Effect of Phosphorus Fertiliser on Soil Organic Matter Composition of Hill Country Pasture

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ABSTRACT

Soil organic matter is important as storage for carbon and nutrients, supporting soil structure, and as a filter for pollutants entering the soil ecosystem. The recovery of soil organic matter in depleted soils can take decades, or even hundreds of years. It has been assumed that in non-eroding pasture, soil carbon levels either increase or not change over time. However, some recent studies have suggested that fertiliser addition to pasture soils may contribute to decreases in soil carbon content. My hypotheses were:

1. As P fertiliser loadings increase the soil carbon content and C:N ratio will decrease.
2. Changes in C pools will be greater in the more active pool (readily available carbon, and microbial biomass carbon) within the soil total carbon

The study was undertaken at a long term fertiliser trial, established in 1980, at the Whatawhata Hill Country Research Station west of Hamilton, New Zealand. The fertiliser trial has P fertiliser application rates maintained since 1984. Olsen P, total C, total N, labile carbon, respirable carbon, specific respiration rate, microbial biomass C, microbial quotient, mineralised N, microbial biomass N, microbial N quotient, and mineralised N per microbial biomass nitrogen, C:N ratio, and soil pH were measured on soil samples collected from 12 paddocks with six P fertiliser loading (0, 10, 20, 30, 50, 100 kg P ha^{-1} yr^{-1}).

As expected, the available P (Olsen P) increased significantly ($P<0.001$) with increasing P fertiliser application rate. Total carbon, labile carbon, and total nitrogen all decreased significantly ($P<0.05$) with increasing P fertiliser application. No significant relationships were found between P fertiliser and respirable carbon, microbial carbon, microbial (C) quotient, microbial specific respiration, microbial nitrogen, microbial (N) quotient, mineralised N, or C:N ratio. The first hypothesis was rejected as the C:N ratio did not change with increased P fertiliser application. However, both C and N decreased with increased P fertiliser application. The second hypothesis was, therefore, accepted in part because there was a decrease in labile carbon (readily available carbon) and total carbon, with P fertiliser application, but no relationship was evident for the respirable carbon and microbial biomass.

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CHAPTER 1

INTRODUCTION

1.1. BACKGROUND – The Role of Fertilisers and Soil Organic Matter in New Zealand Agriculture

Soil conservation and soil health have become increasingly important in the context of environmental sustainability of agriculture because farming impacts both the farm environment and the wider environment. With fertiliser use and other technological inputs, high crop yields are possible on soils with low organic matter. However with limited land suitable for agriculture, and a limit to energy-intensive fertiliser inputs, the maintenance of soil organic matter has been identified as the most important property determining the state of soil health (Gregorich *et al.,* 2006).

During the initial stages of land use change from forest or cropped land to pasture in New Zealand, topsoil C and N have been reported to have increased mainly as a result of increased fertility (Walker *et al.,* 1959, Jackman, 1964a), and have been shown to accumulate organic matter and plant nutrients associated with the organic fraction (Broadbent *et al.,* 1964). As a drive to continually increase production, pasture management in New Zealand in the past two decades has undergone rapid increase in intensification mainly by use of higher animal stocking rates and fertiliser inputs (MacLeod and Moller, 2006).

Soil fertility varies across New Zealand but soils were naturally low in nutrients. Hence, phosphatic fertilisers and other essential soil nutrients have been commonly

used to supply the essential elements not immediately available in unfertilised soils. The 10 year period (1975 – 85) resulted in 1 million tonnes of fertiliser applied annually to New Zealand hill country, particularly in the North Island (Gillingham *et al.,* 1990). Over a 17 year period (1987 – 2004), fertiliser use on pastures and crops had significantly risen from 1.1 million Mg yr^{-1} (1987) to 3.2 million Mg yr^{-1} (2004), and with a 10-fold increase reported for N fertiliser use over the 20 years (Sparling and Schipper, 2004). A large portion (52 %) of N inputs to land for New Zealand, estimated at 503 Gg (for 2001), came from nitrogen fixation by pasture legumes, mainly white clover, compared to 24 % from N fertiliser (Parfitt *et al.,* 2006).

The increase in pasture legumes (typical of high fertility pasture) and hence N fixation and transfer to grasses, is mainly encouraged by the application of phosphatic fertilisers (Morton & Roberts, 1999). Phosphorus is an element considered as a macro-nutrient essential for plant nutrition. A deficiency of P constrains the ability of a plant to complete its "vegetative or reproductive stage of its life cycle" (During, 1984). Phosphorous, is thus provided for pasture development and maintenance through superphosphate fertiliser application (Morton & Roberts, 1999). However, with increased nutrient loadings through fertiliser use, there is still not much information as far as the fertiliser's effects on soil organic C and N (Schipper *et al.,* 2007).

Soil organic matter (SOM) was often described as a complex mixture of living organisms, decomposing and remains of dead organisms, nonhumic and humic substances (Tan, 2005) and contains elements of carbon, sulphur, and organic P (Jackman, 1964a). SOM controls many soil properties and cycling of nutrients, provides for storage of nutrients in the soil and contributes to soil structure, which helps reduce erosion and improves the infiltration of water and gases into the soil. Soil organic matter is also important for: enhancing the buffering capacity of soils, retaining pollutants that may otherwise be released into air or water, ease of trafficking and tillage, and for ecosystem support (Sparling *et al.,* 2006; Loveland and Webb, 2003).

The type and amount of SOM may be influenced by land-use, soil type, climate and vegetation. However, there is concern that changes in soil organic carbon (SOC) concentrations have occurred under pastures receiving intensive fertiliser inputs (During 1984; Loveland and Webb, 2003). Decline of organic matter was a concern on all land uses that affected 14-36 % of the 511 sites representative of 98 % of New Zealand's land area (Sparling & Schipper, 2004). A decrease in soil organic matter could cause a serious decline in soil quality, and thereby decrease the productive capacity of agriculture (During 1984; Loveland and Webb, 2003) and further diminish the soil's capacity for environmental protection (Sparling *et al.,* 2006).

Unlike the rapid recovery that can be achieved for soil chemical, and some soil physical characteristics, through fertiliser application and tillage, the recovery of soil organic matter in depleted soils can take decades, or even hundreds of years (Gregorich *et al.*, 2006).

1.2. CHANGES IN SOIL ORGANIC MATTER IN PASTORAL AGRICULTURE

In the past, it has been assumed that SOM in non-eroding pasture has stable carbon both in total amounts and in composition (including Tate *et al.,* 1995). However, few studies have begun to find potential negative effects of intensification of pastoral soils including nutrient and soil losses. Recent studies (including Lambert *et al.,* 2000, Bellamy *et al.,* 2005, and Schipper *et al.,* 2007) have further found unfavorable decreases in C and N under permanent pastures where there is much land intensification. Lambert *et al.,* (2000) found a decrease by 200 kg C ha^{-1} yr^{-1} (about 20 g C m^{-2} yr^{-1}) within the 0-7.5 cm topsoil in pasture that received superphosphate application. Schipper *et al.,* (2007) studied changes in C and N throughout areas with intensive pastoral agriculture in New Zealand and found that within the top 1m depth of the soil, total C decreased by on average 2.1 kg C m^{-2} in the last 20 years or so.

Similarly total N lost 0.18 kg N m^{-2} on average. On an annual basis, C losses were estimated at 106 g C m^{-2} y^{-1} and N losses at 9.1 g N m^{-2} y^{-1} (Schipper *et al.*, 2007).

High levels of organic matter (total C, total N, and mineralisable N) occur in the top 10 cm of pasture topsoils compared to that under long-term indigenous vegetation. Nitrogen (total N and mineralisable N) were typically greater under pastures than under other land uses since conversion of forests to clover-based pastoral agriculture and the use of increased P loadings (Sparling *et al.*, 2004). The continuing accumulation of N under pastures however could be limited in particular as soil C content on many New Zealand pastures have been observed to be either at steady state (Tate *et al.*, 1995) or decreasing on several high fertility pastures (Lambert *et al.*, 2000; Schipper *et al.*, 2007). The soil C content regulates the accumulation and storage rate of N and the time (years) remaining for which soil would reach its maximum storage. When soil C: N ratio reaches a steady state, net N immobilisation would not be occurring and N loss by nitrate leaching would result (Schipper *et al.*, 2004).

With organic C content assumed to be at steady state, and N accumulating under pasture soils, the C:N ratio was found to have declined (Sparling and Schipper, 2002). The average C:N ratios observed had decreased from 16.2 under indigenous forests, to 11.3 under dairy pastures (Sparling *et al.*, 2004). The C:N ratio can decrease in pasture under the increasing use and application of fertilisers because P fertiliser would lead to more N fixation hence providing more inputs of N to soil and into organic matter (Schipper *et al.*, 2004). With recent observations of soil C content declining, this would also decrease the C:N ratio. However, it is unclear whether fertiliser addition to pasture soils leads to a decrease in soil carbon content, and what carbon fractions might be affected within the soil.

1.3. THE WHATAWHATA FERTILISER TRIAL SITE SELECTED

Subsidies on fertiliser to many hill country farms were withdrawn in the mid 1980's which resulted in an initial substantial fall of fertiliser use followed by a gradual and marked increase again in fertiliser use (Sparling *et al.,* 2004; MacLeod and Moller, 2006). Much research information was required by farmers to assist in enabling them to make sensible decisions on efficient fertiliser application to maintain pasture or animal production (Gillingham *et al.,* 1990). The Whatawhata research station, located east of Raglan and west of Hamilton contains a large scale phosphate fertiliser trial established in 1980, and has been maintained ever since, as a result of the farmer's need for fertiliser research information.

My research was carried out at the Whatawhata fertiliser trial farm situated within the Whatawhata research station (Figure 1.1). The Whatawhata site was selected because there is a record of different P fertiliser applications on the pastures soils. This fertiliser trial site provided an opportunity to test recent findings of C losses under long term agricultural intensification through increased nutrient P loading, and to investigate any changes in organic matter fractions.

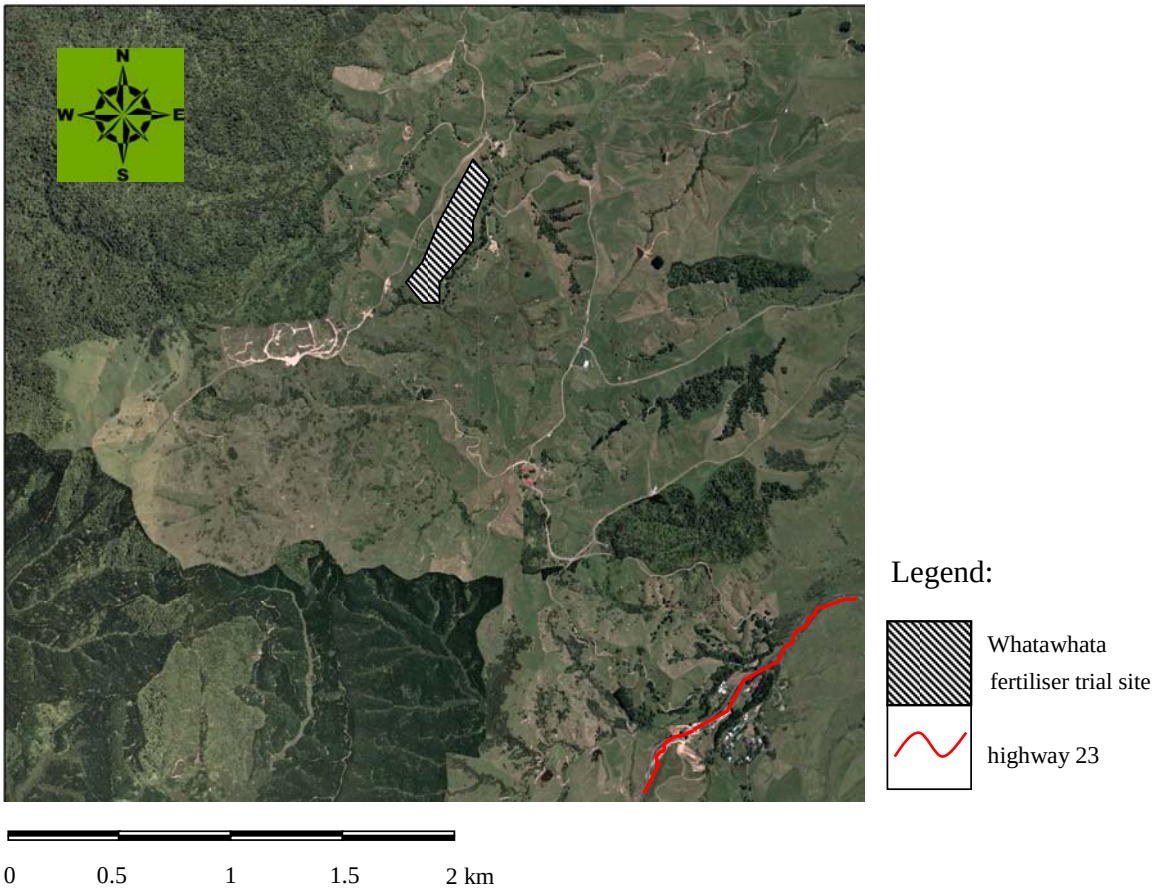

Figure 1.1: *Fertiliser trial site (estimate) within the Whatawhata research station.*

1.4. THESIS OBJECTIVES

The main objective of my thesis was to investigate the effects of fertiliser (P) on soil organic matter (SOM) content and the storage of nutrients in organic fractions (N, C, P). It was hypothesised that:

- As P fertiliser increases C:N ratio will decrease.
- Changes will be greater in the more active pool (readily available carbon, and microbial biomass carbon) than in the soil total carbon pool.

This study sought to contribute to enhancing the understanding of the effects of long term fertiliser application on soil organic matter and the implications for soil quality, sustainable farm production, and for wider environmental protection. The study sought to provide data on the environmental effects of fertiliser application with implications for improved management practices for a more environmentally sustainable agriculture.

1.5. CONTEXT OF THE STUDY

The study was based on the effects of a long term (23 years) P fertiliser trial on soil organic matter content under grazed pasture in New Zealand's north island temperate hill country soils. The study measured plant available P (Olsen P), total C and N, C:N ratio, and fractions of SOM under different fertiliser applications, in the top 7.5 cm of the topsoil.

CHAPTER 2

CHANGES IN SOIL ORGANIC MATTER UNDER FERTILISATION: LITERATURE REVIEW

2.1. SOIL ORGANIC MATTER

2.1.1. Definition and Significance

Soil organic matter (SOM) is the combination of plant and animal components that has been changed to the extent that it no longer contains its original structural form (Oades, 1989). SOM contains living organisms, and dead organisms that are partly decomposed and decomposed plant and animal remains. Below ground components of the plant in grasslands and tundra may reach 75-98 % of plant biomass (Fogel, 1985). SOM comprised both nonhumic substances, and humic substances (Tan, 2005). The non-humic substances are the metabolic products of organisms and include substances such as carbohydrates, amino acids, and lipids. The humic substances which comprise 50 % to 85 % of the total organic matter content include humic acids and fulvic acids, and are high molecular weight compounds that are synthesised by soil microorganisms (Tan, 2005). SOM may often be referred to as humus, and is an amorphous dark coloured material (McLaren and Cameron, 1996). Soil organic matter contains carbon (C), sulphur (S), and organic P (OP) in fractions assumed to be relatively constant (Jackman, 1964). Carbon in soil is the largest actively cycling C pool in terrestrial ecosystems. The soil C to a depth of 1 m, contains approximately 1500-2000 Pg C in different organic forms (Amundson, 2001).

Organic matter levels vary between soils and are influenced in variations of soil forming factors including climate (temperature and moisture), soil acidity, drainage conditions, inorganic nutrients, soil parent material, and human activity. In New Zealand, the majority of mineral soils have topsoil organic matter levels that range from about 3 to 20 % (Mclaren & Cameron, 1996).

Soil organic matter is a dynamic fraction of soil continually undergoing physical and chemical changes that result from decomposition and mineralisation processes within the soil. Normally carbon dioxide (CO_2), water (H_2O), nutrients, and inorganic and organic acids are the end products from the decomposition and mineralisation processes (Tan, 2005). The pool size or soil organic matter content is a "balance between addition and decomposition rates (turnover rates)" (Blair *et al.,* 1995) or "in a state of dynamic equilibrium between the processes of degradation and accumulation" (Cresser *et al.,* 1993). Agricultural management practices can have consequences of apparent changes "in both the pool size and turnover rate of SOM, carbon and therefore, nutrients" (Blair *et al.,* 1995).

Organic matter in soils is an important soil constituent influencing the physical, chemical and biological properties of soils and the cycle of nutrients (Tan, 2005) and in maintaining soil quality (Percival *et al.,* 2000). Organic matter enhances the water-holding capacity of soils in particular when soil has coarse texture (Cresser *et al.,* 1993), and the development of stable soil structures by promoting soil aggregation (Tan, 2005). SOM also provides essential nutrients for plants including N and S; and is the source of food and energy for microorganisms, which have crucial roles in the many biochemical processes in soils such as ammonification, nitrification, N-fixation, and nutrient cycling (Tan, 2005).

2.1.2. Soil Organic Matter Pools

SOM or C has been subdivided into pools in an attempt to identify small changes occurring in total SOM that have been difficult to detect because of the generally high background levels and natural soil variability (Blair *et al.,* 1995).

Initially soil organic matter can be subdivided mainly into three fractions:

(i) *Active*, containing plant, animal, and microbial residues that are decomposing;

(ii) *Living*, containing living soil biota or biomass made up of microorganisms, animals and plant roots; and

(iii) *Recalcitrant or passive*, containing organic matter that is chemically and physically resistant to biodegradation

The radiocarbon ages or half lives range from less than 1 year for the easily decomposable pool (contains easily oxidisable components), approximately 5 to 25 years for the biomass (living soil biota) pool (Loveland and Webb, 2003; Schloter *et al.,* 2006), and more than 2,500 years for the resistant or chemically stabilised pool (Jenkinson and Powlson, 1981). Over 90 % of SOM is in the resistant forms, nevertheless carbon continually flows from one pool to another (Cresser *et al.,* 1993). Studies have been carried out on the fractionation of the major pools that further determined soil organic matter content in the forms of natural ^{13}C abundance (Balesdent *et al.,*1990), microbial biomass C (Sparling, 1992), and labile C (Lefroy *et al.,* 1993).

The three main pools generally comprise non-living and living (Schloter *et al.,* 2006) or ‘inert’ and ‘active’ fractions respectively (Loveland and Webb, 2003). The sizes of the ‘active’ and ‘inert’ fractions are important for the rate of SOM turnover (Loveland and Webb, 2003).

The active pool of SOM which is the plant and animal residues decomposing and imparted to the soil in the preceding 5 years (or at the most 25 years), comprises a

small percentage of the total soil organic carbon and contains all forms of decomposing organisms including microorganisms (Schloter *et al., 2006*). The SOM pool is also the source of many essential plant micronutrients (Loveland and Webb, 2003). Fine roots and mycorrhizas are the major contributors to returning organic matter to the soil rather than leaf and branch litter (Fogel, 1983).

The non-living pool or fraction is composed of materials of different ages and origins which includes the humified fraction (Schloter *et al.,* 2006). The humified pool of SOM regulates different aspects of soil quality which includes the outcome of ionic and non-ionic compounds, soil cation exchange capacity and the permanent stability of microaggregates (Herrick and Wander, 1997). The 'inert' pool or the older SOM generally does not provide a nutritional contribution to crops.

The ratio of the short-lived SOM and the long-term SOM is assumed to be relatively constant (with time) when SOM is at or close to equilibrium (Loveland and Webb, 2003). However such equilibrium may take decades to establish and can be significantly affected by short-term agricultural practices (Loveland and Webb, 2003).

a. Soil Carbon

Only a small portion of carbon from the total global source of carbon (more than 10^{19} kg) is actively responsible for the changes in the carbon cycle, while most of the global carbon is either being stored in sediments, as carbonate in oceans and in igneous rocks and fossil fuels (Bohn, 1976). The active carbon pool comprises carbon stored in living organisms, in the atmosphere, and in soil organic matter. The mass of carbon in soil organic matter, globally, amounts to approximately 3 x 10^{15} kg, which is about five times the size of the atmospheric pool (Bohn, 1976), and approximately double that in living plants within soils (McLaren and Cameron, 1996). In New Zealand, the amounts of soil C (0-1 m depth) typical for grassland and forest sites, ranged from 44 to 268 t ha^{-1} (Tate *et al.,* 1995).

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The land-based carbon cycle involves conversion of atmospheric carbon dioxide to plant material by the process of photosynthesis followed by decomposition of plant and animal remains into the soil. During decomposition, carbon transformations are facilitated by microbial activity, oxidising carbon to carbon dioxide which is returned to the atmosphere. Some carbon may be further assimilated by a plant as carbonate or bicarbonate ions or leached from the soil and may eventually reach the ocean. Annually, large carbon movements and interchange from one phase to another take place in the land cycle including the movement of 10 % of carbon from plants, and 5 % of carbon from soil organic matter (McLaren and Cameron, 1996). In New Zealand, annual input of C in the topsoil (0-23 cm) averaged to 3 t for grassland soils, lower than that in forest soils (Tate *et al.,* 1995).

Total carbon in soils is the carbon that comes from both organic and inorganic C. The organic C component exists within the soil organic matter fraction, while inorganic C is mainly found in carbonate minerals. Unlike organic C that is present in all agricultural soils, inorganic C does not persist in some soils because of its termination during formation of carbonate minerals initially present in some parent materials. When inorganic C is lacking, a total C analysis can be used to measure organic C in soils. Organic C existing within the soil organic fraction contains microorganisms, residues of plants and animals at various stages of decomposition, the more stabilised "humus" derived from residues, and elemental forms of C such as carbonized compounds including charcoal, graphite and coal (Nelson *et al.,* 1996). Soil organic C accumulation is controlled by the chemical stabilisation of organic matter, but relates poorly to clay content (Percival *et al.,* 2000). The proportion of carbon relative to nitrogen and phosphorus in the organic matter of mineral soils is in the ratio of approximately 110:9:1 by weight (Allison, 1973). For example, every 1 t of C stored could store about 100 kg of N, hence extra C accumulated in soil will have the benefit of providing for additional N storage (Schipper *et al*., 2004). Soil carbon (C) is determined as a common indicator of the sustainability of agricultural systems and changes can occur in the total and active (labile), C pools (Blair *et al.,* 1995).

b. Labile Carbon

The labile carbon is the active, decomposable component of soil organic matter that is mostly found in the 'humic' pool, and includes the active microbial biomass and the partly decomposed plant material (Tate *et al.,* 1995). The labile C comprises a larger pool than soil microbial biomass alone, and has been found in soils sampled in deep profiles indicating its mobility and a source that may also be responsible for denitrification and methonogenesis processes (Blair *et al.,* 1995). Labile C represents approximately 93 % of the total C (0-23 cm depth) in New Zealand's indigenous forests and grasslands, and has a turnover period of approximately 8-40 years (Tate *et al.,* 1995). Labile C is often determined to show if there are any changes in the lability (estimate of turnover rate) of soil carbon which is also a measure of sustainability of agricultural systems (Blair *et al.,* 1995). Labile C was observed to have a strong positive relationship with temperature (Tate *et al.,* 1995).

c. Soil Microbial Biomass

The soil microbial biomass represents a minor fraction of both organic and inorganic nutrient pools in the majority of ecosystems, and is considered as the "living fraction" of organic matter (Jenkinson and Ladd, 1981). The soil microbial biomass omits macrofauna (larger than 5 x 10^3 μm^3) and plant roots (Jenkinson and Ladd, 1981). Bardgett *et al.,* (1997) observed on hill grassland soil, about 50 % of microbial biomass and 40-70 % of its activity within the surface 0-5 cm soil, and reduced amounts at lower depths (5-10 cm). The study also found that removal of sheep grazing from grasslands (with brown earth and podzolic soils) was found to significantly reduce microbial biomass and activity in the surface soil.

Carbon and nitrogen microbial mediated processes such as mineralization and immobilization are significant as they underpin soil functions and fertility (Schloter *et al.,* 2006), global C change, and soil organic matter turnover (Horwath *et al.,* 1994). Because soil microbial biomass has a relatively fast rate of turnover of 1-2 years (Sparling, 1992), it has been used as a subpool of SOM, and an important indicator of the changes of organic matter than the total soil organic matter content (Anderson and Domsch, 1989; Sparling 1992; Blair *et al.,* 1995), and can be used to provide an early

detection of changes in soil conditions as well as the direction of change, that may be gradually occurring in the total soil organic matter (Sparling 1992; Schloter *et al.,* 2006). Measurements of microbial size and activity provide a more sensitive measure and indicator of changes in soil resulting from changing management practices (Brookes *et al.,* 1985; Sarathchandra *et al.,*1988; Sparling, 1992; Haynes, 1999). The microbial carbon (C_{mic}), and the microbial quotient (C_{mic}/C_{org} ratio), where the microbial carbon is divided by soil organic (or total) carbon (C_{org}), provide a responsive index and important measures for monitoring soil organic matter changes as a consequence of land management (Ross and Cairns,1982; Anderson and Domsch, 1989). Typically, microbial quotients are in the range of 1 to 5 % (Sparling, 1992). Changes in the microbial quotient provide an indication of organic matter inputs to soils, how efficient organic matter is being transformed to microbial C, losses of C from the soil, and the fixing of organic C by the soil mineral fractions (Sparling, 1992). Under continuous grass/clover pasture of 5 years, the microbial quotient was observed to decline with depth, indicating the lower proportion of readily available carbon substrate with depth (Haynes, 1999).

The ratio of soil basal respiration to microbial biomass, known as the microbial metabolic quotient, specific respiration rate, or qCO_2 is increasingly being used as an alternative measure of changes in microbial biomass as a result of ecosystem disturbance based on Odums' theory of ecosystem succession (Wardle and Ghani, 1995). When the qCO_2 declines, it reflects the increase in the efficiency of the soil microflora at conserving C, and occurs during succession and following recovery from disturbance, as equilibrium conditions are approached. Schipper *et al.* (2001) measured increased in qCO_2 and microbial biomass still at 20 years of vegetative succession stage following recovery from volcanic deposition and at 52 years of vegetative succession stage following recovery from land slips. The qCO_2 usually declines when pH, clay content and amounts of microbial biomass increase (Wardle and Ghani, 1995). The qCO_2 tended to increase with soil depth, indicating a decline in the proportion of readily available substrate with increased depth (Haynes, 1999). There are also limitations in using qCO_2 e.g. it can be insensitive to distinguish between the effects of disturbance and stress (Wardle and Ghani, 1995).

A modeling approach that examined carbon and nitrogen cycling in the plant/soil/microorganisms/humic substances, calculated the turnover of microbial C for each microbial group as:

$$\text{Turnover of microbial C} = \frac{\text{microbial death (C)}}{\text{biomass (C)}}$$

(McGill *et al.,* 1981).

On average, the microbial population had a turnover of 5.5 of which fungal activity dominates with a turnover rate of 7.7 generations per year while most of the bacterial population only regenerated once in a year (McGill *et al.,* 1981).

The diversity of the microbial community and its interaction with the diversity of plants (primary producers) provide key functions that are the basis of all ecosystems (Loreau, 2001), and thus have follow-on effects for agricultural management (Schloter *et al.,* 2006). Bacteria and fungi exist in very high density and diversity in soils and along with other microorganisms control soil processes including mineralization of natural compounds and foreign substances. Microbial diversity can be affected during rapid changes in environmental conditions specifically by modifying their activity rates, biomass, gene expression, and community structure (Schloter *et al.,* 2006). The status of the soil nutrient, in addition to, temporal and seasonal changes also affects the size and activity of the microbial biomass (Sarathchandra, 1988).

2.1.3. Decomposition of Organic Matter in Soils

When dead plant materials and dead organisms are added to the soil, a complex series of events occur that have a strong effect on the properties and fertility of soil. Heterotrophic organisms in the soil break down the plant and animal remains and utilise the organic compound components as a source of food. During the process of decomposition and digestion of the organic components, excretion products are

produced which then become food for other organisms. When organisms involved in the decomposition die, they also become sources of food and are added to the food pool. From the decomposition process, carbon in organic compounds is often broken down and released as CO_2, under aerobic conditions (McLaren & Cameron, 1996).

Plant residues consisting of litter, branches, root fragments and exudates, are the most important input to SOM, contributing approximately 6 tonnes C $ha^{-1}yr^{-1}$ for temperate forests and 3 tonnes C $ha^{-1}yr^{-1}$ for temperate grasslands. The root system of the plant residues contributes between 60 and 70 % of the carbon input (Fogel, 1983) which is known as 'rhizo-deposition'. The root system comprises both soluble amino acids, organic acids, carbohydrates, and insoluble material such as sloughed-off cells (Cresser *et al.,* 1993).

Cellulose, a simple polymer, containing glucose, is usually responsible for more than half of plant residue carbon, followed by hemicelluloses (20 %), Lignin (18 %) and the remaining from proteins and amino acids (Cresser *et al.,* 1993). Cellulose is depolymerised by specialised microorganisms in the soil in particular fungi (*Trichoderma, Fusarium* and *Aspergillus*), and to a lesser extent bacteria (*Bacillus* and *Pseudomonas*). The decomposition of cellulose under aerobic conditions normally produces CO_2, while organic acids (acetic acid) are often produced under anaerobic conditions:

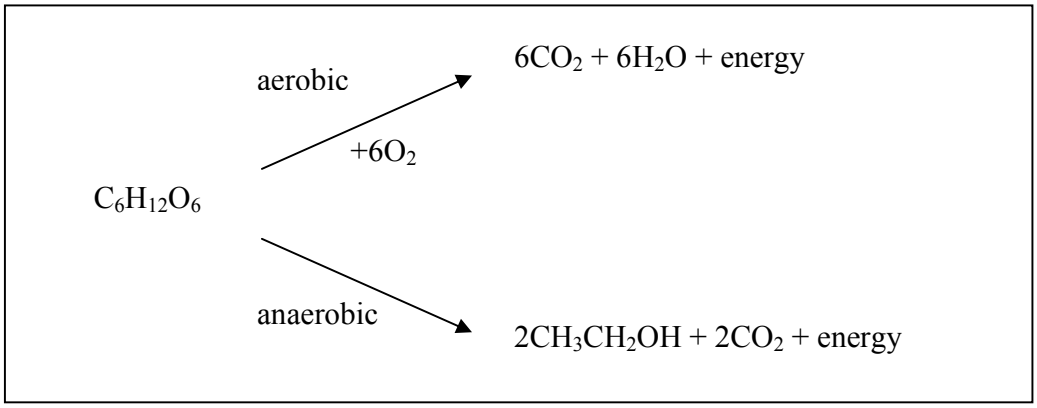


Figure 2.1: *Cellulose decomposition under aerobic and anaerobic conditions (Cresser et al., 1993)*

The decomposition of cellulose, catalysed by cellulase enzymes, is typical of the decomposition of many organic polymers in the soil, where initially it is depolymerised by specialised microbial enzymes, ultimately releasing simpler units of the polymer which becomes a substrate for a much broader group of soil microorganisms. The decomposition process also involves simple non-specialised enzymes (Cresser *et al.,* 1993).

An initial flush of decomposition takes place within a year during which two thirds of most plant residues that are more readily decomposed, are broken down. A much slower, but steady breakdown of the more stable humic substances that are more protected from rapid microbial attack then continues (Cresser *et al.,* 1993). Carbon mineralisation controlling the rate of SOM degradation is a key process of the soil C cycle (Schloter *et al.,* 2006).

The major factors affecting decomposition of organic materials include:

- substrate quality, characterized by the chemical composition of the decomposing matter (affected by indices such as Nitrogen content and C:N ratio),
- soil moisture, which affects microbial growth,
- temperature, which is a major factor affecting microbial growth rate,
- soil pH, which affects decomposition of specific microbial groups,
- pesticides,
- growing plants which provide the rhizosphere much favoured by microbial growth, cultivation, clay contents of soils (where SOM content tends to increase, and decomposition tends to decrease, with increasing clay contents), and
- the physical inaccessibility of soils

  (Haynes *et al.,* 1986).

2.1.4. Organic Matter Turnover in Soils

The process of continual decomposition and renewal of organic matter is known as turnover (Cresser *et al.,* 1993).

Organic matter or soil C turnover can be calculated as:

$$\text{Turnover (yr at steady state)} = \frac{\text{soil organic carbon (t ha}^{-1}\text{)}}{\text{input (or output) of organic carbon (t ha}^{-1}\text{ yr}^{-1}\text{)}}$$

(Cresser *et al.,* 1993).

For example a study by Jenkinson and Ladd (1983) on an arable soil under continuous cultivation in southern England with 26 tonnes of SOM per ha^{-1} and an annual carbon input of 1.2. tonnes, had a turnover of 22 years i.e. the organic matter in the arable soil is turning over every 22 years (Cresser *et al.,* 1993). The amount of organic carbon input (i.e. humus as formed from decomposing plant residues) is the same as the amount of carbon lost (as CO_2) from the soil when humus or organic carbon is decomposed (McLaren & Cameron, 1996). Turnover applies to the total organic matter content of soil, and also to individual fractions. The different fractions have different turnover rates because they have different rates at which they are decomposed and renewed (Cresser *et al.,* 1993). Turnover times of 10 to 40 years have been observed for soil organic matter in ecosystems that range from tropical forests to continuous arable crops, however there are organic materials in the soil known to have a turnover time ranging from less than one year to those of several thousand years (McLaren & Cameron, 1996). Total soil C turnover times determined from radiocarbon content of SOM, mainly ranged from 30 to 125 years (Tate *et al.,* 1995).

In temperate ecosystems, environmental changes from impacts of land use and climate, on soil C storage will not be immediately evident because of the delayed response attributed to the complicated pathways and interactions of C in soil (Tate *et*

al., 1995). Long turnover times of total soil C can be attributed to the accumulation of Al-humus (Tate, 1992), and irregular waterlogging was also found as mainly responsible (Tate *et al.,* 1995; 1993).

2.1.5. Soil organic matter steady state

Generally for undisturbed ecosystems and soils, a steady state is assumed to have been achieved and whereby annually, the net amount of C fixed by photosynthesis is matched by a similar quantity released to the atmosphere as CO_2 (Oades, 1989).
When the steady state or equilibrium of soils were approached, the values of C:N at all depths (0-7.5 cm, 7.5-15 cm, 15-30 cm) were found to be relatively uniform and low (Jackman, 1964). The steady state status of soils is affected by land use disturbances e.g. ploughing, which redistributes the organic matter content within the soil profile. A shorter period of time would be needed for som to reach its steady state, when the shallower the ploughing and the closer the soil is to its steady-state. The organic matter contents of soils can also differ significantly between soil types e.g. allophonic soils have more abundant organic matter contents compared to non-allophanic soils (Jackman, 1964).

2.1.6. Soil Organic matter accumulation and loss

A change in soil organic matter turnover can increase or decrease the soil organic matter reserves until a new equilibrium is reached. The net change in soil carbon content is a balance between inputs and losses. Relatively over long term periods, changes of SOM under permanent pasture, and the resulting changes in the supply of available nutrients, have been restricted to the top 15 cm of the soil (Jackman, 1964). Inputs to soil C include above-ground plant litter, roots, and dung from grazing-animals and losses include soil respiration and erosion (Lambert *et al.,* 2000). A change in SOM may occur, driven by a change in soil conditions (e.g. soil pH, aeration, temperature), which alters the rate of microbial processes in the different

soil organic matter fractions, and by changes in the quality of the substrate entering the soil.

Formation of peat is an example of an extreme situation of soil organic matter accumulation consisting mainly of un-decomposed or partially decomposed plant remains. Organic matter accumulation occurs when conditions are unfavourable for decomposition including poor drainage, high acidity, and low aeration. Acidity or low pH generally restrains turnover of organic matter. For example, low pH in soils in a forest in north-east Scotland has resulted in the accumulation of a humus layer on a podzol beneath conifers, by 10 cm over 40 years (Billett *et al.,* 1988). The humus layer accumulation was also a result of the high lignin content of litter that decreased the quality of a substrate for decomposition by microorganisms (Cresser *et al.,* 1993).

Annual gross input to soil C in pasture was approximately 9 t ha^{-1} (Parfitt *et al.,* 2002). Managed pastures, and some cropping systems, in New Zealand contain about 30 to 130 Mg ha^{-1} of organic C in the top 20 cm of soil (Percival *et al.,* 2000; Parfitt *et al.,* 2002).

Loss of SOM occurs when conditions that favour decomposition are stimulated e.g. peat soils have demonstrated rapid loss of soil organic matter when drainage and aeration were carried out. The loss of soil organic matter is also accelerated by lime and fertiliser additions required for sustaining crop productivity (Cresser *et al.,* 1993).

The status of soil C under pastures has become quite a controversial matter particularly since the conversion of native forest to grazed pasture. Different studies have been conducted under different historical land use management (Lambert *et al.,* 2000), giving varying results.

Grassland studies (Walker *et al.,* 1959) found that organic matter and nutrients in organic forms were accumulated under New Zealand conditions. Within some soil profiles of the North Island, accumulation of organic matter under managed pasture has previously been measured in soils growing pastures of different ages. Jackman

(1964a) found accumulation of organic matter was generally greater in the top 7.5 cm, followed by the 7.5-15 cm depth, while no change in organic matter was observed in the 15-30 cm depth.

On the contrary, other studies have measured either no increase or decreases in soil organic C (Lambert *et al.,* 2000, Parfitt *et al.,* 2000, Bellamy *et al.,* 2005, Schipper *et al.,* 2007). Losses of carbon have been reported which include approximately 200 kg C ha^{-1} yr^{-1} during the experimental period (Lambert *et al.,* 2000), and a loss of soil C within the 1m depth on average of 2.1 kg C m^{-2} in the last 20 years or so (Schipper *et al.,* 2007). Bellamy *et al.,* (2005) found carbon loss with increasing rate of loss of more than 2 % yr^{-1} in soils with carbon contents more than 100 g kg^{-1} with soil carbon content. Parfitt *et al.,* (2002) found soil total C content as not changed with time (25 year timeframe).

Possible reasons for observed losses in soil C include ongoing degradation of C since the clearing of original forest a century earlier or the possible downward trend of C towards a new and lower equilibrium for grassland (Lambert *et al.,* 2000). Despite these findings, very little was known about what really is or are causing the changes in C in the context of long-term pasture initially transformed from forest (Schipper *et al.,* 2007).

