Nitrogen and carbon mineralisation in agricultural soils of South Australia

by

ANGELA CLOUGH

Bachelor of Science (Honours) (Adel.)

Department of Agronomy and Farming Systems
Roseworthy Campus, Adelaide University,
South Australia, Australia

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SUMMARY

Changes in the form of soil nitrogen (N) are difficult to measure on a routine basis suitable for predicting the amount of soil N that becomes available to a crop in a growing season. However, soil organic carbon (SOC) is relatively simple to measure. Thus, the relationship between SOC and N is used in models to predict the change in available soil N over time and hence anticipate a crop's fertiliser N requirements.

Previous Australian studies have highlighted two potential weaknesses in these fertiliser requirement prediction models. Firstly, studies with soils amended with calcium carbonate (CaCO₃) or lime showed that the presence of CaCO₃ reduced C mineralisation rates. Secondly, using total OC in models may not be valid because not all OC is necessarily available for mineralisation due to chemical and physical protection. The effect of these two factors on N fertiliser requirement models has not been determined and might be important in regions with calcareous soils and a history of burning. The objective of the research presented in this thesis was to fill this knowledge gap and apply the information to calcareous Xeralfs in South Australia.

Field experiments were established at two sites with different CaCO₃ contents to determine whether CaCO₃ affected net N mineralisation rates under two cropping treatments. Results were inconclusive with high variability between replicates (coefficient of variation (cv) up to 637%) despite intensive soil sampling equivalent to 900 samples ha⁻¹. High variability was due to the net N mineralisation rates having high spatial variability (cv > 100%) and being very close to zero (< 0.5 μg⁻¹ N g soil⁻¹ day⁻¹). All further experiments were conducted under laboratory conditions.

Before starting a long term in vitro mineralisation experiment, two technical experiments were conducted to establish techniques for generating mineralisation rates that represented field conditions as closely as possible. The first technical experiment compared two Xeralfs subjected to three soil treatments; sieving only, sieving plus repacking, and intact soil cores.
Recovery of total CO$_2$-C from sieved plus repacked cores averaged at least 94% of that recovered from intact cores. Total amounts of C mineralised were highest in the sieved only treatment and least in the intact treatment. Net N mineralisation was statistically the same across all soil treatments in the calcareous soil. In the non-calcareous soil net N mineralisation was in the order intact < repacked, sieving only.

The other technical experiment showed that mineralisation of glucose-C could be used on mildly alkaline and calcareous Xeralfs to monitor C mineralisation rates without glucose-C becoming trapped in the soil. Further, evolved CO$_2$ was not derived from CaCO$_3$. Using results from the two technical experiments, a 231 day incubation compared the net N and C mineralisation rates in four Xeralfs with different CaCO$_3$ contents collected from two locations in South Australia.

The 231 day incubation showed that mineralisation of SOC and labelled glucose-C differed between soils. Net N mineralisation rates produced a similar trend to C mineralisation. The effect of soil type suggested that the form or availability of SOC differed between soils. The total proportion of C mineralised was up to 8.9% less in soil with added CaCO$_3$ compared to non-calcareous soil. However, the effect of CaCO$_3$ was only due to differences in the mineralisation rates of glucose-C, not SOC. Differences in net mineralisation rates of substrate N and native soil N were less discernible than for substrate C.

Explanations for the effect of SOC and CaCO$_3$ on mineralisation rates were determined using high energy ultra-violet (UV) photo-oxidation followed by cross polarisation magic angle spinning $^{13}$C nuclear magnetic resonance analysis (CP/MAS $^{13}$C NMR). Analysis of <53 µm soil fractions showed that between 17% and 40% of SOC was in a condensed aromatic form, most likely charcoal. Adjusting the total amounts of C mineralised during the 231 day incubation to account for the presence of charcoal reduced the differences between soils. However, the soils with CaCO$_3$ still mineralised less OC than the non-calcareous soils which indicated that physical protection of OC by calcium – organic matter (Ca-OM) bridging also reduced mineralisation rates (Muneer and Oades, 1989c).
The concept that organic material remaining after photo-oxidation may be physically protected within Ca-OM aggregates was investigated by treating soils with a mild acid prior to photo-oxidation. More organic material was protected in the calcareous than the non-calcareous soils regardless of whether the calcium (Ca) occurred naturally or was an amendment. Acid treatment showed that the presence of exchangeable Ca reduced losses of organic material upon photo-oxidation by 7% due to calcium bridging. The finding that OM is protected in the presence of Ca has implications for C sequestration in soils. Larger amounts of C may be retained in calcareous soils than in non-calcareous soils with low exchangeable Ca. Further research is warranted to quantify this difference between soils.

The implications of these findings for N fertiliser prediction models is that in soils with charcoal, the active OC pool is overestimated when a conventional method such as Walkley-Black is used to determine the soil's OC content. Overestimation leads to higher rates of N mineralisation being assumed than actually occurs and therefore predicted N fertiliser requirements are underestimated. Using UV photo-oxidation to assess soils’ OC content overcomes the problem of overestimating OC. However, presently the method is too expensive and time consuming to be used in routine analysis. With regards to the effect of CaCO₃ on N fertiliser prediction models, the effect is small and unlikely to have an effect that impacts upon N fertiliser recommendations in calcareous soils similar to those used in this study. This conclusion may not hold true in highly calcareous soils (> 50%) such as those farmed at the southern tip of Yorke Peninsula, and far western Eyre Peninsula, South Australia. The effect of calcium on N mineralisation in highly calcareous soils may be significant and warrants further investigation especially where the soil has high amounts of exchangeable calcium.
DECLARATION

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

I give consent to this copy of the thesis, when deposited in the University Library, being available for loan and photocopying.

[Signature]

Angela Clough

[Date]
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CHAPTER 1

Literature Review

1.1 Soil organic matter decomposition

1.1.1 Definition of soil organic matter

Soil organic matter (SOM) can be said to be both the product of living matter and the source of it (Thaer, 1808 cited by Boyle et al, 1989). The term SOM covers a range of materials that are widely variable in their susceptibility to degradation. SOM such as sugars and starches decompose rapidly (the labile fraction) whilst lignins decompose extremely slowly (the recalcitrant fraction) (Oades, 1988). Humus is almost totally resistant to microbial degradation. This variability leads initially to a period of rapid decomposition followed by a period of slow decomposition, that is, two-phase decomposition.

Soil organic matter has been defined in terms of chemical fractionation (Greenland, 1965; Stevenson and Elliott, 1989). However, the relationship between chemical extractants and the dynamics of SOM is unclear because SOM that is physically protected, and therefore slow to decompose, is present in the extracts (Jenkinson, 1971; Oades and Ladd, 1977).

Soil organic matter is also defined in terms of physical fractionation (Cambardella and Elliott, 1993). Physical fractionation divides SOM into particle size fractions based on a four step process of wet sieving (2000μm - 53μm), gentle sonication, sieving of sonication products (250μm - 20μm) and density flotation (0.2 - 20 μm). The size fractions recognised by Cambardella and Elliott (1993) were: > 2000 μm (large macroaggregates), 250 – 2000 μm (small macroaggregates), 53 – 250 μm (microaggregates), 20 – 53 μm (silt), 2 – 20 μm (fine silt), 0.2 – 2 μm (course clay) and < 0.2 μm (fine clay).

1.1.2 Factors affecting soil organic matter decomposition

1.1.2.1 Aggregate stability

Microorganisms positively affect aggregate stability by mechanically binding soil particles and
producing chemicals with binding properties (Lynch, 1981). Mechanical binding is facilitated by clay adhering to the walls of fungal hyphae and bacteria. Microorganisms also affect aggregate stability by what appears to be a simplistic method of adsorption of bacterial and fungal products (Gupta and Germida, 1988). There is some photographic evidence of materials produced by earthworms and microbes binding soil particles (Greenland and Hayes, 1978). The materials were listed as being worm casts, flagella and predominantly polysaccharide mucilage. This was supported by findings that soil aggregation increased when organic residues are added to soil containing microbes but not when added to sterile soil (Martin et al., 1967). Carbohydrates in particular have been shown to persist despite being subjected to periodate treatment for 6h. Whether their persistence is a cause or effect of aggregate stability has been difficult to establish (Cheshire et al., 1983).

The quantity and type of residual plant material in the soil affects aggregate stability (Tisdall and Oades, 1982). When OM was removed from a soil sample, dispersion can be achieved with a lower energy input then when OM is retained (Edwards and Bremner, 1967). Equally, aggregate stability has been shown to increase in the presence of living roots due to an associated increase in microbial biomass (Lynch, 1981). The stage of decomposition of organic amendments has been shown to be a factor with older residue giving less aggregation than fresh residue (Martin et al., 1955). When the C:N ratio is low, microbes use the soil-binding materials produced when the organic amendments were initially decomposed as an energy source. Hence aggregate stability initially increases but is eventually reduced by the presence of an active microbial population. Increased aggregate stability has been shown to be accompanied by a reduction in SOM decomposition (Lynch, 1981).

Aggregate stability is also increased by inorganic compounds or elements binding with SOM. Positively charged clays, calcium (Ca) and aluminium (Al) ions form covalent bonds with OM hence reducing the susceptibility of OM to decomposition (Oades, 1995).

1.1.2.2 Disturbance

Disturbance by cultivation or sieving reduces aggregate stability (Tisdall and Oades, 1980),
the size of soil aggregates (Gupta and Germida, 1988) and increases the accessibility of microorganisms to SOM (Lynch, 1981). In the immediate term, as shown by sieving, the increase in accessibility leads to an increased in the microbial population. This is accompanied by an increase in the rate of SOM decomposition as signified by a flush in carbon dioxide (CO₂) evolution (Ross et al., 1985). However, available SOM increases in soils that are cultivated because carbon inputs are higher in cropped soils than in soils under native vegetation (Robertson et al., 1997). Other immediate effects of sieving are an increase in extractable inorganic P and N content (Ross et al., 1985). The increase in N and P has been attributed to the death of microorganisms during sieving and the subsequent release of N and P compounds. Ross et al. (1985) also noted that with particularly wet soils (about 75% moisture content) sieving was accompanied by smearing and an increase in denitrification.

1.1.2.3 Temperature

Decomposition of SOM is facilitated by microorganisms that can only survive and function between about 10°C and 30°C (Oades, 1995). At temperatures below 10°C and above 35°C, microbial activity and SOM decomposition are very slow. An increase in mean annual air temperature of 9°C within the function range of microbes was shown to double the rate of decomposition (Ladd et al., 1985) at field sites in South Australia (Ladd et al., 1985), England and Nigeria (Jenkinson and Ayanaba, 1977). Under laboratory conditions, decomposition rates as represented by CO₂ evolution doubled for every 10°C increase in temperature (Howard and Howard, 1993).

1.1.2.4 Moisture

Like temperature, soil moisture also affects SOM decomposition because the process is facilitated by microorganisms. At low soil water potentials, that is when soil pores > 10 μm radius are filled with air (McGill and Myers, 1987), the decomposition process is most rapid since both water and oxygen are available to bacteria (Ladd et al., 1985). In dry conditions such as 5% to 10% water holding capacity (WHC), water is more tightly bound in soils and therefore decomposition and mineralisation rates are low (Howard and Howard, 1993). Conversely, in waterlogged soil, decomposition is slowed due to a low proportion (< 15%) of
air filled pore spaces (AFPS) (Miller and Johnson, 1964). Limited AFPS restricts oxygen supply through slowing the diffusion of oxygen through the soil hence anaerobic conditions prevail. The optimal moisture status for decomposition and mineralisation is considered to be about 60% water filled pore spaces (WFPS) (Howard and Howard, 1993; Sierra 1997).

In natural systems and in the laboratory, soils are subjected to wetting and drying cycles. The wetting phase of these cycles have been shown to produce a flush of microbial activity, increase in microbial population and decomposition rates whilst during the drying phase the opposite occurs including the death of microorganism (van Schreven, 1967; van Veen et al., 1985). The cycles produce a flush of activity partially because aggregates that contain OM are disrupted during drying thus more OM is exposed to microbes during the wetting phase (Sorensen, 1974). In addition, microorganisms that were killed in the drying phase due to either dehydration or lack available substrate provide another source of OC and organic N (Stark and Firestone, 1995). The intensity of these flushes decreases with the number of wetting and drying cycles as OM that is not bound within aggregates is decomposed (van Schreven, 1967).

Aggregate size, disturbance, temperature and moisture content all interact to determine the decomposition rate of OM. Decomposition of OM as a whole can be segmented into various components that specifically deal with the elements found in OM. The two elements of particular interest in this review are C and N. As such, the dynamics of these two elements in soil will be discussed in detail.

1.2 Nitrogen cycling

1.2.1 Overview of the nitrogen cycle

The N cycle is essentially the transformation and movement of N atoms through various N containing compounds via microbial and enzymatic processes (Fig 1). Agricultural production makes N cycling more complex than N cycling in natural ecosystems. This is because in agriculture, N is imported and exported from the local on-farm system that is continuously
undergoing change in the form of cultivation, compaction and amendment. The key elements of the N cycle and the agricultural practices that influence it will be discussed with particular reference to calcareous soils.

1.2.2 Nitrogen mineralisation

The underlying reason for N fertiliser needing to be applied to cereal crops is a lack of available N in the soil. Available N, that is N in the forms ammonium (NH₄⁺), nitrite (NO₂⁻) and nitrate (NO₃⁻), is formed by specific components of the SOM decomposition process known as
N mineralisation and nitrification (Stevenson, 1982). These processes can be summarised by Equation 1:

\[
\text{--- mineralisation } \rightarrow \\
R - \text{NH}_2 \leftrightarrow \text{NH}_4^+ \leftrightarrow \text{NO}_2^- \leftrightarrow \text{NO}_3^- \\
\text{--- nitrification } \rightarrow 
\]

where R -NH₂ is N in amino acids.

N mineralisation is the process by which organic N is converted to inorganic N, that is N in the form of amino acids is converted via soil microorganisms to ammonium. Nitrification is the process by which ammonium is converted to nitrite then to nitrate. When the rate of movement of N from the organic to the inorganic pool is measured, the value is known as the gross N mineralisation rate.

In the field, the conversion of ammonia to nitrite is facilitated by *Nitrosomonas* sp. (Jarvis et al., 1996). Nitrite does not usually accumulate in soils, particularly alkaline soils, instead it is quickly converted by *Nitrobacter* sp. to nitrate. These bacteria are obligate aerobes using CO₂ and carbonates as their C source (Bartholomew and Clark, 1965). The optimal pH range for nitrification *in vitro* is between 6 and 8. Nitrification rates are significantly reduced below pH 6 and are negligible below pH 4.5. At pH>8, the presence of ammonium inhibits nitrification. However, nitrification rates measured *in situ* are not related in soil pH (Robertson, 1982).

Not all of the ammonia produced is converted to nitrites and nitrates. Some of the ammonia is used directly by plants (Salisbury and Ross, 1985), some is fixed and some is used by microbes to synthesise tissue. A small amount of ammonia may be volatilised although this is more likely to occur with ammonia that is broadcast as an ammonium-based fertiliser than with ammonia that is produced through mineralisation, especially on sandy soils with low water-holding capacity (Havin and Tisdale, 1999).
The rate of N mineralisation is dependent upon the same soil properties and climatic factors that affect decomposition as previously described (aggregate size, soil disturbance, temperature and moisture). As with decomposition, N mineralisation rates are higher in disturbed soils than undisturbed soils (Stenger et al., 1995). Comparative experiments showed that mineralisation rates were 1.5 to 3 times higher in a disturbed than an undisturbed loam soil incubated at 21°C for 84 days (Stenger et al., 1995). Nitrogen mineralisation rates are also increased by reducing aggregate size due to organic material being freed from aggregates (Elliott, 1986). Increasing soil temperature from 10°C to 35°C produced a 6-fold increase in the amount of mineral N present in the soil after 14 days incubation (Kladivko and Keeney, 1987). Keeping WFPS at about 65% was shown to optimise N mineralisation by providing the water needed for microbial activity without reducing oxygen diffusion rates through the soil pores (Sierra, 1997). Conversely, N mineralisation rates are reduced in waterlogged soils due to slow diffusion of oxygen because pore spaces are filled with water (Stevenson, 1982).

In addition to the afore mentioned factors that effect both decomposition and mineralisation, mineralisation rates are affected by the OC: N ratio in the soil (Oades, 1989). As a general rule, an OC:N ratio above 20:1 will lead to N immobilisation due to competition for the N by microbes (van Veen et al., 1985). Soil amendments also effect N mineralisation rates through changing soil properties. For instance, amending an acid Mollosol with 6 to 12 t gypsum (CaSO₄·2H₂O) ha⁻¹ decreased soil pH and subsequently the N mineralisation potential whilst amending the same soil with 1 t lime (CaCO₃) ha⁻¹ had the opposite effects (Carter, 1986).

1.2.3 Nitrogen immobilisation

Nitrogen immobilisation is the conversion of inorganic N to organic N, that is, the assimilation of ammonium and nitrate into organic compounds (Stevenson, 1982). Hence, the immobilisation process is the reverse of the mineralisation process (Eq 1). Immobilisation is promoted when plant residues with a high (>20:1) C:N ratio are added to soil. Immobilisation also occurs in the presence of other C and N substrates such as glucose and ammonium sulphate solutions. Amending both calcareous sand and calcareous clay with wheat straw in
controlled conditions resulted in inorganic N being immobilised for the first 32 days of the incubation period (Ladd et al., 1977a). Although an undesirable process for plant growth in the short term because N is rendered unavailable to plants, immobilisation is the consequence of retaining cereal residue. However, residue retention is necessary for maintaining aggregation in soils, and high OC% and nutrient reserves sufficient for sustainable agriculture (Larson et al., 1972; Powlson et al., 1987; Campbell et al., 1991a). Net N immobilisation is usually temporary and the long term benefits of improved soil structure theoretically exceed the short-term costs of immobilising N resources.

The immobilisation and mineralisation processes are reversible and in practice operate concurrently. Thus immobilised N is re-mineralised to inorganic N. Re-mineralisation of the organic N contained in soil biota occurs when the soil biota die and themselves become a source of organic N for microbes. The amount of N re-mineralised during the first growing season after incorporation of labelled plant residues has been measured to be in the range 2% - 10% of total immobilised N. The amount of re-mineralised N in subsequent years was less ranging from 1% to 3% total mineralised N (Hauck, 1981). Soil biota can be a significant source of organic N with up to 5% of total organic N contained in soil biota at any one time (Anderson and Domsch, 1978). Death of soil biota can occur due to various events such as the C source used for energy being exhausted, lack of oxygen in soil pores due to waterlogging, desiccation during drying soil or physical shearing as occurs when wet soil is sieved (Ross et al., 1985).

Without using isotopes to track the movement of N between pools, only the net effect is measured and the rate of movement is known as the net N mineralisation rate. The distinction between gross and net N mineralisation is important to note. Comparative experiments on a loamy sand showed that gross N mineralisation in the 0-5 cm layer was 3 to 4 times higher over a period of 144 h than net N mineralisation (Sparling et al., 1995).

1.2.4 Nitrogen inputs

The major sources of N in agriculture are fertilisers and N fixation by leguminous pastures or
grains. In Australia, fertiliser-N is commonly in the form of urea (CO(NH$_2$)$_2$) or liquid ammonia (NH$_3$) or as a multi-nutrient high analysis fertiliser such as monoammonium phosphate (MAP), diammonium phosphate (DAP), (NH$_4$)$_2$SO$_4$ or NH$_4$NO$_3$. Ammonium-based fertilisers are readily available to plants (Salisbury and Ross, 1985). However, availability of ammonium-based fertilisers is reduced through fixation in soils abundant in 2:1 layer clays such as illite or vermiculite (Nommik, 1981) and through volatilisation if the fertilisers are not incorporated into soil. Urea-N is converted in soil to ammonium-N through hydrolysis (Havin and Tisdale, 1999). Hydrolysis is catalysed by the enzyme urease that is produced by soil borne bacteria, fungi and actinomycetes. The reaction is as follows:

$$CO(NH_2)_2 + H^+ 2H_2O \rightarrow 2NH_4^+ + HCO_3^-$$

The efficiency of each previously listed fertiliser as a source of N depends upon the soil moisture content, soil pH, OM content, microbial activity and application method. The various soil conditions which promote loss of applied N will be discussed in a later section.

Nitrogen fixation by grain and pasture legumes is also a source of N in agricultural systems. An $^{15}$N analysis of Medicago littoralis var. Harbinger that was grown with ($^{15}$NH$_4$)$_2$SO$_4$ at 10% enrichment showed that about 65% of legume-N was derived from N fixation (Ladd et al., 1981). Availability of N from leguminous material is affected by the rate of decomposition and mineralisation and therefore by the same climatic and soil factors previously described. In the N model by Payne and Ladd (1993a), N input to subsequent crops through N fixation increased as the quantity of leguminous biomass increased and as harvest index was reduced. The rates of decomposition in South Australian Xeralfs from the Barossa and Mid-North regions have been comprehensively studied in experiments using $^{15}$N to track decomposition of legume derived N and subsequent uptake by the following crop. After 7 months in the field about 31% of incorporated and ground $^{15}$N labelled Medicago littoralis had been released into the soil as inorganic N through mineralisation and was present in the top 90 cm of soil (Ladd et al., 1983). This accounted for about 6% of total inorganic N in the soil profile. After a further 8-9 months in the field, 88% to 93% of $^{15}$N was recovered in the top
90cm of soil and the mature wheat crop (Ladd et al., 1981). Recovery of legume derived $^{15}$N in the following wheat crop accounted for between 20% and 28% of $^{15}$N in the incorporated legume (Ladd et al., 1983). This proportion was equivalent to between 82% and 88% of $^{15}$N that had been mineralised between incorporation of the legume and sowing of the wheat. These studies show that legumes are a source of N to cereals but decomposition and recovery of fixed N is a slow process.

1.2.5 Nitrogen losses

Lysimeter studies in North America showed that crops recovered between 45% and 75% of available N in one growing season (Allison, 1955). In field experiments, between 20% and 35% of the non-utilised N was recovered in the leachates. Provided N is not leached below the maximum rooting depth, or lost by another means, inorganic N can be utilised by crops in following seasons. Permanent N losses through leaching are considered negligible in soils with a calcareous horizon (Stevenson, 1986). This could be expected to apply to many South Australian soils in the regions of Eyre Peninsula, Yorke Peninsula and the Murray Basin because calcrite formations are common in B and C horizons (Milnes and Hutton, 1993). However, on a South Australian calcareous sandy-loam where CaCO$_3$ content increased with depth, only 78 – 80% of urea-N was recovered in soil (0-90 cm) and in the crop one year after application (Ladd and Amato, 1986). Most of this discrepancy was considered to be due to N leaching to a depth greater than 90 cm.

Nitrogen can also be lost from dryland agricultural systems through volatilisation; that is the conversion of an N source (usually fertiliser) to gaseous ammonia followed by loss to the atmosphere (Fenn and Hossner, 1985). Most reactions are dependent upon chemical reactions whilst others, such as the loss of ammonia from urea is mediated by urease. The likelihood of fertiliser N being lost to the atmosphere is directly affected by soil pH. Soil alkalinity has been shown to result in up to 62% of applied urea-N being volatilised from sandy loam (pH 10.6) under laboratory conditions (Rao and Batra, 1983). In addition, volatilisation has been shown to be higher in soil with high CaCO$_3$ content (Fenn and Miyamoto, 1981), soil moisture content up to field capacity and soil temperature particularly
between 8°C and 20°C (Rao and Batra, 1983). Field studies in an alkali (pH 8.4) calcareous (20% CaCO₃) soil demonstrated that low moisture content caused urea-N to be hydrolysed at a slower rate and therefore losses of ammonia were less (Gezgin and Bayrakli, 1995). For instance, during the first 12 days of the field study, only 6.4% of urea N was volatilised despite the general finding that most volatilisation occurs in the first 2 to 10 days (Gould et al., 1986). Fertiliser type also affects volatilisation with acid fertilisers, such as ammonium nitrate and ammonium sulphate, being particular susceptible to loss of ammonia (Fenn and Hossner, 1985). Volatilisation of fertiliser N has been shown to be higher where the fertiliser is applied to the surface or incorporated at shallow depths. For instance, 60% of surface applied ammonium sulphate N was volatilised from the soils used by Rao and Batra (1983) whilst less than 10% was volatilised when the fertiliser was incorporated at a depth of 7.6 cm.

Loss of N through denitrification is primarily caused by anaerobic bacterial respiration, with aerobic denitrifiers also contributing to a lesser extent (Bartholomew and Clark, 1965). The denitrification process can be summarised by Equation 2:

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2
\]

Denitrification occurs when oxygen is excluded from the soil such as during prolonged waterlogging or in fine textured, structureless soils or microsites. In these conditions of reduced aeration, denitrification rates are optimised in soil with pH ranging from 6 to 8, at a temperature between 15°C and 35°C and where there is a readily available OC supply (Bartholomew and Clark, 1965). At a moisture regime suitable for microbial activity (60% WFPS), denitrification of crop residue derived N was found to be negligible (0 – 0.06 mg N kg⁻¹ d⁻¹) in a loam soil with pH 6.5 incubated at 25°C (Aulakh et al., 1991). However, at a suboptimal moisture regime (90% WFPS), denitrification in the same soil incubated under the same conditions increased between four and ten fold depending upon the type of crop residue used.

Where N fertilisers have been added, losses of N attributed to denitrification have been
estimated to be near 30% in cropping systems although actual losses are dependent on the type of fertiliser-N applied. (Hauck, 1981). Losses can be over 50% in rice paddies where N fertiliser is broadcast (De Datta et al., 1989). Denitrification rates measured in the top 15cm of a massive clay soil of the Denchworth series incubated with 110 kg fertiliser N ha$^{-1}$ gave estimated losses of NO$_2$-N to be 12 kg ha$^{-1}$ per day (Iqbal, 1992). Measured rates of denitrification should only be taken as a guide to a soil's potential for denitrification. The spatial variability for soil denitrification can be very high with coefficients of variation in the range 100-500% (Parkin, 1991) and there are no reliable methods of measuring denitrification rates in soil especially clay soils (Hauck, 1986; Smith, 1991).

1.3 Quantifying N mineralisation

1.3.1 Net nitrogen mineralisation potentials

Laboratory incubations under optimal conditions for N mineralisation (sieved soil, mixed with sand and incubated moist between 30$^\circ$C and 35$^\circ$C) have been used to measure potential rates of net N mineralisation (Cabrera and Kissel, 1988a; Stanford and Smith, 1972; Xu et al., 1996). Potential rates were shown to vary between 0.7 and 7.85 mg N week$^{-1}$ depending upon the amount and type of plant residues in each soil (Stanford and Smith, 1972) even when soils were incubated under identical conditions. A potential net N mineralisation study with 123 sites in South Australia (Xu et al., 1996) showed similar variability between sites to the values measured by Stanford and Smith (1972) with the amount of mineralisable N varying from 14 to 121 kg N ha$^{-1}$ over a period of 4 weeks.

Incubation methods, such as those just discussed, are time consuming. Therefore many chemical methods have been developed with the aim of measuring soils' potentially available N. Twelve chemical and 5 biological methods were reviewed and compared (Gianello and Bremner, 1986) to ascertain which chemical methods gave results that most closely resembled net N mineralisation potentials determined by incubation for up to 12 weeks. The comparison showed that two rapid chemical methods were able to account for 90% of the variation in N mineralisation potentials obtained by the biological methods in 30 soils from
Iowa. The two methods were; heating soil at 100°C in 2M KCl for 4 h, and steam-distilling soil with pH 11.2 phosphate-borate buffer.

Regardless of the biological or chemical method used, all in vitro methods with sieved and dried soil can only be used to ascertain a soils' potential available N and do not reflect the amount of N mineralised in situ (Cabrera and Kissel, 1988a, 1988b; Sierra, 1992; Mary and Recous, 1994). The discrepancy is due to the combined effect of soil disturbance during the sieving process causing organic material to become available for mineralisation, and due to soils being incubated in ideal temperature and moisture regimes. Methods designed to minimise soil disturbance and changes in temperature and moisture content from that in the field need to be used when attempting to estimate net N mineralisation in the field.

1.3.2 Benefits of measuring nitrogen mineralisation in situ

Measuring N mineralisation directly either in the field or in undisturbed soil cores has been recognised as providing mineralisation rates that more closely reflect the actual rates that occur in the field (Cabrera and Kissel, 1988a). Comparative experiments have shown there are large discrepancies between net N mineralisation rates measured in the field and those measured using the classical method of sieving soil followed by repeated wetting and drying as practiced by Stanford and Smith (1972). Comparisons in net N mineralisation rates for five soil types showed that the amount of N mineralised in 104 days was overestimated by up to 343% when the method of Stanford and Smith (1972) was used instead of measuring net N mineralisation in undisturbed soil cores (Cabrera and Kissel, 1988a). Large discrepancies are to be expected given that disturbing soil allows the soils' net N mineralisation potential to be measured whereas undisturbed soil measures net N mineralisation rates in a sub-optimal environment.

Although sieving and drying soils has been used extensively to measuring soil's net N mineralisation potential (Stanford and Smith, 1972) the data are unsuitable for use in models that are designed to predict how much N will the mineralised during a growing season. If net N mineralisation potential data are used in models designed for this purpose, net N
mineralisation rates will be overestimated. The consequence of this overestimation would be an underestimation of the amount of N fertiliser recommended to make up the shortfall between crop demand and supply from soil N. Given the limited usefulness of mineralisation potential values in N models several techniques have been developed to measure net and gross N mineralisation either in undisturbed soil cores or in the field.

1.3.3 Techniques for measuring nitrogen mineralisation in situ

Methods of measuring N mineralisation that have been designed to provide a means of measuring N mineralisation in the field include; measuring accumulation of mineral N in ion exchange resins placed in the field (Binkley and Matson, 1983), burying bags of disturbed soil in the field (Raison et al., 1987; Poovarodom et al., 1988) and storing undisturbed soil cores in the field in plastic bags, metal cans or capped plastic tubes (Eno, 1960; Poovarodom et al., 1988). The limitations of using these types of methods as a means of measuring N mineralisation in situ soil have been reviewed in some detail (Raison et al., 1987; Rees et al., 1994).

