

**The effects of drying and rewetting cycles
on carbon and nitrogen dynamics in
soils of differing textures and
organic matter contents**

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This thesis is submitted in partial fulfilment of the requirements for the Lincoln University

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**The effects of drying and rewetting cycles on carbon and
nitrogen dynamics in soils of differing textures and
organic matter contents**

By T. Harrison-Kirk

Many researchers have reported differences in soil C and N dynamics between soils of different textures and/or soil organic matter contents. However, it has proven difficult to determine the exact relationships and mechanisms between C and N dynamics and soil texture/SOM. There are few studies that consider how these soil physical and chemical conditions influence the effects of drying and rewetting on the mineralisation of C and N and the microbial transformations that follow.

The objectives of this study were: 1) To determine the effects of repeated drying and rewetting cycles on C and N dynamics in soils of differing textural class and organic matter levels. 2) To use C & N mineralised at constant moisture contents to calculate mineralisation during dry/wet cycles for comparison with actual mineralisation.

Two soil types with contrasting textures were chosen and 6 paddocks on each soil type were selected to produce an OM gradient for each soil.

Three moisture treatments were chosen to simulate moist (field capacity at -0.01 MPa), moderately dry (120% of wilting point at -1.5 MPa) and very dry (80% of wilting point at -1.5 MPa) field conditions. The dry moisture treatments were then combined with a rewet treatment where they were either rewet or maintained dry (+ or – rewet), resulting in a total of five dry/rewet treatments.

Soils were packed into funnel tops to a BD of 1.1 g/cm³ and sealed in glass jars fitted with septa to allow gas sampling. Drying was achieved using silica gel which allowed continued gas measurement during drying periods. Gas samples were collected throughout the

experiment and analysed for CO₂ by IRGA and N₂O by GC. At the start and end of the study, soils were analysed for Min N, MBC, MBN, HWC, DOC, POM, total C and total N. The correlation between calculated and actual C mineralisation data indicates that the intercept is not consistent with the origin and that the slope is not consistent with the 1:1 line.

While those paddocks with high %C had high cumulative C mineralisation, there didn't appear to be any strong relationship between soil texture or OM content and the difference between actual and calculated C mineralisation.

A plot of calculated C mineralisation rates against the actual C mineralisation rates shows that much of the error in the calculated cumulative data arises from an underestimation of the mineralisation flush when the dry soil is rewetted, especially during the first dry-rewet cycle, and an over estimation of the rate at which respiration decreases as the soil dries.

In order to use C mineralisation data from soils held at constant moisture contents to accurately predict C mineralisation in soils exposed to dry-rewet cycles, knowledge of the stress history for the soil would be required e.g. size, duration and frequency of rainfall events, dry rates etc.

The N₂O-N emission data is inherently more variable than the C mineralisation data. The fine-textured soils tend to have much higher N₂O-N emissions than the coarser soils, probably due to the creation of anoxic sites upon rewetting in the fine-textured soils.

The data indicates that prediction of N₂O-N emissions in soils exposed to dry-rewet cycles using emission data from soils held at constant moisture contents would be very inaccurate, primarily due to the inherent variability of N₂O-N emissions in soils.

Keywords: soil; drying; rewetting; cycles; carbon; nitrogen; mineralisation

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1. Introduction

In most terrestrial ecosystems the surface soils are exposed regular cycles of drying and rewetting. Increased rates of carbon (C) and nitrogen (N) mineralisation following the rewetting of a dried soil have been reported in many studies (Birch, 1958; Kieft et al., 1987; Orchard and Cook, 1983; Soulides and Allison, 1961) with elevated rates persisting for up to two weeks following rewetting (Beare et al, submitted 2008). Understanding how drying and wetting affect C and N transformations is important in predicting soil organic matter (SOM) dynamics, determining the effects of climate change on greenhouse gas (GHG) emissions from soils and estimating plant available nutrients.

Many researchers have reported differences in soil C and N dynamics between soils of different textures and/or soil organic matter contents. However, it has proven difficult to determine the exact relationships and mechanisms between short-term C and N dynamics and soil texture/SOM. For instance, it has been suggested that differences in C and N between fine- and coarse-textured soils may be due to differences in SOM input, rather than decomposition dynamics, since fine-textured soils tend to be more fertile than coarse-textured soils. Soil texture also affects soil water dynamics which in turn impacts on the soil microbial community and nutrient cycling, hence observed differences in C and N dynamics may be a function of soil water storage due to soil texture, rather than a function of texture directly. There are few studies that consider how these soil physical and chemical conditions influence the effects of drying and rewetting on the mineralisation of C and N and the microbial transformations that follow.

1.1 Objectives

- 1) To determine the effects of repeated drying and rewetting cycles on short-term C and N dynamics in soils of differing textural class and organic matter level with respect to:
 - C mineralisation
 - nitrous oxide (N₂O) emissions
 - net N mineralisation
- 2) Use C mineralised or N₂O emitted at constant moisture contents to try to calculate mineralisation/emissions during dry/rewet cycles.

1.2 Hypotheses

Hypothesis 1: That calculated C mineralisation and N₂O emissions will not equal the actual C mineralisation and N₂O emissions.

Hypothesis 2: The difference between the calculated and the actual C mineralisation and N₂O emissions will vary with soil texture & OM content.

2. Review of the literature

Soils are subject to seasonal variations in temperature and moisture, which can cause changes to soil physical and chemical properties. Natural variations in moisture conditions can result in drying and wetting cycles, periodically intensified by rain, condensation, capillarity, solar radiation and wind, amongst others. Soils under irrigation can also develop cyclical moisture variation, as the amount of water available to plants is decreased by plant uptake which is, in turn, a function of the irrigation cycle. The intensity of the dry-wet cycles will depend on the crop, tillage system, climate and soil type (Oliveira et al., 2005).

It is well known that wetting a dry soil can cause a flush of C and N mineralisation (Birch, 1958). This response to rewetting can vary with soil texture, quality and quantity of SOM, temperature, frequency of drying and wetting cycles and the amount of water added to rewet the soil. Since dry-wet cycles of soil occur naturally due to fluctuating moisture contents, they are likely to be important in the cycling of both SOM and plant nutrients (Wu and Brookes, 2005).

Wetting of a dry soil has been found to cause both growth of microbial communities and rapid increases in C and N cycling rates. An important effect on microorganisms appears to be a shift from a dormant state to one of high metabolic activity, with the response in soil respiration several magnitudes higher than the response in microbial biomass. The wetting may also release a relatively small ephemeral pool of C that can be depleted by the microbial population within a matter of days (Saetre and Stark, 2005).

Mechanisms of dry-wet cycle effects on SOM mineralisation

The main mechanisms thought to be involved in the effects of dry-wet cycles on the mineralisation of SOM and nutrients are (a) the pulse is largely a result of the mineralisation of nonbiomass SOM, rendered accessible to microbial attack by the rewetting event. In this theory the drying and rewetting process disrupts soil aggregate structure, releasing organic matter from physical protection within aggregates and producing a pulse of microbial

activity as this material is mineralised (Appel, 1998; Denef et al., 2001b; Sorensen, 1974; Utomo and Dexter, 1982), and (b) that microbial C, not SOM-C, is the major substrate mineralised to produce the rewetting CO₂ pulse (Bottner, 1985; Kieft et al., 1987). The rapid increase in soil water potential associated with the rewetting of a dry soil causes microbes to experience osmotic shock. In general, microbes either lyse completely or adjust to the water potential shock by releasing intracellular osmoregulatory solutes. The compounds released into the soil are taken up by surviving microorganisms and mineralised, producing the respiration pulse (Fierer and Schimel, 2003).

Previous research has supported one or the other of these mechanisms with some studies suggesting that much of the C and N mineralised after drying and rewetting coming from killed microbial biomass and other work suggesting that, although the magnitude of the increase in extractable organic C was similar to that of the decrease in microbial biomass C, only a part of the increased extractable C was so derived (Wu and Brookes, 2005). Some studies have combined these two proposed mechanisms, suggesting that both biomass C and SOM-C contribute to the rewetting CO₂ pulse (Gestel et al., 1991; Scheu and Parkinson, 1994; Van Gestel et al., 1993; Veen et al., 1985).

Drying and rewetting may also decrease the turnover time of the biomass and hence immobilised nutrients, such as N, P and S, may also have an accelerated turnover rate during dry-wet cycles. Wu and Brookes (2005) have suggested that only about 28-40% of the increased CO₂ evolved by dry-wet cycles came from killed biomass and the majority from non-biomass SOM, including microbial metabolites. This suggests that although the amount and turnover of microbial biomass may be significantly changed by dry-wet cycles, non-biomass SOM is the larger source of C which is mineralised during dry-wet cycles.

To adapt to low soil water contents, soil microorganisms accumulate compatible organic solutes in their cytoplasm. When the soil is rewetted, the microbes must quickly lower their internal concentration of these solutes, or risk bursting from excessively high turgor pressure. Kieft et al. (1987) estimated that 16-60% of viable microbial biomass may be released upon soil rewetting. The energy released may then be consumed by nearby microorganisms. In contrast, Appel (1988) found that in some agricultural soils, very little of the N mineralised following soil rewetting came from the microbial biomass, and it was concluded that drying and rewetting increased the decomposability of non-biomass SOM.

However, there is still uncertainty regarding the mechanisms responsible for the mineralisation pulse observed when a dry soil is rewetted. It has also been speculated that

the pulse of CO₂ released after a dry-wet cycle is the result of the mineralisation of highly enriched cytoplasmic solutes by cells responding to the water potential shock (Fierer and Schimel, 2003). The rewetting induced mineralisation of cytoplasmic C, with limited cell lysis, may explain why a number of studies have observed an increase in respiration after a dry-wet cycles with no significant reduction in microbial biomass (Bloem et al., 1992; Fierer and Schimel, 2002; Lundquist et al., 1999a; Magid et al., 1999; Scheu and Parkinson, 1994).

However, results of Saetre and Stark (2005) indicate that the labile C pool released by soil rewetting is more than just microbial cytoplasm. They concluded that the labile substrate pool used initially by microbes was probably largely of microbial origin (e.g. released cytoplasmic material and dead cells), but later in the incubation microbes may have relied more heavily on substrates derived from plant material.

In the future, many areas of the globe may experience greater variability in the timing of precipitation events, leading to many soils experiencing more frequent dry-wet events. If nonbiomass SOM-C is the main source of the rewetting CO₂ pulse, an increase in the frequency of soil dry-wet events will increase the amount of SOM-C accessible to microbial attack, potentially decreasing the amount of C sequestered in a soil over time. However, if microbial biomass is the source of the rewetting pulse, an increase in the frequency of dry-wet events may increase the level of physiological stress for soil microbes, potentially reducing C mineralisation and increasing C sequestration over time (Fierer and Schimel, 2003).

Dry-wet cycles also affect soil physical properties such as aggregation. The rewetting of a dry soil can result in the rapid intake of free water during which air becomes entrapped and compressed in soil pores, causing swelling or inflation of the soil aggregates. This can cause macroaggregate disruption (slaking) and could lead to enhanced macroaggregate turnover and loss of macroaggregate associated SOM. However the effects of dry-wet cycles on soil aggregates is not clear, because water-stable aggregates decrease and increase during dry-wet cycles (Mikha et al., 2005). Denef et al. (2001a) observed that most aggregates become slake resistant after two dry-wet cycles, SOM remained occluded in macroaggregates and physically protected from microbial decomposition.

Studies have shown a decrease in macroaggregates with the rewetting of a dry soil (Degens and Sparling, 1995; Denef et al., 2001a; Denef et al., 2001c), while others have shown a lack of significant effect of dry-wet cycles on the distribution of aggregate size classes and

aggregate-associated C and N (Mikha et al., 2005). Many soil factors affect soil-aggregate stability, such as the methods used to dry and rewet the aggregates, soil texture and total C content, in particular both clay and SOM can promote the stabilisation of soil aggregates (Mikha et al., 2005). Drying of soils can also increase the effectiveness of labile organic binding compounds (e.g. polysaccharides) in forming aggregates. Therefore, in soils experiencing short-term fluctuations in water content, the influence of labile organic binding agents on soil aggregation may be enhanced and extended compared with that in soils with little variation in water content (Degens, 1997). It has been proposed that the mechanical disruption of soil aggregates by drying and wetting may be as important as chemical and biological factors in causing the flush of microbial activity (Mikha et al., 2005).

By altering the structure of macro- and microaggregates, the dry-wet process can make physically protected SOM extractable (Denef et al., 2001c; Utomo and Dexter, 1982). A dry-wet cycle can break bonds between soil particles or clay leaves, releasing SOM protected within microaggregates (Degens and Sparling, 1995; Denef et al., 2001c). Studies have also shown that the SOM-C released by dry-wet events is likely to be stable and highly resistant to decomposition (Degens and Sparling, 1995; Lundquist et al., 1999a; Magid et al., 1999) as recurrent dry-wet events could successively deplete the quantity and quality of labile SOM held within aggregates. Soils rarely exposed to intense dry-wet events could release more labile SOM on rewetting than a soil exposed to frequent dry-wet events, resulting in a larger pulse of mineralisation from these soils (Fierer and Schimel, 2003).

Effects of dry-wet cycles on N dynamics

Wetting of very dry soils usually causes a release of inorganic N, which may be taken up by plants (Saetre and Stark, 2005). Numerous studies have demonstrated increased net N mineralisation following wetting of a dry soil, yet the mechanisms responsible for this phenomenon are not completely resolved (Saetre and Stark, 2005). Microbial activity can be re-initiated rapidly (< 1 h) following wetting of a dry soil. Microbial decomposition of substrates with low C:N ratios results in the net release of inorganic N, and the process of soil drying and wetting seems to increase the availability of these substrates. (Saetre and Stark, 2005).

Increased net N mineralisation may occur due to either an increase in gross N mineralisation or a decrease in gross N immobilisation by microbes (Saetre and Stark, 2005). Net N mineralisation rates can be rapid in the first days following soil rewetting. Both N mineralisation and microbial N immobilisation may be stimulated immediately after soil

wetting; however, net N mineralisation is often observed as gross N mineralisation is stimulated to a greater degree than N immobilisation (Saetre and Stark, 2005).

In contrast, Mikha et al. (2005) found the flush of C mineralised after each wetting event was accompanied by a reduction in net N mineralisation. This resulted in a significant reduction in soil inorganic N in soils exposed to repeated dry-wet cycles compared to soils kept continuously moist. They concluded that the microbes were assimilating inorganic N to meet microbial demand due to multiplication, growth and maintenance of the living and active biomass (Mikha et al., 2005). Franzluebbers et al. (1994) proposed that repeated dry-wet cycles could cause a reduction in net N mineralisation, either because of chemical reactions during the drying period which reduce the amount of available N, reduced size or activity of the microbial biomass, or because of a change in microbial species composition, such that more N was retained in the microbial biomass.

The reduction in N mineralisation in soils exposed to repeated dry-wet cycles observed by Mikha et al. (2005) does not agree with other studies in which there was an increase in mineralisation after rewetting (Bottner, 1985; Cabrera, 1993b; Kieft et al., 1987; Scheu and Parkinson, 1994). The disagreement in N mineralisation upon rewetting of a dry soil could be related to the contribution of organic residue to the C and N flush after rewetting or in methodological differences between studies, especially where soil physical disruption and/or changes in temperature accompanied soil drying. Soil physical disruption could cause aggregate breakdown and release protected SOM, which contributes to the C and N flush on rewetting (Mikha et al., 2005).

Saetre and Stark (2005) found a generally good qualitative correspondence between the patterns of C mineralisation, gross N mineralisation and gross N immobilisation rates, indicating that microbial consumption of C substrate and biomass production was the primary driver of N fluxes. However, the relationship varied with time and vegetation type, suggesting that additional factors besides substrate C:N may also influence N flux rates, such as the decomposability of the SOM which in-turn could influence substrate-use efficiencies.

Effects of repeated dry/wet cycles

Repeated drying and rewetting of soil has been shown to reduce microbial activity, resulting in reduced cumulative mineralised C and N. Mikha et al. (2005) and Franzluebbers et al. (1994), observed a reduction in cumulative mineralised C and N from soils exposed to repeated dry-wet cycles. Results indicated that the increased microbial activity on rewetting

was not sufficient to compensate for the reduction in C mineralisation during the drying period and as the number of dry-wet cycles increase the differences in cumulative C mineralisation between a soil exposed to dry-wet cycles and a soil kept continuously moist increases. It has been proposed by Magid et al. (1999) that microorganisms lose some of their ability to degrade complex substrates during desiccation. This ability is partially regained upon rewetting, but not to the extent maintained by microorganisms under continuously moist conditions. In contrast, Fierer and Schimel (2002) found that, over a two month incubation, soils that received multiple dry-wet events had average respiration rates that were either 10% higher (for a loam soil under oak) or 10% lower (for a clay loam under grass) than their respective continuously moist controls. This indicated that the CO₂ pulse after each wetting event must have been large enough to either almost compensate (in the case of the grass soil) or overcompensate (in the case of the oak soil) for the reduced respiration rates during the drying periods.

The stress history plays an important role in the magnitude of the rewetting CO₂ pulse from subsequent dry-wet events. The extent of the CO₂ pulse upon rewetting is significantly reduced with repeated dry-wet cycles. This observation cannot be explained by reductions in the size of the microbial biomass pool. Two possible explanations have been proposed: (i) if drying-rewetting releases physically protected organic matter, there simply may be less organic matter available for release following a series of dry-wet cycles, reducing the CO₂ pulse, or (ii) after several dry-wet events, the microbial community may adjust to the water potential shock encountered during rewetting. This adjustment would lessen the mortality rate and reduce the size of the flush of labile substrate available for mineralisation by the surviving microorganisms (Fierer and Schimel, 2002).

The reduction in the size of the pulse with repeated cycles could be due to changes in the physiological state of the microbial community, making them less susceptible to desiccation (Mikha et al., 2005). Studies have reported that microbial communities can adjust to dry-wet cycles by withstanding changes in osmotic potential (Gestel et al., 1993; Harris, 1981; Lundquist et al., 1999a). Harris (1981) also reported that microorganisms' ability to withstand desiccation depends upon their cell walls and growth type. Slow growing soil organisms are less susceptible to drying conditions than fast growing ones (Mikha et al., 2005). Studies by Mikha et al. (2005) and Franzluebbers et al. (1994) showed that repeated dry-wet cycles did not significantly reduce the size of the soil microbial biomass, indicating that the size of the microbial biomass was not a limiting factor in C and N mineralisation.

Although no change in the microbial biomass was observed in response to repeated dry-wet cycles, a change in species composition could still result (Mikha et al., 2005). However, the inability to definitively identify the cause of the rewetting pulse makes it difficult to explain the relationship between dry-wet stress history and the size of the rewetting pulse (Fierer and Schimel, 2002).

Longer-term effects of dry/wet cycles

While it is well known that drying-rewetting yields a short-term (days) increase in C and N mineralisation rates, less is known about the longer term implications of dry-wet stresses for ecosystem C and N dynamics. For instance, after the short-term pulse of C and N, do soil processes return to their pre-stress state? If they do then it is relatively unimportant to include dry-wet dynamics into soil process models due to their relatively short duration and limited magnitude in annual C and N transformations. If however, a dry-wet event causes a change in the equilibrium for soil C and N transformations (relative to the unstressed soil), or if the recovery to pre-stress basal rates is slow, then incorporating dry-wet events into models that adequately predict C and N fluxes becomes more important, and more difficult. If the frequency of dry-wet events controls soil processes, then the variability in rainfall in a given period, not just average rainfall, would have to be incorporated into models of soil C and N dynamics (Fierer and Schimel, 2002).

The longer-term effects of drying-wetting stress history show greater responses compared to the short-term effects. A number of studies (Fierer and Schimel, 2002; Franzluebbers, 1999; Magid et al., 1999; Schimel et al., 1999) have found that dry-wet cycles can retard long-term (> 6 weeks) C mineralisation rates. A decrease in the supply of remaining mineralisable SOM following a period of frequent dry-wet cycles would be the most obvious explanation for the reduction in respiration rates. Presumably, after a single dry-wet cycle the pool of potentially mineralisable SOM would increase either due to a release of physically protected organic matter or a 'priming effect' caused by the release of labile substrates during biomass turnover. So, with time, a series of dry-wet cycles would serve to reduce the total supply of available SOM (Fierer and Schimel, 2002). Another potential explanation for the reduction in long-term respiration rates is a change in the composition of the microbial community. Not all members of the microbial biomass are equally adept at mineralising SOM. The loss of some microbes during drying-wetting or a change in the physiologies of the surviving population could reduce the ability of the microbial biomass to mineralise SOM, as has been demonstrated by Schimel et al. (1999).

Frierer and Schimel (2002) found that when a soil under oak from an area of Mediterranean climate (relatively wet winters and very dry, hot summers) was exposed to dry-wet cycles, the microbial biomass increased. This effect was not evident in the short-term and only became apparent six weeks after the last wetting event, once the soils seemed to have approached an equilibrium state. This finding is contrary to a number of other studies which have shown a decrease in microbial biomass after exposure to dry-wet cycles (Gestel et al., 1996; Sorensen, 1983). It has been proposed that microbes found in soils that commonly experience extreme fluctuation in moisture content (such as those from a Mediterranean climate) may be better adapted to frequent dry-wet cycles, compared to microbes from soils that seldom experience such extremes (Frierer and Schimel, 2002).

The frequency of dry-wet cycles also has ramifications for soil N dynamics. Frierer and Schimel's (2002) results also suggested a substantial increase in the autotrophic nitrifier population after the application of frequent dry-wet events. This observed increase was surprising given that nitrifiers are generally considered to be highly sensitive to moisture stress and that other studies have observed a decrease in nitrification rates with repeated dry-wet cycles (Fierer and Schimel, 2002).

It has been proposed that while nitrifier activity may, in general, be sensitive to periods of low moisture, nitrifiers from soils that commonly experience extreme fluctuation in moisture content (such as those from a Mediterranean climate) may be able to survive drying periods. This low nitrifier mortality during drying coupled with the ability of nitrifiers to thrive on the flush of NH_4^+ released during rewetting, could lead to an overall increase in nitrifier biomass and activity in soils exposed to frequent dry-wet cycles (Fierer and Schimel, 2002).

This increase in nitrifier biomass would be expected to correspond to an increase in nitrification rates in these dry-wet stressed soils. However it was found that, six weeks after the last dry-wet cycle, soil nitrate concentrations were actually lower in soils with a history of multiple dry-wet events. The microbial uptake of nitrate may have exceeded any enhanced nitrate production. Even though nitrate is not the preferred form of N for microbial uptake, when ammonium concentrations are low, nitrate consumption can be substantial (Fierer and Schimel, 2002). Nitrate concentrations may also be lowered in frequently dry-wet soils due to pulses of denitrification following rewetting. A number of studies have shown a short-term increase in NO and N₂O emissions following a dry-wet event (Boyer and Groffman, 1996; Davidson et al., 1993; Scholes et al., 1997).

Dry/wet cycle effects on microbial community composition

Although the effects of dry-wet cycles on microbial biomass have been well studied, less is known about the effects on microbial community composition or structure. Physiological stress, such as that imposed by drying and rewetting, could reduce total microbial diversity by favouring a portion of the microbial community best adapted to coping with the given stress. Alternatively, microbial diversity may increase due to dry-wet events by enhancing the spatial and temporal heterogeneity of the soil environment, promoting species coexistence (Fierer et al., 2003).

Frequent dry-wet cycles may alter the specific composition of microbial communities by selecting for microbes that can survive rapid changes in soil water potential. Actively growing microbes have been found to be more susceptible to dry-wet stress than slower growing microbes, possible due to differences in cell wall characteristics (Bottner, 1985; Gestel et al., 1993; Van Gestel et al., 1993). Rapid changes in soil water potential may also select for gram-positive bacteria and fungi which have thicker, more rigid cell walls, and compatible solutes that enhance osmoregulatory capabilities (Harris, 1981; Schimel et al., 1999). Alternatively, other studies have suggested that frequent dry-wet cycles may select for fast-growing microbes that are able to grow rapidly on the labile substrates released into the soil on rewetting (Denef et al., 2001c; Jager and Bruins, 1975; Lundquist et al., 1999a; Scheu and Parkinson, 1994).

Differences in soil abiotic conditions, such as temperature and soil moisture, have been shown to influence microbial community structure. The microbial community structure can also be a function of organic matter availability, organic matter quality and soil nutrient status. Above ground plant communities can also directly contribute to differences in microbial community composition (Fierer et al., 2003). Studies have shown that moisture regime can influence the structure of the microbial community, but the differences between litter and soil types are often greater in magnitude than any moisture effects (Fierer et al., 2003; Lundquist et al., 1999a).

Fierer et al. (2003) observed that the soil bacterial community in a soil under oak changed in response to dry-wet stress while the soil bacterial community in a soil under grass was largely unaffected. The possible explanation for this observation was that the grass soil microbial communities were, in their natural state, already selected for by frequent dry-wet cycles, compared to the oak soil which had been covered by a thick litter layer and canopy shading leading to lower natural soil moisture variability. Microbial processes (respiration

and nitrification) were also more strongly affected by dry-wet stresses in the oak soil than in the grass soil. Other studies have reported that exposure to dry-wet cycles is often more stressful for microbial communities not preadapted to a high degree of variability in natural soil moistures (Gestel et al., 1993; Kieft et al., 1987; Lundquist et al., 1999a; West et al., 1988).

Since C is often the primary nutrient limiting microbial biomass growth in soils, it could be expected that frequent dry-wet events would select for soil microbial communities that can adjust to sudden increases in water potential without rapidly mineralising a large proportion of their accumulated cytoplasmic solutes. Work has shown considerable variability in the physiological responses of different soil isolates to a rapid increase in water potentials. Besides mineralisation, other mechanisms bacteria and fungi can use to remove accumulated solutes include: the rapid conversion of the solutes to less osmotically active forms inside the cell, or leaving the solutes largely intact and having strong enough cell walls to sustain the increased turgor pressure generated upon rewetting (Fierer and Schimel, 2003; Harris, 1981). Soils that are exposed to frequent dry-wet events may have a microbial community that utilises the more efficient mechanism to adjust intracellular water potentials, mineralising a smaller proportion of cytoplasmic C on rewetting than soils rarely exposed to dry-wet events (Fierer and Schimel, 2003).

3. Materials and Methods

3.1 Site selection

Two soil types commonly found on the Canterbury Plains of New Zealand were selected: Lismore silt loam (NZ classification: Brown soil, USA classification: Udic Ustochrept), and Temuka clay loam (NZ classification: Gley soil, USA classification: Mollic Haplaquept). The Crop and Food Research Land Management Index (LMI) database was used to identify paddocks on these soil types with differing soil organic matter (SOM) contents. For each soil type six paddocks were selected to represent a SOM gradient.

3.2 Sample collection and preparation

Samples were collected of the soil A horizon from six sites within each paddock, avoiding wheel marks, stock camps, fence lines and other such artefacts. At each sampling site a soil

sample 15 x 15 cm square and 7.5 cm in depth was collected with a spade and placed in a crush resistant container for transport to the laboratory.

At the laboratory the samples were passed through a 4 mm sieve and large pieces of OM and fresh plant material were removed. The samples from the six sites within each paddock were combined and mixed to form one sample per paddock, this bulk soil sample was air-dried 25°C and then stored at room temperature until the beginning of the experiment.

Soils from each paddock underwent preliminary analysis for a range of soil properties (Table 1). The percentage of sand, silt, and clay was determined using the pipette method (Gee and Or, 2002), with sand defined as particles > 53 µm, silt between 53 and 2 µm and clay as < 2 µm. Total carbon (C) and nitrogen (N) content was measured on a LECO CNS-2000 analyser, and moisture contents at field capacity (FC) and wilting point (WP) (water potentials of -0.01M Pa and -1.5M Pa respectively) were determined using tension tables and pressure plate apparatus.

Table 1. Some chemical and physical properties of the soils used.

Paddock		%C	%N	% sand	% silt	% clay	FC	WP
ID	Land use						(-0.01 MPa) (%ww)	(-1.5 MPa) (%ww)
LIS 2	Pasture	4.9	0.44	24	58	15	42.1	14.8
LIS 3	Pasture	4.7	0.41	19	62	15	32.8	12.0
LIS 4	Cropping	2.9	0.26	21	62	15	30.7	10.0
LIS 5	Cropping	2.6	0.24	19	63	15	29.9	10.2
LIS 6	Cropping	2.2	0.22	21	59	17	27.8	9.8
LIS 7	Pasture	4.4	0.40	26	56	17	35.8	14.2
TEM 1	Pasture	6.2	0.61	16	52	27	53.1	22.3
TEM 2	Cropping	4.1	0.38	10	59	28	39.5	13.8
TEM 3	Cropping	3.7	0.31	8	69	21	39.3	11.6
TEM 4	Cropping	3.1	0.27	21	50	26	32.2	14.1
TEM 5	Cropping	3.2	0.28	20	54	24	34.5	12.0
TEM 6	Cropping	2.0	0.19	23	57	18	32.3	9.3

3.3 Experimental Design

Three moisture treatments were selected for the experiment; FC, 120% of WP (WP x 1.2) and 80% of WP (WP x 0.80). They were chosen to simulate moist, moderately dry and very dry field conditions. The dry moisture treatments were then combined with a rewet treatment where they were either rewet or maintained dry (+ or – rewet), resulting in a total of five dry/rewet treatments (Table 2).

Table 2. Dry/rewet treatments.

Treatment name	Treatment ID	Moisture Treatment	Rewet treatment
Continuously moist	WW	FC	Maintained at FC
Moderately dry, rewet	MDW	120% WP	+ rewet
Very dry, rewet	VDW	80% WP	+ rewet
Moderately dry	MD	120% WP	- rewet
Very dry	VD	80% WP	- rewet

A pre-experiment was carried out using a sub-set of the soils to determine the length of the period required to slowly dry the soils down to the target moisture contents using silica gel desiccant (chromatography grade, Sigma-Aldrich, Cat. #288624). A sample of the silica gel desiccant was sealed in an incubation jar containing elevated levels of CO₂, the CO₂ levels were monitored for several days to ensure the gel did not adsorb CO₂ which would lead to an underestimation of C mineralised during the drying phase. Under these test conditions, the silica gel used was found to not adsorb CO₂.

The experiment consisted of three phases; the pre-incubation phase, the treatment phase and the post treatment phase. The experiment commenced with all samples at field capacity for a 14 day pre-incubation phase, this allowed the soils to reach an equilibrium before the dry/rewet treatments were imposed. The treatment phase consisted of three 20 day dry/wet cycles in which the soil is dried down for 16 days, then rapidly rewet and incubated moist for 4 days before the next drying cycle began. Those soils that reached their target moisture contents prior to the end of the 16 day drying period (such as the MDW treatments) had the silica gel removed and were incubated dry until the start of the rewet period. The MD and VD treatments were dried down to their respective moisture contents under the same drying conditions as their corresponding dry-rewet treatments, they were then incubated in their dry

states for the rest of the treatment phase of the experiment. Following the completion of the treatment phase, all the soils were adjusted back to FC and incubated for a further 18 day post treatment phase (Fig. 1).