2.2. SOIL NITROGEN

2.2.1. Introduction

Nitrogen (N) exists naturally in many forms and predominates in the earth's crust (18 x 10^{15} tonnes) and in the atmosphere (3.8 x 10^{15} tonnes) as nitrogen (N_2) gas (McLaren and Cameron, 1996). Nitrogen can occur in the biosphere in solid, liquid and gaseous phases, and either as simple inorganic or complex organic compounds (Floate, 1987). Nitrogen in soil is one of the major plant nutrients and the most

vulnerable to microbial transformations compared to phosphorus and potassium (Alexander, 1977). As a key building block of the protein molecule, nitrogen is a crucial component of plants, animals, and microorganisms (Alexander, 1977).

Nitrogen in soils exists in three main forms: (i) organic N associated with plant material, soil organisms and soil humus, (ii) ammonium N (NH_4^+) fixed by clay minerals, and (iii) mineral N in the forms of ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) in soil solution. Over 95 % of soil N is in soil organic matter and not available to plants, while
1-6 % is present as fixed N in clay minerals, and may be partly available to plants, and 1-2 % is mineral N which is the plant available form of N (McLaren and Cameron, 1996). Inorganic N or mineral N is constantly formed by decomposition and mineralisation of organic-N through the nitrogen cycle (Tan, 2005). Because N dominates in the organic form, it needs to be broken down to produce the mineral forms (NH_4^+ and NO_3^-) that are available for plant uptake (McLaren and Cameron, 1996).

Agricultural systems range from those without external N inputs through to systems with high intensification with large inputs including from nitrogen fertiliser. Systems with low N inputs are often reflected by low productivity and a negative N balance (Ledgard, 2001). Nitrogen supplied from soil is the main factor that controls herbage production from grass-dominant pastures (Floate, 1987). Nitrogen is cycled through several processes which mainly involve nitrogen fixation, mineralization, nitrification, denitrification, volatilization, and immobilization (McLaren and Cameron, 1996).

2.2.2. Nitrogen Under Pasture

Nitrogen cycles through different forms within the soil/plant/animal/atmosphere system under pasture. Nitrogen flows in the soil system are influenced by factors determining inputs and outputs of N. Nitrogen inputs to the soil N system are from

atmospheric N fixed by legumes (mainly clover), N returned by via dung and urine by grazing animals that feed on the clover (Morton and Roberts, 1999), and from decomposing plant material. Other inputs can be from N fertilisers normally added to increase production and profitability. All N returned to the soil is transformed through biochemical processes of micro-organisms from organic to mineral forms (NH_4^+ and NO_3^-) that can be used by plants (Morton and Roberts 1999; Floate, 1987). In grazed pastures, N that is normally recycled within a plant to SOM via plant litter is inhibited, but instead is consumed by herbivores and passed to SOM via animal excreta. Usually less than 15 % of N remains by an animal after ingestion (though a larger amount is retained in intensive dairying systems), while the rest is excreted in dung and urine (Floate, 1987). Usually, in most grasslands, N is limited, and with long residence times of grasslands, there is potential for significant internal cycling (including redistribution by grazing animals) and for reactive nitrogen (Nr) (includes inorganic reduced forms of N: NH_3, NH_4^+, inorganic oxidised forms: NO_x, N_2O, and NO_3^-, and organic compounds: urea, and proteins) accumulation (Galloway *et al.,* 2003). The quality of SOM under pastures is enhanced through the growth of legumes (supplying N), that in turn depend on the P status of the soil normally obtained by the soil parent material or P fertiliser (Parfitt *et al.,* 2003).

Decomposition of plant material is accelerated more rapidly in the rumen of an animal compared to decomposition in the soil, thus grazing animals tend to speed the nitrogen cycle. N returned to soil in urine is usually hydrolysed rapidly to ammonia (NH_3) (approximately less than 1 hour to 1 day) and hence becomes available to plants. However, because NH_3 is volatile, the rapid conversion of urine to ammonia may cause significant losses of nitrogen (Floate 1987). Leaching from urine patches are also a source of N loss particularly when the concentration of N in solution (urine) excels the plant capacity to utilise it. Conversely, N cycled via dung tends to be at a slower rate and causing less leaching and volatilisation losses (Floate, 1987).

Factors controlling the processes in the nitrogen cycle include soil fertility (availability of phosphorus, potassium and sulfur), temperature, water supply (moisture), and soil acidity (Floate, 1987). The heterogeneity within ecosystem

environments determines how nitrogen is cycled for example, the differences in soil conditions over distances (spatial dimensions); while the variability of the time scale with differences in residence times in some pools, also affects the nitrogen cycle (Floate, 1987).

2.2.3. Nitrogen Pools

a. Soil Nitrogen Pools

(i) Total and Organic N

Total Nitrogen in a grassland environment is a total of all forms of nitrogen. The abundance of organic N content in New Zealand much owed to the historical land use changes which included the conversion of indigenous forests to form the grass-clover pastures in the mid 19th century (Jackman, 1964b). Over 90% of N in soils is stored as organic N (Jackman, 1964a). Within the top 0-36 cm total nitrogen was estimated at about 375 g N m^{-2}, of which about 99.5 % is organic N and only 0.5 % mineral N (Woodmansee *et al.,* 1981). In New Zealand, total N (including mineralisable N) were larger under pastures compared to other land uses (Sparling and Schipper, 2004). Of the organic N, about 88.8 % is relatively inert or inactive, and about 10.7 % is in the form of living and dead biomass (plant, microbe, and animal parts) (Woodmansee *et al.,* 1981). The soil pool of organic nitrogen relatively ranges from 1000 to 10,000 kg ha^{-1} (Figure 2.2). The large organic pool of nitrogen can only be used by plants after it has been mineralised (Floate, 1987). Total N has been measured as more significantly correlated to biomass and activity compared to total C with biomass and activity, which emphasised the importance of N availability as strong regulators for microbial metabolism, while total C only indicates the size of the organic matter pool (Kaiser *et al.,* 1992).

Estimations of long-term immobilisation rates of N into SOM have been reported in a number of studies for New Zealand. Estimated storage rates include 119 kg N $ha^{-1}yr^{-1}$ of pasture soil previously converted from prodocarp/broadleaf forest vegetation (Walker *et al.,*1959), between 2 and 78 kg N $ha^{-1}yr^{-1}$ under long term pasture

depending on soil type and management (Jackman, 1964b), and 19 kg N $ha^{-1}yr^{-1}$ in long term pasture with high phosphorus fertiliser loadings, because of its effect on stimulation of N-fixation by clover, however minimal effect (net immobilization) observed at lower phosphorus inputs (Lambert *et al.*, 2000). More recently and contrary to these findings, soil total N measured to 1 m depth from soil profiles of permanent pastures (about 20 years) has been found to decrease, on average by 1.8 t ha^{-1} over 20 years or 90 kg N ha^{-1} yr^{-1} (Schipper *et al.*,2007).

(ii) Inorganic N

Inorganic nitrogen, normally in soil solution, is the smallest pool relative to organic N, ranging from approximately 10 to 100 kg ha^{-1} (Figure 2.2). Inorganic or mineral nitrogen occurring predominantly as ammonium (NH_4^+) and nitrate (NO_3^-) are the only source of N in soil available for plant uptake (Floate, 1987).

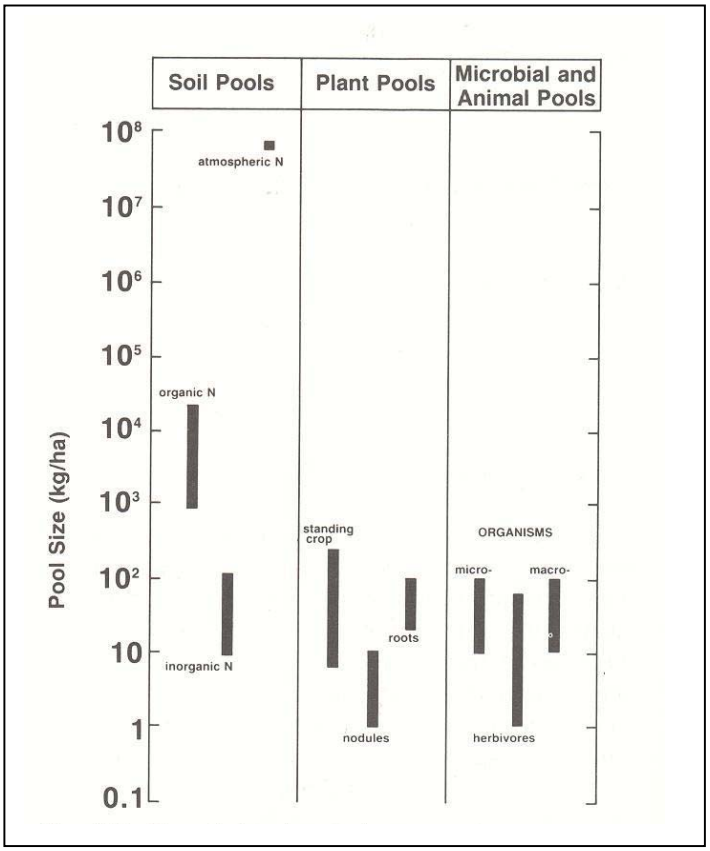


Figure 2.2: *The comparative size and typical range of values (kg N ha^{-1}) of Nitrogen pools in grassland ecosystems (Floate, 1987)*

Both NH_4^+ and NO_3^- can be utilized by most plant species. However, plant species vary in their ability to use NH_4^+. For most plants, when more NH_4^+ is absorbed than NO_3^-, their root rhizosphere would become acidic (Malhi *et al.,* 1988).

b. Plant Pools

Nitrogen in the plant biomass pool consists of roots, stems, leaves and reproductive organs, and typically ranges in a grassland ecosystem from approximately 1 to 500 kg ha^{-1} (Figure 2.2). Legume is normally separated from grass because of its significant role in nitrogen fixation (Floate, 1987), hence its N component would normally be significant on its own.

c. Microbial and Animal Pools

Generally, the microbial and animal pools consist of N ranging from approximately 10 to 100 kg ha^{-1}. The microbial pool has N that ranges from about 10 to 100 kg ha^{-1} (Figure 2.2) (Floate, 1987). In colder environments such as New Zealand, nitrogen in microorganisms may be up to 150 kg ha^{-1} (Woodmansee *et al.,* 1981). Microbial N values for an alluvial yellow-brown loam (specifically a Waihou fine sandy loam, a Typic Vitrandept), that has been studied for seasonal and fertiliser P effect, ranged between 130 and 220 ug N g^{-1}, which comprised about 1.5 % to 2.2 % of the total N in the soil (Sarathchandra *et al.,* 1988).

Seasonal changes affect the size of "microbial N". Over the winter period, microbial N has been found to accumulate (while biomass size was declining) and followed by a rapid decline observed for spring and autumn (Sarathchandra *et al.,* 1988). The accumulation may have been caused by the relative increase in the bacterial fraction compared to fungi (Alexander, 1977), while the decline in spring may have been caused by a series of reactions which follows: bacterial growth stimulated by root development (which released easily available C), associated increase in N mineralisation by bacteria, scavenging of these bacteria by protozoa and eventually releasing of about 1/3 of the bacterial N into soil (Clarholm, 1985).

Microbial N pool varies and is influenced by factors affecting soil micro-organisms which include climate, availability of nutrients, and predation. Microbial deaths are mostly caused by drought, freezing and thawing, lack of nitrogen, and predation (Woodmansee *et al.,* 1981).

2.2.4. Nitrogen Transformations

Nitrogen transformations, mainly mineralisation/immobilisation processes (Figure 2.4) are governed mainly by bacteria and fungi in soil which regulates soil fertility and plant nutrition (Jones, 1979; Ledgard, 2001). All of these processes contribute to the N-cycle (Figure 2.3).

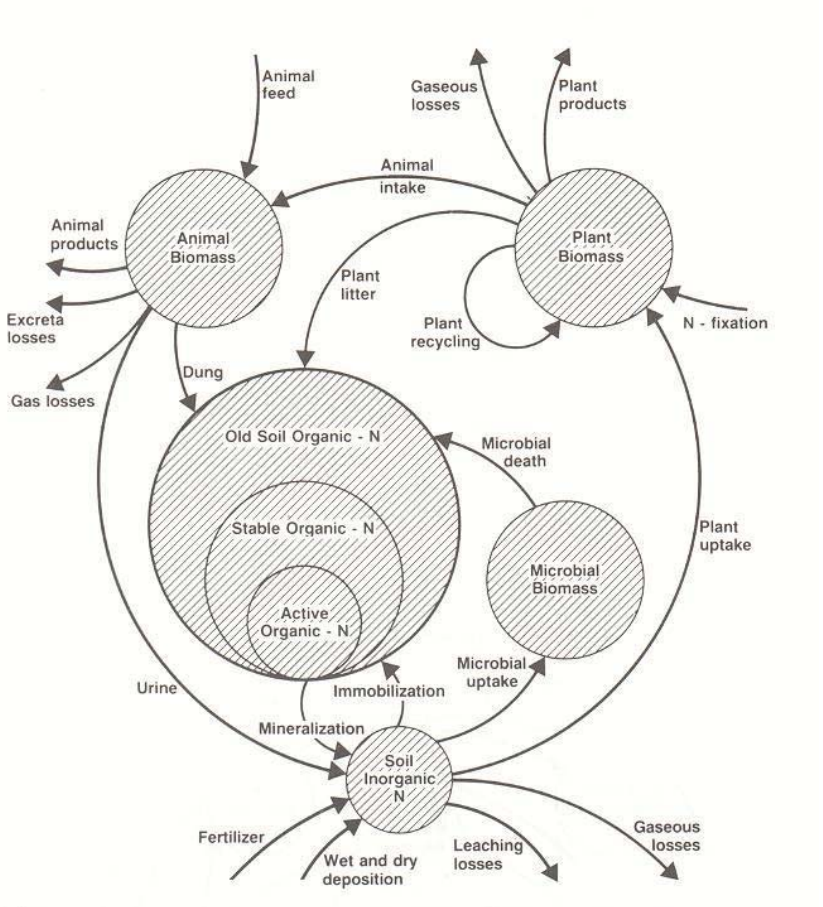


Figure 2.3: *A typical nitrogen cycle for managed grassland ecosystems with the main components and pathways (after Floate, 1987)*

a. Fixation

Nitrogen fixation is the most significant factor influencing the amount and rate of nitrogen flow through grassland ecosystems (Floate 1987). Pasture production in New Zealand have much reliance on nitrogen obtained from clover based pastures (particularly white clover), for providing forage for animal production (Morton and Roberts, 1999). Generally only about 200 – 400 kg N ha^{-1} yr^{-1} can actually be fixed across a range of legumes as found in more recent studies (Ledgard, 2001).

Factors affecting N fixation include soil pH, fertility, and available moisture (Jones, 1979). However, legume growth and therefore N fixation can be limited from lack of fertility (essential nutrients) of the soil (Jones, 1979; Floate, 1987), soil pH, and available moisture (Jones 1979; Ledgard, 2001), and from pests, where a decrease in pasture N_2 fixation below their potential can result, for example between 20-200 kg N $ha^{-1}yr^{-1}$ (Ledgard, 2001).

b. Immobilisation and Mineralisation

Mineralisation is the conversion of organic nitrogen into ammonium. The processes of mineralisation and immobilisation can occur concurrently within a location in a soil as plants and microorganisms take up inorganic nitrogen during periods of rapid plant growth (Woodmansee *et al.,* 1981).

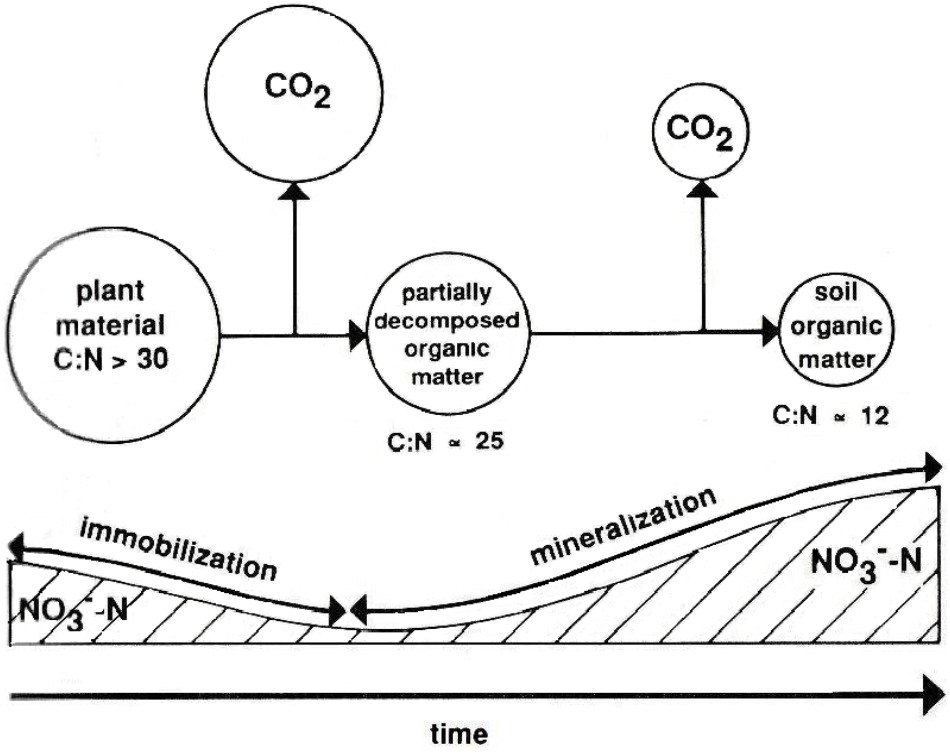


Figure 2.4: *Decomposition of plant material, immobilization and mineralization of nitrogen, and relative C:N ratios (from McLaren and Cameron, 1996)*

Mineralisation of organically bound N is necessary to supply N to plants. The process is a series of reactions mediated by soil micro-organisms whereby organic N (from soil organic matter and other organic materials) is converted into inorganic or mineral N. The soil micro-organisms can then absorb mineral N into their biomass, by immobilisation (McLaren and Cameron, 1996).

Mineralisation caused by soil micro-organisms involves the breakdown of complex proteins in organic compounds into amino acids (amino-N) which are then transformed to ammonia (ammonification), which simultaneously provides energy to soil micro-organisms:

$R\text{-}NH_2 + H_2O \rightarrow NH_3 + R\text{-}OH + energy$

The ammonia released is then converted to ammonium by hydrolysis, and then further to nitrite (NO_2^-) and nitrate (NO_3^-). NH_4^+ and NO_3^- pools have high turnover (turned over once a day) in annual grassland (Davidson *et al.,* 1990). The mineralisation of N indicating microbial size and activity can provide useful characteristics of soil health under different treatments (Barkle *et al.,* 2001).

Immobilisation of N differs with plant species, state and rate of growth, soil conditions, and abundance of available N (Allison, 1973). Net immobilisation occurs when there is more mineral N assimilated by soil microbes than is released by mineralisation. On the other hand, net mineralisation occurs in soil when there is more mineral N released from organic matter than is required by micro-organisms. (McLaren and Cameron, 1996). Net immobilisation (N accumulation) in organic matter (litter) is normally followed by a slow net mineralisation (N release) mainly because as C:N ratio continues to decrease during decomposition, N would no longer be limiting to microbial growth and activity, and switch from net immobilisation to net mineralisation (Haynes *et al.,* 1986). During the decomposition of litter, the amount of N is known to increase (Haynes *et al.,* 1986) .The increase in N resulted when, where initially it was generally being limited in organic residues, and being in demand by the decomposer microorganisms, it was utilised and incorporated into microbial cells, and later converted into the more resistant humic substances. In this way, most of N is retained in the decomposing material while C is continuously reduced through loss as CO_2, and thus causing the C:N ratio of litter to decrease over the decomposition process (Haynes *et al.*, 1986).

Net immobilisation (N accumulation) would generally occur where C:N ratio of litter is high (>25 to 30:1; and with N content < 1.4 to 1.8 %) and includes cases where organic residues (with high C:N ratio) are incorporated into agricultural soils, because microorganisms utilising most of N in organic matter, would accumulate N from outside the litter (organic matter) system (Allison, 1973). The incorporation of N from outside the litter system would generally cause a deficiency of inorganic (mineral) N in the surrounding soil, and where the heterotrophic decomposer biomass normally outstrips the nitrifier organisms and plants for NH_4^+-N (Allison, 1973).

Thus, N fertilisers are commonly added when organic residues are applied to soil, to maintain the supply of mineral N that would otherwise be insufficient to plants during decomposition (Haynes 1986). Under a range of soils studied under permanent pasture, immobilisation was shown to dominate in the top 0-7.5 cm soil (Jackman, 1964).

Net mineralisation (N release) normally occurs in organic matter (litter) that have high N content e.g. legume residues, where N is not limiting to microbial growth and activity, thus net immobilisation does not occur, but instead a net mineralisation takes place shortly following organic matter decomposition. Net mineralisation occurs generally where C:N ratio of organic matter is less than 25 to 30. Following the net mineralisation phase, the concentration of N retained in the decomposing matter would continue to increase relative to C, and is normally incorporated into humic polymers by the actions of the decomposer microflora (Haynes, 1986). Under permanent pastures, unlike immobilization, mineralization was shown to predominate in the 7.5-15 cm soil depth (Jackman, 1964a).

c. Nitrification

Nitrification is the biological conversion of ammonium (NH_4^+) through oxidation to nitrite (NO_2^-), and nitrite to nitrate (NO_3^-). Nitrification is brought about by autotrophic bacteria (including *Nitrosomonas, Nitrosolobus,* and dominantly *Nitrobacter*) who obtain their energy (ATP) through the oxidations of NH_4^+ and NO_2^- (Haynes *et al.,* 1986) (Figure 2.5). Environmental factors can have strong impacts on nitrification because the nitrification process can only be accomplished by a few species of bacteria, which can easily be affected by changes occurring to ecosystems induced by these factors. Nitrification can also lead to subsequent losses of N as gaseous loss of N_2 and N_2O via denitrification and leaching as NO_3. Three broad factors regulate nitrification: "(1) ubiquitous factors (substrates and products, pH, aeration and moisture and temperature); (2) regulatory factors in natural ecosystems (allelopathy, limiting supply of NH_4^+ and other nutrient deficiencies); and (3) man-made factors (trace element toxicities, pesticide residues, and specific inhibitors)" (Haynes, 1986).

Significant nitrification occurs at soil temperature 10 ^{o}C and increases sharply as soil temperature increases attaining a maximum rate over 30 ^{o}C (Jones, 1979). For example, chemoautotrophic nitrification with a pH optimum of pH 6 to 7 (Haynes *et al.,* 1986) or around pH 7-8 (Bollman, 2006), had a maximum temperature of around 40 ^{o}C, however, some indigenous autotrophs may also be present with a maximum pH in the range of pH 4 to 5, and temperature of 50 to 60 ^{o}C (Haynes *et al.,* 1986). Generally, nitrification also reaches its maximum rate at soil moisture potentials that ranges from -10 to -33 kPa (Haynes *et al*, 1986).

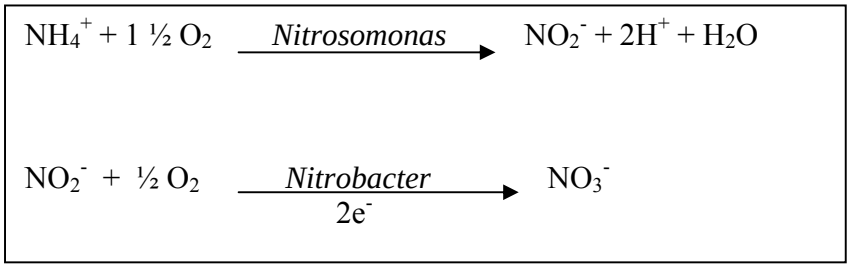


Figure 2.5*: Autotrophic Nitrification Processes*

d. Denitrification

Denitrification is the conversion of nitrate to nitrous oxide (N_2O) and eventually to nitrogen gas (N_2) (Steele *et al.,* 1984). Denitrification is favored by poor drainage with low oxygen (anaerobic) conditions (Jones, 1979). The biological process of denitrification involves anaerobic respiration of bacteria whereby nitrogen oxides (NO_3^-, NO_2^-, N_2O) are substituted for oxygen (O_2) as terminal electron acceptors resulting in the reduction of these plant-available anion species (NO_3^-, NO_2^-) to gaseous products (N_2 and/or N_2) (Steele *et al.,*1984). Conditions of very low O_2 and temperatures above 5 ^{o} C are required for the ongoing denitrification by most bacteria (Steele *et al.,* 1984).

Large losses of N were found in soils which contain high organic carbon content (Steele *et al.,* 1984). A continual N deficiency is widespread throughout New Zealand (Steele *et al.,* 1983) and denitrification is of concern in agricultural soils as it will exacerbate N deficiency (Steele *et al.,* 1984).

2.3. C:N RATIO

The ratio of carbon (C) and nitrogen (N) shows the extent of decomposition of organic matter and whether microbial activity is causing mineralisation or immobilisation (McLaren and Cameron 1996; Cornforth, 1998). The C:N ratio is the ratio of the percentage of organic C (% C_{org}) and percentage of organic N (% N), and it provides an index for measuring humification and humus quality (Tan, 2005). The C:N ratio also gives an indication of the quality of N in soil organic matter where a high ratio reflects low N quality while a low ratio shows a high N quality (Parfitt *et al.,* 2003). The C:N ratio varies for plant material and soil biomass. Plant materials have C:N ratios ranging from approximately 20:1 to 100:1 depending on plant species, stage of growth, and the nutrient status of the soil. In comparison, soil biomass (living organisms) has a lower and more stabilised C:N ratio ranging from 9:1 to 4:1 (McLaren and Cameron, 1996, Cornforth 1998).

In plant material a C:N ratio of approximately 25:1 (with N content of 1.7 %) (Haynes *et al.*, 1986), results in organic matter decomposition with no net immobilisation or net mineralisation taking place (Paul and Juma, 1981; McLaren and Cameron, 1996) (Figure 2.4.). A C:N ratio of less than 25:1 (with N content > 1.4 to 1.8 %) (Hayne, 1986), usually favours net mineralisation. For instance, clover has a C:N ratio of 20:1 and when it breaks down in soil, it results in net mineralisation of N, and for wheat straw (C:N ratio of 80:1) when incorporated into the soil will cause immobilisation of soil N. But over time, as wheat decomposes, N will then be released. The C:N ratios for other fractions of soil organic material ranged from approximately 5 :1 for soil bacteria to 10:1 for soil fungi (McLaren and Cameron, 1996).

The C:N ratio is usually high in fresh plant material such as wheat straw (about 80:1) and decreases to as low as 8 to 15 in decomposed material (Tan, 2005) such as humus (10:1) (McLaren and Cameron, 1996). Humus is the very stable form of organic matter remaining after most plant and animal residues have been decomposed by soil

microorganisms (Cornforth, 1998). Humus enhances the cation exchange capacity of soils (Tan, 2005). The C:N ratio of soil organic matter normally stabilises at a value dependant on climatic conditions and management practices (Tan, 2005). Normally it becomes constant approximately between 8:1 and 16:1 (McLaren and Cameron, 1996).

With organic C content assumed to be at steady state, and N accumulating under pasture soils, the average C:N ratios were found to have decreased from 16.2 (under indigenous forests), to 15.5 (under forestry), 11.8 (under drystock pasture), and 11.3 under dairy pastures (Sparling *et al.,* 2004). The C:N ratio of topsoils generally stays above 10 because of the presence of a constant sufficient amount of inorganic available N whilst not affecting organic N (Jackman, 1964a), however, the C:N ratio deceases with depth (Oades, 1989). The minimum of C:N ratio is likely to depend on fertiliser including nitrogen loading and will differ with land use and soil type. The C:N ratio can decrease in pasture under the increasing use and application of fertilisers such as P fertiliser that would lead to more N fixation that provides more inputs of N to soil and into organic matter (Schipper *et al.,* 2004).

2.4. PHOSPHORUS UNDER PASTURE

Managed grasslands differ from natural grasslands which affect phosphorus cycling (Gillingham, 1987). In a managed grassland ecosystem, the amount of soil phosphorus is affected by phosphate fertiliser inputs, increased pasture growth (due to phosphorus and other fertilisers), and by higher stocking rates that recycle phosphorus through faecal matter deposits from the grazing animal (Figure 2.6) (Gillingham, 1987).

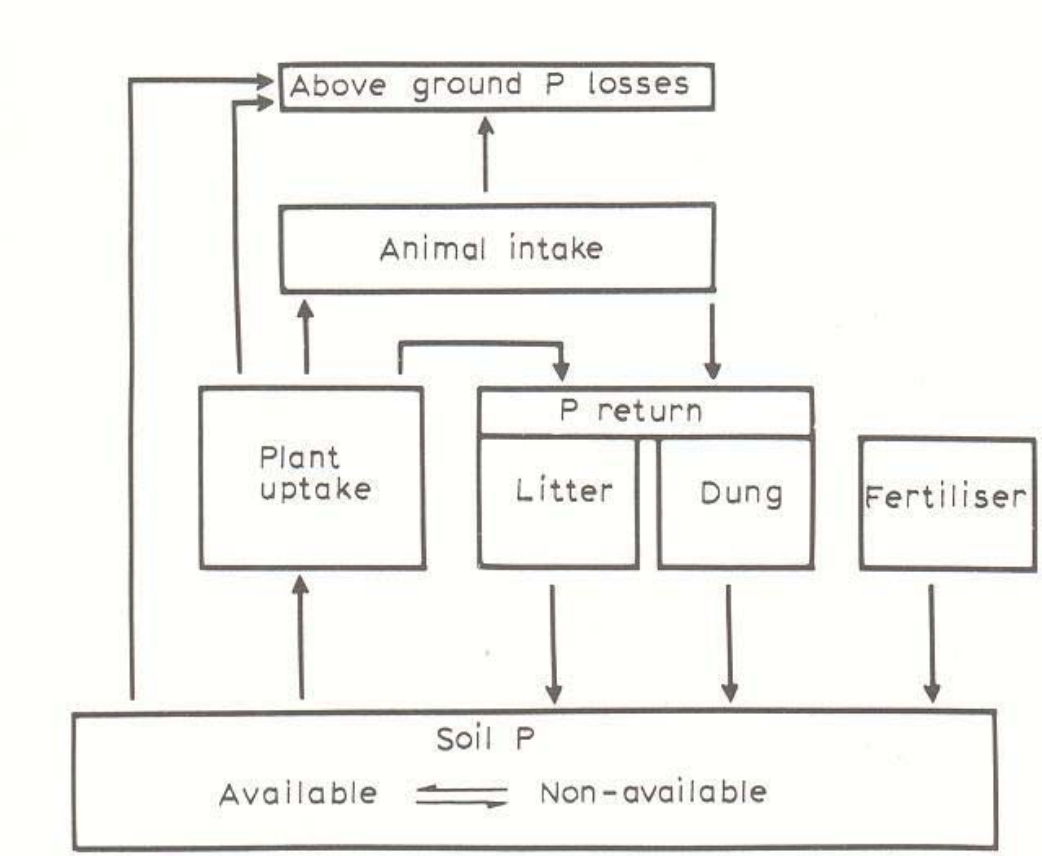


Figure 2.6: *Phosphorus cycling showing major pathways in a grazed pasture ecosystem (Gillingham, 1987)*

2.4.1 Soil Phosphorus

Phosphorus in soils occurs in organic and inorganic forms (Kuo, 1996). Soil phosphorus comprises all phosphorus beneath the soil surface despite its origin and forms including parent material and fertiliser, (Gillingham, 1987). Phosphorus in soils exist in organic forms to the extent of 20-50 % of the total but can also reach as much as 75 %. The proportion of organic P of the total increases as the N content increases and the pH increases (Allison, 1973). Mineralisation of organic matter provides plant-available phosphorus and contributes to the soil phosphorus "pool" (Gillingham, 1987). The inorganic and organic phosphorus content of soils is subject to being influenced by factors which includes the nature of the parent material; nature

of processes that affect soils such as climate, vegetation and microbes; soil texture; temperature and moisture; and ages of a soil (Sauchelli, 1965). On newly developed cultivated land, and well topdressed pasture, the quantity of SOM tend to increase in the top 10-15 cm, and also tend to increase markedly in the phosphorus and other nutrient contents (During, 1984).

a. P Balances

Phosphorus (P) lost from the paddock system in animal produce and through animal transfer is referred to as the Animal Loss Factor (ALF). The ALF expressed in kg P per stock unit (SU) is defined as consumption of 550 kg DM yr^{-1}. Animal losses of P per SU increased with increasing slope independent of fertiliser rate (Rowarth *et al.,* 1992a).

The size of P pools (soil, plant P uptake, faecal P return, and total P) for above and below ground was determined for different slopes in a paddock and at varying rates of P fertiliser input using data collated from previous studies at the Whatawhata fertilizer trial except (Rowarth *et al.,* 1992b). The study found a surplus of P for the above-ground components of the P cycle on the 0 – 10^{o} slopes while a P deficit was observed on all other slopes (11-20 ^{o}, 21-30 ^{o}, or 31+).

b. Total P

At the Whatawhata fertiliser trial, total P in the soil pool increased with increasing slope (Rowarth *et al.,* 1992b). Changes in total P (kg ha^{-1} yr^{-1}) in soil on easy and steep slopes were greater at high rates of fertiliser addition than at low rates, however, conversely the reverse was observed for campsites (0-10 ^{o} slopes) where increases in organic P were greater at low rates than at higher rates of fertiliser application. Total average annual soil P increased on the easy slopes (11-20 ^{o}) with increasing fertiliser addition which ranged from 5.9 for the 10 kg P ha^{-1} yr^{-1} to 93.5 for the 100 kg P ha^{-1} yr^{-1} (Rowarth *et al.,* 1992b).

c. Available Phosphorus and Above Ground Losses of Phosphorus

Potentially available phosphorus in soil to plants can be indicated from the total amount of phosphorus that is in equilibrium with phosphorus in solution (Gillingham, 1987).

(i) Plant Uptake of Phosphorus

Assimilation of the relatively immobile nutrient phosphorus by plants takes place mainly from the surface soil, and is dependent on the distribution pattern of the plant root. Hence the competitive ability of many pasture species is attributed to their denser root system that has a greater advantage of exploring a larger volume of soil (Gillingham, 1987). Cultivars of white clover (*Triflium repens)* have better demonstrated their suitability to the soil conditions (lower fertility and harsher environmental conditions) of New Zealand hill country (Gillingham, 1987). The phosphorus uptake by pasture varies seasonally as largely regulated by growth conditions. Plant growth and phosphorus concentration decreased most in drier (moisture stress) periods, and are also influenced by soil type e.g. yellow-grey earths are more prone to moisture stress in summer (Saunders and Metson, 1971).

The phosphorus concentration in plants varies with plant species, type of tissue, age of tissue, and age and physiological condition of the plant. Generally less phosphorus is contained in older plant tissues than in young leaves (Gillingham, 1987). The amount of water-soluble inorganic phosphorus in plant leaves increases with an increase in total phosphorus content (Bromfield and Jones, 1972). To obtain maximum yields, the minimum phosphorus concentrations in some plant tissues are between 2.8 – 3.6 μg P g^{-1} for ryegrass (*Lolium perenne)* and 3 - 4 μg P g^{-1} for white clover (*Trifolium repens*) (Gillingham 1987). Phosphate requirements for most grasses are similar to ryegrass (Gillingham, 1987). Plant uptake of P at the Whatawhata fertiliser trial has been found to be proportional to P losses observed from other slopes that were positively correlated with increasing P fertiliser addition (Rowarth *et al.,* 1992c).

(ii) Animal Intake of Phosphorus

Most of the phosphorus in plant material in managed pasture, is consumed by animals or used as feed. A further source of phosphorus is also provided by supplementary feeding at certain times of the year and also up to 12 % is ingested daily from soil during grazing (Gillingham, 1987). Concentration of P in leaves is generally higher than P found in stems. The P concentration was significantly ($r = 0.98$, $P < 0.01$) higher (by 0.02 %-0.03 %) in pre-grazed pasture compared to post-grazed pasture that contained 0.92% P (Rowarth *et al.,* 1992a).

d. Phosphorus Returns

Phosphorus is mainly returned to soil as either animal excreta or as dead plant material.

(i) Excreta

Animal faeces form the majority of almost all phosphorus excreted. The physiological state of the grazing animals also affects, and thus determines the fraction retained in the animal and ultimately released or exported. The amount of phosphorus in returned animal excreta is measured from the amount of herbage consumed and the phosphorus content of the herbage consumed (Gillingham, 1987).

(ii) Dead plant material

Organic matter decomposition and thus the rate of cycling of P in pastures is accelerated by microbial animals including earthworms (Sharpley and Syers, 1977). Phosphorus is also released when litter (dead leaves and stem) is decomposed.

e. Organic Phosphorus

Organic soil phosphorus may exist as a component of either living or dead organic matter. Soil organic phosphatic compounds mainly comprise of nucleoproteins, phosphatides including lecithin, and phytin (calcium-magnesium salt of inosite-phophoric acid). The nucleoprotein comprises the largest proportion of the phosphatic compounds present in microorganisms, while phytin dominates (75 %) of the total phosphorus content of plants (Sauchelli, 1965).

