrophenol g^{-1} soil h^{-1}: right column) activity following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia that was sterilised (●) or sterilised and reinoculated with non-sterile soil (○). Protease and phosphodiesterase activity was also measured in control samples (no cadaver) of sterile (■) and sterile soil reinoculated with non-sterile soil (□). Soils were sterilised by autoclaving at 121 °C for 30 minutes on two consecutive days. In between autoclave treatments, soils were incubated at 22 °C for 24 hours. Following sterilisation soils were calibrated to a matric potential of –0.05 megapascals. Bars represent standard errors where n = 4.

7.2.8. Phosphodiesterase activity

Phosphodiesterase activity was affected by cadaver burial and autoclaving (Figure 7.9). Cadaver burial resulted in an increase in phosphodiesterase activity in FA and PR soil ($P < 0.01$). However, autoclaving apparently had a greater influence than cadaver burial in WB soil as a greater rate of phosphodiesterase activity was observed in non-sterile samples relative to sterile samples during the initial 14 days of burial. A similar trend was observed in FA control samples. Conversely, sterile PR control samples were associated with a rate of phosphodiesterase activity greater than in non-sterile PR control samples ($P < 0.05$).

7.2.9. Soil pH

Cadaver burial resulted in an increase in soil pH to approximately 9 in all soils (Figure 7.10). This increase was delayed in sterile PR soil. A decrease in pH was observed in sterile FA, sterile PR and non-sterile PR control samples during the latter 14 days of incubation.

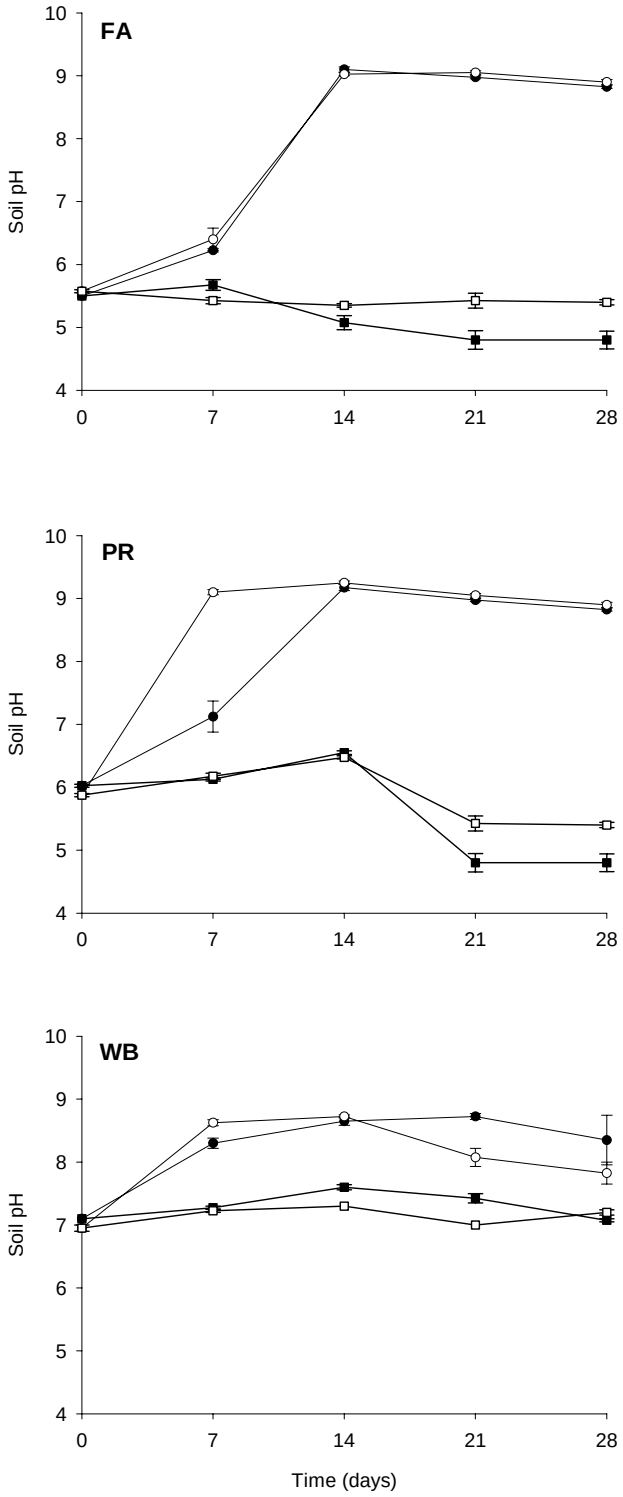

Figure 7.10. pH of soil following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver in 500 g (dry weight) sieved (2 mm) soil from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia that was sterilised (●) or sterilised and reinoculated with non-sterile soil (○). Soil pH was also measured in control samples (no cadaver) of sterile (■) and sterile soil reinoculated with non-sterile soil (□). Soils were sterilised by autoclaving at 121 °C for 30 minutes on two consecutive days. In between autoclave treatments, soils were incubated at 22 °C for 24 hours. Following sterilisation soils were calibrated to a matric potential of –0.05 megapascals. Bars represent standard errors where n = 4.

7.3. Discussion

The current results show that the burial of a cadaver in the presence of soil microorganisms can result in greater cadaver decomposition than in the absencce of soil microorganisms (Figure 7.2). This challenges the current paradigm that views burial in soil as a means to slow cadaver decomposition (Fiedler and Graw, 2003). All differences in mass loss were observed between day 14 and day 28 (Figure 7.2) and, thus, coincide with the established view that the soil microbiota begin to participate in cadaver decomposition during the latter phases of breakdown (Evans, 1963; Micozzi, 1986; Janaway, 1996). This phenomenon was also reflected by a decrease in CO_2-C evolution (in FA and WB soils) (Figure 7.4), and phosphodiesterase activity (in FA soil) (Figure 7.9) during the latter phases of decomposition. These phenomena might also indicate that the activity of cadaveric microbes can significantly decline following early phase decomposition. This would make sense if the majority of cadaveric microbes were anaerobic because the onset of intermediate phase decomposition is typically associated with the rupture of the skin and a reintroduction of oxygen to the system (Johnson, 1975).

Some of the current results, however, contrast with the concept that the participation of the soil microbiota is restricted to late phase cadaver decomposition. The relatively rapid increase in the rate of CO_2-C evolution (Figure 7.4) during the initial 8-9 days of incubation in non-sterile PR and WB soil relative to sterile soil provides evidence to show that the soil microbial biomass can be active during early phase cadaver decomposition. Therefore, it is possible that while cadaver burial can immediately trigger soil microbial activity, the effects of this metabolism are not detectable via measurements of mass loss for a number of days. This may be due to

the soil microbial biomass multiplying on and within the tissues of the cadaver, largely replacing the mass loss of the cadaver. This might also explain negative ACE values (Table 7.2).

The dynamics of the soil microbial biomass in non-sterile WB soil were interesting. While mass loss was similar to previous chapters, C_{mic} continued to increase by day 28. This might mean the composition of microbial population in autoclaved and reinoculated WB soil is different than in field fresh soil. This was further shown easily apparent by the lack of fungal hyphae on day 28, which differed from the results in previous chapters. The composition of the soil microbial biomass will be dealt with in greater detail in Chapter 9.

Interestingly, the burial of a cadaver in sterile soil also resulted in a flush of CO_2-C evolution (Figure 7.4), an increase in enzyme activity (Figure 7.9) and soil pH (Figure 7.10). This might be due to the activity of cadaveric microorganisms residing on the skin and interacting with the soil matrix (including dead soil microbial cells). The interaction between cadaveric microbes and the soil matrix may also explain the similarity in cadaver mass loss between sterile and non-sterile PR soil (Figure 7.2). In addition, the coarse texture of PR soil might have allowed decomposition fluids (i.e. mass) to drain away relatively rapidly following the rupture of the skin. This would likely require the participation of cadaveric, keratinophilic microbes possibly represented by the hyphae observed above the anus and mouth in sterile soils (Figure 7.7).

It is well known that biocidal treatment can affect physicochemical properties of soils (Jenkinson, 1966; Coûteaux, 1992). Some of these effects are indirect and result from the removal of the biological component of soils while others are a direct influence of the imposed biocidal treatment (Tiwari et al., 1988). In the current study autoclaving had a significant effect on C_{mic} and enzyme activity (Table 7.1). Enzyme activity has previously been observed to be greatly decreased by autoclaving and this has been attributed to the death of the microbial biomass and enzyme denaturation (Tiwari et al., 1988). In addition, autoclaving can alter soil organic matter so that it becomes less resistant to microbial attack (Jenkinson, 1966). This is typically associated with a narrowing of the C:N ratio, which indicates that the fraction of soil organic matter rendered more available is more rich in N (Powlson, 1977). This might explain the decreased rate of protease activity in WB soil following reinoculation (Figure 7.9), as an increase in available N might not require the catalysis of protein to peptide.

In spite of the autoclave-induced effects many of the current results were similar to those observed in previous chapters. The decomposition pattern was characterised by early, intermediate and late phase decomposition and is similar to cadaver decomposition observed and described in Chapter 4 and other taphonomic literature (Payne, 1965; Payne et al., 1968; Micozzi, 1986; Kocárek, 2003). In the current experiment the transition from early phase to intermediate decomposition was apparently enhanced by the presence of the soil microbial biomass and/or an interaction between the cadaver, cadaveric microbes and the soil matrix (observed in PR soil) (Figure 7.2).

The measurement of soil pH (Figure 7.10) again proved a reliable marker of gravesoils. This phenomenon has been observed in each of the previous chapters and may hold great potential for the search and location of clandestine graves. In contrast the measurement of qCO_2 represented no more than a crude indicator of the presence of cadaver burial. In contrast, the ACE was able to show a difference between soil treatments, where cadavers in a soil-free environment and sterile PR soil were associated with more efficient breakdown. This might be due to a predominance of anaerobes in these settings, which makes the ACE a misleading measure, such as reported in Chapter 5. These findings provide more evidence for the concept that soil-borne microbes play a more pronounced role in clayey soils while cadaveric microbes play a greater role in sandy soil. The negative ACE value was due to a negative mass loss value. Negative mass loss was almost certainly caused by the adherence of soil to the skin.

In conclusion, the presence of soil microorganisms can result in an increase in the rate of cadaver decomposition relative to cadaver decomposition in the absence of soil microorganisms. However, soil type appears to have an influence on this process. These results support the concept that the soil microbial biomass is capable of making a significant contribution to cadaver decomposition (Chapter 6). Although it is possible that the soil microbial biomass might participate in cadaver decomposition immediately following burial, these effects were not observed via measurements of mass loss until the latter phase of incubation.

CHAPTER 8

SEASONAL VARIATION IN CADAVER DECOMPOSITION FOLLOWING BURIAL IN A FIELD SETTING

The tropical savannas near Townsville, Queensland, Australia can be defined, in part, by their seasonality. The year is divided into a warm, wet season and a cooler dry season. These changes in temperature and moisture can have a significant effect on processes associated with the decomposition of an organic resource (Manlay et al., 2004; Sarmiento et al., 2004). This is due to evidence that microbial activity and decomposition rates tend to be low during the cool dry season (Gupta and Singh, 1981; Chen et al., 2002) and increase at the onset of the warm wet season (Holt et al., 1990). Although the decomposition of cadavers in tropical savanna ecosystems has received little attention (Morris et al., 1982) it is believed that seasonal changes in temperature and moisture can affect the processes associated with the decomposition of these resources.

To date, the effect of season on cadaver decomposition in soil has only been investigated in temperate ecosystems (Morovic-Budak, 1965; Mant, 1987). These studies were anecdotal in nature and reported that cadaver decomposition tends to be more rapid during the warmer summer months. This phenomenon is generally attributed to the increased activity of arthropods and vertebrate scavengers (Rodriguez and Bass, 1985; Mann et al., 1990) but is also due to the influence of temperature on autolytic and putrefactive processes that take place inside the cadaver (Gill-King, 1997). To date, seasonal effects on the soil microbial biomass and chemistry associated with cadaver decomposition has not been investigated.

In order to examine the effect of season on cadaver (*Rattus rattus*) decomposition in a tropical savanna setting, field trials were conducted at the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in Queensland, Australia during the dry season (October 2002) and wet season (March 2003). Details concerning site and soil characterisation are presented in Chapter 3.

A 20 m x 10 m study area was laid out at the FA and WB sites while an 18 m x 12 m study area was used at the PR site. Each study area was divided into 2m x 2m plots. Cadavers were buried at a depth of 2.5 cm in the centre of each 'test' plot. In 'control' plots (i.e. no cadaver) cadaver burial was simulated to account for any effects caused by soil disturbance. Following burial/soil disturbance, cadavers and soil were harvested as described in Chapter 2. In addition, a 10 g soil subsample was collected and frozen at –20 °C for phospholipid fatty acid analysis described in Chapter 9. Soil matric potential of the detritisphere was calculated following each harvest by converting gravimetric water content (g H_2O g^{-1} soil) to volumetric water content (%) (Chapter 3). Site temperature data was collected from nearby weather stations that conducted temperature readings over a 24 hour period beginning at 9 am on each day of the incubations. The experiment was replicated six times. Each treatment was set up with sufficient replicates for four sequential harvest events conducted at intervals of seven days over a period of 28 days. This resulted in a total of 144 samples for each season. This experiment was conducted in order to test the hypothesis that cadaver decomposition in a tropical savanna soil is greater during the wet season. In addition, this experiment was conducted in order to determine if the cadaver effects observed under laboratory conditions can be detected in a field setting.

8.2. Results

8.2.1. Site temperature and soil moisture

The air temperature (Table 8.1) and soil moisture content (Figure 8.1) at each site was greatest during the wet season. In FA soil volumetric water content in the wet season ranged from 19.85% (day 0) to 1.8% (day 28). This equalled a matric potential of –0.3 megapascals (MPa) on day 0 and –15 MPa on day 28. In PR soil volumetric water content ranged from 12.8% (-0.109 MPa) on day 14 to 1.7% (-0.75 MPa) on day 28. Volumetric water content in WB soil ranged from 32.2% (-0.165 MPa) on day 0 to 24.1% (-1.05 MPa) on day 28. The effect of cadaveric moisture was not detected by gravimetric moisture measurements. Volumetric water content remained constant during the dry season at 3.0% (FA: -15 MPa), 2.9% (PR: -15 MPa) and 22.2% (WB: -3 MPa).

Table 8.1. Air temperature at the FACE/Yabulu (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savannas of Queensland, Australia during dry (October 2002) and wet (March 2003) season. Data was calculated from measurements taken over a 24 hour period from 9am each day.

Site	Season	Mean maximum temp. (°C)	Mean minimum temp. (°C)
FA	Dry	22.9	16.7
	Wet	30.5	27.0
PR	Dry	26.9	16.4
	Wet	30.7	23.1
WB	Dry	20.5	13.7
	Wet	31.6	27.5

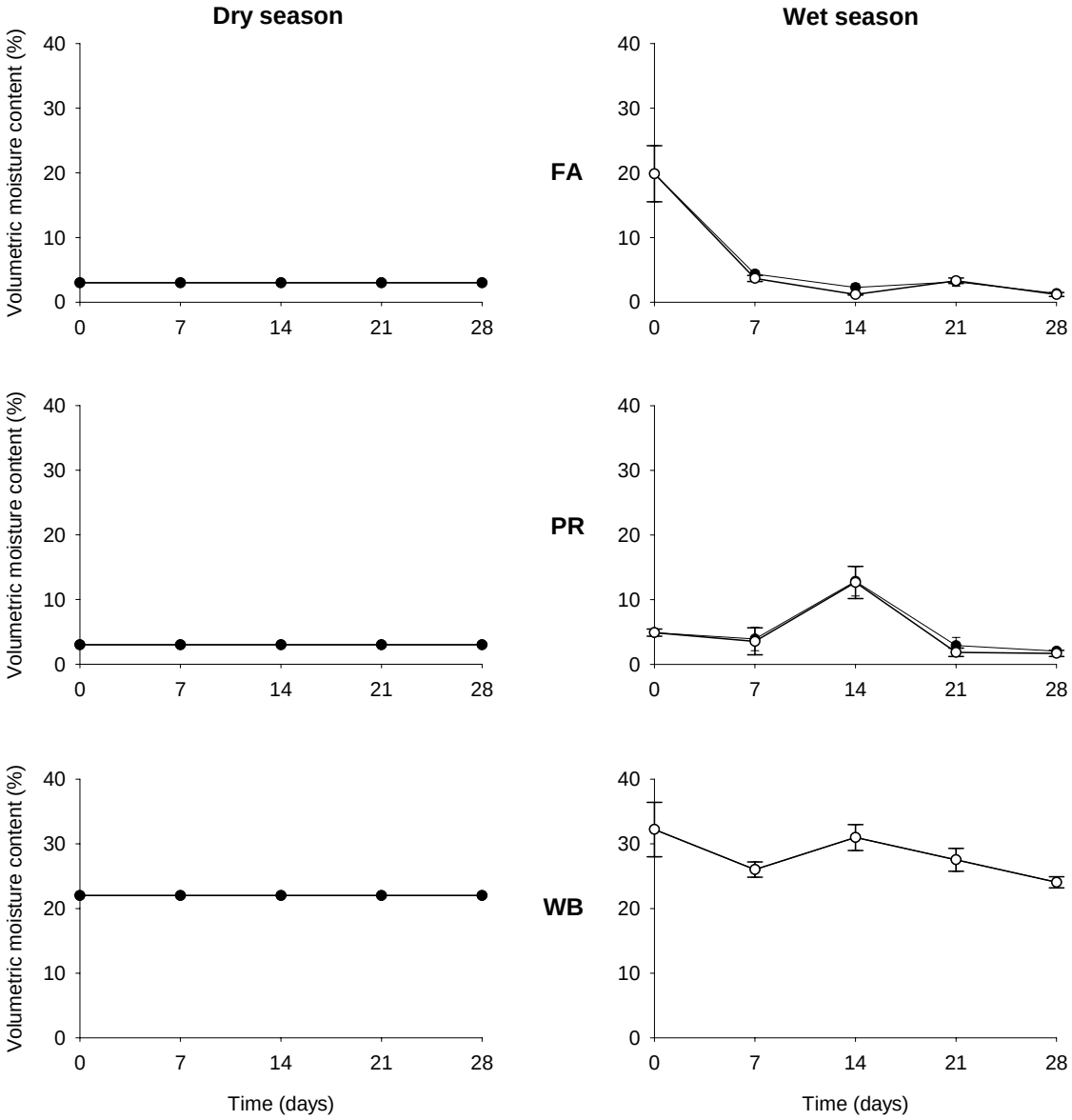

Figure 8.1. Volumetric moisture content (%) of soil at the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in Queensland, Australia during the dry season (October 2002) and wet season (March 2003) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and without a cadaver (○). Bars represent standard errors where n = 6.

8.2.2. Cadaver mass loss

Cadaver mass loss in the wet season was greater than in the dry season in FA ($P < 0.001$) and PR ($P < 0.01$) soils but season effect was observed in WB soil (Figure 8.2). Cadaver decomposition in WB soil was greater than in FA and PR soil ($P < 0.001$). Exhumed cadavers were more desiccated than in the laboratory experiments and came very close to 100% mass loss, comprising desiccated skin, bone and hair.

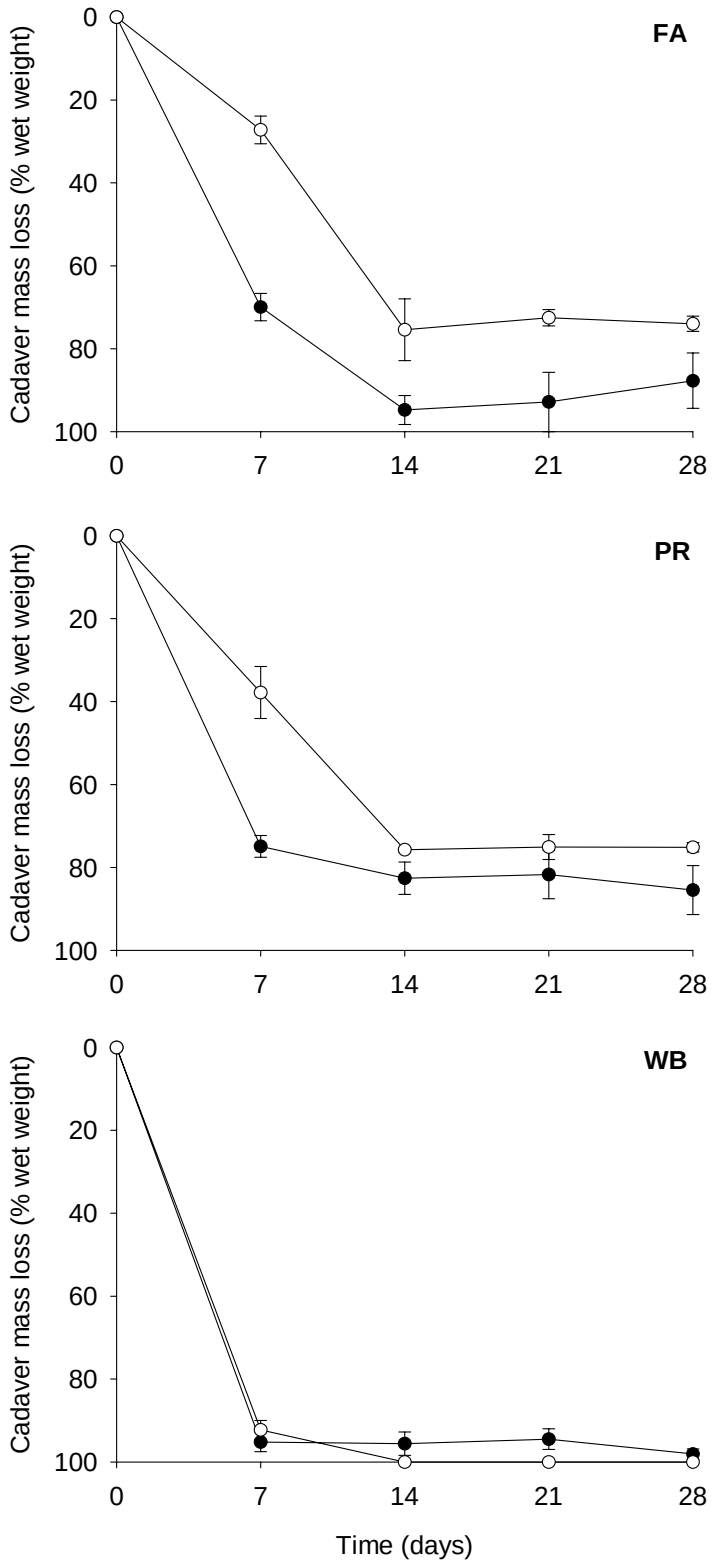

Figure 8.2. Mass loss (% wet weight) of a juvenile rat (*Rattus rattus*) cadaver following burial (2.5 cm) at the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in Queensland, Australia during the dry season (○: October 2002) and wet season (●: March 2003). Bars represent standard errors where n = 6.