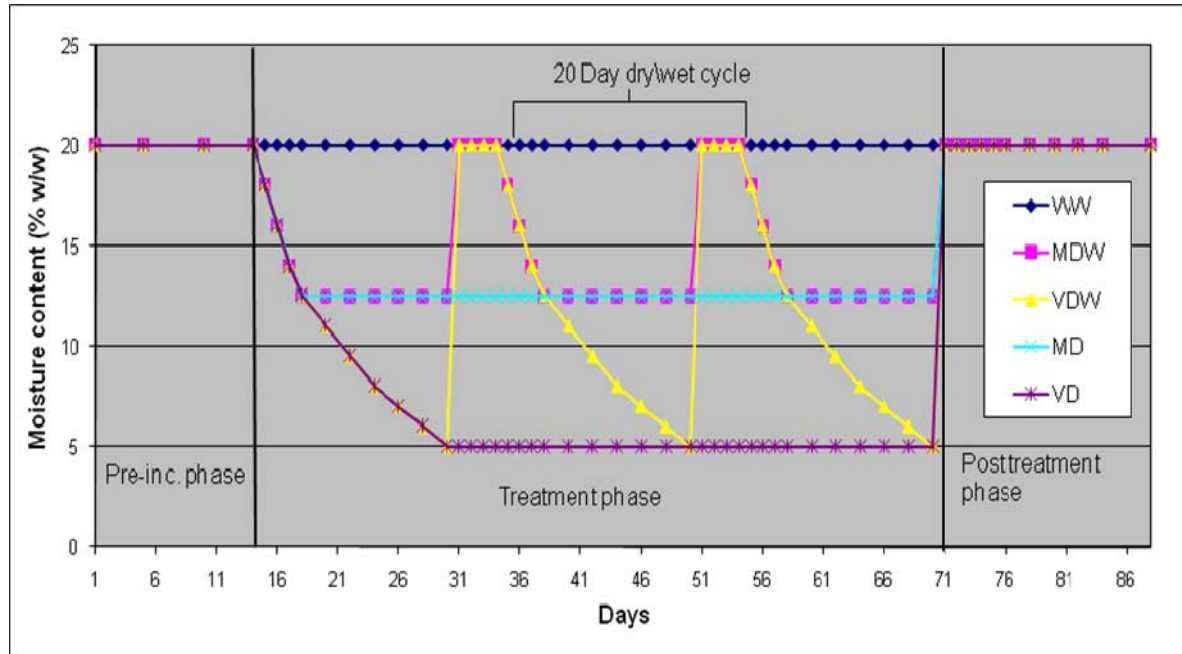


Figure 1. A schematic diagram representing the dry/wet treatments and rewetting cycles.

Four replicates of each soil were prepared for each dry-rewet treatment and an additional four replicates of each soil were prepared for removal after the pre-incubation phase for destructive sampling and analysis, giving a total of 288 samples. Due to the limitations of space and equipment and the time requirements of this number of samples, it was decided (after discussion with a Biometrician) to split the experiment into two incubation runs, with two reps in each run.

3.4 Experiment set-up

The air-dried soils were wetted to FC by spreading the soils onto trays and spraying with N-free nutrient solution (100 mg Ca L^{-1} , 24 mg Mg L^{-1} , 113 mg S L^{-1} , 0.5 mg P L^{-1} , and 4 mg K L^{-1} , added as KH_2PO_4 , K_2SO_4 , MgSO_4 and CaSO_4 (Cabrera, 1993a)) the soils were mixed and refrigerated overnight to allow even moisture distribution. The next day the soils were mixed again and packed into plastic Buchner funnel tops (55 mm diameter x 30 mm high) to a depth of 25 mm and a dry bulk density (BD) of 1.1 g/cm^3 . The holes in the base of the

funnel tops aided soil drying and gaseous exchange, while a glass fibre filter paper placed over the holes retained any fine particles. The soil packed funnel tops were weighed and sealed in 1L glass preserving jars. These jars had a rubber septa inserted in the lid to allow for headspace gas sampling (Fig. 2a). The incubation was carried out at 22°C.

Drying of the soils will be achieved by adding a pottle containing 40 g of silica gel desiccant to the jars. A piece of plastic mesh was placed on top of the pottle to support the soil sample and ensure the silica gel did not come into direct contact with the soil. This arrangement allowed for continuous gas measurement while the soils are drying (Fig. 2b & 2c). Moisture loss was by monitored by weighing the soil samples periodically over the drying period, the silica gel was replaced at the same time.

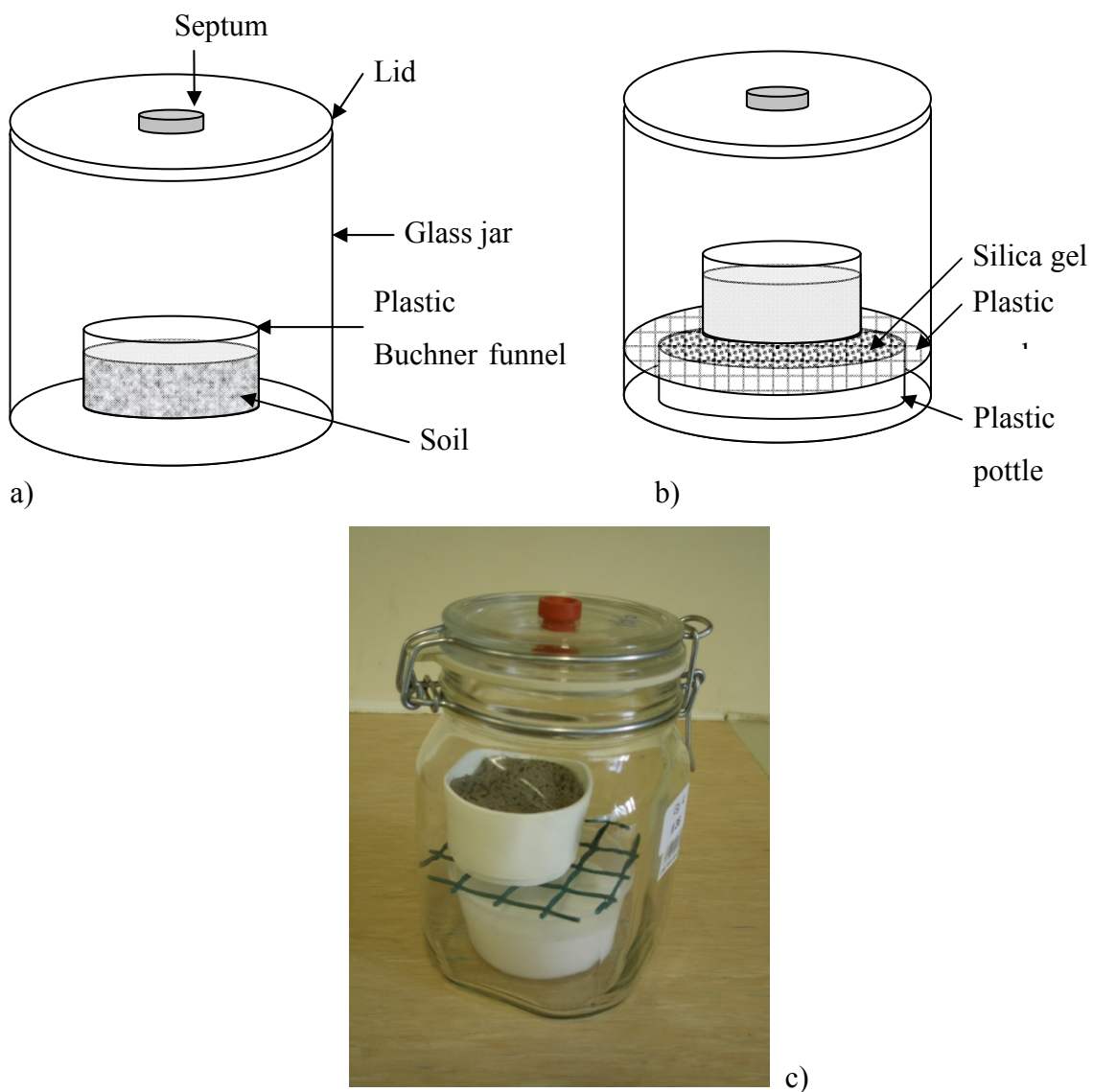


Figure 2. The incubation set-up used when the soils were a) moist or b) being slowly dried down; c) a photograph of the actual set-up.

Rapid rewetting was performed by dripping deionised (DI) water onto the soil surface via a syringe barrel fitted with a 23 gauge needle. The surface of the soil sample was protected from impact damage by the water droplets using a glass fibre filter paper placed on top of the soil prior to rewetting commencing, and by suspending the needle tip only 2-3 cm from the soil surface (Fig. 3). A final moisture adjustment was made after removing the filter paper by weighing the soil sample and adding any additional water using a transfer pipette.

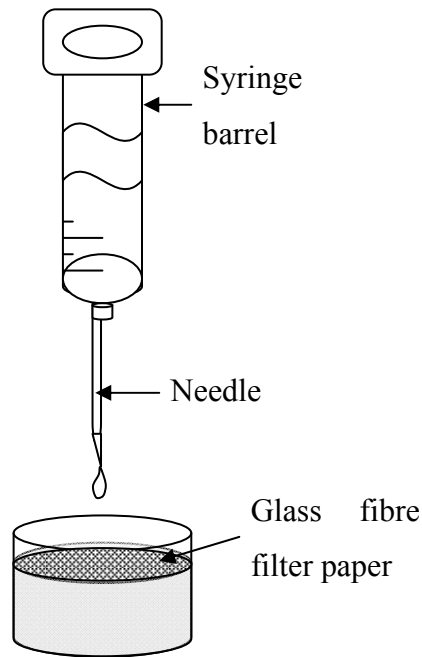


Figure 3. The set-up used for rapid rewetting of the dry soils.

3.5 Gas sampling and analysis

Gas samples were collected by inserting a needle through the septum in the jar lid and withdrawing a sample using a 20 ml gas tight syringe (Fig. 4). The headspace was mixed before sampling by pumping the syringe four times. After collection of the gas samples, the jars were opened and placed in front of a fan for a period of approximately 30 seconds to allow them to return to ambient conditions, before being sealed and returned to the incubator.



Figure 4. Collection of gas samples (infrared gas analyser in the background).

The gas samples were analysed for CO₂ and N₂O. Samples for CO₂ analysis were injected directly into a LI-COR Li-7000 infrared gas analyser (IRGA) at the time of sampling (Fig. 4). Samples for N₂O analysis were injected into evacuated 6ml vials and analysed in batches on a Shimadzu GC-17A gas chromatograph fitted with FID and ECD detectors.

Gas samples for CO₂ analysis were collected periodically throughout the pre-incubation and post treatment phases of the experiment as required. During the treatment phase of the experiment, samples were taken on days 2, 5, 8, 11, 14, 16, 17, 18 and 20 of each of the three dry-rewet cycles shown in Figure 1.

Gas samples for N₂O analysis were also collected periodically throughout the pre-incubation and post treatment phases of the experiment, although not as frequently as for CO₂ analysis. During the treatment phase of the experiment, samples were taken on days 2, 5, 11, 17, 18 and 20 of each of the three dry-rewet cycles shown in Figure 1.

3.6 Soil Analyses

At the end of the pre-incubation stage soil samples were removed from their jars, passed through a 4 mm sieve and analysed for:

- Mineral (inorganic) nitrogen (Min N)
- Microbial biomass carbon and nitrogen (MBC & MBN)
- Hot water extractable carbon (HWC)
- Dissolved organic carbon (DOC)
- Particulate organic matter (POM)

- Total C and N
- Gravimetric moisture content (MC)

This data served as a baseline.

Immediately after all the soils were returned to FC at the end of the treatment phase, a small sub-sample was removed from each of the incubated soils using a 10 mm diameter cork borer. These sub-samples were crumbled and analysed for:

- Min N
- HWC
- DOC

These measures were performed to see if there were changes in these parameters over the post treatment phase of the incubation. This is sampling time two.

At the end of the incubation all soil samples were removed from the incubation jars, passed through a 4 mm sieve and analysed for:

- Min N
- MBC & MBN
- HWC
- DOC
- POM
- Total C and N
- MC

This is sampling time three.

Mineral N concentrations were determined by 1 hour extraction with 2 M KCl and subsequent analysis of the filtered extract for NH₄-N and NO₃-N on a FIAstar 5000 analyser (Keeney and Nelson, 1982).

Microbial biomass C and N were determined using the chloroform fumigation technique (Vance et al., 1987). The total organic carbon (TOC) in the extracts was measured directly on a Shimadzu TOC-V CSH analyser, while the total N was determined using the persulphate oxidation method (Cabrera and Beare, 1993) and measured on FIAstar 5000 analyser.

Hot water extractable C was determined using the procedure described by Ghani *et al.* (2003). Total organic C was measured in both cold water and hot water extracts on a Shimadzu TOC-V CSH analyser. The fraction of soil organic carbon in the cold water extracts was classified as dissolved organic C (DOC), while the fraction in the hot water extracts was classified as hot water extractable C (HWC).

Two size fractions of particulate organic matter (POM) were measured; 1000-250 and 250-53 μm diameter. Briefly, 20g of moist soil was dispersed in sodium hexametaphosphate (Calgon) solution by shaking overnight. The soil suspension was then washed through a stack of sieves with screen sizes of 1000, 250 and 53 μm . The material retained on the 250 and 53 μm sieves was dried at 60°C overnight, weighed, ground and analysed for total C and N on a LECO CNS-2000 analyser.

Soil total C and N content was measured on a LECO CNS-2000 analyser, and gravimetric moisture content was determined by weight loss following drying at 105°C overnight.

4. Water Filled Pore Space

The target gravimetric moisture contents used in the trial were converted to water filled pore space (WFPS). Those paddocks with high % clay and/or high %C have higher WFPS at field capacity (FC), particularly Tem 1 which has 100% of pore space filled at FC. Any differences in WFPS between the paddocks are not as evident under drier conditions, except again for Tem 1 which retains markedly higher WFPS than the other paddocks (Table 3).

Table 3. Water filled pore space (%) (WFPS)			
Paddock	1.2 x WP		0.8 x WP
	FC	(MD)	(VD)
LIS 2	79	33	22
LIS 3	62	27	18
LIS 4	58	23	15
LIS 5	56	23	15
LIS 6	52	22	15
LIS 7	67	32	21
TEM 1	100	50	33
TEM 2	74	31	21
TEM 3	74	26	17
TEM 4	61	32	21
TEM 5	65	27	18
TEM 6	61	21	14

5. Statistical Analysis – Chemical Analyses

The data was analysed using a mixed model for each response variable. Some variables appeared to be strongly heteroscedastic. Although it appears that the paddocks from which the soil was sampled were quite variable in their composition, a weighted analysis did not solve the problem. A log transformation of that data was then used to stabilise the variances where necessary. Results are presented on the log scale in these situations for ease of interpretation. All analyses were carried out using the REML (Restricted Maximum Likelihood) algorithm in GenStat v.10 (VSN International)

It was hypothesised that the organic matter content (represented by %C) and texture (represented by %Clay) in the soil may affect the behaviour exhibited in response to the wet-rewet cycles with which it was treated. In most cases one or both of these variables was instrumental. However, in some situations there was strong evidence that although these linear continuous variables did aid in explaining some of the variability in the data; it was not sufficient. This was shown by the significant Lack of Fit of the model when the saturated (or most complete) model was fitted. The saturated model is that in which Paddock is fitted as a predictive factor even though it is not replicated. This indicates that other factors (e.g. landuse, management history, or vegetation type) may contribute to the variability in the data. Where necessary the saturated model was used to obtain estimates of means and standard errors.

The most appropriate model for each variable was fitted, and in figures showing the means some measure of the precision of these estimates were created to describe the behaviours indicated by this model. Models with categorical factors are presented with Least Significant Differences (LSD), and models with continuous predictors are presented with Confidence Limits (CL).

6. Results – Chemical Analyses

The form of the model and statistical importance of each term in the model is given in Table 4 & 5. Overall results suggest that Paddock, or %C and/or %Clay, strongly affected the response of the soil. This can be seen in the graphs (e.g. 5 & 6) where different paddocks are not only higher or lower than others (even at baseline), but also the response to the dry-rewet cycles is quite different. In variates where data was collected only at baseline and at

the termination of the trial, a discussion of differences between “Treatments” is taken to include the baseline as a separate treatment.

Table 4. The statistical significance of each term of the mixed model in explaining the variation in each of the chemical analysis where %C or %Clay affects the response of the soil.

Term	%C	%Clay	%Clay .%C	T*Trt	%C*T *Trt	%Clay *T*Trt	%C* %Clay*Trt
HWC	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Total C	<0.001	<0.001	0.57	<0.001	0.037	<0.001	-
Total N	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-
MBC		<0.001	0.010	<0.001	0.052	<0.001	-
Log (C in POM 53µm)		<0.001	0.047	0.002	-	<0.001	-
Log (N in POM 53µm)		<0.001	0.211	<0.001	-	0.002	-
Log (C in POM 250µm)		<0.001	<0.001	<0.001	<0.001	0.060	-
Log (N in POM 250µm)		<0.001	0.007	<0.001	0.005	<0.001	-
Log (C in total POM)		<0.001	0.003	0.001	<0.001	<0.001	-
Log (N in total POM)		<0.001	0.102	<0.001	<0.001	0.004	-
T = sampling time, Trt = dry/rewet treatment. POM 53µm = Particulate OM of the size fraction 53 – 250 µm, POM 250 µm = Particulate OM of the size fraction 250 – 1000 µm, and total POM is the sum of the fractions.							

Table 5. The statistical significance of each term of the mixed model in explaining the variation in each of the chemical analysis where paddock affects the response of the soil.

Term	Paddock*Time	Paddock*Trt	T*Trt	Paddock*T*Trt
Min N	<0.001	<0.001	0.066	0.093
Log (DOC)	<0.001	<0.001	0.009	0.132
MBN	-	<0.001	-	-

6.1 Mineral Nitrogen

Mineral N was measured at the end of the pre-incubation phase (baseline), at the end of the treatment phase (Time 2) and at the end of the incubation (Time 3). This was done in order to compare changes over time with respect to the response to the dry-rewet treatments and after the treatments were removed. There was strong evidence of a difference between paddocks, and that this difference was not consistent with either sampling time or dry-rewet treatment ($p < 0.001$ for each) (Fig. 5 & 6). Figure 5 indicates that mineral N concentration is

larger at times 2 and 3 when compared to the baseline; particularly for paddocks Lis 2, 3, 7 and Tem 1. Most soils also show an increase in mineral N concentration from Time 2 to Time 3.

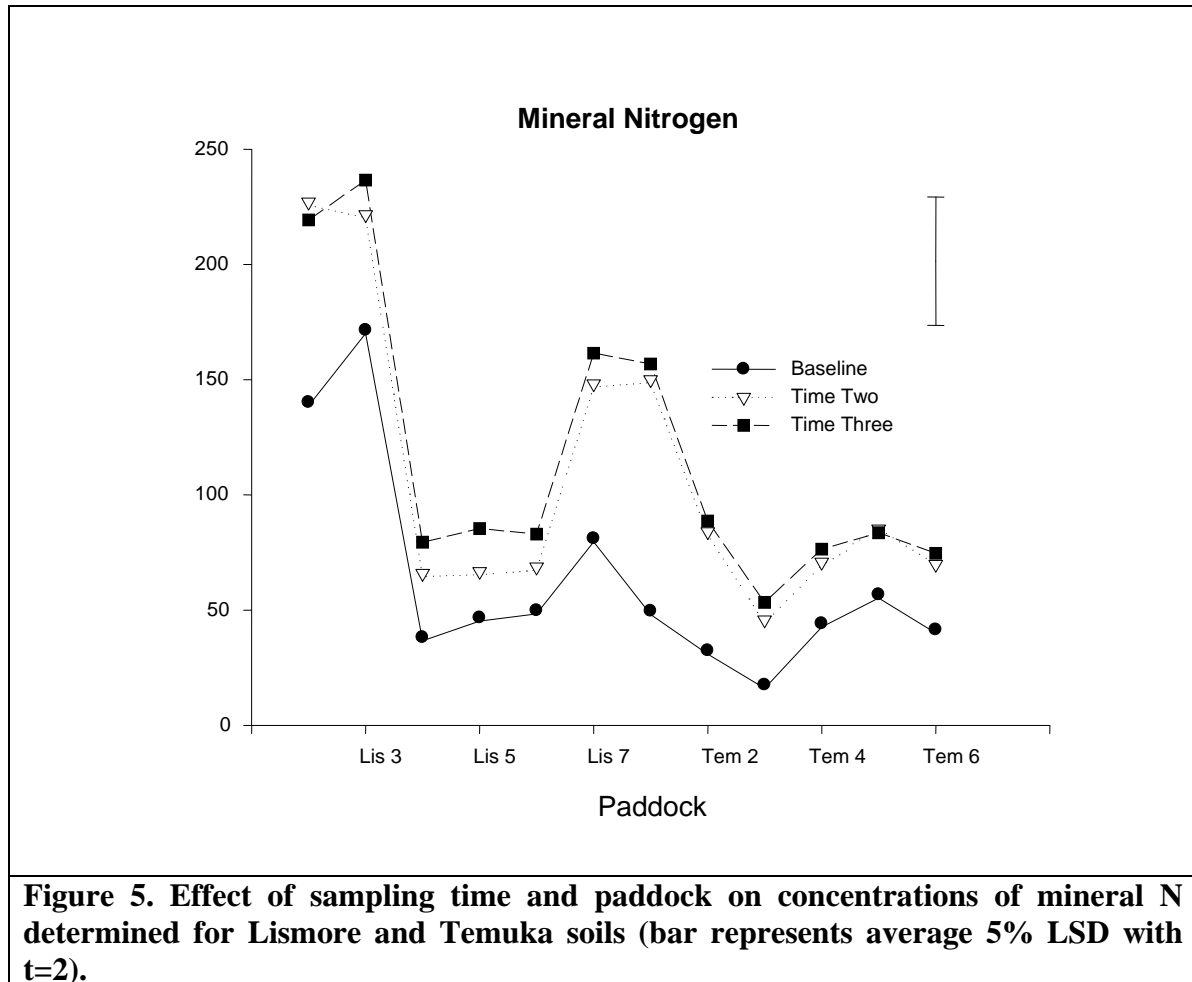
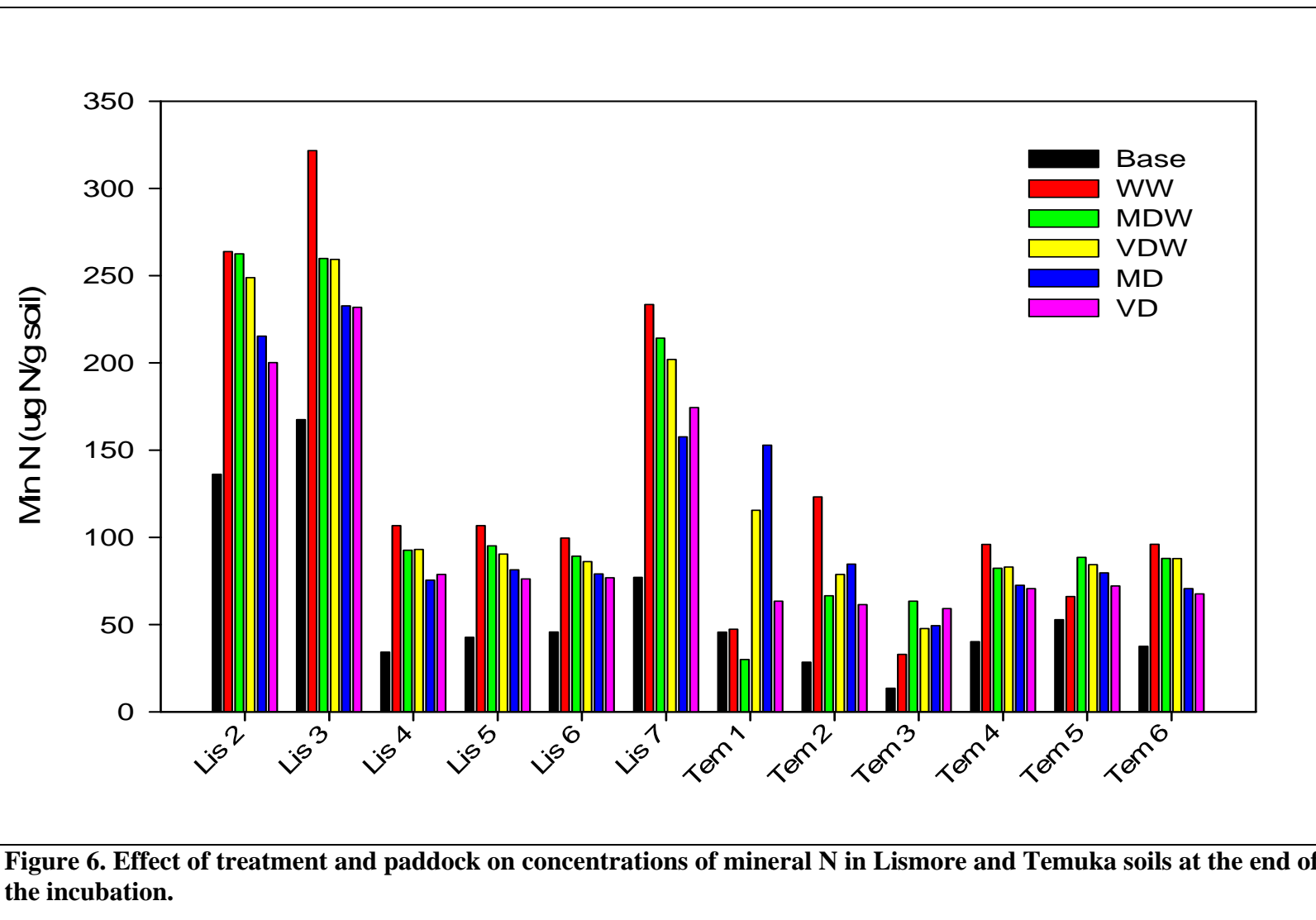


Figure 6 shows a complex story that indicates that the different levels of %C and %Clay in the soils interact to influence the effect of dry-rewet treatments on mineral N. The baseline measurements are consistently lower than the Time 3 measurements, but are higher for Paddocks Lis 2 and 3 than for other paddocks. WW treatments usually results in the highest concentration of mineral N, and the VD treatment is often the lowest, although still higher than baseline. However, paddock Tem 1 is quite unusual in its response to the treatments in that the VDW and MD treatments resulted in the highest mineral N concentrations with WW and MWD being very low.

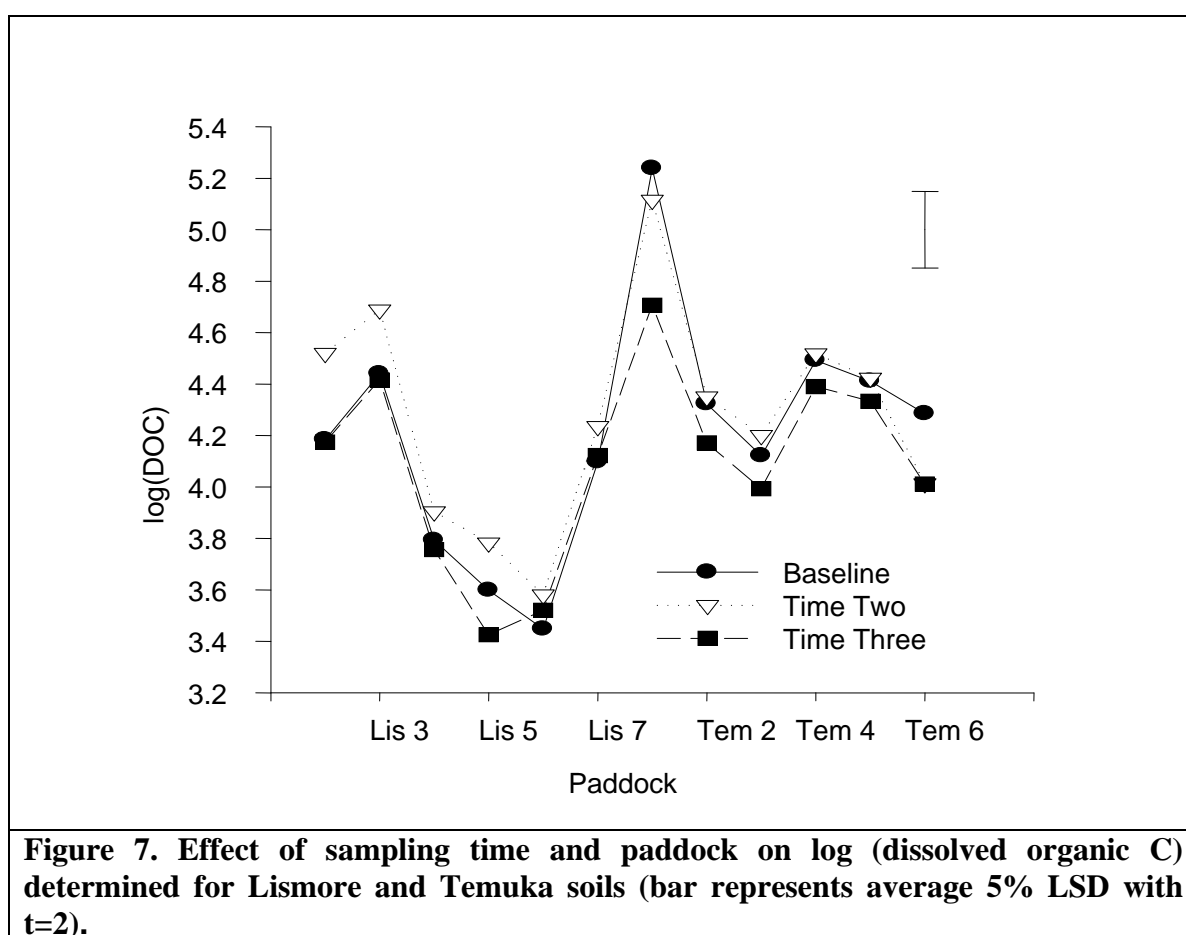
Graphs for individual paddocks showing mineral N concentrations at each sampling time and for all of the treatments can found in the Appendix (Fig. A1 and A2)



6.2 Dissolved Organic Carbon

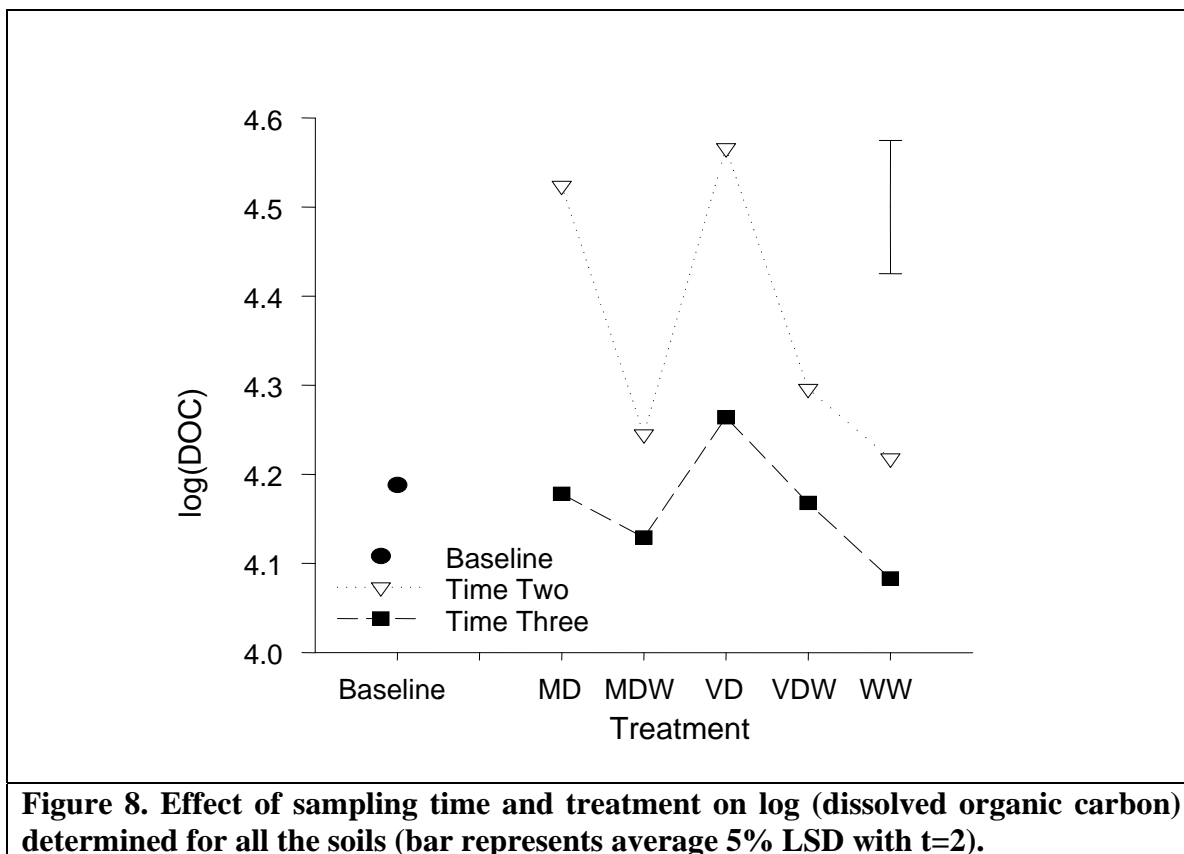
DOC required a log transformation to standardise variances. There was strong evidence of a difference between Paddocks, and that this difference was not consistent with either Time or Treat ($p < 0.001$ for each) (Fig. 7, 9). There is also some evidence ($p = 0.009$) that the effect of Time is not the same for each Treatment (Fig. 8).

