Drying/rewetting cycles in southern Australian agricultural soils: effects on turnover of soil phosphorus, carbon and the microbial biomass.

A thesis submitted in fulfilment of the degree of Doctor of Philosophy
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Dedicated to my parents, Robert and Betty Butterly

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Abstract

Phosphorus (P) limitations to agricultural productivity commonly occur in Australian soils and have largely been overcome by the use of inorganic fertilisers. However, studies have shown that most of the P taken up by plants is from native P pools. The turnover of P and native soil organic matter may be strongly affected by drying and rewetting (DRW). Rewetting dry soil results in a pulse of respiration activity and available nutrients. In Mediterranean-type climates surface soils naturally undergo recurrent DRW cycles. In southern Australia, soils experience DRW due to erratic rainfall within the growing season, and short, high intensity thunderstorms also during summer periods. The principal objective of this thesis was to determine the significance of dry-rewet events, for altering P availability and cycling in agricultural soils in Australia.

Soils representing a wide range of soil types and climatic zones of southern Australia, showed large flushes in carbon (C) mineralisation after a single DRW event. For some soils these were comparable with reported values, however large variability in flush size between soils was observed. Soils that commonly experience DRW did not appear to be more resilient to DRW than soils from areas with fewer DRW events. Even when soils had relatively small respiration flushes, as a result of low soil organic matter, a high proportion of the soil C was mineralised after rewetting. Soil physiochemical properties (total C, total N, organic C, humus, microbial biomass P, organic P, sand and silt) were correlated to the size of the flush, hence nutrient availability and soil texture appear to primarily determine flush size. Therefore, the influence of climate on DRW may relate to determining the quantity of organic matter and microbial biomass that is available for turnover.

Different size and composition of the microbial biomass within the same soil matrix were achieved by adding three different C substrates (glucose, starch and cellulose at 2.5 g kg^{-1}) at 5 times over 25 weeks. The treatments showed disparate responses to DRW, due to greater biomass (larger flushes) and effects of community composition, highlighting the central role of the soil microbes in DRW processes. When subjected to multiple DRW events these soils showed smaller rewetting respiration flushes with subsequent rewetting events. In contrast, the amount of P released after rewetting was

the same. This study showed that increases in P after rewetting were transient and rapid immobilisation of P by microbes occurred, which may limit the availability to plants. The composition of the microbial community was changed by DRW with a reduction in fungi and gram negative bacteria, showing that certain species are more susceptible to DRW than others.

Closer investigation at 2 hourly intervals after rewetting confirmed the transient nature of P flushes. The response in microbial respiration after rewetting was immediate, with the highest activity occurring within the first 2 h. Phosphorus availability was increased by DRW but remained stable over the following 48 h incubation period. The study highlights the rapid nature of changes in available nutrients after rewetting. Furthermore, while potentially only a small component of the P flush that occurred, the DRW soil had higher levels of P than most incubated soil at 48 h, this would be potentially available for plant uptake or movement with the soil solution.

Long-term water regimes (continuously moist or air-dry, or DRW occurring at different times during incubation) that were imposed on two soils from different climatic regions over a 14 wk period, did not alter available nutrient (P and C) pools or the size of the microbial biomass. However, these long-term water regimes determined the respiration response of the soils to experimental DRW. The largest flushes occurred in the treatment with the longest dry period, and confirm findings of reported studies that the response of a soil at rewetting is determined by the length of the period that it is dried. Microbial biomass was little affected by experimental DRW, but showed large changes in C:P ratio. Thus, changes in physiological state or community composition may be more affected by DRW than the size of the microbial biomass. Microbial communities were altered by DRW irrespective of climatic history (warm wet summer and temperate Mediterranean), however these changes were not related to specific groups of organisms. In addition, the disparate respiration responses and inhibition of phosphatase by DRW, indicate that functional changes may be induced by DRW but can not be sufficiently explained by quantifying available nutrient pools or the microbial biomass.

The use of wheat seedlings bio-indicators of P availability after the long-term water regimes, confirmed that plant available P was altered by DRW, indicated by differences in growth, although the large variability in seedling growth made it difficult to quantify these differences. However, the distribution of labile P, available at planting, in soil and plant pools at harvest, showed that long-term water regimes increased P allocation in plant tissue in one soil and decreased it in another. Furthermore, only a small fraction of the labile P present at planting was taken up by plants, which confirms the superior ability of soil microbes to immobilise P that is released by DRW. Nevertheless, since the long-term water regimes increased P availability, this may be transported via surface water or leaching.

DRW is important for C and P turnover in soils of southern Australia. However, P flushes occur rapidly after rewetting and are transient. Therefore, DRW appears to have only minor consequences for P availability to plants.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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January 2008

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Chapter 1

Review of Literature

Chapter 1. Introduction & Review of Literature

1.1 PHOSPHORUS IN AGRICULTURE

Phosphorus (P) is an important nutrient for the productivity of agricultural systems. P is essential for the synthesis of the nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Marschner 1995). Therefore P has a fundamental role in the storage and translation of genetic information that is imperative for cell function. In addition, phospholipids are important structural components within cell membranes. P is critical for cellular energy production due to the formation of high energy phosphoanhydride bonds in Adenosine triphosphate (ATP) and Adenosine diphosphate (ADP) molecules (Oberson and Joner 2004). Also, phosphate esters such as glucose-6-phosphate and phosphoglyceraldehyde are important for energy transfer within metabolic biosynthesis and degradation pathways (Marschner 1995).

Australian soils are highly weathered, nutrient deficient and have low total P concentrations compared with other areas in the world (Nash and Halliwell 1999). In addition, only a small proportion of total soil P exists in the soil solution, which is the pool that is immediately plant available (Holford 1997). Soils which have not had fertiliser added generally do not release P fast enough to support the requirements of fast growing crop plant species (Schachtman *et al.* 1998). Nevertheless, isotope studies in southern Australian by McLaughlin *et al.* (1988a) showed that a large proportion of P taken-up by wheat plants was from native organic matter. This suggests that P cycling via organic matter decomposition and microbial biomass turnover is an important component of P supply in agricultural soils of southern Australia.

Inadequate supply of P to crop and pastures will result in nutrient deficiency. Phosphorus deficiency in wheat results in stunted growth and depressed tillering (Elliott *et al.* 1997). In addition, limited P supply in wheat has been shown to reduce both the leaf area and photosynthetic capacity (Rodriguez *et al.* 1998). The reduction in the size and mass of leaves leads to greater chlorophyll concentration and leaves appear darker in colour (Marschner 1995). Not surprisingly, the limitation to plant growth especially at tillering in cereal crops is associated with a depression in yield.

Inorganic P fertilisers have been important to ensure adequate yields in Australia over the past 100 years (Bertrand *et al.* 2003). Application of P fertiliser, mainly as superphosphate ($Ca(H_2PO_4)_2$) to crops and pastures has been a standard practice within farming systems. New forms and methods of application of P fertilisers such as liquid fertilisers are being developed in order to increase fertiliser efficiency (Holloway *et al.* 2001; Lombi *et al.* 2004). The long history of P fertiliser use in combination with poor P fertiliser efficiency has resulted in the accumulation of substantial amounts of P in some soils (Bertrand *et al.* 2003).

1.2 FORMS OF PHOSPHORUS IN SOIL

1.2.1 Inorganic phosphorus

Apatite is the most common primary P mineral found within the earth's crust (Frossard *et al.* 1995). Dissolution of apatite by acidification results in the release of inorganic P (P_i) as orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}). Inorganic P within the soil occurs as either phosphate anions adsorbed to positively charged soil components or as poorly soluble precipitates and in the soil solution (Richardson 2001). The form in which P_i exists in soil solution is dependant on the pH with $H_2PO_4^-$, and H_3PO_4 dominating below pH 6.0 and HPO_4^{2-} present only in small proportions (Schachtman *et al.* 1998). Soil pH also determines how P reacts with other elements. Calcium phosphates dominate in alkaline soils while in acid soils iron and aluminium phosphates are more abundant (Barber 1995).

At least 170 different P minerals have been found to occur naturally (Holford 1997). Precipitated forms of P include mono, di and tri-Ca phosphates and hydrates, hydroxy and fluoro apatites and various amorphous forms of Al-P and Fe-P including berlinite, variscite and strengite (Richardson 2001).

Inorganic P compounds may also enter the soil from other sources. Inorganic P within micro-organisms and plant tissue is released into the soil via decomposition and turnover of organic matter. Forms of P_i within the soil originating from organisms

include polyphosphates that regulate biosynthesis and are used to store P within cells and pyrophosphates which are by-products of metabolic pathways (Bünemann *et al.* 2008; Gressel and McColl 1997). The biggest contribution of P_i into soils within southern Australia would occur from the application of inorganic fertilisers and that originating from parent material.

1.2.2 Organic phosphorus

A large proportion of P within soils is in an organic form (Dalal 1977). Recent studies show that organic P (P_o) comprises 13-68% of total P (P_t) in calcareous soils of Western Australia (Samadi and Gilkes 1998) and 34-48% in alkaline soils of South Australia (Bertrand *et al.* 2003). In acid soils of New South Wales and Victoria 35-60% of P_t is present as P_o (McLaughlin *et al.* 1990).

Soil P_o originates from plant residues and animal wastes and the soil microbial biomass (Nash and Halliwell 1999). The majority of soil P_o exists as orthophosphate monoesters, of which 60% is in the form of inositol phosphates (Turner *et al.* 2002b). It is widely believed that the predominant form of P_o in the soil is phytic acid or phytate ($C_6H_6(OHPO_3)_6$ (Barber 1995). Phytate functions as a P storage compound in plants (Tiessen *et al.* 1994). However, recent studies using nuclear magnetic resonance (NMR) suggested that phytate is not the major form of P_o (Smernik and Dougherty 2007).

Lesser amounts of other phosphate esters such as phospholipids, nucleic acids and sugar phosphates have been identified in soil (Stewart and Tiessen 1987). Phosphate esters are present in plants and animals as well as micro-organisms. Phospholipids are components of cellular membranes and can comprise up to 30% of the microbial biomass (Magid *et al.* 1996) and are also the major part of P_o within plant tissue (Bieleski 1973). A large proportion of P_o within soils is not yet identified (Richardson 2001).

In arable soils the microbial biomass accounts for, on average, 3% of the total P_o within the soil (Magid *et al.* 1996). Microbial P consists mostly of nucleic acids (60%), acid soluble P_i and P_o esters (20%) and phospholipids (20%) (Oehl *et al.* 2001a). Within microbial cells, RNA is the dominant nucleic acid and comprises 30-50% of cellular P

(Alexander 1977). Even though the microbial biomass only constitutes a small proportion of the P_o within the soil it is very important as it mediates the mineralisation and turnover of P_o within the soil.

The proportion of the different forms of P_o within the soil is a function of which forms are added and their respective decomposition rate. Organic matter inputs mostly contain orthophosphate diesters however, characterisation of P within the soil shows that P_o is predominantly orthophosphate monoesters (Turner *et al.* 2002b). For example, phospholipids are a major component of the P_o in plant residues (Bieleski 1973) but only found in small quantities within the soil. Phospholipids and sugar phosphates are rapidly degraded within the soil. In contrast, soils accumulate phosphate monoesters which adsorb to soil and are largely unavailable for mineralisation (Hayes *et al.* 2000).

1.3 THE PHOSPHORUS CYCLE

Phosphorus cycling through soil, plants and animals (Figure 1.1) is dependent on the input, output, transfer and transformation between the different pools of the cycle (Gressel and McColl 1997). There are three main pools of P, being available P, labile P and stable P_i/P_o. The available P pool is often considered central to the P cycle, as it is the fraction that is directly available for uptake by plants and the microbial biomass. This pool consists of P_i in the soil solution. In agricultural systems the application of fertilisers is aimed at directly increasing this plant available pool.

The available pool is replenished by desorption from the solid phase and decomposition of the labile P pool (Holford 1997). Labile P is in equilibrium with P_i in the soil solution. Therefore labile P is the potentially available fraction of P_t, which can occur as either inorganic orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) or in organic forms. Labile P_o components such as phospholipids and nucleic acids are rapidly mineralised to supply the soil solution with P_i. Inputs of labile P_o can occur from residues and from the turnover of the microbial biomass. Between 35 and 85% of labile P has been found to be in an organic form (Kelly *et al.* 1983).

The majority of the P_t will exist as a stable inorganic/organic pool. This pool contains P_i compounds that are poorly soluble and P_o compounds that are resistant to mineralization by micro-organisms in the soil. As the term suggests this pool is very stable within the soil and contributes very little, if at all, to the supply of P_i for the available pool within the soil.

The processes involved in soil P cycling are precipitation-dissolution and sorption-desorption reactions which control the abiotic transfer of P between the solid phase and soil solution, and biological immobilisation-mineralisation processes that control the transformations between inorganic and organic forms (Frossard et al. 2000). These processes can be classified into two groups according to their effect on increasing or decreasing the P concentration in the soil solution.

NOTE: This figure is included on page 5 of the print copy of the thesis held in the University of Adelaide Library.

Figure 1.1: The phosphorus cycle. Adapted from Stewart and Tiessen (1987).

1.3.1 Processes decreasing phosphorus in soil solution

1.3.1.1 Sorption and precipitation

Surface adsorption and precipitation are major abiotic P retention processes that decrease the availability of applied P (Samadi and Gilkes 1999). Sorption is the process by which P_i is removed from the soil solution by reaction with the solid phase of the soil. P sorption occurs with positively charged compounds that contain hydroxyl (Fe and Al oxides), carboxyl (organic matter) or silanol (clays) groups (Hinsinger 2001). Phosphorus sorption in soil is strong in many Australian soils such as the highly calcareous soils of the Eyre Peninsula, or in soils with high amounts of Al oxide and low $CaCO_3$ content such as those in the Wimmera or Mallee (Bertrand *et al.* 2003).

Phosphorus sorption is determined by the characteristics of the compounds that are adsorbed. Adsorption of P monoesters is related to the charge density of the PO_4^- group and the spatial conformation of the molecule (Frossard *et al.* 1995). Therefore, not only is the quantity of reactive sites important in determining P sorption but also their spatial organisation, with some compounds having sorption sites with greater accessibility (Frossard *et al.* 1995). It has been suggested that in calcareous soils with low amounts of Ca and P, soil solution P concentrations will be controlled by sorption reactions (Tunesi *et al.* 1999). However, at high concentrations of P and Ca, precipitation processes will dominate. Thus, long-term sorption of P in calcareous soils is governed by calcium carbonate content.

Precipitation differs from adsorption in that P reacts with other ions in solution to form insoluble complexes. This process is dependant on the concentration of positively charged ions within the soil, which is directly affected by pH. In alkaline soils P precipitates as Ca phosphates whereas in acid soils P ions precipitate as Fe and Al phosphates such as strengite or variscite (Hinsinger 2001). The concentrations of active $CaCO_3$ and Fe and Al oxides are very important in determining to what extent soluble P is converted into less soluble compounds (Samadi and Gilkes 1998). Precipitation of added fertiliser P is the main mechanism for P retention in the highly calcareous soils of the Eyre Peninsula (Bertrand *et al.* 2003).

1.3.1.2 Plant uptake

Only P in the soil solution is immediately plant available. Plants actively take up P from the soil solution across the plasma membrane. Only orthophosphate ions $H_2PO_4^-$ and HPO_4^{2-} appear to be taken up by transport systems of plant roots (Schachtman *et al.* 1998). If P supply is sufficient to satisfy P requirement for metabolic processes it may then be transported from the cytoplasm into vacuoles via a number of P transporters (Schachtman *et al.* 1998). Plants and micro-organisms are in direct competition with each other for P within the soil solution (Holford 1997).

Plant uptake of P is affected by the concentrations of cations (Al, Ca, Mg and Fe) and the pH of the soil solution. High concentrations of mobile cations result in precipitation reactions reducing the amount of P that is available to the plant. Soil pH affects plant P uptake by altering the chemical form of the P within the soil solution and by changing the solubility of P minerals and the number of potential P binding sites. Plants prefer the mono-valent form of P ($H_2PO_4^-$) and thus uptake rates are highest between pH 5 and 6 (Schachtman *et al.* 1998).

Movement of P through the soil occurs via diffusion and takes place much more slowly than that of other macronutrients, such as nitrate that moves via mass flow. Even in soils with adequate levels of available P, the soil around roots can become P depleted during periods of high P uptake (Schachtman *et al.* 1998). Hence the supply of P to the root is a major constraint to plant uptake.

A number of adaptations exist which allow plants to overcome the limited P availability and to facilitate the transport of the available P that already exists within the soil. Both plants and micro-organisms are known to manipulate the soil in order to create favourable conditions for growth. Plants, bacteria and fungi within the soil are able to solubilise various forms of precipitated P (Richardson 2001) and have the ability to access sparingly soluble P compounds (Gyaneshwar *et al.* 2002). Plants and micro-organisms produce and secrete phosphatases that mineralise P_o in order to increase the P_i concentration within the soil solution (Horst *et al.* 2001). Similarly, both plants and micro-organisms have the ability to modify the pH of their external

environment by releasing protons and bicarbonate which directly alters the bioavailability of P (Hinsinger 2001). For example, under P limited conditions the highly dense cluster roots of white lupin release organic acids and phenolic substances which chelate Fe/Al thereby increasing P availability (Wasaki *et al.* 2003). A reduction in pH, via proton release, surrounding roots or microbial cells directly increases the solubility of various forms of precipitated P (Ca-P) in alkaline soils (Richardson 2001).

Some plant species can modify root growth and physiology to allow more efficient use of plant available P through better exploration of the soil profile. These include modified root growth, production of root hairs and enhanced expression of P_i transporters (Vance *et al.* 2003). A large number of plants form a symbiosis with mycorrhizal fungi in order to increase utilisation of P existing in the soil profile. Fungal hyphae extend from the root cortex into the soil and facilitate the transport of P and other mineral nutrients to the plant in exchange for carbohydrates to the fungus (Strack *et al.* 2003). These modifications are important in most soils as transport of P to the root rather than the efficiency of plant uptake is the process that limits the acquisition of P (Horst *et al.* 2001).

1.3.1.3 Immobilisation

Soil micro-organisms take up P_i from the soil solution via an energy dependent process (Stewart and Tiessen 1987). Immobilisation is sometimes referred to as assimilation. The microbial biomass may take up P following the addition of organic matter or in response to changes in physiology caused by water fluctuations or other disturbances (Frossard *et al.* 2000). In soils with low SOC, P immobilisation is often limited by carbon (C) availability. Phosphorus applied as plant residues and fertiliser is rapidly immobilised by the microbial biomass as residues also provide a C source for the microbes (McLaughlin *et al.* 1988b). Water fluctuations or other disturbances release additional C and P substrate that can be mineralised and immobilised by soil microbes.

1.3.2 Processes increasing phosphorus in the soil solution

1.3.2.1 Dissolution and desorption

Dissolution of P minerals, such as apatite, occurs naturally in soils (Frossard *et al.* 1995). This process requires acidic conditions and as a result does not occur in alkaline soils unless mediated by H^+ secretion from plant roots or soil microbes. Solubility of Fe and Al phosphates increases with increasing pH, whilst solubility of Ca phosphates decreases at pH greater than 8 (Hinsinger 2001). Therefore pH-dependent P dissolution will depend on the form of P minerals that exist in the solid phase (Frossard *et al.* 1995).

In agricultural systems the largest fluctuations in available P are brought about by the addition of mineral fertilisers. Agricultural fertilisers are manufactured by mimicking the natural dissolution process. Superphosphate is formed by the reaction of rock phosphate with acid (Nash and Halliwell 1999). Phosphorus fertilisers such as superphosphate and ammonium phosphates are water-soluble and dissolve rapidly after application, releasing PO_4^{3-} into the soil solution (Di *et al.* 2000).

Desorption of P from soil surfaces also results in increased P concentration in the soil solution. Desorption is an important process as the majority of the P taken up by plants is derived from labile, weakly adsorbed P (Frossard *et al.* 1995). Many soils in southern Australia have large amounts of sorbed P. The ability of a soil to desorb P is inversely related to the P sorbing capacity (Frossard *et al.* 1995). The buffering capacity of a soil is a function of the sorption capacity and sorption strength and will control the rate of desorption and diffusion (McDowell *et al.* 2001).

1.3.2.2 Mineralisation and turnover

The two main biotic processes that influence P_i release into the soil solution are P_o mineralisation and turnover through the microbial biomass (Frossard *et al.* 2000). In addition to mediating the turnover of P_o, soil microbes also act as a significant reservoir of P within soils (Magid *et al.* 1996). The size and activity of this biomass is therefore very important in P cycling.

Mineralisation is the process by which P_o is dephosphorylated by phosphatase enzymes to release orthophosphate (Nash and Halliwell 1999). Phosphatases can be of plant or microbial origin (Yadav and Tarafdar 2001). Plants secrete phosphatase enzymes into the rhizosphere. Different plant species have varying abilities to mineralise P_o compounds (Chen *et al.* 2004). Soil micro-organisms contribute a significant proportion of phosphatase enzymes within the soil (Richardson 2001). After release from cells, phosphatases enzymes may be adsorbed to soil particles but still remain active within the soil (Oehl *et al.* 2001b).

Turnover encompasses the transformation and movement of P between different pools. The turnover of organic matter therefore involves a number of processes including the decomposition of residues, mineralisation of P_o and the growth and death of the microbial biomass. Turnover of the microbial biomass is affected by edaphic factors, especially C availability and changes in soil water content which are discussed in more detail in the following sections.

1.4 DRY-REWET CYCLES AND FLUSH EFFECTS

Soil water has been shown to be a major factor determining the turnover of soil organic matter and is therefore an important part of nutrient cycling (Thomsen *et al.* 1999). Water within the soil is important for the transport of soluble nutrients and providing a medium for the movement of micro-organisms within the soil profile. Rapid changes in the water content of soils can occur during the summer within the Mediterranean climate experienced in southern Australia due to short high-intensity thunderstorms. Also, given the low and erratic rainfall within this environment, coupled with relatively high evapotranspiration, soils commonly undergo large fluctuations in water content even within the winter.

Wetting of dry soil occurs more rapidly than drying and may not allow sufficient time for microbes to adjust to the changes in osmotic potential. Recent studies suggest that wetting is more important than drying in contributing to the turnover of the microbial biomass (Kieft *et al.* 1987; Turner *et al.* 2003). As the soil is rewet there is a sudden

influx of water into cells that may result in cell lysis and release of cellular contents into the soil.

Drying and wetting cycles are known to result in changes in nutrient availability (P as well as nitrogen (N) and C). The 'flush' of available nutrients after rewetting dry soil occurs from a sudden increase in the organic substrates, which are then mineralised by the microbial biomass (Fierer and Schimel 2002; Franzluebbers *et al.* 2000; Sorensen 1974). There are three mechanisms by which this 'flush' is thought to occur. This includes the release of contents from cells killed by cell lysis, export of osmo-regulatory substrates from living cells and the exposure of occluded organic matter from breakdown of soil aggregates. Therefore the flushes can be considered to be either biological in origin through effects on the size and activity of microbial biomass or physiochemical via changes in the physical stability of soil organic matter or aggregates within the soil (Figure 1.2).

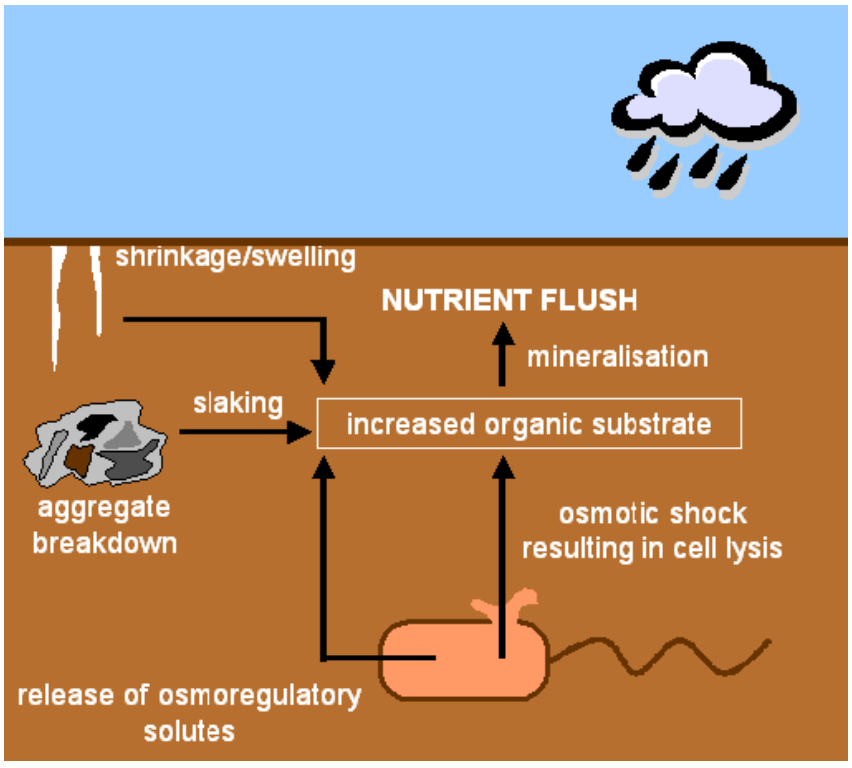

Figure 1.2: Nutrient cycling during drying and rewetting.

1.4.1 Biological effects

1.4.1.1 Increased substrate via cell lysis

Changes in microbial biomass size with temporal changes in soil water content have been reported (Bottner 1985; Campbell *et al.* 1973). Drying has been shown to result in death of a large proportion of the microbial biomass (He *et al.* 1997), mainly consisting of 'active' soil microbes (Bottner 1985). Actively growing microbes have thinner cell walls making them more susceptible to cell lysis (Van Gestel *et al.* 1993). Phosphorus released during cell death is predominantly P_o (Turner and Haygarth 2001) which is then mineralised by the surviving soil organisms resulting in an increase in P_i (Sparling *et al.* 1985). A portion of the microbial biomass will be able to survive the desiccation stress by regulating the concentration of internal osmotic solutes (Csonka and Hanson 1991).

1.4.1.2 Increased substrate via osmo-regulation

In soils frequently subjected to wetting and drying, death or turnover of the microbial biomass may not be the primary mechanism for the increased nutrient flush. Van Gestel *et al* (1993) observed higher biomass C turnover during drying and rewetting in temperate soils than tropical soils and attributed this to better adaptation of soil microbes within the tropics to drying and rewetting. In soils where a high proportion of the adapted microbial population remains alive other mechanisms may dominate. The export of intracellular compounds by the microbial biomass during osmo-regulation has been suggested (Fierer and Schimel 2003; Halverson *et al.* 2000).

In order to overcome changes in soil water potential, microbes are able to manipulate the concentrations of intracellular compounds (Csonka and Hanson 1991). At low water potentials that occur in soil during drying microbial cells accumulate intracellular compounds. The compounds used in this process are referred to as 'compatible solutes' as they have no net charge under physiological conditions and are compatible with cellular function, even at high concentrations (Sleator and Hill 2002). Compatible solutes used in osmo-regulation are generally amino acids and carbohydrates. Enteric bacteria are known to use potassium ions, glutamate, trehalose, proline, glycine betaine and proline betaine (Miller and Wood 1996). Some of these

compounds such as glutamate are rapidly synthesised by soil micro-organisms while other compounds are derived from extracellular sources within soil (Csonka and Hanson 1991). The ability of micro-organisms to adjust to changes in soil water potential varies and therefore microbes have different abilities to tolerate drought (Section 1.5).

During rewetting of a dry soil, high cellular concentrations of these solutes would cause a rapid influx of water into cells. Soil microbes can balance this osmotic gradient through the release of these compounds into the surrounding environment. *Pseudomonas* spp. have been shown to release up to 26% of amino acids and 60% of low molecular weight carbohydrates following rewetting (Halverson *et al.* 2000). The mechanisms of solute release and whether this process is energy dependent is still unclear (Halverson *et al.* 2000). Once these compounds are released into rewetted soil they provide an important source of substrate for surviving microbes.

1.4.1.3 Substrate utilisation following rewetting

Increases in substrate concentration can therefore occur either from cell death during drying, the release of osmoregulatory substances from cells during rewetting, the release of cellular contents of cells unable to adapt to rewetting or exposure of previously occluded organic matter (see below). All of these processes result in increased availability of substrates within the soil, which are then mineralised by surviving microbial biomass producing flushes of available nutrients. The increase in nutrient availability following rewetting only occurs for a short time. A rapid pulse in microbial activity following rewetting takes place within a 48 h period and can persist for up to 6 days (Fierer and Schimel 2003). Phosphorus is released immediately after rewetting, followed by a period with no net release of P (1 week) and then constant but lower rates of mineralisation than observed in the initial flush (Grierson *et al.* 1998). Rewetting not only stimulates mineralisation but also the growth of the surviving microbes which immobilise the available nutrients and restore population densities similar to levels seen prior to drying (Bottner 1985; Wu and Brookes 2005).

1.4.2 Physical/chemical effects

1.4.2.1 Shrinkage/swelling and slaking

In addition to biological processes, changes in the water content of soil may also affect the physical processes. Changes in soil water potential as a result of drying and rewetting affect the physical structure of the soil. Shrinking and swelling is thought to disrupt soil aggregates and release organic matter that was previously physically protected (Denef et al. 2001a). Slaking is the term used to describe the disruption of soil aggregates caused by high internal air pressure as a result of a sudden influx of water (Bossuyt *et al.* 2001). Unequal swelling and compression of entrapped air facilitates the disintegration of soil aggregates (Olsen and Court 1982). Soil micro-organisms are then able to mineralise this formerly occluded material.

1.4.2.2 Changes in chemical reactivity

Changes in soil water content will also have other effects on nutrient availability. For example, increased P sorption capacity has been shown to occur following air drying of soils (Haynes and Swift 1985b). The mechanisms of the increase in P sorption following air-drying remain unclear. Increases in sorption have been proposed to occur from either a shorter time to reach absorption equilibrium or from changes in the structure of organic matter Al/Fe associations (Haynes and Swift 1985a).

An increase in number of sorption sites via the breakdown of soil aggregates is more likely than a change in P affinity of existing sites (Olsen and Court 1982). However, a study with no visible aggregates at the start of the experiment and a long equilibration time (96 h) of continuous shaking, still showed an increase in P sorption following air drying (Haynes and Swift 1985a). Therefore the increase in sorption may not be only explained by additional binding sites from degraded aggregates. Also, soil drying increases extractable Fe/Al which are known to form strong complexes with P thereby reducing its availability (Haynes and Swift 1985b).

Changes in chemical reactivity following drying and rewetting may increase nutrient availability. When dry soil is rewetted organic substrate desorption from soil surfaces

may occur (Seneviratne and Wild 1985) and has been suggested as a possible source of additional substrate causing the flush following rewetting.

1.5 FACTORS INFLUENCING FLUSH DYNAMICS

Drying and rewetting of soil and the subsequent flush in microbial activity and available nutrients may be a significant process in the cycling of P (McNeill *et al.* 1998; Turner *et al.* 2002a; Turner and Haygarth 2003) as well as C (Adu and Oades 1977; Fierer and Schimel 2002; Miller *et al.* 2005) and N (Cui and Caldwell 1997; Kruse *et al.* 2004; Mikha *et al.* 2005; Murphy *et al.* 1998). The size of the flush will depend on the size of the organic pool, the quality of organic matter and the properties of the soil biota (Van Gestel *et al.* 1993). The size of flushes following rewetting have been shown to be related to the size of the microbial biomass (Sparling *et al.* 1985; Turner *et al.* 2003). Similarly, the increase in P_i following air drying of soil has been attributed to the P content of the microbial biomass (Sparling *et al.* 1985). A single dry/wet cycle can potentially kill one third of the microbial biomass (Bottner 1985; Wu and Brookes 2005). Sudden increases in water potential in a grassland soil released up to 58% of the microbial biomass C (Kieft *et al.* 1987).

The flush will not only be affected by the size of the microbial biomass but also the inherent properties of the microbial community. The extent of the flush via cell death may decrease if the soil microbes are able to adapt to the physiological effects of drying and rewetting (Kieft *et al.* 1987). Sparling *et al.* (1985) observed that a greater decrease in soil microbial biomass occurred in field soils not normally subjected to water stress compared to those soils that experienced regular drying and wetting cycles. Resistance to drying is affected by intrinsic properties of the microbial biomass such as cell age, physiological state and cell wall characteristics (Harris 1981; Van Gestel *et al.* 1993). In many cases it may be expected that smaller flushes would occur if the microbial biomass is more resilient to the effects of drying and rewetting. However, large flushes have still been observed following rewetting with no change in the size of the microbial biomass pool which suggests that cell lysis did not occur (Fierer and Schimel 2003). Other studies have also observed considerable flushes in N (Bloem *et al.* 1992), C (Lundquist *et al.* 1999a) and N and C (Fierer and Schimel 2003; Mikha *et al.* 2005) with no associated change in soil microbial biomass. In these studies

without an apparent decrease in microbial biomass, the increase in labile substrate may occur from the release of compatible solutes as microbes undergo osmo-regulation (Halverson *et al.* 2000).

Soils with higher organic matter content have been associated with better water retention during drying and less desiccation of the microbial biomass (He *et al.* 1997). Contrastingly soils with high microbial biomass, often associated with high levels of organic matter have been shown to exhibit greater flushes following rewetting (Bloem *et al.* 1992; Franzluebbers *et al.* 2000). These conflicting results show that a better understanding of flush dynamics as affected by the quantity and quality of organic matter is required.

In addition to organic matter other soil properties will be important in determining the flushes that occur in different soils types. Soil texture will be important for flush dynamics by altering the ability of the soil to retain water and also influencing the soil's capacity to protect organic matter from mineralisation (Baldock and Skjemstad 2000). Drying of soil has been shown to increase P sorption (Haynes and Swift 1985b). Contrastingly, drying soil generally decreases its adsorption capacity by favouring crystallisation and a reduction in the surface area for adsorption (Haynes and Swift 1985b). Alkaline and calcareous soils are common in southern Australia and known for their high sorbing capacity. As these soils are able to sorb a large proportion of the P that becomes available during a flush, the quantity which can be accessed by soil microbes or plants may be relatively small despite a large release of P upon rewetting. The interaction between sorption and buffering capacity and changes in P availability during drying and rewetting needs further examination.

Information concerning the duration of flush effects and the influence of repeated drying and rewetting cycles on flush dynamics are inconsistent. Bottner (1985) observed that the proportion of the biomass killed from repetitive cycles slowly increased and re-established populations became less active after each cycle. In contrast, CO_2 loss from soils increased with the number of wetting events indicating higher activity and thus adaptation of soil microbes (Fierer and Schimel 2002). Repeated drying and rewetting has been shown to increase mineralisation of non-living soil organic matter components (Wu and Brookes 2005). The adsorption and

desorption of released P was larger for repeated dry/wet cycles than for constantly moist soil (Olsen and Court 1982).

Repeated dry rewet cycles did not have any effect on soil organic matter (SOM) mineralisation in a sandy loam soil (Degens and Sparling 1995). This lack of response might mean that either the microbial population had adapted to the changes in water availability or that soil aggregates had become stable, thus exposure of occluded organic matter or increase in sorption area did not occur. Drying and rewetting was found to enhance aggregate turnover in the first two dry/wet cycles, however after this point aggregates became more stable and slake-resistant (Denef et al. 2001a). Soils frequently exposed to extremes in water potential or those with poor structure may be expected to have smaller flushes than those with good aggregation. Smaller respiration flushes with increasing number of drying and rewetting events are most commonly reported (Bottner 1985; Chow *et al.* 2006; Fierer and Schimel 2002; Mikha *et al.* 2005). Reductions in the rewetting flush are thought to occur via stabilisation of soil aggregates and changes in microbial biomass size and physiology, as discussed earlier, or by adaptation of the microbial community.

Adaptation of the soil microbial biomass most likely occurs by a change in community structure, with organisms able to withstand the effects of drying and rewetting becoming dominant. Drying and rewetting has been shown to alter soil microbial communities with no change in biomass (Steenwerth *et al.* 2005). Fierer *et al.* (2003) showed that soil microbial communities within oak soils were altered by a dry-rewet event, while those within grassland soils were largely unaffected and attributed this difference to previous soil water regimes. Grassland soils are thought to be more exposed to severe changes in water content than oak soils due to less vegetation cover. Adaptation of soil microbes to climate has been proposed for smaller rewetting flushes in areas that commonly experience drying and rewetting (Kieft *et al.* 1987). Fungi are the most resilient of the soil microbes to desiccation, followed by gram positive bacteria which can form thick-walled spores and are usually found deep within soil aggregates (Halverson *et al.* 2000; Harris 1981; Turner *et al.* 2003). Fungal and bacterial species contribute to different functions within the P cycle and react differently to environmental and nutritional factors (Oberson and Joner 2004). Fungi are responsible for the formation of aggregates, which may be important in providing protection of

bacteria from the effects of drying and rewetting. Changes in microbial community structure may therefore affect the P cycling during flushes.

In conclusion, the majority of the P that is released during the flush is P_o and is derived from cellular contents of the microbial biomass (Turner and Haygarth 2001). Soil micro-organisms then mineralise this P_o to P_i which is then either taken up by soil microbes, plants or subject to physiochemical reactions. The dynamics of the various P pools following a flush remains unclear. Examination of changes in available P during a flush and the proportion that can be taken up by plants are of particular interest. The effects of a drying event may be short-lived, as remoistening for 1 day almost reversed the effect of drying a soil at 60°C (Barrow and Shaw 1980). More information on how different P pools and other nutrients (C and N) change during drying and rewetting is needed, particularly the labile pool which includes the microbial biomass. It would be useful to ascertain the extent to which the pools change and whether or not these changes are transient or last for longer periods.

1.6 AIMS

The overall aim of the work described in this thesis was to investigate and characterise the function of soil micro-organisms in P (and C) dynamics during soil drying and rewetting events. The principal objective was to determine the significance of dry-rewet events, characteristic of the climate in southern Australia, for altering P availability and cycling in agriculturally important soils. In particular, the work aimed to;

- Quantify the flush in soil respiration (CO_2) and available P, C and N in a range of agricultural soils following a single drying rewetting. In addition, to determine the relationship between the magnitude and rate of the CO_2 flush and soil physiochemical properties (Chapter 3).
- Investigate the influence of repeated dry-rewet cycles in soil on P and C dynamics, particularly changes in P pools and availability. Further, to determine the activity, size and composition of the microbial biomass in order

to identify the role of the microbial biomass during repeated dry-rewet cycles (Chapter 4).

- Examine short-term (hourly) fluxes in respiration following rewetting and quantify temporal changes in resin extractable P, dissolved organic P, extractable organic C and total dissolved N in order to determine the relationship of these to short-term respiration flushes (Chapter 5).
- Determine if the previous long-term water regime alters available nutrient pools (P, N and C) or the supply of nutrients (mineralisation activity) in response to drying and rewetting and if soil microbial communities are altered by long-term changes in soil water content (Chapter 6). In addition, to determine the significance of dry-rewet induced changes in nutrients for plant availability (Chapter 7).

Chapter 2

General Methods

Chapter 2. General Methods

Experimental methodologies which are commonly used throughout this thesis are outlined below. Modifications or specific techniques are further described in the methods sections of each chapter.

2.1 INCUBATION SYSTEM

Soil cores were constructed using PVC tube (37 mm ID x 50 mm in height, IPLEX Pipelines) fitted at one end with nylon mesh (0.75 µm, Australian Filter Specialists). Pre-incubated soil (usually 20 g DW) was packed into each soil core to a bulk density (BD) of 1.4 g cm^3 and individual cores were transferred into gas tight incubation chambers (Ball® Quart Wide Mouth Mason Jars, Jarden Corporation) fitted with stainless steel septum ports to facilitate sampling of headspace gasses. Septum ports were fitted with replaceable general purpose septum (#6518, Altech Associates). To maintain headspace humidity small polycarbonate vials containing 8 ml reverse osmosis (RO) water were added to each chamber. Headspace CO_2 concentrations were analysed using a Servomex 1450 infra-red gas analyser (Servomex, UK) and specific details of the sampling schedule are described in respective chapters.

2.2 WATER-FILLED PORE SPACE

Water-filled pore space (WFPS) was determined according to Franzluebbers (1999) using the following:

$$WFPS = (SWC \times BD) / Porosity \quad \text{(Equation 2-1)}$$

$$Porosity = 1 - (BD/PD) \quad \text{(Equation 2-2)}$$

where, SWC is the gravimetric soil water content (g g^{-1}), BD is the bulk density (g cm^{-3}) and PD is the particle density (2.65 g cm^{-3}).

2.3 MICROBIAL BIOMASS CARBON AND NITROGEN

Microbial biomass C (MBC) and N (MBN) were determined using a 24 h chloroform fumigation followed by 1 h of extraction of 20 g (DW) soil with 40 ml 0.5 M K_2SO_4

(Vance *et al.* 1987). Total C and N in the extracts were measured with a Formacs Series Combustion TOC/TN Analyser (Skalar, The Netherlands). Microbial biomass C and MBN are reported as the difference between fumigated and non-fumigated sub-samples.

2.4 RESIN/MICROBIAL BIOMASS PHOSPHORUS

Microbial biomass P (MBP) was determined using 2 g (DW) soil by simultaneous liquid fumigation and extraction with anion-exchange resin membranes (BDH #55164) in bicarbonate form for 16 h as described by Kouno *et al.* (1995), but using hexanol as the fumigant instead of chloroform and eluting the resins with 0.1 M NaCl/HCl. Additional soil samples were included and spiked with P solution to determine P sorption. The concentration of P in the eluate was determined colorimetrically (Murphy and Riley 1962). Phosphorus within non-fumigated extracts was denoted as resin P (P_{resin}). Microbial biomass P was calculated as the difference between fumigated and non-fumigated sub-samples.

2.5 EXTRACTABLE ORGANIC CARBON

Extractable organic C (EOC) was determined by shaking 20 g (DW) soil with 40 ml 0.5 M K_2SO_4 on a flat bed shaker for 1 h, followed by vacuum filtration using Whatman 42 (2.5 µm pore diameter). The remaining K_2SO_4 extract was stored frozen and later analysed for total C (Section 2.3). When MBC measurements were also being determined, non-fumigated 0.5 M K_2SO_4 extracts (Section 2.3) were used to represent EOC.

2.6 TOTAL DISSOLVED NITROGEN

Total dissolved N (TDN) was determined by shaking 20 g (DW) soil with 40 ml 0.5 M K_2SO_4 on a flat bed shaker for 1 h, followed by vacuum filtration using Whatman 42 (2.5 µm pore diameter). The remaining K_2SO_4 extract was stored frozen and later analysed for total N (Section 2.3). When MBN measurements were also being determined, non-fumigated 0.5 M K_2SO_4 extracts (Section 2.3) were used to represent TDN.

2.7 DISSOLVED INORGANIC PHOSPHORUS

Dissolved inorganic P (DIP) within freshly filtered 0.5M K_2SO_4 extracts (Section 2.3 and 2.5) was quantified colorimetrically using malachite green (Ohno and Zibilske 1991).

2.8 TOTAL DISSOLVED PHOSPHORUS, DISSOLVED ORGANIC PHOSPHORUS

Total dissolved P (TDP) within EOC extracts was performed by adding 0.5 ml of K_2SO_4 extract with 1 ml digestion mix (containing 5g Ammonium Persulphate (SIGMA) in 50 ml of 0.9 M H_2SO_4) in a 2.0 ml eppendorf vial. Lids of eppendorf vials were pierced and these were autoclaved at 121°C at 1.1 bar for 1 h. After cooling the pH of the digested solutions was neutralised using 1M NaOH and the total P content was determined colorimetrically using malachite green (Ohno and Zibilske 1991). Dissolved organic P (DOP) is reported as the difference between total P and inorganic P (Section 2.7). Glucose-6-P (SIGMA) was used as a positive control during all digests.

2.9 DISSOLVED INORGANIC NITROGEN

Dissolved inorganic N (DIN) (NO_3 + NH_4) was determined following Rayment and Higginson (1992) except 10 g (DW) soil was extracted with 40 ml of 0.5M K_2SO_4 (instead of 2M KCl) on an end-over-end shaker for 30 min. Also, extracts were centrifuged at 2000 RPM (537 *g*) for 5 min and vacuum filtered through Whatman 42.

2.10 AROMATICITY OF EXTRACTABLE ORGANIC CARBON

Aromaticity of EOC was assessed by calculating specific UV absorption (Chin *et al.* 1994). Absorption of undiluted EOC extracts was determined photometrically at 280 nm and normalised per unit of EOC (A280 mg EOC^{-1}).

2.11 pH

For pH measurement, soil was shaken with H_2O in a ratio of 1:5 for 60 min, with 3 analytical replicates per treatment. After allowing sediment to settle for 30 min, pH in the supernatant was measured with a Thermo ORION 960 pH/conductivity electrode.

2.12 TOTAL CARBON AND NITROGEN

Total C and N were determined by dry combustion of finely ground samples using a LECO CN analyser 2000.

2.13 TOTAL PHOSPHORUS

Total P of soil was determined by digestion with perchloric and nitric acid in a 1:6 ratio, followed by colorimetric determination of inorganic P (Murphy and Riley 1962).

Chapter 3

Rewetting CO_2 flushes in Australian agricultural soils and the influence of soil properties

Chapter 3. Rewetting CO_2 flushes in Australian agricultural soils and the influence of soil properties

3.1 INTRODUCTION

Surface soils frequently undergo drying and rewetting (DRW) cycles which are known to be important for the turnover of C (Adu and Oades 1977; Fierer and Schimel 2002; Sorensen 1974), P (Magid and Nielsen 1992; Sparling *et al.* 1985) and N (Fierer and Schimel 2002; Kruse *et al.* 2004). When a dry soil is rewet, flushes of CO_2 and available nutrients occur due to increased levels of microbial activity and mineralisation.

Although not proven unequivocally, a number of possible mechanisms for the flush of CO_2 at rewetting have been proposed. Firstly, DRW can increase the turnover of soil organic matter (SOM) through the disruption of macro-aggregates and the release of physically protected SOM (Denef *et al.* 2001b). Other studies have proposed that microbial biomass C is the primary source of the CO_2 pulse (Van Gestel *et al.* 1993; West *et al.* 1989; Wu and Brookes 2005). Death of soil microbes can occur via desiccation during drying (Bottner 1985) or alternatively through osmotic shock which occurs when soil is rewet. Rewetting is thought to be more detrimental for microbial cells than drying as the change in soil water potential occurs more rapidly, often causing cell lysis (Kieft *et al.* 1987). Microbial mediated CO_2 flushes can also occur with no apparent cell death (Fierer and Schimel 2003). Soil microbes maintain internal water potentials during drying by accumulating intracellular solutes (Harris 1981). Upon rewetting these osmoregulatory compounds are either metabolised or released into the surrounding environment (Halverson *et al.* 2000). In any case, the availability of labile substrate is increased at rewetting and mineralised by surviving microbes producing the CO_2 flush.

Although a number of field and laboratory studies have examined DRW effects on biogeochemical cycles, most have only considered one or two soils. Without investigating soils from different climatic and management histories it is impossible to ascertain the importance of DRW for a range of environments. The relative importance of DRW in different soils may be related to the inherent soil properties, particularly those that influence the proposed mechanisms accounting for enhanced CO_2 emission; such as the quantity and degradability of SOM, the availability of SOM for

mineralisation by soil microbes and the resilience of the microbes to withstand fluctuations in water potential. Higher SOM content may increase C availability, microbial biomass size and also influence bulk density and water holding capacity of a soil. Increased capacity of a soil to buffer against fluctuations in water content will greatly affect microbial survival. Also, soils that are well structured and have higher clay contents will provide greater protection of micro-organisms to water stress but may also reduce their accessibility to C and other nutrients. Although the interaction of soil parameters and processes may be quite complex, basic soil properties may provide a valuable tool in predicting the response of soils to DRW.

Agricultural soils of southern Australia regularly experience DRW. These soils are old, heavily weathered and eroded and commonly have low SOM due to the limitations on net primary productivity imposed by low rainfall. Coarse textured soils are common in many areas and are likely to have quantitatively smaller responses to DRW cycles than finer textured soils (Austin *et al.* 2004). Furthermore, in soils that are frequently exposed to DRW, soil microbial communities may have adapted to the negative effects of changes in soil water potential (Kieft *et al.* 1987). The importance of DRW in the turnover of C and nutrients within Australian soils remains unclear.

The experiment described in this chapter used a range of agricultural soils, representing major soil types and climatic histories within southern Australia. The experiment tested the hypothesis that flushes in respiration (CO_2) and available nutrients following a single DRW event differed in different soils and that the magnitude of the flushes (CO_2 and nutrients) were related to soil physiochemical properties.

3.2 METHODS

3.2.1 Overview

Soils used in this experiment were taken to represent a large number of soil types and thus physiochemical properties and climatic zones across the agricultural areas of southern Australia. Thirty-two different soils were obtained from 18 locations and subjected to a single DRW event (6 d drying period then rewet to 70% WFPS). To

enable the large number of soils to be used the experiment was conducted in 2 parts. Firstly, changes in microbial respiration activity were determined by measuring CO_2 flushes at 0, 6, 12, 18, 24, 30, 36, 54, 72 and 90 h following rewetting. Secondly, changes in available nutrients were measured immediately after rewetting (1 h) and at the end of the incubation period (90 h).

3.2.2 Soil collection and physiochemical properties

Thirty-two soils were collected from 18 locations representing a range of climatic areas and soils types, and hence physiochemical properties. At Harden, Junee Reefs, Waite, Waikerie and Otterbourne (Yass) were collected from long term rotation trials during summer-autumn 2004 (Wakelin *et al.* 2008). At each site, soil was collected from a number of different management practices. A total of 30 soil cores (0-10 cm) were taken randomly within each experimental plot and bulked. Soils from Walpeup were taken from the long-term rotation trial MC14 (Latta and O'Leary 2003) from surface (0-10 cm) soils of conventional tillage treatments in March 2004. West Australian soils (0-10 cm) were collected in 2004 from farmed (Gillingarra, Meckering) and unfarmed (virgin) areas (Cunderdin, Lancelin, Northam, West York, Wongan Hills and Yallanbee). At each WA site, composite samples were obtained by taking a number of samples and bulking. Bordertown and Keith soils were collected in April 2005. Keith soil was collected to a depth of 0-20 cm from within a paddock that had not received fertiliser for 25 years. Bordertown soil was taken to a depth of 10 cm from a farmed paddock in which the previous crop was wheat. At Monarto, soil was collected (0-20 cm) from a number of sites within two farmed (fallow/wheat) paddocks and mixed to create a composite sample. As all of the soils used in the incubation study were previously stored air-dry for different periods it was decided to include a duplicate set of Waite soils that were collected immediately prior to the incubation to compare the effects of air drying and storage. These soils designated 'Waite fresh' were taken from the same locations within the Waite long-term rotation trial and arboretum as the other Waite soils. The location of sampling sites, soil classification, and land-use details are listed below (Table 3.1).