In brief, N mineralisation rates are highly dependent upon soil disturbance, soil temperature and soil moisture content. Any procedure that alters these parameters from field conditions will greatly change N mineralisation rate (Raison et al., 1987). Storing soil in plastic bags does not allow for field fluctuations in moisture content over time and disturbing soil changes soil aeration and porosity. Measuring accumulation of mineral N in ion resins relies upon water being present to move mineral N through the soil and mineral N that is absorbed by roots is omitted from the measurement.

Two alternative methods that are considered to be capable of accurately measuring gross and net N mineralisation under field conditions are described in detail in the following section of this review.

The first method is known as the isotopic dilution method which was developed to measure gross in situ N mineralisation on a daily time increment (Davidson et al., 1991). The method
involves uniformly injecting ($^{15}$NH$_4$)$_2$SO$_4$ into intact soil cores and measuring changes in the $^{14}$N : $^{15}$N ratio of the ammonium pool over a few days. Injecting N as a solution resulted in cores having up to an eightfold increase in their gravimetric soil water content compared to the surrounding soil, an increase in downward movement of water due to the soil’s high sand content and thus an overestimation of gross N mineralisation (Sparling et al., 1995). This limitation was overcome by modifying the method to inject N in a gaseous form (NH$_3$) into sandy soil (Murphy et al., 1997) and heavier textured loams (Murphy et al., 1999). The only drawback of this method is that it is unsuitable for monitoring long term changes in mineral N and injecting gas into soil requires specialist equipment.

A simpler in situ exposure method for tracking net N mineralisation over an extended period of time such as a growing season or a calendar year was initially developed for use in forests (Raison et al., 1987). However, the in situ exposure method has since been used to measure net N mineralisation, surface leaching and crop N uptake in cereal cropping systems (Stein et al., 1987; Rees et al., 1994). The method as described by Stein et al. (1987) involves inserting paired PVC tubes (Covered tube and Open tube) into soil to a depth of 10cm and leaving them for several weeks. At sampling time (t), the soil cores are removed and the tubes are reinserting into the soil for the next incubation period. A soil sample from a neighbouring unconfined site (Field) is taken at the beginning of each sampling period. Net changes in the amount of mineral N in the 0-10 cm layer are calculated as follows:

- Net N mineralisation = N$_{cov}$(t+1) – N$_{field}$
- Surface leaching of mineral N = N$_{cov}$ – N$_{open}$
- Crop uptake of mineral N = N$_{open}$ – N$_{field}$

Questions have been raised over the effect that capping the tubes to exclude rainfall has on net N mineralisation estimations (Rees et al., 1994). Moisture content has been found to be higher in covered cores than in the surrounding soil and subsequently net mineralisation rates were significantly overestimated (Rees et al., 1994). Severing living roots when inserting cores into soil is also considered to be potentially problematic in that the severed roots
artificially introduce an additional source of C (Rees et al., 1994). The problem of severed roots was considered by Raison et al. (1987) and dismissed as being unsubstantiated by empirical evidence.

When using this *in situ* exposure method, within site variability can be high. A compilation of data sets from virgin soils in north Queensland showed coefficients of variation ranging between 10% and 20% for both N and C where soil samples were taken at 10 m intervals (Spain et al., 1983). Given the prospect of high spatial variability, large numbers of paired tubes must be used in a small area to produce significant treatment differences. Stein et al. (1987) used 10 paired tubes per plot but plot size was not stated in their paper. Raison et al. (1987) used 16 sampling points in a 0.25 ha plot to obtain significant differences between fertiliser treatments.

An argued shortfall in all the *in situ* methods hereto described is that they only measure changes in mineral N content in the 0-10 cm layer. This approach could be said to be of limited value because plants are capable of taking up water from depths of at least one metre (Fischer and Kohn, 1966). Subsequently it has been suggested that porous ceramic cups be used to sample inorganic N in solution at depth. However, the cups preferentially sample large pores in their immediate vicinity thus creating sampling errors (Hauck et al., 1994). Additionally, those measuring N mineralisation rates are interested in changes in N availability not the total amount of N available to plants in the soil profile. Therefore, it is only necessary to sample the soil layers where change is likely to occur. By measuring microbial biomass N and extractable inorganic N, Sparling *et al.* (1995) found that 90-95% of net N mineralisation activity occurred in the 0-5 cm layer in soil samples treated with a single rewetting. Even with double rewetting treatments the amount of inorganic N in the 5-10 cm layer was only in the order of 3.5 μg g⁻¹ N. Thus, measuring changes in mineral N content that are due to mineralisation in the 0-10 cm layer appears to be valid.

1.3.4  Laboratory techniques for estimating *in situ* nitrogen mineralisation

A major challenge of measuring net N mineralisation *in situ* is the logistics given that intensive
sampling is required to overcome spatial variability (Macduff and White, 1985; Hauck et al., 1994). Several laboratory techniques have been trialled to avoid the difficulties of in situ measurements yet maintain conditions as close as possible to those found in the field. One such technique was used to estimate in situ net N mineralisation in eucalypt plantations (O'Connell and Rance, 1999). Soil samples taken from the field were packed in PVC tubes (38 mm diameter) to field bulk density and exposed to a set temperature (4, 12, 20, 28, 35 or 42°C) at one of 10 soil moisture contents for period of up to 56 days. The rates of mineralisation at each temperature and moisture content were related to field temperature and moisture content as measured hourly and every four weeks, respectively. The predicted in situ net N mineralisation rate was compared to rates obtained using the method described by Raison et al. (1987). The modelled rates and actual rates were well related ($r^2 = 0.97$) and predict in situ net N mineralisation rates to within 20% of actual rates (O'Connell and Rance, 1999).

1.4 Carbon cycling

1.4.1 Overview of the carbon cycle

The concentration of OC present in the 0-10 cm layer of Australian soils varies from less than 1% in cultivated Red Brown Earths to more than 14% in forested alpine humus soil (Stace et al., 1968). The rate that C cycles between atmospheric CO₂ and organic material is determined by the same environmental factors than effect N cycling; soil moisture content, soil temperature and soil disturbance (Fig. 2) (Oades, 1995). In addition, the rate of C cycling is highly dependent on the rate of photosynthesis by plants and the type of OM present in the soil; that is the quality of OM.

1.4.2 Carbon inputs in soil

Carbon is inputted into agricultural soils indirectly through photosynthesis and through the addition of amendments to the soil. Amendments may include organic matter, lime fertiliser, plant residues, exudates from plants and microbes, and through the death of roots on living plants. A significant proportion (20% – 50%) of C that is placed below ground by a plant can
be lost to the soil while the plant is still growing (Paustian et al., 1990). Oades (1995) claimed that fine roots might have a 100% annual turnover. Inputs of C to an acid soil through sloughed root cells and due to plants producing exudates was measured to be about 3% and 4% of all accumulated C for wheat and barley, respectively (Gregory and Atwell, 1991). These proportions equated to an annual addition of about 16 kgC ha\(^{-1}\) for wheat and 24 kgC ha\(^{-1}\) for barley.

![Diagram of the carbon cycle]

**Fig 2. The carbon cycle**

Total SOC has been shown to increase in agricultural systems when soil is subjected to no tillage (Chan et al., 1992; Franzluebbers et al., 1994). A ten year field experiment in Alabama showed that SOC in the 0-10 cm layer increased from 0.6% to 1% with no tillage compared to conventional cultivation (Wood et al., 1991). The increase in SOC was attributed to increased
aggregate stability. Similarly, a nine year field experiment with continuous wheat showed that SOC increased by 33% in the 0-5 cm layer when no tillage was practiced instead of conventional cultivation (Franzluebbers et al., 1994). The increase in SOC content was attributed to less C mineralisation due to a lack of soil disturbance under the no-tillage practice. In addition, the amount of SOC present after nine years increased by 95% when the time the field was under crop was increased from 6 months to 10.5 months each year, and when N fertiliser was applied. The increase in SOC due to higher cropping intensity and using N fertiliser was attributed to higher biomass production.

1.4.3 Carbon mineralisation
Carbon mineralisation is the process of converting C in organic matter to CO₂ (Ajwa et al., 1998). This process is facilitated by heterotrophic bacterial species and many other soil organisms such as fungi and protozoa that oxidize OC to CO₂ (Stevenson, 1986). The CO₂ generated by the heterotrophs and other soil organisms is subsequently used by autotrophs as an energy source whilst converting ammonium to nitrite and nitrite to nitrate (Bartholomew and Clark, 1965). Given that micro-organisms are an integral part of both the C and N cycle it would be expected that the cycles are closely related. This relationship was demonstrated by laboratory incubations of soils subjected to various rotation and fertiliser regimes (Campbell et al., 1991b). There was a significant relationship between C mineralisation and total N content ($r^2 = 0.61$) and between C mineralisation and potentially mineralisable N ($r^2 = 0.85$).

Due to C mineralisation being a microbial mediated process, the rate of C mineralisation (alias respiration) is dependent upon soil temperature, soil moisture and soil disturbance (Oades, 1995) through the same mechanisms previously described for the overall process of SOM decomposition. A flush in C mineralisation as denoted by increased CO₂ evolution occurs when soils are suddenly exposed to higher temperatures (up to 35°C), more optimal moisture regimes (about 60% WFPS) or when soils are disturbed (van Schreven, 1967; Sorensen, 1974). These three parameters effect the mean turnover time of OC which is considered to average about 7 years in arable soils (Bolin et al., 1979). In vitro, the maximum recovery of evolved CO₂ from readily utilised C sources like glucose is about 65% to 75%
The quality of OM also effects the rate of C mineralisation. Simple molecules such as glucose are readily available energy sources for bacteria and have been shown to be almost completely mineralised after only a few days incubation. For instance, a Vertisol incubated at 25°C and 40% WHC for 24 h only retained 17% of the initial added glucose (Ladd et al., 1992). Only about 0.5% of initial glucose was present after 8 days incubation. Similar results were found for 22 other Australian soils subjected to similar incubation conditions (Amato and Ladd, 1992).

In vitro studies have shown that plant residue C is mineralised at a far slower rate than glucose-C (Amato and Ladd, 1992). Plant residues contain readily decomposed material like hemicellulose and carbohydrates, and more complex substrates such as lignins (Oades, 1995). In a laboratory incubation, the proportions of vetch, soybean, corn and wheat residues recovered as CO$_2$-C after 35 days incubation at 60% WFPS ranged from 46% to 55% (Aulakh et al., 1991). Carbon mineralisation rates differed between plant residues due to differing C : N ratios (Aulakh et al., 1991). For instance, the in vitro incubation with incorporated plant residues showed that maximum rate of CO$_2$ evolution occurred later in the incubation period as C:N ratios increased (Aulakh et al., 1991). Maximum rates of CO$_2$ evolution occurred after 2 to 3 days for vetch (8:1), 3 days for soybean (43:1), 5 days for corn (39:1) and 8 days for wheat (82:1).

Carbon mineralisation is slower in sub-optimal conditions as shown in the same experiment by Aulakh et al. (1991) conducted at higher water content. At 90% WFPS, 31% of wheat residues and 40% of corn residues were recovered as CO$_2$-C over a period of 35 days. Less CO$_2$-C recovery with the higher water regime was attributed to one of two potential causes that had previously been identified (Suchomel et al., 1990; Aulakh and Doran, 1991). One possible cause was that a high proportion of WFPS lead to a lack of aeration thus lower microbial activity and less CO$_2$ evolution. Alternatively, the low proportion of air filled pores lead to evolved CO$_2$ being trapped within the soil. Thus although, CO$_2$ was evolved, it was not
recovered in the headspace of the closed jars that housed the soil.

The effect of OM quality on C mineralisation rates was demonstrated in a sandy loam that was collected from an environment naturally subjected to repeated wetting and drying cycles throughout the year (Degens and Sparling, 1995). Subjecting the soil to wetting and drying cycles in vitro did not result in a flush a C mineralisation as has often been observed upon applying that treatment to soils (van Schreven, 1967; Sorensen, 1974). Degens and Sparling (1995) proposed that there was no response to the treatment because readily utilised OC had already been mineralised in the field and only recalcitrant material remained.

Temperatures outside the range considered suitable for microbial activity (10-35°C) also lower C mineralisation rates. This was demonstrated by a field experiment at a site with a mean annual temperature of 7.9°C (Wolters, 1991). In contrast to laboratory studies with plant materials (Aulakh et al., 1991), fresh beech leaves buried in the field for one year and protected from macrofauna and mesofauna only lost between 16% and 22% of initial C.

1.4.4 Carbon dioxide fixation and carbon immobilisation

The initial rate of fixation of atmospheric CO₂-C by photosynthetic organisms is dependent upon the rate of photosynthesis (Salisbury and Ross, 1985). In turn, the rate of photosynthesis is dependent upon the amount of plant available water, solar radiation, temperature and availability of other nutrients. As discussed in the previous section about C mineralisation, the amount of time that C remains in plant residue, photosynthetic bacteria and blue-green algae before decomposition is related to the type of OM (Oades, 1995). In addition, decomposition rates are dependent on the size and shape of the material (Oades, 1995).

The microbial biomass is recognised as an integral part of the C cycling process since all SOC passes through it at some point during the cycle. At any particular point in time, the microbial biomass in a soil contains between 1% and 5% of the total SOC (Oades, 1995). Carbon is immobilised in microbes, fungi, and as exocellular products of these organisms in
several chemical structures, principally alkyl, O-alkyl and carboxyl carbon (Baldock et al., 1990). These structures have been shown to account for the other 30% of readily available C substrate, in this case glucose, that is not respired by microbes during laboratory incubations (Baldock et al., 1990).

Immobilisation of C can also occur by CO₂ being converted to other inorganic C forms such as carbonates (Anderson, 1982). This process occurs in alkaline soils where CO₂ reacts with water to form soluble carbonates or hydrogen carbonate salts. The carbonate ions may in turn react with Ca ions to form CaCO₃ which has a low solubility (Weast, 1969). Without acidification, C will not be released from carbonates to progress in the C cycle.

1.4.5 Carbon losses from soil
Physical removal of C from soil occurs when CO₂ is respired by microbial organisms or roots and diffused through air filled pore spaces to the soil surface. As previously mentioned, this loss accounts for about 65% to 75% of readily available C sources as utilised by microbes in laboratory incubations (Baldock et al., 1990; Ladd et al., 1992). In the field, loss of C to the atmosphere through root and microbial respiration has been shown to be less than in the laboratory. A field study with labelled CO₂ gas showed that the highest rates of respiration from barley roots and microbes occurred at tillering with 25% of ¹⁴CO₂ recovered in the headspace 24 h after labelling (Gregory and Atwell, 1991). At grain filling, respiration of CO₂ was least with 2.1% recovered after 24 h. Over the entire growing season, loss of C from the plants and soil totalled 235 kg ha⁻¹ for barley and 132 kg ha⁻¹ for wheat.

Some C is also lost from agricultural systems at harvest when grain is removed from the field. However, the amount of C removed through harvest is very low relative to the amount of total OC present in soil. On a larger scale, OC is lost from soil due to the actions of cultivation and removal of crop residues, especially in the first few years of cropping (Dalal and Mayer, 1986; Chan et al., 1992). As with any other form of disturbance, the immediate effect of soil cultivation is more OM becoming available for decomposition and therefore accelerated C mineralisation. Cultivation can also be accompanied by soil erosion and produces greater
fluctuations in soil temperature and moisture which effect microbial activity and can lead to flushes of C mineralisation. A ten year field experiment in Australia showed that cultivation intensity did not significantly effect the rate of OC decline (Chan et al., 1992). Both one and three cultivations prior to sowing lead to OC losses of up to 31% compared to direct drilling practice. The same Australian field experiment also showed that removing crop residue by burning the stubble resulted in significant loss of OC from the 0-10 cm layer relative to retained stubble treatments.

A 68 year field experiment on a Red Brown Earth at Urrbrae showed that decline in SOC is affected by cropping rotation (Grace et al., 1995). Eleven rotations were included in the experiment. The largest decline in SOC occurred in the rotations that included a fallow and no long term pasture where SOC fell from an initial value of 2.75% in 1925 to an average of 1.22% in 1993. The smallest decline of only 0.29% occurred in the rotation that started as wheat – pea rotation in 1925 and was converted permanent pasture to in 1950. Rapid decline in SOC for rotations containing a fallow was attributed to accelerated decomposition of native OM and crop residues in fallow periods where water is stored in the soil. Conversely, slow decline in SOC where pastures were included in the rotation for at least two consecutive years was due to the continuous growth, death and decomposition of roots from pasture plants.

The rate of loss of OC due to cropping also varies depending upon initial OC and soil aggregation (Dalal and Mayer, 1986). For six soils from southern Queensland, the rate of loss of OC from the 0-10 cm layer was found to be inversely related to the soil's clay content ($r = 0.97$).

1.4.6 Techniques for measuring carbon mineralisation in situ and in vitro
Directly measuring C mineralisation is a relatively simple process because C in the form CO$_2$ is generally released from soil rather than being cycled within the soil as is the case for N. The relative simplicity of measuring CO$_2$ means that evolution of CO$_2$ can be used to measure decomposition of OM or utilisation of organic substrates (eg labelled glucose) and as an
indicator for movement of N between N pools. Mineralisation of C can be measured by collecting CO$_2$ in alkali traps (NaOH or KOH) placed in close proximity to the soil and analysing either for total CO$_2$-C or labelled substrate CO$_2$-C (van Gestel et al., 1991; Franzluebbers, 1999). Alternatively, CO$_2$ can be collected from the headspace directly above the soil and the concentration determined by gas chromatography (Ajwa et al., 1998).

In acid soils, evolved CO$_2$ is derived either from OM or from a C source added to the soil as an amendment. In calcareous soils, C can be derived from OM or carbonates that react with organic acids produced during decomposition of OM (Oades, 1989). Despite the inability to distinguish unlabelled mineralised C from carbonate derived C without using delta $^{13}$C values, some incubation experiments with calcareous soils assume that all CO$_2$ is derived from OM or the C substrate (van Gestel et al., 1991; Ajwa et al., 1998). This assumption does not seem to be unreasonable as a search of the literature failed to find any evidence that carbonate derived CO$_2$ confounded SOM studies. The inability to distinguish substrate derived CO$_2$ from carbonate derived CO$_2$ becomes important when calcareous soil is acidified to retrieve all of the unmineralised labelled C source at the completion of an experiment (Watwood et al., 1991). Therefore to account for all substrate C in calcareous soils a minimum requirement is to label the substrate and have an alkali trap large enough to trap all carbonate, OM and substrate derived CO$_2$ (Amato, 1983; Watwood et al., 1991).

Another proposed complication of studying SOM dynamics in calcareous soils is the possibility that substrate derived CO$_2$ may be retained within the soil solution as carbonates (bicarbonate, carbonic acid or carbonate ions) or be fixed in soil as carbonates rather than diffusing to the soil surface for recovery (Guerin, 1999). However, acidification of two slightly alkaline soils (pH 7.5 and 7.8) containing the substrate endosulfan showed that substrate derived $^{14}$CO$_2$ was not retained in soil solution or fixed as carbonates. Despite this result, Guerin (1999) recommended that the risk of underestimating C mineralisation due to retention of substrate derived CO$_2$ be assessed prior to commencing mineralisation experiments with alkaline soils.
1.5 Physical and chemical protection of organic carbon

1.5.1 Microbial and fungal attack

Microbes and fungi can only use OC as an energy source if their exocellular enzymes can physically access the SOM (Ladd and Foster, 1988). However, physical access by soil biota can be restricted because OM and soil aggregates form complex spatial arrangements (Fig. 3). Fragments of OM with large surface area : volume ratios that are either free in solution between aggregates or only loosely bound to soil aggregates can be readily accessed by fungi and bacteria (Skjemstad et al, 1993). Organic matter fragments with smaller surface area : volume ratios or OM with limited exposure to the soil solution are more difficult for microbes and fungi to access.

Some OM is lodged in pores within soil aggregates. This OM is not accessible to fungi that have diameters ranging between 3 μm and 50 μm and are located in larger pores between microaggregates (<250 μm) (Ladd and Foster, 1988). Bacteria are relatively small (0.5 – 2 μm diameter) and are able to access fragments of OM contained in the larger pores within soil aggregates (Ladd and Foster, 1988). However, OM lodged in the larger pores is still relatively protected from microbial attack because only a small proportion of the OM is exposed to the soil solution (Skjemstad et al., 1993). The smallest pores that bacteria can access has been suggested to be those with a diameter of at least 0.48 μm (McGill and Myers, 1987). Other submicron pores within soil microaggregates are too small for bacteria to access (Foster, 1981). Any OM in these submicron pores is physically protected from microbial attack until the microaggregate itself breaks down.

The most recalcitrant OM is fully encased by soil within the aggregate and cannot be assessed by any microbes until the soil aggregate is disturbed. The concept that OM enclosed within aggregates is less accessible than OM loosely bound to the edge of aggregates was supported by radiocarbon dating the OC found within soil aggregates (Skjemstad et al., 1993). By discriminating between OC afforded physical protection within soil aggregates, Skjemstad et al. (1993) estimated that readily oxidised OC could be
considered modern whilst physically recalcitrant OC in clay fractions was about 320 years old.

Fig 3. Representation of organic matter (dark grey) associated with soil aggregates (light grey). A, organic matter free in solution or as small particles, B, larger particles of organic matter either free in solution or loosely bound to soil aggregates, C, organic matter lodged in small pores in soil aggregates, D, organic matter fully enclosed by the soil aggregate (Skjemstad et al., 1993).

1.5.2 Clay content

Clays are known to have an association with OM. The clay fraction of soil has been found
associated with the residual products of substrate-C that has been utilised by microbes (Baldock et al., 1990). Analysis of the clay fraction of a fine sandy loam showed that the fraction contained 73% of residual substrate-C. Clays are known to form soil aggregates through clay-OM bonding (Edwards and Bremner, 1967). Clay-OM bonding occurs by non-organic adsorption mechanisms such as hydrogen (H) bonding and van der Waals forces (Parfitt and Greenland, 1970). The bonding of clay to OM causes OM to be protected from microbial attack due to physical protection (Craswell and Waring, 1972). Protection of OC due to clays has been demonstrated in C mineralisation studies which showed that the total amount of SOC mineralised after 24 days in vitro incubation decreased from 34 to 22 mg C g⁻¹ total C as clay content increased from 30 to 350 mg g⁻¹ soil (Franzluebbers, 1999).

The effectiveness of adsorption between clays and organic polymers is reduced in soils where the clays are saturated with calcium, sodium, aluminium or potassium ions (Martin et al., 1955; Parfitt and Greenland, 1970). However, polyvalent cations are thought to have an important role in binding SOM through cation bridging (Oades, 1988). Cation bridges are of particular importance in calcareous soils.

1.5.3 Calcium bridging

Peterson (1947) experimented with the concept that Ca ions acted to provide a link between clay and OM to form water-stable granules in soils. Peterson theorised that Ca was capable of acting as a bridge between carboxyl groups of uronide particles and clay particles, thus forming Ca-humates. The Ca linkage mechanism is illustrated as follows:

\[
\text{clay - Ca - OOC - R - COO - Ca - OOC - R - COO - Ca - clay}
\]

Using H-pectin as the polyuronide with various quantities of CaCO₃, Peterson (1947) measured the thixotropy of the suspensions and found evidence of Ca linkage. Twenty years later a similar theory was proposed based on the assumption that the mechanism for aggregation is merely the reverse of the mechanism for dispersion (Edwards and Bremner, 1967). Dispersion was achieved by shaking soil with a suspension of a sodium (Na) saturated
cation-exchange resin. This caused the monovalent cations to replace the polyvalent Ca and iron (Fe) cations thus weakening and rupturing the inter-particle bonds. Therefore, the proposed theory was that clay particles were linked with OM through polyvalent cations such as Ca, Fe and Al.

Drawing on the earlier work by Peterson (1947), Oades (1988) proposed that Ca ions bridge cells and cell debris to clays thus forming chemically stable aggregates that restrict microbial access and therefore SOM decomposition. This theory was supported by laboratory experiments (Gaiffe et al., 1984) which demonstrated that removing Ca from soil stimulates OM decomposition and C mineralisation.

Muneer and Oades (1989a) showed that adding Ca, particularly in the form of gypsum, inhibited C mineralisation in Urrbrae fine sandy loam amended with 120mg of labelled glucose cm⁻³ and incubated for 730 days. Initially CO₂ release was higher in the amended soils than in the control however after only 20 days both gypsum and lime treatments inhibited C mineralisation. Particle size distribution was also affected by amending the soils with Ca and incubating for 730 days. The amended soils had a greater percentage of particles in the 50-250 mm diameter range and less in the <50 mm diameter range than the control soils. This change resulted in a reduction in the amount of dispersible clay thus improving the soil's structural stability.

Expanding their experiments into the field and using labelled straw instead of glucose Muneer and Oades (1989b) found the field and laboratory results to be complimentary. They concluded that losses of OM could be reduced and a soil's structural stability could be improved by adding Ca as lime or gypsum. Field work by Oades (1988) gave compatible results, demonstrating that adding CaCO₃ to a Rhodoxeralf soil initially resulted in increased CO₂ production but eventually C mineralisation was reduced.

Muneer and Oades (1989c) proposed a model based on a previous model by Tisdall and Oades (1982) and their own work illustrating the role of Ca in linking clay and organic matter.
and the wider ramifications. The model proposed that Ca was involved in:

- the stability of aggregates both larger and smaller than 250μm,
- stability of quasi-crystals and domains,
- crosslinking functional groups in humic macromolecules,
- linking clay aggregates to humic material to form microaggregates that are subsequently bound together by roots, hyphae and bacteria to form large stable aggregates.

Note needs to be taken that not all research in this area has produced the same results. A more recent five year field experiment on the same soils concluded that there was no significant accumulation of soil OC that could be attributed to applying 0.8 t calcium ha⁻¹ in the forms of lime or gypsum (Baldock et al., 1994). Increases in soil OC were measured, however, they were due to the annual incorporation of wheat straw. Given these conflicting results, this topic was mooted as an area that required further research (Baldock et al., 1994).

1.5.4 Charcoal

Charcoal is a form of OC that is said to be chemically protected (Skjemstad et al., 1996). That is, charcoal is considered inert in terms of its role in C cycling and as such it needs to be considered as a passive fraction of the C cycle (Parton et al., 1987). Several Australian soils have been shown to contain significant amounts of charcoal relative to total OC (Skjemstad et al., 1996, 1999a). For instance, a Krasnozem from Queensland was shown to have 30% of its OC in the form of charcoal. Based upon these findings, Skjemstad et al. (1996) concluded that charcoal is a major component of OC in many Australian soils. The finding that charcoal can be a major component of SOC has emphasised the need to differentiate between inert OC and active OC. Inert OC is any form of OC that cannot be moved between C pools (eg charcoal) and active OC is any OC that is labile.

1.5.5 Measuring organic carbon using the UV technique

Techniques used to measure OC are based upon two main assumptions, namely, that all active OC can be accounted for by the chosen technique, and secondly, inert forms of OC, like charcoal, are not detected. The second assumption is based on comments in the
literature which state that charcoal is virtually undetected by the oxidation methods of Walkley and Black (1934) and Heanes (1984) (Piper, 1944; Heanes, 1984). However, this assumption has been challenged by Skjemstad et al. (1999a) who found that both the Walkley-Black and the Heanes method detected various proportions of charcoal derived from plant material. The proportion of charcoal recovered was inconsistent and depended upon the type of plant material used in the experiment and the charcoal's particle size. Given the factors determining recovery rates, a correction factor could not be created to convert total OC recovery to active OC recovery.

The ultra-violet (UV) photo-oxidation method can discriminate between active OC and charcoal as well as the amount of physical protection afforded to organic material by soil aggregates (Skjemstad et al., 1993) and theoretically by other OM bonding materials such as Al or Ca ions. The method uses UV light as a substitute for the microbial decomposition of SOM in a suspension. Decomposition of SOM using this method occurs gradually in four steps.

Initially, SOM that is either free in solution or adsorbed to exposed surfaces is decomposed. This is followed by the decomposition of SOM that is loosely bound to soil aggregates. Given that SOM itself is part of the binding material in soil aggregates, these aggregates may start to breakdown. Thus SOM lodged in small pores becomes exposed to UV light and decomposed. Finally, SOM that is fully encased in a soil aggregate may become exposed to UV light as the soil aggregate breaks apart. After a period of up to 2 h, the only SOM remaining is either physically protected within aggregates or in the form of charcoal. The presence of charcoal in some soil samples after the decomposition is complete has been confirmed using nuclear magnetic resonance (Skjemstad et al., 1996)

1.6 Modelling nitrogen availability through turnover of organic carbon

Total OC is a simple parameter to measure and there is a close relationship between the C and N cycle. Therefore several total SOC-based models have been developed to predict the
amount of N that becomes available to plants during the cropping season (Payne and Ladd 1993a, 1993b). The models vary in their complexity.

1.6.1 Simple nitrogen prediction models
The simplest model assumes that the same proportion of SOC is available in all soils and that mineralisation rates are constant at a wide range of soil temperatures and regardless of soil texture. The only factors used to predict the amount of N mineralised over a growing season is initial OC content and growing season rainfall. This simple model has been used in extension programs for grain growers (Evans, 2000). However, as explained in the previous discussion, these assumptions are unlikely to be valid.

A somewhat more involved model was developed in South Australia for use by grain growers who crop in the southern part of Australia (Payne and Ladd, 1993a, 1993b). The growers’ model accounts for N inputed through growing leguminous crops and pastures in previous years and the difference in N mineralisation rates due to clay content. Payne and Ladd (1993a) suggested that SOC contents determined using the Walkley-Black method be increased if clay content was below 10% and decreased if clay content above 40%. Soils with modified OC% outside the range 0.6% to 2% are not accommodated by the model and no adjustments were made for chemically protected OC. Seasonal differences in soil temperature and moisture content are not accounted for in the model thus it is assumed that net N mineralisation rates do not vary between seasons. The growers’ model is based upon N mineralisation rates measuring in situ and in vitro for South Australian non-calcareous Xeralfs principally in the Mid-North of the State (JN Ladd, personal communication). The validity of the growers’ model for crops grown on the calcareous soils of Yorke Peninsula was specifically questioned by Payne and Ladd (1993a). These calcareous soils were considered to mineralise less N than expected given their OC contents. No reason was given for the low net N mineralisation rates.