Measurements taken at Time 2 are usually slightly higher than either the Baseline or Time 3 measurements. There are large, and significant, differences in measurements taken from different paddocks, with Lis 4, 5 & 6 (low %C, low %Clay) having low log (DOC); and Tem 1 (high %C, high %Clay) having higher log (DOC). The other paddocks appear very similar, indicating that perhaps the effects of %C and %Clay are additive (i.e. these paddocks have a combination of medium %C, %Clay; high %C, low %Clay; and vice versa) (Fig. 7).



It appears that there is not a large change between measurements taken at the Baseline or at Time 3, but there is a decrease from Time 2 to Time 3. The decrease in log(DOC) level

over time is greater for the MD and VD treatments than for the other three dry-rewet treatments (Fig. 8). Graphs for individual paddocks showing untransformed DOC results at each sampling time and for all treatments can found in the Appendix (Fig. A3 and A4).



Untransformed DOC data for each paddock at time 3 again indicate the strong difference in response for the paddocks, and the lesser effect of treatment within each paddock. In the Lismore paddocks the dry treatments (MD & VD) result in the higher levels of DOC, and the WW treatment results in lower levels. This pattern is not so consistent for the Temuka paddocks, where the VD treatment still often results in higher DOC levels, but WW is not always lowest (Fig. 9).

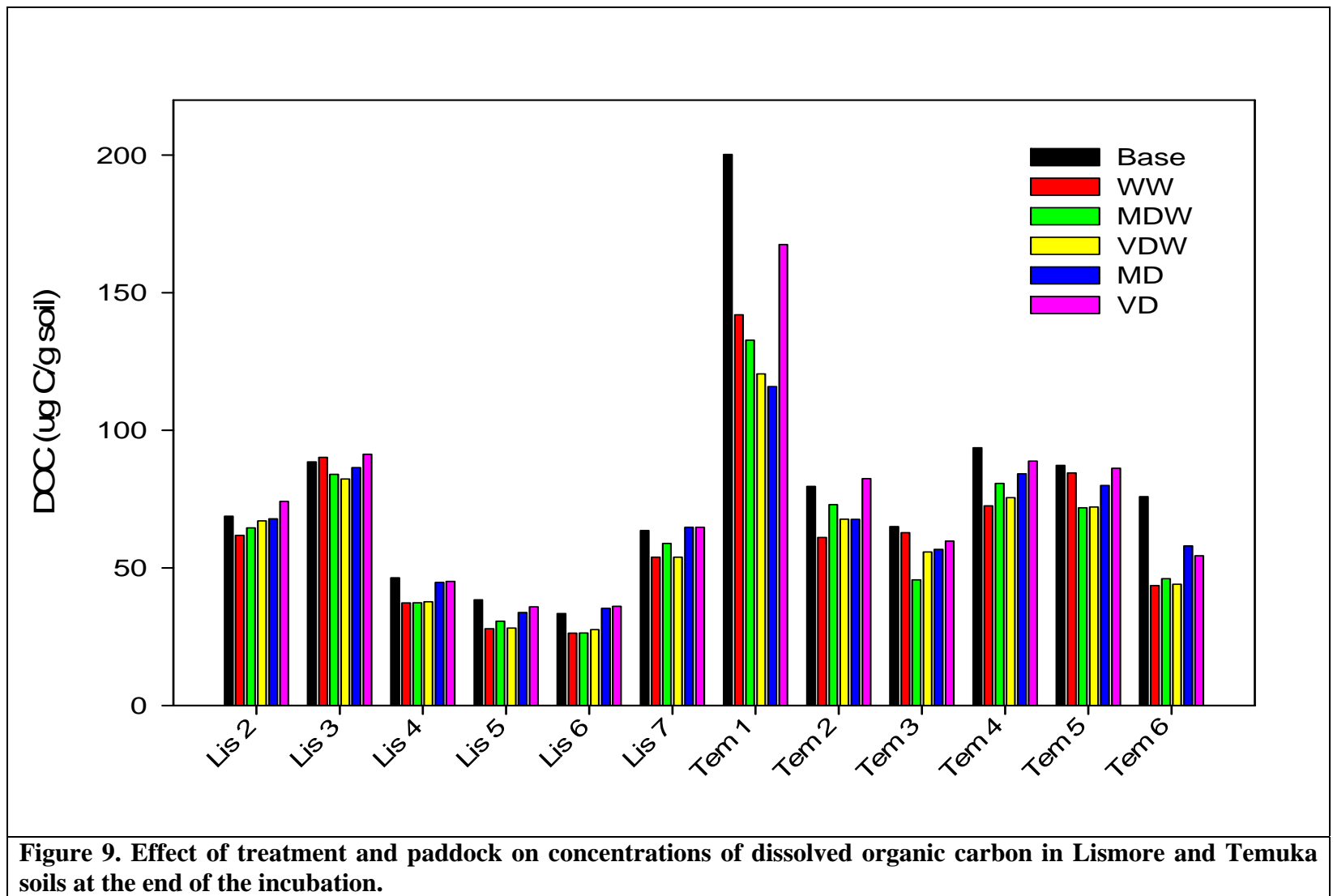


Figure 9. Effect of treatment and paddock on concentrations of dissolved organic carbon in Lismore and Temuka soils at the end of the incubation.

6.3 Hot Water Extractable Carbon

There is evidence ($p < 0.001$) that there is a treatment and time effect; and that this effect is not consistent with %C and %Clay. For Lismore paddocks there is little difference between HWC at Baseline and Time 2, but Time 3 HWC is slightly lower. Temuka paddocks show a different pattern with HWC at Baseline and Time 3 very similar and Time 2 slightly higher, however, these differences between sampling times are small (Fig. 10). Graphs for individual paddocks showing HWC results at each sampling time and for all treatments can found in the Appendix (Fig. A5 and A6).

At the end of the trial all of the treatments in the Lismore paddocks showed a decrease in HWC from baseline, in contrast most of the Temuka paddocks showed an increase in HWC relative to Baseline for at least one of the treatments. Across all the paddocks the dry treatments (MD & VD) often had the highest HWC (Fig. 11) but again the differences are quite small.

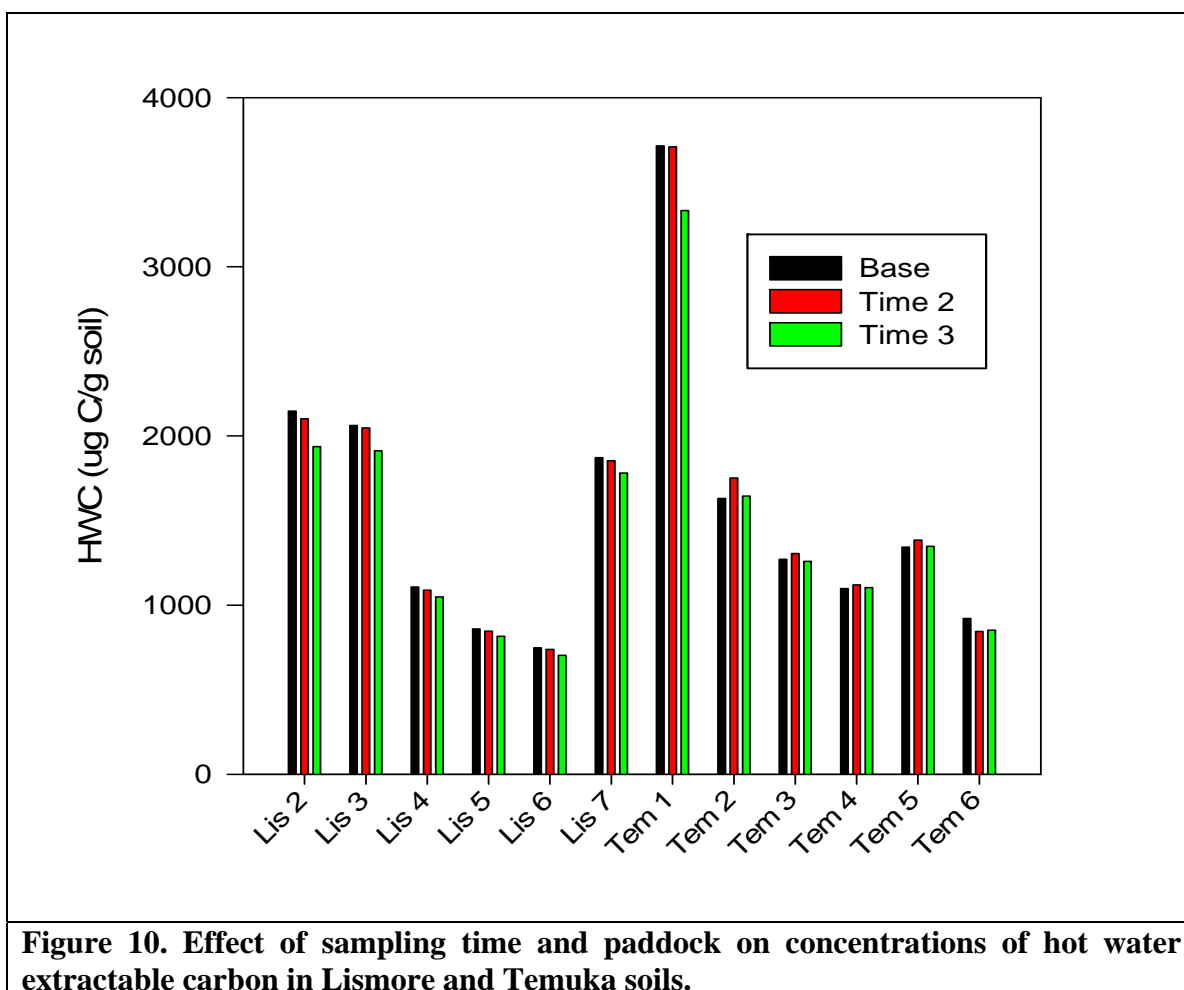


Figure 10. Effect of sampling time and paddock on concentrations of hot water extractable carbon in Lismore and Temuka soils.

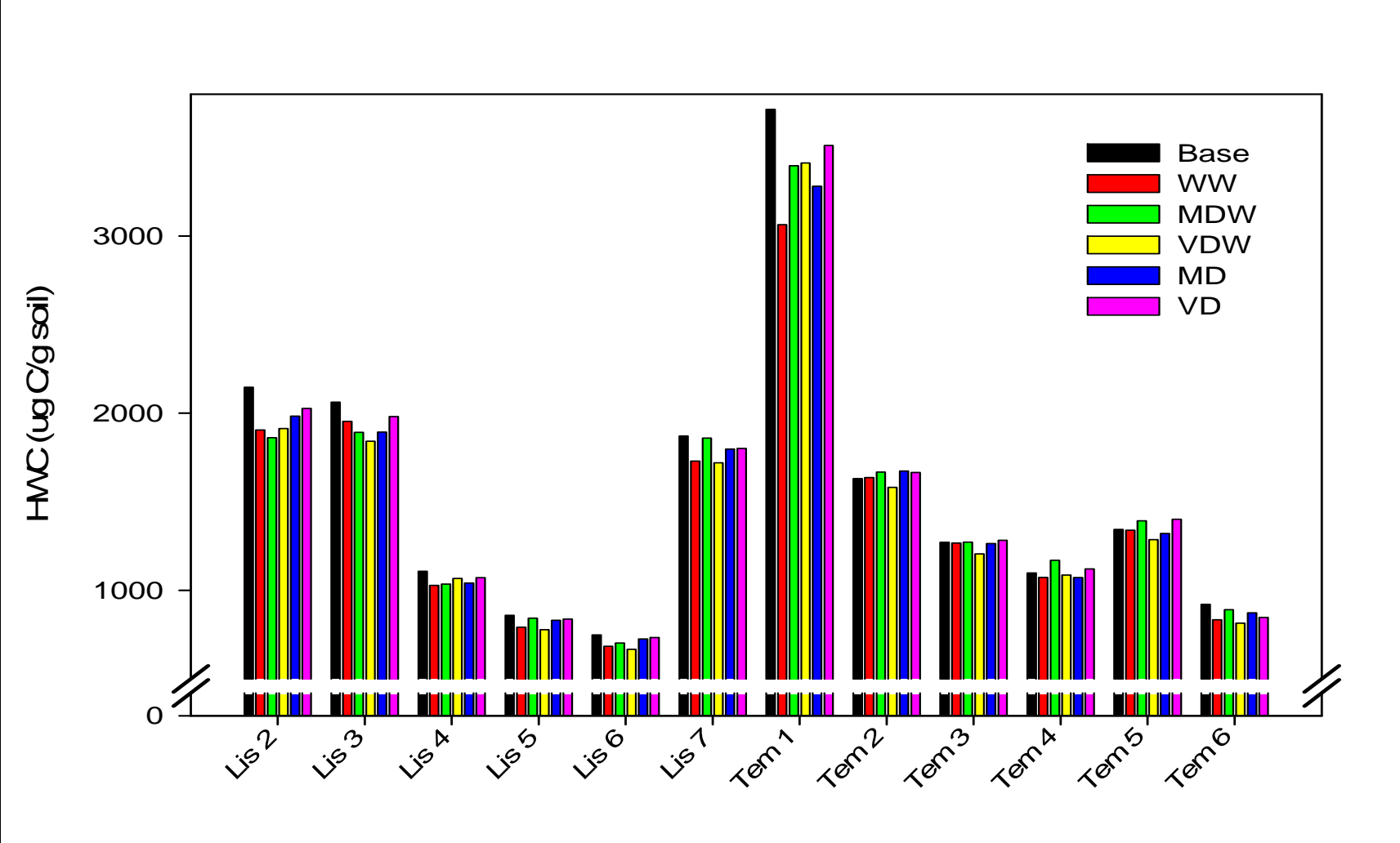


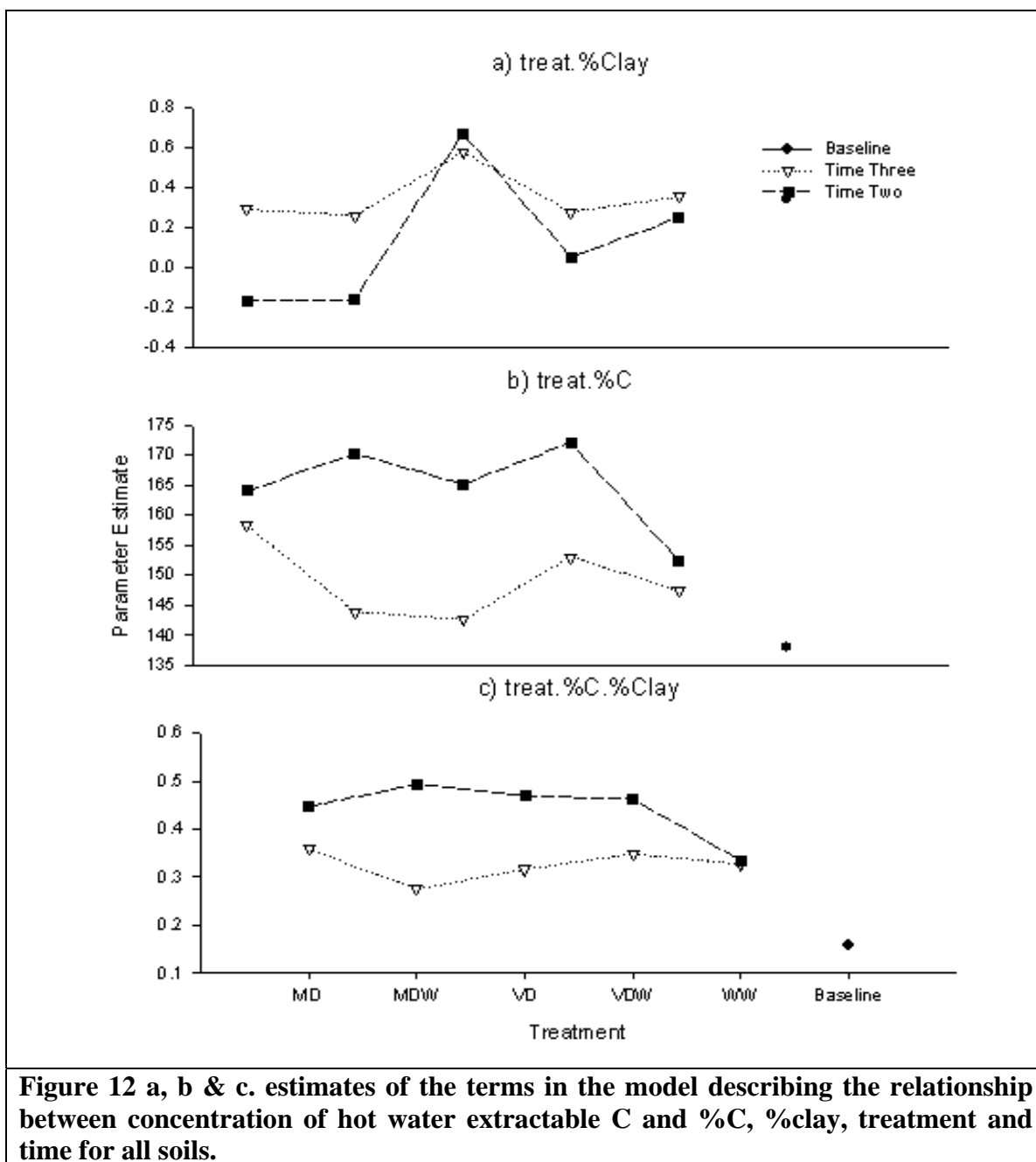
Figure 11. Effect of treatment and paddock on concentrations of hot water extractable carbon in Lismore and Temuka soils at the end of the incubation.

To better understand the impact each continuous variable (%C and %Clay) has, in conjunction with the categorical variables (treatment), the model estimates (from the REML variance components analysis) are presented in Figure 12 a, b & c. Predictions and mean estimates of HWC from the model are based on these parameter estimates.

Percent clay has relatively small effect on HWC. For each increase in %Clay, the estimated HWC increases by ~0.4 (ug C/g soil) depending on which treatment is under assessment. However this differs between sampling times and for MD and MDW at Time 2, the estimated HWC decreases by ~0.2 (ug C/g soil). Apart from this, there is not much difference in the effect of %Clay at times 2 or 3 (Fig. 12a).

Percent C has a much larger affect and the model indicates that for each increase in %C, the estimated HWC increases by 140-170 (ug C/g soil) depending partially on treatment, but mostly on Time (Time 3 has lower estimates than time 2) (Fig. 12b).

The model also indicates that the relationship between %C, %Clay and the categorical factors is not linear (positive values in Fig. 12c).



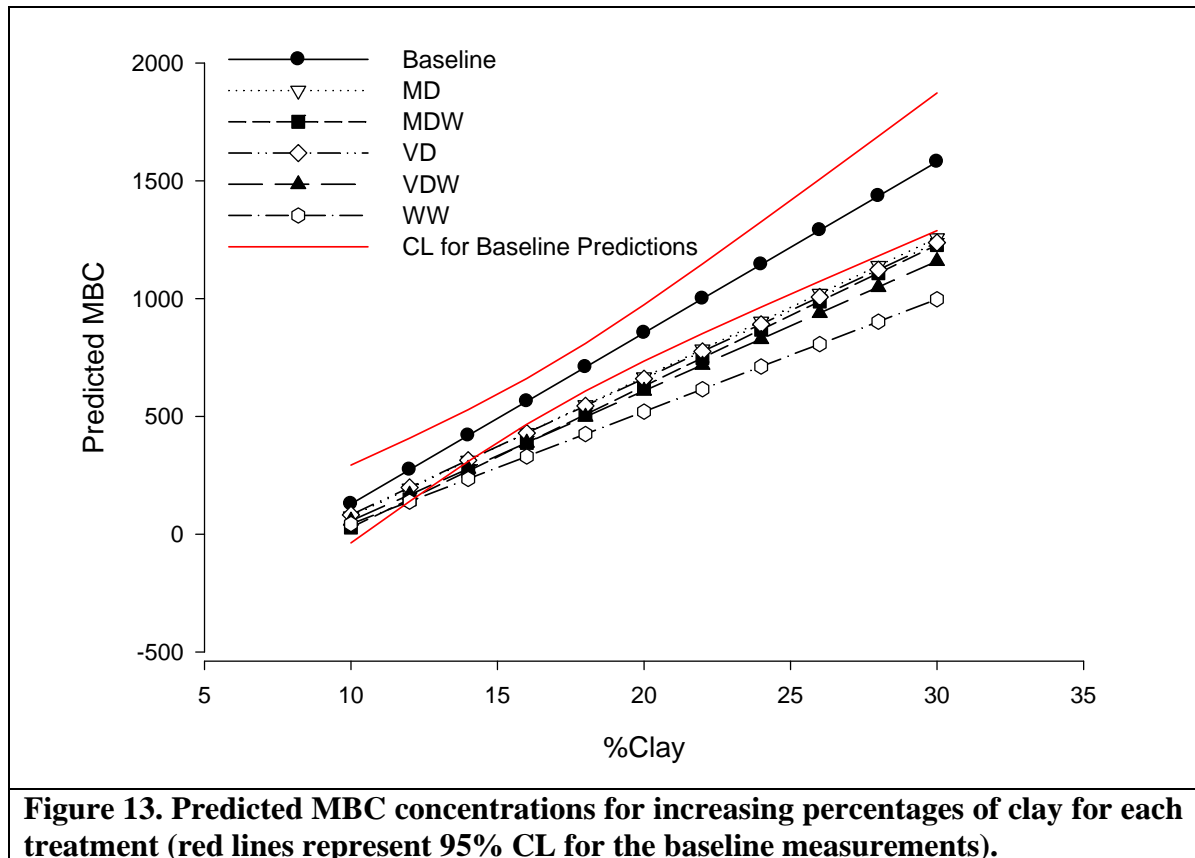
6.4 Microbial Biomass Carbon and Nitrogen

MBC

There is strong evidence that there is a strong treatment effect; and that this effect is not consistent with %C ($p < 0.001$). Overall the predicted MBC concentration (from the REML analysis) increases with increasing levels of %C across all the dry-rewet treatments for all the soils (Fig. 13). However, there is also some evidence that %C and %Clay interact in their effect on MBC (Fig. 15). Further, at higher levels of %C, MBC increases with

increasing %Clay, however at the lowest levels of %C, MBC decreases with increasing %Clay (Fig. 15).

By the end of the incubation MBC concentrations for all treatments decreased from Baseline, with the lowest concentrations in the WW treatment and the highest concentrations in the dry treatments (MD & VD) in almost all of the paddocks (Fig. 14).



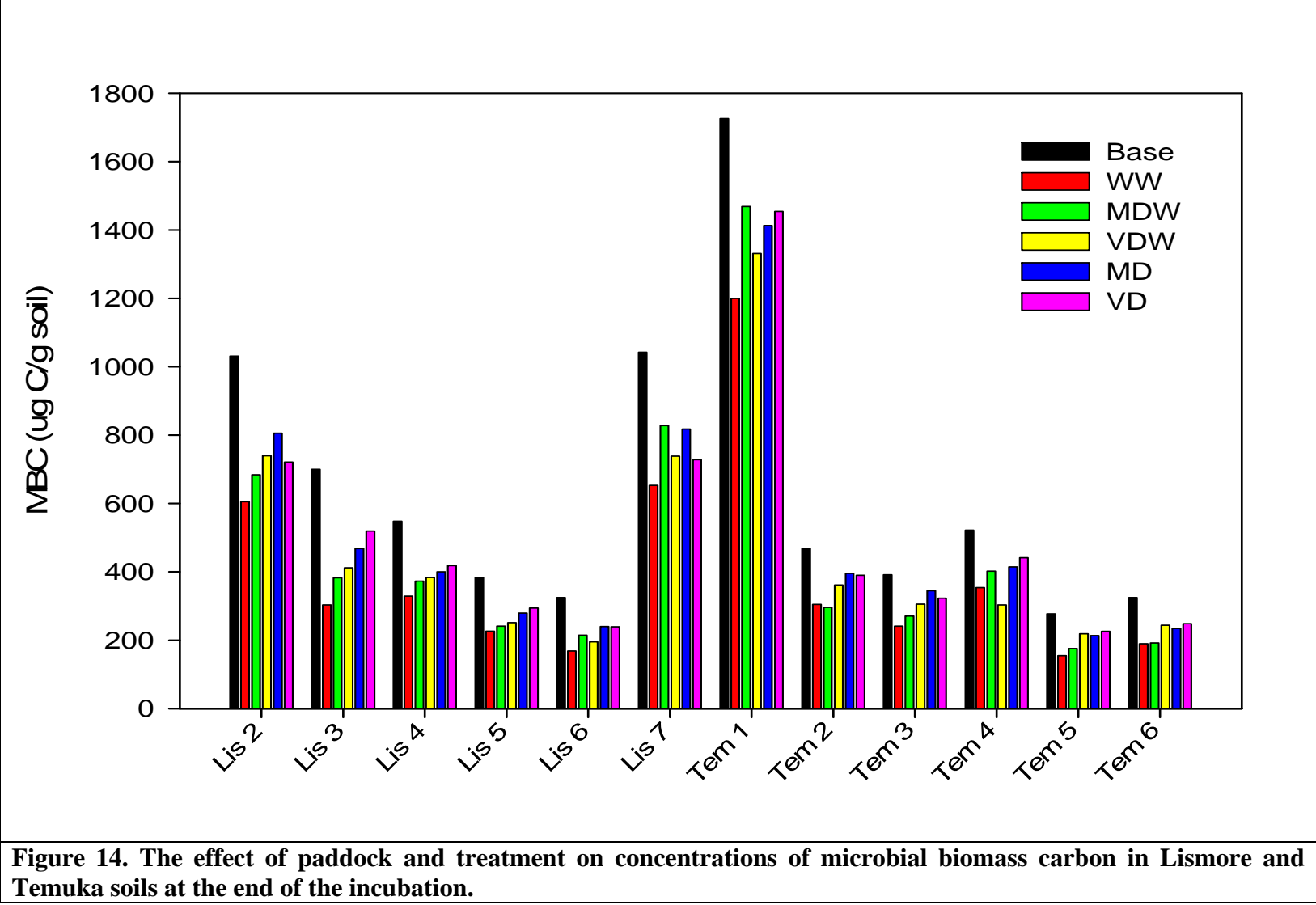


Figure 14. The effect of paddock and treatment on concentrations of microbial biomass carbon in Lismore and Temuka soils at the end of the incubation.

