AN INVESTIGATION OF PASTURE LEGUME ROOT AND SHOOT PROPERTIES THAT INFLUENCE THEIR RATE OF DECOMPOSITION IN SOIL

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Abstract

Agriculture is the largest source of GHG emissions (47.8 %) in New Zealand. Emissions are increasing annually, driven by increasing relative productivity. Irrespective of the climate regime, grassland soils have historically sequestered large amounts of atmospheric C into SOM (soil C) raising interest in the potential for agricultural emissions to be mitigated through acceleration of soil C sequestration. Soil C sequestration is a direct result of the rate of deposition (excreta, plant litter, and roots) exceeding the rate of decomposition and can be raised by: 1) increasing the rate of input (manipulating the drivers of vegetation); or 2) increasing the longevity of C in the system.

This PhD study tests the hypothesis that C sequestration in pasture soils can be accelerated, by selecting pasture species that contribute slower decomposing litter to soil.

A series of laboratory incubation studies were conducted to measure the decomposition rate (CO_2 emissions) of plant shoots and roots with high (*Lotus pedunculatus)* and low (*Trifolium repens*) tannin contents. In addition the effects of residue management (fresh and freeze dried), application to soil (fresh - surface, freeze dried - surface, and freeze dried - mixed) and rate of application (2, 5, 10 mg C. g^{-1} soil) were evaluated. The effect of species, plant management, plant part, and rate of application on C emissions were all statistically significant ($P < 0.05$), with large variance in CO_2 emissions associated with all treatments.

Plant species and plant part influenced the amount of C retained in the soil, although not entirely as expected. *Lotus pedunculatus* shoot material retained significantly more C than *Trifolium repens* shoot material at all rates of application (2, 5, 10 mg C. g^{-1} soil); whereas *Trifolium repens* root material retained significantly more C than *Lotus pedunculatus* root material at all rates of application ($P < 0.05$). Notably plant roots and particularly *Trifolium repens* roots had slow decomposition rates compared to shoot materials.

Research showed that soil and plant residue preparation greatly influenced the total amount of C retained for both shoot and root treatments, with more C retained under conventional incubation techniques (dried - mixed application) than with fresh applications. This indicates that CO_2-C retention in a field situation may be overestimated if predicted using conventional laboratory incubation techniques. However from a research perspective it is infinitely easier to work with pre-dried incubation materials (timing, handling, chemical analysis) so it is highly likely that this style of incubation practice will continue to be the preferred method of research. Care must therefore be taken when extrapolating the results from such incubation studies.

A four compartment (2 soil C pools, persistent and labile; and 2 plant C residue pools, fast and slow) computer simulation model was developed and provided an excellent explanation of the CO_2 emissions from the incubation of fresh shoot and root material. The measurement of the metabolisable energy (ME) or lignin contents of plant shoot and root were successful in parameterising (allocating C to) the fast and slow plant residue pools. Plant tannin content was not able to explain CO_2 emission rates. The experimental and modelling studies provide evidence that grazed pasture rotations in mixed farming systems could be manipulated, by careful plant pasture species selection, to accelerate soil C sequestration. Litter and root metabolisable energy (ME) or lignin contents could be useful in species selection, but further research into other pasture species and pasture management techniques is required. Field studies should focus on the role of clover (*Trifolium repens)* roots in building pasture soil C content.

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List of Abbreviations

$BaCl_2$	barium chloride
BD	bulk density
C	carbon
^{12}C	carbon-12 (natural, stable isotope of carbon)
^{13}C	carbon-13 (natural, stable isotope of carbon)
^{14}C	carbon-14 (radioactive isotope of carbon)
Ca	calcium
CEC	cation exchange capacity
cf	meaning "compare / compared with"
CFC-13	chlorotrifluoromethane
CH_4	methane
cm	centimeter
C : N	carbon to nitrogen ratio
$^{14}CO_2$	carbon dioxide-14 (radioactive isotope of carbon)
CO_2	carbon dioxide
CO_2-C	carbon in the form of carbon dioxide
CT	condensed tannin
ctrl	control
d	day
D	disturbed
DM	dry matter
EGT	Egmont Silt Loam soil
FC	field capacity
FD	freeze dried
Fe	iron
g	gram
GHG	greenhouse gas
GWP	global warming potential
H_2O	hydrogen dioxide (water)

ha	hectare
HCA	hydroxycinnamic acids
HCl	hydrochloric acid
HSG	high sugar grasses
IPCC	Intergovernmental Panel on Climate Change
IRMS	isotope ratio mass spectrometry
K	potassium
KCl	potassium chloride
kg	kilogram
KH_2PO_4	potassium dihydrogen phosphate
KOH	potassium hydroxide
L	liter
LAI	leaf area index
LT	*Lotus pedunculatus* (lotus)
LTF	lotus fresh, surface treatment
LTM	lotus dried, mixed in treatment
LTS	lotus dried, surface treatment
m	meter
M	molar
me	milliequivalent
ME	metabolisable energy
Mg	magnesium
mg	milligram
MJ	mega joules
ml	milliliter
mm	millimeter
Mn	manganese
MO	microorganism
MSFAT	Nutrition Laboratory, Massey University School of Food and Advanced Technology
Mt CO_2-e	million tonnes of carbon dioxide equivalent
N	nitrogen

^{14}N	nitrogen-14 (stable, non-radioactive isotope of nitrogen)
^{14}N	nitrogen-14 (isotope of nitrogen, frequently used in NMR)
Na_2CO_3	sodium carbonate
$NaHCO_3$	sodium bicarbonate
NaOH	sodium hydroxide
NH_4^+	ammonium
nm	nanometer
NMR	nuclear magnetic resonance
NO_3^-	nitrate
O_2	oxygen
OC	organic carbon
OD	oven dried
OM	organic matter
P	phosphorus
ppb	parts per billion
ppm	parts per million
PWP	permanent wilting point
S	sulphur
SRO	short range order amorphous clay minerals
SO_4^{2-}	sulphate
SO_4-S	sulphate sulphur
SOM	soil organic matter
T_0	time zero
T_x	after “x” days of soil respiration / incubation, where x equals number of days
TK	Tokomaru Silt Loam soil
trt	treatment
U	undisturbed
µg	microgram
USEPA	United States Environmental Protection Agency
UV	ultra violet
vs	versus

W	watt
WC	*Trifolium repens* L. (white clover)
WCF	white clover fresh, surface treatment
WCM	white clover dried, mixed in treatment
WCS	white clover dried, surface treatment
WHC	water holding capacity
wt	weight
w : v	weight to volume
y	year
Zn	zinc
θ	volumetric water content
<	less than
>	greater than
%	percent / percentage
$^{-1}$	suffix meaning “per”, i.e. g^{-1} means “per gram”

Chapter 1

Introduction to the thesis

1.1 Background to the thesis

Soil organic carbon (SOC) has long been considered an indicator of soil quality and environmental sustainability, with the terrestrial soil C pool estimated at 2,500 Gt C (Dungait et al. 2012a). A rise in atmospheric CO_2 is recognised globally to be the leading cause of accelerated global warming; fossil fuel consumption is the leading contributor of atmospheric CO_2, with agricultural activities also a significant contributor (IPCC, 2013). The drastic effects of global warming (e.g. drought, sea level rise, and increased intensity of storms) have highlighted the necessity to mitigate where possible. Global ecosystems are significantly affected by any increase in atmospheric CO_2 concentration and, as the ocean has limited ability to reabsorb CO_2, the onus is on finding suitable means to sequester CO_2 into the terrestrial system.

Agriculture has a large potential to reduce CO_2 emissions and increase C sinks as, irrespective of the climate regime, grassland soils have historically sequestered large amounts of atmospheric C. There is widespread agreement that with careful management of this terrestrial pool there is a possibility to invoke a shift towards long term C storage; even small increases in the soil C pool can significantly reduce atmospheric CO_2 concentrations, therefore there is potential to alter the global C balance (Nave et al. 2018; Tate, 1992).

As an agricultural nation it is therefore of little surprise that New Zealand has focused on agriculture as a means to reduce our global C footprint (Ministry for the Environment, 2018). In New Zealand, the agricultural sector is the largest source of GHG emissions, contributing 48 % of national emissions (Ministry for the Environment, 2021; Pinares-Patino et al. 2009); a third of which are emitted by ruminant animals (CH_4); and a sixth from the breakdown of faecal, urinary and inorganic fertiliser N associated with pastoral agriculture (N_2O) (Ministry for the Environment, 2018; Ministry for the Environment, 2009).

Globally, New Zealand’s total GHG emissions are miniscule (0.2 % of estimated anthropogenic emissions) (Ministry of Agriculture and Forestry, 2006) and New Zealand product is considered sustainable on a global emissions scale, yet the total quantity of emissions from the agriculture sector is increasing (an increase of 17.1 % from 1990 to 2019); driven by increased animal productivity (Ministry for the Environment, 2021). While the total amount of GHG emitted from New Zealand pastoral agriculture has increased, the amount per unit of product has declined (Leslie et al. 2008). The agricultural sector therefore has a unique potential to mitigate their emissions by developing techniques to sequester CO_2 in the terrestrial pool, either by increasing SOM or through afforestation.

In order to manipulate the C cycle in pastoral systems it is important to understand the drivers that control the amount of C stored in those soils, and the fluxes in and out of the soil**.** There are six key flows of C in a pastoral system – photosynthesis, shoot respiration, translocation to the roots, shoot tissue and root turnover and exudates (plant litter), animal intake and excreta return (Parsons & Chapman, 2000). In grazed pasture systems, the two major fluxes influencing total C input are that of excreta (dung and urine, from herbage eaten) and plant litter and roots turnover (Denef & Six, 2006; Rasse et al. 2005). Pastoral management has historically perturbed the natural C cycle through a focus on increasing relative productivity (through the application of fertiliser and breeding of highly productive, digestible pasture species). Stored C longevity can be manipulated by altering soil processes, total microbial biomass and community structure, and / or the food source supplied to that microbial community (Treseder et al. 2011; Thornley, 1998). Data suggests that the type and diversity of plant species in grasslands has an important role for C transfer into the soil and is able to modify C storage under a given land use scheme (Steinbeiss et al. 2008; Tilman et al. 2006). Changing the management of pastoral species could therefore potentially increase C longevity in the soil.

It is well known that differences in the chemical composition of plant species play important roles in regulating soil processes through functions such as litter decomposability (i.e. litter longevity), mineralisation, microbial community composition and function (Crowther et al. 2019; Cornelissen, 1996; Hobbie, 1992) and interaction with soil minerals. However, understanding of the specific roles that each of these play is still limited, and in order to establish the particular effects that diverse plant species, plant functional groups, and traits have on soil functions comprehensive experiments are required that separate these factors so that the direct effects of each can be seen (Hector et al. 2000). The flows of C through grazed grassland are large and the C balance highlights the issue that the total amount of C sequestered every year is often very small. However, over time, the C sequestered has the potential to add up to a very large quantity, so it is very important to understand how these C dynamics can be influenced. As plants are the primary source of C to the system there may be potential to exploit changes in plant traits or management that can cause a shift in soil sequestered C. The amount of C sequestered in a soil can be raised by either: 1) Increasing the rate of input (by manipulating the drivers of vegetation); or 2) Increasing the longevity of C once in the system (by manipulating the drivers of loss – e.g. respiration (CO_2) and decomposition (Parsons et al. 2013; Parsons et al. 2009).

Our understanding about the influence of different plant species on C flows for legume-based hill pastures is scarce even though it represents more than 50 % of New Zealand’s terrestrial ecosystem. Grazed pastures are a complex agro ecosystem and, as pastoral plant species supply the input of C to this system, the role of plants requires further investigation. The intention of this research is to provide an increased understanding into the role of pasture plants, and in particular plant traits such as tannin content, on the turnover time of soil C.

This research aims to quantify short term C loss as a result of pasture residue decomposition, and attempts to provide a simple decomposition simulation model to predict C loss from pastoral soils.

1.2 Research hypotheses

This study investigated the following primary (i to iii) hypotheses:

(i) That litter and roots from high tannin plants have a slower rate of decay and leave greater amounts of C residues in soils.

(ii) That differences in tissue chemistry between shoot and root material will lead to systematic differences in litter decomposition rates; therefore the application of different plant biomass (shoot and root) to the soil will alter the rate and total amount of litter decomposed.

(iii) That the method of application of plant biomass to the soil will alter the rate and total amount of plant biomass decomposed, an important factor when trying to extrapolate laboratory studies to field dynamics.

The testing of the above primary hypotheses led to further experimentation to test the following secondary hypotheses:

(iv) That increasing the rate of plant residue-C applied to the soil will not result in an equivalent proportional increase in the rate of CO_2-C loss from the soil.

(v) That soil order (i.e. Pallic Soil vs Allophanic soil) differences (structure and hydrous oxide metal content) will influence both the rate and extent of OM decomposition.

(vi) That disturbing the root-soil matrix will result in an initially higher rate of CO_2 evolved compared to an undisturbed sample; however, with time a similar amount of CO_2 will be evolved from both samples.

(vii) That differences in the chemical composition of plant materials can be used to partition plant litter C into theoretical pools allowing the accumulated CO_2-C emitted during a short term laboratory incubation to be predicted by a simple soil C decomposition model.

1.3 Research objectives

This research set out to examine the role of pasture plant traits, species, and plant functional groups in carbon and nitrogen cycling with a view to understanding and mitigating greenhouse gas emissions from soil. A glasshouse trial and a series of laboratory incubations were conducted:

(i) To generate plant shoot and root material through glasshouse studies for decomposition studies, and chemically characterise the plant tissue to determine how specific plant traits differ.

(ii) To test if plant species chemistry can influence the rate of decomposition and the amount of residue C remaining after a period of decomposition.

(iii) To test if plant shoot and root tissue type and chemistry can influence the rate of decomposition and the amount of residue C remaining after a period of decomposition (not including contribution from root exudates).

(iv) To quantify the effect of three constrasting techniques of applying plant residues to soil incubation (fresh - surface application, freeze dried - surface application, freeze dried - mixed in application) on the rate of decomposition of plant residues and the amount of residue C remaining after a period of decomposition.

(v) To quantify the effect of three C application rates (2, 5, 10 mg C. g^{-1} OD soil) on the rate of decomposition and the amount of plant residue C remaining after a period of decomposition.

(vi) To characterise the effect of soils of contrasting mineralogical properties on plant root decomposition.

(vii) To characterise the effect of soil disturbance on plant root decomposition.

(viii) To develop a soil C decomposition model to predict short term C loss (CO_2-emissons) from soils incubated with litter and roots of pasture legumes; and to test, using regression analysis, whether differences in plant chemistry explain the variance observed in soil respiration rates.

1.4 Thesis structure

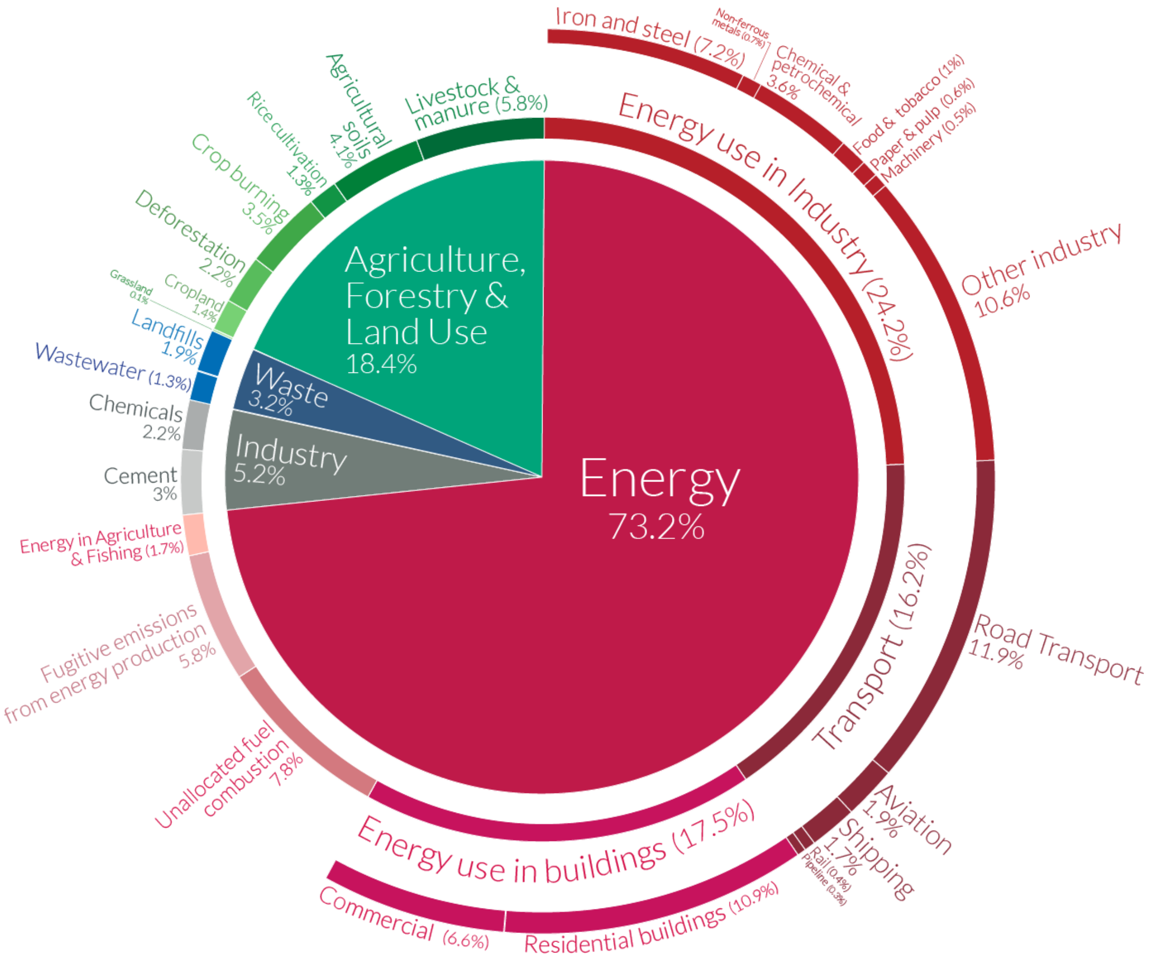
Chapter 1 Introduction	Significance of the PhD research.
Chapter 2 Review of literature	A summary of the current literature on CO_2 emissions, factors affecting C loss, and common decomposition measurement methods.
Chapter 3 Incubation study #1	A laboratory study that quantifies the loss of CO_2 from lotus and white clover shoot material applied to Tokomaru soil as fresh and freeze dried material.
Chapter 4 Incubation study #2	A laboratory study that simulates lotus and white clover root decomposition in disturbed and undisturbed conditions in both Egmont and Tokomaru soils.
Chapter 5 Incubation study #3	A laboratory study that simulates lotus and white clover shoot and root decomposition at 2, 5, 10 mg C g^{-1} OD soil in Tokomaru soils.
Chapter 6 Modelling decomposition	Development of a simple predictive decomposition simulation model.
Chapter 7 Research conclusions and implications	Conclusions and implications of experimental research.

Chapter 2

Review of literature

2.1 Greenhouse Gases

Global warming has been identified as one of the greatest challenges of our century; with impacts such as drought, sea level rise and more intense storm damage forecast across the world. The cause of global warming is the build up of GHG's in the Earth's atmosphere. GHG's absorb and re-emit infrared radiation in the atmosphere; trapping heat, warming the surface of the Earth, ultimately resulting in fluctuations in both wind and precipitation patterns (Kiehl & Trenberth, 1997). Accelerated global warming is considered to be caused by anthropogenic (human-related) activities that emit additional quantities of GHG's to the Earth's atmosphere (Figure 2.1), resulting in a global rise in temperature – 0.74 °C over the last 100 y with an average increase of 1.5 – 4.5 °C predicted by 2050 (Fernando et al. 2012; Carter et al. 2007; IPCC, 2007).



OurWorldinData.org – Research and data to make progress against the world's largest problems.
Source: Climate Watch, the World Resources Institute (2020). Licensed under CC-BY by the author Hannah Ritchie (2020).

Figure 2.1 Global GHG emissions by sector: global gas emissions in 2016 were 49.4 billion tonnes CO_2 equivalent (Ritchie, 2020).

In most developed nations, GHG emissions are dominated by CO_2 emissions from power generation, industrial processes and transport (Leslie et al. 2008). Fossil fuels now appear to be the main factor contributing to the rise in atmospheric CO_2 (roughly 75 % of the increase since pre-industrial times) (Carter et al. 2007); while agriculture accounts for about 50 % and 60 % of global anthropogenic emissions of CH_4 and N_2O respectively (Pinares-Patino et al. 2009; IPCC, 2007). In many cases emissions from agriculture have been decreasing, yet between 1750 and 2005 concentrations of CO_2 increased from 280 to 379 ppm, CH_4 from 715 to 1,732 ppb, and N_2O from 270 to 319 ppb (Snyder et al. 2009;

IPCC, 2007). In order to mitigate anthropogenic GHG emissions, an understanding of the source and magnitude of these emissions is required (Table 2.1).

Table 2.1 Sources of natural and anthropogenic GHG in the Earth's atmosphere.

Natural Greenhouse Gases	Anthropogenic Greenhouse Gases
Water (H_2O) Sources: Water vapour in the Earth's atmosphere	Synthetic gases i.e. Chlorofluorocarbons (CFC), sulphur hexafluoride (SF_8) Sources: Industrial processes and refrigeration
Carbon dioxide (CO_2) Sources: Decay of plant matter	Carbon dioxide (CO_2) Sources: Burning of fossil fuels, deforestation and landuse change
Methane (CH_4) Sources: Wetlands	Methane (CH_4) Sources: Landfills, agriculture (especially from the digestive systems of grazing animals (as enteric fermentation) as well as from wastewater management
Nitrous oxide (N_2O) Sources: Soils and ocean	Nitrous oxide (N_2O) Sources: Soils, fertilisers and wastewater management
Ozone (O_3) Sources: Produced and destroyed by chemical reactions in the atmosphere	Ozone (O_3) Sources: Photochemical reactions from industry, vehicle exhaust

Modified from the Fertilizer and Lime Research Centre (2012).

Of the total incoming light that reaches the Earth's atmosphere, approximately 50 % is absorbed by the Earth's surface; a further 20 % is absorbed by gases (UV light by stratospheric ozone, and infrared by CO_2 and H_2O); and the remaining 30 % is not absorbed but rather reflected into space by cloud, ice, snow and sand (Atmosphere, Climate & Environment Information Programme, 2005 as cited in Fertilizer and Lime Research Centre, 2012). GHG's differ in their warming influence on global climate due to their different radiative properties and lifetime in the atmosphere (Pinares-Patino et al. 2009); this has been termed the GWP. As CO_2, CH_4 and N_2O differ in their effectiveness in trapping heat and in their turnover rates in the atmosphere, calculation of their GWP depends on the timeframe considered (Table 2.2) (Snyder et al. 2009). The USEPA defines the GWP for a particular GHG as "the ratio of heat trapped by one unit mass of the GHG to that of one unit mass of CO_2 over a specified time period" (USEPA, 2006 as cited in Fertilizer and Lime Research Centre, 2012). The IPCC generally considers a time frame of 100 y to be an appropriate measure of the GWP of a molecule. For a 100 y timeframe, unit masses of CH_4 and N_2O are considered to have 25 and 298 times the GWP, respectively, as a unit of CO_2 (IPCC, 2007).

Table 2.2 Global Warming Potential for some greenhouse gases (W. m^{-2}. ppb^{-1}).

GHG	Life span (years)	Radiative efficiency (W. m^{-2}. ppb^{-1})	SAR (100-years)*	20-years	100-years	500-years
CO_2	Variable	1.4×10^{-5}	1	1	1	1
CH_4	12	3.7×10^{-4}	21	72	25	7.6
N_2O	114	3.03×10^{-3}	310	289	298	153
CFC-13	640	0.25	NA	10,800	14,400	16,400

* Note: The IPCC has published several reference values for GWPs for GHGs over time. Currently the GWP of CH_4 is thought to be 25, however many countries including New Zealand, still use the IPCC's Second Assessment Report (SAR 100-years) of 21.

Fertilizer and Lime Research Centre (2012)

2.2 Global reservoirs of C

The total global reservoir of C is vast - approximately 46,000 Gt (MacKay, 2009), yet only a small fraction is actively involved in the annual fluxes of the C cycle between the principle pools - atmosphere, terrestrial system (vegetation and soils), oceans and fossil fuels (Figure 2.2). The terrestrial soil C pool is estimated at 2,500 Gt C (1,550 Gt C as SOC and 950 Gt C as soil inorganic C (i.e. carbonates)) (Dungait et al. 2012a). Nearly all C captured during photosynthesis in the terrestrial system is released back to the atmosphere either through plant respiration (60 Gt C. y^{-1}) or microbial respiration / decomposition of plant residues (60 Gt C. y^{-1}) (Lal et al. 2007).

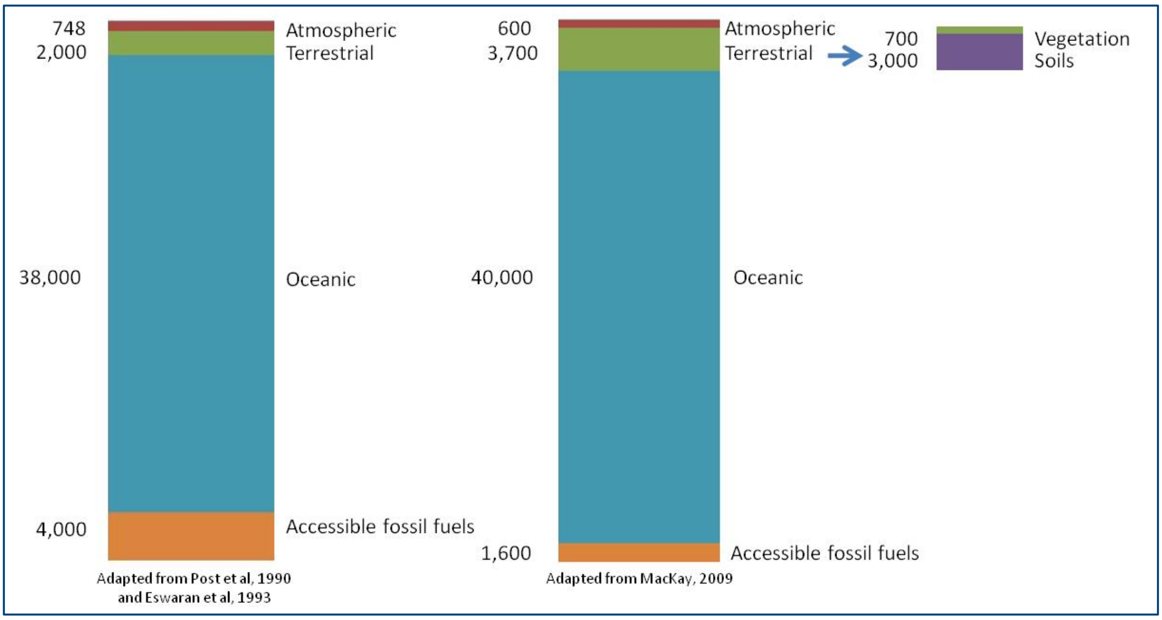


Figure 2.2 Principal reserves of C (Gt) comparing 1980 estimates (Eswaren et al. 1993; Post et al. 1990) to 2006 estimates (MacKay, 2009).

Global ecosystems are significantly affected by any increase in atmospheric CO_2 concentration and the data indicates that even small changes per unit area in the soil C pool could significantly impact atmospheric concentrations and therefore the global C balance (i.e. a 10 % reduction in global soil C would cause the atmospheric pool to increase by around 50 % (or 300 Gt C) (Tate, 1992). As part of the COP21 United Nations Framework Convention on Climate Change, held in Paris in 2015, it was agreed to limit global warming to < 2°C compared with pre-industrial levels. This accord was backed by a progressive international research agenda that aspires to increase soil C stocks by 0.4 % per year (Minasny et al. 2017). Whether or not this target is feasible, agriculture is considered to have a large potential to reduce CO_2 emissions and increase C sinks.

Although there are uncertainties as to how global warming will affect C sequestration and decomposition (and therefore the total soil C pool), the potential impact on C fluxes has been extensively reviewed (e.g. Parsons et al. 2011; Soussana & Lüscher, 2007) and modelled (e.g. Thornley & Cannell, 1997). Cole et al. (1997) estimated that it would be possible to increase the amount of C stored in the world's agricultural soils by 0.44 - 0.88

billion tonne annually over a 50 y period, recovering 50 - 65 % of the estimated historic loss of C from currently cultivated soils (Snyder et al. 2009); indicating that careful management could significantly reduce atmospheric CO_2 concentrations.

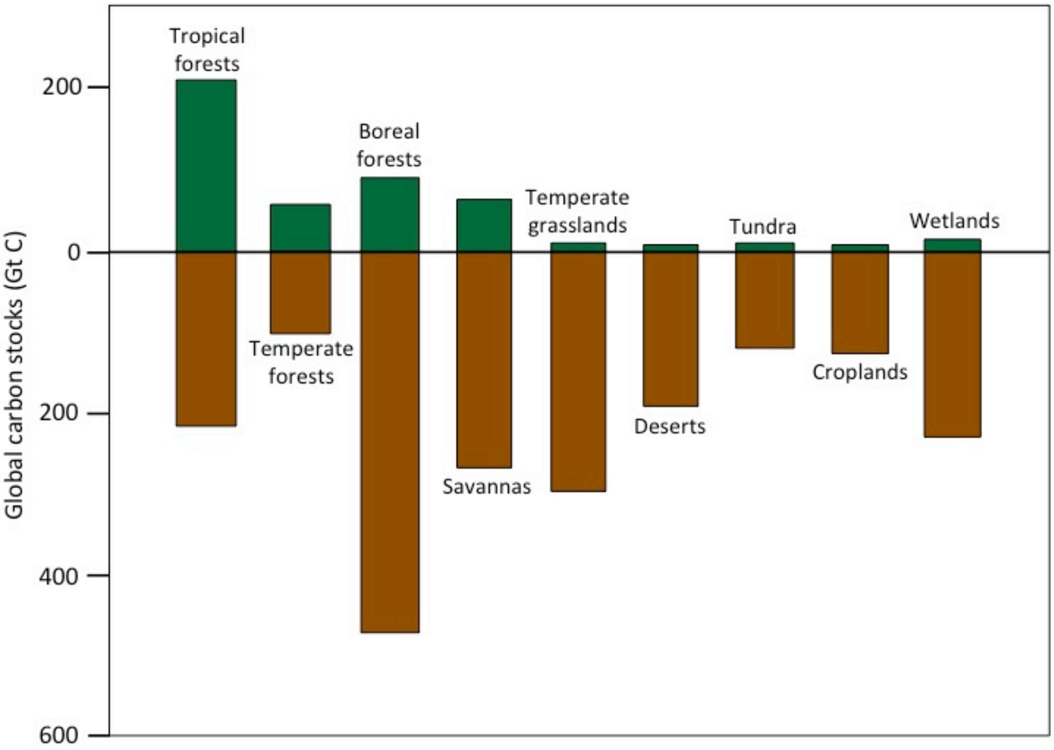


Figure 2.3 **Estimates of terrestrial C stocks for the main biomes above (green bar) and below (brown bar) ground (redrawn from: Allard, 2003 based on data from IPCC, 2001).**

New Zealand’s agriculture sector contributes 48 % to national emissions (only 0.17 % of estimated global anthropogenic emissions), which directly accounts for approximately 10 % of gross domestic product and over 65 % of New Zealand’s total exports by value. However agricultural emissions are on the rise (17.1 % from 1990 to 2019, which equates to 5.8 million tonnes of CO_2 equivalent), driven by production demand and consequent intensification of pastoral farming systems (Ministry for the Environment, 2021).

New Zealand product is considered sustainable on a global emissions scale as, even with intensification, the amount of GHG per unit of product has declined (Leslie et al. 2008). There exists an unique potential to mitigate agricultural emissions through sequestration of CO_2 in the terrestrial pool (trees and soil organic matter, SOM). Techniques need to be developed to increase SOM under grazed pasture systems, New Zealand’s dominant land use.

2.3 Grazed pasture systems

There are six key flows of C in a grazed pasture system – photosynthesis, shoot respiration, translocation to the roots, shoot tissue and root turnover and exudates (plant litter) (Reid et al. 2015), animal intake and excreta return (Parsons & Chapman, 2000). The amount of C in the soil system will be relative to the rate of input (plant residues, excreta, etc.) versus the rate of output (decomposition, leaching) (Chapin et al. 2012 as cited in Beidler et al. 2020) (Figure 2.5). While the flows of C through grazed grassland are large (Figure 2.4), the total amount of C sequestered every year is often very small, so it is very important to understand how these C dynamics can be influenced over time in order to ultimately understand and measure the flows of C in to and out of soils.

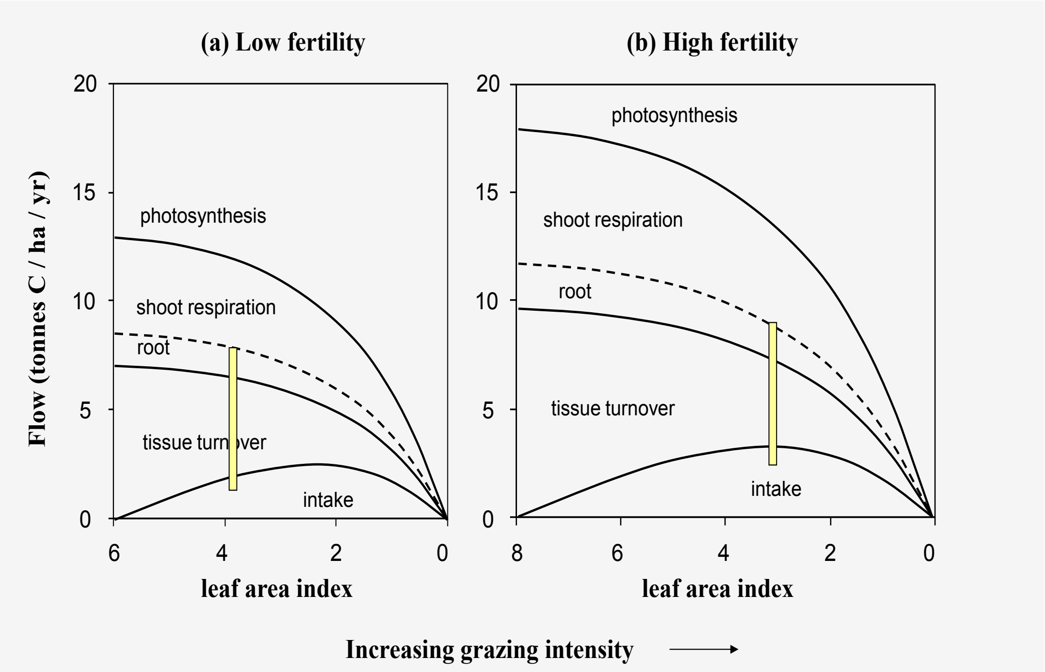


Figure 2.4 **The major flows of C (t C. ha^{-1}. y^{-1}) through plants and animals in grazed pastures in relation to the intensity of grazing, as defined by the LAI sustained (*cf* annual mean vegetation cover) under (a) low fertility and (b) high fertility conditions. Vertical bars show examples of the potential total flow of C to soil (source: Parsons & Chapman, 2000; Thornley, 1998).**

In essence, factors that increase plant growth (e.g. fertility – nutrient availability, warmth, brightness and moisture; higher post- grazing residuals; and greater biomass) will increase flows of C into soil.

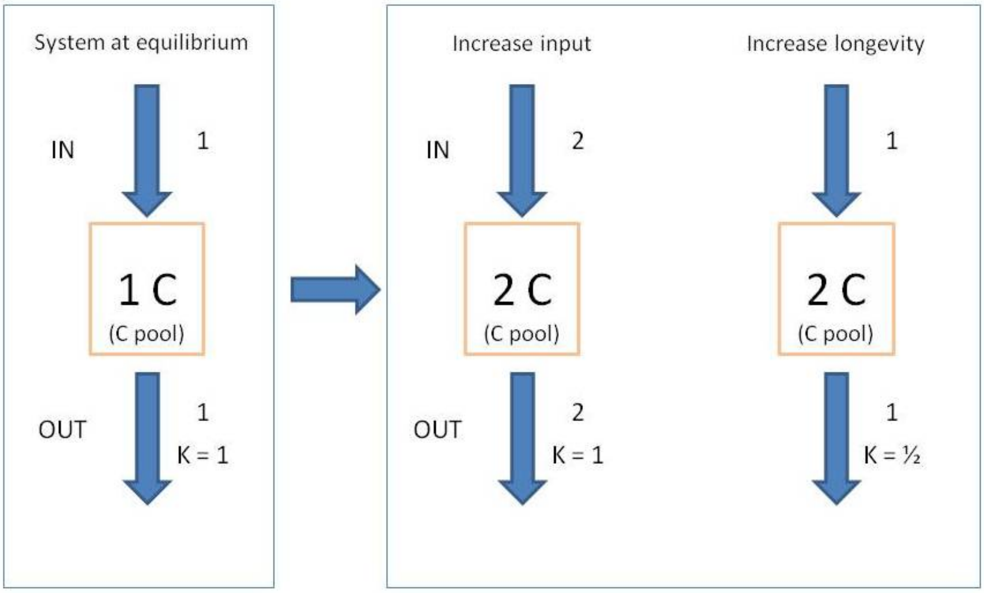


Figure 2.5 Two possible ways to increase C in the soil pool (K = rate constant, longevity = 1 / K) (adapted from: Parsons et al. 2009; Parsons et al. 2013).

Increasing the input of C to the soil pool will increase the outputs from that pool whilst simultaneously increasing the amount of C remaining in the pool, provided that the rate constant (K) (or proportion of the state) remains the same (Figure 2.5), as the flow out of the soil C pool equals the rate constant multiplied by the state (flow out = K.S). Alternatively, the C pool can be increased by increasing the longevity of C in the soil (reducing the rate constant). Therefore to successfully measure whether there has been an actual increase in the soil C pool both inputs and losses must be measured.

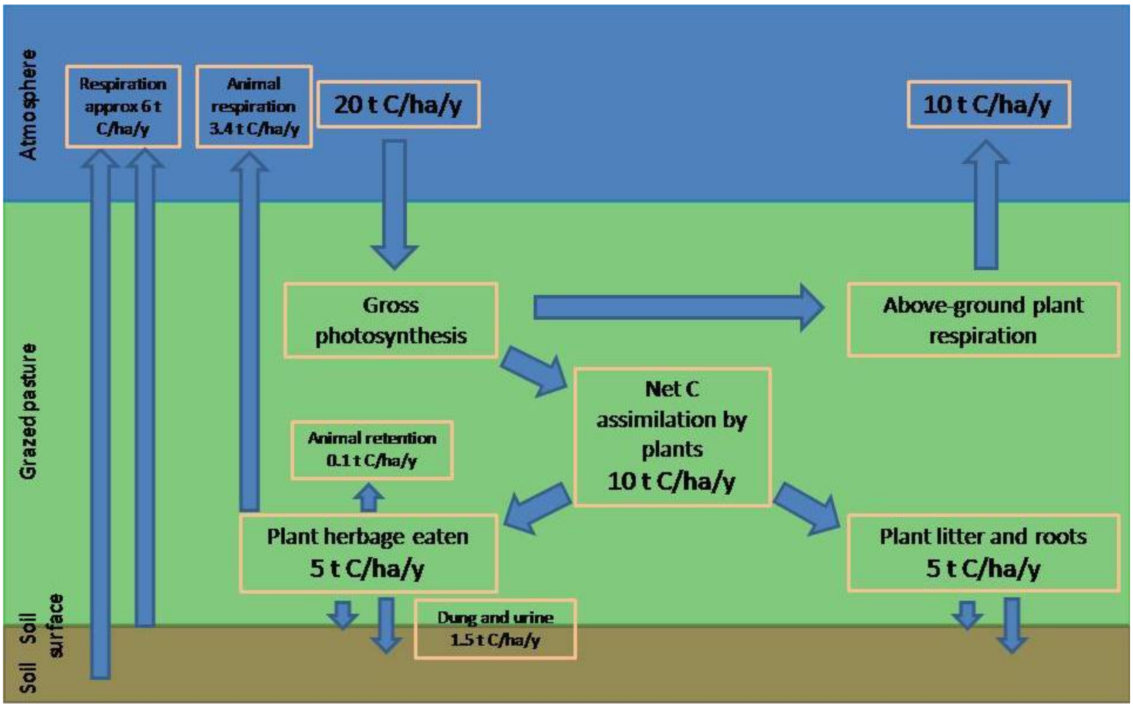


Figure 2.6 Annual flows of C in a grazed pasture. This is a general example of a C balance for dry stock, grazed pasture systems (net balance is almost zero) (adapted from: Parsons & Chapman, 2000).

As gross photosynthesis by plants is the sole input of C to the pastoral system (apart from fixation by soil chemoautotrophs) there may be potential to exploit changes in plant traits or management that can cause a shift in soil sequestered C (Figure 2.6). The amount of C sequestered in a soil can be raised by either: 1) Increasing the rate of input (by manipulating the drivers of vegetation); or 2) Increasing the longevity of C once in the system (by manipulating the drivers of loss – e.g. respiration (CO_2) and decomposition - Figure 2.5).

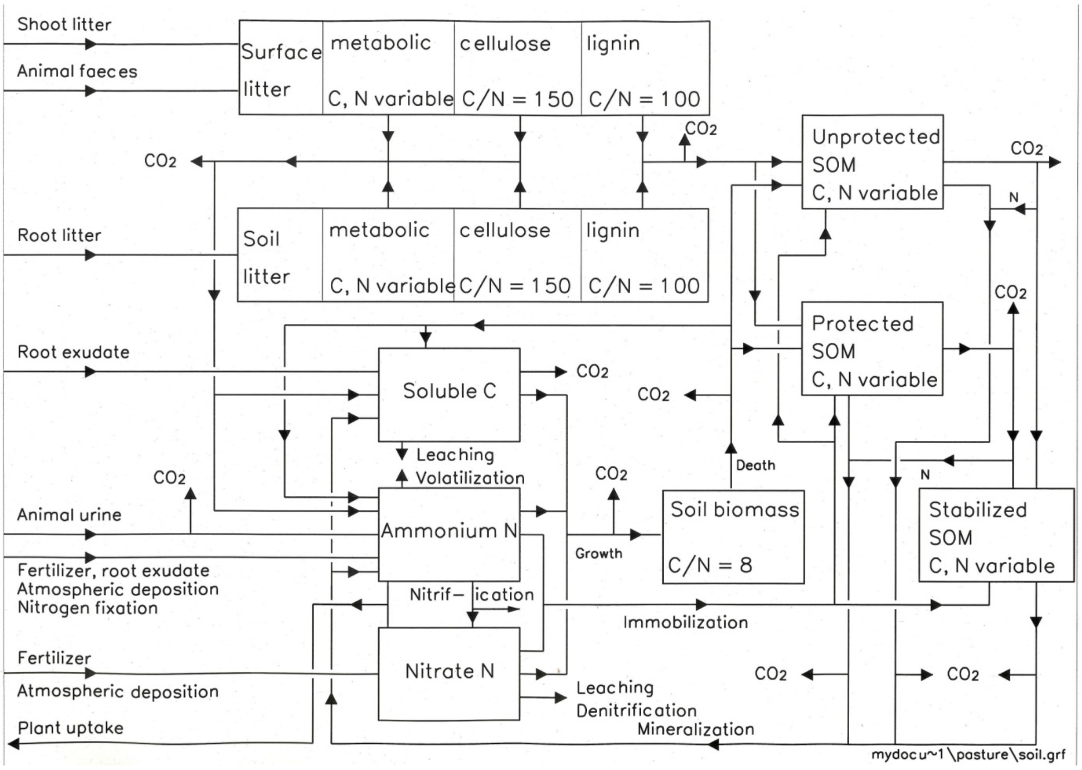


Figure 2.7 **Soil and litter submodel - an example of the fate and transformation of C and N in pastures (source: Thornley, 1998).**

In order to manipulate the C cycle in grazed pasture systems it is important to understand the drivers that control the amount of C stored in those soils, and the fluxes in and out of the soil (e.g. photosynthesis and LAI, fertiliser, stocking rate / grazing intensity, and water supply) (Figure 2.7). There are many factors that affect plant potential for photosynthesis (LAI, water availability, temperature, etc). These have been reviewed extensively elsewhere (e.g. Campbell et al. 2007; Jones & Rawson, 1979; Gifford, 1974). Plants may respire 30 – 80 % of photosynthetically assimilated C (Loveys et al. 2002). Animals in the system reduce total C inputs to the soil (only 30 % of total herbage eaten is returned as dung and urine), as well as reduce the longevity of C in the system (fast cycling) (Figure 2.8). Carbon taken up by animals is released in respiration (approximately 70 %) rather than left in roots / shoots where it would have remained for a longer time in the biosphere (Parsons et al. 2011; Parsons et al. 2009). After grazing, any remaining C can have considerable longevity: leaves (weeks), live roots (months), leaf and root litter (days to months, with a small proportion decades to centuries) (Parsons et al. 2011).