1.6.2 Complex nitrogen prediction models
Complex N prediction models have been developed and refined as more quantitative
information about N cycling in soils becomes available (van Veen et al., 1984; Parton et al., 1993). Models such as CENTURY (Parton et al., 1987), SUNDIAL (Smith et al., 1996) and APSIM (Probert et al., 1998) account for most N losses, the effect of OM quality and soil conditions that effect the microbial biomass. The flexibility of these models provides site specific N mineralisation rates since they can accommodate changes in soil temperature, soil moisture and various soil textures.

In addition, the complex models are built with separate sub-routines for the various types of SOM. For instance, the CENTURY model which is based upon long term data from field experiments at Rothamsted separates SOM into five pools each with a different turnover time (Paustian et al., 1992). Two pools are for plant litter and animal manure, and three pools are for SOM. The litter and manure is divided into a structural pool with a turnover time of about one to five years and a metabolic pool with a turnover time of about one to 12 months (Parton et al., 1987). SOM is fractionated into one of three pools primarily based upon the SOM’s turnover time. The active fraction has the shortest turnover time of one to five years and includes live microbes, metabolites and readily decomposable SOM such as carbohydrates. The slow fraction is composed of SOM that is physically protected or is in chemical forms that are more resistant to decomposition, for instance, cellulose. The turnover time for the slow fraction is about 20 to 40 years. The third SOM pool is the passive or inert fraction which consists of SOM that is highly resistant to decomposition due to physical protection within aggregates or chemical protection in recalcitrant forms such as charcoal. The turnover time for SOM in the passive fraction is estimated to be between 200 and 1500 years (Parton et al. 1987).

Using the CENTURY model requires many initial site parameters that are fairly simple to measure such as soil bulk density, intial C and N content, clay and sand content, soil depth and monthly rainfall data. The model also requires the initial percentage of SOM in each pool. However, initial proportion of SOM in each pool can only be estimated because methods for measuring SOM fractions are based upon chemical or physical fractionation not functional fractionation. Estimates are based on site-specific values such as soil texture and residue
lignin content.

The proportion of OM that was classified for the CENTURY model by Jenkison and Rayner (1977) as chemically stabilised (passive fraction) was only 4%. Other work with CENTURY model assumed that 8% of SOM had a turnover time of 1500 y (passive fraction) (Parton et al., 1993). However, as previously mentioned, more recent studies have shown that some soils contain far higher proportions of chemically recalcitrant OC than previously estimated (Skjemstad et al. 1996, 1999a).
1.7 Objectives of this research

The objective of this research was to investigate two main hypotheses:

A. Calcium carbonate decreases N mineralisation rates in South Australian soils under field and cultural conditions.

B. The chemical cause of the reduction in N mineralisation is calcium forming bonds with organic material thus slowing N mineralisation rates.

Hypothesis A was researched by conducting experiments that met the following objectives:

- Measure and compare *in situ* net N mineralisation in soil under two cropping treatments on the Yorke Peninsula and in the Mid-North of South Australia.
- Determine if the presence of CaCO$_3$ affects *in situ* net N mineralisation in soils measured for the first objective.
- Measure and compare *in vitro* net N and C mineralisation rates in soils from Yorke Peninsula and in the Mid-North of South Australia and determine if the presence of CaCO$_3$ affects mineralisation rates.

Hypothesis B was researched by conducting experiments that met the following objectives:

- Find evidence for a mechanism involving calcium that explains the differences in mineralisation rates between calcareous and non-calcareous soils from Yorke Peninsula.
- Determine whether differences in mineralisation rates between soils from Yorke Peninsula and the Mid-North are due to chemical protection of OC as charcoal.
- Quantify the effect of physical and chemical protection of OC on N availability in soils from Yorke Peninsula.
Chapter 2
Long term *in situ* N mineralisation study using the method of Raison *et al* (1987) - Found high spatial variability

Estimating *in situ* N and C mineralisation *in vitro*

Chapter 3
Risk assessment for using $^{14}$C in alkaline soils
- Found $^{14}$C was suitable

Chapter 4
Assessing techniques for minimising the effects of soil disturbance
- Use repacked moist soil cores

Chapter 5
Long term *in vitro* C and N mineralisation study
- Found differences between soils

Chapter 6
Physical and chemical protection of OC
- Found differences between soils due to physical protection of OC in Ca-OM aggregates and chemical protection of OC as charcoal

Chapter 7
Implications for predicting N fertiliser requirements using OC based models
CHAPTER 2

In situ net nitrogen mineralisation in agricultural soils with different contents of calcium carbonate

2.1 Introduction

The N cycling models developed in South Australia for the purpose of predicting N fertiliser requirements in the State's annual cereal-based cropping systems (Payne and Ladd, 1993a, 1993b; Xu et al., 1996) are primarily based on net N mineralisation rates determined for Rhodoxeralfs (Red Brown Earths) in the Barossa and Mid-North regions. Several in vitro techniques (Ladd et al., 1977b, 1994) have been used to determine N mineralisation rates for the South Australian N models (Payne and Ladd, 1993a, 1993b; Xu et al., 1996). The common elements amongst the various in vitro techniques are that the soil is collected from the field, repeatedly disturbed and kept moist during incubation.

As previously discussed, these actions maximise N mineralisation rates and allow potential rates in the field to be determined. The shortcoming with in vitro techniques becomes evident when attempts are made to relate potential N mineralisation rates to N mineralisation rates which occur in the field (Cabrera and Kissel 1988a; Rees et al. 1994). Net N mineralisation has been shown to be higher in sieved soil than in undisturbed cores in the field (Cabrera and Kissel, 1988a; Sierra, 1992) and laboratory (Ross et al., 1985; Cabrera and Kissel, 1988b). In Cabrera and Kissel's (1988a) experiments, net N mineralisation predicted from laboratory incubations was overestimated by between 67% and 343% compared to field studies. Sieving soils and incubating at a low bulk density (< 1 Mg m⁻³) relative to field bulk density has also been shown to increase CO₂ release during the first few days of incubation particularly in clay soils (Franzluebbers, 1999) although not necessarily by a significant amount (Hassink, 1992). Overestimates of C and net N mineralisation has been attributed to microbes' having greater access to OM after soil aggregates are disrupted by wetting and drying (van Schreven, 1987) or sieving (Sierra, 1992). Additional
explanations relate to more OM becoming available through incorporation of organic residues upon sieving (Raison et al., 1987), decomposition of cut root material, and decomposition of microbes killed during the action of sieving (Ross et al., 1985).

The issue of overestimation becomes critical when the purpose of measuring N mineralisation rates is to include the data in models designed to predict how quickly organic N becomes available to plants. Overcoming the limitations of in vitro techniques requires N mineralisation to be measured in situ. A technique developed for forest ecosystems (Raison et al., 1987) is considered to most closely reflect N mineralisation in field conditions (Cabrera and Kissel, 1988a) and has been shown to be related to in vitro measurements through soil temperature and moisture (O'Connell and Rance, 1999).

In addition to the potential inadequacy of Payne and Ladd’s (1993b) prediction model because of the methods used to acquire net N mineralisation data, the validity of using these models in the calcareous soils of Yorke Peninsula is in doubt (Payne and Ladd, 1993a). In part, this uncertainty arose because calcium had been found to reduce net N mineralisation rates in laboratory studies (Muneer and Oades, 1989a) and in some field studies (Muneer and Oades, 1989b, 1989c) where Ca was applied to non-calcareous soil. Restricting usage of the N prediction model to non-calcareous soils in South Australia would severely limit the model's application because large areas of wheat and barley production occur on calcareous and alkaline soils in the Mallee, Yorke Peninsula and Eyre Peninsula.

The experiments presented in this section were designed with two purposes; to determine if in situ net N mineralisation rates differed between the Red Brown Earths of the Barossa and the Xeralfs of Yorke Peninsula and to determine if the presence of naturally occurring calcium in the field affected in situ net N mineralisation rates. These aims were approached by using the exposure method (Raison et al., 1987) to measure in situ net N mineralisation rates at two sites on Yorke Peninsula and one site in the Barossa under oat (Avena sativa L. cv Wallaroo) and
fallow treatments. A field pea (*Pisum sativum* L. cv Alma) treatment was included in preparation for the following season.

### 2.2 Materials and methods

#### 2.2.1 Locations and soils

Three field sites differing in their CaCO₃ contents were selected in two regions of South Australia with wheat and barley based dryland cropping systems. Two of the field sites were selected in a single paddock on a commercial farm (Kenton Farm) near the township of Arthurton on Yorke Peninsula (34° 11' S 137° 51' E). Initial site selection was based upon a fizz test (1M HCl) in the field for the presence of carbonates. The fizz test showed that the sites differed in their carbonate content. Field observations were confirmed in the laboratory by measuring the CaCO₃ equivalent (CCE%) (Allison and Moodie 1965) in soils taken from each site. The sites, hereafter referred to as Low CCE and High CCE, had 0.4% and 9.8% CCE, respectively. The two sites on Yorke Peninsula were selected to assess the effect of Ca on net N mineralisation rates.

A third site with very low CaCO₃ content was selected at Turretfield Research Centre near the township of Rosedale (34° 55' S 138° 83' E) in the Barossa Region. The key feature of these three sites was that they differed in their CaCO₃ contents.

#### 2.2.2 Initial soil sampling and soil analysis

Prior to commencing the experiments in winter, the Yorke Peninsula and Turretfield sites had been fallowed through the summer with sheep grazing the stubble from the previous winter wheat crop. Two weeks before establishing the field experiments, 15 soil samples (0-100 cm) were taken in a 10 m x 10 m grid pattern over each site. Deep cores were divided into 6 increments (0-10, 10-20, 20-40, 40-60, 60-80 and 80-100 cm) and assessed for inorganic-N content after air drying the soil at 40°C for 72 h (Fig 2.1). After air drying, soil was passed through a 2 mm sieve.
Figure 2.1. Amount of inorganic N (nitrate-N plus ammonium-N) and the standard deviation at each depth for 6 depth increments prior to sowing at the three field sites (n = 15).
Figure 2.2. Rainfall from the 1 July 1994 to 31 December 1994 as measured at Turretfield Research Centre, Barossa Valley, and Kenton Farm, Yorke Peninsula. Turretfield season and YP season show the length of growing season from sowing to harvest.
and 10 g (air dried weight) was extracted by end over end shaking for 1 h in 100 mL of 2M KCl. The extract (25 mL) was filtered through Whatman No.42 filter paper and shaken with 1 g Chelex 100 resin (Bio-Rad Laboratories Inc.) for 45 minutes to remove Mg$^{2+}$ and Ca$^{2+}$ ions which would have otherwise interfered with the hydrazine sulphate mediated reduction process used for determining nitrate and ammonium by colourmetric analysis (Ananth and Moraghan, 1987; Kempers and Luft, 1988). After shaking the extract was decanted and analysed for nitrate and ammonium (Kempers and Zweers, 1986; Rayment and Higginson, 1992) using an Alpkem auto-analyser with WinFLOW III software (Perstorp Analytical, USA). All inorganic-N results were adjusted for the moisture content of the air-dried soil. Moisture contents for air dried and field moist soil was determined by placing 25 g to 30 g of soil in a pre-weighed plastic container, weighing the soil in the container and placing in a drying oven at 105°C. Soil samples were left in the drying oven until they reached a stable weight. Drying time was about 2 days for air dried soil and 4 days for field moist soil. Soil samples were re-weighed after drying. Moisture content was calculated as follows:

\[
\text{% Moisture Content} = \frac{(\text{wet soil weight} - \text{dry soil weight}) \times 100}{\text{dry soil weight} - \text{container weight}}
\]

General soil characteristics for the three sites were determined from soil samples taken prior to commencing the experiments and before application of any fertiliser for the upcoming season (Table 2.1).

2.2.3 Germination rates

The germination rates of oat and pea seed used in the field experiments were quantified in the laboratory so the seeding rates could be calculated. Two hundred randomly selected seeds of each type were placed on sheets of paper towel moistened with distilled water. The paper towel was loosely wrapped, placed in a plastic bag with about 10 mL of free distilled water and sealed. The sealed plastic bags were placed in an incubator at 30°C. After 3 days incubation, the seeds were removed and the germination rate was determined by counting how many seedling had a
radical at least 1 cm long. The germination rates were 78% for oat and 88% for pea.

Table 2.1. Soil characteristics in the 0 to 10 cm layer prior to field experiments for the two sites on Yorke Peninsula and the site at Turretfield.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Units</th>
<th>Low CCE</th>
<th>High CCE</th>
<th>Turretfield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td></td>
<td>Haploxerafl</td>
<td>Rhodoxerafl</td>
<td>Rhodoxerafl</td>
</tr>
<tr>
<td>Bulk density</td>
<td>Mg m$^{-3}$</td>
<td>1.02</td>
<td>1.00</td>
<td>1.20</td>
</tr>
<tr>
<td>Clay</td>
<td>%</td>
<td>40</td>
<td>24</td>
<td>35</td>
</tr>
<tr>
<td>pH (water)</td>
<td></td>
<td>8.1</td>
<td>8.5</td>
<td>6.9</td>
</tr>
<tr>
<td>Organic carbon</td>
<td>%</td>
<td>1.92</td>
<td>2.26</td>
<td>1.29</td>
</tr>
<tr>
<td>Total N</td>
<td>%</td>
<td>0.159</td>
<td>0.250</td>
<td>0.180</td>
</tr>
<tr>
<td>CCE</td>
<td>%</td>
<td>0.4</td>
<td>9.8</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Extractable P</td>
<td>mg kg$^{-1}$</td>
<td>43</td>
<td>49</td>
<td>63</td>
</tr>
<tr>
<td>Extractable K</td>
<td>mg kg$^{-1}$</td>
<td>660</td>
<td>362</td>
<td>640</td>
</tr>
<tr>
<td>Electrical conductivity</td>
<td>dS m$^{-1}$</td>
<td>0.18</td>
<td>0.18</td>
<td>0.07</td>
</tr>
<tr>
<td>Exchangeable Ca</td>
<td>cmol kg$^{-1}$</td>
<td>30.70</td>
<td>33.70</td>
<td>12.35</td>
</tr>
<tr>
<td>Exchangeable Mg</td>
<td>cmol kg$^{-1}$</td>
<td>4.93</td>
<td>3.34</td>
<td>4.56</td>
</tr>
<tr>
<td>Exchangeable Na</td>
<td>cmol kg$^{-1}$</td>
<td>0.64</td>
<td>0.60</td>
<td>0.45</td>
</tr>
<tr>
<td>Exchangeable K</td>
<td>cmol kg$^{-1}$</td>
<td>2.03</td>
<td>1.16</td>
<td>1.90</td>
</tr>
<tr>
<td>Cation exchange capacity</td>
<td>cmol kg$^{-1}$</td>
<td>38.30</td>
<td>38.80</td>
<td>19.26</td>
</tr>
<tr>
<td>Extractable Cu</td>
<td>mg kg$^{-1}$</td>
<td>2.4</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Extractable Zn</td>
<td>mg kg$^{-1}$</td>
<td>57.1</td>
<td>18.3</td>
<td>20.1</td>
</tr>
<tr>
<td>Extractable Mn</td>
<td>mg kg$^{-1}$</td>
<td>195</td>
<td>12</td>
<td>37</td>
</tr>
<tr>
<td>Extractable Fe</td>
<td>mg kg$^{-1}$</td>
<td>36</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

2.2.4 Experimental design and agronomy

The experiments at each site were arranged in a complete randomised block design with three crop treatments; oat, field pea and fallow. Each treatment had five replicates. All plots were 10 m x 10 m. The experiments at Turretfield and Yorke Peninsula were sown on the 5th and 6th of July 1994, respectively.

Seeding rates were based upon guidelines established for the local regions (Taylor et al., 1991) and anticipated germination rates of 80% and 90% germination for oat and pea, respectively. The oat crop was sown at 70 and 90 kg seed ha\(^{-1}\) and the pea crop was sown at 85 and 110 kg seed ha\(^{-1}\) at Yorke Peninsula and Turretfield, respectively. Based upon these seeding rates the target crop densities were 35 pea plants m\(^{-2}\) and 180 oat plants m\(^{-2}\) at Yorke Peninsula sites and 45 pea plants m\(^{-2}\) and 230 oat plants m\(^{-2}\) at the Turretfield site.

One day prior to sowing, the Turretfield site was harrowed and cross-harrowed to evenly distribute residual wheat straw. Immediately prior to sowing, soil was conventionally cultivated to a depth of 5 cm with one pass at all sites. In the same operation, P was applied at 20 kg P ha\(^{-1}\) as triple superphosphate. Seed was sown at a depth of 5 cm using a 10 row cone seeder with 18 cm row spacings and trailing harrows. Due to the width of the seeder and the plots, each plot was sown in six side by side sections.

Emergence density within each plot was monitored by counting the number of plants in 9 x 1 m rows within each plot at 31 and 42 days after sowing (DAS). Only a maximum of three rows were counted from any one of the six sections within a plot. Throughout the experiment, fallow plots were kept free of vegetation by periodically spraying with glyphosate (Roundup by Monsanto Australia Ltd) applied at 2 L ha\(^{-1}\). Oat were not sprayed for weeds at any time during the experiment.

At maturity, grain and straw yields were determined by harvesting one 1 m\(^2\) area within each plot.
by hand. The remaining grain within each plot was harvested on the 12th and 9th of December 1994 using a plot harvester. Daily rainfall data was recorded at both locations throughout the experiment (Fig 2.2).

2.2.5 In-season soil sampling and net nitrogen mineralisation

The season was divided into three periods for the purpose of measuring rates of net N mineralisation. The length of an in situ incubation period was based upon in-season rainfall. Periods were longer when rainfall was limited as the daily change in inorganic N was expected to be relatively small. Net N mineralisation rates were measured in oat and fallow plots using the method described by Raison et al. (1987) for forest systems and adapted by Stein et al., (1987) for use in cereals. Since pea plots were only included as a preparatory treatment for measuring net N mineralisation in the following season under a cereal, net N mineralisation was not measured in this season.

The methodology consisted of taking three soil samples from the 0-10 cm layer at each sampling time. Two samples were contained in 15 cm length tubes (75 mm diameter) made from polyvinylchloride (PVC) stormwater pipe and the third was taken using a 7.5 cm auger. Based upon inorganic-N results for the deep cores taken prior to sowing, nine random sampling points were installed in each plot. Intensive sampling was deemed necessary to overcome the high variability in inorganic-N at each site. At each sampling point a pair of tubes (15 cm apart) were hammered into the soil to a depth of 10 cm using a one piece core driver especially designed to fit 75 mm diameter tubing. Cores were only placed between plants. After installation, one of the tubes was capped with stormwater capping (L) which had four holes drilled in the sides (3 mm diameter) of the cap at equidistance points. These holes allowed air movement across the soil surface inside the core. The other tube in each pair was left open (NL) to measure the amount of inorganic-N leached from the 0-10 cm layer. Directly after installing each pair of tubes, a soil sample was taken within 30 cm of the pair to a depth of 10 cm using a 75 mm auger. The auger sample (NT) provided a baseline for the commencing sampling period. Net N mineralisation and
leaching were calculated using the following equations from Stein et al. (1987):

\[
\text{Net N mineralisation} = L_{i+1} - NT_i \\
\text{Leaching} = L_i - NL_i
\]

where \(L\) is inorganic N in the capped core and \(NT\) is inorganic N in the auger sample and \(NL\) is inorganic N in the open core.

After sampling, soils were stored at 4°C prior to air-drying at 40°C for 3 days and analysing for nitrate-N and ammonium-N using the same procedure described for the analysis of the initial soil samples.

At the Yorke Peninsula sites, the \textit{in situ} incubation periods for LowCCE were for 28, 30 and 57 days during the season covering the dates 4th August to 1st September, 2nd September to 1st October, and 2nd October to 26th November, respectively. Dates for initiation and termination of incubation periods were one day later for HighCCE. At Turretfield, \textit{in situ} incubation periods were for 28, 29 and 56 days during the season covering the dates 2nd August to 30th August, 31 August to 29 September, and 30th September to 24th November, respectively.

2.2.6 \textbf{Statistical analysis}

Differences in grain yields between sites were analysed using the two sample unpaired t-test procedure in SAS version 6.12 (SAS Institute Inc., 1996). The two sample paired t-test procedure was used to determine if there was any difference in the soil moisture content of samples taken at each site at the three sampling times. The General Linear Model (GLM) procedure in the same statistical program was used to compare the amount of inorganic N in each sample type (\(L\), \(NL\) and \(NT\)), the rates of N mineralisation within the three incubation periods and the overall net N mineralisation rate for fallow and oat treatments within each site. Differences in inorganic N and N mineralisation between sites could not be statistically compared because standard errors differed
between sites by more that two fold (Gomez and Gomez, 1984). Attempts were made to map the variability in inorganic N within each plot. However, the variability was too high to enable a curve to be fitted to the semivariogram that was needed to allow point specific data to be krigged and interpolated for mapping.

2.3 Results

2.3.1 Seasonal characteristics
The long term median annual rainfall for the middle region of Yorke Peninsula (Maitland, 34° 38' S 137° 68' E) and for Turretfield is 485.9 mm and 459.1 mm, and the median in-season rainfall from July to December (inclusive) for the same locations is 254.1 mm and 241.6 mm, respectively (Bureau of Meteorology, 1995). Compared to these long term medians, rainfalls during the season were relatively low. The annual and in-season rainfalls at the sites on Yorke Peninsula were 261 mm and 123.5 mm, respectively. Between sowing and the first emergence density count, 42 mm of rain fell and a further 9.5 mm fell between the first and second emergence density counts. At Turretfield, the annual and in-season rainfalls were 467.9 mm and 156.2 mm, respectively. Between sowing and the first emergence density count, 38.8 mm of rain fell and a further 10 mm fell between the first and second emergence density counts.

2.3.2 Emergence and plant densities
At 31 DAS, seedling emergence density at the Yorke Peninsula sites relative to target densities were 59% and 75% for pea and 63% and 22% for oat at Low CCE and High CCE, respectively (Table 2.2). At Turretfield, emergence densities relative to target densities 31 DAS were 97% and 95% for pea and oat, respectively (Table 2.2). After a further 11 days, the emergence density had increased to 91% and 80% for pea and 73% and 47% for oat at Low CCE and High CCE, respectively (Table 2.2). At 42 DAS, emergence densities at Turretfield were 109% for pea and 95% for oat.
Table 2.2. Emergence density (plants m$^{-2}$) of field pea (cv Alma) and oat (cv Wallaroo) at Yorke Peninsula (Low CCE and High CCE) and Turretfield field experiments as measured by counting 9 x 1 m rows at two times (31 and 42 days after sowing (DAS)).

<table>
<thead>
<tr>
<th></th>
<th>Low CCE</th>
<th>High CCE</th>
<th>Turretfield</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Field pea</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First count</td>
<td>21.5 (17.0)</td>
<td>27.5 (15.7)</td>
<td>45.7 (15.7)</td>
</tr>
<tr>
<td>Second count</td>
<td>33.1 (14.5)</td>
<td>29.1 (10.4)</td>
<td>51.2 (12.0)</td>
</tr>
<tr>
<td><strong>Oat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First count</td>
<td>113.5 (37.3)</td>
<td>39.4 (40.0)</td>
<td>22.2 (17.9)</td>
</tr>
<tr>
<td>Second count</td>
<td>131.9 (12.5)</td>
<td>84.4 (12.5)</td>
<td>221.7 (15.5)</td>
</tr>
</tbody>
</table>

* co-efficients of variation are given in parentheses.

Plant densities at harvest on Yorke Peninsula were 93% and 96% for pea (Table 2.3) and 79% and 48% for oat (Table 2.4) at Low CCE and High CCE, respectively. At harvest at Turretfield, plant densities had fallen to 73% and 49% of the target densities for pea and oat, respectively (Tables 2.3 and 2.4). Pea plants at both Yorke Peninsula sites were visibly water stressed in the first few weeks of the season as indicated by purpling on the edges of the leaves.

2.3.3 *Straw and grain yields and yield components at Yorke Peninsula*

Yield components showed that pea plants grown at Low CCE were smaller and produced fewer pods and less grain than plants grown at High CCE. This resulted in pea plants grown at Low CCE attaining lower grain and straw yields than pea plants at High CCE ($P = 0.320$).

Oat grain yield at Low CCE were 9.3% lower than grain yield at High CCE, however, the plant density was higher at Low CCE as indicated by the crops’ emergence density at 42 DAS. The higher plant density at Low CCE was negated by plants producing fewer tillers, heads, spikelets and grain per plant than oat plants grown at High CCE. Viable grains from Low CCE also had a
slightly lower grain weight than viable grains from High CCE.

Table 2.3. Grain and straw yields (kg ha⁻¹), number of plants m⁻² and yield components (plant⁻¹) of field pea (cv Alma) at Yorke Peninsula (Low CCE and High CCE) and Turretfield field experiments as measured after harvesting by hand on 1st and 6th of December, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Low CCE</th>
<th>High CCE</th>
<th>Turretfield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain yield</td>
<td>1008 (21.1)</td>
<td>1533 (14.6)</td>
<td>1352 (19.6)</td>
</tr>
<tr>
<td>Straw yield</td>
<td>1448 (23.3)</td>
<td>1888 (18.6)</td>
<td>1736 (14.0)</td>
</tr>
<tr>
<td>Harvest index</td>
<td>0.44 (23.2)</td>
<td>0.45 (8.7)</td>
<td>0.44 (19.3)</td>
</tr>
<tr>
<td>Number of plants</td>
<td>33.7 (13.2)</td>
<td>34.9 (16.7)</td>
<td>34.3 (13.5)</td>
</tr>
<tr>
<td>Pods</td>
<td>4.43 (22.1)</td>
<td>5.55 (17.9)</td>
<td>5.15 (18.1)</td>
</tr>
<tr>
<td>Viable grains</td>
<td>13.97 (26.9)</td>
<td>18.45 (15.4)</td>
<td>16.70 (14.7)</td>
</tr>
<tr>
<td>Total grains</td>
<td>13.97 (26.9)</td>
<td>18.45 (15.4)</td>
<td>20.03 (16.2)</td>
</tr>
<tr>
<td>100 grain wt</td>
<td>21.78 (12.3)</td>
<td>23.73 (4.9)</td>
<td>20.23 (9.3)</td>
</tr>
</tbody>
</table>

* Co-efficients of variation are given in parentheses.

2.3.4 Straw and grain yields and yield components at Turretfield

Pea grain and straw yields at Turretfield was between the two yields attained at Yorke Peninsula despite the higher seeding density and emergence density at 42 DAS. Only 83.4% of pea seed was viable due to the seeds being infested by pea weevils (Bruchus pisorum L.).

Oat grain yield was also lower at Turretfield than the Yorke Peninsula sites despite the higher seeding density and emergence density at 42 DAS.
Table 2.4. Grain and straw yields (kg ha\(^{-1}\)), number of plants m\(^2\) and yield components (plant\(^{-1}\)) of oat (cv Wallaroo) at Yorke Peninsula (Low CCE and High CCE) and Turretfield field experiments as measured after harvesting by hand on 1\(^{st}\) and 6\(^{th}\) of December, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Low CCE</th>
<th>High CCE</th>
<th>Turretfield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain yield</td>
<td>1603 (*12.7)</td>
<td>1768 (25.2)</td>
<td>1139 (18.0)</td>
</tr>
<tr>
<td>Straw yield</td>
<td>1939 (11.6)</td>
<td>1560 (22.1)</td>
<td>2178 (24.1)</td>
</tr>
<tr>
<td>Harvest index</td>
<td>0.45 (8.4)</td>
<td>0.53 (2.2)</td>
<td>0.35 (19.7)</td>
</tr>
<tr>
<td>Number of plants</td>
<td>142.3 (19.6)</td>
<td>86.6 (35.4)</td>
<td>113.6 (17.6)</td>
</tr>
<tr>
<td>Tillers</td>
<td>1.69 (15.3)</td>
<td>2.03 (18.9)</td>
<td>4.06 (20.1)</td>
</tr>
<tr>
<td>Heads</td>
<td>1.67 (15.4)</td>
<td>1.97 (19.5)</td>
<td>1.57 (15.1)</td>
</tr>
<tr>
<td>Spiklets</td>
<td>20.07 (7.1)</td>
<td>26.97 (23.4)</td>
<td>21.42 (17.0)</td>
</tr>
<tr>
<td>Viable grains</td>
<td>40.78 (12.4)</td>
<td>66.10 (25.9)</td>
<td>43.91 (18.2)</td>
</tr>
<tr>
<td>1000 grain wt</td>
<td>27.71 (4.3)</td>
<td>30.91 (1.3)</td>
<td>22.85 (3.5)</td>
</tr>
</tbody>
</table>

* co-efficients of variation are given in parentheses.

2.3.5 Soil moisture inside and outside cores

At some sampling times and sites there were significant differences in the soil moisture contents from within uncapped cores and from neighbouring unconfined soil (Table 2.5). At Turretfield, the soil moisture content of soil in uncapped cores was significantly higher (\(P < 0.05\)) than soil from neighbouring unconfined soil only at the first sampling time. At Low CCE, soil from uncapped cores had a significantly higher moisture content (\(P < 0.05\)) than unconfined soil at the second sampling time and a significantly lower soil moisture content at the third sampling time. At High CCE, the soil moisture content was significantly higher in uncapped cores (\(P < 0.05\)) than neighbouring unconfined soil at only the second sampling time.
Table 2.5. Average moisture content (%) of soil in the 0 to 10 cm layer at Yorke Peninsula (Low CCE and High CCE) and at Turretfield, at three sampling times during the growing season and by three soil sampling types (L, UL and NT).

<table>
<thead>
<tr>
<th>Sample time and type</th>
<th>Low CCE</th>
<th>High CCE</th>
<th>Turretfield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>L₁</td>
<td>26.8 (2.7)</td>
<td>23.5 (1.0)</td>
<td>13.5 (2.0)</td>
</tr>
<tr>
<td>UL₁</td>
<td>19.2 (3.8)</td>
<td>18.7 (1.8)</td>
<td>11.5 (2.4)</td>
</tr>
<tr>
<td>NT₁</td>
<td>19.9 (2.0)</td>
<td>19.6 (1.7)</td>
<td>9.8 (1.9)</td>
</tr>
<tr>
<td>L₂</td>
<td>20.9 (3.2)</td>
<td>20.3 (1.5)</td>
<td>9.2 (2.1)</td>
</tr>
<tr>
<td>UL₂</td>
<td>20.2 (3.9)</td>
<td>19.9 (2.2)</td>
<td>8.4 (2.8)</td>
</tr>
<tr>
<td>NT₂</td>
<td>17.7 (1.7)</td>
<td>18.3 (1.3)</td>
<td>7.4 (1.7)</td>
</tr>
<tr>
<td>L₃</td>
<td>14.5 (2.3)</td>
<td>14.3 (2.8)</td>
<td>8.0 (2.2)</td>
</tr>
<tr>
<td>UL₃</td>
<td>10.3 (1.0)</td>
<td>10.1 (1.9)</td>
<td>5.2 (0.8)</td>
</tr>
<tr>
<td>NT₃</td>
<td>10.9 (1.9)</td>
<td>10.5 (2.6)</td>
<td>5.1 (1.4)</td>
</tr>
</tbody>
</table>

* standard deviations are given in parentheses.