Table 3.1: Soil sampling locations, soil classifications and land-use details.

NOTE: This table is included on page 29 of the print copy of the thesis held in the University of Adelaide Library.

[1]The Australian Soil Classification (Isbell 2002)

All soils were air dried and sieved to ≤ 2 mm for physiochemical analyses. Total C and N (Chapter 2.12), Total P (Chapter 2.13) and mid infra-red diffuse reflectance analysis (MIR) predictions (Janik *et al.* 1998) were performed on air dried samples. Microbial biomass P (MBP) and resin extractable P (P_{resin}) (Chapter 2.4) and microbial biomass C (MBC) and nitrogen (MBN) (Chapter 2.3) were determined at the end of the pre-incubation period (Section 3.2.3). Chemical properties for each soil are summarised below (Table 3.2).

3.2.3 Soil incubations

Twenty g of each air dried soil were packed into PVC cores (Chapter 2.1) to a bulk density of 1.4 with 4 replicates. Cores were wet to 70% WFPS and pre-incubated for 14 d at 25°C.

3.2.4 Drying and rewetting

At the end of the 14 d pre-incubation period, sets of each soil were kept either constantly moist (70% WFPS) or dried and rewet. Drying was achieved by placing soil cores in sealed plastic containers containing self-indicating silica gel (BDH Chemicals). The silica was changed every day for the first three days with regeneration of the silica at 110°C overnight. The drying period continued for 6 d and the gravimetric water content of all soils was < 1% (w/w). At rewetting, water was added drop-wise in a circular motion out from the centre of the core to a final gravimetric water content equating to 70% WFPS.

3.2.5 Quantifying respiration flush

Immediately after rewetting cores were transferred into individual incubation chambers (Chapter 2.1) and CO_2 measurements were taken at 0, 6, 12, 18, 24, 30, 36, 54, 72 and 90 h following rewetting. Changes in respiration activity were quantified by measuring headspace CO_2 concentrations using a Servomex 1450 infra-red gas analyser (Servomex, UK). For each sampling time, CO_2 within the headspace of incubation chambers was quantified, chambers were then opened to equilibrate CO_2 to atmospheric concentrations; the chambers were closed and measured again.

Table 3.2: Soil physiochemical properties

NOTE: This table is included on page 31 of the print copy of the thesis held in the University of Adelaide Library.

[1, 2 & 3]As described in Chapter 2 and [4]by mid infra-red diffuse reflectance analysis (Janik et al. 1998)

Respiration rates for each of the 9 sampling times and the cumulative respiration over the entire incubation period were calculated for each individual core.

3.2.6 Quantifying changes in available nutrients

Changes in available nutrients were quantified in DRW soils, immediately after rewetting (1 h) and at the end of the incubation period (90 h) by extracting 5 g of soil with 20 ml of 0.5M K_2SO_4 in an end-over-end shaker for 30 min and then centrifuged at 2000 RPM (537 *g*) for 5 min. Supernatants were vacuum filtered through Whatman #42 and dissolved inorganic P (DIP) was immediately determined within filtered extracts (Chapter 2.7). The remaining solution was stored frozen and later analysed for extractable organic C (EOC) (Chapter 2.5), total dissolved nitrogen (TDN) (Chapter 2.6), dissolved inorganic N (DIN) (NO_3 + NH_4) (Chapter 2.9) and total dissolved P (TDP) and dissolved organic P (DOP) as previously described (Chapter 2.8). As the soils used did not contain carbonates and inorganic N was a small fraction of the total soil N, the total soil C:N was therefore taken to represent that of the organic material.

3.2.7 Modelling of respiration data

Carbon mineralisation kinetics were determined for each individual soil core. Cumulative CO_2-C data was fitted to both a one-pool (Equation 3.1) and a two-pool (Equation 3.2) C mineralisation model:

$$C_{min} = C_o\,(1\text{-}exp^{-kt}) \qquad \text{(Equation 3.1)}$$

$$C_{min} = C_s\,(1\text{-}exp^{-st}) + C_l\,(1\text{-}exp^{-lt}) \qquad \text{(Equation 3.2)}$$

where C_{min} is the cumulative carbon released after incubation time t, Co is the potentially mineralisable fraction of organic carbon (mg CO_2-C g^{-1}), k is the proportional rate constant for mineralisation of C from the potentially mineralisable fraction of C (day^{-1}), t is the duration of the incubation over which carbon mineralisation was measured, C_s and C_l represent the size of the stable and labile fractions of organic carbon respectively (mg CO_2-C g^{-1}) and s and l are the proportional rate constants for mineralisation of C from the stable and labile fractions, respectively (day^{-1}). Both models assume that mineralisation occurs at an exponential rate;

however, the two-pool model differentiates between 'slow' and 'fast' mineralisation components.

The models were fitted to cumulative CO_2-C mineralisation data for each individual soil core using least-square non-linear curve fitting in Microsoft EXCEL®. Solver was used to find the best fit for each model using a 5% tolerance level and with precision and convergence values of 10^{-6} and 10^{-4} respectively. An *F*-ratio test was used to determine if the two-component model provided a statistically better fit than the one-component model (Equation 3.3):

$$\text{(Equation 3.3)} \qquad F = \frac{(RSS_1 - RSS_2) / ((n_1 - p_1) - (n_2 - p_2))}{RSS_2 / (n_2 - p_2)}$$

where RSS_1 is the residual sum of squares of the one-pool model, RSS_2 is the residual sum of squares of the two-pool model, n is the number of sampling times and p is the number of model parameters. A significant ($P < 0.05$) *F-ratio* test (Equation 3.3) indicated that the two-pool model provided a statistically better fit than the one-pool model.

Models were fitted to C_{min} data expressed in terms of mg C_{min} per unit mass of soil and also per unit mass of soil C. While C_{min} expressed on a soil mass basis highlights the relative differences between the soils, C_{min} per unit mass of soil C indicates differences in the mineralisability of soil C between soils.

3.2.8 Statistical analyses

C_{min} was not extrapolated beyond the study period. Calculations of total C_{min} at the end of the study period (90 h) were determined using the model and are denoted as Co_{90h}. Soils from Harden, Otterbourne Waite, Waikerie and Monarto (11 soils in total) were not included in this analyses as the missing data points (36, 54 and 72 h) resulted in erroneous over estimations of *k*. Statistical analyses of model parameters *k* and log transformed Co_{90h} for mineralisable C fraction (mg CO_2-C g soil^{-1}) and C mineralisability (mg CO_2-C g soil C^{-1}) and also available nutrients (mg kg^{-1}) were performed by two-way ANOVA (site and DRW as the main factors) using

STATISTICA (StatSoft, Inc). Significant differences (P<0.05) between means were then tested using post-hoc Tukey test.

3.3 RESULTS

3.3.1 Soil properties

Physiochemical properties of the soils varied widely (Table 3.2). Total C contents ranged from 5.3 to 58.5 g C kg^{-1}, total N from 0.5 to 4.4 g N kg^{-1} and total P from 28 to 678 mg P kg^{-1}. Overall, the soils had coarse texture, with sand contents between 43 and 79%. The maximum clay content was 26.6% with only one other soil having a clay content greater than 20%. Of the 17 soil properties, only total C and total N were significantly correlated (r^2 = 0.76). Weak correlations (r^2 < 0.5) of total N, total C or humus were observed with sand (negative relationship) and silt (positive relationship) content.

3.3.2 Modelling of microbial respiration (C_{min}) data

The one-pool model provided a statistically better fit of cumulative C_{min} data than the two-pool model. It is possible that the incubation period of 90 h (3.75 d) used in this experiment was not sufficiently long enough to have captured the mineralisation of a more recalcitrant C pool. Any further reference to modelled data represents only that of the one-pool model fitting and analyses. C_{min} data expressed per unit mass of soil and per unit mass of soil C are shown in Appendix 3.1 and 3.2. An illustration of the model fitting of four soils is shown below (Figure 3.1). Predicted model parameters Co_{90h} and k were independent and are therefore considered separately.

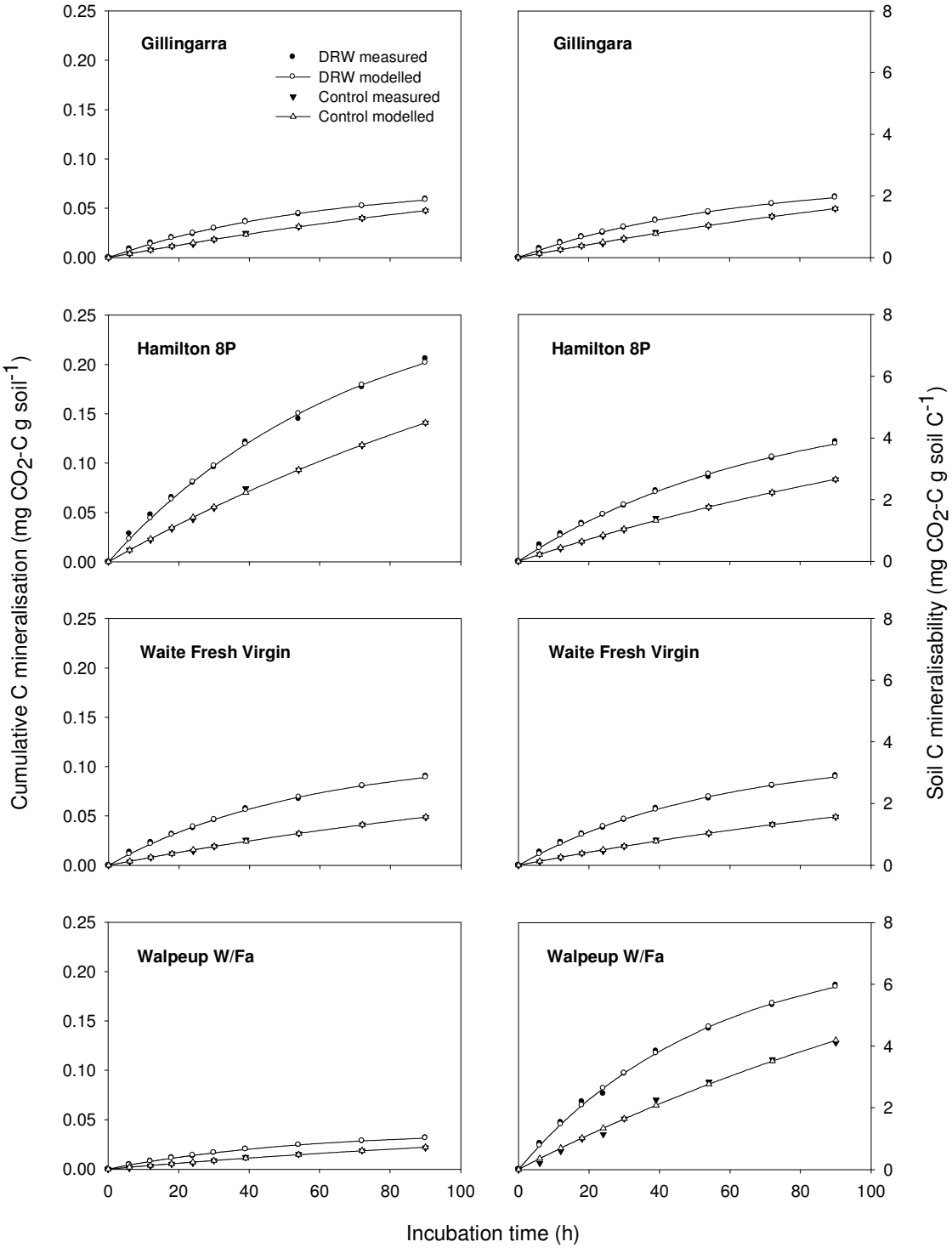

Figure 3.1: Cumulative C mineralisation (left) and soil C mineralisability (right) for measured (solid) and modelled (hollow) data in soil subjected to DRW (circles) and constantly moist (triangles) controls.

3.3.3 Effect of DRW on C_{min} parameters

Compared to constantly moist, higher Co_{90h} following DRW occurred in Hamilton (8P and 23P), Harden CC, Junee Reefs (Cocksfoot and Lucerne), Monarto, Waite Fresh (PPa, Virgin and W2/Pa/Fa) and Waite stored (Virgin and W2/Pa/Fa) and Walpeup W/Fa soils (Table 3.3). Waikerie Pa/W, Pea/W and Northam all showed decreases in Co_{90h} as a result of the DRW, however these were not significant. DRW increased k in all soils except Otterbourne 125. Waikerie soils (Pa/W and Pea/W) showed a decrease in k in DRW soils and were the only soils in which a decrease in k occurred.

Table 3.3: Mineralisable C fraction (Co_{90h}) and proportional mineralisation rate constant (k) from one-pool C mineralisation model fitting (mg CO_2-C g soil^{-1}) in soils subjected to DRW and constantly moist controls. Significant differences (P<0.05) between DRW treatments using Tukey pairwise comparisons are indicated with ***.

Soil	Co_{90h} (mg CO_2-C g soil^{-1})			k (day^{-1})		
	Moist	DRW	Tukey	Moist	DRW	Tukey
Bordertown	0.064	0.080		0.005	0.012	***
Cunderdin	0.025	0.031		0.008	0.016	***
Gillingarra	0.048	0.058		0.005	0.017	***
Hamilton 1P	0.125	0.155		0.005	0.011	***
Hamilton 23P	0.102	0.140	***	0.007	0.018	***
Hamilton 8P	0.141	0.202	***	0.006	0.014	***
Harden CC	0.031	0.044	***	0.010	0.020	***
Harden DD	0.036	0.046		0.011	0.022	***
Junee Reefs Cocksfoot	0.044	0.058	***	0.004	0.014	***
Junee Reefs Lucerne	0.040	0.054	***	0.004	0.013	***
Keith	0.020	0.020		0.009	0.018	***
Lancelin	0.022	0.027		0.011	0.018	***
Meckering	0.039	0.040		0.004	0.013	***
Monarto	0.025	0.036	***	0.023	0.029	***
Northam	0.041	0.034		0.005	0.017	***
Otterbourne 125	0.061	0.069		0.013	0.015	
Otterbourne 250	0.037	0.046		0.017	0.024	***
Otterbourne Nil	0.034	0.044		0.011	0.019	***
Waikerie Pa/W	0.036	0.035		0.017	0.011	***
Waikerie Pea/W	0.033	0.032		0.015	0.010	***
Waite Fresh PPa	0.080	0.112	***	0.008	0.018	***
Waite Fresh Virgin	0.049	0.089	***	0.006	0.018	***
Waite Fresh W2/Pa/Fa	0.040	0.068	***	0.007	0.014	***
Waite PPa	0.054	0.066		0.011	0.019	***
Waite Virgin	0.054	0.082	***	0.012	0.023	***
Waite W2/Pa/Fa	0.035	0.058	***	0.014	0.022	***
Walpeup Virgin	0.109	0.121		0.004	0.012	***
Walpeup W/Fa	0.022	0.031	***	0.006	0.019	***
Walpeup W/Pa	0.062	0.071		0.004	0.015	***
West York	0.047	0.060		0.007	0.020	***
Wongan Hills	0.026	0.030		0.006	0.016	***
Yallanbee	0.081	0.106		0.005	0.015	***

The responses of Co_{90h} and k to DRW in the different soils can be categorised into four groups (Figure 3.2). Twelve soils showed increases in Co_{90h} and k (A), 17 showed increases in k but not Co_{90h} (B), 1 showed no change in either Co_{90h} or k (C), and 2 showed a decrease in k with no change in Co_{90h} (D).

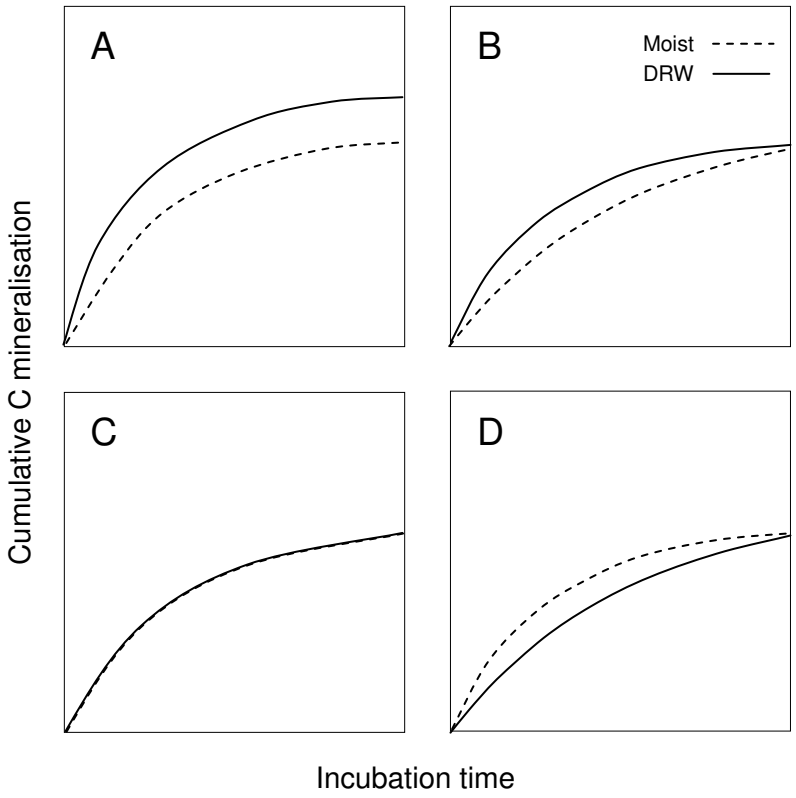

Figure 3.2: Carbon mineralisation responses to DRW; (A) increase in Co_{90h} and k, (B) increase in k and no change in Co_{90h}, (C) no change in either Co_{90h} or k and (D) decrease in k with no change in Co_{90h}.

3.3.4 Carbon mineralisability

Normalisation of C_{min} data (C_{min} per unit SOM) was performed to indicate variation in C mineralisability between different soils (Table 3.4). Similar to results expressed per unit of soil, DRW increased Co_{90h} compared to moist incubated controls, except for Northam and Waikerie which showed a decrease. However, the increases in Co_{90h} were only significant in Hamilton 8P, Harden CC, Junee Reefs Cocksfoot and Lucerne, Monarto, Waite Fresh Virgin, Waite Fresh W2/Pa/Fa, Waite Virgin, Waite W2/Pa/Fa and Walpeup W/Fa and W/Pa soils. Proportional rate constants (k) were not altered by normalisation and were therefore the same as C_{min} per unit mass of soil.

C mineralisability was significantly different between soils (P<0.05) indicating that the flush in C_{min} following rewetting is not solely controlled by soil C content. The highest C mineralisability in either moist incubated or DRW treatments occurred in Waikerie, Walpeup and Wongan Hills soils and the lowest at West York, Gillingarra, Otterbourne (250). In addition, at some locations land-use or agronomic practices altered C mineralisability. At Walpeup, C mineralisability was higher in W/Pa than W/Fa and these were greater than in the virgin soil. Otterbourne 125 and Hamilton 23P showed higher C mineralisability than other levels of fertilization at a given site. Also, both freshly sampled and stored Waite soils had higher C mineralisability in the W2/Pa/Fa treatment than PPa or virgin soil. Agronomic management had no significant effect on C mineralisability at Waikerie, Harden and Junee Reefs.

3.3.5 Effect of DRW on nutrient availability

Most soils showed no change in EOC during the incubation period after rewetting (Figure 3.3). EOC was reduced in Harden (CC and DD), Junee Reefs (Cocksfoot and Lucerne), Otterbourne Nil, Hamilton 1P and Hamilton 8P. Increases in EOC were seen in Walpeup W/Fa and Wongan Hills soils.

Table 3.4: C mineralisability (Co_{90h}) determined by one-pool C mineralisation model fitting (mg CO_2-C g soil C^{-1}) in soils subjected to DRW and constantly moist controls. Significant differences (P<0.05) between DRW treatments using Tukey pairwise comparisons are indicated with ***.

Soil	Co_{90h} (mg CO_2-C g soil^{-1})		
	Moist	DRW	Tukey
Bordertown	2.32	2.90	
Cunderdin	2.45	3.05	
Gillingarra	1.59	1.94	
Hamilton 1P	2.71	3.36	
Hamilton 23P	1.79	2.46	
Hamilton 8P	2.66	3.81	***
Harden CC	2.62	3.69	***
Harden DD	3.01	3.79	
Junee Reefs Cocksfoot	3.02	4.05	***
Junee Reefs Lucerne	2.58	3.53	***
Keith	2.61	2.65	
Lancelin	3.08	3.87	
Meckering	2.96	3.08	
Monarto	2.52	3.56	***
Northam	2.75	2.28	
Otterbourne Nil	1.63	2.10	
Otterbourne 125	2.80	3.15	
Otterbourne 250	1.57	1.97	
Waikerie Pa/W	4.77	4.55	
Waikerie Pea/W	3.98	3.80	
Waite Fresh PPa	1.72	2.40	
Waite Fresh Virgin	1.57	2.86	***
Waite Fresh W2/Pa/Fa	2.46	4.14	***
Waite PPa	1.85	2.27	
Waite Virgin	1.69	2.56	***
Waite W2/Pa/Fa	2.20	3.60	***
Walpeup Virgin	4.02	4.48	
Walpeup W/Fa	4.19	5.92	***
Walpeup W/Pa	5.92	6.81	***
West York	1.16	1.68	
Wongan Hills	4.01	4.55	
Yallanbee	1.80	2.37	

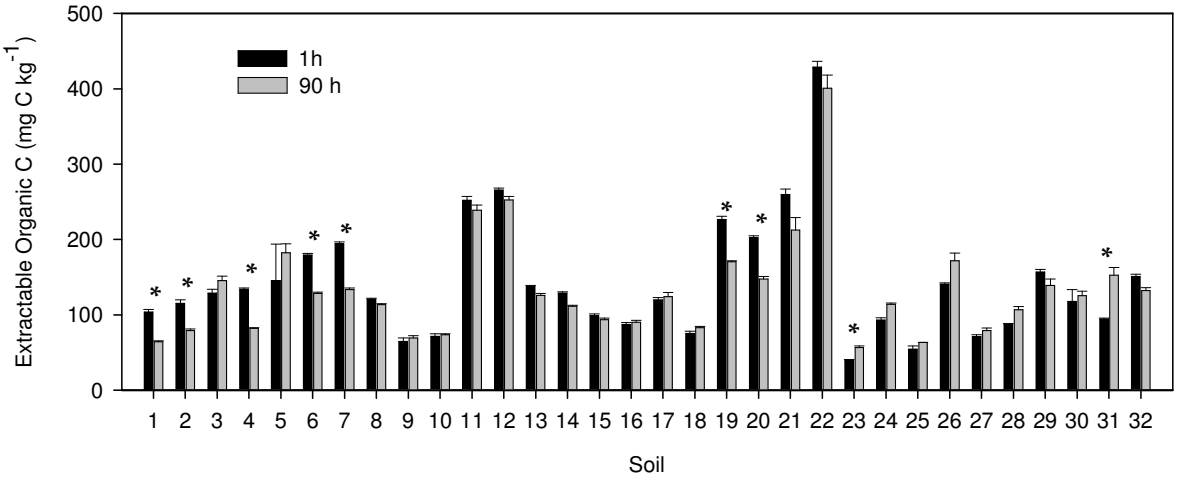

Figure 3.3: Extractable organic carbon (EOC) in soils immediately after rewetting (1 h) and at the end of the incubation period (90 h). Significant (*) differences (P<0.05) between sampling times.

Changes in N availability were greater than either C or P. Significant increases after rewetting in TDN were not observed in 8 of the 32 soils; Otterbourne 125, Waikerie W/Pea, Lancelin, Meckering, Northam, West York, Wongan Hills and Yallanbee (Figure 3.4). In addition, all soils except Keith, Walpeup W/Fa, Lancelin, Northam, West York and Yallanbee showed increases in DIN from 1 to 90 h after rewetting (Figure 3.5).

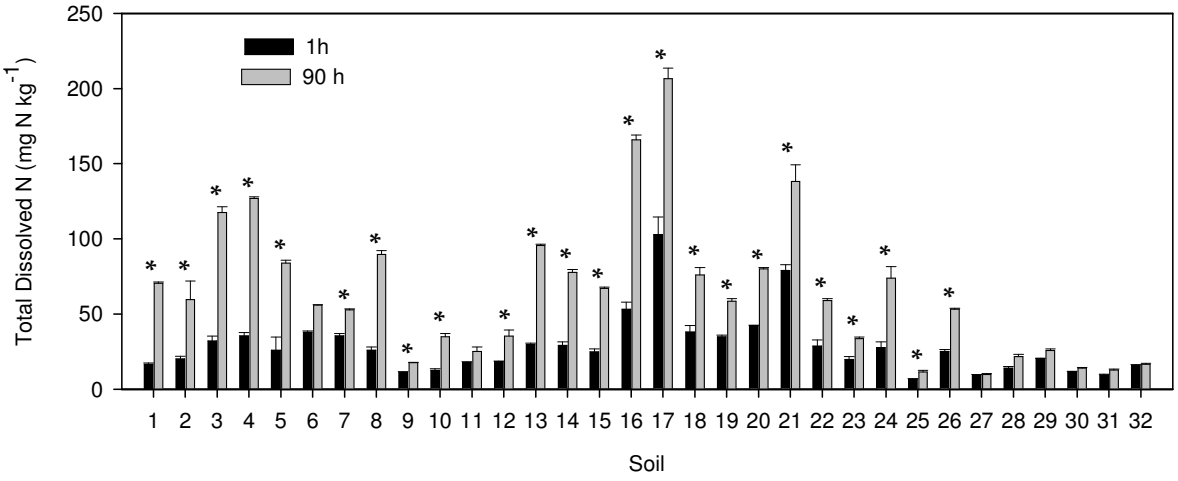

Figure 3.4: Total dissolved N (TDN) in soils immediately after rewetting (1 h) and at the end of the incubation period (90 h). Significant (*) differences (P<0.05) between sampling times.

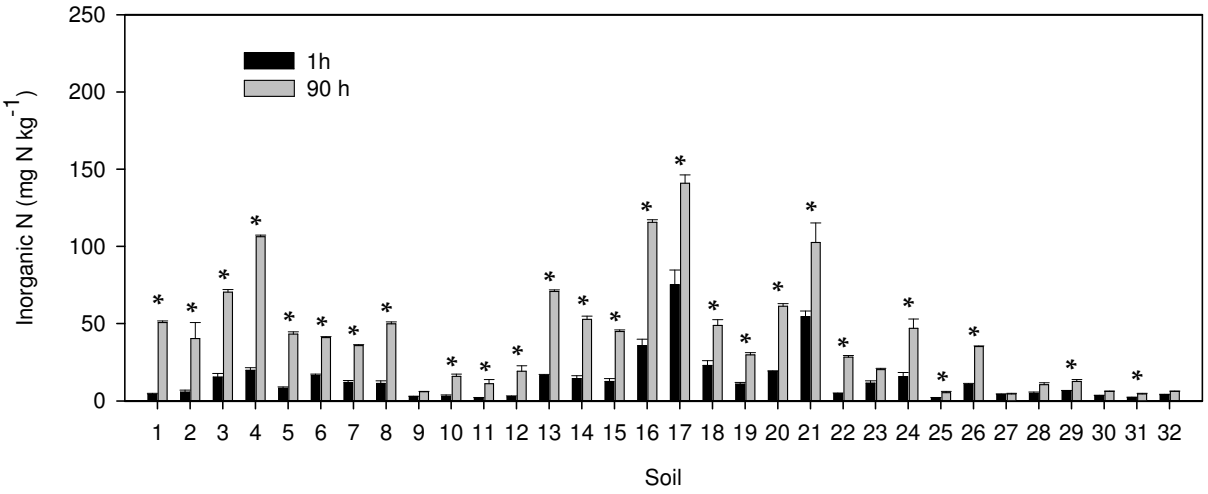

Figure 3.5: Dissolved inorganic N (DIN) in soils immediately after rewetting (1 h) and at the end of the incubation period (90 h). Significant (*) differences (P<0.05) between sampling times.

P availability was relatively constant during the period after rewetting, in contrast to C and N. Only Harden CC showed a significant increase in TDP during moist incubation (Figure 3.6). No significant changes in DIP were seen (data not shown).

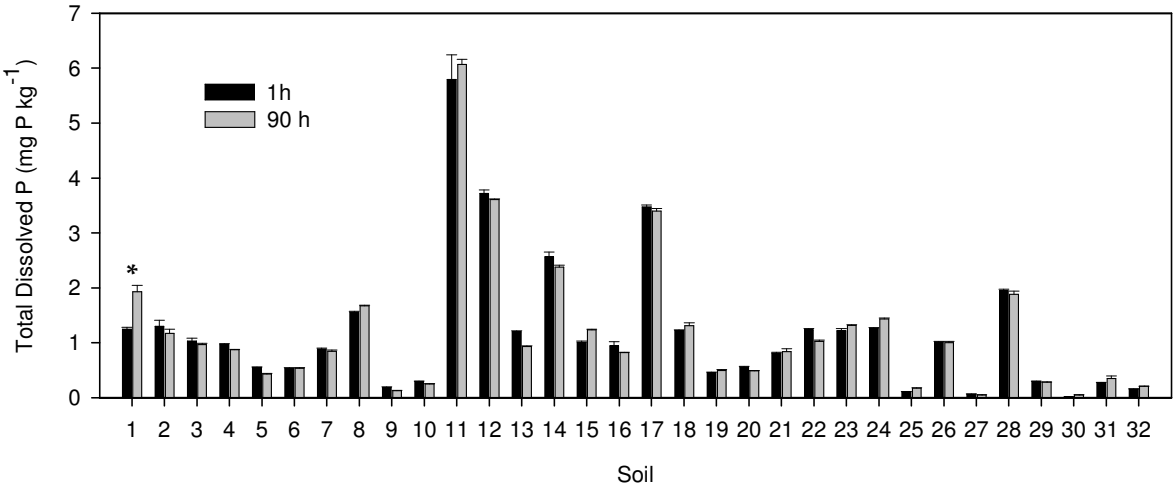

Figure 3.6: Total dissolved P (TDP) in soils immediately after rewetting (1 h) and at the end of the incubation period (90 h). Significant (*) differences (P<0.05) between sampling times.

Regression analyses showed that nutrient availability at 1 h was weakly correlated with mineralisable C fraction (Co_{90h}). In DRW soils Co_{90h} was strongly correlated with TDN (r^2=0.353) and DIN (r^2=0.256) and less correlated with EOC (r^2=0.189). Phosphorus availability was not related to Co_{90h}. In addition, no relationships were observed between nutrient availability and k (data not shown).

3.4 DISCUSSION

3.4.1 DRW and C_{min}

This study showed that DRW is an important process for the turnover of soil C in a wide range of Australian soils. The response of the 32 soils to a single DRW event varied and could be categorised into four different types of response (Figure 3.2). Approximately 40% of the soils studied showed significant increases in both the proportional rate of mineralisation (k) and the quantity of C that was mineralised

(Co_{90h}) following the DRW event (Table 3.3). An increase in C_{min} in DRW soils has been reported in a number of studies (Austin *et al.* 2004; Fierer and Schimel 2003; Mikha *et al.* 2005). The microbial biomass has been suggested as the main source of labile substrate upon rewetting (Bottner 1985; Wu and Brookes 2005). The sudden change in osmotic potential that occurs when dry soil is rewet causes microbial cell lysis (Kieft *et al.* 1987) or results in the release of labile osmoregulatory substrates that are accumulated during drying (Halverson *et al.* 2000). In addition, DRW increases macro-aggregate turnover and releases stabilised C making it accessible for microbial degradation (Denef *et al.* 2001b). Regardless of the source, the increase in *k* and Co_{90h} in these soils highlighted that DRW resulted in an increase in C_{min} due to release of labile substrate.

Seventeen soils showed a significant increase in *k* with no change in Co_{90h} (Table 3.3). This highlighted that the mineralisable C fraction was the same between DRW and moist incubated soils and may be due to the DRW event not releasing additional C substrate. The increase in *k* in DRW soils indicated that microbes in these soils simply utilised this C pool faster than those in moist incubated soils. This suggested that in these soils DRW increased the availability of labile substrate but did not increase the amount of C mineralised during the 90 h period. However, Co_{90h} will not only be determined by substrate quantity but also its degradability and availability to micro-organisms. It is possible that DRW released substrate through the mechanisms explained earlier but this could not be utilised by soil microbes. Degens and Sparling (1995) found that DRW did not increase total C_{min} and suggest that organic C stabilisation with soil surfaces may have reduced C availability to micro-organisms. Also, if additional substrate was released it may have stimulated rapid growth of the microbial biomass which later became C or nutrient limited resulting in reduced respiration activity. As this study made no attempt to quantify changes in the microbial biomass it was not possible to explain the changes in *k* that were observed.

DRW did not significantly alter Co_{90h} or *k* for the Otterbourne 125 soil (Table 3.3). Of the studies that have examined DRW, few have reported no effect of DRW on SOM turnover. The apparent resistance of this soil to DRW could occur from two possible mechanisms. Firstly, it has been suggested that frequent exposure of soils to DRW may deplete easily mineralisable C fractions and result in a more recalcitrant SOM pool

(Degens and Sparling 1995). However, this soil is located at the same site to other soils in the current study and thus climate and DRW regime do not explain the different response. Examination of the chemical composition of the organic materials within this soil would be required to determine if C mineralisability was responsible for the different response of this soil compared to others at the same location. Magid *et al.* (1999) found that DRW did not increase SOM decomposition and suggested that native SOM may be more resistant to decomposition than many studies imply. It was proposed that the high temperatures and physical disruption of soil aggregates which occur in many DRW studies may have a greater influence on C_{min} than the DRW event itself (Magid et al. 1999). Secondly, assuming that the availability of labile SOM was increased by DRW, other factors such as nutrient availability could have limited microbial mineralisation in this soil. The soil nutrient status and physical parameters of the Otterbourne 125 soils did not indicate any limitations to C mineralisation (Table 3.2). The Otterbourne 125 soil had a low predicted pH; however, this was not dissimilar to pH values within other soils. Furthermore, no single property explained why this soil behaved differently to other soils at this location with different management histories.

DRW significantly decreased *k* in the Waikerie soils with no change in Co_{90h}. Therefore microbial activity was negatively affected by the DRW indicated by the lower proportional C_{min} rates compared to moist incubated soils. The response of the Waikerie soils appeared to be related to C availability as soil total C (7.6 - 8.4 g kg^{-1}), organic C (0.6 – 1.6%) and microbial biomass C (62 – 89 mg kg-1) were amongst the lowest values measured in all the soils (Table 3.2). A decrease in *k* but no change in Co_{90h} shows that while initial rates are lower, these are sustained over a longer period of time, which could be explained by a lower microbial biomass. The DRW event may have caused a lower microbial biomass, however the activity of the microbes may also be C limited. DRW has been shown to kill the active component of the microbial biomass (Bottner 1985; Van Gestel *et al.* 1993) and therefore changes in *k* after rewetting may be limited by the ability of the surviving microbes to return from an 'inactive' state.

3.4.2 C_{min} and soil properties

It is well established that C_{min} is determined by the physical and chemical properties of the soil environment, the chemical composition of SOM and the capability and capacity of soil microbes to mineralise SOM (Baldock 2007; Krull *et al.* 2003). Regression analyses were performed to determine if soil properties could be used to describe the C_{min} response to DRW. The size of the C_{min} flush following rewetting was determined for each soil using Co_{90h} per g soil basis (ΔCo_{90h} = Co_{90h} DRW – Co_{90h} moist).

Total N, organic P and total C were highly correlated with ΔCo_{90h} (Figure 3.7). Total P and P_{resin} were poorly correlated with ΔCo_{90h} (data not shown). Greater C_{min} fluxes in soils with higher SOM have been explained by increased availability of labile substrates and larger microbial biomass (Steenwerth *et al.* 2005). However, Wang *et al.* (2003) found that total C and MBC were poorly correlated to respiration in a range of Australian soils. The disparity between DRW studies with respect to SOM is likely to be confounded by other functions that the SOM provides, such as stabilising soil aggregates (Haynes and Swift 1985b) and influencing the soil rewetting rate (Six *et al.* 2004). In addition, total nutrient concentrations in soils are often poor indicators of nutrient availability. Total C estimates do not provide information about the proportion of recalcitrant or physically protected C compounds which may not contribute to Co_{90h}. Also, total P overestimates P availability in soil due to sorption and precipitation reactions with minerals and soil surfaces (Holford 1997). However, poor correlations between P_{resin} and ΔCo_{90h} were also observed despite P_{resin} providing a better estimation of soil P availability than total P. This could indicate that a P limitation does not occur in some soils. The low correlation between P_{resin} and ΔCo_{90h} may also indicate that P_{resin} values measured prior to DRW were a poor indicator of P availability after rewetting. Available P indices measured during the incubation period are discussed later.

Examination of C fractions, POC and humus show that these were less correlated (r^2= 0.40 and 0.46) with ΔCo_{90h} than total OC (Figure 3.7). Therefore the C_{min} flush following rewetting did not appear to be primarily regulated by either fraction, which would have been indicated by stronger correlations than total OC with ΔCo_{90h}. Other studies have shown that C_{min} was highly correlated with SOM and POC over similar

incubation times (3 d) (Franzluebbers *et al.* 2000). However, SOM and POC values in the current study were extremely small and had a much narrower range than those of Franzluebbers (2000). POC undergoes rapid decomposition and contributes to the flux of C, N and P (Salas *et al.* 2003). Since the turnover time of POC is much shorter than humus, the relative proportions of these might therefore be expected to determine C_{min} responses to DRW. Predictions of POC and humus by MIR provide good estimations of actual physical fractions (Janik *et al.* 2007) and conceptual C pools (Skjemstad *et al.* 2004). As a proportion of OC, POC ranged between 0.23 and 67.2% while humus ranged from 32.6 and 98.7% (Table 3.2). However, the relative contribution of these C pools to ΔCo_{90h} remains unclear. Since MIR estimates do not account for differences in composition of these C pools, further chemical fractionation is required to determine the specific C compounds that contribute to ΔCo_{90h}.

MBP had a stronger relationship with ΔCo_{90h} (Figure 3.7) than either MBN (r^2=0.36) or MBC (r^2=0.22) (data not shown). The importance of MBP in determining the amount of P released at rewetting has been demonstrated in Australian soils (Turner and Haygarth 2001). High correlations of MBC with short-term C_{min} flushes (3 d) have been shown across a wide range of soils (Franzluebbers *et al.* 2000). However, the MBC in the current study was much lower (max 504 mg kg^{-1}) than those (up to 6000 mg kg^{-1}) reported by Franzluebbers (2000) due to higher total organic C. In addition, the range of values of MBC in the current study was also less. The higher correlation with MBP than either MBC or MBN may have indicated that the microbial biomass was P limited, however this was not supported by the P_{resin} data. At low MBC levels, the nutritional status of microbes could be a better indicator of respiration potential than the actual size. Substrate availability rather than MBC has been shown to be the principal determinant of respiration during moist incubation (Wang *et al.* 2003). Also, microbial biomass measurements in the current study were taken prior to DRW, as this was thought to represent the potential labile pool that would be available to contribute to the flush at rewetting. Since the CO_2 flush at rewetting will be determined by the surviving microbes, biomass estimates after rewetting may show stronger correlations with ΔCo_{90h}.

The influence of soil texture on the C_{min} after DRW was evident in the current study with strong correlations between ΔCo_{90h} and sand and silt (Figure 3.7). The size of ΔCo_{90h} was significantly reduced at higher sand contents, with low or negative ΔCo_{90h} values occurring above 70% sand. ΔCo_{90h} was greater with increasing silt content. The relationships between these parameters and C_{min} are contrary to other studies. Higher C_{min} in sand has been reported due to greater accessibility of microbes to organic matter, greater amounts of particulate material and increased susceptibility of soil microbes to changes in water potential (Parfitt and Salt 2001; van Veen and Kuikman 1990). Conversely, finer textured soils have a significant protective effect on SOM decomposition via stabilisation with minerals and clay surfaces and physical occlusion of SOM within aggregations (Baldock and Skjemstad 2000; Krull *et al.* 2001). Therefore, the importance of texture appears to relate to the retention of organic matter within these soils rather than textural controls on the rewetting flush in C_{min}. Clay content was poorly correlated (r^2=0.18) (data not shown) with ΔCo_{90h}. The lack of correlation with clay content and ΔCo_{90h} could reflect the low clay content of these soils. MIR predictions showed that the average clay content of the soils was 13% with only two soils having clay contents of 20% or greater (Table 3.2). Furthermore, Wang *et al.* (2003) concluded that the release of substrate following DRW potentially exceeds any substrate limitation induced by higher clay content and hence clay content may be less important after DRW than in incubations under stable conditions.

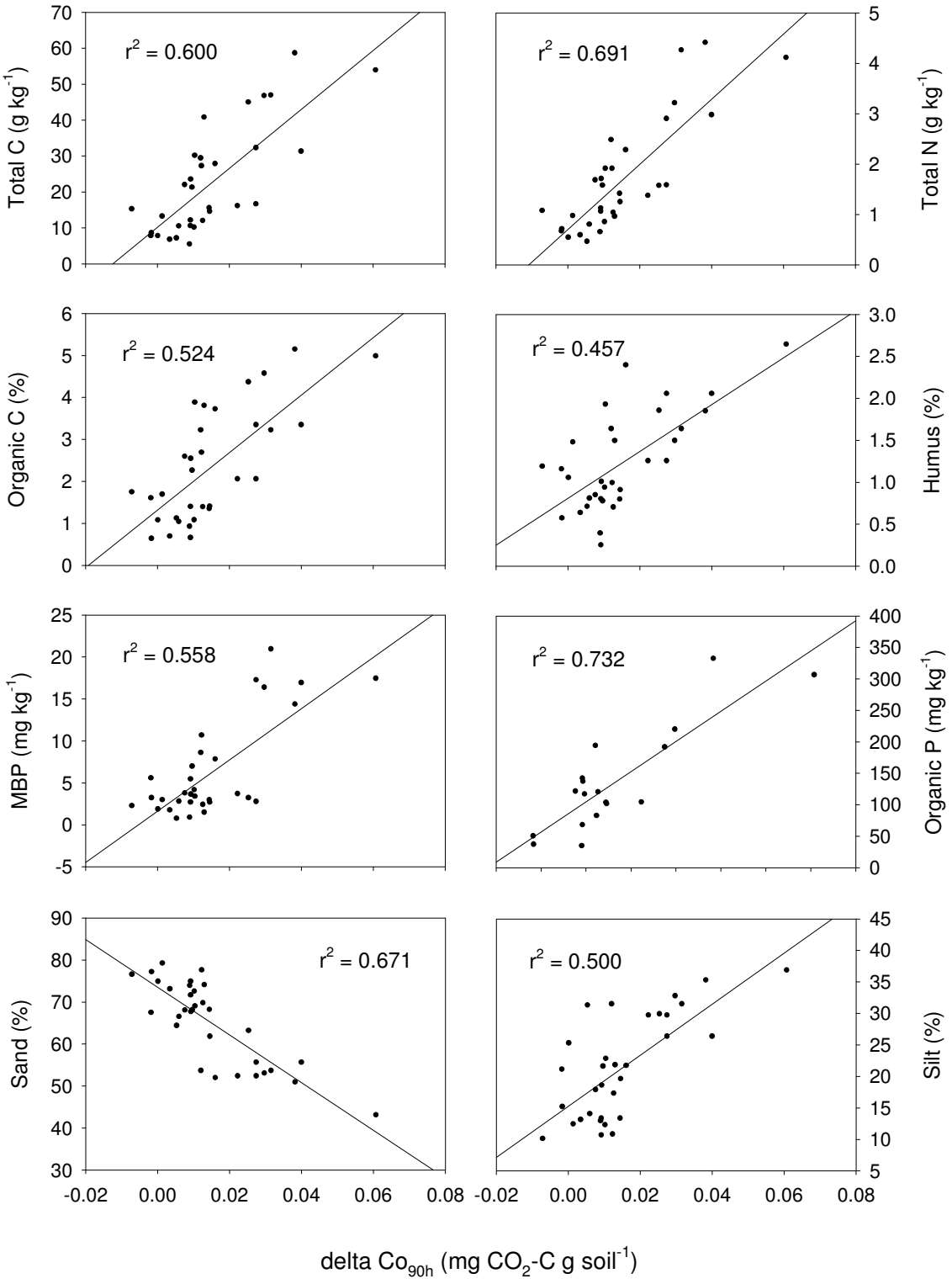

Figure 3.7: Correlations between ΔCo_{90h} (DRW Co_{90h} – Moist Co_{90h}) and eight soil properties.

No soil properties were correlated with *k* for either moist incubated or DRW treatments (data not shown). Texture has been shown to be important in determining the rates of SOM decomposition but not the extent of decomposition (Gregorich *et al.* 1991). Similarly, clay and sand content but not C or N content were significantly related to values of *k* (Riffaldi *et al.* 1996). Values of *k* were higher in the DRW than moist incubated soils (Table 3.3). However, the values of *k* for DRW (*k* = 0.010 - 0.029) or moist incubated soils (*k* = 0.004 - 0.023) varied very little despite the large range in values of Co_{90h}. Values obtained for *k* are at the lower end of those reported in other studies (Riffaldi *et al.* 1996).

Pulses in mineralisation occurred after rewetting in a wide range of soils with different climatic histories. Therefore, the influence of climate appears to be less important than soil properties and management in determining a soil's response to DRW. Given the correlation between SOM (total C, organic C and humus) and MBP with ΔCo_{90h}, the importance of climate for DRW appears to be in determining the quantity of SOM and microbial biomass that is available for mineralisation.

3.4.3 DRW and nutrient availability

The relationship between C_{min} and nutrient availability was investigated by regression analyses. The change in nutrient availability was determined as the difference between nutrient concentration at 1 h and 90 h (Δ_{90h} = 90 h – 1 h). Changes in EOC availability after DRW (Δ_{90h}) were variable with some soils showing EOC increases (up to 58.2 mg kg^{-1}) and others decreases (up to 60.7 mg kg^{-1}). DRW increased TDN with Δ_{90h} values between 0.8 and 112.7 mg N kg^{-1}. Inorganic N Δ_{90h} values were between 0.2 and 86.4 mg N kg^{-1}. Changes in soluble P fractions (TDP and DOP) were negligible. Regression analyses showed that the availability of nutrients (Δ_{90h}) was not correlated with ΔCo_{90h}.

3.4.4 Mineralisability of soil carbon

Mineralisability of soil C was different between soils as shown by normalised C_{min} data (C_{min} per unit SOM) (Table 3.4). Responses in C_{min} to DRW events will therefore not simply be a function of the size of the soil organic matter pool but also the composition and chemical nature of organic material. Regression analyses did not

show any relationship between POC or humus fractions with either Co_{90h} DRW, Co_{90h} moist or ΔCo_{90h} (ΔCo_{90h} = ΔCo_{90h} DRW - ΔCo_{90h} moist). Therefore, understanding C_{min} responses induced by DRW within different soils will require more detailed information of soil organic matter than just the size of these conceptual pools. Future studies should involve characterisation of the chemical structure of the organic materials present within different soils, which can be achieved using solid state ^{13}C nuclear magnetic resonance (NMR) (Baldock *et al.* 2004).

3.5 CONCLUSIONS

A single DRW event altered C_{min} in a range of Australian agricultural soils and highlights that DRW is an important process for the turnover of soil C and nutrients. The effect of DRW was either to increase the amount of mineralisable C (Co_{90h}) and proportional mineralisation rate (k), increase k but not Co_{90h}, decrease k but not Co_{90h} or not change k or Co_{90h}, with most soils being categorised by the first two responses. The size of the flush in C_{min} in some soils (e.g. Hamilton and Waite) was comparable to those reported in other studies, however there was large variation in the size of the flush between soils. The size of the mineralisation flush (ΔCo_{90h}) was positively correlated with total C and N as well as the organic P content of the soil. Also, the C_{min} flush after rewetting was negatively correlated with sand content, highlighting the importance of soil texture in determining the response to DRW. The role of microbial biomass size in determining ΔCo_{90h} was not clear in the current study as MBC was only quantified prior to DRW. Further studies should examine temporal changes in the size of microbial biomass pools (MBC, MBP and MBN) following rewetting. Predicted POC and humus fractions were less correlated with ΔCo_{90h} than either total or organic C pools. In addition, neither POC nor humus content explained the differences in mineralisability that was observed between the soils. Further analysis of the chemical structure of soil organic matter, for example using ^{13}C NMR, is required to determine the contribution of specific C compounds to C_{min} following DRW. Therefore, only a few soil physiochemical properties (total C, total N, organic C, humus, MBP, organic P, sand and silt) were related to the size of the flush, however this highlights that nutrient availability and soil texture appear to be the principal factors that determine C_{min} flushes at rewetting. Furthermore, soils from climatic areas that commonly

experience DRW did not appear to be more resilient to DRW than soils from areas that are less frequently DRW. Therefore, the influence of climate appears to be related to determining the size of SOM and microbial biomass pools that are present within the soil, rather than water regime history. The current study showed that ΔC_{90h} was not related to changes in nutrient availability. Further research is required to understand the link between flushes in respiration (C_{min}) after rewetting and nutrient availability. In particular, to investigate these respiration and nutrient flushes during multiple DRW events, since soils naturally undergo recurrent DRW.

Chapter 4

Repeated drying/rewetting of soils with different microbial biomass and community composition

Chapter 4. Repeated drying/rewetting of soils with different microbial biomass size and community composition

4.1 INTRODUCTION

Drying and rewetting (DRW) cycles are known to be important for the turnover of soil carbon (C), phosphorus (P) and nitrogen (N). Rewetting of dry soil induces the mineralisation of soil organic matter (SOM) producing a pulse of CO_2 and available nutrients (Chapter 3) (Fierer and Schimel 2002; Wu and Brookes 2005). The increase in labile substrate is thought to occur from the enhanced availability of SOM, physical release of occluded SOM from soil aggregates (Denef *et al.* 2001b), lysis of active microbial cells (Fierer and Schimel 2003; Franzluebbers *et al.* 2000) and/or the mineralisation of microbial osmoregulatory compounds (Halverson *et al.* 2000). While a number of studies have investigated DRW and C cycling, less consideration has been given to P (Grierson *et al.* 1998; Turner *et al.* 2003; Turner and Haygarth 2001).