Organic P is of little immediate use in agriculture since it is too insoluble for plant assimilation. New Zealand's cool climate temperature and generally low soil pH favours the accumulation of organic P which could continue for many years (Walker and Adams, 1959). Jackman (1964b) found that over a range of soils 45 to165 kg P ha^{-1} accumulated in organic form within 10 years of development. Organic P accumulation can cease as a result of liming. Liming (application of calcium carbonate) is commonly used in agriculture to raise the soil pH (During, 1984) which is required for good pasture establishment and maintenance (Morton & Roberts, 1999) by making inorganic P more available while ceasing the accumulation of organic P (During, 1984).

f. Inorganic Phosphorus

Inorganic soil phosphorus has four major forms: (i) water-soluble compounds such as monocalcium phosphate, (ii) phosphorus adsorbed on amorphous surface coatings of clay minerals and colloids, (iii) occluded and chemisorbed phosphorus that tends to develop a stronger chemical bond after adsorption, and (iv) phosphorus precipitated in discrete, largely insoluble, forms. The forms of inorganic soil phosphorus are in dynamic equilibrium with one another, nevertheless occluded and precipitated phosphorus forms the major proportion of "permanently" fixed phosphorus (Gillingham, 1987). Phosphorus movement within a managed grassland ecosystem (Fig. 2.6) includes the removal of phosphorus from soil solution by plants. Phosphate uptake by plants promotes the release of phosphorus from inorganic and organic sources to maintain an equilibrium concentration of phosphorus in soil solution (Gillingham, 1987). Inorganic phosphorus had been shown to leach from dead hayed off pasture plants during decomposition (Jones and Bromfield, 1969). However the loss of inorganic phosphate by leaching was largely prevented by the presence of microbial activity (Jones and Bromfield, 1969).

g. Erosion Loss

Inorganic P from fertiliser has been observed as not leached to depth from the soil types found at Whatawhata (Rowarth, 1992c). Hence, erosion may be a possible factor contributing to P loss. If amounts of P supplied was in excess than required by

plants, it may have likely that P would be washed into ground surface waters (Cornforth, 1998), and this effect would have been more pronounced in steep hill country farms.

2.4.2. P Fertilisers

a. Normal Superphosphate and Triple Superphosphate

Superphosphate fertiliser, has for many years been used as the standard fertiliser, and has the main phosphate mineral composed of monocalcium phosphate monohydrate [$Ca(H_2PO_4)2.H_2O$] (Lindsay, 1979). The phosphate fertilisers (single superphosphate and triple superphosphate) are soluble (readily available to plant) P and S fertiliser, contains appreciable quantities of Ca, and are often mixed with K or N. Single superphosphate has been the traditional and major P fertiliser used in New Zealand, however alternative forms of phosphatic fertilisers have been investigated when costs of single superphosphate fertilisers escalated in the early 1980s (Percival *et al.,* 1984). TSP generally contains more P (Morton & Roberts, 1999) of up to 48 % compared to 20 % found in single superphosphate (Leikam and Achorn, 2005). Production of TSP derived from reacting phosphate rock (PR) with phosphoric acid, is relatively simple compared to the production of single superphosphate which was from acidulating PR with sulfuric acid. With the more simple production and higher P content, TSP has increasingly replaced single superphosphate (Leikam and Achorn, 2005).

To maintain both the desired level of production which is normally set at 90 % of maximum growth, and the size of the P cycling pool, it is assumed that fertiliser P required is equal to the sum of the P losses within the soil and by the grazing animal (Gillingham *et al.,* 1984, Rowarth *et al.,* 1992a). Any pasture on a hill block usually contains a broad range of growth rates as influenced by factors related to both soil and slope. Contrasting sites according to soil and slope are likely to have differing phosphate (P) requirements to sustain the 90 % of maximum production (Gillingham, 1984).

b. Reactivity and Fixation in Soil

A fertilised soil is not an equivalent system because there is normally intense reactivity occurring at sites applied with phosphate fertiliser. Water soluble phosphate fertilisers react with existing soil minerals, clays and hydrous oxides which are able to fix phosphorus. The chemical reactions within a soil are controlled by properties of a fertiliser including solubility, and the character of the soil. Compounds formed tend to transform rapidly (even when not in thermodynamic equilibrium) particularly in the presence of soil moisture or when a water-soluble fertiliser compound is introduced. Some major phosphate compounds that typically form in soil as reaction products from phosphate fertiliser include brushite ($CaHPO_4.2H_2O$), amorphous ($(Fe, Al)_3PO_4.nH_2O$)), $NH_4Fe(HPO_4)_2$, and taranakite $[(H_6(NH_4)_3Al_5(PO_4)_8.18H_2O]$ (Sauchelli, 1965). Pasture assimilate inorganic P from the soil solution where it normally exists in very low concentrations (0.01 to 0.05 ppm) and accumulate it in the above ground tissue to 2500 to 5000 ppm (or 0.25 to 0.5 % on a dry matter basis) (During, 1984).

Organic matter in mineral soils has also been shown to maintain soil phosphorus in its available form whereby the favoured mechanism may have been an anion exchange preventing or replacing phosphate compounds of iron and aluminium (Sauchelli, 1965).

In fertilised soils, plants normally utilise initially 20 to 30 % of phosphorus from the applied phosphates in the first year with recovery gradually decreasing in the succeeding years. (Sauchelli, 1965). Not all phosphorus is fixed by plants from the applied phosphates in fertilisers. The remaining component of phosphorus is either consumed by microorganisms, precipitated by soluble cations, or adsorbed by the colloidal complex of the soil such as on clay mineral surface, and thus making it more stable and less available to plants (Sauchelli, 1965). Thus, P fertilisers (including P from dung) are a crucial part of the P cycle because they replace P uncontrollably lost from the system such as those complexed in stable organic forms (During, 1984).

c. Phosphorus Requirements for Pasture Production

Generally, for a typical good quality grass-clover pasture producing 12 to 14 tonnes of herbage DM ha^{-1} yr^{-1}, the total annual uptake of phosphorus amongst other nutrients was 40-60 kg ha^{-1} (During, 1984). However, several environmental factors affect phosphorus requirements. Pasture established on the same land slope may mask different soil types originated from either sedimentary or various volcanic ash parent materials that also vary in physical and moisture retention properties, and thus may also have different phosphate (P) requirements to maintain maximum pasture production (Gillingham, 1984). At the Whatawhata fertiliser trial, on a Yellow Brown Earth-Brown granular loam soil, pasture production was measured on two contrasting slopes. The maximum pasture growth response achieved (14, 900 kg DM ha^{-1}) on easy (10 – 20 ^{o} slope) strata required 50 kg P ha^{-1} yr^{-1}, while only 30 kg P $ha^{-1}yr^{-1}$ was needed on steep (30 – 40 ^{o} slope) strata that yields less maximum growth and about half of that of the easy slope (7, 700 kg DM ha^{-1}) (Gillingham *et al.,* 1984). The difference in P requirements on the two slope strata was related to their varied moisture status. It was found that steep slopes which have a lower moisture content (as they dry out more quickly in spring and rewet slowly in autumn in comparison to land on easier slopes) only required the fertiliser loading of 30 kg P ha^{-1} yr^{-1} to satisfy the requirements of the pasture dominated by browntop-ryegrass-subterranean clover-lotus species (Gillingham, 1984). Another study by Lambert *et al.,* (1983) also found a similar pattern of decreasing herbage accumulation relative to increasing slope for the 15 -27 ^{o} slope studied, however, slope did not have an effect on the seasonal spread of annual herbage accumulation and neither did fertiliser (P) treatment.

Pasture species determined at the Whatawhata fertiliser trial site in spring 1982, found that ryegrass dominated on the easy slopes, while lower-fertility grasses (other than brown top and ryegrass) which include weeds, moss, dead matter and other grasses dominated on the steep slopes. There was a greater legume content on the steep slopes compared to the easy slopes which implied the status of available N on soils on the Steep slopes was possibly lower compared to those on the easy slopes (Gillingham, 1984). After 4 years (1984-88) of the fertiliser trial, Gillingham *et al.,*

(1990) found on the easy slopes, a 14 % reduction in pasture production in paddocks that previously received high fertiliser inputs, compared to pasture production also on easy slopes with low fertiliser inputs. However, Dodd *et al.* (1999) found that after 15 years of the trial (1984 -1999), a significant decrease (up to 16 %) was observed also on the easy slopes under all fertiliser treatments but not in paddocks with a history of high fertiliser inputs only. The decline in pasture production was observed only in paddocks on the steep slopes which previously received high fertiliser inputs (Dodd and Ledgard, 1999). Species (mainly key fertility species of ryegrass and white clover) abundance continued to decrease in the residual treatment compared with the maintenance treatment. For over 15 years, species abundance had decreased by 15 % in the residual treatment compared to the maintenance treatment (Dodd and Ledgard, 1999).

d. Effect on Nutrients & Organic Matter Content

With fertiliser application, pastures have also had marked increases in the nitrogen, sulphur and phosphorus content (During, 1984). Phosphorus is required to facilitate the establishment of legume in pasture, particularly clover in temperate regions. The legumes are needed to 'fix' atmospheric nitrogen biologically which results in increase of soil nitrogen, which increases fertility required for the desirable grasses to dominate pasture. Usually, coupled with pasture establishment, is the improvement of the soil structure and of its water-holding capacity, equally desired for a high level of fertility for grasses (Lazenby, 1965).

e. Effects of Withholding

At Whatawhata, ceasing fertiliser addition for each treatment on some paddocks created a 'residual' or a withholding treatment that was compared with the 'maintenance' treatment where fertiliser addition was continued. Withholding of fertiliser P had an effect on Olsen P levels and pasture production ((Dodd and Ledgard, 1999).

A previous study by Sauchelli (1965), found a 'residual response', indicative of the effects of soluble phosphate fertilisers applied initially, to remain available in western

soils after several years. In a loam type soil, soluble phosphates that have been incorporated were found to remain after 10 yrs in a form easily removed, indicating the persistency of their potential fertilising action (Sauchelli, 1965).

f. Olsen P

Available P in soil is the amount (fraction) of labile inorganic phosphate in the soil that can rapidly move into the soil solution. The Olsen-P test is sometimes used in conjunction with the P retention test to predict phosphate fertiliser requirements (McLaren and Cameron, 1996). Olsen P tests have been used as a monitoring indicator for changes in available P (Rowarth *et al.,* 1992), or changes in soil fertility over time, in particular where land management has been involved, to monitor its impact on soil nutrients (Wheeler, 2004).

Naturally Olsen P concentrations are low in New Zealand soils (Sparling and Schipper, 2004). Target soil test ranges (P level for an average of 97 % or near maximum pasture production) for Olsen P are 20-30 for both ash and sedimentary soils, and 35-45 for pumice soils (Morton and Roberts, 1999). A study at the Whatawhata fertiliser trial found a weak relationship between Olsen P status and relative production, however, the effect of Olsen P on yield is more pronounced when differing slopes are compared (Gillingham *et al.,* 1984). For example, maximum production on Easy slopes had an Olsen P of 15, while an Olsen P of 10 was optimum for maximum pasture production on steep slopes.

Investigation of the P fertiliser withholding effect at Whatawhata found no significant difference in Olsen P between the maintenance and residual treatments at the lower P fertiliser rates (10, 20 and 30 kg P ha^{-1} yr^{-1}) where Olsen P levels were in the range of 10-15 units over the 15 years (1984 – 1999) of measurement. However the difference is more prominent in the higher treatments (50 and 100 kg P ha^{-1} yr^{-1}) where soil Olsen P significantly increased in the maintenance treatment compared to the residual treatment. Slope did not affect Olsen P levels, for example, soil Olsen P tests at Whatawhata were found to be similar on steep and easy slopes (Dodd and Ledgard, 1999).

2.5. EFFECT OF ALLOPHANE

Allophane refers to "a group of clay-size minerals with short-range order which contain silica, alumina and water in chemical combination."(Parfitt, 1990). The types of allophane found in New Zealand include Proto-imogolite allophane and Imogolite-like allophane characterised by an Al:Si ratio of 2-4, and Pumice allophane characterized by an Al:Si ratio of approximately 1, and those found as stream deposits. The properties exerted by allophane are influenced by the structure and shape of the allophane particles, the large surface area and the chemical groups existing at the surface. Of the allophane groups, the most reactive are the $Al(OH)H_2O$ groups which reacts in acidic conditions to become $Al(OH_2)+H_2O$ or $Al(OH)(OH)^-$ in alkaline conditions (Parfitt, 1990).

Organic matter which bears a negative charge, upon interaction, increases the negative charge on allophane while decreasing the positive charge (Parfitt, 1990). As much as 25 % C is related with allophonic soil clay portions (Parfitt, 1990). Allophane in highly allophanic soils reacts with organic matter to form strong complexes with Al and is accountable for the slow rates of mineralisation of C and N in these allophonic soils compared with the non-allophanic soils (Broadbent *et al.,* 1964). Further, Ross and Cairns (1982) determined that biochemical activities per unit of organic carbon are much lower in allophanic soils than in non-allophanic soils.

2.6 SOIL PH

In acidic soils, the concentration of H^+ ions exceeds that of OH^- ions. Several compounds contribute to the development of soil acidity, including inorganic and organic acids that were produced by the decomposition of soil organic matter, respiration of plant roots producing CO_2 that the produces H_2CO_3 in water, and H^+ ions also present in water. A large fraction of H^+ ions in soils are normally absorbed

by the clay complex as exchangeable H^+ ions, and their degree of dissociation as H^+ into the soil solution also contributes to the acidity of the soil (Tan, 1993).

Soil acidity or pH strongly controls plant growth by indirectly affecting element availability and mobility (Tan, 1993), specifically the nutrient ions that are essential for plant growth (including NH_4^+, NO_3^-, NO_2^-, HPO_4^{2-}, $H_2PO_4^-$ polyphosphates, Fe^{2+}, Fe^{3+}, SO_4^{2-}, S^{2-}) (Cresser *et al.,* 1993; Floate, 1987). Soil acidity influences the rate of biological soil processes, mainly through limiting the size (numbers) and activity of microorganisms, which has a strong effect on the proportional amounts of old, stable and active organic nitrogen. Raising soil pH by liming can accelerate the decomposition rate (Floate, 1987) by increasing microbial activity (Jackman, 1960). In New Zealand, higher pH generally exists under agricultural land as a result of widespread application with lime following clearance of the forests (Sparling and Schipper, 2004).

Acidic soils are normally found in humid regions including New Zealand, and normally associated with large amounts of soluble Al, Fe, and Mn (Tan, 1993). Comparatively, alkaline soils usually occur in semiarid to arid regions, and in contrast are normally associated with low amounts of soluble Al, Fe, and Mn (Tan, 1993). High concentrations of Al and Mn are toxic to plant growth which are normally distinguished as inhibiting phosphorus uptake and translocation (Haynes, 1982), and severely restricting root extension (Cresser *et al.,* 1993).

Soil pH also affects the cation exchange capacity (CEC) of soils and soil organic matter (Cresser *et al.,* 1993). Soil acidity also influences microbial activity of the soil (Jackman, 1960). Microbial activity increases as pH is raised and results in the increase in the rate of organic matter breakdown mainly due to the release of available carbon which is the limiting factor to microbial activity. Available nitrogen is also released by raising soil pH, however, it is unclear that the release of available phosphorus from organic forms occurs when pH was raised (Jackman, 1960).

Several similar pH optimum ranges which promote high pasture production have been reported which include a pH 6 to 7 (Tan, 1993), pH of 4.5 to 7.5 (McLaren and Cameron, 1996), and pH 5.8 – 6.0 (Morton and Roberts, 1999, Edmeades, 1998). The soil pH range reported by Morton and Roberts (1999) was the same for ash, sedimentary, and pumice soils. An optimum pH close to 7 is generally found in most nitrogen-fixing systems. A pH of less than 5.5 will affect certain bacteria such as *Rhizobia meliloti* but some other leguminous and non-leguminous systems have been known to still function at or below pH 4.5 (Granhall, 1981).

To increase soil pH as required for increasing of pasture production, lime is normally applied to soil (Morton and Roberts, 1999). However liming has not been found to show that it enhances P availability including in Northern Yellow-Brown Earths (Ultic Soils) and thus lime and P requirements should be considered independently (Edmeades *et al.,* 1984).

Soil acidity or alkalinity as measured by pH develops different chemical relationships with phosphorus e.g. as reported by Sauchelli (1965), phosphorus forms tend to be influenced at different pH:

- *pH 2 to 5*: The strong existence of iron and aluminum in clays at low pH precipitates and retain phosphorus on clays as phosphates;
- *pH 4.5 to 7.5*: Phosphorus is adhered on the surface of clay particles but at pH 6.5 to 7.5, available P for plant utilisation is at its optimum.

Generally, a pH less than pH 6.5 (where aluminum and iron is excess) and that above pH 7.5 (where calcium and magnesium ions are dominates), decreases the solubility and thus availability of phosphorus when phosphorous forms less soluble compounds with these elements and ions. However at higher pH of pH 8.5 to 10, sodium ions that are released react with phosphate ions to from sodium phosphates which are comparatively more soluble than calcium and magnesium phosphate compounds (Sauchelli, 1965).

2.7. SUMMARY AND CONCLUSION

Soil organic matter continuously undergoes decomposition and mineralisation processes within the soil. The pool size of soil organic matter determined by addition and decomposition rates can be impacted by agricultural management practices by causing changes in both the pool size and turnover rate of SOM, C and therefore nutrients. Specifically, carbon and nitrogen processes such as mineralisation and immobilisation are significant as they underpin soil functions and fertility. The soil microbial biomass as the active fraction of SOM strongly influences the SOM levels and nutrient cycling which are key to a sustained productivity of agricultural systems. Soil carbon in pasture soils have recently been found to decrease however, reasons for this loss are unclear. My study using long term pastures at Whatawhata would determine the state of soil organic matter under the influence of P fertiliser agricultural regime and would contribute to previous studies conducted at Whatawhata and other related pasture studies.

CHAPTER 3

SITE DESCRIPTION, EXPERIMENTAL DESIGN, & FIELD SAMPLING

3.1. INTRODUCTION

This chapter describes the Whatawhata research site and the experimental design. The rationales for different approaches or methods used in this thesis are discussed.

3.2. SITE LOCATION

The research site is AgResearch's large scale fertiliser trial area at Whatawhata Research Station, which has latitude of 37 ^{o} 48'S and an altitude of 200 m (Gillingham *et al.*, 1990; Rowarth *et al.*, 1992). The study site is located within the Whatawhata district in the North Island of New Zealand, approximately 22 km west of Hamilton and 125 km south of Auckland (Figure 3.1).

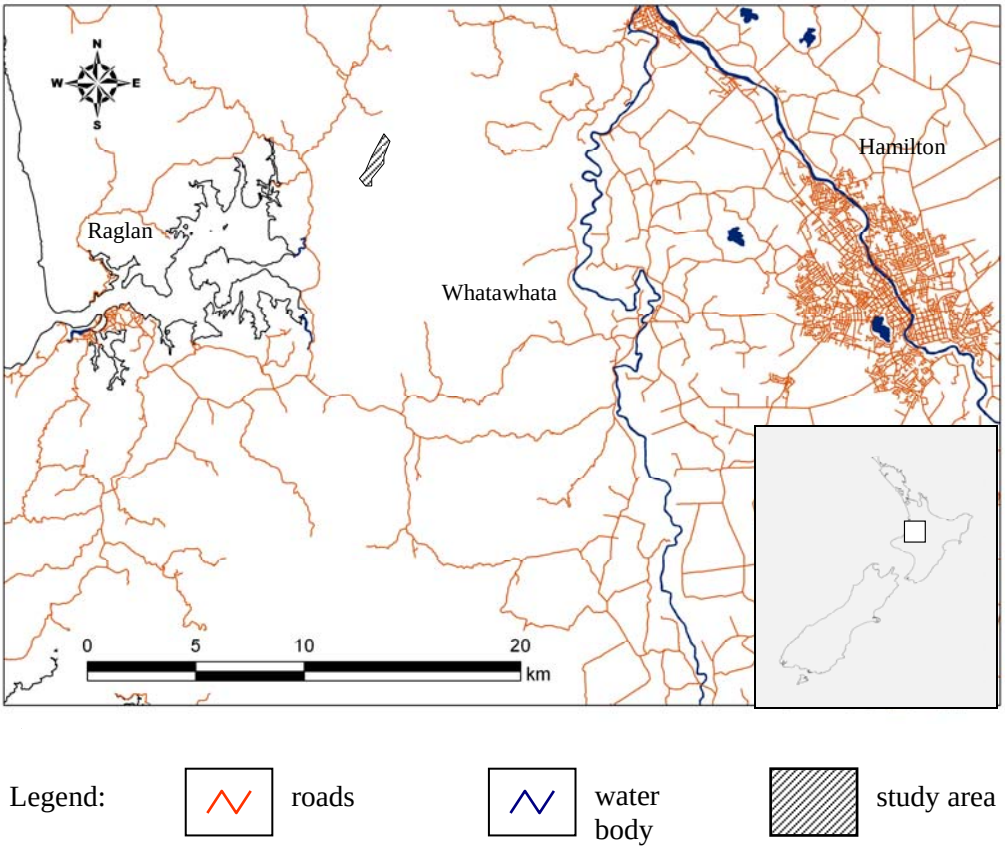


Figure 3.1: *The fertiliser trial study site (estimate) location at Whatawhata.*

3.3. PHYSICAL ENVIRONMENT

3.3.1. Soil and parent materials

The soil at the Whatawhata Research station comprises predominantly of clay soils developed from argillaceous greywacke, with some recent volcanic ash on the gentle slopes (Bruce, 1978) and is typical of many North Island areas (Farrelly, 1986). The prevalent soil at the Whatawhata research station site is the Kaawa hill soil, a Northern Typic Yellow Ultic Soil (Typic Haplohumult). Other Whatawhata soils include the Waingaro Steepland soils related to yellow-brown earths (Brown Soils), Naike hill soils, a granular brown loam (Granular soil)

developed on halloysite-rich beds of Hamilton Ash, and Dunmore silt loam, a yellow-brown loam (Allophanic soil) from moderately weathered volcanic ash from the 'Mairoa' ash beds (Bruce, 1978).

Soils sampled in this study at the Whatawhata fertiliser trial were Kaawa Hill soil (Typic Yellow Ultic Soil) derived from greywacke/argillite and some weathered volcanic ash. Areas which contained allophane and were avoided in the sampling area were Dunmore yellow-brown loams (Entic Dystrandept) with a P sorption capacity > 80 %; and Naike Hill soils (Brown Granular Loams) (Bruce, 1978), with P sorption capacities between 60 and 80 % (Rowarth *et al.*, 1992b).

Ultic Soils are typically acid soils distinguished by clayey and/or organic illuvial characteristics in subsoil horizons. Weathering products of siliceous sediments or acid igneous rocks, the Ultic Soils normally contain blends of clay minerals that include kaolinite, halloysite, aluminium-interlayered vermiculite and smectite, while several are also developed in the weathering products of limestones and greensands. Ultic Soils commonly occur in the northern North Island, and regions of Wellington, Marlborough and Nelson (Hewitt, 1998).

Ultic Soils are commonly characterised by the presence of plant toxic aluminium usually present in the B horizons (Hewitt, 1998), are of medium phosphate retention group (Cornforth, 1998), have medium to high CEC values, low levels of magnesium and potassium and low nutrient reserves. Low levels (less than 3 mg 100 g^{-1} of extractable phosphorus, indicative of inorganic phosphorus), and less than 20 mg 100 g^{-1} of total phosphorus are common (Hewitt, 1998). Most Ultic Soils are poorly drained, but a few are also well drained, and are generally susceptible to compaction during wet periods. The surface horizons are mainly silty and are vulnerable to erosion (Hewitt, 1998).

3.3.2. Topography & Vegetation

The Whatawhata Research Station has a wide range of topography, slopes, altitude and soil types (Farrelly, 1986). The main vegetation on the Waingaro

steepland soils and the Dunmore silt loam soils was originally 'tawa' (*Beilschmiedia taw*) dominated dicotylous-podocarp forest (Radcliffe *et al.*, 1968; Gillingham 1973, 1980). Before World War II, the farm area was initially developed from native bush, remnants of which remain in steep gullies. The gullies now also have manuka, fern, gorse and blackberry. Most of the land used was densely covered with manuka, bracken, hard fern and club moss (*Lycpodium fastiguiatum*) before pasture development. Weeds that used to persist at Whatawhata farm station include 'Inkweed', 'Nodding thistles' 'Blackberry' and 'Gorse' which was the most persistent (Farrelly, 1986).

The fertiliser trial site has been in pasture since at least the 1940s (Farrelly, 1986). During pasture development, the Whatawhata Research Station was oversowed with white and subclovers, *Lotus pedunculatus*, grasses of average fertility including crested dogstail, browntop, Danthonia, and grasses of higher fertility which included ryegrass and cocksfoot. Vegetation that existed during the trial period (since 1980) and during follow up studies on the pasture trial area ranged from ryegrass/white clover predominant on flat areas to browntop/suckling clover on steep sites (Rowarth and Gillingham *et al.*, 1992). More herbage has been found to accumulate on north-facing slopes than on southerly slopes (Radcliffe *et al.*, 1968; Gillingham, 1973, 1980).

3.3.3. Soil Profile Descriptions

Soil profile descriptions were carried out on paddocks 13 (100 kg P^{-1} ha^{-1}), and 18 (0 kg P^{-1} ha^{-1}) following the method of Milne *et al.*, (1995), and Oyama (1970) for description of the soil colour. Soil located for the soil profile sites were non-allophanic as verified by an allophane test. The two soils described (Figure 3.2, Tables 3.1 and 3.2) were similar. The main differences between them were that the Ap horizon for paddock 13 had more root abundance in the top 0 - 3 cm topsoil, few curled up earthworms, and it had a distinct wavy boundary, while the soil profile in paddock 18 had many, microfine and extremely fine roots only, throughout the Ap horizon, with no visible earthworms, and it had a distinct

smooth boundary (between Ap and Bw horizons). The Bw horizons had small colour differences.

(a)

(b)

Figure 3.2: *Soil profiles at (a) paddock 13 showing Ap to Bt horizons, and (b) paddock 18 showing Ap to Bw horizons only.*

***Table 3.1**: Soil Profile for Paddock 13: Kaawa Hill Soil*

Site location	Whatawhata fertiliser trial, Whatawhata research station; Paddock number 13; 0.989 ha; about 25 m from boundary fence with paddock 12, about 30 m (diagonal) from the paddock's gate, about 20 m straight from gate 11 fence, and about 50 m from the neighbouring ridge.
GPS coordinates	E2692244 and N6377390 with an elevation of 119 m
Aspect	Westerly
Slope	15 ^{o}
Vegetation of site	clover and ryegrass, cocksfoot grass (*D. glomerata* L), scotch thistles (*Cirsium vulgare*), and california thistles (*Cirsium ravens*)
Parent material	Greywacke/Argillite and some weathered volcanic ash
Soil Classification	Typic Yellow Ultic Soil
Soil profile description	
Ap 0 - 20 cm	dark brown (10 YR 3/4) clay loam; moderately developed, very coarse prismatic, breaking to strongly developed fine prismatic structure; brittle, very plastic, and very sticky; many, microfine and extremely fine roots, with top 3 cm very strongly mattered with plant roots and base of plant, and root is abundant.; few pinkish curled up earthworms; and distinct wavy boundary;
Bw 20 - 45 cm	yellowish-brown (10 YR 5/6) clay loam; strongly developed, medium-coarse blocky, breaking to strongly developed very fine to fine polyhedral structure; brittle, very plastic, and very sticky; many, microfine and extremely fine roots; slightly moist; and distinct smooth boundary;
Bt 45 – 80 cm	bright yellowish-brown (10 YR 6/8) clay with few bright brown (7.5 YR 5/8) mottles; strongly developed, very coarse, breaking to strongly developed medium-coarse blocky structure; semi-deformable, very sticky, and very plastic; common microfine and extremely fine roots; distinct clay skins covering all the ped faces; and distinct smooth boundary;
Bt(g) 80 – 90+ cm	bright yellowish-brown (10 YR 7/6) clay, with common, microfine to very fine bright brown (7.5 YR 5/8) mottles and with common, microfine to very fine light grey (5YR 8/2) redox segregation (pale mottles); strongly developed, very coarse, breaking to strongly developed medium-coarse blocky structure; semi-deformable, very plastic, and very sticky; and no roots.

Table 3.2: *Soil Profile for Paddock 18: Kaawa Hill Soil*

Site location	Whatawhata fertiliser trial, Whatawhata research station; Paddock 18; 0.773 ha; about 50 m from boundary fence with paddock 17, about 45 m from boundary fence with paddock 16, and about 60 m to the end of the paddock's fence.
GPS coordinates and altitude	E2692152 and N6377231 with an elevation of 129 m
Aspect	westerly
Slope	17 ^{o}
Vegetation of site	summer grasses and a few dandelions (*Taraxacum officinalis aggr.*), california thistles, and cocksfoot grass
Parent material	Greywacke/Argillite and some weathered volcanic ash
Soil Classification	Typic Yellow Ultic Soil
Soil profile description	
Ap 0 – 20 cm	dark brown (10 YR 3/4) clay loam; strongly developed polyhedral, breaking to very fine to fine structure; brittle, very sticky, and very plastic; many, microfine and extremely fine roots; and distinct smooth boundary;
Bw 20 - 40+ cm	brown (7.5 YR 4/6) clay loam, with few very fine bright reddish brown (5 YR 5/8) mottles; coarse to very coarse breaking to fine to very fine structure; brittle, very sticky, and very plastic; and many, microfine and extremely fine roots.

3.3.4. Bulk Density

The soil bulk density was determined for three replicates from the 0-5 cm depth in each of paddocks 18 (0 kg P ha^{-1} yr^{-1}) and paddock 13 (100 kg P ha^{-1} yr^{-1}) (Figure 3.3). Using pre-weighed steel rings (of about 6 cm diameter and 5 cm height), each ring was hammered into the soil, using a piece of wood and a small mallet. The ring and soil enclosed were removed and the soil at the top and bottom of the ring were cut to the level of each of the ring's end. The soil sample volumes (in the rings) were placed immediately in a plastic bag for determination of dry bulk

density in the laboratory. Determination of the soil dry bulk density for each of the paddocks was described in section 4.3. The bulk densities averaged to 1.15 g cm^{-3} in paddock 13, and 1.25 g cm^{-3} in paddock 18.

Figure 3.3: *Samples collected to a depth of 5 cm for bulk density measurement*

3.3.5. Soil pH

Reported soil pH ranged from pH 5.2 for Waingaro steepland soils sampled at 0 – 23 cm depth, to 5.6 for the Kaawa Hill soils sampled at 0-13 cm depth (Bruce, 1978). Soils sampled at 0 – 7.5 cm at the Whatawhata fertiliser trial since 2004 had pH averaging around 5.2 (Ian Power pers comm. 20/11/2007).

3.3.6. Climate

The nearest climate station was near the Whatawhata Hill Country Research Station entrance buildings.

a. Temperature

The mean annual ground temperature at Whatawhata (1952-1980) at 10 cm soil depth was at 13.7 ^{o}C, which was the same as the mean annual air temperature, but lower than average temperatures recorded at soil depths beyond 10 cm i.e. 15.3 ^{o}C at 30 cm and 1 m (New Zealand Meteorological Service, 1983). The warmest month was February with a mean temperature of 18.1 ^{o}C, while the coldest was in July with a mean temperature of 8.4 ^{o}C. Temperature tends to fall rapidly in autumn and early winter (Bruce, 1978).

b. Precipitation/Moisture

Generally, the climate at Whatawhata is warm to humid with a mean annual rainfall (1952-1980) of about 1600 mm ((New Zealand Meteorological Service, 1983). Rainfall data for Whatawhata were measured at the bottom of the research station and so the mean annual rainfall at the trial area should be expected to be higher because of its higher altitude and being located on a more westerly aspect. Flooding was not frequent but two major floods were recorded in 1907 and 1958 where rivers rose 16 ft above normal causing flooding of farmlands and settlements (Farrelly, 1986).

3.3.7. Land Use History

a. Establishment of the Whatawhata Hill Country Research Station

Whatawhata was cleared from native forest when pastoral farming started in the early 19th century. Sheep farming dominated the farm industry and along with wheat formed the basis of the farm economy up to the 1880s. The Whatawhata Hill Country Research Station was established in 1949 to provide practical, scientifically researched, solutions to efficient livestock (sheep and cattle) hill country farming (Farrelly, 1986).

Plantations along the farm were established for eucalyptus (1950s), mostly on lower fertility areas. *Pinus radiata*, *Cupressus macrocarpa* and Japanese Poplars were planted on steep land and areas difficult to access. Trees and shrubs were mainly planted to provide shelter, control erosion, and for land beautification. An

agroforesty programme introduced in 1971 at Whatawhata integrated forestry and animal grazing to further complement the study of animal production on hill country. A pasture research programme in 1973 investigated soil fertility for improving the productivity of hill country pastures to the levels of those areas of the lowlands. The soil fertility research programme consisted of testing and evaluation of a variety of legumes and grasses that had potential for use on North Island hill land (Farrelly, 1986).

From 1980, the overall research programme changed at Whatawhata. The focus shifted onto soil fertility and agroforestry, and the role of fertiliser in developing hill country soils for higher pasture production and for the formulation of recommendations for phosphate fertiliser use (Farrelly, 1986).

b. Phosphate Fertiliser Application (Pre-1980)

Soil fertilisation started in 1950. Aerial topdressing by aircraft enabled superphosphate to be applied to accessible and also to areas previously inaccessible. In a 10 year period from 1952-1962, most of the farm received 2 cwt super per acre per year (approximately 251 kg P ha^{-1} yr^{-1}). Investigation at the time demonstrated a pasture response to molybdenum on the steeper yellow brown earth (mudstone soil types) (Farrelly, 1986). Prior to commencement of the fertiliser trial programme in the early 1980s; the area had annual topdressing with c.400 kg ha^{-1} of single superphosphate which in some instances included molybdenum and potassium (Rowarth *et al.,* 1992b).

c. Phosphate Fertiliser Trial Programme

The phosphate fertiliser trial site was established across 20 paddocks situated in a north-westerly aspect block and encompassing steep and easy contours (Figure 3.4). The trial site has a total area of 21.4 ha (Rowarth *et al.,* 1992b) with paddocks ranging from 0.243 to 1.223 ha (Rowarth *et al.,* 1992c). The paddocks have varied topography from flat to more than 30 ^{o} slopes, specifically comprised of four slope groups: campsites (0-10^{o}), easy (11-20 ^{o}), moderate (21-30 ^{o}), and steep (31 ^{o}+) (Rowarth *et al.,* 1992b).

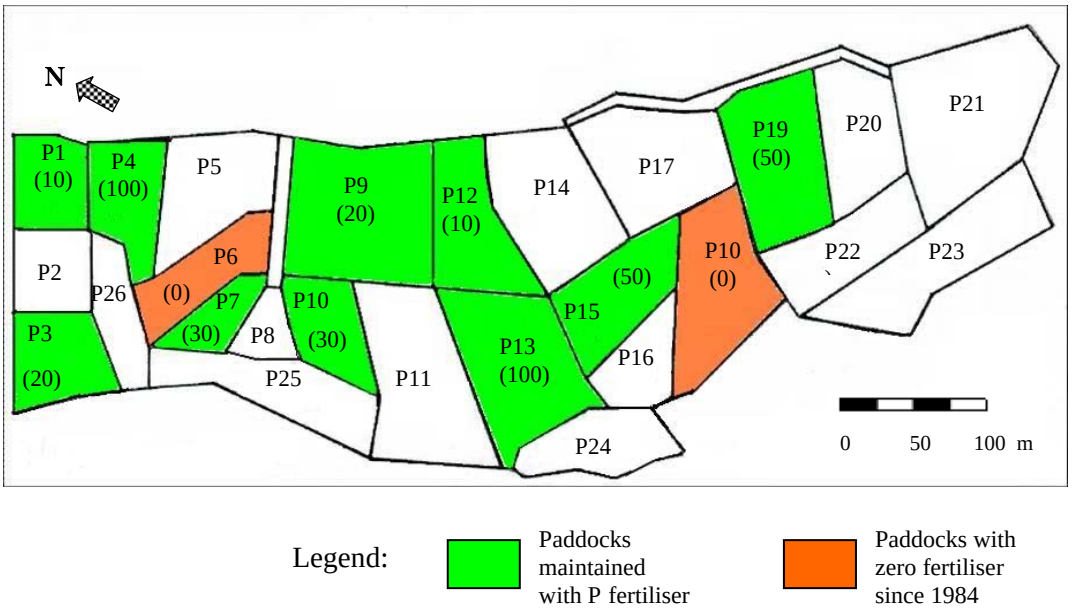


Figure 3.4: *Whatawhata fertiliser trial paddock subdivision plan. Paddock numbers are denoted as "P" and fertiliser P application rates (in kg P ha^{-1} yr^{-1}) are in parenthesis "( )". (modified from AgResearch 2007).*

The 20 paddocks were topdressed by single superphosphate applied annually in late summer to autumn at rates of 10, 20, 30, 50 and 100 kg P ha^{-1} yr^{-1} to 4 replicates initially for four years (1980-1984) (Gillingham *et al.*, 1990). Another five paddocks were also topdressed with the five different treatments from 1980-1984 and were used as preconditioning areas for stock to avoid nutrient transfer onto the trial paddocks. After four years, in 1984, the trial was decreased to 2 replicates of each treatment (Gillingham *et al.*, 1990), which continued to receive phosphate fertiliser at the prescribed rates from 1984 to date (Table 3.3 and Table 3.4). Of the four paddocks that received 10 kg P ha^{-1} yr^{-1} from 1980-1984, two have been maintained at the 10 kg P ha^{-1} yr^{-1} rate and the other two received no fertiliser applications. In this thesis, the two paddocks that received no fertiliser since 1984 are referred to as zero fertiliser treatments. Triple superphosphate has been used since 1989 instead of single superphosphate fertiliser (Dodd and Ledgard*et al.*, 1999) (Table 3.4).

Fertiliser nitrogen (N) and potassium (K) have never been applied since 1980. Calcium (Ca) and sulphur (S) were applied within the single superphosphate fertiliser (Table 3.3), whereas only Ca and P were applied as contained in the triple superphosphate applications (Table 3.2) (Ian Power, pers comm., 2007).