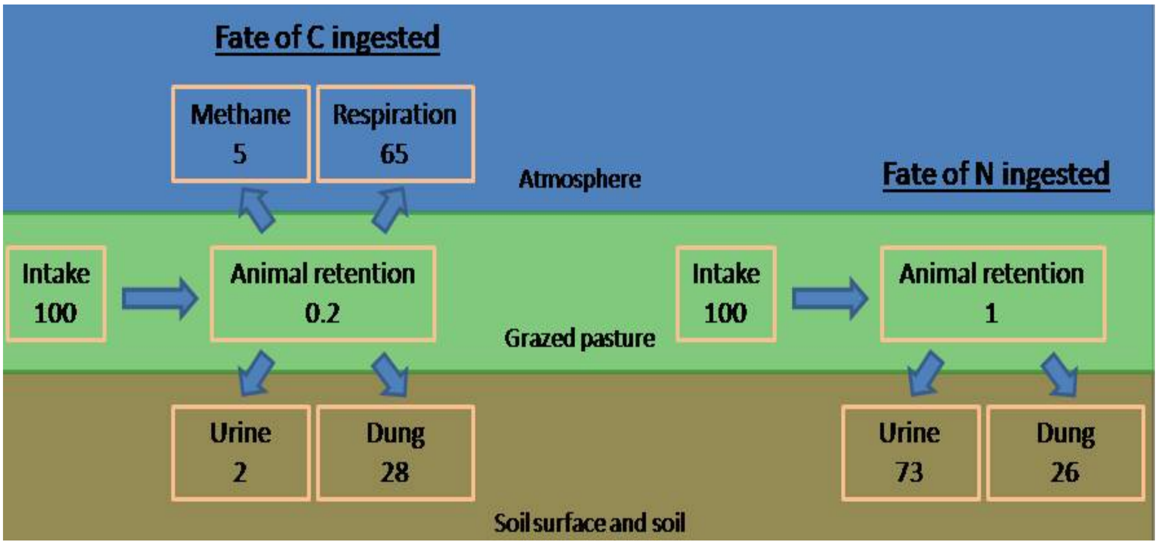


Figure 2.8 Fate of plant herbage eaten under a dry stock pasture system. Grazing animals 'uncouple' the C and N cycles (shown as % of intake) (adapted from: Parsons et al. 2011).

It is well known that differences in the chemical composition of plant species are important in regulating soil processes through functions such as litter decomposability (i.e. litter longevity), mineralisation, microbial community composition and function (Freschet et al. 2012a, 2012b; Cornelissen, 1996; Hobbie, 1992), and interaction with soil minerals. At the time of writing, research data suggests that the type and diversity of plant species in grasslands also has an important role for C transfer into the soil; and is able to modify C storage under a given land use scheme (Steinbeiss et al. 2008; Tilman et al. 2006).

The expression of plant traits can differ between plant species and plant functional groups (i.e. grasses and legumes), and the manipulation of these traits has been shown to influence both the drivers and longevity of C. Previous short-term (up to 4 months) and long-term studies (3 - 5 y) have shown that the decomposition rate of plant residues is negatively correlated with plant traits including C:N ratio, lignin (Berg et al. 1993; Van

Vuuren et al. 1993; Taylor et al. 1989) and lignin:N ratio (Cotrufo et al. 1994; Melillo et al. 1982); and positively correlated with the percentage of sugars, free amino acids and soluble materials (Prescott & Preston, 1994; Taylor et al. 1989). Carbon to nutrient ratios (specifically C:N ratio) (Cotrufo et al. 1994; Melillo et al. 1982); the amounts of phenolics (e.g. tannins) (Kuiters, 1990; Nicolai, 1988); lignin / fibre composition; high sugars; and high suberin, cutin and the presence of endophytes (which will not be considered in this review) may all impact on decomposition. However, understanding of the specific roles that each of these play is still limited. To establish the particular effects that diverse plant species, plant functional groups and traits have on soil functions, comprehensive experiments are required which separate these factors so that the direct effects of each can be seen (Hector et al. 2000).

There is a large variation in the length of time for, and rate at which, C may accumulate in soil. There is evidence that the longevity of stored C, and the amount of soil C stored, may be increased when vegetation and soil management practices are modified, and inadvertently total microbial biomass and community structure are also modified (Balesdent et al. 2000; Paustian et al. 2000; Post & Kwon, 2000; Post et al. 1990). For example, there is evidence that different plant species (e.g. *Lolium perenne, Trifolium repens, Lotus pedunculatus*) can affect the fate of soil C in the short term (Bardgett, 2011). Historically there has been much debate over the relative proportion of C contributed to the soil pool from shoot and root litter / exudates (plant parts), with recent evidence strongly in favour of root inputs (Denef & Six, 2006; Rasse et al. 2005). However, it is commonly agreed that the amount of C present in any particular soil is dictated by the quality and quantity of plant litter deposited. It is generally accepted that shoots, roots and root exudates all have an impact on the amount of C sequestered; and pasture C balances suggest that both surface applied and root OM may contribute substantially to soil OM (Parsons et al. 2011). Although the importance of each source varies both spatially and temporally, it has been proposed that root material has a longer residence time (slower decomposition rate; longer longevity) than shoot litter, and therefore is more likely to be stabilised on soil aggregates due to the spatial location of root litter input (Denef & Six, 2006).

Animal excretal returns contribute 33 % of the total annual C return to a pastoral system, whereas plant litter and roots contribute 77 % (Figure 2.5). Given plant production is the major input of fixed C to the soil pool, plant traits could affect:

(i) The rate and total amount of decomposition of uneaten plant litter and roots;

(ii) The partitioning of C between ingestion and excretion by grazing ruminants (i.e. animal retention and respiration);

(iii) The quantity and quality of excretal returns, and the subsequent rate and total amount of decomposition.

Understanding about the influence of different plant species on C flows for legume-based hill pastures is scarce even though it represents more than 50 % of New Zealand's terrestrial ecosystem. Grazed pastures are a complex agro ecosystem and, as pastoral plant species supply the input of C to this system, the role of plants requires further investigation. Changing the management of key pastoral species could potentially increase C longevity in the soil; there needs to be more research to increase understanding of the role of key pasture plant species, plant traits, and plant parts within the C cycle; in particular the factors that could be manipulated to lead to an increase in soil C. The agricultural industry has a unique potential to mitigate GHG emissions by developing techniques to sequester CO_2 in the terrestrial pool through increasing SOM.

2.4 Soil origin and microbial community

There is a wealth of literature available that discusses the stabilisation of C in soil through the chemical reaction of organic material with soil minerals (Shen et al. 2018; Kleber et al. 2015; Kögel-Knabner et al. 2008). Particular focus is the reactive surfaces of clays and physical aggregation (micro or macro), which protect OM from decomposition, as modelled in Figure 2.7 (Heckman et al. 2018). The chemical nature of organic material may influence the type of microorganism (MO) that is the first decomposer, the nature of the hydrolysis reaction, and the first decomposition products. Subtle differences in the chemical nature of litter and decomposition products alter their adsorption on soil clay surfaces (Huang et al. 2002; Six et al. 2002; Six et al. 2000).

Regardless of how C is stabilized, the effect of plant traits will always start at the extracellular enzyme level (degradation through the depolymerisation and hydrolysis of plant material) (Dungait et al. 2012a and citations therein); thereby determining the decomposition and subsequent C stability of a substrate. The nature of the substrate and its subsequent accessibility will affect decomposer diversity (e.g. bacterial to fungal ratio; MO diversity) and the resulting decomposition of residue (Liang & Balser, 2010; Buurman et al. 2007). The modern concept proposed by Dungait et al. (2012a) is one of physical protection. This concept suggests that the decomposition products of compounds within litter may stabilize the remaining litter, or alternatively compounds within the litter (e.g. polysaccharides) may contribute to the formation of soil aggregates which occlude litter thereby protecting residues from decomposition. Litter occluded within aggregates may be protected as it is inaccessible to MO and their catalytic enzymes, or alternatively because microbial activity is limited by environmental constraints (e.g. rate of oxygen diffusion to the site).

McNally et al. (2017) recently sampled a wide range of New Zealand soils to examine commonly measured characteristics that might indicate the soil C sequestration potential under two major land uses, grazed pasture and continuous cropping. Shen et al. (2018)

took this one step further and investigated the different organic C fractions stored in these soils, as a step towards understanding whether different land uses have a common influence on the OM quality and quantity of soils. Their results showed that the soil organic C fraction is driven by the abundance of short-range order, amorphous clay minerals (SRO), C input (associated with root density), chemical changes in soil caused by fertiliser amendments, and other anthropogenic activities (i.e. soil cultivation). They also found that soils with abundant SRO were richer in microbial-derived versus plant-derived OM, and concluded that more detailed studies using paired sites (with similar pedogenesis but differing in land use / management) should be undertaken.

2.5 Plant characteristics and the fate of fixed C

2.5.1 Plant species

As there is increasing evidence that different plant species (e.g. *Lolium perenne*, *Agrostis capillaris, Trifolium repens*), plant functional groups (e.g. legumes, grasses) and traits within any one species (e.g. high tannins, fibre composition) can alter soil microbial community composition and function (Parsons et al. 2009; Chung et al. 2007), it is timely to consider how the physical and biochemical characteristics of these components leads to differences in the amount, fate and longevity of C in soil (Bardgett, 2011). There is strong evidence suggesting that the potential decomposition rate of leaf litter depends largely on the physico-chemical properties (e. g. lignin (short term), cellulose, hemicellulose, and tannin) of the leaves of the species considered (e.g. Couteaux et al. 1995; Gillon et al. 1994; Aber et al. 1990; Broadfoot & Pierre, 1939). Plant litter and exudates act as a primary resource for decomposer organisms and as a source of nutrients for subsequent plant growth (McNaughton et al. 1989). These initial decomposition products may themselves turn over, along with the plant-derived molecules, and these products make up an important part of SOM (Liang and Balser, 2010; Buurman et al. 2007). The quality of the initial litter is therefore a powerful determinant of the biomass of soil organisms and their microbial metabolism, which in turn regulates key processes such as decomposition and nutrient flow (Findlay et al. 1996), and ultimately the longevity of C in the soil (Ayres et al. 2006; Bardgett et al. 1998).

2.5.2 Plant part: shoot versus root

The majority of decomposition studies have been performed with above-ground plant material with only a few studies considering roots (i.e. Gorissen & Cotrufo, 2000). However, Magid et al. (1996) have shown that decomposition rates may differ between leaf and root material; with studies by Gorissen (1996) and Cotrufo & Gorissen (1997) indicating that roots appeared to have lower decomposition rates than shoots. Franck et al. (1997) found that, in general, roots decomposed more slowly than shoot litter; and a slower decomposition rate for the roots of *Festuca vivipara* were also reported for litter bag experiments (Robinson et al. 1997), which was attributed to the lower initial quality of the root tissue (a higher C:N ratio and a higher lignin content) compared to the shoot tissue. Although Gorissen & Cotrufo (2000) observed an interaction between tissue type and grass species, the average decomposition of root material was 34 % lower than the decomposition of leaf material. These studies support the observation that root tissue decomposes more slowly than leaf tissue, leading to a longer residence time and total amount of root C in soil (Denef & Six, 2006).

2.5.3 Plant tissue chemistry

Bardgett et al. (1998) proposed that three aspects of litter quality may influence the growth and activity of soil organisms: (i) C to nutrient ratios of the litter (especially C:N) (also considered by Cornelissen, 1996; Cotrufo et al. 1994; Melillo et al. 1982)); (ii) the proportion of high molecular-weight carbohydrates contained in the litter which are of a recalcitrant nature (e.g. cellulose, hemicellulose and lignin) (also considered by Lavelle et al. 1995; Berg et al. 1993; Van Vuuren et al. 1993; Taylor et al. 1989); and (iii) the total amount and type of phenolics present (e.g. tannins) (also considered by Kuiters, 1990; Nicolai, 1988). Although several studies agree with the findings by Bardgett et al. (1998), these parameters are considered of lesser importance than accessibility of the substrate to decomposers; with physical protection (soil aggregation) increasing the longevity of organic residues in soil (Dungait et al. 2012a). Regardless, litter quality may vary greatly within functional or taxonomic groups (Cornelissen, 1996); therefore a change of plants within a system effectively manipulates the fundamental biology of a system, and therefore the longevity and fate of C in that system.

The decomposition of plant material determines the rate at which nutrients (from decaying plant litter) are immobilized or released into soil (Fanin et al. 2016; Cleveland et al. 2014; Tracy & Sanderson, 2007). Factors which influence microbial communities also alter the rate of decay: soil temperature, O_2 diffusion, moisture, pH and reactive properties, available minerals, and the C:N ratio of the plant residue are the key environmental influences. Likewise, the rate of substrate decomposition (CO_2 evolution) is dependent upon its chemical composition (Table 2.3), the age of the plant, the size of the substrate, and the surrounding physical and chemical conditions (Alexander, 1961).

Table 2.3 Initial review of the chemical composition (g. kg^{-1} DM) and OM digestibility of the leaves of subtropical and temperate grasses, herbs and legumes.

Species	Total N	Soluble sugar (a)	Pectin (a')	Neutral detergent fibre (NDF)	Acid detergent fibre (ADF)	Cellulose (b)	Hemicellulose (b')	Lignin	Ratio (a+a')/(b+b')	Organic matter digestibility (OMD)	Source
Subtropical grasses											
Kikuyu	27.4	53	5	581	249	234	332	15	0.10	0.671	Jackson et al, 1996
Paspalum	25.0	77	8	615	264	242	351	22	0.14	0.636	Jackson et al, 1996
Smooth witchgrass	15.9	91	4	558	271	249	287	22	0.18	0.675	Jackson et al, 1996
Crowfoot	13.0	69	10	640	303	290	337	13	0.13	0.612	Jackson et al, 1996
Summer grass	25.0	60	6	483	253	234	230	19	0.14	0.738	Jackson et al, 1996
Temperate forages											
Perennial ryegrass[a]	36.8	117	6	384	196	184	188	12	0.33	0.839	Jackson et al, 1996
Perennial ryegrass[b]	45.2	74	10	406	194	184	212	10	0.43	0.850	Jackson et al, 1996
Perennial ryegrass[c]	-	126	-	589	330	-	-	-	-	-	Miller et al, 2001
High sugar ryegrass[c]	-	165	-	544	300	-	-	-	-	-	Miller et al, 2001
Perennial ryegrass[d]	-	189	-	488	-	-	-	-	-	-	Miller et al, 2001
High sugar ryegrass[d]	-	351	-	380	-	-	-	-	-	-	Miller et al, 2001
Yorkshire fog	34.4	72	4	468	210	194	258	16	0.36	0.658	Jackson et al, 1996
Chicory	19.7	111	98	168	124	104	44	20	1.69	0.897	Jackson et al, 1996
Plantain	17.5	170	60	231	166	115	65	51	1.39	0.859	Jackson et al, 1996
Red clover	46.9	95	39	181	127	115	54	12	1.06	0.855	Jackson et al, 1996

[a] Dissected from the same pastures as the subtropical grasses.

[b] Grown in the same area as the other temperate forages.

[c] Fed to dairy cattle in production experiment

[d] Collected for degradation studies

The effects of N addition on decomposition vary with litter chemistry. Plant litter which has a low C:N ratio (high N) is easily decomposable by soil MO (Berg, 2000; Hobbie, 1992; Melillo et al. 1982), as microbial growth and enzyme production will not be limited by N availability (Aber et al. 1990). This may account for the difference in decomposition rates between different plant species and plant functional groups (Cornelissen, 1996; Hobbie, 1992). It has been suggested that the addition of a high amount of N (low C:N ratio) may support decomposition by cellulose decomposers (e.g. bacteria) over lignocelluloses decomposers (e.g. fungi) in both forest soils and under intensive grassland systems, due to the suppression of fungal lignolytic enzyme synthesis (Berg, 2000), with the opposite true for less intensive, low N grassland systems (Bardgett et al. 1999b). Alternatively, some studies have reported that N compounds (amino acids, ammonium and nitrate) may be incorporated into compounds associated with phenolic and lignin molecules to create molecules which are resistant to microbial decomposition (Talbot et al. 2012). Regardless, changes in the availability of C compared to N have the potential to shift the balance of soil microbial population and function (Parsons et al. 2011; Bardgett et al. 1999a; Bardgett et al. 1999b; Bardgett et al. 1996).

2.6 Specific plant traits – a consideration of both rumen and soil decomposition studies

A review of the literature for pasture plants highlights that there have been many studies done on the effect of plant traits on their rumen digestibility and fewer studies on the decomposition of both plant residues and roots in soils. To assist with understanding how plant traits may affect the pasture C cycle, both the effect of plant traits on rumen function and on decomposition in soil have been reviewed. Each section will consider the rumen as a model for decomposition as affected by plant traits (decomposability, microbial protein, change in micro flora), the impact of rumen decomposition on the quantity and quality of excretal C compounds entering the soil decomposition pathway, and any evidence for a direct effect of plant traits on the decomposition of both roots and plant litter.

Under normal, high intensity pasture systems there is a large amount of available protein N in the diet, relative to the amount of C supply. This imbalance results in large losses of N from the rumen (as ammonia) most of which is subsequently excreted in the urine (Ellis et al. 2011). This high input of N to the soil may lead to very rapid decomposition of existing litter, as microbial growth and enzyme production will no longer be limited by N availability (Aber et al. 1990). Likewise it may also lead to a shift in microbial community diversity (particularly in urine patches) towards bacterial decomposition over fungal decomposition.

It is reasonable to expect that fungi would be the initial colonisers of any litter return to the soil surface (due to microbial access), but once cell lysis has occurred that there would be an increase in bacterial activity (Ruess & Ferris, 2004). This bacterial activity may coincide with a period of rapid decomposition, with an initial lag phase in decomposition while fungal colonisation occurs. Fungal colonisation itself may lead to more recalcitrant microbial products (e.g. chitin and glomalin) (Wright & Upadhyaya, 1998) or microbial by-products that are more likely to be protected in soils (Buurman et al. 2007; Gonalez-Perez et al. 2007). Ultimately this increases soil C longevity.

2.6.1 Fibre composition

Fibre (e.g. cell wall polysaccharides, lignin and hydroxycinnamic acids) is the structural component of plants (Table 2.3), and is an important source of energy for grazing animals. The digestibility of fibre in the rumen determines the availability of both energy and nutrients for the animal (Baumont et al. 2000; Faville et al. 2010), ultimately determining animal performance (Allen, 1996). It is recognised that grazed pasture systems in New Zealand currently fail to provide sufficient nutrition for optimal ruminant production (Faville et al. 2010), partly due to an inaccessibility of fibre for microbial degradation in the rumen (Kristensen et al. 2005). For this reason, research for at least the last 100 y has focused on increasing the efficiency with which the rumen MO degrade fibre (Krause et al. 2003).

Rumen fermentation is unique because it relies on MO that produce fibrolytic enzymes within the host's anaerobic gut (Krause et al. 2003). Forage fibre consists of a significant fraction of fiber that poses resistance to degradation by rumen MO (up to 33 % of grasses and 50 % of legumes total fiber fraction). This resistant fraction is closely related to the content of lignin within the forage (ranges from 5 to 25 % of the plant cell wall (Allen & Mertens, 1988; Smith et al. 1972). It is also understood that the lignin fraction physically protects cell wall polysaccharides, preventing microbial degradation (Allen & Mertens, 1988).

Microbial constraint on fibre digestion is the critical factor limiting the productivity of ruminants (Allen & Mertens, 1988). Anaerobic fungi inhabit the rumen and actively degrade plant cell walls through the production of enzymes including cellulases, hemicellulases, and xylanases (Akin & Borneman, 1990). Rumen fungi extensively colonize lignin rich forages and have an active role in the degradation of fibre (Bauchop, 1979, 1981). This ability to degrade lignified tissues, to partially degrade more resistant tissues, and to penetrate the cell wall cuticle indicates that rumen fungi are more adapt at cell wall degradation than rumen bacteria (Akin & Borneman, 1990). This may be why fungi are more prevalent in the rumen under high fibre diets (Bauchop, 1979).

Lignin and hydroxycinnamic acids (HCAs) are key fibre components (Faville et al. 2010). Lignin binds polysaccharides, physically protecting against microbial enzymes and limiting cell wall degradation (Jung & Deetz, 1993). HCAs further obstruct cell wall degradation (Grabber et al. 2004) by the formation of cross-links between polysaccharide chains and lignin in the cell wall (Hatfield et al. 1999). To date, plant breeding programmes in New Zealand have focused on the production of palatable, easily digestible, grasses (e.g. HSG). The selection of forage plants with naturally low concentrations of cell wall HCAs for breeding (Faville et al. 2010; Casler & Diaby, 2008; Casler et al. 2008; Casler & Jung, 1999) have high potential to improve forage degradability in grasses, however this may have a negative effect on soil C sequestration.

2.6.2 Lignin

The composition and structure of lignin varies considerably within cell walls, plant tissues and plant parts, and among different plants; despite only being comprised of three building blocks - guaiacyl, syringyl, and p-hydroxyphenyl units (the latter only a minor component of lignin). This variation in composition and structure may greatly influence cell wall degradability, as well as aspects like total concentration and the presence of cross-linking to other cell wall components (Grabber et al. 2004).

Lignins are one of the only components in cell walls, which appear to be resistant to bacterial and fungal degradation in the rumen (Grabber et al. 2004). It may also restrict the enzymatic degradability of structural polysaccharides by hydrolytic enzymes (Grabber et al. 2009; Grabber, 2005), impairing lignocellulosic conversion into feed utilization by livestock (Grabber et al. 2009). Forage digestibility has been reported to have negative, positive, and no correlation to the concentration of lignin and / or to the ratio of guaiacyl and syringyl units in lignin (Grabber, 2005 and citations therein; Guo et al. 2001 and citations therein; Baucher et al. 1999). In forage crops, an increase in the amount of guaiacyl lignin has been correlated with a decrease in cell wall degradability, which would be in keeping with its condensed, highly cross-linked nature (Guo et al. 2001 and citations therein). However, other reports have suggested that the proportions of guaiacyl, syringyl, and p-hydroxyphenyl units in lignin are probably not a direct factor controlling total digestibility (Grabber, 2005; Guo et al. 2001); although a reduction in the deposition of any of these units would improve litter digestibility in the rumen if total lignin concentration was also reduced (Grabber, 2005; Guo et al. 2001 and citations therein).

As plants mature the lignin composition of forages increases (e.g. in the stems of alfalfa and other legumes), and there will be a shift towards a higher lignin syringyl / guaiacyl ratio (Guo et al. 2001 and citations therein; Buxton & Russell, 1988). Concurrently there will be a decrease in the enzymatic degradability of leaf cell walls and plant stems due to lignin accumulation, and the progressive lignification of both primary and secondary vascular and sclerenchyma cell walls (Grabber, 2005; Guo et al. 2001 and citations therein). Although guaiacyl rich lignin protects primary cell wall polysaccharides more efficiently than syringyl rich lignin, its highly branched nature means it may not penetrate the secondary wall to the same extent as syringyl rich lignin, thereby having less impact on secondary wall degradation (Guo et al. 2001). The relationship between lignin and digestibility is discussed in more detail in Guo et al. (2001).

As cell wall development is so complex, it may be difficult to identify the underlying mechanisms controlling cell wall degradability (Grabber, 2005). Other variables that may limit microbial and / or enzyme accessibility, affecting total digestibility (or perhaps even yield no net change in digestibility (Grabber, 2005)) may include a variation in the concentration of protein, non-structural carbohydrates, minerals, fibre components, and other phenolic compounds; or just come down to cell wall structural differences (Guo et al. 2001 and citations therein). The interactions between lignin and the cell wall are complex, but regardless the implication derived from rumen studies is that the presence of lignin will decrease total digestibility of litter, which will have flow on effects in excrement to the soil and subsequent microbial activity. Likewise lignin is also likely to reduce degradability in the soil through its direct influence on soil MO and their enzymes.

Lignin (the second largest source of terrestrial C) has been shown to physically protect cellulose (the largest C source) and hemicellulose from enzymatic hydrolysis (Kirk & Farrell, 1987), making it an essential part of the earth’s C cycle. Litter decay rates are often correlated with the initial lignin:N or lignin:cellulose content of litter, suggesting that interactions between lignin and more labile compounds are important controls over litter decomposition and therefore C longevity in soils (Talbot et al. 2012; Zhang et al. 2008; Trofymow et al. 2002; Moore et al. 1999; Aerts, 1997; Cortez et al. 1996; Aber et al. 1990; Melillo et al. 1982)

Lignin comprises approximately 20 % of plant litter input to the soil (Thevenot et al. 2010; Kirk & Farrell, 1987); and may influence interactions with labile litter components (e.g. polysaccharides and N in the cell wall), if they are physically or chemically protected from microbial attack and subsequent decomposition (Talbot et al. 2012). Rates of decomposition are only weakly correlated with total lignin content (Jung & Casler, 1991) which suggests that other mechanisms may control lignin, polysaccharide, and protein loss from decomposing plant tissue. However, the mechanisms behind the interaction of lignin with decomposition processes are poorly understood.

Three mechanisms have been proposed to explain the influence of lignin on short term decomposition processes: (i) resistance to degradation by extracellular enzymes (Kirk & Farrell, 1987); (ii) physical protection of cellulose and hemicellulose (Boerjan et al. 2003; Jung & Deetz, 1993) and; (iii) chemical protection of hemicellulose and protein in the cell wall (Talbot et al. 2012; Grabber et al. 2004). In higher plants, lignin not only provides strength and rigidity to plant structures by the formation of cross-links (Faville et al. 2010; Kirk & Farrell, 1987), it also provides protection against the biodegradation of carbohydrates (i.e. enzymatic hydrolysis) and tolerance to environmental stresses (Thevenot et al. 2010).

There is a large amount of contradiction within literature with regards to lignin decomposition rates in soils (Thevenot et al. 2010). A common perception is that lignin is slowly degradable in soils (Marschner et al. 2008), making it relatively recalcitrant and therefore limiting soil C cycling (St. John et al. 2011; Adair et al. 2008; Zhang et al. 2008). However, many studies have found no evidence of selective preservation of lignin in soils (Amelung et al. 2008; Nierop & Filley, 2007; Dignac et al. 2005; Gleixner et al. 2002; Rumpel et al. 2002; Gleixner et al. 1999). Chain configurations identified in lignin macromolecules (Chen & Sarkanen, 2010) and distinctive rates of decomposition for these monomers (Bahri et al. 2008; Dungait et al. 2008; Bahri et al. 2006) suggest there are mechanisms behind rapid SOM turnover (Dungait et al. 2012b). Therefore, low residual lignin contents in both tropical SOM (Vancampenhout et al. 2009) and at Rothamsted (Van Bergen et al. 1997) may be attributed to fast decomposition rates.

Furthermore, with the exception of recognizable fragments of plant material, unmodified lignin is rarely found in surface soil indicating that it may turn over more rapidly in soil than in total SOM (Dignac & Rumpel, 2006; Van Bergen et al. 1997). Using ^{13}C stable isotope analysis of lignin monomers extracted from soils, Rasse et al. (2006) and Dungait et al. (2010) determined that most lignin decomposes within one year; Dignac et al. (2005) and Heim & Schmidt (2007) determined that the remainder lignin decomposes within decades; while a more recent review by Thevenot et al. (2010) concluded that most lignin is likely to be decomposed within 5 y.

It is well established that basidiomycetes are key organisms in the decomposition of lignin, in particular white rot fungi which completely mineralize it to CO_2 and H_2O under aerobic conditions (Robertson et al. 2008). However, the role of other soil organisms in partial lignin breakdown and modification has also been recognized under both aerobic and anaerobic conditions (Chen et al. 1985), i.e. actinobacteria which target associated polysaccharides and are therefore responsible for up to 15 % of lignin degradation in soil (Dignac et al. 2005).

As there is a clear morphological distinction between grasses (monocotyledons) and legumes (dicotyledons) in pasture systems, it is important to investigate the difference in amount of lignin and HCAs present in each, as this will determine the availability of cellulose / hemicellulose for decomposition and therefore the longevity of C in soil. Grasses (e.g. perennial ryegrass) have a specifically high level of cross-linking HCAs (mainly ferulic acid) which bind lignin to cellulose and hemicelluloses (Pigden & Heaney, 1969). In contrast, dicotyledons (e.g. white clover) have a much lower percentage of HCAs and hence fewer cross-links, which may lead to higher rates of decomposition. Several recent studies using litterbags have found the rate of total C loss in grass litter is related to HCAs linking polysaccharides to lignins in the cell wall (Talbot et al. 2012; Hatfield et al. 1999). Therefore the amount of HCAs present in plant tissue could determine the relationship between lignin and decomposition by altering the magnitude of physical and chemical protection of labile litter components.

2.6.3 Plant polyphenols

Compounds rich in polyphenols (i.e. tannins and lignin) were considered waste products until the mid-1950's; classified as 'secondary' metabolites due to their apparent lack of purpose in primary metabolism. In 1959, Fraenkel proposed that plants produce polyphenols as a chemical defence against other organisms; a theory which has gained widespread acceptance (Northup et al. 1998). Polyphenols may comprise up to 60 % of plant dry matter (Cates & Rhodes, 1977); therefore such a large 'investment' of photosynthate must be of benefit to the producer. For example, the presence of concentrated polyphenols in vegetation subjected to highly leached, infertile soil conditions has been interpreted as a defence mechanism as limited soil nutrient availability creates a greater 'cost' for replacing foliage lost to herbivory (McKey et al. 1978; Janzen, 1974).

In the past there has been considerable research into the role that polyphenols play in both rumen and soil decomposition studies – with the focus mainly on both lignins and tannins. It is evident from these studies that polyphenols can slow decomposition by binding to proteins and can be toxic to rumen microbes. However many of these processes are reversible as they are pH dependent, although it is unlikely you would get such a drastic change in pH in the well buffered rumen.

It has been suggested that heavily defended plant species produce litter that is more recalcitrant for decomposers, than litter produced by less heavily defended species, as foliar defensive traits (such as total phenolics, lignin and condensed tannins) reduce decomposer performance (Kurokawa & Nakashizuka, 2008). Although some recent ecological studies have shown no significant correlation between foliar concentrations of polyphenols and degree of herbivory (Hartley & Lawton, 1987; Faeth, 1985; Laine & Henttonen, 1985; Coley, 1983); this is likely because not all phenolics are water soluble, and of those that are, not all precipitate proteins in a similar way to tannins (Reed, 1995). Studies of leaf palatability to invertebrates and leaf digestibility in rumen extracts have been performed alongside litter decomposition bioassays for the same plant species; and have shown a positive relationship between palatability or digestibility and decomposability (da Silva et al. 2010; Cameron & LaPoint, 1978). Other studies have reported beneficial effects of polyphenols on herbivores, such as protection from predators and disease (Hunter & Schultz, 1993; Taper & Case, 1987); although polyphenol-rich vegetation is still generally considered to decrease pasture, decomposer and herbivore productivity (Kuiters, 1990), and contribute to negative feedbacks which further diminish soil fertility (Chapin III et al. 1993; Muller et al. 1987). These studies confirm that herbivory has the ecological or evolutionary potential to adapt to plants with high tannin or lignin content and alter such processes as C and nutrient cycling.

Ruminants rely on the production of microbial protein within the rumen for their N requirements (Brooker et al. 1995). Microbial protein is generated from the degradation of dietary protein by MO within the rumen, which increases microbial biomass and therefore the generation of microbial protein. The supply of microbial protein is therefore dependent on the amount and type of forage intake. Many forage plants produce compounds that are toxic and that reduce productivity in grazing ruminants (e.g. oxalic acid, fluoroacetate and polyphenolic compounds) (Brooker et al. 1995). Polyphenolic compounds can complex with dietary and / or bacterial protein, to form an insoluble protein-tannin precipitate. This precipitate has an inhibitory effect on microbial enzymes involved in protein and fibre degradation, making it poorly digestible in the rumen and lower digestive tract (Kumar & Singh, 1984). Alternatively, polyphenolic compounds may pose benefits due to the increased use of endogenous N in the rumen, improved efficiency of protein utilization, and increased secretion of salivary glycoproteins (Reed, 1995). Other cell wall components (e.g. cellulose or lignocellulose) may restrict bacterial access to plant protein, thereby also inhibiting microbial growth and the synthesis of microbial protein.

Polyphenols can strongly influence plant-litter-soil interactions, including: regulating nutrient cycling and OM dynamics, ameliorating chemical and physical infertility factors, and altering soil properties. Tannins, rich in polyphenols, can form recalcitrant complexes with many substrates, including cellulose, starch and proteins; and may also form complexes with enzymes (both internal enzymes of decomposer invertebrates, and extracellular enzymes of microbes) leading to reduced microbial activity (Horner et al. 1988). Lignin, also rich in polyphenols, has long been considered resistant to enzymatic degradation, although the perception of lignin as a long-term recalcitrant compound is now under question (Dungait et al. 2012a).

A high rate of tissue turnover is generally associated with plant species that have an intrinsically high growth rate and nutrient content (especially N), and which produce both easily-decomposable litter and exudates. These plants tend to have a high percentage of monomeric secondary metabolites in their tissue (Horner et al. 1988); and are commonly limited by C fixation rates rather than by nutrients, for example rapidly growing perennials and plants living in nutrient-rich habitats (Parsons et al. 2009; De Deyn et al. 2008; Personeni et al. 2005; Personeni & Loiseau, 2004). Carbon sequestration under these species is dependent upon the quantity of litter produced outweighing the rapid decomposition of this low C:N material. Pine trees are also well known for the polyphenol content of their needles, which is known to vary with season (Nerg et al. 1994), but their needles have a comparatively high C:N ratio.

In contrast, slow growing plants tend to have relatively resistant plant tissues and produce nutrient poor, recalcitrant litter. The tissue of these plants (e.g. many woody perennial species) tends to have a high percentage of polymeric secondary metabolites (Horner et al. 1988); and the plants themselves are commonly limited by nutrients, rather than C (Parsons et al. 2009; De Deyn et al. 2008; Personeni et al. 2005; Personeni & Loiseau, 2004). These plants may result in increased C sequestration due to the presence of polyphenols, including tannins, which slow the rate of initial decomposition. Decomposition may be slowed further due to secondary decomposition products in the form of microbial metabolites (exudation and excretion, or cell death and decay (Hofman & Dusek, 2003)) which enter the SOM pool and which may themselves be more stable (Dungait et al. 2012b).

Polyphenol rich compounds have been shown to change the structure of the microbial community in soils. These changes have been shown to be compound-specific, with each of the tested compounds selecting for a distinct population (Schmidt et al. 2013). As the response of MO appears to be compound-specific, previous soil characteristics (e.g. previous levels of N fertilisation, type of plant cover) may dictate the responses of soil MO to the addition of polyphenol rich substrates. For instance, if the addition of polyphenols could be used to change the ratio of bacteria to fungi in the soil, the increase in fungi would result in an increase in fungal protein, which has been shown to play a major role in the stabilisation of soil aggregates (although early research is recognised here which suggests that bacteria may be more important than fungi in the initial stabilization of aggregates, Harris et al. 1964). In particular, the production of glomalin, a glycoprotein produced by the hyphae of arbuscular mycorrhizal fungi, has been shown to be highly correlated with aggregate stability in a variety of soils from different geographic locations, and with a range of different textural characteristics (Wright & Upadhyaya, 1998). The authors suggested that the response seen may be due to the contribution of glomalin to soil particle hydrophobicity, or alternatively that its insoluble, hydrophobic, glue-like nature may initiate aggregation and protect aggregating material (soil minerals, MO and SOM). Regardless, an increase in aggregation would lead to an increase in SOM stabilisation and C sequestration, according to Dungait's theory (2012a) of physical protection.

2.6.4 Tannins

Tannins are widely occurring plant polyphenols which can co-precipitate a range of organic molecules, including proteins (Fierer et al. 2001; Bradley et al. 2000). Tannins are estimated to be the fourth most abundant biochemical after cellulose, hemicellulose and lignin (Kraus et al. 2003; Hernes & Hedges, 2000), and are often more plentiful in rapidly cycling soft tissues (e.g. leaves and needles) than lignin (Benner et al. 1990a; Benner et al. 1990b; Hedges & Weliky, 1989). Consequently tannins are believed to play a key role in belowground N cycling (due to their ability to form recalcitrant complexes with organic N) (Nierop et al. 2006); and may also lead to the development of recalcitrant organic fractions since they can form complexes with carbohydrates and cellulose (Horner et al. 1988). Tannins are known to influence litter decomposition, nutrient cycling, N sequestration, microbial activity, humic acid formation, metal complexation and pedogenesis (Nierop et al. 2006; Kraus et al. 2003).

Plant tannins are divided into two major groups consisting of condensed tannins and hydrolysable tannins, both of which are important for different biological processes (Coulis et al. 2009; Schweitzer et al. 2008). Tannins are produced by plants and formed within the plant via the phenylpropanoid pathway. There is evidence that low soil pH and low fertility will increase the proportion of condensed tannins within a species; *Lotus pedunculatus* (cv. Grasslands 'Maku') grown on a high fertility soil in the North Island of New Zealand contained condensed tannins accounting for 2 - 3 % DM, whereas condensed tannin content was approximately 2.5 times higher when grown in an acid soil of low fertility (8 - 11 % DM) (Barry & Forss, 1983).

Condensed tannins may be either procyanidin or prodelphinidin depending on the frequency and distribution of OH groups on the aromatic B ring (Figure 2.9) (Kaal et al. 2012). The number and placement of these aromatic OH groups dictate the reactivity of condensed tannins (Slabbert, 1992 as cited in Kaal et al. 2012) and their protein precipitation capacity (Kraus et al. 2003; Hagerman et al. 1998; Kumar & Horigome, 1986;

Porter et al. 1986 as cited in Fierer et al. 2001). For example, condensed tannins from *Lotus corniculatus* and *Lotus pedunculatus* have considerably different chemical structures – those from *Lotus pedunculatus* are predominantly prodelphinidin-type subunits (relative molecular mass 2200), whereas those from *Lotus corniculatus* are predominantly procyanidin-type subunits (relative molecular mass 1900) (Foo et al. 2000; Aerts et al. 1999; Barry & McNabb, 1999). These two species have significantly different effects upon ruminant activity and forage nutritive value (Aerts et al. 1999; Barry & McNabb, 1999), therefore it is total condensed tannin concentration and the variation in chemical structure and molecular mass, that likely leads to differences in how tannins are observed to react both in the rumen and in the soil (Kraus et al. 2004; Fierer et al. 2001; Hagerman et al. 1998).

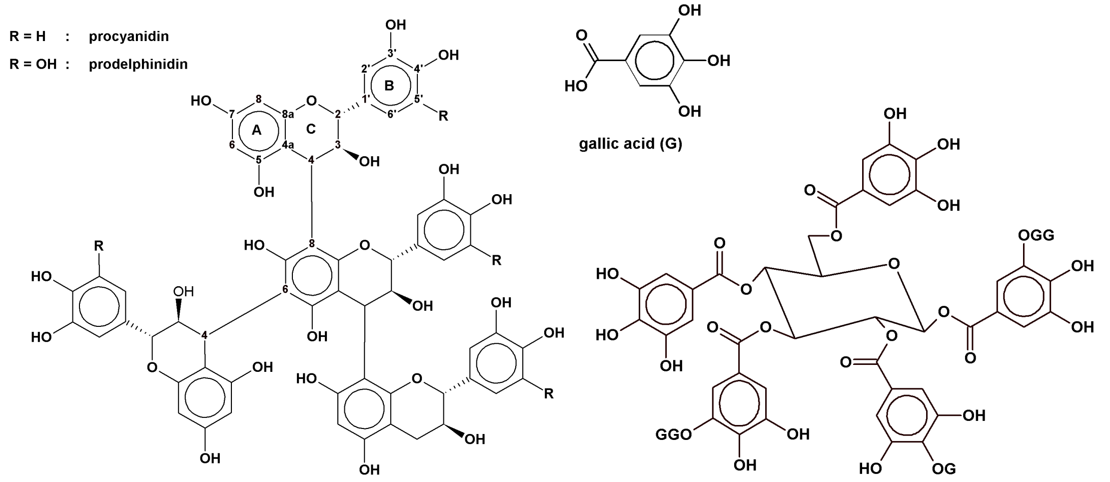


Figure 2.9 Chemical structure of condensed tannins (left) and hydrolysable tannins (right) (source: Kaal et al. 2012).

Different plant species and plant functional groups invest different amounts of C resources into growth and defence (Herms & Mattson, 1992; Bazzaz et al. 1987). Plant species which produce high amounts of defence compounds, such as tannins, tend to produce unpalatable, poor quality litter which negatively affects animal feed intake, feed digestibility, and efficiency of production (Kosola et al. 2006; Forkner et al. 2004; Reed, 1995). Condensed tannins are present in many plant species (Table 2.4 & 2.5), including some pasture legumes (Jones et al. 1976); with many species containing very low concentrations of condensed tannins (e.g. perennial ryegrass, yorkshire fog, white clover in its flowers but not its leaves). In contrast *Lotus corniculatus* (2.1 - 4.6 % DM) (Harris et al. 1998; Jackson et al. 1996; Terrill et al. 1992; Waghorn et al. 1987), sulla (4.5 % DM) (Terrill et al. 1992), sainfoin (2.5 - 3.5 % DM) (Aufrere et al. 2008), willow (1.8 - 4.2 % DM leaves and shoots < 5 mm) and poplar (0.6 - 2.6 % DM leaves and shoots < 5 mm) (Kemp et al. 2001) all contain effective condensed tannins at concentrations within the ideal range of 3 - 5 % of DM; while *Lotus pedunculatus* has relatively high concentrations of condensed tannins (6.3 - 7.7 % DM) (Terrill et al. 1992; Barry & Duncan, 1984) which, although not effective in increasing animal production, do prevent bloat (Waghorn et al. 1990; Terrill et al. 1992).

Table 2.4 Condensed tannin content of legumes, herbs and grasses.