8.2.3. Microbial biomass (C_{mic})

An increase in C_{mic} following cadaver burial was observed during both seasons. This increase was enhanced during the wet season (Figure 8.3). During the dry season, an increase in C_{mic} in FA soil was observed on day 28 while an elevated level of C_{mic} in PR and WB soils was observed at each harvest. In the wet season an greater C_{mic} was observed at each harvest except day 28 in FA and PR soils. In addition, a soils effect was observed during both seasons. During the dry season C_{mic} in WB was greater than in PR, which was greater than in FA ($P < 0.05$). During the wet season C_{mic} following cadaver burial in PR was greater than in WB, which was greater than in FA ($P < 0.05$).

8.2.4. Protease activity

Cadaver burial resulted in an increase in protease activity during wet and dry season and this increase was enhanced during the wet season in FA and PR test samples ($P < 0.01$) (Figure 8.4). Elevated rates of protease activity during the dry season were only observed during the latter half of incubation. No seasonal effect was observed in WB test samples or in control samples. During the wet season, protease activity was greatest in FA and PR test samples ($P < 0.05$).

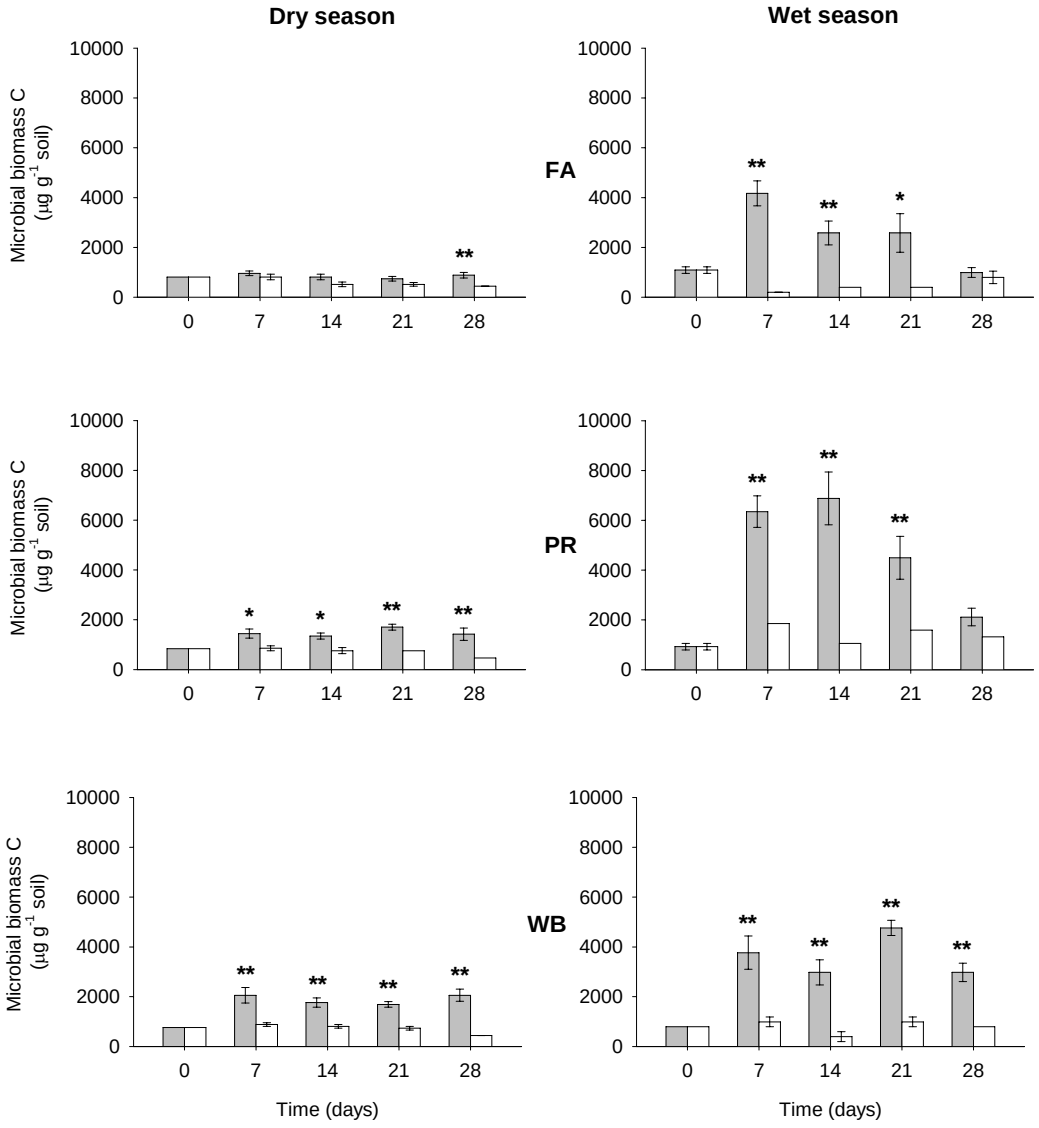

Figure 8.3. Microbial biomass C (μg C_{mic} g^{-1} soil) in soils at the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in Queensland, Australia during the dry season (October 2002) and wet season (March 2003) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (■) and without a rat cadaver (□). * denotes significant difference between test and control samples within time: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

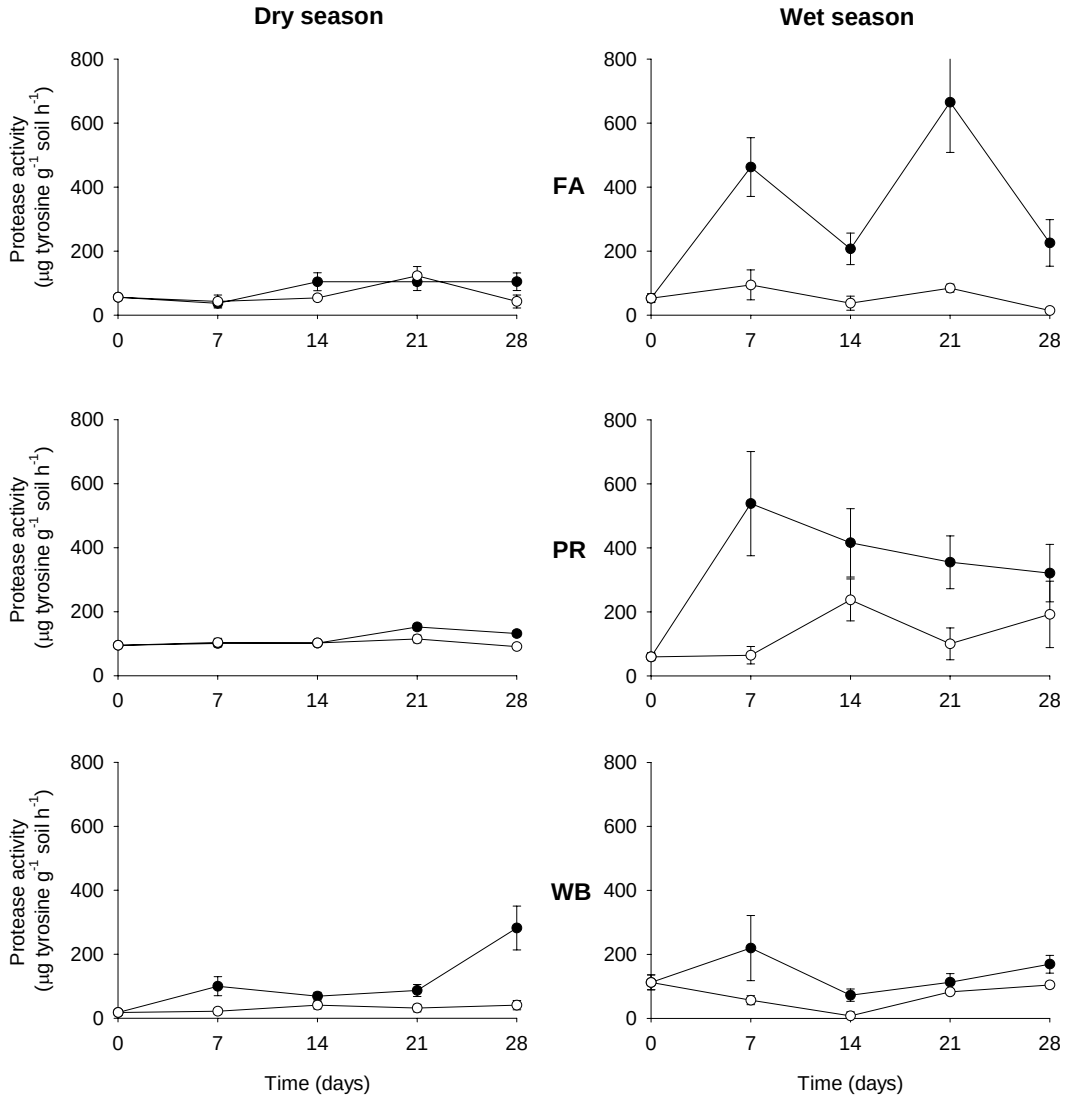

Figure 8.4. Protease activity (µg tyrosine g^{-1} soil h^{-1}) in soils at the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in Queensland, Australia during the dry season (October 2002) and wet season (March 2003) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and without a rat cadaver (○). Bars represent standard errors where n = 6.

8.2.5. Phosphodiesterase activity

During the dry season cadaver burial resulted in an increase in the rate of phosphodiesterase activity in PR and WB soil ($P < 0.05$). During the wet season, cadaver burial resulted in an increase in phosphodiesterase activity in FA ($P < 0.001$) and PR ($P < 0.01$) soil (Figure 8.5). Phosphodiesterase among test samples was always greater during the wet season. In addition, phosphodiesterase activity in PR and WB control samples during the wet season was greater than during the dry season ($P < 0.05$). Phosphodiesterase activity was always greatest in WB soil ($P < 0.05$).

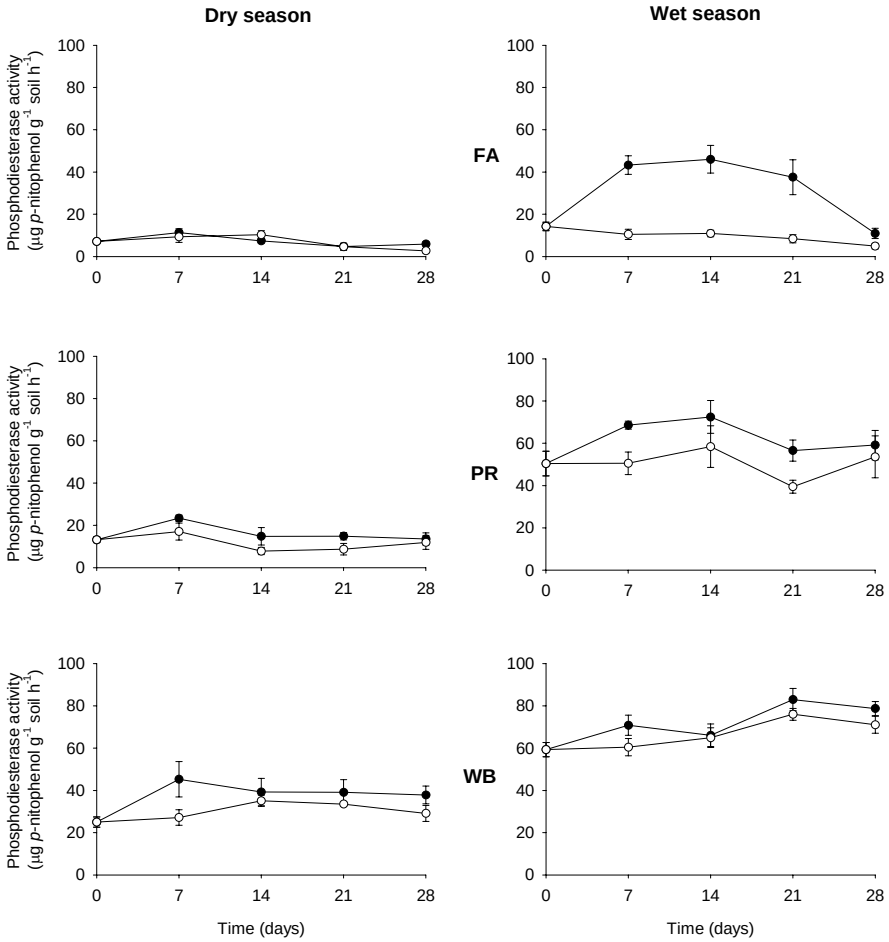

Figure 8.5. Phosphodiesterase activity (µg *p*-nitrophenol g^{-1} soil h^{-1}) in soils at the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in Queensland, Australia during the dry season (October 2002) and the wet season (March 2003) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and without a rat cadaver (○). Bars represent standard errors where n = 6.

8.2.6. Ammonium-N (NH_4^+-N)

Cadaver burial resulted in an increase in the concentration of NH_4^+-N at each harvest during wet and dry season ($P < 0.05$) (Figure 8.6). The concentration of NH_4^+-N in test samples was always greater during the wet season. A soil type effect was not observed following cadaver burial during the dry season. However, cadaver burial in the wet season resulted in a greater concentration of NH_4^+-N in WB and FA soils relative to PR soil ($P < 0.05$).

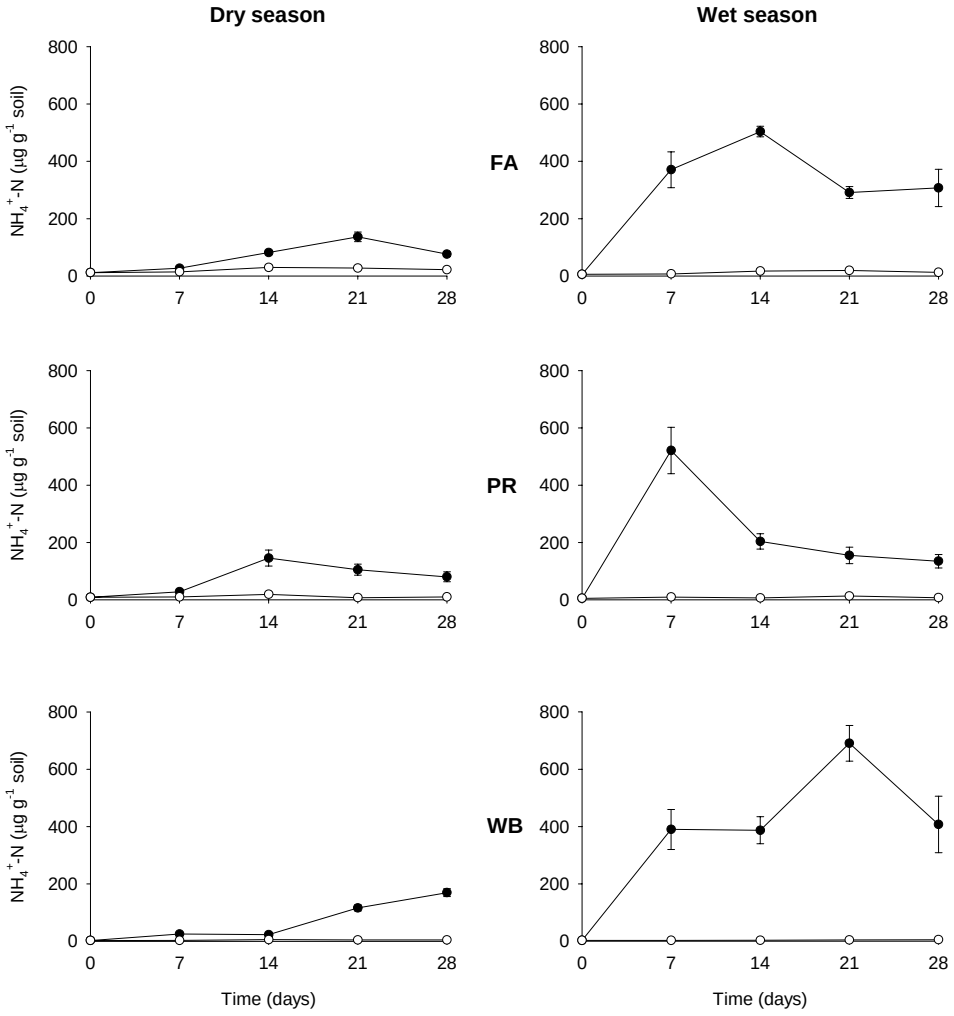

Figure 8.6. Concentration of ammonium-N (µg NH_4^+-N g^{-1} soil h^{-1}) in soils at the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in Queensland, Australia during the dry season (October 2002) and the wet season (March 2003) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and without a rat cadaver (○). Bars represent standard errors where n = 6.

8.2.7. Nitrate-N (NO_3^--N)

Cadaver burial had little effect on the concentration of NO_3^--N, as the only cadaver effect was an elevated concentration of NO_3^--N on day 14 in WB test samples and on day 28 in PR soils (Figure 8.7). Both of these increases took place during the dry season. A seasonal effect was observed in WB soil as the concentration of NO_3^--N in test samples in the dry season was greater than in the wet season. Soil type had no effect on the concentration of NO_3^--N although PR soil tended to have the highest mean concentration of NO_3^--N.

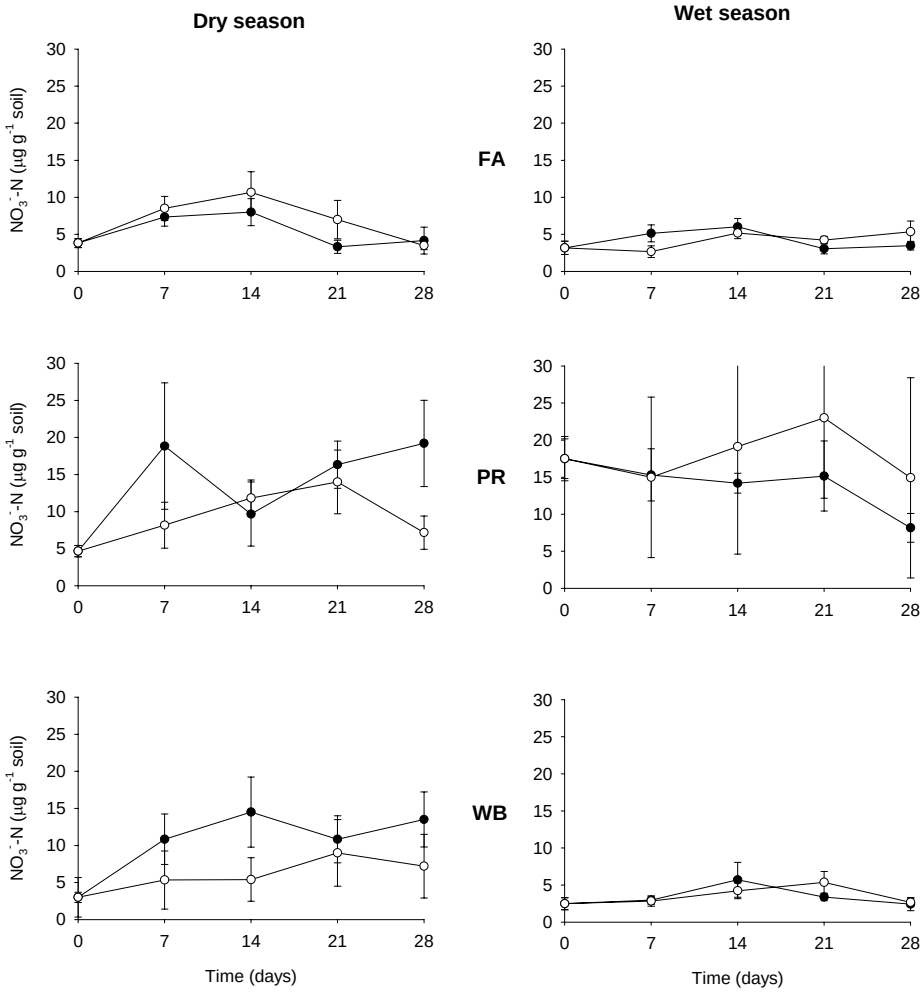

Figure 8.7. Concentration of nitrate-N (µg NO_3^--N g^{-1} soil) in soils at the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in Queensland, Australia during the dry season (October 2002) and the wet season (March 2003) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and without a rat cadaver (○). Bars represent standard errors where n = 6.