Table 3.3: *Single superphosphate fertiliser composition applied to paddocks in the Whatawhata fertiliser trial (1984-1988). Data supplied by Ian Power (pers comm., 16/11/2007).*

Paddock number	**kg P ha^{-1} yr^{-1}**	**kg Ca ha^{-1} yr^{-1}**
P6, P18	0	0
P1, P12	10	7
P3, P9	20	14
P7, P10	30	20
P15, P19	50	34
P4, P13	100	69

Table 3.4: *Triple superphosphate fertiliser composition applied to paddocks in the Whatawhata fertiliser trial (1989-date). Data supplied by Ian Power (pers comm., 16/11/2007).*

Paddock number	**kg P ha^{-1} yr^{-1}**	**kg Ca ha^{-1} yr^{-1}**	**kg S ha^{-1} yr^{-1}**
P6, P18	0	0	0
P1, P12	10	24	13
P3, P9	20	48	25
P7, P10	30	72	37
P15, P19	50	119	62
P4, P13	100	237	124

d. Grazing Management

Grazing treatments were introduced in May 1984 and performed through rotational grazing in the fertiliser trial paddocks as follows: Five mobs comprised of about 200 Romney-cross ewes or wethers were first placed in the preconditioning paddocks for 2 days, moved to the first replicate of the lowest Residual P rate treatment (10 kg P ha^{-1} yr^{-1}) for 2-3 days, then on to the second replicate of the same treatment also for the same amount of days, then moved onto the same manner to the first and second replicates of the Maintenance paddocks of the same P rate treatment i.e. also of 10 kg P ha^{-1} yr^{-1}. Grazing continued with the Residual and Maintenance paddocks for the next P rate treatments of 20, 30, 50 and 100 kg P kg P ha^{-1} yr^{-1} respectively and then the mobs were removed from the trial area (Gillingham *et al.,* 1990). Higher stock numbers were placed on paddocks that received higher rates of P fertiliser because of increased dry matter (DM) produced on the higher rate P paddocks (Rowarth *et al.*, 1992c). After grazing in each paddock, pasture height was about 2-3 cm (Gillingham *et al.*, 1990). Grazing stopped when the pasture cover declined to 1000 kg DM ha^{-1}. Pasture was then left for re-growth for 4-6 weeks (Rowarth *et al.*, 1992c). The total trial area was continuously grazed in the early months of spring i.e. September-October (Gillingham *et al.,* 1990).

e. Research Programmes at Whatawhata Fertiliser Trial

The research programmes utilising the Whatawhata Fertiliser Trial have consisted of a series of trials and include the following studies:

- 1980 – 1984: The application of superphosphate on paddocks at five different treatment rates (10, 20, 30, 50, 100 kg P $ha^{-1}yr^{-1}$) for four years (Gillingham *et al.,*1984);
- 1984 – 1988: The investigation of the residual effects of phosphate application withheld from some paddocks, on pasture production, pasture species composition, stock grazing days and Olsen P status (Gillingham *et al.,*1990);
- 1988 – 1999: The follow up study on the effects of withholding superphosphate application on pasture production, pasture species composition and Olsen P status conducted both at Whatawhata and Te Kuiti research stations (Dodd and Ledgard *et al.,* 1999);

- 1992: above-and below-ground P balances (Rowarth *et al.*, 1992b)
- 1992: phosphate balances on four slope groups at Whatawhata station were investigated as the effect of different P fertiliser rate treatments by Rowarth *et al* (1992c).

3.4. EXPERIMENTAL DESIGN

Twelve paddocks (numbers 1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 18, 19) were sampled representing the six fertiliser regimes maintained since 1984 (Table 3.3 and Table 3.4). Paddocks sampled received either: 0, 10, 20, 30, 50, or 100 kg P ha^{-1} yr^{-1} since 1984. Two paddocks (6 and 18) had no fertiliser application since 1984 but previously had fertiliser application (10 kg P ha^{-1} yr^{-1}) from 1980-1984. They are listed as "0" P in this thesis.

Selected sites within paddocks selected were "easy" slopes (11-20 ^{o}). The slopes measured with an Abney level were confirmed on a re-visit to the Whatawhata fertiliser trial. Selecting "easy" slopes which tend to form the majority of area in hill country could improve soil testing in terms of P because they are the most responsive to P within a paddock than other areas of different slopes (Rowarth *et al.***,** 1992a). "Easy" slopes have less eroding characteristics hence the added fertiliser is less likely to be washed off. Sites were selected to avoid areas near gateways, troughs or other obvious areas of stock congregation. Sampling sites on "easy" slopes were also selected so as to be consistent to the similar "easy" slope group that have been used in previous studies at Whatawhata including in Gillingham *et al.,* (1988 and 1990), Rowarth *et al.,* (1992 b,c), and Dodd & Ledgard (1999), and for ease of comparison of results obtained from this research to those from such previous studies. The "easy" slopes were also used to avoid allophanic soil material that occurs on flatter slopes in the area.

Soil sampling was constrained to the north-west to south-west aspect within the fertiliser trial paddocks. Samples were collected from similar aspects to ensure

that erosion or downhill effects of rainfall or leaching or exposure to other climate factors such as sun and prevailing wind were similar for all the soils sampled.

Soil sampling was carried out along 50 m transects across some paddocks but in some smaller paddocks it was physically impossible to use the whole 50 m transect, so two 25 m transects were used in parallel about 1-1.5 m apart (Figure 3.5). Samples were collected using regular distances along each transect, and avoiding thick grass (which indicates urine patches) as much as possible. Samples collected systematically tend to provide more accurate results compared to simple random sampling, because the samples tend to be distributed more evenly over the site of interest (Tan, 2005).

The sampling depth used was 7.5 cm (Morton & Roberts, 1999) which is within the range of where the most soil organic matter generally occurs the most i.e. within the top 10 cm in pasture soil (Sparling *et al.*, 2004). It has also been assumed that plant residues input under permanent pasture would mostly be found close to the surface of the soil (Jackman, 1964a) and hence should comprise the majority of the organic matter.

The GPS locations of sampling transect and soil description were recorded (Table 3.5) as this is crucial for potential future studies such as re-sampling.

Table 3.5: *GPS coordinates and elevation of sampling site locations at the Whatawhata fertiliser trial for paddocks under different fertiliser P application rates. The coordinates, marked using 'WGS84' coordinate system with an eTrex GPS (Garmin Ltd, 2003), showed an easting and northing of the start and end of each transect.*

Treatment (Kg $Pha^{-1}yr^{-1}$)	Paddock number code	Easting	Northing	Elevation (m)
10	P6	E2692322	N6377271	116
		E2692343	N6377281	117
10	P18	E2692131	N6377039	135
		E2692130	N6377030	136
10	P1	E2692486	N6377438	120
		E2692474	N6377438	125
10	P12	E2692305	N6377138	134
		E2692305	N6377140	135
20	P3	E2692364	N6377456	96
		E2692362	N6377454	97
20	P9	E2692322	N6377200	123
		E2692325	N6377202	124
30	P7	E2692336	N6377401	103
		E2692321	N6377392	107
30	P10	E2692292	N6377289	115
		E2692280	N6377296	115
50	P15	E2692170	N6377085	120
		E2692165	N6377084	125
50	P19	E2692163	N6376927	162
		E2692168	N6376931	161
100	P4	E2692443	N6377312	128
		E2692440	N6377330	122
100	P13	NR*	NR*	NR*

* NR: not recorded

3.5. FIELD SAMPLING

3.5.1. Time of Sampling

A one-off field sampling was undertaken to collect samples for determination of microbial biomass and soil chemical analyses. Field sampling was conducted early November (16.11.2006, during spring) so that samples could be obtained while soils were still moist but warm enough for microbes to be active. The

sample timing was important to capture the optimum conditions for the microbial biomass.

3.5.2. Collection of Samples

Topsoil was sampled using the stainless steel foot corer (7.5 cm depth) with an internal diameter of 25 mm. Approximately fifty cores were collected about 0.5 m apart and bulked by transect (if using 50 m transect or two 25 m transects) (Figure 3.5). Sufficient sample was collected to provide for laboratory analysis and for further analysis if required.

Figure 3.5: *Samples collection were made along the marked transects*

CHAPTER 4

METHODS

4.1. ALLOPHANE TESTS

Prior to establishing sampling transects, allophane tests were carried out in the field, to ensure that allophanic soils were avoided (Figure 4.1). Allophanic soils in New Zealand with higher extractable aluminium (Al) have high (≥ 85%) phosphate retention (Jackman, 1964b, McLaren & Cameron, 1996; Hewitt, 1998). The high phosphate retention is likely to reduce the effects of the added P fertiliser (Milne *et al.,* 1995). Allophane also tends to react with organic matter and form complexes that slow down the rates of mineralisation of C and N in soils with a high allophane content compared with non-allophanic soils (Broadbent *et al*. 1964). Further, biochemical activities per unit of organic carbon are much lower in allophanic soils than in non-allophanic soils (Ross *et al*., 1982). Hence for consistency, soil samples were only collected from non-allophanic sites.

An allophane test indicates the presence of reactive hydroxy-aluminium groups that exist in complexes of allophane and aluminium humus (Milne *et al.,* 1995). The allophane test kit contained a saturated aqueous NaF solution, and a filter paper (Whatman No. 42 cut in approximately 5 x 5 cm pieces) which had been treated with phenolphthalein indicator and dried. The procedure followed that described in Fieldes and Perrott (1966). About 5 g of soil from each of 2-3 sites selected along a proposed transect was placed on the filter paper and a few drops of saturated 0.85 M NaF was applied to wet the soil. Soils containing a substantial amount of allophane turn the

indicator in the range of pale red to dark red colour in about 60 seconds after application of the solution.

Figure 4.1: *Soil testing for the presence of allophane.*

4.2. LABORATORY PREPARATION

4.2.1 Soil Sieving

Samples collected from the field were stored in labeled polyethylene bags overnight in a fridge at 4 ^{o}C. The soil samples from the fridge were removed the following day and left to stand at room temperature for about 1 hour before sieving. Soils were initially sieved using a 6.7 mm sieve to remove stones, leaves, roots, and other debris, and followed by sieving with a 4.0 mm sieve to further remove plant material and break down larger soil aggregates. Sieving with a 4 to 6 mm mesh size does not affect

the soil microbial biomass size or activity (Jenkinson & Powlson, 1980). Samples were sieved in the order from the lowest to the highest P treatments so as to avoid cross contamination from the higher P concentration treated samples to the lower P concentration treated samples. The 6.7 mm and 4.0 mm sieves were thoroughly cleaned with a brush between sieving of each sample. Sieving of soil samples collected from the field was necessary to ensure penetration of the soil by a fumigant, and to enable obtaining of a representative sample for any of the analysis.

4.2.2. Preparation of Sub-samples

Following sieving each soil sample was sub-sampled as follows:

(i) About 200 g of soil was placed in a polyethylene bag and stored moist at 4 ^{o}C. These soil sub-samples (not fumigated and not incubated), were extracted with 2M KCl for subsequent analysis of nitrate and ammonium (for day 0) (section 4.11.2).

(ii) About 500 g of soil was placed in a tray and left on the lab bench for air drying. This portion was analysed for Olsen P, total C and N, and other organic fractions (sections 4.5, 4.6).

Air drying was appropriate as opposed to oven drying as air drying has minimal effect on analysis of total carbon, total nitrogen, and soil P content (Tan, 2005). After air-drying the samples were stored in tied polyethylene bags to avoid contamination in the lab, until analysis.

(iii) The remainder of the sample was pre-incubated in polyethylene bags at room temperature (25 ^{o}C), with a moist cotton ball loosely fitted into the neck of each bag to maintain the moisture status of the soil samples, until treatment the next day. These sub-samples were used to determine microbial biomass using the fumigation-incubation method (section 4.7.2),

microbial respiration measurement (section 4.7.3), and extraction of N with 2 M KCl (section 4.11.2) for subsequent analysis of nitrate and ammonium after incubation (day 14).

The moist samples received priority for lab preparation and analysis because the microbial processes in particular microbial respiration, and N mineralisation (to be measured) can change over time in soil, hence the soil samples require that they retain their original moisture status as much as possible (Tan, 2005). When preparation for analysis was not possible in a day then all moist samples were stored at 4 ^{o}C (Ross, 1988) and in the dark to preserve their moisture status (Jenkinson & Powlson, 1976a) and to prevent condensation and minimise microbial growth (Tan, 2005).

4.3. MOISTURE CONTENT AND MOISTURE FACTOR

Moisture content was determined as described in Topp and Ferré (2002). Two replicates of about 5 g each, from each sample, were weighed into pre-weighed aluminum containers and placed in an oven at 105 ^{o}C for 24 hours. After 24 hours, the soil and dishes were removed from the oven and placed immediately into desiccators for approximately 30 minutes for cooling and then reweighed. Gravimetric water content Θ_M was calculated as follows:

Gravimetric water content Θ_M :

$$= \frac{\text{(mass of moist soil + container) – (mass of oven dry soil + container)}}{\text{(Mass of oven dry soil + container) – (Mass of container)}}$$

(Topp and Ferré, 2002).

The moisture factor for moist or airdried soils was determined according to Blakemore *et al.,* (1987). The moisture factor was calculated as:

$$\text{Moisture Factor (MF)} = \frac{\text{(Wt 'Moist' or 'Air-dry' soil (g))}}{\text{(Wt Oven-dry soil (g))}}$$

$$= \frac{\text{[(Wt of Moist or 'Air-dry' soil + container) – (Wt of container)]}}{\text{[(Wt Dry Soil + container) – (Wt of container)]}}$$

4.4. BULK DENSITY

The soil bulk density was determined only for paddock 18 (0 kg P ha^{-1} yr^{-1}) and paddock 4 (100 kg P ha^{-1} yr^{-1}). Three samples collected from each paddock with metal rings of approximately 6 cm diameter and 5 cm height were determined in the laboratory for bulk density.

(a) The soil in the ring (pre-weighed), placed in a pre-weighed aluminium tray was weighed (M_{s1+r+t}) and placed to dry in the oven at 105 ^{o}C overnight;

(b) The soil, ring, and tray were removed from the oven the next day and placed to cool to room temperature in a dessicator and reweighed (M_{s2+r+t});

(c) The mass of oven dry soil contained in the ring was calculated as:

$$M_s = (M_{s2rt}) - M_r - M_t$$

Where:

M_s is mass of oven dry soil (g)

M_{s2rt} is mass of dry soil and ring and tray (g)

M_r is mass of ring (g)

M_t is mass of tray (g)

(d) The soil dry bulk density (ρ_b) was calculated as:

$$\rho_b = M_s / V_r$$

where:

ρ_b is dry bulk density (g cm^{-3})

M_s is mass of oven dry soil (g) (determined in c)

V_r is volume of ring (cm^{-3}) determined as:

Vr = area of end x height = $\pi r^2 h$

Where:

Vr is volume of ring/cylinder

π is 3.14

r is radius of ring (1/2 x diameter) (cm)

h is ring height (cm)

4.5. OLSEN P

Olsen P was determined by extraction with sodium bicarbonate ($NaHCO_3$) (Olsen *et al.,*1954) following the method in Blakemore *et al.,* (1987). The test for Olsen P, commonly used on New Zealand's pasture, provides a sensitive indicator of the P available to plants (Morton and Roberts, 1999; Lambert *et al.,* 2000), and gives the best relationship with production (Edmeades, 1998).

4.5.1 Reagent Preparation

Several reagent solutions were prepared, as required for the extraction of Olsen P from the soil sub-samples. All reagent solutions were prepared for a total of 36 sub-samples (triplicates of 12 soil samples) as follows:

a. 0.5 M Sodium Hydrogen Carbonate ($NaHCO_3$) solution

(i) 0.2 % superfloc solution:

The 0.2 % superfloc solution was first prepared as it was required for the preparation of the 0.5 M $NaHCO_3$. The 0.2 % superfloc solution was prepared from dissolving 0.2 g of polyacrylamide into 100 mL deionised water.

(ii) 0.5M $NaHCO_3$ solution:

Each of the 36 soil sub-samples needed 40 mL each or a total of 1,440 mL or approximately 1.5 L of 0.5 M $NaHCO_3$ solution for all 36 sub-samples. Two litres of 0.5 M $NaHCO_3$ solution was prepared by dissolving 82.0g of $NaHCO_3$ in deionised water and was diluted to 1960 mL. The pH of the $NaHCO_3$ was adjusted immediately to 8.5 by the drop wise addition of 50 % NaOH followed by addition of 2 mL of 0.2 % superfloc solution. The solution was then made up to 2 L. Because the solution was light sensitive, it was wrapped in aluminum foil immediately after preparation.

b. Murphy and Riley reagent solution A

The Murphy and Riley reagent solution A was prepared from the following solutions:

(i) 2.5M H_2SO_4 solution:

Concentrated H_2SO_4 of 344 mL was added to 1 L of deionised water contained in a 2 L volumetric flask which was previously settled in a cold water bath. The 2.5 M H_2SO_4 solution was left to cool.

(ii) Ammonium molybdate and Antimony potassium tartrate solution:

30 g of ammonium molybdate was dissolved in 400 mL deionised water. Separately, 0.6672 g of antimony potassium tartrate was dissolved in 50 mL deionised water.

(iii) Murphy and Riley reagent solution A:

Both of the solutions (ammonium molybdate and antimony potassium tartrate) were added to the 2.5 M H_2SO_4 solution [see 4.5.1.(b)(i)], made up to 2 L solution and transferred to a dark bottle. A further 500 mL of deionised water was added and the solution mixed thoroughly to give the Murphy and Riley reagent solution A.

c. Murphy and Riley reagent B

The Murphy and Riley reagent B was prepared on the day of the experiment as it does not keep for more than 24 hours. For each of the 36 sub-samples, 8 mL of the Murphy and Riley reagent solution B was required. Preparation of the Murphy and Riley reagent B involved dissolving 5.28 g of ascorbic acid in 500 mL of reagent A [see 4.5.1 b (iii)] using the empirical ratio of 1.056:100. The Murphy and Riley reagent B solution prepared was more than sufficient for all 36 sub-samples.

d. 0.5 M Sulphuric Acid (H_2SO_4)

Approximately 3 L and 250 mL of 0.5 M H_2SO_4 was required for all the 36 sub-samples and 6 standards to be prepared. A 2 L solution was prepared only as there was sufficient amount of 0.5 M H_2SO_4 available in the laboratory. The 2 L of 0.5 M H_2SO_4 was prepared by adding 56 mL of concentrated H_2SO_4 to approximately 1.5 L deionised water contained in a 2 L flask (settled in a cold water bath), and made up to 2 L with deionised water.

4.5.2 Standards Preparation

Six standards of 0, 1, 2, 3, 4, and 5 ppm P were prepared as follows:

a. Preparation of 100 ppm P

A 100 ppm P solution was prepared to make up the standards. 0.4401 g of potassium dihydrogen phosphate (KH_2PO_4) was dissolved in deionised water, and 28 mL of concentrated H_2SO_4 added to it as a preservative, and made up to 1 L.

b. Standards

From the 100 ppm P solution (4.5.2. a): 0, 1, 2, 3, 4, and 5 mL were pipetted into 100 mL flasks and each diluted to 100 mL of 0.5 M H_2SO_4, creating 0, 1, 2, 3, 4, and 5 ppm P standards respectively.

From each standard, 2 mL was taken into a 20 mL flask and followed by addition of 0.6 mL of 0.5 M H_2SO_4, 1.6 mL of Murphy and Riley reagent, and diluted to 20 mL with deionised water. The standards were then left for approximately 10-15 minutes to allow for colour development before measuring colour absorbance with the spectrophotometer at 880 nm.

4.5.3 Sample Preparation

The soil sub-samples were prepared as follows:

(i) Two grams of air-dried soil sub-samples were weighed and placed each into a 50 mL centrifuge tube;

(ii) 40 mL of the 0.5 M $NaHCO_3$ [4.5.1.(a)(ii)] extracting solution, was added to each of 2 g of air-dried soil sub-sample [4.5.3.(i)] and shaken on the end-over-end shaker for 30 minutes;

(iii) The soil sub-samples mixed with 0.5 M $NaHCO_3$ extracting solution in (ii) were filtered through filter paper (whatman no 41) into 20 mL vials;

(iv) From each of the filtered extract obtained in (iii), 10 mL was pipetted into a 100 mL volumetric flask;

(v) 3 mL of 0.5 M H_2SO_4 was added dropwise to each of the extracts in (iv) and the flask was swirled until the gas escaped. Approximately 80 mL of deionised water was added;

(vi) 8 mL of Murphy and Riley Reagent B solution [4.5.1.(c)] was added to the soil sub-sample extract solution in (v), made up to 100 mL with deionised water, and mixed well;

(vii) The soil extract solution mixed in (vi) was left for approximately 15-20 minutes to allow for colour development before reading absorbances on the spectrophotometer at 880 nm (Figure 4.2).

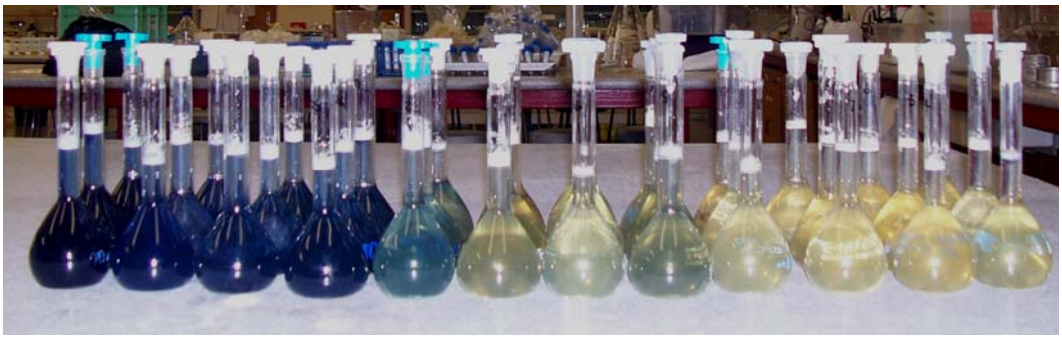


Figure 4.2: *The soil sub-sample extract solutions developed colour and were ready for Olsen P measurement on the spectrophotometer.*

4.5.4. Absorbance Measurements

Colour absorbances of the standards and sample solutions prepared (see 4.5.2. and 4.5.3. respectively) were measured on the spectrophotometer (Metertek SP-830, Metertech Inc., GBC Scientific, New Zealand, 1996) set at 880nm. The 0 ppm P standard was used for zeroing the spectrophotometer before continuing to read absorbances of the 1, 2, 3, 4, and 5 ppm P standards.

Again the 0 ppm P standard was read in the spectrophotometer as a baseline or blank before reading absorbances of samples. Actual absorbances of samples were then corrected by subtracting from the blank (0 ppm P) absorbance reading.

4.5.5. Calculation of Olsen P

Olsen P was calculated as follows:

(i) Standard Curve:

A standard curve was constructed from absorbances obtained from 0, 1, 2, 3, 4, and 5 ppm P standards (absorbance measurements described in 4.5.4) and its linear equation determined as:

$$y = 0.0507\ x$$

where,
y was absorbance,
x was P concentration in ppm or ug g^{-1} soil,
and 0.0507 was the slope.

(ii) P concentration per 2 g soil:

Absorbances of samples were corrected from the blank absorbance (4.5.4). Using the linear equation derived from the standard curve in (i), the concentration of P for each sample was determined from rearranging the equation in (i) as follows:

$$x = y/0.0507$$

(iii) Mass of P extracted from Samples:

The mass of P extracted from each of 2 g of soil sub-sample was calculated as follows:

$$A = B \times C$$

Where:
B was concentration of P in µg g^{-1}soil (equivalent to µg mL^{-1}),
C was 40 mL of the 0.5 M $NaHCO_3$ extracting solution,
A was mass of P (µg soil)

(iv) P concentration per gram soil:

The concentration of P (Olsen P) per gram soil was calculated as:

$$D = A/2$$

Where:

A was mass of P (µg soil) determined in 4.5.5. (iii),

2 was mass of soil used in g,

D was concentration of P (Olsen P) in µg g^{-1} soil

(v) P concentration per gram oven dried soil:

The concentration of P (Olsen P) per gram soil was corrected for oven dried soil mass as described:

$$E = D/F$$

Where:

D was concentration of P (Olsen P) in µg g^{-1} soil determined in 4.5.5. (iv),

F was Moisture Factor,

E was the P concentration (Olsen P) in µg g^{-1} oven dried soil

The Olsen P values were averaged for the triplicate samples for each of the 12 paddocks.

4.6. DETERMINATION OF TOTAL CARBON & TOTAL NITROGEN

4.6.1 Analysis of Total Carbon & Total Nitrogen

About 2 g of air-dry soil was taken from each of the 12 air-dried soil samples after mixing. Each sub-sample was ground using the ball mill grinder (Retsch, ISO 9001;

mm 2000) for 1 minute. Twelve samples of about 0.25 g (+/- 0.01 g) each were weighed from each sub-sample and analysed for Total Carbon and Nitrogen using the LECO-TruSpec CN Determinator (LECO Corporation, 2006).

The TruSpec CN determinator instrument determines Carbon and Nitrogen content of a range of materials including feeds, fertilizers, and soils. The instrument connects to an external PC and operates by a Windows-based software program to control the system operation and data management.

Samples analysed using the instrument undergo three phases: purge, combust, and analyze. The purge phase flushed any atmospheric gases from the sample that may have entered during sample loading. During the combustion phase the sample is placed in a hot furnace (950 ^{o}C) and flushed with oxygen to ensure complete combustion. The products flow through a secondary furnace (850 ^{o}C) for further oxidation and removal of particulates. In the analysis phase, the combustion gases are mixed with introduced oxygen and then flushed through the CO_2 infrared detector (where carbon is measured as carbon dioxide) and the 3 cc aliquot loop (where oxygen is removed and NO_x changed to N_2). Carbon dioxide and water are then removed and nitrogen content left is then determined using a thermal conductivity cell (LECO Corporation, 2006).

4.6.2 Calculations for Total Carbon & Total Nitrogen

Values of Carbon or Nitrogen in percentages obtained from the Leco CN determinator instrument are converted to milligram per gram soil as follows:

z % C = z grams of carbon or nitrogen per 100 grams soil

(where z is the number of grams of carbon or nitrogen)

= (z g C 100 g^{-1} soil) /100

= z g C g^{-1} soil

= (z g C g^{-1} soil) x 1000

= z mg C g^{-1} soil.

4.7. DETERMINATION OF LABILE CARBON

Changes in soil carbon (C) can also be measured in terms of active or labile or easily respired or oxidised C which is an indication of the ease of decomposition of organic matter in soil. Labile carbon in the Whatawhata fertiliser trial samples were determined using the method of oxidation of the soil organic C by potassium permanganate ($KMnO_4$) with the use of the 333 mM $KMnO_4$ (Lefroy *et al.,* 1993; Blair *et al.,* 1995). This method would determine the labile C which is easily oxidised by 333 mM $KMnO_4$ and the non-labile C which is not oxidised by 333 mM $KMnO_4$. The labile organic matter pool is an important food and nutrient source for plant and microbial growth while the non-labile or "resistant" C pool is important for soil structure and buffering capacity (Blair *et al.,* 1995).

4.7.1 Reagent Preparation

Analytical grade potassium permanganate was used to prepare a 333 mM solution of $KMnO_4$. For two sample batches to be measured separately, two $KMnO_4$ solutions were also prepared. For batch number one, 52.633 g of $KMnO_4$ was used to prepare a 1 L reagent solution and 26.31 g of $KMnO_4$ was used to prepare a 500 mL reagent solution for batch number two. The $KMnO_4$ reagent solution was prepared one day before the experiment as the $KMnO_4$ (in solid crystal forms) required at least 18 hours to dissolve completely. The $KMnO_4$ aided by a magnetic stirrer was left overnight to dissolve in a glass beaker covered with parafilm and wrapped in aluminium foil in the dark. All glassware used had been thoroughly cleaned including being acid-washed to remove any material that could be easily oxidized and result in the unfavorable production of MnO_2. The presence of MnO_2 and light causes degradation of $KMnO_4$ (Blair *et al.,* 1995).

On the following day, the reagent solution was filtered three times through glass fibre wool to remove traces of MnO_2. The final filtered $KMnO_4$ reagent solution was then used to prepare standards, reagent blanks, and soil sub-samples.

4.7.2 Sample Preparation

Air-dried soil samples from 12 paddocks collected from Whatawhata fertiliser trial area were hand-ground (using a mortar and pestle) and triplicates of 500 mg soil from each paddock treatment were weighed into 50 mL plastic falcon (centrifuge) tubes. A further 12 sub-samples of 500 mg were also weighed from each paddock treatment sample to be used as soil blanks. Using an autopipette, 25 mL of newly prepared 333 mM $KMnO_4$ was added to all 36 soil samples. The extra 12 soil blank sub-samples received no $KMnO_4$ but only 25 mL of deionised water. Three 25 mL of only $KMnO_4$ reagent were also prepared as reagent blanks. Two batches of samples of all 36 sub-samples were prepared each with different reagents and standards.

The centrifuge tubes containing treated soil samples (soil + $KMnO_4$), soil blanks (soil + water only), and reagent blanks ($KMnO_4$ solution only) were all tightly capped and shaken using an end-over-end shaker for 1 hour. The tubes were then centrifuged for 5 minutes at 2000 rpm using a centrifuge (GP8, International Equipment Company, USA, 1998).

The clear supernatant from the centrifuged samples was diluted by 0.25 % by taking 0.25 mL of supernatant and diluting with deionised water to 100 mL.

4.7.3 Standards Preparation

The Standards were prepared from the freshly made 333mM $KMnO_4$ reagent into 50 mL falcon (centrifuge) tubes as follows:
0 mM $KMnO_4$: 3 mL water
111 mM $KMnO_4$: 1 mL $KMnO_4$ + 2 mL water
222 mM $KMnO_4$: 2 mL $KMnO_4$ + 1 mL water
333 mM $KMnO_4$: 3 mL $KMnO_4$

All standards were sealed and mixed on a vortex (SRM1, Ratek Instruments Pty Ltd, Boronia, Victoria) for about two minutes and then diluted by 0.25 % by taking 0.25 mL from each standard solution and diluting with deionised water to 100 mL.

4.7.4. Spectrophotometer absorbance measurements

The samples were all mixed and their absorbances read on a split beam spectrophotometer (Metertek SP-830, Metertech Inc., GBC Scientific, New Zealand, 1996) at 565 nm (Figure 4.3). The absorbances of the diluted standards were first measured and a standard curve produced before proceeding to measure the rest of the samples. The absorbance of the rest of the diluted samples were then determined within 30 minutes after standardising the spectrophotometer.

Figure 4.3: *Colour absorbance measurements of $KMnO_4$ on the spectrophotometer for all soil sub-samples, for determination of $KMnO_4$ concentration oxidised by labile carbon.*

4.7.5. Calculations for Labile Carbon

Absorbance values of soil samples were corrected by subtracting absorbance of soil blanks from absorbance of samples. From the standard curve constructed from absorbance readings of $KMnO_4^-$ standards of 0, 111, 222, and 333 mM (described in 4.9.4), a linear line equation of y = 0.0033x was obtained with an R^2 of 0.999. The fraction of labile carbon is determined following calculations below:

(i) Determination of KMnO4- Concentrations:

The concentrations of $KMnO_4^-$ (in mMol/L) used in the oxidation of carbon (labile C) from the digested soil samples were determined using the standard curve linear line equation as follows:

$$x = \frac{y}{0.0033}$$

Where:

'x' is $KMnO_4^-$ concentration in mMol L^{-1};
'y' is absorbance;
'0.0033' is the slope of the standard line.

(ii) Calculation of $KMnO_4^-$ (mM/L) consumed by soil sub-sample:

$$A = B - x$$

Where:
'A' is $KMnO_4^-$ (mM L^{-1}) consumed by the soil sub-sample;
'B' is the mean for reagent blanks (mM L^{-1});
'x' is $KMnO_4^-$ (mM L^{-1}) obtained from (i).

(iii) Calculation of $KMnO_4^-$ (mM) per tube of 25 mL $KMnO_4$ reagent per soil sub-sample:

$$C = A \times 0.025\ L$$

Where:

'C' is $KMnO_4$ consumed per tube (mM per 25 mL reagent per tube);

'A' is $KMnO_4$ consumed by each soil sub-sample (mM L^{-1}) obtained from (ii);

'0.025 L' is the volume of $KMnO_4^-$ reagent added to each soil sub-sample per tube (mixed in falcon tube for digestion).

(iv) Calculation of $KMnO_4$- consumed in mM per mg soil:

$$D = \frac{C}{500}$$

Where:

'D' is $KMnO_4$ consumed in mM per mg soil;

'C' is $KMnO_4$ consumed per tube (mM per 25 mL reagent per tube) obtained from (iii);

'500' is weight of soil sub-sample in mg.

(v) $KMnO_4$ Conversion Calculation:

$$E = D \times 9$$

Where:

'E' is $KMnO_4$ consumed as mg carbon per mg soil;

'D' is $KMnO_4$ consumed in mM per mg soil obtained from (iv);

'9' is the equivalent mass (milligrams) of carbon oxidised from 1 mM MnO_4 consumed (Blair *et al.,* 1995).

(vi) Carbon Consumed Conversion Calculation:

$$F = E \times 1000$$

Where:
'F' is Carbon consumed expressed in mg C g^{-1} soil;
'E' is $KMnO_4$ consumed as mg C mg^{-1} soil) obtained in 'v';
'1000' is conversion factor for milligram to gram.

4.8. DETERMINATION OF MICROBIAL BIOMASS CARBON

4.8.1. Description of Fumigation Method

The Fumigation Incubation (FI) method was selected to determine microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN). The FI method was used rather than the more contemporary method of fumigation-extraction (FE) because microbial biomass N can be estimated more accurately with the incubation method than with the extraction method (Sparling pers. comm., November 2006). Also the FI method showed less variability with the k_C and k_N factors, compared to the FE method (Sparling and Zhu, 1993). Mineralised C (determined from CO_2 evolution) and mineralised N (determined from an increase in NH_4^+ pool) from the fumigated and unfumigated samples were used to estimate the size of the soil biomass C and N (Jenkinson & Powlson, 1976a; Anderson & Domsch, 1978; Sarathchandra, 1984; Shen *et al.,* 1987, Ross, 1987; Jenkinson & Ladd, 1981; Sparling and Zhu, 1993, Wu *et al.,* 1996).

4.8.2. Soil Fumigation

a. Fumigation of samples:

1. The fumigation procedure was carried out in a fume cupboard in large dessicators. Four, approximately 25 g (oven dry weight equivalence) fresh sub-samples from each paddock soil sample were weighed. Two samples from the four sub-samples were weighed into 20 mL beakers to be fumigated. The other two remaining sub-samples were weighed and placed into 200 mL plastic pottles and these were to be used as the control (i.e. not fumigated). The twenty four samples (replicates of 12 soil samples) in the 20 mL glass beakers, to be fumigated, were split between two dessicators containing 30 mL purified $CHCl_3$, and lined with damp paper towels at the bottom to maintain humidity. Purified $CHCl_3$ was prepared as described in section 4.7.2. (ii).

2. Each dessicator was sealed and evacuated using a vaccum pump until the $CHCl_3$ had boiled for 1-2 minutes. Boiling the purified $CHCl_3$ ensured that the $CHCl_3$ vapour dispersed throughout the dessicator and through the soil samples.

3. After boiling, the dessicator tap was closed off (to maintain the vacuum inside the dessicator) while the vacuum pressure pump was running for about 2-3 minutes to clear $CHCl_3$ from the pipes and seals before being turned off and disconnecting the vacuum pump tubes from the dessicator.

4. The dessicator was then left in the dark for 24 hours.

5. The unfumigated samples were covered with plastic sheets and left at room temperature (about 25 ^{o}C) also for 24 hours.

b. Purification of Chloroform:

Reagent grade chloroform ($CHCl_3$) normally contains ethanol (1-2 % w/w) as a stabiliser, however, it has to be removed so that the $CHCl_3$ can be used as a fumigant for microbial biomass determinations. Otherwise ethanol tends to remain in the soil after $CHCl_3$ is removed and may act as substrate to microbial growth during the incubation period (Williamson *et al*., 1995). Hence ethanol removal was essential so that it did not affect microbial metabolism and growth. Ethanol was removed from reagent grade chloroform following the method described in Williamson *et al*., (1995) with modifications noted in parenthesis [ ]:

1. A 30 ml [instead of 20 ml] sample of reagent grade chloroform ($CHCl_3$) was washed with 60 ml [instead of 40 mL] of deionised water [instead of double-distilled water] in a separating funnel.

2. The funnel was shaken for 1 minute with the pressure regularly released. After settling for about 10 seconds, the $CHCl_3$ which was the bottom layer was let to run into a 25 mL beaker. The remaining water phase was discarded. The washing step was repeated three times [instead of two times].

3. The final washed $CHCl_3$ was stored in a 100 mL glass beaker and 1-2 g of anhydrous K_2CO_3 added as dessicant [instead of using 2-3 g $CaCl_2$]. The purified $CHCl_3$ was then covered with a watch glass before transferring it to the dessicator for sample fumigation.

c. Evacuation of Chloroform:

After 24 hour incubation, the purified chloroform contained in a beaker was removed from the desiccator containing the samples. Chloroform vapour present in the soil samples was removed by evacuating the dessicator with a vacuum pressure pump for 5 minutes. The vacuum was then turned off and the dessicator allowed to refill with air for 30 seconds. The evacuation procedure was repeated five times.

d. Incubation:

Beakers containing soil samples (fumigated and non-fumigated/control) were then incubated in sealed 1 L Agee storage jars for 14 days at 25 ^{o}C. It has also been found that by the end of 10 days, the effects of fumigation had largely subsided (Jenkinson and Powlson, 1980). This research used 14 days to ensure that the full effects of fumigation on microbial activity had occurred.

Agee jars in which incubation was carried for both fumigated and unfumigated soil samples, were injected with 2 mL of deionised water to maintain humidity in the soil samples (Jenkinson and Powlson, 1980).

Fumigated soils were not inoculated with moist unfumigated soil as described in Jenkinson *et al*., (1976b). A number of studies have indicated that reinoculation of the fumigated soil samples is usually not essential since some of the microorganisms would still escape the fumigation treatment (Parkinson and Paul, 1982), and also there were sufficient substrate for microbial activity present in the fumigated soils for the period of incubation (Graham Sparling, pers comm., 2007).

4.8.3. Respired Carbon

Carbon dioxide (CO_2) is the final end product of microbial decomposition of organic matter in soil when the soil is adequately aerated. Thus the production of CO_2 is often measured as an indicator of decomposition rates of organic matter in soil (Cresser *et al.,* 1993).