Species		Condensed tannin (% DM)					Total N (% DM)	Tannin: protein ratio	Source
		Vanillin-HCl	Butanol-HCl						
		Extractable	Extractable	Protein-bound	Fibre-bound	Total			
Legumes									
Canary clover*	*Dorycnium rectum*	-	8.300	5.400	0.600	14.300	3.25	0.700	Terrill et al, 1992
Hairy canary clover*	*Dorycnium hirsutum*	-	12.100	6.500	0.100	18.700	2.60	1.150	Terrill et al, 1992
Prostrate canary clover*	*Dorycnium pentaphyllum*	-	10.000	2.300	0.300	12.600	3.57	0.560	Terrill et al, 1992
Red clover	*Trifolium pratense*	0.140	0.040	0.060	0.070	0.170	-	-	Jackson et al, 1996
White clover[a]	*Trifolium repens* cv. Grasslands Kopu	-	-	-	-	0.140	-	-	Harris et al, 1998
White clover (in heavy flower)[b]	*Trifolium repens* cv. Grasslands Kopu	-	-	-	-	0.360	-	-	Harris et al, 1998
Big trefoil	*Lotus pedunculatus*	-	-	-	-	6.300	-	-	Barry & Duncan, 1984
Big trefoil*	*Lotus pedunculatus*	-	6.100	1.400	0.100	7.700	4.82	0.260	Terrill et al, 1992
Birdsfoot trefoil*	*Lotus corniculatus*	-	0.700	1.300	0.100	2.100	4.34	0.080	Terrill et al, 1992
Birdsfoot trefoil[c]	*Lotus corniculatus* cv. Grasslands Goldie	-	-	-	-	2.160	-	-	Harris et al, 1998
Birdsfoot trefoil	*Lotus corniculatus*	-	-	-	-	2.200	-	-	Waghorn et al, 1987
Birdsfoot trefoil[d]	*Lotus corniculatus* cv. Grasslands Goldie	-	-	-	-	2.490	-	-	Harris et al, 1998
Birdsfoot trefoil	*Lotus corniculatus*	-	2.710	0.610	0.180	3.500	-	-	Wang et al, 1994
Birdsfoot trefoil	*Lotus corniculatus*	3.680	3.580	0.860	0.180	4.620	-	-	Jackson et al, 1996
Narrow leaf birdsfoot trefoil*	*Lotus tenuis*	-	0.200	0.300	0.100	0.600	4.57	0.020	Terrill et al, 1992
Sainfoin (vegetative)	*Onobrychis viciifolia* L.	-	-	-	-	3.500	-	-	Aufrere et al, 2008
Sainfoin (early flowering)	*Onobrychis viciifolia* L.	-	-	-	-	2.500	-	-	Aufrere et al, 2008
Sainfoin (hay taken at flowering)	*Onobrychis viciifolia* L.	-	-	-	-	0.600	-	-	Aufrere et al, 2008
Sulla*	*Hedysarum coronarium*	-	3.300	0.900	0.300	4.500	3.69	0.200	Terrill et al, 1992
Crownvetch*	*Coronilla varia*	-	1.600	1.300	0.200	3.000	4.68	0.100	Terrill et al, 1992
Serradella*	*Ornithopus sativus*	-	0.400	2.000	<0.100	2.400	2.86	0.130	Terrill et al, 1992
Perrennial lupin*	*Lupinus polyphyllus*	-	0.110	0.030	0.030	0.170	4.01	0.008	Terrill et al, 1992
Cicer milkvetch*	*Astragalus cicer*	-	0.040	0.060	0.060	0.160	5.00	0.005	Terrill et al, 1992
Lucerne	*Medicago sativa*	0.420	0.000	0.050	0.000	0.050	-	-	Jackson et al, 1996
Herbs									
Sheep's burnet*	*Sanguisorba minor* ssp. muricata	-	0.100	0.140	0.100	0.340	2.92	0.019	Terrill et al, 1992
Chicory	*Chichorium intybus*	0.330	0.100	0.040	0.030	0.170	-	-	Jackson et al, 1996
Chicory*	*Chichorium intybus*	-	0.140	0.260	0.020	0.420	2.44	0.028	Terrill et al, 1992
Plantain	*Plantago lanceolata*	2.510	1.090	0.130	0.190	1.410	-	-	Jackson et al, 1996
Grasses									
Perennial ryegrass[e]	*Lolium perenne*	0.100	0.050	0.000	0.040	0.090	-	-	Jackson et al, 1996
Perennial ryegrass*	*Lolium perenne*	-	0.110	0.000	0.000	0.110	5.21	0.003	Terrill et al, 1992
Perennial ryegrass[f]	*Lolium perenne*	0.095	0.080	0.050	0.050	0.180	-	-	Jackson et al, 1996
Yorkshire fog*	*Holcus lanatus*	-	0.140	0.010	0.010	0.160	-	-	Terrill et al, 1992
Yorkshire fog	*Holcus lanatus*	0.310	0.230	0.000	0.000	0.230	-	-	Jackson et al, 1996
Yorkshire fog (wild ecotype)*	*Holcus lanatus*	-	0.110	0.030	0.040	0.180	3.26	0.008	Terrill et al, 1992
Kikuyu	*Pennisetum clandestinum*	0.107	0.060	0.030	0.001	0.091	-	-	Jackson et al, 1996
Paspalum	*Paspalum dilatatum*	0.090	0.050	0.067	0.057	0.174	-	-	Jackson et al, 1996
Smooth witchgrass	*Panicum capillare*	0.165	0.100	0.050	0.020	0.170	-	-	Jackson et al, 1996
Crowfoot	-	0.247	0.023	0.017	0.020	0.060	-	-	Jackson et al, 1996
Summer grass	*Digitaria sanguinalis*	0.280	0.045	0.050	0.055	0.160	-	-	Jackson et al, 1996
Tall fescue	*Festuca arundinacea* ssp. Kentucky 31	-	-	-	-	0.500	-	-	Puchala et al, 2005

* Freeze dried forage samples

[a] Not a pure sward, but clover comprised 74% of total DM

[b] Not a pure sward, but clover comprised 70% of total DM

[c] Not a pure sward, but lotus comprised 75% of total DM

[d] Not a pure sward, but lotus comprised 73% of total DM

[e] Grown in the same area as other temperate forages (perennial ryegrass, yorkshire fog, chicory, plantain, red clover, lucerne, birdsfoot trefoil).

[f] Dissected from the same pasture as kikiyu, paspalum, smooth witchgrass, crowfoot, and summer grass.

Table 2.5 Condensed tannin content of grains, protein concentrate meals and other forage species.

Species		Condensed tannin (% DM)					Total N (% DM)	Tannin: protein ratio	Source
		Vanillin-HCl	Butanol-HCl						
		Extractable	Extractable	Protein-bound	Fibre-bound	Total			
Grains									
Sorghum (low tannin)	*Sorghum* spp.	-	0.080	0.000	0.060	0.140	1.71	0.013	Terrill et al, 1992
Sorghum (high tannin)	*Sorghum* spp.	-	0.440	0.660	0.080	1.180	2.02	0.093	Terrill et al, 1992
Brewers' grains	-	-	0.070	0.050	0.020	0.140	4.65	0.005	Terrill et al, 1992
Barley	*Hordeum vulgare*	-	0.110	0.000	0.000	0.110	1.95	0.009	Terrill et al, 1992
Triticale	*X. Triticosecale*	-	0.090	0.000	0.000	0.090	2.50	0.006	Terrill et al, 1992
Protein concentrate meals									
Cottonseed	*Gossypium* spp.	-	0.210	1.000	0.390	1.600	7.38	0.035	Terrill et al, 1992
Rapeseed	-	-	0.070	0.370	0.150	0.590	6.34	0.015	Terrill et al, 1992
Copra	-	-	0.060	0.280	0.140	0.480	3.60	0.021	Terrill et al, 1992
Soya bean	*Glycine max*	-	0.100	0.000	0.000	0.100	8.40	0.002	Terrill et al, 1992
Other forage species:									
Willow									
Tangoio willow[a]	*Salix matsudana* x *alba*	-	-	-	-	4.180	-	-	Kemp et al, 2001
Tangoio willow[b]	*Salix matsudana* x *alba*	-	-	-	-	9.200	-	-	Kemp et al, 2001
Tangoio willow[c]	*Salix matsudana* x *alba*	-	-	-	-	7.100	-	-	Kemp et al, 2001
Moutere willow[a]	*Salix matsudana* x *alba*	-	-	-	-	3.950	-	-	Kemp et al, 2001
Matsudana willow[a]	*Salix matsudana*	-	-	-	-	1.790	-	-	Kemp et al, 2001
Poplar									
Veronese poplar[a]	*Populus deltoides* x *nigra*	-	-	-	-	0.980	-	-	Kemp et al, 2001
Veronese poplar[b]	*Populus deltoides* x *nigra*	-	-	-	-	2.200	-	-	Kemp et al, 2001
Veronese poplar[c]	*Populus deltoides* x *nigra*	-	-	-	-	2.200	-	-	Kemp et al, 2001
Louisa Avanza[a]	-	-	-	-	-	2.610	-	-	Kemp et al, 2001
Tasman[a]	*Populus deltoides* x *nigra*	-	-	-	-	2.360	-	-	Kemp et al, 2001
Selwyn[a]	*Populus deltoides* x *nigra*	-	-	-	-	1.920	-	-	Kemp et al, 2001
Argyle[a]	*Populus deltoides* x *nigra*	-	-	-	-	1.130	-	-	Kemp et al, 2001
Weraiti[a]	*Populus deltoides* x *nigra*	-	-	-	-	1.110	-	-	Kemp et al, 2001
Pakaraka[a]	*Populus deltoides* x *nigra*	-	-	-	-	0.930	-	-	Kemp et al, 2001
Otahoua[a]	*Populus deltoides* x *nigra*	-	-	-	-	0.930	-	-	Kemp et al, 2001
Toa[a]	*Populus* x *euramericana* x *yunnanensis*	-	-	-	-	0.600	-	-	Kemp et al, 2001

[a] Leaves plus stems 5 mm or less, measured in summer

[b] Bark from 5 year old trees, measured in summer

[c] Bark from 10 year old trees, measured in summer

Condensed tannins react with plant proteins in the rumen, forming an insoluble complex and thereby reducing protein degradation by rumen bacteria. The passage of plant protein to the intestine is increased per unit of N consumed, as is the availability and absorption of essential amino acids (60 % increase) compared with equivalent condensed tannin-free forage (Widdup et al. 2004; Waghorn et al. 1990). This complex is pH dependent: as long as pH remains in the range 3.5 – 7.0 it will remain stable and insoluble, but outside this range dissociation and solubilisation of protein will occur (Barry & Forss, 1983). Ruminant nutrition studies conducted in New Zealand with *Lotus* species indicate that the optimal concentration of condensed tannins in forage diets is approximately 2.2 % DM. The depression of rumen fibre digestion starts to occur at 4.0 % DM, with intake and growth depression occurring in the range of 6 - 10 % DM (Waghorn et al. 1987; Barry et al. 1986; Barry, 1985; Barry & Duncan, 1984). Using rumen as a model, it is likely that the optimal concentration of condensed tannins in litter returned to the soil will also be in the vicinity of 2.2 % DM.

Ruminants eating forage which contains tannins excrete about 20 % more of their surplus dietary N in dung and correspondingly less N in urine (Crush & Keogh, 1998; Waghorn et al. 1994). As approximately 30 % of animal C intake is excreted as dung and urine (Parsons et al. 2011), if animal digestion is decreased due to the negative effects of tannins, a higher percentage of C should be returned to the soil. This has the effect of increasing the longevity of C in the terrestrial system (even if only by days or weeks) as it is not immediately lost to the atmosphere as animal respiration.

While plant tannins are well known for the role they play in herbivore and pathogen defence, they also impact on terrestrial nutrient cycling (Hattenschwiler & Vitousek, 2000; Kuiters, 1990; Horner et al. 1988). There is evidence from rumen studies that tannins may act as regulators of soil microbial communities, as herbivore digestion and microbial decomposition are regulated by MO with similar enzymes (Bais et al. 2004; Kraus et al. 2003; Hattenschwiler & Vitousek, 2000; Bardgett, et al. 1998). This means that litter with high concentrations of condensed tannins may strongly prohibit the activities of both rumen and soil MO (Hattenschwiler & Vitousek, 2000), and therefore have a role in the mediation of nutrient cycles - affecting the partitioning of C above- and below-ground (Fischer et al. 2006; Binkley & Giardina, 1998; Northup et al. 1998), reducing digestion and decomposition rates, and ultimately increasing C longevity. However there have been relatively few studies into the ability of tannins to affect microbial community structure. In ruminants, tannins in forage cause a shift towards microbial populations that can degrade tannins, but similar, distinct results have not been found in soils (Ammar et al. 2009). It is likely that the effects in soils will depend on differences between the soils, the ecosystems, or the tannins themselves (Schmidt et al. 2013); and there has been evidence from research that the effects of tannins do strongly depend upon plant species and tissue type (i.e. tannin structure and type) (Nierop et al. 2006; Kraus, et al. 2003; Fierer et al. 2001).

Tannins tend to remain at relatively high concentrations in senescent plant material, which means they enter the soil within litter (Lin et al. 2007; Hattenschwiler & Vitousek, 2000). A number of incubation studies with forest floor litter and / or the topsoil of forest soils have been carried out to investigate the impact of tannin amendments in relation to C and N dynamics (Kraus et al. 2004; Fierer et al. 2001; Bradley et al. 2000). The reported extent of the effect of tannins on C and N cycling differ (Nierop et al. 2006), which may be explained by their chemically heterogeneous nature (Figure 2.9); but the general trend is that litter high in tannin content results in reduced decomposition rates and nutrient availability (Nierop et al. 2006; Bais et al. 2004; Kraus et al. 2004; Kraus et al. 2003; Hattenschwiler & Vitousek, 2000; Kilburtji et al. 1999; Northup et al. 1998; Schimel et al. 1998; Schimel et al. 1996; Basaraba & Starkey, 1966; Gallardo & Merino, 1993, 1992; Horner et al. 1988; Nicolai, 1988; Benoit et al. 1968). Kraus et al. (2004) highlighted that the structural characteristics of tannins need to be accounted for and reported when their effect on soil processes is considered. Many of these studies also reported a rapid loss of tannins (approximately 80 %) from conifer and deciduous litter within the first year of decomposition (Lorenz et al. 2000), which may have been due in part to the presence of simple phenolics or low molecular mass fractions that were easily decomposed (Madritch et al. 2007).

Despite the wide use of tannins in ecosystem studies, there is limited information available about conditions that affect their decomposition in soils. On entering the soil system, tannins can either remain in soil solution, precipitate out, adsorb into soil particles (e.g. clay, humus, allophane), complex with proteins or metals, and / or undergo biotic or abiotic degradation (Kraus et al. 2003 and citations therein). Soil characteristics (e.g. pH, mineralogy, nutrient concentrations, and microbial community) and the specific structure of tannin are all likely to influence how tannins are processed in soil, however it is difficult to find research that supports this. A review by Dungait et al. (2012b) suggests that low pH and low oxygen conditions may slow decomposition, which is supported by Kraus et al. (2003 and citations therein) who proposed that high rainfall conditions are likely to result in tannins leaching from surface layers; whereas under cool, low rainfall conditions tannins are more likely to remain and influence the soil system. However Fierer et al. (2001) demonstrated that different soil types respond differently to tannin additions and therefore more research is required to definitively separate the possible factors at work.

Research has shown that plants are more likely to accumulate condensed tannins and phenolics in their litter under low fertility conditions (Kraus et al. 2003; Booker & Maier, 2001; Gebauer et al. 1998) and / or highly acidic soil conditions (pH < 4) (e.g. Spodosols and Utisols under the Mendocino Ecological Staircase) (Northup et al. 1995 as cited in Kraus et al. 2004), which would ultimately result in relatively high tannin inputs through both litter deposition and foliar leaching to the soil (Kraus et al. 2004 and citations therein; Schofield et al. 1998).

There are various theories about the mechanisms behind tannin activity in soils:

(i) Tannins may physically protect other compounds (e.g. cellulose) from microbial attack (Schweitzer et al. 2008; Kraus et al. 2004; Hattenschwiler & Vitousek, 2000);

(ii) They may form complexes with proteins and other compounds which are unavailable for microbial uptake (Nierop et al. 2006; Fierer et al. 2001; Bradley et al. 2000; Hattenschwiler & Vitousek, 2000);

(iii) They may cause toxicity which:

   a. Restricts soil organism activity (e.g. nematodes, arthropods, enchytraeids and earthworms) (Kosola et al. 2006; Bradley et al. 2000; Northup et al. 1998);

   b. Restricts microbial activity (Juntheikki & Julkunen-Tiitto, 2000; Nierop et al. 2006; Kraus et al. 2003; Horner et al. 1988; Benoit & Starkey, 1968a, 1968b; Basaraba & Starkey, 1966; Goldstein & Swain, 1965; Davies et al. 1964; Handley, 1961);

   c. Deactivates microbial exoenzymes (including proteases, phosphatases, and glycosidases) (Schmidt et al. 2013); and / or

d. Alters soil community composition therefore altering microbial products (Schweitzer et al. 2008; Madritch et al. 2007; Nierop et al. 2006).

Tannins have long been considered to be stable in soils, but studies using pyrolysis-GC / MS have only definitively identified the presence of tannins in litters and A-horizons of soils (Kaal et al. 2012), indicating that they are not as stable as previously thought. Pyrolysis-GC / MS studies have also shown that in both allophanic and non-allophanic soils there is little protection of primary plant derived material (this has not been proven for secondary OM); rather it is the microbial by-products which are protected in soils (Buurman et al. 2007; Gonalez-Perez et al. 2007). Therefore any influence from plant species on soil C may be due to their influence on the microbial population rather than the stability of the plant material itself. This, in turn, would suggest that tannins also influence total decomposition and soil litter stability through the microbial population rather than through any inherent stability of the tannins themselves.

In summary, it is known that tannins precipitate proteins thus slowing down (protein) decomposition; affect soil biogeochemical processes including microbial activity (toxicity) and enzyme activity by binding to substrates; and, in turn, influence soil processes including OM decomposition, N mineralization, and nitrification. Therefore to fully understand the importance of tannins and their structural characteristics in the decomposition of pastoral species (both shoot and root litter) and subsequent interactions in soil, there needs to be further decomposition studies carried out under different soil conditions with a range of soil types and high and low tannin containing litters.

2.7 Common decomposition measurement methods

Several methods are currently used to study decomposition processes, with residue losses measured with the litterbag technique, C dynamics quantified by stable isotope labelling, and CO_2 effluxes commonly measured by both chamber and micrometeorological techniques. Soil CO_2 efflux is the result of both soil CO_2 production (mainly root and microbial respiration) and transport of CO_2 to the atmosphere (Janssens et al. 2000). Chamber methods may be open or closed, dynamic or static; while micrometeorological methods include the eddy covariance techniques, the flux-gradient method, the aerodynamic method and the Bowen ratio / energy balance (Denmead, 2008; Liang et al. 2004; Janssens et al. 2000; Dugas, 1993), of which only eddy covariance will be discussed here.

The litterbag method was developed to shed light on decomposition processes in litter rich, undisturbed forest soil systems (Magid et al. 1997). A mesh bag allows residues to be introduced to the soil, while still remaining confined from the soil environment. Due to its simple design, this method has been extended to include investigations of arable systems (Christensen, 1985; Harper & Lynch, 1981; Douglas et al. 1980). Issues with the technique include the likely entrance of soil into the bag which will confound weight loss estimations, and the generation of a microenvironment which differs considerably from one in which residues are in intimate contact with the soil (Magid et al. 1997; Tian et al. 1992).

Stable isotope labelling (Stewart & Metherell, 1999) and radioactive isotope labelling (Saggar & Hedley, 2001) are proven techniques available to quantify C and N dynamics in naturally functioning ecosystems (Dungait et al. 2012b; Dungait et al. 2009). Using stable isotope labelling is a relatively safe and cost effective method which can be used to accurately interpret complex systems. In isotope labelling, isotope ratio mass spectrometry (IRMS) is used to determine either a natural or artificial increase in the abundance of ^{13}C or ^{15}N relative to the abundance of more common ^{12}C and ^{14}N (Dungait et al. 2012b; Grilo et al. 2011; Dungait et al. 2009). New insights have been gained into soil C dynamics using these stable ^{13}C isotopes, challenging perceptions of labile or stable pools. An advantage of stable isotope labelling is that unlike radioisotopes (e. g. ^{14}C), their incorporation into the soil does not affect the viability of organisms. This means that components in the C and N pools can be labelled and traced over time (Grilo et al. 2011). A review by Dungait et al. (2012b) concluded that microbial and enzyme accessibility to SOM controls its decomposition, rather than its intrinsic chemical recalcitrance which poses interesting questions as to the effect of a high plant-tannin concentration on SOM decomposition.

At present chamber methods are the best technique available for measuring CO_2 flux as they are widely applicable and do not present uncertainty if vegetation is present (Norman et al. 1997). Chamber methods are categorized as either static or dynamic based on the presence or absence of air circulation (Janssens et al. 2000). There are three different principles: (i) closed-static chamber systems (both chemical trap and gas sampling), (ii) closed-dynamic chamber systems, and (iii) open chamber systems (Norman et al. 1997).

A closed-static chamber system with a chemical trap determines the total mass of CO_2 released from the soil by trapping evolved CO_2 in solutions such as NaOH, KOH or soda lime (NaOH and $Ca(OH)_2$) (Keith & Wong, 2006; Grogan, 1998; Jensen et al. 1996). The CO_2-enriched solution is precipitated with $BaCl_2$ and titrated with dilute HCl, with the total amount of CO_2 trapped equalling the CO_2 flux. If chemical traps are applied carefully, they will allow a relatively accurate comparison of *in situ* soils, although the sensitivity range must be wide enough to capture the true range of effluxes being measured (Nay et al. 1994).

Static chambers have no air motion, so molecular diffusion is the dominant process driving soil CO_2 efflux. (Rochette et al. 1992). It is important that the chemical trap is of a large enough capacity, or is changed frequently, to ensure that the concentration of CO_2 does not build up in the chamber headspace. If it does the concentration gradient between the soil and chamber will decrease, thereby reducing the rate of soil respiration and the measured flux (Janssens et al. 2000). If static chambers are closed for too long the supply of O_2 within the chamber will be depleted, reducing the rate of respiration; and in such cases static chambers tend to underestimate soil CO_2 fluxes (Norman et al. 1997). High rates of soil CO_2 evolution may also limit static chamber measurements if the trap is not large enough due to the diffusional limitation of the trapping of CO_2 in the alkali solution (Grogan, 1998).

Several studies have shown that soil-surface CO_2 fluxes may be underestimated by up to 100 % when the trap size and diffusional limit are not carefully attended to (Magid et al. 1997; Nay et al. 1994; Norman et al. 1992; Rochette et al. 1992). Cooler soil temperature inside the static chamber may also contribute to lower fluxes so it is important to keep the temperature constant (Janssens et al. 2000). However provided best practices are followed - the trap capacity is large enough, it is changed frequently, and the temperature is kept constant - CO_2 build up, and the issues associated with that build up, can be successfully avoided.

A closed-static chamber system with gas sampling may also be used to provide an estimation of CO_2 flux. Syringes are used to remove gas samples at different time periods and these samples are then analysed using gas chromatography (Hedley et al. 2006; Liang et al. 2004; Pumpanen et al. 2004; Pumpanen et al. 2003; Bekku et al. 1997). Static chambers are inexpensive and easy to use but they are labour intensive and over long periods may lead to changes in the soil microclimate inside the chamber. These systems tend to underestimate surface fluxes to a greater extent than infrared gas analysis in a closed-dynamic system, making them more vulnerable to error (Norman et al. 1997). In general, static techniques tend to overestimate small fluxes and under estimate large fluxes (Nay et al. 1994), and are therefore often regarded as inferior to dynamic chamber systems (Janssens et al. 2000; Norman et al. 1992).

In closed dynamic chamber systems the air is circulated continuously between the chamber and an infrared gas analyser in an isolated loop; the CO_2 flux will correspond to the rate of soil CO_2 released (Rayment, 2000; Rochette et al. 1997; Rochette et al. 1992). Closed dynamic chambers are labour intensive but they do allow CO_2 fluxes to be measured quickly with minimal chamber interference. In comparison, open dynamic chambers are designed for fresh air (with a known CO_2 concentration) to circulate through the chamber and replace the CO_2-enriched air removed for infrared gas analysis and flux calculation (Fang & Moncrieff, 1998, 1996; Kanemasu et al. 1974). This technique is generally very accurate although it is possible to over- or under-estimate CO_2 flux (Liang et al. 2004; Pumpanen et al. 2004; Pumpanen et al. 2001; Kanemasu et al. 1974).

Dynamic chamber systems overcome several chamber problems inherent in static chamber systems due to their short sampling period which results in negligible changes in soil temperature and water content and limited build up of CO_2 in the chamber. Dynamic systems also thoroughly mix the air in the chamber headspace, which prevents the build up of a thick soil boundary layer. However, a comparison experiment conducted by Jensen et al. (1996), which estimated field-scale CO_2 flux from unplanted soils over a 24 hour period, found that a closed-static chamber (alkali-trapping of CO_2) provided the best integrative measure of CO_2 flux when compared to a closed-dynamic chamber (infrared gas analysis every 2 minutes). It was also found that the estimate from the dynamic chamber would only be improved by continual monitoring.

At the field scale there are a range of micrometeorological techniques (of which eddy covariance is one of the most common) which can be used to estimate soil CO_2 efflux while avoiding chamber-related issues (Janssens et al. 2000; Dugas, 1993). The basic concept of these techniques is that eddies transport gas from the soil surface to the measurement height; and that the vertical flux is identical to the efflux from the soil (Mosier et al. 1996). Eddy covariance systems typically measure CO_2 flux at a height of 1 - 2 m above the ground. The advantage of this technique (and other micrometeorological techniques) is that it does not affect the emission of gases from the soil (Dugas, 1993), it can measure soil CO_2 efflux continuously over long time periods and larger surface areas are considered, thereby sampling spatial heterogeneity (Janssens et al. 2000).

The successful application of any micrometeorological technique is dependent on the presence of a relatively uniform upwind-fetch and atmospheric steady-state conditions (Baldocchi, 2003; Janssens et al. 2000). The presence of vegetation between the soil and the measurement height may alter the measured fluxes (Norman et al. 1997; Rochette et al. 1992), as total net flux is measured meaning soil CO_2 flux can only be estimated (Norman et al. 1992). In bare soil, however, this method can be used for direct measurement of soil CO_2 flux (Dugas, 1993). The typical error of a eddy covariance system is in the range of 10 - 20 %, with large variability observed in the night-time fluxes (Norman et al. 1997). In view of all this, it is unlikely that micrometeorological techniques will replace chamber methods as the most common means of measuring soil CO_2 efflux (Norman et al. 1997).

There are many methods for measuring soil CO_2 efflux, which exhibit a wide variance in accuracy, spatial and temporal resolution, and applicability. There is currently still no standard or reference to test accuracy and there is a degree of uncertainty to all measurement techniques (Janssens et al. 2000). This means that the choice of a specific technique is often a trade-off between requirements (accuracy and resolution) and feasibility (applicability and cost). At present chamber methods are considered the best technique available for measuring CO_2 flux in decomposition studies as they are widely applicable and do not present uncertainty if vegetation is present (Norman et al. 1997).

Classical chamber methods which measure CO_2, either by infrared gas analysis or alkali trapping, remain useful tools (Davidson et al. 2002) despite the increasing popularity of micrometeorological techniques (Baldocchi, 2003). The key advantage of chamber methods is that they allow CO_2 fluxes to be measured directly from the soil, are relatively inexpensive and easy to use (Grogan, 1998), and offer the possibility of replicated measurements over space. However, a large number of soil chamber CO_2 flux measurements may be required due to the high spatial variability of the flux and the small surface area of each measurement (Dugas, 1993). In comparison, micrometeorological techniques have superior spatial and temporal integration of CO_2 flux (Dugas, 1993); although they are limited by comparatively high purchase and installation costs (Baldocchi, 2003; Norman et al. 1997).

Since there are strengths and weaknesses to both chamber and micrometeorological techniques depending on their intended use (Hedley et al. 2006; Keith & Wong, 2006; Liang et al. 2004; Grogan, 1998; Jensen et al. 1996), it has been suggested that a combination of chamber and micrometeorological techniques might be the most effective way to accurately measure CO_2 flux. For example, some studies have simultaneously used eddy covariance and chamber methods to separate net ecosystem CO_2 exchange from soil respiration (Dore et al. 2003; Lavigne et al. 1997), as well as to correct fluxes obtained by eddy correlations during night periods (Dore et al. 2003; Anthoni et al. 1999). However, most chamber methods and all micrometeorological techniques are only useful to obtain the total CO_2 flux without differentiation of the CO_2 sources (Kuzyakov, 2006).

2.8 Conclusions

Current literature indicates that increasing soil C is a proven GHG mitigation strategy, and the models available clearly show that this could be achieved. For pastoral lands this would require an increased input of C to the soil or a decrease in the decomposition rate of soil C.

An extensive review of literature supports the view that the potential to increase soil C in grazed pasture soils could be facilitated by selecting pasture species with key physical and biochemical differences (e.g. fibre composition or plant polyphenol content: lignin and tannin). The quality of initial litter is a powerful determinant of the biomass of microorganisms and their microbial activity, which in turn regulates key processes such as litter degradation and nutrient flow.

Rumen studies have provided information on the influence of plant biomass quality on the extent of decomposition by rumen microbes. Plants with a higher lignin or tannin content have been shown to have a negative impact on the digestability of animal feed, with a slower degradation rate in the rumen. In the rumen, polyphenols can slow degradation by binding with proteins to create an insoluble protein-tannin precipitate (more resistant to decay) or by exhibiting a toxicity effect on rumen microbes, both leading to reduced microbial activity. If the type of plant cover in a pastoral system is modified, for example to increase the addition of polyphenol-rich substrates (e.g. tannins), this may change the structure of the soil microbial community, the response of soil MO, and subsequently the rate of C decomposition, however at the time of writing there is no evidence to support this.

Conclusive evidence to support the hypothesis that pasture species high in tannins could lead to increased soil C is not available for New Zealand pasture soils. To test this hypothesis, plant litter and root decomposition studies in incubated soil will be undertaken in static chambers capable of measuring soil respiration (see Section 1 Introduction for more detail of research hypotheses and objectives).

Chapter 3

The extent of decomposition of lotus (*Lotus pedunculatus*) and white clover (*Trifolium repens*) shoot material after 125 d of incubation in a Pallic soil, influenced by incubation technique.

3.1 Introduction

In Chapter 2.2, grazed pasture rotations in mixed farming systems were discussed as a way of building soil organic matter (SOM) and soil carbon stocks. To accelerate carbon sequestration in soils under pasture the opportunity exists to select pasture species that contribute litter to soil that specifically slows plant litter decomposition rates (carbon use efficiency). In grazed pasture systems carbon enters the decomposition cycle in several ways (Figure 2.2):

- Uneaten shoot litter
- Grazed animal excreta return
- Root exudates
- Root litter

Shoot material traits

The objective of the experimental work described in this chapter was to test the concept that plant traits of uneaten shoot litter can influence the rate of decomposition (soil microorganism carbon use efficiency) and the amount of residue carbon remaining after a period of decomposition. Plant chemical properties (traits) identified as likely to slow decomposition rates included the total amount and type of phenolics present (e.g. tannins), carbon to nutrient ratios of the litter (especially C:N), and a high proportion of high molecular-weight carbohydrates such as cellulose, hemicellulose and lignin (Chapter 2.2).

Two pasture legumes that differ in their ecological niche: *Lotus pedunculatus* (LT) in low fertility pastures, *Trifolium repens* L. (WC) in high fertility pastures; and also differ in their specific plant traits, were selected and an experiment was designed to test whether these species and method of application of those species (hereby termed management) will influence the amount of carbon dioxide (CO_2-C) sequestered in a Pallic topsoil (Tokomaru Silt Loam).

Hypotheses

Two hypotheses are presented:

(i) A high percentage of tannins, as found in a common pasture species, will decrease the rate and total amount of litter decomposed in soil, therefore retaining a greater amount of C, compared to that of a comparable pasture species which contains relatively low tannin content;

(ii) The method of application of residues to the soil will alter the rate and total amount of litter decomposed. Decomposition is likely to be incomplete when there are inhibiting factors present; i.e. spatial location of decomposition residues (access to living organisms); hydrolysis conditions (dry-wet; access to enzymes; clays and metals); and microbial respiration.

Litter placement method

This study compares different ways of adding amendments to the soil – fresh, dried and ground; mixed in or surface application. The methods of application consider that the use of surface applied fresh dried litter, and subsequent placement in or on the soil surface might influence SOM accessibility by fungi versus bacteria, and therefore the extent of decomposition that occurs.

This concept acknowledges that bacteria and fungi both contribute to the turnover of litter in many different ecosystems. Fungi have been shown to have a strong potential to degrade common compounds found in leaf litter (e.g. polysaccharides, lignin and polyphenols) (Rihani et al. 1995); and their predominant role in decomposition may be explained by their ability to explore soil space and reserves of SOM which would otherwise be inaccessible (Fontaine et al. 2011). Bacteria are less efficient at degrading lignin, but in some cases may be able to use intermediates produced by fungal decomposition (Ruttimann et al. 1991). In other words, fungi can make C available to bacteria, which would otherwise be unavailable. However, there may also be antagonistic interactions between fungi and bacteria, with studies on beech leaf litter (Moller et al. 1999) and on decaying wood (Tsuneda & Thorn, 1995), suggesting that bacteria may actually inhibit greater fungal degradation.

Experimental design

These conceptual hypotheses were translated into a working experimental hypothesis, that: in soil-plant litter incubations less CO_2 will be evolved from a soil-plant residue system where there is protection of the OM from decomposition leading to a greater conservation of plant or decomposer residues.

Experimental objectives

(i) To chemically characterise the shoot material of *Lotus pedunculatus* (LT) and *Trifolium repens* L. (WC) legume pasture species, to determine how their specific plant traits differ.

(ii) To determine the impact of high and low tannin pasture species on plant litter decomposition, by conducting an incubation experiment and measuring the rate and total amount of CO_2 evolution.

(iii) A simple, low cost alkali trap incubation procedure was adopted. This technique was initially introduced by Lundegardh (1927), and has been adapted and used in numerous studies since (i.e. Stotzky, 1965; Jensen et al. 1996; Janssens & Ceulemans, 1998; Ghatohra, 2012).

3.2 Materials and methodology

3.2.1 Soil collection and preparation

Tokomaru silt loam topsoil (TK) was collected to a depth of 7.5 cm from a permanent pasture site in Rongotea, New Zealand (40°17’ S, 175°24’ E, 24 m above sea level). The site had been under sheep grazed, permanent ryegrass-white clover pasture management (C_3 pasture ecosystem) for more than 50 years (Parfitt et al. 1984; Roberts & Thompson, 1984). This soil has developed from loess derived mainly from greywacke rocks. It is rich in both silt and clay, and is dominated by 2:1 clay minerals (Baisden et al. 2010; Herath et al. 2013). The 30 year mean rainfall at the site is 969 mm y^{-1}, with a minimum daily air temperature of 9 °C and a maximum of 17.6 °C, and 1750 total sunshine hours.

The soil was air dried until it passed easily through a 5 mm sieve. After sieving, the soil was dried in a glasshouse to a volumetric water content (ϴ) of 0.25, just above calculated permanent wilting point (PWP) ~ 0.17, and stored in a chiller (temperature < 4 °C). Prior to incubation the soil was sieved to 2 mm. PVC cylinders (65 mm diameter x 80 mm length) were packed to 60 mm depth with 265 g moist soil (ϴ 0.25; 199 g OD equivalent), to a dry bulk density (BD) of 1.0 g. cm^{-3} (see Figure 3.1 as example) ready for incubation (Section 3.2.4).

Post incubation soil sampling

Post incubation, the soil still in the PVC tube was placed in a piston microtome and the moist soil was sliced into 0.5 cm slices for the entire, approximately 6.0 cm, profile. Samples were immediately frozen and freeze dried. The sections in the upper profile 0.5 – 3.0 cm, containing the added amendments, were analysed for pH, total phosphorus, total sulphur, total C and total extractable mineral N (NO_3^- and NH_4^+). Restricting sampling to the top 3.0 cm ensured the 2.0 cm zone into which amendments were mixed was analysed, and magnifies the C addition with respect to the background soil C concentration.

3.2.2 Soil characterisation

The following analyses were conducted on the soil pre- (Table 3.1) and post-incubation (Table 3.9), and the methodology is described below:

Soil moisture

Soil moisture contents were determined by heating a known weight of moist soils to constant weight at 105 °C in an oven. Soil chemical analysis was conducted on air dried soil < 2mm diameter in particle size.

Soil pH

Soil pH was determined by the method described by Blakemore et al. (1987) with a modified ratio of 5 g of soil in 12.5 ml of deionized water. Soil was agitated well then left to settle overnight. The next morning pH of the solution (above the soil layer) was measured using a glass pH electrode and a digital pH meter.

Total C, N and S

Subsamples were finely ground in a Tema Mill ring grinder (400V, 6P 50 #62, Rocklabs: Auckland, New Zealand); a small amount (~ 150 mg) was then weighed along with sulphanilamide and tungsten oxide, combusted at 1150 °C in the Elementar (vario MACRO cube) and the resulting gases measured automatically for C, N and S content.

Olsen P

Soil extractable P was determined by the Olsen method described by Blakemore et al. (1987). Air dried, 2mm particle size soil subsamples were extracted (1:20, w:v) with 0.5 M $NaHCO_3$, pH 8.5 for 30 minutes. Phosphorus in the extract was determined colorimetrically by the method of Murphy & Riley (1962) using an UV / visible spectrophotometer at 712 nm.

Sulphate Sulphur

Subsamples were extracted in KH_2PO4 using a method and colorimetric finish described by Wakinson and Kear (1996).

Mineral N

Ammonium and nitrate were extracted with 2M KCl using a 1:10 soil:extractant ratio and a 1 hour end-over-end shake followed by filtration and colorimetric determination as described by Blakemore et al (1987).

Exchangeable cations

Subsamples were extracted with a solution comprised of NH_4^+, acetic acid, caesium chloride (CsCl) and strontium nitrate for K^+, Ca^{2+} and Mg^{2+}. The solution pH was stabilized at pH 7 with acid / NH_4^+ as appropriate. The resulting solution was read on the Atomic Absorption Spectroscopy (AAS) described by Blakemore et al (1987).

Table 3.1 Chemical analysis of Tokomaru Silt Loam topsoil (TK) (depth 7.5 cm, air dried, < 2mm sieve size) at T_0 pre-incubation.

Analysis Type	**TK soil**	
	Mean	***SD***
C (%)[1]	4.0	*0.021*
N (%)[1]	0.43	*0.0045*
S (%)[1,2]	0.052	*0.0026*
Olsen P (mg. L^{-1})[2,3]	42.4	*5.5*
K (me. 100 g^{-1})[2,3]	0.31	*0.027*
Ca (me. 100 g^{-1})[2,3]	10.1	*0.75*
Mg (me. 100 g^{-1})[2,3]	1.0	*0.10*
CEC (me. 100 g^{-1})[2,3]	15.8	*1.1*
pH[2,3]	5.5	*0.037*
Sulphate Sulphur (mg. kg^{-1})[2,3]	16.3	*3.5*

Analyses performed by: [1]Elementar (vario MACRO cube); [2]Standard Soil Testing, Fertilizer & Lime Research Centre, Massey University; [3]Hills Laboratory. Results presented on the basis of three replicates.

Chemical analysis of the soil showed that it presented no limiting soil fertility conditions for decomposition, with chemical fertility (Roberts & Morton, 2009) in the medium to high range (Table 3.1).

3.2.3 Plant material harvest, preparation and characterisation

Lotus (LT) shoot material was harvested from a seeded road cutting on Old West Road, Palmerston North (40°40’63.92”, 175°61’07.21”), and white clover (WC) from Massey

University’s No. 4 Dairy Farm (40°23’57.73”S, 175°36’36.56”E). Two harvests of this material were made a week apart, to provide material for both the freeze dried (first harvest) and fresh (second harvest) treatments.

Freeze drying

The fresh shoot material collected was immediately frozen at -20 °C. Frozen samples were loaded into the freeze dryer (Cuddons 0610, Blenheim) at -14 °C (shelf temperature), the condenser was set to -30 °C. Once the vacuum was sufficient, approximately 1 millibar, the shelf temperature was adjusted to 20 °C to speed up the sublimation process. The samples were dried under vacuum for approximately 72 hours.

The freeze dried material was ground in a Cyclotec 1093 sample mill (Foss Tecator, Hoganas, Sweden) by passing through a 1 mm screen, and stored ready for use. Subsamples of the freeze dried shoot material were taken for full chemical analysis. Total C was determined by an ignition method using an Elementar (vario MACRO cube), as per the method described for soil samples in Chapter 3.2.2. A typical animal “feed” profile, which included lignin (Tecator Fibertec) and metabolisable energy (ME) (Alderman equation), and condensed tannin content (Butanol-HCl) was analysed by the Nutrition Laboratory, Massey University School of Food and Advanced Technology (MSFAT). The samples were also sent to Hill Laboratories (Hamilton New Zealand) for full elemental analysis which also included lignin and ME content.

Fresh

LT and WC shoot material were collected from the field late afternoon (when temperatures had started to cool). This material was taken immediately back to the laboratory, cut into pieces < 2 cm in length and placed into the prepared incubation chambers.

3.2.4 Incubation preparation

The PVC cylinders (65 mm x 80 mm), packed with soil, were pre-incubated for one week, according to standard procedure, before the application of shoot material at T_0 (Figure 3.1). This conditioned the soil microbial population, reducing any soil disturbance effect (Saggar et al. 2000; Saggar et al. 1981; Alexander, 1961). Each PVC cylinder was placed in an incubation chamber (1.8 L sealed, air-tight preserving jar) with a 30 ml 1M trap to capture emitted CO_2, and a 10 ml H_2O vial to maintain a H_2O saturated atmosphere (Ghatohra, 2012; Saggar et al. 2000; Bardgett & Saggar, 1994; Van Gestel et al. 1993). Extra chambers were also set-up to account for control soil respiration; and for the CO_2 already present in the enclosed space / any CO_2 entry during venting.

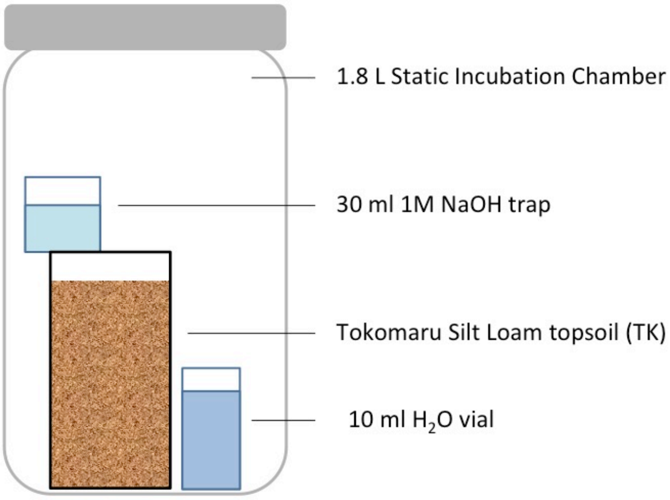


Figure 3.1 Static Incubation Chamber setup. Pre-incubation of Tokomaru Silt Loam (TK) topsoil.

After the pre-incubation period, the treatments were applied: (i) LT and WC; (ii) three different application methods: fresh on surface (F), freeze dried on surface (S), freeze dried and mixed in (M); (iii) applied at the rate of 2.44 mg C. g^{-1} OD soil. The rate of 2.44 mg C. g^{-1} OD soil represents the estimated amount of C in un-utilised pasture shoot material that is returned to the soil as litter each year. Pasture utilisation was estimated at 82 % (Holmes et al. 1987) and total dairy pasture eaten as 15,000 kg DM / ha; the rate of application reflects the unit surface area in this experiment (tube diameter 65 mm, depth 80 mm, weight OD soil 199 g). As the topsoil was analysed to have a rate of 40 mg C. g^{-1} OD soil, the application was concentrated to ensure a rate of C addition that would provide a detectable soil C change. The application was concentrated (~ 10 mg C. g^{-1} OD soil) by applying the litter to the soil surface or mixing into only the top 2.0 cm of the soil (Figure 3.2).