Surface soils within Mediterranean climates undergo repeated DRW cycles from the irregular distribution of growing season rainfall and also summer rainfall events. However, few studies have examined the influence of repeated DRW on available nutrient pools and the soil microbial biomass. Repeated DRW cycles have been shown to increase mineralisation of non-living SOM components (Wu and Brookes 2005). Other studies have shown decreases or no effect of repeated DRW cycles on SOM mineralisation (Degens and Sparling 1995; Mikha *et al.* 2005). Degens and Sparling (1995) suggested that native SOM may be recalcitrant in soils which frequently experience DRW cycles. Therefore the response of soil to DRW may be related to the time since labile material was added to the soil (Cosentino *et al.* 2006; Sorensen 1974). Furthermore, although SOM turnover was higher in DRW than moist incubated soils, doubling the number of rewetting events over a 16 week period had no effect on total mineralisation (Miller *et al.* 2005).

Smaller CO_2 flushes with increasing number of rewetting events are most commonly reported (Bottner 1985; Chow *et al.* 2006; Fierer and Schimel 2002; Mikha *et al.* 2005). Reductions in the rewetting flush with repeated DRW events may occur with no change in microbial biomass size. In these cases, smaller flushes are thought to occur

via a reduction in the release of SOM either from reduced SOM availability (Fierer and Schimel 2002) or increased stability of soil aggregates which can occur after 2 DRW cycles (Denef *et al.* 2001a). However, smaller flushes with subsequent DRW events may also be due to a reduction in microbial biomass size and from re-established populations being less active (Bottner 1985). In addition, changes in the physiological state of the decomposer community may also occur, thereby reducing their susceptibility to changes in water potential (Mikha *et al.* 2005).

Few studies have examined the influence of repeated DRW on microbial community composition. It has been suggested that naturalised soil communities which frequently experience DRW may adapt to withstand the negative effects of fluctuations in water potential (Kieft *et al.* 1987; Steenwerth *et al.* 2005; Van Gestel *et al.* 1993). Fierer *et al.* (2003) showed that bacterial community structure was altered in an oak but not grassland soil and suggest that the different responses of the soils may reflect water regime histories. Oak soils are thought to experience less severe changes in water content than grassland soils due to vegetation cover. Also, DRW altered microbial communities in sub-surface but not surface layers, suggesting microbial adaptation to DRW occurs at the soil surface (Lundquist *et al.* 1999b). These authors also showed that microbial communities within surface soils from conventional and organic systems showed similar responses to DRW, despite having distinct community structures. However, reduced microbial diversity within intensive agricultural systems appears to increase the susceptibility of the soil microbes to DRW compared with less disturbed ecosystems (Steenwerth *et al.* 2005). This study showed that reduced diversity also resulted in loss of microbial function, therefore reducing CO_2 and nutrient flushes at rewetting. Thus, the effect of multiple DRW events on soils with different microbial community compositions is unclear. Also, examination of soil microbial responses to DRW is often complicated by the use of different soils.

The experiment described in this chapter utilised a soil that had been repeatedly amended with different carbon sources (glucose, starch and cellulose) to give different forms and availability of P and also different sizes and community composition of the microbial biomass within the same soil. This experiment aimed (i) to investigate the influence of repeated DRW cycles on P and C dynamics, particularly changes in P pools and availability, (ii) to determine the activity, size and composition of the

microbial biomass in order to identify the role of microbial biomass and community composition during repeated DRW cycles. It was postulated that smaller P, C and respiration flushes occur with repeated DRW cycles due to reductions in the size and activity of the microbial biomass. Also, it was hypothesised that soils with disparate microbial communities would respond differently to repeat DRW.

4.2 METHODS

4.2.1 Overview

The experiment consisted of a full factorial design with four amended soils and two DRW treatments. The soils were amended with glucose (G), starch (S), cellulose (C) and non-amended (N) 5 times over a 25 week period and then kept constantly moist (M) or DRW (D). A total of three DRW cycles were imposed consisting of one week of drying and one week of moist incubation. Controls were kept moist throughout. The abbreviations used were as follows (Table 4.1).

Table 4.1: Summary of abbreviations used

	Non-amended	Glucose	Starch	Cellulose
Moist	MN	MG	MS	MC
DRW	DN	DG	DS	DC

4.2.2 Soil sampling

Soil was collected from a long-term field experiment on a Chromic Luvisol (FAO/ISRIC/ISSS 1998) in Wagga Wagga, NSW (35°05'S, 147°20'E) (Heenan *et al.* 2004). The experimental plots are separated from each other by border plots, which have not been fertilised or tilled since the beginning of the trial in 1979. A composite sample was made out of soil samples collected in July 2005 from several border plots in blocks 1, 3 and 5 (0-5 cm depth). The soil was initially sieved to 4 mm to separate plant residues and soil, and sieved again at 2 mm the following week. After sieving, the moist soil (19 % gravimetric water content) was stored at 4^{o}C for two weeks until

the beginning of the experiment. Mid infra-red diffuse reflectance analysis (MIR) predictions (Janik *et al.* 1998) show the soil to be a clay loam (32 % clay, 9 % silt and 59 % sand) with a pH of 5.3.

4.2.3 Carbon sources and nutrient solution

The C sources added were glucose (D-Glucose, 10117.4Y, BDH Chemicals), starch (starch from corn, 73% amylopectin and 27% amylose, S4126, Sigma) and cellulose (Sigmacell® Cellulose Type 20, particle size 20 µm, S3504, Sigma). These were chosen to provide the same essential C source (glucose) that would become available at different rates. These were added in powdered form at a rate of 2.5 g C kg^{-1} at each amendment. At each amendment all treatments received 10 ml of a nutrient solution that added the following nutrients (in mg kg^{-1}): N (125), K (25), S (20.1), Ca (5), Mg (5), Fe (0.35), Mn (0.1), B (0.05), Cu (0.01), Zn (0.01), and Mo (0.002). The pH of the nutrient solution was adjusted to 5.5. Phosphorus was not added as synthesis of P compounds by micro-organisms was quantified by another study using the same soils (Bünemann *et al.* 2008).

4.2.4 Carbon amendment procedures

Amendments were mixed into the soil by hand and the water content was adjusted to 70% of WHC (23% gravimetric). The soil was lightly packed into a PVC column with a hole (13mm diameter) at the base that was covered with a fine mesh. The column was loosely covered with aluminium foil and incubated at 25°C in the dark, with weekly replacement of soil water loss. A single column was used for each treatment. After 4 weeks the column was placed on a funnel and leached with 500 ml H_2O under suction in order to remove excess mineral N and other accumulated substances that might inhibit microbial activity. Five weeks after the first amendment the soil was amended again. In total, five amendments were performed over a total incubation period of 25 weeks. No leaching was performed at the end of the last incubation period.

4.2.5 Sample preparation

Five weeks after the last amendment, the soil was removed from the PVC column and homogenised by passing it through a 4 mm sieve. Soil sub-samples were air-dried for measurement of pH and ground for C, N and P analysis. The incubation study described below was conducted using the moist soil.

4.2.6 Soil Incubation

Amended soils were adjusted to a water content of 70% WFPS. Soil (18 g) was packed into individual PVC cores (Chapter 2.1) with 4 replicates for each treatment. Cores were then incubated at 25°C for 5 days after packing. To simulate drying and rewetting, soil cores were dried and kept dry for a total of 1 week after which they were rewet and incubated moist (70% WFPS) for 1 week. This procedure was repeated 3 times. To impose drying, cotton pouches (60 mm x 60 mm) were constructed containing 8 g self-indicating silica gel (BDH Chemicals). Silica pouches were added to each individual chamber and were changed at 1, 2, 3 and 4 d when chambers were vented during respiration measurements. Standard curves and blank chambers (no soil core) were included with silica pouches when necessary. The water content of moist incubated cores was monitored gravimetrically and adjusted to 70% WFPS as required.

4.2.7 Soil respiration

Respiration was quantified by measuring headspace CO_2 concentrations within each chamber using Servomex 1450 infra-red gas analyser (Servomex, UK) (Chapter 2.1). Cumulative CO_2 measurements were started three days prior to onset of the first DRW cycle and continued until the end of the experiment. At each measurement CO_2 within the headspace of incubation chambers was quantified directly using a septum port within the lids.

At the start of the first drying phase, CO_2 within the chambers was quantified, chambers were then opened briefly to allow CO_2 to return to atmospheric concentrations, to record soil pot weights, to remove water from reservoir and to add silica pouches. The chambers were then closed, the headspace CO_2 measured and they

were incubated at 25°C. After 1 d the headspace CO_2 was measured again, and the chambers were opened to equilibrate headspace gases, record changes in soil gravimetric water content and change silica pouches. Chambers were then closed and initial CO_2 was measured. This procedure was repeated at 2, 4 and 7 d. Silica pouches were regenerated in an oven at 105°C overnight.

At the beginning of the moist incubation (day 7 of the drying phase) CO_2 was measured as previously stated. However, this time chambers were opened, soil cores were weighed then rewet to 70% WFPS, 8 ml of water was added to reservoirs, chambers were quickly closed and CO_2 measured again. At 2, 4 and 7 d after rewetting CO_2 within each chamber was measured, the chambers were then opened and returned to atmospheric CO_2 concentrations, closed and remeasured. This procedure was repeated 3 times over a 6-week period.

4.2.8 Microbial biomass and nutrient analyses

Characterisation of soil pH (Chapter 2.11), total C and N (Chapter 2.12) and total P (Chapter 2.13) were carried out on air-dry and ground soils at the beginning of the study as previously described. Total C equals organic C since no inorganic C was detected.

Concentrations of available nutrients were determined during the initial characterisation and at each of the four experimental sampling times. Moist incubated soils were only sampled initially and at three times to correspond with the periods of moist incubation of the DRW treatment (four times in total). Resin extractable P (P_{resin}) and microbial P (MBP) (Chapter 2.4) were determined as previously described. Microbial C (MBC) was estimated as outlined in Chapter 2.3 but 5g of soil was extracted in 20 ml of 0.5M K_2SO_4 (instead of 10 g soil and 40 ml extractant used previously). Microbial C is reported as the difference between chloroform fumigated and non-fumigated samples. Total C of non-fumigated samples was used to represent extractable organic carbon (EOC). Aromaticity of the EOC was assessed by calculating specific UV absorption (absorption at 280nm normalised per mg C) (Chin *et al.* 1994).

Phosphorus fractions in the non-fumigated 0.5M K_2SO_4 extracts were also determined. Immediately after extraction, inorganic P (P_i) was determined colorimetrically using malachite green (Chapter 2.7). Extracts were then digested and analysed for total dissolved P (TDP) (Chapter 2.8). Dissolved organic P (DOP) is reported as the difference between the total (TDP) and P_i fractions.

4.2.9 Microbial community composition by fatty acid methyl ester analysis

Fatty acid methyl esters (FAME) were extracted at the beginning (following amendment) and the end of the experiment described here following the protocol by Pankhurst *et al.* (2001). Briefly, 3 g of frozen soil were placed in a Teflon® tube and saponified with 6 ml of 3.75 M NaOH in 50% (v/v) aqueous methanol by boiling in a water bath for 30 min. After cooling, the tubes were centrifuged for 3 min at 2000 RPM and 3 ml of the supernatant were transferred to a glass tube. An aliquot (50 µl) of internal standard [tridecanoic acid (13:0)] and 6 ml of 3.25 M HCl in 45% (v/v) aqueous methanol were added, vortexed and incubated at 80°C for 10 min. After cooling, the solution was extracted with 1.5 ml of 1:1 hexane-methyl-tert butyl ether by shaking end-over-end for 10 min. The extract was centrifuged at 2000 RPM for 3 min and the top phase transferred to a new tube, washed with 4 ml of 0.024 M NaOH by shaking for 5 min, followed by centrifuging for 3 min. The top phase was collected into a gas chromatography (GC) vial and evaporated under N_2. Prior to GC analysis, the residue was resuspended in 0.2 ml of 1:1 hexane-methyl-tert butyl ether and 10 µl of nonadecanoic acid (19:0) standard was added. The FAME were separated by capillary GC (HP 5890, Hewlett Packard) with a flame ionisation detector. The GC was equipped with a HP 25 m × 0.2 mm fused silica capillary column and hydrogen was used as the carrier gas. The temperature program was ramped from 170 to 250°C at 5°C min^{-1}. The FAME peaks were identified by the MIDI program based on their chain length (MIDI; Microbial ID, Newark, DE, USA). The peak areas were normalized against the two internal standards, thus correcting for the efficiency of the methylation reaction, extraction efficiency and recovery in GC analysis. Fatty acid nomenclature was used as described by Frostegård *et al.* (1993).

Since FAME's can be of microbial as well as plant origin (Drenovsky *et al.* 2004), only fatty acids that have been clearly related to soil micro-organisms were used for the

statistical analysis, using the selection of Zak *et al.* (2000). Fungi were represented by the fatty acid 18:2ω6c, gram-positive bacteria (G+ve) by i15:0, a15:0, i16:0 and i17:0, gram-negative (G-ve) bacteria by 16:1ω7c, 16:1ω5c, cy17:0 and cy19:0, actinomycetes by 10me18:0 and total bacteria by the sum of the two previous groups of fatty acids plus 14:0, 15:0, 17:0, a17:0, 17:1ω8c and 18:1ω9c. The relative abundances of individual FAME's were calculated as weight percentages (wt %) of the total weight (μg/g soil) of these selected FAME's. Estimation of functional diversity was performed using richness and evenness indices (Zak *et al.* 1994).

4.2.10 Statistical analyses

For each amended soil, 2-way analysis of variance (ANOVA) was used to determine the effects of DRW x sampling time on soil chemical properties using GENSTAT 8th Edition (Lawes Agricultural Trust). Significant differences ($P<0.05$) between means were then tested using post-hoc Tukey test. FAME data were analysed by principal component analysis using CANOCO 4.5 (CANOCO, Microcomputer Power).

4.3 RESULTS

4.3.1 Respiration rate

Respiration rates fluctuated with changes in soil water content (Figure 4.1). As soils dried, respiration rates decreased until they were no longer detectable (after about 3 d). Rewetting after one week of dry incubation produced a rapid pulse in respiration with the magnitude of the flush being greatest in glucose amended soil followed by cellulose, starch and smallest in non-amended soil. Each treatment except cellulose amended soil showed a slow decline in respiration rate in the week of moist incubation with the rates remaining higher than in the moist incubated soils. Respiration activity in the cellulose amended soil was lower in rewet soil than the constant moist control. The size of the rewetting respiration flush was smaller with each subsequent DRW cycle in all treatments. At the third cycle the rewetting flush was approximately one third of that measured in the first cycle. Soils held at constant water content showed a gradual decline in respiration activity. These basal respiration

rates were lowest in the non-amended soil, 2-3 times higher in the glucose and starch amended soils and 3-6 times higher in the soil amended with cellulose.

Cumulative respiration was highest in the glucose amended soil followed by cellulose, starch and was lowest in the non-amended soil (Figure 4.2). DRW significantly increased cumulative respiration compared to the moist controls in the non-amended soil and the soils amended with glucose or starch. DRW reduced cumulative respiration in the soil amended with cellulose compared with constantly moist soil. The moist incubated cellulose amended soil had a much higher basal respiration rate than all other moist incubated treatments.

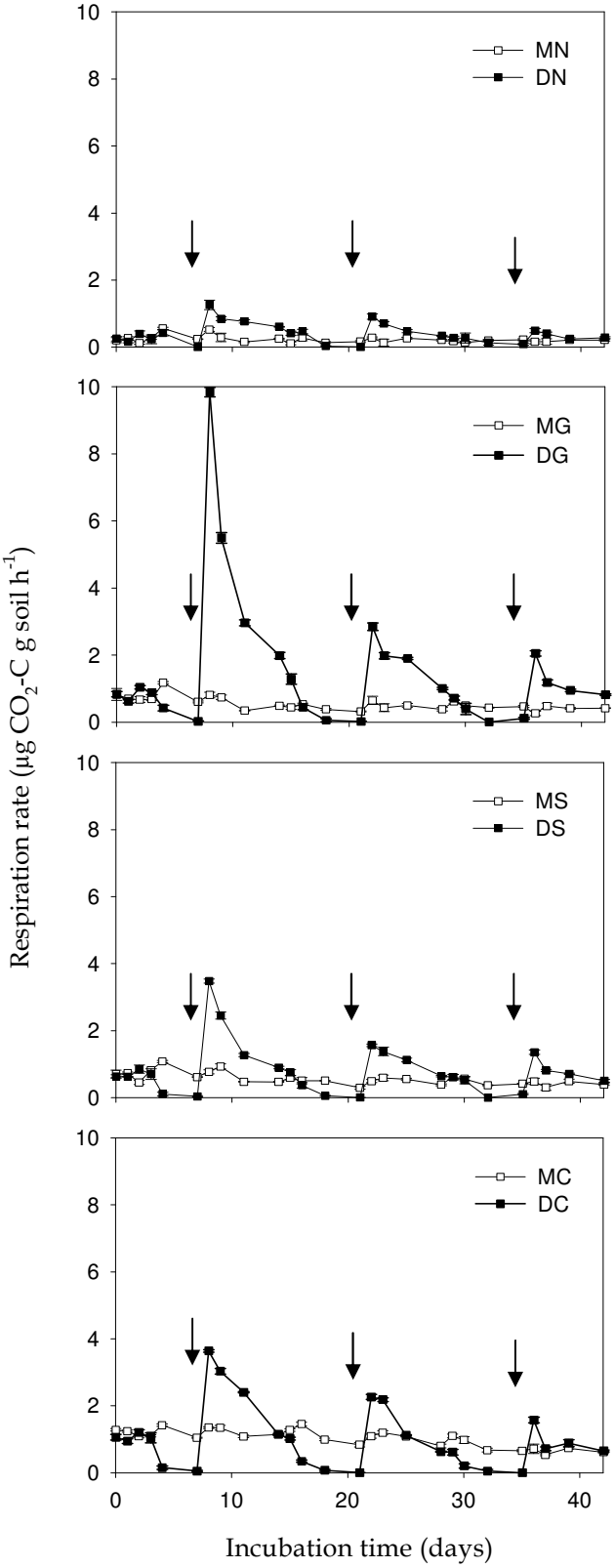

Figure 4.1: Respiration rates in moist (M) and DRW (D) soils previously amended with glucose (G), starch (S), cellulose (C) and non-amended (N). Bars indicate standard errors of the mean. Arrows indicate rewetting events.

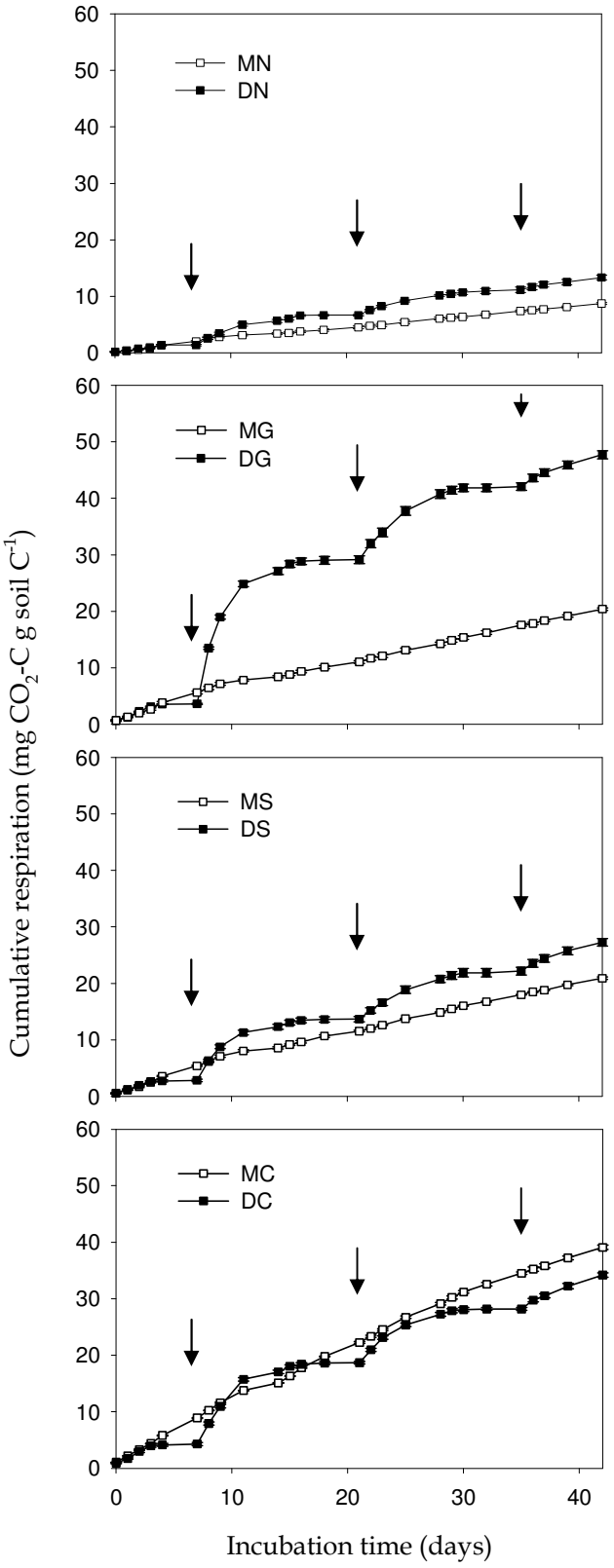

Figure 4.2: Cumulative respiration activity in moist (M) and DRW (D) soils previously amended with glucose (G), starch (S), cellulose (C) and non-amended (N). Error bars indicate standard error of the mean. Arrows indicate rewetting events.

4.3.2 Carbon availability

At the end of the 25 week period, total C content of amended soils was only slightly higher than the unamended soil indicating that most of the C added (12.5 g C kg^{-1}) had been mineralised. Total C contents for glucose, starch and cellulose amended and the non-amended soils were 29.6, 28.4, 29 and 26.7 g C kg^{-1} respectively.

The amount of EOC in moist incubated soils was very similar between treatments (Figure 4.3). Extractable organic C in these soils gradually decreased throughout the incubation period. Rewetting of dry soil produced an immediate flush of EOC in all treatments. At the first rewetting event the increase in EOC was between 135-160 mg C kg^{-1} for glucose, starch and cellulose amended soils and approximately 98 mg C kg^{-1} for the non-amended soil. In all treatments, lower amounts of EOC were released with subsequent (2^{nd} and 3^{rd}) DRW events. However, even though the flushes of EOC were smaller they were still significant. During periods of moist incubation EOC decreased in the DRW soils. For each soil the amount of EOC at the end of the moist incubation phase was similar for each of the 3 DRW cycles and in the amended soils it was generally higher than in the constantly moist controls.

The aromaticity of the EOC was assessed by SUVA (Table 4.2). In the moist incubated controls the aromaticity slowly increased and was inversely proportional to the amount of EOC. In soils subjected to DRW the aromaticity of EOC was reduced immediately after rewetting. However, there was no significant difference between DRW and constantly moist soils at the end of the 7 d of moist incubation within each treatment.

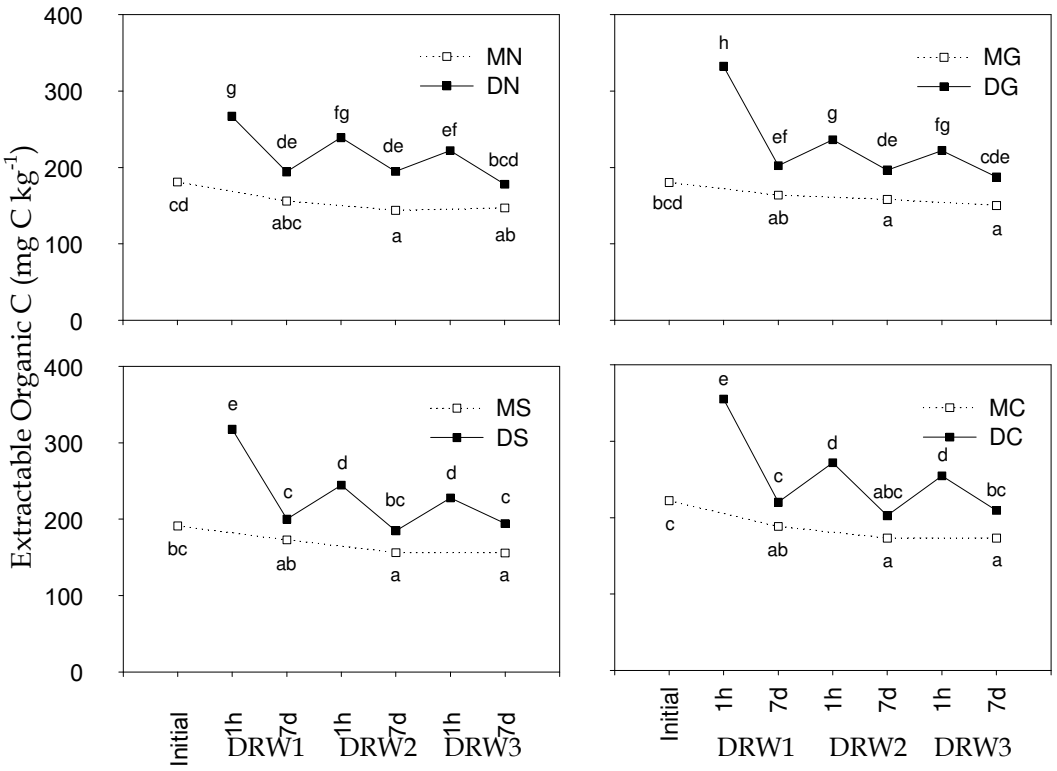

Figure 4.3: Extractable organic C (EOC) in moist (M) and DRW (D) soils previously amended with glucose (G), starch (S), cellulose (C) and non-amended (N) for soil extractions at the end of pre-incubation (initial) and at 1 h and 7 d after each of 3 DRW cycles. Letters indicate significant differences (P<0.05) between DRW treatments for each amended soil.

Table 4.2: Extractable organic C (EOC) degradability as indicated by specific UV absorbance (SUVA A250 nm/mg C ml^{-1}) in moist (M) and DRW (D) soils previously amended with glucose (G), starch (S), cellulose (C) and non-amended (N).

		N		G		S		C	
		Moist	DRW	Moist	DRW	Moist	DRW	Moist	DRW
Initial		0.0536		0.685		0.734		0.748	
DRW1	1h		0.550		0.547		0.574		0.454
	7d	0.617	0.755	0.755	0.904	0.817	0.914	0.871	0.738
DRW2	7d	0.672	0.751	0.785	0.935	0.896	0.990	0.942	0.800
DRW3	7d	1.076	0.760	1.133	0.683	1.306	1.058	1.190	1.175

4.3.3 Phosphorus availability

Resin P was different in each of the four soils (Figure 4.4). The moist incubated soils had average P_{resin} contents of 2.2, 12.6, 8.2 and 19 mg P kg^{-1} in soils amended with glucose, starch, cellulose and non-amended respectively. Generally P_{resin} in the moist incubated controls remained constant throughout the entire incubation period.

Increases in P_{resin} occurred immediately after rewetting in all treatments (Figure 4.4). The greatest absolute increases in P_{resin} were found in the non-amended soil where up to 7 mg P kg^{-1} was released immediately after rewetting. In soils amended with starch and cellulose the increases were around 5-6 mg P kg^{-1} soil. The glucose amended soil showed the least fluctuations in P_{resin} (2-3 mg P kg^{-1}) as a result of the DRW but were still significant given the low levels of resin P in this soil. In most cases any increase in the resin extractable P of these soils immediately after rewetting had disappeared following the week of moist incubation. However, in the glucose amended soil the reductions in P_{resin} during periods of moist incubation were smaller than in the other treatments. The overall effect of DRW in this soil was a P_{resin} of 6.4 mg P kg^{-1} which was 2.5 times higher than that of the moist control. In contrast to EOC, the amount of P_{resin} released following rewetting did not appear to decrease with subsequent DRW cycles.

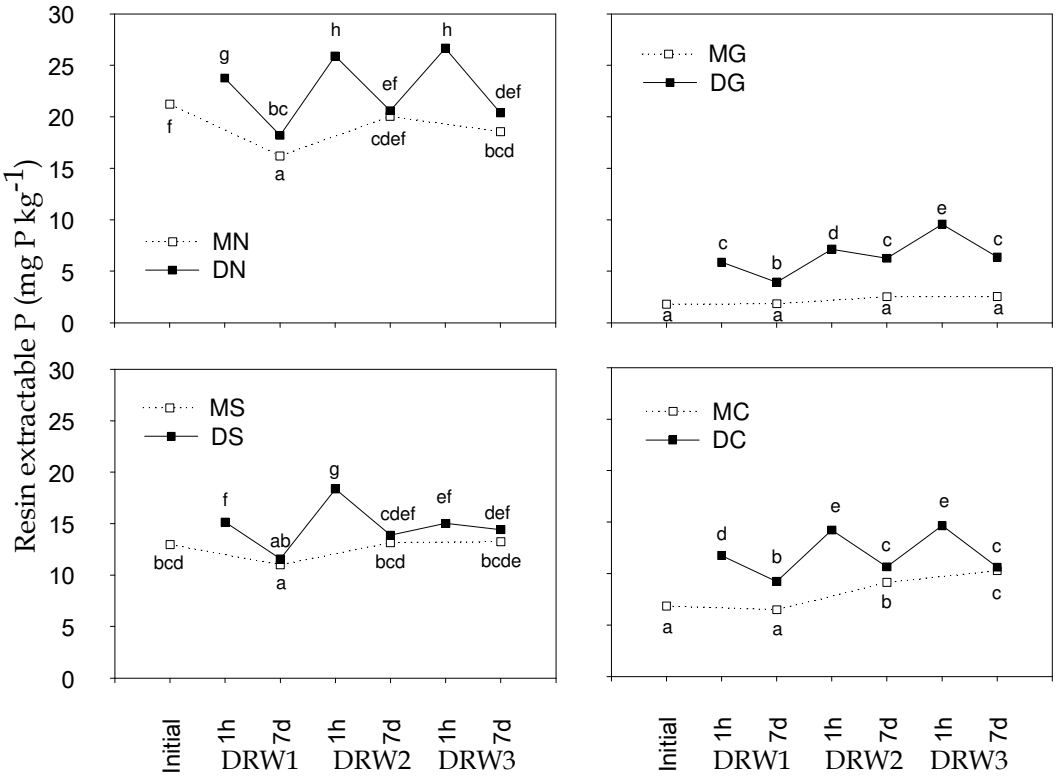

Figure 4.4: Resin extractable P (P_{resin}) in moist (M) and DRW (D) soils previously amended with glucose (G), starch (S), cellulose (C) and non-amended (N) for soil extractions at the end of pre-incubation (initial) and at 1 h and 7 d after each of 3 DRW cycles. Letters indicate significant differences (P<0.05) between DRW treatments for each amended soil.

Moist incubated soils showed a slow decrease in TDP (Figure 4.5). In DRW soils there was a significant increase in TDP following the first rewetting event (except the starch amended soil). After this point there was a general decrease in TDP until the third rewetting event. At the third rewetting event the increase in TDP in the starch and cellulose amended soils was not significant compared to the moist control. In the glucose amended soil the DRW treatment had significantly higher TDP levels compared to the moist control.

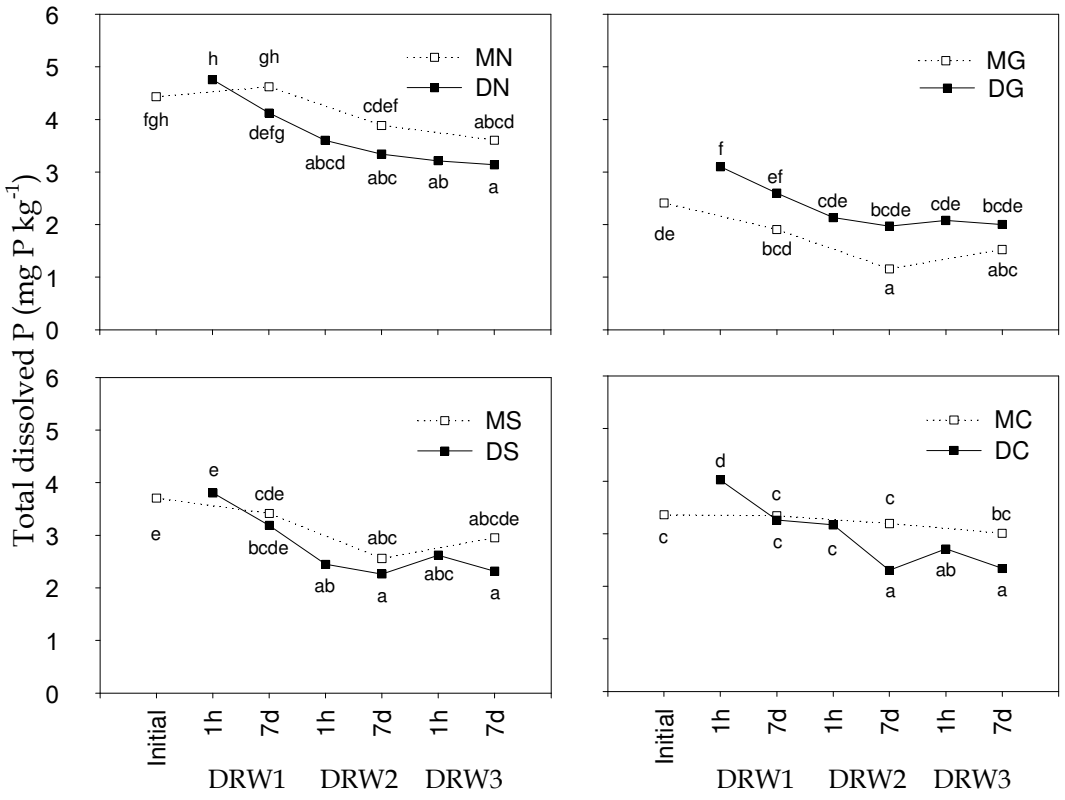

Figure 4.5: Total dissolved P (TDP) in moist (M) and DRW (D) soils previously amended with glucose (G), starch (S), cellulose (C) and non-amended (N) for soil extractions at the end of pre-incubation (initial) and at 1 h and 7 d after each of 3 DRW cycles. Letters indicate significant differences (P<0.05) between DRW treatments for each amended soil.

A high proportion of TDP (50-75%) was present in an organic form (DOP) (Figure 4.6). All treatments, except the starch amended soil showed significant increases in DOP immediately after the first rewetting event. No significant increase in DOP was seen in the second rewetting event in any treatment. The largest flush in DOP occurred in starch and cellulose amended and the unamended soils in the third DRW cycle.

Generally DOP was released at rewetting and decreased during periods of moist incubation. The patterns of DOP fluctuations were not as evident in the glucose amended soil. Hence, the glucose amended soil was the only one not to show a reduction in DOP as a result of the DRW. Furthermore, fluctuations in DOP show similar trends to EOC.

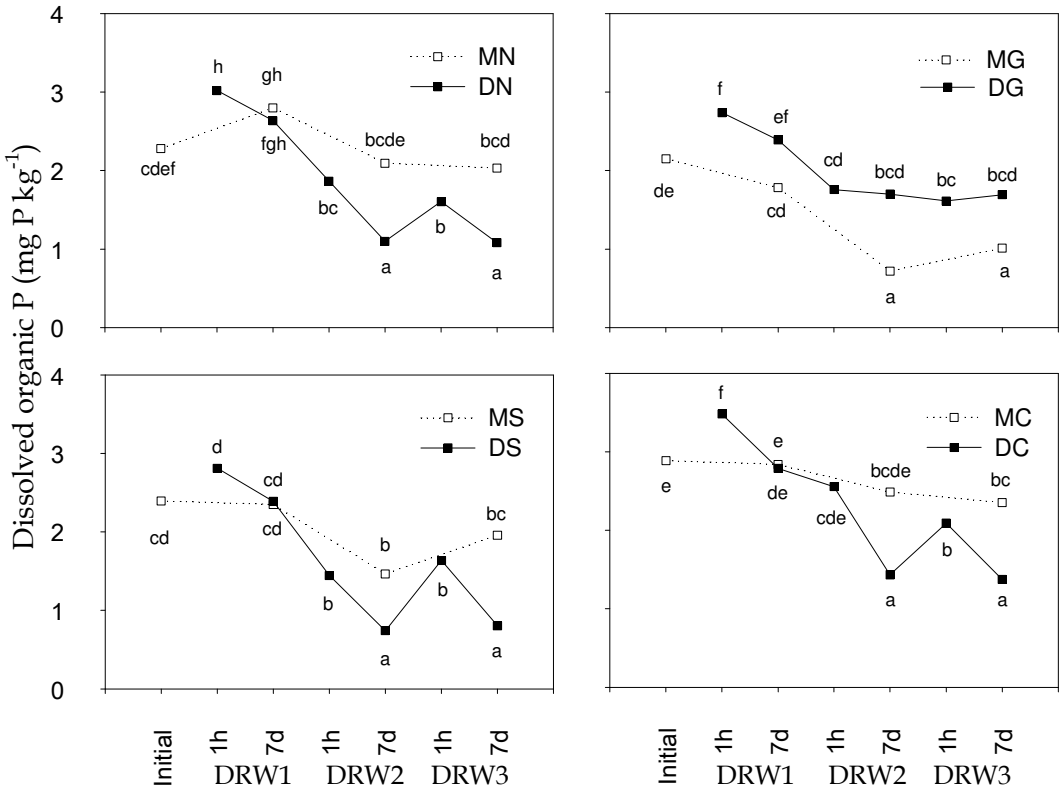

Figure 4.6: Dissolved organic P (DOP) in moist (M) and DRW (D) soils previously amended with glucose (G), starch (S), cellulose (C) and non-amended (N) for soil extractions at the end of pre-incubation (initial) and at 1 h and 7 d after each of 3 DRW cycles. Letters indicate significant differences (P<0.05) between DRW treatments for each amended soil.

4.3.4 Microbial biomass size and composition

The addition of carbon altered microbial biomass size as indicated by MBC and MBP (Figure 4.7 and Figure 4.8). Soil amended with glucose had significantly greater MBC (347 mg C kg^{-1}) than all other treatments. Microbial biomass C in soils amended with starch and cellulose was similar with mean MBC contents of 166 and 162 mg C kg^{-1} respectively being less than half of that present in the glucose amended soil. Not surprisingly, soils that received no additional carbon had the smallest microbial biomass with only 73 mg C kg^{-1}.

Microbial biomass P shows similar treatment differences as MBC (Figure 4.8). The glucose amended soil had the largest MBP with a mean of 24.2 mg P kg^{-1}. In the starch and cellulose amended soils MBP was similar with mean values of 10.7 and 10 mg P kg^{-1} respectively. In the non-amended soil MBP was very low (3.9 mg P kg^{-1}).

Moist incubated soils showed a slow gradual decline in MBC over the incubation period except in non-amended soil. The effect of DRW on the size of the microbial biomass was similar for both C and P. The first DRW event resulted in a large reduction of MBC and MBP indicating the death of a large proportion of the biomass, with the largest absolute reduction observed in the glucose amended soil. During periods of moist incubation there was some increase in the size of the biomass but never to the levels that were seen prior to the initial DRW event. The glucose amended soil did not show any increase in biomass during moist incubation. The second and third DRW cycles did not have such a large impact on the microbial biomass as the first cycle with only small decreases seen immediately after rewetting.

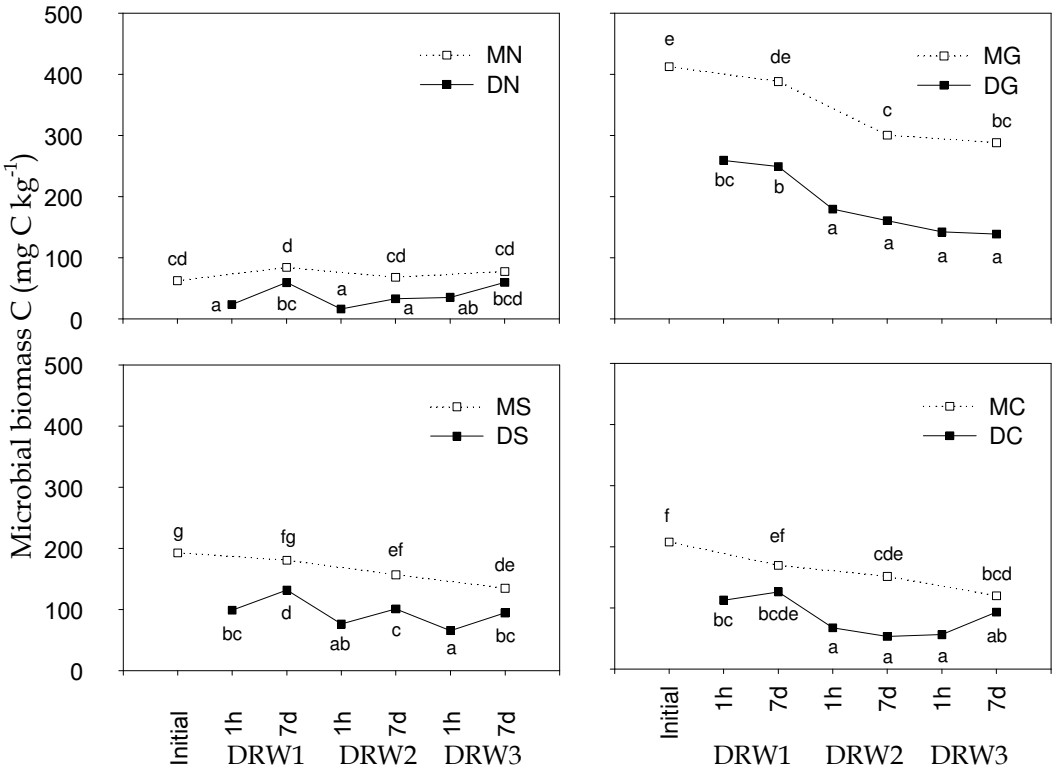

Figure 4.7: Microbial biomass C (MBC) in moist (M) and DRW (D) soils previously amended with glucose (G), starch (S), cellulose (C) and non-amended (N) for soil extractions at the end of pre-incubation (initial) and at 1 h and 7 d after each of 3 DRW cycles. Letters indicate significant differences (P<0.05) between DRW treatments for each amended soil.

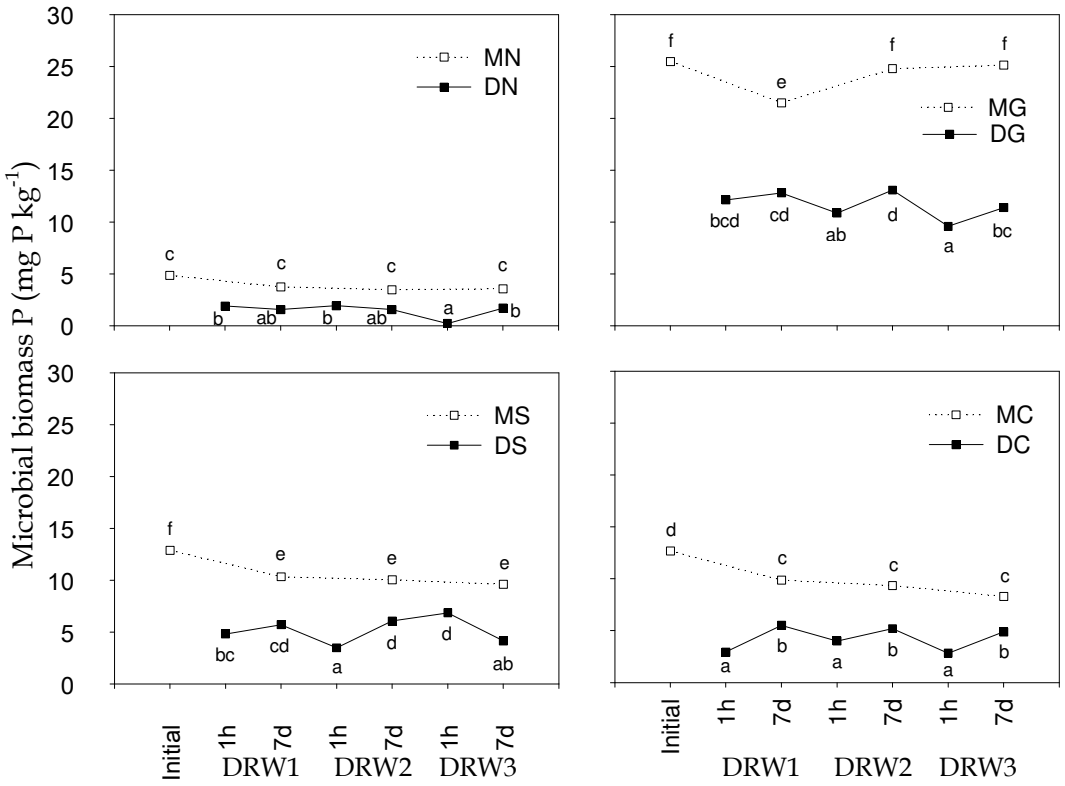

Figure 4.8: Microbial biomass P (MBP) in moist (M) and DRW (D) soils previously amended with glucose (G), starch (S), cellulose (C) and non-amended (N) for soil extractions at the end of pre-incubation (initial) and at 1 h and 7 d after each of 3 DRW cycles. Letters indicate significant differences (P<0.05) between DRW treatments for each amended soil.

Principal component analysis (PCA) of signature FAME showed that the addition of C changed the microbial community structure as amended soils were significantly different from the non-amended soil (Figure 4.9). The first principal component axis explained 85.1% of the variance in FAME profiles. Soils further to the right such as the glucose amended soil had a high proportion of fungal fatty acid (Figure 4.10). The non-amended soil to the left was dominated by fatty acids indicative for gram positive bacteria. FAME's commonly used to identify actinomycetes were not detected in these soils.

Principal component analysis showed clear changes in microbial community composition as a result of DRW. Soils amended with glucose and starch had the greatest changes in microbial community composition. The change was smaller but still significant in cellulose amended soil. The effect of DRW for each of these soils was a reduction in fungal fatty acid and an increase in signature fatty acids for G+ve bacteria. Unlike the other treatments, the cellulose amended soil showed a large shift along the principal component axis 2 after DRW and the shift was associated with a reduction in fatty acids indicative for G-ve bacteria. The community composition in the non-amended soil was unaffected by DRW (Figure 4.9).

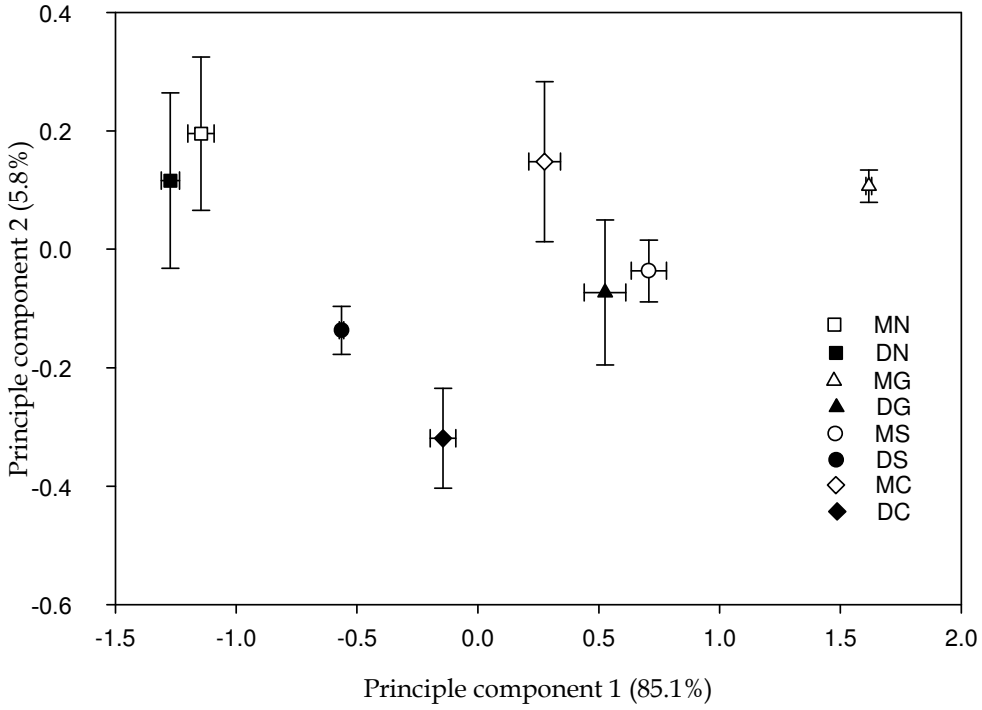

Figure 4.9: Principal component analysis of fatty acid methyl ester (FAME) profiles as indicator of microbial community composition within moist (M) and DRW (D) soils previously amended with glucose (G), starch (S), cellulose (C) and non-amended (N) at the end of the study. Bars indicate stand error of the mean.

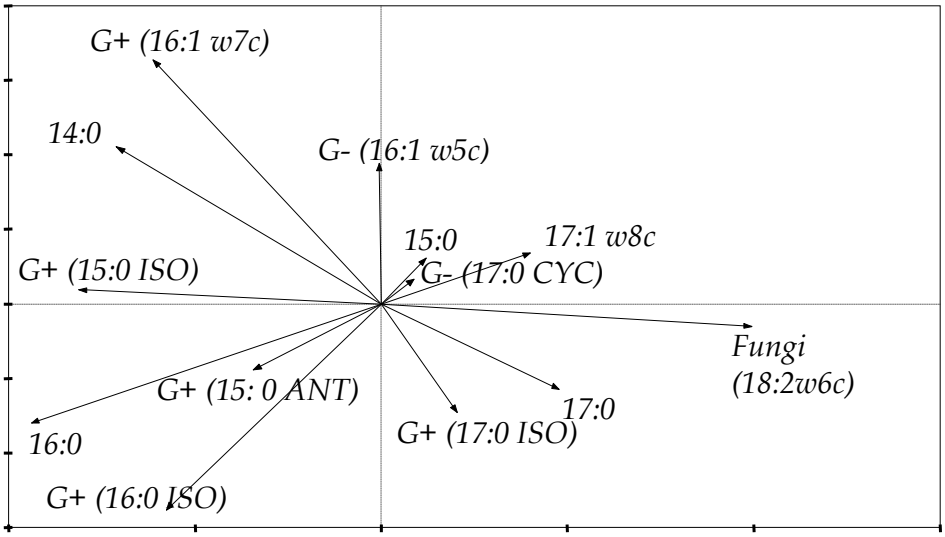

Figure 4.10: Vector plot of PCA showing signature fatty acid methyl ester (FAME) associated with fungi, gram positive (G+ve) and gram negative bacteria (G-ve).