Production of CO_2 from the incubated samples (fumigated and unfumigated or controls) was measured by injecting headspace samples into a Infrared gas analyser (model: LI-6262, LI-COR, SN: IRG3-1068). The IRGA analyser functions on the principle that heteroatomic gas molecules absorb specific electromagnetic wavelengths (Anderson, 1982). My research used the IRGA method described by Clegg *et al*., (1978) with a few adaptations. The system was composed of an IR gas

analyzer, milli-volt (mV) recorder, flow meter, drying columns, and a tygon tubing that connects the system. Nitrogen gas (N_2) was used as the carrier gas of the sample (CO_2) gas.

CO_2 concentration was measured on day 7 (to test the method) and then again on day 14. A CO_2 standard curve was created from injections of 1 mL, 0.8 mL, 0.6 mL, 0.4 mL, and 0.2 mL of 1 % CO_2 gas. A linear relationship between CO_2 concentrations and the analyser/recorder response was used to calculate CO_2 concentrations in incubations. Replicates of standards for 0.5 mL and 0.3 mL were also produced as required for sample measurements at day 7 and day 14 of incubation. A sample of 0.5 mL and 0.3 mL (for day=7) and a 0.5 mL (day=14) of CO_2 gas was obtained from each sample and injected through a short section of surgical tubing in the sample line (Clegg *et al*., 1978). The injected CO_2 gas sample was carried by the N_2 carrier gas, passed through the drying column where water was removed, passed through the IR gas (CO_2) analyser where CO_2 was detected, and the response was traced by the mV recorder and recorded as peaks. Each peak height was then measured (in mm) to provide a quantitative measure of the amount of CO_2.

4.8.4. Calculation for Microbial Biomass Carbon

For each sample, soil respiration (% CO_2) was calculated as follows:

% CO_2 = % CO_2 (sample) - % CO_2 (blank)

$$\% CO_2 = \left[\frac{A}{C}\right] - \left[\frac{B}{C}\right]$$

Where:

A = Sample peak height (mm)

B = Blank peak height (mm)

C = Standard peak height (mm)

Volume of CO_2 in 1L jar (mL CO_2) = Soil respiration x 1000 mL

Where:

Soil respiration is % CO_2 measured as $\frac{\text{x mL } CO_2}{\text{100 mL air}}$

Vol. CO_2 (mL) at Standard Temperature and Pressure (STP) = Vol. CO_2 in 1000 mL jar x 0.916 ml (for 1 mL CO_2 at laboratory temperature and pressure).

- At STP conditions temperature is at 0 ^oC (273.15 K), and atmospheric pressure is at 760 mmHg
- Standard laboratory temperature was assumed to be 25 ^oC (298 ^oK), and pressure at 760 mmHg.
- 1 mL CO_2 at laboratory temperature and pressure = 0.916 mL at STP, using the ideal gas equation: $[P_1V_1/T_1] = [P_2V_2/T_2]$

Mass of CO_2 at STP (mg CO_2) = CO_2 (mL) at STP x $\frac{\text{1.96 mg STP}}{CO_2 \text{ (mL) at STP}}$

- Avagadro's law states that "at constant pressure and temperature, the volume of a gas is directly proportional to the number of moles of the gas present" (Chang, 2002).
- At STP 1 mole of an ideal gas occupies 22.414 L(Chang, 2002) Hence:
  - 1 mol CO_2 = 44g
  - 44 g CO_2 will occupy 22.4 L at STP
  - 22.4 L of CO_2 will weigh 44 g at STP
  - 22,400 mL CO_2 = 44,000 mg CO_2
  - Thus 1 mL CO_2 = 1.96 mg CO_2 at STP

Mass CO_2-C (mg) = mass (mg CO_2) x 0.273 (from Molecular Weight of C / Molecular weight of CO_2).

Mass (µg) CO_2 g^{-1} of soil (ODW) = $\frac{[\text{mass } CO_2\text{-C (mg)}]}{[25 \text{ g}]} \times 10^{-3}$

The total microbial biomass C (MBC) was calculated according to the equation outlined in Parkinson and Paul (1982). This is summarised as:

$B = F/k_c$

Where: B is the soil microbial biomass C (μg C g^{-1} soil);

F is the flux of CO_2-C (μg C g^{-1} soil), derived from CO_2-C evolved by fumigated soil during incubation, less that evolved by unfumigated soil incubated for the same time under the same conditions:

[CO_2-C evolved from fumigated soil – CO_2-C evolved from nonfumigated (Control)];

and

k_c with a value of 0.45, known as the k factor, is the fraction of biomass C mineralised to CO_2 during the whole incubation period at 25 ^oC (Jenkinson and Ladd, 1981; Horwath and Paul, 1994).

The value of B (soil microbial biomass carbon in μg C g^{-1} soil) obtained is equivalent to per oven dry weight of soil, which was corrected using the moisture factor for each sample to obtain the dry mass of soil;

i.e. $$\text{Dry Mass of Soil} = \frac{\text{Mass of Wet Soil (g)}}{\text{M.F}}$$

4.9. CARBON MICROBIAL QUOTIENT DETERMINATION

The microbial quotient (C_{mic}/C_{org}) represents a proportion of the soil microbial biomass carbon (C_{mic}) from the soil total carbon (C_{org}). The microbial quotient is calculated as follows:

$$\text{Microbial Quotient (\%)} = \frac{C_{mic}\ (\text{mg C } g^{-1}\ \text{soil})}{C_{org}\ (\text{mg C } g^{-1}\ \text{soil})} \times 100$$

Most soils in New Zealand have a negligible content of free carbonate due to soil pH below 7.0, hence the total carbon value obtained from the Leco furnace determination was treated as the total organic carbon (C_{org}) content of the soil (Metson *et al.,* 1979).

4.10. DETERMINATION OF MICROBIAL CARBON SPECIFIC RESPIRATION RATE

The microbial specific respiration rate represents the respiration rate per mass of microbial biomass carbon. The rate shows the efficiency of CO_2 respiration per microbial biomass carbon as opposed to total microbial biomass carbon.

The microbial specific respiration rate is calculated by first determining the microbial respiration rate per gram soil:

(i) Respiration Rate (μg CO_2-C hr^{-1} g^{-1} soil):

$$a = \frac{b}{c}$$

Where:
'a' is the Respiration rate in μg CO_2-C per hour per gram soil;
'b' is the average mass of CO_2-C in μg per g soil obtained at STP per 25 g (ODW) soil. The average mass of CO_2-C was determined for both the control (non-fumigated) soil and the fumigated soil samples incubated for 14 days; and
'c' is time given as 336 hours from the 14 days of incubation period.

(ii) Specific Respiration Rate (μg CO_2-C $hr^{-1}mg^{-1}$ MBC):

$$d = \frac{a}{e} \times 1000$$

Where:
'd' is the microbial specific respiration rate in μg CO_2-C per hour per milligram microbial biomass carbon (MBC):
'a' is the microbial respiration rate in μg CO_2-C per gram soil obtained from (i); and
'e' is the microbial biomass carbon (MBC) in μg C per gram soil.

Soil microbial activity was calculated from the soil microbial biomass carbon and microbial respiration, and following on the use of the metabolic quotient or specific respiration (qCO_2) and the C biomass/total organic C (B_c/TOC) ratio (Schloter *et al.,* 2006).

4.11. DETERMINATION OF MINERALISED NITROGEN

4.11.1 Introduction

Mineralised N is the amount of inorganic N released during a set time of incubation from unfumigated soils, and indicates N mineralised during organic matter decomposition. Mineralised N is also a sensitive measure of biomass (Jenkinson & Ladd, 1981).

4.11.2 KCl Extraction of N

To determine available mineralisable N and Microbial N mineralised from the soil, mineral N (NH_4^+ and NO_3^-) needs to be extracted from the soil samples for analysis.

2 M KCl (Blakemore *et al*., 1987) was used to extract NO_3^- and NH_4^+ from soil (Fig. 4.4). Three litres of 2 M KCl was prepared for time (day) = 0 for 24 samples (non-fumigated only), and 5 litres was prepared for time (day) = 14 required for a total of

48 samples (24 non-fumigated or control, and 24 fumigated). The methods of Blakemore *et al.,* (1987) were generally used:

- Replicates of two 25 g (ODW), field-moist, were weighed into 200 mL plastic pottles, and then 100 mL of 2 M KCl added.
- The sealed samples were then mixed using an orbital shaker for 30 minutes. After settling for few minutes the samples were filtered through folded filter paper (Whatman no. 40) into 30 mL vials and were filled up to the rim.

The samples were then stored at -18 ^{o} C to preserve until they could be analysed. The filtered 2 M extracts are normally stable for several months when stored at low temperatures (Keeney and Nelson, 1982).

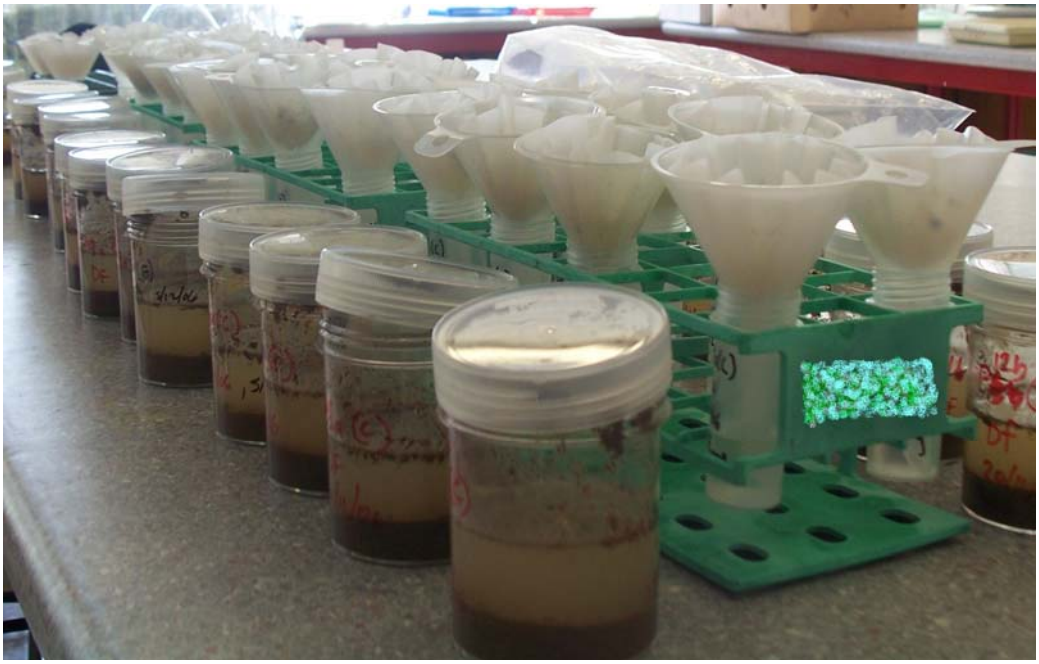


Figure 4.4: *Filtering of extracts (mineral N) from soil sample solutions (mixed with 2 M KCl).*

4.11.3. Analysis of Mineral N (NH_4^+, NO_3^-)

Duplicates of the KCl extract samples from each paddock were analysed for nitrate and ammonium on a Skalar SAN Plus segmented flow analyser (Skalar Analytical B.V., Breda, Netherlands). The nitrate method uses cadmium reduction to nitrite

followed by diazotisation with sulphanilamide and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride to form an azo dye measured colourimetrically at 540 nm. The ammonium method is based on the modified Berthalot reaction. Ammonia is chlorinated to monochloramine which reacts with salicylate and is then oxidised to form a green coloured complex which is measured colourimetrically at 660 nm.

4.11.4. Calculation of Mineralised N

Mineralised N is calculated by first determining inorganic N concentration (NH_4^+ and NO_3^-) extracted by the volume of 2 M KCl used [(i) a] and corrected for the soil mass used [(i) b]; determining Total mineral N ($\sum NH_4^+$-N + NO_3^-N) (ii); and determining Mineralised N, from the difference in Total mineral N, only from non-fumigated samples before and at the end of the incubation period (iii).

(i) Calculation for N concentration from NH_4^+ and NO_3^- (analysed by the SKALAR Flow Analyser):

a. Mass of N extracted:

$$C = A \times B$$

Where: A is N concentration extracted (ppm equivalent to μg g^{-1})

B is volume of 2 M KCl extractant used (100 mL)

C is mass of N extracted (μg)

b. N Concentration:

$$E = C/D$$

Where: C is mass of N extracted (μg)

D is soil mass used (25 g ODW)

E is N concentration (μg N g^{-1})

(ii) Total mineral N

Total extractable mineral N was obtained from the sum of NH_4^+-N and NO_3^--N for unfumigated and fumigated samples:

$$F = E + e$$

Where: e is NO_3^-- N concentration (μg g^{-1} soil)

E is NH_4^+-N concentration (μg g^{-1} soil)

F is the Total mineral N (i.e. ∑(NH_4^+-N + NO_3^--N) (μg g^{-1} soil)

(iii) Mineralised N

Mineralised N was derived from unfumigated samples as:

$$G = \sum F_{T14} - \sum F_{T0}$$

Where: $\sum F_{T0}$ is the total mineral N (unfumigated) before incubation or day 0

$\sum F_{T14}$ is the total mineral N (unfumigated) after incubation or day 14

Mineralised N was calculated for all laboratory duplicate samples and averaged for each paddock soil sample.

4.12. DETERMINATION OF NITROGEN FLUSH AND MICROBIAL BIOMASS NITROGEN

Following calculations of Mineralised N [(4.11.4 (iii)], Microbial Biomass Nitrogen (MBN) was calculated at the end of incubation by first determining Nitrogen (N) flush (i), and then dividing the N flush by a k_N factor to obtain the Microbial Biomass Nitrogen (MBN) (ii).

(i) N flush Calculation:

$$I = \sum H_{T14} - \sum F_{T14}$$

Where: F_{T14} is the Total mineral N (unfumigated) after incubation [as in 4.11.4 (iii)]

H_{T14} is the Total mineral N (fumigated) after incubation

I is N flush

(ii) Microbial Biomass Nitrogen Calculation:

$$K = I/J$$

Where: I is N flush (μg g^{-1} soil)

J is k factor of 0.68

K is the Microbial Biomass Nitrogen (μg g^{-1} soil)

MBN was calculated for all laboratory replicate samples and averaged for each paddock soil sample.

4.13. MICROBIAL NITROGEN QUOTIENT DETERMINATION

The nitrogen microbial quotient (N_{mic}/N_{org}) represents a proportion of the soil microbial biomass N (N_{mic}) from the soil total nitrogen (N_{org}). The N microbial quotient is calculated as:

$$N = L/M \times 100$$

Where: L is the Microbial Biomass Nitrogen (mg g^{-1} soil)

M is the Total N (mg g^{-1} soil)

N is the proportion of Microbial Biomass N from Total N (%)

4.14. DETERMINATION OF NITROGEN MINERALISED PER MICROBIAL BIOMASS UNIT

Following calculations of Mineralised N (4.11.4) and Microbial Biomass Nitrogen (MBN) (4.12), Nitrogen Mineralised per microbial biomass N unit was calculated as follows:

$$c = a / b$$

Where: a is the average mineralisation rate (μg g^{-1} soil hr^{-1}) [obtained from average mineralised N (μg g^{-1} soil) / 336 hours (of incubation)];

b is the average microbial biomass nitrogen (MBN) (μg g^{-1} soil)

c is the average mineralised N rate per biomass N unit (ug N hr^{-1} μg^{-1}), c was then converted to μg N hr^{-1} g^{-1} biomass N by multiplying by 10^6.

4.15. pH DETERMINATION

Soil pH measurements were carried out for all 12 soil samples from the Whatawhata Fertiliser trial. Measuring soil pH is important in providing useful links with other soil properties (Blakemore *et al.,* 1987). The pH measurements were conducted using air-dried soil samples following the method of Blakemore *et al.,* (1987) with pH determination in water.

a. pH Meter Calibration:

The pH Meter (Mettler Toledo, MP 220, GBC Scientific, New Zealand) was calibrated with pH 7.0 and a pH 4.0 buffer solutions before measuring the pH of the soil samples.

b. pH Measurement in Water:

- Approximately 10 g of air dried soil from each sample was weighed into a 100 mL beaker and 25 mL water added;

- The soil sample with water was mixed on a high speed mixer for 15 seconds;
- All samples were left for 30 minutes (instead of overnight);
- With the pH electrode, the pH of the supernatant of the homogenised sample was then measured and recorded.

A duplicate measurement was carried out first for one sample which after obtaining pH results within 0.1 pH unit, the subsequent pH measurements were then carried out for the remaining samples without replicates.

4.16. DATA ANALYSIS

Data analysis was carried out using 'Microsoft Office' Excel (v 2003) regression analysis, and 'Statistica' (v 8, StatSoft Inc. 2004-2007) one way analysis of variance.

CHAPTER 5

RESULTS & DISCUSSION

5.1. INTRODUCTION

This chapter presents results obtained from laboratory experiments on soils collected from Whatawhata Research Station. Measurements were made of microbial and chemical properties of moist and air-dried soil samples, including Olsen P, on soils from the six different P fertiliser treatments. The relationships between the varying soil phosphorus and the microbial, carbon, and nitrogen fractions of soil organic matter are investigated. Results of nitrate and ammonium levels as a function of the P application treatments are also presented. Full data sets are included in Appendices A-H.

5.2. PHOSPHORUS

Soil samples from the Whatawhata P fertiliser trial were analysed for soil available inorganic phosphorus, measured as Olsen P. There was a significant ($p<0.001$) relationship, and a strong positive correlation ($R^2=0.82$) between the Olsen P and fertiliser P application rate (Figure 5.1).

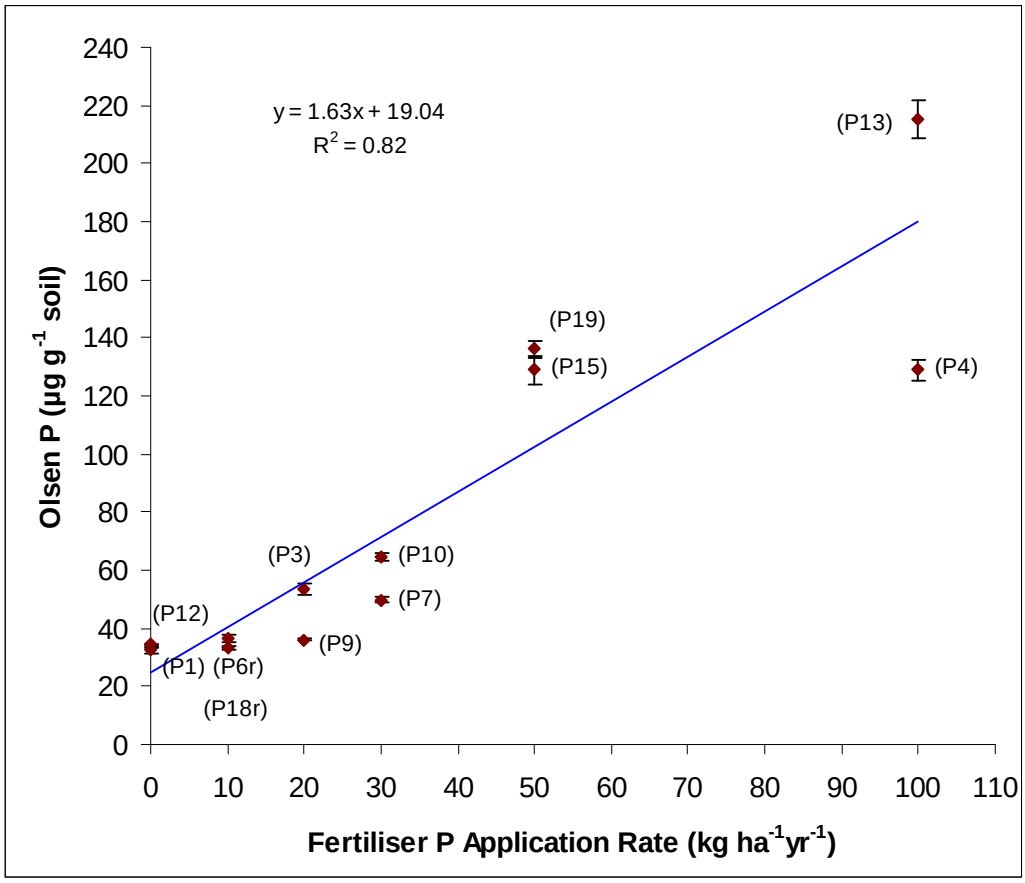


Figure 5.1: *Phosphorus concentration (Olsen P) in soils sampled from twelve paddocks with six P fertiliser application rates. Error bars represent one standard deviation of the mean (n = 3).*

5.3. CARBON

5.3.1. Total Carbon

Total carbon was found to be significantly ($p<0.05$) negatively correlated ($R^2 = 0.44$) with fertiliser P application rate (Figure 5.2).

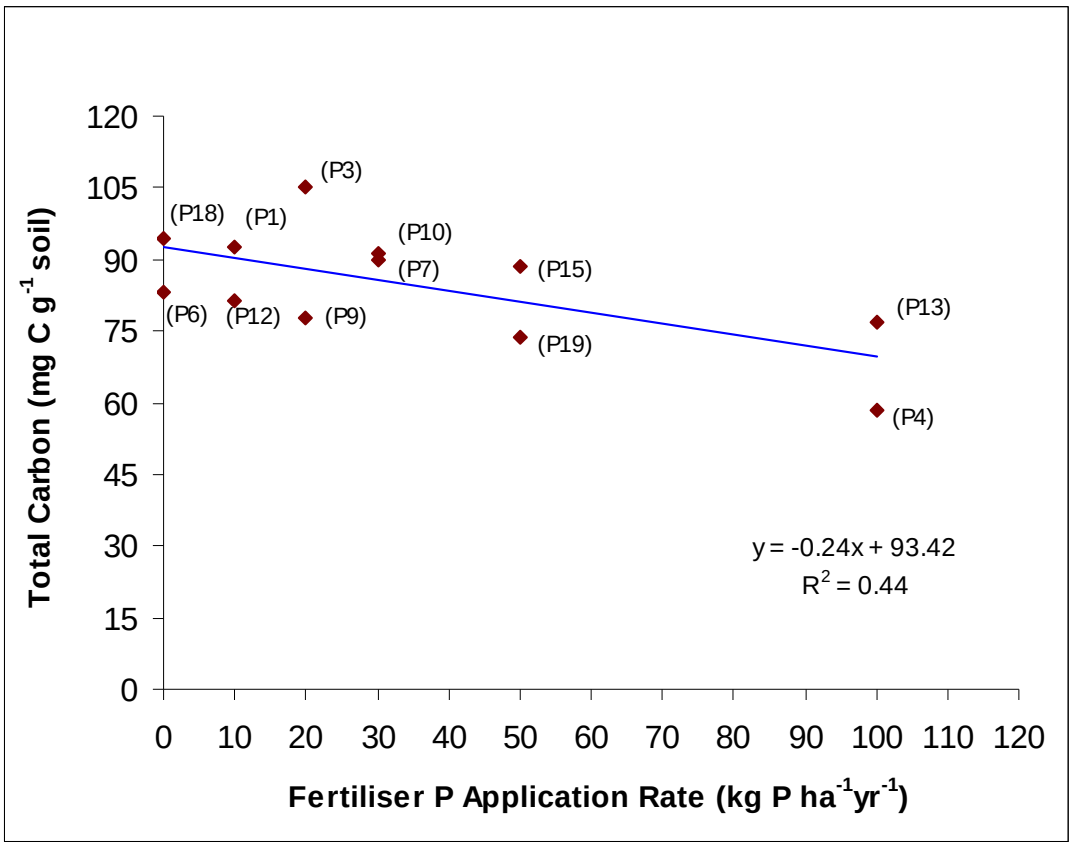


Figure 5.2: *Total Carbon from twelve paddocks with six P fertiliser treatment rates.*

5.4. LABILE CARBON

5.4.1. Labile Carbon

a. Labile C and P fertiliser

Labile carbon (C), determined using the ease of oxidation of organic carbon by $KMnO_4^-$ solutions, was found to range from 11.03 mg C g^{-1} soil to 23.36 mg C g^{-1} soil. A significant ($p<0.01$) negative correlation ($R^2 = 0.4$) was found between labile C and P fertiliser treatment rate (Figure 5.3).

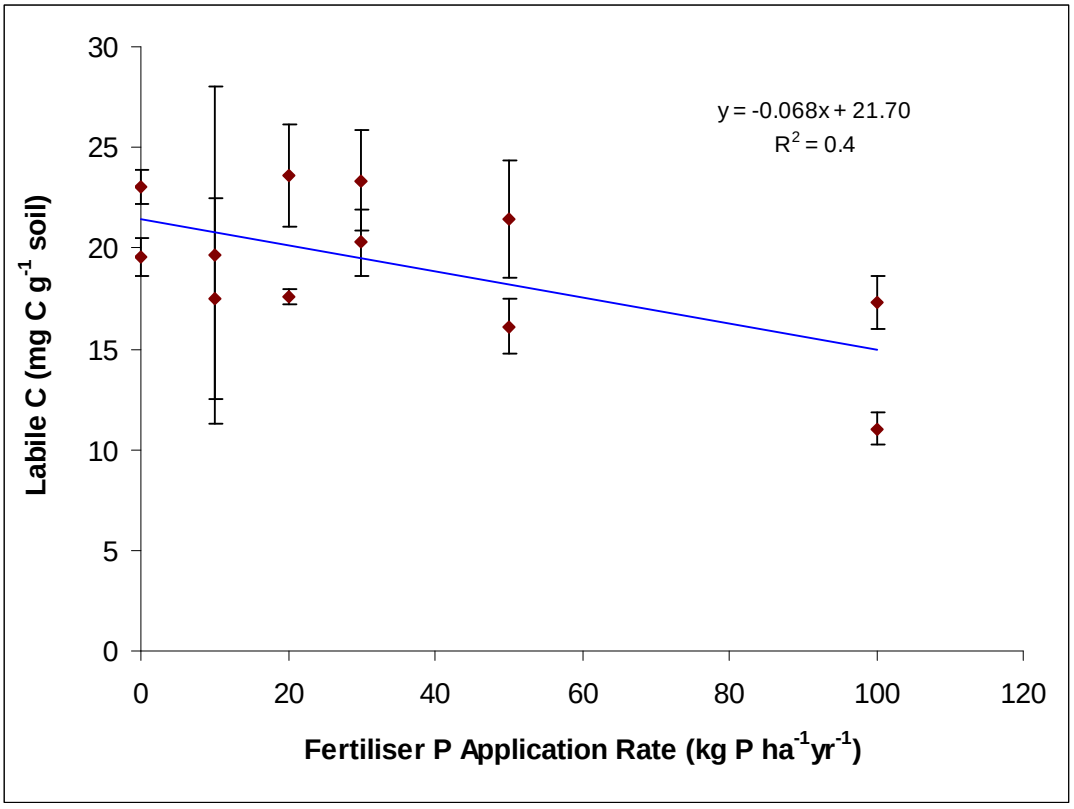


Figure 5.3: *Labile carbon distribution in soils with different fertiliser P application rates. Error bars are for one standard deviation of the mean of lab replicates (n=3).*

b. Proportion of Labile C in Soil Carbon

Labile C across all samples made up about 20 % of the total C (Table 5.1).

Table 5.1: *Proportion of microbial carbon and labile carbon from soil total carbon for varying fertiliser P treatments.*

P Fertiliser Application Rate (kg $Pha^{-1}yr^{-1}$)	**Paddock Number**	**Proportion of Labile Carbon** (C_{lab}/C_{org}) (%)
0	P6	23.55
0	P18	24.41
10	P1	21.29
10	P12	21.40
20	P3	22.40
20	P9	22.58
30	P7	25.95
30	P10	22.17
50	P15	24.23
50	P19	21.82
100	P4	18.81
100	P13	22.46

5.5. MICROBIAL BIOMASS CARBON & RESPIRATION

5.5.1 Respirable Carbon

Respirable carbon (CO_2-C in unfumigated soil after incubation at day 14) showed no significant ($p>0.05$) relationship with fertiliser P application rate. There was also variability between paddock samples measured ($R^2 = 0.27$), (Figure 5.4).

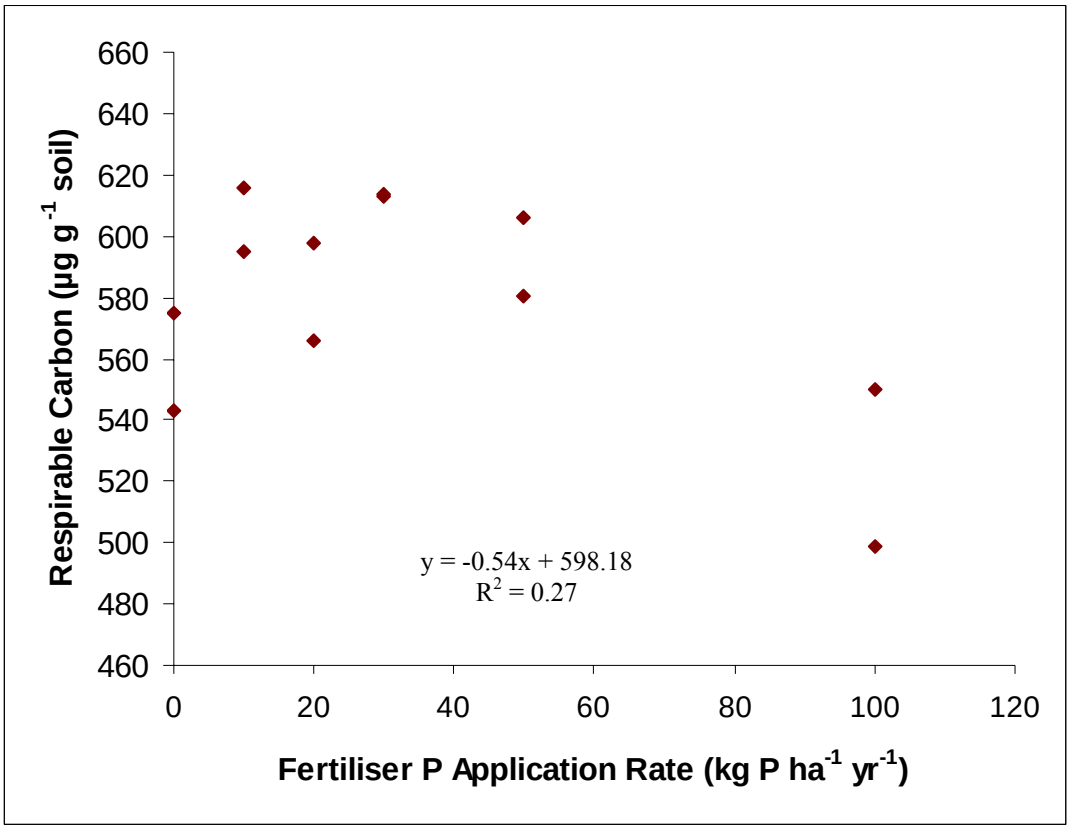


Figure 5.4: *Distribution of respirable carbon in soils with varying P application rates (data are means of two laboratory replicates).*

5.5.2. Microbial Biomass Carbon

There was no significant relationship between the microbial biomass carbon (MBC) and the fertiliser P application rate, and high variability was also observed ($R^2 = 0.06$) (Figure 5.5).

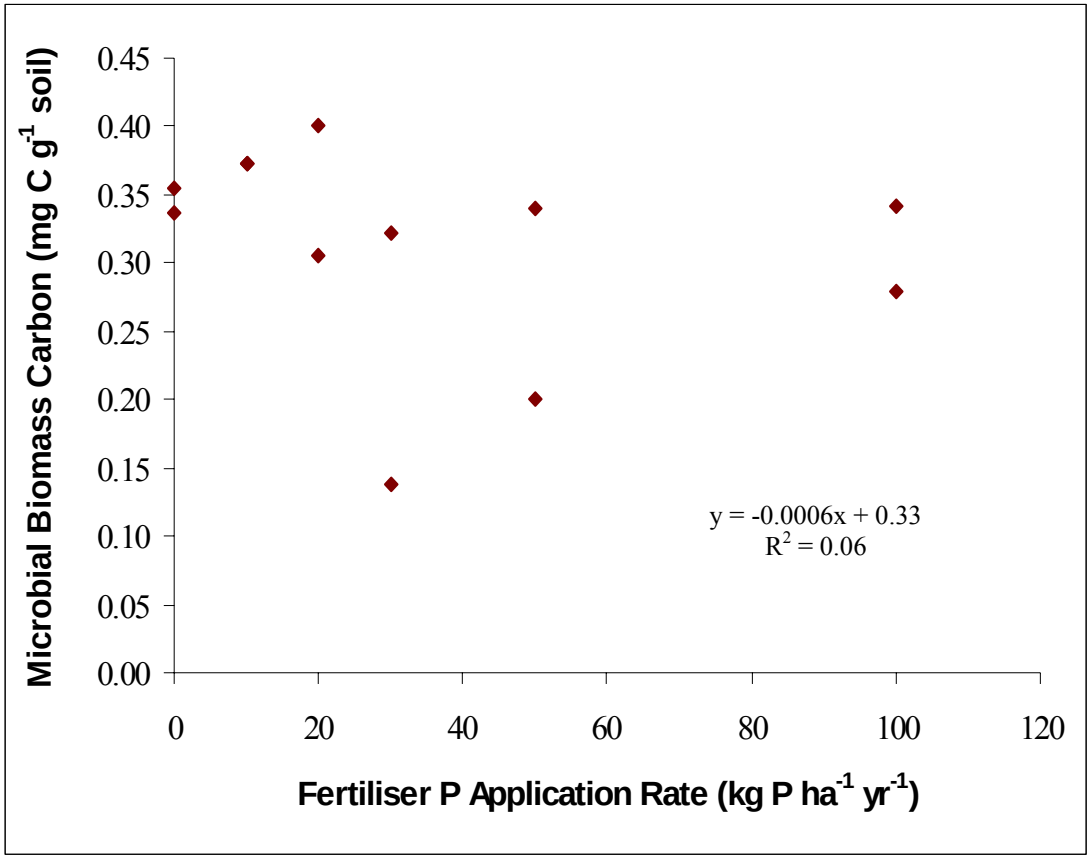


Figure 5.5: *Microbial biomass carbon of twelve soil samples with six P treatment rates (data are means of two laboratory replicates).*

5.5.3. Microbial Quotient

The microbial quotient (C_{mic}/C_{org}) is the proportion of the soil microbial biomass carbon (C_{mic}) in the soil total organic carbon (C_{org}). The microbial quotient showed no significant relationship with P fertiliser application rate (Figure 5.6).

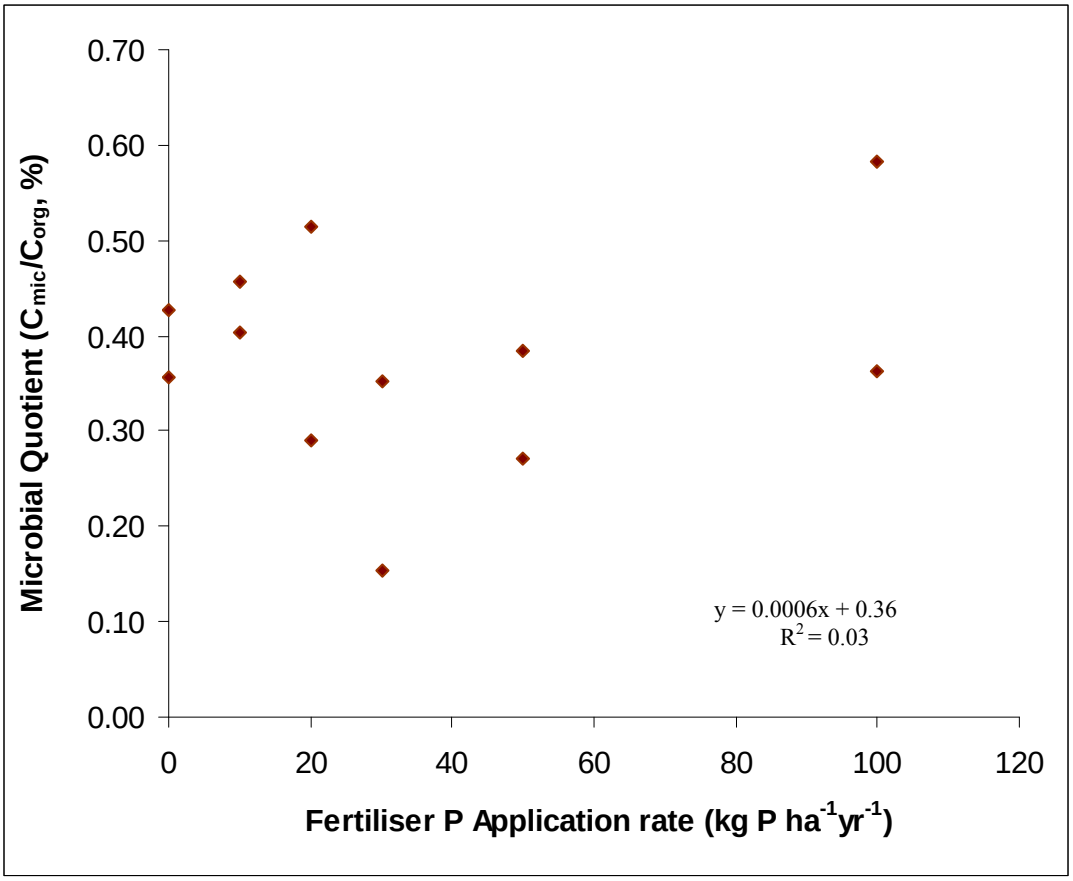


Figure 5.6: *Microbial quotient (ratio of microbial carbon to total carbon) from 12 soils sampled with six P fertiliser treatment rate (data are means of two laboratory replicates).*

5.5.4. Specific Respiration Rate

The specific respiration rate (also known as the microbial metabolic quotient, or qCO_2) is a measure of the ratio of soil basal respiration to microbial biomass, and also indicates changes in microbial biomass as a result of ecosystem disturbance (Wardle and Ghani, 1995). There was no significant relationship observed between the rate of application of P fertiliser and the microbial specific respiration rate, (Figure 5.7) ($R^2 = 0.003$). The variability in some soil samples was high.