The top 2.0 cm of soil in all cylinders was disturbed (this depth was chosen to provide an easily analysable zone at incubation end). For the mixed (M) applications, the top 2.0 cm of soil was shaken with the freeze dried (FD) material and then repacked to a bulk density of 1.0 g. cm^{-3}. The two surface applications (fresh, F, and freeze dried - ground, S) were distributed evenly over this disturbed soil surface. All treatments were run in triplicate.

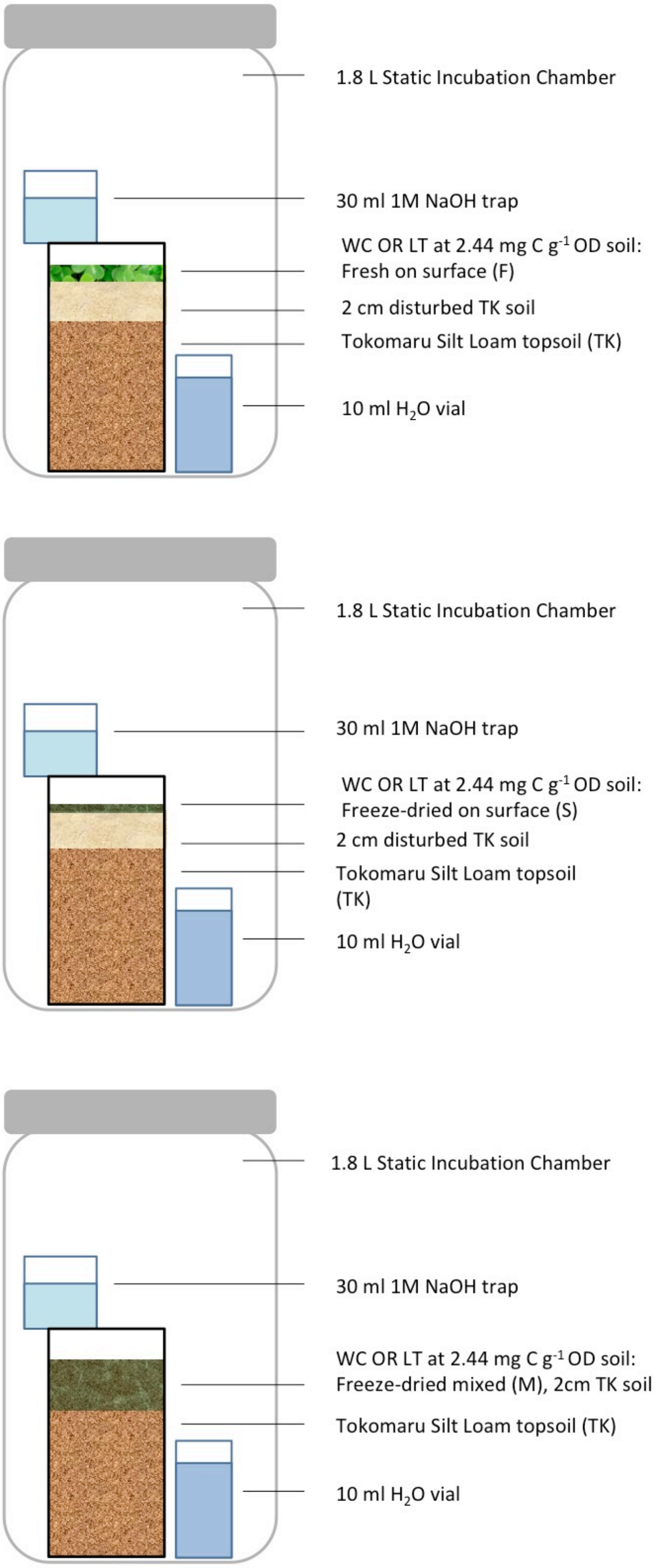


Figure 3.2 Static Incubation Chamber setup. Incubation of Tokomaru Silt Loam (TK) topsoil with addition of lotus (LT) and white clover (WC) shoot material, applied to the soil as fresh (F), surface (S) and mixed in (M) treatments.

The chambers were arranged by blocks and incubated in dark conditions at an average temperature of 21.7 °C (18.3 – 25.0 °C). The soils were at estimated volumetric water content of 0.25 at incubation T_0.

3.2.5 Measurement of CO_2 flux

Static NaOH CO_2-trap chamber method

Soil CO_2 efflux was determined in the sealed chamber headspace by absorbing CO_2 using 30 ml 1M NaOH solution stored in a polypropylene cup. The polypropylene cup was elevated so the surface of the NaOH solution was approximately 5 cm above the soil surface in the centre of the chamber. The chamber headspace volume was 1478 ml. The seal between the chamber top and base was gas-tight.

After every absorption interval the chambers were opened, NaOH solution from each chamber was taken out and stored in a plastic P35 container for titration to calculate the amount of CO_2 emitted during that particular absorption interval. The amount of CO_2 emitted was further divided by the interval duration to calculate the hourly emission rate. The chambers were vented and the alkali traps were initially changed daily (for 40 d) to ensure they were not saturated and the O_2 in the chamber was never exhausted. As the daily rate of emitted CO_2 decreased, the trap change interval was reduced to every 10 d (to ensure sufficient O_2 would be present at all times in the chamber). Each chamber was vented for 5 minutes at each trap change to replenish the O_2 concentration in the incubation chambers. After venting, fresh alkali solutions were placed in each chamber for the next interval, and the procedure was repeated after every absorption interval.

The soil moisture content was maintained just above PWP (ϴ 0.25) by a light application of water (by weight) to the soil surface, when required, at the time of the trap change. Aerobic conditions were maintained at all times, including during the period of peak respiration (T_8 to T_{17}). At no stage did the CO_2 effluxes consume more than 10 % of the O_2 in the incubation chambers (maximum 9 % was used) (Appendix 1).

Total duration of incubation was 125 d (T_{125}). At T_{125} the soil was sliced into 0.5 cm depths, frozen and freeze dried for further analysis. After freeze drying, subsamples were collected from soil slices 0.5 - 3.0 cm and analysed for pH, phosphorus, sulphur, total C and total extractable mineral N (NO_3^- and NH_4^+).

Determination of CO_2 evolved

The total amount of CO_2 absorbed in the NaOH solution was determined by back-titrating a 5.05 ml aliquot of NaOH with standardised 0.2M HCl, after precipitation of any carbonates with a 5.05 ml aliquot of 1M $BaCl_2$. Two drops of phenolphthalein were added as an indicator to determine the endpoint. All the chemicals used were of analytical grade, and solutions were prepared using deionised water. The solution of 0.2M HCl was standardised again Na_2CO_3 as per the method outlined by Lambert et al. (1949).

Based on the titration data, the amount of C emitted, the rate of C emitted (mg C. g^{-1} soil. h^{-1}), and the amount of accumulated C evolved (mg C. g^{-1} OD soil) were determined. Results are expressed on the basis of OD (105 °C) soil weight unless otherwise stated, and are the means of three replicate determinations.

3.2.6 Statistical analysis

Statistical differences between the treatments applied were determined by analysis of variance two-way (ANOVA) using Minitab® 16.1.0. Post hoc analysis was performed using the Duncan and Bonferonii tests at *P = 0. 05*.

3.3 Results

3.3.1 Shoot material characterisation

Lotus (*Lotus pedunculatus*) shoot (LT) had markedly higher condensed tannin (CT) content (8.7 % vs 0.1 %) than white clover (*Trifolium repens* L.) shoot (WC) (Table 3.2).

Of the total CT detected in WC, almost all of it was Protein Bound CT, whereas for LT it was a mixture of Free CT, Protein Bound CT and Fibre Bound CT. These results are similar to those reported by other authors (Harris et al. 1998; Terrill et al. 1992; Barry & Duncan, 1984). WC had a higher metabolisable energy (ME) than LT, and LT had over twice the lignin content of WC. LT varied significantly from WC in Fe, Mn and Zn foliage concentrations. LT had triple the concentration of Mn and twice the concentration of Zn than WC, while WC had twice the concentration of Fe than LT. Other chemical characteristics (i.e. % C, % N, % P, % S, ME) were very similar.

Table 3.2 Chemical composition of freeze dried lotus (LT) and white clover (WC) shoot tissue (mean and standard deviation presented), as measured pre-incubation.

Analysis Type	LT shoot		WC shoot	
	Mean	*SD*	Mean	*SD*
C (%)^{1}	44.7	*0.14*	41.8	*0.050*
N (%)^{1,2}	3.9	*0.059*	3.8	*0.036*
C:N^{1,2}	11.4	-	11.0	-
P (%)^{2}	0.24	*0.021*	0.29	*0.014*
S (%)^{1,2}	0.21	*0.0026*	0.16	*0.0066*
K (%)^{2}	1.7	*0.21*	2.8	*0.0000*
Ca (%)^{2}	0.71	*0.042*	1.1	*0.035*
Mg (%)^{2}	0.32	*0.014*	0.26	*0.014*
Fe (mg. kg^{-1})^{2}	72.0	*3.5*	140.0	*36.1*
Mn (mg. kg^{-1})^{2}	98.0	*2.8*	30.0	*4.2*
Zn (mg. kg^{-1})^{2}	38.0	*4.2*	22.0	*2.8*
CT Total (%)^{3}	8.7	*0.07*	0.10	*0.0000*
Lignin (%)^{2,3}	6.7	*0.82*	2.6	*0.41*
ME (MJ. kg^{-1})^{2,3}	10.6	*0.46*	11.7	*0.32*

Analyses performed by: ^{1} Elementar (vario MACRO cube); ^{2} Hills Laboratory; ^{3} MSFAT. Results presented on the basis of three replicates.

Plant amendments of LT and WC were made at an equivalent rate of 5.49 mg dry matter (DM). g $^{-1}$ oven dried at 105 °C (OD) soil and 5.84 mg DM. g^{-1} OD soil respectively, equating to a rate of 2.44 mg C. g^{-1} OD soil. The difference in DM application meant that 0.216 mg N. g^{-1} OD soil and 0.012 mg S. g^{-1} OD soil were applied to LT treatments while 0.223 mg N. g^{-1} OD soil and 0.009 mg S. g^{-1} OD soil were applied to WC treatments.

3.3.2 Effect of species and management on CO_2 evolution over 125 d

The control (no added amendment) had a steady efflux of CO_2, which was at a consistent rate throughout the 125 d (Figure 3.3). All other treatments showed an increase in microbial activity (respiration rate, CO_2 emission) with the introduction of the plant amendments. Within the first 10 d of amendment all treatments, except lotus fresh (LTF), had reached their peak respiration rate and respiration had started to slow down (Figure 3.4). Microbial activity was slower to start in the LTF treatment and did not reach peak respiration until around 20 d. The initial rate of respiration in the treatments determined the end amount of CO_2-C emitted, i.e. the treatments with the greatest respiration in the first 10 d were the treatments that emitted the most CO_2 over the course of the 125 d. However, LTF was the exception to this; despite a slower increase in respiration rate, elevated respiration occurred over a longer period of time, which meant that it emitted more CO_2 with time than other LT treatments.

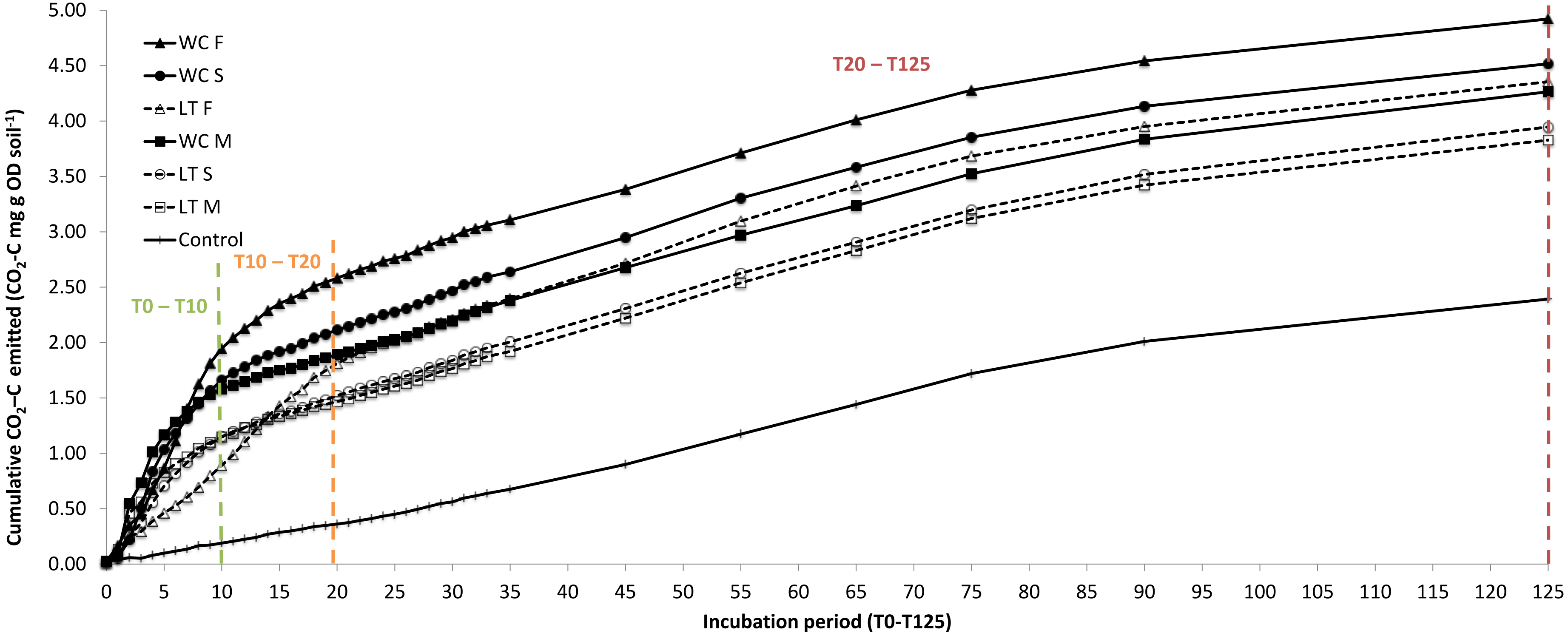


Figure 3.3 The cumulative emission of CO_2-C (mg. g^{-1} OD soil) over 125 d from Tokomaru Silt Loam (TK) topsoil alone (Control) and after addition of lotus (LT) and white clover (WC) shoot material at T_0, applied to the soil as fresh (F), surface (S) and mixed in (M) treatments.

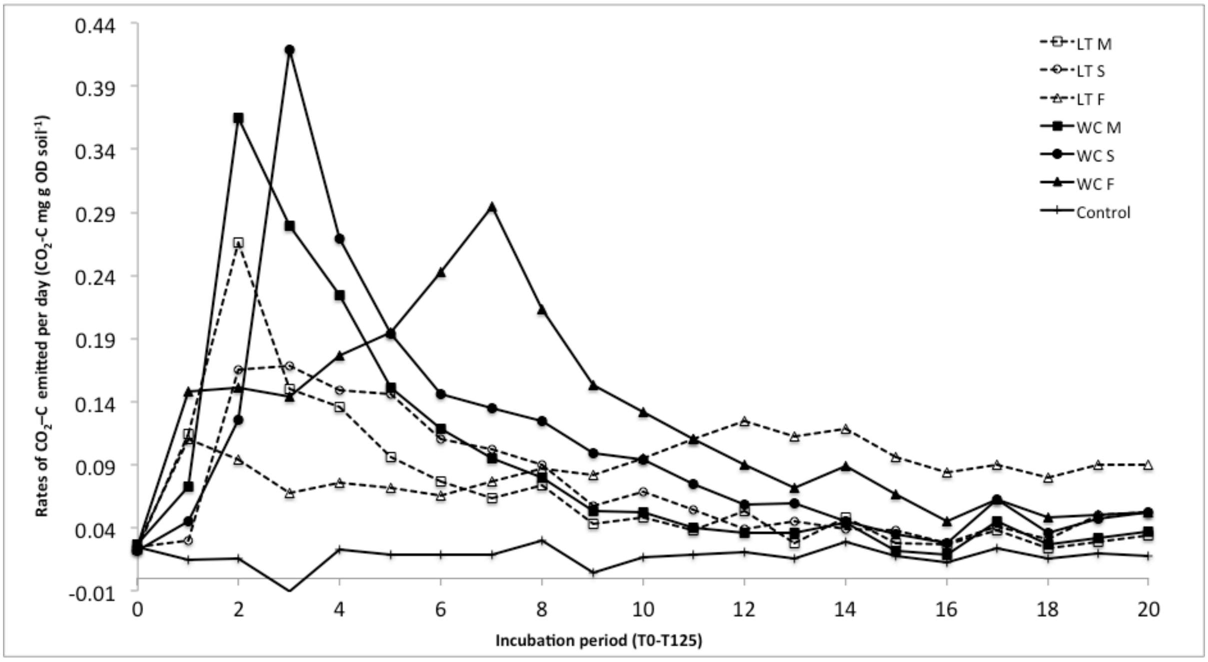


Figure 3.4 Rates of CO_2-C (mg C. g^{-1} soil) emitted per day, for the first 20 d of incubation, from TK soil alone (control) and after addition of LT and WC at T_0, applied as fresh (F), surface (S) and mixed in (M) treatments. The incubation chambers were held at an average temperature of 21.7 °C.

3.3.3 CO_2 evolved incubation end (T_{125})

The analysis of variance in the accumulated amount of CO_2-C evolved showed significant effects of species and / or management of residue but was not replicated at two of the three distinct phases of growth seen during the incubation (Figure 3.3), and over the length of the entire incubation period (T_{125}). Species and management explain 97.68 % of the variance in total accumulated CO_2-C (mg. g^{-1} OD $soil_{T125}$) emitted when control (background) respiration is removed from consideration, with species accounting for 51.7 % of the variation in C emitted and management accounting for 45.7 % (Table 3.3).

Table 3.3 The statistical significance of species, management of residues and treatment replicate on total accumulated CO_2-C loss (mg C. g^{-1} OD soil) from the Tokomaru Silt Loam topsoil (TK), after amendment with lotus (LT) and white clover (WC) shoot material (treatment – control).

Time	Species	Management	Replicate	Species (%)	Management (%)	R-Sq (%)
T_0-T_{125}	P = 0.00	P = 0.00	NS	51.7	45.7	97.7
T_0-T_{10}	P = 0.00	NS	NS	84.8	0.4	85.3
T_{10}-T_{20}	NS	P = 0.00	NS	2.7	84.1	86.8
T_{20}-T_{125}	NS	NS	NS	19.6	15.0	37.3

NS - Not significant, P > 0.05

Within both plant species, the greatest amount of CO_2-C emitted, according to the management treatment applied was: fresh > surface > mixed (Table 3.5, Figure 3.5). All management treatments within LT resulted in a lower amount of C being released than the WC, except in the case of LTF, which overtook WCM emissions in the later stages of decomposition (Figure 3.3). The WCF treatment emitted the greatest and LTM the lowest amount of CO_2-C during the 125 d incubation (Table 3.5). When the accumulated release of CO_2-C (T_0 -T_{125} d) from a treatment minus the control soil is expressed as a percentage of the added plant C, there was 21 - 25 % higher emissions when fresh materials were placed on the soil surface (WCF and LTF) than when dried ground material (WCM and LTM) were mixed into the soil (Table 3.4). The > 100 % value in the WCF treatment may have resulted from added plant C “priming” the accelerated decomposition of SOM – C.

Table 3.4 **Average total CO_2-C emitted, as a percentage of C added, from the Tokomaru Silt Loam topsoil (TK) after amendment with lotus (LT) and white clover (WC) shoot material, from T_0 to T_{125}.**

Treatment	CO_2-C $\sum_{T125}^{T0} \mathit{control}$
WCF	103.7 % ± 1.8 %
WCS	87.1 % ± 3.2 %
LTF	80.4 % ± 3.8 %
WCM	76.7 % ± 1.3 %
LTS	63.7 % ± 1.8 %
LTM	58.8 % ± 2.3 %

Each value in the table represents the mean of three replicates with standard deviation (±).

Table 3.5 **Accumulated CO_2-C emitted (mg. g^{-1} OD soil) from the Tokomaru Silt Loam topsoil (TK), after amendment with lotus (LT) and white clover (WC) shoot material over the entire incubation period T_0 to T_{125}.**

Treatment	Cumulative CO_2-C (mg. g^{-1} OD soil)
WCF	4.9 ± 0.04
WCS	4.5 ± 0.08
LTF	4.4 ± 0.09
WCM	4.3 ± 0.03
LTS	3.9 ± 0.04
LTM	3.8 ± 0.06
Control	2.4 ± 0.02

Each value in the table represents the mean of three replicates with standard deviations (±).

Within each management practice, LT emitted less CO_2-C than WC resulting in a higher amount retained in the soil. The final amount of C released from the WCF treatment was more than the total amount of C added in the shoot litter amendment (Figure 3.5).

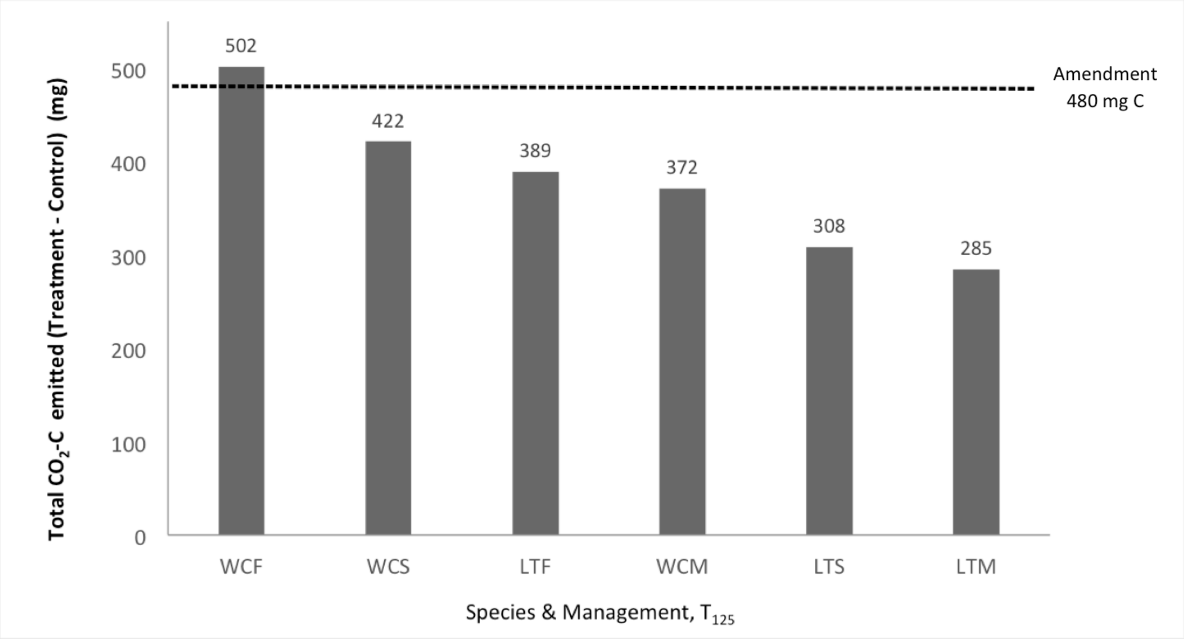


Figure 3.5 Cumulative CO_2-C – Control (mg C) emitted from the Tokomaru Silt Loam topsoil (TK) at T_{125}, after amendment with lotus (LT) and white clover (WC) shoot material, applied to the soil as fresh (F), surface (S) and mixed in (M) treatments.

The rate of CO_2-C evolution accelerated rapidly when the plant litter was applied to all treatments (Figure 3.3). There was no apparent "lag phase" but three phases of growth can be inferred from the pattern of CO_2-C evolved:

1. Rapid period of decomposition with no apparent limitation by substrate accessibility (T_0-T_{10})
2. Substrate limited (T_{10}-T_{20})
3. Return to basal level of CO_2 respiration (T_{20}-T_{125})

3.3.4 Phase I: Rapid period of decomposition with minimal limitation by substrate accessibility, T_0 – T_{10}

The rapid period is characterised by decomposition rates that ranged from 0.030 and 0.418 mg CO_2-C. g soil^{-1}. d^{-1} at their peak. At the end of the rapid decomposition phase (T_{10}) the effect of species was statistically significant ($P < 0.05$), accounting for 84.8 % of the variance but the effect of management was not yet significant ($P > 0.05$) (Table 3.3).

The CO_2 emission from the LTF treatment behaved as though it was substrate limited from T_0, with no rapid decomposition phase exhibited. At the end of the rapid decomposition phase the differing treatments had emitted 28.7 to 72.0 % of total C added (Table 3.6). This indicates that decomposition in the first 10 d of incubation will greatly influence the total C lost.

Table 3.6 Average total CO_2-C emitted as a percentage of C added from the Tokomaru Silt Loam topsoil (TK) with lotus (LT) and white clover (WC) shoot material, from T_0 to T_{10}.

Treatment	$CO_2\text{-C} \sum_{T10}^{T0} - control$
WCF	72.0 % ± 0.7 %
WCS	60.3 % ± 0.7 %
LTF	28.7 % ± 0.3 %
WCM	57.1 % ± 0.6 %
LTS	39.4 % ± 0.4 %
LTM	39.3 % ± 0.1 %

Each value in the table represents the mean of three replicates with standard deviation (±).

3.3.5 Phase II: Substrate limited, $T_{10} – T_{20}$

From T_{10} to T_{20} the rate of CO_2 released from all treatments declined to a slower rate than those seen in the rapid decomposition phase, appearing to be substrate limited. Management significantly ($P < 0.05$) influenced the amount of CO_2 evolved in the 10 d period (T_{10} -T_{20}), accounting for a total of 84.1 % of the variance (Table 3.3).

At the end of the substrate limited phase the differing treatments had emitted 45.3 to 91.0 % of total C added (Table 3.6 and Table 3.7). The WCF treatment emitted 35 % more CO_2-C than the LTF treatment.

Table 3.7 Average total CO_2-C emitted as a percentage of C added from the Tokomaru Silt Loam topsoil (TK) with lotus (LT) and white clover (WC) shoot material, from T_{10} to T_{20}.

Treatment	$CO_2\text{-C} \sum_{T20}^{T10} - control$
WCF	18.9 % ± 0.1 %
WCS	11.5 % ± 0.8 %
LTF	30.7 % ± 0.8 %
WCM	5.6 % ± 0.2 %
LTS	8.2 % ± 0.6 %
LTM	6.0 % ± 0.5 %

Each value in the table represents the mean of three replicates with standard deviation (±).

3.3.6 Phase III: return to basal level of CO_2 emissions, $T_{20} – T_{125}$

Between T_{20} and T_{125} the CO_2 efflux from all treatments were similar. Species and management were both no longer statistically significant ($P < 0.05$) (Table 3.3).

Although the rate of decomposition for both species and all treatments are initially different, the final rate (gradient) of CO_2 emissions in all instances is similar to that of the control soil (approximately 0.020 mg CO_2-C. g^{-1} soil. d^{-1}). Rates less than those in the substrate limited phase appear to be this basal respiration. During Phase III, the accumulated CO_2-C emissions above the control soil were equivalent to less than 20 % of the added plant C (Table 3.8). Finally, a stacked column graph (Figure 3.6) can be used to illustrate clearly how the LTF treatment has a markedly different decomposition pattern to the other treatments.

Table 3.8 Average total CO_2-C emitted as a percentage of C added from the Tokomaru Silt Loam topsoil (TK) with lotus (LT) and white clover (WC) shoot material, from T_{20} to T_{125}.

Treatment	$CO_2\text{-C} \sum_{T125}^{T20} - \mathit{control}$
WCF	12.7 % ± 1.2 %
WCS	15.3 % ± 2.4 %
LTF	21.0 % ± 2.9 %
WCM	14.1 % ± 1.5 %
LTS	16.1 % ± 1.3 %
LTM	13.5 % ± 2.8 %

Each value in the table represents the mean of three replicates with standard deviation (±).

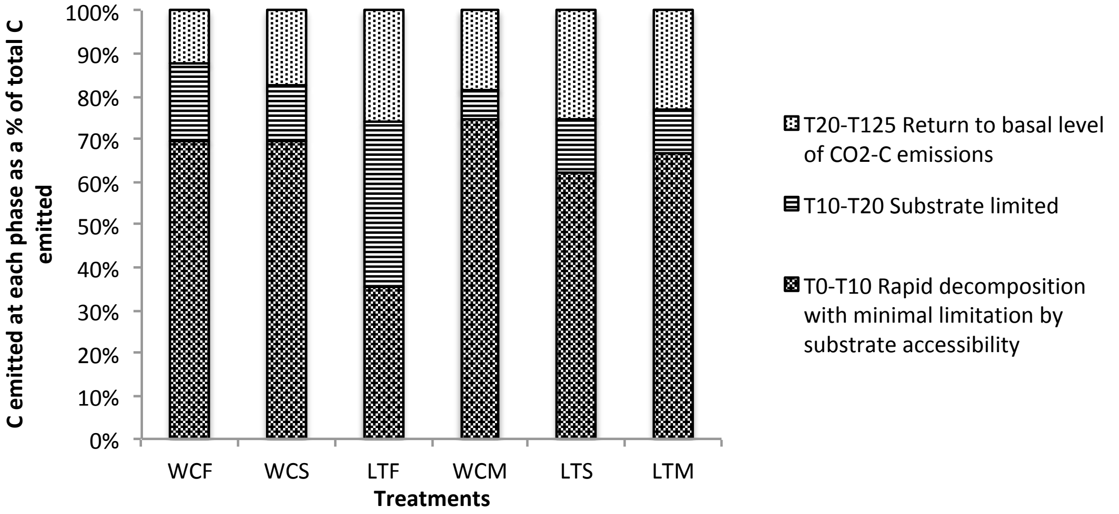


Figure 3.6 Total cumulative CO_2-C emitted at each phase of growth as a percentage of the total cumulative CO_2-C emitted by T_{125} (less control).

3.3.7 Visual observations support the difference in initial emissions

Fungal colonisation was visible on the WCS and LTS treatments from T_2, with the population visually decreasing after T_{33} (Figure 3.7). Both fresh treatments also showed the presence of fungal colonisation – in the WC 5 d after amendment (T_4), but not until 10 d after amendment (T_9) in the LT. There was no visible fungi colonisation for the mixed treatments.

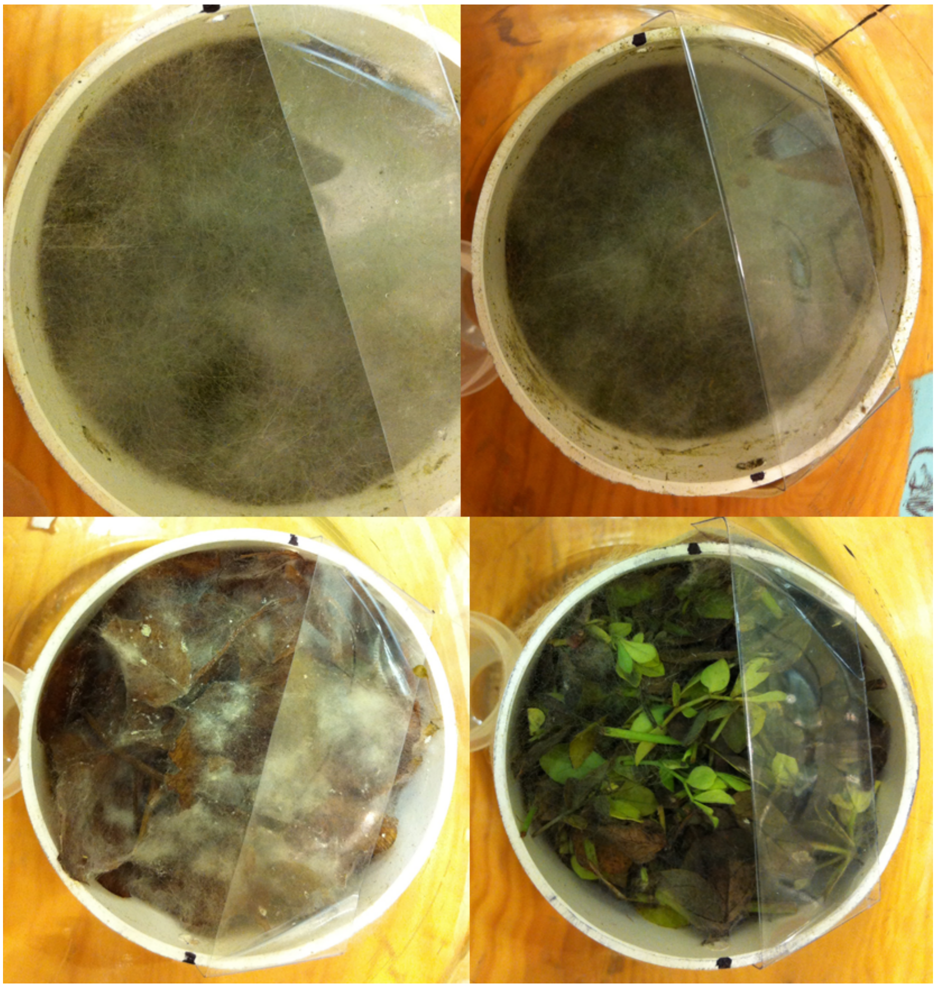


Figure 3.7 Fungal colonisation of WCS (top left) and LTS (top right) at T_3. Fungal colonisation of WCF (bottom left) and LTF (bottom right) at T_9 and T_{11} respectively.

3.3.8 Post incubation soil analysis

Soil pH were 0.3 – 0.7 units lower than in the initial T_0 sample (pH 5.5 – 5.4, Table 3.1 and Table 3.9). Soil pH had decreased from control in the two fresh treatments while it had increased in the mixed and surface treatments. Extractable phosphate and sulphate values were slightly higher than the T_0 values (42 µg P. g^{-1} soil and 16 µg S. g^{-1} soil, respectively) but the treatments had minor effects on the values with small increases in the LTS and WCF treatments for P and in the LTF and WCF treatments for S.

Table 3.9 Soil pH, Total C, Olsen P, Soluble SO_4-S and Total extractable mineral N in the top 3 cm of soil, post incubation (expressed on an oven dry basis) and the Control soil at T_0 (prior to incubation).

Treatment	Total C (mg C. g^{-1} OD soil)		pH		Olsen P (ug P. g^{-1} OD soil)	
	Mean	*SD*	Mean	*SD*	Mean	*SD*
LTM	42.0	*1.0*	5.1	*0.05*	49.3	*2.5*
LTS	41.2	*1.1*	5.1	*0.02*	55.3	*5.1*
LTF	39.9	*0.8*	4.8	*0.02*	49.2	*1.8*
WCM	39.7	*0.2*	5.1	*0.09*	48.1	*2.3*
WCS	39.1	*0.4*	5.0	*0.02*	48.7	*1.7*
WCF	39.2	*0.5*	4.7	*0.06*	51.3	*1.0*
Control	38.3	*0.4*	5.0	*0.01*	49.5	*5.1*
Time 0	40.5	*0.6*	5.4	*0.02*	40.9	*1.2*

Treatment	Ext. SO_4^{2-} (ug S. g^{-1} OD soil)		Ext. Min. N (ug N. g^{-1} OD soil)			
			Ext. NO_3		Ext. NH_4	
	Mean	*SD*	Mean	*SD*	Mean	*SD*
LTM	15.9	*4.5*	385	*8.2*	58	*8.2*
LTS	16.3	*1.5*	391	*12.7*	65	*4.8*
LTF	20.3	*3.7*	411	*15.6*	65	*9.0*
WCM	14.7	*0.9*	423	*29.6*	67	*14.4*
WCS	18.2	*2.8*	435	*21.0*	71	*9.3*
WCF	21.6	*0.3*	462	*10.1*	51	*20.6*
Control	15.4	*0.2*	333	*11.4*	35	*4.1*
Time 0	7.9	*0.5*	113	*4.0*	29	*2.5*

Calculating CO_2 loss by soil analysis at T_{125} is difficult to achieve as the 2.44 mg C added per g soil (only 6 % of total soil C) is difficult to detect. C analysis had a standard deviation ranging from 1 – 2 % of the mean. This deviation was 0.4 – 0.8 mg C. g^{-1} soil equivalent to 16 – 32 % of the amendment C, which meant that estimating differences in the residual amount of amendment C between treatments was inaccurate. In addition, the difference in soil C in the top 3 cm between T_0 and the incubated Control indicated that approximately 2 mg C. g^{-1} soil of soil derived C had been mineralised during the incubation. The soil in the whole 8 cm column would have been a source of the CO_2-C measured by NaOH trap, therefore, a comparison between the C evolved as CO_2 and the difference in soil C in the T_0 minus Control samples was not calculated and reported.

The plant material added had a C:N ratio that would suggest mineralisation was to be expected (C:N < 12, Table 3.2). Vigil & Kissel (1991) indicate that residues with a C:N ratio less than 40 and N concentration greater than 2 % are likely to mineralise and release N.

A significant amount of mineralisation (Table 3.9) occurred over the decomposition period (125 d), with 86 – 90 % of total N recovered as NO_3^- (even in the control soils). As the majority of N extracted was present as NO_3^-, this is a strong indication that the incubation was an aerobic process. 10 – 14 % of the N recovered was present as NH_4^+. Over the incubation period more C was lost than N, indicating that the C:N ratio would have decreased over the incubation period. The N lost was seen as an accumulation in mineral N.

Approximately a quarter of the N added was recovered as mineral N. Only the top 3.0 cm of the soil column (6.0 cm deep) was sampled. It was assumed that because NO_3^- is mobile, the N unaccounted for had moved to lower sections of soil that were not sampled. A small amount of N may have undergone denitrification, but it was not expected to be significant due to the aerobic nature of the incubation. Soil gravimetric water contents were maintained at 0.33 g. g^{-1} dry weight soil (< 70 % FC). It was calculated that less than 9 % of O_2 in the incubation chambers was consumed by CO_2 effluxes at any stage. Incubations with readily decomposable litter in soils approaching field capacity would need to consider the possibility of anaerobic fermentation and CH_4 emission.

The LTM treatment emitted 59 % C and had the lowest amount of mineral N present in the soil column at incubation end whereas the WCF treatment emitted 104 % C and had the highest mineral N. Nitrate is appearing in proportion to the amount of CO_2-C evolved, but it is not being quantitatively recovered as it is assumedly lower in the profile than the depth of soil sampled. At this stage further analysis of NO_3^- was not pursued due to the costs of additional sampling. Oxidation of organic N and S to NO_3^- and SO_4^{2-} are acidifying reactions (Bolan & Hedley, 2003), and treatments with higher concentrations of both extractable NO_3^- and SO_4^{2-} - (e.g. WCF, Table 3.9) had the lowest soil pH values.

The idea of restricting the sampling to the top 3.0 cm was that this includes the 2.0 cm zone into which amendments were mixed, and magnifies the C addition with respect to the background soil C concentration. The analysis was still unsatisfactory: CO_2 evolved can be measured more sensitively than the change in total soil C and N mineralisation at incubation end. Error in correlating sampled C and N residues was suspected. There are two possible reasons: 1. The FD sampled soils were not completely dry; or 2. The soil placed in the incubation chambers at T_0, was dryer than expected, given the water content calculated from sampled soil.

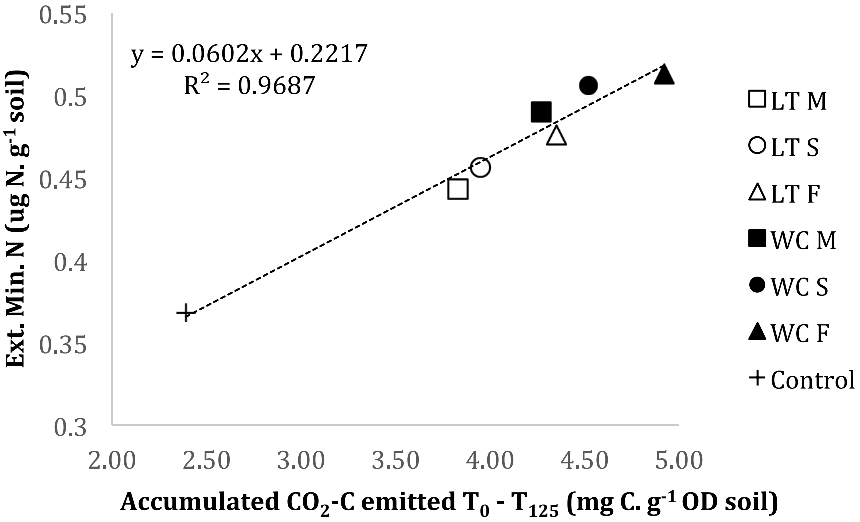


Figure 3.8 The relationship between accumulated CO_2-C (mg. g^{-1} OD soil) emitted T_0 - T_{125} and recovered extractable mineral N (ug N. g^{-1} soil) T_{125} across all treatments: LT and WC shoot material applied to the soil as fresh (F), surface (S) and mixed in (M) treatments.

There is a consistent relationship between the amount of CO_2-C evolved and N mineralisation (Figure 3.8). A quantitative relationship cannot be reported as the CO_2-C measured is evolved from the whole soil column whereas the mineral N measured is from only the top 3.0 cm of soil. It is possible that there may have been less decomposition at depth (which is the basis of the inversion tillage theory); as even in a small column there is a delay in O_2 diffusion down the column and CO_2 diffusion up the column.

3.4 Discussion

The incubation has produced very interesting results showing that plant species and how the plant litter is incorporated into, or placed on, the soil (management) have very significant effects on the extent of decomposition and even the extent to which the added material primes (accelerates) the "basal" decomposition rate of native SOM. Over the 125 d of the incubation, the extent of "litter" decomposition in this experiment is consistent with other incubation studies. In a 112 d incubation study (22 °C with soils held at 65 % of field capacity, FC), Zunino et al (1982) found that 55 % - 62 % of added ^{14}C (as wheat straw) was evolved as $^{14}CO_2$ from non-allophanic soils ranging in texture from sandy loams to clays. These results are similar to that seen in the LTM and LTS treatments at T_{125} (Figure 3.3; Table 3.4); however the LTF and WC treatments decomposed to a greater extent, similar to the results of Walela et al. (2014) who found that in a 105 d incubation using an Alfisol topsoil at 60 % FC and 25 ^{0}C with added mineral N, 76.5 %, 80.7 % and 82.3 % of native pastures standing dead biomass, oat straw and oat roots decomposed.

Similarly, as found by Zunino et al (1982) and other researchers (e.g. van Gestel et al. 1993 and Walela et al. 2014), much of the decomposition occurs in the first month (T_{20}) (Figure 3.3, Table 3.6 & Table 3.7) and the differences between plant species effect on the rate of CO_2 evolution are more distinguishable (Figure 3.3) in this period. In the experiment of Zunino et al (1982) they found that smaller molecules glucose, cellulose and dextrose, were decomposed more rapidly than wheat straw and microbial cells.

3.4.1 Effect of species

The most significant effect on the short-term rate of decomposition and the final extent of decomposition was plant species with 85 % of the variation in the rate of CO_2-C evolved in the first 10 d being accounted for by the litter being WC or LT (Table 3.3). Within each management practice, LT released 18 - 24 % less CO_2-C than WC resulting in a higher amount retained in the soil. These results are consistent with the initial hypothesis that the high tannin and lignin content of LT compared to WC would influence the rate of decomposition in soil.