Glucose addition significantly reduced richness and evenness indices (Table 4.3). Richness and evenness was significantly increased in glucose and starch amended soils as a result of DRW. At the end of the DRW cycles richness and evenness were not significantly different between all treatments.

Table 4.3: Richness and evenness of signature fatty acid methyl ester (FAME). Letters indicate significant differences for each parameter (P<0.05).

	Richness		Evenness	
	Control	DRW	Control	DRW
N	0.74bc	0.81bcd	2.01bc	2.18bcd
G	0.63a	0.77bcd	1.72a	2.07bcd
S	0.74b	0.83d	1.99b	2.25d
C	0.82cd	0.80bcd	2.23cd	2.16bcd

4.4 DISCUSSION

4.4.1 Carbon mineralisation

Rewetting dry soil increased cumulative respiration in soils amended with glucose and starch and the non-amended soil (Figure 4.2). Increased mineralisation as a result of DRW has been previously shown and is thought to result from increased turnover of the microbial biomass or the release and mineralisation of soil organic matter (Fierer and Schimel 2002; Wu and Brookes 2005). Drying/rewetting reduced cumulative respiration in the cellulose amended soil. This soil had much higher rates of basal respiration in the moist controls than all other soils, which have been suggested to be due to the higher proportion of G-ve bacteria which are known to be fast growing organisms (Harris 1981; Van Gestel *et al.* 1993). However, this does not appear to be the case in the current study. A reduction in cumulative respiration in DRW soils occurs when the respiration flush after rewetting is not large enough to compensate for the reduced activity during dry periods (Franzluebbers *et al.* 1994; Mikha *et al.* 2005).

Rewetting of dried soil resulted in an immediate flush of respiration activity which was between 2-10 times the basal rates in the moist controls, with large differences in the magnitude of this flush between the different soils. Changes in activity of soil microbes in response to fluctuations in soil water are well documented (Fierer and Schimel 2003; Franzluebbers 1999). The rewetting CO_2 flush seen in this study was much greater than those reported (Fierer and Schimel 2003; Lundquist *et al.* 1999a; Mikha *et al.* 2005; Wu and Brookes 2005) and is surprising given the higher levels of microbial biomass in the published studies (160 – 1400 mg C kg^{-1}) than the current study (20 – 400 mg C kg^{-1}). A higher susceptibility of the microbial biomass to DRW within the current study is discussed later (Section 4.4.3).

The flush in respiration activity seen after rewetting was associated with the release of EOC (Figure 4.3). Between 135 and 160 mg EOC kg^{-1} was released in the first rewetting event in the amended soils and equates to a 1.7 fold increase in EOC with the non-amended soil having a significantly smaller EOC increase. Similar increases in available C following rewetting of dry soil have been reported (Lundquist *et al.* 1999a).

Extractable organic C released at rewetting was mineralised during moist incubation indicated by a reduction in EOC availability. Concurrent with this EOC utilization was the increase in EOC aromaticity and a slow decline in microbial respiration activity. This suggested that soil microbes were utilising labile C compounds resulting in an increase in the proportion of more recalcitrant forms which could ultimately limit C availability during the moist period.

The level to which EOC was depleted at the end of the three moist incubation periods was similar within each soil (~200 mg C kg^{-1}). This remaining portion is likely to be less degradable. EOC is known to contain both labile and stable pools, with the stable components having longer turnover times than the incubation periods of this study (Cookson *et al.* 2005). Besides aromatic structures these stable components may contain alkyl compounds with double bonds which also make them more resistant to mineralisation (Kalbitz *et al.* 2003).

A reduction in the size of the respiration flush in subsequent DRW cycles was observed in all soils. The most likely cause of this was a reduction in C availability as less EOC was released with subsequent DRW cycles. The similarity in EOC degradability at the end of moist incubation periods, described above, further implied that a reduction in EOC supply rather than degradability was responsible for the decrease in cumulative respiration. Bottner (1985) showed that smaller respiration flushes may be partly caused by a reduction in the proportion of the biomass surviving with each DRW cycle. In this study most of the microbial biomass was killed in the first rewetting event (50 - 75%) and the size of the surviving microbial biomass was not proportional to the decrease in respiration activity. In addition to reduced C availability, changes in microbial community (discussed later) may also explain smaller rewetting CO_2 flushes.

The large differences in cumulative respiration between soils did not appear to be regulated by C supply. At any time after rewetting the amount of EOC released was very similar between the amended soils and was lower in the non-amended soil, yet these soils show marked differences in cumulative respiration. Furthermore, aromaticity of the EOC following each rewetting event was also similar between soils eliminating C degradability as a factor for the variation. Even when accounting for

differences in microbial biomass size after rewetting, normalised (mg CO_2-C mg MBC^{-1}) respiration rates were higher in the glucose amended soil after the first rewetting event and for the cellulose amended soil over the entire incubation period compared to starch and non-amended soils (data not shown). Hence differences in the ability of the microbial biomass to utilise C appeared to exist. Also, differences in C utilization are most likely much greater, since it is unlikely that all of the microbial biomass present was equally contributing to the respiration flush. The soil microbial communities within each soil were different (discussed later) which would contribute to differences in cumulative respiration between soils.

In this study the source of the rewetting C flush was not clear. The microbial biomass would seem the likely source of the available C released at rewetting. The microbial biomass has been suggested as the main source of available C during DRW (West *et al.* 1989; Wu and Brookes 2005). The largest reduction of the MBC occurred at the first rewetting event with large flushes in respiration and EOC. However, flushes in respiration and EOC occurred in subsequent DRW cycles with, in most cases, no significant change in MBC. Furthermore, the amount of EOC measured after rewetting was very similar between soils and yet these were undergoing very different changes in MBC. Microbial C potentially contributed between 17 - 29% and 26 - 46% in the first and second rewetting events respectively and much less in the third DRW. In this study the flush in respiration seen after rewetting did not appear to be simply the turnover of MBC and therefore appeared to involve increased availability and mineralisation of soil organic matter (Wu and Brookes 2005).

4.4.2 Phosphorus availability

MBP content of the soils was altered by the addition of the amendments. The glucose amended soil had a much larger pool of MBP (~25 mg P kg^{-1}) than the other treatments, with the non-amended soil having an extremely low MBP (<5 mg P kg^{-1}). P_{resin} within these soils was inversely proportional to MBP (R^2=0.82). The differences in these parameters between the soils imply that the addition of the C amendments resulted in immobilisation of P_{resin} into the microbial biomass.

Drying and rewetting resulted in significant fluctuations in available P in all soils. The largest flushes in P_{resin} were around 7 mg P kg^{-1} in the non-amended soil, representing a 35-40% increase compared to the constantly moist soil. Turner *et al.* (2002a) showed increases of up to 1.76 mg kg^{-1} water extractable P in Australian agricultural soils which constituted 45-545% increase in P. Contrary to respiration activity, the flushes in P_{resin} were generally smallest at the first rewetting event and increased at subsequent DRW events. Increases in P_{resin} following rewetting were short-term and in most cases had disappeared after a week of moist incubation. Only the glucose amended had a significantly higher P_{resin} at the end of the incubation in DRW compared to constantly moist soil. Consequently, changes in available P resulting from DRW appear transient, presumably as a result of competing microbial assimilation and sorption by soil surfaces.

Changes in MBP did not reflect the measured changes in P_{resin}. The largest flushes in P_{resin} were seen in the non-amended soil which had the smallest MBP. The small decrease in MBP seen in this soil could not explain the large flush of P_{resin} after the first rewetting event. Similarly this soil did not show significant changes in MBP in the subsequent rewetting events where the greatest changes of P_{resin} were measured. Furthermore, the largest reduction in MBP (~13 mg P kg^{-1}) following rewetting occurred in the glucose amended soil which only corresponded to an increase in P_{resin} of 5 mg P kg^{-1}. The lack of relationship between these pools indicated that the source of the P flush is non-biomass in origin, presumably from the solubilisation of organic matter and release of adsorbed P_i. This finding agrees with other studies that have shown increases in bicarbonate extractable P were due to increased solubility rather than the release from microbial biomass (Magid and Nielsen 1992; Turner and Haygarth 2003).

Total dissolved P constituted a smaller pool of the P within the soil than P_{resin} (TDP was 20-30% P_{resin}). In addition, despite the large fluctuations in both P_{resin} and MBP (up to 13 mg P kg^{-1}) at rewetting, changes in TDP were relatively small (< 1 mg P kg^{-1}). The dissolved P fraction was primarily in an organic form. Fluctuations in DOP as a result of DRW occurred, with the release of DOP into the soil solution immediately after rewetting and mineralisation during periods of moist incubation. However, since the amount of DOP was very small any increase in P_i from this mineralisation was not

detectable. The glucose amended soil did not show these clear patterns of DOP release and mineralisation. Fluctuations in DOP were more pronounced in the later DRW cycles and could occur for a number of reasons including increased enzyme activation or adaptability of the microbial community.

Although it appeared that the microbial biomass was not directly contributing to the rewetting flush, significant changes in MBP and MBC were seen during DRW. Particularly at the first rewetting event, the large changes in the size of MBP and MBC suggested that significant turnover of the biomass occurred, most likely from osmotic shock and cell lysis at rewetting (Kieft *et al.* 1987). It has been proposed that the rewetting nutrient flush can occur without cell lysis through the rapid remineralisation of internal organic osmoregulatory solutes that are accumulated during drying (Halverson *et al.* 2000). However, even though the fumigation-extraction techniques used to determine MBP and MBC in this study do not quantify individual cells, it is unlikely that the large changes in MBP and MBC could be achieved by just remineralisation of internal solutes. Internal organic osmoregulatory compounds have been shown to only comprise up to 15% of the total biomass (Kieft *et al.* 1987). Fierer and Schimel (2003) also support this theory and estimated that only 2-4% of MBC was mineralised following rewetting in oak and grassland soils. However, MBC and MBP were reduced by 40% and 52% respectively in the glucose amended soil during the first rewetting and appear to be too large to just represent mineralisation or transport of such compounds.

Furthermore, it has been shown that P released from the microbial biomass at rewetting is primarily P_o (Turner and Haygarth 2001). Assuming all of the MBP released in the first rewetting event is P_o this should have resulted in large increases in DOP. However, only small increases in DOP were observed and suggest that organic compounds released during microbial turnover were being stabilized within the soil. This could then explain why the glucose amended soil was the only soil to show a net increase in DOP due to DRW. In this soil the large release of P_o from the biomass during the first rewet could mean that high amounts of adsorbed P_o are able to buffer the soil solution in the subsequent DRW cycles. Hence, the large reductions in DOP during moist incubation after rewetting that were seen in the other soils did not occur.

4.4.3 Microbial biomass size and community composition

The glucose amended soil was dominated by fungi and the starch and cellulose amended soils were quite similar, with the cellulose having a higher proportion of G-ve bacteria. No indicator fatty acids for actinomycetes were detected. Glucose significantly reduced FAME richness and evenness which was anticipated as the objective was to favour particular groups of organisms. However, addition of the other C substrates did not result in changes to these taxonomic indices.

The initial rewetting event caused a reduction in the size of the microbial biomass as a result of DRW in all soils. However, subsequent rewetting events had little effect. Interestingly, the microbial biomass never returned to the levels seen prior to DRW even after 7 d moist incubation periods. Other studies have shown that MBC is able to recover from DRW in as little as 1.3 d (Wu and Brookes 2005). It is possible that the addition of C amendments and favourable incubation conditions during the 25 week pre-incubation resulted in a large active microbial biomass that was killed in the first rewetting event. Active fast-growing 'zymogenous' organisms are more susceptible to dry conditions than slow growing 'autochthonous' ones (Bottner 1985; Van Gestel *et al.* 1993). Fast-growing organisms are less physiologically capable of surviving the rewetting and therefore the surviving microbes are usually inactive or dormant fractions (Bottner 1985; Cortez 1989). After the first DRW event the biomass was composed of microbes that survived the initial shock and are potentially more resistant to the subsequent rewetting events.

DRW did not significantly change the FAME composition of the non-amended soil. The microbial community in this soil potentially reflected that of the indigenous state which may be more resilient to the DRW either because of the community composition or because the rate of turnover was closer to a steady state. In the amended soils the overall effect of DRW was a reduction in fungal fatty acid (18:2w6c) and a dominance of fatty acids from G+ve bacteria (i15:0; a15:0; i16:0). This finding agrees with (Hamer *et al.* 2007) who found a shift towards G+ve bacteria and an increase in bacteria:fungi ratio in forest soils when rewet after a 4 wk drying period. A higher tolerance of G+ve bacteria and fungi to changes in water potential is thought to occur due to the thickness, rigidity and permeability of their cell walls (Harris 1981). However, this

study showed that fungi were in fact reduced by DRW. A higher susceptibility of fungi than bacteria to DRW has been previously proposed as fungi are associated with larger pores that are unable to retain water as soil is dried and are also located on the external surfaces of aggregates (Denef *et al.* 2001a). DRW caused a significant increase in richness and evenness (Table 4.3) in glucose and starch amended soils. This is in contrast to Fierer *et al.* (2003) who proposed that reduced richness or diversity occurs after DRW due to perturbation favouring a portion of the microbial community. However, the increase in diversity seen in glucose and starch amended soils may have occurred if these soils were dominated by a single group of fast growing organisms and the DRW event reduced the size of this group.

Although the microbial community was altered by DRW it was difficult to establish if this was the explanation for reductions in the rewetting CO_2 flush or that subsequent rewetting events had much less effect on the microbial biomass. It is most likely that other mechanisms also occurred such as changes in the physiological state of microbial cells which would also decrease their susceptibility to DRW (Mikha *et al.* 2005).

4.5 CONCLUSIONS

Microbial biomass size and composition greatly altered the response of the soils to DRW, with large differences in total respiration between soils. However, increases in EOC at rewetting and EOC degradability were similar between soils and suggested a disparate capacity of the microbial communities to utilise the available C. Smaller respiration flushes with subsequent rewetting events were observed concomitant with a reduction in the release of EOC. The dissimilarity between the amounts of EOC released, P_{resin} and reductions in microbial biomass suggest that the source of the rewetting flush was non-biomass in origin and that organic compounds released via microbial death were possibly undergoing sorption reactions with soil surfaces. Although highly variable, MBC only contributed up to 46 % of the total C mineralised during a DRW event. Significant flushes in available P occurred after rewetting and unlike the respiration flush and C availability, did not reduce with subsequent rewetting events. A reduction in the CO_2 flush and increased stability of the microbial biomass with subsequent rewetting events was potentially due to shifts in the

microbial community composition combined with changes in cell physiology. The stability of the microbial community in the non-amended soil to DRW stress potentially highlighted the robust nature of the indigenous microbial fauna to changes in water potential. This study showed that in the same soil matrix, differences in size and composition of microbial biomass will influence the response of a soil to DRW. Further examination of how soil water regime affects soil microbial communities and their function is required, particularly if indigenous microbes of Australian soils are adapted to resist fluctuations in soil water availability that are commonly experienced. In addition, since P flushes were transient, further examination of the amount of P released immediately after rewetting and short-term changes in P (due to microbial assimilation and soil sorption) is required to determine if these are important for agricultural production.

Chapter 5

Short-term fluctuations in respiration activity and phosphorus, nitrogen and carbon immediately after rewetting

Chapter 5. Short-term fluctuations in respiration activity and phosphorus, nitrogen and carbon immediately after rewetting

5.1 INTRODUCTION

Drying and rewetting (DRW) of soil stimulates organic matter decomposition producing a rapid flush in CO_2 and available carbon (C), nitrogen (N) and phosphorus (P). Rewetting increases the availability of labile organic compounds from living and non-living sources which are rapidly mineralised by surviving soil microbes, producing the flush in available nutrients. Most studies which have examined the influence of DRW on nutrient cycling have focussed on C (Degens and Sparling 1995; Franzluebbers *et al.* 2000; Mikha *et al.* 2005; Miller *et al.* 2005; Wu and Brookes 2005) and N (Bottner 1985; Kruse *et al.* 2004; Murphy *et al.* 1998), however few have considered P (Chepkwony *et al.* 2001; Grierson *et al.* 1998; McNeill *et al.* 1998; Nguyen and Marschner 2005; Turner and Haygarth 2001).

In most cases, DRW studies examine changes in C, N and P at daily or weekly intervals. Although elevated levels of microbial mineralisation may extend for these periods, most of the activity occurs within the first 2 days (Fierer and Schimel 2003; Kieft *et al.* 1987; Steenwerth *et al.* 2005) (Chapters 3 and 4). Particularly, in soils which have not received recent organic matter additions or those with low SOM contents, CO_2 and nutrient flushes after DRW peak and decline very rapidly. Steenwerth *et al.* (2005) showed that the flush in respiration activity occurred in the first 6 hours following rewetting of a grassland soil. Closer examination of the period immediately after rewetting is needed to determine the relationship between these CO_2 flushes and C, N and P availability. Knowledge of the magnitude and duration of nutrient flushes after rewetting events is imperative to ascertain the importance of these in production agriculture and nutrient cycling.

Since P is very stable or insoluble, competing sorption reactions and microbial immobilization will, in most soils, result in the rapid removal of P that is released into the soil solution at rewetting. Flushes in resin extractable P (P_{resin}) and dissolved organic P (DOP) have been observed in previous experiments (Chapter 3 and 4). However, the use of single-point extractions (as used for DOP) to quantify soluble P

pools only provides a snap-shot of the actual P availability. Anion exchange resins provide a continual P sink. Therefore, measurements made using these may better represent actual P fluxes after DRW. In addition, these membranes remove P from the soil solution via exchange reactions and therefore more closely simulate P removal by plant roots and micro-organisms than do chemical extractants.

The experiment described in this chapter aimed to develop higher resolution curves for respiration (greater than 6 h intervals used previously) and determine the suitability of a modified anion exchange resin method for determining short-term changes in P. It was hypothesised that short-term respiration flushes are related to P_{resin}, DOP, EOC and TDN availability.

5.2 METHODS

5.2.1 Soil incubation

An incubation study was undertaken to investigate short-term flushes in microbial respiration and available nutrients after a single DRW event. Soil collected from Wagga Wagga (147°20'E, 35°05'S) (Chapter 4.2) was packed into soil cores (Chapter 2.1) which were either incubated with or without added C to represent an 'active' and 'stable' microbial biomass. Carbon was added as glucose (BDH Chemicals) solution at a rate of 2.5 g C kg^{-1}. All soil cores were then adjusted to 70% WFPS and pre-incubated for 10 d at 25°C. In total 120 cores were incubated consisting of 2 C treatments (with or without glucose), 2 DRW treatments (DRW/constantly moist) and 10 sampling times with 3 replicates.

5.2.2 Drying and rewetting

At the end of the pre-incubation period, sets of each soil were kept either constantly moist (70% WFPS) or dried and rewet. Drying was achieved by placing soil cores in sealed plastic containers containing self-indicating silica gel (BDH Chemicals). To ensure rapid drying, silica was exchanged daily for the first few days with a second quantity that was regenerated at 110°C overnight. The soils were dried to ~3% gravimetric water content over a 7 d period. At rewetting, water was added drop-wise

in a circular motion out from the centre of the core to a final gravimetric water content equating to 70% WFPS. Immediately after rewetting cores were transferred into individual chambers (Chapter 2.1) and incubated at 25°C. Incubation chambers were sampled at 2, 4, 6, 8, 11, 15, 19, 26, 33 and 49 h following rewetting.

5.2.3 Flush in microbial respiration

At each sampling, microbial respiration was determined by quantifying headspace CO_2 concentration within each chamber using a Servomex 1450 infra-red gas analyser (Servomex, UK) (Chapter 2.1). Total microbial respiration (Cumulative CO_2) data was fitted to C mineralisation models as previously described (Chapter 3.2.7). Respiration rates for each sampling interval were calculated as the difference between subsequent measurements. Controls without soil were included throughout the incubation.

5.2.4 Flush in available nutrients

After gas analyses, soil cores were removed from chambers for nutrient analyses. Soil (10g) was extracted with 40mL 0.5M K_2SO_4 by shaking end-over-end for 1 h and then centrifuging at 2000 RPM for 5 min. Supernatants were vacuum filtered through Whatman #42. Dissolved inorganic P (DIP) in filtered extracts was determined using malachite green (Ohno and Zibilske 1991) with a 30 min colour development period (Chapter 2.7). The remaining solution was stored frozen and later analysed for extractable organic carbon (EOC) (Chapter 2.5), total dissolved nitrogen (TDN) (Chapter 2.6), total dissolved P (TDP) and dissolved organic P (DOP) as previously described (Chapter 2.8).

The P_{resin} method of Kouno et al. (1995) was modified to determine short-term fluxes of P. The size and preparation of the anion exchange resins (BDH #55164) were as previously described (Kouno et al. 1995) except tubes containing 2 g soil and 30 ml of RO water were shaken horizontally for 1 h with 2 anion exchange resins per tube. Resins were then removed, rinsed free of soil with RO water and transferred to individual clean tubes. Resins were eluted with 30 ml 0.1 M NaCl/HCl by shaking horizontally for 2 h. The concentration of P in the eluate was determined colorimetrically (Murphy and Riley 1962).

Two resin strips were used in this experiment as previous tests had shown that 2, 3 or 4 strips per tube with a 1 h extraction did not significantly change the amount of P measured (Appendix 5.1). Therefore movement of P from the soil into solution and from the soil solution to the membrane was the limiting factor and apparently not the capacity of the strips to bind the P. Standard extractions (1 resin for 16 h) were performed at two sampling times (8 and 33 h) to check the consistency between the standard and modified extraction techniques. The same linear relationship between the 1 h and 16 h shaking times was always observed (data not shown).

5.2.5 Statistical analyses

Lines of regression were fitted using Sigma Plot v 9.0 (Systat Software Inc.). Three-way analysis of variance (ANOVA) was used to determine the effects of DRW x time x glucose addition on soil chemical properties using GENSTAT 8th Edition (Lawes Agricultural Trust). Significant differences ($P<0.05$) between means were tested using least significant difference (LSD) analyses (n=3). Outputs of carbon mineralisation models were subject to 2-way ANOVA with time and glucose addition as factors and significance between means was tested using a post-hoc Tukey Test ($P<0.05$, n=3).

5.3 RESULTS

5.3.1 Respiration rate

DRW resulted in a large increase in respiration rate (Figure 5.1). In the first hour after rewetting, respiration rates in the soil with glucose were 4.6 times higher after DRW than the moist incubated control. Smaller increases in respiration rate occurred in the soil without glucose where the respiration rate was 2.7 times higher in the DRW soil compared to the moist incubated control. For both treatments the respiration rates were highest in the first hour and decreased exponentially. The decline in respiration rate appeared to stabilize after 12 h in the treatment without glucose and approximately 24 h in the soil with glucose. Respiration rates were more stable after these times. However, in both soils the respiration rates in the DRW treatment remained significantly higher than the moist soil until 49 h.

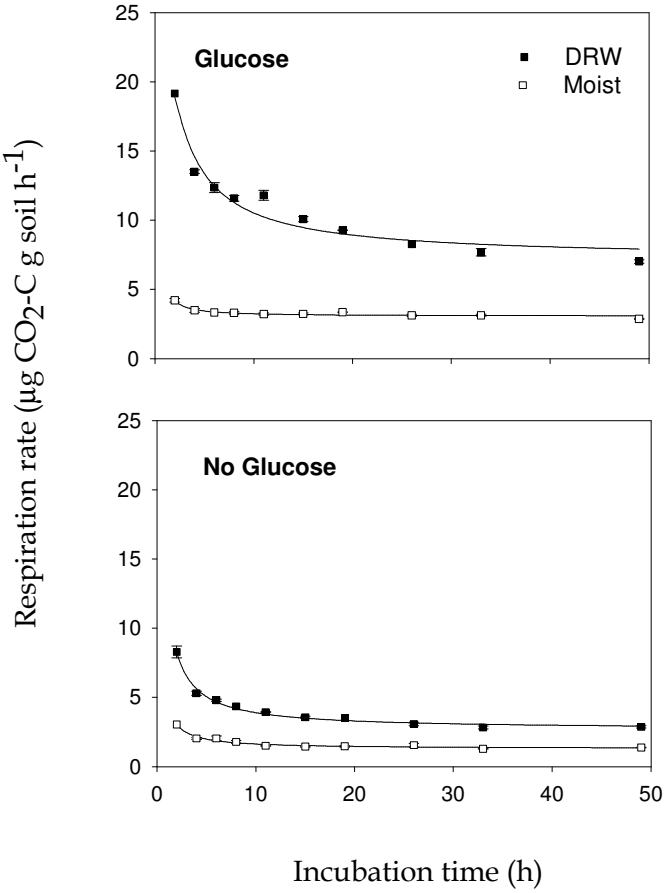

Figure 5.1: Respiration rate in soils subject to DRW (black squares) and moist incubated controls (white squares) and incubated with (top) and without (bottom) glucose. LSD = 0.407 (n=3).

The addition of glucose significantly increased basal cumulative C mineralisation (C_{min}) in the moist-incubated soil and was twice as high as the soil with no added glucose (Figure 5.2). The increase of C_{min} in the moist incubated soils was very stable and appears almost linear. In both soils DRW significantly increased C_{min} compared to the moist controls. The DRW soils with and without added glucose had total C_{min} of 0.343 and 0.140 mg CO_2-C g soil^{-1} respectively. In the soil with glucose, the increase in C_{min} after DRW was 2.4 times the moist incubated control. The soil without glucose showed an increase in C_{min} of 2.1 times after DRW compared to the moist incubated control. The effect of DRW on C_{min} was still evident at 49 h and it appeared as though elevated C_{min} would continue for some time.

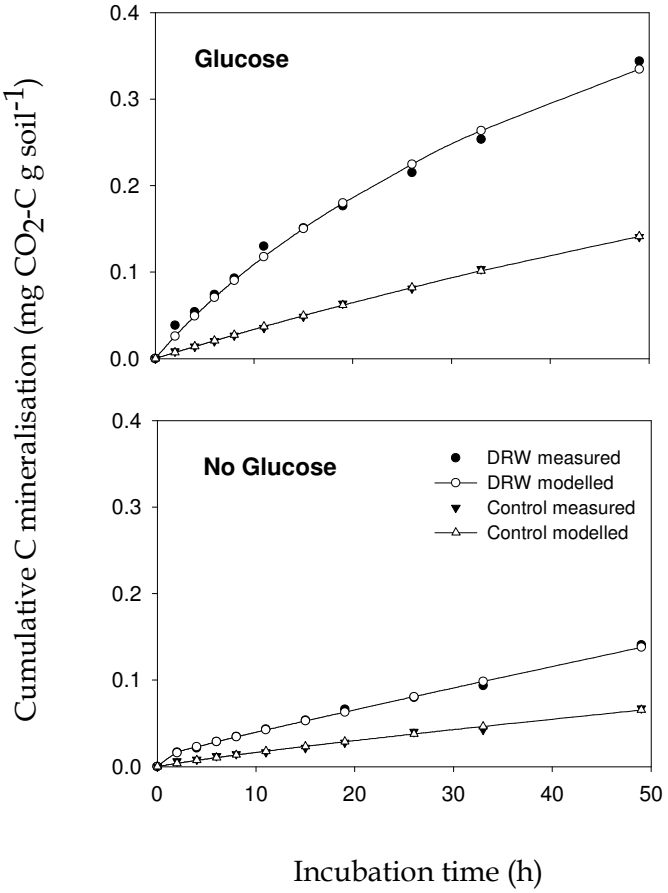

Figure 5.2: Cumulative respiration for measured (black) and modelled (white) data in soil subjected to DRW (circles) and constantly moist (triangles) controls and incubated with (top) and without (bottom) glucose. LSD = 0.007 (n=3).

The one-pool model provided a statistically better fit of cumulative C_{min} data than the two-pool model. C_{min} was not extrapolated beyond the study period. Calculations of C_{min} at the end of the study period (49 h) were determined using the model and are denoted as Co_{49h}. Model output indicates that mineralisable C fraction (Co_{49h}) was significantly increased by DRW and the addition of glucose (Table 5.1). However, the proportional mineralisation rate constant (k) was not different between moist incubated soils with and without glucose. DRW significantly increased k compared to moist incubated soils and the increase in k by DRW was significantly greater in the soil incubated with glucose. Therefore DRW increased C_{min} rate (k) and total C_{min} (Co_{49h}).

Table 5.1: Predicted mineralisable C fraction (Co_{49h}) and proportional mineralisation rate constant (k) of one-pool C mineralisation model fitting (mg CO_2-C g soil^{-1}) in soil subjected to DRW and constantly moist and incubated with and without glucose. Letters indicate significant differences (P<0.05) for each parameter.

Soil	DRW	Co_{49h} (mg CO_2-C g soil^{-1})	k (day^{-1})
Glucose	Moist	0.140b	0.0084a
Glucose	DRW	0.344c	0.0269c
No Glucose	Moist	0.067a	0.0074a
No Glucose	DRW	0.141b	0.0171b

5.3.2 Nutrient availability

DRW resulted in the release of EOC (Figure 5.3). Adding glucose before pre-incubation had no effect on EOC at DRW as the amounts of EOC in moist incubated soils was not significantly different. The increase in EOC in the DRW soils remained higher than the moist controls over the entire incubation period. Therefore DRW resulted in an increase in C availability that extends beyond the incubation period.

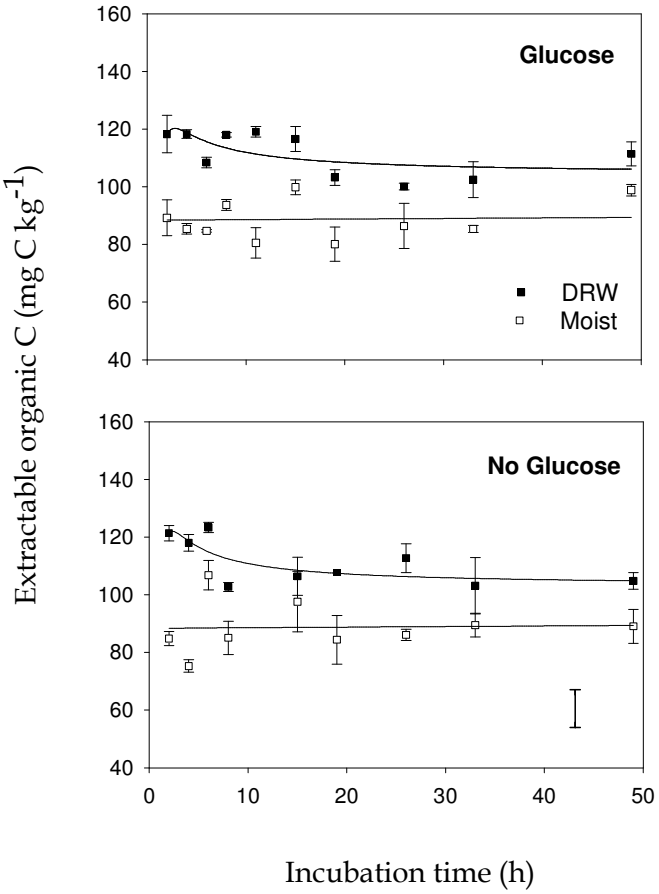

Figure 5.3: Extractable organic C (EOC) in soils subject to DRW (black squares) and moist incubated controls (white squares) and incubated with (top) and without (bottom) glucose. Bars indicate standard error of the mean. LSD = 13.02 (n=3).

Unlike EOC, DON was significantly affected by the addition of the glucose. The soil with added glucose had a mean DON of only 37.1 mg N kg^{-1} which was significantly smaller than the soil without glucose which had a mean DON of 133.4 mg N kg^{-1}. However, DON was less affected by DRW than the addition of the glucose. Drying/rewetting decreased DON slightly but this was not significant compared to moist controls. Nitrogen levels were very stable throughout the incubation period, indicating that there was no net mineralisation of DON.

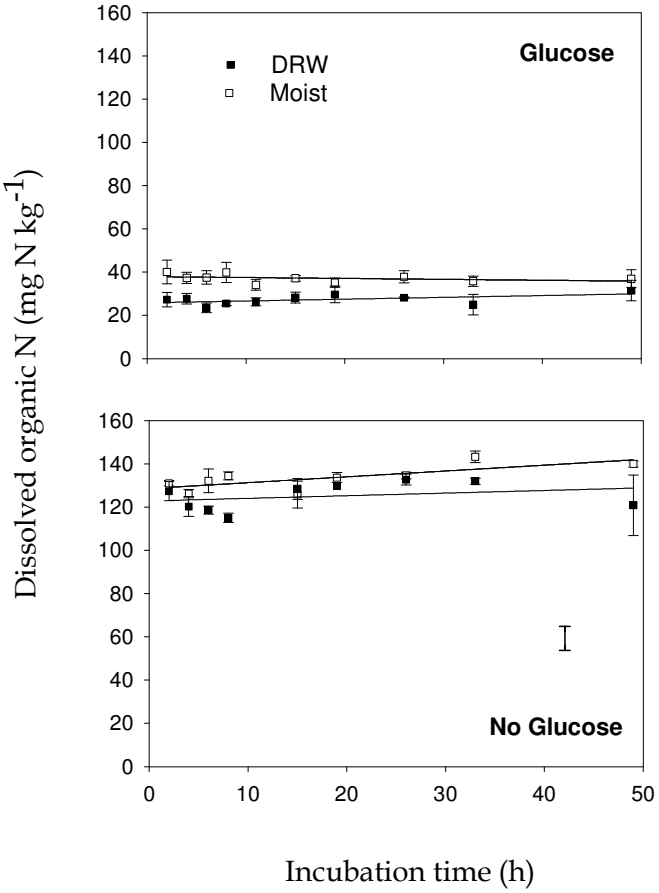

Figure 5.4: Dissolved organic N (DON) in soils subject to DRW (black squares) and moist incubated controls (white squares) and incubated with (top) and without (bottom) glucose. Bars indicate standard error of the mean. LSD = 11.14 (n=3).

Compared to soil without glucose, P_{resin} was reduced by the addition of glucose by 3 mg P kg^{-1} at the end of the pre-incubation (Figure 5.5). In both soils the moist incubated controls showed gradual increases in P_{resin} over the incubation period. Drying/rewetting significantly increased P_{resin} compared to the moist incubated controls by 73% in the soil with glucose and by 85% in the soil without glucose. Unlike the moist controls, P_{resin} in the DRW soils did not change over the incubation period and after 49 h was at the same levels as the moist controls. Therefore the effect of DRW on P availability appeared to be transient.

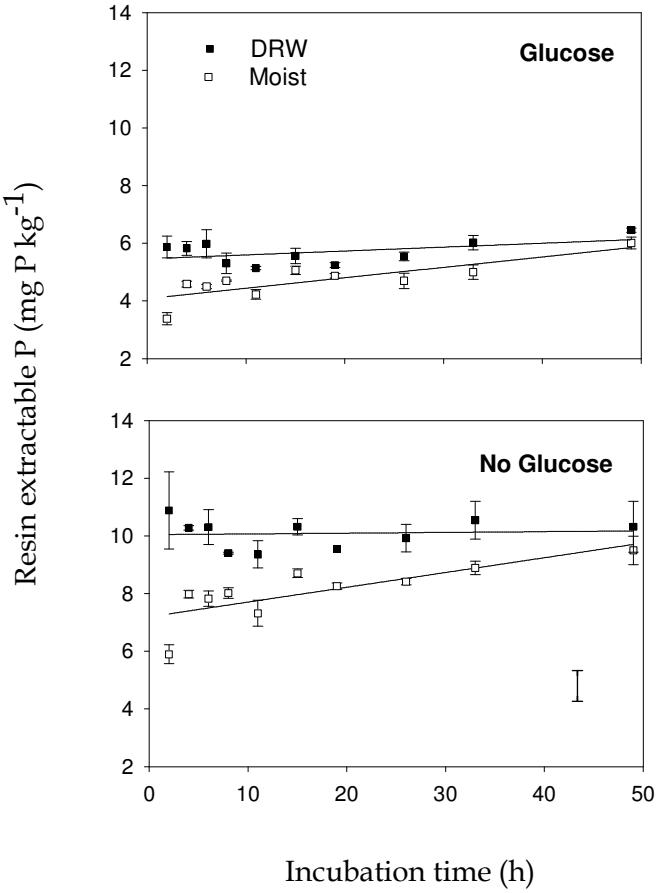

Figure 5.5: Resin extractable P (P_{resin}) in soils subject to DRW (black squares) and moist incubated controls (white squares) and incubated with (top) and without (bottom) glucose. Bars indicate standard error of the mean. LSD = 1.10 (n=3).

Despite the differences in P_{resin} between soils with and without glucose, DIP in solution was not affected by the glucose addition (Figure 5.6). However, DRW significantly increased DIP, with mean DIP concentrations in the moist controls of 0.8 mg P kg^{-1} compared to 2.1 mg P kg^{-1} in the DRW soils. DRW had therefore more than doubled DIP concentrations. The DIP pool was very stable over the incubation period.

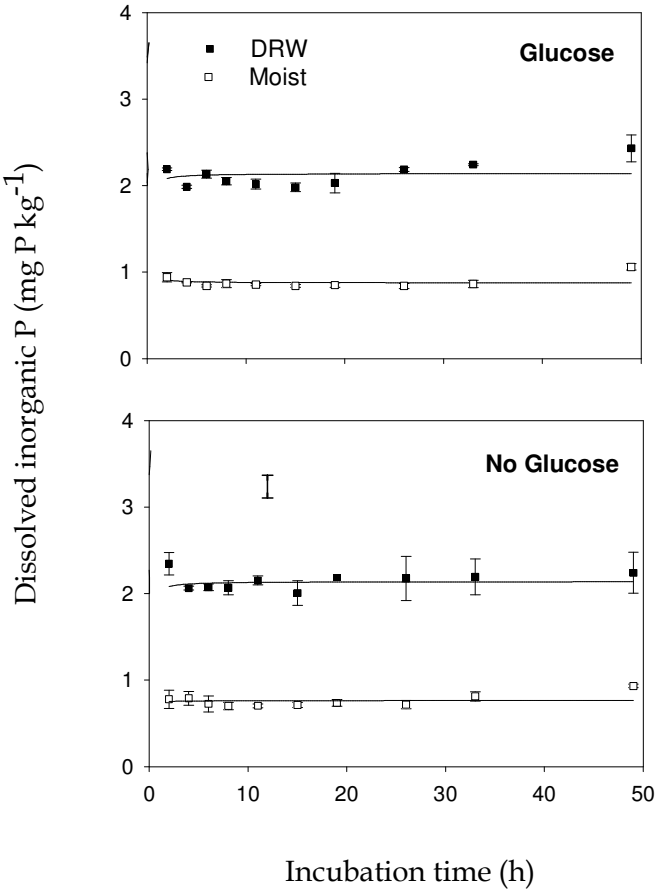

Figure 5.6: Dissolved inorganic P (DIP) in soils subject to DRW (black squares) and moist incubated controls (white squares) and incubated with (top) and without (bottom) glucose. Bars indicate standard error of the mean. LSD = 0.25 (n=3).

Dissolved organic P was a much smaller P pool in the soil solution, being less than half that of DIP (Figure 5.7). Similar to DIP, the DOP concentration was not significantly altered by the addition of glucose. DRW reduced DOP in the soil solution. Together with the increase in DIP this suggested that mineralisation of this P pool was occurring at rewetting. However, the differences in DOP between DRW and moist incubated soils were small (sometimes not significant) and therefore the increase in DIP cannot be explained by a decrease in DOP alone.

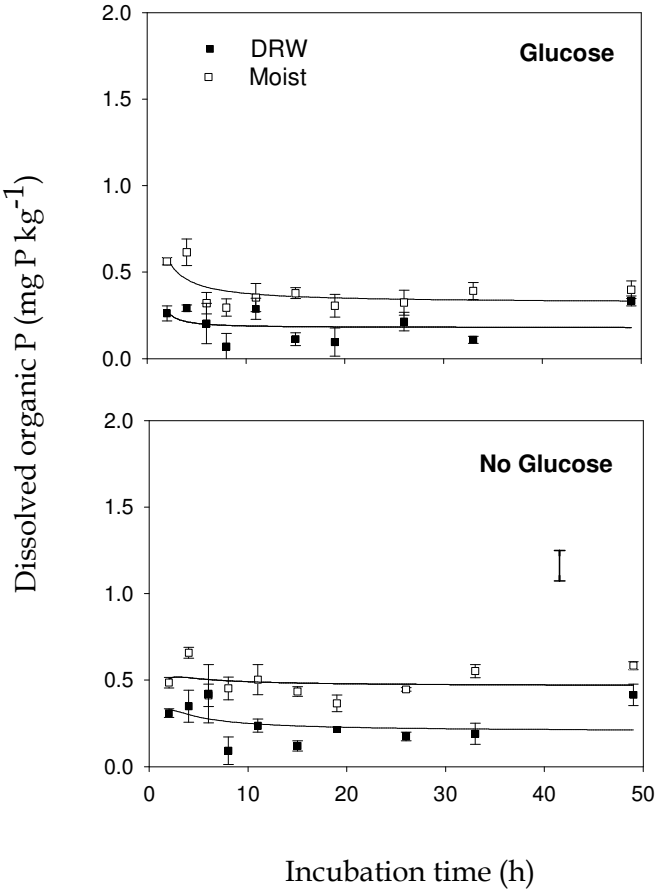

Figure 5.7: Dissolved organic P (DOP) in soils subject to DRW (black squares) and moist incubated controls (white squares) and incubated with (top) and without (bottom) glucose. Bars indicate standard error of the mean. LSD = 0.175 (n=3).

5.4 DISCUSSION

5.4.1 DRW and carbon mineralisation

Large differences in basal respiration of most incubated soils with and without glucose were observed (Figure 5.1). These differences in microbial activity between the soils possibly reflect a larger and more active biomass in the soil incubated with glucose (Figure 5.1 and Figure 5.2). Although no attempt was made to quantify microbial biomass size in this experiment, it is likely that the addition of the glucose increased the microbial biomass (Chapter 4). Furthermore, the large reduction in DON (Figure 5.4) and P_{resin} (Figure 5.5) in the soil incubated with glucose suggested microbial immobilization of these nutrients with the increase in C availability from the glucose.

Increases in respiration rates following DRW occurred immediately (Figure 5.1) and were associated with an increase in EOC availability (Figure 5.3). The larger rewetting respiration flush in the soil incubated with glucose could be due to either a greater microbial biomass size. This suggests the importance of the size and composition of microbial communities at the time of rewetting in determining the rewetting flush. Interestingly, the differences in rewetting respiration flush did not appear to be related to EOC supply, since the amount of EOC released at rewetting and it's utilisation during the incubation were the same for each soil (Figure 5.3). Although respiration rates decreased exponentially, the DRW soils always had significantly higher respiration rates than the constantly moist soils (Figure 5.1). Therefore the effect of DRW on microbial activity most likely extends for much longer than the incubation period used in this study. Increases in respiration activity following DRW in previous incubation studies were between 1.5 and 4 d (Chapter 3) and for more than 7 d in soils with a large microbial biomass (Chapter 4).

The rapid decline in respiration rates following rewetting highlighted the importance of examining short-term responses to rewetting. It appeared that no lag in microbial response after rewetting had occurred. Hence, actual respiration rates in the current study could have reached even higher levels than those measured at 2 h. For example, average respiration rates in the DRW soil incubated with glucose could have potentially reached more than 25 μg CO_2-C g soil h^{-1} in the first hour and would have been only 12.3 and 11.7 μg CO_2-C g soil h^{-1} if measurements were taken at 6 h or 11 h respectively. Obviously, the extremely rapid and large rewetting flushes that have occurred would have been completely overlooked. However, even the 2 h time frame in the current study may be grossly generalising the actual fluxes that occur. Recent studies have shown that microbial utilisation of simple sugars and amino acids takes place very rapidly after addition with maximal respiration rates after glucose addition occurring between 10 and 30 min (Jones and Murphy 2007). However, the response time in the study of Jones and Murphy (2007) may not occur after DRW since not all C compounds released during DRW will be as labile as simple sugars and amino acids. Also, some soil organic matter components derived from plants, such as lignin, breakdown extremely slowly (Baldock 2007). In the current study it is possible that the EOC was less labile (not contributing to respiration flush) or that highly labile

components had already been depleted prior to the first measurement, since the amount of EOC (Figure 5.3) at 2 h did not explain the large differences in respiration flush between soils incubated with and without glucose. The respiration response of a native soil without C addition would therefore reflect the relative contribution of highly labile C compounds and more stable C compounds that may be released from the soil or plant residues.

Cumulative C_{min} was higher in the soil incubated with glucose and was increased by DRW (Figure 5.2). C_{min} in the soil with glucose was starting to stabilise, indicating that this soil had reached maximal C_{min} rate. In the soil that did not receive glucose, C_{min} was still almost linear at 49 h. Modelling cumulative C_{min} data shows that the addition of glucose significantly increased the mineralisable C fraction (Co_{49h}) (Table 5.1). This is likely to represent a higher microbial biomass C as discussed earlier as EOC was not different between soils. Drying/rewetting increased the size of Co_{49h} and EOC. Therefore EOC may be contributing to the labile C pool at rewetting. However, other studies show that components of EOC can be poorly degradable by microbes (Kalbitz *et al.* 2003). Furthermore, since DRW significantly increased Co_{49h} and mineralisation rate (k), DRW released additional labile C substrate, although the source of this was not clear. The fact that k was significantly higher in the DRW soil with glucose is presumably due to a larger microbial biomass.

5.4.2 DRW and nutrient availability

Incubation of soil with glucose resulted in a reduction of DON (Figure 5.4) and also P_{resin} (Figure 5.5). Increased C availability would have induced microbial demand for N and P and thus resulted in mineralisation of DON and uptake of N and P. Dissolved inorganic P and DOP fractions were not significantly altered by glucose addition which was unexpected given such a large decrease in DON (90 mg kg^{-1}). Soil microbes have N:P ratio of 7 (Cleveland and Liptzin 2007). However, given the large decrease in N shown above, it was surprising that P_{resin} was only reduced by 3 mg P kg^{-1} after incubation with glucose as this would suggest an N:P ratio of 30. Resin extractable P may under-estimate microbial P uptake as concurrent P release and immobilisation could be occurring and thus P never reaches the available pool.

Drying/rewetting induced rapid mineralisation of P at rewetting as indicated by increases in P_{resin} and DIP and a reduction in DOP. The larger flush of P_{resin} in the soil without glucose indicated reduced immobilisation of P, possibly due to a smaller microbial biomass in this soil. Interestingly, any changes in DON and soluble P fractions (DIP and DOP) occurred prior to the first measurements at some point in the first 2 h after rewetting, and these nutrient pools remained unchanged for the rest of the incubation. The static nature of these nutrient pools may indicate low nutrient demand by soil microbes and is concomitant with lower microbial activity during these periods. Similarly, the higher P_{resin} induced by rewetting did not change over time. The slow increase in P_{resin} in the moist incubated controls was difficult to explain and indicated either mineralisation of soil P, release of P from microbial cells, or to a lesser extent, changes in physiochemical equilibria. The lack of utilisation of soluble and labile nutrients in the moist soils was indicative of microbial C limitation.

5.5 CONCLUSIONS

The current study demonstrated that the large flushes in C_{min} that occur after rewetting a dry soil are due to increased mineralisation rate of microbes and not an increase in mineralisable C. The rapid flush in C_{min} following rewetting, followed by elevated but steadier rates of C_{min}, suggested distinct phases in microbial activity. Further examination of C_{min} in the initial 2 h period at higher resolution is required to better understand these fluxes. Similarly, flushes in available nutrients occurred within 2 h and were quite stable after this point. Therefore, the extent of the nutrient flushes within the initial 2 h could not be quantified. While determining absolute changes in nutrients at smaller time scales may prove difficult, inferences can be made from flushes in microbial activity (C_{min}) as this parameter represents an integration of all soil processes (physical, chemical and biological). The current study showed that DRW substantially increased DIP which would potentially be available for plant uptake or could move in soil solution. In both soils it was evident that the DRW flush would extend for much longer periods than those used in this study. While the anion exchange resins are sufficient for quantifying changes in P availability using the intervals used here (2 h), the method may not have the sensitivity for very short periods (15-30 min) that would be required to characterise the initial (2 h) period after

rewetting. While DRW is important for short-term (hourly/daily) changes in P availability, it is not clear if long-term (monthly) DRW regimes alter the size of P pools. Knowledge of P dynamics after DRW at both time scales is imperative to assess the implications of DRW for agricultural systems.

Chapter 6

Long-term effects of drying/rewetting on nutrient pools and the size and composition of the microbial biomass

Chapter 6. Long-term effects of drying/rewetting on nutrient pools and the size and composition of the microbial biomass

6.1 INTRODUCTION

Surface soils naturally experience recurrent drying and rewetting (DRW) cycles. These cycles are a feature of Mediterranean-type climates which have relatively low rainfall (<600 mm annually) and hot dry summers. Sporadic summer rainfall events are characterised by high intensity thunderstorms which only last for brief periods. Rewetting dry soil produces a flush of CO_2 and nutrients as a result of increased microbial mineralisation. The sudden influx of water at rewetting causes soil structural changes and aggregate breakdown increasing organic matter availability to micro-organisms (Denef et al. 2001a). In addition, the sudden change in water potential releases organic substrate from the microbial biomass either through cell lysis (Fierer and Schimel 2003; Turner *et al.* 2003) or the release of labile osmo-regulatory compounds (Halverson *et al.* 2000; Kieft *et al.* 1987). Investigations of DRW processes have shown dissimilar responses of soils from different climatic histories and land-use (Fierer *et al.* 2003; Lundquist *et al.* 1999b). An understanding of how water regime history influences a soil's response to DRW is pertinent given erratic seasonal rainfall and imminent changes in climate.

The disparate responses of soils to DRW may relate to the amount and distribution of rainfall or to inherent soil properties. Compared to soils in Mediterranean climates, soils within high rainfall areas are potentially exposed to fewer DRW events and also shorter, less severe drying intervals. The ability of DRW events to enhance or retard soil organic matter (SOM) decomposition may partially depend on the duration in which a soil is dry (Lundquist *et al.* 1999a). The influence of DRW may also be determined by soil properties. Total C, total N, microbial biomass P and texture were shown to be strongly correlated to the size of CO_2 flushes after rewetting in a range of soils (Chapter 3). Soils within high rainfall areas commonly have higher organic matter contents which may result in reduced wetability due to the hydrophobicity of the SOM (Caron *et al.* 1996). Also, higher levels of SOM enhance water retention and reduce the impact of drying on the microbial biomass (He *et al.* 1998). Soil texture will alter the

response of a soil to DRW by regulating the accessibility of SOM to microbes and the ability of the soil to retain water.