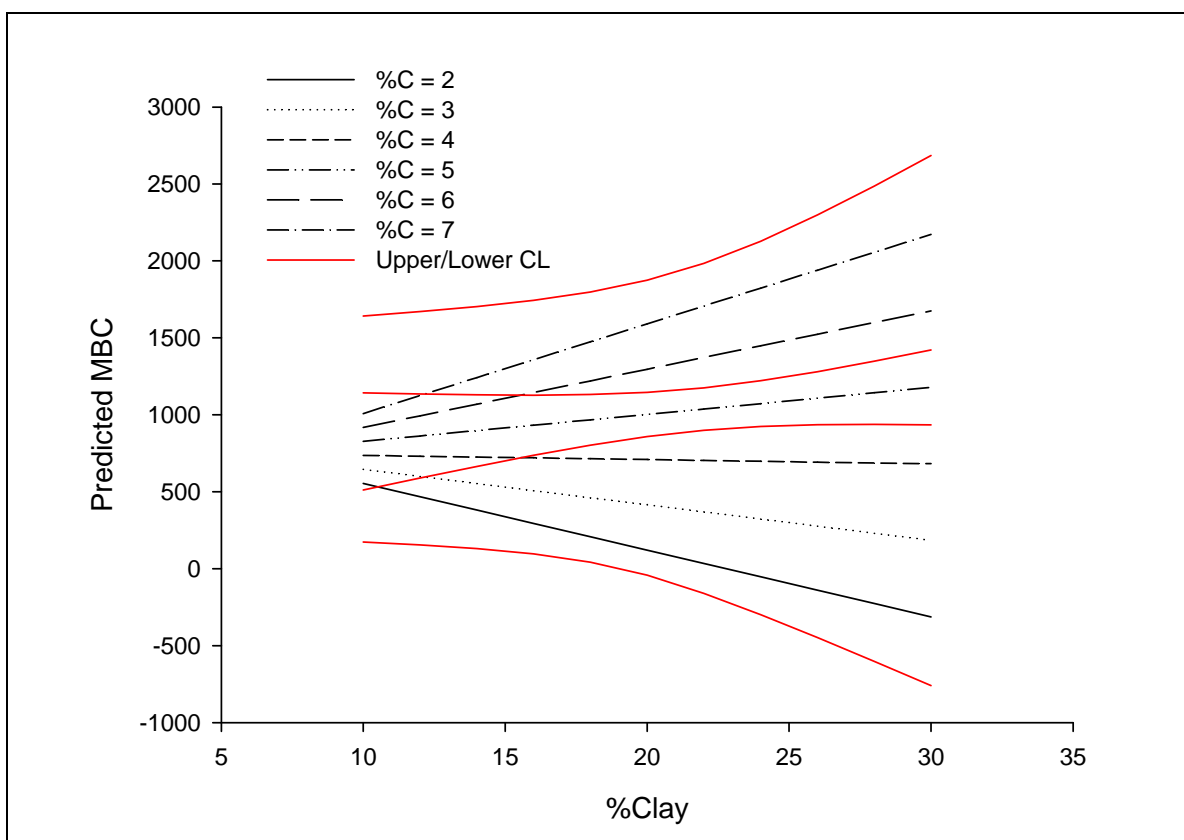


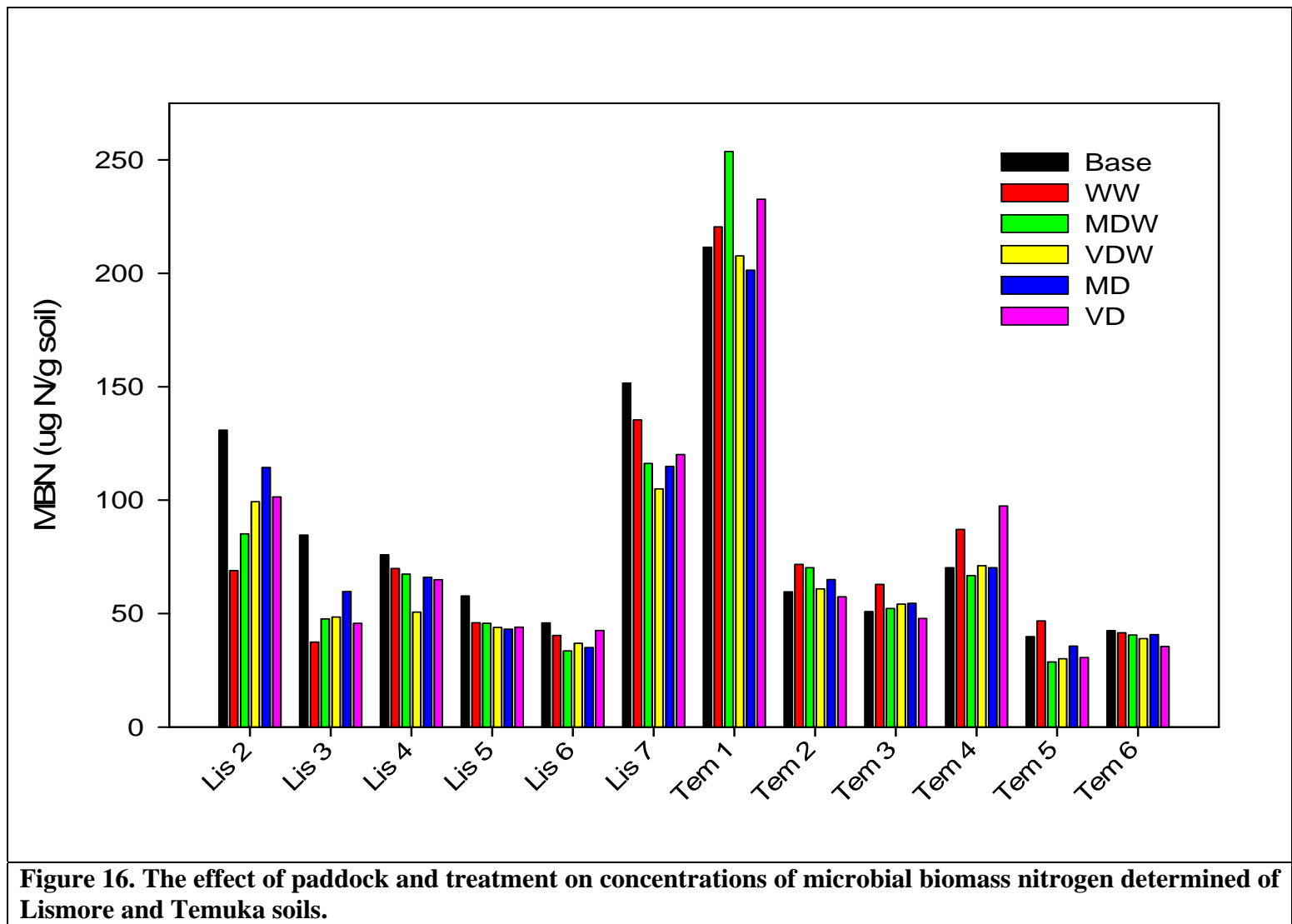
Figure 15. Predicted MBC concentrations for increasing percentages of clay and carbon (red lines represent upper and/or lower 95%CL).

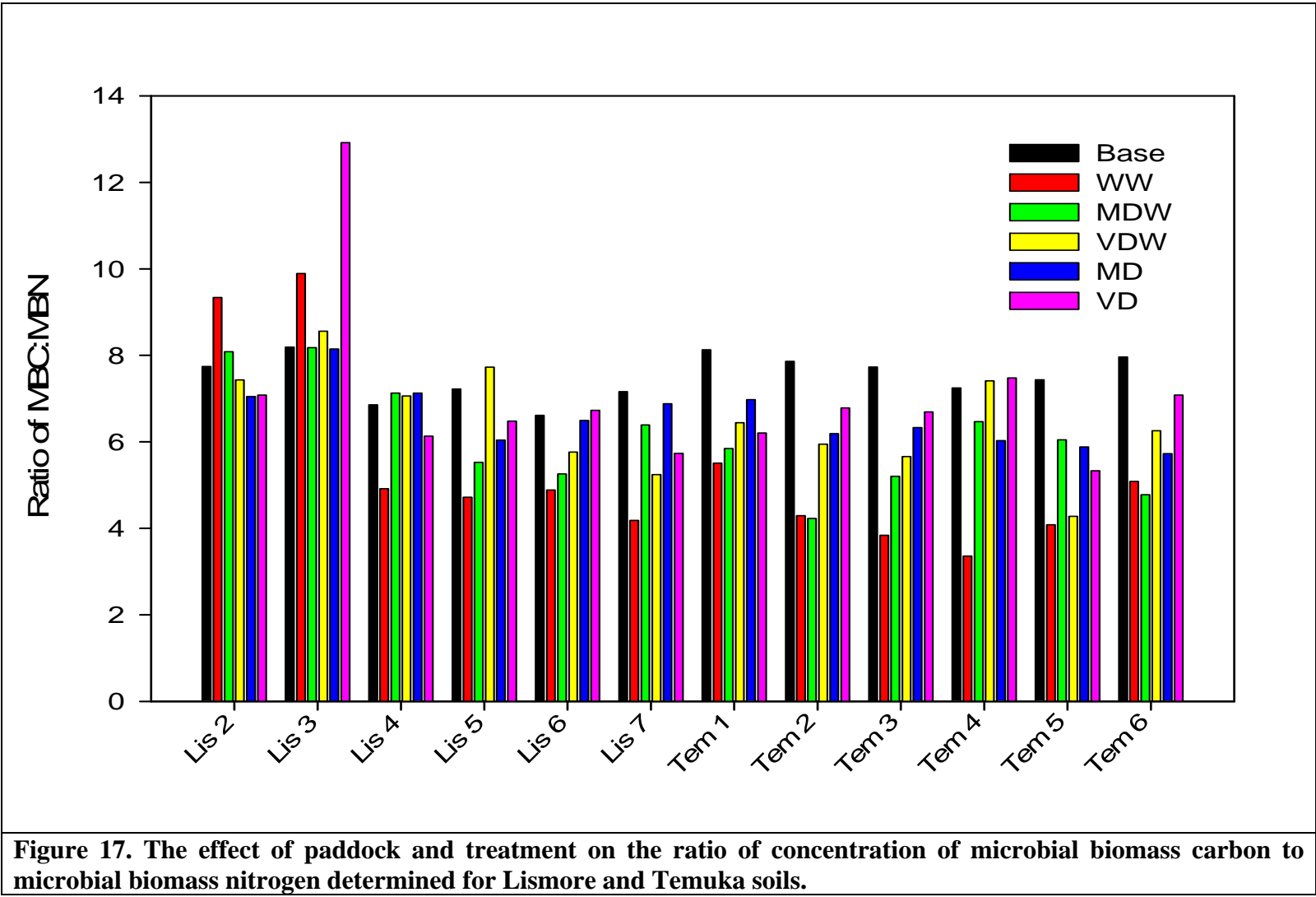
MBN

There is strong evidence ($p < 0.001$) that there is a difference in response for each paddock; and that this is not consistent for each treatment (Fig. 16). Again there are large differences in MBN response for each paddock, with paddock Tem 1 being particularly high. There is evidence that MBN of all treatments drops after baseline for the Lismore paddocks, tending to remain highest in the dry treatments (MD & VD). There does not appear to be any consistent pattern for the Temuka paddocks although some treatments show an increase in MBN from baseline, particularly WW.

Ratio of MBC to MBN

The ratio of MBC:MBN was very similar for all paddocks at Baseline. There is strong evidence of a treatment effect at the end of the trial. In the majority of the paddocks many of the dry-rewet treatments showed a decrease in MBC:MBN from baseline, with WW having the lowest ratio and the dry treatments (MD & VD) tending to have the highest, paddocks Lis 2 & 3 do not follow this pattern (Fig. 17).





6.5 Total Carbon and Nitrogen

Total C

There is some evidence of a treatment effect on the concentration total C measured at the end of the trial, but the changes are small and not consistent with %Clay ($p < 0.001$) or %C ($p = 0.037$). In general there is a decrease in total C from baseline, this decrease tends to be greatest in the WW treatment and smallest in the dry treatments (MD & VD). This trend is more consistent in the Lismore than the Temuka soils (Fig. 18).

Total N

There is evidence of a treatment effect on the total N measured at the end of the trial, and that this effect is not consistent with %Clay or %C ($p < 0.001$, respectively). There is also evidence that the dry treatments (MD & VD) have higher total N than the other dry-rewet treatments (Fig. 19), but as with Total C the changes are small.

6.6 Particulate Organic Matter

POM 53 μ m Carbon and Nitrogen

The patterns of response for C and N in the 53 μ m POM fraction are similar. In the paddocks with high C content (Lis 2, 3, 7 & Tem 1) there is evidence of a response to the dry-rewet treatments for both C and N, however this response is not consistent. The Temuka paddocks tend to show an increase in C and N from Baseline, particularly in the MDW, VWD and VD treatments, this pattern is not evident in the Lismore paddocks where the VD treatment shows a marked decrease from Baseline in Lis 2, 3 & 7 (Fig. 20 & 21).

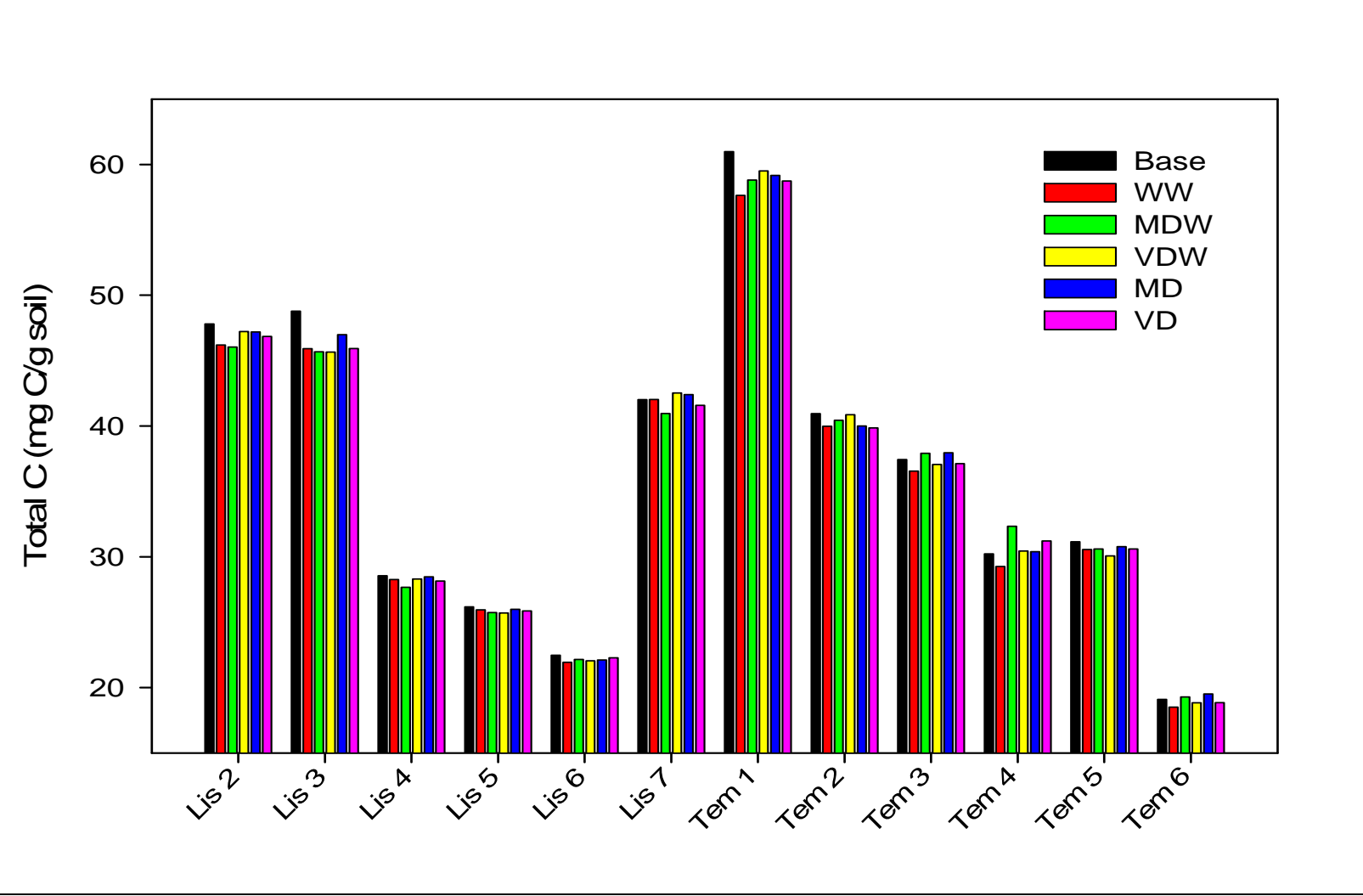


Figure 18. The effects of paddock and treatment on the amount of total carbon in Lismore and Temuka soils.

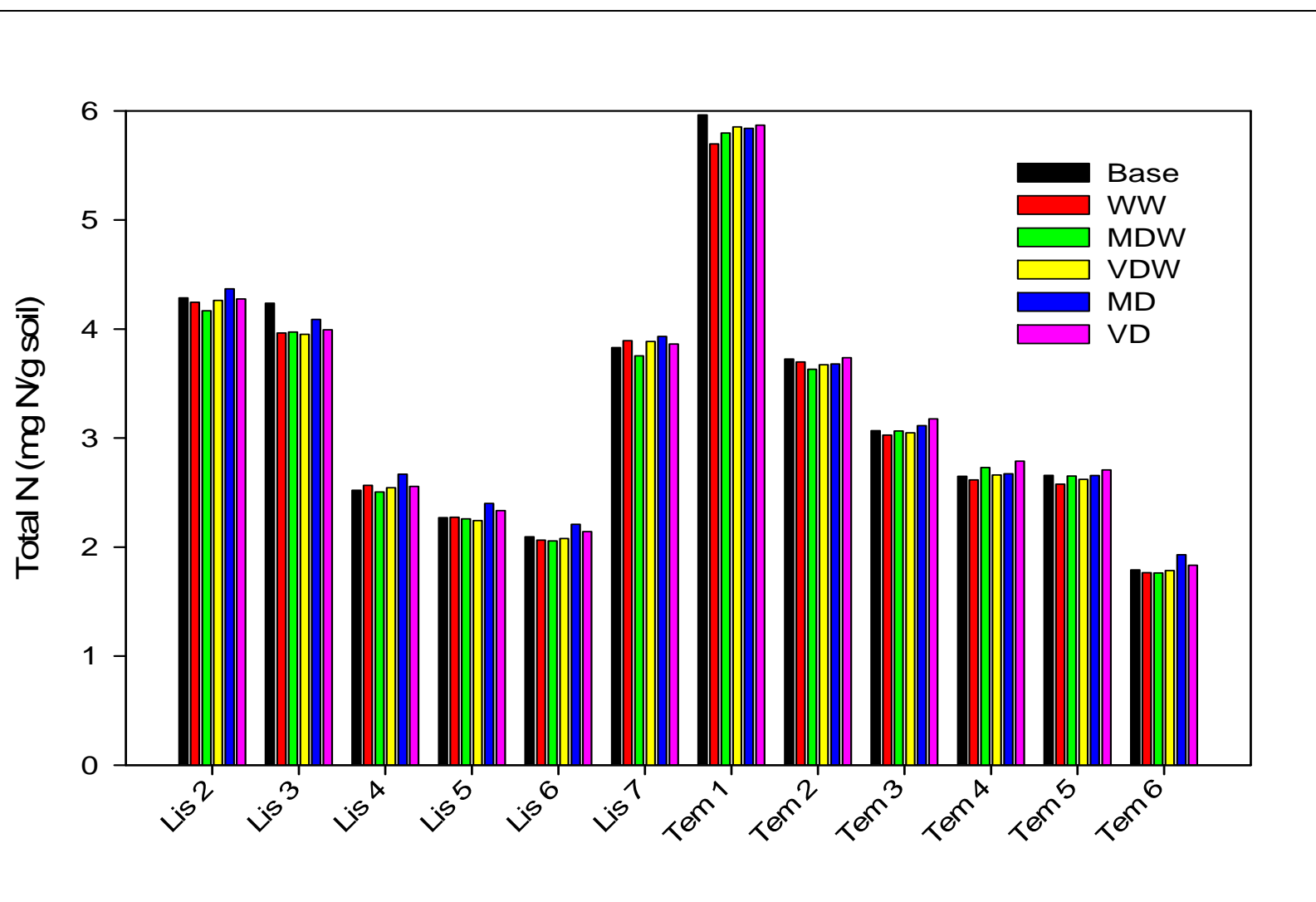


Figure 19. The effects of paddock and treatment on the amount of total nitrogen in Lismore and Temuka soils.

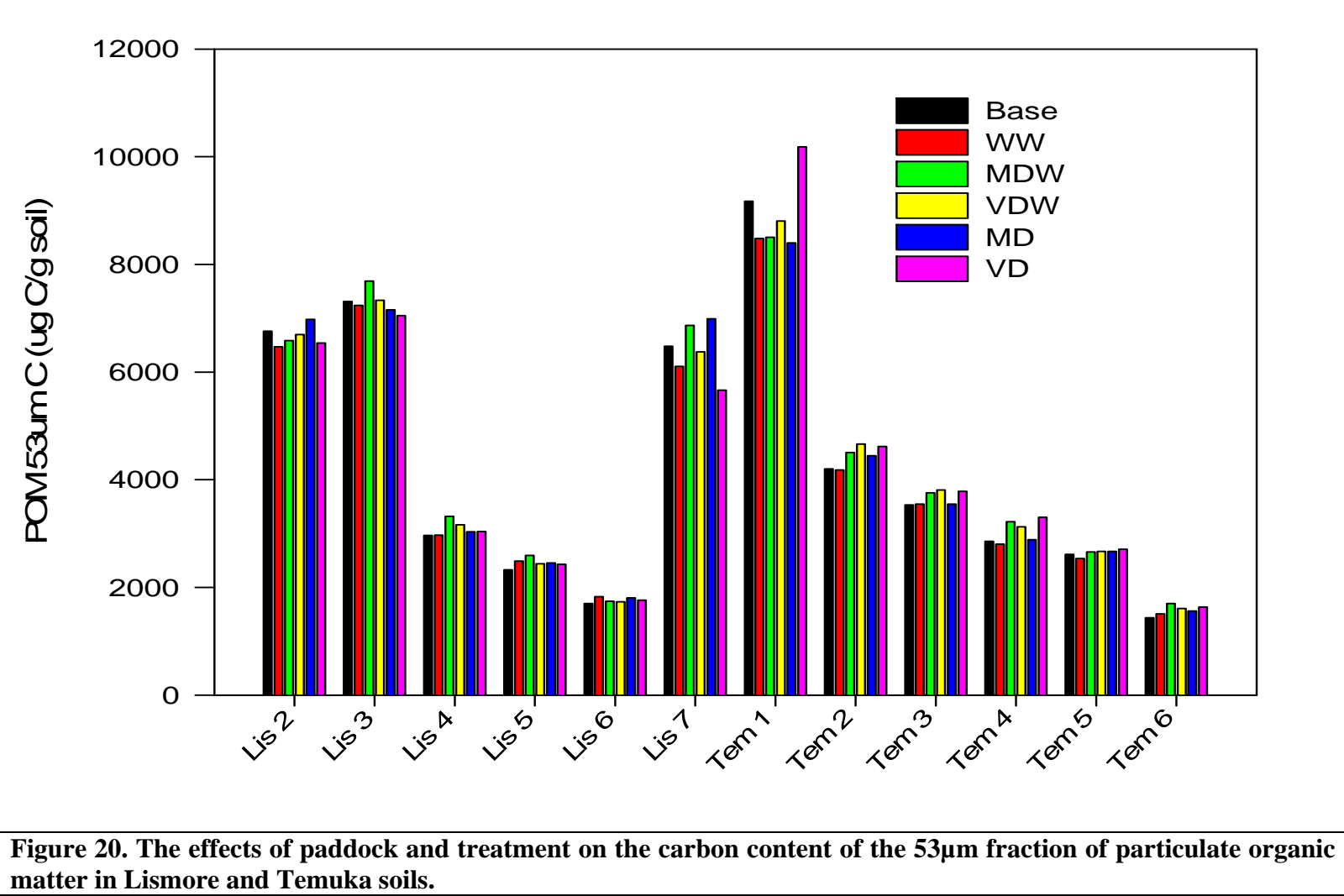


Figure 20. The effects of paddock and treatment on the carbon content of the 53µm fraction of particulate organic matter in Lismore and Temuka soils.

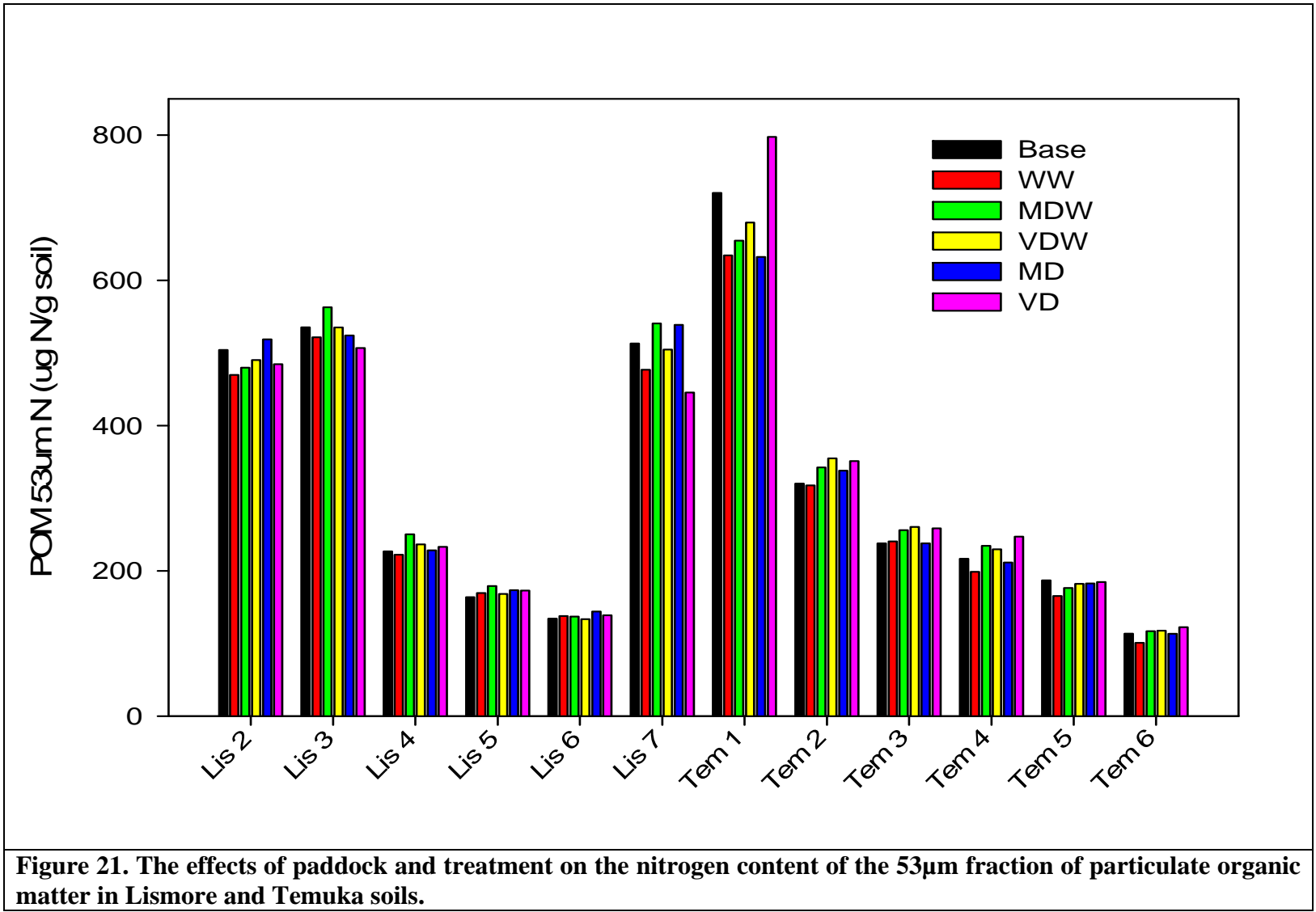


Figure 21. The effects of paddock and treatment on the nitrogen content of the 53µm fraction of particulate organic matter in Lismore and Temuka soils.

POM 250 μ m Carbon and Nitrogen

Like the 53 μ m POM fraction, the patterns of response for C and N in the 250 μ m POM fraction are similar and the paddocks with high C content (Lis 2, 3, 7 & Tem 1) show a decrease from baseline in both C and N concentrations across the dry-rewet treatments, but the pattern of response is not consistent.

In the Lismore paddocks the treatments tend to show a decline from Baseline. In the Temuka paddocks, with the exception of Tem 1, the treatments show a slight tendency to increase from Baseline. As with the 53 μ m POM fraction, the Temuka paddocks tend to show the greatest increase in C and N from Baseline in the MDW, VWD and VD treatments. (Fig. 22 & 23).

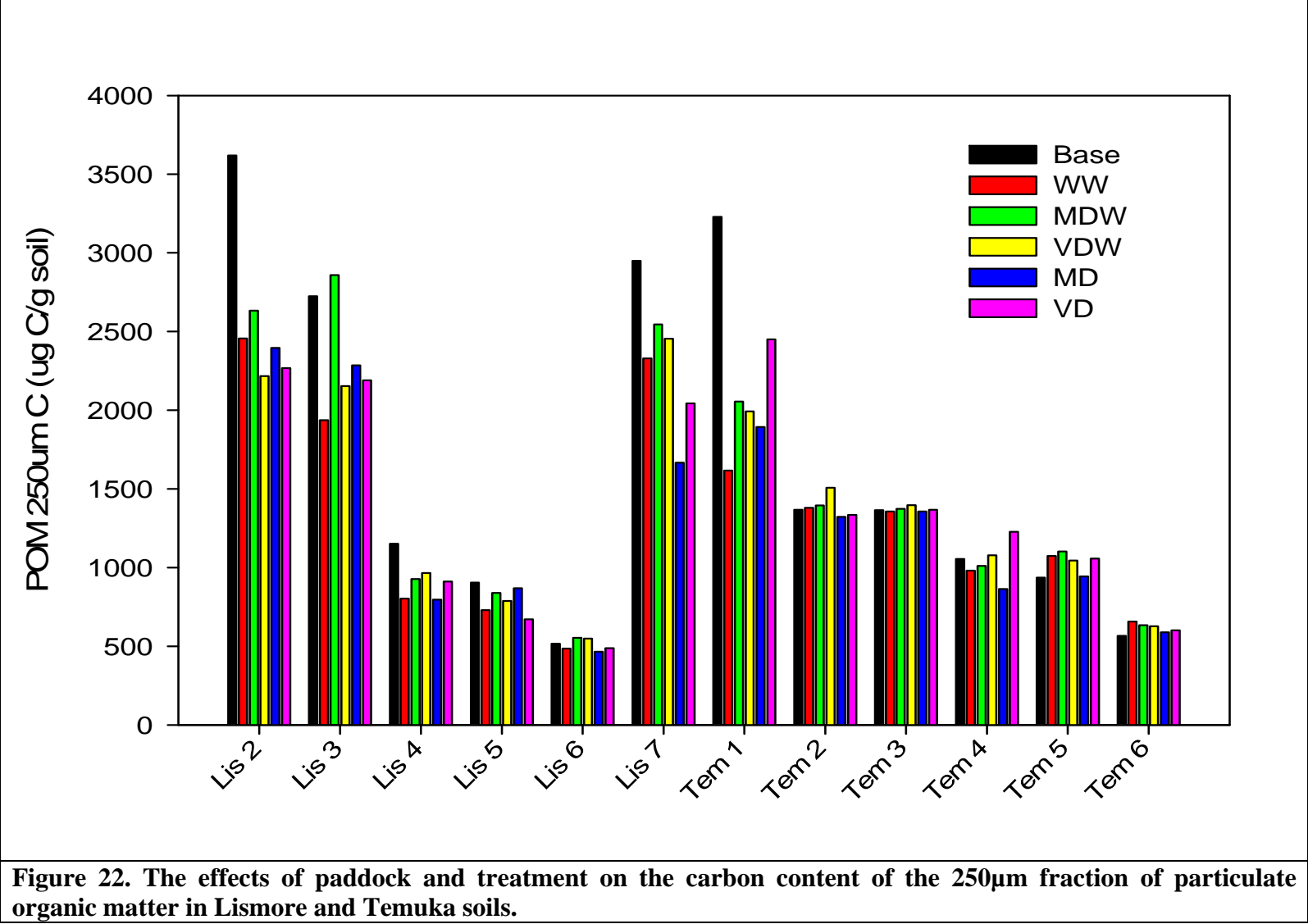


Figure 22. The effects of paddock and treatment on the carbon content of the 250µm fraction of particulate organic matter in Lismore and Temuka soils.