8.2.8. Soil pH

Cadaver burial resulted in an increase in soil pH in all soils during both seasons ($P < 0.05$) (Figure 8.8). This increase was greater during the wet season in FA and PR soil ($P < 0.05$).

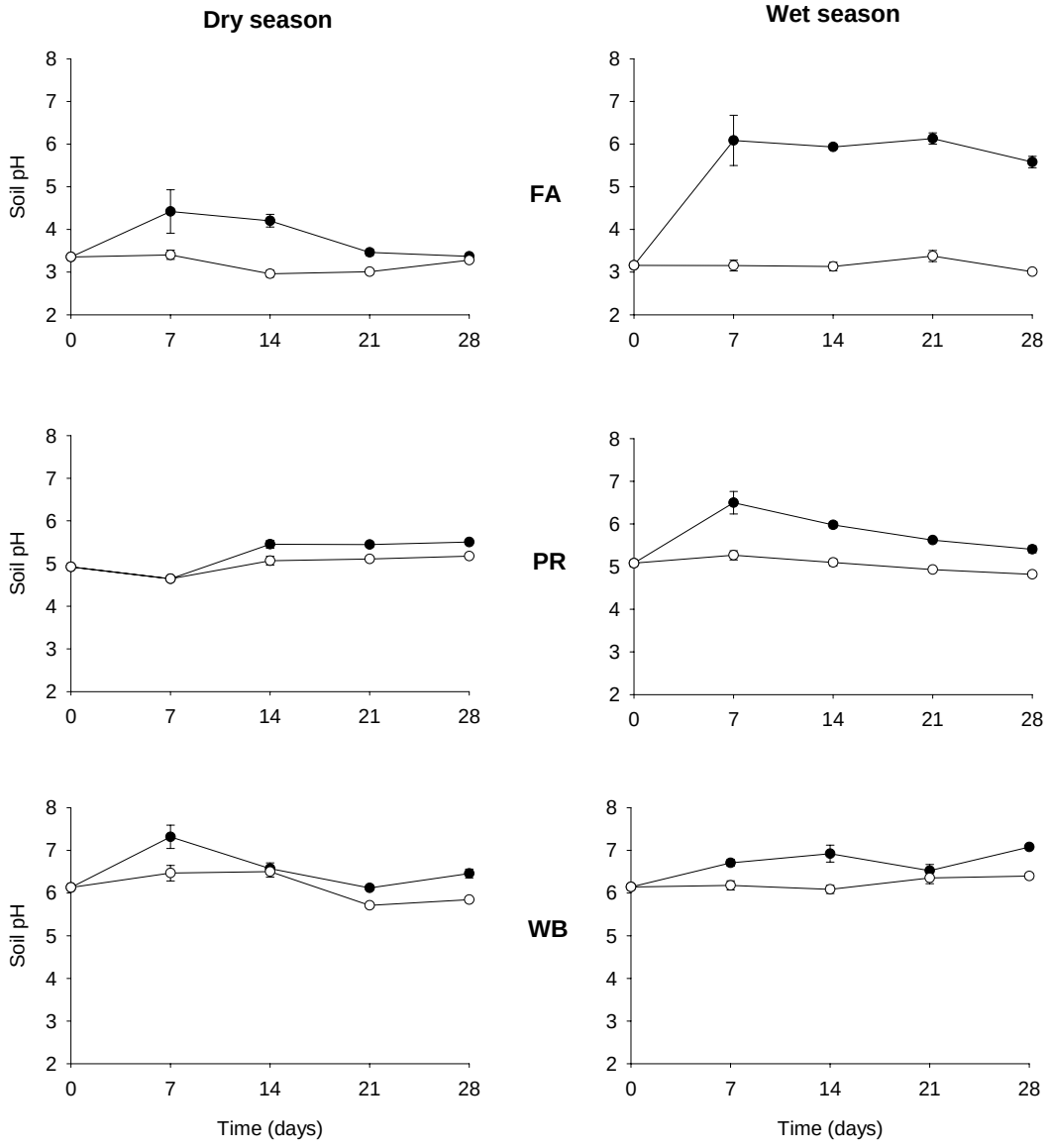

Figure 8.8. Soil pH at the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in Queensland, Australia during the dry season (October 2002) and the wet season (March 2003) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and without a rat cadaver (○). Bars represent standard errors where n = 6.

8.3. Discussion

The current results show that a cadaver buried in a tropical savanna soil during the wet season can undergo a more rapid rate of decomposition than in the dry season. This phenomenon was associated with greater levels of C_{mic} (Figure 8.3), protease activity (Figure 8.4), phosphodiesterase activity (Figure 8.5), NH_4^+-N (Figure 8.6) and soil pH (Figure 8.8). The increase in the rate of mass loss and associated processes was probably related to the greater increases in available moisture and temperature observed during the wet season. An increase in the rate of cadaver mass loss during the wet season was not surprising since previous studies have reported an increase in the rate of cadaver decomposition during warmer months (Morovic-Budak, 1965; Mann et al., 1990; Fiedler and Graw, 2003). Also, the matric potential of FA and PR soil (Figure 8.1) did not exceed the moisture content that resulted in a decrease in the rate of cadaver decomposition (FA: -0.01 MPa; PR > -0.01 MPa) observed in Chapter 5. Therefore, the matric potential of the soils during the wet season would possibly allow for an increase in microbial activity (e.g. metabolism, mobility) and diffusion of nutrients to, and products from, the soil microbial population. The increase in temperature also probably enhanced microbial metabolism. An increase in temperature of 7 °C was observed to result in an increase in microbial metabolism under laboratory conditions (Chapter 4). Consequently, the difference in temperature observed between seasons (Table 8.1) would almost certainly have resulted in an increase in microbial activity and contributed to the increase in cadaver mass loss (Figure 8.2).

The increase in the rate of cadaver mass loss during the wet season is also possibly related to an increase in arthropod activity that is typically observed during the wet season (Janzen, 1973; Poulin et al., 1992). Interestingly, arthropod activity, along with the physical nature of Vertosols, is possibly the reason for the similarity in cadaver mass loss between seasons at the WB site (Figure 8.2). When WB soil (Vertosol) dries it forms relatively large clods that have substantial voids between them. Such voids, forming a network of inter-connected pores, might have allowed for greater access of arthropods.

A number of differences were observed between cadaver decomposition between field and laboratory settings. These differences are likely due to the fact that the incubation chambers used in the laboratory incubations are enclosed systems that are maintained at a constant temperature and moisture content. First, cadaver mass loss was greater in a field setting relative to a laboratory setting. This phenomenon is probably due to an increase in the extent of desiccation (observed upon exhumation) and an increase in the number of decomposer organisms (e.g. arthropods) in a field setting. Thus, in a relatively dry field setting, the initial stages of cadaver decomposition might be primarily due to the dehydration of a cadaver and this loss of mass can be enhanced by an increase in the activity of decomposer organisms associated with the wet season.

The macroscopic presence of fungal hyphae was not observed at any of the field sites, while in the laboratory incubations fungal hyphae was typically observed by day seven. Again, this is probably due to the relatively low, constant matric potential in the laboratory incubations. In addition, soil pH was consistently greater

in the laboratory incubations. The relatively small increases in pH observed in the current study are similar to those found by Rodriguez and Bass (1985) in Knoxville, Tennessee, USA. Despite these differences, an increased level of C_{mic} (Figure 8.3), enzyme activities (Figure 8.4; Figure 8.5), NH_4^+-N (Figure 8.6) and soil pH (Figure 8.8) indicates that biological and chemical markers of cadaver decomposition can endure for at least 21 days after approximately 95% of cadaver mass has been lost (Figure 8.2).

The increase of soil pH during cadaver decomposition has been attributed to the presence of NH_4^+-N (Hopkins et al., 2000) and, in a field setting, this effect could have been diminished as a result of NH_3 volatilisation, which might have been restricted by the enclosed system used in the laboratory incubations. The levels of inorganic N observed in the current study were similar to the results in Hopkins et al. (2000) and Towne (2000) although their work was carried out in different ecosystems, over longer periods of time and in association with much larger animals (*Sus scrofa, Bos bison, B. taurus*). Interestingly, cadaver burial had no effect on the concentration of NO_3^--N (Figure 8.7). The concentration of NO_3^--N is typically low in tropical savanna soils (Singh et al., 1989) and the current results may be due to the inability of nitrifiers to function in the presence of a cadaver and/or in association with the relatively high matric potential that characterise the current soils. In addition, Lata et al. (2004) have suggested that the plants in tropical savanna soils might be able to outcompete soil microorganisms for NH_4^+-N.

To conclude, cadaver decomposition can be more rapid following burial in the wet season. The increased rate of mass loss was associated with the growth of the soil

microbial biomass and an increase in protease and phosphodiesterase activity. These biological changes took place in conjunction with an increase in NH_4^+-N and soil pH. The increases observed were probably due to the increase in temperature and moisture associated with the wet season and may have also been related to a change in the structure of the soil microbial community, which is the subject of Chapter 9.

CHAPTER 9

THE SOIL MICROBIAL COMMUNITY IN ASSOCIATION WITH CADAVER DECOMPOSITION IN SOIL

The amendment of soil with an organic resource can result in a shift in the structure of the soil microbial community (Bossio et al., 1998) and these changes can be further influenced by abiotic variables such as temperature and moisture (Bossio and Scow, 1998; Waldrop and Firestone, 2004). In addition, changes in microbial community structure has been observed to coincide with changes in microbial community function such as enzyme activity (Waldrop et al., 2000) and carbon dioxide evolution (Zogg et al., 1997; Bossio et al., 1998). These changes have been observed following the amendment of soil with organic resources such as manure (Bossio et al., 1998), however, structural and functional dynamics of the microbial community in association with cadaver decomposition in soil has received little attention (Rhodes et al., 1998; Tibbett and Carter, 2003).

It is currently understood that cadaver burial can result in the growth of the soil microbial biomass (see previous Chapters and Hopkins et al. (2000)), which is likely to be composed, in part, of cadaver-borne microbes as demonstrated in Chapter 7 and Rhodes et al. (1998). In addition, the fungal fraction of the microbial community has been observed to display an apparent successional sequence in association with cadaver decomposition (Tibbett and Carter, 2003). This is due, in part, to the presence and form of nitrogen. However, it is currently unknown if cadaver burial in a tropical savanna soil can result in a shift in the structure of the microbial community in soil and, if so, if it is related to the functional processes associated with cadaver decomposition, such as enzyme activity.

A number of methods have been developed that provide phenotypic or genotypic data concerning the structure of the soil microbial community. Nucleic acid analyses can provide genotypic information concerning the structural composition of a microbial population. Methods such as Terminal Restriction-Fragment Length Polymorphism (Tiedje et al., 1999) and Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (Muyzer et al., 1993) can be used to identify soil microbial community structure to species and strain level. As such, nucleic acid analysis is the only approach available to estimate the absolute diversity of soil microbiota (Torsvik et al., 1990a; Torsvik et al., 1990b) and, in many cases, is the only approach for monitoring the dynamics of specific taxa (Sinsabaugh et al., 1999).

One method to assess the whole soil microbial community is via phospholipid fatty acid (PLFA) analysis. PLFAs are not used for storage and are rapidly decomposed. Thus, their presence represents the living, active component of the soil microbial community (Tunlid and White, 1992). Any change in PLFA pattern reflects a change in microbial community structure. Unfortunately, PLFA has limited capability to identify microorganisms to species and strain level. This is because an individual species may contain numerous PLFAs and many PLFAs are associated with more than one species (Lechevalier, 1977; Zelles, 1999). Because of this, and the fact that extremely little work has been done on PLFAs in tropical savanna soils, PLFAs in the current study were not used as biomarkers of functional groups. However, PLFAs were used to assess microbial community structure whereby any

observed variation in PLFA profile represented a shift in the structure of the soil microbial community.

9.2. Methods

9.2.1. Experimental design and sampling

PLFA analysis was conducted on the subsamples of the detritisphere collected and frozen (-20 °C) during the incubations at the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites described in Chapter 8. This analysis, conducted on subsamples harvested on day 0, day 7, day 14 and day 28, was replicated four times. The current experiment was conducted in order to test the hypothesis that cadaver burial, season, soil type and time of harvest are associated with a shift in the structure of the soil microbial community.

9.2.2. Phospholipid fatty acid analysis

9.2.2.1. Lipid Extraction

PLFA extraction was based on the method of Pankhurst et al. (2001). Soil (10 g dry weight) from the field trial (Chapter 8) was amended with 19 ml of one-phase extraction buffer comprised of chloroform:methanol:phosphate buffer (0.8:1:2 by vol.). The soil/buffer suspension was mixed on a rotating shaker for 2 hours followed by centrifugation at 2000 rpm for 5 minutes. The supernatant was removed and placed in a 50 ml culture tube with a Teflon-lined cap (Crown Scientific, Beverly, Australia; Catalogue no. 9826-16). Centrifuged soil samples were then amended with 5 ml of one-phase buffer, vortexed for 15 seconds and centrifuged at 2000 rpm for 5 minutes. Supernatants were added to respective 50 ml glass culture tubes (Crown Scientific, Beverly, Australia; Catalogue no. 9826-16) and the volume

of the supernatants calculated for each sample. The volume of the supernatant was used to calculate the volume of chloroform and phosphate buffer required to conduct phase separation. The required ratio of phosphate buffer:chloroform:methanol equalled 0.9:1:1 (by volume). Following the addition of phosphate buffer and methanol the culture tubes were sealed with a Teflon-lined cap and vortexed for 1 minute. The cap was then removed and the solution was dried under nitrogen (N_2). The dried sample represented the lipid extract.

9.2.2.2. Lipid fractionation

Lipid extract was resuspended in 1 ml chloroform ($CHCl_3$) and applied to a column of silicic acid conditioned with $CHCl_3$. Neutral lipids were firstly eluted with 5 ml $CHCl_3$, followed by glycolipids, which were eluted with 10 ml acetone. Finally, phospholipids were eluted with 5 ml methanol into a clean culture tube and dried under N_2 at 40 °C. Fractionation blanks were made by passing only reagents (5 ml $CHCl_3$, 10 ml acetone, 5 ml ethanol) through a silicic acid column.

9.2.2.3. Acid methylation

Dried phospholipid fractions were amended with 1.5 ml acidified (1% H_2SO_4) methanol (Christie, 1989). Samples were sealed with Teflon-lined caps and incubated over night at 60 °C. Following incubation, samples were allowed to cool to room temperature. Samples were then amended with 3 ml purified water and 5 ml petroleum ether prior to being vortexed for 15 seconds. This resulted in the formation of an upper ether layer and a lower aqueous layer. The upper ether layer was transferred to a clean culture tube using a pipette. The aqueous layer was amended with 5 ml of petroleum ether and the extraction and removal of the ether

layer was repeated. The transferred ether layer was dried under N_2 at 40 °C. Following drying, 10 µl of C 19:0 standard [0.037 g nonadecanoic acid methyl ester (Sigma N5377) dissolved in 50 ml hexane] and 100 µl hexane were added to the culture tube. The resulting solution was pipetted into 200 µl gas chromatography (GC) inserts (Agilent Technologies, Forest Hill, Australia; Catalogue no. 5181-1270) placed in 2 ml GC vials (Agilent Technologies, Forest Hill, Australia; Catalogue no. 5182-0715) with caps (Agilent Technologies, Forest Hill, Australia; Catalogue no. 5182-0717).

PLFAs were separated by capillary GC using an automated procedure developed by MIDI (MIDI, Inc., Newark, DE). The system comprised a GC (HP 5890) – Hewlett Packard HP with flame ionisation detector, HP-IB communications, HP 3365 ChemStation software and computer. The HP 3365 ChemStation software operated the sampling, analysis and integration of the chromatographic sample. The GC was equipped with a HP 25 m x 0.2 mm fused silica capillary column. The temperature was ramped from 170 °C to 250 °C at 5 °C $minute^{-1}$. Hydrogen was the carrier gas. The PLFA peaks were identified by the MIDI program based on their equivalent chain length. The peak areas were normalised against the 19:0 internal standard.

9.2.2.4. Fatty acid nomenclature

The number before the colon identifies the C chain length of the fatty acid. The number after the colon represents the number of C=C double bonds and their location relative to the methyl end (ω) of the molecule. For example, 16:1ω5 is a 16-C fatty acid with one double bond beginning at the fifth C from the methyl end. The letters *c* and *t* represent *cis* and *trans* isomers of the double bond. The letters *i*

and *a* designate methyl branching in the first (*iso*) or second (*anteiso*) C, respectively, from the methyl end. Branches at other positions are identified by a number indicating the position of the methyl branch relative to the carboxylic end of the molecule. For example, *i*16:0 has a methyl branch on the first C from the ω end and 10Me18:0 has a methyl branch on the tenth C from the carboxylic end. The prefix *cy* represents a cyclopropane fatty acid.

9.2.3. Statistical analysis

PLFA profiles were assessed via a multivariate analysis of variance with permutations using Bray-Curtis dissimilarities (McCardle and Anderson, 2001). This was done with the PERMANOVA_2factor program (Marti J. Anderson, University of Auckland). Observed variation in total PLFA profiles was explained using distance-based multivariate analysis with forward selection of explanatory variables in a linear regression model using permutations. Explanatory variables comprised soil biophysicochemical characteristics measured in Chapter 8 (microbial biomass C, ammonium-N, nitrate-N, phosphodiesterase activity, protease activity, soil pH). This analysis was conducted using the DISTLMforward 1.3 program (Marti J. Anderson, University of Auckland). Visual representations of the total PLFA profiles were presented in non-metric multidimensional scaling (MDS) plots using Bray-Curtis distances. These plots were generated using PRIMER 6 β (PRIMER-E, Plymouth, United Kingdom). All multivariate statistics were generated following log(x + 1) transformation.

9.3. Results

A number of PLFAs were consistently detected during analysis. These included saturated (14:0, 15:0, 16:0, 17:0, 18:0), branched (i15:0, a15:0, i16:0, i17:0, a17:0), cyclopropyl (cy17:0, cy19:0), hydroxyl (i15:0 3OH, 16:1 2OH, 16:0 2OH), monounsaturated (16:1ω5c, 16:1ω7c, 16:1ω9c, 16:1ω3c, 17:1ω6c, 18:1ω11c, 18:1w6c, 18:1ω9c, 18:1ω3c, 19:1ω8t) and polyunsaturated (18:2ω6c, 18:4ω3c) PLFAs. Some hydroxyl (16:0 2OH) and monounsaturated (16:1ω9c, 16:1w3c, 17:1ω6c, 18:4ω3c, 18:1ω11c, 18:ω3c, 18:1ω6c) PLFAs were only observed during the wet season. Of these, 16:1ω9c, 18:1ω6c were restricted to WB soil. These data are presented in Appendix C.

9.3.1. Effect of cadaver burial on PLFA profiles

A shift in PLFA profiles following cadaver burial was observed in each soil except PR soil during the dry season (Table 9.1). The shift observed in the PR soil during the wet season was due to the presence of a cadaver. The shifts observed in FA and WB soil during the dry season were due to an interaction between cadaver treatment and time of harvest. Explanatory variables explained from 24.4% to 46.3% of the observed variation (Appendix E: Table E.1). However, these variables were only significantly related to shifts in PLFA profiles in FA and WB soil during the wet season and WB soil during the dry season (Appendix E: Table E.1).

Table 9.1. Statistical analysis of PLFA profiles between test samples (+ cadaver) and control samples (soil without cadaver) in soils from the Yabulu/FACE (FA), Pallarenda (PR) or Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver or soil disturbance during the dry (October 2002) and wet (March 2003) season.

		Cadaver		Time of harvest		Cadaver x Time of harvest	
Soil	Season	F	*P*	F	*P*	F	*P*
FA	Dry	1.34	0.226	8.60	**<0.001**	2.64	**0.010**
	Wet	0.99	0.364	9.25	**<0.001**	0.88	0.524
PR	Dry	0.72	0.613	1.53	0.125	0.92	0.491
	Wet	2.83	**0.021**	3.14	**0.004**	2.91	**0.005**
WB	Dry	1.68	0.130	3.40	**0.002**	1.96	**0.051**
	Wet	0.72	0.643	1.83	**0.044**	1.45	0.142

The variation in PLFA profile in FA soil during the wet season was observed on day 7 (Figure 9.1) and was significantly related to soil moisture ($P < 0.001$) (Appendix E: Table E.1). This can be observed in Figure 9.1 and in Chapter 8 (Figure 8.1) as matric potential of test and control soils on day 7 was greater ($P < 0.05$) than on day 14 and day 28.