It has been proposed that soils which naturally undergo frequent DRW may be more adapted to the effects of water stress and would therefore exhibit reduced responses to DRW. Fierer *et al.* (2003) showed that soil microbial communities within oak soils were altered by DRW while those within grassland soils were largely unaffected. These authors attributed this difference to previous water regimes as drying occurred more frequently in grassland soils than those in oak woodlands. Also, adaptation of microbial communities to DRW within surface layers has been proposed as these exhibit fewer changes in phospholipid profiles than communities deeper within the soil profile (Lundquist *et al.* 1999b). Although adaptation of soil microbial communities to climate has been proposed (Kieft *et al.* 1987), this is not conclusive as other studies have reported changes in bacterial communities irrespective of climatic parameters (Van Gestel *et al.* 1993). These authors suggest that the ability of microbes to withstand DRW is determined by inherent properties of the microbes. Significant flushes after rewetting occurred in a range of soils, despite these having different climatic histories (Chapter 3). It is not known how exposure to different water regimes alters the composition of indigenous microbial communities or the extent of the DRW flush.

The experiment described in this chapter aimed to determine the effect of different long-term soil pre-treatment water regimes and subsequent DRW patterns on the long-term nutrient availability and soil microbial community composition. It was postulated that long-term soil pre-treatment water regimes alter (i) labile nutrient pools (P_{resin}, DOP, EOC, DON and microbial biomass) and (ii) potential P, N and C mineralisation patterns (microbial biomass size, activity and enzyme activity) in subsequent DRW.

6.2 METHODS

6.2.1 Overview

Soils were collected from two field sites representing distinct climatic histories. Hamilton is an area with a higher annual rainfall (688 mm) which is evenly distributed (warm wet summer). Crystal Brook is drier (472 mm) and has a distinct hot dry summer (temperate Mediterranean). Soil cores from each site were subject to 4 different pre-treatment soil water regimes over a 14 week period: maintained at field capacity; 3 DRW events evenly spaced (intermittent); 3 DRW events at the end of incubation (false break, to the start of the growing season) and maintained air-dry. The two DRW treatments both had 3 DRW events but with intermittent representing sporadic rainfall events and a false break situation where the rainfall events occur close to the start of the growing season (break of season = start of winter rain).

6.2.2 Field sampling and processing

Surface soil (0-5cm) was collected from two field sites; Hamilton, VIC (37°49'S, 142°04'E) and Crystal Brook, SA (33°21'S, 138°12'E) just prior to end of the 2006 growing season. The Hamilton soil is classified as a Chromosol and the Crystal Brook soil a Calcarosol (Isbell 2002). The Hamilton site was a permanent grass pasture at the Victorian Department of Primary Industries Research Station. The Crystal Brook site was a paddock in a wheat rotation. At each site, several soil samples were taken in a grid pattern across the paddock, transported to the laboratory and stored at 5°C. Within 48 h after sampling, soils were processed by passing them through a 4 mm sieve to remove plant roots and foreign material and bulked for each site. Sub-samples were taken from each soil and dried for chemical analyses. Mid infra-red diffuse reflectance analysis (MIR) predictions (Janik *et al.* 1998) are shown below (Table 6.1).

Table 6.1: Initial physiochemical properties of soils from Hamilton and Crystal Brook.

	Hamilton	Crystal Brook
pH[1]	5.2	7.3
Total C[2]	4.83	1.85
Sand[2]	44.3	53.3
Silt[2]	39.8	14.3
Clay[2]	16	32

[1]measured in CaCl (1:5); [2]predicted by MIR (%)

6.2.3 Determination of field capacity

The gravimetric water content (θ_g) at field capacity (θ_{fc}) was determined for each soil using the hanging water column - constant head burette technique (Reynolds and Clarke Topp 2007). Briefly, soil cores were packed as for experimental cores (Section 6.2.4) with 4 replicates for each soil, saturated for 24 h and placed on a covered 1 bar pressure plate cell (Soilmoisture Equip Corp, USA) and held at a suction of $-1\psi_m$ (-0.33 bar) at 25°C. Gravimetric water content of each core was recorded daily and continued for 7 d until stable. The θ_g corresponding to θ_{fc} was used to adjust water contents during incubation as required.

6.2.4 Pre-treatment soil water regimes

Soil cores were constructed using 20 g soil and bulk density 1.4 as previously described (Chapter 2.1) and pre-incubated at field capacity (θ_g = 0.448 for Hamilton and θ_g = 0.227 g g^{-1} for Crystal Brook) for 1 week. Soil cores were then exposed to 4 different pre-treatment soil water regimes for 13 weeks, intended to mimic different soil water conditions commonly experienced during summer periods (Table 6.2). Abbreviations of the different pre-treatments are shown below (Table 6.3).

Table 6.2: Pre-treatment soil water regimes

Week	Field capacity	DRW Intermittent	DRW False Break	Air-dry
0	Pre-incubation			
1	FC	AD	AD	AD
2	FC	AD	AD	AD
3	FC	AD	AD	AD
4	FC	FC	AD	AD
5	FC	AD	AD	AD
6	FC	AD	AD	AD
7	FC	AD	AD	AD
8	FC	FC	AD	AD
9	FC	AD	AD	AD
10	FC	AD	FC	AD
11	FC	AD	AD	AD
12	FC	FC	FC	AD
13	FC	AD	AD	AD
14	FC	AD	FC	AD

FC = Field capacity; AD = Air-dry

Table 6.3: Summary of abbreviations used.

	Field capacity	DRW intermittent	DRW false break	Air-dry
Hamilton	H_m	H_{int}	H_{fb}	H_d
Crystal Brook	C_m	C_{int}	C_{fb}	C_d

Constantly moist ($_m$) soil cores were held at field capacity using pressure plate apparatus described earlier (Section 6.2.3). The θ_g of these cores was checked periodically during maintenance of the plate system. The two DRW treatments each received 3 DRW events but with intermittent ($_{int}$) representing sporadic rainfall events and a false break ($_{fb}$) situation where rainfall events occurred close to the start of the growing season and are separated by shorter dry periods. During rewetting periods, soil cores were adjusted gravimetrically to θ_{fc} and transferred to large plastic containers with lids and water reservoirs to maintain headspace moisture. Containers were opened periodically to allow ventilation. Water contents of all treatments except H_m/C_m were adjusted gravimetrically. Soil cores for the constantly dry ($_d$) treatment were allowed to dry at room temperature for 2 d until they were air-dry (θ_g = 0.026 g g^{-1}) and were kept uncovered.

6.2.5 Experimental drying and rewetting

To determine how soils from each of these pre-treatment soil water regimes responded to further DRW at the end of the 13 wk pre-treatment, all cores (except H_m/C_m) were allowed to air-dry for one week (θ_g = 0.032 g g^{-1} for both Hamilton and Crystal Brook soils)(Table 6.4). After this point, cores were rewet to θ_{fc}, placed in glass incubation chambers (Chapter 2.1) and incubated for 14 d at 25°C. On day 14, half of the cores for each treatment were dried by adding silica pouches (Chapter 4.2.6) to the chambers and the other half were incubated at θ_{fc}. Silica pouches were changed periodically and drying continued for 7 d. At the end of this period, dry soil cores were rewet to θ_{fc} and incubated with moist cores for 14 d at 25°C as before.

Table 6.4: Experimental DRW for Hamilton (H) and Crystal Brook (C) soils . Sampling occurred at 1 h and 14 d after the two DRW events (weeks 16 and 19).

Week	DRW	H_m/C_m		H_{int}/C_{int}		H_{brk}/C_{brk}		H_d/C_d	
15	1	FC	FC	AD	AD	AD	AD	AD	AD
16		FC	FC	FC	FC	FC	FC	FC	FC
17		FC	FC	FC	FC	FC	FC	FC	FC
18	2	FC	AD	FC	AD	FC	AD	FC	AD
19		FC	FC	FC	FC	FC	FC	FC	FC
20		FC	FC	FC	FC	FC	FC	FC	FC

FC = Field capacity; AD = Air dry

6.2.6 Respiration rate

Changes in respiration rate of individual incubation chambers were quantified by measuring headspace CO_2 concentration using a Servomex 1450 infra-red gas analyser (Servomex, UK) (Chapter 2.1). Measurements were taken at 1, 2, 3, 4, 6, 10 and 14 d during the 2 moist incubation periods and at 1, 2, 3, 4 and 7 d during the drying period. Respiration rates were calculated for each interval. Cumulative respiration data were fitted to carbon mineralisation (C_{min}) models as described previously (Chapter 3.2.7).

6.2.7 Microbial biomass and nutrient analyses

Measurement of soil pH (Chapter 2.11), total C and N (Chapter 2.12) and total P (Chapter 2.13) were carried out at the beginning of the study as previously described.

Concentrations of available nutrients were determined after field sampling and at each of the four experimental sampling times (Table 6.4). Resin extractable P (P_{resin}), microbial biomass P (MBP) (Chapter 2.4) and microbial biomass C (MBC) were determined as previously described (Chapter 2.3). Microbial biomass C is reported as the difference between chloroform fumigated and non-fumigated samples. Total C of non-fumigated samples was used to represent extractable organic carbon (EOC).

Phosphorus fractions in the non-fumigated K_2SO_4 extracts were also determined. Immediately after extraction, inorganic P (P_i) was determined colorimetrically

(Chapter 2.7). Extracts were then digested and analysed for total dissolved P (TDP) (Chapter 2.8). Dissolved organic P (DOP) is reported as the difference between the total (TDP) and P_i fractions.

6.2.8 Phosphomonoesterase activity

Acid phosphomonoesterase activity was measured as previously described (Eivazi and Tabatabai 1977; Tabatabai and Bremner 1969). Acid rather than alkaline phosphomonoesterase assay was chosen due to the neutral to acidic pH of the soils used in this experiment (Table 6.1). Briefly, soil (1 g) was shaken with 0.25 ml of toluene; 4 ml modified universal buffer (MUB) and 1 ml of 15 mM *p*-nitrophenyl phosphate (FLUKA) substrate dissolved in MUB buffer. The MUB was prepared from a stock solution (12.1 g tris, 11.6 g maleic acid, 14 g citric acid and 6.3 g boric acid dissolved in 500 ml of 1 M NaOH and adjusted to 1 L with RO water) whereby 200 ml of the MUB stock solution was adjusted to pH 6.5 under continuous stirring using 0.1 M HCl and made up to a final volume of 1 L with RO water. A pH of 6.5 has been shown to be the optimal pH for phosphomonoesterase in most soils (Eivazi and Tabatabai 1977). Samples were vortexed and incubated at 37°C in a stationary water bath for 1 h. The reaction was terminated by adding 1 ml 0.5 M $CaCl_2$ and 4 ml 0.5M NaOH after which samples were vortexed and centrifuged at 3000 RPM (1207*g*) for 3 min. Supernatants were diluted in RO water and the *p*-nitrophenol released during incubation was determined photometrically at 400 nm. Controls were performed as described in the assay procedure except that substrate was added after the addition of $CaCl_2$ and NaOH and immediately prior to filtration.

6.2.9 Microbial community composition

Microbial community composition was assessed by Phospholipid Fatty Acid (PLFA) extraction (Bardgett *et al.* 1996; Frostegård *et al.* 1993). Briefly, 2 g (wet wt) soil was extracted with 1.5 ml 0.15 M citrate buffer (adjusted to pH 4 and reduced to account for water within the soil), 1.9 ml chloroform, 3.8 ml methanol and 2 ml Bligh and Dyer reagent (1:2:0.8; chloroform, methanol and citrate buffer). Samples were incubated in the dark for 2 h and vortexed every 20 min (5 times in total). To stop extraction, samples were vortexed and centrifuged at 2000 RPM for 15 min. Supernatants were transferred to a clean vial. Soil pellets were re-extracted with 2.5 ml Bligh and Dyer

reagent by vortexing and centrifuged a second time. Organic and aqueous phases of the combined supernatants were separated by adding 3.1 ml chloroform and 3.1 ml citrate buffer, vortexing and centrifuging at 2000 RPM for 2 min. The lower organic phase was transferred to a clean vial and stored at -20°C overnight.

On the second day, the lipid phase was dried under N_2 at 40°C. Samples were then resuspended in 1 ml of chloroform and vortexed and transferred into individual silica bonded columns (Supelco, Supelclean LC-Si-SPE). Vials were rinsed a further two times with 0.5 ml chloroform and the suspension transferred to the columns as before. Lipid fractionation was achieved by eluting neutral lipids with 5 washes (~6 ml in total) of chloroform, glycolipids with 5 washes (~6 ml in total) of acetone, and finally phospholipids into new vials by adding 5 washes (~6 ml in total) of methanol. Phospholipid suspensions were then dried under N_2 at 40°C. Alkaline methanolysis was performed by dissolving the sample in 1 ml of methanol-toluene (1:1 v/v) solution with the addition of 1 ml 0.2M methanolic KOH, vortexing and incubating at 37°C in the dark within a stationary water bath for 15 min. Methanolysis was stopped by the addition of 2 ml hexane-chloroform (4:1 v/v), 0.3 ml 1M acetic acid and 2 ml autoclaved RO water, vortexing and centrifuging at 2000 RPM for 10 min. The upper organic phase was transferred to a clean vial and the residual aqueous phase was re-extracted with 2 ml hexane-chloroform (4:1 v/v) solution and the combined organic phases were dried under N_2 gas at 40°C. Finally, samples were resuspended in 170 µl dichloromethane and 30 µl of internal C19:0 standard (0.01 M methylnonadecanoate in dichloromethane) and quickly transferred to gas chromatograph (GC) vials under a N_2 gas headspace.

Analyses of PLFA extracts were performed by GC using a HP 6890 (Hewlett Packard) fitted with flame ionisation detector (FID) and a fused-silica column (Supelco, SP-2560; 75 m x 180 µm and a film thickness of 0.14 µm) and using helium as carrier gas (20cm/sec). The GC oven temperature program was; 140°C for 5 min and 240°C for 15 min with a ramp of 4°C per min). Injector and detector temperatures were set to 250°C and 260°C respectively. Separated FAME were identified by retention time against a qualitative Supelco 37 component FAME mix (Supelco). Samples containing unknown PLFA peaks were then analysed by gas chromatography/mass spectrometry (GC/MS) with column and run conditions as for GC/FID above and with ionisation energy of

70eV. Unknown peaks were identified using mass spectral library NIST02 (HP Chemstation). Fatty acid nomenclature was used as described by Frostegård *et al.* (1993). In total, 29 fatty acids were included in the multivariate analyses. Signature fatty acids were used to represent gram positive (G+ve) bacteria (iC15:0 and iC16:0); gram negative bacteria (G-ve) (C16:1ω7c and C18:1ω7); actinomycetes (10ME-C17:0 and 10ME-C18:0); fungi (C18:1ω9c; 18:2ω6t; C18:2ω6c; C18:3ω6c) and arbuscular mycorrhizal fungi (C16:1ω5c) (Marschner 2007; Olsson *et al.* 1997; Zelles 1999). All remaining fatty acids (C14:1 ω5c; 6ME-C14:0; C15:0; C15:1ω5c; C17:0; C16:1ω7t; C17:1ω7c; C18:0; C18:1ω9t; C18:1ω8; C20:0; C20:3ω6c; C22:1ω9c; C20:4ω6c; C23:0; C22:2ω6c; C20:5ω3c) were assumed to represent bacteria.

6.2.10 Statistical analyses

For each soil (Hamilton and Crystal Brook), 3-way analyses of variance (ANOVA) was used to determine the effects of water pre-treatment x experimental DRW x sampling time on soil chemical properties using GENSTAT 8th Edition (Lawes Agricultural Trust). Carbon mineralisation was not extrapolated beyond the study period. Calculations of C_{min} at the end of the two experimental moist incubation periods (14 d) were determined using the model and are denoted as Co_{14d}. C_{min} model outputs (Co_{14d} and k) were analysed using a 3-way ANOVA with soil x water pre-treatment x DRW as main factors, except for the first incubation period where a 2-way ANOVA was used with soil x water pre-treatment as main factors. Significant differences (P<0.05) between means were then tested using a post-hoc Tukey test. The PLFA data was analysed separately for each soil by principal component analysis using CANOCO (CANOCO, Microcomputer Power).

6.3 RESULTS

6.3.1 Soil respiration

Respiration during the experimental DRW was significantly different between pre-treatment soil water regimes in both Hamilton and Crystal Brook soils (Figure 6.1). The highest respiration rates immediately after the first experimental rewetting were in H_d, which had respiration rates five times higher than the H_m treatment. H_{int} and H_{fb}

were similar and had respiration rates approximately double those of the H_m treatment. In the Crystal Brook soil, the dry treatment, C_d, also had the highest respiration rate following rewetting, with rates four times those in the C_m treatment. The elevated rates of respiration following the first rewetting event lasted up to 6 d in the Hamilton soil, approximately 2.5 d for C_d and less than 2 d for C_{int} and C_{fb} (Figure 6.1).

Respiration rates after the second experimental DRW event showed similar patterns in both Hamilton and Crystal Brook treatments (Figure 6.1). The rewetting flush in respiration was highest in the treatments that had not previously been exposed to DRW (H_m and C_m). Respiration rates in the soils that had been previously subjected to DRW events during pre-incubation (3x) and during the experiment (1x) (H_{int}, H_{fb}, C_{int} and C_{fb}) were similar to those in the first experimental rewetting event. For H_d and C_d treatments, the second experimental DRW respiration flush was significantly smaller than the first. Respiration rates after the second rewetting were half (C_d) and one third (H_d) of the rates at the first rewet. The duration of elevated respiration activity following rewetting in the Crystal Brook soil was shorter than the Hamilton soil and was similar in each rewetting event.

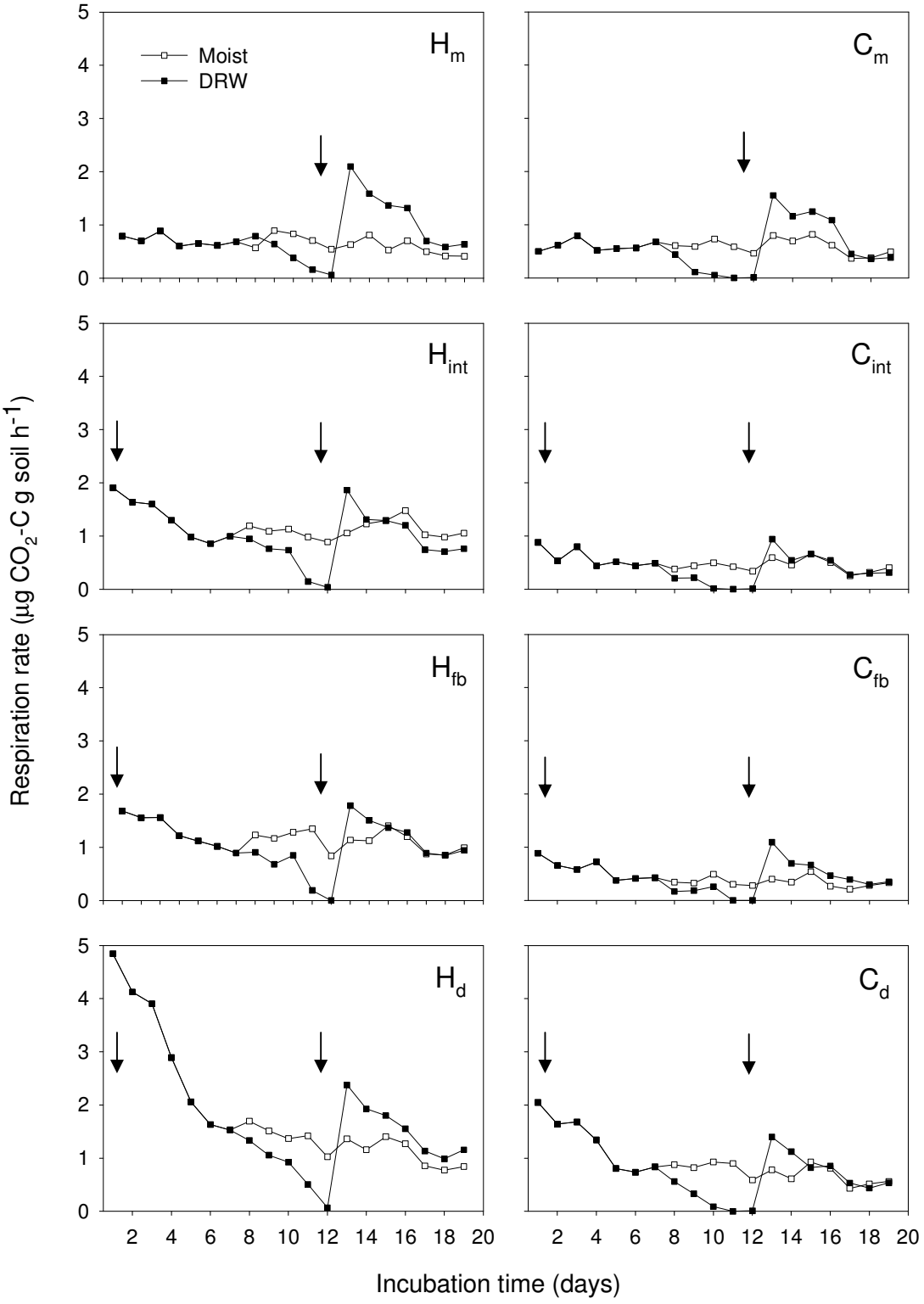

Figure 6.1: Microbial respiration activity in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d; Table 6.3) and either subjected to experimental DRW (v) or moist incubated (□). Arrows indicate the timing of rewetting events.

6.3.2 Modelling of microbial respiration data

Cumulative respiration data were fitted to one-pool C_{min} models expressed per unit of soil (mg CO_2-C g $soil^{-1}$) (Appendix 6.1 and 6.3). Modelling of C_{min} data showed that the water pre-treatments significantly altered the amount (Co_{14d}) and the rate (k) of C_{min} after experimental rewetting (Table 6.5). The constantly dry pre-treatments (H_d and C_d) had significantly higher Co_{14d} and k values than all other pre-treatments. In the Hamilton soil, Co_{14d} for H_d was 2.3 times higher than for H_{int} and H_{fb} and 4.2 times higher than for H_m. Similarly, the values for k were highest in H_d, not significantly different between H_{int} and H_{fb} and smallest in H_m. For the Crystal Brook soil, Co_{14d} was similar in C_{int} and C_{fb} treatments and the moist control (C_m), however Co_{14d} in the C_d treatment was significantly higher. The C_m treatment had a significantly lower k value than C_{int}, C_{fb} and C_d. Overall, Co_{14d} was significantly higher in H_{int}, H_{fb} and H_d than in C_{int}, C_{fb}, and C_d. However, Co_{14d} of the H_m and C_m were not different and k values were not different between soils in any of the corresponding treatments.

Except for C_m treatment, Co_{14d} was not significantly different for the Crystal Brook treatments after the second DRW (Table 6.5). Although values for k were higher in the DRW pre-treatments than moist controls after the second DRW, these differences were not significant. Therefore, differences between the pre-treatments in the Crystal Brook soil observed after the first DRW were not present after the second DRW. For the Hamilton soil, Co_{14d} in the H_m treatment was still significantly lower than the other pre-treatments, with no difference between H_{int}, H_{fb} and H_d. The effect of DRW in the second experimental moist incubation period was an increase in k for H_m and H_{int} and an increase in Co_{14d} for H_m and H_d.

Table 6.5: Predicted mineralisable C fraction (Co_{14d}) and proportional mineralisation rate constant (k) of one-pool C mineralisation model fitting (mg CO_2-C g soil^{-1}) in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d; Table 6.3) and either subjected to experimental DRW or moist incubated. Letters indicate significant differences (P<0.05) between treatments for each parameter and DRW event.

	DRW 1*		DRW 2			
			Moist		DRW	
	Co_{14d} (mg CO_2-C g soil^{-1})	k (day^{-1})	Co_{14d} (mg CO_2-C g soil^{-1})	K (day^{-1})	Co_{14d} (mg CO_2-C g soil^{-1})	K (day^{-1})
H_m	0.005^{a}	0.196^{ab}	0.004^{abc}	0.212^{abc}	0.008^{fg}	0.319^{de}
H_{int}	0.009^{b}	0.262^{cd}	0.008^{fg}	0.184^{a}	0.008^{fg}	0.290^{bcde}
H_{fb}	0.009^{b}	0.236^{bc}	0.007^{efg}	0.203^{ab}	0.008^{g}	0.257^{abcde}
H_d	0.021^{c}	0.305^{d}	0.008^{efg}	0.229^{abcde}	0.011^{h}	0.269^{abcde}
C_m	0.004^{a}	0.168^{a}	0.004^{abc}	0.245^{abcde}	0.006^{def}	0.329^{e}
C_{int}	0.004^{a}	0.241^{bc}	0.003^{ab}	0.240^{abcde}	0.003^{abc}	0.315^{cde}
C_{fb}	0.004^{a}	0.258^{cd}	0.002^{a}	0.247^{abcde}	0.004^{abc}	0.325^{e}
C_d	0.009^{b}	0.291^{d}	0.005^{bcd}	0.216^{abcd}	0.006^{cde}	0.307^{cde}

*H_m and C_m treatments were not DRW in the first cycle

Model fitting of normalised respiration data (expressed as mg CO_2-C g soil C^{-1}) (Appendix 6.2 and 6.4) showed that during the first experimental incubation period Co_{14d} values for C_m were significantly higher than H_m (Table 6.6). After the first experimental DRW, the C_d treatment had a significantly higher Co_{14d} than the H_d treatment. Values of Co_{14d} were not different between H_{int}/C_{int} or H_{fb}/C_{fb} treatments. At the second experimental DRW event, normalised respiration data showed that for both moist and DRW treatments, C_m and C_d had significantly higher Co_{14d} values compared to H_m and H_d treatments (Table 6.6). Therefore, soil C mineralisability within the C_m and C_d treatments was higher than H_m and H_d treatments.

Table 6.6: Predicted C mineralisability (Co_{14d}) of one-pool C mineralisation model fitting (mg CO_2-C g soil C^{-1}) in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d; Table 6.3) and either subjected to experimental DRW or moist incubated. Letters indicate significant differences (P<0.05) between treatments for each DRW event.

	DRW 1*	DRW 2	
		Moist	DRW
	Co_{14d} (mg CO_2-C g $soil^{-1}$)	Co_{14d} (mg CO_2-C g $soil^{-1}$)	Co_{14d} (mg CO_2-C g $soil^{-1}$)
H_m	0.097^{a}	0.082^{a}	0.164^{abcd}
H_{int}	0.182^{b}	0.162^{abc}	0.155^{abc}
H_{fb}	0.179^{b}	0.150^{abc}	0.169^{bcd}
H_d	0.417^{c}	0.152^{abc}	0.215^{cd}
C_m	0.224^{b}	0.220^{cde}	0.337^{f}
C_{int}	0.216^{b}	0.168^{bcd}	0.188^{bcd}
C_{fb}	0.215^{b}	0.124^{ab}	0.208^{bcd}
C_d	0.483^{d}	0.248^{de}	0.301^{ef}

*H_m and C_m treatments were not DRW in the first cycle

6.3.3 Carbon availability

Pre-treatment water regimes did not significantly alter EOC availability in either Hamilton or Crystal Brook soils (Figure 6.2). The EOC concentrations in Hamilton soil were between 310 and 431 mg C kg^{-1} and between 125 and 170 mg C kg^{-1} for Crystal Brook. The difference in EOC between these soils reflects differences in total C content (Table 6.1). Although only significant for H_d and H_{int}, all treatments showed a reduction in EOC availability during the first two-week moist incubation period, indicating EOC utilisation. The Hamilton treatments showed the largest decrease in EOC (34-44%), with smaller decreases in the Crystal Brook treatments (7-25%). The response of the pre-treated soils to the second experimental DRW cycle was variable (Figure 6.2). Generally the second rewetting resulted in higher EOC in DRW than moist incubated soils; however this was only significant for H_m. The largest increase in EOC at the second rewetting occurred in the H_m (106 mg C kg^{-1}) and C_m (78 mg C kg^{-1}) pre-treatments which was the first DRW for these. Extractable organic C at the end of the experimental incubation study was not different between DRW pre-treatments. Generally EOC in the Crystal Brook soil was more stable during DRW than the Hamilton soil.

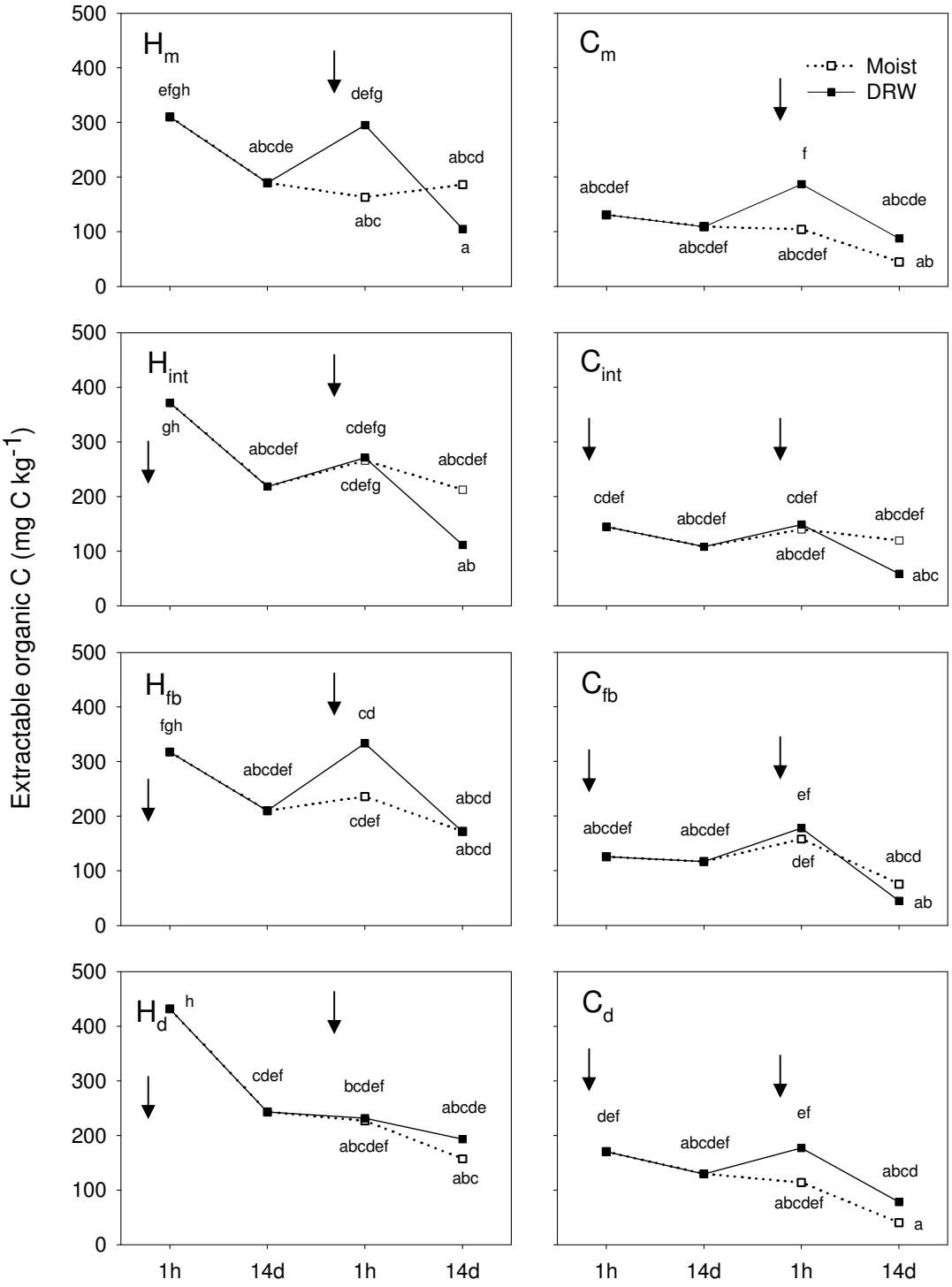

Figure 6.2: Extractable organic C (EOC) in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d; Table 6.3) and either subjected to experimental DRW (solid line) or moist incubated (dotted line). Arrows indicate the timing of rewetting events. Letters show significant differences (P<0.05) between water regimes and DRW for each soil.

6.3.4 Phosphorus availability

Resin extractable P was not affected by the pre-treatments for either Hamilton or Crystal Brook (Figure 6.3). The P_{resin} content of the Hamilton soil was between 17 and 20.5 mg P kg^{-1} and was much lower than the 37.5 to 44.7 mg P kg^{-1} in the Crystal Brook soil. In the H_m treatment, there was a significant increase in P_{resin} during experimental moist incubation, however all other treatments remained unchanged. Increases in P_{resin} during moist incubation were much larger in the Crystal Brook soil (although not significant for C_{int} and C_{fb}).

In the second experimental DRW cycle, P_{resin} in H_d treatment was significantly decreased by DRW compared to the moist control, however the P_{resin} concentration of the other treatments was unaffected by DRW (Figure 6.3). The effects of the second experimental DRW on P_{resin} availability were much more prominent in the Crystal Brook soil. Between 10 and 16.6 mg P kg^{-1} was released during moist incubation in the Crystal Brook treatments. At 1 h after rewetting there was less P_{resin} in DRW than moist incubated treatments (not significant for C_{int} and C_d) and the P_{resin} concentration of the C_m and C_d treatments was lower at the end of the study. The effect of DRW was greatest in the C_m treatment which was not previously exposed to DRW. The P_{resin} content of the Hamilton soils showed less variation in response to DRW than the Crystal Brook soil.

Total dissolved P (TDP) concentration was not affected by pre-treatment water regime in either Hamilton or Crystal Brook soils (Figure 6.4). Similarly, TDP content of the Hamilton soil was not affected by the experimental DRW. The low concentrations (1-1.3 mg P kg^{-1}) and lack of change in this P pool suggest that P is limiting in the Hamilton soil. The concentration of TDP in the Crystal Brook treatments was higher than in the Hamilton soil and ranged from 2.1 to 2.5 mg P kg^{-1}. The TDP content was not significantly different between pre-treatment soil water regimes in the Crystal Brook soil. An increase in TDP of between 0.5 and 1 mg P kg^{-1} was observed in these soils after experimental DRW, during moist incubation (although not significant for C_d and C_{int} treatments).

For both Hamilton and Crystal Brook soils, the second experimental DRW event had no effect on the TDP concentration, as TDP in DRW and moist incubated treatments did not differ either immediately (1 h) or after the 14 d moist incubation period (Figure 6.4). Total dissolved P in C_m, C_{int} and C_{fb} increased during the moist incubation while TDP in the C_d treatment did not significantly change.

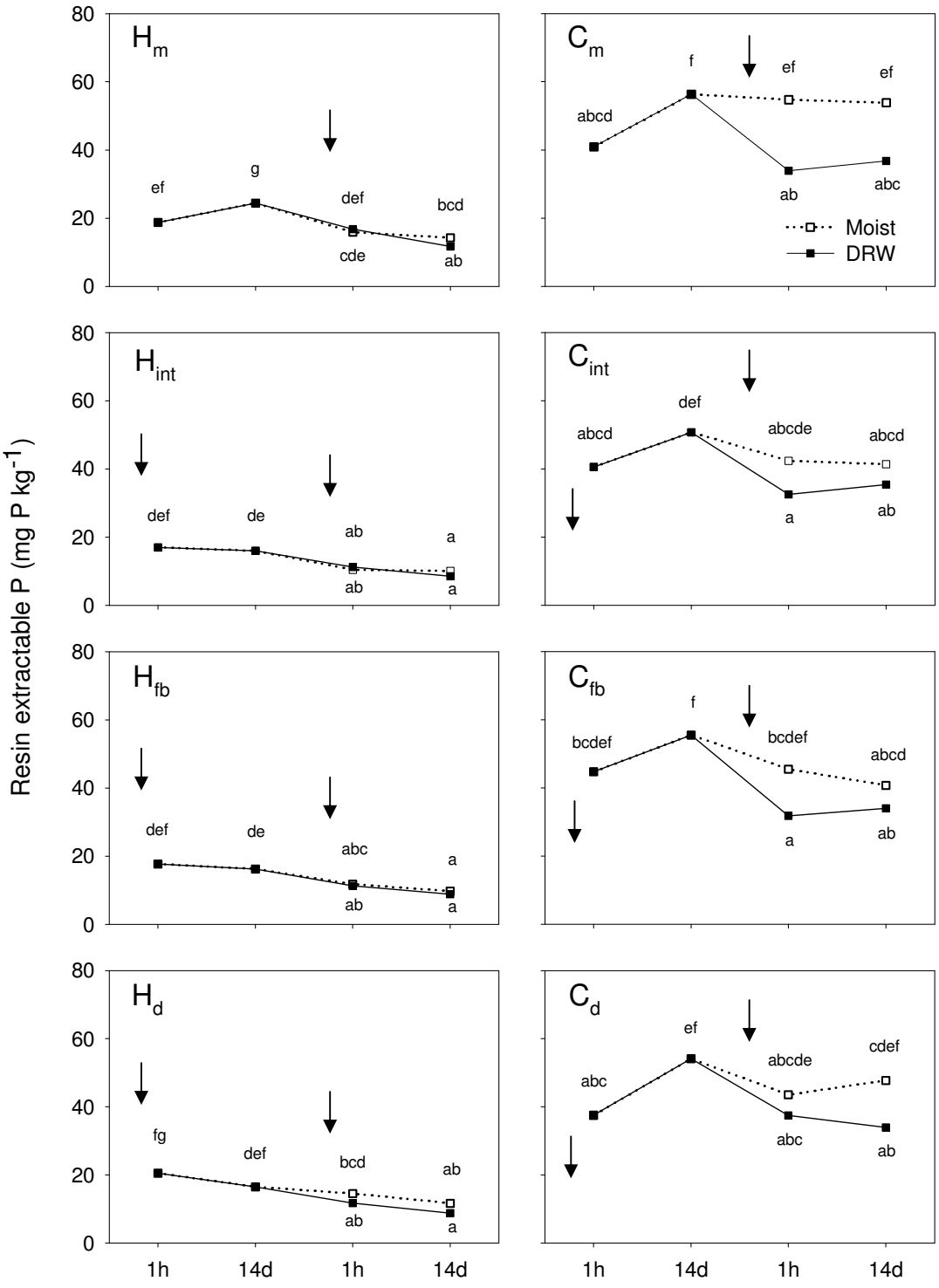

Figure 6.3: Resin extractable P (P_{resin}) in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d; Table 6.3) and either subjected to experimental DRW (solid line) or moist incubated (dotted line). Arrows indicate the timing of rewetting events. Letters show significant differences (P<0.05) between water regimes and DRW for each soil.

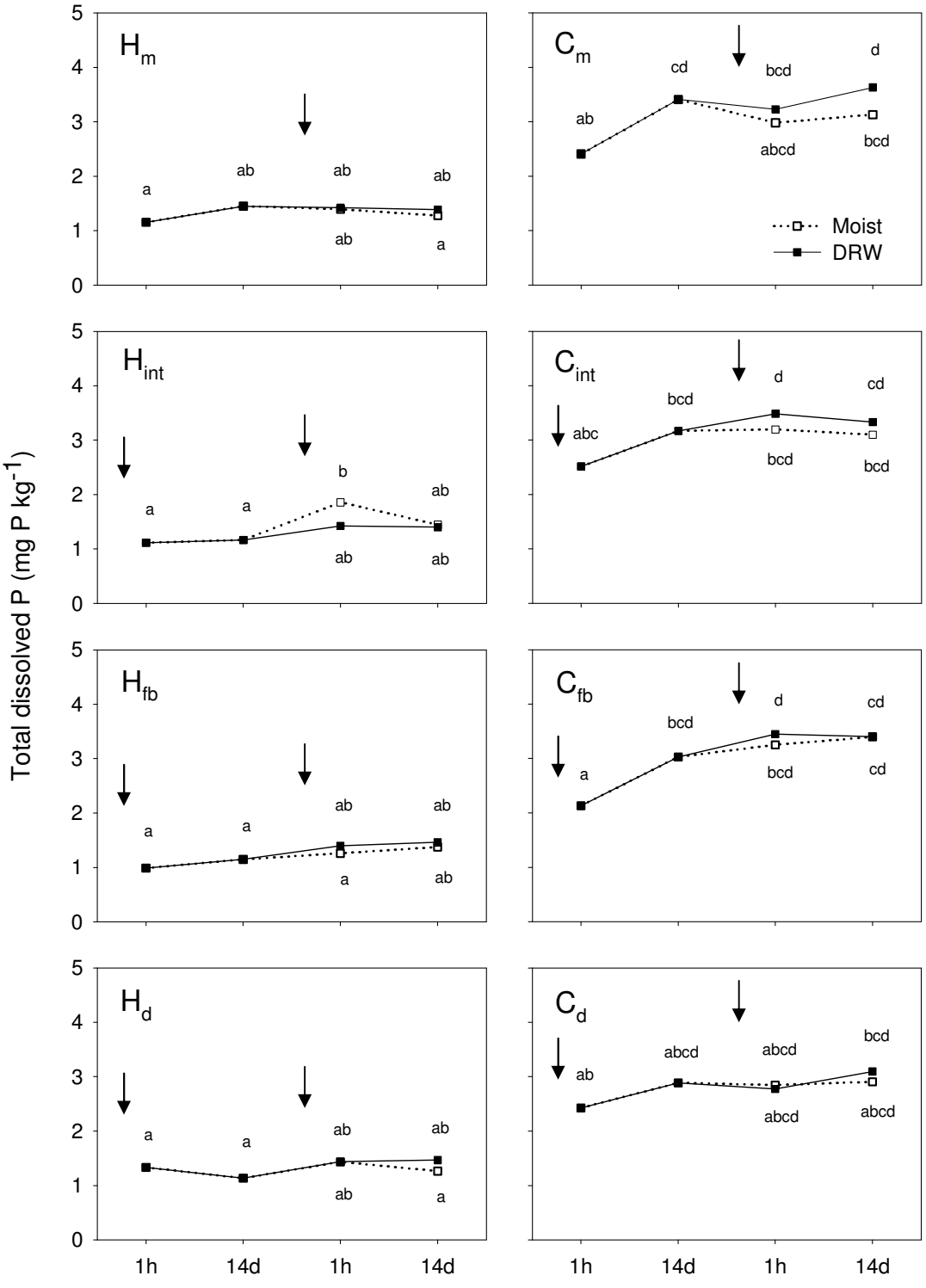

Figure 6.4: Total dissolved P (TDP) in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d; Table 6.3) and either subjected to experimental DRW (solid line) or moist incubated (dotted line). Arrows indicate the timing of rewetting events. Letters show significant differences (P<0.05) between water regimes and DRW for each soil.

Dissolved organic P (DOP) concentrations were higher in Hamilton than in the Crystal Brook treatments (Figure 6.5). The concentration of DOP was not affected by the pre-treatments for the Hamilton soil, since the amount of DOP was not different between pre-treated soils at 1 h. Increases in DOP of between 13 - 18 % occurred in H_m, H_{int} and H_{fb} during the experimental 14 d moist incubation period. However, in the Crystal Brook soil, DOP increased between 2.3 and 15 times in C_m, C_{int} and C_{fb} treatments during moist incubation. Interestingly, both H_d and C_d treatments showed a decline in DOP during the moist incubation. The second DRW cycle did not alter DOP concentrations of the Hamilton soil (except H_{int}) (Figure 6.5). The increase in DOP in the H_{int} treatment was unexpected and cannot be explained. Although the increases in DOP at the second DRW in the Crystal Brook soils were not significant, the DRW treatments were consistently higher than the moist controls, suggesting that DOP was released at rewetting.

Acid phosphatase activity was significantly higher in the H_m treatment than in H_{int}, H_{fb} and H_d (Figure 6.6). Therefore, phosphatase activity was almost 1.5 times lower when the soil had experienced DRW in the pre-incubation period. Phosphatase activity declined during the first 14 d experimental moist incubation period in the H_m treatment, however remained higher than the H_{int} and H_{fb} soils. Phosphatase activity in H_{int}, H_{fb} and H_d treatments did not change significantly during the moist incubation period. Similarly, the C_m treatment had significantly higher phosphatase activity than the other Crystal Brook treatments. Phosphatase activity was stable during experimental moist incubation within all Crystal Brook treatments and was still significantly higher in C_m at the end.

The second experimental DRW event significantly reduced phosphatase activity in Hamilton DRW treatments compared to moist incubated controls (Figure 6.6). Thus, in the Hamilton soil, DRW appeared to inhibit phosphatase activity, as the moist incubated treatments showed increased phosphatase activity during moist incubation. However, this inhibition appeared to be short-term since by 14 d H_d and H_m treatments were back at levels similar to the moist controls.

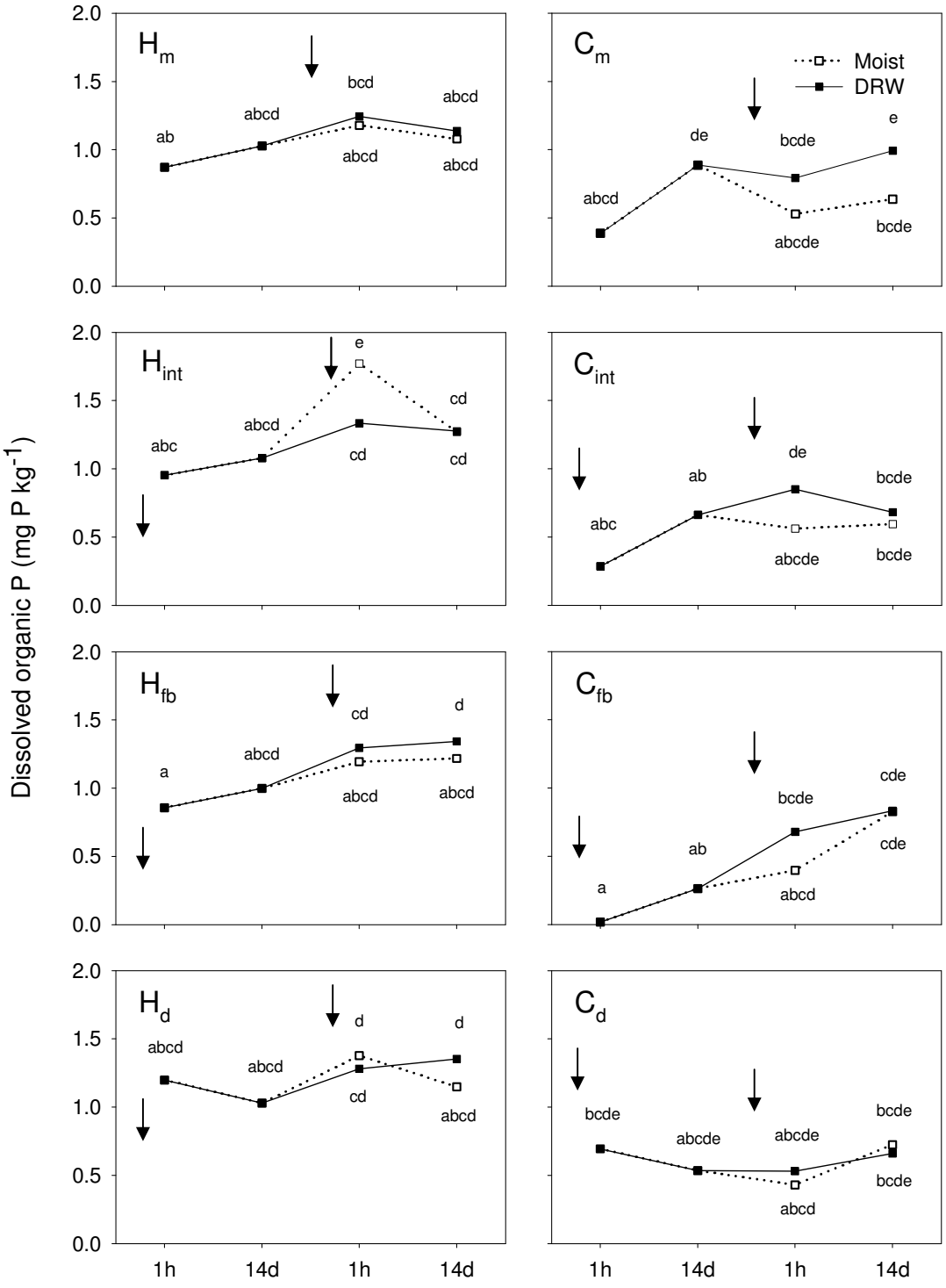

Figure 6.5: Dissolved organic P (DOP) in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d; Table 6.3) and either subjected to experimental DRW (solid line) or moist incubated (dotted line). Arrows indicate the timing of rewetting events. Letters show significant differences (P<0.05) between water regimes and DRW for each soil.