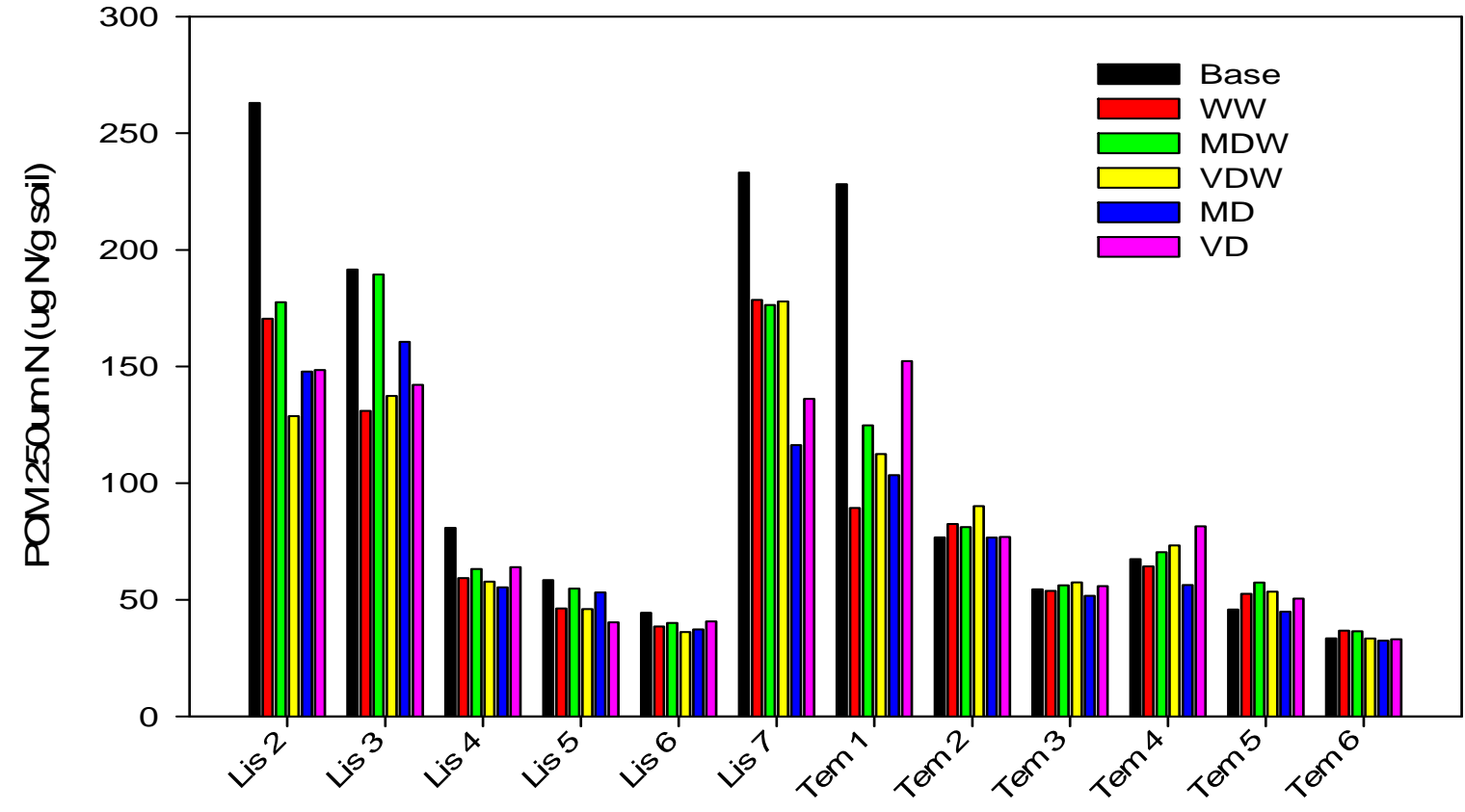


Figure 23. The effects of paddock and treatment on the nitrogen content of the 250µm fraction of particulate organic matter in Lismore and Temuka soils.

7. Data Calculation & Statistical Analysis - Carbon Mineralisation & Nitrous oxide emissions

Calculated Data

The moisture contents and average respiration/emission rates (average of rates over treatment phase (days 32 to 70) of the incubation) of the constant moisture content treatments (WW, MD & VD) were used to produce calculated rates and cumulative mineralisation/ emission data. The moisture contents of the MDW and VDW treatments were recorded whenever gas measurements were made during the treatment phase of the incubation. These moisture contents were converted into a proportion of the constant moisture contents, e.g. if the constant moisture content for WW is 42.1% and MDW soil is at 28.9%, then, as a proportion of the constant soil moisture, it is at 0.68. This proportion was in turn used to calculate the respiration/emission rate by multiplying the constant treatment rate by the proportion figure, e.g. if the WW rate is 0.21 and the moisture proportion is 0.68, then the calculated rate is 0.14.

Calculated respiration/emission rate data was produced to correspond to every actual measured data point over the entire treatment phase of the incubation. To produce the calculated cumulative data, the calculated respiration/emission rates were multiplied by the number of hours in the interval between measurements and then summed.

Statistical Method

In order to investigate the relationship between the calculated and observed cumulative C mineralisation and N₂O-N emission data for the samples that underwent a series of drying and rewetting cycles (VDW and MDW treatments) there were three approaches used:

- The first was simply a graphical exploration the data to get a better understanding of the correlation between the observed and calculated cumulative mineralisation rates.
- The second was an ANOVA with treatment and the “method of obtaining the data” (i.e. actual or calculated) as predictive factors. This would indicate whether there is a significant difference in values, and where the means for the two groups lie in relation to one another.
- The third was to use a series of F-tests to better understand how well the calculated data fits the the observed in a regression framework, testing the intercept and the slope both separately and together.

The rates of C mineralisation and N₂O-N emission at the end of the trial and cumulative values of C mineralised and N₂O emitted was analysed using ANOVA in GenStat v. 10. For these data sets there was evidence that the assumption of heteroscedasticity was not met, so a log transformation was used and results presented on the log scale.

8. Results - Carbon Mineralisation & Nitrous oxide emissions

8.1 Actual versus calculated data

Carbon mineralisation

Graphical exploration of the data indicates that one paddock (Tem 1) has very high values in both the predicted and the observed variates (Fig. 24). It also indicates that the variability of data increases with increasing mean; indicating that either a log transformation or removal of the outlying paddock will be required. There does not appear to be any reason to remove this paddock apart from the fact that it will be very influential.

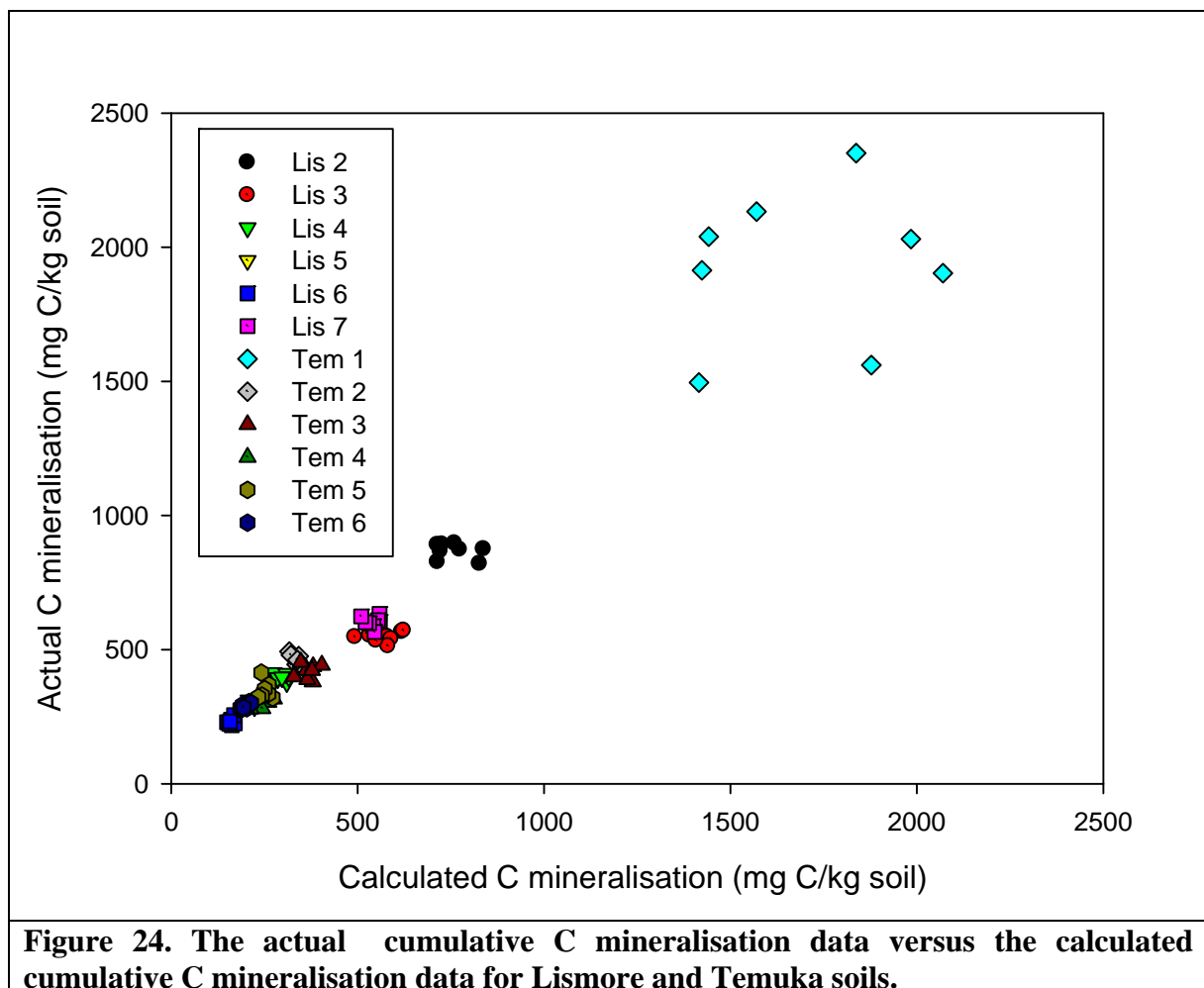
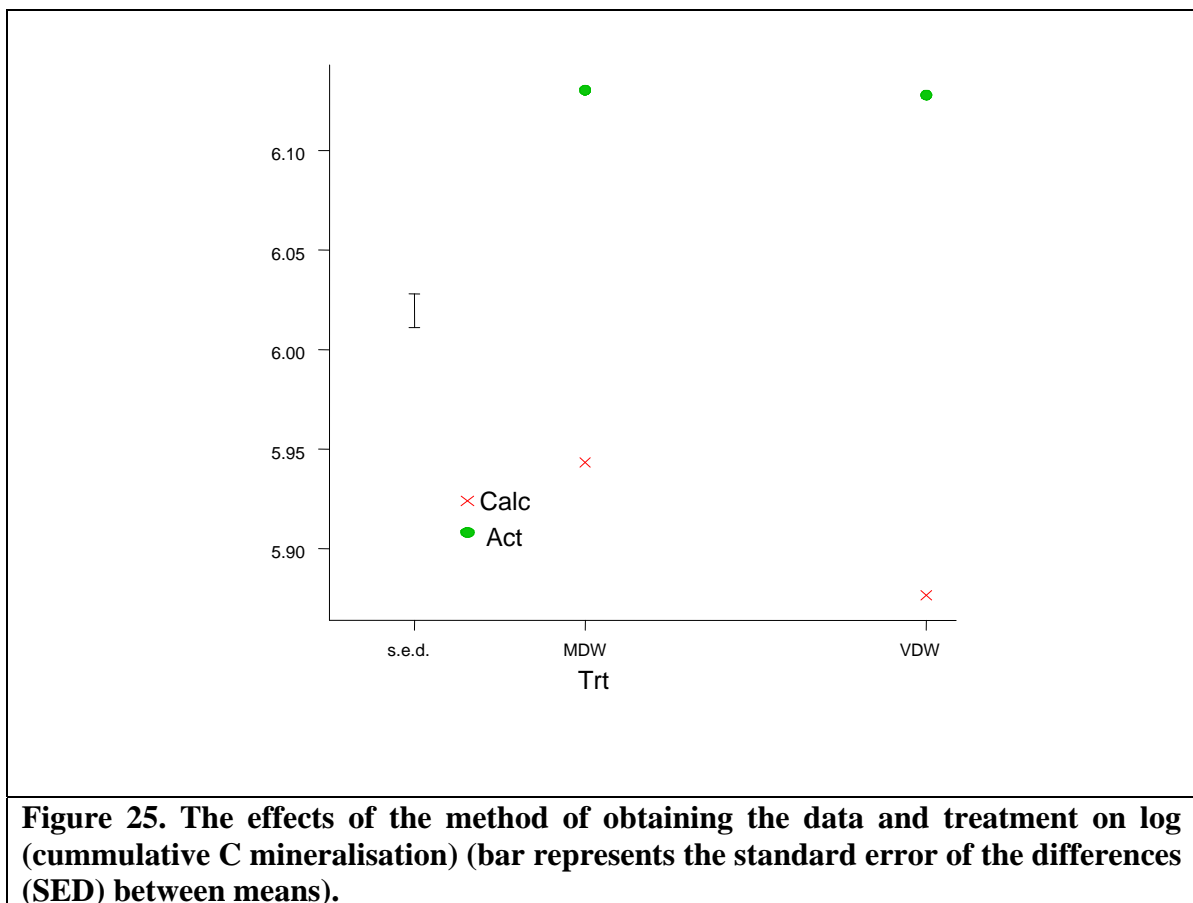
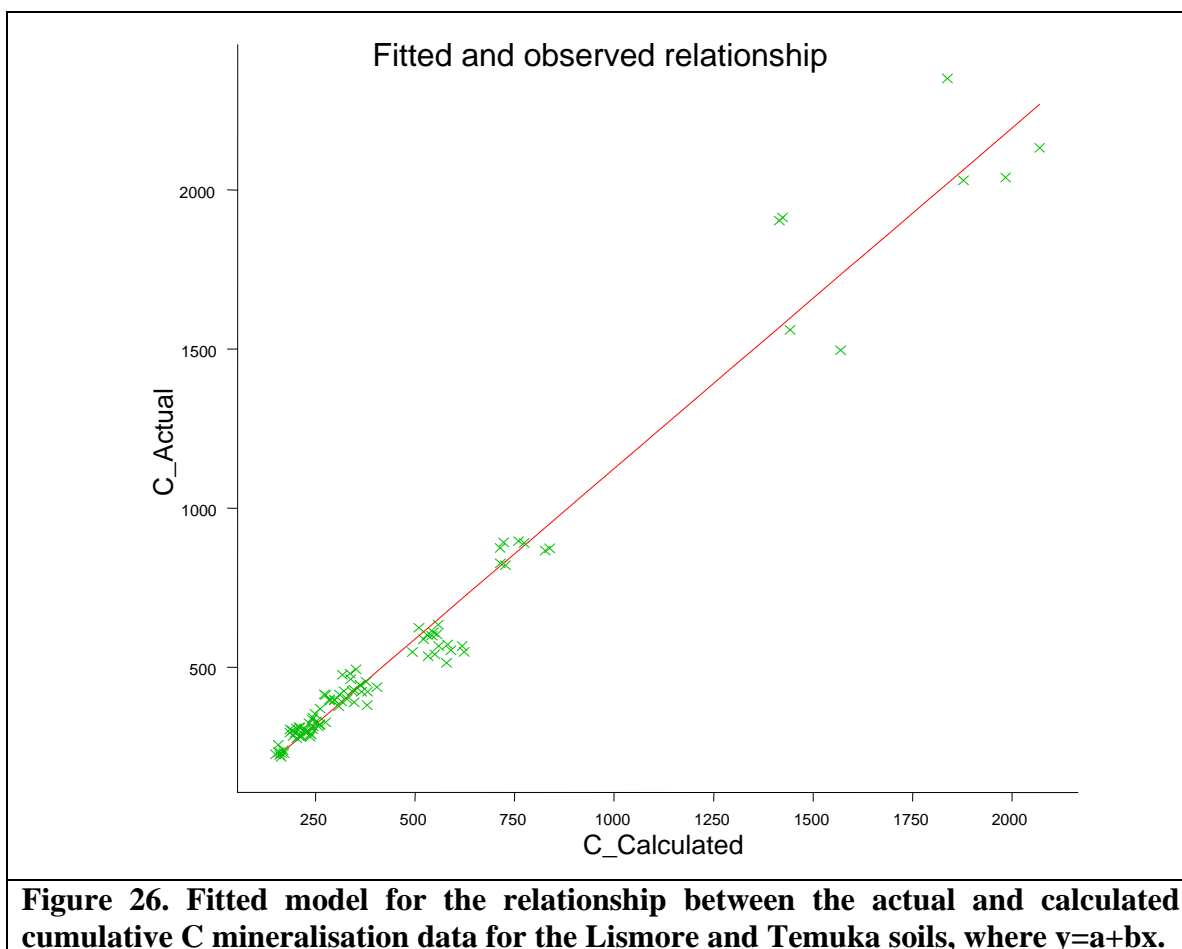


Figure 24. The actual cumulative C mineralisation data versus the calculated cumulative C mineralisation data for Lismore and Temuka soils.

Analysis of the log transformed data indicates that the actual values were higher than the calculated values, and that this difference was statistically significant. There was also evidence of a difference between MDW and VDW for the calculated values, but not the observed values (Fig. 25).

Investigating the nature of the correlation between the calculated and observed C mineralisation data, the model indicates that not only is the intercept not consistent with the origin ($p < 0.0001$) but that there is evidence that the slope is not consistent with the 1:1 line ($p < 0.001$). The actual estimates for the intercept and slope for the model $y = a + bx$ are $a = 52.9$; $b = 1.07$ (still quite close to 1). The fitted model can be seen in Fig. 26, the R-sq for this model is 96.6%.





Looking at individual paddocks, with the exception of Lis 3, the calculated data is lower than the actual data and the percentage difference between the calculated and actual data is greater for the VDW treatment than the MDW treatment, with a range of -7 to -28% for MDW and -10 to -36% for VDW respectively (Table 6).

While those paddocks with high %C had high cumulative C mineralisation (especially Tem 1), but there doesn't appear to be any evidence of a strong relationship between %Clay or %C and the percent difference between actual and calculated cumulative C mineralisation (Table 6 & Fig. 27).

Table 6. The calculated and actual amounts of C mineralised for each paddock and the percentage difference between the two values (n=4).

Paddock	MDW			VDW		
	Calculated (mg C/kg)	Actual (mg C/kg)	% diff.	Calculated (mg C/kg)	Actual (mg C/kg)	% diff.
Lis 2	774	888	-13	746	847	-12
Lis 3	594	557	7	549	537	2
Lis 4	308	394	-22	286	400	-29
Lis 5	230	296	-22	211	309	-32
Lis 6	166	229	-27	156	234	-33
Lis 7	545	609	-10	536	598	-10
Tem 1	1762	1896	-7	1643	1961	-16
Tem 2	351	448	-22	328	461	-29
Tem 3	378	408	-7	359	430	-17
Tem 4	260	318	-18	233	289	-20
Tem 5	260	361	-28	244	335	-27
Tem 6	206	284	-27	189	296	-36

Plotting the calculated C mineralisation rates against the actual C mineralisation rates shows that much of the error in the calculated cumulative data arises from an underestimation of the mineralisation flush that occurs when the dry soil is rewetted, especially during the first dry-rewet cycle, and an over estimation of the rate at which respiration decreases as the soil dries, especially during the initial drying phase. There is evidence that the fit becomes improves for the second dry-rewet cycle, but there may be an overestimation the mineralisation flush by the third cycle (Fig. 28). Figure 28 shows the average actual and calculated C mineralisation rates across all the paddocks, plots for individual paddocks can be found in the Appendix (Fig. A7 & A8).

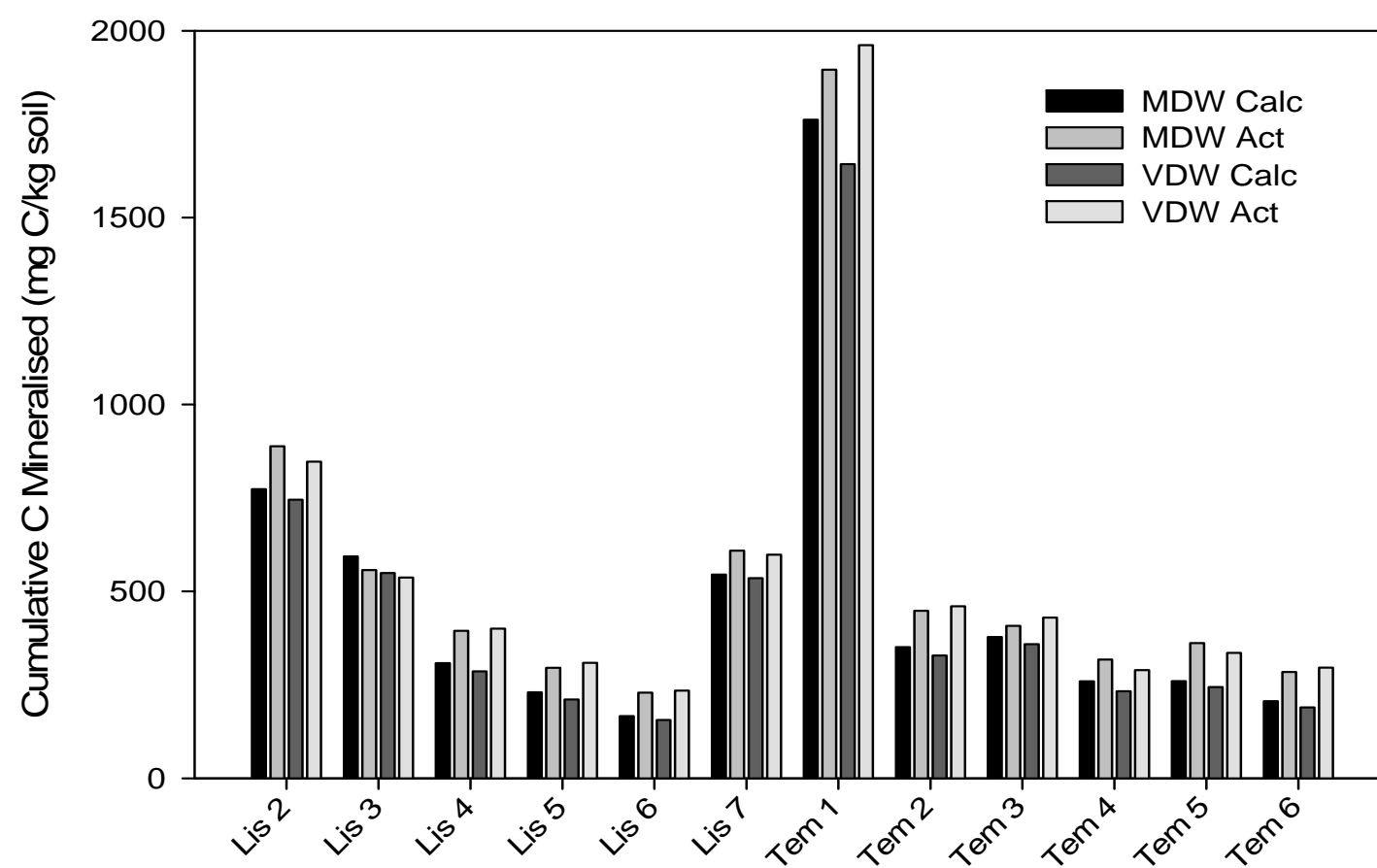
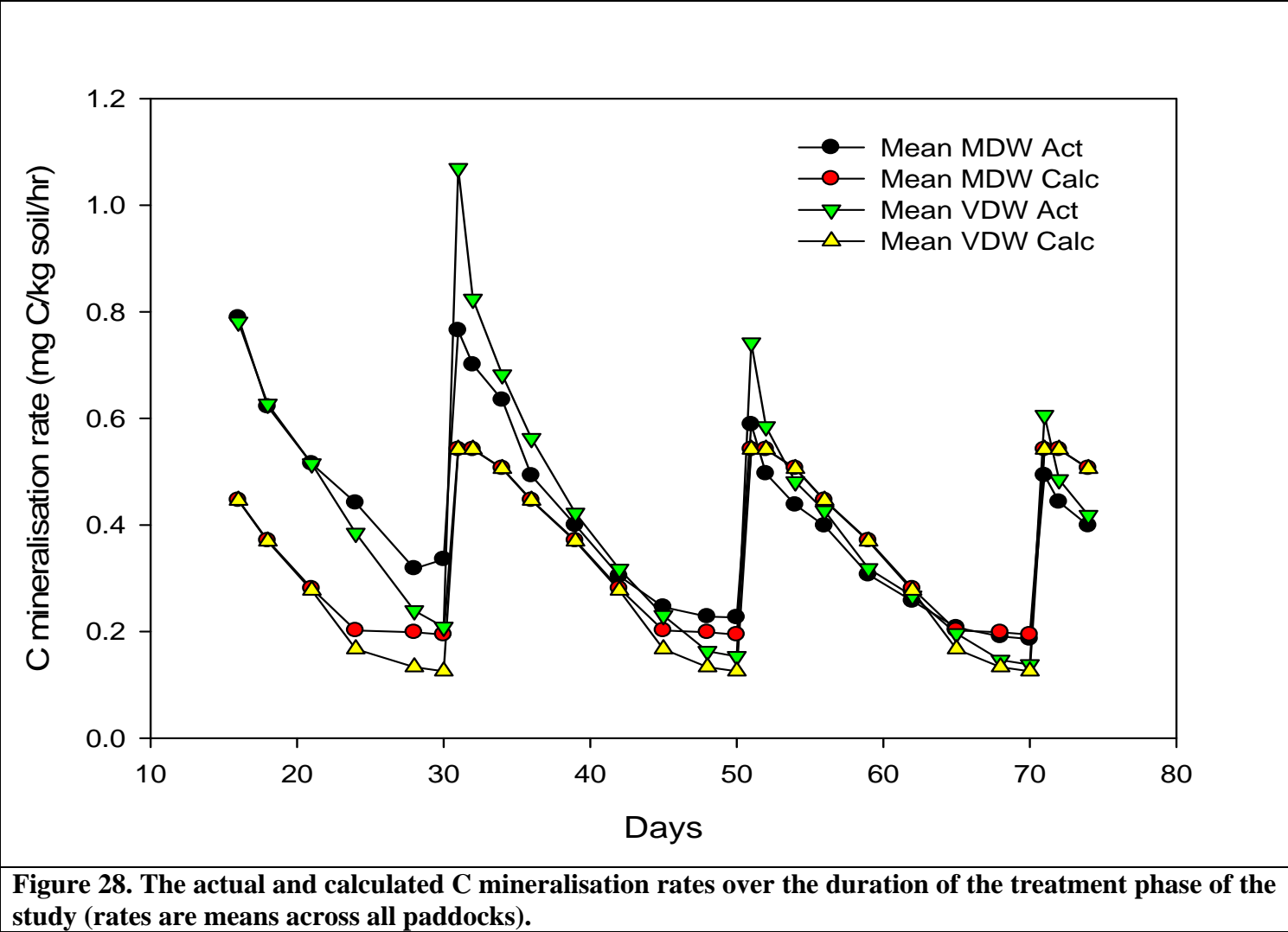


Figure 27. The actual and calculated amounts of C mineralised over the treatment phase in the MDW and VDW treatments for each paddock.