2.3.6 Effect of crop treatment on net nitrogen mineralisation rates

2.3.6.1 Within each site

Of the four occasions inorganic N was measured within each plot at each site, eight, three and four sampling times at Low CCE, High CCE and Turretfield, respectively, showed that the amount of inorganic N present under oat was significantly less than in fallowed soil (Table 2.6). However, these differences did not tend to translate into differences in net N mineralisation rates either within each incubation period or over the whole experiment (Fig 2.3). Only in the first incubation period at High CCE were net N mineralisation rates different between crop treatments. The lack of difference in net N mineralisation rates was primarily due to high variability between replicates at each site as shown in Figure 2.3. This stemmed from high coefficients of variation for inorganic N measured at each sampling time. Coefficients of variation ranged between 19% and 240% at
Low CCE, 13% and 112% at High CCE, and 44% and 637% at Turretfield. Despite the high variability, one constant for the three sites was that the net effect of changes in the form of N was mineralisation in all incubation periods and both treatments except the second period under fallow at Turretfield. Following on from net changes in N form within incubation periods, the overall effect was net N mineralisation at all sites.

Table 2.6. Difference between the amount of inorganic N g soil$^{-1}$ under fallow and oat treatments (Fallow minus Oat) in Low CCE, High CCE and Turretfield at all sampling times.

<table>
<thead>
<tr>
<th></th>
<th>Low CCE</th>
<th>High CCE</th>
<th>Turretfield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μg inorganic N g soil$^{-1}$</td>
<td>μg inorganic N g soil$^{-1}$</td>
<td>μg inorganic N g soil$^{-1}$</td>
</tr>
<tr>
<td>Initial N</td>
<td>-4.73 ns</td>
<td>5.87 s</td>
<td>-1.27 ns</td>
</tr>
<tr>
<td>First L</td>
<td>4.15 s</td>
<td>-2.47 ns</td>
<td>-3.85 ns</td>
</tr>
<tr>
<td>First NL</td>
<td>4.51 ns</td>
<td>-0.84 ns</td>
<td>10.11 ns</td>
</tr>
<tr>
<td>First NT</td>
<td>5.35 s</td>
<td>0.70 ns</td>
<td>15.42 s</td>
</tr>
<tr>
<td>Second L</td>
<td>3.66 s</td>
<td>0.10 ns</td>
<td>4.26 ns</td>
</tr>
<tr>
<td>Second NL</td>
<td>4.51 s</td>
<td>-1.72 ns</td>
<td>4.89 ns</td>
</tr>
<tr>
<td>Second NT</td>
<td>6.10 s</td>
<td>3.54 ns</td>
<td>15.56 s</td>
</tr>
<tr>
<td>Third L</td>
<td>13.94 s</td>
<td>11.84 s</td>
<td>38.43 s</td>
</tr>
<tr>
<td>Third NL</td>
<td>15.80 s</td>
<td>12.70 ns</td>
<td>31.29 ns</td>
</tr>
<tr>
<td>Third NT</td>
<td>13.27 s</td>
<td>12.57 s</td>
<td>33.29 s</td>
</tr>
</tbody>
</table>

s = significant difference in the amount of inorganic N in soil under the two crop treatments, ns = non-significant difference in the amount of inorganic N in soil under the two crop treatments

2.3.6.2 Between sites

In the first incubation period, net N mineralisation rates varied between the Yorke Peninsula sites. The mineralisation rate under oat at Low CCE was only one quarter the rate under oat at High
CCE. Under fallow at Low CCE the rates were more than double at High CCE. However, during the second and third incubation periods, the differences between sites were smaller. Over the whole cropping period, the net N mineralisation rates at Yorke Peninsula were 0.28 and 0.20 μg inorganic N g⁻¹ soil day⁻¹ under fallow and 0.15 and 0.20 μg inorganic N g⁻¹ soil day⁻¹ under oat at Low CCE and High CCE, respectively. Using the bulk density (0-10 cm) at each site, these net N mineralisation rates were translated into the cumulative amount of inorganic N that became available to plants during the three incubation periods. At Yorke Peninsula, the increase in inorganic N was 33 and 23 kg inorganic N ha⁻¹ under fallow and 18 and 23 kg inorganic N ha⁻¹ under oats at Low CCE and High CCE, respectively.

Net N mineralisation rates at Turretfield were similar to the rates at Low CCE for fallow treatment and similar High CCE for oat treatment during the first incubation period. Net N mineralisation rates were comparatively low in the second incubation period and high in the third incubation period, especially under the fallow treatment. However, over the whole cropping period, these fluctuations at Turretfield tended to counteract each other such that net N mineralisation rates were slightly higher than rates measured at Yorke Peninsula. Net N mineralisation rates at Turretfield were 0.34 and 0.26 μg inorganic N g⁻¹ soil day⁻¹ under fallow and oat, respectively. Higher net N mineralisation rates at Turretfield were accentuated by the site's relatively high bulk density such that the amount of inorganic N which became available to plants over the incubation periods was at least 39% higher at Turretfield than Yorke Peninsula. Net N mineralisation rates at Turretfield translated to 46 and 35 kg inorganic N ha⁻¹ becoming available to plants during the three incubation periods under fallow and oats, respectively.

2.3.7 Effect of crop treatment on inorganic nitrogen loss through leaching

2.3.7.1 Within sites

At all three sites the amount of inorganic N leached from the 0 – 10cm layer each day approached zero (Fig 2.4). Only in the initial incubation period at Turretfield were net N leaching
Figure 2.3. Net inorganic N mineralisation rate for three periods (Min1, Min2 and Min3) and the overall net inorganic N mineralisation rate (MinT) during the 1994 growing season under two treatments (Oat and Fallow) at three sites in South Australia. Error bar represent standard deviation.
rates significantly different ($P < 0.01$) between crop treatments (Fig 2.4). As with the variability in net N mineralisation rates, the lack of difference in net N leaching rates was primarily due to the amount of inorganic N leached being very low and high variability between replicates at each site which stemmed from high coefficients of variation for inorganic N measured at each sampling time.

Unlike net N mineralisation, there was no consistent trend towards either upward or downward movement of inorganic N in the sampled soil layer within incubation periods. This lack of consistency was due to only very small amounts of inorganic N moving either up or down the soil profile on a daily basis.

### 2.3.7.2 Between sites

Over the whole cropping period, the net inorganic N leaching rates at Yorke Peninsula were 0.02 and 0.05 μg inorganic N g soil$^{-1}$ day$^{-1}$ under fallow and 0.01 μg inorganic N g soil$^{-1}$ day$^{-1}$ under oat at Low CCE and High CCE, respectively. Net inorganic N leaching rates at Turretfield were 0.03 and 0.09 μg inorganic N g soil$^{-1}$ day$^{-1}$ under fallow and oat, respectively. At High CCE, leaching rates translated into small losses from the 0-10 cm layer of < 1 kg inorganic N ha$^{-1}$ under fallow and 1.3 kg inorganic N ha$^{-1}$ under oats. Inorganic-N loss through leaching at Low CCE was also minimal at 1.9 kg inorganic N ha$^{-1}$ under fallow and 5.7 kg inorganic N ha$^{-1}$ under oats. The amount of inorganic N leached at Turretfield was 3.7 kg inorganic N ha$^{-1}$ under fallow and 12.9 kg inorganic N ha$^{-1}$ under oats.

After accounting for any leached inorganic N, the amounts of inorganic N that became available in the 0-10 cm layer over the season under the fallow treatments were 31 kg inorganic N ha$^{-1}$ at Low CCE, 22 kg inorganic N ha$^{-1}$ at High CCE and 42 kg inorganic N ha$^{-1}$ at Turretfield. Similarly, the amounts of inorganic N that became available under the oat treatments were
Figure 2.4. Net inorganic N leaching rate for three periods (Lch1, Lch2 and Lch3) and the overall net inorganic N leaching rate (LchT) during the 1994 growing season under two treatments (Oat and Fallow) at three sites in South Australia. Error bar represents standard deviation.
12 kg inorganic N ha\(^{-1}\) at Low CCE, 22 kg inorganic N ha\(^{-1}\) at High CCE and 23 kg inorganic N ha\(^{-1}\) at Turretfield.

2.4 Discussion

2.4.1 Crop growth

Low plant densities for oat relative to expected target density was due to seed viability being less than expected based upon the germination rates determined prior to sowing. Particularly low plant densities for oat sown at High CCE may have been due to seed being sown too deep (> 6 cm) in the soil. The reduction in plant densities during the season at Turretfield are attributed to the low rainfall (107.4 mm) after 42 DAS causing some plants to die prior to harvest. Although this amount of rain, was higher than that which fell after 42 DAS at Yorke Peninsula (72 mm), the seeding rate and plant density was also higher hence the crop as a whole had a higher demand for water. The low oat grain yields (less than 2 t ha\(^{-1}\)) reflect the relatively low in-season rainfall.

Grain yields for pea and oat were low due to no N being applied as a fertiliser and relatively low in-season rainfall. The difference in pea grain yields between Yorke Peninsula sites may have been because Low CCE had a higher clay content than High CCE and therefore a greater proportion of water in the soil was unavailable to plants grown at Low CCE. Less available water may have resulted in plants grown at Low CCE being more water stressed throughout the season than plants grown at High CCE despite the same amount of rainfall occurring at both sites. As with pea plants at Low CCE, lower values for the oat yield components at Low CCE may also have been due to the site having a higher clay content and therefore less available water.

As with the crops grown on Yorke Peninsula, grain yields from pea and oat were limited at Turretfield due to lack of N fertiliser and relatively low in-season rainfall. Comparable oat and pea grain yields between the two locations were due to the number of plants per hectare at Turretfield being less at harvest than at the second emergence density count and comparable with the plant...
densities at harvest at the Yorke Peninsula sites. The reduced plant density of oat at Turretfield was compensated by each plant producing at least double the number of tillers produced by oat plants at the Yorke Peninsula sites. However, the relatively high number of tillers did not translate into higher yields primarily due to low numbers of heads per plants and low seed weights. This indicates that water and nitrogen was limiting during the heading and grain filling stages.

2.4.2 Differing soil moisture contents
Every method for measuring net N mineralisation has advantages and disadvantages. Differences in soil moisture contents inside and outside of soil cores are considered to be the inherent shortfall of in situ methods that use confined soil (Adams et al 1989; Rees et al 1994). Therefore finding significant differences in soil moisture content between confined and uncapped cores about half of the time, as occurred in this research, is not surprising. Adams et al (1989) found no difference in the moisture content of two forest soil samples from unconfined soil and soil contained within perforated cores. However, perforated soil cores were unlikely to be suitable for use in annual cropping situations since crop roots would enter the cores and extract inorganic N.

2.4.3 Effect of variability
High variability of inorganic N, and subsequently net N mineralisation, within a site is not uncommon in experiments where few samples are collected from a large area (Raison et al., 1987; Sierra, 1996, 1997; Stoyan et al., 2000). For instance the amounts of nitrate recovered from in situ studies in a Mollisol had coefficients of variation between 28% and 234% with 25 replicates (Sierra, 1996). Similarly, an in situ N mineralisation study in a podzolic soil required 24 cores in an area of 0.25 ha to reduce the standard error to a range of 10% to 15% (Raison et al., 1987). This limitation was recognised prior to field experiments at Turretfield and Yorke Peninsula hence the sampling density was relatively high. However, unlike an experiment in a forest system (O'Connell and Rance, 1999) where 9 samples were taken in a 15 m x 15 m area, intense sampling did not reduce the variability to a manageable level. Adams et al. (1989) suggested that
high variability was due to roots cut upon insertion of cores into the soil becoming a source of mineralisable N. However, this appears to be an unlikely explanation since in forest soils, mineralisation of cut roots was found to have no effect on overall net N mineralisation rates (Raison et al., 1987).

High variability, in terms of co-efficient of variation, in this experiment was an artefact of the net N mineralisation values being small. This has previously been recognised in other N mineralisation studies using in situ and intact cores as the reason why variability was high and had wide ranging values (Sierra, 1996). High variability in inorganic N was also simply an artefact of small amounts of inorganic N being present in the soil (Frazer et al., 1990). The actual range of change in inorganic N for a treatment with a coefficient of variation of 1216% was -1.41 to 1.25 µg inorganic N mg⁻¹ total initial N.

2.4.4 Effect of season

Low rainfall at both sites relative to long term averages showed that the season was unusually dry. Seasonal constraints were evident by the average oat grain yields attained by farmers across South Australia being only 82% of the average over the previous 8 seasons (Australian Bureau of Statistics, 1995). Compared to the state-wide average grain yield attained by farmers in the same season (0.93 t ha⁻¹), oat grain yield were high on the Yorke Peninsula and moderate at Turretfield. Grain yield averages were not available for field peas and they are considered to be a minor crop.

A dry season would have lowered the moisture content of the soil and therefore reduced mineralisation rates (Miller and Johnson, 1964). Subsequently, net N mineralisation rates measured during the season were probably lower than would be expected during a season with median rainfall. Seasonal differences in net N mineralisation rates due to variation in soil moisture content and temperature have been reported for three forest soil in Western Australia (O’Connell and Rance, 1999). Annual rates of net N mineralisation in the forest soils ranged from 69 to 113,
72 to 81 and 79 to 84 kg inorganic N ha\(^{-1}\) year\(^{-1}\) for the three sites. Seasonal variation may partially explain why the amount of inorganic N that became available under oat at Turretfield was less than half the amount that became available under wheat a comparable site at Tarlee, South Australia, during the 1991 cropping season (Ladd et al., 1994). During the experiment at Tarlee on a calcic Rhodoxeralf, net N mineralisation was measured over 112 days using a similar technique to that used at Turretfield and leaching was prohibited by sealing the bottom of the tubes. Without N fertiliser, 55 kg inorganic N ha\(^{-1}\) became available in the continuous wheat treatment (WW) and 72 kg inorganic N ha\(^{-1}\) became available in the wheat – sown pasture rotation (WSP).

2.4.5 Extrapulating within a soil group

Despite high variability prohibiting statistical differences to be drawn between site, it was possible to compare the experimental sites and sites used in earlier studies in Xeralfs (Ladd et al., 1994). Given the differences in net N mineralisation rates between the sites at Yorke Peninsula, Turretfield and Tarlee, directly extrapolating from one site to another would be invalid. The inability to extrapolate was not unexpected give that the amount of potentially mineralisable N for 123 sites throughout the wheat and barley cropping areas of South Australia ranged from 15 to 120 kg N ha\(^{-1}\) (Xu et al., 1996).

Some models have shown that extrapolations can be made between some sites due to OC% being positively related to net N mineralisation rate. For instance, in the long term rotation experiment at Tarlee, higher net N mineralisation rates were considered to reflect higher OC% (Ladd et al., 1994). Also, a net mineralisation model based upon laboratory incubated soil from the 123 sites in South Australia (Xu et al., 1996) found that OC% was positively and linearly related to percentage net N mineralisation rates. However, difference between Tarlee and Turretfield could not be attributed to OC% since WW at Tarlee had 0.96% OC and Turretfield had 1.29 OC%. The concept that higher OC concentration leads to higher net N mineralisation rates was also contrary to the rates measured at Yorke Peninsula and Turretfield where the Yorke Peninsula
Peninsula sites had lower net mineralisation rates but higher OC% than Turretfield. Differences between sites were also unlikely to be due to pH since Xu et al. (1996) found that the pH (range: 5.0 to 8.9) of the 123 soils assessed did not significantly influence the percentage net N mineralisation rate in the 0-10 cm layer. In addition, for the comparison between Tarlee and Turretfield, the sites’ pH were similar at 6.9 and 6.8 (1:5 H2O extraction), respectively.

These unexplained differences between Turretfield and Tarlee indicated that extrapolating between sites and seasons, even within the same soil group in South Australia, would be invalid. For the sites at Yorke Peninsula, extrapolating from other sites would be particularly difficult because the relatively low net N mineralisation rates completely contradicted expectations based upon the principle that OC% is positively related to the amount of mineralisable N.

2.4.6 Effect of Calcium

Low net N mineralisation rates on Yorke Peninsula compared to soils at Tarlee and Turretfield supported the concept that naturally occurring calcium may decrease net N mineralisation rates as found in Urrbrae sandy loam amended with calcium (Muneer and Oades, 1989a, 1989b). However, comparing the Low CCE and High CCE sites at Yorke Peninsula showed that there was little difference between the two sites despite the latter site having almost 10 times the amount of CCE. Indeed, under the oat crop, net N mineralisation was higher in soil at High CCE than Low CCE. This contradiction may have been due to net N mineralisation rates being limited by the dry season thus making differences difficult to detect.

Alternatively, lack of difference may have been because although CCE differed between sites, exchangeable Ca was very similar (Table 2.1). Thus free Ca was equally available at both sites to form Ca-OM bridges and protect OM from microbial attack and therefore reduce mineralisation. In addition, relatively low net N mineralisation may have occurred because the quality of OM was poor compared to OM at Turretfield and Tarlee. That is, OM at Yorke Peninsula may have been composed of less readily decomposable material such as carbohydrates and more recalcitrant...
OM such as charcoal. Higher amounts of charcoal at Yorke Peninsula than Turretfield is a possibly since burning cereal residues is commonly practiced in the former region but not the later.

2.5 Conclusions

The conclusion from this research was that the field experiments measured net N mineralisation rates that were too spatially variable to allow any statistical differences to be detected between treatments within sites or between sites. Further, net N mineralisation rates measured at one site could not be applied to other sites even if the climate and cultural practices were similar.

2.6 Implications for future experiments

The results from the field experiment had four major implications for subsequent experiments:

• Having nine sampling points did not reduce variability in inorganic N or net N mineralisation rates to the point that statistical differences could be found between treatments or sites despite large differences. This indicated that measuring net N mineralisation in situ in these soils was not a practical option. Thus all other experiments on these soils were conducted under laboratory conditions to overcome field variability and seasonal influences. Soils were also amended with readily available N and C sources to overcome high variability due to low net N mineralisation rates. Laboratory experiments also have the advantage of allowing C mineralisation to be frequently assessed throughout the experiment using non-destructive sampling techniques to measure CO₂-C release from the soil. Measuring CO₂ evolution provides an indicator for gross N mineralisation since there is a direct relationship between the amount of CO₂ evolved from a soil and the change in the amount of inorganic N present (Miller and Johnson, 1964).

• Given all experiments needed to be conducted in laboratory conditions, the question becomes "What laboratory technique are most representative of field conditions?"
Although not shown statistically, net N mineralisation rates and inorganic N availability on Yorke Peninsula appeared to be lower than at Turretfield. The reason for this difference was not explained in the field experiments. Was the difference due to climatic conditions or soil properties such as the presence of relatively high amounts of exchangeable Ca or a high proportion of recalcitrant OM?

These questions were answered in subsequent laboratory experiments where SOM dynamics were assessed through measuring C and N mineralisation.
Assessing the validity of using alkali traps as a means of measuring carbon mineralisation in calcareous soil

3.1 Introduction

Carbon mineralisation in soil is often measured by collecting the CO₂ respired by microbes in an aqueous alkali solution such as NaOH or KOH (van Schreven, 1967; Muneer and Oades, 1989a; van Gestel et al., 1991; Franzluebbers, 1999). The alkali solution technique (alias alkali trap) can be used to measure mineralisation of total organic carbon, that is soil organic carbon plus substrate-C, or mineralisation of labelled substrate-C only.

Using the alkali trap technique to measure total C mineralisation relies upon two key assumptions. Firstly, the technique assumes that all CO₂ collected in the trap was derived from microbial respiration and not from any other source within the soil. Another C source that is common in many alkaline soils is carbonate-C. Under acidifying conditions, carbonates can be converted to CO₂ and released from the soil (Anderson, 1982). The release of carbonate derived CO₂ leads to an overestimation of total C mineralisation. Measuring C mineralisation of a labelled C substrate rather than mineralisation of total OC can partially circumvent invalidation of this first assumption.

The second assumption is that all mineralised C is released from the soil. That is, no respired CO₂ is converted to bicarbonates or carbonic acid and retained in the soil solution, or precipitated as carbonates. This second assumption may be invalid in alkaline (pH < 7) soils (Anderson, 1982) with the two-way reaction (Equation 1) shifting to the right due to lack of H⁺ ions.

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^- \leftrightarrow \text{H}^+ + \text{CO}_3^{2-}
\]  

(Equation 1)
Validating these assumptions prior to conducting detailed C mineralisation studies with alkaline soils was suggested to be a logical procedure (Guerin, 1999). However, few C mineralisation studies that used calcareous soils with unlabelled substrates reported a validation component (Ladd et al., 1977b; Knaebel and Vestal, 1988; Guerin, 1999) or made mention that some CO₂ may have been derived from carbonates (van Schreven, 1967; Wolters, 1991; Howard and Howard, 1993). Alternatively, some biodegradation studies in alkaline soils have acidified the soil after incubation to release all CO₂ derived from labelled substrates (Pignatello, 1987; Watwood et al., 1991). However, acidification is unsuitable where the aim is to measure the amount of C mineralised from natural unlabelled SOC. This preliminary experiment was designed to test whether measuring C mineralisation by capturing \(^{14}\)CO₂ and total CO₂ in alkali traps was a method which did not breach the two given assumptions in two soils with pH > 7 and carbonates.

3.2 Materials and methods

3.2.1 Soil preparation

Two surface soils (0-10 cm) with different calcium carbonate contents were collected from a commercial farm near Arthurton, Yorke Peninsula, and used in this incubation. General soil characteristics of the sites are given in Chapter 2 and particular characteristics of the soil samples used in this incubation are given in Table 3.1. The CCE in the Low CCE soil was less than 1% and for most studies the soil would be considered to be non-calcareous since no visible bubbles of CO₂ were released when the soil was subjected to 1M HCl. On the other hand, the High CCE contained 9% CCE which was highly reactive with 1M HCl. Given the low amount of carbonates in Low CCE these two soils were used to determine if carbonate-C was recovered from High CCE in alkali traps thus breaching the first assumption previously mentioned.

At both sites, the previous crop was barley that was harvested in November. Soil samples were taken in mid April in standing stubble. At each site, 20 soil cores were collected from the 0-10 cm layer within a 20 m x 20 m area using an auger (75 mm diameter). Samples from each site were
bulked, mixed and passed through a 2 mm sieve. Stones and large pieces of organic residues were removed. Sieved samples were stored field moist in plastic bags at 4°C for two weeks.

Table 3.1. Characteristics of the soil samples used in the 216 h incubation.

<table>
<thead>
<tr>
<th></th>
<th>Unit</th>
<th>Low CCE</th>
<th>High CCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density</td>
<td>Mg m⁻³</td>
<td>1.06</td>
<td>1.07</td>
</tr>
<tr>
<td>OC</td>
<td>%</td>
<td>2.33</td>
<td>2.49</td>
</tr>
<tr>
<td>CCE</td>
<td>%</td>
<td>0.4</td>
<td>9.1</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>8.1</td>
<td>8.5</td>
</tr>
<tr>
<td>Exchangeable Ca</td>
<td>cmol kg⁻¹</td>
<td>31</td>
<td>34</td>
</tr>
<tr>
<td>Moisture content</td>
<td>%</td>
<td>10.3</td>
<td>10.8</td>
</tr>
</tbody>
</table>

A Determined by Walkley-Black Method (Walkley and Black, 1934), B Calcium carbonate equivalent (CCE) (Allison and Moodie, 1965), C pH (H₂O 5:1), D Determined by extraction in 1M Ammonium chloride at pH 7.0 (Rayment and Higginson, 1992), E Gravimetric moisture content determined by drying at 105°C.

3.2.2 ¹⁴Carbon labelled straw extract

Labelled C was used to determine if CO₂ was retained in these two alkaline soils. Five grams of ¹⁴C labelled wheat straw was mixed with 250 mL of warm RO water and shaken by hand for 15 minutes. The solution was filtered through a 0.2 μm disposable filter (Millipore Corporation, USA) and the filtrate collected. The number of counts in the filtrate was measured using a scintillation counter operating at 88% efficiency. Scintillation vials were prepared by adding 0.1 mL of extract to 9.9 mL of scintillation cocktail (Optiphase Hisafe 3, Fisons Chemicals, England) and briefly shaking the vials by hand to mix the two solutions. Prepared vials were left to equilibrate in the dark for 16 h prior to counting. The results obtained in counts per minute (cpm) were corrected for background radiation and counter efficiency and were expressed as disintegrations per minute.
3.2.3 Experimental design and incubation

The experiment was arranged as a complete factorial randomised block design with two soils incubated for three periods with four replicates. Twelve x 45 g (dry weight equivalent) of each sieved soil were placed loosely in 70 mL plastic sampling jars. The soil samples were brought to 50% WFPS and inoculated with $^{14}$C (3159 dpm mL$^{-1}$) by adding 9 mL of straw filtrate to each sample. Immediately after inoculation, each soil sample was sealed in a 1 L glass jar that contained 10 mL of 2N NaOH. Each jar had a septum in the lid. Two sealed jars containing only the alkali traps were also incubated to measured absorption of background CO$_2$ in the jars. Samples and blanks were incubated in a constant temperature room at 25°C throughout the experiment.

3.2.4 Carbon dioxide sampling and analysis

Alkali traps in all sample and blank vessels were changed after three incubation times; 48, 120 and 216 h after commencement of the incubation. Immediately after each trap was removed a fresh alkali trap containing 10 mL of 2N NaOH was introduced and the jar was sealed. After removing the first alkali trap and sealing the jar, a needle attached to a syringe containing 0.5N H$_2$SO$_4$ was inserted into the jar through the septum. Volumes of 2.5 mL and 7.5 mL of acid were slowly added to each sample of Low CCE and High CCE, respectively. This action acidified the soils to pH < 6 and promoted the conversion of CO$_3^{2-}$ to CO$_2$. After a further 20 minutes the fresh (second) alkali trap was removed and a third new alkali trap (10 mL of 2N NaOH) was sealed into the jar to collect any additional CO$_2$ evolved due to the addition of acid. This second fresh alkali trap was removed after 72 h after the first incubation period (48 h), 96 h after the second period (120 h) and 72 h after the third period (216 h).

Immediately after each sampling session, the traps were analysed for $^{14}$C using a scintillation counter. Scintillation vials were prepared by adding 2 mL of 2N NaOH to 8 mL of scintillation
cocktail and briefing shaking the vials by hand to mix the two solutions. Prepared vials were left to equilibrate in the dark for at least four hours prior to counting. The results obtained were expressed in dpm after correcting for background radiation, counter efficiency (88%) and the total volume of the alkali trap (10 mL).

After collection, alkali traps were stored at room temperature in a CO₂-free dessicator and total CO₂-C was determined for all traps at the end of the incubation trial. Total CO₂-C was determined by titration using a TTT85 titrator, an ABU80 autoburette and a TTA60 titration assembly (Radiometer, Copenhagen), and a 645 Multidosmat (Metrohm) filled with 0.0511M HCl. The volume of the trap used in the titration varied from 200µl to 1000µl depending upon the amount of CO₂ trapped. Results were corrected for background CO₂-C using the blanks from each corresponding incubation period and adjusted to give the total CO₂-C present in each alkali trap.

3.2.5 Statistical analysis
Prior to adding acid, recovery of ¹⁴C (dpm) was expressed as a cumulative total and as a rate relative to the incubation period (h). Cumulative CO₂-C recovery was expressed relative to initial SOC and as a rate relative to the incubation period (h). After adding acid, ¹⁴C and CO₂-C recovery were expressed as the amount of C recovered within each incubation increment as a total and as a rate relative to the incubation period (h).

Differences in ¹⁴C and CO₂-C recovery within each soil over the three sampling times was assessed by analysis of variance (ANOVA) after logarithmically transforming the data. Although soil samples, were randomised during the incubation period, results were not assessed for soil x time interactions because standard deviations differed between soils more than two-fold even when values were logarithmically transformed. These large differences in standard deviations invalidated the use of ANOVA as a statistical means of comparing values. Instead, soils were compared by t-test with consideration given to whether the standard deviation between soils differed or was the same. For the t-test, standard deviation was deemed different at P < 0.05. All
statistical analyses were performed using the package SAS version 6.12 (SAS Institute Inc., 1996). Values were considered significantly different at $P < 0.05$.

3.3 Results

3.3.1 Recovery of respired carbon dioxide-C

The initial alkali trap removed from each incubation chamber measured the amount of $^{14}$C and total unlabelled C respired by microbes and released from the soil as CO$_2$-C. In both soils, cumulative release of $^{14}$CO$_2$-C increased with time ($P < 0.001$) (Table 3.2) and the rate of $^{14}$C release decreased over time ($P < 0.001$) as $^{14}$C derived from the labelled substrate became more scarce. There was no difference between replicates for either Low CCE ($P = 0.868$) or High CCE ($P = 0.361$). Overall, neither the cumulative amount of $^{14}$CO$_2$-C released ($P = 0.239$) nor the rate of $^{14}$C release ($P = 0.526$) differed with soil type. Average cumulative amounts of $^{14}$CO$_2$-C release were 9776 and 9078 dpm and average rates of $^{14}$CO$_2$-C release were 103.7 and 90.9 dpm h$^{-1}$ for Low CCE and High CCE soils, respectively.

Table 3.2. Cumulative recovery of $^{14}$C in alkali traps directly after incubation and prior to addition of acid (Data was logarithmically transformed for analysis).