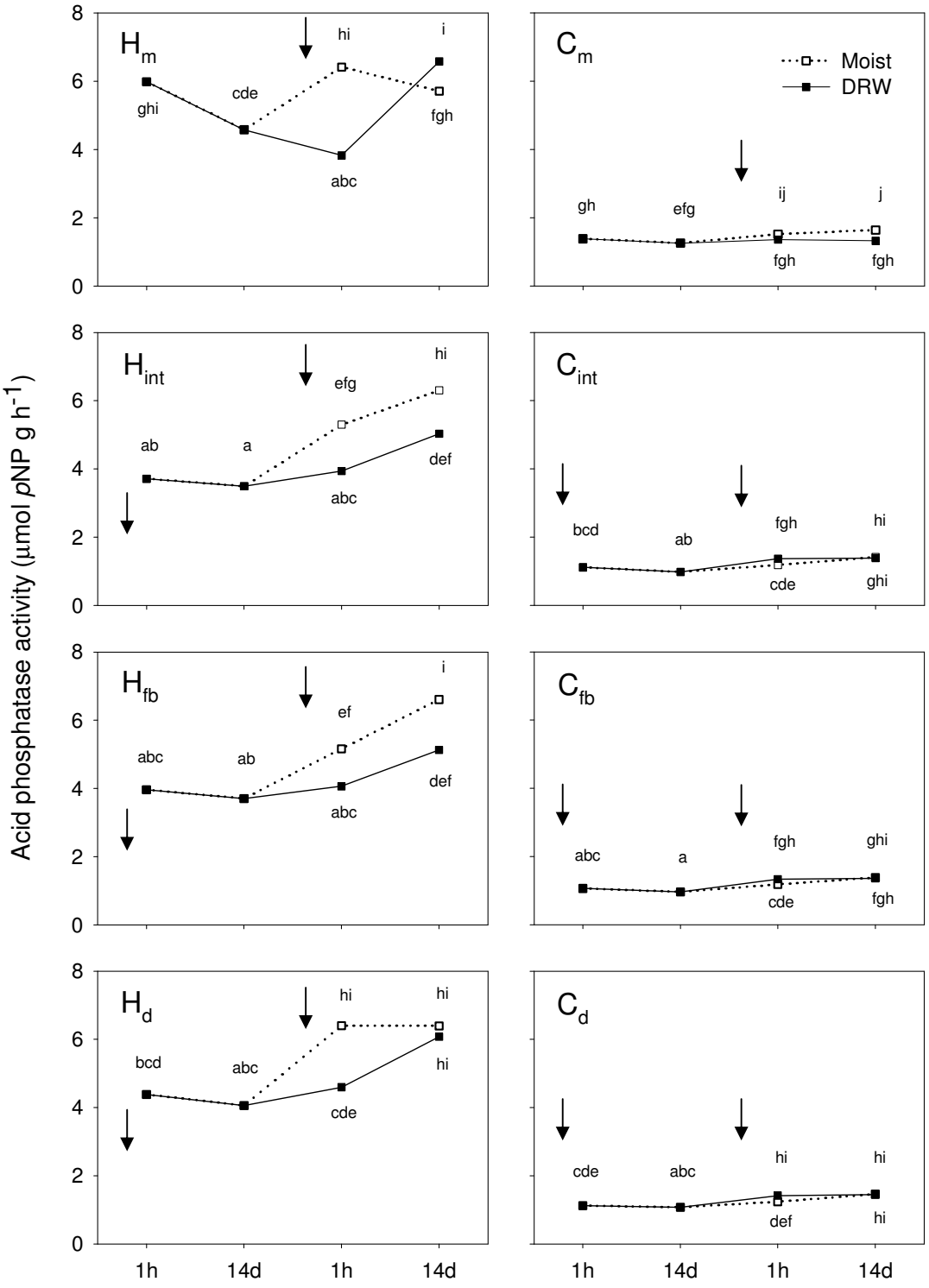

Figure 6.6: Acid phosphatase activity in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d; Table 6.3) and either subjected to experimental DRW (solid line) or moist incubated (dotted line). Arrows indicate the timing of rewetting events. Letters show significant differences (P<0.05) between water regimes and DRW for each soil.

Crystal Brook treatments C_{int}, C_{fb} and C_d showed significant increases in phosphatase activity after the second DRW event compared to moist controls. However, the C_m treatment had significant decrease in phosphatase activity. The changes in phosphatase activity due to DRW were extremely small in this soil. The low levels of phosphatase activity in the Crystal Brook soil are most likely a result of the higher available P concentration in this soil.

6.3.5 Microbial biomass size and community composition

Microbial biomass C and MBP were not affected by pre-treatment water regime in either Hamilton or Crystal Brook soil (Figure 6.7 and Figure 6.8). Although not significant, MBC declined during the experimental moist incubation and the largest decrease (670 mg C kg^{-1}) occurred in the H_m treatment which had remained moist. Furthermore, during this period, MBP increased (only significant for H_d and C_{int}) (Figure 6.8) and consequently the C:P of the microbial biomass decreased during the moist incubation period.

Generally after the second rewetting event, MBC in the DRW treatments increased compared to the moist controls (Figure 6.7), however these increases were not significant. Microbial biomass P was generally reduced by the second DRW event when compared to moist controls (only significant for H_m and C_d treatments). At the end of the incubation, neither MBC nor MBP in the Hamilton soil were significantly different between pre-treatment water regime or experimental DRW treatments. For the Crystal Brook treatments MBC, but not MBP was significantly increased by DRW. Microbial biomass C levels in the experimental DRW treatments were 2.4 to 3.1 times higher than the moist incubated controls. In the Crystal Brook soil MBP was not affected by experimental DRW (Figure 6.8) and therefore the C:P of the microbial biomass was significantly increased by the two experimental DRW events.

The pre-treatment water regime significantly altered the microbial community composition in the Hamilton soil (Figure 6.9). The microbial community composition in the H_m treatment was clearly differentiated from the other treatments that had been subjected to DRW during the pre-incubation. The DRW interval (1, 3 and 15 wks) had no significant effect on microbial community composition as H_d, H_{int} and H_{fb} were not

different. The second experimental DRW event only showed a significant effect on microbial community composition in the H_d and H_m treatments, which had received only one DRW cycle during pre-incubation and the experimental period. Vector plots of signature PLFA did not indicate any effects of DRW on specific organism groups. For the Crystal Brook soil, the changes in microbial community composition that occurred after DRW were less pronounced than in Hamilton soil, indicated by the small explanation of principal component (PC) axis 2 and the large errors associated with PC axis 1 (Figure 6.10). The microbial community composition of C_{fb} and C_m treatments was the most affected by DRW and appeared to be due to changes in abundance of fungal fatty acids.

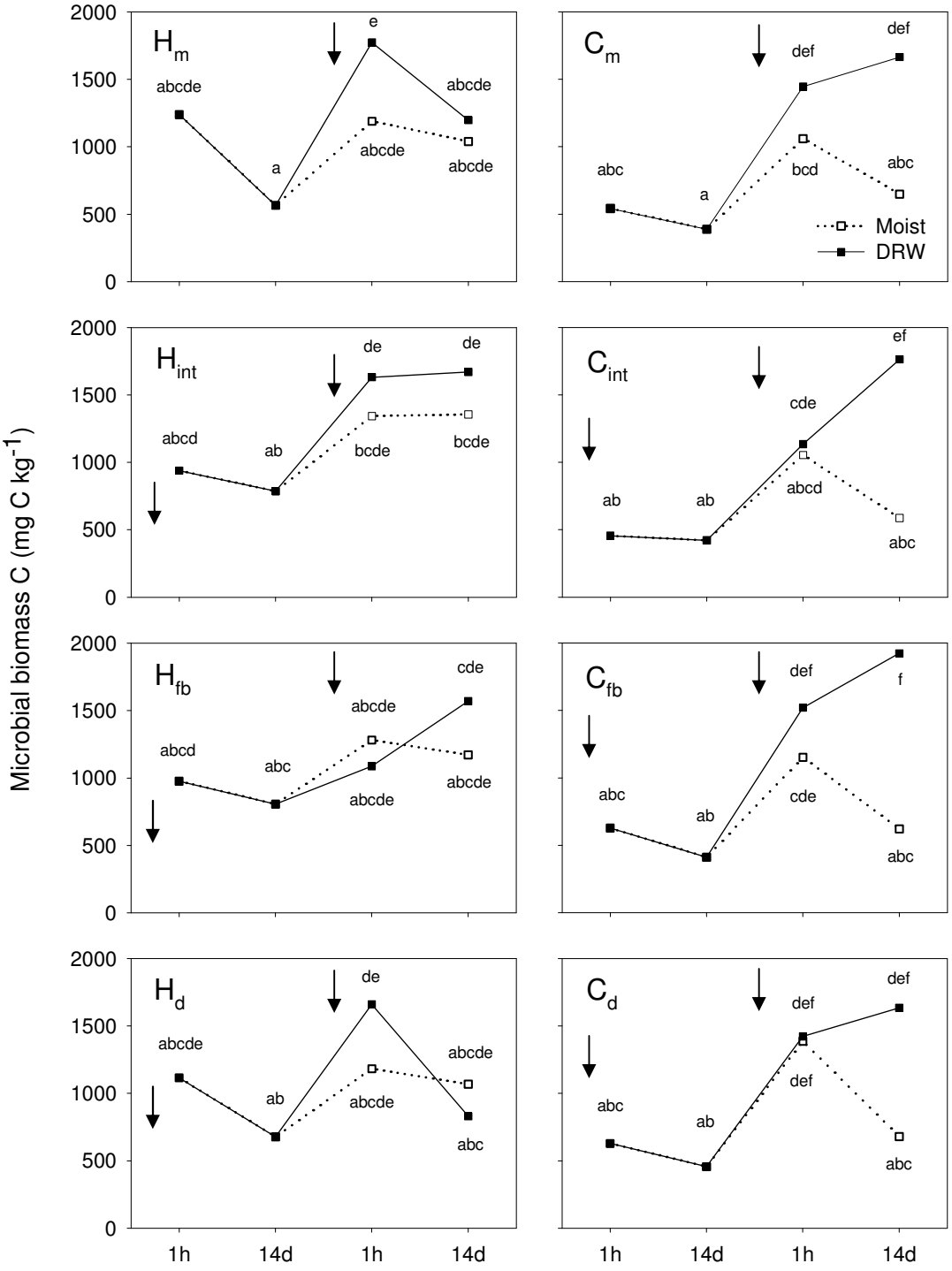

Figure 6.7: Microbial biomass C (MBC) in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d; Table 6.3) and either subjected to experimental DRW (solid line) or moist incubated (dotted line). Arrows indicate the timing of rewetting events. Letters show significant differences (P<0.05) between water regimes and DRW for each soil.

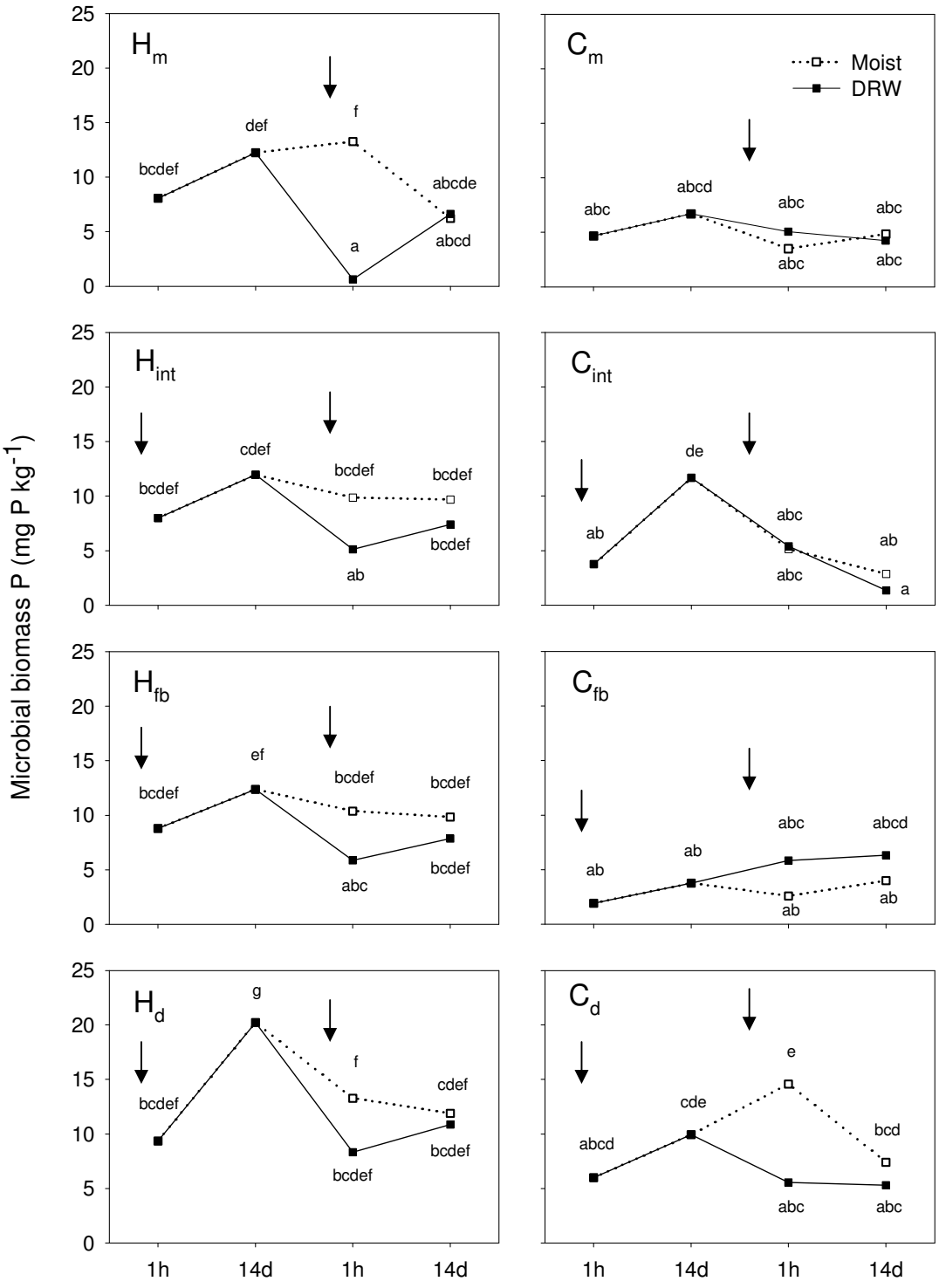

Figure 6.8: Microbial biomass P (MBP) in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d; Table 6.3) and either subjected to experimental DRW (solid line) or moist incubated (dotted line). Arrows indicate the timing of rewetting events. Letters show significant differences (P<0.05) between water regimes and DRW for each soil.

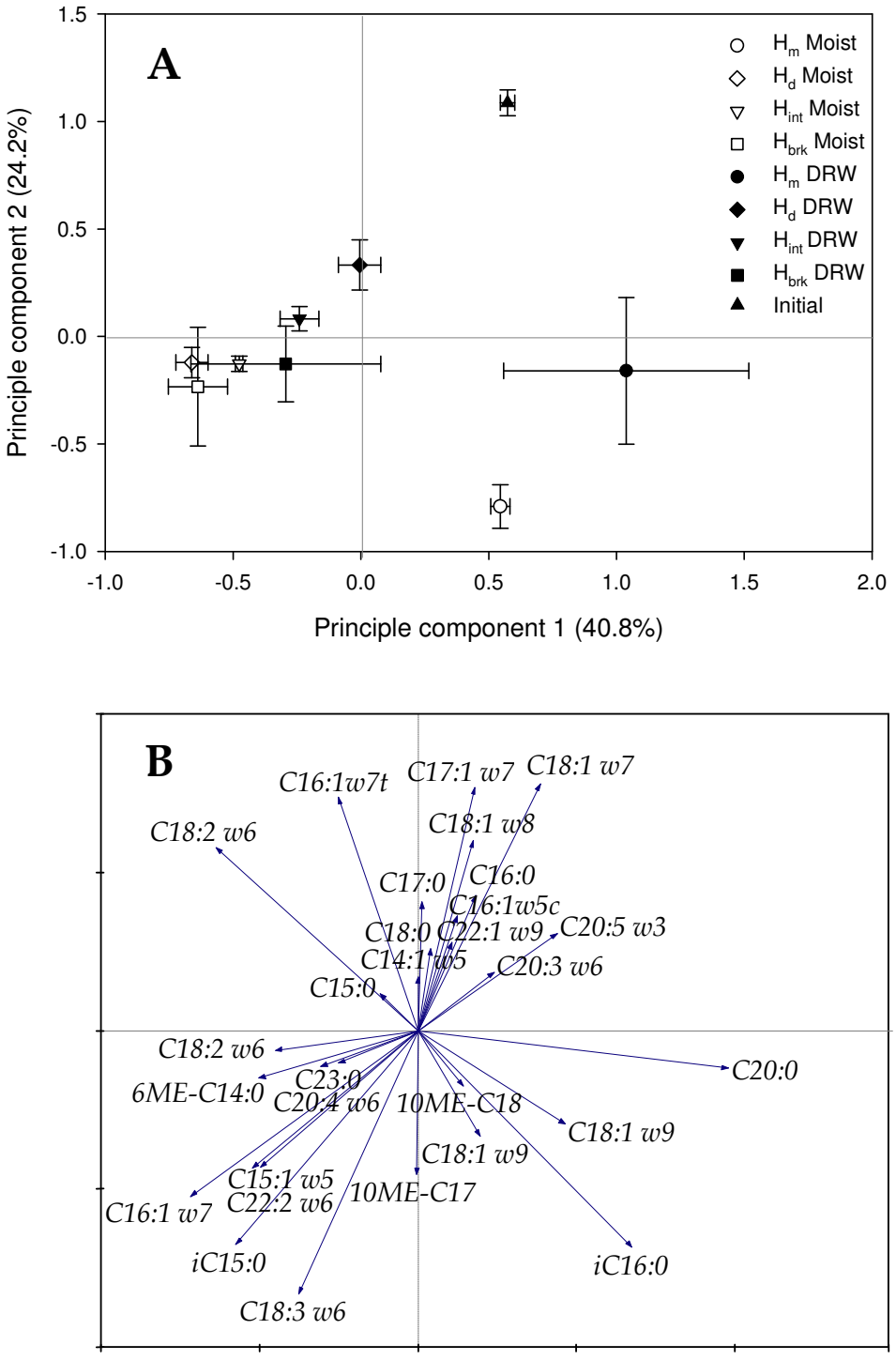

Figure 6.9: Principal component analysis of phospholipid fatty acid profiles within Hamilton (H) soil before pre-treatment (initial) or with four pre-treatment water regimes (m, int, fb, and d; Table 6.3) and subjected to either experimental DRW (black symbols) or moist incubated (white symbols) (A). Bars indicate standard errors of the mean. Vector plots of associated signature PLFA (B).

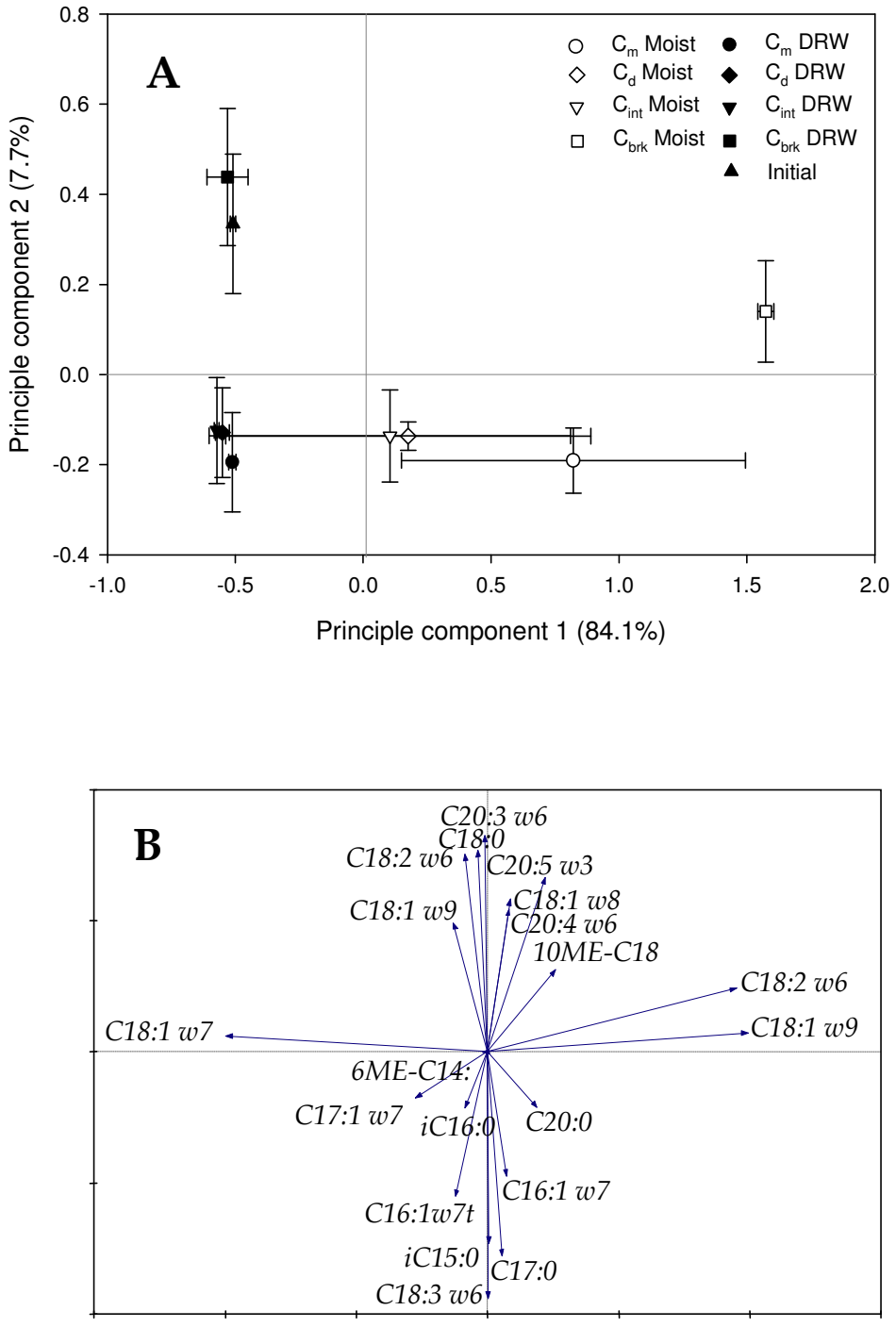

Figure 6.10: Principal component analysis of PLFA profiles within Crystal Brook (C) soil before pre-treatment (initial) or with four pre-treatment water regimes (m, int, fb, and d; Table 6.3) and subjected to either experimental DRW (black symbols) or moist incubated (white symbols) (A). Bars indicate standard error of the mean. Vector plots of associated signature PLFA (B).

6.4 DISCUSSION

6.4.1 Effect of pre-treatment water regime

The response in microbial respiration to rewetting was determined by pre-treatment soil water regime. The largest flushes in respiration occurred in the H_d and C_d treatments that had been dry for the longest periods. However, microbial respiration responses in H_{int} and C_{int} were similar to those in H_{fb} and C_{fb}, respectively, despite these having different intervals (1 and 3 wks) since the last DRW event. It has been proposed that the size of respiration flush at rewetting is determined by the length for which a soil is left to dry (Birch 1960). This appeared to apply to longer drying periods however, the shortest interval used by Birch (1960) was 3 wks. The lack of a difference between 1 and 3 wks since the last DRW event in the present experiment suggested that this may not apply to short-term intervals.

Carbon and P availability (EOC, P_{resin}, TDP, and DOP) were not affected by pre-treatment water regime. Similarly, microbial biomass size was not altered by water pre-treatments, as the constantly moist soil and those rewet after 1, 3 and 15 wks dry incubation did not differ in microbial biomass C and P (Figure 6.7 and Figure 6.8). This indicated that nutrient pools and microbial biomass were not affected by fluctuating soil water regimes. Bottner (1985) also found that the microbial biomass was resilient to the effects of DRW using similar dry/wet periods as the current study. Wu and Brookes (2005) showed that even though a large proportion of the microbial biomass is killed by DRW it is largely restored after 1.3 d. This finding is contrary to many studies that report biomass sensitivity to DRW (Bottner 1985; Van Gestel *et al.* 1993)(Chapter 4).

As explained above, such recovery of the microbial biomass after DRW has not been observed in previous experiments (Chapter 4). The soils in the previous studies had been air-dried and sieved to 2 mm. In contrast, the freshly sampled soils used in the current study were only coarsely sieved and not air-dried and therefore may have retained micro-aggregate structure better than 2-mm sieved air dried soils. This may have provided better protection of the microbial communities to desiccation. In addition, the microbial communities of freshly sampled soil may more closely

represent indigenous microbial communities than those modified with C substrates (Chapter 4). The adaptation of indigenous microbial communities to the changes in osmotic potential have been proposed (Lundquist *et al.* 1999b; Van Gestel *et al.* 1993), primarily by the selection of more tolerant organisms such as fungi or G+ve bacteria (Harris 1981). However, there was no evidence of resistance to DRW by soil microbes in previous experiments (Chapter 4).

Phosphorus pools (MBP, P_{resin}, TDP and DOP) were not altered by pre-treatment water regimes (Figure 6.3, Figure 6.4, Figure 6.5 and Figure 6.8). Recent studies have shown that rewetting dry soil increases soluble P concentrations, primarily organic P forms derived from the microbial biomass (Styles and Coxon 2006; Turner *et al.* 2003; Turner and Haygarth 2001). However, these studies estimate P flushes using single extractions, which only provides information about P availability in the short-term. Grierson *et al.* (1998) showed the immediate increase in P concentration after rewetting is followed by a 3-4 d period where P remains stable. These authors reported that P mineralisation kinetics after the stable period, were similar between DRW and moist incubated soils, possibly due to recovery of the microbial biomass. The results shown here support those of Grierson *et al.* (1998) in that the effects of DRW on P availability appear to be short-lived. Site management and soil properties have an over riding influence on P availability. The high P_{resin} of the Crystal Brook soil reflects P fertilization history, while the higher levels of TDP in the Hamilton soil are probably due to the higher SOM content.

The differences in respiration rate between pre-treatments water regime were not explained by either available nutrients or microbial biomass size. Other studies have shown that pulses in respiration activity following DRW are poorly correlated with microbial biomass size (Fierer and Schimel 2002) and that EOC does not always represent C availability to microbes (Lundquist *et al.* 1999a). Therefore, changes in available nutrient pools may not be suitable indicators of DRW-induced changes in nutrient cycling. However, as DRW changes the respiration rate and phosphatase activity, this suggested that certain functions in the soil may be affected by DRW.

6.4.2 Effect of DRW and the influence of pre-treatment soil water regime

Pre-treatment soil water regimes influenced the respiration response of the soils to subsequent DRW. The largest CO_2 flushes occurred in the soil that was dried for the longest period (15 wks). This was also confirmed in the second DRW event as H_m and C_m that were exposed to DRW for the first time and had been dried for only 1 wk, had much smaller CO_2 flushes than the H_d and C_d treatments at the first rewetting after 15 wks air-dry incubation (Figure 6.1). Increased solubility of SOM with longer drying period has been suggested to occur (Bartlett and James 1980). The smaller CO_2 flushes in the H_d and C_d treatments at the second rewetting event are in agreement with previous studies where the size of the respiration flush decreases with increasing number of DRW cycles (Franzluebbers *et al.* 1994; Mikha *et al.* 2005) (Chapter 4). The reduction in CO_2 flushes with subsequent rewetting events could occur from changes in cell physiology or increasing the proportion of drought-tolerant microbes thereby reducing the contribution of the microbes to the labile nutrient pool at rewetting (Mikha *et al.* 2005). However, microbial biomass size did not appear to be related to respiration rate (Figure 6.7). Smaller CO_2 flushes with subsequent rewetting events may have occurred simply because less C was available for release (Cosentino *et al.* 2006; Fierer and Schimel 2002). Furthermore, in the H_{int}/H_{fb} and C_{int}/C_{fb} treatments that had been subjected to DRW in the pre-incubation, the size of the CO_2 flush was not different between rewetting events. The small and short-lived CO_2 flushes in these treatments suggested that the effects of DRW may be greatly reduced after a small number of DRW cycles. Few studies have examined a greater number of DRW cycles than have been used here. However, Fierer and Schimel (2002) showed that CO_2 flushes after 6, 9 and 15 DRW events were not significantly different, supporting the hypothesis that multiple DRW reduce flush size. The reasons for the reduced effects of DRW are not clear however, they could occur from changes in microbial community composition or lack of C, as discussed earlier or via physical stabilisation of aggregates (Denef et al. 2001a).

Model fitting to microbial respiration data highlights the different mineralisation responses of the two soils to DRW (Table 6.5). In the Hamilton soil, DRW caused an increase in the mineralisable C fraction (Co_{14d}) and the mineralisation rate (k). In the Crystal Brook soil there was no increase in Co_{14d} but only an increase in k. The

different responses of the soils is possibly related to the SOM content of the soils, with the Hamilton soil having 2.6 times more soil C than the Crystal Brook soil (Table 6.1). The lower SOM content may also explain the relatively short rewetting CO_2 flushes in the Crystal Brook soil. Respiration flushes were very short-lived in the Crystal Brook treatments. Only in the soils rewet for the first time, were the respiration rates higher than the moist controls for more than 2 d. For C_d, the increase in Co_{14d} in the DRW treatment compared to the moist control showed that a longer drying interval (more than 3 wks) was required to increase the amount of C available at rewetting. The values of *k* were significantly altered by the water pre-treatments as seen after the first experimental rewetting event. After the second experimental DRW event values of *k* were not different between pre-treatments water regimes in the Crystal Brook soil, confirming that the effect of the water pre-treatments had no effect at the second DRW event. In the Hamilton soil at the second DRW event, the H_d treatment still had higher Co_{14d} than the other treatments showing that this treatment had a residual effect. Also in the moist and dry pre-treatments, significantly higher C mineralisability was observed in the Crystal Brook soil (Table 6.6).

The concentrations of P_{resin} and TDP were not altered by experimental DRW in the Hamilton soil despite strong rewetting CO_2 flushes. Cui and Caldwell (1997) showed that rewetting flushes were associated with increases in C and N but not P. In the Crystal Brook soil, a reduction in P availability occurred after the second DRW event (Figure 6.3). Microbial immobilisation did not appear responsible for this decrease, as microbial P contents were also reduced. The lack of change in TDP and DOP in both soils indicated that DRW had minimal consequences for environmental P losses, since the concentrations of soluble P forms remained low. Resin extractable P was reduced immediately after rewetting in the Crystal Brook soil (only significant for C_m and C_{fb}) and these reductions in P_{resin} availability extend for 14 d in C_m and C_d treatments. This suggested that plant P availability could be reduced by DRW in this soil.

The variability of MBC in the Hamilton soil made it difficult to assess DRW impacts on microbial biomass size (Figure 6.7). However, the high levels of SOM in the Hamilton soil could mean that there is sufficient C for microbes and therefore microbial biomass size may be limited by other nutrients, such as N or P. During the first moist incubation period (and to a lesser degree in the second period), the C:P ratio of the

microbial biomass in the Hamilton soil decreased strongly. This P immobilisation was likely to be the reason for the stable P_{resin} concentrations, as any P which was released during DRW would have been taken up by the microbial biomass. This is in contrast to the Crystal Brook soil, where MBP did not increase after DRW. On the other hand, in the Crystal Brook soil large increases in C:P occurred during the second moist incubation period. This indicates that microbial biomass within this soil was limited by C and not P, and that C mineralisation during moist incubation was facilitating microbial growth. Increases in growth of the microbial biomass with DRW have been reported and are suggested to be due to adaptation of the microbes present within the soil (Fierer and Schimel 2002). The responses in microbial biomass did not differ between pre-treatments water regimes. Also, since large fluctuations in biomass size and C:P occurred in the Crystal Brook soil, the microbial biomass in this soil (from a drier climate) did not appear to be any better adapted than the biomass present within the Hamilton soil (from a wetter climate).

6.4.3 Changes in microbial community composition

The pre-treatment soil water regimes significantly altered the microbial community composition in the Hamilton soil (Figure 6.9). The H_m treatment was clearly differentiated from the other treatments that had been subjected to DRW during pre-treatment. However, there were no differences in microbial community composition between H_d, H_{int} and H_{fb} although their DRW interval (1, 3 and 15 wks) and number of rewetting events (1 and 3) differed. Therefore, it did not appear as though increasing the duration or number of DRW cycles had an effect on microbial community composition. The second DRW event only had a significant influence on microbial community composition in the H_d and H_m treatments, which had been subjected to fewer DRW cycles. This confirmed that it is mainly the presence or absence of DRW events, but not their frequency that influence microbial community composition. In the Hamilton soil, DRW did not cause obvious changes in the abundance of certain microbial groups.

For the Crystal Brook soil, the changes in PLFA that occurred after DRW were not as marked as in the Hamilton soil (Figure 6.10). Lundquist *et al.* (1999b) showed that DRW-induced changes in soil microbial communities in surface layers were minimal

and suggested that adaptation to DRW exists in surface soils. Although the microbial community composition appeared to be largely unaffected by DRW, the total biomass and C:P are changed, which may suggest that all species within the community were affected. However, it has been suggested that microbial communities in soils under intensive agriculture, such as Crystal Brook, are less robust than those from less disturbed grassland systems (such as Hamilton) due to greater microbial diversity (Fierer *et al.* 2003; Steenwerth *et al.* 2005). This does not appear to apply to the current study as the Hamilton soil showed the largest change in community composition.

In the current study it was not possible to determine if changes in specific groups of organisms occurred after DRW. Both C_{fb} and C_m treatments showed the greatest changes in microbial community composition and there was an indication of increased abundance of fungi. Steenwerth *et al.* (2005) also showed that in agricultural soils no specific PLFA markers were found to be linked to changes in microbial community after DRW. A number of studies have demonstrated the effect of DRW on total microbial biomass however, specific changes in the fungi:bacteria are conflicting (Gordon et al. 2008; West et al. 1987). It has been suggested that fungi and G+ve bacteria have a greater capacity to survive DRW due to the inherent properties of their cell walls (Harris 1981; Schimel *et al.* 1999). The greater survival of fungi after DRW has been shown in previous studies (Cosentino *et al.* 2006). However, even though it is not possible to link changes in microbial communities with specific organism groups, DRW-induced changes in community composition still occurred. Changes in soil microbial communities exposed to multiple DRW events have been shown to reduce functional diversity and decomposition rates (Schimel *et al.* 1999). Although pre-treatment soil water regimes appeared to have caused functional changes, indicated by different phosphatase and respiration rates, these were not accompanied by shifts in specific groups of organisms. However, only broad groups of organisms are differentiated by PLFA and therefore species changes within these groups remain undetected. Further work examining the effects of DRW would need to consider molecular techniques that can better link changes in soil microbial communities with changes in ecosystem function (Nocker *et al.* 2007).

6.5 CONCLUSIONS

Pre-treatment soil water regime altered the patterns of microbial respiration during subsequent DRW cycles. The largest CO_2 flushes at rewetting occurred in the soil that had been dry for the longest period. Altering the distribution of the 3 DRW events during the pre-treatment regime (intermittent / false break) did not influence the flush at the subsequent rewet event. Therefore, short-term drying intervals (1 and 3 wks) appeared to have similar effects on microbial respiration. Available nutrients (C and P) and microbial biomass were not affected by long-term water regime history. However, in one soil P availability decreased after rewetting; therefore it remains to be determined if these changes have implications for plant P availability or leaching. Microbial biomass size was also little affected by long-term water regime, however large changes in C:P occurred during periods of moist incubation. Shifts in microbial C:P may indicate changes in microbial physiology and community composition. PLFA analyses showed that the greatest change in microbial community composition occurred in the Hamilton soil. Indigenous microbial communities within the Crystal Brook soil may be more adapted to DRW given the greater exposure to naturally occurring DRW events, however since microbial biomass was altered it would appear that all species within the community were affected. In addition, the disparate respiration responses between pre-treatment soil water regimes and inhibition of phosphatase activity by DRW, suggest that functional changes occurred. These are not sufficiently explained by measured nutrient pools, microbial biomass size or changes in microbial community composition using PLFA. The use of plants as bio-indicators of P availability may be better than extraction based methods to quantify the effects of long-term water regime on soil P. Extraction based methods may not reflect actual plant P availability and unlike plants do not integrate temporal changes in P.

Chapter 7

Determining the changes in phosphorus availability after long-term drying and rewetting using a bio-assay

Chapter 7. Determining the changes in phosphorus availability after long-term drying and rewetting using a plant bio-assay

7.1 INTRODUCTION

Drying and rewetting (DRW) is known to be important for the turnover of soil organic matter and nutrients (Fierer and Schimel 2002; Franzluebbers *et al.* 2000; Turner and Haygarth 2003). Changes in phosphorus (P) availability following DRW in the other experiments described in this thesis have varied, with no change in soluble P (Chapter 3), increases in resin extractable P (P_{resin}) and dissolved organic P (DOP) (Chapter 4) and increases in P_{resin} and soluble inorganic P (Chapter 5). While these studies showed that DRW may alter P availability in the short-term, in most cases this P was rapidly immobilized by the surviving microbes after rewetting. A subsequent study to investigate the long-term effects of water regime history, showed that neither nutrient availability nor microbial biomass size were altered in the long-term (Chapter 6). However, the pre-treatment water regimes showed disparate responses in microbial respiration at rewetting and phosphatase activity was inhibited by DRW. This suggested that functional changes in these soils had occurred due to the water regimes. However, temporal changes in P in various soil extracts may not adequately reflect P availability to plants. Extraction-based methods to estimate P availability may over or under estimate actual plant P availability. Also, temporal changes in plant P availability may not be well defined by extractions as these are point measurements. The use of wheat seedlings as a bio-indicator of P availability may provide an integrative estimation of any actual differences in the capacity of a soil to provide P to plants.

The experiment described in this chapter aimed to (i) quantify P plant availability using wheat seedlings and to (ii) determine if P pools (root, shoot, P_{resin}, microbial biomass P, total dissolved P and dissolved organic P) are influenced by pre-treatment water regime. It tested the hypothesis that long-term soil pre-treatment water regimes altered nutrient availability and hence would affect plant biomass.

7.2 METHODS

7.2.1 Overview

Soils were collected from two field sites representing different climatic histories (as described in Chapter 6). Soil from each site was subject to 4 different pre-treatment soil water regimes over a 14 wk period; constant field capacity; 3 DRW events evenly spaced (intermittent); 3 DRW events at the end of incubation (false break) and constantly air-dry (Chapter 6). Young wheat seedlings were used to determine P bioavailability. Seedlings were planted at 1 h and 14 d after rewetting following either the first or the second DRW event.

7.2.2 Field sampling and processing

Field sampling and soil preparation was performed as previously described (Chapter 6.2.2). Field capacity (θ_{fc}) was determined for each soil as outlined in Chapter 6.2.3.

7.2.3 Pre-treatment soil water regimes

Soils (1.8 kg) were placed in plastic containers (36 cm x 23 cm) and adjusted to θ_{fc}. Soil was lightly packed to give an approximate bulk density of 1.4 g/cm^3. For each soil, single containers were used for each of the 4 pre-treatment water regimes (field capacity, DRW intermittent, DRW false break and air dry) (Table 6.2, Chapter 6) representing soil water conditions commonly experienced during summer periods in southern Australia (8 containers in total). The field capacity treatment was kept at constant water content by covering with a lid and adjusting the water content gravimetrically when necessary. For each of the DRW treatments, lids were removed and soil was allowed to air-dry. At rewetting the soils were returned to θ_{fc} gravimetrically using RO water and mixed by hand to distribute the water evenly. To remove the effect of the disturbance due to mixing, moist incubated soils were also mixed at the same time. During periods of moist incubation containers were covered with lids. All containers were periodically opened to allow ventilation. The 4 pre-treatment water regimes and abbreviations used were as follows (Table 7.1).

Table 7.1: Pre-treatment water regimes and abbreviations

	Field capacity	DRW intermittent	DRW false break	Air-dry
Hamilton	H_m	H_{int}	H_{fb}	H_d
Crystal Brook	C_m	C_{int}	C_{fb}	C_d

7.2.4 Experimental drying and rewetting

To determine how each soil pre-treatment water regime responded to DRW, all pre-treatments (except H_m/C_m) were allowed to air-dry for one week (Hamilton 3.5 g g^{-1} and Crystal Brook 3.1 g g^{-1}). After this point, the soils were rewet, thoroughly mixed and 70 g of each soil were transferred into 50 ml Falcon® tubes for planting (denoted 1 h). The remaining soil was lightly packed in the tub and incubated at θ_{fc} for 14 d. At 14 d, 70 g soil from each pre-treatment water regime was removed as before and transferred to a new set of 50 ml Falcon® tubes with 4 replicates (denoted 14 d).

The remaining soil was divided in half and either kept at θ_{fc} or dried. Drying was achieved by removing lids from containers and allowing the soil to air-dry. Drying continued for 7 d after which the soils were rewet to θ_{fc}. Falcon® tubes were prepared once again at 1 h and 14 d as above for both experimental DRW treatments and 4 pre-treatments (4 replicates). All incubations (except those with seedlings) were carried out at 25°C. A summary of the experimental DRW and planting times are as follows (Table 7.2).

Table 7.2: Experimental DRW for Hamilton (H) and Crystal Brook (C) soils. Seedlings were planted at 1 h and 14 d after the two DRW events (weeks 16 and 19).

Week	DRW	H_m/C_m		H_{int}/C_{int}		H_{brk}/C_{brk}		H_d/C_d	
15	1	FC	FC	AD	AD	AD	AD	AD	AD
16		FC	FC	FC	FC	FC	FC	FC	FC
17		FC	FC	FC	FC	FC	FC	FC	FC
18	2	FC	AD	FC	AD	FC	AD	FC	AD
19		FC	FC	FC	FC	FC	FC	FC	FC
20		FC	FC	FC	FC	FC	FC	FC	FC

FC = Field capacity; AD = Air dry

7.2.5 Preparation and growth of wheat seedlings

Wheat seedlings were used as a bio-assay of P availability. Wheat cv. Goldmark was used as it is considered to be P efficient (Osborne and Rengel 2002). Wheat seeds were surface sterilized using sodium hypochlorite (30% v/v), then rinsed 4 times with RO water and imbibed in the last change of water overnight on a flat-bed shaker. After 24 h the germinated seeds were rolled in sheets of waxed paper (10 cm^2), stood in 1 cm of water to keep wet and allowed to grow under natural light for 5 d. At planting the seedlings were removed from the paper, the seed was excised and 1 seedling was transplanted in each Falcon® tube containing soil. Seedlings were grown in a growth room with 16 h light/8 h dark cycles and mean minimum and maximum temperatures of 16°C and 23°C respectively for 26 d. The water content of the planted Falcon® tubes was maintained at θ_{fc} with RO water each day.

7.2.6 Harvesting wheat seedlings

At harvest, shoots were cut at the soil surface. Soil was tipped out of the tubes and roots carefully removed. Root material was washed with RO water and excess water removed. Root and shoot fresh weights were determined immediately after root washing. Plant samples were then dried for 3 d at 45°C after which the samples were weighed again to obtain the dry weight (DW).

7.2.7 Microbial biomass and nutrient analyses

At the start of the experimental period, dried soils were rewet to θ_{fc}, all soils were mixed by hand and samples were taken for microbial biomass P (MBP), resin P (P_{resin}) (Chapter 2.4), microbial biomass C (MBC) (Chapter 2.3), extractable organic C (EOC) (Chapter 2.5) and total dissolved P (TDP) (Chapter 2.8). Samples for soil nutrients and microbial biomass were also taken at planting (1 h and 14 d after DRW) and when plants were harvested.

7.2.8 Tissue phosphorus determination

Dried shoot and root samples were digested in 7 ml of 6:1 nitric: perchloric acids and the P concentration of the digests was determined photometrically using a phospho-molybdate reagent (Hanson 1950).

7.2.9 Phosphorus budget

Phosphorus budgets, per tube, were calculated for each pre-treatment soil water regime to determine the relationship between the size of the labile P pool at planting and its distribution in different soil (MBP, P_{resin} and TDP) and plant (shoot and root P content) fractions at harvest. Labile P was calculated the sum of MBP, P_{resin} and TDP. Phosphorus recovery was calculated to determine if the labile P present at planting was recovered within labile P and shoot and root P content at harvest.

7.2.10 Statistical analyses

For each soil (Hamilton and Crystal Brook), 3-way analyses of variance (ANOVA) was used to determine the effects of pre-treatment soil water regime x experimental DRW x sampling time on soil chemical properties using GENSTAT 8th Edition (Lawes Agricultural Trust). Significant differences (P<0.05) between means were then tested using a post-hoc Tukey test.

7.3 RESULTS

7.3.1 Plant growth

Plant growth in the Hamilton soil was much lower than in the Crystal Brook soil (Figure 7.1). Growth of seedlings planted at 1 h after rewetting in the Hamilton soil was greater in DRW pre-treatments compared to the moist incubated control, however this was only significant for shoot DW in H_d and root DW in H_{int} (Figure 7.1). When seedlings were planting 14 d after rewetting, plant growth was less than when the seedlings were planted at 1 h. There was no significant difference in plant growth between pre-treated Hamilton soils when planted at 14 d after rewetting. In the Crystal Brook soil, shoot DW was reduced in the C_d pre-treatment compared to other moisture pre-treatments. In contrast to the Hamilton soil, plant growth was increased in the Crystal Brook soils when seedlings were planted at 14 d after DRW compared to planting at 1 h. No significant differences in plant growth were observed between pre-treatment water regimes for seedlings planted at 14 d (Figure 7.1).

After the second experimental DRW, there was no significant difference in the growth of seedlings between pre-treatment water regime or experimental DRW treatments for either Hamilton or Crystal Brook soils (Figure 7.2). However, in the Hamilton soil, plant growth was greater if the seedlings were planted at 14 d after the experimental DRW than when planted at 1 h. Also, when planted at 14 d, the growth of the seedlings appeared to be affected by pre-treatment water regime with dry matter production being H_d>H_{fb}>H_{int}>H_m. In the Crystal Brook soil, there appeared to be an effect of the pre-treatment water regime, with C_{int}, C_{fb} and C_d having lower growth than C_m when planted at 1 h or 14 d after the experimental DRW (Figure 7.2).

Tissue P concentration was not significantly different between pre-treatment water regime or experimental DRW for either soil (Table 7.3 and Table 7.4). Therefore, total P content of shoot and root tissue (Appendix 7.1 and 7.2) showed the same relationships as plant biomass.

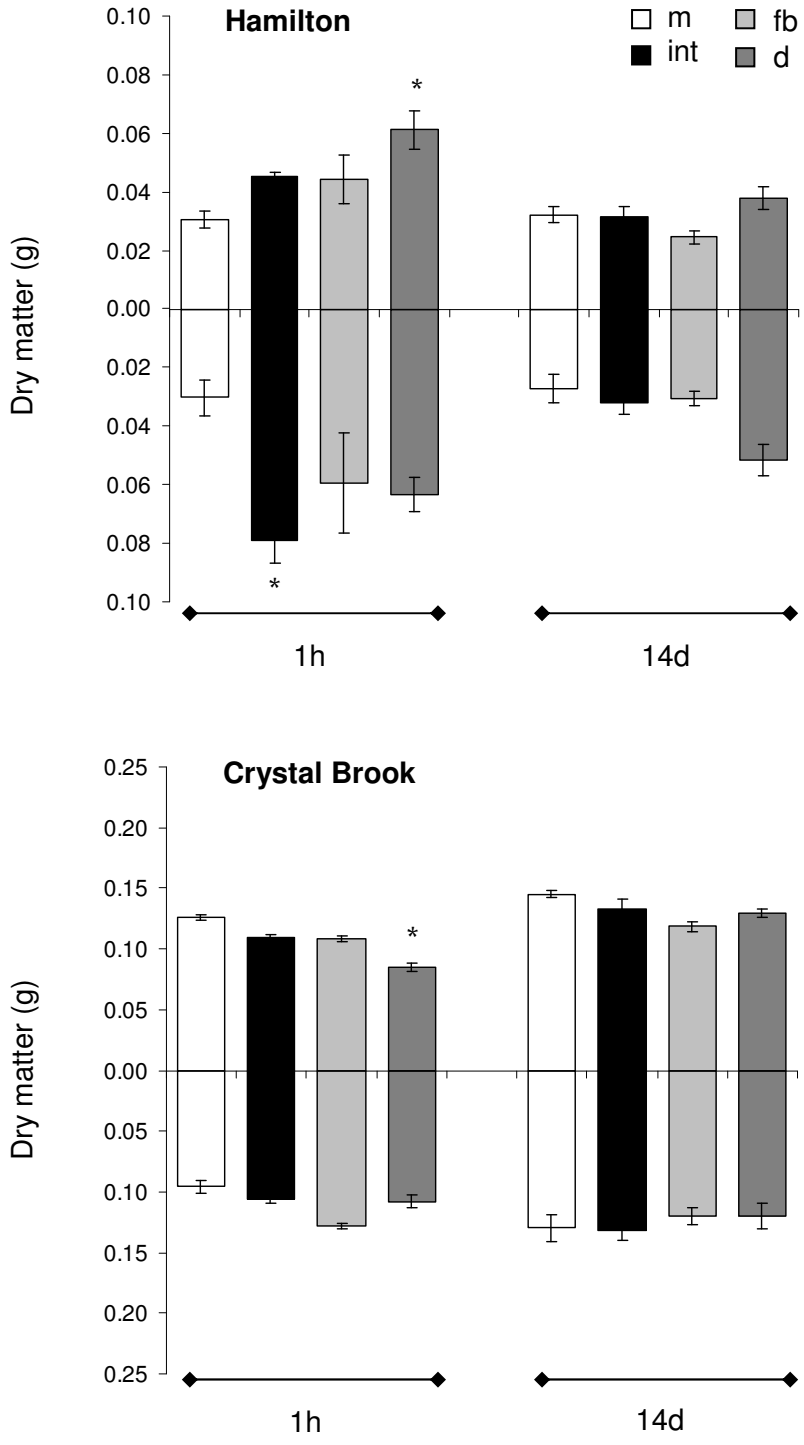

Figure 7.1: Shoot (above) and root (below) dry matter (g) of wheat seedlings planted in Hamilton (top) and Crystal Brook (bottom) soils at 1 h and 14 d after the first DRW with four pre-treatment soil water regimes (m, int, fb and d; Table 7.1). Bars indicate standard error of the mean (n=4). Significant differences (*) between treatments and moist controls (P<0.05).