The lower amount of cumulative CO_2-C evolved by the LT was consistent with higher residual soil C (Table 3.9) at T_{125}. Demonstrating a significant change in soil C content is difficult because the amount of C added in the treatments (2.44 mg C. g^{-1} OD soil) makes up only 6 % of the native soil C content (40 mg C. g^{-1} OD soil), although the application was concentrated to ensure a rate of C addition that would provide a detectable soil C change (~ 10 mg C. g^{-1} OD soil). Whilst the results support the hypothesis that a "high tannin" pasture species will decrease the total amount of shoot litter decomposed in soil compared to that of a comparable "neglible tannin" pasture species, the results do not conclusively show cause and effect. Other properties of the two litters e.g. higher lignin and Mn content in the LT may be responsible (this will be discussed more fully later in the thesis).

There are two theories as to why tannin content may influent litter decomposition rates: 1. That the tannin-rich shoot litter is more recalcitrant and resistant to decomposition (Nierop et al. 2006; Madritch et al. 2007; Schweitzer et al. 2008); 2. That the tannin-rich shoot litter decomposes readily but forms secondary microbial products that are themselves more resistant to decomposition (Buurman et al. 2007). Existing literature reporting differences in soil respiration rates caused by different pasture legume litter could not be found at the time of writing. The wider effect of vegetation type on litter decomposition rates is well documented for forest systems (e.g. Bonifacio et al. 2008) and for vegetation with different lignin content including oat straw, native pasture (standing dead biomass) and forest litter and roots (e.g. Walela et al. 2014). The reduced respiration rates for LT (Table 3.4), with a higher lignin: nitrogen ratio than WC in all management systems compared to WC (Table 3.2), is entirely consistent with the results of Walela et al. (2014), who found that the initial lignin:nitrogen ratio of the litter, native woodland leaves, senesced native pasture and oat straw, influenced both the primary decomposition phase and the % C remaining after a 105 d laboratory incubation varying from 43.3 % for native woodland leaves to 19.3 % for oat straw.

3.4.2 Effect of method of application

The method of application of shoot litter to the incubation had an obvious effect on the amount of CO_2-C lost from the different treatments, with results consistent between species. Enriquez et al. (1993) reviewed 256 published reports on decomposition rates of different plant materials and came to the conclusion that moist plant material decomposes substantially faster than dry material, provided nutrient concentrations were similar. In this study, within both plant species, the total amount of cumulative CO_2-C released according to the management treatment applied was: fresh > surface > mixed (Figure 3.3; Table 3.5) which agrees with the conclusions of Enriquez et al. (1993). For the WCF treatment (fresh - surface application) more than

100 % of the added C was respired by T_{125}, as opposed to only 87.0 % from the WCS (freeze dried - surface application) treatment and 76.4 % from the WCM treatment (freeze dried - mixed with soil). For the LTF treatment 80.1 % of added C was respired, while LTS and LTM emitted 63.2 % and 58.5 % respectively.

A study by van Schreven (1968) agrees with the results seen in this incubation, finding that after 12 weeks less CO_2 was produced from incubated dried and ground plant material than from the fresh finely chopped plant material (both a mixture of *Lolium perenne* L., *Festuca pratensis* Huds., *Phleum pratense* L. and *Trifolium repens* L.), with most of the difference being seen in the first 3 weeks. This study also found that the net production of mineral nitrogen was much less in the soil samples (taken from a peaty region in Noordoostpolder, Netherlands) where dried plant material was added; with the amount dependent on both pre-treament of the soil (fresh or dried) and pre-treatment of the plant material (fresh or dried).

With both plant species, the total amount of cumulative CO_2-C released for the freeze dried treatments was dried - surface > dried - mixed (Figure 3.3; Table 3.5). This can be related to the more accessible material in the surface and fresh treatments, making the material more disposed to colonisation by fungi, than the mixed treatment (as visually seen from T_2 to T_{33}), and this colonisation contributed to the higher CO_2 loss from the surface treatments. The mixed treatment has more initial contact with soil and therefore may be less accessible and lead to more chemical stabilisation of both primary organic matter and secondary decomposition products by the soil.

3.4.3 Evidence of Priming

The final amount of CO_2-C released from the WCF treatment was more than the total amount of C added in the shoot litter amendment. The CO_2-C release pattern (Figure 3.3) suggests that the residue was exhausted between T_{45} and T_{55}**.** This indicates that the addition of organic material to soil results in a small amount of increased decomposition of native SOM ("priming effect") (Sorensen, 1974; Kuzyakov 2002; Kuzyakov 2010; Mazzilli et al. 2014), as compared to control soils without addition. According to Kuzyakov (2010) a priming effect is any short-term change in SOM turnover intensity (e.g. decomposition), which can be caused by a change in a number of different soil-environmental and plant-mediated factors, including the state of soil fertility (Jenkinson et al. 1985; Kuzyakov et al. 2000), and plant cultivation and subsequent plant root growth (rhizosphere priming) (Kuzyakov, 2002; 2010). Kuzyakov (2002, 2010) further suggest that there are seven possible mechanisms alone that control rhizosphere priming, which are related to fresh, living root.

A priming effect is confirmed in Figure 3.5 which shows that a small amount of C has been released over and above that which is expected from the native SOM without shoot litter addition. SOM priming is thought to be controlled by the size and activity of microbial populations in response to the addition of accessible decomposable material – possibly due to either an abundant production of enzymes or due to a shift in the microbial population (Fontaine et al. 2011), as well as a result of direct physical (mechanical soil disturbance) and chemical effects (pH, nutrient availability, etc (Hamer & Marschner, 2005; Kuzyakov, 2010)); however little is known about its many underlying mechanisms (Kuzyakov et al. 2000; Brant et al. 2006).

In practical farming situations, the cultivation of plants may stimulate SOM decomposition. Kuzyakov (2002) commented that the growth of plants (rhizosphere effects and litter contributions) may either increase (3 – 5 fold) or, occasionally, decrease (10 - 30 %) the rate of microbial SOM decomposition compared to that in root-free soil; with an acceleration of SOM turnover most commonly observed.

The total CO_2-C emitted here would seem to support Kuzyakov's observation of an increased rate of SOM turnover. The control data may therefore underestimate the amount of natural C being released by the soil under plant amendment and subsequently not fully account for the priming seen. Further study (i.e. an incubation using C^{14} analysis) may help determine where the CO_2 has evolved from (i.e. native SOM or added C).

However, the rate in the period T_{20} to T_{125} of CO_2-C emissions for all treatments is similar to that of the basal respiration of the control soil which indicates that the rate of release of CO_2-C is not that different to that which would be expected from the native SOM, without shoot addition (natural state). This indicates that it is likely that all treatments exhibit similar, but small, priming effects, although this will need further investigation.

3.4.4 Stages of decomposition

In the results section, C emission rates were categorised simply in terms of likely substrate availability. In terms of microbial ecology the addition of fresh substrate to soil has been shown to cause a response from the soil microflora, resulting from the changes in substrate utilisation by bacterial and fungal populations and nutrient availability. The activity response to substrate addition to soil has been found to follow a multiphasic curve with slow initial evolution followed by a stage of rapid release – with four more-or-less distinct phases of microbial growth, including predation by secondary decomposers (Blum & Mills, 1991; Hu & van Bruggen, 1997; Jurgens & Sala, 2000). This multiphasic curve may be the result of two mechanisms:

(i) The microbial community structure changes, due to microbial succession, from a bacteria-dominated community to a fungi-dominated community, or vice-versa.

(ii) Bacterial populations with low C requirements become dominant, as decomposition progresses and C becomes scarce.

For example, bacteria and fungi have been shown to have a dominant role during the initial and secondary stages of cellulose decomposition, respectively (Hu & van Bruggen, 1997); the initial phase due to bacteria, and the second phase due to fungi. In terms of microbial succession, it has been suggested that copiotrophic bacteria populations may increase on cellulose addition to soil because of their high growth rates; and then as available C is depleted oligotrophs become more competitive and deplete C compounds to inaccessible levels for copiotrophic bacteria; which results in the release of fungi-activated cellulolytic enzymes and a dominance of fungi-cellulose decomposition (Hu & van Bruggen, 1997). In N-limiting conditions, fungal populations may also hold a competitive advantage over bacterial populations due to their high N use efficiency and relatively high C:N ratios of fungal biomass.

Background soil respiration (slow continuing decomposition of residues in soil sample)

Air drying and subsequent rewetting and incubation of soils strongly influences microbial biomass and activity (Van Gestel et al. 1993 and references therein), which has been demonstrated in many studies through the increase in CO_2 evolution (increased C mineralised), compared to continuously moist control soils (Birch, 1958; Soulides & Allison, 1961; Sorensen, 1974). It has been suggested that this flush of CO_2 from the soil is partly due to the release of amino acids and other compounds from humic material; and partly due to the death of a portion of soil microorganisms, rendering substrates available for the surviving soil microorganisms (Stevenson, 1956; Soulides & Allison, 1961; Jenkinson, 1966; Sorensen, 1974, 1983). For this reason, in the present study, the soils were incubated for a period of 7 d prior to the addition of plant material. The data in this experiment suggests that pre-incubation avoided a "flush" caused by rewetting the soil, and the soil had returned to a level of activity similar to the control soil by the time the plant materials were added.

Increased readily available substrate added then lag phase

Any increase in the rate of CO_2 production, as a response to the addition of substrate, can be interpreted as an increase in microbial biomass (Anderson & Domsch, 1975; Smith et al. 1985). Many studies have reported slow, initial CO_2 evolution (otherwise termed the lag phase), after the addition of a utilisable substrate to a slow-growing or steady-state microbial population, followed by a substantial increase in endogenous metabolism resulting in a stage of rapid CO_2 release (Ribbons & Dawes, 1963; Smith et al. 1985; Hu & van Bruggen, 1997). Excluding the LTF treatment, there was no lag phase evident in the results presented herein (Figure 3.3 and 3.4).

Successful primary colonisers exhibit Log (logarithmic or exponential) phase growth

The fastest rates of CO_2-C emission (0.030 to 0.418 mg CO_2-C. g^{-1} soil. d^{-1}) for both species and all treatments were between T_1 and T_{10}, and were clearly associated with the addition of shoot litter at T_0 (Figure 3.3). This is consistent with incubation studies by Zunino et al. (1982) who reported ~ 30 % of added C evolved as CO_2 within the first two weeks in sandy loam soils, ~ 60 % by week 16 (i.e the fastest rates were within the first two weeks after addition of wheat straw litter to those soils). All LT treatments exhibited an immediate increase in rates of CO_2-C emitted for the first 2 d after addition of plant material (0.030 to 0.267 mg CO_2-C. g^{-1} soil. d^{-1}), at which point the rate of CO_2-C release declined to a lower rate of exponential increase. Likewise, the WC treatments also had an initial flush in rate (0.072 to 0.418 mg CO_2-C. g^{-1} soil. d^{-1}) which then dropped to a relatively lower rate of CO_2-C release (Figure 3.3 and Figure 3.4). There are potentially two scenarios as to why no lag phase was seen – either it was too short to be seen within the 24 hour period of measurement; or alternatively resident microorganism activity was limited only by substrate, and immediately responded to the addition of substrate with increased growth and activity. It may be that the initial increase seen in CO_2-C release was due to the release of free sugars by leaching from the shoot litter, before cell lysis and the release of more resistant compounds including lignin, and tannins in the LT (Gessner, 1991). The consistently slower rate of CO_2-C release in the LT species may be the result of the release of tannins complexing with other plant compounds and their impact on microbial activity (Waghorn et al. 1990; Widdup et al. 2004)).

The delay in the peak of C emission rates for the fresh surface applied treatments (T_2 to T_7 for WC and T_2 to T_{12} for LT) compared to immediate peak in the other treatments (Figure 3.4) may be explained by accessibility of substrate, and the colonisation strategy of micro-organisms. Hu & van Bruggen (1997) suggested that the initial phase of decomposition was due to the activity of bacterial populations, and the second phase to fungal populations. This theory could explain the initial two day flush in CO_2-C release in all treatments after plant amendment, and would also tie in with the theory of initial release and use of free sugars followed by the release of more resistant compounds which would require fungal intervention to access and decompose (Gessner, 1991). Shi et al. (2013) proposed a theory which would suggest that in the more slowly decomposing LT residues, initial respiration is lower meaning microbial biomass is building up more slowly than in the WC residues (which build up rapidly but then declines because of lack of substrate, followed by turnover of a proportion of the microbial biomass). However, this respiration in the LT treatments will be sustained for longer as more substrate-C is left for microbial growth in later stages of the experiment (as seen visually in the LTF treatment, Figure 3.3).

Predation and a succession of secondary decompositions with declining readily available substrate

As mentioned in detail above, it has been suggested that substrate availability will determine microbial succession – whether the shift is within the microbial community towards a dominance of species more suited to the conditions (i.e. from copiotrophic bacteria at high C availability to oligotrophs at lower C availability); or whether it is a shift in the community composition itself (i.e. from a bacteria-dominated community to a fungi-dominated community) (Hu & van Bruggen, 1997; Jurgens & Sala, 2000). However, the microbial community composition was not specifically analysed so it is impossible to make comment on what succession may have occurred over the incubation period, except to comment on the visual observations made.

Fungi colonisation was first visible on the surface treatments 2 d after plant amendment (T_2), and became visible on the fresh treatments at T_5 for the WC and T_{10} for the LT; with the population visually decreasing on all treatments after T_{33}. Beare et al. (1992) found that surface litter of rye (*Secale cereal*) supported a higher ratio of fungal to bacterial biomass and fungivore to bacterivore biomass (ratios were 2.7 and 2.2 times greater than buried litter, respectively), by the end of their 10 month study period (Beare et al. 1992); with exclusion experiments indicating that fungi had a greater influence over surface litter decomposition, while bacteria were more important in buried litter decomposition. This study visually agrees with the findings by Beare et al. (1992), as the dried - mixed treatments were the only treatments that did not exhibit surface fungal growth. However, what Beare et al. (1992) also found, was that the buried litter resulted in a greater abundance and biomass of all microbial and faunal groups compared to that of the surface litter. Both that study and this implies that the placement of litter will strongly influence microbial community structure, and therefore the resulting rates / timing of decomposition.

These results also suggest that the use of fresh litter in incubation studies may give greater insight into the mechanisms behind C stabilisation compared to that of incorporated litter - not on the basis of total CO_2-C emitted, but in the type of residue which remains in the soil. We propose that the stabilisation mechanism for soil C may be a result of the type of decomposer community, on the basis that surface litter has been shown to give off slower rates of CO_2-C than that of buried litter (even if only initially), and had a tendency towards fungal-dominated decomposer communities. Previous research has shown that fungal colonisation leads to an increase in microbial protein which is considered more recalcitrant than plant protein (i.e. chitin and glomalin) (Wright & Upadhyaya, 1998); fungal products have been linked to soil aggregate stability (Wright & Upadhyaya, 1998); while Dungait et al. (2012a) has proposed that physical protection (i.e. aggregation) will lead to stabilisation of SOM in soil; and Buurman et al. (2007) has provided evidence of stabilisation of microbial by-products. If we wish to simulate actual scenarios of what would occur in the field as a result of shoot litter fall as grazing residues, then fresh surface litter will provide the most insight into the type of microorganism population and likely microbial products that will form. This will allow us to investigate the relative recalcitrance of the products formed and assess their likely contribution to C stabilisation.

Return to "basal" level of soil respiration

Although the rate of decomposition for all treatments is initially different, they return to a rate of release similar to that of the control soil from T_{25} (~ 0.018 mg CO_2-C. g^{-1} soil. d^{-1}) (Figure 3.3). Shi et al. (2013) reported a control soil (basal) respiration rate (in a 2 mm sieved sandy clay loam soil) of 0.014 mg CO_2-C. g^{-1} soil. d^{-1}. It is not clear whether Shi et al. (2013) have presented these results per g moist soil (soil at 50 %

WHC) or per g dry soil. If expressed on a dry soil basis then the basal respiration rates between the two studies / soils are comparable.

The influence of a rapid initial decomposition phase on total CO_2-C emitted

Shi et al. (2013) reported respiration rates during the incubation of shoots and roots of grasses and cereals. They found that rates in the first 10 d determine the end difference in cumulative respiration between treatments; with longer incubation times having no significant effect on the differences seen. This incubation clearly follows this trend with all treatments, except LTF, having emitted 62 to 75 % of the total C to be emitted by T_{125} (Table 3.4 and Table 3.6). The differences were clearly established in the rapid decomposition phase, with only LTF establishing a difference in the substrate limited phase (the decomposition curve indicating that, although respiring at a slower rate, that the LTF treatment may not be limited by substrate until after T_{15}). LTF emitted only 36 % over the first 10 d, but by 20 d was within the treatment range of 74 to 88 % of the total C emitted over T_{125}. It is possible that secondary decomposition may be responsible for the differences seen in this fresh treatment, with fungal colonisation only visually starting 5 d after significant colonisation in the WCF treatment (Figure 3.7), although this has not been proven.

At the end of the rapid decomposition phase (T_{10}), treatments had emitted between 28.7 to 72.0 % of the total C added (excluding control soil respiration) (Table 3.6). The total C lost in the rapid decomposition phase when considered as a percentage of total C added, can be used to predict the percentage of added C emitted long term. With the exception of the LTF treatment (which appeared to be subject to some form of substrate limitation in the early stages of decomposition), the relationship between C emitted in the rapid decomposition phase and C emitted long term appeared to follow a 1:1.44 trend (Figure 3.9).

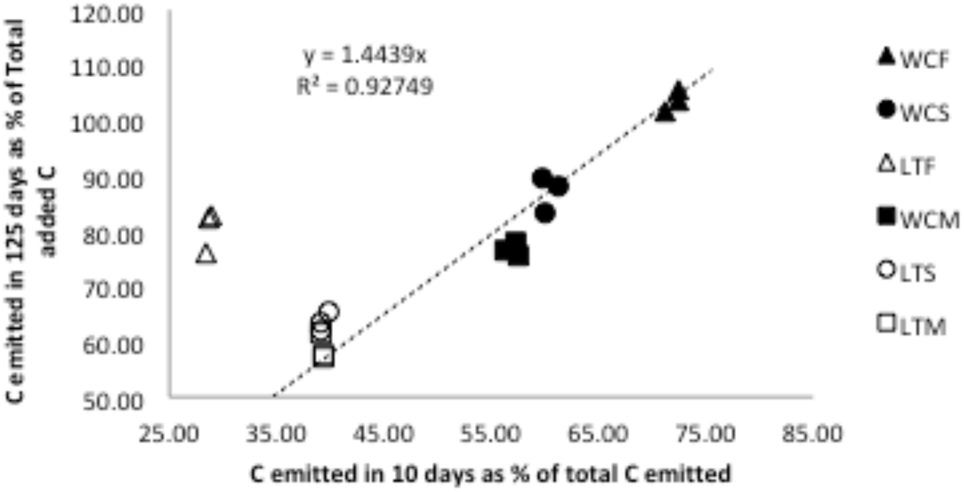


Figure 3.9 C emitted in 10 d as % total C added compared to C emitted in 125 d as % total C added.

The rate of C release for both LTM and WCM treatments up until T_2 was between 38 and 74 % higher than the other surface treatments (fresh and mixed), but by T_3 the rate of C release for the surface application of both LT and WC had overtaken. This is in line with a study which compared no-tillage compared to conventional tillage, and found that the decay rates of buried litter (1.4 - 1.7 % d^{-1}) were approximately 2.5 times faster than the rates of surface litter (0.5 - 0.7 % d^{-1}) (Beare et al. 1992). It was also in line with the expectation that because the material was dried, ground and mixed into the soil, giving it a larger surface area (smaller particle size equals lower activation energy (Chouchene et al. 2010)) and potentially higher degree of contact with soil microorganisms than the fresh material due to incorporation in the soil, it should theoretically decompose at a quicker rate. The bulk of litter input to soil ecosystems is in the form of fresh leaves, yet current understanding of organic matter decomposition is based largely on experimental studies using pre-dried leaves (Gessner, 1991). This paradox points to the critical role that this experiment will play in evaluating to what extent those experiments with dried leaves reflect natural litter decomposition.

From T_3 to T_5 the WCM and WCS treatments emitted a higher rate of CO_2-C (0.279 - 0.151 and 0.418 - 0.194 mg CO_2-C. g^{-1} soil. d^{-1} mixed and surface respectively) than the

WCF treatment (0.144 - 0.194 mg CO_2-C. g^{-1} soil. d^{-1}), which is what was expected from the hypothesis (Figure 3.4). A study considering the decomposition of alder leaves *(Alnus glutinosa* (L.) Gaertn.) in a stream ecosystem, found that soluble organic C, carbohydrates and phenolics were lost quickly from dried leaves, but were retained for several days in fresh leaves (Gessner, 1991) which agrees with what was observed. From T_5 the rate of CO_2-C emitted from the WCF treatment overtook the other WC treatments. For the fresh treatment more than 100 % of the added C was respired by T_{125}, as opposed to only 87.1 % from the WCS treatment and 76.7 % from the WCM treatment (Table 3.4).

Likewise, the LTM and LTS treatments emitted a higher rate of CO_2-C (0.023 - 0.136 and 0.024 - 0.149 mg CO_2-C. g^{-1} soil. d^{-1} respectively) than the LTF (0.026 - 0.076 mg CO_2-C. g^{-1} soil. d^{-1}) for the first five days after plant amendment (T_0 to T_4). The rate of release from the LTF treatment overtook the rate of release from the LTM at T_7, and at T_9 for the LTS treatment. This comparatively higher rate of release from the LTF treatment carries on until T_{20} (when it settles to approximately 0.090 mg CO_2-C. g^{-1} soil. d^{-1}), with the cumulative amount of CO_2-C released for the LTF treatment overtaking the cumulative amount of CO_2-C released from both the LTM and LTS treatments at T_{14}.

Overall the LTF emitted 80.4 % of added C, while LTS and LTM emitted 63.7 and 58.8 % respectively (Table 3.4). Therefore, although the rate of colonisation may have delayed the rate of C emitted from the fresh treatment, it appears to have had no impact on the total amount of C emitted long term. Within both plant species, the total amount of cumulative CO_2-C released according to the management treatment applied was: fresh > surface > mixed (Figure 3.3; Table 3.5). However, given the practical reality that any amendment within a pastoral system would be the result of fresh litter being dropped during grazing, LTF and WCF probably best simulate grazed pasture, and there is the risk that the conventional practice of drying, grinding and mixing in residues for incubation studies over-estimate the amount of C stored under different treatment regimes compared to a field situation.

3.5 Conclusions

This incubation experiment simulated white clover and lotus shoot decomposition, applied as fresh - surface, freeze dried - mixed and freeze dried - surface treatments in TK soil. Plant species, LT or WC, have a marked effect on the decomposition of shoot material added to soil. The effect of plant species was clearly seen in the first 10 d of incubation, and whilst there was no clear effect in the middle to later stages of incubation, this early effect meant that there was a significant difference at incubation end (T_{125}). Simulating the return of ungrazed shoot material to soil showed that lower CO_2-C was evolved over 125 d from LT shoot residues than WC shoot residues, resulting in a higher residual soil C at incubation end – i.e. less decomposition occurred under LT shoot residues.

Method of application of the shoot material also influences the degree of decomposition in 125 d; a significant effect in the middle stage of incubation (T_{10} – T_{20}) leads to a significant difference at incubation end (T_{125}). Irrespective of species, fresh material added to the soil surface was more completely decomposed than dried ground material added to the soil surface or mixed into the top 2.0 cm of soil. These differences were clearly established in the rapid decomposition phase, within the first 10 d.

Overall the LTM treatment resulted in the highest amount of estimated added plant C retained in the soil (41 %), while the WCF treatment resulted in the lowest amount retained (- 4 %). Overall more C was retained under the LT species than under the WC species, and under the freeze dried - mixed treatment. Plant species and method of litter application clearly affect the decomposition of shoot litter and subsequently the amount of C sequestered, however it is unknown if the plant species trends established for shoot litter decomposition extend to root litter decomposition. An incubation study considering root litter decomposition should be conducted. Freeze dried plant material applied to the soil surface or mixed with the soil both underestimate the rapid decomposition phase and subsequent total decomposition of plant material in this incubation study. It is important therefore to consider conducting an experiment using fresh plant material in order to estimate the amount of C that would be lost from fresh material in the field.

Chapter 4

The extent of decomposition in soil of lotus (*Lotus pedunculatus*) and white clover (*Trifolium repens*) root material in two contrasting soils: a Pallic soil and an Allophanic soil, after 157 d of incubation.

4.1 Introduction

Experimental research conducted in Chapter 3 showed that plant species and the form and manner of litter addition to soils have a significant effect on shoot litter decomposition rates. Irrespective of placement treatment, virtually all *Trifolium repens* L. (WC) shoot litter treatments decomposed faster and to a greater extent than their comparative *Lotus pedunculatus* (LT) treatments. Irrespective of plant species *Fresh* litter placed on the surface of soil emitted more CO_2 > dried ground litter place on the soil *surface* > dried ground litter *mixed* with soil. These results suggest that in LT dominated pastures, compared to WC, more undecomposed shoot tissue may lead to accelerated soil C sequestration. However, it is unknown if the plant species trends established for shoot litter decomposition extend to root litter decomposition (Charlton & Stewart, 1999).

Root material traits

Root tissue and exudates are a significant part of the plant-soil C cycle yet the contribution of root tissue is often overlooked when estimating C turnover in grazed systems. In general, root systems are much less frequently studied than shoot systems (Chapter 2.2.1). Although there is a sizeable body of literature on roots and rhizomes of herbage grasses, there is a lack of integration of data on root growth dynamics with that of shoot growth dynamics. At the date of writing, no published studies of combined root and shoot turnover / decomposition for pasture legumes exists. Bloomfield (1993) has also raised concerns that in incubation experiments to study the relative decomposition rates of shoot litter and roots, roots need to be left insitu, in undisturbed soil. Whilst this is technically difficult to achieve the experimental design (Section 4.1, below) will attempt to measure the relative rates of root decomposition in disturbed and undisturbed soil conditions.

In the literature, terms used to describe below-ground root residue and root exudate inputs include rhizodeposition and below-ground litter; hereafter referred to as rhizodeposition. Roots (approximately 40 % C by weight) constitute a substantial proportion of annual plant productivity and C input to soils (Jackson et al. 1997); with below-ground estimates in terrestrial ecosystems ranging from 15 % to 83 % of total annual DM production (Coleman, 1976; Bernard et al. 1988; Aerts et al. 1992; Konings et al. 1992).

This level of assimilate transfer is supported by other studies (e.g. Kuzyakov & Domanski, 2000) in which pasture plants have been shown to translocate between 30 – 50 % of all assimilates below ground, equating to around 2200 kg C. ha^{-1} in one vegetative period. Despite the variance reported, it has been estimated that roughly 50 % of all assimilated C is incorporated in root tissue; 33 % respired as CO_2 by roots and rhizosphere MO (utilizing exudates and fine roots) a few days after assimilation; with the remaining 17 % contributing to SOM and MO (Kuzyakov & Domanski, 2000). Meharg and Killham (1990) showed rhizodeposition as low as 8 %, with other studies as high as 80 % (Zagal, 1994; Meharg and Killham, 1990; Dormaar & Sauerbeck, 1983). Freschet et al. 2013 considered data from 32 studies, where plant tissues from the same species had been decomposed in the same site or microcosm, and estimated that for grasslands, rhizodeposition inputs (as fine roots) make up approximately 33 % of annual residue inputs (26 % for leaf litter; 41 % for fine stems). There was evidence that this material decomposes around twice as slowly as leaf litter derived from the same species. Freschet et al. 2012a, 2012b make the point that the chemical and structural traits influencing decomposition vary between plant organs (leaves, stems and roots) as well as across species.

Fresh litter return in a grazed pasture system therefore represents only a small percentage of the C input into the soil system, with the majority of C input derived from rhizodeposition. The type of plant species has been shown to significantly affect the amount of soil C sequestered from decomposing shoot material (Chapter 3), therefore one might assume that root material may have a similar effect.

Decomposition rates of OM

Decomposition rates of OM in soils are highly dependent upon the structural nature and chemical attributes of the soil. The origin of the soil parent material has a strong legacy effect on soil physical properties such as structure, aggregation, porosity, water filled holding porosity, and soil surface area; all of which strongly influence SOM decomposition (McNally et al. 2017; Russell, 1988). Extrapolation of the results of the shoot decomposition study in Chapter 3 is limited because the study was conducted in only one soil (Pallic soil). The two soils chosen for this incubation study include a Pallic soil, from the same source as Chapter 3, and an Allophanic soil (see Chapter 4, Section 2.1). These soils differ in their parent materials and clay mineralogy.

It is generally expected that Allophanic soils will immobilise more partly decomposed C (Wang et al. 2016); it has recently been proposed that the C immobilised may actually be terminal decomposition products (small decomposed molecules at the end of decomposition) rather than fresh C inputted to the soil (Shen et al. 2018). It is hypothesised that these small organic molecules complex with Fe and Al of SRO minerals reducing / inhibiting enzymatic hydrolysis. Therefore, soil structure and hydrous oxide metal content (level of free Fe and Al in soils) will influence both the rate and extent of OM decay (Shen et al. 2018). Herath et al. (2014) found that the Allophanic soil (Egmont Black Loam) has an abundance of microaggregates which is believed to facilitate more efficient decay of primary OM, and production of secondary OM. This secondary OM is thought to interact with reactive surfaces from volcanic origin (allophane, Al^{3+}), thus becoming stabilised and resulting in a dominance of secondary OM. In contrast, the Pallic soil (Tokomaru Silt Loam) has a lower presence of these reactive surfaces, which means that as OM decomposes it is not stabilised to the same extent, and instead is completely decomposed to CO_2; thus there is a greater fraction of primary OM in soils formed from sedimentary materials.

Hypotheses

Four hypotheses are presented:

(i) Differences in tissue chemistry between shoot and root material will lead to systematic differences in litter decomposition rates;

(ii) A high percentage of tannins, in LT tissue, will decrease the rate and total amount of litter decomposed in soil, therefore retaining a greater amount of C, compared to that of a comparable pasture species, WC, which contains relatively low tannin content;

(iii) Soil origin (Pallic Soil vs Allophanic soil), structure and hydrous oxide metal content will influence both the rate and extent of OM decomposition (Suárez-Abelenda et al. 2015; Herath et al. 2014);

(iv) Disturbing the root-soil matrix will result in an initially higher rate of CO_2 evolved compared to an undisturbed sample; however, with time a similar amount of CO_2 will be evolved from both samples (see Experimental design – below).

These conceptual hypotheses were transferred into working experimental objectives similar to those described in Chapter 3. The exception was that plant root material needed to be generated for plant analysis prior to decomposition studies.

Experimental design

In Chapter 3, variability was reduced by mixing plant material with the soil. However, Bloomfield et al. (1993) highlighted that estimating root decomposition rates is complicated, as any analysis disturbs the intact soil structure. In order to cover the concerns raised by Bloomfield, that the nature of the incubation environment is an important determinant of extended decomposition; roots need to be left insitu. However, there is a concern that the natural structure effect of the soil may create a higher degree of variability of CO_2 evolution where roots are left insitu, whereas homogenisation of the soil plus roots may offer lower levels of variability so that treatment effects (plant type) can be realised. As a way to measure the soil disturbance effect on decomposition the incubations were established in two different formats, simply disturbed (D) and undisturbed (U) soil plus root samples (the incubation detail is covered in the methodology).

The practical circumstances at which major root turnover occurs is at pasture renewal, which in New Zealand constitutes herbicide treatment (usually Glyphosate), followed by shallow tillage, no-tillage or moldboard ploughing. The incubation technique chosen was designed to simulate pasture renewal, the U treatments simulated no tillage, whereas the D treatments simulated mixed soil / Mouldboard plough.

Experimental objectives

(i) To generate plant root material through glasshouse studies for plant analysis prior to decomposition studies.

(ii) To chemically characterise the tissue of *Lotus pedunculatus* (LT) and *Trifolium repens* L. (WC) roots, and determine how their specific plant traits differ.

(iii) To characterise the effect of soils of contrasting mineralogical properties on plant root decomposition.

(iv) To test if plant root tissue chemistry can influence the rate of decomposition and the amount of residue C remaining after a period of decomposition.

4.2 Materials and methodology

4.2.1 Soil collection and preparation

Tokomaru silt loam topsoil (TK), the Pallic soil, was collected as described in Chapter 3.2.1.

Egmont silt loam topsoil (EGT), the Allophanic soil, was collected to a depth of 7.5 cm from a permanent pasture site in Hawera, New Zealand (39°37′ S, 174°21′ E, 66 m above sea level). The EGT soil has developed on andesitic volcanic ashes of Oakura and Okato tephras and allophane is dominant in its mineralogy (Suarez-Abelenda et al. 2015). The two soils are classified as Typic Fragiaqualf and Typic Hapludand (Soil Survey Staff, 2006), respectively.

The climatic conditions are similar for both the TK and EGT sites, with a mean annual rainfall in the range between 1000 and 1300 mm. y^{-1} and a mean annual soil temperature of approximately 12 °C (Baisden et al. 2010). Both sites have been under permanent pasture for at least 50 y (Parfitt et al. 1984; Roberts & Thompson, 1984).

Soils were prepared and stored as outlined in Chapter 3.2.1. After chilling, a sub sample of the two soils was taken for use in the glasshouse trial (described in Chapter 4.2.2 to 4.2.3).

4.2.2 Soil characterisation

Chemical analysis methods are explained in more detail in Chapter 3.2.2. Analysis of both soils showed that they presented no soil fertility limiting conditions for plant growth (Morton & Roberts, 2009) and decomposition. Results are reported in Table 3.1 and Table 4.1.

Table 4.1 Chemical analysis of Egmont Silt Loam (EGT) and Tokomaru Silt Loam (TK) topsoils (depth 7.5 cm, air dried, < 2mm sieve size) prior to (control) and after lotus (LT) and white clover (WC) growth in the glasshouse trial (EGT LT, EGT WC, TK LT and TK WC).

Analysis Type	**EGT Ctrl**		**EGT LT**		**EGT WC**		**TK Ctrl**		**TK LT**		**TK WC**	
	Mean	***SD***	**Mean**	***SD***	**Mean**	***SD***	**Mean**	***SD***	**Mean**	***SD***	**Mean**	***SD***
C (%)^{1}	9.0	-	-	-	-	-	4.0	*0.021*	-	-	-	-
N (%)^{1}	0.91	-	-	-	-	-	0.43	*0.0045*	-	-	-	-
S (%)^{1,2}	0.14	-	-	-	-	-	0.052	*0.0026*	-	-	-	-
Olsen P (mg. L^{-1})^{2,3}	43.5	*0.80*	29.2	*4.6*	34.0	*5.5*	45.1	*0.5*	44.7	*2.2*	62.0	*1.7*
K (me. 100 g^{-1})^{2,3}	0.38	*0.019*	0.16	*0.038*	0.19	*0.022*	0.36	*0.0061*	0.25	*0.023*	0.27	*0.045*
Ca (me. 100 g^{-1})^{2,3}	11.6	*0.18*	8.2	*0.45*	6.8	*0.36*	10.7	*0.26*	6.0	*0.76*	5.4	*0.27*
Mg (me. 100 g^{-1})^{2,3}	1.0	*0.028*	0.19	*0.084*	0.20	*0.047*	1.1	*0.033*	0.16	*0.013*	0.19	*0.049*
CEC (me. 100 g^{-1})^{2,3}	28.8	*1.9*	26.0	*0.49*	25.7	*2.3*	18.0	*2.4*	14.3	*1.3*	11.2	*0.70*
pH^{2,3}	5.2	*0.023*	5.2	*0.021*	5.1	*0.075*	5.2	*0.058*	5.1	*0.081*	5.0	*0.044*
Sulphate Sulphur (mg. kg^{-1})^{2,3}	49.3	*0.58*	10.7	*1.5*	43.4	*4.6*	15.1	*1.1*	4.6	*0.47*	5.7	*0.38*

Analyses performed by: ^{1}Elementar (vario MACRO cube); ^{2}Standard Soil Testing, Fertilizer & Lime Research Centre, Massey University; ^{3}Hills Laboratory. Results presented on the basis of three replicates.

4.2.3 Cultivation and preparation of root material - Glasshouse trial set up and maintenance

Seed of *Lotus pedunculatus* cv barsille (LT) were sourced with rhizobium inoculum from Lincoln University in December 2012. The seed was inoculated with a water-inoculum slurry of Nodulaid (Peat based, Group D for inoculation of *Lotus pedunculatus*), produced by Becker Underwood and chilled overnight. Seed of *Trifolium repens* cv maku (WC) were also sourced (pre-inoculated) from Lincoln University in December 2012.

A total of 180 pots of TK and EGT soil were prepared with 500 g of soil (Bulk density 1.0 g. cm^{-3}) and arranged in blocks in the Soil Department glasshouse at the Plant Growth Unit (PGU), Massey University, Palmerston North. These pots were left sitting in water until they became saturated, then were drained to FC. The weights of random reference pots were taken and used as indicators to schedule watering during plant growth.

The LT (30 seed / pot) and WC (15 seed / pot) seeds were then sown on the surface, which was regularly sprayed with water to keep the surface soil moist. Seed application rate was higher in the LT treatment as the seed were known to have lower viability. The pots were also covered with wet newspaper to allow germination in dark, moist conditions. Three days after sowing, germination of both LT and WC seedlings had started so the newspaper covers were removed. After two weeks the pots were thinned down to the ten strongest seedlings in each pot, seedlings were transplanted between pots (within soil types) if required.

The pots were watered with a modified low-N nutrient solution (Middleton and Toxopeus, 1973) twice a week, with additional watering (distilled water) as required to maintain water content as close to FC as possible (using the reference pots) (Figure 4.1). The foliage in the pots was periodically cut to simulate optimal grazing practices; this foliage was weighed, bagged, oven dried at 70 °C and re-weighed to give % DM yield.

Low-N Nutrient Solution
420 ml Major A
420 ml Major B
42 ml Minor
24 ml Fe Citrate
Made up to 9L with distilled water

Major Element Stock Solution - A (high pH)

Constituent	Weight (g) in 2.5 L
KH_2PO_4	7.50
K_2HPO_4	8.17
K_2SO_4	4.34

Major Element Stock Solution - B (low pH)

Constituent	Weight (g) in 2.5 L
$MgCl_2.6H_2O$	5.09
$CaCO_3$	1.28
HCL (M)	25 mL
Na_2SO_4	3.89

Minor Element Solution

Constituent	Weight (g) in 1.0 L
H_3BO_3	0.0043
$CoCl_2.6H_2O$	0.0008
$CuCl_2.2H_2O$	0.0019
$MnCl_2.H_2O$	0.0214
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.0015
$ZnCl_2$	0.002

Ferric Citrate Solution

Constituent	Weight (g) in 2.0 L
Fe Citrate	0.117

Figure 4.1 Low-N nutrient solution (modified from Middleton and Toxopeus, 1973) applied at 50 ml / pot, twice weekly over one-year glasshouse trial.

As part of Massey University's Plant Growth Unit's Best Management Practice, the plants in the glasshouse were subject to a rigorous spray programme to minimize any risk of pest infestation. The glasshouse was covered with shade cloths over the summer months. The plants were maintained under these glasshouse conditions for just over one year before three pots for each treatment, with the highest % DM shoot yield, were selected and harvested for the root incubation studies. The remainder pots were on grown.

4.2.4 Root material harvest and preparation

Three pots of each treatment (LT TK, LT EGT, WC TK, WC EGT), with the highest % DM shoot yield, were selected. The pots were sprayed with Glyphosate, and left for 24 hours prior to harvest (Figure 4.2).

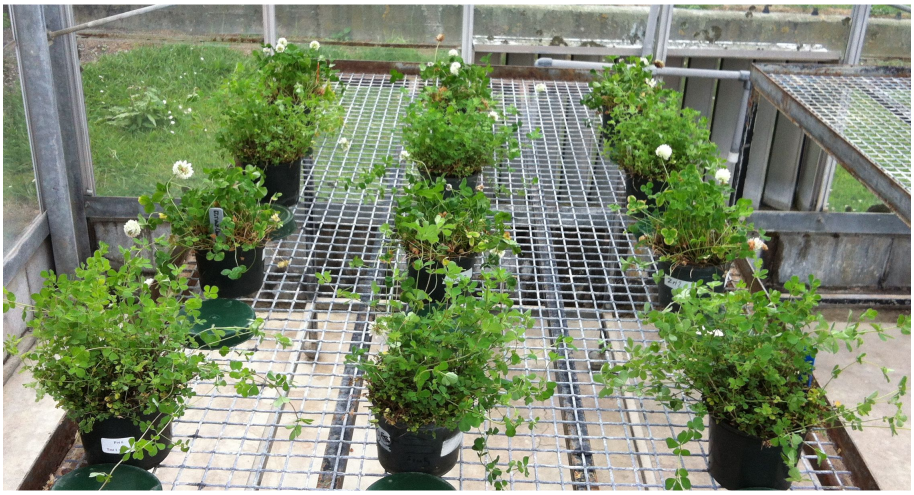


Figure 4.2 Glasshouse trial: Pots of LT and WC selected for harvest, 24 hours after treatment with Roundup.

Figure 4.3 Harvested foliage from LT (left) and WC (right) grown in TK and EGT soils in the one-year glasshouse trial.

After this period the foliage was harvested (cut off at the soil surface) from each pot (Figure 4.3), weighed and dried as described in Chapter 3, Section 2.3. The soil mass was carefully removed from the pot and cut like a cake into eight equal triangular slices (Figure 4.4). Four slices were used for incubation and four slices were set aside for analysis (see root characterisation and incubation preparation below) (Figure 4.4 and Figure 4.5).

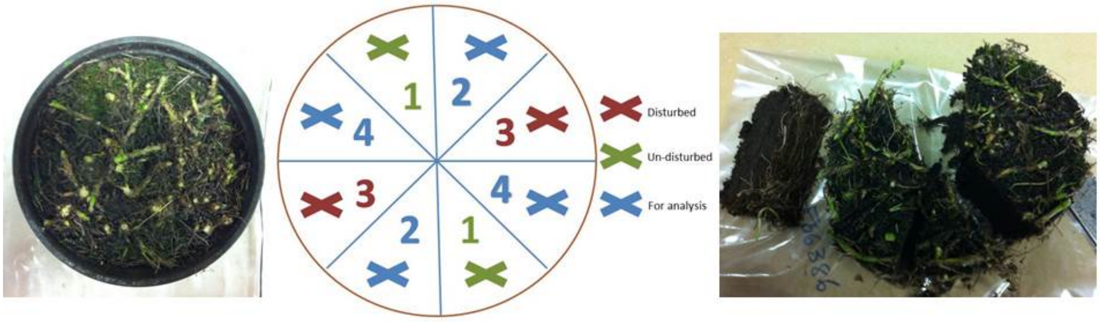


Figure 4.4 Harvested pot, 8 slices prepared for treatments (Disturbed and Undisturbed) and analysis.

Of the four slices put aside for incubation, two slices were placed intact and undisturbed together in an incubation chamber, and two slices were chopped into 1 cm pieces, crumbled up, sieved through a 5.6 mm sieve, and placed together in another incubation chamber (Figure 4.5).

Figure 4.5 Ready for incubation: intact (undisturbed) soil slices (left); chopped and sieved (disturbed) soil slices (right).

4.2.5 Root material characterisation

Of the four slices put aside for analysis, two slices were combined and wet sieved for total root and stolon content (hereby termed roots), which were chilled and frozen at -20 °C. Frozen samples were freeze dried as detailed in Chapter 3, Section 2.3. The freeze dried root material was then ground for C analysis and analysed by Elementar (vario MACRO cube) as per the method described in Chapter 3.2.3.

The root mass was expressed per unit freeze dried soil (mg root. g^{-1} FD soil). The mean mg root. g^{-1} FD soil was calculated from 6 observations per plant species in each soil. These mean values were used to estimate the root mass and C content (mg C. g^{-1} FD soil) present in the soil slices added to each incubation chamber.