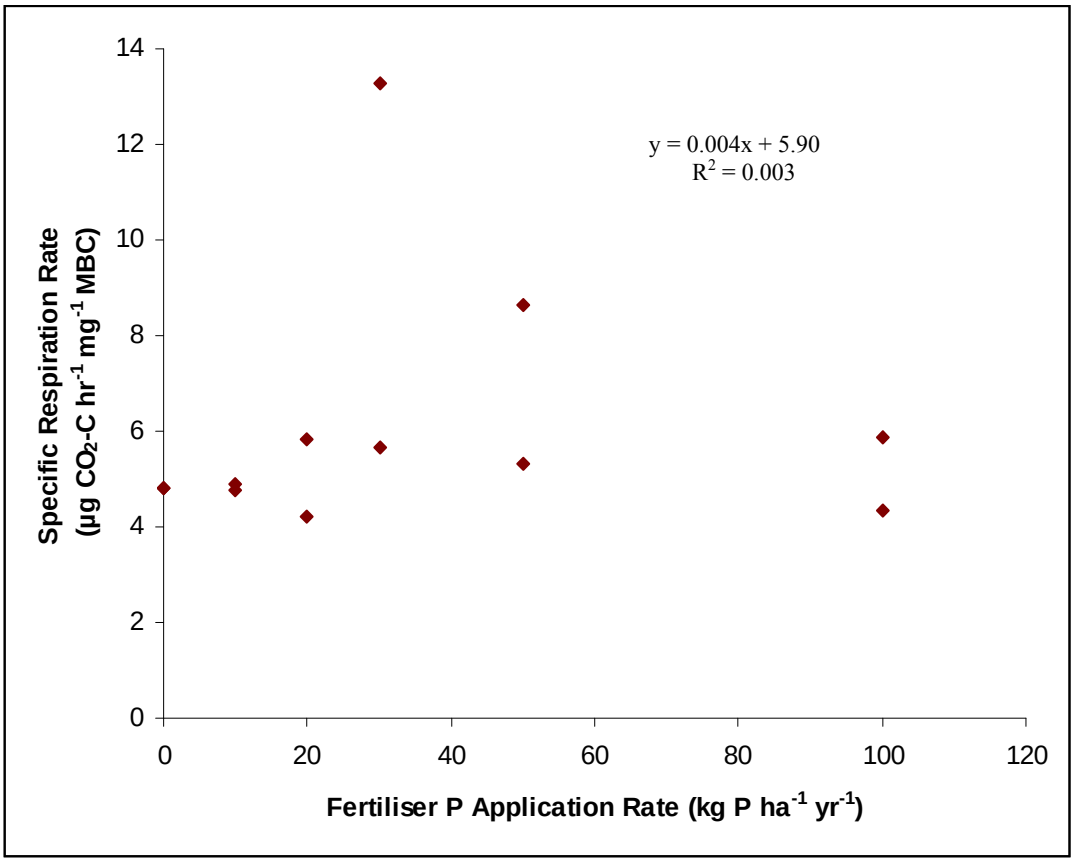


Figure 5.7: *Microbial Specific Respiration Rate (or respiration efficiency rate) showing CO_2 respired per hour per mg of microbial biomass carbon for soils of varying P fertiliser treatments (data are means of two laboratory replicates).*

5.6. NITROGEN

5.6.1. Total Nitrogen

A significant ($p<0.01$) negative correlation ($R^2 = 0.52$) was found between total nitrogen and P fertiliser application rate (Figure 5.8)

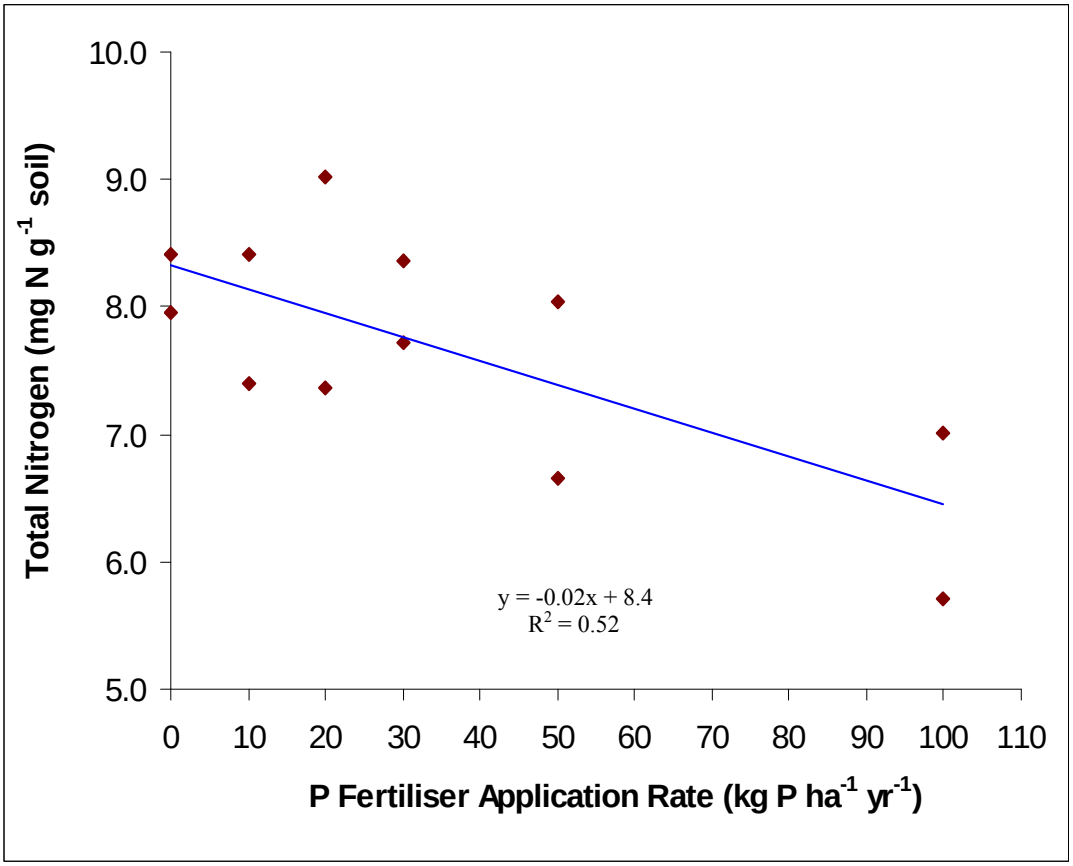


Figure 5.8: *Total N in 12 soil samples with 6 P application rates.*

5.6.2. Mineral N and Mineralised N

Mineral N was determined as the total of NH_4^+ and NO_3^- (i.e. $\sum(NH_4^+\text{-N} + NO_3^-\text{-N})$) in both non-fumigated and fumigated samples before (T_0) and after (T_{14}) incubation (Table 5.2). Mineral N observed increased in the order: Fumigated (T_{14}) > Unfumigated (T_{14}) > Unfumigated (T_0).

Table 5.2: *Total Mineral N (i.e. $\sum[NH_4^+\text{-N} + NO_3^-\text{-N}]$) in soil samples in both unfumigated samples before incubation (T_0) and after incubation (T_{14}), and in fumigated samples after incubation (T_{14}). Data are means of 2 replicates.*

Fertiliser P Application Rate (kg P ha^{-1} yr^{-1})	**Paddock Number**	**Average Unfumigated** $\sum[NH_4^+\text{-N} + NO_3^-\text{-N}]$ **T = 0 day** (µg g^{-1} soil)	**Average Unfumigated** $\sum[NH_4^+\text{-N} + NO_3^-\text{-N}]$ **T = 14** (µg g^{-1} soil)	**Average Fumigated** $\sum[NH_4^+\text{-N} + NO_3^-\text{-N}]$ **T = 14 days** (µg g^{-1} soil
0	6	19	101	209
0	18	31	79	138
10	1	12	70	201
10	12	13	86	248
20	3	17	95	167
20	9	17	60	222
30	7	11	63	172
30	10	25	85	187
50	15	15	50	170
50	19	20	40	161
100	4	21	110	186
100	13	26	58	194

Mineralised N was determined as the difference between the total of NH_4^+ and NO_3^- in the unfumigated samples both before (T_0) and after (T_{14}) incubation i.e. Mineralised N = $\sum(NH_4^+\text{-N} + NO_3^-\text{-N})$ (T_{14}) - $\sum(NH_4^+\text{-N} + NO_3^-\text{-N})$ (T_0). No significant relationship was observed between mineralised N and fertiliser P application rate (Figure 5.9), ($R^2 = 0.04$).

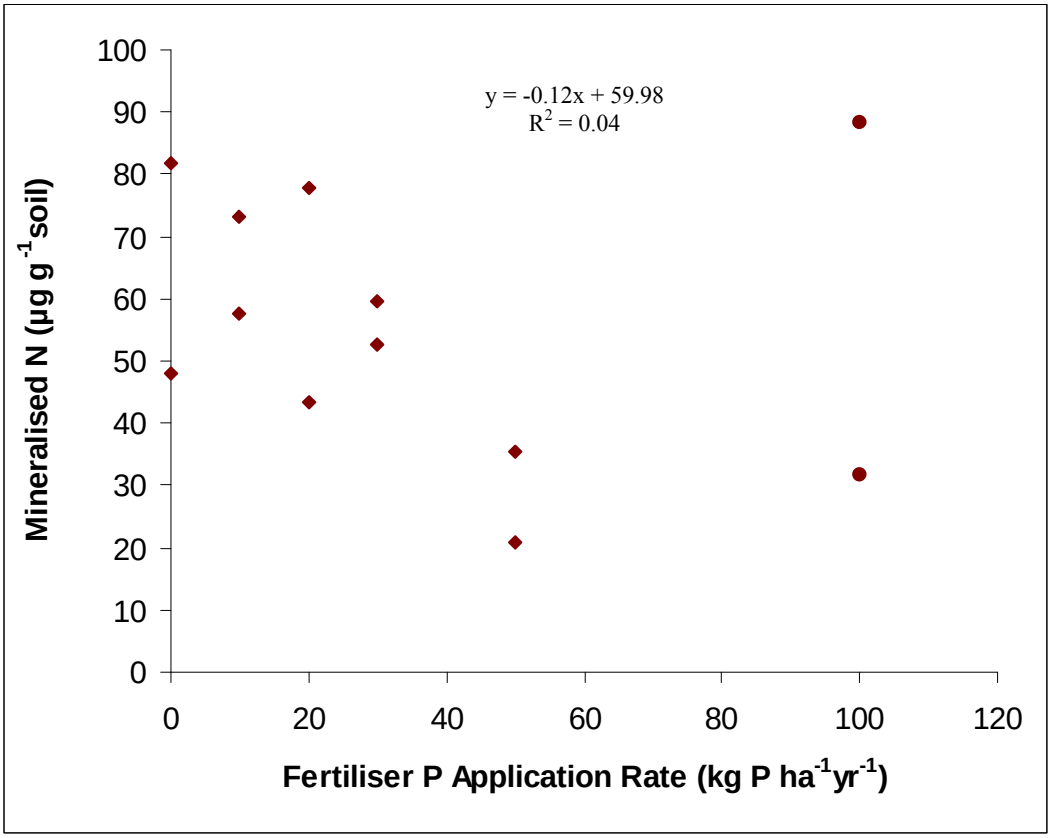


Figure 5.9: *Mineralised nitrogen versus fertiliser P application rate (data are measured of 2 sample replicates).*

5.6.3. N Flush and Microbial Biomass N

Nitrogen (N) Flush which is the amount of N mineralised from the microbial biomass was the difference between total mineral N after incubation (T_{14}) in fumigated and non-fumigated samples i.e.

N flush = $\sum(NH_4^+\text{-N} + NO_3^-\text{-N})$ (fumigated) - $\sum(NH_4^+\text{-N} + NO_3^-\text{-N})$ (unfumigated). Microbial biomass N is calculated from the N flush using the correction factor of k = 0.68. No significant correlation was found between the amount of microbial biomass nitrogen (MBN) and P fertiliser treatment (Figure 5.10).

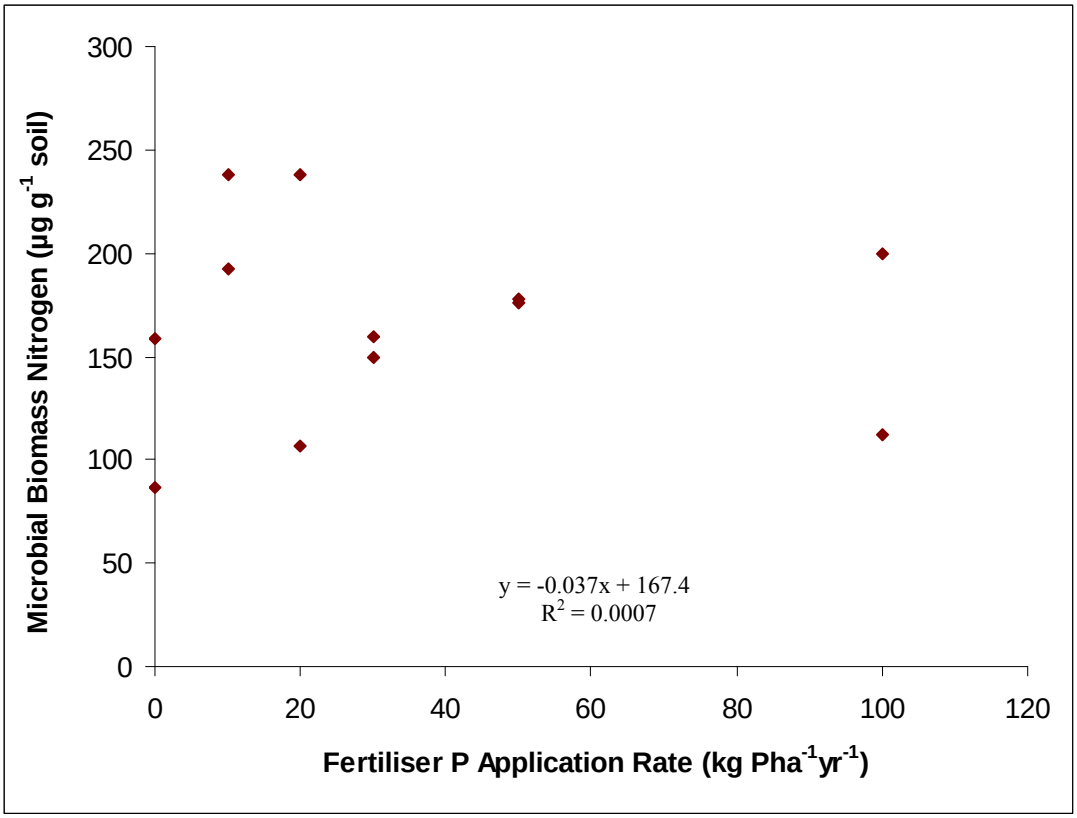


Figure 5.10: *Microbial biomass nitrogen versus fertiliser P application rate (data are means of two replicates).*

5.6.4. Microbial Biomass Nitrogen Quotient

The Microbial Biomass Nitrogen (N) Quotient determined as a proportion of microbial N of the soil total N, showed no significant correlation with P application rate (Figure 5.11).

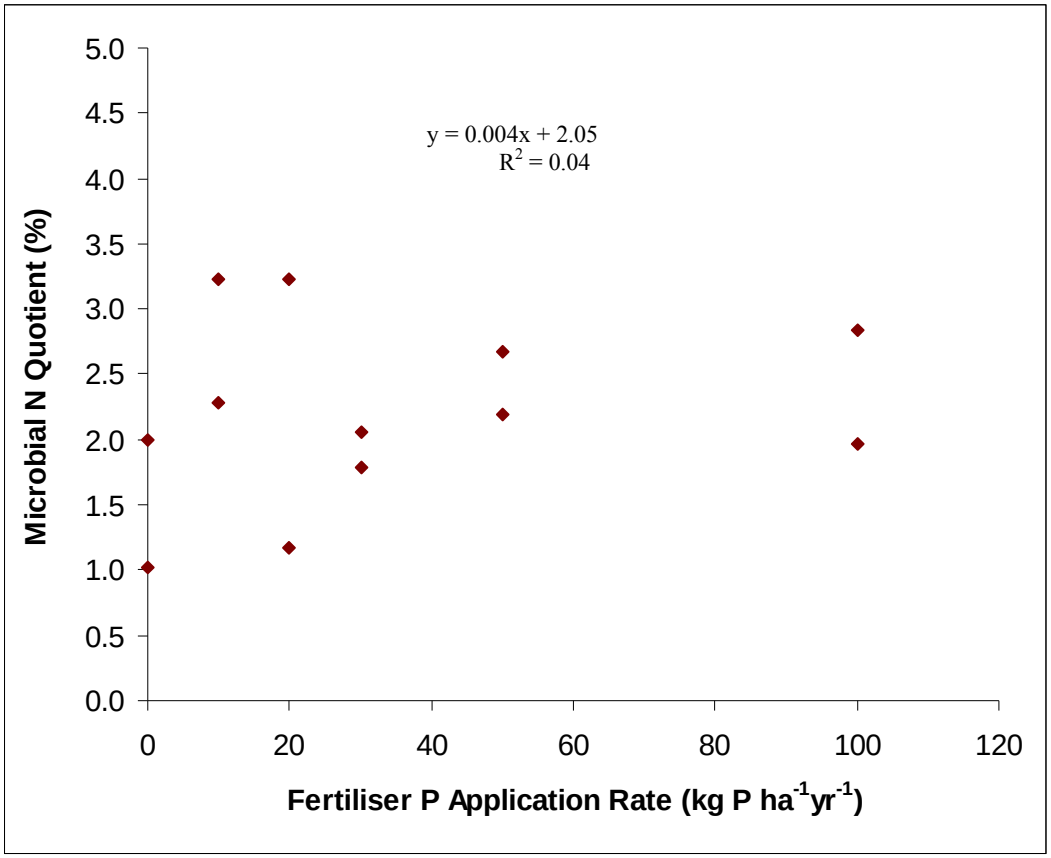


Figure 5.11: *Microbial N Quotient showing proportion of microbial N from total N in soils with different P application rate (data are measured of 2 replicates).*

5.6.5. Nitrogen Mineralised Per Biomass Unit

No significant relationship was observed for mineralised N per biomass N (MBN) in soils and different P application rates (Figure 5.12). There was a high variability in soil samples particularly the 20 and 100 kg P ha^{-1} yr^{-1} treatments ($R^2 = 0.002$).

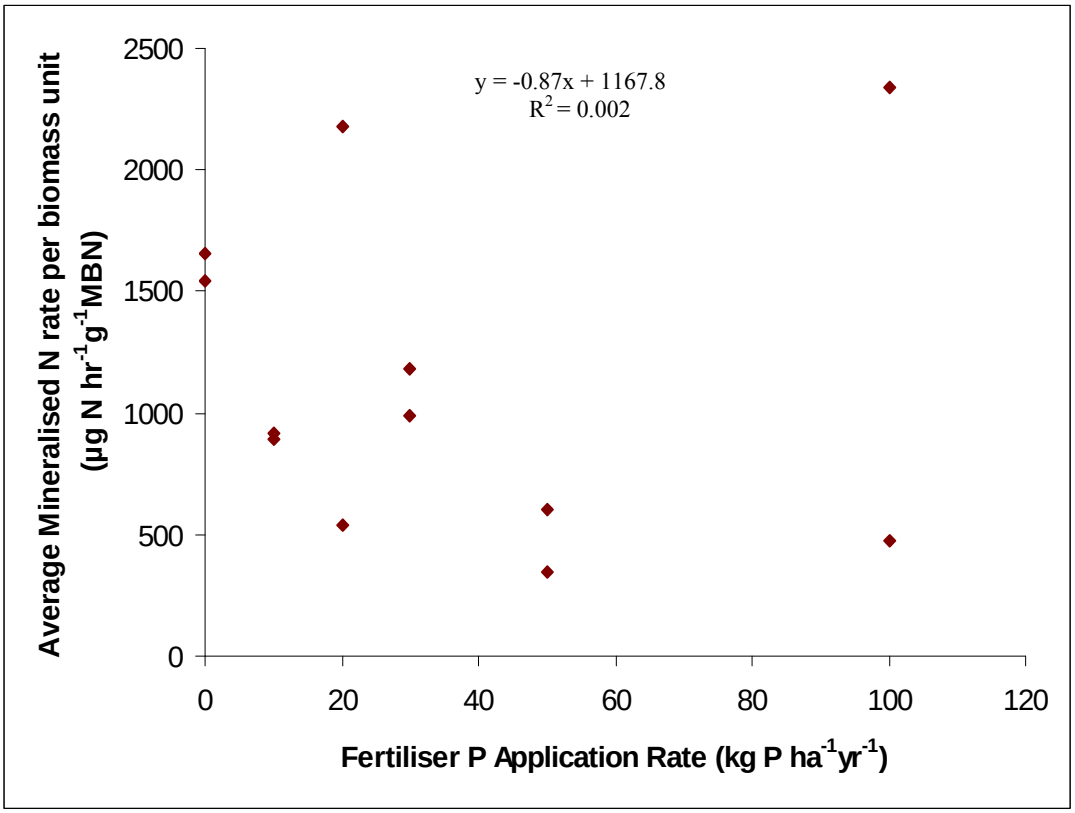


Figure 5.12: *Distribution of N mineralised per biomass N in soils with varying fertiliser P application rate (data are means of two replicates).*

5.7. C:N RATIO

The C:N ratio is the ratio of carbon contained in the soil organic matter, divided by the concentration of nitrogen in the soil organic matter (Cornforth, 1998), which shows the extent of decomposition and quality of organic matter (McLaren and Cameron, 1996). There was no significant correlation between C:N ratio and fertiliser P application rates and there was high variability in the soil samples (Figure 5.13).

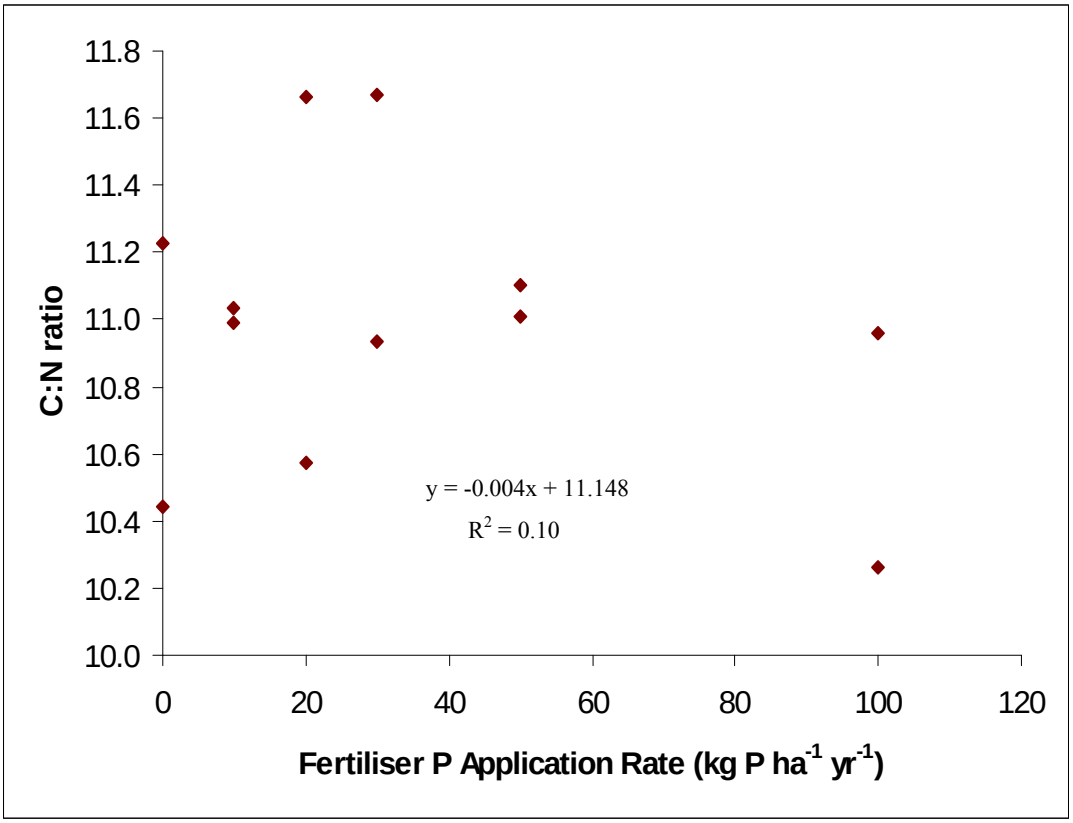


Figure 5.13: *C:N ratio of soils sampled from 6 paddocks of varying fertiliser P treatments.*

5.8. pH

Soil pH ranged from pH 4.7 to 5.1, with a mean pH of 5 for all 12 paddocks. No significant relationship was found between soil pH and fertiliser P application rates, and variability also existed in the soil samples measured ($R^2 = 0.08$), (Figure 5.14).

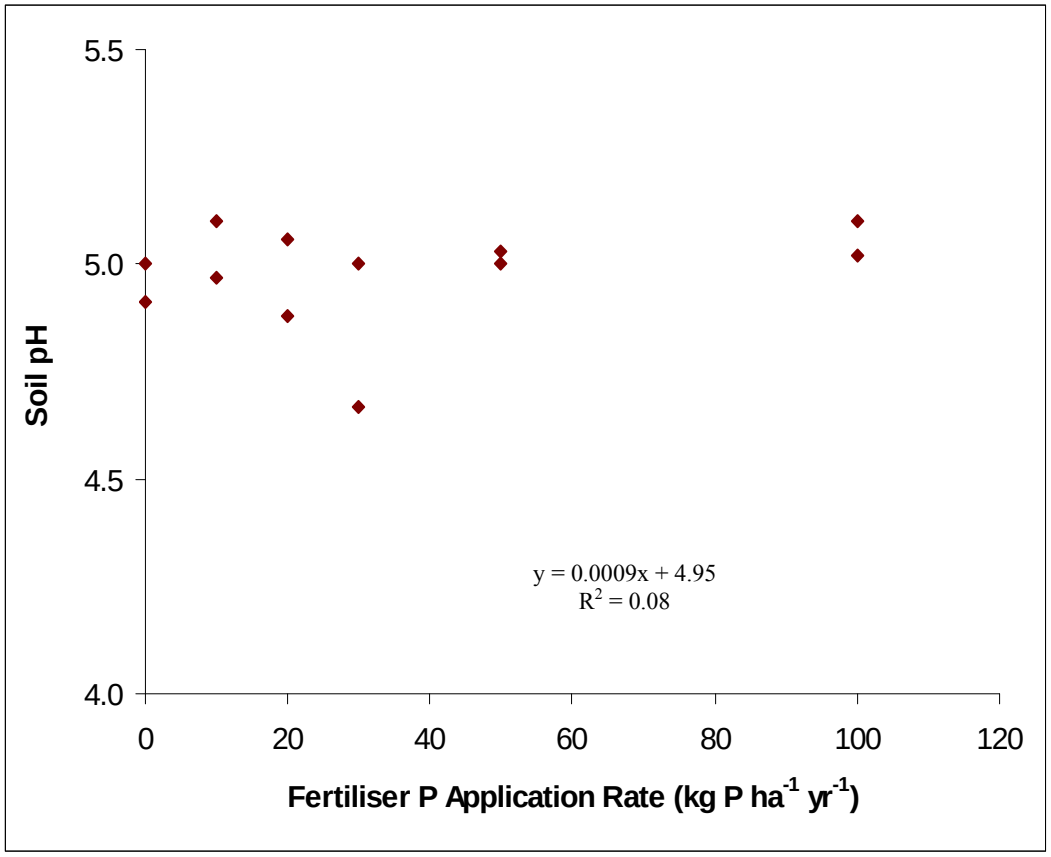


Figure 5.14: *Soil pH for the 12 soils sampled with 6 main P treatments.*

5.9. DISCUSSION

5.9.1. Summary of Main Findings

The available P (Olsen P) increased significantly ($p<0.001$) with increasing P fertiliser application rate (Figure 5.1).

Analysis of carbon and microbial carbon showed that:

- Total carbon was significantly ($p<0.05$) negatively correlated with fertiliser P application rate (Figure 5.2);
- Labile carbon was significantly ($p<0.01$) negatively correlated with P fertiliser application rate (Figure 5.3);
- Respirable carbon showed no significant relationship with P fertiliser application rate (Figure 5.4);
- Microbial biomass carbon showed no significant relationship with fertiliser P application rate (Figure 5.5);
- Microbial (C) quotient and microbial specific respiration rate showed no significant correlation with P fertiliser application rate (Figures 5.6 and 5.7 respectively);

Analysis of nitrogen and microbial nitrogen showed that:

- Total nitrogen was significantly ($p<0.01$) negatively correlated with P fertiliser application rate, (Figure 5.8);
- Mineralised nitrogen showed no significant correlation with P application rate, (Figure 5.9);
- Microbial biomass nitrogen and microbial N quotient showed no significant change with various P fertiliser treatments (Figures 5.10 and 5.11);
- Mineralised N per microbial biomass nitrogen showed no significant change with fertiliser P application rate (Figure 5.12);

Determination of C:N ratio showed that:

- C:N ratio did not change significantly with fertiliser P application rate (Figure 5.13).

Determination of Soil pH showed that:

- Mean soil pH was 5 (range from 4.7 to 5.1) across 12 paddocks, and there was no relationship found between soil pH and P fertiliser application rate (Figure 5.14).

5.9.2. Phosphorus

The increasing Olsen P level with increasing fertiliser P application rate observed in this research confirmed expectations and was consistent with trends for Olsen P determined in earlier research for 'easy' (10-20^{o}) slopes (Rowarth *et al.,* 1992b; Dodd and Ledgard*,* 1999) and recent analysis of archived Whatawhata fertiliser trial samples (up to 2006 samples) (Schipper *et al.,* unpublished data).

Olsen P ranged from about 35 μg g^{-1} in the 0 P treatments to approximately 215 μg g^{-1} in the 100 kg P ha^{-1} yr^{-1} treatment. My Olsen P results were all about 2 to 4 times higher (including the 0 P treatment) than those reported for the same paddocks and slopes in Dodd and Ledgard (1999). My higher Olsen P values were presumably because there had been a further 10+ years of P fertiliser addition since Dodd's and Ledgard's (1999) work. However, Olsen P for the 0 P treatment was unexpectedly higher even when no fertiliser has been added since 1984.

On average, the optimum Olsen P values to achieve near maximum (97 %) pasture production are at 20 units (for sedimentary soils), 22 units (for ash soils), and 38 units for pumice soils (Morton & Roberts, 1999). A previous study at the Whatawhata fertiliser trial showed that 50 kg P ha^{-1} yr^{-1} and a mean Olsen P of 15 units (0-7 cm depth) was required for maximum growth (14, 900 kg DM ha^{-1}) for the easy slopes (Gillingham *et al.,* 1984). Olsen P levels of less than 10 previously found resulted in deterioration of pasture quality as indicated by increased in pasture moss and dead matter content (Gillingham, *et al.,* 1990). Olsen P found for the 50 kg P ha^{-1} yr^{-1} treatments after 10 years of application was 60 μg g^{-1} soil

(Dodd and Ledgard, 1999), while an average of approximately 132 μg g^{-1} soil was found in this current study (after just over 20 years of application). Thus, there has been a build up of Olsen P above that required for maximum pasture growth, in the continuous application of the 50 kg P ha^{-1} yr^{-1}, and even more-so in the 100 kg P ha^{-1} yr^{-1} fertiliser treatment.

The variability observed in the results may be attributed to the variability in the soil samples. Olsen P has been reported as highly variable, both temporally and spatially (Friesen & Blair, 1984). An established soil testing error index gave 15-20 % variability for the Olsen P test meaning that the true or average value for the whole field where the soil sample is obtained lies within plus or minus 20 % of the value obtained from the laboratory (Cornforth, 1998). Soils have a high variability at different points in the field and this variability is highest in grazed pastures compared to cropped soils (Cornforth, 1998).

5.9.3. Soil Carbon

a. Total Carbon

Total C content for the easy slopes ranged from 58.65 to 105.3 g C kg^{-1} with an average of 84.5 g C kg^{-1}. The Total C contents were similar to those measured for archived samples from the Whatawhata fertiliser trial for easy slopes (Schipper *et al.,* unpublished data). The total C contents of the Whatawhata fertiliser trial (although limited to easy slopes in this study) were higher than total C contents (of 42 to 63 g kg^{-1}) found across a range of soils of New Zealand hill pasture under conventional and organic management (Parfitt *et al.,* 2003), and the average of 56 to 67 g kg^{-1} found for pastoral land and indigenous forests (Sparling & Schipper, 2004).

Assuming a bulk density of 1 g cm^{-3}, and that C was evenly distributed in the A horizon (i.e. top 20 cm of soils), and taking the mean of total C in the 0 kg P ha^{-1} yr^{-1} of 88.74 mg C g^{-1} soil, and taking the mean of the total C in the 100 kg P ha^{-1} yr^{-1} of 67.78 mg C g^{-1} soil, there was about 24 % lower C content in the high P treatment (100 kg P ha^{-1} yr^{-1}) than the low P treatment (0 kg P ha^{-1} yr^{-1}).

The significant ($p<0.05$) negative correlation between total carbon and P fertiliser application showed that increased P fertiliser is associated with a small decrease in total carbon. A decrease in soil total carbon has been observed in soils under long term grazed pastures with increasing agricultural intensification (Bellamy *et al.,* 2005; Schipper *et al.,* 2007), and my results for total C were consistent with these previous findings.

b. Labile Carbon

I found a significant ($p<0.01$) negative correlation between labile C and fertiliser P application rate (Figure 5.3). The negative relationship was as expected, because labile C as a sub-pool of total C should also reflect the change found in total C along the P gradient. However, the negative relationship of labile C with P fertiliser application was not consistent with all past findings. For example, a previous study by Conteh *et al.* (1997) also using the ease of oxidation with $KMnO_4^-$ method in organic soils, showed an increase in labile C content of both the cultivated and native soil with the presence of wheat and fertiliser application, compared to native soil with fertiliser application and no wheat planted. Conteh *et al.* (1997) suggested that the increase in labile carbon was related to the growth of the plant (wheat), which showed an increased amount of roots relative to treatments in which no fertiliser was added, and that the roots themselves also exude carbon compounds which also could be labile (Conteh *et al.,* 1997). Hence it was expected that the higher P treatment soils would have higher amount of labile carbon because of the presumed greater pasture growth and decomposition rates. However, the negative and significant change observed for labile C indicates pasture growth rate and thus organic matter decomposition decreased with increasing fertiliser application rate. The decrease in labile C may have been attributed to factors affecting pasture decomposition including higher grazing and stock transfer (associated with higher pasture growth) which would have decreased the amount of plant litter for decomposition (Simpson *et al.,* 1974; Rowarth *et al.,* 1992c; Ledgard, 2001).

The proportion of labile C of total C (about 20 %) was greater than the microbial biomass C of the total C (0.5 %) (Table 5.1 and Figure 5.6) which is as expected

since labile C made up a larger pool of soil organic carbon than microbial biomass.

c. Respired C, MBC, and Specific respiration

(i) Respirable Carbon

Soil respiration (Figure 5.4) did not show a significant difference under the different P treatments. It was expected that the respirable C from microbial respiration would increase along the P gradient with increasing fertility.

The lack of relationship between fertility and respiration was in contrast to the finding by Grayston *et al.* (2001). These authors compared microbial biomass and activity in unimproved, semi-improved, and improved grasslands, the main difference of these grasslands being their pasture composition and fertility status. As soil fertility increased i.e. moving from unimproved to improved grasslands, microbial biomass decreased while soil respiration increased. The lower carbon mineralisation from microorganisms in the low nutrient sites were suggested as a result of the more recalcitrant substrates in the corresponding low fertility grass rhizospheres (Grayston *et al.,* 2001). Microbial biomass responded to low substrate availability in soil by showing characteristics similar to that of an inactive population e.g. low maintenance energy, low respiration rate and slow mean cell-division rate (Brookes *et al.,* 1985). Thus, the availability of carbon substrates from the sampled sites may have limited microbial respiration observed.

(ii). Microbial Biomass Carbon

No significant relationship was found between soil microbial biomass carbon and fertiliser P application rate (Figure 5.5). There were no previous MBC measurements that have been reported at Whatawhata. The MBC values found in this study were slightly lower than the range of values previously reported in pastures (Table 5.3). The non-significant trend of MBC was not expected since the microbial biomass as the active and more sensitive sub-pool of total carbon (Brookes *et al.,* 1985), was expected to easily show changes that were observed in total C. The lack of change suggests that the microbial biomass was not affected

by fertiliser P but that other factors may be limiting the microbial biomass. One factor that might limit the size of the MBC is pH.

Low soil pH in the fertiliser trial might limit organic matter decomposition, and the size of the microbial biomass (Brookes *et al.,* 1985). Acidification in grassland soils may have undesirable effects on plant composition and productivity that may lead to instability of organic matter and nutrients (Pearson, 1987). Low pH has been suggested as linked to the decreased activity of the re-colonising microbial population in soils fumigated with acid (Vance *et al.,* 1987). High pH (normally brought about by liming), have been found to increase phosphorus retention in the surface soil (Russell, 1960b). Increasing soil pH raises microbial activity of the soil and the rate of organic matter decomposition (Jackman, 1960). High pH is associated with high fertility, where exudation from plant species in grasslands are increased, followed by the incorporation of root exudates by microbial communities, which favour bacterial growth and thus increase of microbial biomass (Grayston *et al.,* 2001; Rangel-Castro *et al.,* 2005).

The non-significant relationship found for the MBC and P fertiliser was consistent with findings of Sarathchandra *et al.* (1988) who also found no significant relationship between biomass C and fertiliser P, and for both fertilised and non-fertilised plots (of alluvial yellow-brown loam soil under grazed pasture).

Table 5.3: *Selected microbial biomass values for different landuses from the literature in comparison with data from Whatawhata trial*

Source	**Landuse**	**Microbial biomass carbon (mg C g^{-1} soil)**
Lynch and Panting, 1980	Cropping (wheat)	0.232 – 0.368
Powlson, 1980	Rough pasture	0.790
Jenkinson and Powlson, 1980	Arable field	0.272 - 0.315
Bolton *et al.,* 1985	Cropping (wheat)	0.124 – 0.314
Steele *et al.,* 1984	Permanent pasture	0.936 - 2.743
Sarathchandra *et al.,* 1984	Established pasture	0.540 - 1.890
Sparling and West, 1988	Improved pasture	0.392 – 1.484
This study	Improved pasture	0.138 to 0.401

The ratio of CO_2-C flush to N flush found at Whatawhata ranged from 0.57 to 2.58 with a mean of 1.40, which was lower than that found in Jenkinson (1988), reflecting a low MBC. The mean ratio of CO_2-C flush (respired C from which MBC was derived from) to N flush was 5.31 based on a wider range of soils (Jenkinson, 1988).