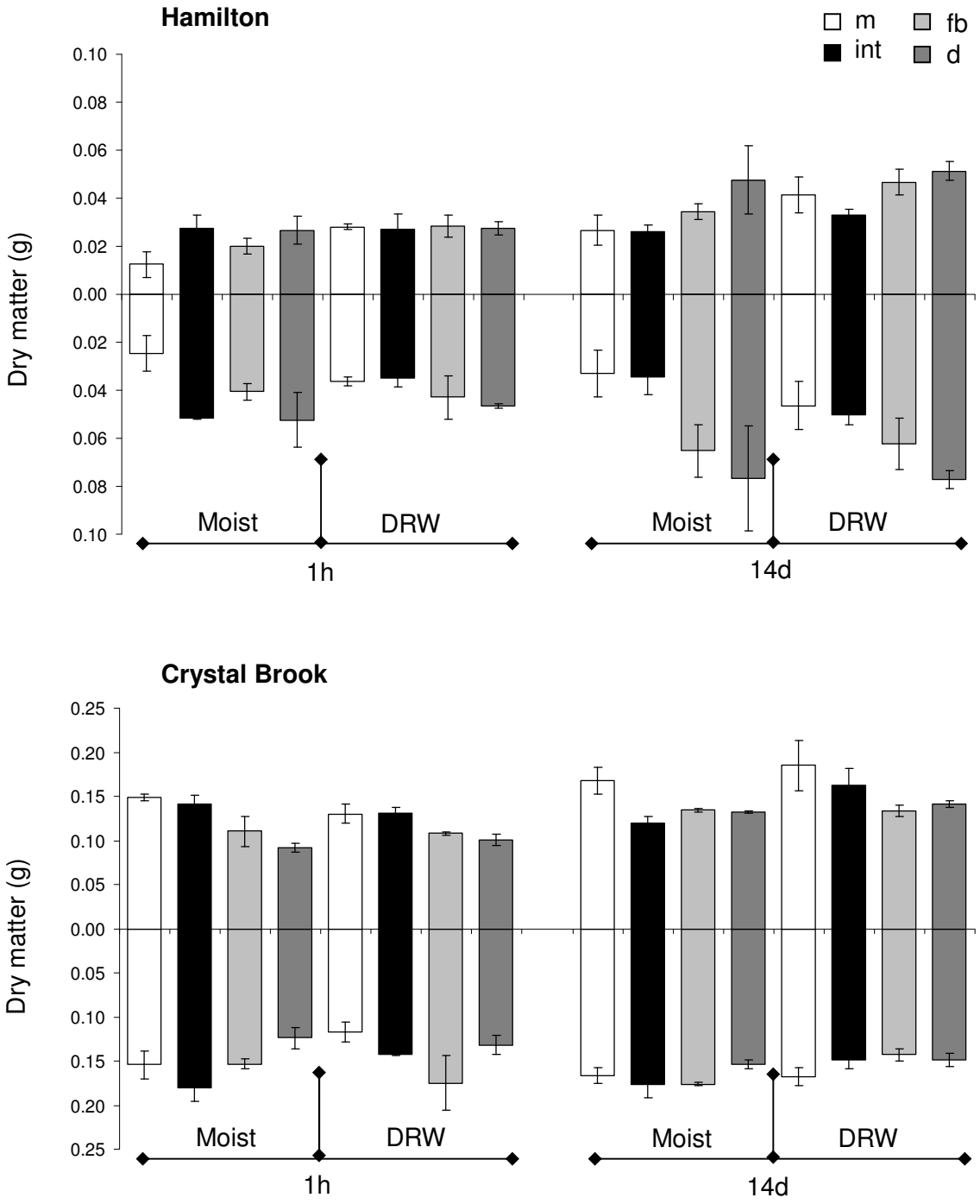

Figure 7.2: Shoot (above) and root (below) dry matter (g) of wheat seedlings planted in Hamilton (top) and Crystal Brook (bottom) soils at 1 h and 14 d after the second DRW or moist controls with four pre-treatment soil water regimes (m, int, fb and d; Table 7.1). Bars indicate standard error of the mean (n=4).

Table 7.3: Shoot and root P concentrations (mg g^{-1}) of wheat seedlings planted in Hamilton (H) and Crystal Brook (C) soils at 1 h and 14 d after the first DRW with four pre-treatment soil water regimes (m, int, fb and d; Table 7.1).

Pre-treat	Sampling	P_{shoot} (mg g^{-1})	P_{root} (mg g^{-1})
H_m	1 h	0.87 ± 0.02	1.25 ± 0.07
H_{int}	1 h	0.80 ± 0.11	0.97 ± 0.05
H_{fb}	1 h	0.98 ± 0.10	1.21 ± 0.15
H_d	1 h	1.04 ± 0.21	1.16 ± 0.07
C_m	1 h	2.75 ± 0.11	1.19 ± 0.29
C_{int}	1 h	1.95 ± 0.45	2.02 ± 0.08
C_{fb}	1 h	2.41 ± 0.08	1.43 ± 0.24
C_d	1 h	1.77 ± 0.37	1.68 ± 0.07
H_m	14 d	1.18 ± 0.04	2.32 ± 0.36
H_{int}	14 d	1.18 ± 0.12	1.23 ± 0.23
H_{fb}	14 d	0.95 ± 0.10	1.37 ± 0.27
H_d	14 d	0.78 ± 0.27	1.28 ± 0.11
C_m	14 d	2.88 ± 0.21	2.08 ± 0.14
C_{int}	14 d	2.77 ± 0.18	2.03 ± 0.15
C_{fb}	14 d	3.27 ± 0.06	0.66 ± 0.08
C_d	14 d	3.26 ± 0.08	n.d

Values are mean ± standard error (n=4)
H_m and C_m were not DRW
n.d. = no data

Table 7.4: Tissue P concentrations (mg g^{-1}) of wheat seedlings grown in Hamilton (H) and Crystal Brook (C) soils planted at 1 h and 14 d after the second DRW or moist controls with four pre-treatment soil water regimes (m, int, fb and d; Table 7.1).

Pre-treat	Sampling	Experimental water regime	P_{shoot} (mg g^{-1})	P_{root} (mg g^{-1})
H_m	1 h	Moist	1.04 ± 0.21	0.54 ± 0.14
H_{int}	1 h	Moist	1.01 ± 0.30	0.98 ± 0.03
H_{fb}	1 h	Moist	0.42 ± 0.18	0.73 ± 0.15
H_d	1 h	Moist	0.92 ± 0.06	1.04 ± 0.09
C_m	1 h	Moist	2.84 ± 0.11	1.94 ± 0.13
C_{int}	1 h	Moist	2.61 ± 0.07	1.90 ± 0.06
C_{fb}	1 h	Moist	2.94 ± 0.10	1.87 ± 0.06
C_d	1 h	Moist	2.86 ± 0.12	1.82 ± 0.11
H_m	1 h	DRW	0.71 ± 0.25	1.23 ± 0.04
H_{int}	1 h	DRW	0.89 ± 0.11	1.15 ± 0.06
H_{fb}	1 h	DRW	0.63 ± 0.17	1.07 ± 0.03
H_d	1 h	DRW	0.53 ± 0.21	0.97 ± 0.01
C_m	1 h	DRW	3.19 ± 0.35	2.07 ± 0.19
C_{int}	1 h	DRW	2.59 ± 0.14	1.79 ± 0.03
C_{fb}	1 h	DRW	3.03 ± 0.27	1.90 ± 0.27
C_d	1 h	DRW	3.56 ± 0.10	2.10 ± 0.26
H_m	14 d	Moist	0.98 ± 0.01	1.08 ± 0.19
H_{int}	14 d	Moist	1.04 ± 0.08	1.36 ± 0.10
H_{fb}	14 d	Moist	1.15 ± 0.03	1.10 ± 0.03
H_d	14 d	Moist	1.22 ± 0.28	1.32 ± 0.14
C_m	14 d	Moist	3.10 ± 0.27	2.73 ± 0.27
C_{int}	14 d	Moist	2.74 ± 0.14	2.74 ± 0.02
C_{fb}	14 d	Moist	2.88 ± 0.06	2.81 ± 0.11
C_d	14 d	Moist	2.88 ± 0.06	2.90 ± 0.24
H_m	14 d	DRW	1.64 ± 0.19	2.15 ± 0.13
H_{int}	14 d	DRW	1.49 ± 0.08	1.81 ± 0.12
H_{fb}	14 d	DRW	1.42 ± 0.16	1.74 ± 0.09
H_d	14 d	DRW	1.33 ± 0.06	1.68 ± 0.14
C_m	14 d	DRW	1.56 ± 0.11	1.55 ± 0.04
C_{int}	14 d	DRW	1.10 ± 0.04	1.54 ± 0.10
C_{fb}	14 d	DRW	1.09 ± 0.03	1.66 ± 0.09
C_d	14 d	DRW	1.32 ± 0.04	1.49 ± 0.07

Values are mean ± standard error (n=4)

7.3.2 Nutrient availability and phosphorus budget

In both Hamilton and Crystal Brook soils, initial labile P (MBP + P_{resin} + TDP) was higher at 14 d than 1 d (Table 7.5). Phosphorus budgets showed that labile P recovery was reduced if planting was delayed. Plants only took-up a small proportion of the labile P that was available at planting (4.5% for Hamilton and 12.3% for Crystal Brook), so most of the labile P was retained in the soil (Table 7.5) Also, it appeared as though the percentage of labile P present at the start recovered in MBP was reduced when planting was delayed. In the Hamilton soil, a higher proportion of labile P was recovered in plant tissue in H_{int}, H_{fb} and H_d compared to H_m when seedlings were planted at 1 h after DRW. However, when planting was delayed until 14 d after DRW, the effect of the pre-treatments on recovery of labile P in plant tissue, present at the start, were not observed. In the Crystal Brook soil, the effect of the water pre-treatments on labile P recovery in plant tissue was opposite to the Hamilton soil. The C_{int}, C_{fb} and C_d treatments had lower proportions of labile P in plants than C_m and indicated a negative effect of the DRW pre-treatments on plant P availability. Also, unlike the Hamilton soil, the effect of the DRW pre-treatment was still present in the Crystal Brook soil when the seedlings were planted at 14 d.

After the second experimental DRW event the greatest effect of the water pre-treatments was on percentage MBP (Table 7.6). Percentage MBP was higher in H_{int}, H_{fb} and H_d than H_m and this was observed for both experimental DRW treatments (moist/DRW) and both planting times. The H_m treatments had a higher proportion of labile P present as P_{resin} compared to H_{int}, H_{fb} and H_d. Therefore, in the Hamilton soil it appeared as though the DRW pre-treatments increased the microbial biomass, reduced P_{resin} and increased the amount of labile P that ended up in plant tissue. The microbial biomass obtained a higher percentage of the labile P than the wheat seedlings.

In the Crystal Brook soil after the second experimental DRW event, soil P pools were not affected by water pre-treatments (Table 7.7). However, the proportion of labile P recovered in plant tissue was reduced by the DRW pre-treatments when seedlings were planted at 1 h after rewetting. The percent of labile P recovered in plant tissue was higher in C_m than C_{int}, C_{fb} and C_d in both experimental DRW (moist/DRW) and moist incubated treatments. However, when planting was delayed to 14 d the effect of

the water pre-treatment was reduced. Compared to the Hamilton soil, the percentage of labile P recovered in plant tissue was higher in the Crystal Brook soil. The proportion of labile P present at the start of the plant bio-assay, which was recovered in MBP within the Crystal Brook soil, was less than Hamilton soil.

Table 7.5: Allocation of P within soil (MBP, P_{resin} and TDP) and plant (P_{shoot} and P_{root}) pools at harvest, determined as a percent (%) of the labile P at planting and calculated for Hamilton (H) and Crystal Brook (C) soils with four pre-treatment soil water regimes (m, int, fb and d; Table 7.1).

	Labile P[a] at planting (mg P)	Recovery[b] (%)	MBP[c] (%)	P_{resin}[d] (%)	TDP[e] (%)	P_{shoot}[f] (%)	P_{root}[g] (%)	P_{plant}[h] (%)
1h								
H_m	1.9	95.7	48.8	42.4	1.2	1.4	1.9	3.4
H_{int}	1.9	94.8	46.6	41.3	1.1	1.9	3.9	5.8
H_{fb}	2.2	69.3	20.4	42.7	0.9	1.9	3.4	5.3
H_d	2.2	86.2	49.2	30.0	0.8	2.9	3.3	6.2
14d								
H_m	2.2	68.8	18.3	44.7	1.3	1.7	2.8	4.5
H_{int}	2.3	63.3	25.8	33.2	1.1	1.6	1.6	3.2
H_{fb}	2.6	69.8	34.1	30.0	1.2	0.9	3.6	4.5
H_d	2.9	63.7	33.5	26.3	0.7	0.9	2.2	3.2
1h								
C_m	3.5	91.9	10.8	66.1	1.9	9.9	3.2	13.1
C_{int}	3.4	88.0	11.9	62.8	1.8	5.8	5.7	11.6
C_{fb}	3.6	87.7	4.6	69.0	1.8	7.2	5.1	12.3
C_d	3.7	83.4	8.9	64.0	1.4	4.1	5.0	9.1
14d								
C_m	4.5	79.0	8.5	53.6	1.7	9.2	6.0	15.2
C_{int}	4.4	81.8	6.4	59.5	1.5	8.4	6.0	14.4
C_{fb}	4.5	75.5	7.7	55.9	1.5	8.7	1.7	10.4
C_d	4.7	70.7	8.6	51.6	1.4	9.1	n.d.	n.d.

[a] Labile P is the sum of MBP, P_{resin} and TDP in the soil at planting
[b] Labile P + plant biomass P at harvest as a proportion of the labile P at planting
[c] MBP, [d]P_{resin}, [e]TDP, [f]P_{shoot}, [g]P_{root}, [h]P_{plant} at harvest as a proportion of the labile P at planting
n.d. = no data

Table 7.6: Allocation of P within soil (MBP, P_{resin} and TDP) and plant (P_{shoot} and P_{root}) pools at harvest, determined as a percent (%) of the labile P at planting and calculated for Hamilton (H) soil with four pre-treatment soil water regimes (m, int, fb and d; Table 7.1) either DRW or moist incubated and planted with wheat seedlings at 1 h or 14 d after DRW

	Labile P[a] at planting (mg P)	**Recovery[b] (%)**	**MBP[c] (%)**	**P_{resin}[d] (%)**	**TDP[e] (%)**	**P_{shoot}[f] (%)**	**P_{root}[g] (%)**	**P_{plant}[h] (%)**
1 h								
H_m Moist	1.7	85.1	17.4	63.9	2.1	0.8	0.9	1.7
H_{int} Moist	1.7	94.9	44.7	44.2	1.5	1.5	2.9	4.4
H_{fb} Moist	1.8	94.5	43.7	47.4	1.4	0.4	1.6	2.0
H_d Moist	1.9	99.8	49.1	45.4	1.3	1.2	2.7	3.9
14 d								
H_m Moist	1.4	95.7	15.0	73.8	2.3	1.4	3.2	4.6
H_{int} Moist	1.5	98.7	35.5	57.1	2.0	1.5	2.6	4.1
H_{fb} Moist	1.7	99.2	44.9	49.0	1.6	1.1	2.6	3.7
H_d Moist	2.0	90.6	43.9	42.0	1.3	0.7	2.3	3.0
1 h								
H_m DRW	1.5	83.5	23.9	53.2	1.7	1.7	2.8	4.6
H_{int} DRW	1.7	84.7	35.2	43.5	1.8	1.6	2.6	4.2
H_{fb} Moist	1.8	84.8	38.1	40.0	1.6	2.2	2.8	5.0
H_d Moist	2.1	85.4	46.5	31.0	1.5	3.3	3.2	6.5
14 d								
H_m DRW	1.4	87.5	12.8	63.1	3.3	3.5	4.8	8.3
H_{int} DRW	1.5	92.7	33.5	49.8	2.4	3.4	3.7	7.0
H_{fb} Moist	1.8	88.1	36.6	40.7	1.9	5.0	4.0	9.0
H_d Moist	1.8	108.4	56.9	38.2	1.7	5.9	5.8	11.6

[a] Labile P is the sum of MBP, P_{resin} and TDP in the soil at planting

[b] Labile P + plant biomass P at harvest as a proportion of the labile P at planting

[c] MBP, [d]P_{resin}, [e]TDP, [f]P_{shoot}, [g]P_{root}, [h]P_{plant} at harvest as a proportion of the labile P at planting

Table 7.7: Allocation of P within soil (MBP, P_{resin} and TDP) and plant (P_{shoot} and P_{root}) pools at harvest, determined as a percent (%) of the labile P at planting and calculated for Crystal Brook (C) soil with four pre-treatment soil water regimes (m, int, fb and d; Table 7.1) either DRW or moist incubated and planted with wheat seedlings at 1 h or 14 d after DRW

	Labile P[a] at planting (mg P)	**Recovery[b] (%)**	**MBP[c] (%)**	**P_{resin}[d] (%)**	**TDP[e] (%)**	**P_{shoot}[f] (%)**	**P_{root}[g] (%)**	**P_{plant}[h] (%)**
1 h								
C_m Moist	3.4	99.2	11.9	63.3	2.8	12.4	8.7	21.2
C_{int} Moist	3.4	98.7	10.4	65.6	2.1	10.7	9.9	20.6
C_{fb} Moist	3.5	94.2	10.5	64.1	2.1	9.2	8.2	17.5
C_d Moist	3.0	109.6	10.3	79.1	3.7	8.8	7.7	16.5
14 d								
C_m Moist	3.6	97.2	14.6	61.3	3.3	11.4	6.6	18.0
C_{int} Moist	3.5	96.1	6.6	70.2	2.2	9.7	7.3	17.0
C_{fb} Moist	3.6	95.4	12.1	62.5	2.3	9.2	9.3	18.5
C_d Moist	3.5	94.9	9.0	65.9	2.1	10.2	7.7	17.9
1 h								
C_m DRW	3.5	105.3	8.6	66.2	2.0	14.7	13.8	28.5
C_{int} DRW	3.6	99.7	8.1	68.4	1.8	9.0	12.3	21.4
C_{fb} Moist	3.4	103.8	8.0	71.3	2.3	11.3	10.9	22.2
C_d Moist	3.6	99.8	9.6	66.7	1.6	10.5	11.3	21.8
14 d								
C_m DRW	3.2	99.1	6.9	70.0	2.8	11.2	8.1	19.3
C_{int} DRW	3.3	94.9	10.5	66.1	1.9	9.6	6.8	16.4
C_{fb} Moist	3.4	94.7	15.7	61.0	2.1	9.0	7.0	15.9
C_d Moist	3.3	93.9	7.7	69.7	2.1	7.8	6.7	14.4

[a] Labile P is the sum of MBP, P_{resin} and TDP in the soil at planting

[b] Labile P + plant biomass P at harvest as a proportion of the labile P at planting

[c] MBP, [d]P_{resin}, [e]TDP, [f]P_{shoot}, [g]P_{root}, [h]P_{plant} at harvest as a proportion of the labile P at planting

7.4 DISCUSSION

7.4.1 Plant growth

Plant growth was greater in the Crystal Brook soil than the Hamilton soil (Figure 7.1 and Figure 7.2). In addition, P concentration of plant tissue grown in Crystal Brook soil was up to 3 times higher than in Hamilton soil. Differences in plant growth and tissue P concentration reflected the higher P_{resin} in the Crystal Brook soil compared to the Hamilton soil.

Pre-treatment water regime affected the growth of wheat seedlings (Figure 7.1 and Figure 7.2). The growth of the seedlings was variable and in most cases the differences were not significant; however, there were a few important observations. The effect of DRW pre-treatments on plant growth was different in the two soils, with Hamilton showing an increase and Crystal Brook showing a decrease in plant growth when compared to moist controls (H_m and C_m). As the Hamilton soil had higher amounts of soil organic matter, the increase in growth following DRW was likely to be related to the increased availability and mineralisation of organic matter resulting in higher nutrient availability, although this was not reflected in P_{resin} or TDP (Appendix 7.4 and 7.5). The microbial biomass in H_{int}, H_{fb} and H_d after moist incubation periods was up to 3 times larger than that in the H_m soil (Appendix 7.3) which supported the idea that C availability was increased by DRW. The reduction in plant growth by pre-treatment DRW in the Crystal Brook soil was not related to P availability as P_{resin} and TDP were high within this soil (Appendix 7.4 and 7.5). However, DRW may have induced nutrient limitations, other than P to plant growth.

The length of time a soil was dried appeared to influence plant growth. In the Hamilton soil, plant growth was the higher after the first than after the second experimental DRW event and also greatest in the H_d treatment which had the longest dry period (Figure 7.1). The opposite was observed in the Crystal Brook soil. The length of the drying period is thought to be important in determining the ability of DRW to enhance or inhibit mineralisation (Lundquist *et al.* 1999a). The drying periods were the same for these soils, however the apparent effect of nutrient availability was

different. Therefore, factors other than the duration of drying such as texture and SOM content, may also appear to control the plant growth response to DRW.

7.4.2 Nutrient availability and phosphorus budget

Phosphorus budgets showed that most of the labile P present within the soil at planting remained in the soil, with only a small fraction being taken up by plants (Table 7.5, Table 7.6 and Table 7.7). During the initial period after rewetting (28 d), plants were poor competitors for the labile P pool compared to soil micro-organisms. Pre-treatment water regimes increased the percent P within the microbial biomass compared to H_m in the Hamilton soil. This was due to DRW increasing MBP (Appendix 7.3).

The influence of the water pre-treatments on plant growth appeared to be greater than the experimental DRW. For example, greater differences in plant dry matter appear to occur between water pre-treatments than either experimental DRW treatments in both soils, when seedlings were planted at 14 d (Figure 7.2). This showed that important changes in nutrient supply and availability occurred due to long-term water regimes. Further work using plants as bio-indicators of P availability should consider larger growth tubes than those used in the current experiment. This would enable plants to be grown for longer periods than the 26 d used here. Longer intervals of plant growth may be required to better quantify the actual changes in the soils capacity to supply P to plants.

7.5 CONCLUSIONS

While changes in plant available P occurred in response to soil water regimes, as indicated by the differences in growth, the large variability in plant biomass made it difficult to determine any clear implications of DRW for plant nutrient availability. Phosphorus budgets also confirmed that long-term water regimes altered the distribution of P within plant and soil pools (DRW increasing and decreasing plant growth for Hamilton and Crystal Brook respectively). However, since P_{resin} and TDP were the same between pre-treatment water regimes, this indicated that these pools

may poorly reflect actual P availability to plants. The main effect of the long-term water regimes appeared to be via altering the size of the microbial biomass and thus the competition to plants for available P. Plants were poor competitors against soil microbes for P. However, since there were differences in plant growth, it would be useful to have experimental systems (larger volumes of soil) that enabled the plants to be grown over longer periods. Although the plant bio-assay may be limited for short-term P dynamics, due to competing microbial immobilisation, it may be a more useful indicator of plant P availability over longer periods.

Chapter 8

General Discussion

Chapter 8. General Discussion

The focus of this study was to investigate and characterise the function of soil micro-organisms in P (and C) dynamics during soil drying and rewetting (DRW) events. The principal objective was to determine the significance of dry-rewet events, characteristic of the climate in southern Australia, for altering P availability and cycling in agriculturally important soils.

Soils from different climate and management histories showed that DRW is an important process for nutrient cycling within a range of environments of southern Australia. The C mineralisation (microbial respiration) response of the soils to a single DRW event could be divided into four groups; increase in both mineralisable C fraction (Co_{90h}) and proportional mineralisation rate (k); increase in k but no change in Co_{90h}; no increase in k or Co_{90h} and decrease in k with no change in Co_{90h} (Chapter 3). These responses support other studies that have shown increases (Austin *et al.* 2004; Fierer and Schimel 2003; Mikha *et al.* 2005) and no change (Degens and Sparling 1995; Magid *et al.* 1999) in total C mineralisation following DRW. While the first two responses accounted for most of the soils (40% and 53% respectively) there was no clear relationship between Co_{90h} and soil physiochemical properties. In addition, no relationship was found between k and any soil physiochemical property. The size of the flush (ΔCo_{90h} = DRW Co_{90h} – Moist Co_{90h}) was highly correlated with soil C status (total C, organic C and humus) total N, organic P, microbial biomass P and texture (sand and silt content). This suggested that negative or no change in ΔCo_{90h} to DRW may occur in soils with high sand contents (> 70%), low total C contents (around 10 g kg^{-1}), low total N (< 1 g kg^{-1}) and organic P contents (< 50 mg kg^{-1}). However, the variability in the data highlights that more information is required before such properties could be used to predict mineralisation responses of soils to DRW. Furthermore, normalisation of respiration (CO_2 flush per unit SOM) of the different soils showed that the differences in size of the CO_2 flush at rewetting are not simply a function of soil C content. Humus and particulate organic C (POC) fractions did not explain the differences in C mineralisability, highlighting that further chemical characterisation of soil C is required to determine which C compounds are being mineralised. The lack of relationship between C mineralisation fluxes and changes in nutrient concentrations showed that, in this case, CO_2 flushes were not useful to infer

changes in soil nutrient pools. In addition, significant flushes were observed in almost all soils, despite these having distinct climatic histories. Therefore, the effect of climate on DRW dynamics appears only to be important in determining the quantity of soil organic matter and the microbial biomass.

The soil C composition is important not only in determining the availability of C for mineralisation during DRW. When a soil was amended with different C substrates, likely to be present within different organic inputs, responses to DRW were altered, possibly via changes in the size and community composition of the microbial biomass (Chapter 4). When subjected to multiple DRW events, soils amended with glucose and starch showed increased mineralisation compared to moist incubated controls, while mineralisation in the soil amended with cellulose was inhibited by DRW. The different amendments altered the size of the microbial biomass and thus the amount of substrate potentially available at rewetting or, for the cellulose amended soil, altered the basal respiration rate (indicating a change in community composition), thereby reducing the difference between DRW and moist incubated controls. Furthermore, different management histories within the same soil altered the mineralisation response to DRW (Chapter 3) confirming that land-use and management may determine the response of soils to DRW.

A reduction in the size of the mineralisation flush at rewetting occurred with multiple DRW events (Chapter 4 and 6). This was not related to reductions in microbial biomass, since the biomass was greatly reduced after the first DRW event but not in subsequent events, yet the respiration flush continued to decrease. In addition, the microbial biomass was not the primary source of the respiration flush. Microbial C contributed 17 - 29% and 26 - 46% in the first and second rewetting events respectively and much less in the third DRW. Significant release of available P occurred at rewetting, but unlike C, did not reduce in size with subsequent rewetting events. The increase in available P appeared to be transient and had generally disappeared after 7 d. The differences in mineralisation between amended soils were partly due to microbial biomass size. However, the amount of C released at rewetting and its degradability were not different between soils, showing a disparate capacity of the microbes to utilise the available C. Drying/rewetting events caused changes in microbial community composition in all amended soils, which was associated with a

reduction in fungi and increase in G–ve bacteria. The size and composition of the microbial biomass is therefore a primary factor in determining the mineralisation response and nutrient availability after DRW.

Increases in mineralisation after rewetting were transient with the largest changes occurring within 48 h after rewetting (Chapter 3 and 4). Investigation of this period at 2 hourly intervals showed that increases in microbial activity were immediate and highest in the first 2 h (Chapter 5). After this point, a sharp decline in microbial activity occurred until approximately 4-6 h after rewetting followed by elevated but steadier mineralisation rates. This highlighted that examination of mineralisation rates over longer periods may underestimate the actual fluxes that occur. Phosphorus availability was increased at rewetting, however it appears as though the largest changes in available nutrients may have occurred within the first 2 h (with the flush in microbial activity) and were quite stable after this point. Therefore, the extent of the nutrient flushes within the initial 2 h could not be quantified. It would appear that any nutrients that were released within this period were rapidly taken up by the surviving microbial biomass. The P concentration of the soil solution remained elevated for 48 h however after rewetting, but it is likely that this was only a small fraction of the actual P flux that potentially occurred. In addition, results from other experiments (Chapter 3 and 4) indicate that the P seen at 48 h after rewetting is likely to have disappeared within 3 - 7 d. The P flush after rewetting would be available for plant uptake or movement with the soil water. The rapid nature of the P flushes may mean that these have greater implications for environmental water quality than availability to plants, as runoff and leaching processes would occur during the same period as the flush. However, these processes were not examined within the current study.

While the importance of short-term flushes were clearly demonstrated, different water regimes imposed over longer periods did not alter P pools, EOC or microbial biomass size (Chapter 6). However, drying interval was important in determining the size of the rewetting CO_2 flush, with the largest flushes occurring with the longest drying interval (15 wks). Shorter drying intervals (1 and 3 wks) were not different, suggesting that this may not apply for short drying periods. The period of increased mineralisation after rewetting was shorter in the Crystal Brook soil compared to Hamilton. Carbon mineralisation model fitting showed that DRW only increased

proportional mineralisation rate (k) in the Crystal Brook soil, while increased both k and mineralisable C fraction (Co_{14d}) in the Hamilton soil. In addition, smaller mineralisation flushes occurred with subsequent rewetting events confirming previous observations (Chapter 4). Therefore, mineralisation dynamics after rewetting will not only be determined by DRW events (duration and interval) but also the capacity of the perturbation to release additional C substrate for mineralisation. This will be determined by the chemical nature of SOC and the ability of soil microbes to access and mineralise this material.

Changes in P availability were generally transient after DRW and given that P pools were not altered after long-term water regimes, it appears as though inherent soil properties and management will have an over-riding influence on soil P pools (Chapter 6). However, in the short-term, decreases in P concentration in the Crystal Brook soil after the second experimental rewetting event occurred which may have implications for plant available P. This was confirmed using plants as a bio-indicator of P availability in Chapter 7 (discussed later). Changes in available P were generally smaller than seen previously (Chapter 4 and 5) and the large changes in C:P of the microbial biomass demonstrate the superior capacity of micro-organisms to immobilise any P that is released.

The microbial biomass size was resistant to differences in long-term water regime. However, long-term water regime altered microbial community composition. These changes in microbial community composition were not as marked in the Crystal Brook soil (dryer climate) compared to Hamilton soil (wetter climate). Microbial communities in soils with different DRW interval (1, 3 and 15 wks) and number of DRW events (1 and 3) were similar. Thus, although changes in soil microbial communities may occur immediately after rewetting, the ability of DRW to significantly alter microbial community structure may take longer periods (months), whereas changes in microbial biomass only appear to occur over shorter periods (weeks). Also, the changes in community composition were not related to specific groups of organisms. However, microbial respiration and phosphatase activity were altered by long-term water regimes suggesting that DRW induced changes in soil function had occurred.

Quantifying nutrient pools did not adequately define changes in plant nutrient availability that appeared to occur after long-term water regimes (Chapter 6). The use of wheat seedlings as a bio-indicator of P availability showed large variability and thus did not provide clear implications of DRW for plant P availability (Chapter 7). However, P budgets confirmed that long-term water regimes altered the distribution of P within plant and soil pools. Long-term DRW regimes increased plant growth in the Hamilton soil and decreased plant growth in Crystal Brook soil compared to constant moist controls, showing that plant P availability was altered.

The influence of climate on DRW flushes appears to be less important than soil properties and management. Soils from climatic areas that experience recurrent DRW events did not appear to be more resistant to DRW than soils from climates that are DRW less frequently (Chapters 3, 6 and 7). Also, even soils from semi-arid areas, which had low total C contents, showed significant mineralisation of C after DRW. Thus, the influence of climate of DRW flushes appears to be via determining the quantity of soil organic matter and the microbial biomass that is available for turnover, rather than the DRW regime itself. This could explain why the long-term water regimes had little effect on changing soil nutrient pools and microbial biomass (Chapters 6 and 7).

A limitation of the current experiments was the use of small cores (20 g) which require good homogenisation. Air-drying and sieving of field soil that are necessary to achieve this homogeneity, result in the loss of aggregation and will intrinsically alter the response of the soil to DRW. In addition, since temperature was intentionally removed as a factor during drying, this limited the speed at which soils could be dried. This may underestimate actual rates of drying that occur in soils within natural systems and thus alter the effect. Furthermore, the current experiments mostly only consider P and C dynamics after DRW and other nutrients, such as N may also be important in DRW and flush effects. Also, the plant bio-assay of P availability was limited by the use of small volumes of soil. Larger soil volumes would have allowed the plants to be grown for longer periods and may have allowed greater differences to be seen between the treatments.

8.1 CONCLUSIONS

DRW is an important process for P and C turnover within soils of southern Australia. Significant flushes in respiration and available nutrients were observed in a wide range of soils types from different climatic areas and management histories. The implications for P availability and C losses are significant considering the pending changes in climate with predictions of worse drought periods and higher intensity storms. The magnitude of these flushes was not simply a function of the soil C content. A more detailed knowledge of the chemical nature of the soil C pools would be required to predict C turnover during DRW events. Inherent soil properties may provide a better starting point to determine flush dynamics in soils from different areas. However, as flush dynamics were altered by land-use and management, using basic soil properties and traditionally measured 'nutrient pools' will not be sufficient to understand these processes.

Soil micro-organisms are central to flush dynamics after DRW via mineralisation, contribution to the labile nutrient pool and providing a rapid sink for P that is made available during DRW. However, despite large variation, the microbial biomass did not contribute more that 46 % of the C mineralised during a rewetting event. Phosphorus fluxes were generally short-lived, due to microbial immobilisation, although short-term changes in P availability as well as plant growth were altered by DRW. These changes in P availability during DRW will have implications for P cycling in agricultural soils. In addition, the rapid nature of P increases after rewetting implies that the potential exists for P loss with soil water as runoff or via leaching. These processes will become increasingly important in areas that experience higher storm intensity due to changes in climate.

8.2 FURTHER RESEARCH

Commonly measured soil physiochemical properties provided some explanation of the variability of mineralisation responses between different soils after DRW. However, these appeared to be insufficient to adequately define the occurrence, magnitude and duration of rewetting mineralisation flushes. Further characterisation

of the chemical structure of native SOC components than used here (Total C, MIR predictions for organic C, humus and POC pools) is required and can be achieved using ^{13}C nuclear magnetic resonance (NMR). A better understanding of the specific C compounds that are mineralised during DRW would be useful to determine the flush dynamics and also to build the capacity of simulation models to predict soil C loss/accumulation given changes in DRW regime.

The information presented here showed that DRW altered microbial community composition and potentially soil function. Further characterisation of the changes in the community composition is required to determine the resilience of indigenous microbial communities to DRW. It appears as though general characterisation of groups of organisms inferred by FAME/PLFA techniques may not have adequate specificity to detect such changes. Thus the use of genetic (DNA/RNA) techniques such as DGGE with proteomics to examine specific gene expression, may make it possible to link the changes in community structure and function.

Although it was evident that micro-organisms were extremely efficient in rapidly immobilizing P that was released during DRW, changes in P availability and plant growth suggest that DRW has implications for agricultural production systems. The use of P isotopes to quantify plant available P pools may provide a better understanding of changes in P pools during DRW. Furthermore, these research efforts only consider P turnover within soil-microbe-plant systems and do not account for environmental losses. The ability of DRW regime to alter P transfer to surface and ground water via runoff or leaching also warrants investigation.

Chapter 9

References

Chapter 9. References

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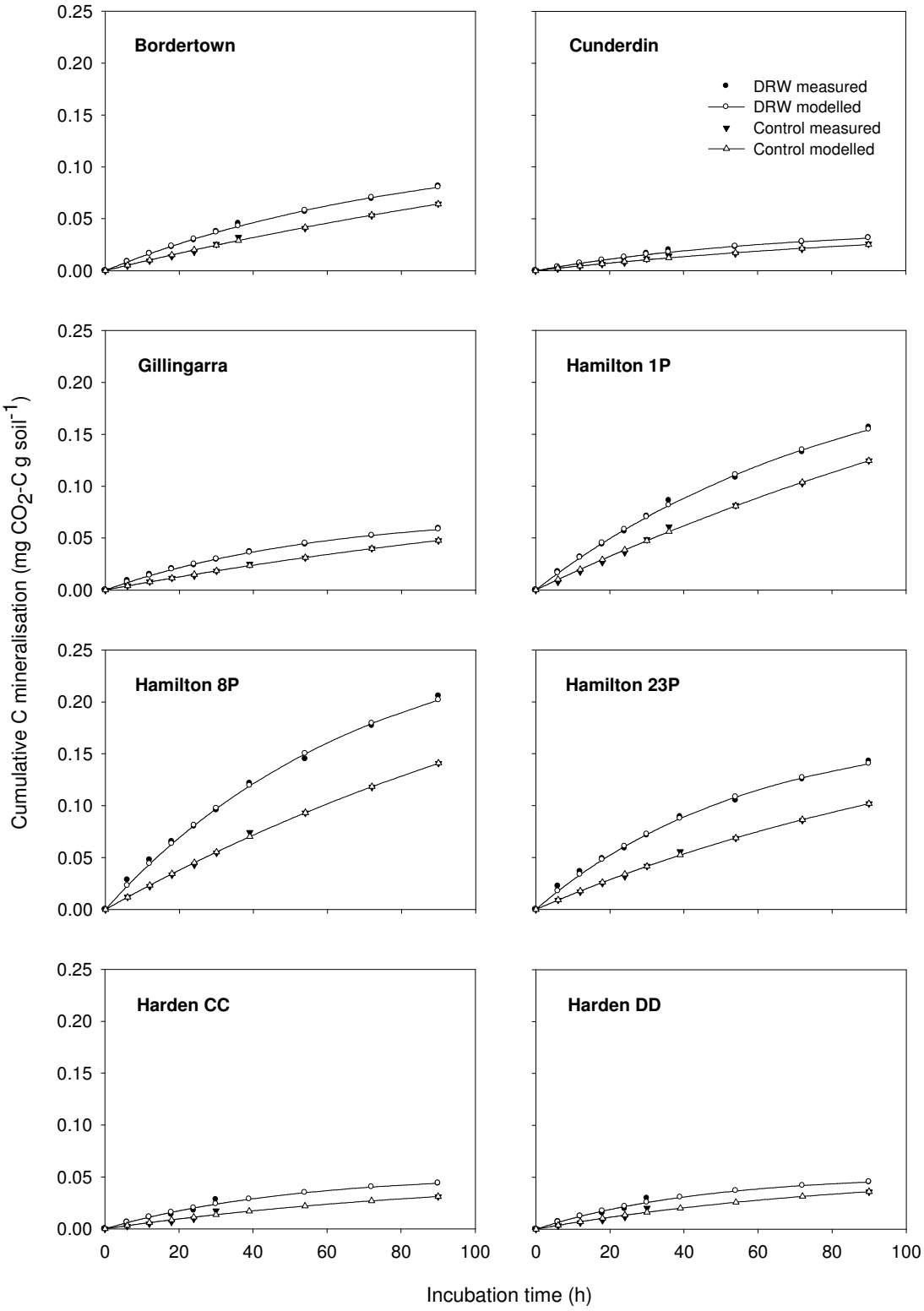
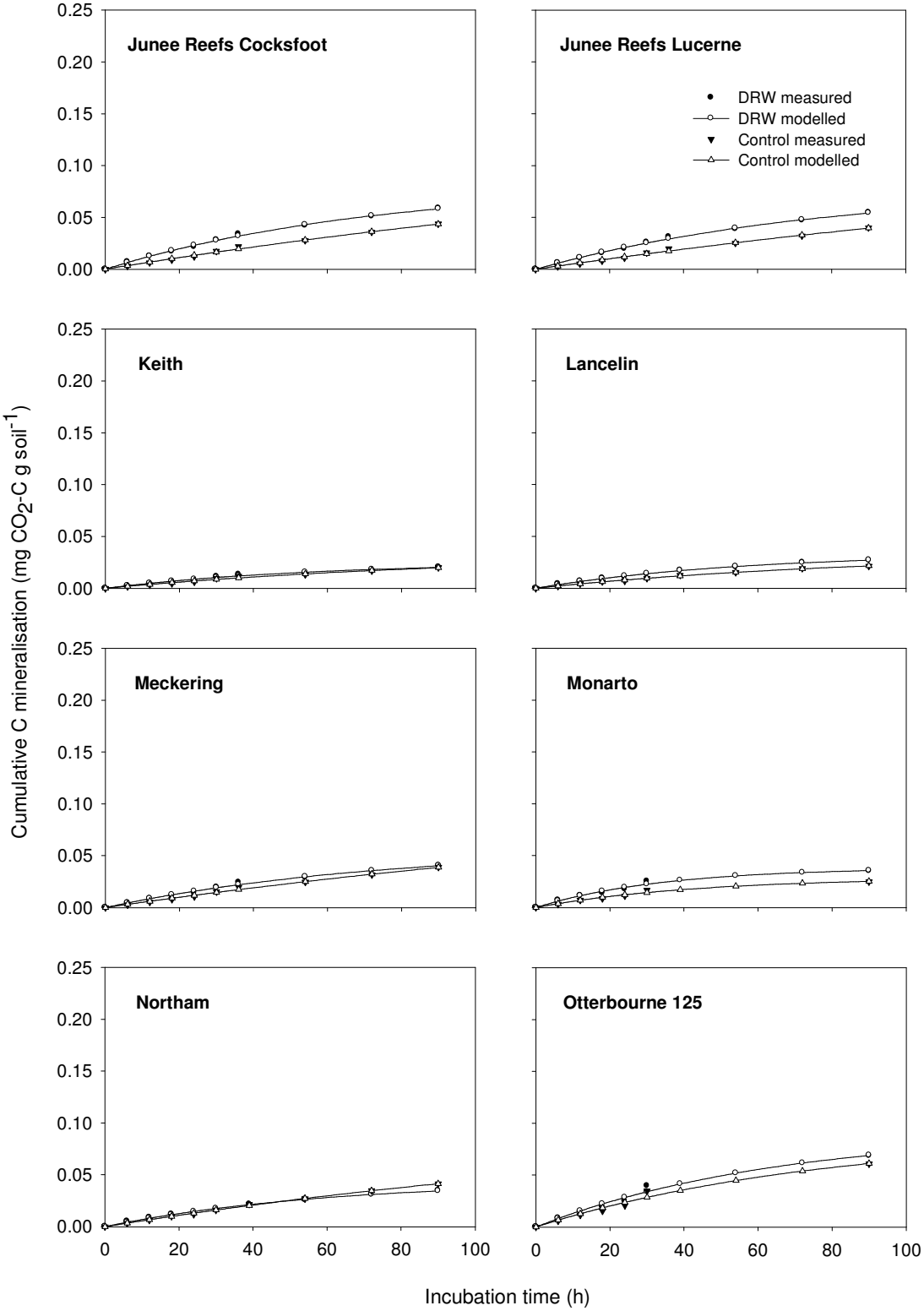
Chapter 10

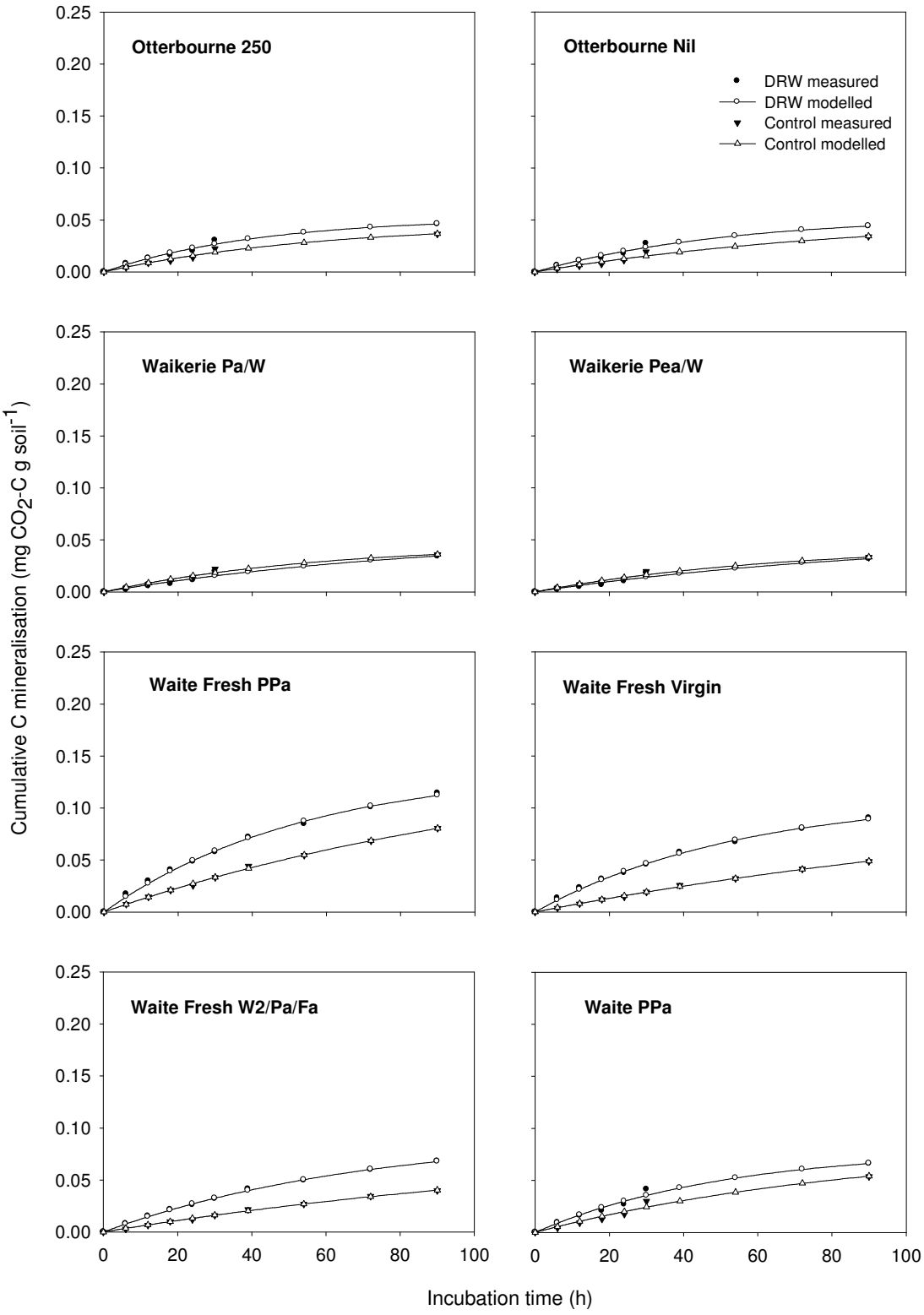
Appendices

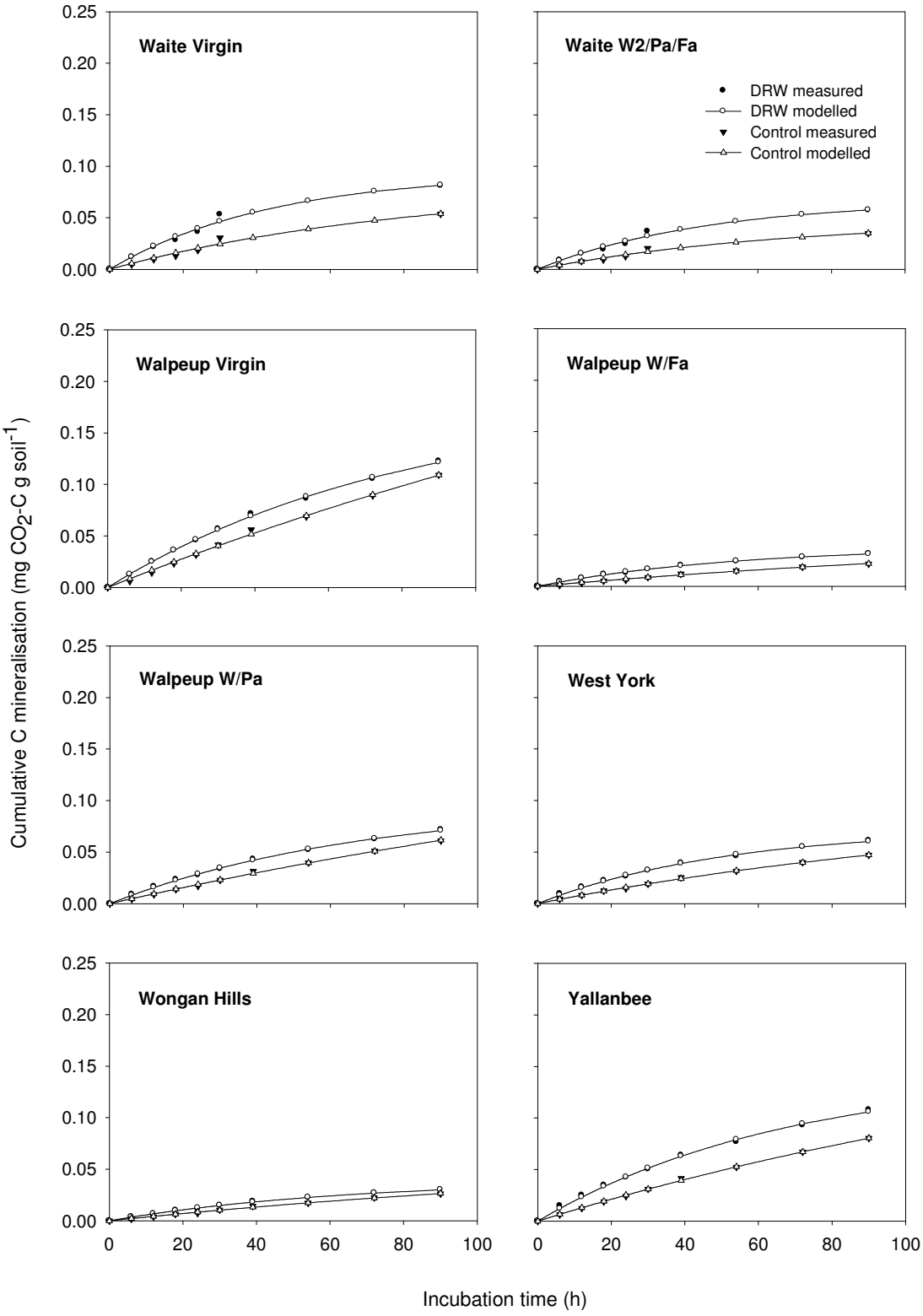
Chapter 10. Appendices

Appendix 3.1: Cumulative C mineralisation for measured (solid) and modelled (hollow) data in 32 soils subjected to DRW (circles) and constantly moist (triangles) controls.