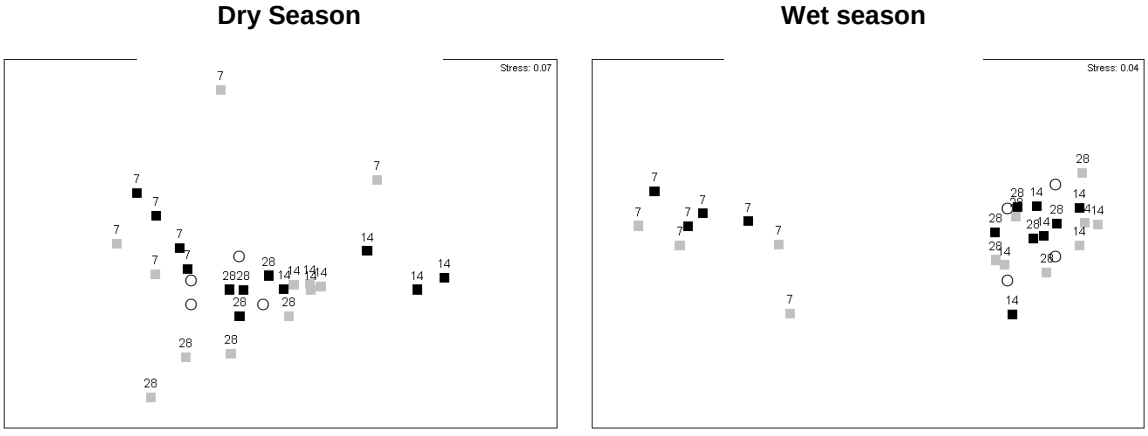

Figure 9.1. Effect of cadaver burial on phospholipid fatty acid profiles in soil from the Yabulu/FACE site in Queensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (■) and without a cadaver (■) during the dry season (October 2002) and wet season (March 2003). ○ represents samples harvested on day 0.

A cadaver effect was observed in PR soil during the wet season (Table 9.1) and this can be seen as a grouping of the PLFA profiles of control soils harvested on day 28 (Figure 9.2). However, none of the explanatory variables were significantly related to this shift (Appendix E: Table E.1).

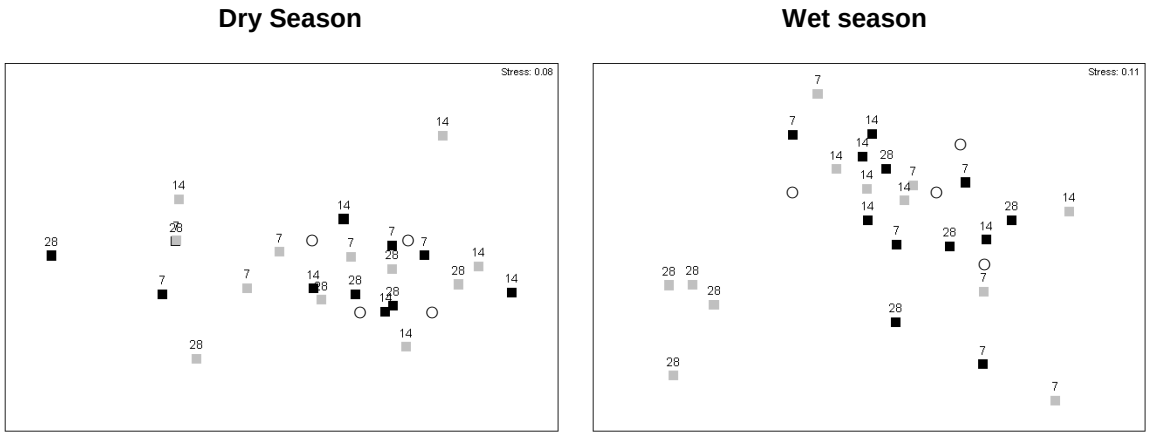

Figure 9.2. Effect of cadaver burial on phospholipid fatty acid profiles in soil from the Pallarenda site in Queensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (■) and without a cadaver (■) during the dry season (October 2002) and wet season (March 2003). ○ represents samples harvested on day 0.

A cadaver-induced shift in PLFA profile following cadaver burial was observed in WB soil during the dry season (Table 9.1). This was significantly related to NH_4^+-N ($P < 0.01$), NO_3^--N ($P < 0.05$) and soil pH ($P < 0.05$) (Appendix E: Table E.1). These results coincide with an increase in NH_4^+-N on day 28 (Chapter 8: Figure 8.6) and a relative decrease in soil pH over time within cadaver treatments (Chapter 8: Figure 8.8) as well as a difference in the concentration of NO_3^--N on day 14 between cadaver treatments (Chapter 8: Figure 8.7). These effects can be seen in Figure 9.3 as samples harvested on day 28 were grouped separately to those harvested on day 7 and 14.

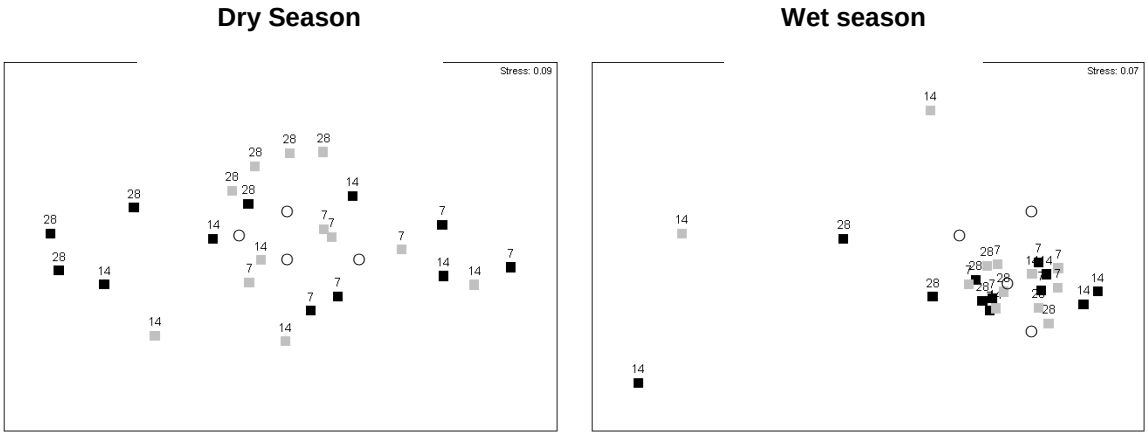

Figure 9.3. Effect of cadaver burial on phospholipid fatty acid profiles in soil from the Wambiana site in Queensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (■) and without a cadaver (■) during the dry season (October 2002) and wet season (March 2003). ○ represents samples harvested on day 0.

The shift in PLFA profile observed in WB soil during the wet season, like FA soil during the wet season, was not related to the presence of a cadaver (Table 9.1). This shift was significantly associated with matric potential ($P < 0.05$) and phosphodiesterase activity ($P = 0.05$) (Appendix E: Table E.1). These findings coincide with an increase in matric potential (Chapter 8: Figure 8.1) and phosphodiesterase activity (Chapter 8: Figure 8.5) in both test and control samples on day 28. These effects, however, are not visually discernable in Figure 9.3.

9.3.2. Effect of season on PLFA profiles

Season had an effect on all PLFA profiles (Table 9.2). In addition, an effect of time of harvest and an interaction between season and time of harvest was observed on all samples except PR soil following cadaver burial (Table 9.2). Soil biophysicochemical characteristics explained from 28.3% to 55.5% of the variation observed in PLFA profiles between seasons (Appendix E: Table E.2).

Table 9.2. Statistical analysis of PLFA profiles between seasons in soils sampled from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savannas of Queensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (+ cadaver) and without a cadaver (- cadaver) during the dry (October 2002) and wet (March 2003) season.

		Season		Time of harvest		Season x Time of harvest	
Soil	Treatment	F	*P*	F	*P*	F	*P*
FA	+ cadaver	18.31	**<0.001**	5.46	**<0.001**	6.90	**<0.001**
	- cadaver	8.59	**<0.001**	2.95	**<0.001**	3.10	**<0.001**
PR	+ cadaver	32.74	**<0.001**	1.68	0.091	1.76	0.076
	- cadaver	23.98	**<0.001**	2.41	**0.005**	2.40	**0.005**
WB	+ cadaver	34.93	**<0.001**	2.60	**0.010**	2.58	**0.011**
	- cadaver	36.40	**<0.001**	2.07	**0.039**	2.08	**0.039**

In FA control samples, protease activity was significantly related ($P < 0.05$) (Appendix E: Table E.2) to the variation observed on day 14 and day 28 of the wet season (Figure 9.4). This is reflected in Chapter 8 (Figure 8.4) by the reduced rate of protease activity on day 14 and day 28 of the wet season.

A similar grouping was observed among test samples but was related to matric potential ($P < 0.05$), microbial biomass C ($P < 0.01$) and NH_4^+-N ($P < 0.001$) (Appendix E: Table E.2). The wet season samples harvested on day 14 and day 28 were associated with a higher matric potential than at day 0 and day 7 (Chapter 8: Figure 8.1) and a higher concentration of NH_4^+-N (Chapter 8: Figure 8.6) than all dry season samples. Wet season samples harvested on day 7 had a similar concentration of NH_4^+-N than the test samples harvested on day 14 and 28 but had a lower matric potential (Chapter 8: Figure 8.1). All dry season samples were associated with a smaller, more consistent concentration of microbial biomass C (Chapter 8: Figure 8.3).

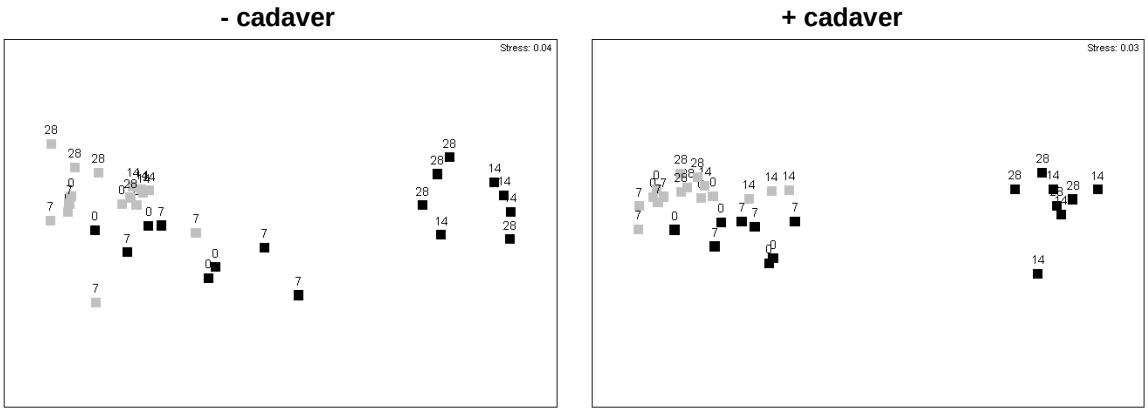

Figure 9.4. Effect of season on phospholipid fatty acid profiles in soil from the Yabulu/FACE site following the burial (2.5 cm) of a rat (*Rattus rattus*) cadaver during the dry season (■: October 2002) or wet season (■: March 2003).

A clear difference in PR control samples between seasons was observed (Figure 9.5). This was significantly associated with matric potential ($P < 0.05$), phosphodiesterase activity ($P < 0.001$) and NO_3^--N ($P < 0.05$) (Appendix E: Table E.2). This coincides with the low matric potential (Chapter 8: Figure 8.1) and elevated phosphodiesterase activity (Chapter 8: Figure 8.5) associated with the wet season and the elevated concentration of NO_3^--N associated with the dry season (Chapter 8: Figure 8.7).

The only soil biophysicochemical parameter significantly associated with the variation observed among PR test samples (Figure 9.5) was phosphodiesterase activity ($P < 0.001$) (Appendix E: Table E.2). This coincided with the elevated rate of phosphodiesterase activity in test samples during the wet season (Chapter 8: Figure 8.5).

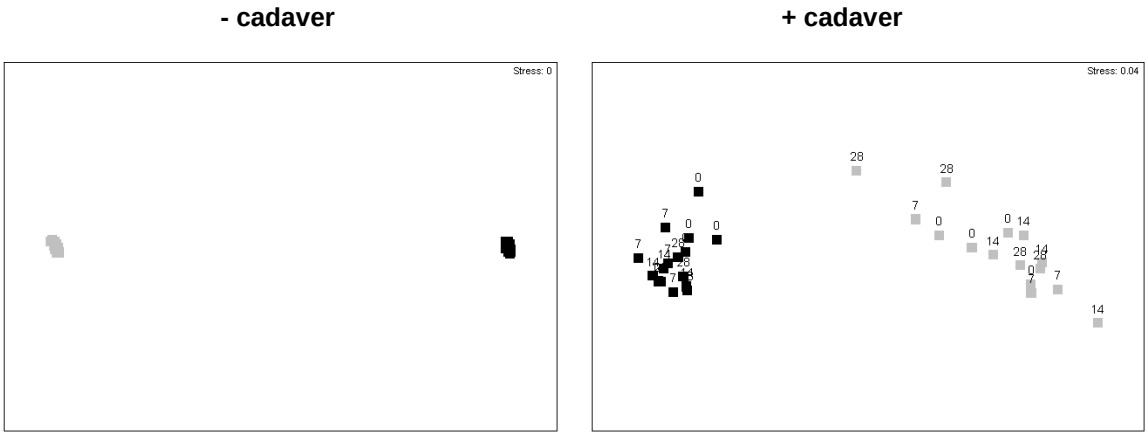

Figure 9.5. Effect of season on phospholipid fatty acid profiles in soil from the Pallarenda site following the burial (2.5 cm) of a rat (*Rattus rattus*) cadaver during the dry season (■: October 2002) or wet season (■: March 2003).

The variation observed in the PLFA profiles in WB control samples between seasons (Table 9.2; Figure 9.6) was significantly related to phosphodiesterase activity ($P < 0.001$) (Appendix E: Table E.2). This coincides with an increase in phosphodiesterase activity following the onset of the wet season (Chapter 8; Figure 8.5). The variation observed between test samples (Table 9.2; Figure 9.6) was significantly related to matric potential ($P < 0.001$), phosphodiesterase activity ($P < 0.001$) and protease activity ($P < 0.05$) (Appendix E: Table E.2). These findings coincide with a low matric potential (Chapter 8: Figure 8.1) and elevated phosphodiesterase activity (Chapter 8: Figure 8.5) associated with the wet season. Protease activity followed opposite trends between seasons (i.e. protease activity during the dry season was low on day 7 and increased over time whereas protease activity during the wet season was highest on day 7) (Chapter 8; Figure 8.4).

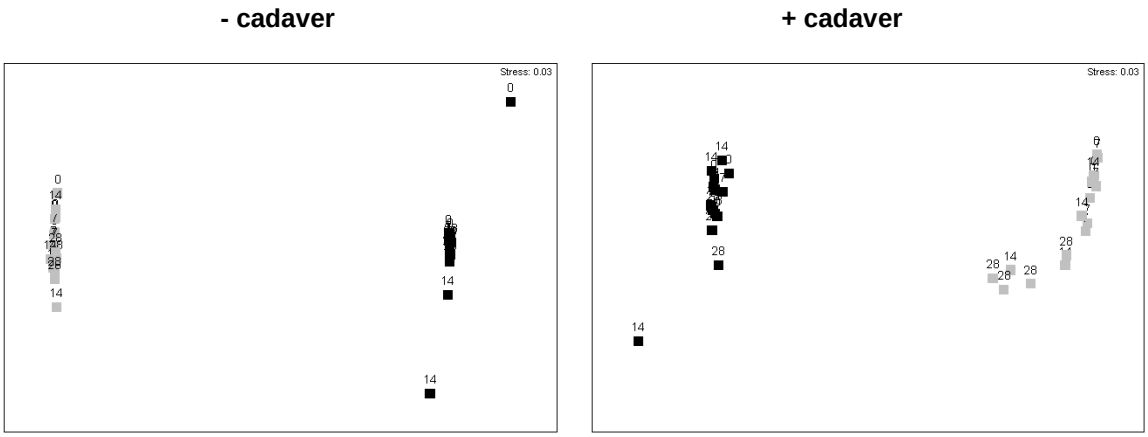

Figure 9.6. Effect of season on phospholipid fatty acid profiles in soil from the Wambiana site following the burial (2.5 cm) of a rat (*Rattus rattus*) cadaver during the dry season (■: October 2002) or wet season (■: March 2003).

9.3.3. Effect of soil type on PLFA profiles

PLFA profiles were consistently different between soils regardless of cadaver treatment (Table 9.3).

Table 9.3. Statistical analysis of PLFA profiles between soils sampled from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savannas of Queensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (+ cadaver) and without a cadaver (- cadaver) during the dry (October 2002) and wet (March 2003) season.

		Soil		Time of harvest		Soil x Time of harvest	
Season	Treatment	F	*P*	F	*P*	F	*P*
Dry	+ cadaver	5.71	**<0.001**	2.40	**0.006**	3.15	**<0.001**
	- cadaver	4.27	**<0.001**	1.69	**0.035**	2.87	**<0.001**
Wet	+ cadaver	16.25	**<0.001**	3.44	**<0.001**	3.49	**<0.001**
	- cadaver	11.27	**<0.001**	3.05	**<0.001**	3.51	**<0.001**

Among control samples, observed variation in PLFA profiles during the dry season was significantly related to NO_3^--N ($P < 0.05$) and soil pH ($P < 0.01$) (Appendix E: Table E.3). While distinct groups cannot be discerned by a visual assessment of Figure 9.7, these results are in agreement with those in Chapter 8 as WB soil had a lower concentration of NO_3^--N (Figure 8.7) (although not significantly so) and a

higher pH (Figure 8.8) than FA and PR soils. The variation in the PLFA profiles of control samples during the wet season (Table 9.3) was significantly associated with matric potential ($P < 0.05$), phosphodiesterase activity ($P < 0.001$) and soil pH ($P < 0.001$) (Appendix E: Table E.3). These statistics concur with the relatively low matric potential (Chapter 8: Figure 8.1), elevated phosphodiesterase activity (Chapter 8: Figure 8.5) and elevated soil pH (Chapter 8: Figure 8.8) observed in WB soil. Unfortunately, the measured soil biophysicochemical parameters did not explain the grouping of FA samples harvested on day 0 and day 7 (Figure 9.7)

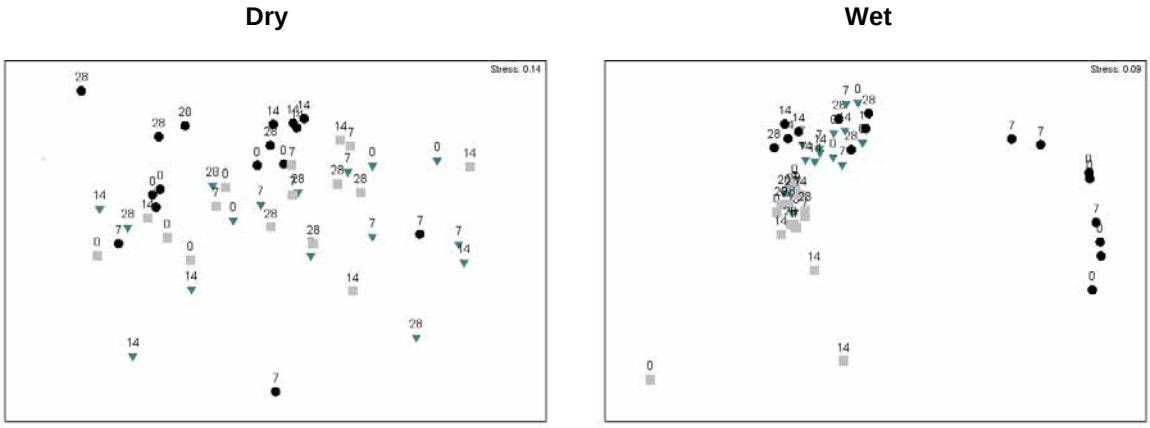

Figure 9.7. Effect of soil type on phospholipid fatty acid profiles from control soils (i.e. no cadaver) at the Yabulu/FACE (●), Pallarenda (▼) and Wambiana (■) sites in Queensland, Australia during the dry season (October 2002) and wet season (March 2003).

The variation in PLFA profiles between test soils during the dry season was related to phosphodiesterase activity ($P < 0.05$), protease activity ($P < 0.001$), NO_3^--N ($P < 0.05$) and soil pH ($P < 0.01$) (Appendix E: Table E.3). A grouping of WB soils can be observed in Figure 9.8, which is consistent with elevated phosphodiesterase activity (Chapter 8: Figure 8.5), low protease activity (Chapter 8: Figure 8.4) and high pH (Chapter 8: Figure 8.8) associated with WB soil following cadaver burial in the dry season. During the wet season the variation in PLFA profiles (Table 9.3) and observed grouping of soils (Figure 9.8) was significantly related to protease activity

($P < 0.01$) and soil pH ($P < 0.001$). The grouping of WB soil (Figure 9.8) coincides with these statistics as a lower rate of protease activity (Chapter 8: Figure 8.4) and higher soil pH (Chapter 8: Figure 8.8) was observed in WB soil relative to FA and PR soil. The measured soil biophysicochemical parameters did not explain the grouping of FA samples harvested on day 0 and day 7 (Figure 9.7)

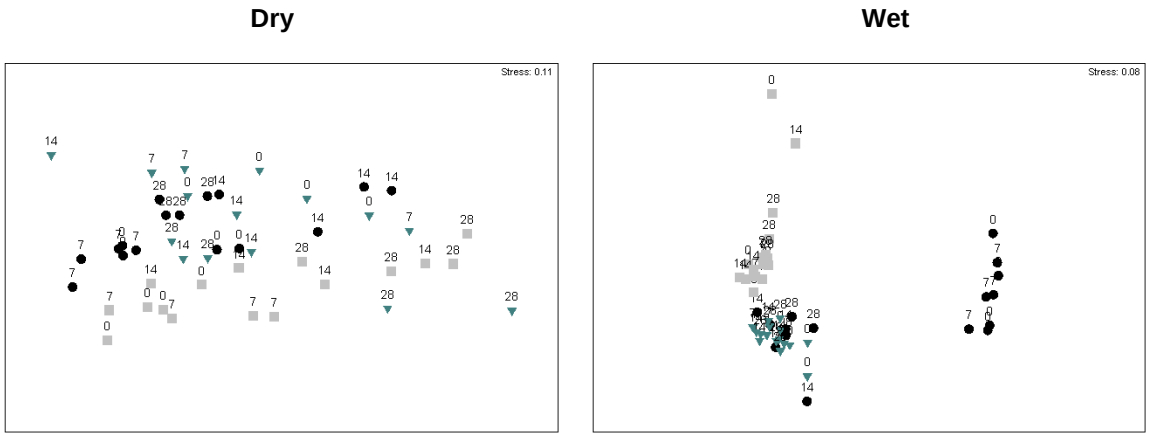

Figure 9.8. Effect of soil type on phospholipid fatty acid profiles from soils at the Yabulu/FACE (●), Pallarenda (▼) and Wambiana (■) sites in Queensland, Australia following the burial (2.5 cm) of a rat (*Rattus rattus*) cadaver during the dry season (October 2002) and wet season (March 2003).