Increase in readily available carbon in improved grasslands stimulated bacterial growth. The availability of carbon sources may vary in different grassland types as demonstrated by the differences in the utilisation of carbon sources by the microbial community (Grayston *et al.,* 2001). Generally, conditions favouring accumulation of organic matter increase both the amount of biomass and the proportion of the soil organic carbon (Jenkinson and Ladd, 1981). It was expected that with greater pasture DM along the P gradient, MBC would also increase, but the lack of change in MBC might be that carbon substrates may not be as readily available to the microbial population.

(iii) Microbial Specific Respiration

The microbial specific respiration rate or metabolic quotient (qCO_2) showed no significant trend with fertiliser P application (Figure 5.7). It was expected that qCO2 would decrease along the P gradient presuming that microbial biomass would be greater in the higher fertility soils. The specific respiration rate was generally greater when the MBC declined and was functioning inefficiently where higher proportion of carbon was used for cell maintenance rather than growth (Wardle and Ghani, 1995). The lack of change in qCO_2 with P fertiliser addition could be attributed to the lack of change also found for MBC along the P gradient.

e. Microbial quotient

There was no significant difference between the microbial quotient and fertiliser P application rate (Figure 5.6). No trend was expected because microbial quotient is directly linked to MBC where no trend was also observed along the P fertiliser gradient.

Usually, the microbial quotient is 1-4 % with an average of 2-3 % (Jenkinson and Ladd 1981). Microbial quotient values reported in previous studies of pasture

soils ranged from 0.76 to 2.4 % (Steele *et al.,* 1984), 1 to 3.9 % excluding peats (Sarathchandra *et al.,* 1984), 3.45 % (Kaiser *et al.,* 1992), 1 to 4 % (Sparling, 1992), and 1.7 to 2.1 % (Haynes & Williams, 1999). In comparison, microbial quotient values found in this research were much lower (0.15 to 0.58 %).

The lower MBC measured at Whatawhata was the reason for the lower values of microbial quotient. The microbial quotient may have been affected by several environmental factors such as clay content, mineralogy, organic matter content, vegetation type, and soil management practice (Sparling, *et al.,* 1992).

5.9.4. Total N, Mineralised N, and Microbial N

a. Total Nitrogen

The results showed a significant ($p<0.01$) negative correlation between total nitrogen and fertiliser P application rate (Figure 5.8). The decrease in total N with increasing P fertiliser application rate was not as expected. Conventionally, increasing P (through increase in fertiliser application) would be expected to result in an increase in clover production and N fixation, thus N concentration should increase in soils with increasing P fertiliser application (Simpson *et al.,* 1974; Walker *et al.,* 1959, Lambert *et al.,* 2000, Sparling & Schipper *et al.,* 2004).

There were large botanical differences between fertiliser treatments. The high fertiliser treatment (100 kg P ha^{-1} yr^{-1}) paddock was observed as had more clover, and also consisted of ryegrass, cocksfoot grass, and other species. The low fertiliser treatment (0 kg P ha^{-1} yr^{-1}) paddock was mostly dominated by summer grasses. Although not all paddocks were examined for pasture species composition, the observation obtained from the two extreme P treated paddocks (Paddocks, 13 and 18), was consistent with normal expectations with higher abundance of clover expected with higher P application rates (Walker *et al.,* 1959; Lambert *et al.,* 2000; Parfitt *et al.,* 2003). Annual pasture DM production (kg ha^{-1}) previously reported for the Whatawhata fertiliser trial increased significantly with increased P application rate on the easy slopes (1984-1988) (Gillingham *et al.,* 1990). Thus, higher total N could be expected in the higher P treatment

paddocks. The negative trend in total N was similar to that for total C, which also showed a negative change with increased P fertiliser application rate. These findings (C and N loss) were consistent with that recently reported for managed permanent pastures in New Zealand (Schipper *et al.,* 2007).

A long-term study at Kybybolite, South Australia (Russell, 1960a) reported that the accumulation of organic nitrogen was not related to the amount and rate of superphosphate applied, but was more dependent on pasture growth and sheep carrying capacity. Gillingham *et al.,* (1990) found most of the pasture growth occurred on easy slopes compared to steep slopes and also related pasture growth to higher stock grazing. Grazing animals have been responsible for the large spatial redistribution of N in hill country pastures (Ledgard, 2001). Previous studies (Hilder, 1965; Simpson *et al.,* 1974) found a large amount of transfer of fertility (soil nutrients) from most areas of a paddock to campsite areas where animals tend to spend most of their time. Grazing pressure; however, would only transfer nitrogen around the paddock area rather than to cause an overall change (Simpson *et al.,* 1974). Higher stock numbers are carried in the higher P treatment paddocks, associated with the higher DM production, (Rowarth *et al.,* 1992c) so more nutrients may be exported from high P treatment paddocks.

A recent study of Bowatte *et al.* (2006) investigating the effects of P fertiliser applications on N fertility in hill country pasture, using a modelling approach, found that addition of P fertilisers to hill country pastures for improving the N status of soils is now questionable, particularly on steep slopes. Bowatte *et al.* (2006) suggested that any increase in clover growth stimulated by P fertiliser application, had minimal effect on the residual N fertility of the soil, attributed mainly to N inputs lost by animal transfer, and through volatilisation and leaching from urine patches. Thus in hill country, the decrease in soil total N with increasing P application rate might be attributed to the higher grazing intensification, animal transfer, and N loss from urine patches through volatilisation and leaching.

b. Mineral N and Mineralised N

The increase in total mineral N observed after incubation (T_{14}) (i.e. $\sum[NH_4^+$-N + NO_3^--N]) compared to that before incubation (T_0) (Table 5.2), indicated that mineralisation of organic N took place during the incubation period. The increase in mineral N after fumigation was mainly from the increase in NH_4^+ but less from NO_3^- (Appendix F) as also observed in previous studies of Voroney and Paul (1984), Shen *et al.,* (1984), and Schnurer *et al.,* (1985). The greater total mineral N in the fumigated samples after incubation compared to total mineral N in the non-fumigated samples after incubation indicated an increase in biomass and activity from the fumigated samples as a result of increased microbial activity when microbial cellular materials are released during decomposition (Jenkinson and Powlson., 1976b; Shen *et al.,* 1984).

Mineralised N was not correlated with fertiliser P application rate, (Figure 5.9). With greater herbage accumulation, there would also be increased supply of N in soil from plant litter and roots (when roots die or exudates), for access to plants (Fogel, 1985; Parfitt *et al.,* 2003). Hence, herbage N is usually correlated with the soil N (Parfitt *et al.,* 2003). It was thus expected that N mineralised should conventionally be greater in the higher fertiliser rate paddocks compared to the lower P fertiliser paddocks. However, the non significant change in mineralised N along the P gradient indicates that other factors may be affecting the mineralisation of N. Factors affecting herbage accumulation in the higher P fertiliser treatment paddocks such as increased animal grazing and export of N from the higher P paddocks would thus be limiting N mineralisation.

During organic matter turnover, the amount of nutrients available to plants may either increase via mineralisation or decrease through immobilisation. Plant nutrients are converted from organic to available forms during mineralisation while the reverse occurs in immobilisation in which available plant nutrients are converted to unavailable organic forms by soil microbial organisms (Cornforth, 1998). Conventionally, both total N and mineralisable N were higher under pastures compared to other land uses, reflecting the extensive N accumulation under pastures, as a result of conversion of original forests to European pastoral agriculture (Sparling & Schipper, 2004).

The relationship between soil net mineralisation with different microbial and chemical parameters has been reported. Parfitt *et al.,* (2003) observed soil net mineralisation was not correlated to microbial biomass C or N, and only weakly related to Olsen P, but strongly related to nematodes and microbial P ($p = 0.001$) (Parfitt *et al.,* 2003). Thus, nematode activity and microbial P could be important regulators of N mineralisation, instead of P fertiliser application as investigated in this study.

c. N Flush and Microbial Biomass Nitrogen

N mineral flush (N flush) is a measure of microbial biomass nitrogen (Jenkinson & Ladd, 1981; Bolton *et al.,* 1985). The N flush determined in this study ranged from 62.3 to 191.0 μg g^{-1} soil, with an average of approximately 113 μg g^{-1} soil. Seven out of the 12 samples have an N flush over 150 μg g^{-1} soil (Appendix F). Previously reported N flushes for pasture soils include 153 μg g^{-1} soil (Jenkinson *et al.,* 1979), 27-158 μg g^{-1} soil (Sarathchandra *et al.,* 1984). Thus, the N flush and therefore microbial biomass N found were within the range of those reported in previous studies, although some sites had higher N flush.

The Microbial Biomass Nitrogen (MBN) of soils from all 12 paddocks showed no significant relationship with fertiliser P application rate (Figure 5.10). The large variability in most soil sample replicates were mainly attributed to the naturally high variability in soils in the field. The non-significant relationship between MBN and P application rate implied that P fertiliser does not have an effect on MBN or soil organic nitrogen for soils at the Whatawhata fertiliser trial.

d. Microbial N Quotient and Mineralised N per microbial biomass unit

The N microbial quotient determined from the results ranged from 1 to 3.2 % which is within the range of the N quotient normally found of 2 to 6% (Graham Sparling pers. comm. Sept.,2007). However, the Nitrogen Microbial Quotient which is the proportion of MBN from total N showed no significant relationship with P application rate (Figure 5.11). The non-significant relationship was expected because no relationship was also observed for MBN.

Microbial activity was found to be limited where litter N was low hence causing low soil N concentration (Pearson, 1987). The mean pasture dry matter production on easy slopes at the fertiliser trial increased with increasing fertiliser P application rate (Gillingham *et al.,* 1990). However, the greater rate of pasture growth in the higher P rates, may also support the higher rate of plant removal by sheep grazing, limiting the amount of litter N in the higher P paddocks, thus may be limiting MBN and mineralised N per microbial biomass unit (Figure 5.12).

5.9.5. C:N Ratio

The amounts of carbon (C) compared relative to other nutrients like nitrogen (N) imply the extent of organic matter decomposition and the probability of whether mineralisation or immobilisation is taking place (McLaren and Cameron, 1996; Cornforth, 1998).

The results for the C:N ratio for all 12 paddocks ranged from C:N ratio of 10.3 to 11.7, with a mean of 11 (Figure 5.14), and showed no significant relationship with P fertiliser application. The mean C:N ratio of 11 found for all paddocks was similar to the mean C:N ratio of 11.8 found under drystock pasture and 11.3 under dairy pasture (Sparling and Schipper, 2004). However, the C:N ratio found in the results for the Kaawa Hill soils sampled, were lower compared to the C:N ratio previously for Kaawa Hill soils (of untopdressed rough pasture) with C:N ratio of 15 (Bruce, 1978). Conventionally, the C:N ratio tends to decline under pastoral agriculture as a result of N accumulation in soil from the inputs from N-fixing legumes, and N fertilisers (Sparling & Schipper, 2002), thus explains the difference in C:N ratio with that in Bruce (1978). Since the C:N ratio at Whatawhata was close to 10, this indicates that the storage of N in organic forms in the topsoils sampled is approaching saturation (Sparling & Schipper, 2004).

The C:N ratio provides an indication of decomposition and the N quality of the SOM. A high ratio reflects low N quality in terms of availability to plants and low decomposition status, while a low ratio shows high N quality and a well decomposed organic matter (Bruce, 1978, Parfitt *et al.,* 2003). The low C:N ratio

(average of 11) indicates a high N quality in soils at Whatawhata. Normally, the N quality is enhanced through the growth of legumes (for N fixation), that in turn depend on the P status of the soil (Parfitt *et al.,* 2003).

5.9.6. Soil pH

Soil pH for all 12 paddocks ranged from pH 4.7 to 5.1, and averaged pH 5 with no significant effect of P fertiliser application (Figure 5.14). The mean pH of 5 found in this study was generally similar to the mean pH determined for 2004 – 2007 (pH of 5.2) also at the Whatawhata fertiliser trial sites (Power, 2007). The mean pH in the results was closer to the mean pH of 5.4 found in indigenous and plantation forest soils but lower than soil pH normally found under agricultural land uses (Sparling & Schipper *et al.,* 2004), with optimum pH of 5.8-6.0 for New Zealand pastures (Edmeades, 1998), and an average of about pH 6.4 in some permanent pastures (Brookes *et al.,* 1984). The fertiliser trial paddocks were reported as not having been limed (Power, 2007), hence the lower pH measurements were expected. The soil pH was similar to that reported for soil (poorly drained clayey non-calcareous) under permanent pasture management with mean soil pH of 5.4 (measured for the 0-10 cm depth) that had not also received fertiliser N inputs over 30 years (Patra *et al.,* 1999).

CHAPTER 6

SUMMARY AND CONCLUSION

6.1. SUMMARY

6.1.1. Experimental Design and Method

My research was carried out at a fertiliser trial at the Whatawhata AgResearch hill country research station. Twelve paddocks with six fertiliser P application rates (0, 10, 20, 30, 50, and 100 kg P ha^{-1} yr^{-1}, with two replicate paddocks per treatment) were sampled at 0-7.5 cm, and analysed in the laboratory for microbial and biochemical characteristics including Olsen P, Total C, Total N, Labile C, microbial respiration (respired C), microbial biomass C (MBC), specific respiration rate (qCO_2), microbial quotient (C_{mic}/C_{org}), mineralised N, microbial biomass N (MBN), microbial biomass N quotient, N mineralised per biomass unit, C:N ratio, and Soil pH.

6.1.2. Summary of Results

Total C was investigated as a function of P fertiliser application. SOM measurements were divided into fractions as total C and the active components measured as labile C and microbial biomass C. As expected the results showed Total C comprised the largest fraction, followed by labile C, and microbial biomass as the smallest pool. Complimentarily, the N components of SOM were measured as Total N, N mineralised, and microbial biomass N.

Olsen P (0-7.5 cm) showed a significant positive correlation with P fertiliser application rate. Significant negative correlations were found between Total C, Total N, and Labile C, and P fertiliser application rate. The C:N ratio, microbial biomass C (MBC), microbial respiration (respired C), its activity (specific respiration rate), mineralised N, N flush, and microbial biomass N (MBN), showed no significant correlation with P fertiliser application rate.

6.2. ADDRESSING THE RESEARCH HYPOTHESIS

Total C has been found in recent studies (Bellamy *et al.*, 2005; Schipper *et al.*, 2007) to be decreasing under permanent pasture in temperate regions. The carbon decrease may be caused by factors such as land use intensification and climate change. This study investigated the effect of land use intensification through a long term fertiliser (P) application on soil organic matter composition and also looked at the C:N ratio because P loading would be expected to increase clover growth, and hence N fixation, and thus increase N in soil.

My research hypothesis stated that:

- As P fertiliser increases C:N ratio will decrease, and
- changes in C pools will be greater in the more active pool (readily available carbon, and microbial biomass carbon) within the soil total carbon.

The significant negative correlations found between pools of total C, total N, labile C, and P fertiliser loading, implied that there were declines of C in the soil organic matter at the Whatawhata fertiliser trial soils. The non-significant relationship between C:N ratio and P fertiliser application rate was attributed to both the parallel declines in total C and total N. Although there was no significant change between the C:N ratio and P fertiliser application rate, total C and N have decreased along the P gradient. The difference of C content of approximately 24 % between the low (0 kg P ha^{-1} yr^{-1}) and high (100 kg P ha^{-1} yr^{-1}) is significant. The C:N ratio of average 11, may

also infer that the storage of N in soil organic matter in the topsoils sampled is approaching saturation (Sparling & Schipper, 2004).

Since C:N ratio did not change along the P gradient, the first hypothesis is rejected as both C and N decreased. The decrease in soil N, as suggested by the study of Bowatte *et al.* (2006) was attributed mainly by N inputs (from clover growth and fixation) lost by animal transfer, and through volatilisation and leaching from urine patches.

Since significant changes were found for labile carbon as well as total carbon, but not the respirable C, and microbial biomass carbon, the second hypothesis is accepted in part because not all of the readily available carbon (both labile carbon and microbial biomass carbon) changed, but only the labile carbon that changed along the P gradient.

6.3. RECOMMENDATIONS FOR FURTHER WORK

Since total carbon, total nitrogen, and labile carbon showed significant decreases with increasing P application rate, further work is required to confirm these findings if fertiliser application should continue in New Zealand.

More study would be required on microbial biomass with a relatively fast turnover rate, and thus an important indicator of changes occurring in soil organic matter (Powlson and Jenkinson 1976; Sparling 1992; Blair *et al.,* 1995). Quantifying the microbial biomass in soils is a complex task. The diversity of various microorganisms such as bacteria, actinomycetes, fungi and protozoa, as well as the different physiological stages of their life cycles, and the soil itself, all add to the complication of direct observations and measurements (Van de Werf and Verstraete, 1987). Minimisation of the variability observed in measurements of the more sensitive microbial biomass, could be achieved in future similar studies, by probably

considering the use of more than one replicate sample from the same paddock, since microbial biomass can vary spatially.

The metabolically active microbial population is important ecologically. Variations in the microbial activity can occur with time of sampling (Ross *et al.,* 1981). To determine the size of the more active biomass as a more sensitive indicator of changes in soil resulting from changing management practices (Brookes *et al.,* 1985; Sarathchandra *et al.,*1988; Sparling, 1992; Haynes, 1999), sampling at different times of the year could be considered, as this could present different results than obtained from the one off sampling in this study. It would be an advantage that other methods of measuring microbial biomass C and activity, namely, the substrate induced respiration (SIR) (Ross, 1987) and the fumigation-extraction technique (Sparling and West, 1988), be explored as alternative procedures and as necessary for greater precision for quantification of the microbial C pool (Kaiser *et al.,* 1992). Other relationships that have been found correlated with microbial biomass in previous studies that were not investigated in this study, including soil properties, pasture composition and growth, climate, nematodes and microbial P (Parfitt *et al.,* 2003), may need to be investigated, as no microbial studies before have been undertaken for soils at Whatawhata fertiliser trial. It would seem that the P is not limiting to microbial activity at the sites, thus further investigation could be undertaken to identify other potential limitations (e.g. K, pH, and other nutrients).

Large differences in soil C and N have been found with depth (Patra *et al.,* 1999) e.g. % N below 1 m depth is between 7 and 35 % (Whitney and Zabowski, 2004). Hence, it may be more comprehensive for future work to determine soil total C and N throughout the profile rather than the 0-7.5 cm depth used in this thesis. A larger sample than currently used in this research is recommended for future work, to be able to confirm the changes found in this study particularly in total C and N.

Phosphate fertiliser may influence many factors, a few of which were investigated in this study. Thus, it is important if other factors such as pasture composition, herbage N, P retention, root nematodes, soil temperature, and other nutrient supplies (Sinclair

et al., 1997), and microbial composition, affecting soil C and N and thus soil organic matter, could be considered in relation to P fertiliser application rates, in future similar studies. It is noted that the soil pH (mean of 5.0) was fairly low. Further work could be undertaken to investigate the effect of increasing soil pH on the soil chemical and biochemical properties tested.

6.4. CONCLUSION

- There was a significant decrease in soil total carbon, total nitrogen, labile C, with increasing fertiliser P application rate. No significant change was found between respirable C, microbial biomass carbon, specific respiration rate, microbial C quotient, mineralised N, microbial biomass nitrogen, N mineralised per microbial biomass nitrogen, microbial N quotient, C:N ratio, or soil pH, and fertiliser P application rate.

- Schipper *et al.* (2007) found declines in C and N but did not say why losses occurred. This study found the effect of fertiliser P application (as a means of agricultural intensification) as significantly correlated with decreased in soil carbon and nitrogen under permanent pasture.

- Although there were changes shown in total C, total N, and labile C, all other measurements particularly the microbial pool measurements did not show any change with fertiliser P application. Therefore, the overall effect of fertiliser P application on soil organic matter at the fertiliser trial was not greatly significant.

- The C:N ratio did not change along the P gradient, therefore, the first hypothesis (As P fertiliser increases C:N ratio will decrease) is rejected, however, both C and N decreased.

- Since significant changes were found for labile carbon (readily available carbon) as well as total carbon, but not the respirable carbon and microbial biomass carbon, the second hypothesis (Changes in C pools will be greater in the more active pool (readily available carbon, and microbial biomass carbon) within the soil total carbon) is therefore, accepted in part.

- The effect of increased stock grazing capacity and animal transfer of herbage and thus nutrients, as well as N volatilisation and leaching may have contributed to the decline in the major pools of C and N with increasing P application rate. Further work is needed to explain these effects.

- Further work should be undertaken at the Whatawhata fertiliser trial to follow up on the findings from this study using a larger sample size with greater replication from each paddock, investigating further the microbial biomass carbon, and also investigating other factors affecting soil C and N such as soil properties (including temperature, pH, P retention), root nematodes, pasture composition, microbial composition, and other nutrients.

APPENDIX A:

Olsen P data

Olsen P Determination

	a	*e*	*f*	*g*	*h*	*i*	*j*	*k*
ID Number	**P Fertiliser Application Rate**	**Corrected "y" Absorbance**	**P Concentration [x=(y+0.0959)/ 0.0728]**	**P Concentration [f*1000/1000 ml]**	**Mass of P extracted [g*40]**	**P Concentration [h/2] [2 is from 2g soil]**	**P Concentration (Olsen P) [i/MF]**	**Mean Olsen P [j/3]**
	(kg P ha^{-1} yr^{-1})	**(Blank = -0.034)**	**(ppm or µg g^{-1})**	**(µg ml-1)**	**(µg)**	**(µg g^{-1} soil)**	**(µg P g^{-1} soil)**	**(µg g^{-1} soil)**
1	0	0.038	1.839	1.839	73.57	36.79	34.70	
2	0	0.037	1.826	1.826	73.02	36.51	34.44	
3	0	0.034	1.784	1.784	71.37	35.69	33.67	34.27
4	0	0.033	1.771	1.771	70.82	35.41	33.10	
5	0	0.032	1.757	1.757	70.27	35.14	32.84	
6	0	0.026	1.674	1.674	66.98	33.49	31.30	32.41
7	10	0.033	1.771	1.771	70.82	35.41	33.41	
8	10	0.034	1.784	1.784	71.37	35.69	33.67	
9	10	0.029	1.716	1.716	68.63	34.31	32.37	33.15
10	10	0.040	1.867	1.867	74.67	37.34	35.22	
11	10	0.044	1.922	1.922	76.87	38.43	36.26	
12	10	0.049	1.990	1.990	79.62	39.81	37.55	36.34
13	20	0.104	2.746	2.746	109.84	54.92	51.32	

Olsen P Determination - Continued

ID Number	P Fertiliser Application Rate (kg P ha^{-1} yr^{-1})	Corrected "y" Absorbance (Blank = -0.034)	P Concentration [x=(y+0.0959)/ 0.0728] (ppm or µg g^{-1})	P Concentration [f*1000/1000 ml] (µg ml^{-1})	Mass of P extracted [g*40] (µg)	P Concentration [h/2] [2 is from 2g soil] (µg g^{-1} soil)	P Concentration (Olsen P) [i/MF] (µg P g^{-1} soil)	Mean Olsen P [j/3] (µg g^{-1} soil)
14	20	0.118	2.938	2.938	117.53	58.76	54.92	
15	20	0.115	2.897	2.897	115.88	57.94	54.15	53.46
16	20	0.042	1.894	1.894	75.77	37.88	35.74	
17	20	0.044	1.922	1.922	76.87	38.43	36.26	
18	20	0.044	1.922	1.922	76.87	38.43	36.26	36.09
19	30	0.097	2.650	2.650	105.99	52.99	49.99	
20	30	0.093	2.595	2.595	103.79	51.90	48.96	
21	30	0.099	2.677	2.677	107.09	53.54	50.51	49.82
22	30	0.155	3.446	3.446	137.86	68.93	63.82	
23	30	0.165	3.584	3.584	143.35	71.68	66.37	
24	30	0.156	3.460	3.460	138.41	69.20	64.08	64.76
25	50	0.380	6.537	6.537	261.48	130.74	123.34	
26	50	0.412	6.977	6.977	279.07	139.53	131.63	

Olsen P Determination - Continued

ID Number	P Fertiliser Application Rate (kg P ha^{-1} yr^{-1})	Corrected "y" Absorbance (Blank = -0.034)	P Concentration [x=(y+0.0959)/ 0.0728] (ppm or µg g^{-1})	P Concentration [f*1000/1000 ml] (µg ml^{-1})	Mass of P extracted [g*40] (µg)	P Concentration [h/2] [2 is from 2g soil] (µg g^{-1} soil)	P Concentration (Olsen P) [i/MF] (µg P g^{-1} soil)	Mean Olsen P [j/3] (µg g^{-1} soil)
27	50	0.412	6.977	6.977	279.07	139.53	131.63	128.87
28	50	0.413	6.990	6.990	279.62	139.81	134.43	
29	50	0.412	6.977	6.977	279.07	139.53	134.17	
30	50	0.433	7.265	7.265	290.60	145.30	139.71	136.10
31	100	0.386	6.620	6.620	264.78	132.39	127.30	
32	100	0.384	6.592	6.592	263.68	131.84	126.77	
33	100	0.408	6.922	6.922	276.87	138.43	133.11	129.06
34	100	0.723	11.249	11.249	449.95	224.97	214.26	
35	100	0.704	10.988	10.988	439.51	219.75	209.29	
36	100	0.752	11.647	11.647	465.88	232.94	221.85	215.13

Olsen P Statistical Analysis

Analysis of Variance; DV: Olsen P					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	94927	1	94927.3	157.1277	0
Residual	20541	34	604.14		
Total	115468				

Regression Summary for Dependent Variable: Olsen P						
F(1,34)=157.13 p<.00000 Std.Error of estimate: 24.579						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(34)	p-level
Intercept			24.7263	5.967669	4.14338	0.000214
P rate	0.9067	0.072333	1.55417	0.123986	12.53506	0

APPENDIX B:

Total Carbon data

Total Carbon Data

Rate of P Fertiliser Applied (kg P ha^{-1} yr^{-1})	Paddock Code	Total Carbon (% or g C/100 g soil)	Total C (g C g^{-1} soil)	Total C (g C g^{-1} soil)	Total C (mg C g^{-1} soil)
0	P6	8.30	0.083	0.083	83.03
0	P18	9.44	0.094	0.094	94.44
10	P1	9.25	0.092	0.092	92.45
10	P12	8.16	0.082	0.082	81.56
20	P3	10.53	0.105	0.105	105.3
20	P9	7.79	0.078	0.078	77.91
30	P7	9.00	0.090	0.090	90.03
30	P10	9.14	0.091	0.091	91.42
50	P15	8.85	0.088	0.088	88.49
50	P19	7.38	0.074	0.074	73.82
100	P4	5.87	0.059	0.059	58.65
100	P13	7.69	0.077	0.077	76.88

Statistical Analysis

Analysis of Variance; DV: Tot C					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	674.712	1	674.7121	7.423366	0.021391
Residual	908.903	10	90.8903		
Total	1583.615				

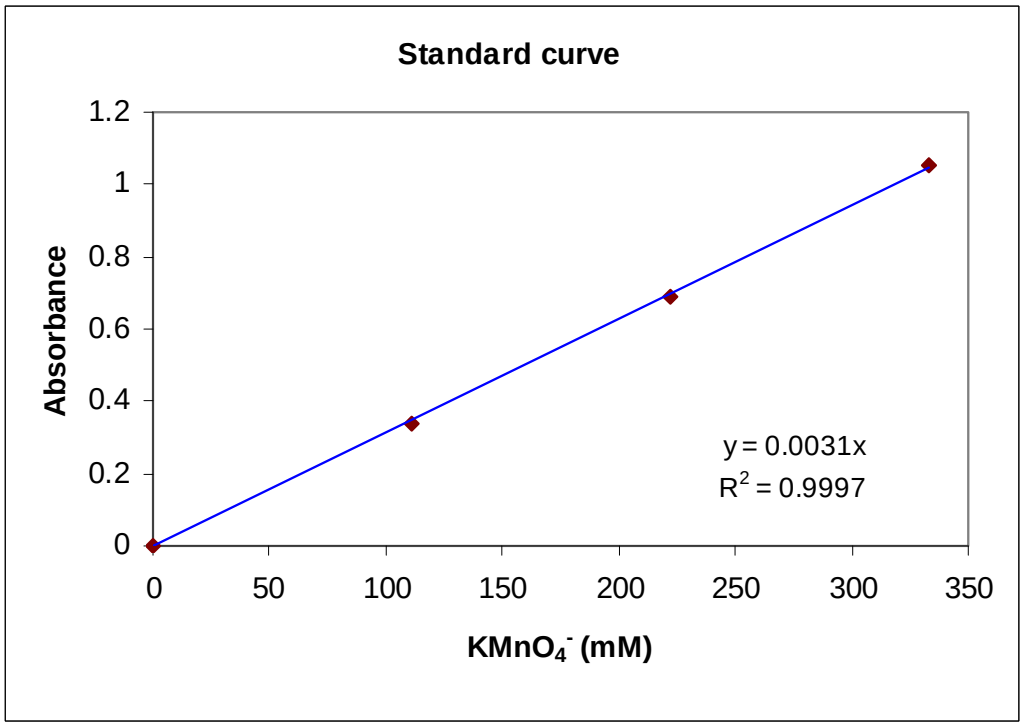
Regression Summary for Dependent Variable: Total C						
F(1,10)=7.4234 p<.02139 Std.Error of estimate: 9.5336						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(10)	p-level
Intercept			92.44146	4.009174	23.05748	0
P rate	-0.65273	0.239571	-0.22695	0.083296	-2.72459	0.021391

APPENDIX C:

Labile Carbon data

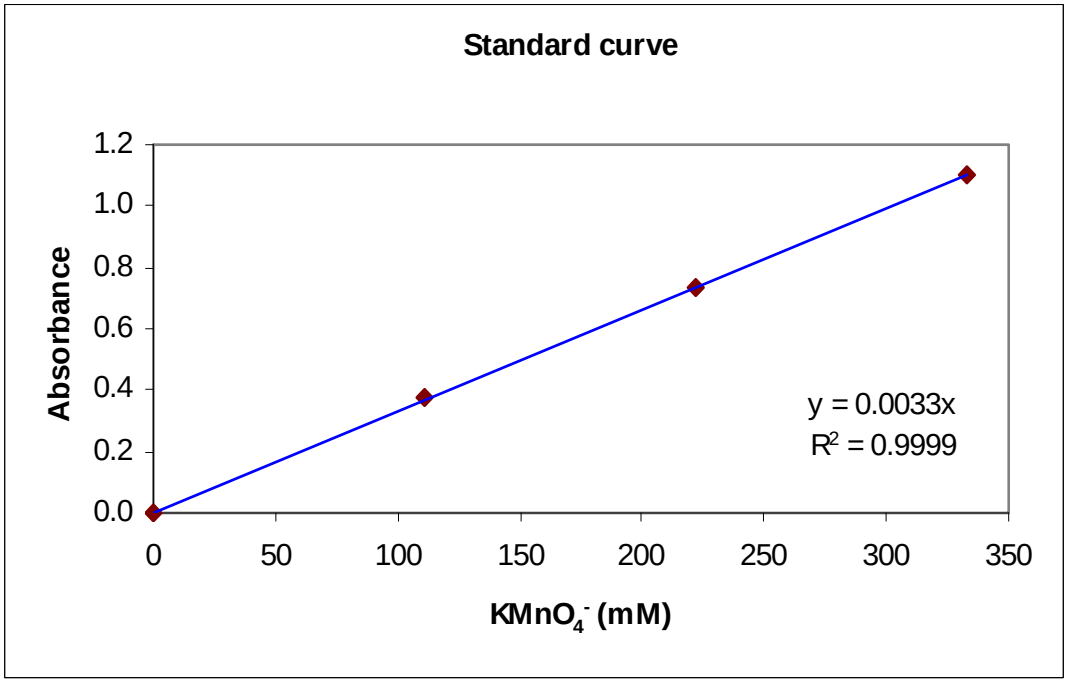
Standards (Batch 1: for samples ID numbers 1-24)

$KMnO_4^-$ STANDARDS			
Water (mL)	333mM (mL)	dilute 0.25 in 100mL (mM)	Abs
3	0	0	0
2	1	111	0.375
1	2	222	0.736
0	3	333	1.099



Standards (Batch 2: for samples ID number 25-36)

$KMnO_4^-$ STANDARDS			
Water (mL)	333mM (mL)	Dilute 0.25 in 100mL (mMol L^{-1})	Absorbance
3	0	0	0
2	1	111	0.34
1	2	222	0.691
0	3	333	1.054



Determination of Labile Carbon Concentration

ID Number	P application rate (kg P ha^{-1} yr^{-1})	Paddock lab replicate	soil weight (mg)	Soil blank	Absorbance corrected	$KMnO4^-$* (mM)	$KMnO4^-$** (mM)	$KMnO4^-$ (mM /0.025 L)	$KMnO4^-$ (mM per mg soil)	$KMnO4^-$*** (mg C per mg soil)	$KMnO4^-$ (mg C per g soil)	Mean (mg C per g soil)
1	0	6a	500	0.003	0.968	293.33	44.646	1.116	0.00223	0.0201	20.09	
2	0	6b	500	0.003	0.980	296.97	41.010	1.025	0.00205	0.0185	18.45	
3	0	6c	500	0.003	0.968	293.33	44.646	1.116	0.00223	0.0201	20.09	19.55
4	0	18a	500	0.004	0.939	284.55	53.434	1.336	0.00267	0.0240	24.05	
5	0	18b	500	0.004	0.950	287.88	50.101	1.253	0.00251	0.0225	22.55	
6	0	18c	500	0.004	0.950	287.88	50.101	1.253	0.00251	0.0225	22.55	23.05
7	10	1a	500	0.002	1.039	314.85	23.131	0.578	0.00116	0.0104	10.41	
8	10	1b	500	0.002	0.920	278.79	59.192	1.480	0.00296	0.0266	26.64	
9	10	1c	500	0.002	0.954	289.09	48.889	1.222	0.00244	0.0220	22.00	19.68
10	10	12a	500	0.003	1.028	311.52	26.465	0.662	0.00132	0.0119	11.91	
11	10	12b	500	0.003	0.977	296.06	41.919	1.048	0.00210	0.0189	18.86	
12	10	12c	500	0.003	0.957	290.00	47.980	1.199	0.00240	0.0216	21.59	17.45
13	20	3a	500	0.001	0.921	279.09	58.889	1.472	0.00294	0.0265	26.50	
14	20	3b	500	0.001	0.953	288.79	49.192	1.230	0.00246	0.0221	22.14	
15	20	3c	500	0.001	0.953	288.79	49.192	1.230	0.00246	0.0221	22.14	23.59
16	20	9a	500	0.002	0.984	298.18	39.798	0.995	0.00199	0.0179	17.91	
17	20	9b	500	0.002	0.989	299.70	38.283	0.957	0.00191	0.0172	17.23	
18	20	9c	500	0.002	0.986	298.79	39.192	0.980	0.00196	0.0176	17.64	17.59
19	30	7a	500	0.002	0.959	290.61	47.374	1.184	0.00237	0.0213	21.32	

Determination of Labile Carbon Concentration - Continued

ID Number	P application rate (kg P $ha^{-1}yr^{-1}$)	Paddock lab replicate	soil weight (mg)	Soil blank	Absorbance corrected	$KMnO4^{-}$* (mM)	$KMnO4^{-}$** (mM)	$KMnO4^{-}$ (mM /0.025 L)	$KMnO4^{-}$ (mM per mg soil)	$KMnO4^{-}$*** (mg C per mg soil)	$KMnO4^{-}$ (mg C per g soil)	Mean (mg C per g soil)
20	30	7b	500	0.002	0.924	280.00	57.980	1.449	0.00290	0.0261	26.09	
21	30	7c	500	0.002	0.949	287.58	50.404	1.260	0.00252	0.0227	22.68	23.36
22	30	10a	500	0.003	0.957	290.00	47.980	1.199	0.00240	0.0216	21.59	
23	30	10b	500	0.003	0.963	291.82	46.162	1.154	0.00231	0.0208	20.77	
24	30	10c	500	0.003	0.980	296.97	41.010	1.025	0.00205	0.0185	18.45	20.27
25	50	15a	500	-0.001	0.884	285.16	54.624	1.366	0.00273	0.0246	24.58	
26	50	15b	500	-0.001	0.910	293.55	46.237	1.156	0.00231	0.0208	20.81	
27	50	15c	500	-0.001	0.923	297.74	42.043	1.051	0.00210	0.0189	18.92	21.44
28	50	19a	500	0.001	0.953	307.42	32.366	0.809	0.00162	0.0146	14.56	
29	50	19b	500	0.001	0.938	302.58	37.204	0.930	0.00186	0.0167	16.74	
30	50	19c	500	0.001	0.936	301.94	37.849	0.946	0.00189	0.0170	17.03	16.11
31	100	4a	500	0.003	0.972	313.55	26.237	0.656	0.00131	0.0118	11.81	
32	100	4b	500	0.003	0.977	315.16	24.624	0.616	0.00123	0.0111	11.08	
33	100	4c	500	0.003	0.983	317.10	22.688	0.567	0.00113	0.0102	10.21	11.03
34	100	13a	500	0.002	0.944	304.52	35.269	0.882	0.00176	0.0159	15.87	
35	100	13b	500	0.002	0.926	298.71	41.075	1.027	0.00205	0.0185	18.48	
36	100	13c	500	0.002	0.933	300.97	38.817	0.970	0.00194	0.0175	17.47	17.27

* $KMnO4^{-}$ concentration not consumed by soil hence absorbed.