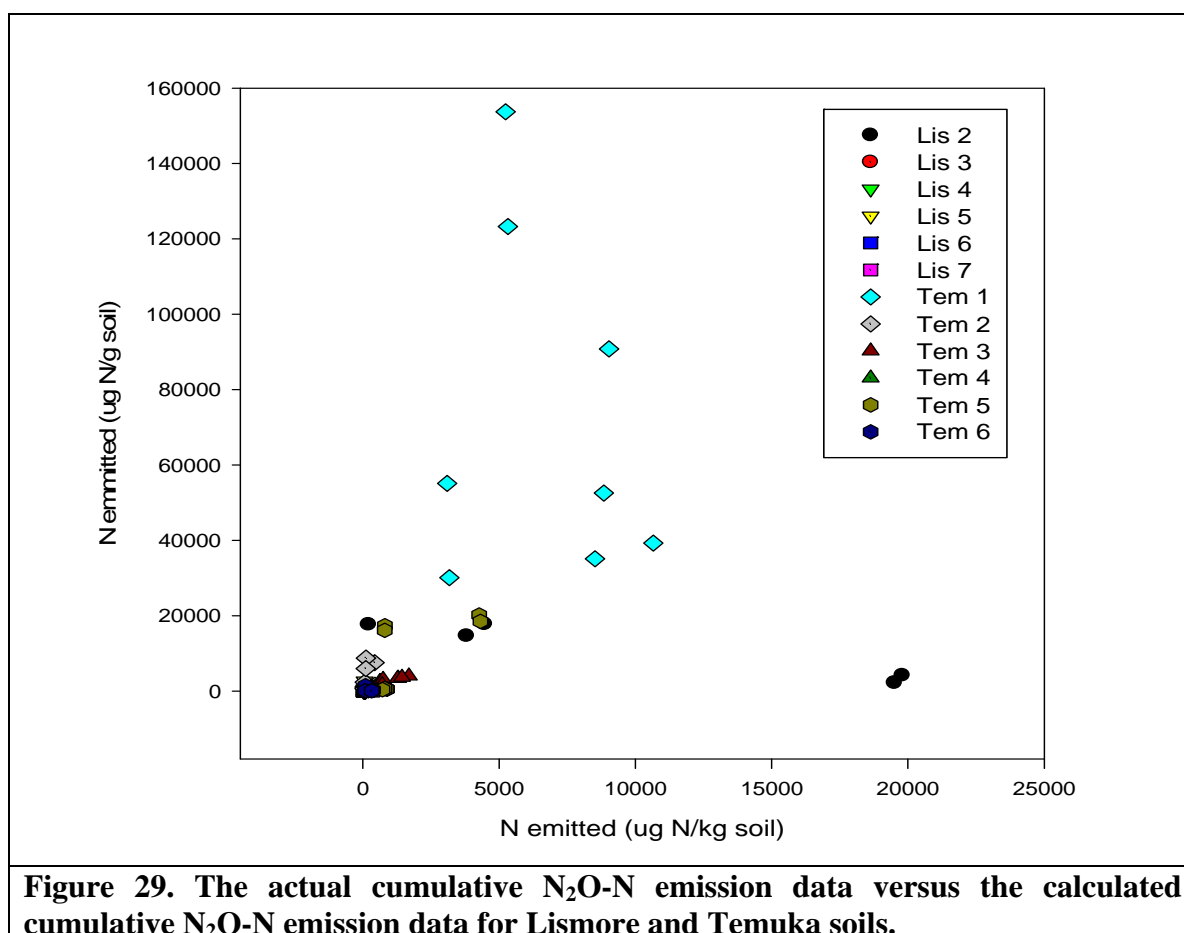


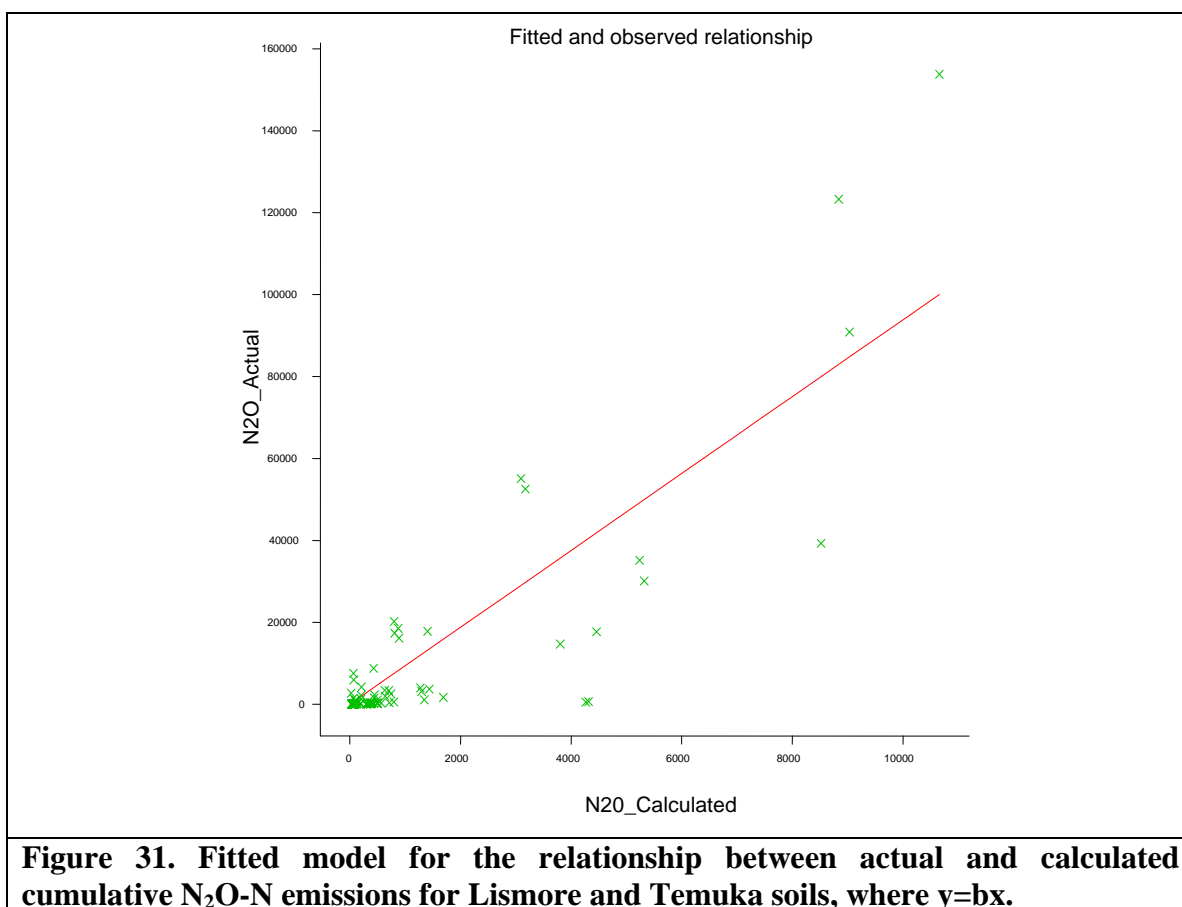
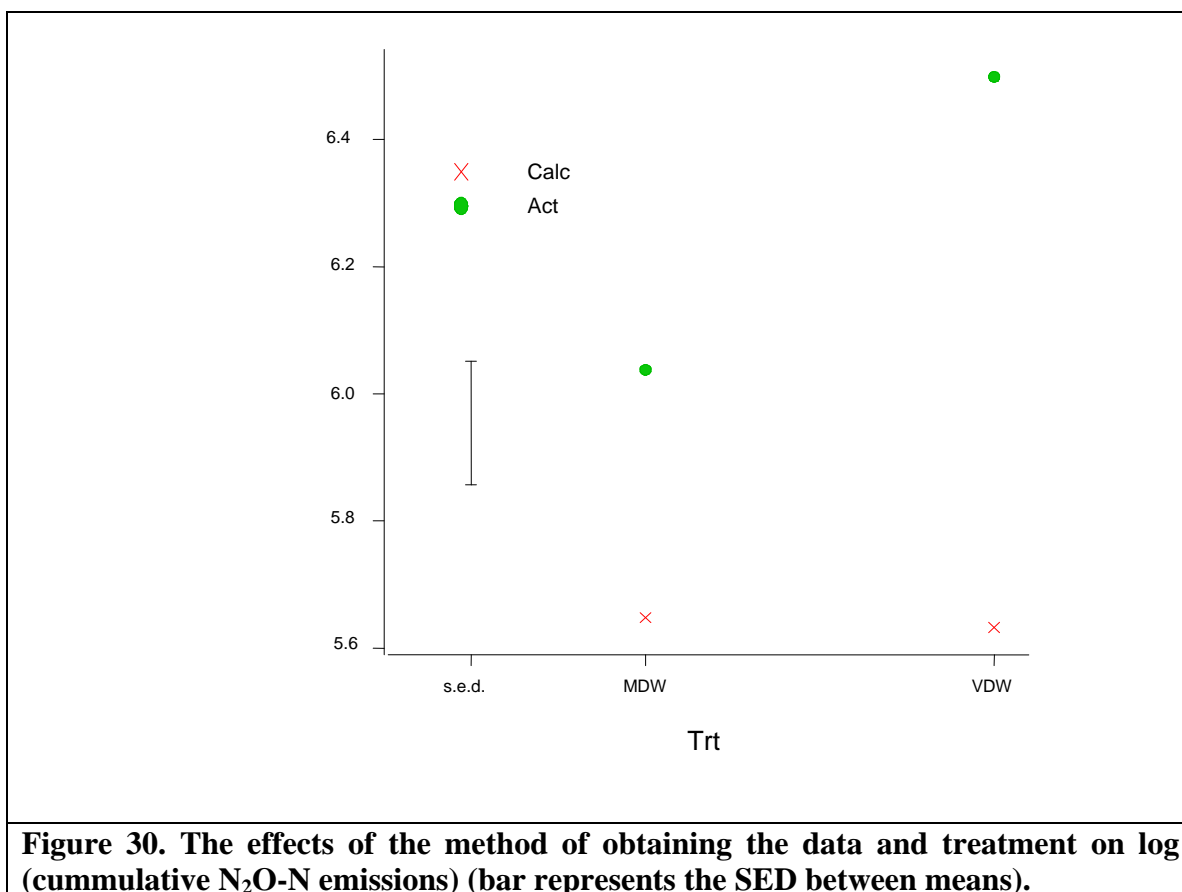
Nitrous oxide-nitrogen emission

Graphical exploration indicates that the N₂O-N emission data is inherently more variable than the C mineralisation data, and paddock Tem 1 still lies as a set of clearly influential points. It also indicates that the relationship between the observed and calculated data is not particularly linear (Fig. 29).

Investigation of the residuals indicated that a log transformation of the data was required. The analysis indicated that the calculated means were lower than the observed means ($p < 0.001$). Further, there was evidence that MDW had lower N₂O-N emission than VDW in the observed data, but not the calculated data ($p = 0.084$) (Fig. 30).

The results of the analysis investigating the nature of the correlation between the calculated and observed N₂O-N emission data indicates that the slope is not consistent with the 1:1 line ($p < 0.0001$), however there is no evidence that the intercept departs from the origin ($p = 0.1372$). There does not seem to be an indication that the slope should be fitted as a non-linear curve. The actual estimate for slope for model $y = bx$ is $b = 9.385$ (i.e. the calculated data is nearly 10-fold lower than the actual data). The R-sq for this model is 74%, far lower than for the C model (Fig. 31).

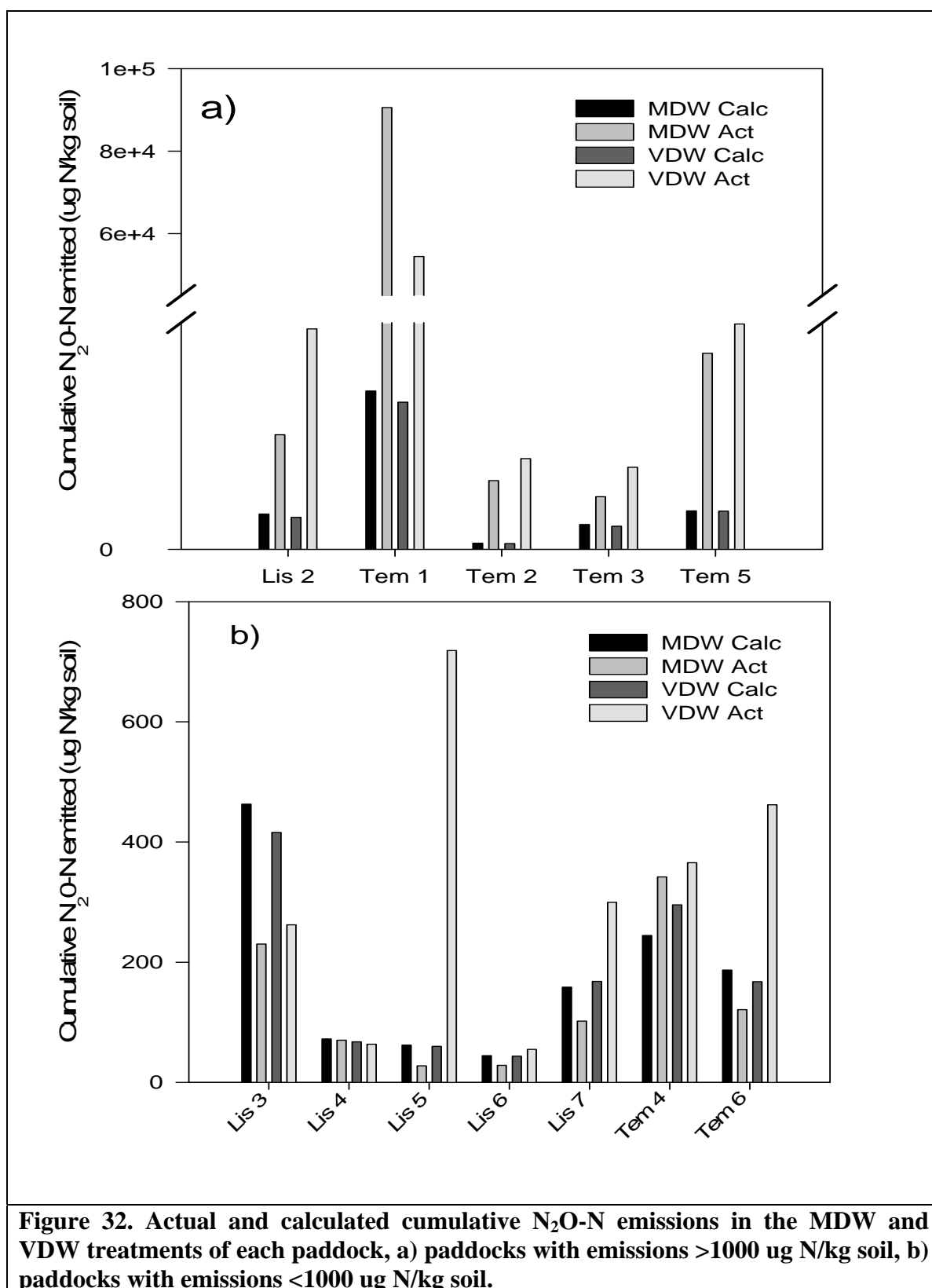




Looking at individual paddocks, with the exception of Lis 2, the calculated data is higher than the actual data for the MDW treatments in the Lismore paddocks. In contrast the actual data is higher than the calculated data in the Temuka paddocks for both treatments. The amount of N₂O-N emitted is greater for the VDW treatment than the MDW treatment in all paddocks, but the percentage difference between the calculated and actual data is not always greater for the VDW treatment than the MDW (Table 7 & Fig. 32).

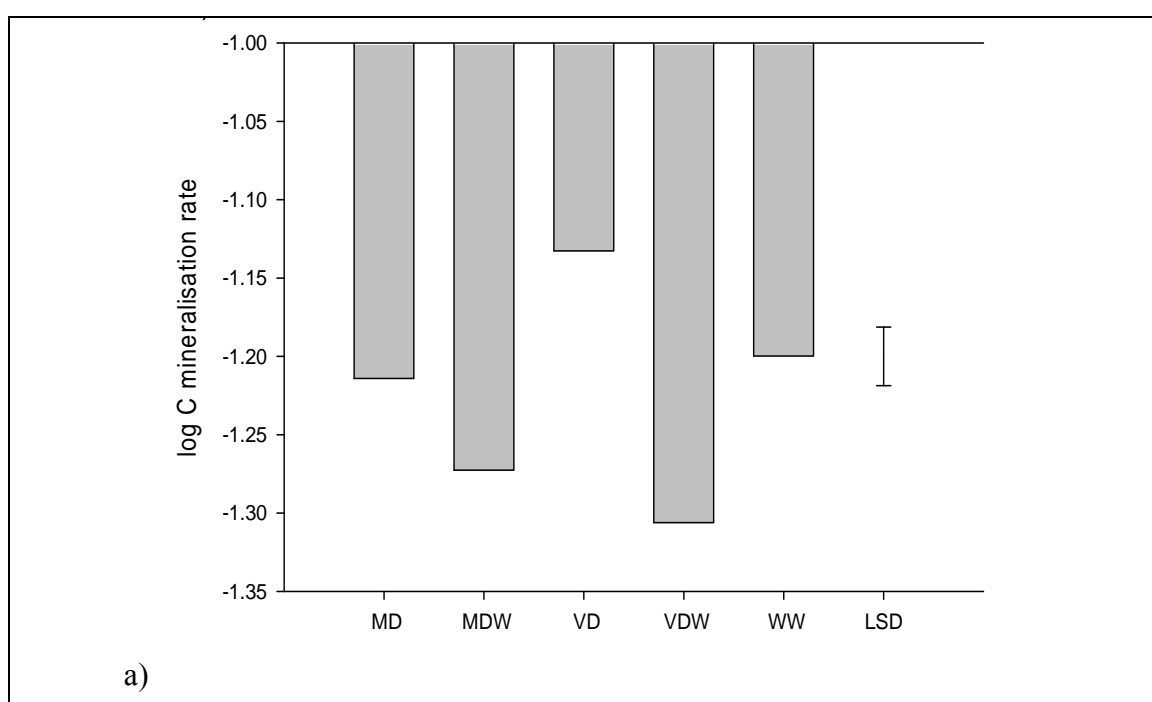
Table 7. The calculated and actual N₂O-N emissions for each paddock and the percentage difference between the two values.

Soil	MDW			VDW		
	Calculated (ug N/kg)	Actual (ug N/kg)	% diff.	Calculated (ug N/kg)	Actual (ug N/kg)	% diff.
Lis 2	1553	5053	-69	1406	9721	-86
Lis 3	463	230	101	416	262	59
Lis 4	72	70	3	67	63	6
Lis 5	61	27	126	60	718	-92
Lis 6	44	28	56	43	54	-21
Lis 7	158	101	55	168	299	-44
Tem 1	6980	90568	-92	6490	54444	-88
Tem 2	265	3025	-91	251	4001	-94
Tem 3	1092	2321	-53	1012	3618	-72
Tem 4	244	341	-29	295	365	-19
Tem 5	1689	8642	-80	1677	9940	-83
Tem 6	186	120	55	167	462	-64



8.2 Rates of C mineralisation and N₂O-N emission

The rate data at the end of the trial showed there was evidence that the assumption of heteroscedasticity was not met, so a log transformation was used. There is strong evidence ($p < 0.001$ respectively) of a difference in the rate data of the treatments for both C and N at the end of the trial. Overall, the VD treatment had the highest rate, MD and WW intermediate and MDW and VDW the lowest, with this pattern consistent for both C and N although the differences are not always statistically significant for the N data (Fig. 33a & b). Graphs of untransformed rates for individual paddocks can be found on the Appendix (Fig. A9 & A10).



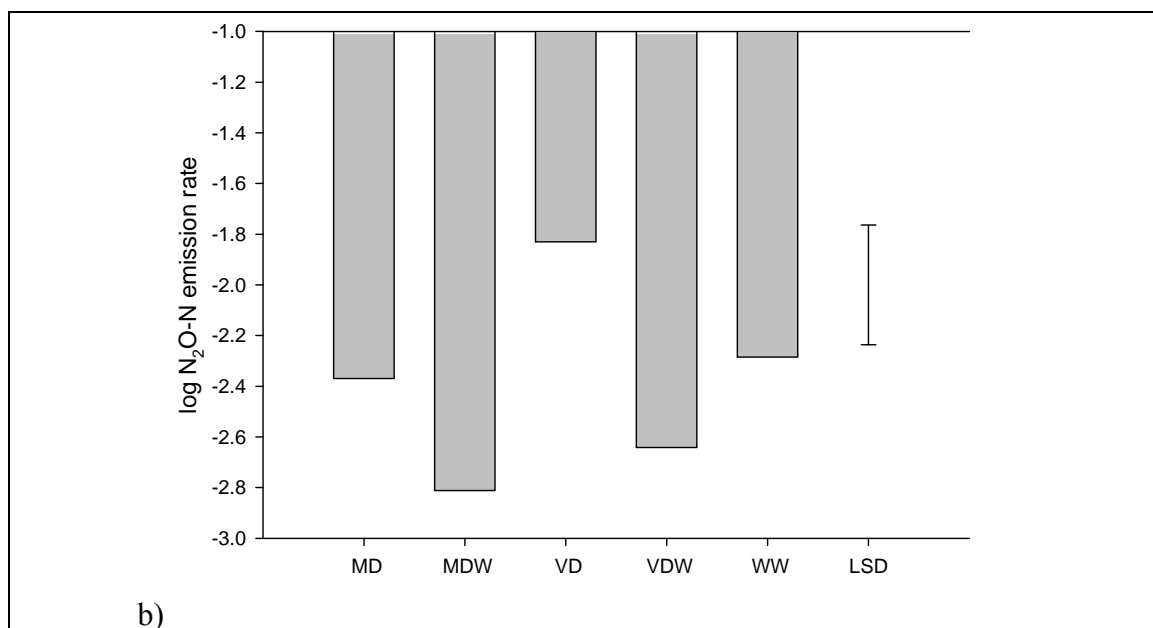
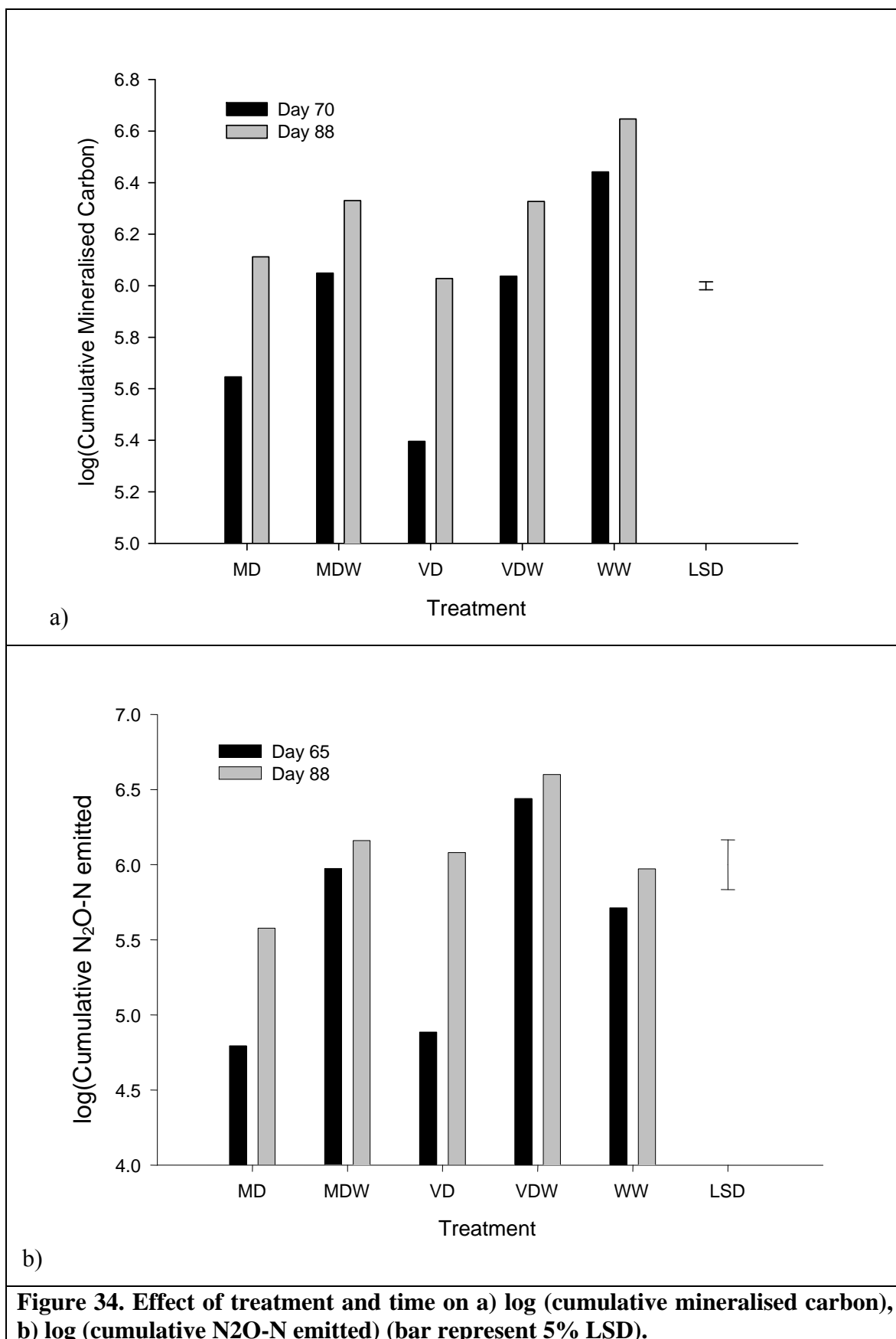


Figure 33. Effect of treatment on a) log (C mineralisation rate) and b) log (N₂O-N emission rate) at the end of the study (bar represents 5% LSD with 188 df).

8.3 Cumulative C mineralisation and N₂O-N emission

The cumulative amount of C mineralised or N₂O-N emitted by the end of the treatment phase of the incubation was compared to the cumulative total at the end of the trial. Again there was evidence of departure from homoscedasticity so the data was investigated using a log transformation.

During this period of the trial (the post treatment phase) all the treatments were returned to field capacity and incubated in a moist state. The cumulative amounts for both C and N continued to increase over this period but the process was not the same for all treatments. For example, the amount of material mineralised/emitted overall is lower for MD and VD than for the other treatments, but a far higher proportion of the mineralisation/emissions that did occur happened in the post treatment phase of the trial. Conversely more Carbon was mineralised for WW than any other treatment; but most of this happened in the first 70 days of the trial (Fig. 34 a & b).



If the cumulative mineralisation/emissions at the end of the treatment phase are calculated as a percentage of the total mineralisation/emissions at the end of the trial, it can be seen that for C these percentages are very similar for all treatments across all the paddocks despite vastly differing amounts of C being mineralised (e.g. Tem 1 WW ~3500 mg C/kg soil, Lis 6 WW ~350 mg C/kg soil). For the WW treatment, 80% of the cumulative total is mineralised by the end of the treatment phase, the MDW and VDW treatments are much the same with cumulative mineralisation at around 75% of the total, MD drops to 60% and VD to only 50% (Table 8).

Table 8. Cumulative carbon mineralised at the end of the treatment phase as a percentage of the total carbon mineralised at the end of the trial.

	Lis 2	Lis 3	Lis 4	Lis 5	Lis 6	Lis 7	Tem 1	Tem 2	Tem 3	Tem 4	Tem 5	Tem 6
	%	%	%	%	%	%	%	%	%	%	%	%
WW	83	83	82	82	82	83	82	81	78	81	78	82
MDW	77	74	75	77	76	76	79	72	75	74	75	77
VDW	76	74	74	75	75	75	80	72	75	72	74	77
MD	65	63	60	60	61	65	73	59	63	63	61	61
VD	58	51	51	50	52	54	62	52	55	50	54	51

The percentage of cumulative N₂O-N emitted for each treatment is much more variable across the paddocks. On average the percentages are similar for WW, MDW and VDW at 81, 84 and 87% respectively; MD drops to 46% and VD to 29% (Table 9).

Table 9. Cumulative N₂O-N emitted at the end of the treatment phase as a percentage of the total N₂O-N emitted at the end of the trial.

	Lis 2	Lis 3	Lis 4	Lis 5	Lis 6	Lis 7	Tem 1	Tem 2	Tem 3	Tem 4	Tem 5	Tem 6	Ave
	%	%	%	%	%	%	%	%	%	%	%	%	%
WW	98	63	76	90	79	89	57	95	62	86	90	89	81
MDW	87	64	79	81	87	86	98	88	98	94	85	67	84
VDW	93	66	60	99	88	93	94	89	99	80	91	96	87
MD	37	57	71	75	70	7	74	11	31	54	19	51	46
VD	24	30	48	12	64	27	51	10	25	24	11	24	29

Graphs of cumulative C mineralisation and N₂O-N emissions for individual paddocks can be found on the Appendix (Fig. A11, A12, A13 & A14).

Discussion

Soil water filled pore space

Aeration is required for C and N mineralisation and factors that influence the diffusion of oxygen through the soil, such as moisture and soil structure, will also alter rates of mineralisation. The optimum moisture level varies between different soils, but mineralisation generally proceeds readily at -0.1 to -1 MPa moisture tension, because neither diffusion of substrates nor the diffusion of gases is restricted. Fine-textured soils have smaller pore spaces and so anoxic sites occur at lower moisture contents than in coarser textured soils (Bollmann and Conrad, 1998; Paul and Clark, 1989).

A number of studies using a wide range of soils show that the percentage of soil pore space filled with water is well correlated with aerobic and anaerobic microbial activity and associated processes of respiration, mineralisation and denitrification (Doran et al., 1990; Franzluebbers, 1999). In general, aerobic microbial activity increases in a linear manner with increased water content between 30 and 60% WFPS and tends to decline above 60 to 70% WFPS (Doran et al., 1990).

Cumulative C mineralisation is at a maximum at WFPS between 53 and 66% and clay content has been found to have no effect on the level of WFPS to achieve maximum C mineralisation (Franzluebbers, 1999). When the moisture treatments used in this study are converted to WFPS it can be seen that at field capacity the majority of the paddocks fall within the range for maximum C mineralisation. The exceptions to this are those paddocks that have high SOM contents, particularly Lis 2, and Tem 1, 2 and 3, which have WFPS above optimum probably due to the ability of SOM to attract and hold water. The C mineralisation in these paddocks does not appear to be adversely affected by these higher levels of WFPS at FC. In particular, in the case of Tem 1 where WFPS is 100% at FC, it may be expected that the moderately dry treatment, with a WFPS of 50%, would have a higher cumulative C mineralisation than at FC, but this is not the case. This may be due to the large amounts of C available for mineralisation and the ability of some soils to maintain up to 93% of maximum C mineralisation even at WFPS of 90% (Franzluebbers, 1999).

Unlike C mineralisation, net N mineralisation decreases sharply when the optimum WFPS level is exceeded to approach zero at values near 80 to 90%. At high WFPS levels, the dramatic decrease in $\text{NO}_3\text{-N}$ is likely to be due to denitrification which occurs at WFPS >70% (Doran et al., 1990; Franzluebbers, 1999).

The optimum WFPS for maximum net N mineralisation has been found to be lower than that for maximum C mineralisation, ranging from 34 to 60% (Doran et al., 1990; Franzluebbers, 1999). Of the coarser textured Lismore soils, only paddock Lis 2 shows a reduced net N mineralisation at FC (WFPS 79%) as indicated by a lower mineral N in the WW treatment compared to the MDW treatment. There is also clear evidence in the Lismore paddocks of greater net N mineralisation with higher levels of SOM. In comparison, in the finer textured Temuka soils, paddocks with a WFPS $\geq 65\%$ show reduced net N mineralisation at FC, and in some cases also in the MDW treatment. Because of the effects of WFPS on net N mineralisation in the Temuka paddocks any effect of SOM levels is masked.

Reduced net N mineralisation is accompanied by high cumulative N_2O emissions due to anaerobic microbial denitrification. Denitrification has been shown to be absent below 63% WFPS and to increase exponentially at WFPS exceeding 70 to 75% (Doran et al., 1990). N_2O emissions were highest in those paddocks with a combination of fine texture (i.e. high clay content), adequate supply of available C (i.e. high SOM content), high levels of initial mineral N and WFPS $>63\%$ at FC.

Those treatments in which the soils were subjected to a series of slow drying and rapid rewetting cycles (MDW and VDW treatments) resulted in the greatest amounts of N_2O being emitted over the course of the study, particularly in the fine-textured Temuka soils. This is likely to be due to the formation of anoxic sites due to rapid rewetting, a sudden flush of available substrates upon rewetting and a corresponding rapid increase in microbial activity.

Soil microbial biomass carbon (MBC) has been found to respond almost linearly to increasing WFPS, with an optimum level ranging from 65 to 100%, with no evidence of reduced MBC near saturation as observed for cumulative C and N mineralisation (Franzluebbers, 1999). The WFPS levels at FC used in this study fall well within this optimum range.

Microbial Biomass

Overall, the soils in this study show increasing MBC with corresponding increases in SOM. This is in agreement with other studies that have found MBC and total C were strongly and positively correlated (Cleveland et al., 2004). Soil organic matter and clay content of the soils appear to have an interesting interaction in their effect on MBC. Where SOM content is high, MBC increases with increasing clay content, this could be due to a combination of

factors including adequate SOM for microbial growth, small pore spaces in fine-textured soils ensuring biomass and SOM are in close proximity and protection of the biomass in micro-pores. However, where SOM content is low, MBC decreases with increasing clay content. Again there are a number of factors that are likely to have contributed to this observation; limited SOM limiting microbial growth, isolation of the biomass from the SOM due to the fine pore structure and the protection of SOM from microbial attack by its inclusion within clay micro-aggregates.

All of the dry-rewet treatments resulted in a decrease in MBC from baseline levels. The soils that were kept continuously moist showed the greatest reduction in MBC, probably due the depletion of C substrates after 88 days incubation with no inputs, resulting in a biomass that is in a maintenance phase rather than actively growing. Compared to the continuously moist treatments, MBC increased with increasing intensity and duration of drying resulting in the highest MBC levels in those soils maintained under very dry conditions and this was consistent across both the coarse and fine-textured soils.