9.4. Discussion

The current study shows that cadaver burial can result in a shift in the structure of the soil microbial community. This finding is supported by other studies that have detected a change in soil microbial community following the amendment of soil with an organic resource (Bossio and Scow, 1998; Bossio et al., 1998). Interestingly, the cadaver-induced shifts detected in FA and PR soil were not related to any of the biophysicochemical parameters measured in Chapter 8. However, the shift that was observed in WB soil was related to soil pH, NH_4^+-N and NO_3^--N. These findings are in agreement with a number of studies that demonstrate abiotic factors such as pH and soil moisture content can influence the soil microbial

community (e.g. Bardgett et al., 1999; Lundquist et al., 1999; Pankhurst et al., 2001). A change in microbial community structure following the burial of a cadaver is not surprising since a cadaver is comprised of a large amount of water and readily decomposable resources such as the internal organs. In addition, a cadaver comes with a heavy microbial inoculum and, indeed, some of the PLFAs (e.g. 16:0, 18:0) may represent the cadaver itself because these PLFAs are present in many animals. However, it was surprising that the cadaver effect was not consistent.

The consistent effect of season and soil type on the structure of the soil microbial community was not a unique finding. Seasonal (Findlay and Watling, 1998; Bardgett et al., 1999; Rajendran and Nagatomo, 1999; Keith-Roach et al., 2002) and soil type related (Bardgett et al., 1997; Bossio et al., 1998) shifts in microbial community have been reported and, in some cases (Bossio and Scow, 1998; Bossio et al., 1998), the seasonal changes in microbial community structure have been observed to be greater than changes due to organic resource (manure, rice straw) amendment. These factors plus variation in plant communities and, thus, the initial composition of the soil microbial community likely contributed to differences in PLFA profiles between tropical savanna soils where seasonal moisture and warm temperature can result in rapid decomposition rates.

Interestingly, some of the variation observed in microbial community structure between season and soil type were significantly associated with protease activity and phosphodiesterase activity and presumably the demand for N and P. These findings provide further evidence that the structure of the soil microbial community can be related to the function of the soil microbial community. Few studies have

shown that changes in function such as CO_2 evolution (Zogg et al., 1997; Bossio et al., 1998) and enzyme activity (Waldrop et al., 2000; Kourtev et al., 2003) can be related to changes in microbial community structure. The seasonal changes observed in the current study might mean that a cadaver buried in the wet season was exposed to a microbial community with a greater ability to decompose organic resources. This may have contributed to the increase in cadaver decomposition observed in the wet season. Similarly, the difference in microbial community structure between soils leads to the suggestion that functional differences might have contributed to the difference in cadaver decomposition between soils. Regardless, it is apparent that season and soil type can be extremely important in determining the composition of the soil microbial community in tropical savanna soils.

The observed change in PLFA profiles over time provides evidence that the structure of the soil microbial community is regulated, in part, by the temporal utilisation of resources, as discussed in Bardgett et al. (1997), and this represents a successional sequence that can be mediated by biotic and abiotic factors. A successional change can occur because of a change in resource quality and/or availability (Swift, 1982). This is likely to have occurred in association with a cadaver as the soft tissues decompose and a greater percentage of recalcitrant materials (e.g. integument, bone) remain. These findings are in agreement with the apparent successional sequence has been associated with the postputrefaction fungi (Sagara, 1995) but further research is required to fully understand the dynamics of the microbial community in association with cadaver decomposition.

Further research into the soil microbial community associated with cadaver decomposition might result in the development of a means to estimate postmortem and postburial intervals. Each of the soils in the current study associated with a cadaver effect also showed a significant interaction between cadaver and time of harvest (Table 9.1). Therefore, it might be possible, following further research, to associate microbial community structure to the period of time that a cadaver has been buried. It is currently believed, however, that methods such as Terminal Restriction-Fragment Length Polymorphism and Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (i.e. those that can be used to identify soil microbial community structure to species and strain level) might be more effective for the dating of clandestine graves.

In conclusion, cadaver burial can result in a shift in the structure in the soil microbial community. However, season and soil type play a more important role in determining the structure and function of the soil microbial community. The current results also showed that the soil microbial community can change over time, possibly representing a successional sequence. However, inconsistent relationships between microbial community structure and the soil biophysicochemical characteristics measured in Chapter 8 lead to the suggestion that the factors regulating the dynamics of soil microbial communities are complex and are probably related to other soil parameters that were not measured during the incubation (e.g. organic C, CO_2 evolution, total P). Furthermore, Bardgett et al. (1999) have suggested that these inconsistencies might also be related to biotic and abiotic factors such as resource availability, predation and heterogeneity of the soil physicochemical environment.

CHAPTER 10

CONCLUSIONS

A cadaver is a substantial source of carbon, nitrogen, energy and water. The breakdown of a cadaver represents a natural disturbance and results in a large pulse of nutrients that can significantly alter soil biology and chemistry. The decomposition of a juvenile rat (*Rattus rattus*) cadaver buried in soil from tropical savanna ecosystems was a relatively rapid process and tended to follow a pattern similar to that observed by Payne et al. (1968) that comprised an “Early Phase”, “Intermediate Phase” and “Late Phase” (Table 10.1). The process of cadaver decomposition is important to ecosystem function because it represents the primary pathway that nutrients sequestered by an animal are released back into a terrestrial ecosystem. While plant material and animal waste represent the majority of organic input into the soil, the effect of cadaver decomposition on soil processes cannot be understated. The current study demonstrated that cadaver decomposition can result in much larger increases in microbial activity, microbial biomass and pH than in association with plant materials or animal waste and these changes can last for a period of up to 28 days (with large ungulates these effects can last for up to 5 years (Towne, 2000)). These effects are not surprising considering that a juvenile rat can contain significantly more water (75%), nitrogen (~2.5%) and phosphorus (~0.5%) than plant and waste material (Widdowson, 1950; Spray and Widdowson, 1950). Thus, the introduction of a cadaver to the soil results in the formation of a ‘cadaver decomposition island’, which represents a change in the size, activity, composition and/or distribution of the soil microbial biomass over an area of soil. This may also effect the food web, possibly resulting in a flush of bacterial and/or fungal feeding

nematodes (Todd et al., 2005) and linkages between aboveground and belowground communities (Wardle et al., 2004). A cadaver decomposition island therefore acts as a highly concentrated hub from which nutrients and energy are released into the wider ecosystem. These islands can be scattered across a landscape and their formation might even act as an emergency resource, such as during drought, when a cadaver represents a rare source of moisture, nutrients and energy available to a terrestrial ecosystem. Swift et al. (1979) suggested that cadaver breakdown might represent a significant pathway of nutrients and energy within a terrestrial ecosystem. The previous chapters show that this is certainly the case but much more work needs to be done in order to fully understand cadaver decomposition in terrestrial ecosystems.

Table 10.1. Relationships between cadaver decomposition phase and measures of cadaver decomposition. These relationships were accelerated or slowed by increases and decreases in temperature and soil moisture.

Measure	Early Phase	Intermediate Phase	Late Phase
Cadaver mass loss	Slow	Rapid	Slow
CO_2-C evolution	Initial increase	Peak leading to gradual decrease	Gradual decrease from peak
Microbial biomass C	Initial increase	Peak biomass	Decrease from peak
Phosphodiesterase activity	Initial increase	Peak	Peak
Protease activity	Initial increase	Increase near peak	Peak
Soil pH	Initial increase or peak	Peak (~ pH 8)	Slight decrease from peak

While several similarities were observed between the decomposition of a juvenile rat cadaver and larger cadavers (e.g. human, pig, adult rat) the current data should only be used as a rough guide to interpret their breakdown. A larger animal will have different absolute and relative amounts of cadaveric components and this

might have a different effect than those observed in the current study. For example, an adult bison (*Bos bison*) in Early Phase decomposition results in the death of aboveground flora and soil nematode communities that begin to re-establish themselves at the onset of Late Phase decomposition (Towne 2000). This is probably due to nitrogen toxicity resulting from the breakdown of an animal with a mass of approximately 1000 kg. None of the results in the current study would suggest such an event. Indeed, they indicate that a small animal has a positive effect on soil microbial communities. Another confounding variable might be the lack of development of the enteric microbial community into the more complex community associated with adult animals (see Discussion: Chapter 6). It is unknown if this would have any effect on the processes associated with cadaver decomposition and it merits attention in the future.

Another problem with the use of the current results to interpret the decomposition of other animals is the use of a 28-day incubation period. In a field setting it was clear that this time period was sufficient to result in skeletonisation. However, this might not have been the case if a larger animal had been used and it clearly was not the case in a laboratory setting. However, a 28-day incubation period resulted in the generation of a great deal of information concerning Early Phase and Intermediate Phase decomposition. Arguably the most important finding from the current work was that cadaver burial in soil can undergo a rate of cadaver decomposition more rapid than a cadaver left to intrinsic processes (e.g. autolysis, putrefaction) (Chapter 7). Until this work was conducted, the established dogma stated that burial in soil was regarded as a means to slow cadaver decomposition (Rodriguez, 1997; Fiedler and Graw, 2003). The current results show that microbial activity can be triggered

within 24 hours of cadaver burial and the rapid mass loss that characterised Intermediate Phase decomposition tended to coincide with peak microbial biomass and activity. These results are likely due to the activity of both cadaveric and soil-borne microbes (Chapter 6; Chapter 7) and the release of decomposition fluids. Regardless of the origin of the microbes, the majority of microbial activity associated with cadaver decomposition was almost certainly aerobic in nature because a significant relationship was observed between CO_2-C evolution and cadaver mass loss. This relationship was not surprising considering that CO_2-C evolution has been observed as the primary gas associated with cadaver decomposition in an aerobic setting (Putman, 1978).

While the data concerning Early Phase and Intermediate Phase decomposition are extremely important it would have been interesting to place an emphasis on Late Phase decomposition and determine the temporal extent of a cadaver decomposition island. This should be done in the future. At present we know that the slowing down of process rates during Late Phase decomposition was probably due to the depletion of cadaveric moisture and readily available resources. Late Phase decomposition was often associated with peak levels of protease activity, which might reflect the depletion of low molecular weight compounds (e.g. amino acids) and the microbial demand for high molecular weight compounds such as protein. It would be interesting to see what other changes come about as the readily available cadaveric fractions are depleted.

Unsurprisingly, the current results show that environmental variables such as temperature and moisture can retard the relationship between soil biology and

chemistry shown in Table 10.1. An increase in temperature resulted in an increase in cadaver decomposition and the speeding up of processes (Chapter 4). Similarly, a decrease in matric potential (i.e. an increase in available moisture) can result in an increase in the rate of cadaver decomposition (Chapter 5). However, the optimal water content was exceeded in more clayey soil, which slowed cadaver breakdown. This was probably due to the onset of anaerobiosis, which was indicated by an uncoupling of the relationship between CO_2 evolution and cadaver mass loss. The current thesis represents the first controlled investigation into the effect of soil moisture on cadaver decomposition. However, the current findings are in accordance with what has been observed in field trials (Turner and Wiltshire, 1999; Hopkins et al., 2000).

The current findings also show that soil type can affect the rate of cadaver decomposition. This was observed as a greater rate of cadaver mass loss in a sandy soil in a laboratory setting and a clayey soil in a field setting (Chapter 8). These results were likely due to the porosity of the soils and, thus, an increase in gas diffusion (aerobic microbial activity, desiccation) and insect activity. These results are in contrast to the findings of Mant (1950), which suggested that soil type would probably have little effect on cadaver decomposition.

The laboratory incubations provided a substantial amount of data that were used to interpret the results from the field experiments. However, a number of discrepancies were observed between the two settings. The first was that cadaver mass loss was much greater in the field. This was probably due to the presence of decomposer organisms not included in the laboratory incubations (i.e. insects) but it might also

reflect the significant influence of moisture in a tropical savanna ecosystem. Cadavers buried in the field were much more desiccated than in the laboratory and this rapid loss of moisture was almost certainly related to the accelerated decomposition.

Unfortunately, metabolic quotient provided little insight into microbial metabolic efficiency associated with cadaver decomposition. However, it was determined that the structure of the soil microbial community can be affected by the presence of a cadaver (Chapter 9). However, soil type and seasonal variation in temperature and moisture appeared to have a much greater effect on the structure of the soil microbial community. In addition, the current study provided more evidence to show that the structure of the soil microbial community can be related to the function of the soil microbial community. Specifically, this relationship was associated with protease and phosphodiesterase activity and, presumably, the microbial demand for nitrogen and phosphorus. Temporal changes in the microbial community of gravesoils were also observed. This putative successional sequence might be associated with the availability of readily available resources.

Cadaver decomposition had a significant effect on soil chemistry. This was observed as an increase in the concentration of ammonium and soil pH. These findings concur with previous research (Vass et al., 1992; Towne, 2000; Hopkins et al., 2001). The pulse of ammonium probably contributed to the increase in pH, which under controlled laboratory conditions resulted in pH 8 to pH 9. Under field conditions, however, the increase in pH was less consistent and was found to range from pH 6 to pH 7 (Chapter 8). This is likely due to greater volatilisation of

ammonia in an open system. In spite of the loss of ammonia to the atmosphere, it is obvious that a cadaver provides a large addition of nitrogen to the soil.

Some of the findings from the current study have the potential to be developed into forensic tools used in the investigation of crime scenes located in terrestrial ecosystems. The successional sequence associated with the soil microbial community might become a means to estimate postmortem interval and/or postburial interval (e.g. Carter and Tibbett, 2003). It is possible that microbial succession is related to the availability of readily decomposable resources. If certain phospholipid fatty acids and/or specific microbial species and strains could be associated with a phase of decomposition then it might be possible to estimate how long a cadaver has been in the ground. Clearly, this would require much more detailed experimental work, which could possibly involve methods not used in the current study such as nucleic acid-based techniques. Ideally, this research would provide similar information to that compiled by forensic entomologists that study the successional sequences of arthropods associated with cadavers placed on the soil surface.

In addition, the increase in soil pH and the concentration of ammonium observed in association with cadaver decomposition might aid in the search and location of clandestine graves, as originally proposed by Rodriguez and Bass (1985). These measurements, particularly pH, are rapid and the facilities required to process samples are not uncommon. Currently, the measurement of soil pH holds the most promise because it could be conducted while a potential crime scene was being surveyed.

While a great deal was learned from the current study it merely represents a small fraction of what needs to be learned about the role of cadaver decomposition in terrestrial ecosystems. More detailed research should be carried out in order to investigate the processes associated with each decomposition phase in greater detail. In addition, similar experiments should be carried out in other ecosystems. Future studies should also focus on defining the compounds that are introduced into the soil upon cadaver decomposition, since a cadaver comprises much different proportions of protein and lipid than plant materials. Also, it would be important to determine the spatial extent to which cadaver burial affects soil physicochemical properties. This would show, for example, the spatial extent of the pulse of inorganic N and other compounds. In terms of forensic application it would be helpful to conduct comparative studies utilising human cadavers with a range of other mammals. All of this work can lead to a greater understanding of the contribution that cadaver decomposition makes to the functioning of terrestrial ecosystems, which has the potential to be become a key area of ecological study.

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APPENDIX A

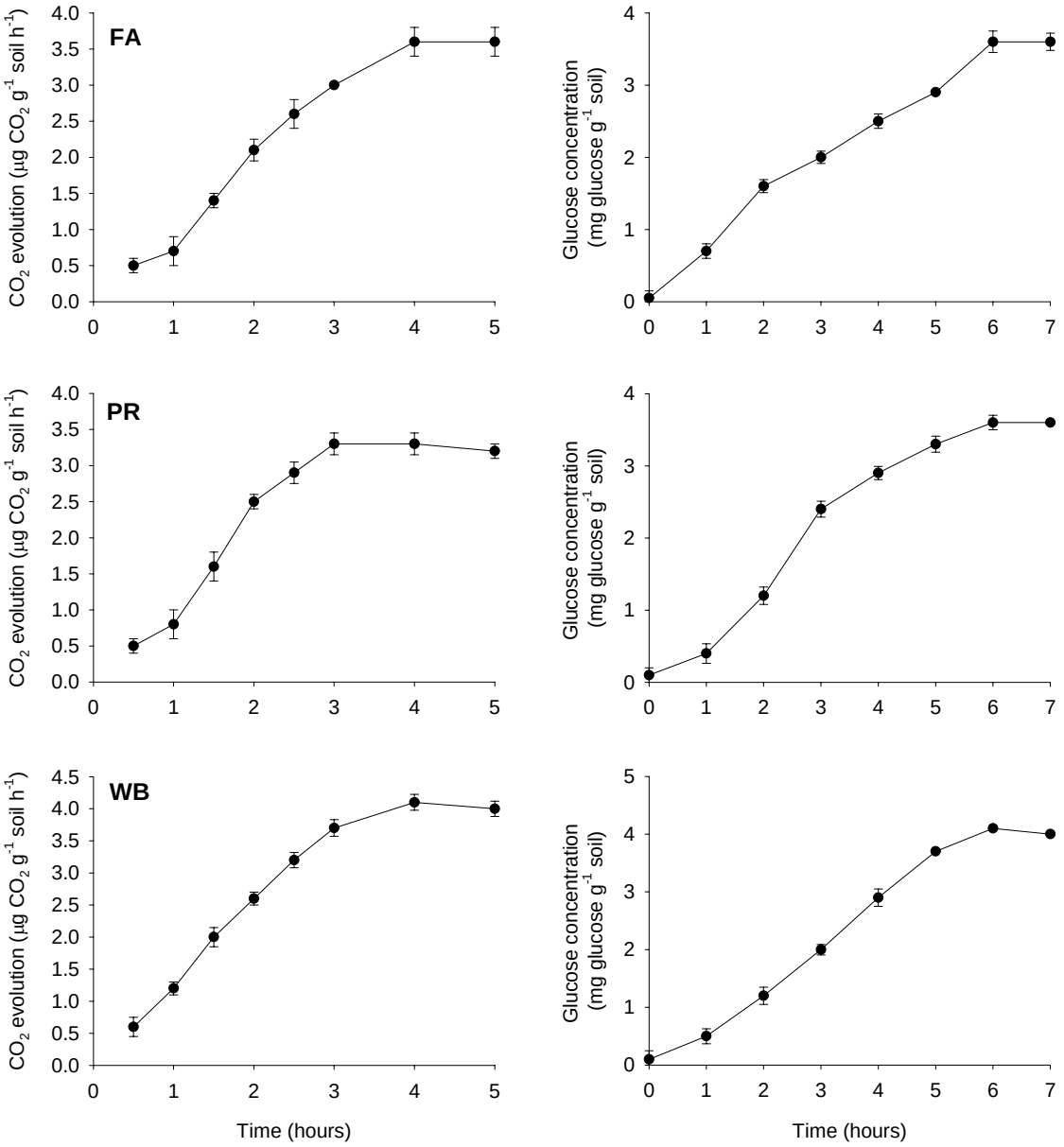

Figure A.1. Results from preliminary incubations for substrate-induced respiration. Soil 5 g dry weight) was amended with glucose solution (6 mg glucose g^{-1} soil) to bring soil to 95% water-holding capacity and incubated at 22 °C in the dark for 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 or 5.0 hours (left column). The time of peak CO_2 evolution was used to designate the time of incubation in order to determine optimal glucose concentration (right column). To determine optimal glucose concentration fresh soils (5 g dry weight) were amended with a range of glucose concentrations (0, 1, 2, 3, 4, 5, 6 or 7 mg glucose g^{-1} soil) to bring soil to 95% water-holding capacity and incubated at 22 °C in the dark for the time period that represented peak CO_2 evolution.

APPENDIX B

Table B.1. Correlation between cadaver mass loss (% wet weight: x) and cumulative carbon dioxide evolution (mg CO_2-C g^{-1} soil: y) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver in soil (500 g dry weight) from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia calibrated to a matric potential of -0.05 megapascals and incubated at 29 °C, 22 °C or 15 °C.