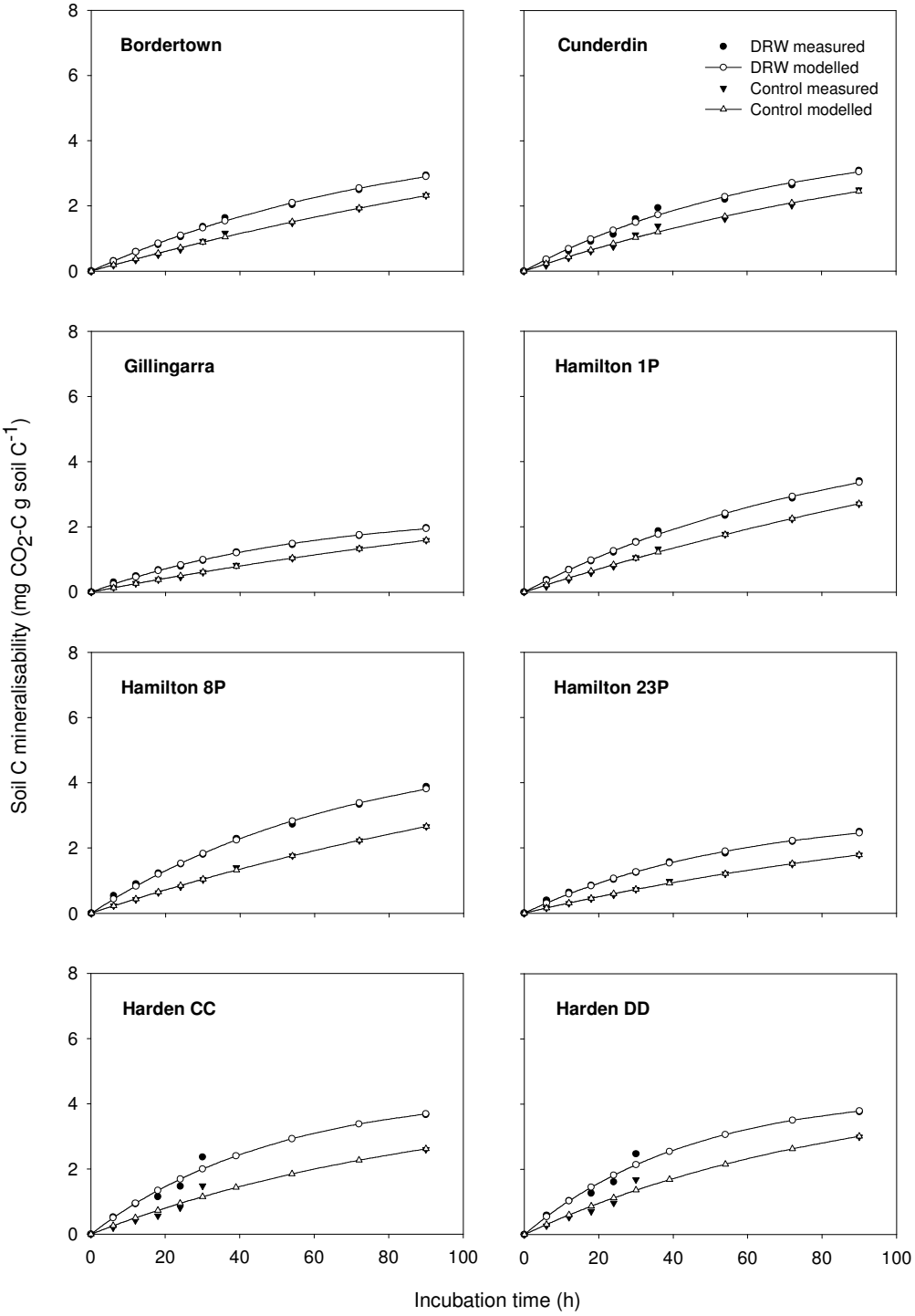


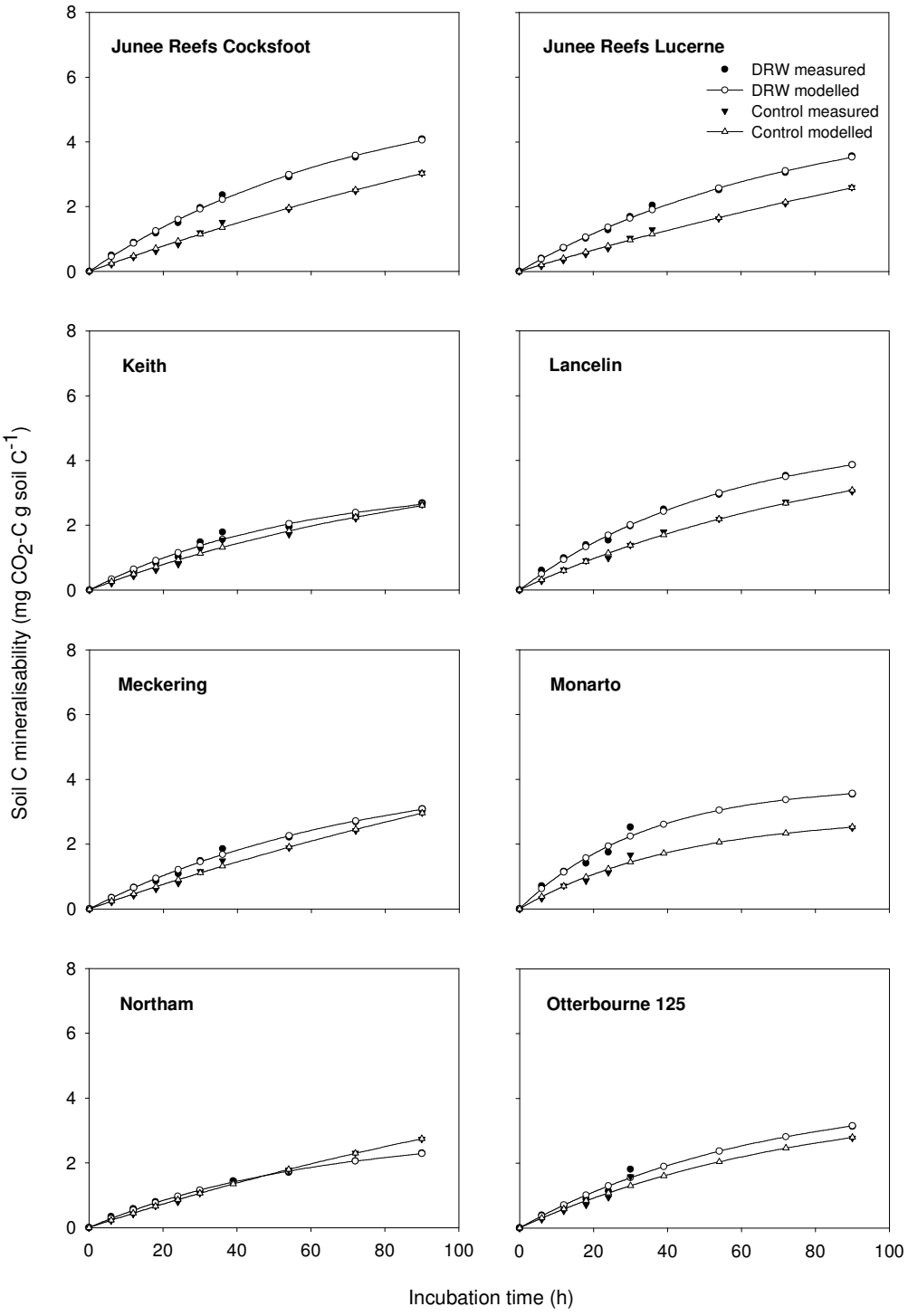

Junee Reefs Cocksfoot
Junee Reefs Lucerne
DRW measured
DRW modelled
Control measured
Control modelled
Keith
Lancelin
Meckering
Monarto
Northam
Otterbourne 125
Cumulative C mineralisation (mg CO2-C g soil-1)
Incubation time (h)
0.00
0.05
0.10
0.15
0.20
0.25
0
20
40
60
80
100

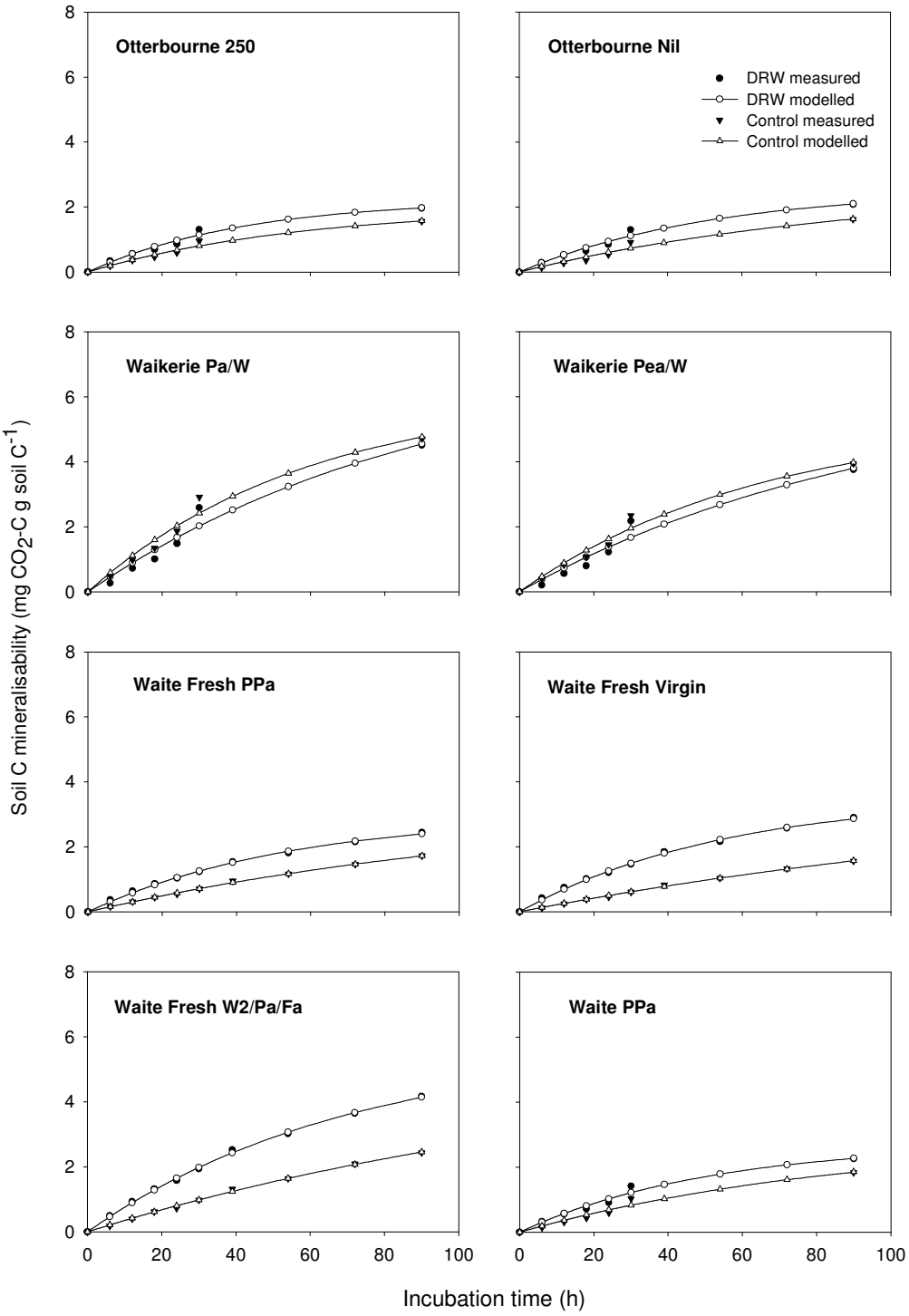

Otterbourne 250
Otterbourne Nil
DRW measured
DRW modelled
Control measured
Control modelled
Waikerie Pa/W
Waikerie Pea/W
Waite Fresh PPa
Waite Fresh Virgin
Waite Fresh W2/Pa/Fa
Waite PPa
Cumulative C mineralisation (mg CO2-C g soil-1)
Incubation time (h)
0.00
0.05
0.10
0.15
0.20
0.25
0
20
40
60
80
100

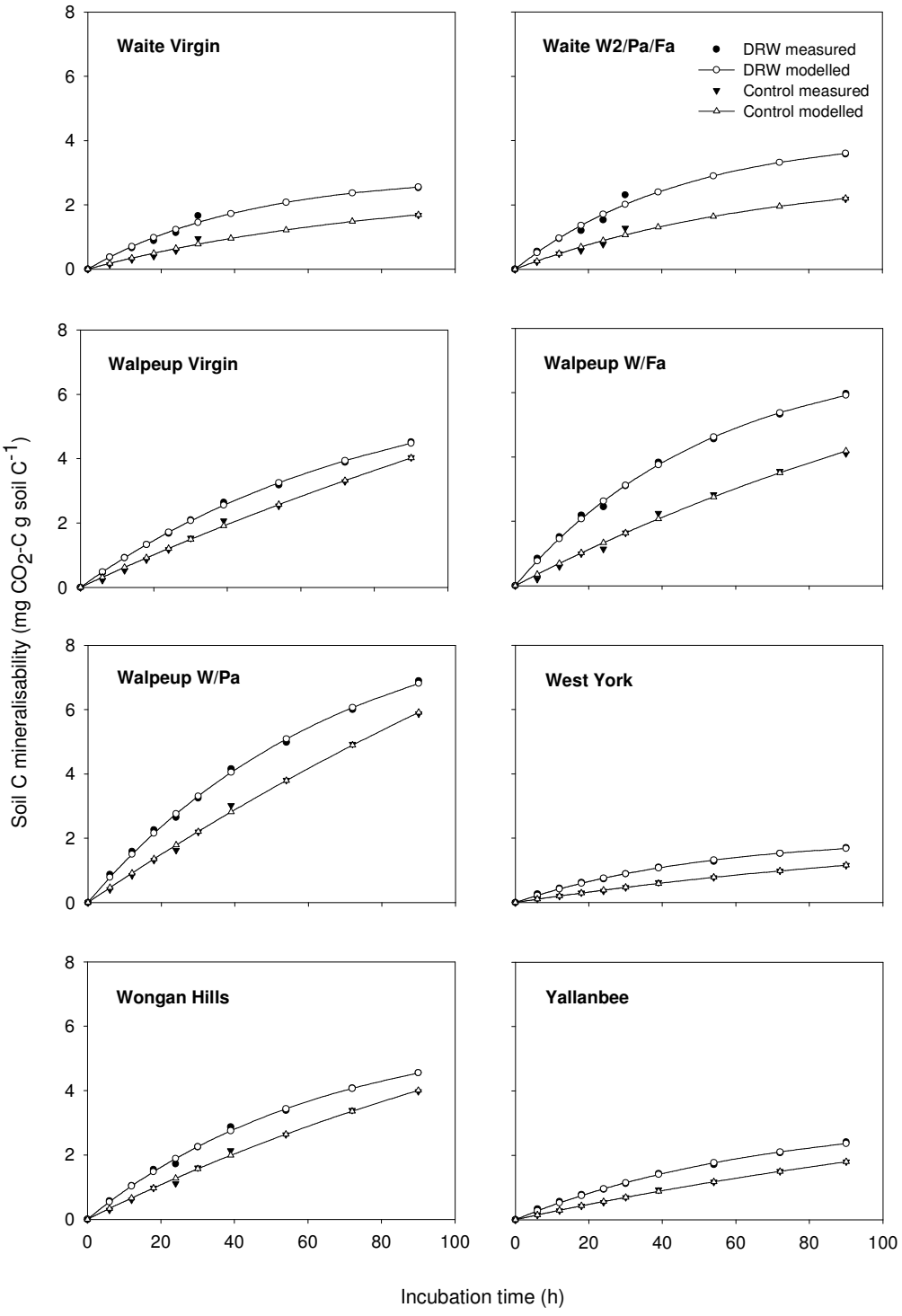

Waite Virgin
Waite W2/Pa/Fa
DRW measured
DRW modelled
Control measured
Control modelled
Walpeup Virgin
Walpeup W/Fa
Walpeup W/Pa
West York
Wongan Hills
Yallanbee
Cumulative C mineralisation (mg CO2-C g soil-1)
Incubation time (h)
0.00
0.05
0.10
0.15
0.20
0.25
0
20
40
60
80
100

Appendix 3.2: Soil C mineralisability for measured (solid) and modelled (hollow) data in 32 soils subjected to DRW (circles) and constantly moist (triangles) controls.

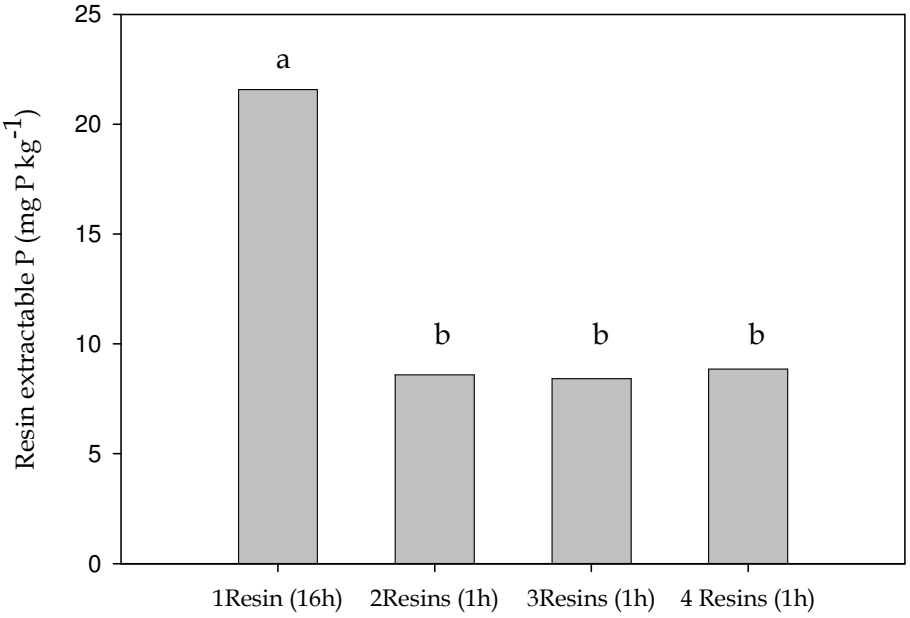



Junee Reefs Cocksfoot
Junee Reefs Lucerne
DRW measured
DRW modelled
Control measured
Control modelled
Keith
Lancelin
Meckering
Monarto
Northam
Otterbourne 125
Soil C mineralisability (mg CO2-C g soil C-1)
Incubation time (h)
0
2
4
6
8
0
20
40
60
80
100

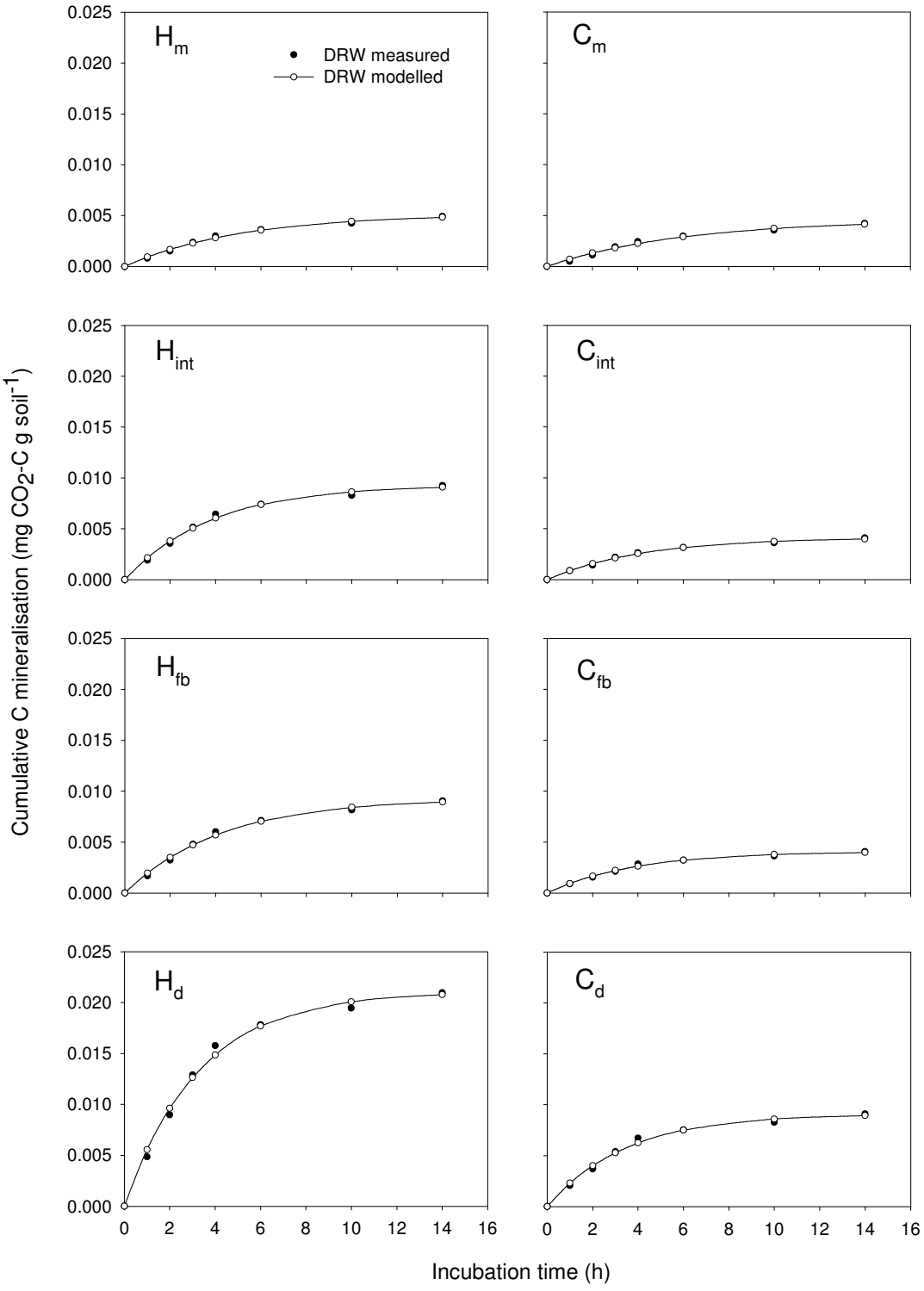

Otterbourne 250
Otterbourne Nil
DRW measured
DRW modelled
Control measured
Control modelled
Waikerie Pa/W
Waikerie Pea/W
Waite Fresh PPa
Waite Fresh Virgin
Waite Fresh W2/Pa/Fa
Waite PPa
Soil C mineralisability (mg CO2-C g soil C-1)
Incubation time (h)
0
2
4
6
8
0
20
40
60
80
100


Waite Virgin
Waite W2/Pa/Fa
DRW measured
DRW modelled
Control measured
Control modelled
Walpeup Virgin
Walpeup W/Fa
Walpeup W/Pa
West York
Wongan Hills
Yallanbee
Soil C mineralisability (mg CO2-C g soil C-1)
Incubation time (h)
0
2
4
6
8
0
20
40
60
80
100

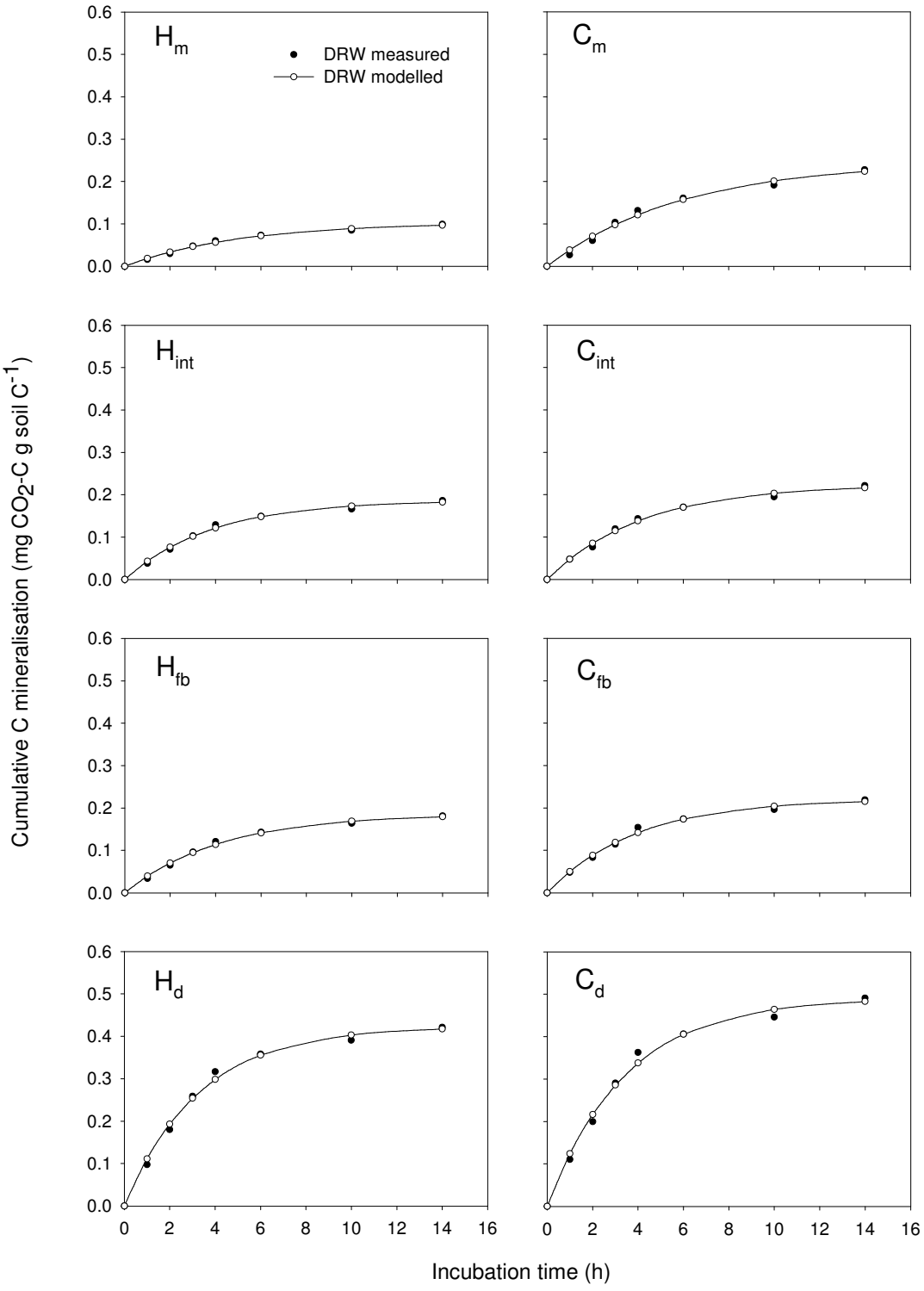
Appendix 5.1: Phosphorus extraction with increasing number of anion exchange resins using a single 1 h extraction as compared to the standard method (16 h shake with 1 resin strip). Letters indicate significant differences between means using post-hoc Tukey test (n=6).



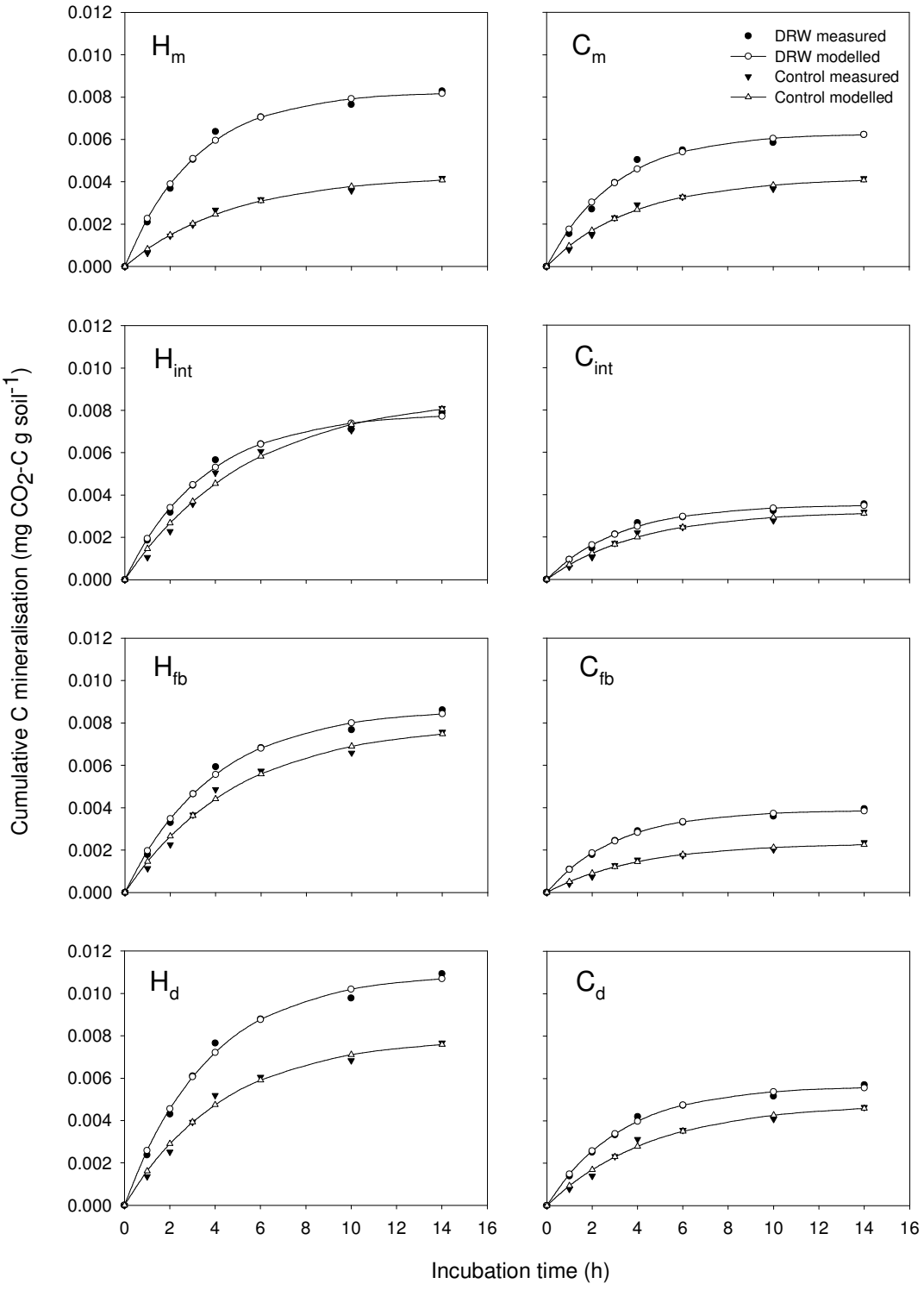
Appendix 6.1: Cumulative C mineralisation in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d) for measured (black) and modelled (white) data.



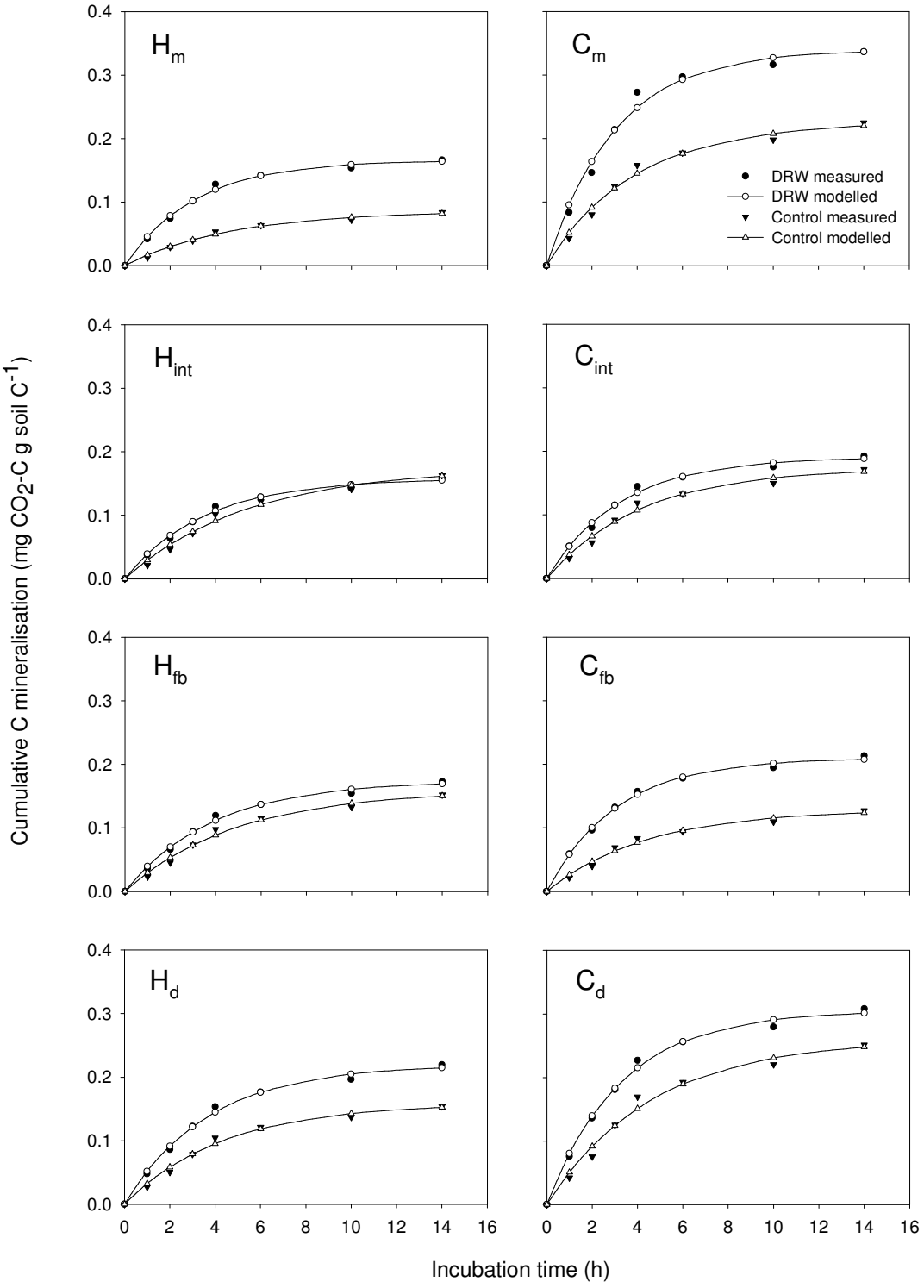
Appendix 6.2: Soil C mineralisability in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d) for measured (black) and modelled (white) data.



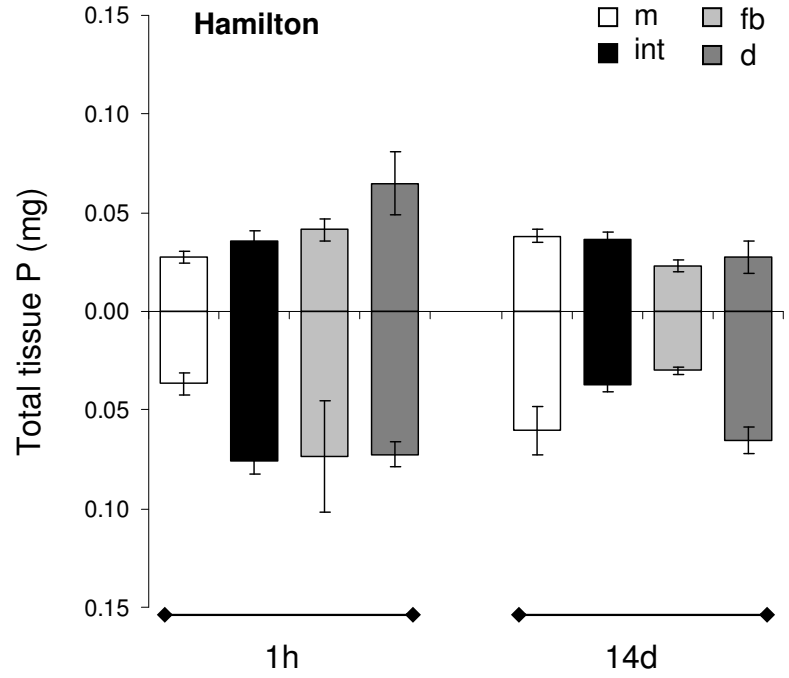
Appendix 6.3: Cumulative respiration in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d) subjected to experimental DRW (circles) or incubated moist (triangles) for measured (black) and modelled (white) data.

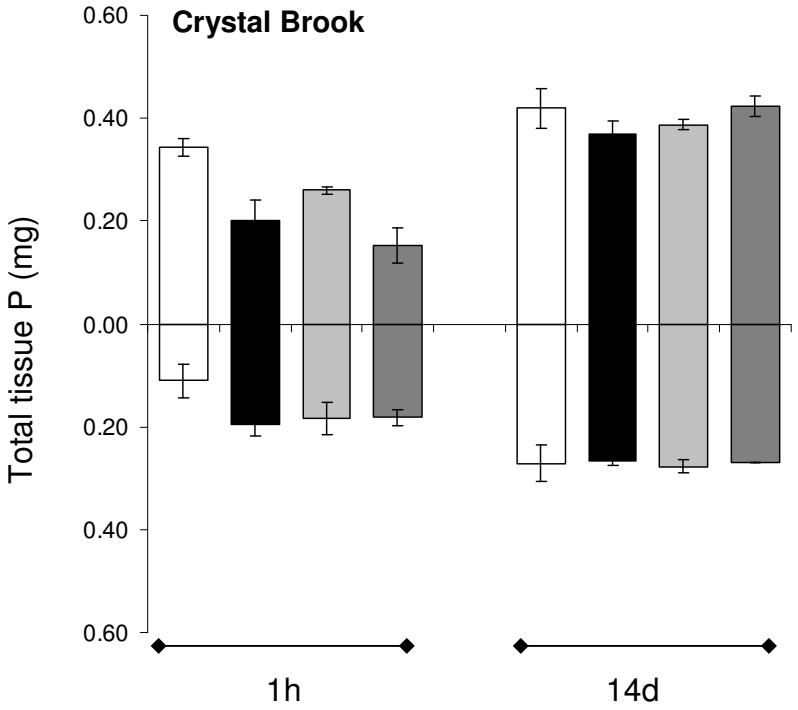


Appendix 6.4: Soil C mineralisability in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d) subjected to experimental DRW (circles) or incubated moist (triangles) for measured (black) and modelled (white) data.

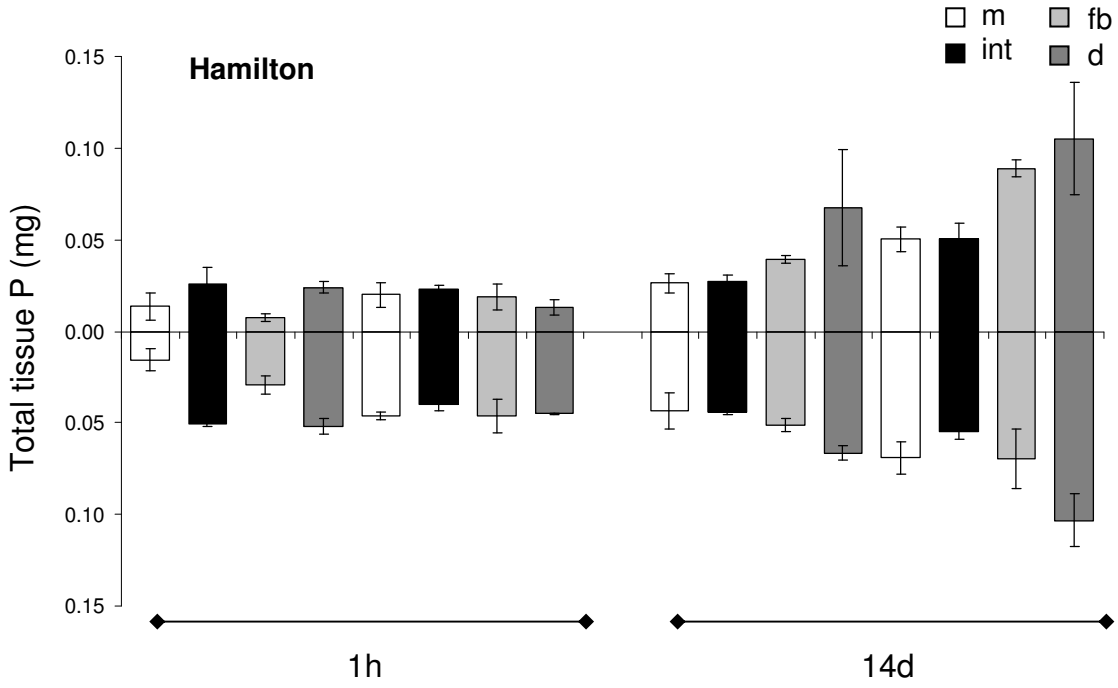


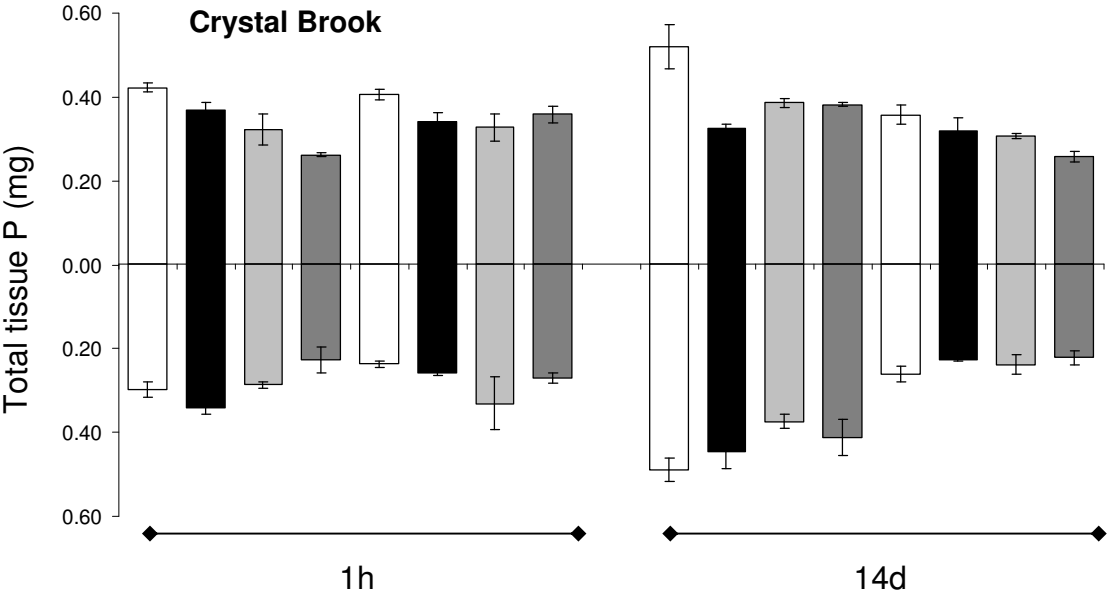
Appendix 7.1: Shoot (above) and root (below) P (mg) of wheat seedlings planted at 1 h and 14 d after DRW in Hamilton (top) and Crystal Brook (bottom) soils with four pre-treatment water regimes (m, int, fb and d). Bars indicate standard error of the mean (n=4).



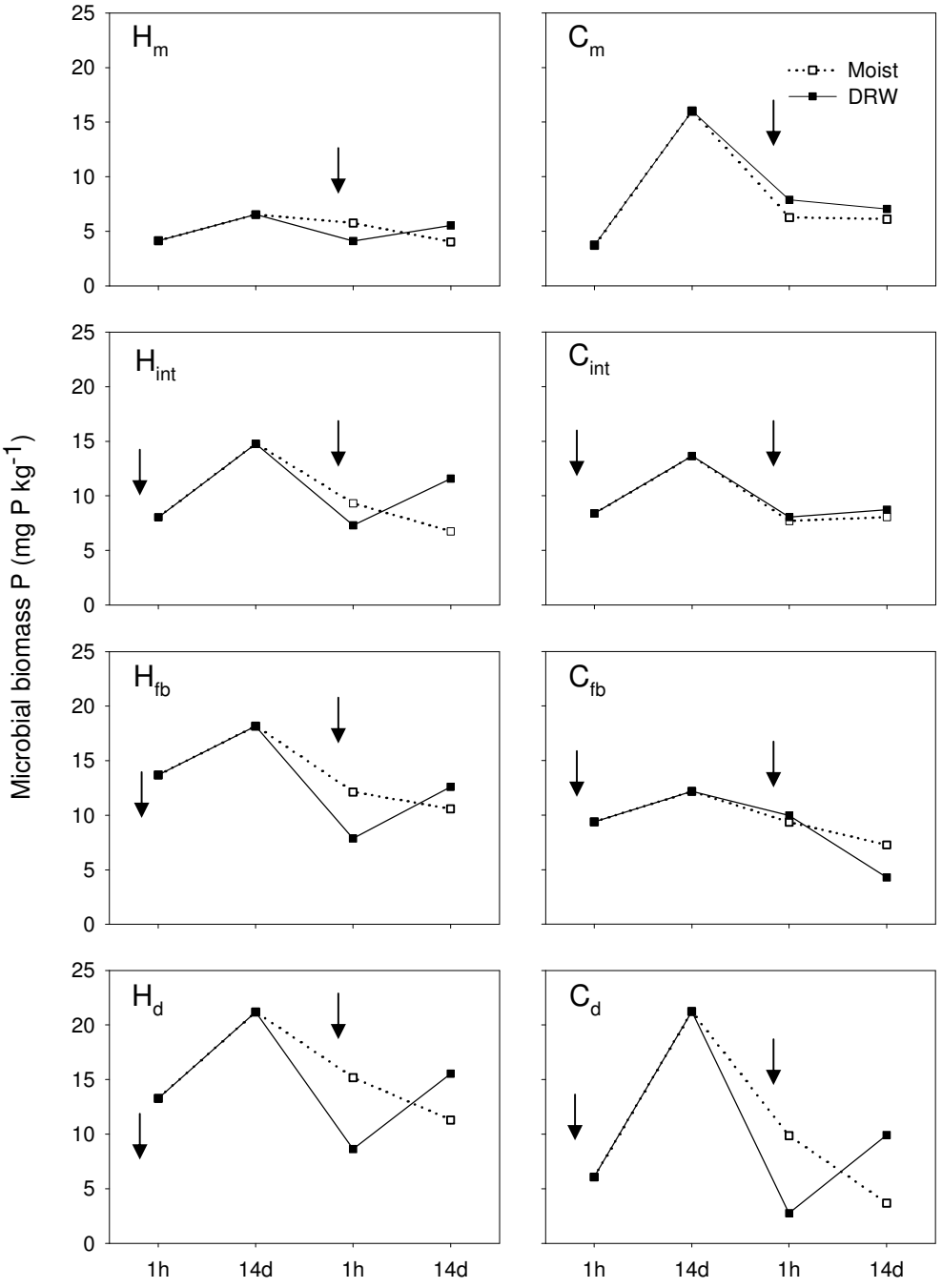


Appendix 7.2: Shoot (above) and root (below) P (mg) of wheat seedlings planted at 1 h and 14 d after DRW in Hamilton (top) and Crystal Brook (bottom) soils with four simulated water regimes (m, int, fb and d). Bars indicate standard error of the mean (n=4).

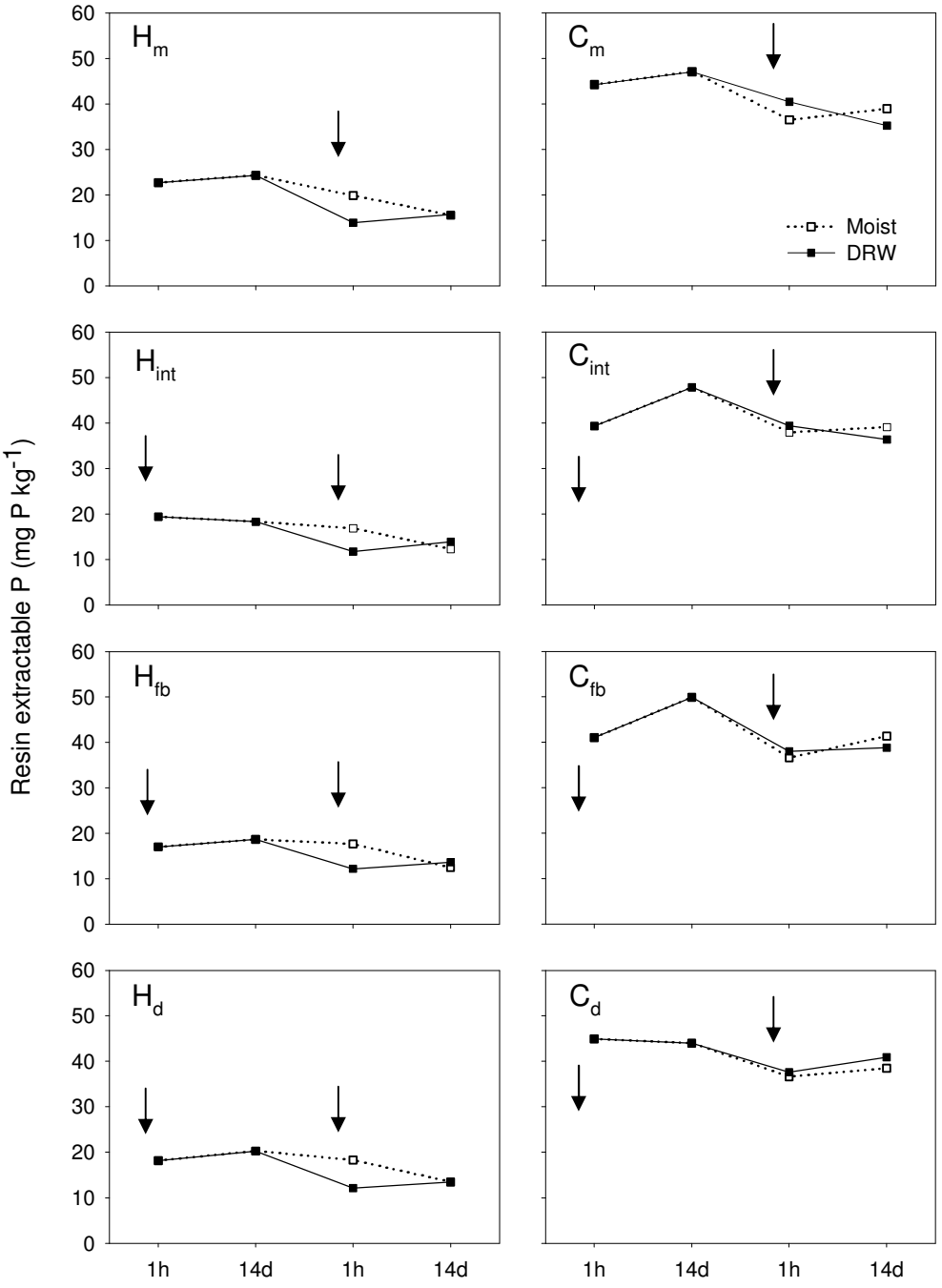




Appendix 7.3: Microbial biomass P (MBP) in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d) and either subjected to experimental DRW (solid line) or moist incubated (dotted line). Arrows indicate timing of rewetting events.



Appendix 7.4: Resin extractable P (P_{resin}) in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d) and either subjected to experimental DRW (solid line) or moist incubated (dotted line). Arrows indicate timing of rewetting events.



Appendix 7.5: Total dissolved P (TDP) in Hamilton (H) and Crystal Brook (C) soils with four pre-treatment water regimes (m, int, fb and d) and either subjected to experimental DRW (solid line) or moist incubated (dotted line). Arrows indicate timing of rewetting events.