Microbial biomass was assessed after completion of the dry-rewet cycles at the end of the treatment phase after all the soils had been returned to FC and incubated for a further 18 days. The fact that those soils that were maintained under dry conditions throughout the treatment phase had the highest MBC at the end of the study indicates that more substrates were available for microbial growth at the end of the treatment phase of the study due to; reduced microbial activity during the treatment phase of the study because of water stress, and a flush of substrates becoming available when the dry soils were rewetted.

The clear pattern of treatment effects seen in the soil MBC levels was not reflected in the MBN levels, this resulted in changes to the ratio of MBC to MBN (MBC:MBN) in the soils. The pattern of treatment effects on MBC:MBN ratio is similar to that for MBC in most of the soils, with the dry-rewet treatments resulting in a decrease in MBC:MBN ratio from baseline levels, the soils that were kept continuously moist having the greatest reduction and highest MBC:MBN ratio in those soils maintained under very dry conditions. An average MBC:MBN ratio for a wide variety of soils has been found to be 8.6 and to range from 3 to 24, so MBC:MBN ratios found in this study can be considered “normal” (Cleveland et al., 2004). The observed changes in MBC:MBN ratio due to the dry-rewet treatments may have been brought about through a number of mechanisms:

- a) The soil microbial biomass consists of a diverse community of organisms with differing C:N ratios e.g. bacteria have an average C:N ratio of 6.5 compared to fungi

with a ratio of 5 to 17 (Cleveland et al., 2004). Microbial community structure and composition can alter in response to a series of dry-wet cycles. The changes in community composition could result in a change in C:N ratio of the microbial biomass (Fierer and Schimel, 2002);

b) Since microbes need to maintain the same water potential inside the cell as that outside. Different microorganisms will accumulate different compounds in their cytoplasm to regulate cellular water potential and these compounds can have differing C:N ratios (Killman et al., 1990);

c) In those soils kept continuously moist, the depletion of C substrates through microbial mineralisation may lead to C limited conditions and consequently a reduction of the C:N ratio in the microbial biomass;

d) Wetting of a dry soil has been found to cause both growth of microbial communities and rapid increases in C and N cycling rates (Saetre and Stark, 2005). Those microorganisms in a dormant state, such as those in the continuously moist soils, may differ in their C:N ratio compared to those microorganisms that are actively growing, such as those exposed to drying and rewetting.

It is not possible to determine which, if any, of these proposed mechanisms is responsible for the changes in MBC:MBN ratio observed in this study.

Labile Carbon Pools

There are considerably larger amounts of C in the hot water extractable C (HWC) than in MBC due to the fact that the HWC method would have extracted not only the MBC, but also root exudates, soluble carbohydrates and amino acids. The C bound to soil enzymes would also be extracted because most soil enzymes would have been denatured at 80°C. Most of these components of SOM are considered labile in nature (Ghani et al., 2003).

Hot water soluble carbon has been proposed as a surrogate measure to estimate MBC as a strong correlation has been found between HWC and MBC (Ghani et al., 2003). However, this is not the case for the data in this study. Our HWC data is more strongly correlated with total C than MBC ($R^2 = 0.87$ vs. 0.75). Hot water soluble carbon also does not show the same pattern of response to the dry-rewet treatments which is clearly evident in the MBC data, although there is still some evidence of lower levels of HWC in the continuously moist treatment and higher levels in the dry treatments. This suggests that the non-microbial SOM material that is extracted as HWC is less sensitive than MBC to the effects of drying and rewetting. Since MBC comprises only one half to a quarter of the total C extracted as

HWC, the response of the MBC component of HWC to the dry-rewet treatments may well be lost due to the lack of response of the non-MBC component.

Over the course of the study there was also little change in HWC with time. Within the paddocks there are only small changes in HWC between baseline, at the end of the treatment phase and at the end of the study, again suggesting that non-microbial SOM is less sensitive than MBC to the effects of drying and rewetting or that the material is not as labile as has been proposed.

Dissolved organic C (DOC) is considered to be part of the highly labile pool of SOM and so may be sensitive to perturbation and stress in the soil-plant system (Doran and Parkin, 1994). DOC has been proposed as an indicator of the C available to soil microorganisms, however the factors controlling its concentration and bioavailability are not well understood (Lundquist et al., 1999b). DOC comprises a relatively small fraction of the total SOM and depending on how it is measured, DOC can represent between 0.05 – 1.7% of total SOM (Gregorich et al., 2003; Matlou and Haynes, 2006; Zsolnay, 2001).

The results of this study show an apparent additive effect of SOM content and texture on levels of DOC extracted from the soils. Perhaps unsurprisingly, soils with high SOM contents have correspondingly higher DOC levels. What is interesting is that the combination of high SOM content and fine soil texture results in higher levels of DOC than a similar SOM content in a coarser textured soil. This is evidenced in the results where the Temuka paddocks have higher DOC levels than the Lismore paddocks even where they have lower SOM levels. This observation may be due to interaction of the organic molecules with clay minerals giving protection against chemical and possibly biological attack, leading to higher DOC levels being retained in the soil (Kaiser and Guggenberger, 2003; Kaiser and Guggenberger, 2007).

Rewetting of the soils at the end of the treatment phase of the study resulted in a sharp increase in DOC levels relative to baseline in those soils that had been maintained in a dry state during the treatment phase. In contrast, those soils that had been subjected to a series of dry-rewet cycles during the treatment phase showed little change in DOC levels at the end of the treatment phase, relative to baseline. While it is not possible to identify definitively the source of peak in DOC levels following rewetting of dry soil, the main mechanisms thought to be involved are; disruption of the soil aggregate structure, releasing SOM from physical protection within aggregates (Appel, 1998; Denef et al., 2001b; Sorensen, 1974; Utomo and Dexter, 1982), and/or the rapid increase in soil water potential

associated with rewetting a dry soil causes soil microorganisms to experience osmotic shock, resulting either complete lysis of microbial cells or the release of intercellular osmoregulatory solutes (Fierer and Schimel, 2003). The lack of response in DOC levels to rewetting in those soils exposed to a series of dry-rewet cycles may be due to the decrease in the amount of physically protected SOM available for release with each subsequent dry-rewet cycle, and/or the microbial community may adjust to the water potential shock lessening the mortality rate (Fierer and Schimel, 2002).

Eighteen days after the end of the treatment phase when all soils were adjusted to FC, DOC levels had decreased in all treatments, but most markedly in the dry treatments that had shown a peak in DOC levels after being adjusted to FC. This suggests that the DOC released following rewetting was highly labile and was rapidly utilised by the soil microbial community and is supported by the corresponding flush of C mineralisation and higher levels of MBC found in the dry treatments.

Total C and N and Particulate Organic Matter

The dry-rewet treatments had little consistent effect on the levels of total C or N in the soils used in this study. Although the treatments did have an effect on other soil C and N pools such as MBC, DOC, Min N and MBN, these pools only make a small fraction of the total C and N present in the soil and there was little change in the overall levels of C and N. This demonstrates that soil total C and N respond much more slowly to changes in the soil-plant system or to short-term stressors, compared to more rapidly cycling pools such as MBC and MBN.

Like total C and N, there was no consistent effect of the dry-rewet treatments on the POM C or N across the soils studied. The largest changes to POM C and N are seen in those paddocks under pasture (Lis 2, 3, 7 and Tem 1), particularly showing decreases in C and N in the POM fraction between 250 – 1000 μm , but there is still no consistent response to the treatments even in these paddocks. POM between 250 – 1000 μm is largely intact plant material. It may be that the OM inputs from the vegetation present in the pasture paddocks is much more labile than that in the cropping paddocks, such that it is easily degraded even when the soils are water stressed. So, changes in POM C and N may have more to do with the source of the OM than the effects of any dry-rewet treatments imposed.

Carbon Mineralisation

Where the soils underwent a series of dry-rewet cycles, the size of the peak in C respiration following rewetting became progressively smaller with each subsequent rewetting cycle. Several earlier studies have also found that stress history plays an important role in the magnitude of the rewetting CO₂ pulse from subsequent dry-wet events, such that the extent of the CO₂ pulse upon rewetting is significantly reduced with repeated dry-wet cycles. This observation cannot be explained by reductions in the size of the microbial biomass pool. Two possible explanations are proposed:

- (i) if drying-rewetting releases physically protected organic matter, there simply may be less organic matter available for release following a series of dry-wet cycles, reducing the CO₂ pulse;
- (ii) after several dry-wet events, the microbial community may adjust to the water potential shock encountered during rewetting. This adjustment would lessen the mortality rate and reduce the size of the flush of labile substrate available for mineralisation by the surviving microorganisms.

However, the inability to definitively identify the cause of the rewetting pulse makes it difficult to explain the relationship between dry-wet stress history and the size of the rewetting pulse (Fierer and Schimel, 2002).

The change in the magnitude of the rewetting pulse with repeated dry-rewet cycles meant that the use of C mineralisation rates from the treatments under constant moisture conditions to estimate mineralisation under dry-rewet cycles, tended to underestimate the cumulative C mineralised over the course of the treatment phase when compared to the actual mineralisation data. This underestimation in cumulative C mineralisation was largely due to; an underestimation of the mineralisation flush when the dry soil is rewetted, especially during the first dry-rewet cycle, and an over estimation of the rate at which respiration decreases as the soil dries, especially during the initial drying phase. There is evidence that the fit of the estimated data improves for the second dry-rewet cycle, but there may be an overestimation the mineralisation peak by the third cycle. The magnitude of the difference between estimated and actual C mineralisation was not influenced by the SOM content or texture of the soils in this study.

A number of studies (Fierer and Schimel, 2002; Franzluebbers et al., 1994; Magid et al., 1999; Schimel et al., 1999) have found that dry-wet cycles can retard long-term C mineralisation rates. This study had similar finding, with the soils that underwent a series of dry-rewet cycles having the lowest C mineralisation rates 18 days after the final rewetting.

The highest rate was in those soils kept in a very dry state for a prolonged period before being returned to FC, while those soils maintained at FC capacity for the duration of the study had rates intermediate to the two.

A decrease in the supply of remaining mineralisable SOM following a period of frequent dry-wet cycles would be the most obvious explanation for the reduction in respiration rates. Presumably, after a single dry-wet cycle the pool of potentially mineralisable SOM would increase either due to a release of physically protected organic matter or a 'priming effect' caused by the release of labile substrates during biomass turnover. So, with time, a series of dry-wet cycles would serve to reduce the total supply of available SOM (Fierer and Schimel, 2002). Another potential explanation for the reduction in long-term respiration rates is a change in the composition of the microbial community. Not all members of the microbial biomass are equally adept at mineralising SOM. The loss of some microbes during drying-wetting or a change in the physiologies of the surviving population could reduce the ability of the microbial biomass to mineralise SOM (Schimel et al., 1999).

The pulse in C mineralisation that occurred when the dry soils were rewetted was not sufficient to compensate for the reduction in mineralisation during the drying period; this finding is consistent with other studies where, as the number of dry-wet cycles increased, the difference in cumulative C mineralisation between soils exposed to dry-wet cycles and soils kept continuously moist increased (Franzluebbers et al., 1994; Mikha et al., 2005).

It is interesting to note that at the cumulative C mineralised end of the treatment phase of the study as a proportion of the total cumulative C mineralised at the end of the study, is consistent across the soils for each of the treatments, despite the absolute amounts of C mineralised varying considerably. This suggests that, although soils may differ in SOM content and texture and consequently have differing rates of C mineralisation, when subjected to the same drying and rewetting conditions the effect on C mineralisation is proportionally the same.

N₂O emissions

The N₂O emission data is inherently more variable than that for C mineralisation. This is likely to be due to combined effects of soil texture and WFPS, as discussed earlier. As a consequence, the use of N₂O emission rates from the treatments under constant moisture conditions, to estimate emissions under dry-rewet cycles, resulted in large discrepancies in the cumulative N₂O emitted over the course of the treatment phase in comparison to the actual emissions data. In the coarser textured soils N₂O emissions are relatively low and the

estimated emissions tend to be higher than the actual emissions. In comparison, the fine textured soils generally have higher N₂O emissions and the estimated emissions are lower than the actual emissions.

At the end of the study, 18 days after the final rewetting event, the pattern of N₂O emission rates for the treatments was similar to that for C mineralisation, with the soils that underwent a series of dry-rewet cycles having the lowest N₂O emission rate, and the highest rate in those soils kept in a very dry state for a prolonged period before being returned to FC. Since N₂O emissions are microbially mediated and dependant on adequate supplies of available C, the reasons for this pattern of treatment response are probably similar to that for C mineralisation, being; a decrease in the supply of remaining mineralisable SOM following a period of frequent dry-wet cycles, and/or a change in the composition of the microbial community.

Conclusion

Of the C and N parameters measured, there was clear evidence of C and N pools that were dynamic, and responded quickly to the changes in soils conditions, and those that were less so. Mineral N, MBC, MBN and DOC all showed a clear response to the dry-rewet treatments and that these responses were influenced by soil texture and SOM content, indicating that these C and N pools are labile and rapidly turned-over. In contrast, total C and N, HWC and POM showed little response to the dry-rewet treatments, indicating that these pools are more recalcitrant and show less of an impact of short-term fluctuations in soil conditions.

In the case of cumulative C mineralisation, the overall correlation of calculated data to actual data appeared quite good, but the calculated data constantly underestimated cumulative C mineralised. When C mineralisation rate data is examined, there was a clear lack of fit of the calculated to the actual data over a series of dry-rewet cycles. The lack of fit of the calculated data was due to an underestimation of the mineralisation flush when the dry soil is rewetted, and an overestimation of the rate at which respiration decreases as the soil dries.

Despite the soils used in this study having a range of different C mineralisation rates and amounts of cumulative C mineralised, there was no evidence of an effect of soil texture or SOM content on the difference between calculated and actual C mineralisation.

The results of this study suggest that estimates of C mineralisation for soils exposed to dry-rewet cycles using data from soils held at constant moisture contents is possible, but knowledge of the stress history for the soil would be required to improve accuracy e.g. size, duration and frequency of rainfall events, drying rates etc.

Calculated cumulative N₂O emissions varied greatly from the actual measured emissions, in some cases by a whole order of magnitude. Unlike C mineralisation, there was some evidence of a texture effect on the difference between calculated and actual N₂O emissions, with an overestimation of emission in the coarse textured soils (where emissions were low), and an underestimation in the fine textured soils (where emissions were high). This study indicates that prediction of N₂O-N emissions in soils exposed to dry-rewet cycles using emission data from soils held at constant moisture contents would be very inaccurate, primarily due to the inherent variability of N₂O-N emissions in soils.

Overall, the results of this study support the first hypothesis that calculated C mineralised/ N₂O emitted would not equal the actual C mineralised/ N₂O emitted. However, the second hypothesis, that the difference between calculated and actual C mineralised/ N₂O emitted would vary with soil texture and organic matter content, was not so well supported. In the differences between calculated and actual data, C mineralisation showed no evidence of any texture or SOM effect, but N₂O emissions did show some evidence of a texture effect which was probably also linked to WFPS since fine-textured soils have smaller pore spaces and so anoxic sites occur at lower moisture contents than in coarser textured soils.

As this was a laboratory study, the findings need to be verified under field conditions. Future work should also focus on other soil factors commonly encountered in field situations which may influence the response to dry-rewet cycles such as compaction, urine addition, vegetation type and landuse, for example. Depending on the duration of any future studies, emphasis should be placed on measuring those C and N pools that have shown the more dynamic responses to changes in soil conditions.

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Appendix

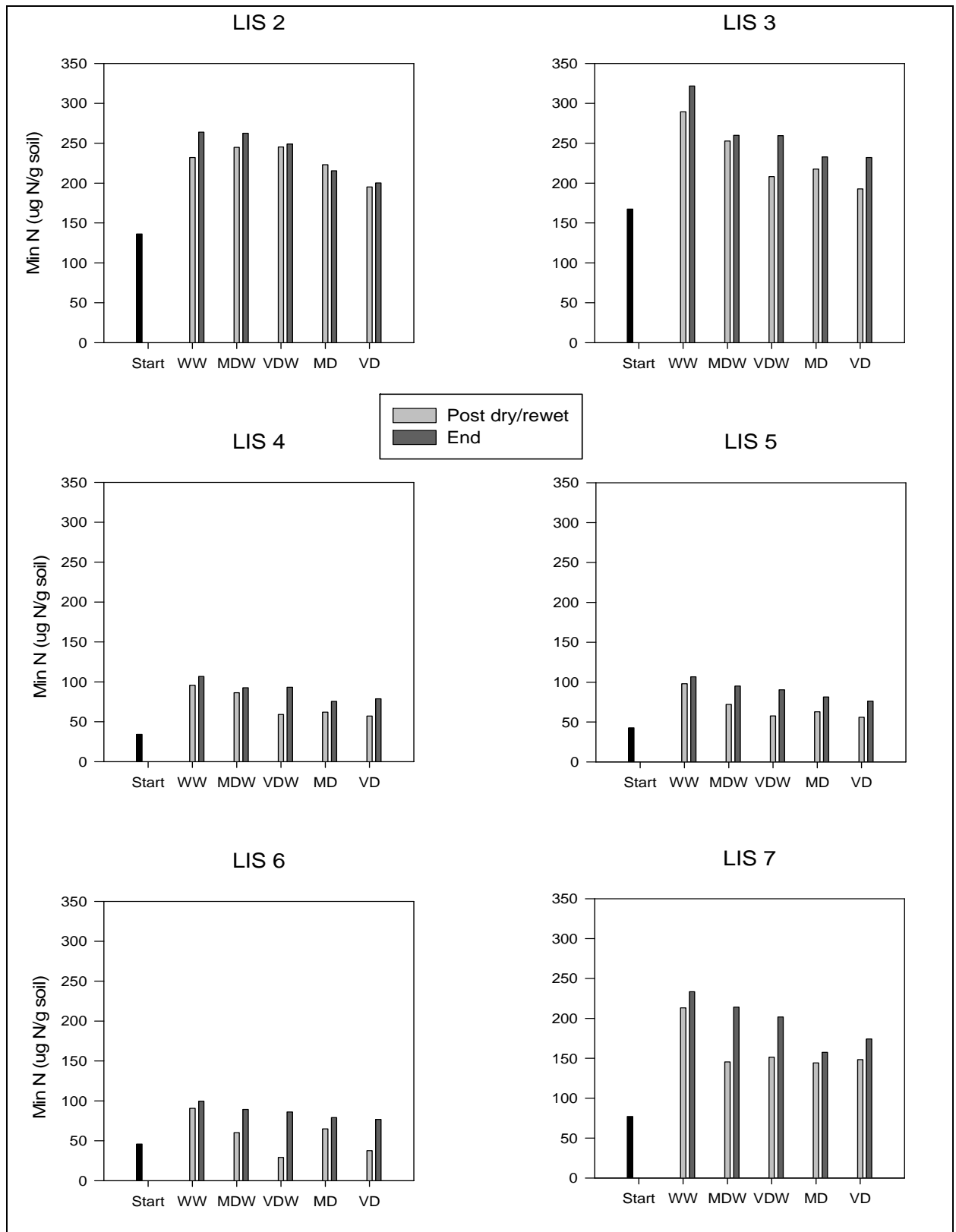


Figure A1. The effect of sampling time and treatment on the concentration of mineral nitrogen in the individual Lismore paddocks.

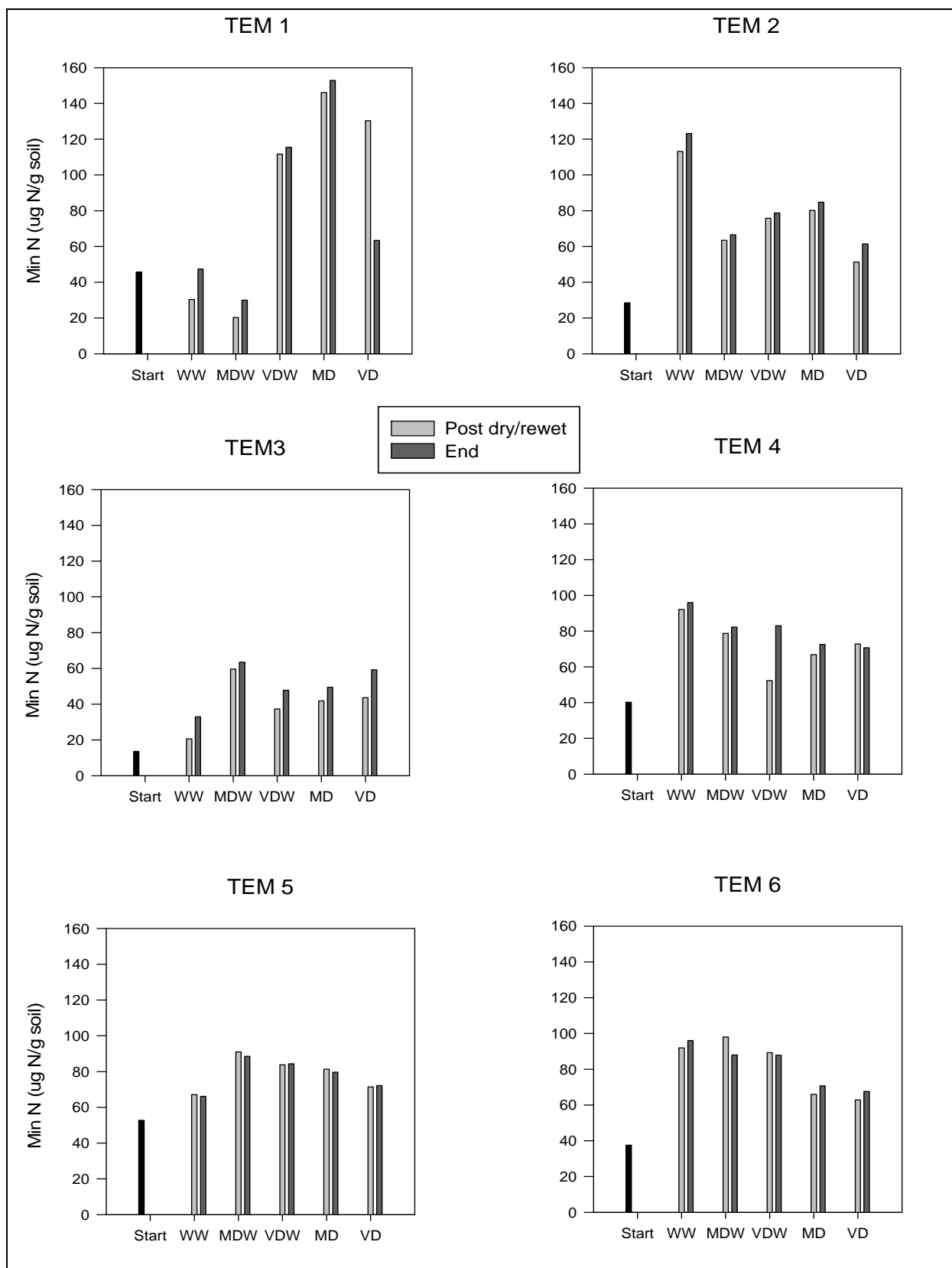


Figure A2. The effect of sampling time and treatment on the concentration of mineral nitrogen in the individual Temuka paddocks.

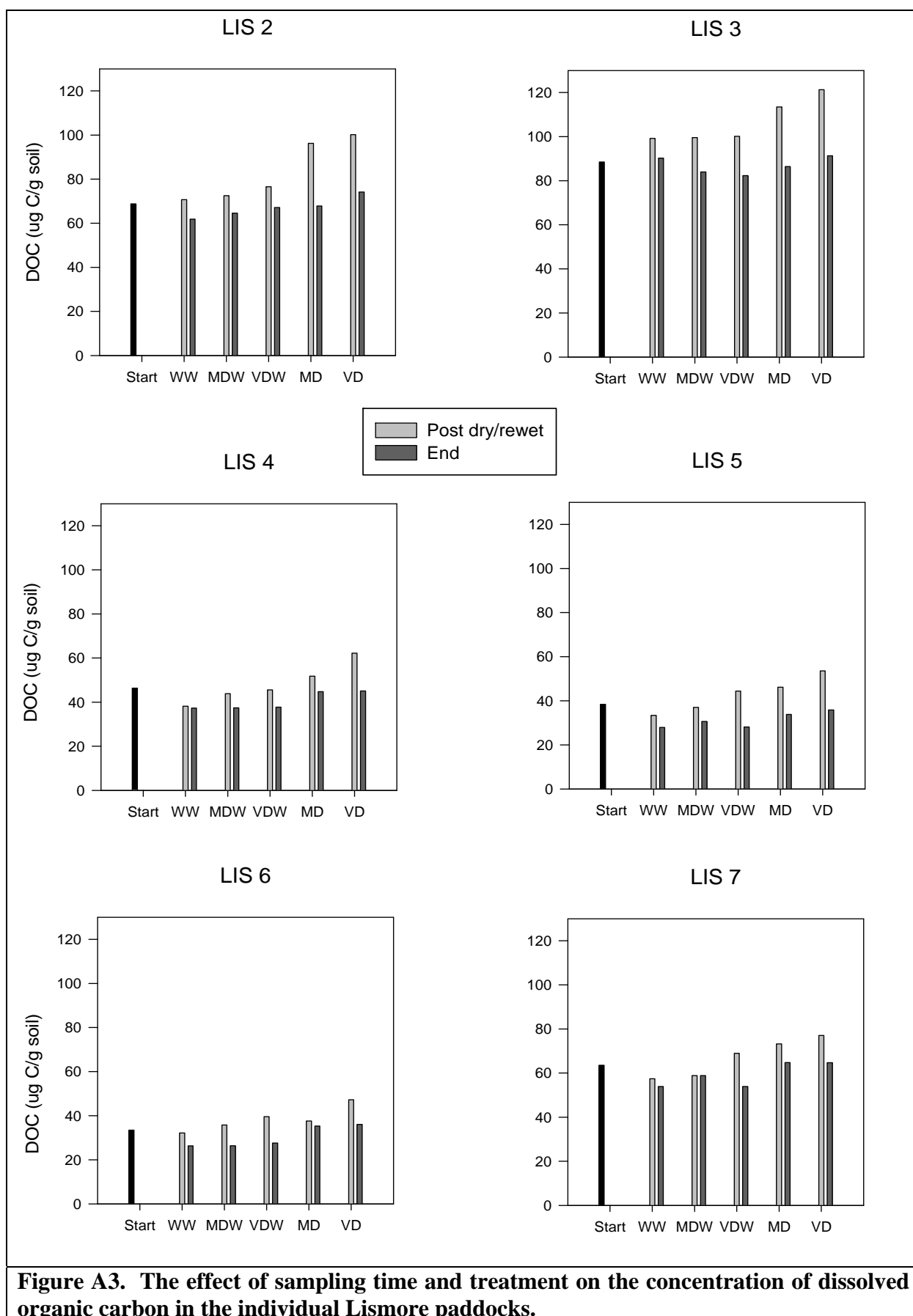


Figure A3. The effect of sampling time and treatment on the concentration of dissolved organic carbon in the individual Lismore paddocks.

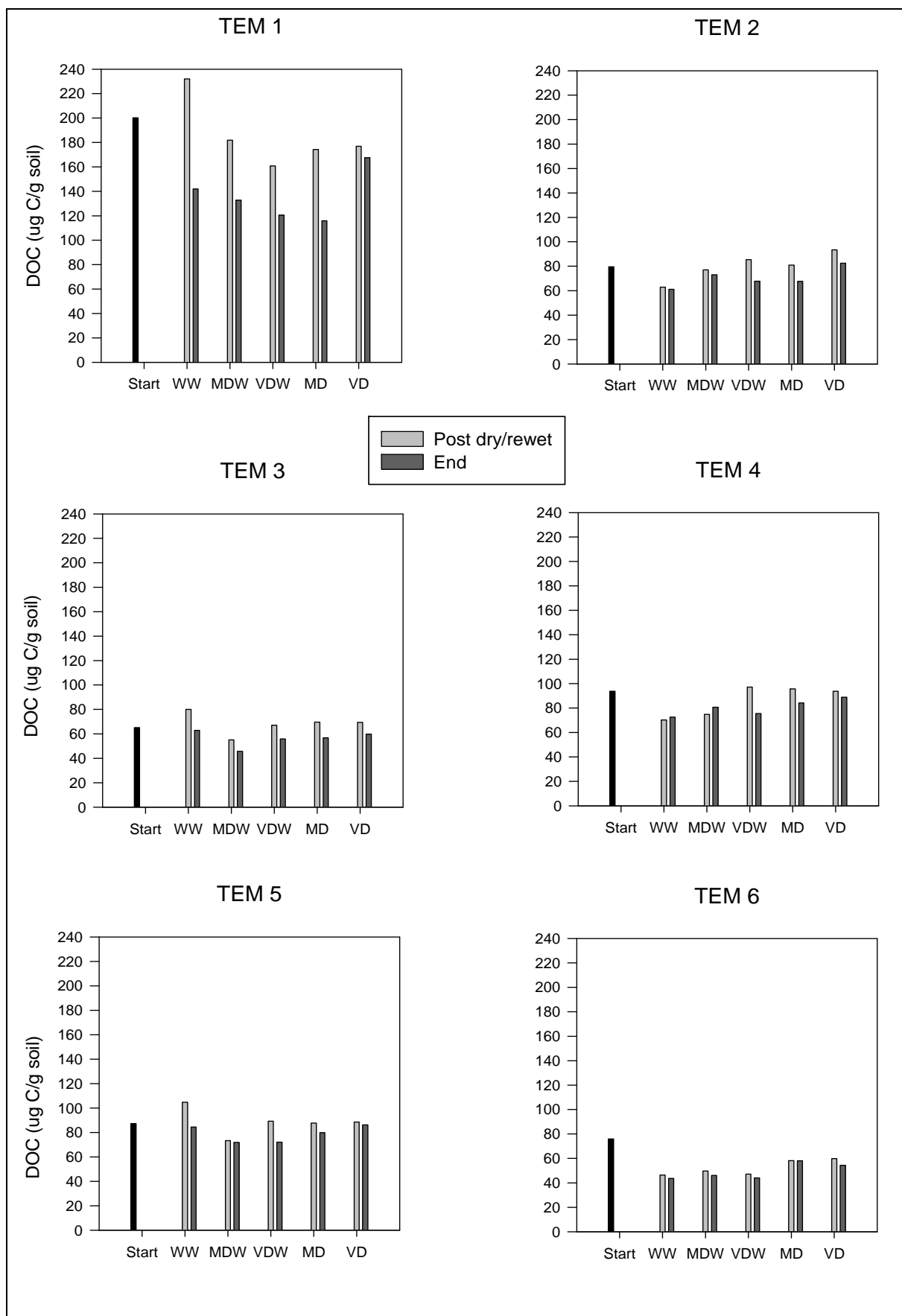


Figure A4. The effect of sampling time and treatment on the concentration of dissolved organic carbon in the individual Temuka paddocks.