<table>
<thead>
<tr>
<th>Incubation period h</th>
<th>Low CCE</th>
<th>High CCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm</td>
<td>dpm</td>
</tr>
<tr>
<td>48</td>
<td>8460 ($^{2.5}$) $^a$</td>
<td>6772 (3.1) a</td>
</tr>
<tr>
<td>120</td>
<td>10341 (2.7) b</td>
<td>9983 (2.3) b</td>
</tr>
<tr>
<td>216</td>
<td>10526 (1.5) b</td>
<td>10479 (1.9) c</td>
</tr>
</tbody>
</table>

$^*$cv (%) is given in parentheses. $^*$Statistical comparisons between incubation times are valid within soil types.

In both soils, cumulative recovery of all CO$_2$-C increased with time ($P < 0.001$) (Table 3.3) whilst
the rate of all CO$_2$-C release decreased with time ($P < 0.001$). There was no difference between replicates for either Low CCE ($P = 0.676$) or High CCE ($P = 0.220$). Total CO$_2$-C recovery was not affected by soil type ($P = 0.785$). There was also no difference between soil types when the total CO$_2$-C recovery ($P = 0.830$) and rate of CO$_2$ recovery ($P = 0.780$) were expressed relative to initial SOC. Average cumulative amounts of CO$_2$-C recovered were 10.55 mg CO$_2$-C g$^{-1}$ SOC for Low CCE and 10.62 mg CO$_2$-C g$^{-1}$ SOC for High CCE. Average rates of CO$_2$-C recovery were 0.096 mg CO$_2$-C g$^{-1}$ SOC for Low CCE and 0.092 mg CO$_2$-C g$^{-1}$ SOC h$^{-1}$ for High CCE.

Table 3.3. Cumulative recovery of CO$_2$-C in alkali traps directly after incubation and prior to addition of acid. (Data was logarithmically transformed for analysis).

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>Low CCE</th>
<th>High CCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td>mg CO$_2$-C g$^{-1}$ SOC</td>
<td>mg CO$_2$-C g$^{-1}$ SOC</td>
</tr>
<tr>
<td>48</td>
<td>6.29 (6.2) $^a$</td>
<td>5.51 (8.0) $^a$</td>
</tr>
<tr>
<td>120</td>
<td>10.03 (1.6) $^b$</td>
<td>10.75 (10.1) $^b$</td>
</tr>
<tr>
<td>216</td>
<td>15.21 (4.0) $^c$</td>
<td>15.62 (2.8) $^c$</td>
</tr>
</tbody>
</table>

$^a$Results are expressed relative to initial organic carbon content present in each soil. $^*$cv (%) is given in parentheses. $^a$Statistical comparisons between incubation times are valid within soil types.

3.3.2 Recovery of carbon dioxide-C after addition of sulphuric acid

Adding sulphuric acid (H$_2$SO$_4$) and bringing the soil pH between 5 and 6, released CO$_2$-C from carbonates in the soil. Any $^{14}$C recovered in the second and third alkali traps after adding the acid is assumed to have been derived from carbonates containing $^{14}$C from the straw extract. In both soils, the amount of $^{14}$C recovered after adding acid was less for each successive incubation increment ($P < 0.001$) (Table 3.4). There was no difference between replicates for Low CCE ($P = 0.456$) or High CCE ($P = 0.284$). Within an incubation increment, the rate of $^{14}$C recovery after
adding the acid tended to decrease for Low CCE although the effect of not significant \((P = 0.182)\) and the rate of \(^{14}\)C recovery significantly decreased over time for High CCE \((P < 0.001)\). Overall, the amount of \(^{14}\)C recovered \((P = 0.048)\) and the rate of \(^{14}\)C recovery \((P < 0.001)\) was less in Low CCE than High CCE. Average amounts of \(^{14}\)C recovered after adding acid were 1094 and 1558 dpm and average rates of \(^{14}\)C recovery were 53 and 347 dpm h\(^{-1}\) for Low CCE and High CCE, respectively. These results indicate that the release and recovery of \(^{14}\)C was affected by soil type.

Table 3.4. Recovery of \(^{14}\)C in alkali traps after addition of acid for each incubation increment. (Data was logarithmically transformed for analysis).

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>Low CCE</th>
<th>High CCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>48</td>
<td>5.5 (3.5) ^a</td>
<td>8.2 (2.5) a</td>
</tr>
<tr>
<td>120</td>
<td>3.8 (2.6) b</td>
<td>5.3 (8.7) b</td>
</tr>
<tr>
<td>216</td>
<td>2.2 (4.0) c</td>
<td>2.9 (3.2) c</td>
</tr>
</tbody>
</table>

*The proportions of \(^{14}\)C recovered from the second and third alkali traps are combined to give one value which is expressed relative to the total amount of \(^{14}\)C added as straw extract (28437 dpm). *cv (%) is given in parentheses. ^Statistical comparisons between incubation times are valid within soil types.

The amount of CO\(_2\)-C recovered after adding acid was less for each successive incubation increment for Low CCE \((P < 0.001)\) and High CCE \((P = 0.003)\) (Table 3.5). There was no difference between replicates for either Low CCE \((P = 0.850)\) or High CCE \((P = 0.490)\). Within an incubation increment, the rate of CO\(_2\)-C recovery decreased with time for Low CCE \((P < 0.001)\) and High CCE \((P = 0.010)\). Expressing CO\(_2\)-C recovery after adding acid relative to the initial amount of SOC did not affect the comparison between soil type. The total amount of CO\(_2\)-C recovered after adding acid was lower in Low CCE than High CCE \((P = 0.0001)\) as was the rate.
of CO$_2$-C recovery ($P = 0.0003$). Average amounts of CO$_2$-C recovered after adding acid were 8.1 and 36.6 mg CO$_2$-C g$^{-1}$ SOC and average rates of CO$_2$-C recovery were 6.2 and 61.4 mg CO$_2$-C g$^{-1}$ SOC h$^{-1}$ for Low CCE and High CCE, respectively. Relatively high total CO$_2$ recovery was largely due to carbon being released from carbonates in the soil.

Table 3.5. Recovery of CO$_2$-C in alkali traps after addition of acid for each incubation increment. (Data was logarithmically transformed for analysis).

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>Low CCE</th>
<th>High CCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td>mg CO$_2$-C g$^{-1}$ SOC</td>
<td>mg CO$_2$-C g$^{-1}$ SOC</td>
</tr>
<tr>
<td>48</td>
<td>12.07 (2.0) a</td>
<td>40.12 (1.1) a</td>
</tr>
<tr>
<td>120</td>
<td>6.92 (2.5) b</td>
<td>34.66 (4.7) b</td>
</tr>
<tr>
<td>216</td>
<td>5.4 (3.0) b</td>
<td>34.29 (6.2) c</td>
</tr>
</tbody>
</table>

*The amounts of CO$_2$-C recovered from the second and third alkali traps are combined to give one value expressed relative to the total amount of initial organic carbon present in each soil. $^*$cv (%) is given in parentheses. $^*$Statistical comparisons between incubation times are valid within soil types.

3.4 Discussion

3.4.1 Retention of $^{14}$carbon labelled carbon dioxide

The release of at least 2.2% of all $^{14}$C from the soil after adding the acid indicated that some $^{14}$C was trapped in the soil either as substrate which reacted with the acid, or retained as respired $^{14}$CO$_2$. Some retention was expected because the high CO$_2$ concentrations in the soil air, relative to atmospheric air, couple with 60% WFPS, drives the conversion of CO$_2$ to H$_2$CO$_3$, Ca(HCO$_3$)$_2$ or CO$_3^{2-}$ under alkaline conditions (Sposito, 1989). The proportion of $^{14}$C retained in High CCE and Low CCE after 9 days incubation was similar to the proportion of glucose derived $^{14}$CO$_2$ retained
in two alkaline and calcareous soils after 64 days incubation at 25°C (Ladd et al., 1977b). After acidifying the alkaline and calcareous soils, the proportion of initial $^{14}$C released was 1.0% for the sandy soil (13% clay) and 2.8% for the clay soil (47% clay). Respired CO$_2$ was also expected to be retained in the clay alkaline soils that Guerin (1999) incubated with endosulfans. However, no endosulfan derived $^{14}$CO$_2$ was released from the two clay alkaline soils after acidification to pH < 6.5. The contrary result for the endosulfan experiment probably occurred because endosulfan is relatively resistant to mineralisation (Guerin, 1999) compared to the wheat straw used in this short-term experiment. Thus the amount of endosulfan derived CO$_2$ may have been undetectable using 2M NaOH or ethanolamine liquid traps.

Higher recovery of $^{14}$CO$_2$ from High CCE after adding the acid, than from Low CCE, showed that more $^{14}$C was retained in High CCE than Low CCE. Higher retention of $^{14}$CO$_2$ in the low clay soil than the high clay soil was contrary to findings from other mineralisation studies with the substrates barley straw (Sorensen, 1975) and glucose (Ladd et al., 1977b). Therefore, another soil characteristic must have caused CO$_2$ to be retained. Higher retention of $^{14}$CO$_2$ in High CCE than Low CCE was unlikely to be due to the soil being more alkaline since the difference between soils was very small. Similarly, higher retention of mineralised $^{14}$C in High CCE due to more $^{14}$CO$_3^{2-}$ reacting with the free Ca$^{2+}$ ions was also unlikely to fully explain the difference between soils. Precipitation of more CaCO$_3$ was rejected as an explanation because the initial amount of exchangeable Ca was similar in both soils. If $^{14}$CO$_2$ retention was highly related to exchangeable Ca$^{2+}$, the proportion of $^{14}$CO$_2$ retained in Low CCE would have been about 91% of that retained in High CCE. Instead, Low CCE retained between 67% and 76% as much $^{14}$CO$_2$ as was retained in High CCE. In addition, higher $^{14}$CO$_2$ retention in High CCE cannot be attributed to higher amounts of CaCO$_3$, per se, because the compound is almost insoluble in cold water (0.014 g L$^{-1}$) (Weast, 1969).

Whatever the reason for higher retention of $^{14}$CO$_2$ in High CCE than Low CCE, the amount of $^{14}$C retained rapidly decreased as the incubation period was extended. Thus, provided the incubation
period is greater than 10 days, the amount of $^{14}$C retained in these soils would not be high enough to affect measurements of substrate-C mineralisation using alkali traps.

3.4.2 *Release of carbon from carbonates*  
A key concern about using alkali traps in incubations with calcareous soils was that C released from CaCO$_3$ during the incubation period may be recovered along with respired CO$_2$, thus overestimating the amount of C mineralised. Comparing total CO$_2$ recovered from the two soils used in this incubation before adding acid showed that carbonate-C was not converted to CO$_2$ in significant quantities and captured by the alkali trap. This result indicated that alkali traps were a valid method of assessing total C mineralisation from organic substrates in these two soils, at least in short term incubations. However, the soil must not be acidified to a pH < 6 as this resulted in higher amounts of CO$_2$ being released from High CCE, presumably derived from CaCO$_3$-C.

3.4.3 *Effect of soil characteristics on carbon mineralisation*  
The total proportion of $^{14}$CO$_2$-C recovered after 9 days incubation (37%) was similar to the proportion of barley straw derived $^{14}$CO$_2$-C recovered after 10 days incubation at 20°C from an alkaline soil (pH 7.7) (39%) and five slightly acidic soils (pH range: 6.2 – 6.8) ($^{14}$CO$_2$-C recovery range: 25% – 40%) (Sorensen, 1975). More $^{14}$CO$_2$ was recovered from labelled glucose incubated for 9 days in a CaCO$_3$ amended Rhodoxeralf at 20°C (Muneer and Oades, 1989a). The proportion of glucose derived $^{14}$CO$_2$ recovered was 48% in soil alone, and 65% in soil plus 10% CaCO$_3$. The higher recovery was due to glucose being more readily decomposable than wheat straw (Oades, 1989).

The net amount of $^{14}$C mineralised and converted of $^{14}$CO$_2$ to carbonates or carbonic acid was the same for Low CCE and High CCE. This similarity indicated that the mineralisation, release and recovery of substrate C was not affected by differences in alkalinity, carbonate content or any other chemical difference between the soils. The same result was presented for the CaCO$_3$ amended Rhodoxeralf after 9 days incubation at 20°C (Muneer and Oades, 1989a). Given that
\(^{14}\)CO\(_2\) retention was less than 3% after nine days incubation, similar total \(^{14}\)CO\(_2\) recovery indicated that the \(^{14}\)C-substrate was equally likely to be mineralised in both soils.

3.5 Conclusions

Alkali traps are a valid method for measuring mineralisation of substrate C and soil OC for studies aimed at comparing the two soils used in this preliminary experiment. Some \(^{14}\)C was retained in both soils, however, the amount decreased to less than 3% of total \(^{14}\)C by the end of the trial. Thus, provided incubations are conducted for more than 10 days, \(^{14}\)C retention is not expected to significantly affect measurements of C mineralisation.

Total C mineralised from substrate and soil organic carbon was not overestimated by release and recovery of carbonate derived CO\(_2\) unless the soil was acidified. Thus provided the pH is kept above 7 during incubations, overestimation of C mineralisation through carbonate-C recovery should not occur.

An additional finding from this experiment was that \(^{14}\)C mineralisation did not differ between soils even after accounting for initial SOC. This indicated that differences between the soil types, including CCE\(\%), did not affect C mineralisation.
CHAPTER 4
Minimising the effect of soil disturbance upon net nitrogen and carbon mineralisation through repacking sieved soil to field bulk density

4.1 Introduction

Variability of N in soil has been found to be particularly problematic in net N mineralisation studies (Raison et al., 1987; Sierra, 1996; 1997) and the field experiment presented in Chapter 2. High variability of C and net N mineralisation rates can be avoided (Macduff and White, 1985) by subjecting soil to wetting and drying cycles, sieving to produce a homogeneous sample (Stanford and Smith, 1972; van Gestel et al., 1991; Zagal and Persson, 1994) and measuring potential C and net N mineralisation under laboratory conditions. Subjecting soils to these disturbances prior to incubation also reduces the number of replicates required to a manageable number (Macduff and White, 1985). However, despite the logistical advantages of sieving, in vitro techniques designed to reduce the need for replication have been criticised for overestimating the net amount of N mineralised under field conditions (Frazer et al., 1990; Mary and Recous, 1994) as discussed in Chapter 3 for in situ and intact cores.

An alternative to sieving only or using intact cores is a compromise technique which has been used to minimise the number of replicates yet apparently give results which reflect net N mineralisation rates in the field (Kladivko and Keeney, 1987; Aulakh et al., 1991; Murphy et al., 1999). The basic technique involves sieving the soil whilst field moist, adding amendments and repacking the soil to a consistent bulk density. Sieving with repacking is assumed to produce mineralisation rates more closely approximating rates occurring in intact cores or the field (Macduff and White, 1985) and allows insoluble amendments to be added to the soil prior to incubation.

Although the sieving with repacking technique is used in research, only a few experiments
(Stenger et al., 1995) have been conducted to test the technique’s validity as a means of reducing variability and approximating mineralisation in intact soil cores. The following incubation study was designed to quantify the impact of sieving and repacking soils to field bulk density upon C and net N mineralisation rates in soils from Yorke Peninsula and compare the results obtained with intact soil cores and loosely packed sieved soil samples. The sieved and repacking soil preparation method will be used in a future in vitro experiment if soil subjected to that technique has C and net N mineralisation rates more closely resembling values from intact cores than from sieved soils.

4.2 Materials and Methods

4.2.1 Soils

The same two soils from Yorke Peninsula (Low CCE and High CCE) were used in this incubation as in the field experiments. Some chemical details of the particular soil samples used in this experiment are given in Table 4.1. Both the soils were from the same paddock, and therefore had the same cropping history, and were subjected to the same cultural practices. However, soil properties differed particularly in clay, CCE and OC contents. The previous crop was barley that had been harvested in November. Stubble was left standing, and the paddock was left fallow until the next cropping season in May. Samples were taken in late May after the breaking rains and prior to sowing or fertiliser applications.

At each site, 12 paired soil cores were collected from a 20 m x 20 m area by driving 75 mm (diameter) x 150 mm lengths of PVC tubing into the 0-10 cm layer using a one piece core driver especially designed to fit 75 mm diameter tubing. Each core was extracted from the surrounding soil by gently rocking back and forth until the core could be extracted by hand. Any incomplete or misshapen cores were discarded on site and a replacement sample was taken. Immediately after extraction, the 24 cores were placed in plastic bags in their PVC tubing and stored at 4°C for 2 weeks.
Table 4.1. Soil characteristics

<table>
<thead>
<tr>
<th>Soil taxonomy</th>
<th>Unit</th>
<th>Low CCE</th>
<th>High CCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(kg m(^{-3}))</td>
<td>Haploxeralf</td>
<td>Rhodoxeralf</td>
</tr>
<tr>
<td>Field bulk density</td>
<td>1.35</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>Stone</td>
<td>(%)</td>
<td>4.0</td>
<td>13.8</td>
</tr>
<tr>
<td>Clay</td>
<td>(%)</td>
<td>42</td>
<td>26</td>
</tr>
<tr>
<td>OC</td>
<td>(%)</td>
<td>1.95</td>
<td>3.45</td>
</tr>
<tr>
<td>Total N</td>
<td>(%)</td>
<td>0.16</td>
<td>0.25</td>
</tr>
<tr>
<td>Inorganic N</td>
<td>(mg kg(^{-1}))</td>
<td>28.1</td>
<td>21.7</td>
</tr>
<tr>
<td>CCE</td>
<td>(%)</td>
<td>0.5</td>
<td>9.8</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>8.1</td>
<td>8.5</td>
</tr>
<tr>
<td>Exch Ca</td>
<td>(cmol kg(^{-1}))</td>
<td>30.7</td>
<td>33.7</td>
</tr>
<tr>
<td>CEC</td>
<td>(cmol kg(^{-1}))</td>
<td>38.3</td>
<td>38.3</td>
</tr>
</tbody>
</table>

A Soil Survey Staff (1989), B Stone is defined as any inorganic material unable to be passed through a 2 mm sieve by hand, C Determined by particle size distribution (Shedrick and Wang, 1993), D OC% determined by difference (total C determined by combustion less CCE-C), E Total N determined by combustion, F Determined using the method described in Chapter 2 based on methods of Kempers and Luft (1988) and Rayment and Higginson (1992), G Calcium carbonate equivalent (CCE) (Alison and Moodie, 1965), H\(_5\)pH (H\(_2\)O 5:1), I Analyzed by South Australian Soil and Plant Analysis Service (Department of Primary Industries Laboratories).

4.2.2 Sample preparation

Twelve cores from each site (one from each pair) were kept intact. The weight and height of the cores were measured to determine average core bulk densities. Average intact core weights (oven dry weight equivalent) and heights were 504 g (cv = 2.3%) and 104 mm (cv = 6.2%) for Low CCE and 450 g (cv = 2.8%) and 105 mm (cv = 4.3%) for High CCE. Soil from all other cores collected at each site was bulked, passed through a 2 mm sieve and the stones (mainly...
limestone) were discarded. A proportion of the sieved soil was packed into new 75 mm (diameter) x 150 mm PVC tubes to the same average field bulk density as intact cores from the same site and a height of 100 mm. All cores were capped at the bottom of the tubes to prevent soil loss. Cores and remaining loose sieved soil were stored in plastic bags at 4°C for two weeks at moisture contents of 8.5% for Low CCE and 8.7% for High CCE. The moisture contents were equivalent to 23% WFPS for Low CCE and 19% WFPS for High CCE.

4.2.3 Experimental design

The experiment consisted of three soil treatments, intact cores (intact), sieved and repacked cores (repacked), and loose sieved soil (sieved) incubated for three periods; 2, 7 and 14 days. Each treatment initially had 12 replicates arranged in a complete randomised block design within a single diurnal growth chamber (20°C with darkness, 28°C with light). All incubation periods commenced on the same day.

4.2.4 Incubation

Twelve x 46 g (oven dry weight equivalent) of sieved soil was placed loosely in 70 mL plastic sampling jars directly prior to initiation of the incubation period. The sieved soil samples and the cores were placed in 1 L and 2 L air-tight glass jars, respectively. All soils were brought to 60% WFPS with RO water and a glucose and ammonium sulphate solution. The total volume of solution required (mL) to bring cores and loose soil to 60% WFPS was calculated using the average bulk density for each treatment and the moisture content of each soil as follows:

\[
\text{Solution required} = \text{SDW} + \left( \text{SDW} \times 0.6 \times \left(1 - \frac{\text{BD}_{2.65}}{\text{BD}}\right) \right) - \text{SWW}
\]

where SDW is soil dry weight, BD is bulk density and SWW is soil wet weight.

The volumes of solution used in in situ and repacked cores were 67 mL for Low CCE and 84 mL for High CCE. The volume of solution used in loose sieved soils was 13 mL with an estimated
bulk density of 1 kg m$^{-3}$.

Soils were inoculated with a glucose and ammonium sulphate solution (1500 $\mu$g C g$^{-1}$ soil and 50 $\mu$g N g$^{-1}$ soil) immediately prior to inserting a 10 mL x 2N NaOH alkali trap into each jar and sealing the jar. Two alkali traps were incubated without soil to measure background CO$_2$ levels. Within blocks, the samples were re-randomised after each CO$_2$ sampling.

4.2.5 Carbon dioxide and inorganic nitrogen sampling and analysis

Alkali traps were collected and replaced every 24 h for the first 7 days and on days 7 and 14 for all soils. Total CO$_2$ content of alkali traps was determined by auto-titration with 0.05N HCl (Radiometer TTT85 titrator and ABU80 autoburette with Metrohm 645 Multidosmat) within 24 h of sampling.

Four replicates from each soil and treatment were destructively sampled for NO$_3^-$-N and NH$_4^+$-N analysis on days 2, 7 and 14. The amount of N$_2$ and N$_2$O was not measured in the incubated soils because previous incubation studies have shown that at 60% WFPS the generation of these compounds is negligible (Aulakh et al. 1991). Immediately after harvest, each sample was passed through a 4 mm sieve and 10 g (moist weight) analysed for NO$_3^-$-N and NH$_4^+$-N content using the same technique and materials detailed in Chapter 2.

4.2.6 Statistical analysis

Net N and C mineralisation were compared between soil types, treatments and time using one and two way ANOVAs in the statistical package GenStat for Windows Release 4.2 (Lawes Agricultural Trust, 2000). Carbon mineralisation data expressed on a daily basis was transformed using log$_e$ before analysis. All other data was analysed without transformations. Values were considered to be significantly different at $P < 0.05$. 
4.3 Results

4.3.1 Cumulative carbon mineralisation
Carbon mineralisation (μg CO₂-C recovered g⁻¹ soil) measured over the 14 day incubation period differed between the two soils (P < 0.001) with 1610 μg CO₂-C g⁻¹ soil recovered from Low CCE and 1861 μg CO₂-C g⁻¹ soil recovered from High CCE. The amount of CO₂-C recovered differed significantly between treatments (P < 0.001) (Table 4.2) with more CO₂-C recovered from the loose soil than either the intact cores or the sieved and repacked cores. There was no significant difference in the average amount of CO₂-C recovered from sieved and repacked cores and intact cores. There was no significant interaction between soil type and soil treatment (P = 0.374).

Table 4.2. Total CO₂-C recovered from Low CCE and High CCE subjected to three soil treatments after 14 days incubation with glucose and ammonium sulphate solution.

<table>
<thead>
<tr>
<th>Soil treatment</th>
<th>Low CCE</th>
<th>High CCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μgCO₂-C g⁻¹ soil</td>
<td>μgCO₂-C g⁻¹ soil</td>
</tr>
<tr>
<td>Intact cores</td>
<td>1443 (3.4) a</td>
<td>1638 (8.7) b</td>
</tr>
<tr>
<td>Sieved and repacked cores</td>
<td>1473 (2.7) a</td>
<td>1741 (1.9) c</td>
</tr>
<tr>
<td>Loose sieved soil</td>
<td>1913 (0.8) d</td>
<td>2202 (2.1) e</td>
</tr>
</tbody>
</table>

*Cv (%) is given in parentheses. ^Statistical comparisons are between all values in the table (P < 0.05).

4.3.2 Daily carbon mineralisation rates
Within the 14 day incubation period and expressed as a function of the amount of soil in each core, the amount of CO₂-C recovered at each time increment differed with soil type (P < 0.001), treatment (P < 0.001) and all interactions (P < 0.001). There was no significant difference between replicates (P = 0.214) During the first three days of incubation CO₂-C recovery from both soils differed between all three treatments with the highest amounts of CO₂-C recovered from the
sieved soil and the least from the repacked soil. After three days incubation CO₂-C recovery from sieved soil declined compared to the other two treatments whilst relatively large quantities of CO₂-C were recovered from the repacked soil. These treatment effects were the same for both soils until day 9. After day 9, the treatment differences were maintained in Low CCE (Fig 4.1) whilst CO₂-C recovery from High CCE (Fig 4.2) was the same for all three treatments.

4.3.3 Cumulative net nitrogen mineralisation

Net N mineralisation (μg inorganic N recovered g⁻¹ soil) measured over the 14 day incubation period differed between the two sites (P < 0.001), soil treatments (P = 0.007) and there was no significant site x treatment interaction (P = 0.278). There was no significant difference between replicates for either soil (High CCE P = 0.53, Low CCE P = 0.97). Significantly less inorganic N was recovered from Low CCE than from High CCE after 14 days incubation (Table 4.3). In High CCE, total net N mineralisation after 14 days did not differ between treatments (P = 0.213). In Low CCE, the net amount of N immobilised differed between all treatments (P < 0.001) with the most N immobilised in soil subjected to the sieved treatment and the least in the intact treatment. In the Low CCE soil, there was less variability within repacked soil cores than within intact soil cores.

Table 4.3. Net N mineralised or immobilised in two soils subjected to three soil treatments after 14 days incubation with glucose and ammonium sulphate solution.

<table>
<thead>
<tr>
<th>Soil treatment</th>
<th>Low CCE (μgN g⁻¹ soil)</th>
<th>High CCE (μgN g⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact cores</td>
<td>-10.03 (*56) a</td>
<td>12.43 (71) a</td>
</tr>
<tr>
<td>Sieved and repacked cores</td>
<td>-24.65 (23) b</td>
<td>-8.57 (294) a</td>
</tr>
<tr>
<td>Loose sieved soil</td>
<td>-34.71 (12) c</td>
<td>0.23 (1216) a</td>
</tr>
</tbody>
</table>

*cv (%) is given in parentheses. ^Statistical comparisons between soil treatments (P < 0.05) are only valid within a soil type.
Figure 4.1. Daily net C mineralisation rate over 14 days incubation for three soil treatments imposed upon Low CCE.
Figure 4.2. Daily net C mineralisation rate over 14 days incubation for three soil treatments imposed upon High CCE.
4.3.4 Incremental net nitrogen mineralisation rates

Daily net N mineralisation rates calculated within the three incubation periods differed with soil type \( (P = 0.004) \) and time \( (P < 0.001) \), but not with treatment \( (P = 0.202) \) or replicate \( (P = 0.898) \). The daily rates of net N immobilisation were higher in Low CCE than High CCE and net N immobilisation occurred at a significantly higher rate during the first two days than in the following 12 days. Some interactions were significant; site x treatment \( (P < 0.001) \), site x time \( (P = 0.790) \), treatment x time \( (0.015) \) and site x treatment x time \( (P < 0.001) \). The effects of treatments upon daily net N mineralisation rates were assessed individually for each soil since soils behaved differently.

For Low CCE, there was no treatment effect \( (P < 0.221) \) but there were significant differences with time \( (P < 0.001) \) and the treatment x time interaction \( (P < 0.001) \) (Table 4.4). There was no significant difference between replicates \( (P = 0.861) \). The treatment x time interaction was due to the repacked and intact soil cores having high net N immobilisation rates during the first two days relative to the sieved soil and the other incubation periods. At each sampling time, the amount of inorganic N in intact cores was relatively similar to the amount in repacked cores compared to the amount in sieved soil (Fig. 4.3). In addition, the change in the amount of inorganic N present over the 14 days was less in intact and repacked cores than sieved soil.

For High CCE, there was also no treatment effect \( (P < 0.011) \), but there were significant differences with time \( (P < 0.001) \) and the treatment x time interaction \( (P < 0.001) \) (Table 4.5). There was no significant difference between replicates \( (P = 0.817) \). The treatment x time interaction was due to soil in the sieved only treatment having a relatively high net N immobilisation rate during the first incubation period compared to the other treatments. Also, all treatments had relatively high immobilisation rates at the first sampling time compared with the two later sampling times. For the first two sampling times, the amount of inorganic N present in intact and repacked cores was similar compared to the amount of N present in sieved soil (Fig. 4.4). At 14 days, similar amounts of inorganic N were present in repacked cores and sieved soils.
Table 4.4. Daily net N mineralisation rates in Low CCE for three treatments incubated with glucose and ammonium solution over three incubation periods.

<table>
<thead>
<tr>
<th>Soil treatment</th>
<th>0 – 2 days (µgN g⁻¹ soil)</th>
<th>3 – 7 days (µgN g⁻¹ soil)</th>
<th>8 – 14 days (µgN g⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact cores</td>
<td>-17.7 ^a</td>
<td>3.4 e</td>
<td>1.2 de</td>
</tr>
<tr>
<td>Sieved and repacked cores</td>
<td>-9.8 b</td>
<td>0.4 d</td>
<td>-1.0 cd</td>
</tr>
<tr>
<td>Loose sieved soil</td>
<td>-2.5 c</td>
<td>-7.7 b</td>
<td>1.3 de</td>
</tr>
</tbody>
</table>

^Statistical comparisons are between soil treatments and time period (P < 0.05) (LSD = 2.76).

Table 4.5. Daily net N mineralisation rates in High CCE for three treatments incubated with glucose and ammonium solution over three incubation periods.

<table>
<thead>
<tr>
<th>Soil state</th>
<th>0 – 2 days (µgN g⁻¹ soil)</th>
<th>3 – 7 days (µgN g⁻¹ soil)</th>
<th>8 – 14 days (µgN g⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact cores</td>
<td>-7.7 ^cd</td>
<td>-1.8 c</td>
<td>3.3 d</td>
</tr>
<tr>
<td>Sieved and repacked cores</td>
<td>-8.8 b</td>
<td>1.8 cd</td>
<td>0.0 cd</td>
</tr>
<tr>
<td>Loose sieved soil</td>
<td>-16.6 a</td>
<td>2.8 d</td>
<td>2.9 d</td>
</tr>
</tbody>
</table>

^Statistical comparisons are between soil treatments and time period (P < 0.05). (LSD = 4.26)
Figure 4.3. Inorganic N immobilisation and re-mineralisation in Low CCE incubated for 14 days after inoculation with a glucose and ammonium sulphate solution.