** $KMnO4^{-}$ concentration consumed and not absorbed.

*** $KMnO4^{-}$ concentration consumed as mg carbon mg^{-1} soil.

Statistical Analysis

Analysis of Variance; DV: Labile Carbon					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	166.1809	1	166.1809	11.02557	0.002154
Residual	512.4589	34	15.0723		
Total	678.6398				

Regression Summary for Dependent Variable: Labile Carbon						
F(1,34)=11.026 p<.00215 Std.Error of estimate: 3.8823						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(34)	p-level
Intercept			21.47597	0.942596	22.78386	0
P rate	-0.49485	0.149029	-0.06503	0.019584	-3.32048	0.002154

APPENDIX D:

Total Nitrogen

Total Nitrogen Data

Rate of P Fertiliser Applied (kg P ha^{-1} yr^{-1})	Paddock Code	Total N (% or g N/100g soil)	Total N (g N g^{-1} soil)	Total N (mg N g^{-1} soil)
0	P6	0.80	0.0080	7.951
0	P18	0.84	0.0084	8.413
10	P1	0.84	0.0084	8.410
10	P12	0.74	0.0074	7.391
20	P3	0.90	0.0090	9.027
20	P9	0.74	0.0074	7.368
30	P7	0.77	0.0077	7.714
30	P10	0.84	0.0084	8.359
50	P15	0.80	0.0080	8.037
50	P19	0.66	0.0066	6.648
100	P4	0.57	0.0057	5.715
100	P13	0.70	0.0070	7.017

Statistical Analysis

Analysis of Variance; DV: Total N					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	4.627056	1	4.627056	10.35992	0.009196
Residual	4.466303	10	0.44663		
Total	9.09336				

Regression Summary for Dependent Variable: Total N						
F(1,10)=10.360 p<.00920 Std.Error of estimate: .66830						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(10)	p-level
Intercept			8.32862	0.281041	29.63485	0
P Rate	-0.71333	0.221622	-0.01879	0.005839	-3.21868	0.009196

APPENDIX E

Respirable Carbon and Microbial Carbon

E.1: Determination of Water Content and Moisture Factor

ID Number	P fertiliser application rate (kg P ha^{-1} yr^{-1})	Paddock sample lab replicate	Container wt (g)	Container + soil (wet) (g)	Container + soil (dry) (g)	Mass of wet soil (g)	Mass of water in soil (g)	Oven Dry Soil (g)	Gravimetric water content Θ_M	Moisture factor (wet soil/dry soil)	Mass of wet soil to give 25 g of dry soil (for CO_2 measurement) (g)
1	0	P6a	2.06	7.06	5.123	5.00	1.937	3.063	0.63	1.63	40.8
2	0	P6b	2.08	7.09	5.146	5.01	1.944	3.066	0.63	1.63	40.9
3	0	P18a	2.09	7.08	4.952	4.99	2.128	2.862	0.74	1.74	43.6
4	0	P18b	2.08	7.09	5.188	5.01	1.902	3.108	0.61	1.61	40.3
5	10	P1a	2.07	7.07	5.165	5.00	1.905	3.095	0.62	1.62	40.4
6	10	P1b	2.08	7.07	5.203	4.99	1.867	3.123	0.60	1.60	39.9
7	10	P12a	2.08	7.08	5.201	5.00	1.879	3.121	0.60	1.60	40.1
8	10	P12b	2.08	7.07	5.147	4.99	1.923	3.067	0.63	1.63	40.7
9	20	P3a	2.07	7.07	5.072	5.00	1.998	3.002	0.67	1.67	41.6
10	20	P3b	2.09	7.09	5.184	5.00	1.906	3.094	0.62	1.62	40.4
11	20	P9a	2.06	7.07	5.096	5.01	1.974	3.036	0.65	1.65	41.3
12	20	P9b	2.09	7.08	5.114	4.99	1.966	3.024	0.65	1.65	41.3
13	30	P7a	2.07	7.07	5.046	5.00	2.024	2.976	0.68	1.68	42.0
14	30	P7b	2.07	7.07	5.112	5.00	1.958	3.042	0.64	1.64	41.1
15	30	P10a	2.08	7.09	4.999	5.01	2.091	2.919	0.72	1.72	42.9
16	30	P10b	2.06	7.07	4.998	5.01	2.072	2.938	0.71	1.71	42.6

E.1: Determination of Water Content and Moisture Factor - Continued

ID Number	P fertiliser application rate (kg P ha^{-1} yr^{-1})	Paddock sample lab replicate	Container wt (g)	Container + soil (wet) (g)	Container + soil (dry) (g)	Mass of wet soil (g)	Mass of water in soil (g)	Oven Dry Soil (g)	Gravimetric water content Θ_M	Moisture factor (wet soil/dry soil)	Mass of wet soil to give 25 g of dry soil (for CO_2 measurement) (g)
17	50	P15a	2.08	7.08	5.078	5.00	2.002	2.998	0.67	1.67	41.7
18	50	P15b	2.10	7.10	5.116	5.00	1.984	3.016	0.66	1.66	41.4
19	50	P19a	2.09	7.09	5.080	5.00	2.010	2.990	0.67	1.67	41.8
20	50	P19b	2.08	7.09	5.100	5.01	1.990	3.020	0.66	1.66	41.5
21	100	P4a	2.1	7.09	5.275	4.99	1.815	3.175	0.57	1.57	39.3
22	100	P4b	2.06	7.07	5.239	5.01	1.831	3.179	0.58	1.58	39.4
23	100	P13a	2.06	7.04	5.071	4.98	1.969	3.011	0.65	1.65	41.3
24	100	P13b	2.09	7.09	5.150	5.00	1.940	3.060	0.63	1.63	40.8

E.2: Respirable Carbon

ID Number	P Treatment (kg P ha^{-1} yr^{-1})	Paddock sample lab replicates	Dry mass of soil (unfumig) (g)	Dry mass of soil (unfumig) (mg)	Mass CO2-C at STP (g per 25g +/- 0.5soil/jar)	Mass CO_2-C at STP (g per 25g +/- 0.5 g soil)	Mass CO_2-C (ug g^{-1} soil)	Mean CO_2-C * (ug g^{-1} soil)
1	0	P6a	24.629	24628.97	14.41	0.5851	585.1	
2	0	P6b	24.983	24982.70	14.12	0.5652	565.2	575.14
3	0	P18a	24.957	24956.84	13.77	0.5518	551.8	
4	0	P18b	25.234	25233.71	13.48	0.5342	534.2	542.98
5	10	P1a	24.101	24101.26	15.05	0.6244	624.4	
6	10	P1b	24.819	24818.83	15.05	0.6064	606.4	615.42
7	10	P12a	23.913	23913.35	14.56	0.6089	608.9	
8	10	P12b	24.384	24384.06	14.16	0.5807	580.7	594.79
9	20	P3a	24.115	24115.31	14.65	0.6075	607.5	
10	20	P3b	24.354	24354.20	14.31	0.5876	587.6	597.54
11	20	P9a	25.635	25635.42	14.12	0.5508	550.8	
12	20	P9b	25.547	25546.85	14.85	0.5813	581.3	566.04
13	30	P7a	23.913	23913.35	15.19	0.6352	635.2	
14	30	P7b	24.384	24384.06	14.41	0.5910	591.0	613.08
15	30	P10a	23.335	23335.10	14.31	0.6132	613.2	
16	30	P10b	23.491	23491.10	14.41	0.6134	613.4	613.33
17	50	P15a	24.115	24115.31	14.8	0.6137	613.7	
18	50	P15b	24.354	24354.20	14.56	0.5978	597.8	605.78
19	50	P19a	24.138	24137.67	13.92	0.5767	576.7	
20	50	P19b	24.241	24241.38	14.16	0.5841	584.1	580.41
21	100	P4a	25.635	25635.42	12.84	0.5009	500.9	
22	100	P4b	25.547	25546.85	12.69	0.4967	496.7	498.80
23	100	P13a	24.329	24328.64	13.48	0.5541	554.1	
24	100	P13b	24.700	24700.32	13.48	0.5457	545.7	549.91

Statistical Analysis

Analysis of Variance; DV: Respirable C					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	3757.47	1	3757.466	3.765941	0.081
Residual	9977.5	10	997.75		
Total	13734.96				

Regression Summary for Dependent Variable: Respirable C						
F(1,10)=3.7659 p<.08100 Std.Error of estimate: 31.587						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(10)	p-level
Intercept			598.18	13.28333	45.03239	0
P rate	-0.52304	0.269524	-0.5356	0.27598	-1.9406	0.081

E.3: Determination of Microbial Biomass Carbon

ID Number	P fertiliser application rate (kg P ha^{-1} yr^{-1})	Paddock sample lab replicate	Mass CO_2-C [unfumigated] (µg g^{-1} soil)	Mean Mass CO_2-C [unfumigated] (µg g^{-1} soil)	Mass CO_2-C [Fumigated] (µg g^{-1} soil)	Mean Mass CO_2-C [Fumigated] (µg g^{-1} soil)	Flush Mean (Fumig-Control) (µg C g^{-1} soil)	Microbial biomass carbon (µg C g^{-1} soil)	Microbial biomass carbon (mg C g^{-1} soil)
1	0	P6a (Ctrl)	585.1		785.9				
2	0	P6b (Ctl)	565.2	575.14	218.9*	785.9	210.8	468.40	0.468
3	0	P18a (Ctl)	551.8		664.6				
4	0	P18b (Ctl)	534.2	542.98	723.8	694.18	151.20	336.00	0.336
5	10	P1a (Ctl)	624.4		764.1				
6	10	P1b (Ctl)	606.4	615.42	802.8	783.41	167.98	373.30	0.373
7	10	P12a (Ctl)	608.9		757.2				
8	10	P12b (Ctl)	580.7	594.79	767.7	762.44	167.65	372.56	0.373
9	20	P3a (Ctl)	607.5		737.8				
10	20	P3b (Ctl)	587.6	597.54	731.7	734.74	137.20	304.88	0.305
11	20	P9a (Ctl)	550.8		748.2				
12	20	P9b (Ctl)	581.3	566.04	745.0	746.60	180.56	401.25	0.401
13	30	P7a (Ctl)	635.2		675.0				
14	30	P7b (Ctl)	591.0	613.08	314.9*	675.00	61.92	137.59	0.138
15	30	P10a (Ctl)	613.2		730.0				
16	30	P10b (Ctl)	613.4	613.33	786.7	758.36	145.03	322.28	0.322

E.3: Determination of Microbial Biomass Carbon - Continued

ID Number	P fertiliser application rate (kg P ha^{-1} yr^{-1})	Paddock sample lab replicate	Mass CO_2-C [unfumigated] (µg g^{-1} soil)	Mean Mass CO_2-C [unfumigated] (µg g^{-1} soil)	Mass CO_2-C [Fumigated] (µg g^{-1} soil)	Mean Mass CO_2-C [Fumigated] (µg g^{-1} soil)	Flush Mean (Fumig-Control) (µg C g^{-1} soil)	Microbial biomass carbon (µg C g^{-1} soil)	Microbial biomass carbon (mg C g^{-1} soil)
17	50	P15a (Ctl)	613.7		815.9				
18	50	P15b (Ctl)	597.8	605.78	701.2	758.54	152.75	339.45	0.339
19	50	P19a (Ctl)	576.7		683.2				
20	50	P19b (Ctl)	584.1	580.41	657.4	670.32	89.91	199.81	0.200
21	100	P4a (Ctl)	500.9		657.4				
22	100	P4b (Ctl)	496.7	498.80	648.2	652.78	153.97	342.17	0.342
23	100	P13a (Ctl)	554.1		689.8				
24	100	P13b (Ctl)	545.7	549.91	661.3	675.52	125.61	279.13	0.279

Statistical Analysis

Analysis of Variance; DV: MBC					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	11891.6	1	11891.59	1.304644	0.266857
Residual	182296.3	20	9114.82		
Total	194187.9				

Regression Summary for Dependent Variable: Microbial Biomass Carbon						
F(1,20)=1.3046 p<.26686 Std.Error of estimate: 95.472						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(20)	p-level
Intercept			347.1064	30.18248	11.50026	0
P rate	-0.24746	0.216652	-0.6914	0.6053	-1.14221	0.266857

E.4: Microbial (C) Quotient

ID Number	P fertiliser Application Rate (kg P ha^{-1} yr^{-1})	Paddock sample code	Microbial Carbon (µg C g^{-1} soil)	Microbial C (mg C g^{-1} soil)	Total Carbon (%) (g C per 100 g soil)	Total C (g C g^{-1} soil)	Total C (mg C g^{-1} soil)	Microbial quotient C_{mic}/C_{org} (%)
1	10	P6	354.81	0.355	8.30	0.083	83.03	0.427
2	10	P18	336.00	0.336	9.44	0.094	94.44	0.356
3	10	P1	373.30	0.373	9.25	0.092	92.45	0.404
4	10	P12	372.56	0.373	8.16	0.082	81.56	0.457
5	20	P3	304.88	0.305	10.53	0.105	105.3	0.290
6	20	P9	401.25	0.401	7.79	0.078	77.91	0.515
7	30	P7	137.59	0.138	9.00	0.090	90.03	0.153
8	30	P10	322.28	0.322	9.14	0.091	91.42	0.353
9	50	P15	339.45	0.339	8.85	0.088	88.49	0.384
10	50	P19	199.81	0.200	7.38	0.074	73.82	0.271
11	100	P4	342.17	0.342	5.87	0.059	58.65	0.583
12	100	P13	279.13	0.279	7.69	0.077	76.88	0.363

Statistical Analysis

Analysis of Variance; DV: Microbial Quotient					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	0.005471	1	0.005471	0.401653	0.540461
Residual	0.136219	10	0.013622		
Total	0.141691				

Regression Summary for Dependent Variable: Microbial Quotient						
F(1,10)=.40165 p<.54046 Std.Error of estimate: .11671						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(10)	p-level
Intercept			0.354632	0.051754	6.852271	0.000044
P rate	0.196505	0.310062	0.000679	0.001071	0.633761	0.540461

E.5: Microbial C Specific Respiration Rate

ID Number	Rate (kg P ha^{-1} yr^{-1})	Mass CO_2-C (µg g^{-1} soil)	Rate [a/336] (µg CO_2-C h^{-1} g^{-1}soil)	µg g^{-1} soil	Resp. rate [b/c] (µgCO2-C h^{-1} $µg^{-1}$ biomass)	Resp. rate [b/c] (µgCO2-C h^{-1} mg^{-1} biomass)
1	0	575.14	1.71	354.81	0.00482	4.824
2	0	542.98	1.62	336.00	0.00481	4.809
3	10	615.42	1.83	373.30	0.00491	4.907
4	10	594.79	1.77	372.56	0.00475	4.751
5	20	597.54	1.78	304.88	0.00583	5.833
6	20	566.04	1.68	401.25	0.00420	4.199
7	30	613.08	1.82	137.59	0.01326	13.262
8	30	613.33	1.83	322.28	0.00566	5.664
9	50	605.78	1.80	339.45	0.00531	5.311
10	50	580.41	1.73	199.81	0.00865	8.645
11	100	498.80	1.48	342.17	0.00434	4.339
12	100	549.91	1.64	279.13	0.00586	5.863

Statistical Analysis

Analysis of Variance; DV: Specific respiration rate					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	0.1813	1	0.181302	0.025277	0.876845
Residual	71.72692	10	7.172692		
Total	71.90822				

Regression Summary for Dependent Variable: Specific respiration rate						
F(1,10)=.02528 p<.87684 Std.Error of estimate: 2.6782						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(10)	p-level
Intercept			5.903728	1.126256	5.241904	0.000378
P rate	0.050213	0.315829	0.00372	0.023399	0.158987	0.876845

APPENDIX F:

Mineral N and Microbial N

F.1.1: Determination of Total Mineral N in samples before incubation (T_0)

	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*
ID Number	**P application rate**	**Paddock sample lab replicate**	**Soil Weight**	**NH_4^+**	**NO_2+NO_3^-**	**mass of NH_4^+-N extracted [d* 100 ml of 2 M KCl]**	**mass of NO_3^--N extracted [e*100ml of 2 M KCl]**	**NH_4^+-N [f/25g soil]**	**NO_3^--N [g/25g soil]**	**∑[NH_4^+-N + NO_3^- -N]**
	(kg P ha^{-1} yr^{-1})		**(g)**	**(ppm N) (or µg g^{-1})**	**(ppm N) (or ug g^{-1})**	**(µg)**	**(µg)**	**(µg/g soil)**	**(µg/g soil)**	**(µg/g soil)**
1	10	P6a	25	1.00	4.03	100.30	402.77	4.01	16.11	20.12
2	10	P6b	25	0.65	4.00	64.70	400.44	2.59	16.02	18.61
3	10	P18a	25	1.03	6.65	103.30	664.51	4.13	26.58	30.71
4	10	P18b	25	0.89	6.99	88.75	698.90	3.55	27.96	31.51
5	10	P1a	25	0.79	2.38	78.77	238.35	3.15	9.53	12.68
6	10	P1b	25	0.57	2.48	56.87	248.04	2.27	9.92	12.20
7	10	P12a	25	0.69	2.66	69.01	266.36	2.76	10.65	13.41
8	10	P12b	25	0.30	2.78	30.07	277.79	1.20	11.11	12.31
9	20	P3a	25	0.14	3.97	13.58	396.80	0.54	15.87	16.42
10	20	P3b	25	0.84	3.51	84.28	350.81	3.37	14.03	17.40
11	20	P9a	25	0.64	3.33	63.63	333.17	2.55	13.33	15.87
12	20	P9b	25	0.69	3.83	68.85	382.70	2.75	15.31	18.06
13	30	P7a	25	1.38	1.24	138.48	124.35	5.54	4.97	10.51
14	30	P7b	25	1.38	1.35	137.62	134.70	5.50	5.39	10.89
15	30	P10a	25	1.49	5.11	149.15	511.11	5.97	20.44	26.41
16	30	P10b	25	1.01	4.97	100.86	497.36	4.03	19.89	23.93
17	50	P15a	25	0.62	2.68	61.52	268.21	2.46	10.73	13.19
18	50	P15b	25	0.66	3.50	66.34	350.42	2.65	14.02	16.67
19	50	P19a	25	2.31	2.46	231.00	246.48	9.24	9.86	19.10
20	50	P19b	25	2.48	2.54	248.43	254.31	9.94	10.17	20.11
21	100	P4a	25	1.08	3.59	108.41	358.74	4.34	14.35	18.69
22	100	P4b	25	1.02	5.02	101.98	502.19	4.08	20.09	24.17
23	100	P13a	25	3.17	3.27	316.89	326.73	12.68	13.07	25.74
24	100	P13b	25	3.31	3.49	330.97	348.53	13.24	13.94	27.18

F.1.2: Determination of Total Mineral N in samples after incubation (T_{14})

	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*
ID Number	**P application rate**	**Paddock sample lab replicate**	**Soil Wt**	**NH_4^+**	**NO_2+NO_3^-**	**mass of NH_4^+-N extracted**	**mass of NO_3^--N extracted**	**NH_4^+-N [f/25g soil]**	**NO_3^--N [g/25g soil]**	**$\sum$[NH_4^+-N + NO_3^--N]**
	(kg P ha^{-1} yr^{-1})		**(g)**	**(ppm N) (or µg g^{-1})**	**(ppm N) (or ug g^{-1})**	**[d* 100 ml of 2MKCl] (µg)**	**[e*100ml of 2MKCl] (µg)**	**(µg/g soil)**	**(µg/g soil)**	**(µg/g soil)**
1	10	P6a (Ctrl)	25	7.49	26.85	748.98	2685.05	29.96	107.40	137.36
2	10	P6b (Ctl)	25	3.67	12.65	366.56	1265.05	14.66	50.60	65.26
3	10	P6a (F)	25	46.79	3.41	4679.07	341.18	187.16	13.65	200.81
4	10	P6b (F)	25	51.28	3.01	5127.95	301.35	205.12	12.05	217.17
5	10	P18a (Ctl)	25	1.49	22.91	148.79	2291.02	5.95	91.64	97.59
6	10	P18b (Ctl)	25	2.05	13.10	205.08	1309.59	8.20	52.38	60.59
7	10	P18a (F)	25	25.32	2.42	2532.24	242.31	101.29	9.69	110.98
8	10	P18b (F)	25	34.76	6.37	3476.23	636.86	139.05	25.47	164.52
9	10	P1a (Ctl)	25	1.57	13.02	156.50	1302.10	6.26	52.08	58.34
10	10	P1b (Ctl)	25	1.70	18.66	170.39	1866.13	6.82	74.65	81.46
11	10	P1a (F)	25	48.77	1.86	4877.15	186.08	195.09	7.44	202.53
12	10	P1b (F)	25	47.73	1.92	4773.26	191.70	190.93	7.67	198.60
13	10	P12a (Ctl)	25	0.75	18.61	75.29	1860.73	3.01	74.43	77.44
14	10	P12b (Ctl)	25	0.73	22.89	72.76	2289.44	2.91	91.58	94.49
15	10	P12a (F)	25	51.91	3.30	5190.84	330.42	207.63	13.22	220.85
16	10	P12b (F)	25	65.07	3.76	6506.54	376.43	260.26	15.06	275.32
17	20	P3a (Ctl)	25	0.73	19.37	72.65	1936.97	2.91	77.48	80.38
18	20	P3b (Ctl)	25	3.15	24.10	315.37	2410.01	12.61	96.40	109.02

F.1.2: Determination of Total Mineral N in samples after incubation (T_{14}) - Continued

ID Number	*a* P application rate (kg P ha^{-1} yr^{-1})	*b* Paddock sample lab replicate	*c* Soil Wt (g)	*d* NH_4^+ (ppm N) (or µg g^{-1})	*e* NO_2+NO_3^- (ppm N) (or ug g^{-1})	*f* mass of NH_4^+-N extracted [d* 100 ml of 2MKCl] (µg)	*g* mass of NO_3^--N extracted [e*100ml of 2MKCl] (µg)	*h* NH_4^+-N [f/25g soil] (µg/g soil)	*i* NO_3^--N [g/25g soil] (µg/g soil)	*j* NH_4^+-N + NO_3^--N (µg/g soil)
19	20	P3a (F)	25	32.38	3.29	3237.70	328.94	129.51	13.16	142.67
20	20	P3b (F)	25	44.59	3.26	4459.43	325.77	178.38	13.03	191.41
21	20	P9a (Ctl)	25	0.64	15.94	63.66	1594.35	2.55	63.77	66.32
22	20	P9b (Ctl)	25	1.01	12.57	100.83	1257.48	4.03	50.30	54.33
23	20	P9a (F)	25	47.06	2.85	4706.18	284.82	188.25	11.39	199.64
24	20	P9b (F)	25	57.85	3.28	5784.96	328.06	231.40	13.12	244.52
25	30	P7a (Ctl)	25	1.09	14.15	108.78	1414.80	4.35	56.59	60.94
26	30	P7b (Ctl)	25	6.10	10.35	609.72	1034.72	24.39	41.39	65.78
27	30	P7a (F)	25	44.11	3.99	4411.08	398.74	176.44	15.95	192.39
28	30	P7b (F)	25	35.55	2.13	3555.12	213.22	142.20	8.53	150.73
29	30	P10a (Ctl)	25	1.57	18.49	157.25	1848.57	6.29	73.94	80.23
30	30	P10b (Ctl)	25	2.25	20.06	224.88	2006.16	9.00	80.25	89.24
31	30	P10a (F)	25	46.40	4.62	4640.10	462.06	185.60	18.48	204.09
32	30	P10b (F)	25	39.67	2.60	3966.81	259.89	158.67	10.40	169.07
33	50	P15a (Ctl)	25	1.89	12.61	189.35	1261.14	7.57	50.45	58.02
34	50	P15b (Ctl)	25	0.99	9.71	98.94	971.15	3.96	38.85	42.80
35	50	P15a (F)	25	40.72	3.46	4072.14	346.35	162.89	13.85	176.74
36	50	P15b (F)	25	37.16	3.70	3716.21	370.15	148.65	14.81	163.45

F.1.2: Determination of Total Mineral N in samples after incubation (T_{14}) - Continued

ID Number	*a* P application rate (kg P ha^{-1} yr^{-1})	*b* Paddock sample lab replicate	*c* Soil Wt (g)	*d* NH_4^+ (ppm N) (or µg g^{-1})	*e* NO_2+NO_3^- (ppm N) (or ug g^{-1})	*f* mass of NH_4^+-N extracted [d* 100 ml of 2MKCl] (µg)	*g* mass of NO_3^--N extracted [e*100ml of 2MKCl] (µg)	*h* NH_4^+-N [f/25g soil] (µg/g soil)	*i* NO_3^--N [g/25g soil] (µg/g soil)	*j* NH_4^+-N + NO_3^--N (µg/g soil)
37	50	P19a (Ctl)	25	1.34	8.58	133.75	857.57	5.35	34.30	39.65
38	50	P19b (Ctl)	25	1.15	9.15	115.40	915.46	4.62	36.62	41.23
39	50	P19a (F)	25	33.98	5.20	3398.44	520.45	135.94	20.82	156.76
40	50	P19b (F)	25	38.68	2.73	3867.75	272.95	154.71	10.92	165.63
41	100	P4a (Ctl)	25	1.34	25.84	133.52	2583.72	5.34	103.35	108.69
42	100	P4b (Ctl)	25	1.36	26.31	136.42	2630.86	5.46	105.23	110.69
43	100	P4a (F)	25	38.86	4.62	3886.02	462.31	155.44	18.49	173.93
44	100	P4b (F)	25	44.87	4.65	4486.67	464.76	179.47	18.59	198.06
45	100	P13a (Ctl)	25	1.38	14.59	137.77	1458.64	5.51	58.35	63.86
46	100	P13b (Ctl)	25	1.21	11.88	121.42	1188.28	4.86	47.53	52.39
47	100	P13a (F)	25	33.42	2.57	3341.66	257.27	133.67	10.29	143.96
48	100	P13b (F)	25	53.42	7.43	5342.49	742.56	213.70	29.70	243.40

F.2: Determination of Mineralised N from unfumigated samples

ID Number	P fertiliser application rate (kg P ha^{-1} yr^{-1})	Paddock sample lab replicates	$\sum[NH_4^+\text{-}N + NO_3^-\text{-}N]$ T = 14 (µg g^{-1} soil)	Mean $\sum[NH_4^+\text{-}N + NO_3^-\text{-}N]$ T = 14 (µg g^{-1} soil)	$\sum[NH_4^+\text{-}N + NO_3^-\text{-}N]$ T = 0 (µg g^{-1} soil)	Mean $\sum[NH_4^+\text{-}N + NO_3^-\text{-}N]$ T = 0 (µg g^{-1} soil)	Mean Mineralised N $[T14_{ctrl} - T0_{ctrl})$ (µg g^{-1} soil)
1	0	P6a (Ctrl)	137.36		20.12		
2	0	P6b (Ctl)	65.26	101.31	18.61	19.36	81.95
3	0	P18a (Ctl)	97.59		30.71		
4	0	P18b (Ctl)	60.59	79.09	31.51	31.11	47.98
5	10	P1a (Ctl)	58.34		12.68		
6	10	P1b (Ctl)	81.46	69.90	12.20	12.44	57.46
7	10	P12a (Ctl)	77.44		13.41		
8	10	P12b (Ctl)	94.49	85.96	12.31	12.86	73.10
9	20	P3a (Ctl)	80.38		16.42		
10	20	P3b (Ctl)	109.02	94.70	17.40	16.91	77.79
11	20	P9a (Ctl)	66.32		15.87		
12	20	P9b (Ctl)	54.33	60.33	18.06	16.97	43.36
13	30	P7a (Ctl)	60.94		10.51		
14	30	P7b (Ctl)	65.78	63.36	10.89	10.70	52.66
15	30	P10a (Ctl)	80.23		26.41		
16	30	P10b (Ctl)	89.24	84.74	23.93	25.17	59.57
17	50	P15a (Ctl)	58.02		13.19		
18	50	P15b (Ctl)	42.80	50.41	16.67	14.93	35.48
19	50	P19a (Ctl)	39.65		19.10		
20	50	P19b (Ctl)	41.23	40.44	20.11	19.60	20.84

F.2: Determination of Mineralised N from unfumigated samples - Continued

ID Number	P fertiliser application rate (kg P ha^{-1} yr^{-1})	Paddock sample lab replicates	$\sum[NH_4^+\text{-}N + NO_3^-\text{-}N]$ T = 14 (µg g^{-1} soil)	Mean $\sum[NH_4^+\text{-}N + NO_3^-\text{-}N]$ T = 14 (µg g^{-1} soil)	$\sum[NH_4^+\text{-}N + NO_3^-\text{-}N]$ T = 0 (µg g^{-1} soil)	Mean $\sum[NH_4^+\text{-}N + NO_3^-\text{-}N]$ T = 0 (µg g^{-1} soil)	Mean Mineralised N [$T14_{ctrl}$ - $T0_{ctrl}$) (µg g^{-1} soil)
21	100	P4a (Ctl)	108.69		18.69		
22	100	P4b (Ctl)	110.69	109.69	24.17	21.43	88.26
23	100	P13a (Ctl)	63.86		25.74		
24	100	P13b (Ctl)	52.39	58.12	27.18	26.46	31.66

Statistical Analysis

Analysis of Variance; DV: Mineralised N					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	183.402	1	183.4017	0.382272	0.550212
Residual	4797.675	10	479.7675		
Total	4981.077				

Regression Summary for Dependent Variable: Mineralised N						
F(1,10)=.38227 p<.55021 Std.Error of estimate: 21.904						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(10)	p-level
Intercept			59.98372	9.211101	6.512112	0.000068
P rate	-0.19189	0.310351	-0.11832	0.191373	-0.61828	0.550212

F.3: Determination of N flush and Microbial Biomass N (MBN)

ID Number	Paddock sample lab replicates	P fertiliser application rate (kg P ha^{-1} yr^{-1})	$\sum[NH_4^+$-N + NO_3^--N] T = 14 (Fumigated) (µg g^{-1} soil)	Mean T14 (µg g^{-1} soil)	$\sum[NH_4^+$-N + NO_3^--N] T = 14 (Control) (µg g^{-1} soil)	Mean T14 (Control) (µg g^{-1} soil)	N Flush (µg g^{-1} soil)	MBN* (µg g^{-1} soil)
0	P6a	0	200.81		137.36			
1	P6b	0	217.17	208.99	65.26	101.31	107.68	158.35
2	P18a	0	110.98		97.59			
3	P18b	0	164.52	137.75	60.59	79.09	58.66	86.27
4	P1a	10	202.53		58.34			
5	P1b	10	198.60	200.56	81.46	69.90	130.66	192.15
6	P12a	10	220.85		77.44			
7	P12b	10	275.32	248.08	94.49	85.96	162.12	238.41
8	P3a	20	142.67		80.38			
9	P3b	20	191.41	167.04	109.02	94.70	72.34	106.38
10	P9a	20	199.64		66.32			
11	P9b	20	244.52	222.08	54.33	60.33	161.75	237.87
12	P7a	30	192.39		60.94			
13	P7b	30	150.73	171.56	65.78	63.36	108.20	159.12
14	P10a	30	204.09		80.23			
15	P10b	30	169.07	186.58	89.24	84.74	101.84	149.76
16	P15a	50	176.74		58.02			
17	P15b	50	163.45	170.10	42.80	50.41	119.69	176.01

F.3: Determination of N flush and Microbial Biomass N (MBN) - Continued

ID Number	Paddock sample lab replicates	P fertiliser application rate (kg P ha^{-1} yr^{-1})	$\sum[NH_4^+\text{-}N + NO_3^-\text{-}N]$ T = 14 (Fumigated) (µg g^{-1} soil)	Mean T14 (µg g^{-1} soil)	$\sum[NH_4^+\text{-}N + NO_3^-\text{-}N]$ T = 14 (Control) (µg g^{-1} soil)	Mean T14 (Control) (µg g^{-1} soil)	Paddock N Flush ID (µg g^{-1} soil)	MBN* lab (µg g^{-1} soil)
18	P19a	50	156.76		39.65			
19	P19b	50	165.63	161.19	41.23	40.44	120.75	177.57
20	P4a	100	173.93		108.69			
21	P4b	100	198.06	186.00	110.69	109.69	76.30	112.21
22	P13a	100	143.96		63.86			
23	P13b	100	243.40	193.68	52.39	58.12	135.56	199.35

* MBN calculated using k = 0.68

Statistical Analysis

Analysis of Variance; DV: MBN					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	17.5	1	17.495	0.006835	0.935742
Residual	25595.87	10	2559.587		
Total	25613.36				

Regression Summary for Dependent Variable: MBN						
F(1,10)=.00684 p<.93574 Std.Error of estimate: 50.592						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(10)	p-level
Intercept			167.4007	21.27556	7.868215	0.000014
P Rate	-0.02614	0.31612	-0.0365	0.44203	-0.08268	0.935742

F.4: Determination of Microbial N quotient

MBN (µg g-1 soil)	MBN (mg g-1 soil)	Total N (g g-1 soil)	Tot N (mg N g-1 soil)	Microbial N Quotient (%)
158.35	0.158	0.0080	7.951	2.0
86.27	0.086	0.0084	8.413	1.0
192.15	0.192	0.0084	8.410	2.3
238.41	0.238	0.0074	7.391	3.2
106.38	0.106	0.0090	9.027	1.2
237.87	0.238	0.0074	7.368	3.2
159.12	0.159	0.0077	7.714	2.1
149.76	0.150	0.0084	8.359	1.8
176.01	0.176	0.0080	8.037	2.2
177.57	0.178	0.0066	6.648	2.7
112.21	0.112	0.0057	5.715	2.0
199.35	0.199	0.0070	7.017	2.8

Statistical Analysis

Analysis of Variance; DV: MBN Quotient					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	0.243421	1	0.243421	0.466834	0.509967
Residual	5.214293	10	0.521429		
Total	5.457715				

Regression Summary for Dependent Variable: MBN Quotient						
F(1,10)=.46683 p<.50997 Std.Error of estimate: .72210						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(10)	p-level
Intercept			2.053648	0.303664	6.762888	0.00005
P rate	0.21119	0.309095	0.004311	0.006309	0.683253	0.509967

F.5: Mineralised N rate per biomass unit

ID Number	Paddock code	P fertiliser application rate kg P $ha^{-1}yr^{-1}$	a Mean Mineralised N [T14ctrl - T0ctrl] (µg g^{-1} soil)	b Mean Mineralisation Rate [a/336 hr] (µg g^{-1} soil hr^{-1})	d Mean MBN (µg g^{-1} soil)	e Mineralised N rate per Biomass N unit [b/d] (µg N hr^{-1} µg^{-1} biomass)	f Mineralised N rate per Biomass N unit [e*1000] (µg N hr^{-1} g^{-1} biomass)
1	P6	0	81.95	0.24	158.35	0.0015	1540.22
2	P18	0	47.98	0.14	86.27	0.0017	1655.25
3	P1	10	57.46	0.17	192.15	0.0009	890.02
4	P12	10	73.10	0.22	238.41	0.0009	912.53
5	P3	20	77.79	0.23	106.38	0.0022	2176.38
6	P9	20	43.36	0.13	237.87	0.0005	542.49
7	P7	30	52.66	0.16	159.12	0.0010	984.90
8	P10	30	59.57	0.18	149.76	0.0012	1183.76
9	P15	50	35.48	0.11	176.01	0.0006	599.98
10	P19	50	20.84	0.06	177.57	0.0003	349.28
11	P4	100	88.26	0.26	112.21	0.0023	2341.00
12	P13	100	31.66	0.09	199.35	0.0005	472.67

Statistical Analysis

Analysis of Variance; DV: Min N Rate per MBN unit					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	9928	1	9927.7	0.020719	0.888406
Residual	4791547	10	479154.7		
Total	4801474				

Regression Summary for Dependent Variable: Min N Rate per MBN unit						
F(1,10)=.02072 p<.88841 Std.Error of estimate: 692.21						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(10)	p-level
Intercept			1167.842	291.0945	4.011899	0.00247
P Rate	-0.04547	0.315901	-0.871	6.0479	-0.14394	0.888406

APPENDIX G

C:N Ratio

Determination of C:N ratio

ID Number	P fertiliser application rate (kg P ha^{-1} yr^{-1})	Paddock sample code	Total C (mg C g^{-1} soil)	Tot N (mg N g^{-1} soil)	C:N
1	0	P6	83.03	7.951	10.4
2	0	P18	94.44	8.413	11.2
3	10	P1	92.45	8.410	11.0
4	10	P12	81.56	7.391	11.0
5	20	P3	105.3	9.027	11.7
6	20	P9	77.91	7.368	10.6
7	30	P7	90.03	7.714	11.7
8	30	P10	91.42	8.359	10.9
9	50	P15	88.49	8.037	11.0
10	50	P19	73.82	6.648	11.1
11	100	P4	58.65	5.715	10.3
12	100	P13	76.88	7.017	11.0

Statistical Analysis

Analysis of Variance; DV: C:N					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	0.153051	1	0.153051	0.830436	0.383588
Residual	1.84302	10	0.184302		
Total	1.996071				

Regression Summary for Dependent Variable: C:N						
F(1,10)=.83044 p<.38359 Std.Error of estimate: .42930						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(10)	p-level
Intercept			11.1093	0.180535	61.53549	0
P Rate	-0.27691	0.303862	-0.00342	0.003751	-0.91128	0.383588

APPENDIX H:

Soil pH data

Soil pH data

ID Number	Rate of P Fertiliser Applied (kg P ha^{-1} yr^{-1})	Paddock sample code	soil weight (g)	pH
1	0	P6	10.002	5.00
2	0	P18	10.004	4.91
3	10	P1	10.005	5.10
4	10	P12	10.005	4.97
5	20	P3	10.004	4.88
6	20	P9	10.005	5.06
7	30	P7	10.002	4.67
8	30	P10	10.002	5.00
9	50	P15	10.005	5.00
10	50	P19	10.001	5.03
11	100	P4	10.001	5.10
12	100	P13	10.004	5.02

Statistical Analysis

Analysis of Variance; DV: soil pH					
	Sums of Squares	df	Mean Squares	F	p-level
Regress.	0.011362	1	0.011362	0.810374	0.389174
Residual	0.140205	10	0.01402		
Total	0.151567				

Regression Summary for Dependent Variable: soil pH						
F(1,10)=.81037 p<.38917 Std.Error of estimate: .11841						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(10)	p-level
Intercept			4.945738	0.049794	99.32383	0
P rate	0.273793	0.304144	0.000931	0.001035	0.90021	0.389174

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