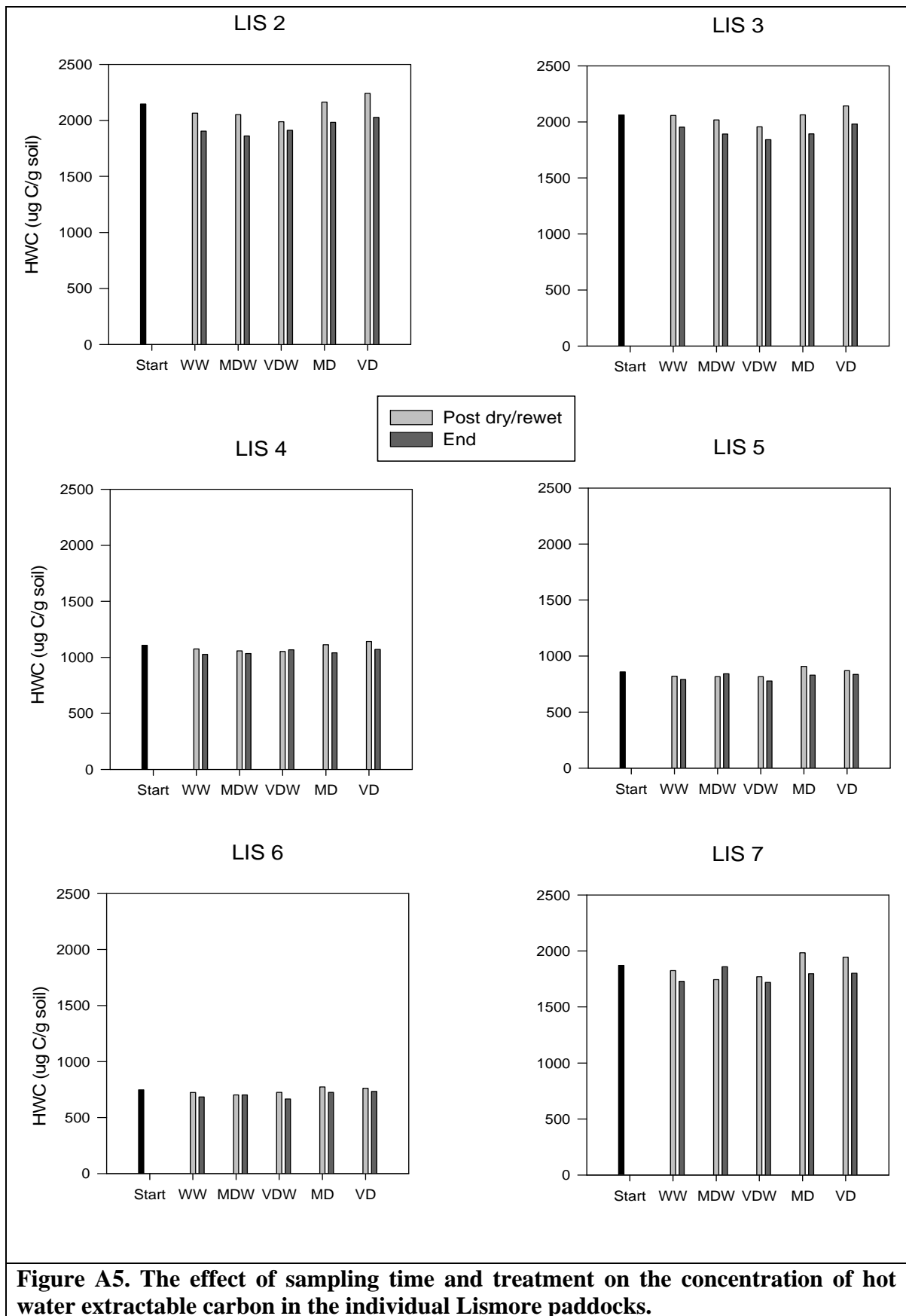


Figure A5. The effect of sampling time and treatment on the concentration of hot water extractable carbon in the individual Lismore paddocks.

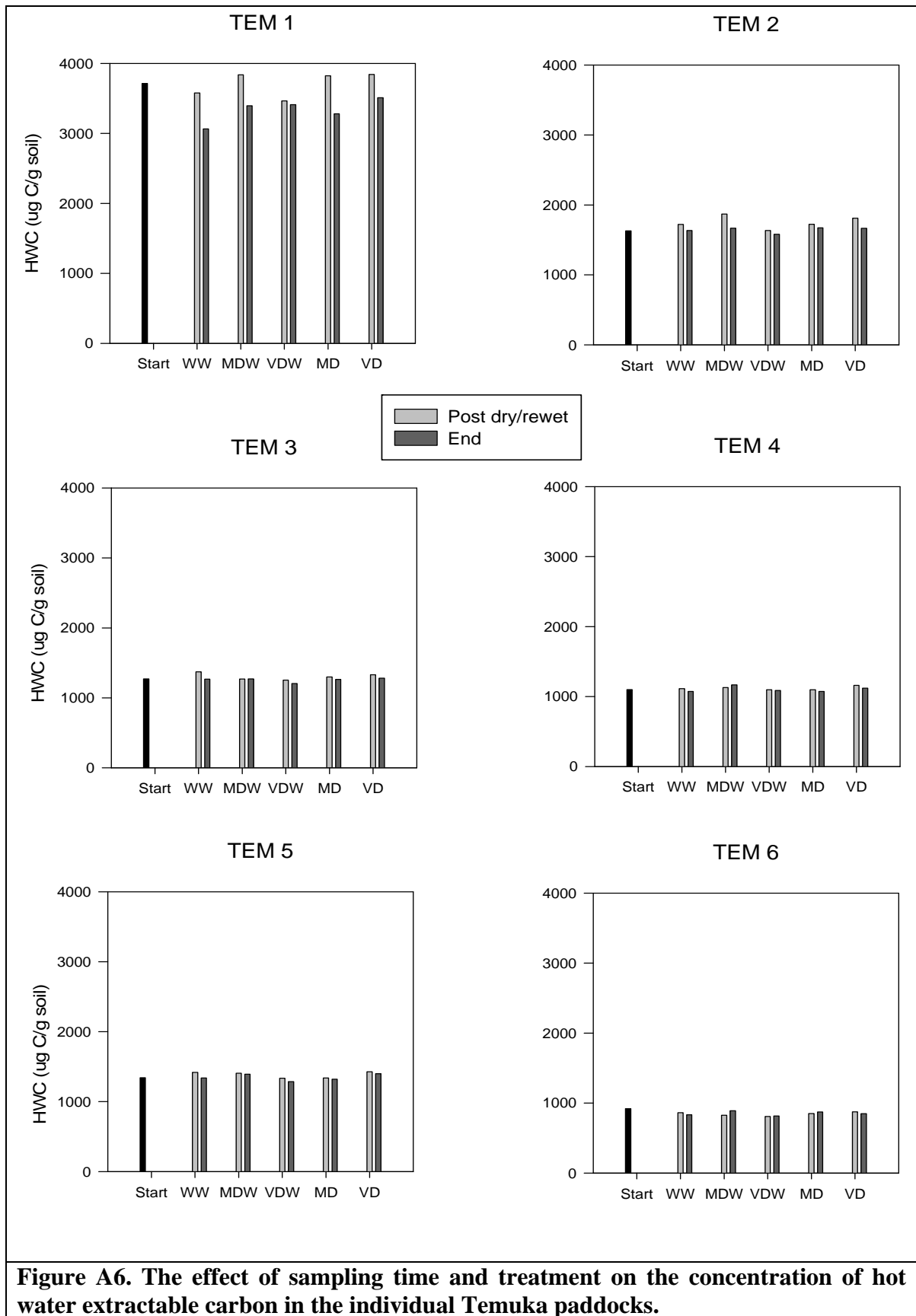


Figure A6. The effect of sampling time and treatment on the concentration of hot water extractable carbon in the individual Temuka paddocks.

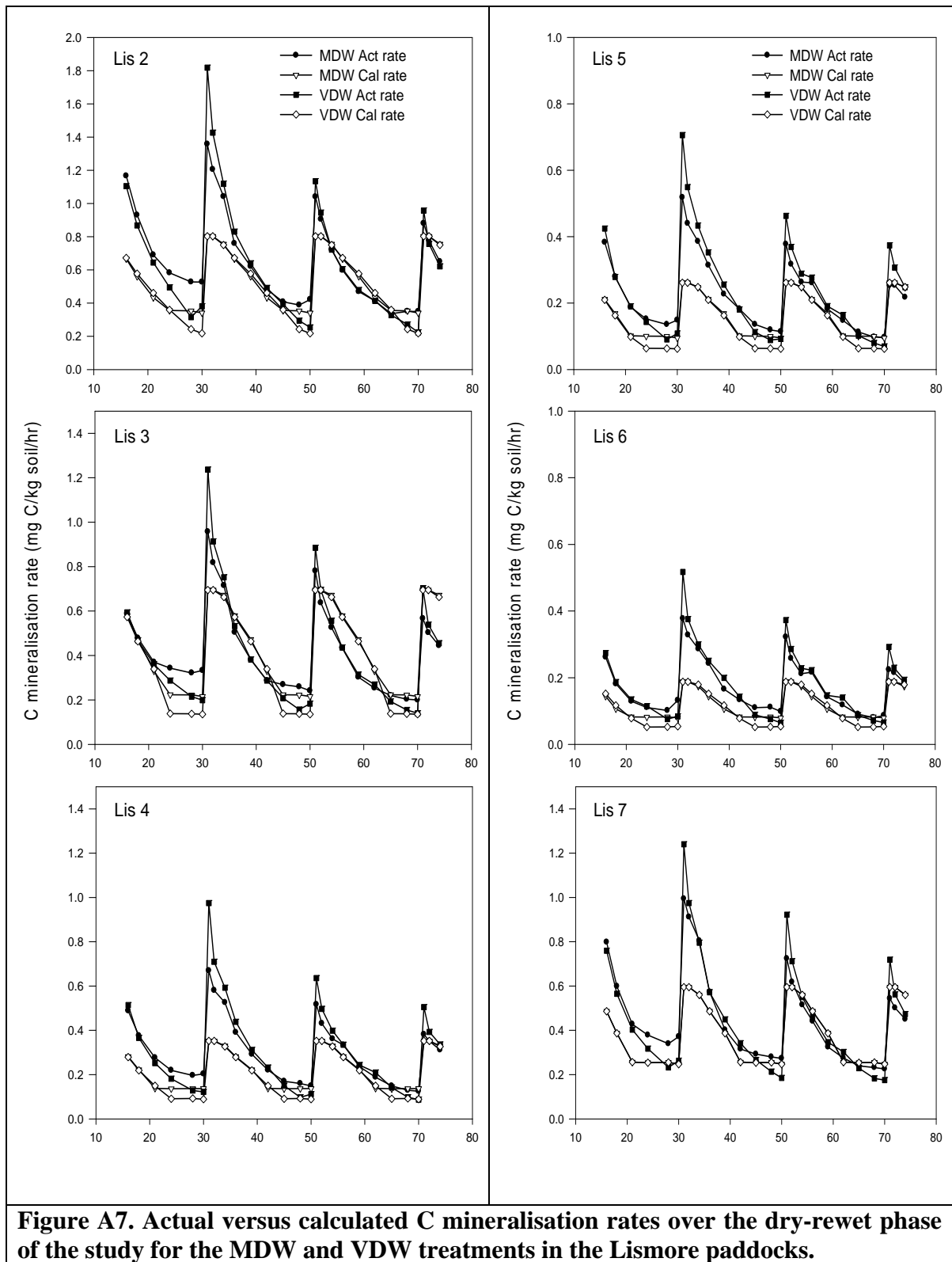


Figure A7. Actual versus calculated C mineralisation rates over the dry-rewet phase of the study for the MDW and VDW treatments in the Lismore paddocks.

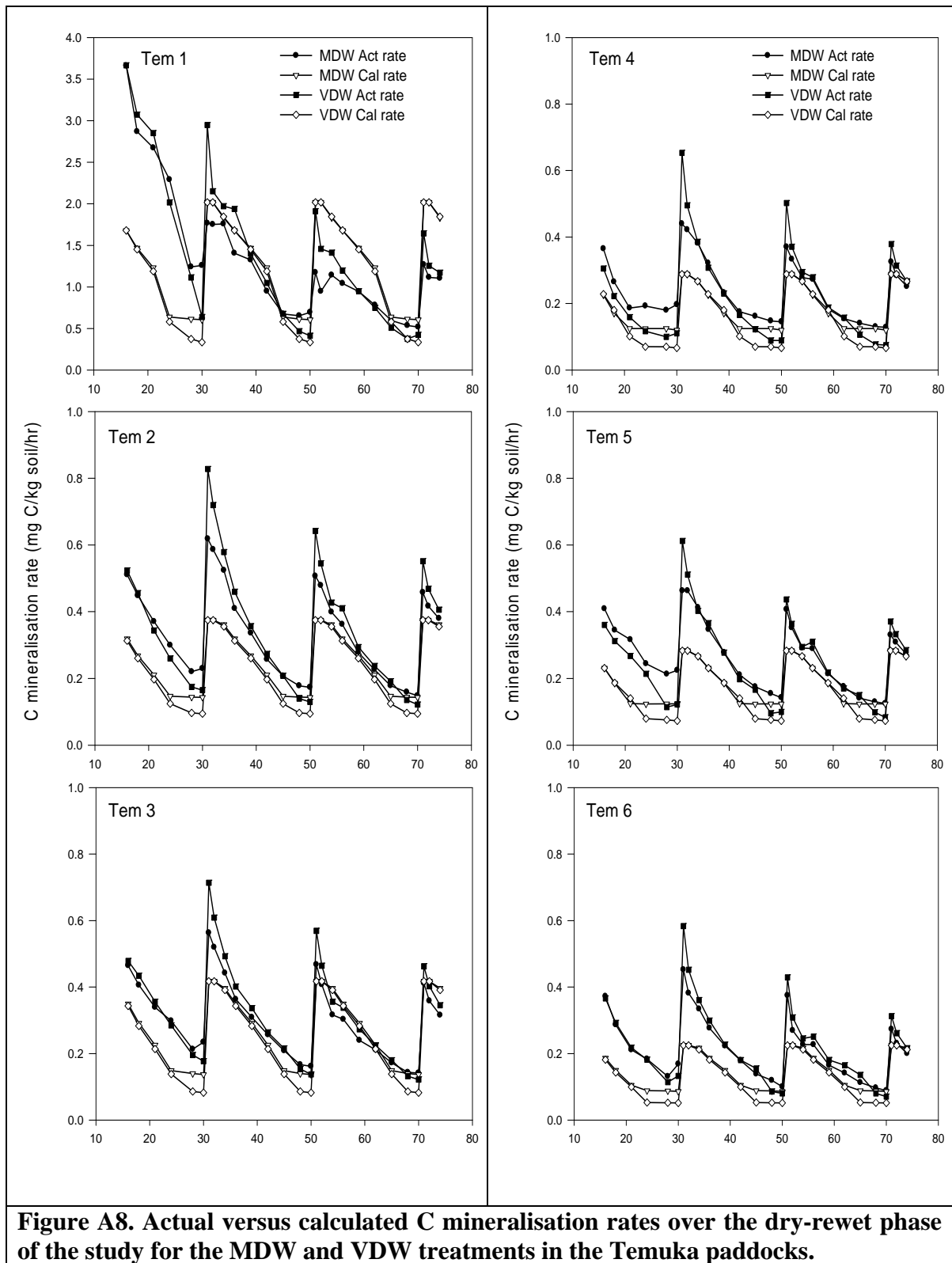


Figure A8. Actual versus calculated C mineralisation rates over the dry-rewet phase of the study for the MDW and VDW treatments in the Temuka paddocks.

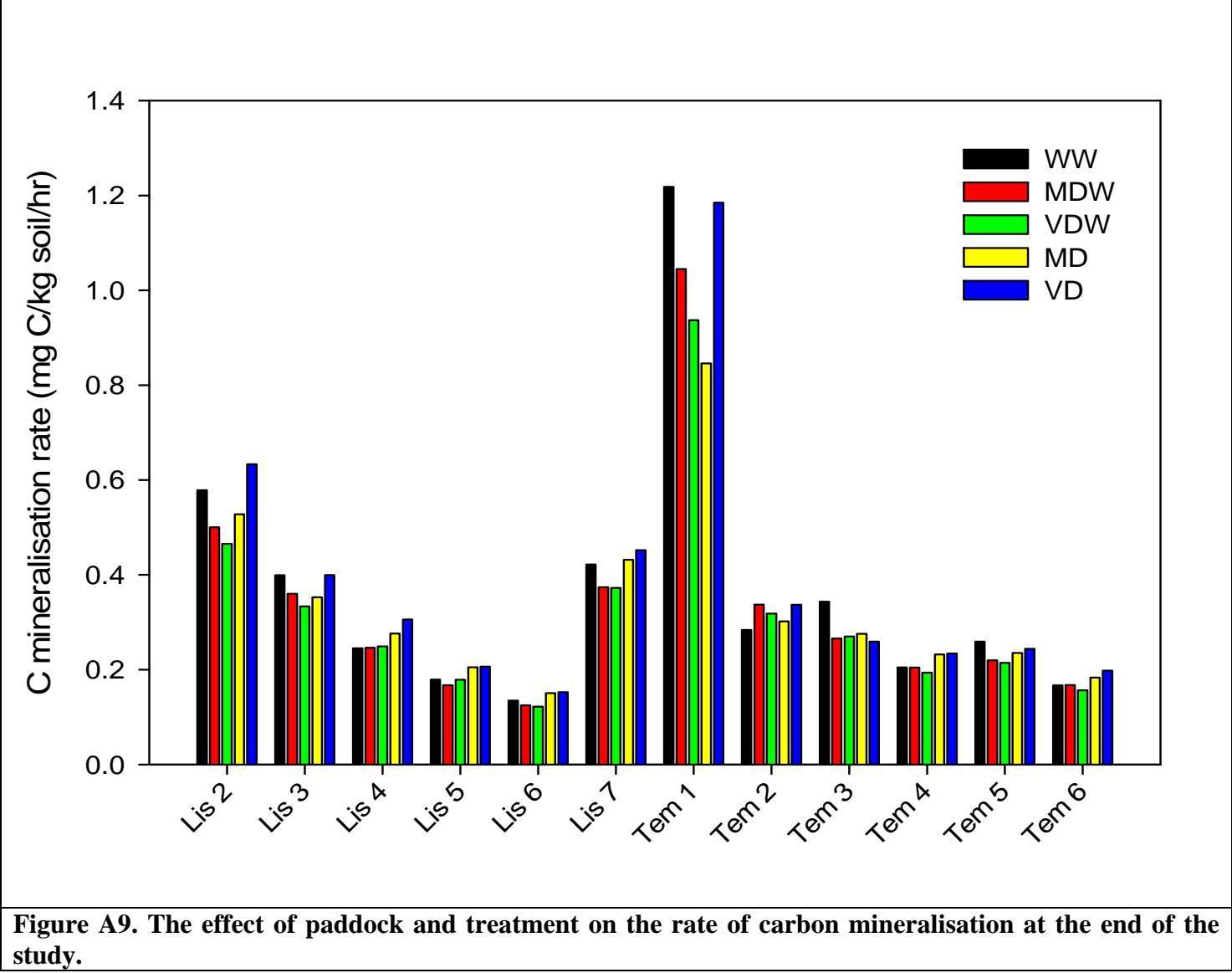


Figure A9. The effect of paddock and treatment on the rate of carbon mineralisation at the end of the study.

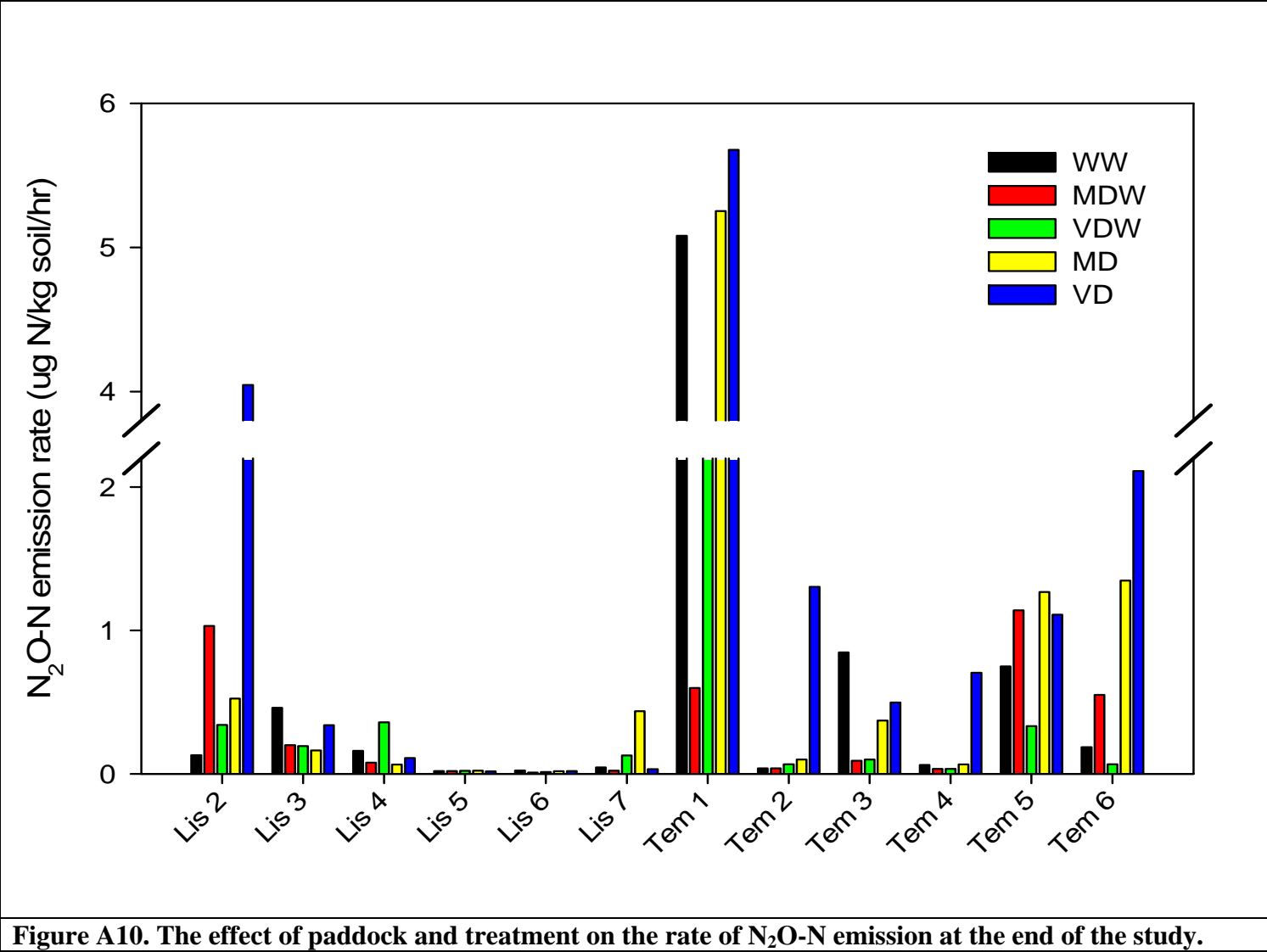


Figure A10. The effect of paddock and treatment on the rate of N₂O-N emission at the end of the study.

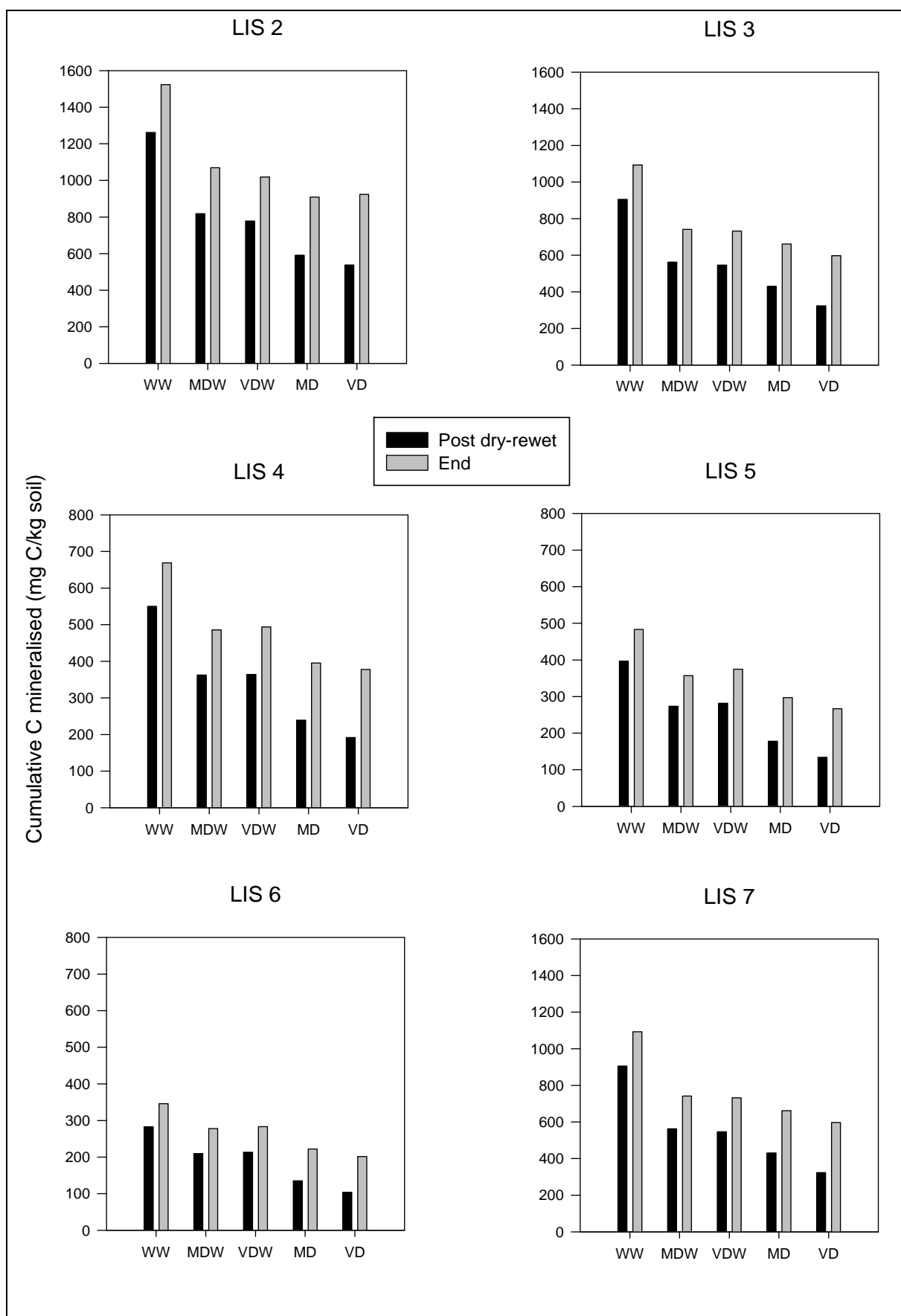


Figure A11. The effect of treatment and time on cumulative C mineralised in the Lismore paddocks.

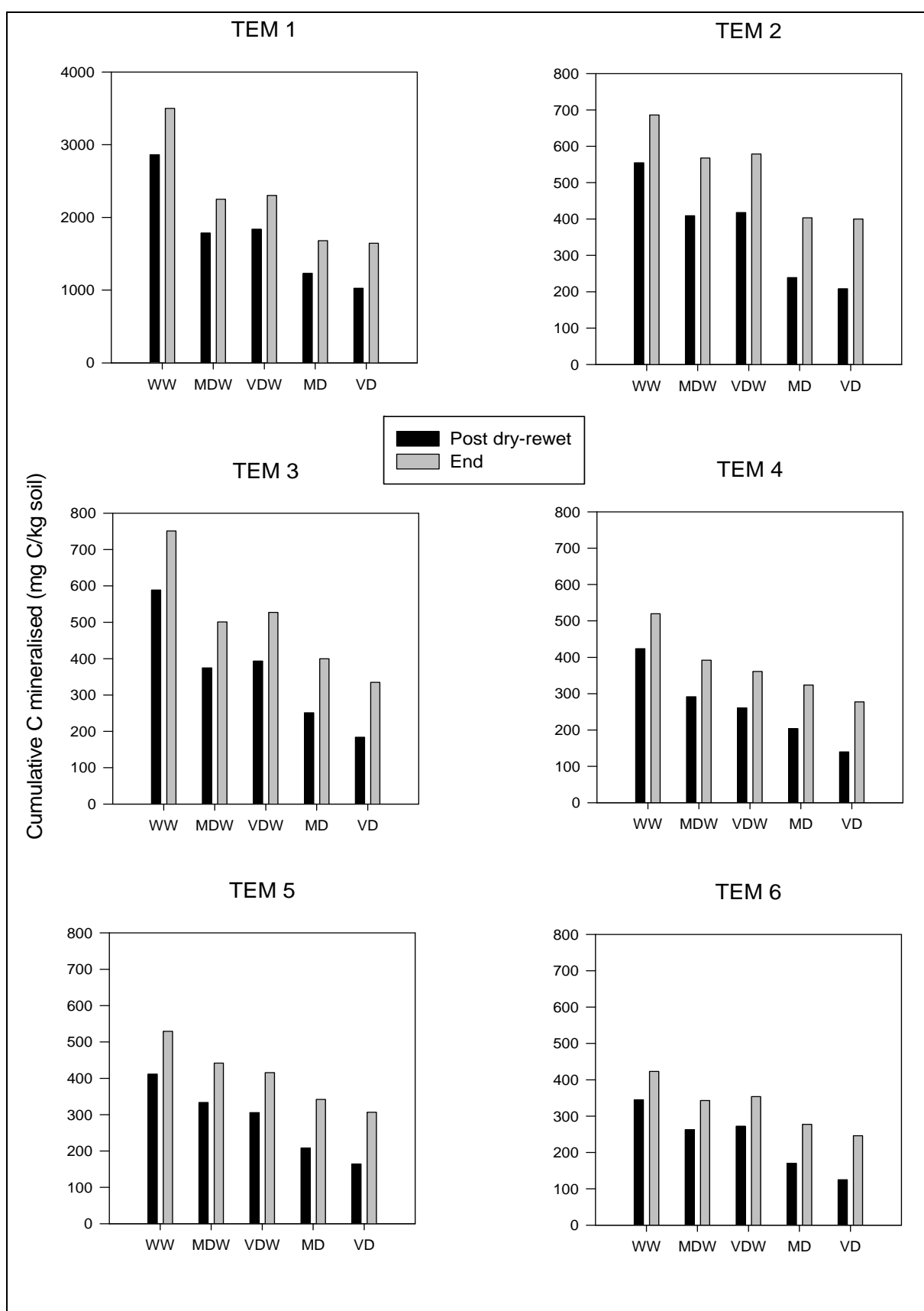


Figure A12. The effect of treatment and time on cumulative C mineralised in the Temuka paddocks.