Figure 4.4: Inorganic N immobilisation and re-mineralisation in High CCE incubated for 14 days after inoculation with a glucose and ammonium sulphate solution.
4.4 Discussion

4.4.1 Effect of soil treatments on carbon mineralisation

Carbon mineralisation is greatly affected by the organic substrate which is being mineralised (Oades, 1989). The substrate, glucose, used in these laboratory incubations is readily decomposable by microbes whereas carbohydrates or lignin that are present in soil organic matter are relatively resilient to decomposition. Due to these differences, comparing total C mineralised or C mineralisation rates between experiments may not be as useful as considering the relative results within each experiment. However, a comparison did show that the total amount of C mineralised in High CCE and Low CCE were not dissimilar to the amount of CO₂-C recovered from other South Australia soils with relatively low soil OC contents. For instance, after 14 days in vitro incubation with glucose and ammonium sulphate a Vertisol had evolved about 670 µg C g⁻¹ soil and an Alfisol had evolved about 800 µg C g⁻¹ soil (Ladd et al, 1992).

Despite many different substrates and laboratory incubation techniques being used in soil disturbance studies, the common finding between all studies is that in the first two to five days of incubation there is a flush of CO₂ release (Adu and Oades, 1978; Aulakh et al., 1991; Ajwa et al., 1998) which is particularly high in sieved soils (Hassink, 1992; Franzluebbers, 1999) due to sieving releasing protected OM and increasing aeration. This flush of CO₂ was also observed in both soils used in this study, particularly in the loose sieved samples. The total amount of CO₂ recovered from both soils incubated as repacked or intact soil cores relative to the sieved soil in the first three days of incubation was similar to results obtain in a previous comparative incubation study with a Kanhapludult (Franzluebbers, 1999). In the Kanhapludult, total CO₂ recovered during the first three days of incubation was about 2.0 to 2.6 times higher in sieved soils which had been dried and re-moistened to 55% WFPS (depending upon mesh size) than in intact soil cores kept field moist at 67% WFPS. Similarly, loose sieved soil from High CCE and Low CCE released 2.2 times more CO₂ than intact soil cores whilst loose sieved High CCE
released 2.7 times more CO₂ than repacked cores and loose sieved Low CCE released 2.9 times more CO₂ than repacked soil cores. Relatively high CO₂ recovery in the first three days from intact soil cores compared to repacked soil cores was possibly due to the glucose solution having contact with a greater volume of soil and hence microbes. Greater soil contact may have occurred because the solution was able to move into the core via biopores that had been formed by earthworms or now decayed roots (Lin et al., 1998). These channels would not have been present in the unstructured soils that were in the repacked cores, hence movement of the solution into the core would have taken longer.

The cumulative results of the experiment were very similar to results from the first three days incubation. This was because CO₂ recovered during the first three days incubation accounted for 42.0%, 31.4% and 66.3% of total CO₂ recovered during the experiment for intact soil cores, repacked soil cores and sieved soil, respectively. This relationship was also reported to be the case in Franzluebbers’ (1999) 24 day incubation where CO₂ recovery during the first three days incubation was highly related to total CO₂ recovery in intact cores \( r^2 = 0.98 \) and sieved soils \( r^2 = 0.99 \). After nine days incubation there was no difference between treatments in High CCE but differences were maintained in Low CCE. The former event also occurred after day 10 in the incubated Kanhapladult (Franzluebbers, 1999).

### 4.4.2 Effect of soil treatments on net nitrogen mineralisation

Like C mineralisation, comparing net N mineralisation experiments is complicated by the amount of readily accessible inorganic N present at the start of incubation. Where soils are amended with a substrate which has a C:N ratio greater than 15:1 (Zagal and Persson, 1994), as was the case in this experiment, the net effect after two to three days incubation is net N immobilisation regardless of whether soil is sieved (Ladd et al., 1977b; Zagal and Persson, 1994) kept intact, or sieved and repacked (Aulakh et al., 1991). Immobilisation within the first few days of incubation occurs because the presence of a readily decomposable C source with a limited N supply leads to more inorganic N being incorporated into the microbial biomass than is produced through
An initial immobilisation effect was observed in the soils subjected to the incubations described in this experiment. However, the reduction in inorganic N was not as dramatic as has been observed in similar incubations with glucose and inorganic N amended soils (Ladd et al. 1992; Zagal and Persson 1994). An explanation for less net immobilisation being observed in the soils from Yorke Peninsula may simply be that inorganic N was measured relatively infrequently in this experiment, that is, only on days 0, 3, 7 and 14. In comparison, inorganic N in glucose and ammonium sulphate amended soils incubated by Ladd et al. (1992) was measured everyday for the first 4 days and every second day thereafter until day 14. The day on which the maximum amount of net immobilisation occurred differed between topsoils occurring on day 1 in the soil with 10% clay and day 12 on the soil with 50% clay (Ladd et al. 1992).

After the first two to three days incubation, the net effect of mineralisation and immobilisation is less predictable because some conditions (e.g. aeration, low C:N) favour mineralisation and others (e.g. high C:N) favour immobilisation. In this study, the amount of inorganic N present tended to increase as organic N was re-mineralised. Similar increases in inorganic N two to ten days after initiation of incubation have been recorded in several short-term incubations where soils were inoculated with a readily decomposable substrate with high C:N (Ladd et al., 1977b; de Bruin et al., 1989). Also, like laboratory incubations with sieved soil (Ladd et al., 1977b; de Bruin et al., 1989), only a small proportion of all the initial inorganic N substrate was re-mineralised by day 14. Re-mineralisation after an initial short period of net N immobilisation occurs because inorganic N quickly becomes limiting to microbial growth and subsequently the microbial population declines. Nitrogen in the microbes has been mineralised to an inorganic form and accumulates due to the loss of CO₂-C from the system through microbial respiration limiting the availability of C.

The effect of treatment on the net amount of N immobilised after 14 days in Low CCE (net N
immobilisation in sieved > repacked > intact) was anticipated based upon the short-term net effects of sieving and aeration on N mineralisation in the presence of a glucose and ammonium sulphate inoculum. Sieving and incubating soil at low bulk density with glucose and ammonium sulphate increased microbial activity as indicated by the flush of CO2, and microbial biomass (Franzluebbers 1999) thus the amount of N immobilised was also increased. When sieved soil was repacked to field bulk density, there was less aeration and hence less N mineralisation. However, there was also less immobilisation due to some of the organic matter freed by sieving becoming inaccessible to microbes. The least amount of N was immobilised in intact cores because the least amount of OM was accessible to microbes therefore the C:N ratio is relatively low.

4.4.3 Variability between replicates

The variability in total CO2 recovered in this incubation study was similar to the variability found in several other studies using various soil handling techniques. For instance, the total amount of CO2 recovered (µg C g⁻¹ soil) from loose sieved soils incubated at 40% water holding capacity with legume varied by 1.8 to 6.1% in a Vertisol and by 3.2% to 6.5% in an Alfisol (van Gestel et al., 1991). CO2 recovery (µg C g⁻¹ soil) in an Argiudoll sieved and repacked with sand was < 5% (Ajwa et al., 1998). As stated by Franzluebbers (1999) and demonstrated by Ross et al (1985), the advantage of using sieved and mixed soils is considered to be the reduced variability compared to using intact cores. This advantage was also demonstrated for total CO2 recovery in both soils used in this study where repacked cores had less variability than intact soil cores (Table 4.2).

Variability has been found to be less problematic in C than N mineralisation studies (van Gestel et al., 1991; Franzluebbers, 1999). This was also the case in this short-term incubation. The effect of soil treatment on variability, and therefore the need for replication, was difficult to interpret due to high variability being related to small values for net N mineralisation rather than the treatments themselves. Variability between replicates for inorganic N relative to total initial N after 14 days
incubation ranged from 12% to 1216%. This broad ranging and high variability has been commonly reported for net N mineralisation studies (Poovarodom et al., 1988; Frazer et al., 1990). For instance, net N mineralisation rates in 25 intact core replicates had coefficients of variation ranging from 36% at 25°C to 310% at 10°C (Sierra 1997). Sierra (1996) was unable to account for this spatial variability in terms of the amount of mineralisable substrate or aeration capacity of the samples.

In Low CCE, the variability between replicates decreased as soil was subjected to more homogenising processes. This has also been reported in other comparative studies (Ross et al., 1985) and was expected given that mixing and sieving reduces spatial variability (Franzluebbers, 1999). In High CCE, variability increased as soil homogeneity increased. Some prior studies have also shown that sieving and repacking soil does not necessarily yield less variability between replicates. For instance, incubations with four replicates of sieved, repacked and leached loam columns had coefficients of variation ranging between 10% and 33% for N mineralisation rates (Stenger et al., 1995). Whilst in the same study with four replicates, intact soil core variability was less than for repacked cores ranging between 0% and 5%.

4.4.4 Differences between soils
The differences in C and net N mineralisation rates between the two soils indicated that they were affected by the soils' properties. The main differences between the two soils were OC%, clay content and CaCO₃ content. Higher recovery of CO₂-C and higher C mineralisation rates in High CCE than Low CCE may have been due to High CCE containing more native OC than Low CCE. As shown in similar in vitro experiments with sieved soil, native OC can contribute almost half of all recovered CO₂-C after 14 days incubation (Ladd et al, 1992). Differences in OC% between the two soils does not appear to have affected net N mineralisation. High CCE had a slightly higher C : N ratio which may have been expected to result in more N being immobilised than in Low CCE. However, the opposite occurred after 14 days incubation with net immobilisation of total N in all treatments for Low CCE and only one treatment for High CCE. Given that the difference in
change of N form is unlikely to be due to differing native C or N the other potential causes, clay content and CaCO₃ content, need to be considered.

High clay content has been demonstrated to cause a reduction in C mineralisation in short (3 to 24 day) incubations (Franzluebbers, 1999) presumably due to soil OM being protected by clay-OM bonding as has previously been postulated (Craswell and Waring, 1972) and indirectly demonstrated (Gupta and Germida, 1988). In this incubation, less CO₂-C was recovered from the soil with higher clay content. However, this result has not been attained in all research about the interaction between clay and C mineralisation (Hassink, 1994; Franzluebbers et al., 1996). Franzluebbers (1999) has hypothesised that differences are due to clay type with low surface charge clays such as kaolinite not attracting OM and hence having no affect on C mineralisation. Net N mineralisation has also been shown to decrease as clay content increases (Hassink, 1994; Strong et al., 1999) and this effect was observed in Low CCE. However, it is unclear why high clay content affects N mineralisation and not C mineralisation.

Adding CaCO₃ to soil decreased C mineralisation in a 120 day laboratory experiment (Muneer and Oades, 1989a) and in field studies (Muneer and Oades, 1989b). The reduction in C mineralisation is considered to occur because OM is chemically protected from microbial attack by Ca-OM bridging (Muneer and Oades, 1989c). Results from this short term incubation did not support the Ca-bridging theory, with less C being mineralised in Low CCE than High CCE irrespective of the soil treatment. However, Muneer and Oades' (1989a) laboratory experiment only resulted in a 4% reduction in cumulative CO₂ after 120 days and there was no reduction in the first 14 days incubation.

The effect of Ca-OM bridging on net N mineralisation, has not been investigated. However, assuming less OC is accessible to microbes in soils with high CaCO₃, the expected outcome would be a lower effective C:N ratio than in soils with little or no CaCO₃. Consequently, soils with high CaCO₃ content would be expected to immobilise less N than soils with low CaCO₃ content.
This hypothesis corresponds to the net change in inorganic N recorded in Low CCE and High CCE after 14 days incubation in this study.

4.5 Conclusions

Given the net effects of the treatments on C and net N mineralisation in Low CCE and High CCE, sieving and repacking soils is a suitable option in circumstances where using intact or in situ cores is not practical. The sieving and repacking technique proved to be particularly suitable for studying C mineralisation with relatively similar amounts of CO$_2$-C being recovered in intact and repacked treatments compared to CO$_2$-C recovered from the sieved soil treatments.

The effects of treatments on the net amount of N mineralised or immobilised was less distinct with high variability making it impossible to clearly distinguish treatment differences, particularly in High CCE. However, in High CCE net N mineralisation rates in intact cores at any particular sampling time were more similar to the rates in repacked cores than sieved soils. In addition, in Low CCE variability was less in the repacked cores than in the intact cores thus supporting the idea of using sieved and repacked cores instead of sieved loose soil in future incubation studies.

An additional finding was that the presence of CaCO$_3$ did not appeared to affect C mineralisation yet did seem at explain differences in soil types for net N mineralisation rates. The role of Ca-bridging in C and N mineralisation will be investigated further using the soil treatment technique validated in this short term incubation.
CHAPTER 5
Quantifying gross and net carbon and net nitrogen mineralisation in agricultural soils
during a 231 day laboratory incubation

5.1 Introduction

The field experiment presented in Chapter 2 showed that net N mineralisation could not be
accurately sampled under field conditions in the soils being studied. Given this restriction, a
long term (231 days) experiment was designed to investigate the same issues presented in
the introductory section of Chapter 2. The first aim was to quantify the difference between the
N mineralisation rates in a soil classified as Xeralfs from Yorke Peninsula and the Mid-North
of South Australia. The second aim was to determine whether naturally occurring CaCO₃
reduces mineralisation rates. The design of the long term experiment acknowledges the
differences between mineralisation rates measured in the field and those measured under
laboratory conditions as was described in Chapter 2. The effects of moving from field to
laboratory studies were minimised by using the information gained in the two technical
experiments discussed in Chapters 3 and 4 when designing the long term experiment.

One of the advantages of laboratory experiments is that more parameters can be frequently
measured to give a more detailed account of the processes that are occurring. This
advantage was utilised in the long term experiment with ¹⁴C and soil OC mineralisation being
frequently measured to give a continuous indication of changes in the C and N pools.
Labelled N was used to ensure no substrate N was unaccounted for during the experiment.
Thus, the objectives of this experiment were to measure gross and net C and net N
mineralisation rates to determine whether rates differed with soil type and to determine
whether mineralisation rates were reduced due to the presence of CaCO₃.
5.2 Materials and methods

5.2.1 Soils
The same two soils from Yorke Peninsula (Low CCE and High CCE) were used in this experiment as in the field experiment and the short term incubations described in Chapters 2, 3 and 4, respectively. Both the soils were from the same paddock, and therefore had the same cropping history, and were subjected to the same cultural practices. However, as mentioned in Chapter 2, soil properties differed particularly in clay, CCE and OC contents. The previous crop was wheat that had been harvested in November. Stubble was left standing, and the paddock was left fallow until the next cropping season in July. Samples were taken in early July after the breaking rains and prior to sowing or fertiliser applications.

The other soil used in this incubation was from Paddock West 7 at The University of Adelaide, Roseworthy Campus (Low CCEUA) (34° 53' S 138° 69' E). Low CCEUA was classified as a Natrixeralf (Soil Survey Staff, 1989). The Roseworthy site was under chemical fallow at the time of sampling and had been under wheat in the previous year.

At each site, 32 intact cores were collected (4 x 12.6 m transects x 8 cores) from the 0-10 cm layer. Collection was facilitated by driving 75 mm (diameter) x 150 mm lengths of PVC tubing into the 0-10 cm layer using the same one piece core driver used to collect soils for the experiment described in Chapter 4. The 8 cores were bulked and transects were treated as replicates. At the Low CCE site, 2 cores, 10 cm apart, were collected at each point along the transects. Some chemical details of the particular soil samples used in this experiment are given in Table 5.1.

5.2.2 Sample preparation
Field bulk density was determined for each core using the same method described in Chapter 4. Immediately after determining field bulk density, the 8 cores from each transect were bulked and transects were treated as replicates. All soils were passed through a 4 mm sieve whilst field moist. The second set of cores from Low CCE was amended with powdered
CaCO₃ (analytical grade) to adjust the CaCO₃ equivalent percentage (CCE%) to that of the High CCE soil and so test the effect of physical addition of CaCO₃. The final CCE% of the amended soil (Low CCE+) was 11% as determined by the method of Allison and Moodie (1965). After mixing the CaCO₃ into the soil, all soils were allowed to equilibrate for 2 weeks at 4°C.

During the equilibration period, the WFPS of each soil was determined using the following calculation:

\[
WFPS (\%) = \frac{H_2O \times BD}{1 - \left(\frac{BD}{2.65}\right)}
\]

where H₂O is gravimetric water content and BD is bulk density.

Table 5.1. Soil characteristics.

<table>
<thead>
<tr>
<th>Soil taxonomy A</th>
<th>Low CCE</th>
<th>High CCE</th>
<th>Low CCE UA H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haploxeralf</td>
<td>7.7 (0.36)</td>
<td>8.3 (0.02)</td>
<td>8.3 (0.03)</td>
</tr>
<tr>
<td>Rhodoxeralf</td>
<td>9.0 (2.08)</td>
<td>7.6 (0.45)</td>
<td></td>
</tr>
<tr>
<td>Natrixeralf</td>
<td>9.8 (0.51)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soil chemistry</th>
<th>Low CCE</th>
<th>High CCE</th>
<th>Low CCE UA H</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH B</td>
<td>7.7 (0.36)</td>
<td>8.3 (0.02)</td>
<td>8.3 (0.03)</td>
</tr>
<tr>
<td>mg kg⁻¹</td>
<td>9.8 (0.51)</td>
<td>9.0 (2.08)</td>
<td>7.6 (0.45)</td>
</tr>
<tr>
<td>Clay D</td>
<td>42 (1.93)</td>
<td>26 (1.93)</td>
<td>19 (1.59)</td>
</tr>
<tr>
<td>OC E</td>
<td>2.69 (0.21)</td>
<td>3.25 (0.17)</td>
<td>2.03 (0.19)</td>
</tr>
<tr>
<td>CCE F</td>
<td>0.5 (0.66)</td>
<td>9.8 (1.36)</td>
<td>1.5 (0.65)</td>
</tr>
<tr>
<td>RlM G</td>
<td>46</td>
<td>65</td>
<td>49</td>
</tr>
<tr>
<td>Illite G</td>
<td>32</td>
<td>20</td>
<td>27</td>
</tr>
<tr>
<td>Kaolinite G</td>
<td>16</td>
<td>9</td>
<td>17</td>
</tr>
</tbody>
</table>

After 2 weeks, soils were moistened with RO water or dried at 30°C, as appropriate, to bring them to 24% moisture content. After standardising the moisture content, soils were allowed to equilibrate for a further 4 weeks at 4°C. After the second equilibration period, a subsample of each soil was packed into 28 x 150 mL phials (No. 40). Each phial contained 100g of soil at a bulk density of 1.2 Mg m⁻³.

5.2.3 Experimental design

The experiment consisted of four soil treatments (High CCE, Low CCE, Low CCE+, Low CCEUA) incubated for up to 231 days after inoculation with a labelled C and N solution. Each soil initially had 28 replicates arranged in a complete randomised block design with four blocks. All incubation periods commenced on the same day. Four replicates (one from each block) of each soil were removed and destructively samples for inorganic N to determine the net amount of N mineralised. At each destructive sampling, the number of CO₂-C samples was also reduced such that each soil had 28 jars for the first sampling and 4 for the last sampling.

5.2.4 Inoculation and incubation

Each repacked soil sample was placed in a 1 L air-tight glass jar. The soil was brought to 60% WFPS by inoculating with a 4.8 mL aliquot of solution. The aliquot contained 134.4 mg glucose-C with 51195 Bq D-glucose-U⁻¹⁴C (Amrad, Inc, USA) and 4.48 mg (NH₄)₂SO₄-N at 10% enrichment with ¹⁵N. The soil was sealed in the jar along with an alkali trap (10 mL 2N NaOH). In addition, two alkali traps were incubated without soil to measure background CO₂ levels. The soils were incubated at 21°C in darkness for up to 231 days.

5.2.5 Residual organic carbon and labelled carbon

Residual total OC and ^¹⁴C present in the soil at the time of destructive soil sampling was determined using the wet combustion method (Amato, 1983). Air dried soil (40°C) was further air-dried at 60°C and fine ground in a Tema Mill. Half a gram of soil was weighed directly into a digestion tube with approximately 1 g of potassium dichromate using a long stemmed funnel. Fifteen mL of combustion acid (3 parts concentrated H₂SO₄ : 2 parts 85% H₃PO₄) was
dispensed into the bottom of the tube. A glass rod (approximately 95 mm) with a stand was inserted into the digestion tube and a 10 mL glass vial containing 5 mL 2N NaOH was placed on the glass rod stand. The digestion tube was sealed with a Subaseal (registered to William Freeman Ltd, manufactured by Sigma-Aldrich, Inc., USA) immediately after constructing the apparatus. Each digestion block held 35 tubes containing soil samples, three blanks and one standard soil in duplicate. After assembling 40 digestion tubes, soils were kept at room temperature for 30 min while the digestion block pre-heated to 100°C. Tubes were placed in the block, allowed to heat to 130°C, kept at that temperature for 2 h and cooled in the block for 16 h. After cooling, the alkali vial was retrieved from the tube with the aid of forceps and covered with Parafilm (American Can Company, Greenwich, CT, USA) to avoid absorption of CO₂ from the atmosphere.

Alkali solutions were analysed for residual total CO₂-C and residual ¹⁴C and using the same scintillation and auto-titration methods and equipment described in Chapter 3. Residual ¹⁴C was expressed relative to the amount of ¹⁴C added to each sample at commencement of the experiment. Residual SOC was calculated by deducting the amount of residual glucose-C from the total amount of CO₂-C recovered in the alkali vial and expressing the remainder relative to initial OC as determined by Walkley and Black method (Walkley and Black, 1934) with a conversion factor of 1.2 applied as suggested by Nelson and Sommers (1982). For calcareous soils, the total amount of CaCO₃-C (naturally occurring plus amended CaCO₃-C) in each soil was deducted from the total CO₂-C recovered to give total residual SOC.

5.2.6 Residual total nitrogen and labelled nitrogen
The amount of total soil N and ¹⁵N present in the soil at the time of destructive sampling was determined by automated N and C analysis mass spectrometry (ANCA-MS). Air-dried soil (40°C) was finely ground in a Tema mill, further dried at 60°C and 50 mg was weighed into a tin foil capsule that was sealed using forceps to twist the foil. The tin foil capsules were combusted and the reaction products were separated by gas chromatography to produce a pulse of pure N for analysis of ¹⁵N by the mass spectrometer (20-20, Europa Scientific, Crewe, UK). One blank (foil only) and two standards with known quantities of ¹⁵N were
included in every 30 samples. Recovery $^{15}$N was expressed relative to the initial amount of $^{15}$N added to each soil sample. Residual soil N was calculated by deducting the amount of substrate N initially added to each sample from the total amount of N recovered and expressing the remainder relative to initial total soil N.

5.2.7 Analysis for recovery of labelled and unlabelled carbon dioxide-C

Alkali traps were collected from each jar and replaced every second day from day 1 to 7, on day 10, every 7 days from day 14 to 42, every 14 days from day 56 to 112, every 28 days from day 140 to 196 and on day 231. Samples were stored in a CO$_2$-free desiccator and total CO$_2$ content of alkali traps was determined by auto-titration within 24 h of sampling using the same procedure and equipment described in Chapter 3. Labelled CO$_2$-C was determined by the same method and equipment described in Chapter 3. The scintillation counter was operating at 88% efficiency and this was accounted for when calculating the total amount of recovered $^{14}$CO$_2$-C. Recovery of $^{14}$C was expressed relative to the initial amount of $^{14}$C present in each sample. In addition, the amount of $^{14}$C recovered at each sampling time was expressed as the daily rate of $^{14}$C mineralisation within each incubation period. Recovery of SOC was calculated by deducting the amount of $^{14}$C recovered from the total amount of CO$_2$-C recovered. Recovery of SOC was expressed relative both as a raw value (mg C) and relative to the initial amount SOC present in each soil type (mg C g$^{-1}$ SOC).

5.2.8 Analysis for net mineralisation of nitrogen and labelled nitrogen

Four replicates of each soil were destructively sampled and analysed to determine inorganic N content on days 0, 14, 56, 112 and 231. Immediately after sampling, each soil was passed through a 4 mm sieve and 10 g (moist weight) was analysed for NO$_3$-N and NH$_4$-N (total inorganic N) using the same procedure (extraction in 2N KCI) and materials described in Chapter 2. Daily net rates of N mineralisation were calculated by the following protocol $(N_t - N_{t-1}) / (d_t - d_{t-1})$ where N is the amount of inorganic N recovered, t is the time of sampling and d is the total number of days that the sample had been incubated prior to destructive sampling.
5.2.9  **Statistical analysis**

The proportion of labelled C that was mineralised and recovered in alkali traps was analysed for the effects of soil type, time and soil type x time interaction using two way ANOVAs in the statistical package SAS version 6.12 (SAS Institute Inc., 1996). Rates of net N mineralisation were also analysed using ANOVA in the same statistical package. Values were considered to be significantly different at $P < 0.05$.

5.3  **Results**

5.3.1  **Changes in soil calcium carbonate content and pH**

Analysing each soil type for CCE content and pH (H$_2$O) at each time of destructive sampling showed there was no significant change with time for any of the soil types (Table 5.2).

5.3.2  **$^{14}$Carbon mineralisation**

The total recovery of labelled C either as residual $^{14}$C in the soil or as mineralised $^{14}$C in the alkali traps, was near 100% for all four soil types at each sampling time (Fig 5.1). This indicated that little labelled C was lost from the system at anytime during the incubation and measurements of $^{14}$C mineralisation were accurate. In all soil types from Yorke Peninsula, there was a flush of $^{14}$C mineralisation in the first three days (Fig. 5.2). In Low CCEUA, the flush occurred between days two and five. The highest rates of $^{14}$C mineralisation occurred in the two to three day period with 6.51%, 8.07%, 8.10%, 8.19% of labelled C recovered from Low CCEUA, High CCE, Low CCE+ and Low CCE, respectively. These proportions of recovered labelled C did not differ between soil types. As the experiment progressed the rate of $^{14}$C mineralisation decreased significantly over time in all soil types ($P < 0.001$) (Fig 5.2). After 100 days incubation, the rate of $^{14}$C mineralisation was very low, there were no significant differences between soil types and the cumulative amount of C mineralised changed very little.
Table 5.2. Calcium carbonate content as measured by CaCO$_3$ equivalent (CCE) and soil pH for each soil type at each destructive sampling time.

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 56</th>
<th>Day 112</th>
<th>Day 231</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low CCEUA</td>
<td>Soil pH</td>
<td>8.3 (±0.3)</td>
<td>8.3 (0.4)</td>
<td>8.2 (0.5)</td>
<td>8.4 (0.5)</td>
<td>8.2 (1.2)</td>
<td>8.2 (0.7)</td>
</tr>
<tr>
<td></td>
<td>CCE %</td>
<td>1.6 (44)</td>
<td>1.4 (35)</td>
<td>1.6 (53)</td>
<td>1.7 (44)</td>
<td>1.5 (36)</td>
<td>1.5 (34)</td>
</tr>
<tr>
<td>Low CCE</td>
<td>Soil pH</td>
<td>7.7 (4.7)</td>
<td>7.6 (5.0)</td>
<td>7.6 (5.2)</td>
<td>7.7 (5.5)</td>
<td>7.6 (6.3)</td>
<td>7.6 (5.5)</td>
</tr>
<tr>
<td></td>
<td>CCE %</td>
<td>0.7 (87)</td>
<td>0.7 (84)</td>
<td>0.7 (83)</td>
<td>0.8 (92)</td>
<td>0.8 (59)</td>
<td>0.8 (91)</td>
</tr>
<tr>
<td>High CCE</td>
<td>Soil pH</td>
<td>8.3 (0.2)</td>
<td>8.3 (0.1)</td>
<td>8.2 (0.2)</td>
<td>8.4 (0.1)</td>
<td>8.3 (0.3)</td>
<td>8.2 (0.1)</td>
</tr>
<tr>
<td></td>
<td>CCE %</td>
<td>9.5 (17)</td>
<td>9.2 (8)</td>
<td>9.2 (16)</td>
<td>10.7 (16)</td>
<td>10.2 (15)</td>
<td>9.8 (14)</td>
</tr>
<tr>
<td>Low CCE+</td>
<td>Soil pH</td>
<td>8.1 (0.5)</td>
<td>8.1 (1.0)</td>
<td>8.1 (0.4)</td>
<td>8.2 (0.7)</td>
<td>8.2 (0.6)</td>
<td>8.1 (0.4)</td>
</tr>
<tr>
<td></td>
<td>CCE %</td>
<td>9.1 (7)</td>
<td>9.2 (6)</td>
<td>8.6 (8)</td>
<td>9.2 (6)</td>
<td>10.0 (11)</td>
<td>9.6 (6)</td>
</tr>
</tbody>
</table>

A Soil pH (H$_2$O 5:1). B Allison and Moodie (1965). *cv (%) is given in parentheses.
Figure 5.1. Total $^{14}$C recovered either directly from the soil (residual) or after conversion of CO$_2$ through mineralisation (mineralised) in four soils incubated for up to 231 days; (a) Low CCEUA, (b) Low CCE, (c) Low CCE+ and (d) High CCE.
Figure 5.2. The daily rate of $^{14}$C recovered as CO$_2$-$^{14}$C relative to initial $^{14}$C from four soils at 19 times during a 231 day incubation under controlled conditions.
Fig 5.3. The proportion of total $^{14}$C recovered as CO$_2$-$^{14}$C from four soils during a 231 day incubation period under controlled conditions. Standard error bars are not visible because they are too small to be distinguished from the point markers.

There was no discernible difference between soil types in the total amount of substrate C mineralised after only 14 days incubation (Fig. 5.3). However, by day 50 the difference between Low CCEUA and the other soil types was distinct. At the end of the incubation period the proportion of total $^{14}$C that had mineralised was 66.2%, 67.5%, 67.6% and 75.1% for Low CCE+, High CCE, Low CCE and Low CCEUA, respectively. The total amount of $^{14}$C mineralised in the soils differed between each soil type and with time, and there was a significant soil type x time interaction ($P < 0.001$) principally due to the relatively high rate of $^{14}$C mineralisation in Low CCEUA. When the soil types from Yorke Peninsula were analysed separately there was a distinct effect of time ($P < 0.001$) but no difference between soil types ($P = 0.187$) and only a trend towards a soil type x time interaction ($P = 0.069$).
Table 5.3. The cumulative proportion (%) of initial SOC recovered in the soil after incubation (Residual SOC) or from mineralisation (Mineralised C) at seven sampling times during an incubation period of 231 days.