The other two slices were chopped to 1 cm, crumbled up and sieved – half the sample was oven dried at 105 °C to calculate moisture content and the other half was freeze dried as above. The freeze dried soil plus root material was then ground for analysis. Subsamples of this freeze dried material were taken for full chemical analysis, as described in Chapter 3, Section 3.2.3.

4.2.6 Incubation preparation

After cutting, the soil slices were immediately added to their respective incubation chambers (Time zero, T_0) (Figure 4.6); at which point a NaOH trap and a H_2O vial were also included (see incubation chamber set up Chapter 3, Section 2.4). The pre-incubation period was not observed due to the insitu nature of the treatments. Extra chambers were also set-up to account for background soil respiration (TK and EGT field soils that had been stored in the chiller at 3 °C), for the CO_2 already present in the enclosed space and any CO_2 entry during venting. The treatment codes applied to the chambers were: (i) LT and WC; (ii) TK and EGT; and (iii) intact - undisturbed (U), and chopped / sieved to < 5.6 mm – disturbed (D). All treatments were run in triplicate.

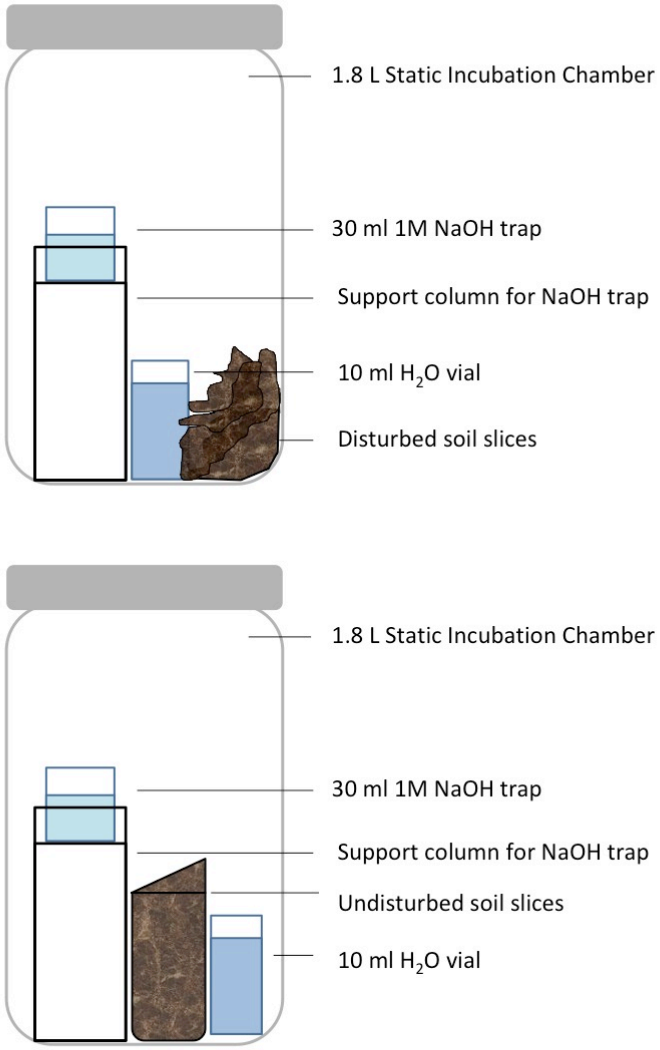


Figure 4.6 Static Incubation Chamber setup. Lotus (LT) and white clover (WC) root material grown in Tokomaru Silt Loam (TK) and Egmont silt loam (EGT) topsoil, disturbed (D) and undisturbed (U) slices.

The variation in slice volume on cutting produced a variation in wet weight in each incubation jar. The treatment codes and soil wet weights added to each jar are presented in Table 4.5.

The chambers were arranged by blocks and incubated in dark conditions at an average temperature of 18 °C (range 14 – 22 °C). The soil moisture content was maintained at an estimated gravimetric water content of 0.35 and 0.50 for TK and EGT soils respectively (70 % FC) throughout incubation.

4.2.7 Measurement of CO_2 flux

Static NaOH CO_2-trap chamber method

Unless stated otherwise, the chamber method follows the description in Chapter 3, Section 2.5. The polypropylene cup was elevated approximately 5 cm above the soil surface in the centre of the chamber. The chamber headspace volume was 1478 ml. The seal between the chamber top and base was gas-tight. The chambers were vented and the alkali traps were initially changed daily (for 23 d), and thereafter monitored and changed as required.

Aerobic conditions were maintained where possible. Total duration of the incubation was 157 d (T_{157}). At T_{157} the soil was removed from the incubation jars, frozen and freeze dried.

Determination of CO_2 evolved

The titration method for determination of the amount of CO_2 trapped (evolved) is explained in more detail in Chapter 3, Section 2.5.

4.2.8 Normalising of data for soil weight

For comparison of treatments, the cumulative CO_2-C from each incubation chamber was divided by the weight of soil in each jar. It was assumed that basal respiration in the control soils was an appropriate "control" for background microbial basal respiration in rooted samples. The limitation of these assumptions are the differences in management of the soils with roots and the control soils. The control soils were stored in the chiller while the glasshouse experiment was underway.

Slicing the soil segments led to a variation in wet soil added as intact or disturbed "soil plus root" slices. CO_2 evolved per chamber was influenced by the weight of soil added. To normalise the data, such that background emission from soil alone could be accounted for, emissions per chamber at any time T_0-T_{157} were first corrected by the dry weight of soil plus root in each incubation chamber. The amount of soil plus root was calculated based on the total estimated dry weight of soil added to each chamber:

Treatment:

$$\text{mg } CO_2\text{-C evolved. g}^{-1} \text{ soil dry weight} = \frac{\sum_{T0}^{T157} \text{Cumulative } CO_2\text{-C evolved (mg)}}{\text{Total dry weight of soil added (g)}}$$

As the amount of root in each treatment varies, the cumulative amount of CO_2-C evolved from the control soils was calculated per gram of dry weight soil added to the control incubation chambers:

Control soil:

$$\text{mg } CO_2\text{-C evolved. g}^{-1} \text{ soil dry weight} = \frac{\sum_{T0}^{T157} \text{Cumulative } CO_2\text{-C evolved (mg)}}{\text{Total dry weight of soil added (g)}}$$

Treatment effects were also compared on the basis of the cumulative amount of CO_2-C evolved per milligram of root C added, after adjusting for the CO_2-C evolved from the control soils:

CO_2 evolved:

$$\text{mg } CO_2\text{-C evolved. mg}^{-1} \text{ root C added} = \frac{\sum_{T0}^{T157} \text{Treatment - Control (mg } CO_2\text{-C evolved)}}{\text{Total estimated root C added (mg)}}$$

4.2.9 Statistical analysis

As the weight of soil in each replicate varies, the data was corrected for weight using the appropriate control soil. There was a large error associated with the variation across pots due to low soil weights yet a high root mass, and was observed as root growth in soil voids. To ensure treatment effects were not confounding other treatment effects, the data was also separately analysed by treatment.

Analysis of variance was conducted using a General Linear Model that incorporated the three fixed effects associated with treatments: species, soil, soil disturbance. The random effects, associated with experimental setup and climatic conditions within the glasshouse, were accommodated by blocking the replicates. Glasshouse conditions were normalised as much as possible by regular movement of the pots.

4.3 Results

4.3.1 Root recovery results

At harvest of the glasshouse trial, the root densities of WC and LT in the EGT and TK soils ranged from 76 to 92 mg of fresh root. g^{-1} wet soil (Table 4.2). The amount of wet root grown in the EGT and TK soils were not significantly different for both plant species, WC and LT (Table 4.3). There was very high variability within the LT and WC root densities sampled, when the EGT and TK soils were considered independently. Likewise, there was very high variability within the EGT soils sampled, when the species LT and WC were considered independently. However, within the TK soils sampled, 75 % of the variability was explained by the difference in root growth between LT and WC species.

Table 4.2 Root recovery and moisture content, and soil gravimetric water content at harvest from Egmont Silt Loam (EGT) and Tokomaru Silt Loam (TK) topsoils growing lotus (LT) and white clover (WC) for just over one year.

Soil Type	Species	Recoverable root (mg wet root. g^{-1} wet soil)		Recoverable root (mg root C. g^{-1} FD soil)		Moisture content of root mass (%)		Soil gravimetric water content	
		Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
EGT	WC	81.0	*15.8*	7.6	*2.2*	14.7	*0.02*	0.75	*0.02*
	LT	90.3	*27.1*	7.8	*2.3*	13.6	*0.02*	0.78	*0.05*
TK	WC	76.2	*6.7*	6.6	*0.6*	16.1	*0.01*	0.39	*0.02*
	LT	92.1	*4.3*	6.8	*0.6*	15.2	*0.02*	0.41	*0.01*
EGT	Control	2.9	*0.3*	-	-	-	-	0.63	*0.03*
TK	Control	10.6	*1.8*	-	-	-	-	0.33	*0.05*

Table 4.3 The statistical significance of root density differences (mg wet root. g^{-1} wet soil) between lotus (LT) and white clover (WC) after just over one year of growing in pots of Egmont Silt Loam (EGT) and Tokomaru Silt Loam (TK) topsoil. Data considered both dependent and independent of soil type and plant species.

Soil	Species	Soil (%)	Species (%)	Error (%)	R-Sq adj (%)
NS	NS	0.2	18.3	81.4	0.5
EGT	NS	-	6.2	93.8	0.0
TK	P = 0.03	-	75.3	24.7	69.1
-	WC	5.5	-	94.5	0.0
-	LT	0.3	-	99.7	0.0

NS - Not significant, $P > 0.05$

The root mass ranged from 14 % to 16 % in moisture content and the soil ranged from 0.39 to 0.78 gravimetric water content (Table 4.2), for TK (0.33 to 0.41) and EGT (0.63 to 0.78), respectively. It was calculated that on a dry soil basis there was 6.6 to 7.8 mg root C. g^{-1} FD soil. The amounts of root C (mg C. g^{-1} FD soil) grown in the EGT and TK soils were not significantly different for both plant species, WC and LT (Table 4.4). There was very high variability within the LT and WC root densities sampled, when the EGT and TK soils were considered independently. Likewise, there was very high variability within the EGT and TK soils sampled, when the species LT and WC were considered independently. No significance was detected due to the very high (86 % - 100 %) variation caused by root growth.

Table 4.4 The statistical significance of root density differences (mg root C. g^{-1} FD soil) in lotus (LT) and white clover (WC) after just over one year of growing in pots of Egmont Silt Loam (EGT) and Tokomaru Silt Loam (TK) topsoil. Data considered both dependent and independent of soil type and plant species.

Soil	Species	Soil (%)	Species (%)	Error (%)	R-Sq adj (%)
NS	NS	13.2	0.3	86.5	0.0
EGT	NS	-	0.2	99.8	0.0
TK	NS	-	2.7	97.3	0.0
-	WC	14.2	-	85.8	0.0
-	LT	12.3	-	87.7	0.0

NS - Not significant, P > 0.05

The root densities (Table 4.2), root C content (presented in Table 4.6) plus the fresh weight of soil slices added to the incubation jars (Table 4.5) were used to estimate the total root weight and C mass added to each incubation jar, which ranged from 469 to 1137 mg C per jar (means presented in Table 4.5). These values were later used to normalize the CO_2-C evolved per mg of root C added.

Table 4.5 Soil weight, estimated root weight and estimated C present in Egmont Silt Loam (EGT) and Tokomaru Silt Loam (TK) topsoil slices, taken from pots growing either lotus (LT) or white clover (WC) for just over one year.

Soil type	Species	Disturbance	Fresh weight of soil slices added to incubation jars		Estimated wet root weight added to incubation jars		Estimated C (mg) added to incubation jars	
			Mean	*SD*	Mean	*SD*	Mean	*SD*
EGT	WC	D	195.3	*9.3*	15.7	*2.6*	846.5	*218.6*
		U	178.1	*14.2*	14.5	*3.6*	780.8	*244.6*
	LT	D	192.4	*15.2*	17.2	*4.7*	847.2	*267.6*
		U	177.0	*35.9*	16.4	*7.3*	784.4	*277.5*
TK	WC	D	149.2	*9.8*	11.4	*1.7*	709.6	*106.7*
		U	159.2	*12.3*	12.2	*1.7*	756.9	*120.7*
	LT	D	169.7	*15.9*	15.7	*2.2*	811.5	*49.5*
		U	173.4	*11.6*	16.0	*1.6*	833.9	*111.9*

4.3.2 Effect of soil weight, root weight and C added on cumulative CO_2 evolved over 157d

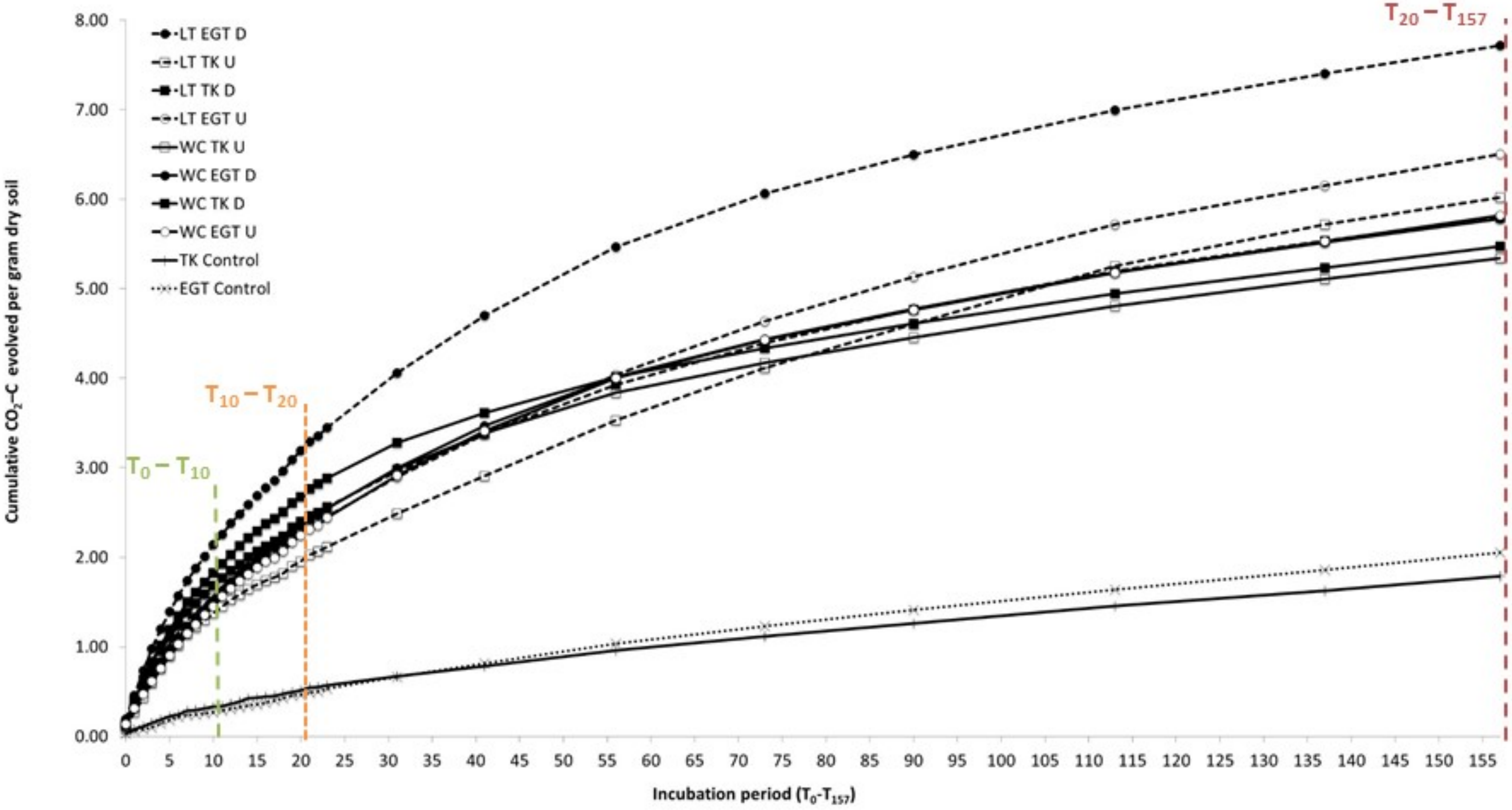


Figure 4.7 **The cumulative emission of CO_2-C (mg CO_2-C. g^{-1} FD soil) over 157 d from disturbed (D) and undisturbed (U) slices of Tokomaru Silt Loam (TK) and Egmont Silt Loam (EGT) topsoils containing roots of lotus (LT) and white clover (WC) grown for just over one year, compared to control soils without fresh root material.**

The control soils (no added amendment) had a steady efflux of CO_2, which was at a consistent rate throughout the 157 d (Figure 4.7). The rate of CO_2-C emission from the background soil in this experiment averages 0.08 - 0.09 mg C. g^{-1} FD. $week^{-1}$ (TK and EGT respectively) for the whole incubation period 0 to 157 d. At 157 d both the EGT control and TK control soils had emitted similar amounts of CO_2-C (between 1.8 and 2 mg C. g^{-1} dry soil), which equated to 12.7 % more CO_2-C emitted from the EGT soil than the TK soil. All other treatments showed an increase in microbial activity with presence of insitu root material.

The wet weight of soil in incubation jars varied within the Egmont soil by 29 % and Tokomaru soil by 27 %. Background CO_2-C emitted per jar was corrected based on the dry weight of soil in each jar. The amount of root weight and root C added to each jar was also estimated based on the weight of soil added to each jar (Table 4.5).

Effect of soil weight

There is a lot of variation, but there is still a slight relationship between the weight of wet soil added to each incubation chamber and the cumulative CO_2-C evolved (Figure 4.8). When Treatment and Control soils were considered this relationship explained 36 % of the CO_2 evolved.

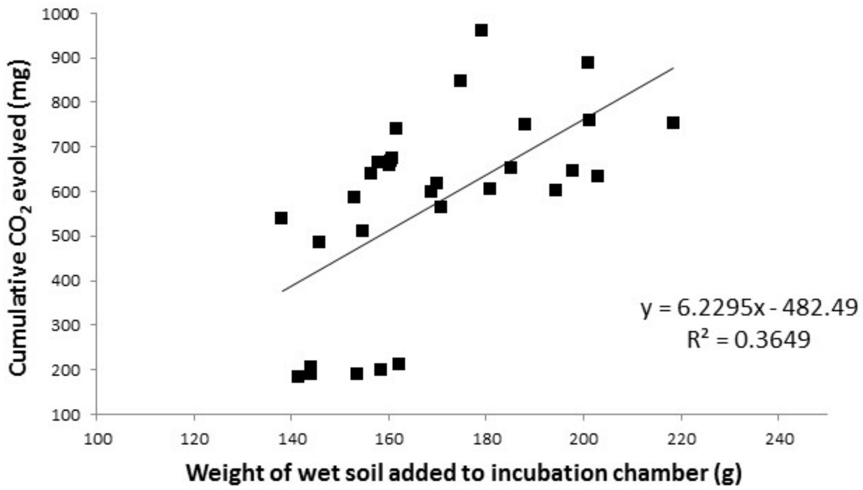


Figure 4.8 The cumulative emission of CO_2-C (mg) at 157 d from disturbed (D) and undisturbed (U) slices of Tokomaru Silt Loam (TK) and Egmont Silt Loam (EGT) topsoils containing roots of lotus (LT) and white clover (WC) grown for just over one year, compared to the weight of wet soil added to each incubation chamber (g). Treatment and Control soils presented.

Effect of estimated root weight

There is a medium correlation between the weight of wet root added to each incubation chamber and the cumulative CO_2-C evolved. When Treatment and Control soils were considered this relationship explained 78 % of the CO_2 evolved. Root weights in soil (or lack of root in the case of control soils) clearly has the dominant effect.

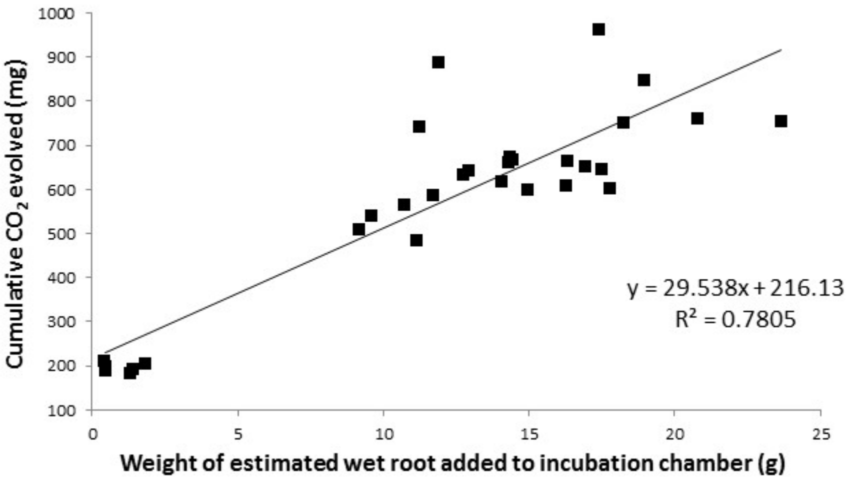


Figure 4.9 The cumulative emission of CO_2-C (mg CO_2-C) at 157 d from disturbed (D) and undisturbed (U) slices of Tokomaru Silt Loam (TK) and Egmont Silt Loam (EGT) topsoils containing roots of lotus (LT) and white clover (WC) grown for just over one year, compared to the weight of wet root added to each incubation chamber (g). Treatment (> 8 g root wt) and Control soils (< 3 g root wt) presented.

Effect of estimated amount of root C added

There is a large amount of variation and very little relationship (3 %) between the amount of estimated root C added to each incubation chamber and the cumulative CO_2-C evolved above that evolved from the incubation of soil alone (ctrl). As soil weight is the only known factor, root weight and root C are estimated; and as there is clearly an effect of soil and root weight on CO_2-C evolved, there is value in considering the cumulative CO_2-C evolved on a soil weight and estimated root C added basis.

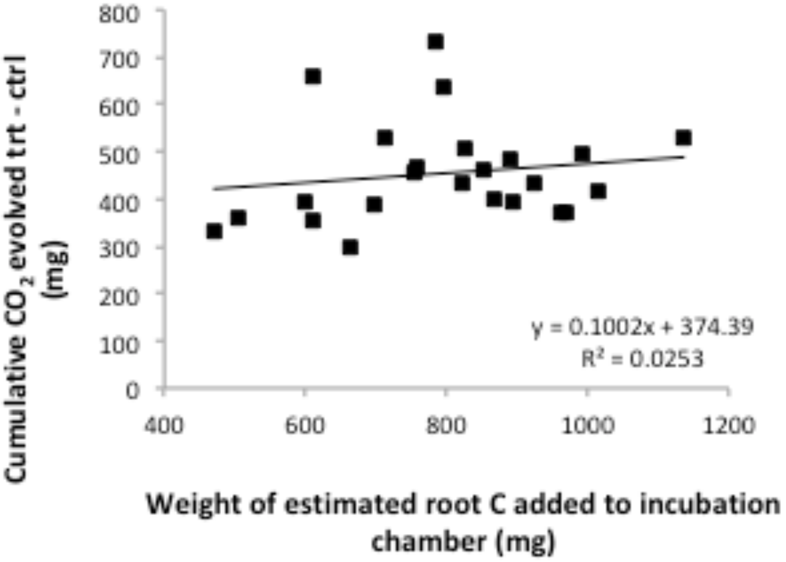
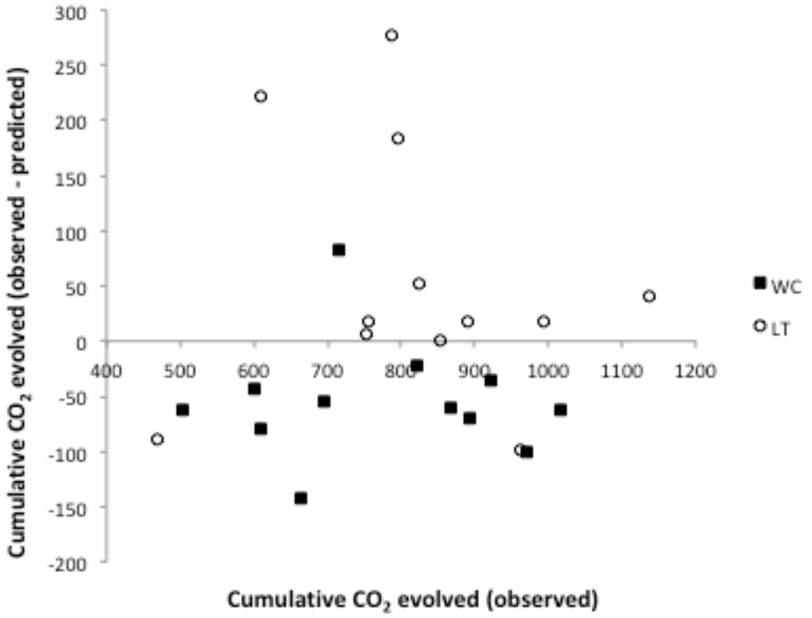


Figure 4.10 **The cumulative emission of CO_2-C (mg CO_2-C) at 157 d from both disturbed (D) and undisturbed (U) slices of Tokomaru Silt Loam (TK) and Egmont Silt Loam (EGT) topsoils containing roots of lotus (LT) and white clover (WC) grown for just over one year, compared to the estimated weight of root C added to each incubation chamber (g).**

When a regression analysis was performed on the dataset presented in Figure 4.10 and an ANOVA run on the residual values unexplained by the regression (Figure 4.11), species was a significant factor (P = 0.01) and did explain 28.1 % of the variation seen. It was noted (Figure 4.11) that the residual values were consistently negative for the WC root samples indicating that the cumulative amount of CO_2 emitted from the estimated amount of C added as WC root samples was consistently lower than that from LT root samples irrespective of the weight of root C added to the soil. Soil type was not shown to be significant.



Soil	NS
Species	P = 0.01
Disturbance	NS
Soil (%)	0.1
Species (%)	28.1
Disturbance (%)	3.3
R-Sq adj (%)	36.9

NS - Not significant, $P > 0.05$

Figure 4.11 Regression analysis. Observed cumulative emission of CO_2-C (mg) less predicted cumulative emission of CO_2-C (mg), compared to the observed cumulative emission of CO_2-C (mg).

4.3.3 Root material characterisation

LT root material had markedly higher CT content (16 fold) than the WC root material (Table 4.6). Of the total CT detected in WC roots, almost all of it was Fibre Bound; whereas for LT it was a mixture of Free CT, Protein Bound CT and Fibre Bound CT. WC root material had higher lignin and ME content than LT (~ 25 % - 30 % higher). Other chemical characteristics fell within similar ranges but WC was significantly higher in Fe, Mn and Zn concentrations than LT. WC root material had roughly twice the Zn and Mn than the LT. LT root material had a hundred times the amount of Fe and four times the amount of Mn than was found in LT shoot material; WC root material had fifty times the amount of Fe and twenty times the amount of Mn than WC shoot material (Chapter 3, Table 3.2).

Table 4.6 **Chemical composition of freeze dried lotus (LT) and white clover (WC) root tissue (mean and standard deviation presented).**

Analysis Type	LT root		WC root	
	Mean	*SD*	Mean	*SD*
C (%)^{1}	38.3	*0.11*	40.2	*0.15*
N (%)^{1,2}	2.5	*0.040*	3.0	*0.020*
C:N^{1,2}	15.3	-	13.4	-
P (%)^{2}	0.39	*0.007*	0.28	*0.007*
S (%)^{1,2}	0.12	*0.080*	0.070	*0.020*
K (%)^{2}	0.30	*0.000*	0.30	*0.000*
Ca (%)^{2}	0.46	*0.035*	0.76	*0.007*
Mg (%)^{2}	0.13	*0.014*	0.12	*0.000*
Fe (mg. kg^{-1}**)**^{2}	6990.0	*226.3*	7690.0	*551.5*
Mn (mg. kg^{-1}**)**^{2}	435.0	*35.0*	720.0	*42.4*
Zn (mg. kg^{-1}**)**^{2}	47.0	*3.5*	97.0	*2.1*
CT Total (%)^{3}	1.6	*0.14*	0.10	*0.000*
Lignin (%)^{2,3}	19.3	*2.1*	23.7	*3.8*
ME (MJ. kg^{-1}**)**^{2,3}	6.2	*0.46*	4.8	*0.93*

Analyses performed by: ^{1} *Elementar (vario MACRO cube);* ^{2} *Hills Laboratory;* ^{3} *MSFAT. Results presented on the basis of three replicates.*

4.3.4 Effect of plant species, soil type and soil disturbance on CO_2 evolution over 157 d

The wet weight of soil in incubation jars varied within the EGT soil by 29 % and TK soil by 27 %. Background CO_2-C emitted per jar was corrected based on the dry weight of soil in each jar. The amount of root weight and root C added to each jar was also estimated based on the weight of soil added to each jar (Table 4.5). All treatments showed an increase in microbial activity (respiration rate, CO_2 emission) from the start of incubation.

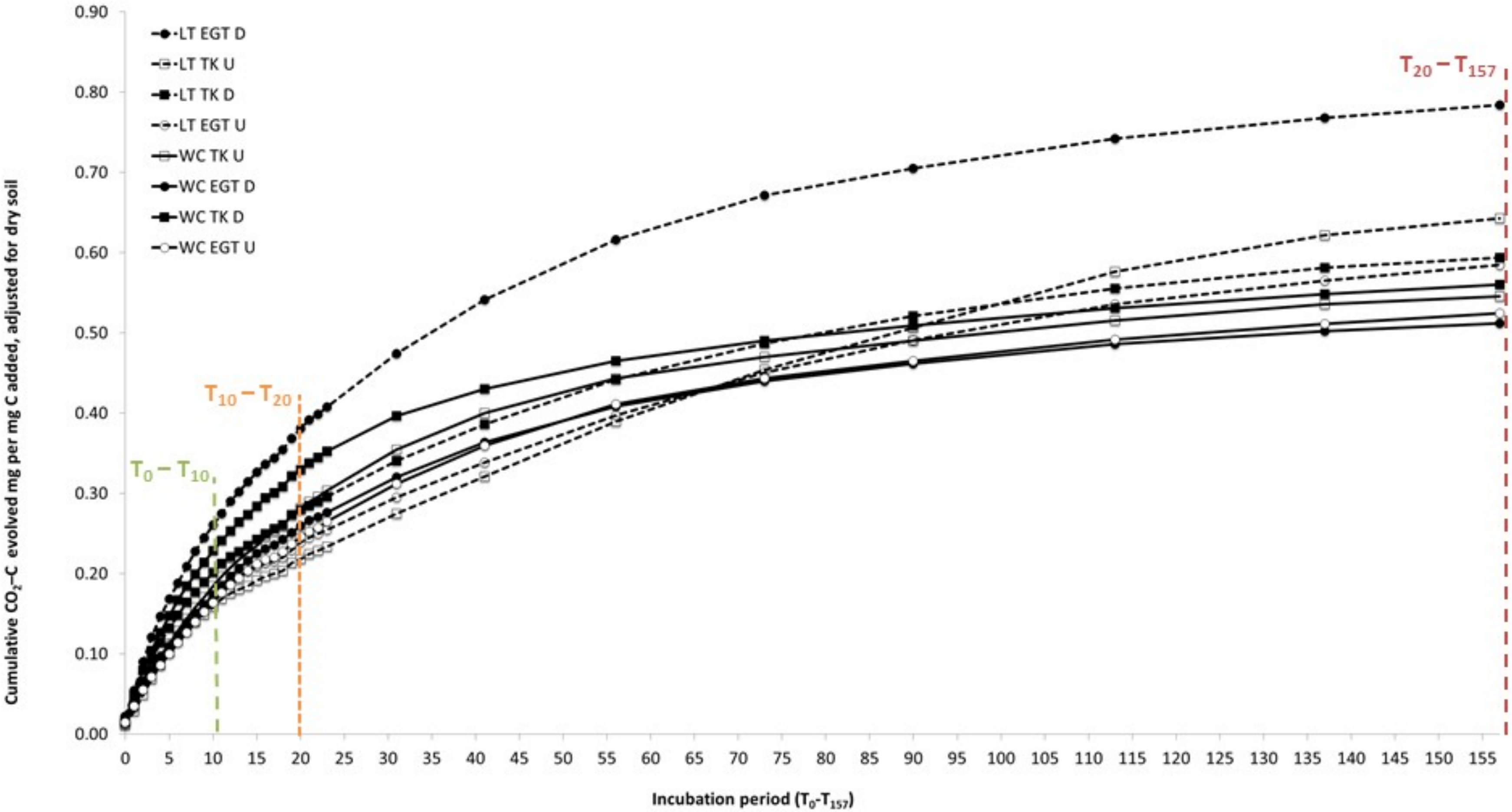


Figure 4.12 **The cumulative emission of CO_2-C (mg CO_2-C. mg^{-1} root C added) over 157 d from disturbed (D) and undisturbed (U) slices of Tokomaru Silt Loam (TK) and Egmont Silt Loam (EGT) topsoils containing roots of lotus (LT) and white clover (WC) grown for just over one year, adjusted for control soils.**

4.3.5 CO_2 evolved incubation end (T_{157})

Variation caused by the fixed treatments was relatively small. Soil, species and disturbance explain only 13.3 % of the variance in total accumulated CO_2-C (mg C. g^{-1} FD $soil_{T157}$) emitted when data is adjusted according to estimated dry soil added to each incubation jar (Table 4.7). None of these factors were significant over the length of the incubation (T_0-T_{157}).

Soil type was not a significant effect at any phase of decomposition considered; disturbance was only significant in the first phase of decomposition ($T_0 – T_{10}$) explaining 18.0 % of total variance; and species was significant only in the last phase of decomposition (T_{20} - T_{157}) explaining 28.3 % of total variance, when all factors were considered together.

Table 4.7 The statistical significance of lotus (LT) and white clover (WC) species, Tokomaru Silt Loam (TK) and Egmont Silt Loam (EGT) topsoils containing roots, and disturbed (D) and undisturbed (U) soil slices on total accumulated CO_2-C loss (mg C. g^{-1} FD soil) over 157 d, adjusted for control soil slices.

Time	Soil	Species	Disturbance	Soil (%)	Species (%)	Disturbance (%)	R-Sq adj (%)
T_0-T_{157}	NS	NS	NS	0.2	11.8	1.3	10.5
T_0-T_{10}	NS	NS	P = 0.04	0.0	0.9	18.0	14.4
T_{10}-T_{20}	NS	NS	NS	0.9	3.0	10.7	10.3
T_{20}-T_{157}	NS	P = 0.01	NS	0.3	28.3	1.6	28.2

NS - Not significant, P > 0.05

However, unlike the results expressed in Chapter 3 for plant shoots, the species trend was opposite for roots with LT emitting comparatively a higher cumulative amount of CO_2-C than WC in both soils and for both D and U treatments (Figure 4.12). Within the EGT soil, D treatments emitted a higher cumulative amount of CO_2-C than U treatments; but the opposite was true within the TK soil.

In Tables 4.8 to 4.13 the CO_2 evolved has been expressed as the CO_2-C mg evolved per mg root C added, adjusted for the dry weight of soil in each jar. LT treatments emitted a greater amount of CO_2-C than WC treatments when considered as a percentage of estimated root C added. LT EGT D emitted the greatest percentage of CO_2-C (78 %), whereas WC EGT D emitted the lowest (51 %) during the 157 d incubation (Table 4.8). When the accumulated release of CO_2-C ($T_0 – T_{157}$) from a treatment minus the control soil is expressed as a percentage of the added plant C, there was a 27 % spread in total emissions across all treatments. For all treatments there was a spread of 1 – 5 % when soil type, species and disturbance were individually considered, with exception of the LT EGT treatment where there was a 20 % difference between the D and U treatment.

Table 4.8 Average total CO_2-C emitted as a percentage of estimated root C added (mg C. g^{-1} C added) from disturbed (D) and undisturbed (U) slices of Tokomaru Silt Loam (TK) and Egmont Silt Loam (EGT) topsoils containing roots of lotus (LT) and white clover (WC) from T_0 to T_{157}.

Treatment	$\sum_{T157}^{T0}$
EGT LT D	78.4 % ± 30.8 %
TK LT U	64.3 % ± 27.3 %
TK LT D	59.4 % ± 4.6 %
EGT LT U	58.5 % ± 11.3 %
TK WC D	56.0 % ± 2.8 %
TK WC U	54.5 % ± 17.0 %
EGT WC U	52.4 % ± 17.6 %
EGT WC D	51.2 % ± 12.8 %

Each value in the table represents the mean of three replicates with standard deviation (±).

Across treatments 25 – 41 % of the total CO_2-C emitted was in the first 10 d of incubation. A further 9 – 18 % was emitted in the following 10 days, with the remaining 41 – 66 % of CO_2-C emitted over the remaining 137 d. 34 – 59 % of the total CO_2-C was therefore emitted within the first 20 d of the incubation which correlates to an initital rapid period of decomposition, as seen in Chapter 3, Section 3.2 and also visually apparent in Figure 4.12. As with the data presented in Chapter 3, there appears to be three phases of microbial growth inferred from the pattern of CO_2-C evolved. The stacked column graph (Figure 4.13) shows that the decomposition patterns across different treatments in this incubation were similar.

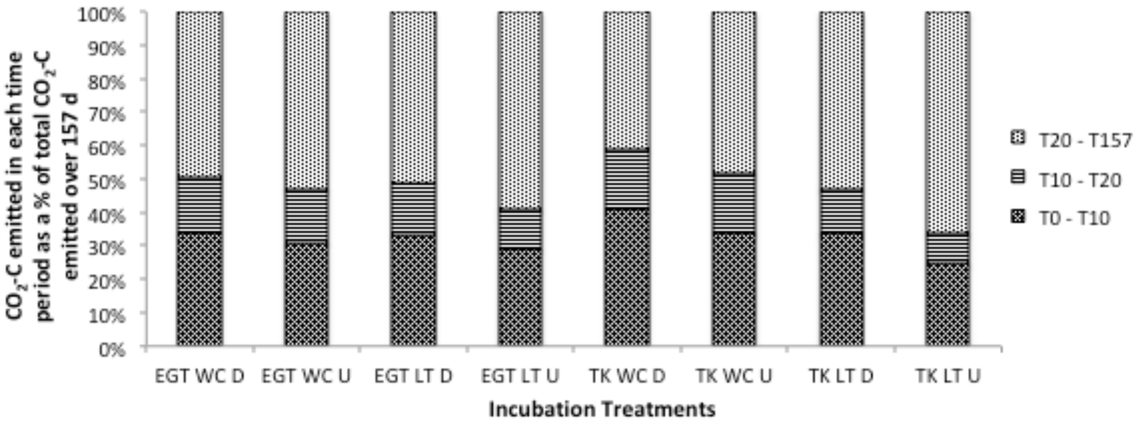


Figure 4.13 Total cumulative CO_2-C emitted at each phase of growth as a percentage of the total cumulative CO_2-C emitted by T_{157}.

4.3.6 Soil type

When analysed within soil type, 6 % of the variance was explained by the treatments in the TK soil and 24 % was explained in the EGT soil. There was a significant effect of soil disturbance (T_0-T_{10}) accounting for 31.8 % and species (T_{10}-T_{20}) accounting for 42.2 % in the early stages of decomposition in the TK soil (Table 4.9). Species was also significant in the later stages of decomposition (T_{20} – T_{157}) accounting for 27.1 %. There was no significant effect of species or disturbance in the EGT soil at the early stages of decomposition, with species only showing significance at the later stage of decomposition (T_{20} – T_{157}) accounting for 30 % of the variation (Table 4.10).

Table 4.9 The statistical significance of lotus (LT) and white clover (WC) species, and disturbed (D) and undisturbed (U) soil slices on total accumulated CO_2-C loss (mg C. g^{-1} FD soil) in Tokomaru Silt Loam (TK) topsoils containing roots over 157 d, adjusted for control soil slices.

Time	Species	Disturbance	Species (%)	Disturbance (%)	R-Sq adj (%)
T_0-T_{157}	NS	NS	5.6	0.4	36.0
T_0-T_{10}	NS	P = 0.02	12.0	31.8	64.0
T_{10}-T_{20}	P = 0.01	NS	42.2	5.7	66.8
T_{20}-T_{157}	P = 0.04	NS	27.1	9.3	50.4

NS - Not significant, $P > 0.05$

Table 4.10 The statistical significance of lotus (LT) and white clover (WC) species, and disturbed (D) and undisturbed (U) soil slices on total accumulated CO_2-C loss (mg C. g^{-1} FD soil) in Egmont Silt Loam (EGT) topsoils containing roots over 157 d, adjusted for control soil slices.

Time	Species	Disturbance	Species (%)	Disturbance (%)	R-Sq adj (%)
T_0-T_{157}	NS	NS	18.4	5.8	60.5
T_0-T_{10}	NS	NS	12.0	13.6	54.8
T_{10}-T_{20}	NS	NS	2.0	16.0	46.6
T_{20}-T_{157}	P = 0.01	NS	30.3	0.1	68.7

NS - Not significant, $P > 0.05$

4.3.7 Plant species

When the CO_2 emissions were analysed within species type there was no significant effect of soil type or disturbance at any phase of decomposition (Table 4.11), and only 2.3 % of the variation was explained by the treatments. In the early stages of decomposition (T_0-T_{20}) soil type explained between 12 – 21 % of the total variance for the WC treatment and 7 – 13 % of the total variance for the LT treatment (Table 4.12); comparatively, disturbance explained 1 – 11 % and 25 – 26 % for the WC and LT treatments respectively. Both soil type and disturbance explained very little of the variance in the later stage of decomposition.

Table 4.11 The statistical significance of Tokomaru Silt Loam (TK) and Egmont Silt Loam (EGT) topsoils containing roots, and management of residues (disturbed (D) and undisturbed (U) soil slices) on total accumulated CO_2-C loss (mg C. g^{-1} FD soil) in white clover (WC) roots over 157 d, adjusted for control soil slices.

Time	Soil	Disturbance	Soil (%)	Disturbance (%)	R-Sq adj (%)
T_0-T_{157}	NS	NS	2.3	0.0	0.0
T_0-T_{10}	NS	NS	21.0	10.6	29.0
T_{10}-T_{20}	NS	NS	12.0	0.7	0.0
T_{20}-T_{157}	NS	NS	1.7	4.8	0.0

NS - Not significant, $P > 0.05$

Table 4.12 The statistical significance of Tokomaru Silt Loam (TK) and Egmont Silt Loam (EGT) topsoils containing roots, and management of residues (disturbed (D) and undisturbed (U) soil slices) on total accumulated CO_2-C loss (mg C. g^{-1} FD soil) in lotus (LT) roots over 157 d, adjusted for control soil slices.

Time	Soil	Disturbance	Soil (%)	Disturbance (%)	R-Sq adj (%)
T_0-T_{157}	NS	NS	3.0	3.8	0.0
T_0-T_{10}	NS	NS	7.4	25.6	13.5
T_{10}-T_{20}	NS	NS	13.1	24.7	27.5
T_{20}-T_{157}	NS	NS	0.1	1.4	0.0

NS - Not significant, $P > 0.05$

4.3.8 Main effects of soil disturbance

When the CO_2 emissions were analysed within management type there was no significant effect of soil type or species at any phase of decomposition; 23.9 % of the variation within the D treatment was explained compared to only 7.3 % in the U treatment. When the CO_2 emissions were analysed within soil disturbance treatments (D or U) there was a significant effect of species by incubation end (T_{20} –T_{157}) in the D treatment, explaining 40.7 % of the variance (Table 4.13). In both D and U treatments no other treatment had a significant effect (Table 4.13, Table 4.14).

Table 4.13 The statistical significance of lotus (LT) and white clover (WC) species, and Tokomaru Silt Loam (TK) and Egmont Silt Loam (EGT) topsoils containing roots, on total accumulated CO_2-C loss (mg C. g^{-1} FD soil) in disturbed (D) treatments over 157 d, adjusted for control soil slices.