Soil	Temperature	Equation	r^2	*P*
FA	29 °C	y = -0.44 + 0.02x	0.877	<0.001
	22 °C	y = 0.02 + 0.03x	0.833	<0.001
	15 °C	y = 0.17 + 0.03x	0.937	<0.001
PR	29 °C	y = -0.55 + 0.03x	0.907	<0.001
	22 °C	y = -0.23 + 0.04x	0.965	<0.001
	15 °C	y = 0.19 + 0.03x	0.878	<0.001
WB	29 °C	y = -0.45 + 0.04x	0.876	<0.001
	22 °C	y = -0.05 + 0.04x	0.871	<0.001
	15 °C	y = 0.00 + 0.04x	0.794	<0.001

Table B.2. Correlation between cadaver mass loss (% wet weight: x) and cumulative carbon dioxide (mg CO_2-C g-1 soil) evolution (y) following the burial (2.5 cm) of a rat (*Rattus rattus*) cadaver in soil (500 g) from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in the seasonally dry tropics of Queensland, Australia incubated soil calibrated to a matric potential of –0.03 MPa, -0.05 MPa, –0.01 MPa.

Soil	Matric potential	Equation	r^2	*P*
FA	-0.3 MPa	y = 0.34 + 0.03x	0.862	<0.001
	-0.05 MPa	y = 0.14 + 0.03x	0.892	<0.001
	-0.01 MPa	y = 0.12 + 0.03x	0.849	<0.001
PR	-0.3 MPa	y = 0.34 + 0.03x	0.889	<0.001
	-0.05 MPa	y = 0.23 + 0.02x	0.953	<0.001
	-0.01 MPa	y = 0.18 + 0.02x	0.954	<0.001
WB	-0.3 MPa	y = 0.54 + 0.03x	0.663	<0.01
	-0.05 MPa	y = 0.23 + 0.03x	0.787	<0.001
	-0.01 MPa	y = 0.56 + 0.02x	0.182	0.166

Table B.3. Correlation between cadaver mass loss (% wet weight: x) and cumulative carbon dioxide evolution (mg CO_2-C g^{-1} soil) associated with a complete juvenile rat (*Rattus rattus*) cadaver (CC), cadaver plus incision (CI) and eviscerated cadaver (EC) following the burial (2.5 cm) in soil (500 g dry weight) from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in the seasonally dry tropics of Queensland, Australia calibrated to a matric potential of -0.05 megapascals and incubated at 22 °C. Where x = cadaver mass loss (%) and y = cumulative CO_2 evolution (g).

Soil	Cadaver treatment	Equation	r^2	*P*
FA	CC	y = 0.68 + 0.02x	0.608	<0.001
	CI	y = 0.67 + 0.03x	0.886	<0.001
	EC	y = 0.45 + 0.03x	0.798	<0.001
PR	CC	y = 0.44 + 0.02x	0.947	<0.001
	CI	y = 0.49 + 0.03x	0.843	<0.001
	EC	y = 0.76 + 0.02x	0.518	<0.01
WB	CC	y = 0.78 + 0.03x	0.736	<0.001
	CI	y = 0.95 + 0.02x	0.564	<0.001
	EC	y = 0.98 + 0.02x	0.386	<0.05

Table B.4. Correlation between cadaver mass loss (% wet weight: x) and cumulative carbon dioxide evolution (mg CO_2-C g^{-1} soil) following the burial (2.5 cm) of a a juvenile rat (*Rattus rattus*) cadaver in a sterile, soil-free environment (soil-free), sterilised soil (sterile) and sterilised soil reinoculated non-sterilised soil (non-sterile) from the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in the seasonally dry tropics of Queensland, Australia incubated at 22 °C in soil calibrated to a matric potential of -0.05 MPa where y = cumulative carbon dioxide evolution (g) and x = cadaver mass loss (%). Soil was sterilised by autoclaving at 121 °C for 30 minutes on two consecutive days. In between autoclave treatments soils were incubated at 22 °C for 24 hours.

Soil	Soil treatment	Equation	r^2	*P*
	SF	y = 0.04 + 0.02x	0.862	<0.001
FA	S	y = 0.52 + 0.03x	0.737	<0.001
	NS	y = 0.43 + 0.02x	0.861	<0.001
PR	S	y = 0.30 + 0.02x	0.871	<0.001
	NS	y = 0.46 + 0.02x	0.803	<0.001
WB	S	y = 0.44 + 0.03x	0.581	<0.001
	NS	y = 0.61 + 0.02x	0.515	<0.01

APPENDIX C

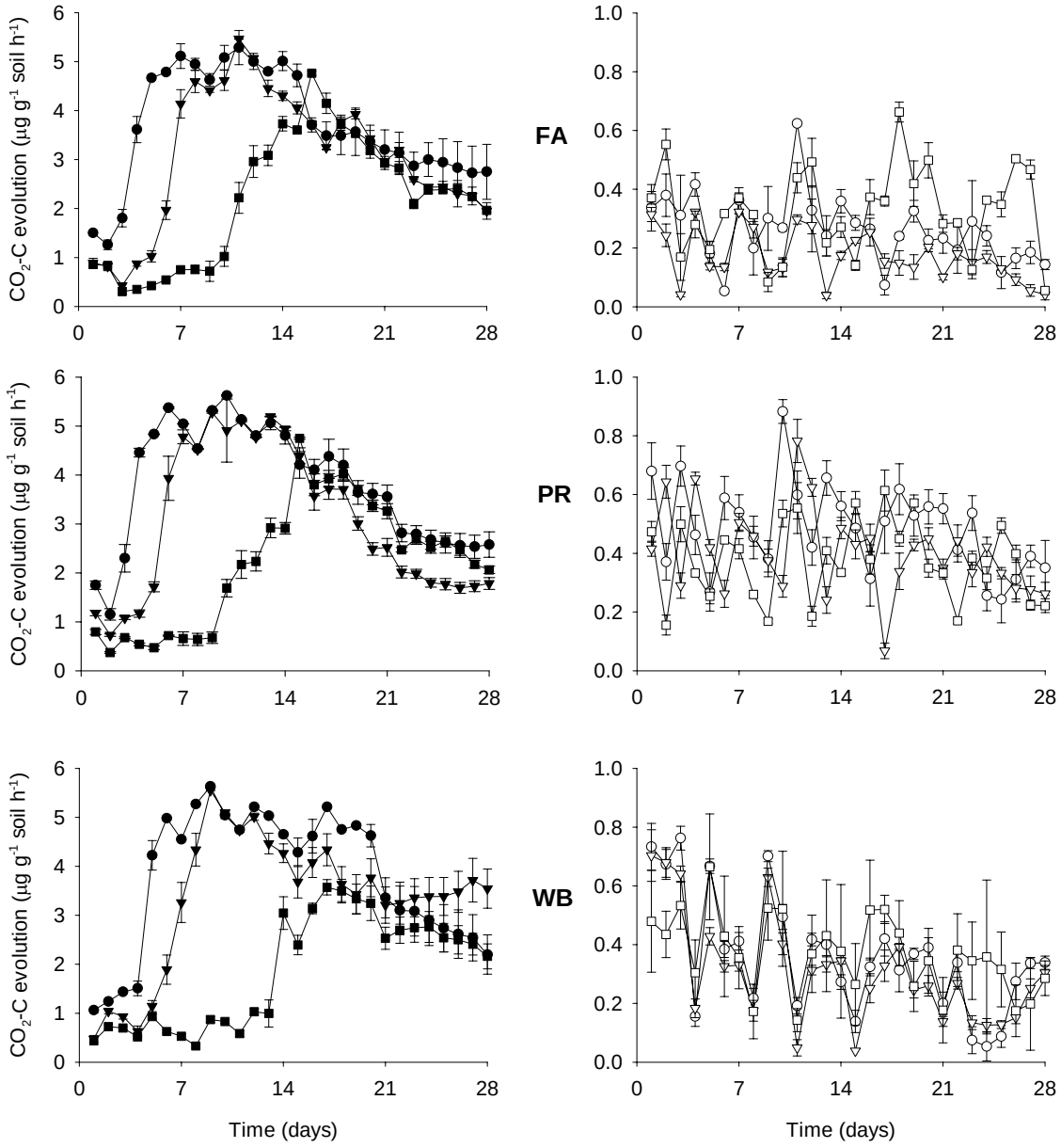

Figure C.1. Daily carbon dioxide evolution (µg CO_2-C g^{-1} soil h^{-1}) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (filled symbols) and in control samples (soil without cadaver: empty symbols) in 500 g (dry weight) soil calibrated to –0.05 megapascals and incubated at 29 °C (●), 22 °C (▼) or 15 °C (■). Bars represent standard error where n = 4. Note different scales were used between cadaver treatments.

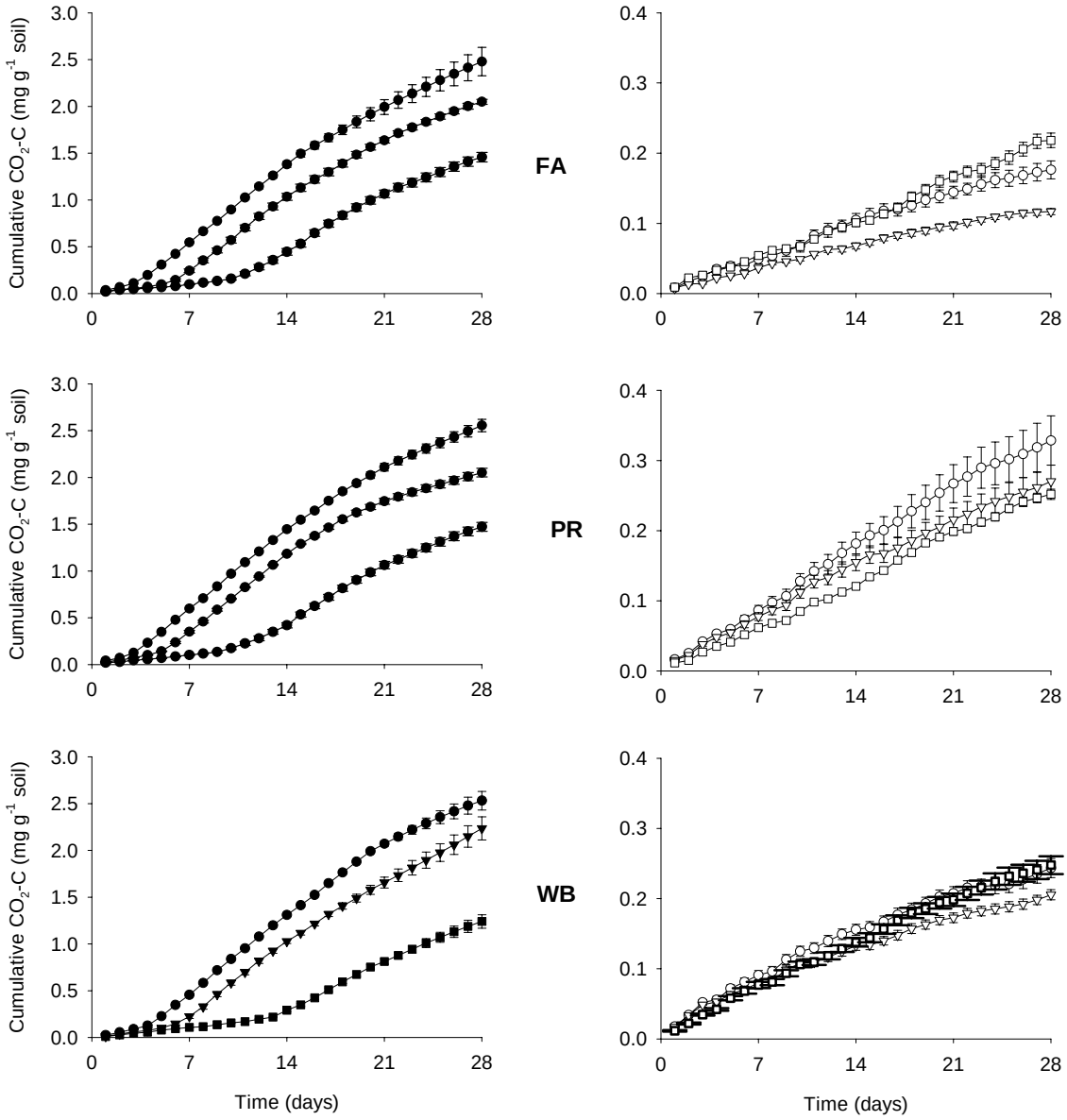

Figure C.2. Cumulative carbon dioxide evolution (mg CO_2-C g^{-1} soil) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (filled symbols) and in control samples (soil without cadaver: empty symbols) in 500 g (dry weight) soil calibrated to –0.05 megapascals and incubated at 29 °C (●), 22 °C (▼) or 15 °C (■). Bars represent standard error where n = 4. Note different scales were used between cadaver treatments.

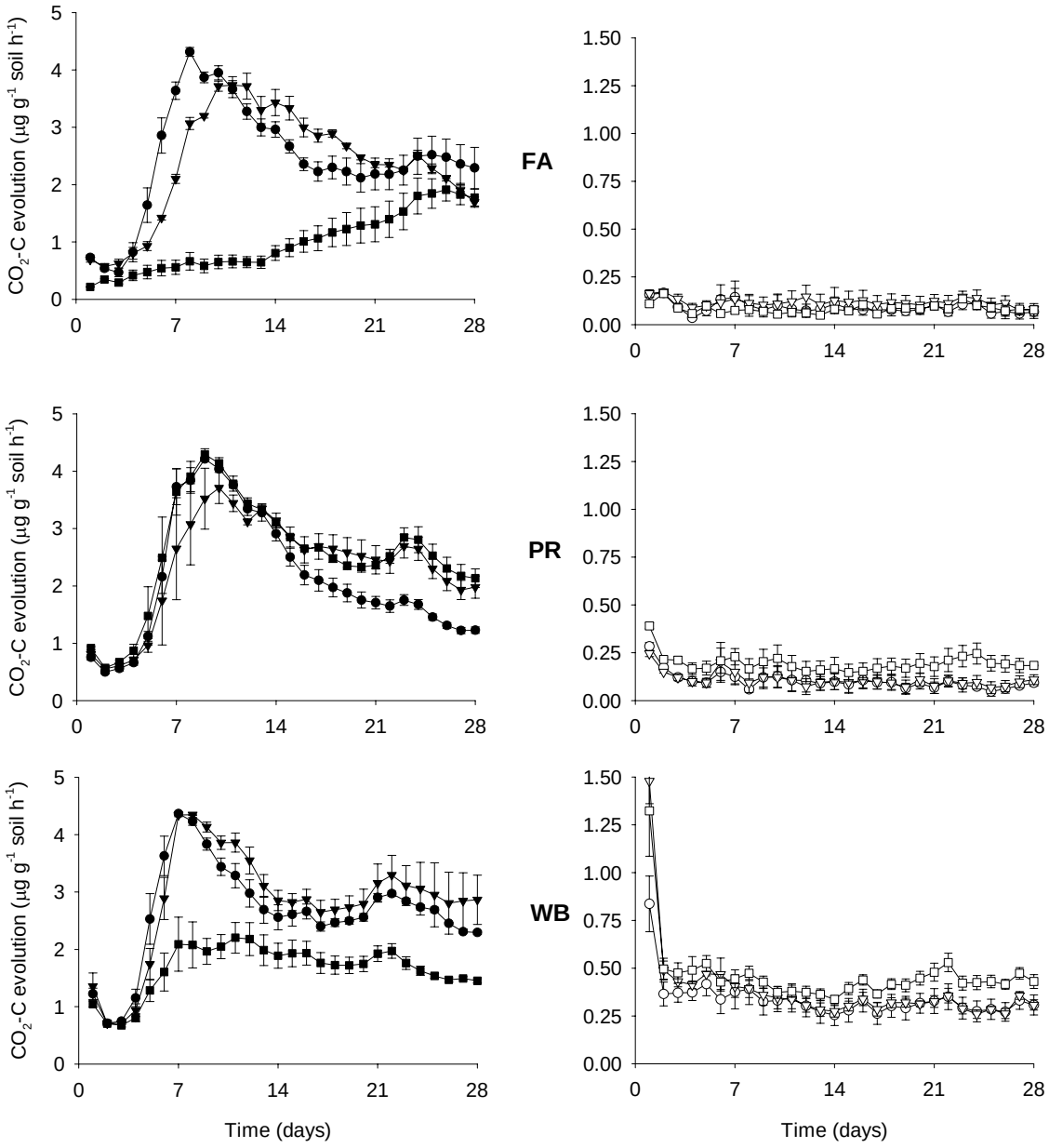

Figure C.3. Daily carbon dioxide evolution (µg CO_2-C g^{-1} soil h^{-1}) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (filled symbols) and in control samples (soil without cadaver: empty symbols) in 500 g (dry weight) soil incubated at 22 °C and calibrated to -0.3 megapascals (MPa) (●), -0.05 MPa (▼) or –0.01 MPa (■). Bars represent standard error where n = 3. Note different scales were used between cadaver treatments.

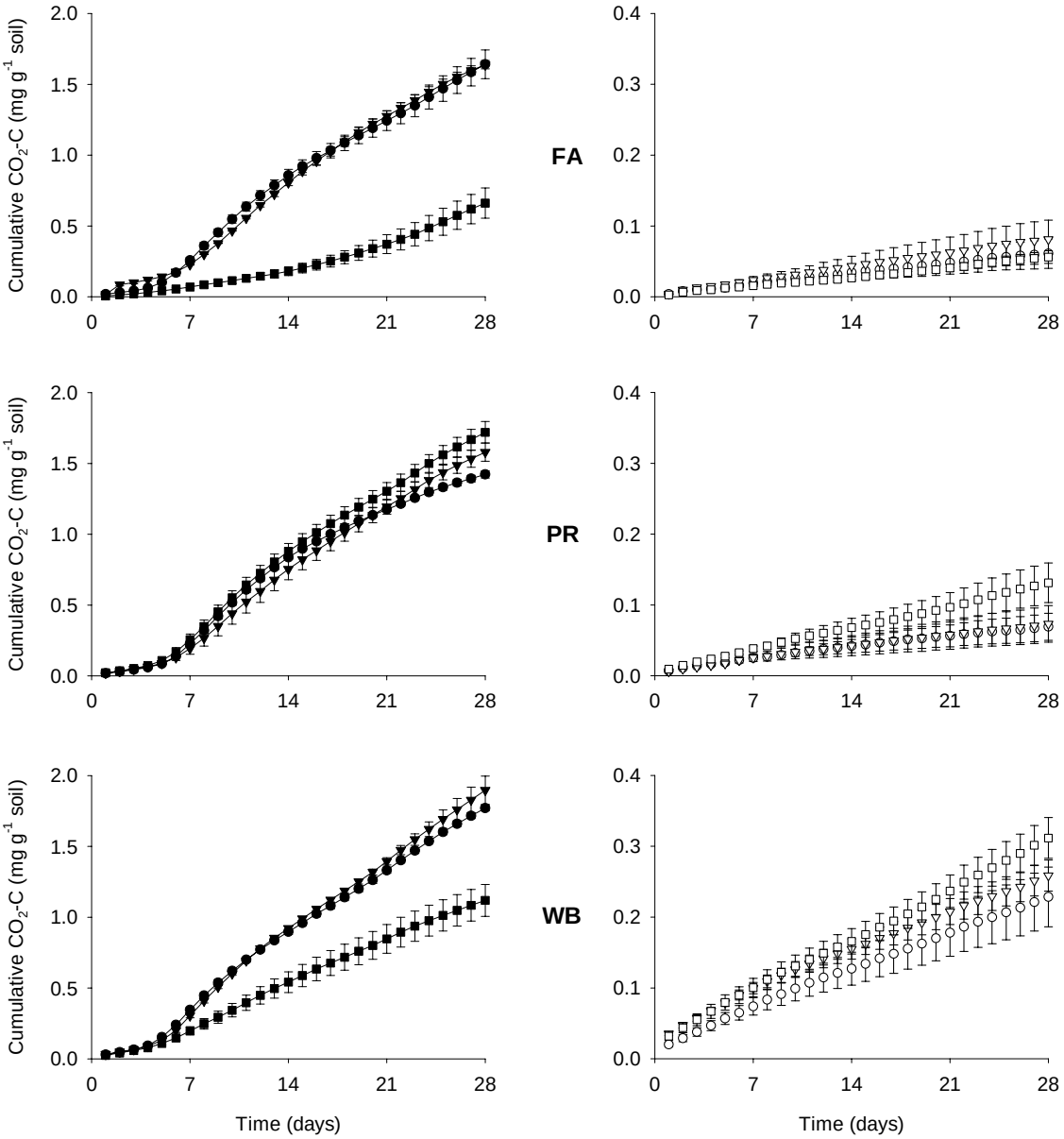

Figure C.4. Cumulative carbon dioxide evolution (mg CO_2-C g^{-1} soil) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (filled symbols) and in control samples (soil without cadaver: empty symbols) in 500 g (dry weight) soil incubated at 22 °C and calibrated to -0.3 megapascals (MPa) (●), -0.05 MPa (▼) or –0.01 MPa (■). Bars represent standard error where n = 3. Note different scales were used between cadaver treatments.

APPENDIX D

Table D.1. Absolute concentration (μg g^{-1} soil) of phospholipid fatty acids (PLFAs) from soil at the Yabulu/FACE site in a tropical savanna ecosystem in Queensland, Australia during the dry season (October 2002). Numbers in brackets represent standard errors where n = 4. Bold represents summed PLFAs per harvest. – denotes PLFA was not detected.