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 56</th>
<th>Day 112</th>
<th>Day 231</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td><strong>Low CCEUA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual SOC</td>
<td>83.7 (6.1)</td>
<td>97.6 (8.3)</td>
<td>88.0 (10.7)</td>
<td>89.5 (5.4)</td>
<td>94.7 (11.0)</td>
<td>98.1 (3.3)</td>
<td>86.1 (7.0)</td>
</tr>
<tr>
<td>Mineralised C</td>
<td>0.0</td>
<td>0.0 (316.0)</td>
<td>0.5 (53.2)</td>
<td>0.8 (48.0)</td>
<td>3.1 (7.6)</td>
<td>5.6 (6.6)</td>
<td>7.7 (5.0)</td>
</tr>
<tr>
<td>Total</td>
<td>83.7</td>
<td>97.6</td>
<td>88.5</td>
<td>90.3</td>
<td>97.7</td>
<td>103.4</td>
<td>93.9</td>
</tr>
<tr>
<td><strong>Low CCE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual SOC</td>
<td>84.4 (6.9)</td>
<td>91.9 (8.1)</td>
<td>86.7 (5.9)</td>
<td>90.9 (3.4)</td>
<td>89.6 (3.8)</td>
<td>89.5 (3.8)</td>
<td>86.4 (3.9)</td>
</tr>
<tr>
<td>Mineralised C</td>
<td>0.0</td>
<td>0.1 (67.9)</td>
<td>0.5 (18.1)</td>
<td>0.8 (24.1)</td>
<td>2.1 (24.4)</td>
<td>3.3 (26.9)</td>
<td>4.9 (23.2)</td>
</tr>
<tr>
<td>Total</td>
<td>84.4</td>
<td>92.1</td>
<td>87.2</td>
<td>91.7</td>
<td>91.6</td>
<td>92.9</td>
<td>91.3</td>
</tr>
<tr>
<td><strong>High CCE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual SOC</td>
<td>96.8 (4.5)</td>
<td>105.6 (3.9)</td>
<td>91.3 (4.9)</td>
<td>94.7 (9.4)</td>
<td>98.5 (5.5)</td>
<td>101.3 (8.5)</td>
<td>90.0 (4.1)</td>
</tr>
<tr>
<td>Mineralised C</td>
<td>0.0</td>
<td>0.2 (31.5)</td>
<td>0.6 (7.5)</td>
<td>0.9 (5.2)</td>
<td>2.2 (8.5)</td>
<td>3.5 (8.0)</td>
<td>5.2 (9.3)</td>
</tr>
<tr>
<td>Total</td>
<td>96.8</td>
<td>105.8</td>
<td>91.9</td>
<td>95.6</td>
<td>100.7</td>
<td>104.8</td>
<td>95.1</td>
</tr>
<tr>
<td><strong>Low CCE+</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual SOC</td>
<td>95.2 (3.7)</td>
<td>90.6 (9.1)</td>
<td>108.7 (5.6)</td>
<td>97.7 (5.9)</td>
<td>99.3 (6.5)</td>
<td>104.6 (4.4)</td>
<td>99.9 (7.5)</td>
</tr>
<tr>
<td>Mineralised C</td>
<td>0.0</td>
<td>0.1 (40.0)</td>
<td>0.7 (13.7)</td>
<td>1.1 (12.0)</td>
<td>2.6 (9.0)</td>
<td>3.8 (4.7)</td>
<td>5.4 (4.4)</td>
</tr>
<tr>
<td>Total</td>
<td>95.2</td>
<td>90.8</td>
<td>109.4</td>
<td>98.8</td>
<td>101.9</td>
<td>108.4</td>
<td>105.3</td>
</tr>
</tbody>
</table>

*cv (%) is given in parentheses.
Figure 5.4. The daily rate of SOC recovered as \( \text{CO}_2 \)-C relative to initial SOC from four soils at 19 times during a 231 day incubation period under controlled conditions.
5.3.3 Soil organic carbon mineralisation

Recovery of SOC was primarily from the soil as opposed to mineralised SOC recovered in the alkali traps. Over 90% of SOC was recovered at most sampling times for all soil types (Table 5.3). As with recovery of labelled C, the recovery rates indicate that little SOC was lost from the system. Values over 100% may be explained by errors in sampling. After 231 days incubation the proportion of mineralised SOC ranged between 4.9% and 7.7% and difference between soil types was not significant.

Mineralisation of SOC was similar to mineralisation of labelled C in so far as their was a flush of mineralisation during the first three days (Fig. 5.4) and the rate of mineralisation decreased as the incubation period was extended (Fig. 5.5) \((P < 0.001)\). Maximum rates of SOC mineralisation occurred on day 1 for Low CCE, High CCE and Low CCE+, and between days two and three for Low CCEUA. The distinct difference between Low CCEUA and the other soils was also noted for labelled C mineralisation. Peak rates of SOC mineralisation did not differ significantly between soil types and were 1.18, 1.30, 1.47 and 1.51 mg C day\(^{-1}\) g\(^{-1}\) initial SOC for Low CCE, Low CCEUA, High CCE and Low CCEUA, respectively. Cumulative recovery was also expressed relative to initial SOC (Fig. 5.6). Expressing mineralised SOC as a function of initial SOC highlighted that a major factor influencing the total amount of SOC mineralised was the amount that was initially present. Expressed relative to initial SOC, the soil type Low CCEUA mineralised significantly more SOC than the other three soil types \((P < 0.001)\) over the same period of time. In addition, there was no significant difference in the amount of SOC mineralised between soil types from Yorke Peninsula regardless of the amount of CaCO\(_3\) present.

5.3.4 Net nitrogen mineralisation

Recovery of labelled N in each soil type was over 90% at most sampling times (Table 5.4). The proportion of total soil N recovered was near 100% in all soil types at all sampling times (Table 5.5). Variation in the proportion of total soil N recovered from each replicate was eight percent or less in all soil types at all sampling times. This variation was less than that found for recovery of labelled N.
Figure 5.5. Total SOC recovered as CO$_2$-C from four soils during a 231 day incubation period under controlled conditions.
Figure 5.6. Total SOC recovered relative to initial SOC (determined by Walkley-Black method) as CO$_2$-C from four soils during a 231 day incubation period under controlled conditions.
Table 5.4. Proportion of $^{15}$N recovered relative to the amount initially added as labelled ammonium sulphate ($\text{(NH}_4\text{)}_2\text{SO}_4$) to four soils after incubation for up to 231 days.

<table>
<thead>
<tr>
<th></th>
<th>Day 0 %</th>
<th>Day 1 %</th>
<th>Day 7 %</th>
<th>Day 14 %</th>
<th>Day 56 %</th>
<th>Day 112 %</th>
<th>Day 231 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low CCEUA</td>
<td>103.3 (15.5)</td>
<td>98.9 (15.3)</td>
<td>88.3 (16.4)</td>
<td>97.6 (6.2)</td>
<td>92.1 (7.2)</td>
<td>85.9 (7.4)</td>
<td>86.0 (14.2)</td>
</tr>
<tr>
<td>Low CCE</td>
<td>118.9 (15.2)</td>
<td>105.9 (26.5)</td>
<td>106.0 (10.7)</td>
<td>102.0 (11.3)</td>
<td>116.0 (21.2)</td>
<td>107.8 (17.1)</td>
<td>131.6 (7.8)</td>
</tr>
<tr>
<td>High CCE</td>
<td>92.8 (13.6)</td>
<td>99.0 (6.1)</td>
<td>99.0 (11.0)</td>
<td>89.2 (6.8)</td>
<td>101.5 (6.1)</td>
<td>106.2 (19.5)</td>
<td>95.9 (5.9)</td>
</tr>
<tr>
<td>Low CCE+</td>
<td>92.4 (3.3)</td>
<td>95.6 (14.6)</td>
<td>93.7 (9.2)</td>
<td>94.3 (16.8)</td>
<td>95.6 (7.5)</td>
<td>86.4 (5.7)</td>
<td>96.2 (14.1)</td>
</tr>
</tbody>
</table>

*cv (%) is given in parentheses.
Table 5.5. Proportion of the total soil N recovered relative to initial total soil N from four soils after incubation for up to 231 days.

<table>
<thead>
<tr>
<th></th>
<th>Day 0 %</th>
<th>Day 1 %</th>
<th>Day 7 %</th>
<th>Day 14 %</th>
<th>Day 56 %</th>
<th>Day 112 %</th>
<th>Day 231 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low CCEUA</td>
<td>100.7 (7.0)</td>
<td>100.3 (7.9)</td>
<td>100.1 (6.1)</td>
<td>101.5 (6.5)</td>
<td>96.5 (6.7)</td>
<td>98.8 (5.5)</td>
<td>100.6 (6.7)</td>
</tr>
<tr>
<td>Low CCE</td>
<td>98.7 (6.5)</td>
<td>95.4 (6.8)</td>
<td>93.3 (7.2)</td>
<td>96.3 (6.3)</td>
<td>95.1 (8.0)</td>
<td>94.3 (4.4)</td>
<td>103.2 (6.1)</td>
</tr>
<tr>
<td>High CCE</td>
<td>97.8 (3.0)</td>
<td>101.5 (5.0)</td>
<td>94.4 (5.6)</td>
<td>100.9 (4.5)</td>
<td>101.6 (6.7)</td>
<td>101.1 (5.4)</td>
<td>105.3 (5.3)</td>
</tr>
<tr>
<td>Low CCE+</td>
<td>102.5 (5.0)</td>
<td>98.5 (4.8)</td>
<td>99.7 (0.0)</td>
<td>100.1 (4.5)</td>
<td>100.4 (6.4)</td>
<td>99.0 (4.1)</td>
<td>103.5 (5.2)</td>
</tr>
</tbody>
</table>

*cv (%) is given in parentheses.
Figure 5.7. The daily rate of net N mineralisation relative to initial total N (TN) at four sampling times during a 231 day incubation period under controlled conditions.
The rate of net N mineralisation differed over time in all soils \((P < 0.001)\) and daily rates of net N mineralisation reduced as the experiment progressed (Fig. 5.7). Daily rates of net N mineralisation between sampling times were not significantly affected by soil type and there was no significant interaction between soil type and time.

However, the overall increase in inorganic N between commencement of the incubation and final sampling at day 231 did significantly differ between soil types \((P < 0.001)\). Final average amounts of inorganic N relative to initial total N were 21.2, 29.2, 32.4 and 40.5 mg N g\(^{-1}\) TN for Low CCE+, High CCE, Low CCE and Low CCEUA, respectively. After 231 days, Low CCE+ had significantly less inorganic N than all other soil types and High CCE had less inorganic N than Low CCEUA. There was no significant difference between the amount of net N mineralised in soil types Low CCE and High CCE.

### 5.5 Discussion

Lack of change in CCE content and pH \((\mathrm{H}_2\mathrm{O})\) during the experiment indicated that organic acids present in the soils did not react with the carbonate and caused CO\(_2\) to be evolved from the carbonates. Lack of change in pH and CCE content indicated that the amount of unlabelled CO\(_2\)-C present in the alkali traps was an accurate measure of SOC mineralisation. That is, unlabelled CO\(_2\)-C was not a combination of CO\(_2\) from SOC and carbonates. The proportions of initial SOC recovered from Low CCEUA, Low CCE and High CCE after incubation were similar to recovery rates of between 92% and 99% reported in other incubation studies (Baldock et al., 1994).

Recovery rates of labelled N near 100% indicated that little substrate N was lost from the soil during the incubation period. Some sampling times had coefficients of variation \((\text{cv} > 5\%)\) which indicated that there was some sampling error during processing to determine how much labelled N was present in the soil at the end of each sampling time relative to initial labelled N. Recovery rates for \(^{15}\text{N}\) were similar to rates reported for other \textit{in vitro} incubations. For instance, 88% (Ladd \textit{et al.}, 1977b) and 93% (Ladd \textit{et al.}, 1977a) for laboratory
incubations of at least 16 days. Ladd et al. (1977a) also observed that $^{15}$N recovery rates declined as incubation experiments continued even though denitrification was an unlikely event.

The initial flush of substrate C mineralisation shown for all soil treatments in this experiment was similar to that shown in the short-term experiment described in Chapter 3 and other C mineralisation studies mentioned earlier (Adu and Oades, 1978; Aulakh et al., 1991; Ajwa et al., 1998). The reduction in the mineralisation rates of substrate C over time that was observed for all soil types was also comparable with observations from other long term C mineralisation studies conducted in the laboratory (Martin, 1989; Muneer and Oades, 1989a; Ladd et al., 1992). In addition, the proportions of substrate C mineralised after 231 days incubation were similar to the proportions mineralised in other incubation studies. For instance, 56% to 66% of $^{14}$C labelled root material was recovered in alkali traps from sandy-loams after 152 days incubation (Martin, 1989) and 66% to 81% of glucose-C was recovered from Xeralfs after 120 days incubation (Muneer and Oades, 1989a). Incomplete mineralisation of substrate C in all soil treatments after the incubation period was completed has also been found in many other C mineralisation studies (Martin, 1989; Muneer and Oades, 1989; Ladd et al., 1992). The proportion of total SOC mineralised over the 231 day incubation was similar to the value obtained in another laboratory experiment using moist intact soil cores where 6.2% of SOC in a Haplustoll was mineralised after 84 days incubation in intact soil cores (Tracy et al., 1990).

Changes in net N mineralisation and immobilisation rates during the course of the incubation period were similar to those observed in other experiments where soils were inoculated with solutions that had high C : N ratios (Ladd et al., 1977b; 1992; Zagal and Perrson, 1994). That is, initially net N immobilisation following by net N mineralisation at the latter stages of the incubation. In this 231 day experiment, the difference in net N mineralisation rates over time was largely due to net N immobilisation occurring in the first 14 days of incubation and very low daily net N mineralisation rates occurring during the last 119 days of incubation. As with the current experiment, the initial flush of net N immobilisation in a calcareous clay (4.4%
CaCO₃) incubated at 25°F with glucose and KNO₃ occurred until day 16 (Ladd et al., 1977b). Also in common, the initial flush of net N immobilisation was followed by net N mineralisation and the rates of net N mineralisation became less as the experiment proceeded (Ladd et al., 1977b; 1992).

The effect of CaCO₃ content on ¹⁴C mineralisation supported laboratory and field work conducted earlier with glucose and CaCO₃ amended soils (Muneer and Oades 1989a; 1989b). Muneer and Oades (1989a) observed a 4% reduction in the total amount of ¹⁴C mineralised after 120 days whilst the equivalent treatment in the current experiment showed a 2.6% reduction in the total amount of ¹⁴C mineralised after 112 days incubation. Consideration of this current experiment in conjunction with the work of Muneer and Oades (1989a; 1989b; 1989c), led to the conclusion that CaCO₃ had a very small adverse affect on mineralisation of readily available C sources. Significantly less (35%) net N mineralisation in Low CCE+ compared with Low CCE extended the findings of Muneer and Oades (1989a) from glucose C mineralisation to the net result of substrate and soil N mineralisation. However, the lack of a significant difference between Low CCE and High CCE in the net proportions of N mineralised suggested that the differences observed would not occur in the field with naturally occurring CaCO₃.

Given the results with ¹⁴C and net N mineralisation, the lack of an effect of CaCO₃ content on SOC mineralisation was unexpected. Observations with SOC mineralisation showed that results for glucose C could not be extrapolated to less readily mineralised forms of OC such as those naturally present in the soil. This finding was inconsistent with the conclusions drawn from a field experiment on a Rhodoxeralf where ¹⁴C wheat straw (3.1 g wheat kg⁻¹ soil) was incubated in disturbed soil cores (Muneer and Oades, 1989b). However, results from this laboratory incubation did support a later experiment on the same soil type (Rhodoxeralf) with incorporated wheat straw (10 t ha⁻¹) incubated for up to four years (Baldock et al., 1994). Like this laboratory experiment, the four year field experiment showed that the presence of CaCO₃ did not retard mineralisation of SOC.
Taking the effects of CaCO₃ on substrate C, SOC and net N mineralisation together, led to the conclusion that C and net N mineralisation in the field would be unlikely to be significantly affected by the presence of CaCO₃ in the soil. Therefore N fertiliser models like that developed by Payne and Ladd (1993a; 1993b) would be expected to be equally effective in calcareous and non-calcareous soils. An exception to this conclusion may be highly calcareous soils where readily available OM sources are present such as demonstrated using the glucose substrate. The relative high proportion of ¹⁴C mineralised in Low CCEUA indicated that the soil had properties that caused substrate C to be more readily mineralised than in the soils from Yorke Peninsula. The same could be said for SOC mineralisation in Low CCEUA even when expressed relative to initial SOC content. Given that both Low CCEUA and Low CCE had minimal amounts of CaCO₃ this chemical soil property could not have caused the difference in C mineralisation rates between the soils with regards to the total proportions of ¹⁴C and SOC mineralised. The difference between Low CCEUA and the other soil types may have been due to Low CCEUA having a relatively low clay content (Table 5.1). As discussed in the literature review and Chapter 3, clay has been shown to form bonds with organic material and reduce C mineralisation through physical protection (Craswell and Waring, 1972; Gupta and Germida, 1988; Franzluebbers, 1999). However, no effect of low clay content was observed in the short-term incubation discussed in Chapter 3 even though short term incubations have shown that high clay content reduces C mineralisation rates (Franzluebbers, 1999).

An alternative explanation for the differences in SOC mineralisation rates between Low CCEUA and the other soil types may be the effect of SOC composition. In this experiment, SOC mineralisation was expressed relative to total initial SOC as determined by the method of Walkley and Black (1934). However, studies designed to differentiate between the various chemical forms of SOC have shown that not all SOC is equally mineralisable (Oades, 1995; Skjemstad et al., 1996). As discussed in the literature review, SOC can be divided into active and inert SOC (Skjemstad et al., 1996). Differences in the proportion of active SOC present in various soils has led to the conclusion that using total SOC values determined by the Walkley-Black method or by difference between total C and inorganic C may not provide an
accurate assessment of the amount of active SOC that is available for mineralisation in these soils (Skjemstad et al., 1996). Given the inconsistency between the effect of CaCO$_3$ on substrate C and the effect of CaCO$_3$ on net N mineralisation and SOC mineralisation, SOC composition in each soil may have played a role. That is, expressing SOC mineralisation relative to total initial SOC would be inappropriate if SOC composition differed between soil types. Depending upon the type of SOC present in each soil, SOC mineralisation may actually be reduced by the presence of CaCO$_3$ as was shown for substrate C mineralisation. A change in the effect of CaCO$_3$ on SOC mineralisation could modify the conclusions drawn from this long term incubation regarding the effect of CaCO$_3$ on N fertiliser models based upon OC content.

In summary, the results of this laboratory experiment indicate that N fertiliser prediction models do not need to account for a soil’s CaCO$_3$ content. However, the experiment also raised two questions. Firstly, was Ca-OM bridging (Muneer and Oades, 1989c) the mechanism responsible for the retardation of substrate C and net N mineralisation in the presence of CaCO$_3$? Secondly, did the composition of SOC differ between soil types thus affecting the amount of active OC present in each soil? These questions were investigated further through the experiments described in the following chapter.
6.1 Introduction

Soil organic carbon content is used as an index for estimating available soil N (Payne and Ladd, 1993a, 1993b). Estimating N fertiliser requirements through OC% relies upon one of two assumptions. Either all OC has the potential to be mineralised in a time frame relevant to agriculture or the same proportion of total OC is inert to mineralisation within all soil types. These assumptions may not necessarily be valid given not all organic compounds in soil are equally susceptible to decomposition. Soluble monomers such as amino acids and glucose are rapidly metabolised by microbes. Polymers such as polysaccharides are utilised at a slower rate due to their size (Oades, 1989) yet can still be incorporated into microbes and microbial products in the course of a cropping season. The effect of differences in OC composition between soils when comparative mineralisation studies are made was discussed in the previous chapter.

Other organic compounds are recalcitrant either due to physical protection through entrapment within aggregates or through covalent bonding, or due to chemical protection. Protection by entrapment has been demonstrated in C and N mineralisation studies which showed a flush of C and N mineralisation when soil aggregates were disrupted through a series of wetting and drying cycles (van Schreven, 1967; Adu and Oades, 1978) or sieving (Hassink, 1992). Physical protection through cation bridging has also been demonstrated in many studies where soils have been amended with cations. Dispersion studies have shown polyvalent cations bonded to clay and OM (Edwards and Bremner, 1967) and amending soils with Ca salts can lead to an increase in aggregate stability (Grant et al., 1992). Laboratory studies have shown that amending soils with Ca salts can lead to reduced C mineralisation (Muneer and Oades, 1989a and Chapter 5) and amending soil in the field with either gypsum or CaCO3 gave similar results (Muneer and Oades,
1989b). Muneer and Oades (1989c) proposed that reduced C mineralisation was due to Ca$^{2+}$ ions forming covalent bonds between clays and OM; a similar mechanism to that proposed by Edwards and Bremner (1967). Most studies on the effects of Ca$^{2+}$ ions upon decomposition of OM have involved amending soils with Ca salts. Relatively few studies have investigated whether findings in amended soils were equally valid for soils naturally high in calcium such as calcareous soils. Given the $^{14}$C mineralisation rates observed in the long term experiment discussed in the previous chapter, the topic warrants further investigation. Organic matter can also be chemically protected from microbial degradation through being within a molecular configuration that is difficult for microbes to decompose. Skjemstad et al. (1996) have shown that up to 30% of OC in an agricultural soil in south eastern Queensland was chemically protected in the form of char. Differing proportions of charcoal in the soils used in the long term experiment previously described may account for the inconsistency between the effect of CaCO$_3$ on $^{14}$C and SOC mineralisation.

This work had two aims. Initially, to determine whether higher amounts of OC are physically protected within aggregates in calcareous soils compared to non-calcareous soils and secondly to determine the proportions of OC in a calcareous soil and two non-calcareous soils which were chemically protected. Both aims were approached using a combination of high energy UV photo-oxidation and solid-state $^{13}$C nuclear magnetic reasonance (NMR) spectroscopy.

### 6.2 Materials and methods

#### 6.2.1 Soils

The soil used for the analysis presented in this chapter were amended and unamended soil from Yorke Peninsula (High CCE, Low CCE and Low CCE+) and soil from Roseworthy (Low CCEUA). Soils were stored for 4 months at 4°C between initial preparation and use in this study.
6.2.2 Sample preparation

Ten grams of each soil (4 soils x 4 replicates) were gently dispersed in 200 mL of RO water with 5 agate marbles on an end-over-end shaker for 16 h. Soils were dispersed by end-over-end shaking instead of the more aggressive sonification method in an attempt to maintain microaggregates formed through Ca-OM bridging. After agitation, samples were washed with RO water through 2 sieves arranged in series and separated into 3 size fractions (>200 μm, 200 - 53 μm, <53 μm). The >53 μm fractions were dried at 80°C and weighed. The <53 μm fraction was stored at 4°C in 600 - 1000 mL of water depending upon the sample. Initial organic carbon content of each <53 μm sample was determined by a modified chromic acid digestion method (Heanes 1984) which has been noted as being largely unaffected by the presence of CaCO₃ (Rayment and Higginson, 1992). For each of the 16 soil solutions, 5 mL was dispensed into a digestion tube. Five mL 0.17M sodium dichromate (Na₂Cr₂O₇) and 20 mL concentrated H₂SO₄ was added to each tube immediately before placing tubes into a digestion block pre-heated to 140°C. After heating for 30 min, tubes were removed from the block and 50 mL RO water was added. A narrow tube was inserted to the bottom of each digestion tube and air was bubbled through for 16 h whilst the solutions cooled. After 16 h, the volume of each tube was made up to 75 mL with RO water, tubes were sealed using a bung and inverted three time to mix the solution. Twenty mL of each solution was poured into a centrifuge tube that was centrifuged for 20 min at 2000 rpm. A subsample was decanted into a curvette and the solution's absorbance was determined at a wavelength of 600 nm using a Pye Unicam PU8600 UV/VIS spectrophotometer (Phillips). Values were converted to organic carbon content by calibrating against a series of 6 glucose standards (0 – 3 mL, 2 mg glucose mL⁻¹) that were included in each digestion batch.

6.2.3 Photo-oxidation

Duplicate aliquots of each soil suspension replicate were subjected to UV photo-oxidation treatment as described by Skjemstad et al. (1993). Aliquots containing 2 - 3 mg OC were transferred to quartz tubes and made to 20 mL. Prior to commencing the treatment, a stainless-steel cold finger water condenser was inserted into each quartz tube. The condensers prevented
the soil suspension from boiling during UV treatment. Throughout the period of UV exposure, samples were supplied with 50 mL min⁻¹ of air through a thin steel tube inserted to the bottom of each quartz tube. The air ensured that the soil particles remained suspended throughout the treatment and facilitated the oxidation process. Soil suspensions were exposed to UV radiation (2.5 kW Hg vapour lamp) for 0.5, 1.0 and 2.0 h. Following UV photo-oxidation, each sample was prepared for OC determination by transferring to a centrifuge tube, adding 0.5 mL saturated Al₂(SO₄)₃ to flocculate the soil particles and centrifuging at 850G for 15 min. After centrifugation, the sample was transferred to a digestion tube, the volume was adjusted to 5 mL and the sample analysed following the method of Heanes (1984) as previously described.

6.2.4 ¹³Carbon Nuclear Magnetic Resonance analysis of ultra-violet photo-oxidised material

Soil samples and the <53 μm fractions were analysed by solid-state ¹³C cross polarisation with magic angle spinning (CP/MAS) NMR spectroscopy. The 4 replicate fractions were bulked for all treatments leading to NMR analysis. The OC content of the bulked replicates was determined by the method of Heanes (1984) prior to UV photo-oxidation treatment. NMR analysis requires at least 10 mg C and therefore 18 aliquots of each of the <53 μm fractions were subjected to UV photo-oxidation for 2.0 h and combined. Samples were treated with 2 % hydrofluoric acid (HF) to remove magnetic materials over a period of 7 days (Skjemstad et al., 1994). The removal of magnetic materials was deemed necessary because Fe is known to be a source of interference that prevents useful spectra from being produced (Skjemstad et al., 1994). After HF treatment, samples were frozen in liquid nitrogen and freeze dried (Dynavac unit at <1 Torr pressure) for 40 h. Six grams of each whole soil were also prepared for NMR analysis by the same HF treatment as described for the soil fractions. The samples (one fraction and one whole soil per soil type) were packed into a zircon rotor and CP/MAS ¹³C NMR spectra were obtained using the same instruments and conditions described in (Skjemstad et al., 1996).

6.2.5  Acid treatment

A volume of each replicate (between 6 and 15 mL) containing 2 - 3 mg OC was adjusted to pH <4
with weak SO₂ solution (6% w/w) to dissolve all CaCO₃. Samples were left to stand overnight and centrifuged at 850 G for 30 min. Samples were not flocculated with saturated Al₃(PO₄)₃ because Al³⁺ ions have been shown to prevent degradation of OM by the UV photo-oxidation method (Skjemstad et al., 1993). The supernatant was removed, 1 mL of Na hexa meta-phosphate added and the pH of each sample re-adjusted to pH 8 with 0.1 M NaOH. All samples were treated with a short, vigorous hand shaking to assist the suspension of soil particles but minimising disruption of the original aggregates. After treatment, samples were subjected to UV photo-oxidation for 2 h as described above.

6.3 Results

6.3.1 Soil fractionation

Initially, the 4 samples were fractionated by size into 3 classes; >200 µm, 200 - 53 µm and <53 µm. The proportion of soil materials in each soil fraction differed. In the low CCE UA sample, 42% of soil material was present in the <53 µm fraction and the other 3 soils averaged 71%. In the <53 µm fraction, the amount of OC present as a proportion of the initial total OC differed among soils (Table 6.1).

6.3.2 Ultra-violet photo-oxidation treatments

All 4 soil types were exposed to UV photo-oxidation for set periods of 0, 0.5, 1.0 and 2.0 h. The overall effect of exposing the soil suspensions to UV photo-oxidation was to reduce the amount of OC present in the sample. There was a significant difference in the amount of OC remaining in the soil fractions after the 4 exposure times with the amount of OC present declining as the UV photo-oxidation treatment was prolonged ($P < 0.001$). Variability in OM susceptibility to degradation was demonstrated for the four <53 µm soil fractions used in the study. Within the 4 soil fractions, the rate of degradation declined the longer the samples were exposed to UV photo-oxidation. Rates of loss ranged from 2.21 to 1.01 mg OC min⁻¹ and from 0.50 to 0.16 mg OC min⁻¹ for between 0 and 0.5 h and 1.0 to 2.0 h of UV photo-oxidation, respectively. There were no
significant differences \( (P = 0.28) \) between any of the replicates within a soil type.

Table 6.1. Mean amount of soil material (mg) and OC (mg) in each size fraction from four x 10 g of each soil.

<table>
<thead>
<tr>
<th>Site</th>
<th>&gt;200 µm (mg)</th>
<th>200 - 53 µm (mg)</th>
<th>&lt;53 µm (mg)</th>
<th>Total OC in &lt;53 µm (mg)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High CCE</td>
<td>698 (*24.0)</td>
<td>2495 (2.3)</td>
<td>6808 (1.8)</td>
<td>229.2 (3.6)</td>
<td>70.45</td>
</tr>
<tr>
<td>Low CCE</td>
<td>503 (11.6)</td>
<td>2304 (7.1)</td>
<td>7193 (2.5)</td>
<td>180.3 (9.8)</td>
<td>67.03</td>
</tr>
<tr>
<td>Low CCE+</td>
<td>499 (7.7)</td>
<td>2063 (6.1)</td>
<td>7438 (1.6)</td>
<td>163.6 (7.9)</td>
<td>82.63</td>
</tr>
<tr>
<td>Low CCE UA</td>
<td>840 (5.4)</td>
<td>4939 (1.6)</td>
<td>4221 (2.4)</td>
<td>130.0 (6.2)</td>
<td>64.05</td>
</tr>
</tbody>
</table>

*cv (%) are given in parentheses.