Time	Species	Soil	Species (%)	Soil (%)	R-Sq adj (%)
T_0-T_{157}	NS	NS	19.7	4.2	21.1
T_0-T_{10}	NS	NS	6.2	0.0	0.0
T_{10}-T_{20}	NS	NS	0.8	5.0	6.3
T_{20}-T_{157}	$P = 0.02$	NS	40.7	8.9	47.7

NS - Not significant, $P > 0.05$

Table 4.14 The statistical significance of lotus (LT) and white clover (WC) species, and Tokomaru Silt Loam (TK) and Egmont Silt Loam (EGT) topsoils containing roots, on total accumulated CO_2-C loss (mg C. g^{-1} FD soil) in undisturbed (U) treatments over 157 d, adjusted for control soil slices.

Time	Species	Soil	Species (%)	Soil (%)	R-Sq adj (%)
T_0-T_{157}	NS	NS	5.8	1.5	19.0
T_0-T_{10}	NS	NS	1.6	0.4	0.0
T_{10}-T_{20}	NS	NS	28.1	0.3	28.1
T_{20}-T_{157}	NS	NS	22.0	1.8	35.8

NS - Not significant, $P > 0.05$

4.4 Discussion

This incubation set out to evaluate the effects of plant species and their roots on their decomposition (measured as CO_2 emitted) in order to ascertain the impact on the amount of C stored in the soil. LT and WC decomposition were simulated in D and U conditions in both EGT and TK soils. There was a large amount of unexplained variation in the results, compared to those presented in Chapter 3. This error appears to exist regardless of whether the soil in the treatments was mixed (D) or not (U). Despite multiple attempts to manipulate the data and remove confounding information, there was still minimal statistical significance seen as a result of the treatments applied.

4.4.1 Effect of species

Overall there was a significant effect of species across all treatments by T_{157}. Within all treatments LT released 5 - 21 % more CO_2-C than WC resulting in a lower amount retained in the soil. Species was significant at both T_0-T_{10} and T_{20}-T_{157} in the TK soil, but only significant T_{20}-T_{157} in the EGT soil. If the rate and total amount of root litter decomposed in soil had been less for the LT treatments than the WC, this would have supported the results from the initial incubation experiment in Chapter 3; which indicated that specific plant traits (like tannin content) can affect soil C decomposition and retention in soils. However, contrary to expectation these results are in direct contrast to the shoot emission results (Chapter 3.5.2), which had been concluded were consistent with the high tannin and lignin content of LT shoots.

The WC roots decomposed less over 157 d than LT roots; LT treatment in EGTD soil emitted the most CO_2, WC treatment in EGTU soil emitted the least CO_2. The results presented here have been based on an estimated amount of added root C (Chapter 4.1.2), so there is a distinct possibility that this may have been greatly underestimated in the case of the LT leading to the results seen. This uncertainty is obviously a limitation of the study, and another incubation study is required to rule out the confounding factor of rate of C addition.

Chemical composition

Hobbie et al. (2010) suggests that traits in shoot litter which influence decomposition may not be present in the root litter, or if present do not have the same effect below-ground as they do above-ground. Silver & Miya (2001) and Scheffer & Aerts (2000) both comment on the fundamentally different physiological functions of roots versus shoots and how this can result in different chemical composition and thus decomposition rates. This situation is present in the results of this experiment, where the proportion of both tannin and lignin is different between shoot and root of each species and that the ratio has changed between the species themselves (refer Tables 3.2 and 4.6). For example, the tannin content of LT roots, while still vastly higher than that of WC roots, is of a magnitude five times less than that in the LT shoots. For the WC the type of tannin has also changed, shifting from protein bound in the shoots to fibre bound in the roots. However, the proportion of lignin in LT is of a magnitude almost three times that in the shoots, while for WC this is a magnitude of nine times. This has shifted from proportionally higher lignin in the LT shoots vs WC shoots to proportionally higher in the WC roots than the LT roots. Other factors have also changed, such as the total ME and the amount of metals such as Fe, Mn and Zn, and these factors also deserve further consideration.

Fresh versus dead root

Bloomfield et al. (1993) made the point that estimating the rate of root decomposition is complicated as any analysis disturbs the natural decomposition environment. Scheffer & Aerts (2000) discussed the further complication that there can be large chemical variation between live and dead roots, depending on the degree to which decomposition has taken place, and yet it is difficult to ensure that the roots sampled have recently died. Research supports that the root systems of grass species, e.g. ryegrass, is constantly turning over (Matthew et al. 2016) with the root: shoot ratio potentially changing during the year, to reflect the variation in the number of leaves available to 'feed' developing roots. This means that any decomposition rates measured using extracted litter or roots may or may not accurately reflect the field situation (Silver & Miya, 2001).

The live versus dead root concept is an interesting one when considered in light of the contrasting results for WC and LT roots in this experiment. Perhaps the results obtained are not directly a result of the chemistry of the LT and WC roots at the start of the incubation, but more a reflection of the amount (history) of decomposition that has already taken place in the pots prior to selection and incubation. Matthew et al. (2016) showed that root biomass deposition differed little under differing grazing management regimes, but that there was marked seasonal variation. Root biomass deposition was found to be about 15 % of above ground herbage accumulation, with similar seasonality, but with bursts in root deposition over a month behind above-ground events. Destructive sampling in field swards provides limited insight into root dynamics because of the slow decomposition rate of dead roots, resulting in a high proportion of dead roots in samples that are difficult to distinguish from live roots

(Matthew et al. 2016 and citations therein). In this study, the root sample chemically analysed was selected randomly, and both live and dead root would have been included in the sample. However the WC roots may have been predisposed to a higher rate of decomposition during plant growth in the glasshouse pots than the LT roots, meaning that once under incubation conditions less fresh root was present in the WC treatment, resulting in a lower rate of recorded decomposition. This may also account for why higher lignin and lower ME was recorded for the WC root sample.

In a study by Gibbs (1986) dead clover roots were completely decomposed after 5 months. Whereas Freschet et al. (2013) estimated that for grasslands, (insitu, excised material in litter bag studies) rhizodeposition inputs make up approximately 33 % of annual residue inputs (26 % for leaf litter; 41 % for fine stems), and that this material decomposes around 2.8 times slower than leaf litter derived from the same species. The point is made that the chemical and structural traits influencing decomposition vary between plant organs (leaves, stems and roots) as well as across species. Limited understanding of the field behaviour of grass root systems is recognised as being partially due to the difficulty in developing suitable measurement techniques. Species obviously has a significant effect on the decomposition of pasture residues, but as this study is limited to working with the estimated amount of added root C, rather than a known amount, further research is required to tease out these answers.

4.4.2 Effect of soil disturbance and soil type

In the early stages of the incubation, the D treatments emitted more CO_2 than the equivalent U treatment. However by incubation end T_{157} there was less of an obvious effect of soil disturbance. When the CO_2 emissions were analysed within soil disturbance treatments (D or U) there was a significant effect of species by incubation end (T_{20} –T_{157}) in the D treatment, explaining 40.7 % of the variance (Table 4.13). In both D and U treatments no other treatment had a significant effect (Table 4.13, Table 4.14).

Variation in CO_2-C emitted appears to exist regardless of whether soil is disturbed or not. This may be due to the actual root weight differing to that of the estimated root weight used to render the data. Despite the confounding variation, disturbance has a significant and important effect in the early stages of decomposition ((T_0-T_{10}) within the TK soil). Take note of Table 4.13 which clearly shows a significant effect of disturbance at T_0 – T_{10}; and Figure 4.10 where all points for the D treatments (solid symbols) had higher values than the associated U treatments (open symbols).

All root incorporated in the soil slices was already dead, sprayed off by Roundup prior to incubation. Root senescence has therefore not been caused by disturbance of the soil slices, and there is no decomposition resistance. The effect seen is one of substrate access. The increase in CO_2-C under D treatments may be because disturbance has increased the surface area of the substrate by breaking it up; or because disturbance has overcome some limiting factor / s to decomposition – aeration, etc. Despite an initial flush from the soil disturbance it is expected that total CO_2 emissions from the D and U samples will equal out. It is possible that the disturbance might result in an increase in the air transport coefficient due to loosening of the soil (change in the physical characteristic of the soil), which physically releases CO_2 previous entrapped in the soil pores from previous microbial activity. This disturbance may also initially stimulate microbial activity, enhancing soil decomposition, with this flush of activity declining on decline of available substrate.

It is commonly accepted that soil OM is an ever evolving continuum of decomposing organic compounds that continue to decrease in size, oxidise, and become more water soluble (Kleber et al. 2015; Hedges & Keil, 1999). An important fraction of OM consists of microbial decomposition products, which also decompose (Liang & Balser, 2010). Hobbie et al. (2010) furthermore pointed out that plant traits may be influential solely based on the microbial community present in the soil which could account for the differences seen between the soil types here.The resulting organic compounds may become chemically protected through interaction with SRO constituents (Kleber et al. 2015) or physically protected within soil aggregates (Shen et al. 2018; Dungait et al. 2012a; Six et al. 2000).

Considering the amount of CO_2-C emitted per mg estimated root added, it appears that there was roughly 4 times the amount of root C added than that of the shoot C added to the incubation in Chapter 3. The rate and total amount of root litter decomposed in soil was higher in the TK soil compared to the EGT soil (with exception of the LT D treatment). This may support the concept of more chemical OM protection in the EGT soil. Shen et al. (2018) reported that soil C fractions considered to have the longest residence times are those chemically associated with mineral surfaces and metal cations forming organo-mineral complexes. Young acid soils (particularly volcanic soils), under high precipitation and good drainage may increase OM preservation as Al is able to form inner-sphere complexes with organic ligands. This may be what happened with the EGT soil in this study, given the results that have been presented here. Previous research (Herath, 2012) had indicated that the higher original OM in the EGT soil is susceptible to natural decomposition stimulation. In comparison Shen et al. (2008) suggested that within allophanic soils (e.g. EGT soil), there is preferential formation of Al hydroxides and the weakening of proton bridging between organic ligands (e.g., carboxylic acid functional groups) as the pH increases; which increases the vulnerability of OM to decomposition.

Hobbie et al. (2010), Silver & Miya (2001), Scheffer & Aerts (2000) and Swift et al. (1979) all suggest that there may be little correlation between shoot and root decomposition of the same species, regardless of any similarities in tissue chemistry, as the site (soil) environmental conditions under which decomposition is taking place will take precedence (i.e. soil moisture, O_2 concentration and pH). In this incubation, environmental conditions after incubation set up (e.g. temperature, light, soil water content, etc.) were held consistent between treatments. This may explain why there was a significant effect of species at incubation stages in the EGT soil but not in the TK soil (i.e. a soil type influence).

The important conclusions to make here is that (i) shoot litter decomposition cannot be extrapolated to root litter decomposition, even if tissue chemistry is similar; (ii) “site” conditions (i.e. soil type, soil disturbance) can have a significant influence on the rate and total amount of CO_2-C decomposition.

4.5 Potential limitations

As has been discussed briefly above, there were limitations to this study which, in some cases, made the results difficult to interpret, these are listed below:

1. Total C is an estimate of the C present in the roots and the soil. Soil sections sampled for total roots may not be representative of the sections incubated. This may have led to an over / under estimation of total C present in the incubation chamber.

2. Senescence of WC and LT plant root material during growth in glasshouse pots varied, possibly leading to different soil and root C status in the soil and root samples taken for incubation experiments.

4.6 Conclusions

This incubation experiment simulated WC and LT root decomposition in D and U conditions in both EGT and TK soils. Despite the confounding treatment results, it was still apparent that much of the decomposition occurs in the first month which was in keeping with the results reported in Chapter 3 and also in line with other decomposition studies (Walela et al. 2014; van Gestel et al. 1993; Zunino et al. 1982). WC roots were decomposed less over 157 d than LT roots. While there was no plant species effect in the early to middle stages of incubation, there was a significant difference by incubation end (T_{157}). When analysed by soil type, there was no significance at any stage in the TK soil, the differences being seen in the EGT soil early in the incubation and by T_{157}. The effect of soil disturbance was significant in both soils in the early stages of decomposition, but this effect lessens with time. However the coefficient of variation within this root study was too high to give any precision around the estimate of treatment effects, compared to the lower coefficient of variation seen in the shoot study in Chapter 3, which allowed precise estimates to be given.

Overall, the WC treatment in U EGT soil resulted in the highest amount of estimated added plant C retained in the soil (49.0 %), while the LT treatment in D EGT soil resulted in the lowest amount retained (25.7 %). Although the trend is different from that seen in the shoot material (Chapter 3.3.2), overall more C was retained under the roots of these species than under the shoots. The results are difficult to interpret as there may be a rate of C effect - the CO_2 evolved for roots is much lower than that of the shoots; this may be caused if the rate of C is higher or if the root material is much more recalcitrant. As there is a species contrast between shoots and roots, including the notable contrast in the compound analysis (e.g. metal compounds), it is important to remove the possible confounding factor that roots have been added at higher rates.

While the shoot incubation made it clear that using freeze dried material can underestimate the amount of C that would be lost from fresh material in the field, this incubation has made it clear that the use of "fresh" roots insitu can lead to uncertainties in estimation of total C present, and limit understanding of the mechanisms at play. While there is value in conducting undisturbed decomposition studies, there exists the potential for huge variability, which is why a further, more controlled study is suggested. It is important therefore to conduct an experiment using freeze dried root and shoot material, added at rates to simulate both residual shoot material but also the, potentially far greater, residual root material. This should allow a comparison between the decomposition of *Lotus pedunculatus* and *Trifolium repens* shoots and roots, and rule out any confounding issue of rate of addition.

Chapter 5

The extent of decomposition in soil of lotus (*Lotus pedunculatus*) and white clover (*Trifolium repens*) shoot and root material at 2, 5, 10 mg C. g^{-1} OD soil after 134 d of incubation.

5.1 Introduction

Estimating the rate of root decomposition continues to be a scientific challenge. Any analysis disturbs their natural environment (Bloomfield et al. 1993) and large rhizosphere microbial population (Killham 1994); and, as shown in Chapter 4, there are problems associated with quantifying root biomass in undisturbed soils as well as problems accurately sampling recently senesced roots (Scheffer & Aerts 2000). The root decomposition study conducted in Chapter 4 was designed to simulate the decomposition of senescing *Lotus pedunculatus* (LT) and *Trifolium repens* (WC) roots after herbicide treatment at pasture renovation. The technique of using soil slices containing “insitu roots” created a large variance in the C mass of the root substrate in replicates of the same treatment, which was believed to cause the large variance (up to 30 %) around treatment means for the extent of root decomposition.

Despite the large variation in root decomposition, there appears to be a large difference between the extent of WC root decomposition during a 157 d incubation (55 – 56 %) in Chapter 4 compared to the extent of WC shoot material (77 % and 100 %, respectively) over 125 d observed in Chapter 3. Whereas this result supports the observations of Shi et al. (2013) and Vidal et al. (2017), that the root materials of some grasses and cereals decompose more slowly than shoot materials; it is not possible to conclude that roots in general decompose slower than shoots, because the extent of LT root decomposition during a 157 d incubation (59 – 64 %) was similar to that of dried WC shoot material (59 %) but lower than fresh shoot material (80 %) observed in Chapter 3. It is important therefore to conduct a more controlled experiment using freeze dried root and shoot material, added at rates to simulate both residual shoot material but also the, potentially far greater, mass of residual root material. This should allow a comparison between the decomposition of LT and WC shoots and roots, and rule out any previous confounding issue of rate of addition.

Hypotheses

Three hypotheses are presented:

(i) Differences in tissue chemistry between shoot and root material will lead to systematic differences in litter decomposition rates. Therefore the application of different plant part (shoot and root) biomass to the soil will alter the rate and total amount of litter decomposed.

(ii) A high percentage of tannins, in *Lotus pedunculatus* tissue, will decrease the rate and total amount of litter decomposed in soil, therefore retaining a greater amount of C, compared to that of a comparable pasture species *Trifolium repens* which contains relatively low tannin content.

(iii) Increasing the rate of residue-C applied to the soil will not result in an equivalent proportional increase in rate of CO_2-C loss from the soil, i.e. more C will be conserved under a higher application of residue-C.

Experimental design

In Chapter 3 variability was minimised by mixing plant material with the soil, whereas in Chapter 4 plant material was incubated insitu and large variability ensued.

As was seen in Chapter 4, and highlighted in literature by Bloomfield et al. (1993), the estimation of root decomposition rates *invivo* is an imprecise process. Taking note of Bloomfield's concerns, and consideration of the higher degree of variability of CO_2 evolved from roots left insitu, in this experiment (Chapter 5) known masses of freeze dried and ground roots were homogenised with the soil prior to incubation to reduce replicate variability so that the treatment effects (plant type, plant part and rate of application) could be realised (the incubation detail is covered in the methodology).

Experimental objectives

(i) To generate plant shoot and root material through glasshouse studies for plant analysis prior to decomposition studies.

(ii) To chemically characterise the tissue of *Lotus pedunculatus* (LT) and *Trifolium repens* L. (WC) shoots and roots, and determine how their specific plant traits differ.

(iii) To test if plant shoot and root tissue type and chemistry can influence the rate of decomposition and the amount of residue C remaining after a period of decomposition (not including contribution from root exudates).

(iv) To quantify the effect of three C application rates (2, 5, 10 mg C. g^{-1} OD soil) on the rate of decomposition and the amount of residue C remaining after a period of decomposition.

5.2 Materials and methodology

5.2.1 Soil collection and preparation

Tokomaru silt loam topsoil (TK), a Pallic soil (Typic Fragiaqualf), was collected and stored as outlined in Chapter 3, Section 2.1. All soil site details were as previously reported. After chilling, a sub sample of the two soils was taken for use in the glasshouse trial (described in Chapter 4, Sections 2.2 to 2.3).

5.2.2 Soil characterisation

Chemical analysis methods are explained in more detail in Chapter 3, Section 2.2. Analysis of the soil showed that it presented no limiting conditions (Morton & Roberts, 2009) for plant growth and decomposition. Results are reported in Table 3.1.

5.2.3 Cultivation and preparation of shoot and root material

Shoot and root material were prepared in the glasshouse trial described in Chapter 4, Section 4.2.3. Pots of *Lotus pedunculatus* and *Trifolium repens* were grown from seed in Tokomaru Silt Loam soil for 2 years. During their growth, these pots were regularly watered to estimated field capacity to minimise periods of water stress, and the plants were regularly cut to simulate optimal grazing strategies. After harvest of the root material for Chapter 4, the remaining pots were maintained under glasshouse conditions for just over two years. At the end of this period, pots with the highest % DM shoot yield, were selected and harvested for the shoot and root incubation studies. One destructive harvest of fresh leaf and stem material (termed "shoot") and roots and stolons (termed "roots") was taken from pots of LT and WC. After harvest, the fresh shoot material was immediately prepared (freeze dried and ground) as per Chapter 3, Section 2.3. The root material was wet sieved (Figure 5.1), then processed and stored as per the shoot material in Chapter 3, Section 2.3.

Figure 5.1 Harvest of root material by wet sieving, ready for freeze drying.

5.2.4 Root material characterisation

Chemical analysis methods of the shoot and root material are explained in more detail in Chapter 3, Sections 3.2.2 and 3.2.3.

5.2.5 Incubation preparation

Prior to incubation the TK soil was retrieved from the chiller and sieved to 2 mm. The freeze dried - ground plant amendment treatments were mixed into a small amount of this soil and then mixed into the remaining bulk of soil (total 200 g OD equivalent) to ensure even distribution within each incubation soil. Once the plant material was mixed in, the soil was adjusted to gravimetric water content of 0.35 (70 % FC) and immediately added to the respective incubation chamber (Time zero, T_0); after which a NaOH trap and a H_2O vial were included (Figure 5.2, for detailed description see Chapter 3.2.5). The pre-incubation period was not observed.

Extra chambers were also set-up to measure background soil respiration (TK field soils that had been stored in the chiller at 3 °C), CO_2 already present in the enclosed space and any CO_2 entry during venting. The treatment codes applied to the chambers were: (i) LT and WC; (ii) shoot and root; and (iii) rate of application (2, 5, 10 mg C. g^{-1} OD soil). All treatments were run in triplicate (Figure 5.2).

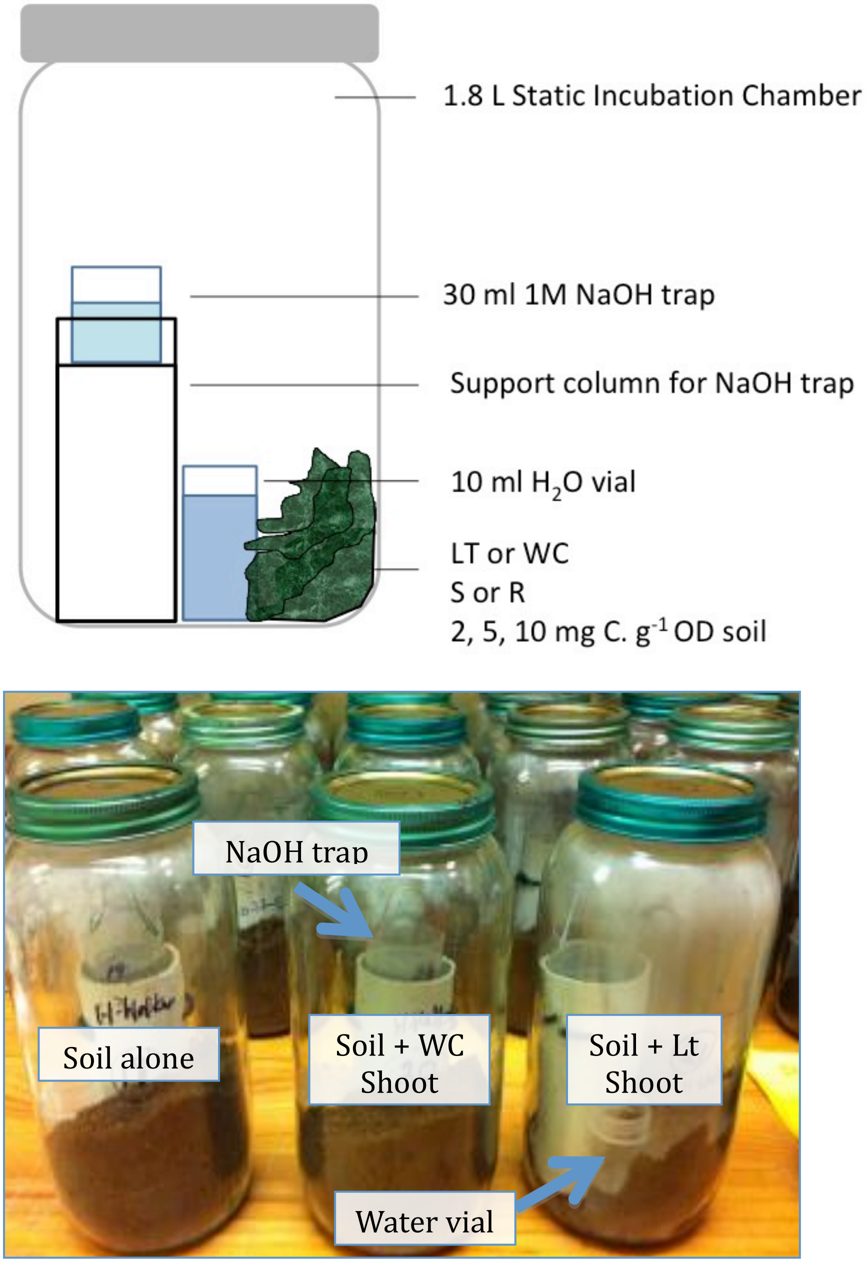


Figure 5.2 **Static Incubation Chamber setup. Top: Lotus (LT) and white clover (WC), shoot (S) and root (R) material grown in Tokomaru Silt Loam (TK) topsoil, applied at 2, 5, or 10 mg C. g^{-1} OD soil. Bottom: Soil in sealed agee jars with NaOH trap and water vial.**

The chambers were arranged by blocks and incubated in dark conditions at an average temperature of 18 °C (range 14 – 22 °C). The soil moisture content was maintained at an estimated gravimetric water content of 0.35 (70 % FC) throughout the incubation (Figure 5.3).

5.2.6 Measurement of CO_2 flux

Static NaOH CO_2-trap chamber method

Unless stated otherwise, the chamber method follows the description in Chapter 3, Section 2.5. The polypropylene cup used to contain the NaOH CO_2 trapping solution was elevated approximately 5 cm above the soil surface in the centre of the chamber. The chamber headspace volume was 1478 ml. The seal between the chamber top and base was gas-tight. The chambers were vented and the alkali traps were initially changed daily (for 16 d) to maximize CO_2 captured and decrease the risk of O_2 depletion in the chamber, and thereafter monitored and changed as required.

Aerobic conditions were maintained where possible. Total duration of the incubation was 134 d (T_{134}). At T_{134} the soil was removed from the incubation jars, frozen and freeze dried.

Determination of CO_2 evolved

The titration method for determination of the amount of CO_2 trapped (evolved) is explained in more detail in Chapter 3, Section 3.2.5. Results are expressed on the basis of OD (105 °C) soil weight unless otherwise stated, and are the means of three replicate determinations.

5.2.7 Statistical analysis

Analysis of variance was conducted using a General Linear Model that incorporated the three fixed effects associated with treatments: species, plant part, rate of C application. The random effects, associated with experimental setup and climatic conditions within the glasshouse, were accommodated by blocking the replicates. Glasshouse conditions were normalised as much as possible by regular movement of the pots.

5.3 Results

5.3.1 Soil analysis results

As reported in Chapter 3, Section 2.2 chemical analysis of the TK soil showed that it presented no limiting conditions for decomposition, with chemical fertility (Roberts & Morton, 2009) in the medium to high range (Table 3.1).

5.3.2 Shoot and root material characterisation

The CT and lignin content of the LT shoot material was markedly higher than that of the WC shoot material; comparatively, WC shoot had higher ME than LT shoot, with the inverse true for root material (Table 5.1). Other indices were very similar. All plant materials exceed the minimum criteria for decomposition being N limited, < 2 % N and C:N ratio > 20:1 (Cabrera et al. 2005).

Table 5.1 Chemical composition of freeze dried lotus (LT) and white clover (WC) shoot (S) and root (R) tissue (mean and standard deviation presented), as measured pre-incubation.

Analysis Type	**LT S**		**WC S**		**LT R**		**WC R**	
	Mean	***SD***	**Mean**	***SD***	**Mean**	***SD***	**Mean**	***SD***
C (%)^{1}	44.7	*0.14*	41.8	*0.05*	38.3	*0.11*	40.2	*0.15*
N (%)^{1,2}	3.9	*0.060*	3.8	*0.04*	2.5	*0.040*	3.0	*0.020*
C:N^{1,2}	11.4	-	11.0	-	15.3	-	13.6	-
P (%)^{2}	0.24	*0.021*	0.29	*0.01*	0.39	*0.007*	0.28	*0.007*
S (%)^{1,2}	0.21	*0.00*	0.16	*0.01*	0.12	*0.080*	0.070	*0.020*
K (%)^{2}	1.7	*0.21*	2.8	*0.00*	0.30	*0.000*	0.30	*0.000*
Ca (%)^{2}	0.71	*0.042*	1.1	*0.04*	0.46	*0.035*	0.76	*0.007*
Mg (%)^{2}	0.32	*0.014*	0.26	*0.01*	0.13	*0.014*	0.12	*0.000*
Fe (mg. kg^{-1})^{2}	72.0	*3.5*	140.0	*36.1*	6990.0	*226.3*	7690.0	*551.5*
Mn (mg. kg^{-1})^{2}	98.0	*2.8*	30.0	*4.2*	435.0	*35.0*	720.0	*42.4*
Zn (mg. kg^{-1})^{2}	38.0	*4.2*	22.0	*2.8*	47.0	*3.5*	97.0	*2.1*
CT Total (%)^{3}	8.7	*0.070*	0.10	*0.00*	1.6	*0.14*	0.10	*0.000*
Lignin (%)^{2,3}	6.7	*0.82*	2.6	*0.41*	19.3	*2.1*	23.7	*3.8*
ME (MJ. kg^{-1})^{2,3}	10.6	*0.46*	11.7	*0.32*	6.2	*0.46*	4.8	*0.93*

Analyses performed by: ^{1} Elementar (vario MACRO cube); ^{2} Hills Laboratory; ^{3} MSFAT. Results presented on the basis of three replicates.

LT, the “high tannin” species selected, had higher CT than WC, the “neglible tannin” species in both shoots and roots (8.7 % CT in the shoot material and 1.6 % CT in the root material vs 0.1 % CT respectively). Of the total CT detected in WC, almost all of it was Protein Bound CT in the shoot material, but Fibre Bound in the root material; whereas for LT it was a mixture of Free CT, Protein Bound CT and Fibre Bound CT in both plant parts. These results are similar to those reported by other authors (Chapter 2, Table 2.0).

LT varied significantly from WC in Fe, Mn and Zn foliage concentrations. LT shoot material had triple the amount of Mn and twice the amount of Zn than WC while WC shoot material had twice the amount of Fe than LT. In comparison, WC root material had roughly twice the Zn and Mn than the LT. Root material in general had roughly a hundred times the amount of Fe and four times the amount of Mn.

Plant amendments of LT and WC shoots and roots were made at rates of 2, 5 and 10 mg C. g^{-1} OD soil. Dry matter amendments of LT and WC were varied to achieve the desired rate of C addition, thus N and S varied as shown in Table 5.2.

Table 5.2 Rate of DM, C, N, and S applied to the lotus (LT) and white clover (WC) shoot (S) and root (R) incubation treatments at plant amendment rates of 2, 5 and 10 mg C. g^{-1} OD soil.

Treatment	Rate of C applied (mg C. g^{-1} OD soil)	Rate of DM applied (mg DM. g^{-1} OD soil)	Rate of N applied (mg N. g^{-1} OD soil)	Rate of S applied (mg S. g^{-1} OD soil)
WC S	2	4.80	0.18	0.01
	5	11.95	0.46	0.02
	10	23.95	0.91	0.04
WC R	2	4.45	0.17	0.01
	5	11.20	0.44	0.02
	10	22.40	0.88	0.05
LT S	2	4.95	0.15	0.00
	5	12.45	0.37	0.01
	10	24.85	0.74	0.02
LT R	2	5.20	0.13	0.01
	5	13.05	0.33	0.02
	10	26.10	0.65	0.03

5.3.3 Comparative incubation conditions Chapters 3 to 5

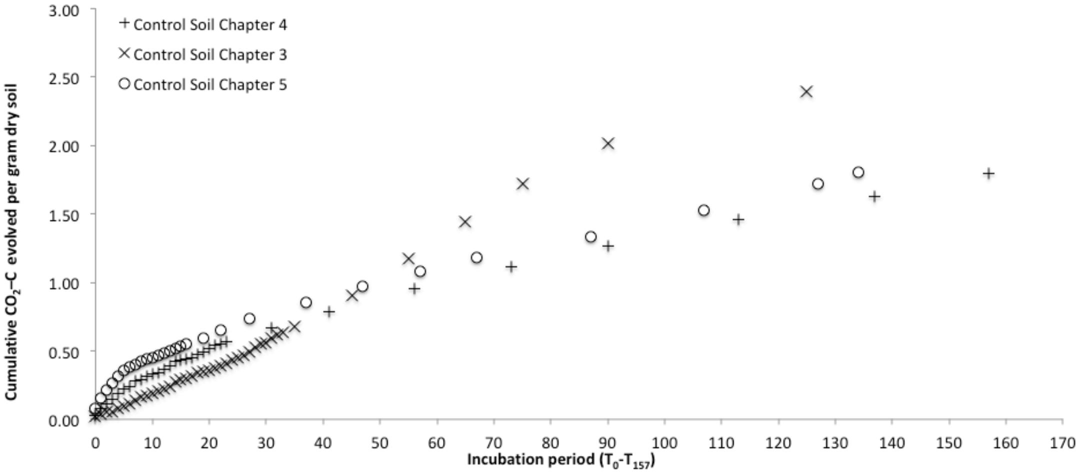


Figure 5.3 **CO_2-C emission from background soil across the incubations presented in Chapter 3, 4, 5.**

In long-term studies (e.g. PhD research) the loss of microflora viability in stored soil samples is a concern. Chilling was used in these studies to attempt to preserve microflora viability. The rate of CO_2-C emission from the Tokomaru soil used in these experiments averaged 0.13 mg CO_2-C. g^{-1} dry soil. $week^{-1}$ when first used in Chapter 3, 0.08 mg CO_2-C. g^{-1} dry soil. $week^{-1}$ in the experiment in Chapter 4 (Figure 4.7), and 0.10 mg CO_2-C. g^{-1} dry soil. $week^{-1}$ in this current experiment, Chapter 5 (Figure 5.3) for the whole incubation periods.

The rate of CO_2 emission of the Control soil data presented in this Chapter was very similar to those recorded for the TK soil in Chapter 3 and Chapter 4, despite differences in the storage time of the soils in a chiller at 3 °C. The soil used in Chapter 3 was freshly collected from the field and contained fine residual root material (not removed in the preparation sieving). By the set up time of the experiments in Chapters 4 and 5 the soil had been stored in the chiller for 383 d and 565 d respectively. The results suggest that adequate microflora viability is retained in the stored soil.

5.3.4 Effect of plant species, plant part and rate of C addition on cumulative CO_2 evolved over 134 d

The treatment effect on CO_2-C evolved (Figure 5.4) was isolated by subtracting the background CO_2-C emitted per control jar. This was achieved by calculating all emissions based on the dry weight of soil in each jar and subtracting the control mean CO_2-C evolved (mg C. g^{-1} OD soil) from each treatment sample mean. The accumulated CO_2-C evolved (mg C. g^{-1} OD soil) for the treatment means are presented in Figures 5.5 and 5.6. All treatments including shoot and root material have accumulated CO_2-C emissions 150 % and 125 % higher; respectively, than the control soils. The majority of the differences caused by plant species, plant part (shoot or roots) and rate of application are obvious from T_5 onwards.

$$Corrected\ Treatment \sum_{T0}^{Tn} \mathrm{CO_2 - C\ evolved\ (mg\ C.\ g^{-1}\ OD\ soil)}$$
$$= (Treatment \sum_{T0}^{Tn} \mathrm{mg\ CO_2 -\ C\ evolved\ .\ \ g^{-1}\ OD\ soil})$$
$$- (control \sum_{T0}^{Tn} \mathrm{mg\ CO_2 - C\ evolved\ .\ \ g^{-1}\ OD\ soil})$$

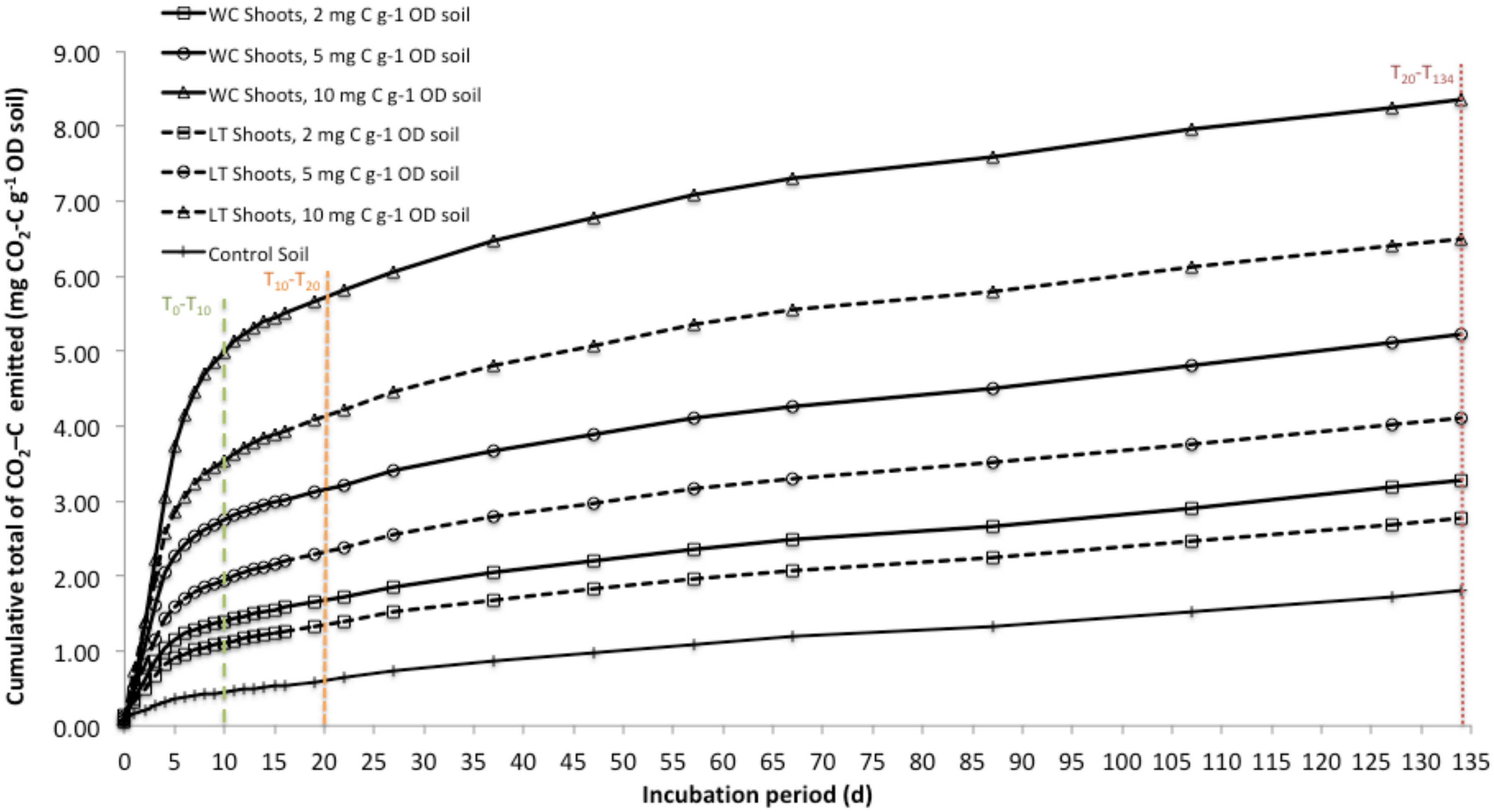


Figure 5.4 **Accumulated CO_2-C (mg C. g^{-1} OD soil) from Tokomaru silt loam (TK) soil alone and after addition of lotus (LT) and white clover (WC) shoot litter, applied to the soil at three rates - 2, 5, and 10 mg C. g^{-1} OD soil. The incubation chambers were held at an average temperature of 18 °C for a period of 134 d.**

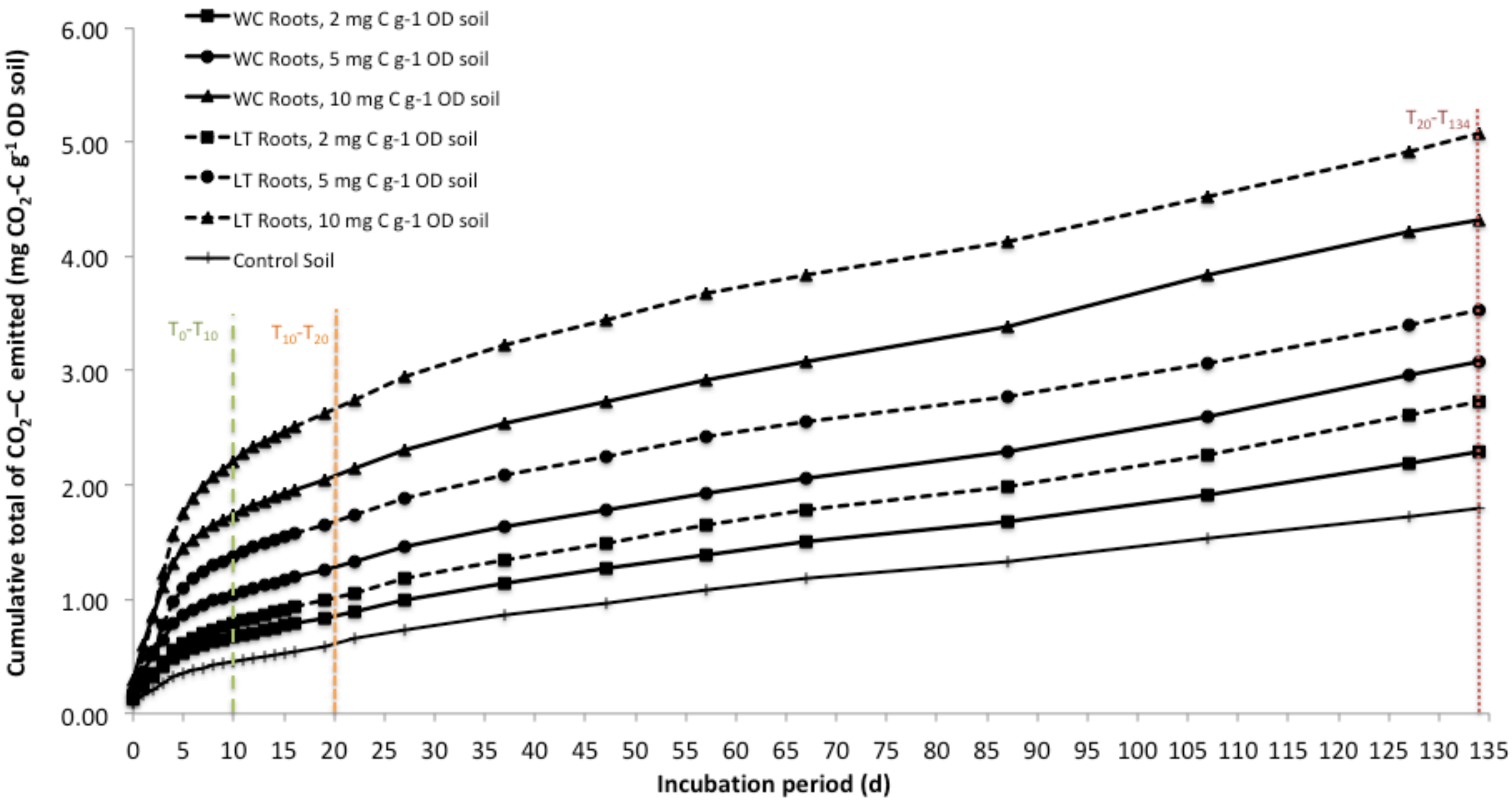


Figure 5.5 Accumulated CO_2-C (mg C. g^{-1} OD soil) from Tokomaru silt loam (TK) soil alone and after addition of lotus (LT) and white clover (WC) root litter, applied to the soil at three rates - 2, 5, and 10 mg C. g^{-1} OD soil. The incubation chambers were held at an average temperature of 18 °C for a period of 134 d.

5.3.5 CO_2 evolved incubation end (T_{134})

The data (accumulated CO_2 evolved (Treatment – Control)) was analysed using the ANOVA model, accounting for effect of species, plant part, rate of application, and replicate. Table 5.3 shows analysis at four time periods; the analysis of variance in the accumulated amount of CO_2 evolved showed significant effects of species, plant part and rate of application. Rate of application accounted for the greatest variation (64 %) in accumulated (T_0 - T_{134}) CO_2 evolved followed by plant part (20 %). The controlled nature of this experiment (unlike Chapter 4) achieved a very low variance (0.03 %) between replicates.

Table 5.3 The statistical significance of species, plant part, rate of application and treatment replicate on total accumulated CO_2-C evolved (mg C. g^{-1} OD soil) from the Tokomaru silt loam soil, after amendment with lotus (LT) and white clover (WC) shoot (S) and root (R) litter (trt - ctrl).