	Day			
	0	7	14	28
Saturated				
14:0	0.01 (0.01)	0.05 (0.03)	-	-
15:0	0.01 (0.01)	0.01 (0.01)	0.02 (0.00)	-
16:0	0.15 (0.05)	0.15 (0.07)	0.20 (0.01)	0.05 (0.00)
17:0	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	-
18:0	0.06 (0.02)	0.09 (0.05)	0.05 (0.00)	0.02 (0.01)
	0.24 (0.08)	**0.29 (0.16)**	**0.27 (0.02)**	**0.08 (0.04)**
Branched				
i15:0	0.09 (0.03)	0.04 (0.01)	0.13 (0.01)	0.05 (0.02)
a15:0	0.03 (0.01)	0.01 (0.01)	0.04 (0.00)	0.02 (0.00)
i16:0	0.05 (0.01)	0.03 (0.01)	0.11 (0.01)	0.05 (0.01)
i17:0	0.04 (0.01)	0.03 (0.01)	0.09 (0.00)	0.05 (0.01)
a17:0	0.02 (0.01)	0.02 (0.02)	0.04 (0.00)	0.02 (0.00)
	0.24 (0.07)	**0.14 (0.07)**	**0.41 (0.02)**	**0.19 (0.04)**
Cyclopropyl				
cy17:0	-	-	0.04 (0.00)	0.01 (0.01)
cy19:0	0.06 (0.01)	0.05 (0.01)	0.14 (0.01)	0.03 (0.03)
	0.06 (0.01)	**0.05 (0.01)**	**0.17 (0.02)**	**0.04 (0.04)**
Hydroxyl				
i15:0 3OH	-	-	-	-
16:1 2OH	0.03 (0.01)	0.03 (0.02)	0.11 (0.01)	0.07 (0.01)
16:0 2OH	-	-	-	-
	0.03 (0.01)	**0.03 (0.02)**	**0.11 (0.01)**	**0.07 (0.01)**
Monounsaturated				
16:1ω5c	-	-	0.01 (0.01)	0.01 (0.01)
16:1ω7c	-	-	-	-
16:1ω9c	-	-	-	-
16:1ω3c	-	-	-	-
17:1ω6c	-	-	-	-
18:1ω11c	-	-	-	-
18:1ω6c	-	-	-	-
18:1ω9c	-	-	-	0.03 (0.03)
18:1ω3c	-	-	-	-
19:1ω8t	-	0.01 (0.01)	0.04 (0.01)	0.01 (0.01)
	-	**0.01 (0.01)**	**0.05 (0.01)**	**0.04 (0.03)**
Polyunsaturated				
18:2ω6c	-	0.02 (0.02)	0.02 (0)	0.01 (0.01)
18:4ω3c	-	-	-	-
	-	**0.02 (0.02)**	**0.02 (0)**	**0.01 (0.01)**

Table D.2. Absolute concentration (μg g^{-1} soil) of phospholipid fatty acids (PLFAs) from soil at the Yabulu/FACE site in a tropical savanna ecosystem in Queensland, Australia during the wet season (March 2003). Numbers in brackets represent standard errors where n = 4. Bold represents summed PLFAs per harvest. – denotes PLFA was not detected.

	Day			
	0	7	14	28
Saturated				
14:0	0.09 (0.06)	0.15 (0.11)	4064 (2482)	542 (542)
15:0	0.01 (0.01)	0.04 (0.01)	516 (301)	162 (162)
16:0	0.43 (0.16)	0.84 (0.46)	26299 (12319)	3455 (2365)
17:0	0.03 (0.01)	0.08 (0.04)	2040 (742)	134 (134)
18:0	0.45 (0.23)	0.84 (0.49)	34432 (20363)	3223 (2607)
	1.01 (0.45)	**1.94 (1.11)**	**67355 (35551)**	**7515 (5781)**
Branched				
i15:0	0.03 (0.00)	0.07 (0.02)	1476 (393)	655 (251)
a15:0	0.01 (0.01)	0.10 (0.06)	758 (307)	178 (178)
i16:0	0.05 (0.02)	0.09 (0.02)	1162 (456)	972 (127)
i17:0	0.03 (0.01)	0.13 (0.08)	1011 (418)	956 (103)
a17:0	0.03 (0.01)	0.21 (0.15)	1235 (424)	363 (122)
	0.15 (0.03)	**0.60 (0.31)**	**5643 (1900)**	**3125 (702)**
Cyclopropyl				
cy17:0	0.01 (0.01)	0.01 (0.01)	247 (247)	-
cy19:0	0.06 (0.02)	0.30 (0.17)	2453 (587)	1365 (612)
	0.07 (0.02)	**0.31 (0.17)**	**2700 (672)**	**1364 (612)**
Hydroxyl				
i15:0 3OH	-	0.06 (0.06)	-	-
16:1 2OH	0.05 (0.01)	0.11 (0.04)	2123 (860)	1154 (174)
16:0 2OH	-	-	-	-
	0.05 (0.01)	**0.17 (0.10)**	**2123 (860)**	**1154 (174)**
Monounsaturated				
16:1ω5c	-	0.06 (0.04)	197 (197)	702 (702)
16:1ω7c	0.01 (0.01)	0.03 (0.02)	333 (333)	-
16:1ω9c	-	-	-	-
16:1ω3c	-	-	-	-
17:1ω6c	-	-	-	-
18:1ω11c	-	-	-	-
18:1ω6c	-	-	-	-
18:1ω9c	0.17 (0.07)	0.46 (0.19)	242 (242)	2106 (931)
18:1ω3c	-	-	-	-
19:1ω8t	0.02 (0.01)	0.04 (0.03)	393 (231)	488 (282)
	0.20 (0.06)	**0.59 (0.20)**	**1165 (396)**	**3296 (838)**
Polyunsaturated				
18:2ω6c	0.01 (0.01)	0.19 (0.13)	656 (407)	186 (186)
18:4ω3c	-	-	-	-
	0.01 (0.01)	**0.19 (0.13)**	**656 (407)**	**186 (186)**

Table D.3. Absolute concentration (μg g^{-1} soil) of phospholipid fatty acids (PLFAs) from soil at the YabuluFACE site in a tropical savanna ecosystem in Queensland, Australia during the dry season (October 2002) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver. Numbers in brackets represent standard errors where n = 4. Bold represents summed PLFAs per harvest. – denotes PLFA was not detected.

	Day			
	0	7	14	28
Saturated				
14:0	0.01 (0.01)	-	0.09 (0.03)	-
15:0	0.01 (0.01)	-	0.03 (0.01)	-
16:0	0.15 (0.05)	0.08 (0.01)	0.67 (0.21)	0.10 (0.01)
17:0	0.01 (0.01)	-	0.03 (0.01)	0.01 (0.00)
18:0	0.06 (0.02)	0.04 (0.00)	0.27 (0.08)	0.01 (0.01)
	0.24 (0.08)	**0.12 (0.01)**	**1.08 (0.33)**	**0.13 (0.01)**
Branched				
i15:0	0.09 (0.03)	0.03 (0.06)	0.16 (0.06)	0.06 (0.00)
a15:0	0.03 (0.01)	-	0.06 (0.02)	0.02 (0.00)
i16:0	0.05 (0.01)	0.03 (0.00)	0.17 (0.06)	0.06 (0.00)
i17:0	0.04 (0.01)	0.01 (0.01)	0.15 (0.05)	0.06 (0.01)
a17:0	0.02 (0.01)	-	0.07 (0.02)	0.03 (0.00)
	0.24 (0.07)	**0.07 (0.02)**	**0.62 (0.21)**	**0.23 (0.01)**
Cyclopropyl				
cy17:0	-	-	0.05 (0.01)	-
cy19:0	0.06 (0.01)	0.06 (0.01)	0.21 (0.06)	0.06 (0.02)
	0.06 (0.01)	**0.06 (0.01)**	**0.27 (0.08)**	**0.06 (0.02)**
Hydroxyl				
i15:0 3OH	0.01 (0.01)	-	0.03 (0.01)	0.01 (0.00)
16:1 2OH	0.03 (0.01)	0.02 (0.01)	0.18 (0.06)	0.08 (0.01)
16:0 2OH	-	-	-	-
	0.04 (0.02)	**0.02 (0.01)**	**0.22 (0.07)**	**0.09 (0.02)**
Monounsaturated				
16:1ω5c	-	-	0.03 (0.01)	0.01 (0.01)
16:1ω7c	-	-	-	-
16:1ω9c	-	-	-	-
16:1ω3c	-	-	-	-
17:1ω6c	-	-	0.02 (0.01)	-
18:1ω11c	-	-	-	-
18:1ω6c	-	-	-	-
18:1ω9c	-	-	-	-
18:1ω3c	-	-	-	-
19:1ω8t	-	-	-	0.01 (0.01)
	-	**-**	**0.19 (0.06)**	**0.02 (0.01)**
Polyunsaturated				
18:2ω6c	-	-	0.07 (0.02)	0.01 (0.00)
18:4ω3c	-	-	-	-
	-	**-**	**0.06 (0.02)**	**0.01 (0.00)**

Table D.4. Absolute concentration (μg g^{-1} soil) of phospholipid fatty acids (PLFAs) from soil at the Yabulu/FACE site in a tropical savanna ecosystem in Queensland, Australia during the wet season (March 2003) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver. Numbers in brackets represent standard errors where n = 4. Bold represents summed PLFAs per harvest. – denotes PLFA was not detected.

	Day			
	0	7	14	28
Saturated				
14:0	0.09 (0.06)	0.08 (0.04)	1317 (764)	206 (206)
15:0	-	0.03 (0.01)	330 (330)	-
16:0	0.42 (0.16)	0.76 (0.31)	3518 (1907)	5573 (3386)
17:0	0.03 (0.01)	0.04 (0.02)	463 (463)	395 (395)
18:0	0.45 (0.23)	0.40 (0.24)	4988 (3792)	5644 (4389)
	1.01 (0.45)	**1.31 (0.62)**	**10618 (4649)**	**11819 (8354)**
Branched				
i15:0	0.03 (0.00)	0.13 (0.03)	2269 (930)	1033 (172)
a15:0	0.01 (0.01)	0.09 (0.02)	1441 (620)	255 (255)
i16:0	0.05 (0.02)	0.11 (0.03)	1668 (788)	1364 (212)
i17:0	0.03 (0.01)	0.06 (0.02)	1076 (788)	1231 (175)
a17:0	0.03 (0.01)	0.07 (0.02)	1070 (509)	725 (282)
	0.15 (0.03)	**0.46 (0.10)**	**7523 (3347)**	**4610 (1020)**
Cyclopropyl				
cy17:0	0.01 (0.01)	0.08 (0.03)	598 (381)	546 (323)
cy19:0	0.06 (0.02)	0.14 (0.06)	2347 (840)	2272 (400)
	0.07 (0.02)	**0.22 (0.09)**	**2945 (1206)**	**2818 (702)**
Hydroxyl				
i15:0 3OH	-	0.01 (0.01)	243 (243)	-
16:1 2OH	0.05 (0.01)	0.06 (0.03)	1006 (745)	1253 (490)
16:0 2OH	-	-	-	-
	0.05 (0.01)	**0.07 (0.03)**	**1249 (981)**	**1252 (490)**
Monounsaturated				
16:1ω5c	-	0.01 (0.01)	384 (228)	-
16:1ω7c	0.01 (0.01)	0.10 (0.05)	1753 (779)	747 (466)
16:1ω9c	-	-	-	-
16:1ω3c	-	-	-	-
17:1ω6c	-	-	-	-
18:1ω11c	-	-	-	-
18:1ω6c	-	-	-	-
18:1ω9c	0.17 (0.07)	0.39 (0.19)	8141 (5279)	3506 (1806)
18:1ω3c	-	-	-	-
19:1ω8t	0.02 (0.01)	0.01 (0.01)	710 (423)	1227 (418)
	0.20 (0.06)	**0.50 (0.23)**	**10989 (6560)**	**5481 (2401)**
Polyunsaturated				
18:2ω6c	0.01 (0.01)	0.02 (0.01)	658 (222)	391 (226)
18:4ω3c	-	-	-	-
	0.01 (0.01)	**0.02 (0.01)**	**658 (222)**	**391 (226)**

Table D.5. Absolute concentration (μg g^{-1} soil) of phospholipid fatty acids (PLFAs) from soil at the Pallarenda site in a tropical savanna ecosystem in Queensland, Australia during the dry season (October 2002). Numbers in brackets represent standard errors where n = 4. Bold represents summed PLFAs per harvest. – denotes PLFA was not detected.

	Day			
	0	7	14	28
Saturated				
14:0	0.03 (0.01)	0.07 (0.04)	0.03 (0.03)	0.06 (0.05)
15:0	0.03 (0.01)	0.02 (0.00)	0.01 (0.01)	0.01 (0.01)
16:0	0.27 (0.12)	0.41 (0.20)	0.32 (0.26)	0.15 (0.04)
17:0	0.02 (0.01)	0.03 (0.01)	0.02 (0.02)	0.02 (0.00)
18:0	0.07 (0.03)	0.51 (0.40)	0.48 (0.46)	0.08 (0.04)
	0.41 (0.17)	**1.04 (0.65)**	**0.85 (0.77)**	**0.32 (0.14)**
Branched				
i15:0	0.16 (0.05)	0.10 (0.03)	0.04 (0.01)	0.06 (0.02)
a15:0	0.06 (0.02)	0.04 (0.01)	0.02 (0.00)	0.03 (0.00)
i16:0	0.15 (0.05)	0.11 (0.03)	0.03 (0.01)	0.05 (0.02)
i17:0	0.06 (0.02)	0.05 (0.01)	0.02 (0.00)	0.03 (0.01)
a17:0	0.08 (0.03)	0.06 (0.01)	0.02 (0.01)	0.03 (0.01)
	0.49 (0.17)	**0.36 (0.08)**	**0.12 (0.02)**	**0.19 (0.05)**
Cyclopropyl				
cy17:0	-	0.01 (0.01)	-	0.01 (0.01)
cy19:0	0.08 (0.03)	0.07 (0.02)	0.02 (0.01)	0.04 (0.01)
	0.08 (0.03)	**0.08 (0.02)**	**0.02 (0.01)**	**0.05 (0.01)**
Hydroxyl				
i15:0 3OH	-	-	-	-
16:1 2OH	-	0.04 (0.02)	0.02 (0.02)	0.01 (0.00)
16:0 2OH	-	-	-	-
	-	**0.04 (0.02)**	**0.02 (0.02)**	**0.01 (0.00)**
Monounsaturated				
16:1ω5c	0.04 (0.02)	0.03 (0.01)	-	-
16:1ω7c	-	-	-	-
16:1ω9c	-	-	-	-
16:1ω3c	-	-	-	-
17:1ω6c	-	-	-	-
18:1ω11c	-	-	-	-
18:1ω6c	-	-	-	-
18:1ω9c	-	0.12 (0.03)	0.05 (0.05)	0.02 (0.02)
18:1ω3c	-	-	-	-
19:1ω8t	-	-	-	-
	0.05 (0.02)	**0.15 (0.03)**	**0.05 (0.05)**	**0.02 (0.02)**
Polyunsaturated				
18:2ω6c	0.05 (0.01)	0.04 (0.01)	0.01 (0.01)	-
18:4ω3c	-	-	-	-
	0.05 (0.02)	**0.04 (0.01)**	**0.01 (0.01)**	**-**

Table D.6. Absolute concentration (μg g^{-1} soil) of phospholipid fatty acids (PLFAs) from soil at the Pallarenda site in a tropical savanna ecosystem in Queensland, Australia during the wet season (March 2003). Numbers in brackets represent standard errors where n = 4. Bold represents summed PLFAs per harvest. – denotes PLFA was not detected.

	Day			
	0	7	14	28
Saturated				
14:0	119 (119)	1365 (1365)	790 (326)	1941 (407)
15:0	-	371 (371)	417 (259)	-
16:0	1986 (405)	7102 (6716)	4524 (2407)	-
17:0	-	732 (504)	668 (250)	444 (288)
18:0	777 (246)	7830 (7830)	-	1231 (259)
	2883 (631)	**17401 (16752)**	**6399 (3208)**	**3616 (919)**
Branched				
i15:0	1074 (226)	2393 (954)	3169 (1048)	2073 (459)
a15:0	334 (195)	935 (411)	1116 (414)	-
i16:0	997 (384)	1869 (624)	3130 (1043)	-
i17:0	646 (234)	1370 (619)	1941 (624)	2209 (460)
a17:0	506 (186)	662 (477)	950 (412)	608 (222)
i17:1 G	-	-	-	3375 (1503)
i17:1 9	1699 (230)	2832 (1967)	2818 (751)	2420 (2420)
a17:1 9	-	-	-	2713 (623)
	5255 (1332)	**10061 (4745)**	**13123 (4225)**	**13399 (2541)**
Cyclopropyl				
cy17:0	-	313 (313)	-	-
cy19:0	790 (310)	4542 (2195)	3957 (695)	115 (115)
	790 (310)	**4856 (2490)**	**3957 (695)**	**115 (115)**
Hydroxyl				
i15:0 3OH	-	-	-	-
16:1 2OH	-	383 (383)	-	-
16:0 2OH	-	398 (237)	780 (459)	2028 (496)
	-	**781 (520)**	**780 (459)**	**2028 (495)**
Monounsaturated				
16:1ω5c	-	1152 (615)	1197 (523)	-
16:1ω7c	341 (341)	-	-	3998 (1945)
16:1ω9c				
16:1ω3c	-	-	-	15678 (4134)
17:1ω6c	755 (323)	1164 (432)	2449 (935)	2980 (1028)
18:1ω11c	614 (406)	285 (285)	861 (861)	5747 (1522)
18:1ω6c				
18:1ω9c	465 (336)	3821 (3060)	1171 (1171)	6050 (2116)
18:1ω3c	-		198 (198)	5349 (2735)
19:1ω8t	138 (138)	130 (130)	631 (219)	3752 (1022)
	2314 (856)	**6553 (2849)**	**6506 (2849)**	**46955 (12336)**
Polyunsaturated				
18:2ω6c	133 (133)	1150 (674)	1072 (715)	-
18:4ω3c	-	-	-	3401 (1054)
	133 (133)	**1150 (674)**	**1072 (715)**	**3401 (1054)**

Table D.7. Absolute concentration (μg g^{-1} soil) of phospholipid fatty acids (PLFAs) from soil at the Pallarenda site in a tropical savanna ecosystem in Queensland, Australia during the dry season (October 2002) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver. Numbers in brackets represent standard errors where n = 4. Bold represents summed PLFAs per harvest. – denotes PLFA was not detected.

	Day			
	0	7	14	28
Saturated				
14:0	0.03 (0.01)	0.07 (0.04)	0.01 (0.01)	0.17 (0.12)
15:0	0.03 (0.01)	0.03 (0.01)	0.01 (0.01)	0.03 (0.02)
16:0	0.27 (0.12)	0.38 (0.20)	0.09 (0.05)	2.37 (1.95)
17:0	0.02 (0.01)	0.04 (0.02)	0.01 (0.01)	0.06 (0.04)
18:0	0.07 (0.03)	0.19 (0.10)	0.05 (0.03)	1.61 (1.12)
	0.41 (0.17)	**0.70 (0.37)**	**0.17 (0.09)**	**4.25 (3.24)**
Branched				
i15:0	0.20 (0.05)	0.18 (0.08)	0.08 (0.02)	0.06 (0.01)
a15:0	0.06 (0.02)	0.07 (0.03)	0.03 (0.01)	0.03 (0.01)
i16:0	0.15 (0.05)	0.15 (0.06)	0.08 (0.02)	0.06 (0.01)
i17:0	0.06 (0.02)	0.08 (0.03)	0.03 (0.01)	0.03 (0.01)
a17:0	0.08 (0.03)	0.11 (0.05)	0.04 (0.01)	0.06 (0.02)
i17:1 G	-	-	-	-
i17:1 9	-	-	-	-
a17:1 9	-	-	-	-
	0.49 (0.17)	**0.58 (0.24)**	**0.26 (0.07)**	**0.24 (0.05)**
Cyclopropyl				
cy17:0	-	0.03 (0.02)	-	-
cy19:0	0.08 (0.03)	0.13 (0.06)	0.03 (0.01)	0.09 (0.04)
	0.08 (0.03)	**0.17 (0.08)**	**0.03 (0.01)**	**0.09 (0.04)**
Hydroxyl				
i15:0 3OH	0.01 (0.01)	-	-	-
16:1 2OH	-	0.02 (0.01)	-	0.03 (0.02)
16:0 2OH		-	-	-
	0.01 (0.01)	**0.02 (0.01)**	**-**	**0.03 (0.02)**
Monounsaturated				
16:1ω5c	0.04 (0.02)	0.06 (0.02)	-	0.01 (0.01)
16:1ω7c	-	-	-	-
16:1ω9c	-	-	-	-
16:1ω3c	-	-	-	-
17:1ω6c	-	-	-	-
18:1ω11c	-	-	-	-
18:1ω6c	-	-	-	-
18:1ω9c	-	0.02 (0.01)	-	4.70 (4.62)
18:1ω3c	-	-	-	-
19:1ω8t	0.01 (0.01)	-	-	-
	0.05 (0.02)	**0.29 (0.14)**	**-**	**4.71 (4.62)**
Polyunsaturated				
18:2ω6c	0.05 (0.01)	0.12 (0.05)	-	0.22 (0.21)
18:4ω3c	-	-	-	-
	0.05 (0.01)	**0.12 (0.05)**	**-**	**0.22 (0.21)**

Table D.8. Absolute concentration (μg g^{-1} soil) of phospholipid fatty acids (PLFAs) from soil at the Pallarenda site in a tropical savanna ecosystem in Queensland, Australia during the wet season (March 2003) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver. Numbers in brackets represent standard errors where n = 4. Bold represents summed PLFAs per harvest. – denotes PLFA was not detected.