As noted in Table 6.1, the <53 µm fractions from the 4 soil types differed in their initial OC contents. After 2 h UV photo-oxidation, soil samples High CCE, Low CCE and Low CCE+ all had similar OC contents of 108.6, 105.3 and 101.7 mg OC, respectively whilst the fourth soil sample, Low CCEUA, had 56.9 mg OC remaining. The effect of the treatment on the 4 soil types can be compared by examining the decline in OC contents proportional to the initial OC contents of the soil fractions. The OC contents in the <53 µm fractions of soil types Low CCE and Low CCE+ declined by similar proportions, 58.4% and 62.2%, respectively, whilst the OC contents of High CCE and Low CCE UA declined by 47.4% and 43.7%, respectively (Fig 6.1).

6.3.3 Photo-oxidation with a sulphur dioxide solution pre-treatment

Pre-treating the <53 µm soil fractions with sulphur (SO₂) solution prior to UV photo-oxidation treatments was expected to reduce the OC contents of the soil fractions further in any soil sample where OM was being physically protected within aggregates formed by Ca²⁺ ions and/or clays. To determine whether this was the case, comparisons were made between the amount of OC
remaining in suspensions of <53 µm fractions exposed to UV photo-oxidation for 2 h with and without pre-treatment with SO₂ solution.

Figure 6.1. OC present in the <53 µm fraction of four soils (10 g samples of each soil) after exposure to UV photo-oxidation for up to 2 h. Results expressed as a percentage of total OC in <53 µm fraction.

The amount of OC remaining in the <53 µm fractions of the 4 soils was significantly less in the samples subjected to treatment with SO₂ solution prior to UV photo-oxidation than that remaining after photo-oxidation without pre-treatment (Fig 6.2). There was no significant difference between replicates (P = 0.058). The amount of OC remaining in the <53 µm suspensions after the dual
treatments were highest for High CCE and Low CCE+ (Table 6.2). The OC remaining in the <53 µm fractions after treatment with SO₂ solution and UV photo-oxidation is assumed to be biologically inert, either in a chemically inert form like char or physically protected within clay aggregates. Based upon these assumptions, the OC contents measured after the dual treatments can be used to calculate the amount of active OC in the <53 µm fractions and the maximum amount of active OC in the whole soil (Table 6.2), assuming that no inert OC was present in the >53 µm fractions.

Figure 6.2. Effect of two treatments; 2 h exposure to UV photo-oxidation with a SO₂ pre-treatment (Treatment 2) and without a SO₂ pre-treatment (Treatment 3) compared to the original amount of OC present in the <53 µm fraction of four soils (10 g samples) (Treatment 1).
Table 6.2. Amount of OC (mg) present in fractions derived from 10g of whole soil.

<table>
<thead>
<tr>
<th>Site</th>
<th>Initial TOC(^a) (mg)</th>
<th>OC in &lt;53 (\mu m) fraction after dual treatments(^b)</th>
<th>Max. active OC in whole soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>High CCE</td>
<td>325</td>
<td>79 (14.0)</td>
<td>247 (4.5)</td>
</tr>
<tr>
<td>Low CCE</td>
<td>269</td>
<td>63 (5.6)</td>
<td>206 (1.7)</td>
</tr>
<tr>
<td>Low CCE+</td>
<td>198</td>
<td>75 (2.2)</td>
<td>124 (1.3)</td>
</tr>
<tr>
<td>Low CCE UA</td>
<td>203</td>
<td>27 (10.5)</td>
<td>176 (1.7)</td>
</tr>
</tbody>
</table>

\(^a\) mg OC determined by chromic acid digestion (Heanes 1984). \(^b\) UV photo-oxidation and SO\(_2\) treatments. * cv (%) are given in parentheses.

The amount of OC remaining in the fractions after SO\(_2\) solution and UV photo-oxidation treatment expressed relative to the amount of OC remaining after UV photo-oxidation alone are 73%, 74%, 60% and 47% for High CCE, Low CCE+, Low CCE and Low CCEUA, respectively. This form of expression assumes that all OC degraded by UV photo-oxidation treatment alone is also degraded when the fractions are subjected to a pre-oxidation treatment with SO\(_2\) solution. That is, any reduction in OC after the dual treatments is additional to the reductions in OC content measured after UV photo-oxidation only. Expressing the OC remaining after dual treatment in this form shows that an additional 13.1%, 16.5%, 23.5% and 22.5% of High CCE, Low CCE+, Low CCE and Low CCEUA, respectively, were degraded after fractions were pre-treated with SO\(_2\) acid.

6.3.4 \(^{13}\)Carbon cross polarisation magic angle spinning Nuclear Magnetic Resonance spectroscopic analysis

The types of OC present in the 4 soils was assessed using solid state \(^{13}\)C CP/MAS NMR analysis after HF treatment. NMR analysis was also conducted on <53 \(\mu m\) fractions that had been exposed to 2 h of UV photo-oxidation. After UV photo-oxidation treatment, the NMR spectra showed OC which remained either due to being physically or chemically protected (Fig 6.3).
Figure 6.3. $^{13}$C NMR spectra for the four soils as whole soils and the <53 μm fraction after 2 h UV photo-oxidation (A: Low CCE UA, B: Low CCE, C: Low CCE+, D: High CCE)
spectra (Fig 6.3) were divided into functional groups at "natural valleys" (Skjemstad et al., 1996) and used to estimate the proportions of each functional group present in each soil (Table 6.3). The proportions of OC in the 6 carbon groups are presented in Table 6.4.

Table 6.3. The percentage of total OC in the six functional groups identified through NMR spectroscopy for four whole soils treated with HF

<table>
<thead>
<tr>
<th>Site</th>
<th>Ketonic/aldehyde</th>
<th>Carbonyl</th>
<th>O-aryl</th>
<th>Aryl</th>
<th>O-alkyl</th>
<th>Alkyl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>High CCE</td>
<td>2</td>
<td>15</td>
<td>5</td>
<td>25</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>Low CCE</td>
<td>2</td>
<td>13</td>
<td>5</td>
<td>26</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>Low CCE+</td>
<td>1</td>
<td>15</td>
<td>5</td>
<td>26</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>Low CCE UA</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>15</td>
<td>39</td>
<td>28</td>
</tr>
</tbody>
</table>

Table 6.4. The percentage of total OC in the six functional groups identified through NMR spectroscopy for four <53 µm fractions treated with HF after 2 h of UV photo-oxidation

<table>
<thead>
<tr>
<th>Site</th>
<th>Ketonic/aldehyde</th>
<th>Carbonyl</th>
<th>O-aryl</th>
<th>Aryl</th>
<th>O-alkyl</th>
<th>Alkyl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>High CCE</td>
<td>2</td>
<td>11</td>
<td>6</td>
<td>46</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Low CCE</td>
<td>1</td>
<td>11</td>
<td>5</td>
<td>43</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Low CCE+</td>
<td>2</td>
<td>14</td>
<td>5</td>
<td>49</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>Low CCE UA</td>
<td>2</td>
<td>11</td>
<td>4</td>
<td>33</td>
<td>26</td>
<td>23</td>
</tr>
</tbody>
</table>
Char estimates from the aromatic carbon group were corrected for residual lignin (Skjemstad et al., 1996) and Bloch decay (Skjemstad et al., 1999b).

Lignin corrections were conducted using equations developed by Skjemstad (unpublished). The equations are for full, partial or zero corrections:

**Full correction**  Applied if lots of lignin present  Charcoal C = Φ - (1.7 x Φ - O)

**Partial correction**  Applied if a little lignin present  Charcoal C = Φ - (Φ - O)

**Zero correction**  Applied if no lignin present  Charcoal C = Φ + (Φ - O)

where Φ is the size of the peak in the Aryl region (145 - 110 ppm) and (Φ - O) is the size of the peak in the O-Aryl region (165 - 145 ppm).

The choice of which lignin correction equation to apply to each spectra is subjective with spectra being judged relative to each other and spectra from other soils.

Corrected char estimates for whole soils and the <53 μm fractions after UV photo-oxidation are given in Table 6.5.

6.4 Discussion

6.4.1 Degradability of organic matter

High energy UV photo-oxidation provides a means of quantifying decomposable OC because the process can degrade all but the most chemically recalcitrant or physically protected organic moieties. Degradation of OC by UV oxidation in the 4 soil fractions did not occur at a uniform rate over 2 h. Degradation of OC in the 4 soil fractions occurred at a rate 4 - 6 times faster during the first 0.5 h than the last 1.0 h. This agrees with the concept that OC is protected against photo-
Table 6.5. The percentage of OC calculated as char from NMR spectroscopic measurements of UV photo-oxidation treated (2 h) fractions after corrections for lignin and Bloch decay.

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Char C in whole soil fraction (%)</th>
<th>Char C in &lt;53 µm soil fraction after UV (%)</th>
<th>Char C in &lt;53 µm fraction (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High CCE</td>
<td>27</td>
<td>81</td>
<td>*88</td>
</tr>
<tr>
<td>Low CCE</td>
<td>30</td>
<td>75</td>
<td>79</td>
</tr>
<tr>
<td>Low CCE+</td>
<td>32</td>
<td>84</td>
<td>86</td>
</tr>
<tr>
<td>Low CCE UA</td>
<td>17</td>
<td>59</td>
<td>34</td>
</tr>
</tbody>
</table>

*milligrams of char C was determined from total OC determined by wet combustion

oxidation to varying degrees within the soil suspension (Skjemstad et al., 1996). Initially, readily decomposable OC is degraded; that is, OC in free OM, OC attached to the outside of soil aggregates and OC which is not chemically protected. When OM attached to the outside of soil aggregates is dislodged by exposure to UV photo-oxidation, the soil aggregates may start to break down and expose OM that was previously physically inaccessible to the UV radiation (and presumably microbial attack). Thus, where a higher proportion of OM is physically protected inside aggregates or a greater quantity of chemically protected OM is in the soil fraction, the slower the rate of OM degradation and the higher the proportion of OC remains after long exposure to UV photo-oxidation.

6.4.2 Retention of organic carbon after ultra-violet photo-oxidation treatment

The amounts of OC remaining after UV photo-oxidation relative to the initial OC in the <53 µm fractions was higher (range 44 - 62%) than retention rates found in soil fractions from some other soils subjected to similar treatments (Skjemstad et al., 1993; 1996). The high OC retention rate in the 4 soil fractions used in this study was partly due to the soils being dispersed by shaking rather
than sonification. Five whole soils which were repeatedly ultrasonicated prior to being divided into size fractions by sieving retained 11 - 43% OC in the clay fractions and 17 - 51% OC in the silt fractions after 2 h of UV photo-oxidation (Skjemstad et al., 1993; 1996). The 3 soil types, High CCE, Low CCE and Low CCE+, retained similar amounts of OC after 2 h of UV photo-oxidation despite having different initial OC in the <53 μm fractions. Similar amounts of remaining OC indicated that the variability in total OC content in the <53 μm fractions was due to differences in readily decomposable OC and the amount of recalcitrant OC was similar among the soil fractions.

Soil type Low CCEUA had a significantly lower amount of initial OC remaining after 2 h of exposure to UV photo-oxidation than the other soils. The relatively low retention of OC indicates that OC in Low CCEUA is relatively decomposable and less physically or chemically protected than in the other soils. This result may partially explain why soils from Yorke Peninsula, the source of soils Low CCE and High CCE, had higher OC% than expected. The crops primarily grown on the Yorke Peninsula sites are cereals which have a high lignin content and burning residue is an historically common practice which leads to high levels of char.

6.4.3 Physical protection of organic matter within micro-aggregates

The two non-calcareous soils retained less OC after dual treatments with SO₂ solution and UV photo-oxidation than when subjected to UV photo-oxidation alone. Degradation of OC in non-calcareous soil fractions after acid treatment indicated that OM was physically protected within micro-aggregates and that the acid treatment, through dissolution processes, increased disaggregation. OM studies using UV photo-oxidation have shown that OM can be trapped inside soil micro-aggregates that are cemented together with minerals such as iron and aluminium oxides and hydroxides (Skjemstad et al., 1993), even after the soils are dispersed by repeated ultrasonification. Upon treating soil fractions with HF, Skjemstad et al. (1993) found that a higher proportion of the OC was degraded after 4 h exposure to UV photo-oxidation. In the same study, flocculating soil fractions with Al³⁺ prior to UV photo-oxidation treatment was shown to increase OC retention. The <53 μm fractions examined in this study are even more likely to contain OM entrapped within micro-aggregates than those fractions analysed by Skjemstad et al. (1993;
1996) because the <53 μm fractions were dispersed using a gentle agitation method specifically for the purpose of retaining aggregates formed by Ca²⁺-clay and OM-clay interactions.

6.4.4 Physical protection of organic matter in the presence of calcium carbonate

Two of the soils used in this study contained free CaCO₃. Based upon previous studies by Muneer and Oades (1989a) the presence of CaCO₃ can be expected to inhibit OM degradation through OM being protected within Ca²⁺-OM aggregates. The High CCE soil that was naturally high in CaCO₃, retained a significantly lower proportion of initial OC than a similar soil from the same region, Low CCE, after 2 h of UV photo-oxidation. Furthermore, retention of OC after 2 h of UV photo-oxidation did not significantly differ between Low CCE+ and Low CCE. The similar retention results obtained in the Low CCE soil and amended Low CCE soil indicate that the mere presence of CaCO₃ did not affect degradability of OM in the <53 μm fractions, at least to photo-oxidation.

The theory that OM was physically protected in the calcareous soils through calcium bridging was further investigated by exposing the calcareous soils to SO₂ solution prior to UV photo-oxidation treatment. As with the non-calcareous soils, the results from the dual treatments showed that the two calcareous soils, Low CCE+ and High CCE, retained less OC than when subjected to UV photo-oxidation alone. Therefore, OM was physically protected within clay-OM aggregates prior to disruption by acid treatment. Higher amounts of OC were retained in the calcareous soils than the non-calcareous soils after dual treatments. The difference in OC retention between the calcareous and non-calcareous soils is not explained through differences in clay content or type in the soil; that is, the calcareous soils were no more likely to have OM-clay aggregates than the non-calcareous soils. The lack of a positive relationship between clay content and OC retention was demonstrated in the Low CCE and Low CCE+ soil samples where the amended soil retained more OC than the non-amended calcareous soil despite having a lower clay content. Higher OC retention in the calcareous soils after treatment with SO₂ solution is assumed to be due a two-step process involving breaking and forming covalent bonds and interaction between OM and...
clay. In the calcareous soils, the acid released the CO$_2$ from CaCO$_3$ leaving Ca$^{2+}$ ions and increasing the soils’ exchangeable Ca content. The acid also reacted with the OM-clay aggregates to free the OM. Immediately after being freed the Ca$^{2+}$ ions formed covalent bonds with the OM thus leading to higher OC retention than in the non-calcareous soil subjected to the same treatment.

The difference in OC retention between the calcareous and non-calcareous soils indicate that the presence of CaCO$_3$ per se does not result in OC being less susceptible to degradation by UV photo-oxidation. Rather the presence of CaCO$_3$ in a soil may be accompanied by high exchangeable Ca. Soils with high exchangeable Ca may have slower OC decomposition rates because any free OM will rapidly bond with the excess Ca$^{2+}$ ions. The effect of high exchangeable Ca$^{2+}$ on OM retention has been demonstrated in pot and field studies. The exchangeable Ca of the non-calcareous Urrbrae fine sandy loam used by Muneer and Oades (1989a, 1989b, 1989c) in their studies on the effect of Ca on C mineralisation rates was only 4.62 cmol(+) kg$^{-1}$ before application of CaCO$_3$ and gypsum. Muneer and Oades (1989b) reported that C mineralisation was decreased more in gypsum treatments than CaCO$_3$ treatments and attributed the difference to gypsum being the more soluble of the two compounds. Seven days after application, the water-soluble Ca (mg 100 g$^{-1}$ soil) was 4.8 and 12.5 in the 0 - 8cm layer and 9.5 and 42.0 in the 8 - 20 cm layer for CaCO$_3$ and gypsum treatments, respectively.

The amount of OC which is physically protected by the Ca ions derived from 10% CaCO$_3$ can be determined by comparing OC retention in the Low CCE and Low CCE+ soil samples. The amount of OC lost upon treating soils with acid in addition to UV was 42.4 mg and 26.9 mg for Low CCE and Low CCE+, respectively. These losses are equivalent to 23.5% and 16.5% of original OC in the <53 μm fractions. Thus a greater proportion of OC was lost from the soil fraction due to acid treatment when CaCO$_3$ was not present. Assuming the difference in OC retention between the two soil fractions was solely due to physical protection within newly formed Ca-OM aggregates, the proportion of OC protected was 7%. The 7% difference is equivalent to 11.45 mg of OC.
protected within Ca-OM aggregates in the <53 μm fraction of the low CCE+ soil sample. Given that the total OC present in the low CCE+ soil was 1.98% the physically protected OC is equivalent to a minimum of 5.8% of total soil OC. The value of 5.8% is a minimum because only the <53 μm fraction was examined. Additional OM could have been protected in >53 μm fractions either through calcium bridging (Muneer and Oades, 1989c) or physical coatings (Chouliaras et al., 1975). White coatings were observed on OM in the >200 μm fractions using light microscopy. The white coating matched the descriptions given by Chouliaras et al. (1975), however they were not chemically identified.

6.4.5 Chemical protection of organic carbon

Physical protection of OC in the 4 soils does not account for all the OC remaining after 2 h of exposure to photo-oxidation. After dual treatments, 27.5 - 78.6 mg OC remained in the <53 μm fractions depending upon the soil type. Using NMR, Skjemstad et al. (1996) have shown that OC which is highly resistant to degradation via UV photo-oxidation is likely to be highly aromatic. Char has been shown to have one major peak at 130 ppm (Shafizadeh, 1984) and OC present after photo-oxidation and HF treatment has been shown by scanning electron microscopy to have a morphology consistent with char and to give a major peak at 130 ppm (Skjemstad et al., 1996). Analysis of the 4 soils used in this study by NMR after photo-oxidation and HF treatment showed between 59% and 84% of the non-degraded OC was probably char.

The proportions of OC shown to be protected as char in the 4 soils (range 17 - 32%) are consistent with findings from other soil studies. Using the same UV photo-oxidation technique, Skjemstad et al. (1996) found 10 - 40% of the total OC in a number of soils was chemically protected and gave a major peak at 130 ppm. In the same analysis, Skjemstad et al. (1996) found between 64 and 97% of the char present in the whole soil was in the <53 μm fraction. The 4 soils used in this study yielded similar results.
6.5 Implications for mineralisation studies and nitrogen fertiliser recommendations

The C and N mineralisation study described in Chapter 5 on the same 3 soil types in controlled conditions for 231 days showed significant differences ($P < 0.001$) between all soils in terms of the amount of total OC mineralised, with Low CCEUA mineralising the greatest amount of OC relative to initial total native OC (Fig 6.4). Given that not all OC is in an active form, the amount of C mineralised would be better expressed relative to total active OC. Active OC was calculated for each soil by totalling the amount of OC present in the $>53 \mu m$ fraction and amount of OC remaining after 2 h of UV photo-oxidation. Expressing cumulative total OC mineralisation relative to active OC as determined by UV photo-oxidation and NMR analysis gave the same order for the three unamended soils (Low CCE UA > Low CCE > High CCE) but the difference between Low CCEUA and the Yorke Peninsula soils was narrowed (Fig 6.5). The concept of expressing OC mineralisation as a function of active OC instead of total OC was also applied to the values obtained in the 231 day incubation for SOC mineralisation. The concept was applied because unlike total OC and net N mineralisation, SOC mineralisation was shown to be unaffected by the presence of CaCO$_3$. Expressing SOC mineralisation relative to active SOC may have been expected to negate this inconsistency. However, expressing SOC mineralisation relative to active OC did not show that the presence of CaCO$_3$ reduced SOC mineralisation rates (Fig 6.6). Indeed, SOC mineralisation increased dramatically in the soil that was amended with CaCO$_3$. These findings contradict those of Muneer and Oades (1989a; 1989b) but support a five year field study on the same soil by (Baldock et al., 1994) and support the general conclusions from this study.

Results obtained for the Yorke Peninsula soils showed that 11.45 mg of OC from a 10 g soil sample was physically protected. Assuming no OC in the $>53 \mu m$ fraction is protected by calcium, these results indicate that only 1 mg OC g$^{-1}$ soil is physically protected within Ca-OM aggregates. The main discrepancy between total OC and active OC is due to the presence of char. Loss of 1 mg OC g$^{-1}$ soil to short term C cycling through physical protection with Ca-OM will not greatly affect N fertiliser recommendations derived from soil OC. Thus these findings do not support the
hypothesis that greater quantities of N fertiliser need to be applied to calcareous than non-calcareous soils to compensate for calcareous soils having lower quantities of active OC than non-calcareous soils.

Figure 6.4. Total OC mineralisation over 231 days of laboratory incubation as a function of total native OC determined by difference between total C recovery and CaCO₃-C recovery.
Figure 6.5. Total OC mineralisation over 231 days of laboratory incubation as a function of total active OC. Active OC was derived by modifying total OC to account for charcoal and recalcitrant OC as determined by UV photo-oxidation and NMR analysis.
Figure 6.6. SOC mineralisation over 231 days of laboratory incubation as a function of total active OC.
CHAPTER 7
Conclusions and recommendations

7.1 Conclusions

Broadly speaking, this study had two main aims. Firstly, to determine if the presence of CaCO$_3$ in soil was the reason behind soil from Yorke Peninsula having relatively high OC contents, given the local farming practices. Secondly, what effect did the composition of the soils' OC have on the mineralisation rates?

7.1.1 Effect of calcium on net nitrogen mineralisation

As discussed in the literature review, rates of net N mineralisation tend to be higher in laboratory incubations than field incubations due to the soil being disturbed, aerated and organic material freed from soil aggregates. This difference puts in doubt the validity of using net N mineralisation rates measured in the laboratory in models used to predict net N mineralisation rates in the field. The field experiment conducted in the beginning of this study (Chapter 2) was designed to measure net N mineralisation rates in undisturbed soils with differing CaCO$_3$ contents incubated at field temperature and moisture content. Results from the field experiment showed that measuring net N mineralisation using the method described by Stein et al. (1987) was not suitable at the sites selected on Yorke Peninsula and at Turretfield. Spatial variability at each site was too high despite intense soil sampling. The coefficients of variation were over 100% due to net N mineralisation rates being close to zero. High variability resulted in there being no significant difference between the sites and little difference between the Oat and Fallow treatments at each site.

Due to the inconclusive results obtained by conducting field incubations, further experiments were conducted in the laboratory under controlled conditions. The long term experiment (Chapter 5) was conducted with soil that was sieved and repacked to field bulk density and inoculated with (NH$_4$)$_2$SO$_4$ and glucose. Given results from the technical experiments (Chapters 3 and 4), this sample preparation technique was considered to produce net N and C mineralisation rates as close as practicable to those obtained in intact soil cores. The net N
mineralisation rates obtained in the long term experiment were significantly affected by soil type with the presence of CaCO₃ (High CCE) reducing net N mineralisation. This result suggested that the presence of CaCO₃ may reduce net N mineralisation rates in the field thus slowing the turnover of OM. However, given (NH₄)₂SO₄ was not distinguished from the less readily available soil N, the measured reduction in net N mineralisation must be considered in conjunction with the effect of CaCO₃ on C mineralisation.

7.1.2 Effect of calcium on gross carbon mineralisation

According to earlier experiments (Muneer and Oades 1989a; 1989b), the presence of CaCO₃ reduces mineralisation of glucose, a readily available C source. However, these earlier experiments had only considered CaCO₃ amended soil, that is, not naturally occurring CaCO₃ as found in soils from Yorke Peninsula. The long term experiment (Chapter 5) with naturally calcareous soil supported earlier work with glucose C being mineralised at a slightly lower (2%) rate in High CCE than in Low CCE.

As an extension of the studies by Muneer and Oades (1989a; 1989b), this long term incubation also investigated the effect of the presence of CaCO₃ on SOC mineralisation. In contrast to results for glucose-C mineralisation, the presence of CaCO₃ at 10% did not affect the mineralisation rate of SOC. This lack of effect contradicted expectations based upon results with glucose-C but supported findings from earlier SOC mineralisation studies with carbonate amended soil (Baldock et al., 1994). Differing responses to the presence of CaCO₃ for glucose C and SOC suggested that CaCO₃ only significantly reduces mineralisation of readily available C, and probably N, sources.

7.1.3 Mechanism for the effect of calcium carbonate on carbon mineralisation

The mechanism responsible for the observation that CaCO₃ reduced C mineralisation rates was investigated through UV photo-oxidation and NMR spectroscopy after the soils were acid treated (Chapter 6). The analysis supported, and gave further details of, the mechanism of Ca-OM bridging originally proposed by Peterson (1947) and revised by Muneer and Oades (1987c). The acid treatment caused OM to be freed from OM-clay aggregates and
simultaneously freed Ca\textsuperscript{2+} ions from CaCO\textsubscript{3}. The freed Ca\textsuperscript{2+} ions immediately formed covalent bonds with the OM thus leading to the physical protection of the OM. This analysis showed that the presence of CaCO\textsubscript{3} in soil is not an indicator per se of the likelihood that OM will be physically protected by Ca-OM bridging. The main chemical property influencing the occurrence of Ca-OM bridging is the presence of high exchangeable Ca in the soil.

7.1.4 Chemical protection of organic matter

Ultra-violet photo-oxidation and NMR spectroscopy showed that a large proportion (up to 32\%) of SOC in the \(< 53\mu m\) fraction was in the form of charcoal, an inert form of C that cannot be mineralised in a time frame relevant to agriculture (Chapter 6). The high proportion of charcoal in the Yorke Peninsula soils is probably due to a series of vegetation burning practices that occurred in the region including the common farming practice of burning stubble, original land clearing by burning native vegetation (Kelly, 1962) and patch burning as practiced by Aboriginal people prior to European settlement. The presence of charcoal explains why total OC contents were unexpectedly high at 270 and 325 mg kg\textsuperscript{-1} for Low CCE and High CCE, respectively using the method of Heanes (1984). Large amounts of charcoal in soil would not be an issue if all soils had the same proportion of TOC as inactive OC. A simple correction could be made to translate TOC to active OC if required. Further, the amount of inactive OC in soils would not have any impact on mineralisation rates when compared across several soils. However, the soils used in Chapter 6 had various proportions of inactive OC ranging from 17\% to 32\% charcoal. Hence, the amount of mineralisation that can be predicted based upon active OC differs with each soil. This study clearly showed that accounting for the inert OC in soil is important for any work comparing rates of SOC mineralisation between soils.

7.1.5 Implications for nitrogen fertiliser applications

The effect of the presence of CaCO\textsubscript{3} on net N and glucose C mineralisation was small, there was no effect of CaCO\textsubscript{3} on SOC mineralisation and the effect of Ca-OM bridging was less than 10\%. Given these results, models designed to predict N fertiliser requirements do not need to account for CaCO\textsubscript{3} content in the soils included in these studies. There may be some
requirement to increase N fertiliser applications in soils with particularly high exchangeable Ca (> 35 cmol(+)/kg⁻¹). However, further studies on soils with high exchangeable Ca would need to be conducted before formulating any recommendations in this matter.

The implications of the presence of charcoal in soils from Yorke Peninsula on the validity of OC-based N fertiliser prediction models are more substantial. The Walkley-Black method (Walkley and Black, 1934), a method of measuring OC that is commonly used in commercial laboratories, has been shown to detect charcoal as well as active OC (Skjemstad et al., 1999a). The method of Heanes (1984) has also been shown to detect active OC and charcoal. Thus OC contents measured by the Walkley-Black method overestimates the amount of active OC present in soils with charcoal and subsequently underestimates the required amount of fertiliser N. This underestimation can be demonstrated using the N fertiliser prediction model developed by Payne and Ladd (1993a, 1993b) for use on soils throughout South Australia and the low to medium rainfall area of Victoria. For instance, consider the following scenario.

A cereal is grown in a soil with 1.8% total SOC to achieve a target grain yield on 4 t ha⁻¹ at 10% protein. According to the tables constructed by Payne and Ladd (1993b), the crop requires 62 kg N ha⁻¹ to achieve this target. The prior crop was a cereal that yields 3 t ha⁻¹. After harvest, the stubble was grazed and one third was ingested. In this situation Payne and Ladd (1993b) estimate that 11 kg N ha⁻¹ has been removed from the system via immobilisation. Thus the total amount of fertiliser N required to achieve the target yield and protein content is 73 kg N ha⁻¹. However, if only two thirds of the SOC is active OC, the active OC content is reduced to 1.2%. Given the lower OC content, the amount of N removed from the system is still 11 kg N ha⁻¹ and the amount of N required to achieve the target yield and protein content is 109 kg N ha⁻¹ (Payne and Ladd, 1993b). Thus, the total amount of N required by the crop is increased from 73 kg N ha⁻¹ to 120 kg N ha⁻¹.

At present, the UV photo-oxidation method is the only chemical method that can distinguish between active OC and inert OC. The Walkley-Black method has been shown to be an invalid
alternative (Skjemstad and Taylor, 1999). The proportion of charcoal detected using the Walkley-Black method has been shown to be dependent upon the size of the charcoal particles, therefore no standard correction factor can be applied to account for detected charcoal. However, the UV photo-oxidation method is too expensive and laborious to be used as a standard commercial procedure. Overcoming the limitations of using OC as a basis for N fertiliser recommendations in soils with charcoal requires that a more commercially applicable method be developed.

7.1.6 Recommendations for future research, development and extension

As a result of this research, I propose that several other related experiments be conducted along with development and extension strategies. They are as follows:

- Experimentation to document the effects of soil disturbance on gross N mineralisation using labelled N in laboratory conditions with destructive sampling every day for the first 14 days incubation.
- Long term (about 250 days) experiments be conducted with several naturally and artifically calcareous soils to measure gross N mineralisation rates and compare with non-calcareous soils. Ideally, confounding factors such as SOC content and clay content need to be kept constant or examined as distinct treatments.
- Experiments designed to define how much exchangeable Ca needs to be present in soil before Ca-OM bridging effects SOM decomposition and C mineralisation rates.
- Develop a rapid and cost effective method for measuring active SOC for use in OC based fertiliser recommendation models applied to charcoal laden soils and soils with high amounts of exchangeable calcium.
- Incorporate into related research an extension module designed to alert land managers to the issue that high SOC content in black soils or soils with high exchangeable Ca may be overestimating available SOC and subsequently soil health.
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