	Day			
	0	7	14	28
Saturated				
14:0	120 (120)	903 (459)	1769 (629)	338 (629)
15:0	-	205 (205)	550 (186)	260 (150)
16:0	1986 (405)	5276 (5008)	3641 (1551)	1076 (1047)
17:0	-	469 (281)	353 (124)	114 (114)
18:0	777 (246)	2781 (2781)	-	-
	2883 (631)	**9634 (8645)**	**6313 (2254)**	**1787 (1428)**
Branched				
i15:0	1074 (226)	2121 (802)	3676 (886)	1956 (495)
a15:0	334 (195)	1118 (283)	1576 (337)	924 (313)
i16:0	997 (384)	1467 (525)	3130 (664)	1877 (461)
i17:0	346 (234)	954 (477)	1553 (264)	1199 (324)
a17:0	506 (186)	303 (303)	786 (284)	470 (257)
i17:1 G	1699 (230)	387 (278)	2702 (375)	1329 (571)
i17:1 9	-	-	-	-
a17:1 9	-	-	-	-
	5255 (1332)	**6350 (2292)**	**13423 (2752)**	**7754 (2177)**
Cyclopropyl				
cy17:0	-	709 (530)	970 (768)	232 (232)
cy19:0	790 (310)	3156 (822)	3436 (582)	2375 (586)
	790 (310)	**3864 (1320)**	**4407 (1332)**	**2607 (720)**
Hydroxyl				
i15:0 3OH	-	-	-	-
16:1 2OH	-	-	-	-
16:0 2OH	-	564 (327)	485 (296)	385 (224)
	-	**564 (327)**	**485 (296)**	**385 (224)**
Monounsaturated				
16:1ω5c	-	539 (350)	894 (187)	655 (282)
16:1ω7c	662 (383)	2076 (344)	3175 (830)	2276 (423)
16:1ω9c	-	-	-	-
16:1ω3c	-	-	-	-
17:1ω6c	756 (323)	452 (271)	1338 (570)	1480 (206)
18:1ω11c	615 (406)	-	-	-
18:1ω6c	-	-	-	-
18:1ω9c	465 (336)	4649 (2447)	438 (401)	2955 (1061)
18:1ω3c	-	-	-	-
19:1ω8t	138 (138)	712 (286)	477 (308)	158 (158)
	2635 (1041)	**8428 (3112)**	**7722 (905)**	**7524 (1620)**
Polyunsaturated				
18:2ω6c	133 (133)	875 (622)	104 (71)	309 (217)
18:4ω3c	-	-	-	-
	133 (133)	**875 (622)**	**104 (71)**	**309 (217)**

Table D.9. Absolute concentration (μg g^{-1} soil) of phospholipid fatty acids (PLFAs) from soil at the Wambiana site in a tropical savanna ecosystem in Queensland, Australia during the dry season (October 2002). Numbers in brackets represent standard errors where n = 4. Bold represents summed PLFAs per harvest. – denotes PLFA was not detected.

	Day			
	0	7	14	28
Saturated				
14:0	-	0.02 (0.01)	0.07 (0.03)	0.01 (0.01)
15:0	-	0.01 (0.00)	0.01 (0.01)	-
16:0	0.08 (0.02)	0.26 (0.08)	0.48 (0.24)	0.19 (0.04)
17:0	-	0.02 (0.01)	0.03 (0.02)	0.01 (0.01)
18:0	0.04 (0.01)	0.18 (0.12)	0.41 (0.17)	0.08 (0.02)
	0.13 (0.03)	**0.48 (0.22)**	**1.00 (0.45)**	**0.30 (0.07)**
Branched				
i15:0	0.04 (0.01)	0.14 (0.02)	0.12 (0.05)	0.13 (0.02)
a15:0	0.02 (0.01)	0.04 (0.01)	0.04 (0.02)	0.04 (0.01)
i16:0	0.02 (0.01)	0.07 (0.01)	0.05 (0.02)	0.07 (0.01)
i17:0	0.03 (0.01)	0.05 (0.01)	0.05 (0.02)	0.06 (0.01)
a17:0	0.02 (0.01)	0.05 (0.01)	0.05 (0.02)	0.06 (0.01)
i17:1 G	-	-	-	-
i17:1 9	-	-	-	-
a17:1 9	-	-	-	-
	0.13 (0.04)	**0.35 (0.05)**	**0.31 (0.12)**	**0.36 (0.06)**
Cyclopropyl				
cy17:0	-	0.02 (0.01)	0.03 (0.02)	0.04 (0.01)
cy19:0	0.02 (0.01)	0.08 (0.02)	0.09 (0.05)	0.06 (0.01)
	0.02 (0.01)	**0.10 (0.03)**	**0.12 (0.07)**	**0.10 (0.03)**
Hydroxyl				
i15:0 3OH	-	-	-	-
16:1 2OH	0.01 (0.01)	0.03 (0.02)	0.04 (0.02)	0.02 (0.01)
16:0 2OH	-	-	-	-
	0.01 (0.01)	**0.03 (0.02)**	**0.04 (0.02)**	**0.02 (0.01)**
Monounsaturated				
16:1ω5c	-	-	-	0.05 (0.01)
16:1ω7c	-	-	-	0.10 (0.01)
16:1ω9c	-	-	-	-
16:1ω3c	-	-	-	-
17:1ω6c	-	-	-	-
18:1ω11c	-	-	-	-
18:1ω6c	-	-	-	-
18:1ω9c	-	0.03 (0.01)	0.04 (0.02)	0.13 (0.02)
18:1ω3c	-	-	-	-
19:1ω8t	-	-	-	-
	-	**0.03 (0.01)**	**0.04 (0.02)**	**0.28 (0.03)**
Polyunsaturated				
18:2ω6c	-	-	-	0.03 (0.01)
18:4ω3c	-	-	-	-
	-	**-**	**-**	**0.03 (0.01)**

Table D.10. Absolute concentration (μg g^{-1} soil) of phospholipid fatty acids (PLFAs) from soil at the Wambiana site in a tropical savanna ecosystem in Queensland, Australia during the wet season (March 2003). Numbers in brackets represent standard errors where n = 4. Bold represents summed PLFAs per harvest. – denotes PLFA was not detected.

	Day			
	0	7	14	28
Saturated				
14:0	2123 (1128)	325 (201)	-	675 (453)
15:0	229 (229)	167 (167)	-	-
16:0	337 (337)	151 (151)	-	-
17:0	1813 (573)	834 (139)	987 (395)	999 (320)
18:0	382 (382)	362 (216)	259 (259)	923 (923)
	4885 (1784)	**1840 (822)**	**1246 (622)**	**2597 (1355)**
Branched				
i15:0	1740 (778)	1501 (461)	271 (271)	861 (140)
a15:0	173 (173)	-	-	-
i16:0	2660 (1376)	2436 (834)	947 (599)	1478 (245)
i17:0	2645 (1261)	2017 (834)	1492 (811)	1637 (213)
a17:0	448 (300)	363 (210)	147 (147)	-
i17:1 G				
i17:1 9	5044 (2768)	2597 (1395)	2271 (1483)	2893 (1080)
a17:1 9	2409 (1305)	2040 (552)	1264 (829)	1717 (199)
	15119 (7698)	**10952 (3598)**	**6392 (4089)**	**8586 (1842)**
Cyclopropyl				
cy17:0	-	-	-	-
cy19:0	6882 (6882)	6479 (5070)	6787 (6787)	9755 (5049)
	6882 (6882)	**6479 (5070)**	**6787 (6787)**	**9755 (5049)**
Hydroxyl				
i15:0 3OH	1198 (733)	-	593 (593)	6231 (6231)
16:1 2OH	244 (244)	-	-	-
16:0 2OH	510 (510)	592 (365)	319 (319)	775 (270)
	1952 (710)	**592 (365)**	**911 (572)**	**7006 (6371)**
Monounsaturated				
16:1ω5c	152 (152)	-	-	-
16:1ω7c	492 (492)	380 (380)	1296 (857)	518 (518)
16:1ω9c	3018 (1873)	2817 (1256)	283 (283)	1764 (610)
16:1ω3c	14000 (6537)	6703 (2682)	5880 (2120)	5699 (962)
17:1ω6c	1486 (1112)	1539 (528)	826 (506)	1005 (244)
18:1ω11c	3461 (2936)	4732 (1530)	3328 (2027)	3987 (352)
18:1ω6c	904 (904)	991 (359)	609 (609)	1093 (86)
18:1ω9c	7294 (3911)	3938 (1207)	5394 (2075)	3594 (432)
18:1ω3c	10373 (7317)	2183 (862)	9337 (5272)	2017 (763)
19:1ω8t	2936 (1904)	1331 (180)	2022 (1071)	2402 (160)
	45478 (23237)	**25941 (8481)**	**29949 (10312)**	**23139 (2675)**
Polyunsaturated				
18:2ω6c	3260 (3260)	-	1356 (1145)	-
18:4ω3c	1362 (789)	1326 (610)	975 (566)	1061 (84)
	4632 (1654)	**1326 (610)**	**2331 (926)**	**1061 (84)**

Table D.11. Absolute concentration (μg g^{-1} soil) of phospholipid fatty acids (PLFAs) from soil at the Wambiana site in a tropical savanna ecosystem in Queensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver during the dry season (October 2002). Numbers in brackets represent standard errors where n = 4. Bold represents summed PLFAs per harvest. – denotes PLFA was not detected.

	Day			
	0	7	14	28
Saturated				
14:0	-	0.02 (0.01)	0.06 (0.04)	0.25 (0.09)
15:0	-	-	0.01 (0.01)	0.04 (0.02)
16:0	0.08 (0.02)	0.22 (0.08)	0.66 (0.37)	1.64 (0.61)
17:0	-	-	0.03 (0.02)	0.06 (0.03)
18:0	0.05 (0.01)	0.11 (0.05)	0.47 (0.29)	1.16 (0.69)
	0.13 (0.03)	**0.35 (0.14)**	**1.23 (0.73)**	**3.15 (1.38)**
Branched				
i15:0	0.04 (0.01)	0.05 (0.01)	0.14 (0.05)	0.20 (0.05)
a15:0	0.02 (0.01)	0.02 (0.01)	0.05 (0.02)	0.08 (0.02)
i16:0	0.02 (0.01)	0.03 (0.00)	0.06 (0.02)	0.11 (0.03)
i17:0	0.03 (0.01)	0.02 (0.01)	0.05 (0.02)	0.09 (0.02)
a17:0	0.02 (0.01)	0.03 (0.01)	0.06 (0.02)	0.11 (0.03)
i17:1 G	-	-	-	-
i17:1 9	-	-	-	-
a17:1 9	-	-	-	-
	0.13 (0.04)	**0.15 (0.04)**	**0.35 (0.14)**	**0.58 (0.16)**
Cyclopropyl				
cy17:0	-	0.01 (0.01)	0.03 (0.02)	0.03 (0.02)
cy19:0	0.02 (0.01)	0.04 (0.02)	0.11 (0.03)	0.11 (0.04)
	0.02 (0.01)	**0.05 (0.02)**	**0.14 (0.05)**	**0.14 (0.06)**
Hydroxyl				
i15:0 3OH	-	-	-	-
16:1 2OH	0.01 (0.01)	-	0.02 (0.01)	0.07 (0.03)
16:0 2OH	-	-	-	-
	0.01 (0.01)	**-**	**0.02 (0.01)**	**0.07 (0.03)**
Monounsaturated				
16:1ω5c	-	-	0.02 (0.02)	0.11 (0.04)
16:1ω7c	-	-	0.07 (0.05)	0.27 (0.09)
16:1ω9c	-	-	-	-
16:1ω3c	-	-	-	-
17:1ω6c	-	-	-	-
18:1ω11c	-	-	-	-
18:1ω6c	-	-	-	-
18:1ω9c	-	-	0.26 (0.19)	0.60 (0.17)
18:1ω3c	-	-	-	-
19:1ω8t	-	-	-	0.02 (0.01)
	-	**-**	**0.35 (0.26)**	**1.00 (0.29)**
Polyunsaturated				
18:2ω6c	-	0.02 (0.01)	0.05 (0.03)	0.15 (0.05)
18:4ω3c	-	-	-	-
	-	**0.02 (0.01)**	**0.05 (0.03)**	**0.15 (0.05)**

Table D.12. Absolute concentration (μg g^{-1} soil) of phospholipid fatty acids (PLFAs) from soil at the Wambiana site in a tropical savanna ecosystem in Queensland, Australia following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver during the wet season (March 2003). Numbers in brackets represent standard errors where n = 4. Bold represents summed PLFAs per harvest. – denotes PLFA was not detected.

	Day			
	0	7	14	28
Saturated				
14:0	2124 (1128)	899 (122)	2923 (1450)	-
15:0	229 (229)	142 (142)	-	-
16:0	337 (337)	-	133 (133)	-
17:0	1814 (573)	356 (206)	1706 (761)	177 (177)
18:0	382 (382)	154 (154)	264 (264)	-
	4885 (1784)	**1550 (541)**	**5027 (2314)**	**177 (177)**
Branched				
i15:0	2141 (549)	1672 (120)	1714 (580)	506 (292)
a15:0	346 (200)	-	-	-
i16:0	-	-	-	-
i17:0	3066 (1012)	1669 (241)	2611 (1201)	857 (337)
a17:0	448 (300)	314 (181)	473 (283)	-
i17:1 G	-	-	-	-
i17:1 9	5704 (2424)	981 (981)	2915 (2915)	982 (676)
a17:1 9	2811 (1104)	1626 (215)	2122 (935)	902 (366)
	14516 (5216)	**6262 (1614)**	**9836 (5501)**	**3247 (1589)**
Cyclopropyl				
cy17:0	-	-	-	-
cy19:0	6882 (6882)	1845 (1090)	1376 (754)	-
	6882 (6882)	**1845 (1090)**	**1376 (754)**	**-**
Hydroxyl				
i15:0 3OH	1648 (617)	128 (128)	1097 (1097)	-
16:1 2OH	244 (244)	-	1320 (800)	-
16:0 2OH	510 (510)	884 (346)	547 (320)	184 (184)
	2402 (347)	**1009 (419)**	**2964 (1702)**	**184 (184)**
Monounsaturated				
16:1ω5c	304 (175)	-	-	-
16:1ω7c	2687 (813)	2042 (603)	1638 (716)	1410 (637)
16:1ω9c	3018 (1874)	2720 (923)	5797 (2003)	-
16:1ω3c	15191 (5804)	9793 (1620)	21784 (9185)	3809 (1445)
17:1ω6c	1486 (1112)	2002 (212)	1815 (748)	615 (206)
18:1ω11c	3875 (2800)	7200 (1118)	11955 (4798)	2753 (971)
18:1ω6c	904 (904)	316 (316)	1592 (853)	-
18:1ω9c	7919 (3556)	4498 (857)	12109 (5896)	1992 (298)
18:1ω3c	10709 (7164)	2931 (866)	26645 (15809)	1992 (298)
19:1ω8t	3086 (1832)	1771 (460)	4504 (1437)	1826 (292)
	50540 (21948)	**35490 (5458)**	**89658 (40601)**	**16181 (4194)**
Polyunsaturated				
18:2ω6c	3261 (3261)	-	-	576 (576)
18:4ω3c	1362 (789)	2218 (666)	1819 (707)	806 (307)
	4623 (1111)	**2218 (666)**	**1819 (707)**	**1382 (346)**

APPENDIX E

Table E.1. Significance (*P*) of the association of soil biophysiochemical parameters and observed variation in PLFA profiles between cadaver treatment during the dry (October 2002) and wet (march 2003) season at the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia.

	FA		PR		WB	
Soil parameter	Dry	Wet	Dry	Wet	Dry	Wet
Soil moisture (% volumetric)	ND	**<0.001 (33.6%)**	ND	0.068 (8.8%)	ND	**0.026 (9.8%)**
Microbial biomass C ($\mu g\ g^{-1}$ soil)	0.214 (5.7%)	0.571 (2.6%)	0.271 (5.1%)	0.506 (3.2%)	0.709 (2.21)	0.583 (3.0%)
Phosphodiesterase activity (μg *p*-nitrophenol g^{-1} soil h^{-1})	0.197 (5.6%)	0.727 (2.2%)	0.388 (4.2%)	0.168 (6.1%)	0.166 (4.8%)	**0.050 (9.2%)**
Protease activity (μg tyrosine g^{-1} soil h^{-1})	0.314 (4.7%)	0.710 (2.3%)	0.875 (1.9%)	0.615 (2.7%)	0.717 (2.2%)	0.279 (4.8%)
NH_4^+-N ($\mu g\ g^{-1}$ soil)	0.098 (7.7%)	0.942 (1.4%)	0.434 (4.0%)	0.652 (2.5%)	**0.005 (14.5%)**	0.686 (2.6%)
NO_3^--N ($\mu g\ g^{-1}$ soil)	0.355 (4.4%)	0.619 (2.5%)	0.503 (3.6%)	0.368 (4.1%)	**0.011 (9.8%)**	0.858 (1.8%)
Soil pH	0.373 (4.3%)	0.881 (1.7%)	0.227 (5.6%)	0.084 (8.0%)	**0.042 (8.5%)**	0.646 (2.7%)
Variation explained (%)	32.4	46.3	24.4	35.4	42.0	33.7

Table E.2. Significance (*P*) of the association of soil biophysiochemical parameters and observed variation in PLFA profiles between dry (October 2003) and wet (March 2003) season following cadaver burial (+C) and in control soils (no cadaver: -C) at the Yabulu/FACE (FA), Pallarenda (PR) and Wambiana (WB) sites in tropical savanna ecosystems of Queensland, Australia.

	FA		PR		WB	
Soil parameter	+C	-C	+C	-C	+C	-C
Soil moisture (% volumetric)	**0.026 (6.0%)**	0.062 (5.6%)	0.690 (1.4%)	**0.042 (4.2%)**	**<0.001 (11.3%)**	0.081 (3.2%)
Microbial biomass C ($\mu g\ g^{-1}$ soil)	**0.007 (7.9%)**	0.486 (2.5%)	0.713 (1.4%)	0.097 (3.6%)	0.176 (3.0%)	0.061 (3.7%)
Phosphodiesterase activity (μg *p*-nitrophenol g^{-1} soil h^{-1})	0.885 (1.1%)	0.431 (2.8%)	**<0.001 (36.4%)**	**<0.001 (25.7%)**	**<0.001 (22.3%)**	**<0.001 (40.6%)**
Protease activity (μg tyrosine g^{-1} soil h^{-1})	0.263 (2.9%)	**0.024 (7.1%)**	0.591 (1.7%)	0.428 (2.3%)	**0.028 (5.5%)**	0.411 (1.8%)
NH_4^+-N ($\mu g\ g^{-1}$ soil)	**<0.001 (19.3%)**	0.762 (1.8%)	0.598 (1.7%)	0.679 (1.7%)	0.136 (3.4%)	0.916 (0.9%)
NO_3^--N ($\mu g\ g^{-1}$ soil)	0.198 (3.2%)	0.168 (4.3%)	0.207 (2.7%)	**0.015 (5.2%)**	0.322 (2.3%)	0.150 (2.7%)
Soil pH	0.333 (2.6%)	0.159 (4.3%)	0.105 (3.4%)	0.256 (2.8%)	0.133 (3.5%)	0.132 (2.7%)
Variation explained (%)	43.0	28.3	48.7	45.5	51.3	55.5

Table E.3. Significance (*P*) of the association of soil biophysiochemical parameters and variation in PLFA profiles between soil types during the wet (March 2003) and dry (October 2002) seasons following cadaver burial (+C) and in control soils (no cadaver: -C) at the Yabulu/FACE, Pallarenda or Wambiana sites in tropical savanna ecosystems of Queensland, Australia.

	Dry		Wet	
Soil parameter	+C	-C	+C	-C
Soil moisture (% volumetric)	0.070 (3.3%)	0.233 (2.4%)	0.368 (1.9%)	**0.031 (3.8%)**
Microbial biomass C ($\mu g\ g^{-1}$ soil)	0.098 (3.1%)	0.220 (2.4%)	0.471 (1.7%)	0.069 (3.1%)
Phosphodiesterase activity (μg *p*-nitrophenol g^{-1} soil h^{-1})	**0.047 (3.7%)**	0.055 (3.9%)	0.266 (2.2%)	<0.001 (12.6%)
Protease activity (μg tyrosine g^{-1} soil h^{-1})	**<0.001 (10.1%)**	0.389 (1.9%)	**0.003 (6.5%)**	0.632 (1.4%)
NH_4^+-N ($\mu g\ g^{-1}$ soil)	0.404 (1.6%)	0.645 (1.4%)	0.399 (1.8%)	0.173 (2.4%)
NO_3^--N ($\mu g\ g^{-1}$ soil)	**0.038 (4.0%)**	**0.011 (5.6%)**	0.283 (2.1%)	0.470 (1.6%)
Soil pH	**0.002 (6.8%)**	**0.005 (6.5%)**	**<0.001 (11.0%)**	**<0.001 (16.4%)**
Variation explained (%)	32.6%	24.1%	27.2%	37.7%