Time	Plant species	Plant part	C application rate	Replicate	Plant species (%)	Plant part (%)	C application rate (%)	Replicate (%)	R-Sq adj (%)
T_0-T_{134}	NS	P = 0.000	P = 0.000	NS	0.79	20.38	63.73	0.03	81.82
T_0-T_{10}	NS	P = 0.000	P = 0.000	NS	1.24	29.45	52.18	0.06	79.40
T_{10}-T_{22}	NS	P = 0.000	P = 0.000	NS	0.05	20.62	65.13	0.02	82.88
T_{22}-T_{134}	NS	NS	P = 0.000	NS	0.28	0.75	86.91	0.04	85.48

NS - Not significant, $P > 0.05$

Even after 10 d, the factors plant part (shoot or root), rate of application and species explain 79 % of the variance in total accumulated CO_2-C (mg. g^{-1} OD $soil_{134\ d}$) emitted when control soil respiration is removed from consideration (Table 5.3). Rate of C application accounted for 52 % of the variation in C emitted, whereas plant part accounted for 29 % (Table 5.4). The effect of plant species was confounded by the effect of plant part, so statistics were also performed to consider the significance of plant species separate to the influence of plant part (Table 5.4 and Table 5.5).

5.3.6 CO_2 evolved in shoot material (T_{134})

Once root and shoot are analysed separately, then species has significant effect on the accumulated CO_2-C (T_0 - T_{134}), albeit a different effect for each species.

Table 5.4 The statistical significance of species, rate of application and treatment replicate on total accumulated CO_2-C evolved (mg C. g^{-1} OD soil) from the Tokomaru silt loam (TK) soil, after amendment with lotus (LT) and white clover (WC) shoot (S) litter (trt - ctrl).

Time	Plant species	C application rate	Replicate	Plant species (%)	C application rate (%)	Replicate (%)	R-Sq adj (%)
T_0-T_{134}	P = 0.000	P = 0.000	NS	8.96	88.76	0.07	96.87
T_0-T_{10}	P = 0.000	P = 0.000	NS	10.00	86.34	0.19	95.08
T_{10}-T_{22}	P = 0.000	P = 0.000	NS	3.70	93.76	0.03	96.45
T_{22}-T_{134}	P = 0.000	P = 0.000	NS	8.12	90.47	0.15	98.22

NS - Not significant, $P > 0.05$

Even as early as 10 d species has a significant effect on the accumulated CO_2-C from shoot incubations. The factors rate and species explain 95 % of the variance in total accumulated CO_2-C (mg. g^{-1} OD soil) emitted when control soil respiration is removed from consideration. Rate of C application accounted for 86 % of the variation in C emitted, whereas species accounted for 10 % (Table 5.4).

5.3.7 CO_2 evolved T_{134} in root material

Similarly species has a significant effect on the accumulated CO_2-C from root incubations. The factors rate and species explain 98 % of the variance in total accumulated CO_2-C (mg. g^{-1} OD $soil_{134\ d}$) emitted when control soil respiration is removed from consideration. Rate of C application accounted for 90 % of the variation in C emitted, whereas species accounted for 8 % (Table 5.5).

Table 5.5 The statistical significance of species, rate of application and treatment replicate on total accumulated CO_2-C evolved (mg C. g^{-1} OD soil) from the Tokomaru silt loam (TK) soil, after amendment with lotus (LT) and white clover (WC) root (R) litter (trt - ctrl).

Time	Plant species	C application rate	Replicate	Plant species (%)	C application rate (%)	Replicate (%)	R-Sq adj (%)
T_0-T_{134}	P = 0.000	P = 0.000	NS	8.34	90.34	0.15	98.35
T_0-T_{10}	P = 0.000	P = 0.000	NS	8.08	90.11	0.02	97.47
T_{10}-T_{22}	P = 0.000	P = 0.000	NS	16.74	79.22	0.03	94.33
T_{22}-T_{134}	P = 0.000	P = 0.000	NS	6.03	87.63	0.89	92.3

NS - Not significant, $P > 0.05$

5.3.8 Rate of C application

In both shoot and root incubations rate of application had the largest effect (explained 86 – 90 % of the variance) on the amount of CO_2-C emitted per weight of dry soil.

At all rates of addition, LT shoot litter resulted in a lower percentage of CO_2-C being released than the WC shoot litter (Table 5.6), i.e. more C is conserved as undecomposed residue under lotus shoot. White clover shoots at an application rate of 2 mg C. g^{-1} OD soil resulted in the greatest amount of CO_2-C released (73.8 %).

Table 5.6 Average total CO_2-C emitted, as a percentage of C added, from the Tokomaru silt loam (TK) soil, after amendment with lotus (LT) and white clover (WC) shoot (S) litter, from T_0 to T_{134}.

Treatment	$CO_2\text{-C}\sum_{T0}^{T134}$ - *control*
WCS 2 mg. g^{-1} OD soil	73.8 ± 1.2 %
WCS 5 mg. g^{-1} OD soil	68.4 ± 0.7 %
WCS 10 mg. g^{-1} OD soil	65.5 ± 1.2 %
LTS 2 mg. g^{-1} OD soil	48.6 ± 2.8 %
LTS 10 mg. g^{-1} OD soil	47.0 ± 2.4 %
LTS 5 mg. g^{-1} OD soil	46.3 ± 1.0%

Each value in the table represents the mean of three replicates with standard deviation (±).

At all rates of addition, WCR litter resulted in a lower percentage of CO_2-C being released than the LTR litter (Table 5.7), i.e. more C is conserved as undecomposed residue under WCR. LTR at an application rate of 2 mg C. g^{-1} OD soil resulted in the greatest percentage of CO_2-C released (46.4 %).

Table 5.7 Average total CO_2-C emitted, as a percentage of C added, from the Tokomaru silt loam (TK) soil, after amendment with lotus (LT) and white clover (WC) root (R) litter, from T_0 to T_{134}.

Treatment	$CO_2\text{-C}\sum_{T0}^{T134}$ - $control$
LTR 2 mg. g^{-1} OD soil	46.4 ± 4.8 %
LTR 5 mg. g^{-1} OD soil	34.4 ± 0.9 %
LTR 10 mg. g^{-1} OD soil	32.8 ± 1.9 %
WCR 5 mg. g^{-1} OD soil	25.6 ± 0.4 %
WCR 10 mg. g^{-1} OD soil	25.1 ± 0.2 %
WCR 2 mg. g^{-1} OD soil	24.5 ± 3.1 %

Each value in the table represents the mean of three replicates with standard deviation (±).

More C is conserved under root treatments than shoot treatments as a whole, at their respective rates of application. WCR left the most residue (75.5 %) in the soil, as a percentage of C added, after 134 d, which correlated to the lowest ME (4.77 MJ. kg^{-1}), highest lignin (23.69 %), and highest Mn (720 mg. kg^{-1}) for all plant treatments (Table 5.1). In comparison, WCS left only 26.2 % as CO_2-C retained, which also correlated to the highest ME (11.72 MJ. kg^{-1}), lowest lignin (2.55 %), and lowest Mn (30 mg. kg^{-1}) of all plant treatments. There was no obvious correlation with the total amount of CT (%).

5.3.9 Period of greatest decomposition, T_0 to T_{10}

Prior to a discussion of the most significant period for decomposition of differing plant parts, the cumulative amounts of CO_2 emitted during specific intervals of time were calculated and the effect of species and rate tested for significance. During all intervals

T_0 - T_{10}, T_{10} - T_{22}, T_{22} - T_{134} there was a significant effect of species and rate on shoot (Table 5.8) and root decompostion (Table 5.9).

Table 5.8 The statistical significance of species, rate of application and treatment replicate on the accumulated CO_2-C evolved (mg C. g^{-1} OD soil) at different incubation intervals from the Tokomaru silt loam (TK) soil, after amendment with lotus (LT) and white clover (WC) shoot (S) litter (trt - ctrl).

Time	Species (Shoot)	Rate	Replicate	Species (%)	Rate (%)	Replicate (%)	R-Sq adj (%)
T_0-T_{10}	P = 0.000	P = 0.000	NS	10.00	86.34	0.19	95.08
T_{10}-T_{22}	P = 0.000	P = 0.000	NS	3.70	93.76	0.03	96.45
T_{22}-T_{134}	P = 0.000	P = 0.000	NS	8.12	90.47	0.15	98.22

NS - Not significant, P > 0.05

Table 5.9 The statistical significance of species, rate of application and treatment replicate on the accumulated CO_2-C evolved (mg C. g^{-1} OD soil) at different incubation intervals from the Tokomaru silt loam (TK) soil, after amendment with lotus (LT) and white clover (WC) root (R) litter (trt - ctrl).

Time	Species (Root)	Rate	Replicate	Species (%)	Rate (%)	Replicate (%)	R-Sq adj (%)
T_0-T_{10}	P = 0.000	P = 0.000	NS	8.08	90.11	0.02	97.47
T_{10}-T_{22}	P = 0.000	P = 0.000	NS	16.74	79.22	0.03	94.33
T_{22}-T_{134}	P = 0.000	P = 0.000	NS	6.03	87.63	0.89	92.3

NS - Not significant, P > 0.05

At the end of the rapid decomposition phase the treatments had emitted 10.9 to 47.5 % of total C added (excluding control soil respiration) (Table 5.10). Plant part, plant species and rate of C application all significantly influenced total CO_2-C emitted. These effects of plant part, plant species and rate of C application were significant during the rapid period of decomposition, and continued throughout the incubation period.

Table 5.10 Average total CO_2-C emitted at T_{10}, as a percentage of C added, from the Tokomaru silt loam (TK) soil, after amendment with lotus (LT) and white clover (WC) root (R) litter, from T_0 to T_{10}.

Plant Part	Treatment	CO_2-C $\sum_{T0}^{T10}$ - $control$
Shoots	WCS 2 mg. g^{-1} OD soil	47.5 ± 0.8 %
	WCS 5 mg. g^{-1} OD soil	46.0 ± 0.4 %
	WCS 10 mg. g^{-1} OD soil	45.4 ± 1.0 %
	LTS 2 mg. g^{-1} OD soil	32.8 ± 0.7 %
	LTS 10 mg. g^{-1} OD soil	30.9 ± 2.8 %
	LTS 5 mg. g^{-1} OD soil	29.9 ± 0.3 %
Roots	LTR 5 mg. g^{-1} OD soil	18.5 ± 0.4 %
	LTR 10 mg. g^{-1} OD soil	17.5 ± 0.2 %
	LTR 2 mg. g^{-1} OD soil	16.9 ± 0.6 %
	WCR 10 mg. g^{-1} OD soil	12.9 ± 0.1 %
	WCR 5 mg. g^{-1} OD soil	11.8 ± 0.3 %
	WCR 2 mg. g^{-1} OD soil	10.9 ± 1.0 %

Each value in the table represents the mean of three replicates with standard deviation (±).

At all rates of addition, root material emitted a lower amount of CO_2-C (10.9 – 18.5 %) than that of the shoot material (29.9 – 47.5 %). At all rates of addition, LT shoot material resulted in a lower amount of C being released (29.9 – 32.8 %) than the WC (45.4 – 47.5 %). However the opposite is true for root material, with LT resulting in a higher amount of C released (16.9 – 18.5 % compared to 10.9 – 12.9 %). This indicates that the initial ranking of CO_2 losses (T_0-T_{10}) for the two species, residue types and rates of C addition are indicative of the longer term trends in CO_2 losses for these treatments (Figure 5.6).

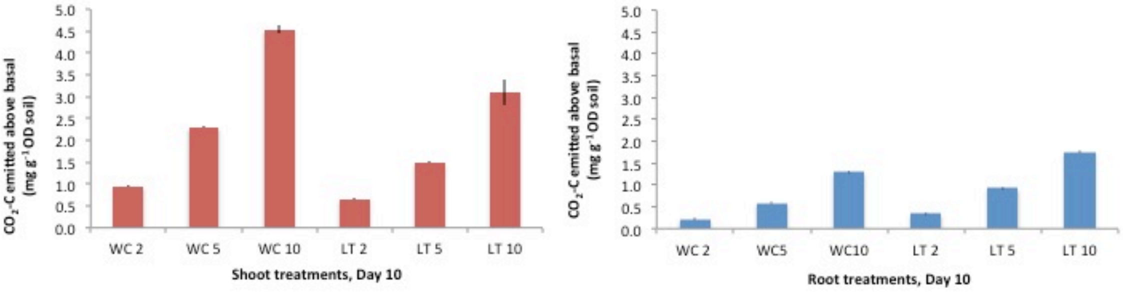


Figure 5.6 CO_2-C emitted, above the control soil rate, from the Tokomaru silt loam (TK) soil, after amendment with lotus (LT) and white clover (WC) root (R) litter, from T_0 to T_{10}.

5.3.10 Period of substrate limited decomposition, T_{10} to T_{22}

From T_{10} to T_{22} the rate of CO_2 released from all treatments declined to a slower rate than those seen in the rapid decomposition phase, appearing to be substrate limited. At the end of the substrate limited phase the treatments had emitted 11.9 to 53.6 % of total C added (excluding control soil respiration) (Table 5.11).

Table 5.11 Average total CO_2-C emitted at T_{22}, as a percentage of C added, from the Tokomaru silt loam (TK) soil, after amendment with lotus (LT) and white clover (WC) root (R) litter, from T_0 to T_{22}.

Plant Part	Treatment	$CO_2\text{-C} \sum_{T0}^{T22} - control$	% emitted between T_{10} - T_{22}
Shoots	WCS 2 mg. g^{-1} OD soil	53.6 ± 0.9 %	6.1
	WCS 10 mg. g^{-1} OD soil	51.5 ± 1.1 %	6.1
	WCS 5 mg. g^{-1} OD soil	51.3 ± 0.4 %	5.3
	LTS 2 mg. g^{-1} OD soil	37.4 ± 0.6 %	4.6
	LTS 10 mg. g^{-1} OD soil	35.5 ± 2.7 %	4.6
	LTS 5 mg. g^{-1} OD soil	34.6 ± 0.3 %	4.7
Roots	LTR 5 mg. g^{-1} OD soil	21.6 ± 0.4 %	3.1
	LTR 10 mg. g^{-1} OD soil	20.9 ± 0.2 %	3.4
	LTR 2 mg. g^{-1} OD soil	20.3 ± 1.4 %	3.4
	WCR 10 mg. g^{-1} OD soil	14.9 ± 0.1 %	2.0
	WCR 5 mg. g^{-1} OD soil	13.6 ± 0.2 %	1.8
	WCR 2 mg. g^{-1} OD soil	11.9 ± 1.3 %	1.0

Each value in the table represents the mean of three replicates with standard deviation (±).

At all rates of addition, root material emitted a lower amount of CO_2-C (11.9 – 21.6 %) than that of the shoot material (34.6 – 53.6 %). At all rates of addition, LTS resulted in a lower amount of C being released (34.6 – 37.4 %) than the WCS (51.3 – 53.6 %). However the opposite is again true for root material, with LTR resulting in a higher amount of C released (20.3 – 21.6 % compared to 11.9 – 14.9 %). At the end of the substrate limited phase the differing treatments had emitted 11.9 to 53.6 % of total C added (Figure 5.7).

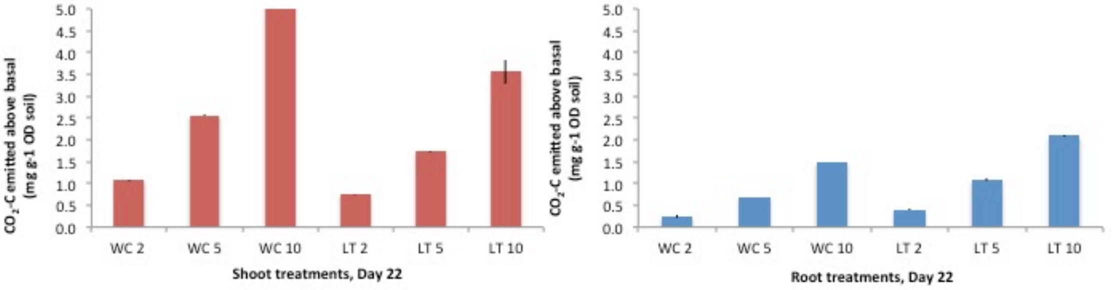


Figure 5.7 **CO_2-C emitted, above the control soil rate, from the Tokomaru silt loam (TK) soil, after amendment with lotus (LT) and white clover (WC) root (R) litter, from T_0 to T_{22}.**

5.3.11 Return to basal-like respiration, T_{22} to T_{134}

There was a rapid decline in CO_2-C evolution rates over time so that by T_{22}, and between T_{22} and T_{134}, the CO_2 efflux from all treatments were of similar magnitude across all amendments. Plant part, plant species and rate of C application were still of significant influence on total CO_2-C emitted ($P < 0.05$). For the shoot treatments approximately 75 % of all CO_2-C was respired by T_{22}, whereas the root treatments had only respired 24 to 46 % (Table 5.12).

Table 5.12 Average total CO_2-C emitted at T_{134}, as a percentage of C added, from the Tokomaru silt loam (TK) soil, after amendment with lotus (LT) and white clover (WC) root (R) litter, from T_0 to T_{134}.

Plant Part	Treatment	$CO_2\text{-C} \sum_{T0}^{T134} - control$	% emitted between T_{22} - T_{134}
Shoots	WCS 2 mg. g^{-1} OD soil	73.8 ± 1.2 %	20.3
	WCS 5 mg. g^{-1} OD soil	68.4 ± 0.7 %	17.1
	WCS 10 mg. g^{-1} OD soil	65.5 ± 1.2 %	13.9
	LTS 2 mg. g^{-1} OD soil	48.6 ± 2.8 %	11.2
	LTS 10 mg. g^{-1} OD soil	47.0 ± 2.4 %	11.4
	LTS 5 mg. g^{-1} OD soil	46.3 ± 1.0%	11.7
Roots	LTR 2 mg. g^{-1} OD soil	46.4 ± 4.8 %	26.1
	LTR 5 mg. g^{-1} OD soil	34.4 ± 0.9 %	12.8
	LTR 10 mg. g^{-1} OD soil	32.8 ± 1.9 %	11.8
	WCR 5 mg. g^{-1} OD soil	25.6 ± 0.4 %	12.1
	WCR 10 mg. g^{-1} OD soil	25.1 ± 0.2 %	10.2
	WCR 2 mg. g^{-1} OD soil	24.5 ± 3.1 %	12.5

Each value in the table represents the mean of three replicates with standard deviation (±).

5.3.12 Visual observations support the difference in initial emissions

Faint fungal colonisation was visible on the WCS 2 mg C. g^{-1} OD soil treatment from T_4, with marked fungal colonisation also visible on the 5 and 10 mg C. g^{-1} OD soil shoot treatments. No fungal colonisation was visible on the WCR treatments. Likewise, no fungal colonisation was visible on the LTS 2 mg C. g^{-1} OD soil treatment, but from T_4 marked colonisation was visible on the 5 and 10 mg C. g^{-1} OD soil LTS treatments. LTR 5 and 10 mg C. g^{-1} OD soil treaments had faint and marked (respectively) fungal colonisation evident from T_4. All colonisation lasted visibly for 2 - 3 weeks before visually disappearing (Figure 5.8).

Figure 5.8 Fungal colonisation visible on the WCS 2 mg C. g^{-1} OD soil treatment at T_4.

5.4 Discussion

As discussed in Chapter 2, Section 2.3 it is critical to understand how C dynamics in a grazed pasture system can be influenced over time in order to understand and measure the flows of C into and out of soils (Beidler et al. 2020). Understanding the factors, which regulate the sequestration and longevity of SOC, is a critical step in predicting the response of global C cycling to future environmental change (Harden et al. 2018; Luo et al. 2016). Factors that increase plant growth (i.e. soil fertility, warmth, moisture) will ultimately increase the flow of C into that soil system - as plant litter, root rhizodeposits, and grazed animal excreta return.

Leaf litter decomposition and associated parameters have long been a focus of research studies, but those on root decomposition are lacking across many ecosystems (Bloomfield et al. 1993), partially due to the difficulty in estimating root decomposition rates invivo. It is known that there is significant allocation of total annual production to below-ground plant parts (anywhere from 15 % to 83 %) in terrestrial ecosystems (Coleman, 1976, Bernard et al. 1988, Aerts et al. 1992, Konings et al. 1992). It is logical, therefore, that below-ground tissues will significantly contribute to C and nutrient turnover in the soil (Scheffer & Aerts, 2000). This incubation provides fundamental data on the effects of shoot and root litter application to soil, without the confounding effects of other CO_2-induced impacts on C mineralization and sequestration, including differences in root biomass, root activity, and soil moisture often encountered in other studies. It evaluated the effects of plant species, plant part, and rate of application on the total amount of decomposition (measured as CO_2-C emitted), in order to ascertain the impact on the amount of C stored in the soil at the end of the incubation period, T_{134}.

5.4.1 Effect of plant species and plant part

In this incubation experiment, the degree of decomposition in both species (emission of CO_2-C) from root material was significantly lower than that of shoot material. Studies by Shi et al. (2013) and Vidal et al. (2017) report slower decomposition of grass and cereal roots than shoots in soil, which is consistent with the incubation results presented here. Likewise Comeau et al. 2013 conducted isotype studies that report larger contributions made by root C (than shoot C) to SOM-C. By the end of the decomposition period (134 d), root material left between 54 – 75 % undecomposed residue in the soil (Table 5.7), compared to shoot material which only left between 26 – 64 % undecomposed (Table 5.6).

Within the shoot treatments, lotus released 32 – 34 % less CO_2-C than clover (as a % of C added) resulting in a higher proportion retained in the soil (Table 5.11). These results are in a similar range to those reported in Table 3.5 (lotus released 18 – 24 % less). Statistical analysis supported these observations, showing that the effect of species and rate of application on total accumulated CO_2-C loss were both highly statistically significant ($P < 0.05$) (Table 5.5). Within the root treatments, clover released 23 – 47 % less CO_2-C than lotus (as a % of C added) (Table 5.11). Statistical analysis supported these observations, showing that the effect of species and rate of application on total accumulated CO_2-C loss were both highly statistically significant ($P < 0.05$) (Table 5.7). These observations support the view that chemical differences in the composition of plant species and plant part regulate how SOM is formed through functions such as litter decomposability mineralization, microbial community composition and function, and interaction with soil minerals (Jackson et al. 2017; Finzi et al. 2015; Keiluweit et al. 2015; Clemmensen et al. 2013; Freschet et al. 2012a, 2012b; Cheng et al. 2005).

However, there are constrasting results reported in the literature. In some studies the presence of plant roots have been shown to significantly stimulate the rate of organic C decomposition (Cheng et al. 2014; Bird et al. 2011). Yet in other studies, they have been shown to have both negative or neutral impacts on organic C decomposition rates (Van der Krift et al. 2002; Loya et al. 2004; Cheng et al. 2014; Saar et al. 2016). While these effects may seem contradictory, it is important to remember that the mechanisms behind SOC cycling are diverse, and there are many ways by which plant roots may impact on this cycling. Freschet et al. 2013 considered data from 32 studies, where plant tissues from the same species had been decomposed in the same site or microcosm, and estimated that for grasslands, rhizodeposition inputs (as fine roots) make up approximately 33 % of annual residue inputs (26 % for leaf litter; 41 % for fine stems). When compared to leaf litter derived from the same species, there was evidence of decomposition processes twice as slow. They point out that the chemical and structural traits influencing decomposition vary between plant organs (leaves, stems and roots) as well as across species, so differences in decomposition are to be expected.

5.4.2 Chemical characteristics of the litter

As dicussed in Chapter 4, Section 4.4 there are differences in both the presence of shoot / root traits and their expression in soil. However, it is relatively unknown whether the species differences in shoot litter and their associated biogeochemical effects are mirrored by differences in root traits and their effects. Roots have fundamentally different physiological functions than shoots, which can result in different chemical composition and thus decomposition rate between the shoot and root material of any considered species (Silver & Miya, 2001; Scheffer & Aerts, 2000).

In this incubation study, more input C is conserved under root treatments than shoot treatments as a whole, at their respective rates of application. WCR left the most residue (75.5 %) in the soil, as a percentage of C added, after 134 d, which correlated to the lowest ME (4.77 MJ. kg^{-1}), highest lignin (23.69 %), and highest Mn (720 mg. kg^{-1}) for all plant treatments. In comparison, WCS left only 26.2 % as CO_2-C retained, which also correlated to the highest ME (11.72 MJ. kg^{-1}), lowest lignin (2.55 %), and lowest Mn (30 mg. kg^{-1}) of all plant treatments. There was no obvious correlation with the total amount of CT (%) (Table 5.1).

Considering the data in Table 5.1, it can be seen that C:N ratios of the plant material added range from 10.96 (WCS) to 15.34 (LTR) across the different plant species and plant parts. WCS (10.96) and LTS (11.37) both provide plant material with a C:N ratio that would suggest mineralization was to be expected (C:N < 12, Table 5.1), while WCR (13.59) and LTR (15.34) both provide plant material that should theoretically result in no net change in mineralization / immobilization (C:N > 12 < 20). However, Vigil & Kissel (1991) indicate that residues with a C:N ratio less than 40 and N concentration greater than 2 % are likely to mineralize and release N. As all plant amendments were in the range of 2.50 – 3.93 % N, some form of mineralization would be expected. LTR had the lowest C:P ratio (101), while LTS had the highest (186). WCR and WCS both had a ratio of 144. Faster mineralisation may be expected for lower C:P ratio material.

Hobbie et al. (2010) found large differences in decomposition of leaf litter and roots of species within the same taxonomic genus (e.g. Stipta grasses, temperate European and North American tree species), independent of environmental constraints. The importance of the litter quality effect on decomposition is well documented (Swift et al. 1979; Couteaux et al. 1995). These differences were attributed to intrinsic litter quality differences. Hobbie et al. (2010) found that the initial litter chemistry was more important in the prediction of decomposition in leaf litter than in roots; with the decomposition rates of roots and leaf litter across the species they sampled were unrelated. Differences in the decomposition rate among the species lotus and white clover, despite the same growth and decomposition conditions, suggest that the composition of these species is influencing decomposition (Hobbie et al. 2010; Wardle et al. 1997; Madritch & Hunter 2002; Zimmer, 2002).

Hobbie et al. (2010) suggested that for their study, this implies that many important plant mechanisms may be overlooked if studies only focus on aboveground traits. Although significant, the effect of plant species in this study was secondary to the that of the rate of C application and plant part. WCR applied at 2 mg C. g^{-1} OD soil emitted

24.5 % of total C added, while WCS applied at the same rate emitted 73.8 % of total C added (Table 5.11). The influence of plant part within these species suggests that, like Hobbie et al. (2010), the differing litter composition between shoot and root is having an influence on decomposition (Cornelissen & Thompson, 1997).

Elias et al. (2020) reported that while litter quality was the most important predictor of litter mass loss, soil origin was also significant, explaining between 5.17 and 15.43 % of total variation. This soil origin difference was shown to be related to the microbial decomposer communities found within the soils (older soils had greater microbial function, relative to those in the younger soils). The most chemically recalcitrant litter (lowest N, highest lignin, lignin:N, and C:N ratio) from moderate logged forest decomposed faster when combined with its “home” soil (Home-Field Advantage) whilst the most labile litter from oil palm decomposed slowest when combined with its “home” soil. This was correlated with lower total soil microbial biomass. Soil disturbance, including the impact on soil microbial community structure, must therefore not be overlooked when considering making changes to plant inputs.

5.4.3 Effect of rate of application

Rate of application of litter to the incubation had the largest effect on the amount of CO_2-C emitted from the different treatments by the end of incubation at T_{134}, with results consistent between species. When the amount of CO_2-C emitted is expressed as a percentage of the amount of C added, it is clear that as the rate of addition increased the percentage of CO_2-C emitted decreased (Figure 5.4). More C was also conserved as undecomposed residue as the rate of C application increased. It has been suggested that one of the ways to increase the amount of C sequestered is to increase the rate of input. Recent meta data analyses showed a direct linear relationship between the rate of C addition in crop residues and soil C accumulation rates (Fujisaki et al. 2018), and increased soil C with increased C input through wastes (Diacono, 2010). Figure 5.9 clearly shows that as the rate of application increased, the amount of C emitted as a % of C added did not proportionally increase, rather there was a slight, but significant, decline in the amount of CO_2-C emitted. Referring back to Figure 2.5, it can be seen that, as predicted, an increased rate of C input to the soil does successfully increase the amount of C retained in the soil pool.

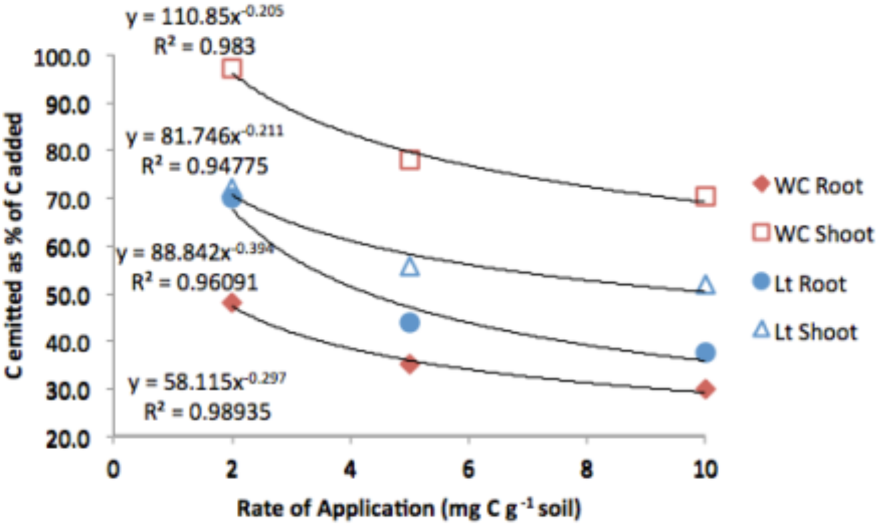


Figure 5.9 **Plot of rate of C application vs. CO_2 emitted as a % of C added as WC and LT root and shoot material.**

It is also possible that an increased input of C may also have impacted on the longevity of C stored within the system. If C input is not limited, microbial activity will flourish (assuming no other limiting factors), and as a result more microbial-derived OM will be produced which may have an increased longevity in the soil system. Pyrolysis GC-MS studies have shown that decomposing organic compounds retain chemical signatures which give a glimpse into the degree of primary plant material and / or the abundance and type of microbial-derived OM (Suarez-Abelenda et al. 2015; Buurman et al. 2007). This microbial-derived OM forms an important part of the soil C system, and may also further decompose alongside plant-derived OM (Liang & Balser, 2010).

5.5 Conclusions

The statistical analysis of the CO_2-C evolved during the incubation clearly showed that rate of application, plant part, and plant species (in that order) were the most significant factors influencing the decomposition rate.

This carefully controlled experiment reduced replicate error, and the effects of rate of C application and plant part on C evolved and C sequestered in the soil were proven to be highly significant. The species effect was proven to be less significant. The results showing the slower decomposition of root material than shoot material are consistent with other laboratory incubation studies and meta analyses conducted on manure and crop residue trials. Of particular interest, and supporting the observation in Chapter 4, is the particularly slow decomposition rate of white clover (*Trifolium repens*) roots. *Trifolium repens* is the common legume in New Zealand pastures and the role of its roots in building pasture soil C content warrants further field studies.

An objective for further research (Chapter 6) is the capability to predict these significant effects on decomposition rates and C sequestration from properties of the plant material and the soil conditions. This goal is also important in the ability to predict the value of green manures and crop residues as sources of N for the next crop (Woodruff et al. 2018). Particular attention should be paid to the relationship between initial litter chemistry and rate of decomposition, and its usefulness for predicting decomposition considered.

Chapter 6

Development of a simple predictive decomposition simulation model

6.1 Introduction

The incubation experiment conducted in Chapter 5 provides soil respiration results showing that plant species (*Trifolium repens* L. (WC) and *Lotus pedunculatus* (LT)) and plant tissue form (root and shoot material) are important determinants of the rate of litter decomposition. Differences in the rate of root and shoot litter decompostion in soils have been reported by others (e.g. Vidal et al. (2017) and Shi et al. (2013)). Although the decomposition of plant material in soils is extremely complex involving a succession of fauna and microflora (Killham, 1994), studies have shown that relatively simple mathematical models can be used to describe the decomposition process. Jenkinson et al. (1990) described the decay rate (the rate of C loss) of plant material from soil as an L-shaped curve (exhibiting rapid loss followed by a much slower loss), which could not be represented by a single decay curve and was fitted best with a two compartment model, where the incoming plant C is split into two different decomposition rates. The two compartment model (Eq. 6.1) from Jenkinson (1977):

$$\mathbf{C = \mathit{A}\, e^{-2.83\mathit{t}} + \mathit{B}\, \mathit{e}^{-0.087\mathit{t}}} \qquad \textbf{Eq. 6.1}$$

gave a good representation of the decay of ^{14}C-labelled ryegrass leaves over a 10 year (y) period. Where *A* and *B* are the fast and slow pools respectively. The fast decomposing pool, *A* with a half life of 0.25 y, represented 71 % of the added plant C and the slow decomposing pool, *B* with a half life of 8 y, represented 21 % of the incoming plant C.

Soil C modelling has advanced since earlier models were developed, with very sophisticated multicompartmental soil C models now regularly calibrated and validated e.g. SOCRATES (Grace et al. 2006), APSIM (Probert et al. 1998), RothC

(Jenkinson et al. 1990) and CENTURY (Parton et al. 1988) all included. These models seek to represent soil C dynamics under vastly different rates of plant input and turnover, at paddock, regional and global scale, and have been very effective in modelling soil C dynamics over long time steps.

There is a need, however, to model the decompostion of crop residues both to predict the effect on soil C stocks and the release of mineralised N to the next crop (e.g. Vigil and Kissel, 1991). A number of previous studies conducted incubations of plant residues in soils then modeled the CO_2 emissions (e.g. Sequaris et al. 2010; Johnson et al. 2007). Sequaris et al. (2010) incorporated fresh ^{14}C-labelled wheat residues into a silt loam soil, and considered the effect of both temperature and soil gravimetric water content on subsequent decomposition of the added residue over 4 – 6 months. The measurements they took were used to calibrate a simple model considering how the inputs and outputs of C into the small-size (< 53 µm) soil particle fractions control total C mineralization. The results were used to simulate decomposition kinetics of fresh OM under different temperature and moisture conditions.

Pereira et al. (2017) describe a three compartment model (nonstructural carbohydrates, cellulose–hemicellulose, and lignin) that simulated the decompostion of vetch, oats and clover residues and their contribution to soil C stocks. Johnson et al. (2007) considered five different crops (alfalfa, corn, cuphea, soybean, and switchgrass) which varied in their chemical composition; and simulated decomposition kinetics of fresh OM in a loam soil under stable environmental conditions: temperature 25°C; 49 % humidity. Johnson et al. (2007) also considered particle size, and plant part (leaf, stem and root organs). The decomposition of these residues was measured as an emission of CO_2 over a set period (1 hour increasing incrementally to 48 hours), and the results of the decomposition study were used to design a four-component model ($r2 = 0.43$), with AI ash, hemicellulose, initial N concentrations and C:N ratio being the best predictors of residue decomposition.

Woodruff et al. (2018) have published a model which simulates the amount of N mineralised from crop residues by using decompostion algorithms that are modified by the concentrations of N, nonstructural carbohydrates, cellulose + hemi-cellulose, and lignin in the crop residue. Soil C dynamics are driven by the quality and digestibility of plant material inputs (Meyer et al. 2015). Therefore the aim of this Chapter is to develop a simulation model capable of predicting the variations in CO_2 emissions observed in the incubation of WC and LT plant materials in earlier Chapters. It will consider the impact of plant chemistry on the decomposition rates of the WC and LT plant materials studied in this thesis.

Hypotheses

(i) The emission of patterns of CO_2 measured in the earlier experiments in this thesis can be predicted by developing and calibrating a multicompartmental soil and plant residue C decompostion model.

(ii) The variable rates of CO_2-C emitted during the incubation different plant residues in the short term laboratory incubation study are a function of plant chemical traits that influence soil C dynamics after plant litter addition.

Experimental objectives

(i) Develop a soil C decomposition model to predict short term C loss (CO_2-emissions) from soils incubated with litter and roots of pasture legumes.

(ii) Test, using regression analysis, whether differences in plant chemistry explain the variance observed in soil respiration rates.

6.2 Methodology

6.2.1 Chemical analysis of plant material using common feed quality indices

Freeze dried shoot and root material were harvested and prepared as described in Chapter 3, Section 3.2.3 and Chapter 5, Section 5.2.3. These freeze dried plant material samples were subject to a full elemental analysis (Chapter 3, Section 3.2.2 and 3.2.3) including lignin and condensed tannin content and metabolisable energy (ME); a very common feed quality measure in the agricultural industry (Waghorn, 2008) (Table 6.1).

Table 6.1 Chemical composition of freeze dried lotus (LT) and white clover (WC) shoot and root tissue (mean and standard deviation presented), as measured pre-incubation and presented in Chapter 5.

Analysis Type	LT S		WC S		LT R		WC R	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
C (%)[1]	44.7	*0.1*	41.8	*0.1*	38.3	*0.1*	40.2	*0.2*
N (%)[1,2]	3.9	*0.1*	3.8	*0.0*	2.5	*0.0*	3.0	*0.0*
C:N[1,2]	11.4	2.3	11.0	1.3	15.3	2.8	13.6	7.5
P (%)[2]	0.2	*0.0*	0.3	*0.0*	0.4	*0.0*	0.3	*0.0*
S (%)[1,2]	0.2	*0.0*	0.2	*0.0*	0.1	*0.1*	0.1	*0.0*
K (%)[2]	1.7	*0.2*	2.8	*0.0*	0.3	*0.0*	0.3	*0.0*
Ca (%)[2]	0.7	*0.0*	1.1	*0.0*	0.5	*0.0*	0.8	*0.0*
Mg (%)[2]	0.3	*0.0*	0.3	*0.0*	0.1	*0.0*	0.1	*0.0*
Fe (mg. kg^{-1})[2]	72.0	*3.5*	140.0	*36.1*	6990.0	*226.0*	7690.0	*552.0*
Mn (mg. kg^{-1})[2]	98.0	*2.8*	30.0	*4.2*	435.0	*35.0*	720.0	*42.0*
Zn (mg. kg^{-1})[2]	38.0	*4.2*	22.0	*2.8*	47.0	*4.0*	97.0	*2.0*
CT Total (%)[3]	8.7	*0.1*	0.1	*0.0*	1.6	-	0.1	-
Lignin (%)[2,3]	6.7	*0.8*	2.6	*0.4*	19.3	2.1	23.7	3.8
ME (MJ. kg^{-1})[2,3]	10.6	*0.5*	11.7	*0.3*	6.2	0.5	4.8	0.9

[1] Elementar (vario MACRO cube); [2] Hills Laboratory; [3] MSFAT.

6.2.2 Modeling soil C

The RothC (Figure 6.1) and Century models use annual time steps, to simulate at the fate of crop and pastoral C inputs into natural ecosystems. The RothC model considers the input of two plant C pools that are transformed during decompostion to three soil C pools (BIO, HUM and IOM).

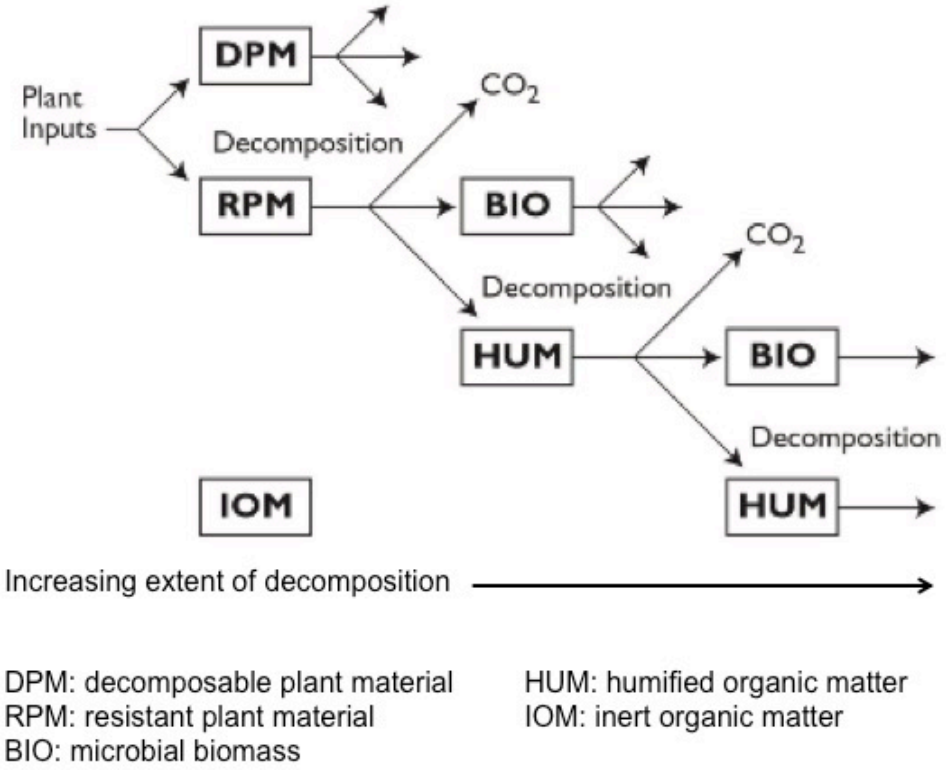


Figure 6.1 Schematic representation of the RothC model (modified from Skjemstad et al. 2004; from original depiction in Jenkinson et al. 1990).

The CO_2 emission results from the laboratory incubations (Chapters 3 and 5 using TK Soil) to be modelled here consider a shorter time step, a four month period, so a model suited to a shorter time step (daily) was required. Using the RothC and rumen models as a basis, the new model was designed initially around two plant litter C pools (labile and more persistent residues), but this was later extended to four plant litter pools to improve model to data fit. The model was designed to be used in short term incubation scenarios, and used as the basis of a simple testing technique to see if plant material properties might lead to a decrease in CO_2 released from plant materials and therefore an increase in the amount of plant residue derived C stored in soils .

6.2.3 Designing a simple two compartment model for freshly collected soil

A two compartment dynamic model (Figure 6.2) was developed to explain the CO_2 emission trends when freshly collected TK soil (control, no additional plant litter added) is incubated in the laboratory. The two compartments reflect labile pasture residues (A, e.g. fine roots that make it through the sample preparation process, any microbial biomass that senesces prior to incubation, and any aggregate protected OM exposed during sample preparation) and more persistent SOM residues (C). It was assumed that the size of the labile residue pool was negligible in comparison to the more persistent SOM pool; therefore, the persistent SOM pool was based on the measured total soil C.

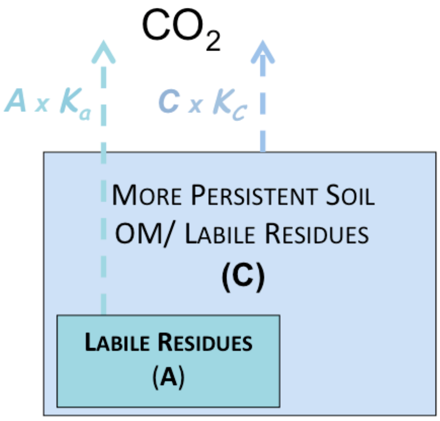


Figure 6.2 Two compartment dynamic model developed to explain the behavior of freshly collected soil when incubated.

The model (Eq. 6.2) was designed in a very simple manner, running on a daily time step, to simulate the disappearance of these two soil C pools (C and A), given the CO_2 release constants (K_a and K_c). The model was fitted to CO_2 emission measurements from Chapter 5, where the laboratory incubation results are reported as the total amount of accumulated CO_2 emssions between measurement days. The CO_2 emissions data was accumulated over time, and simulated CO_2 emissions were predicted. The variance bewteen observed (measured) and predicted (modelled) data was calculated. The variance was minimised by varying the values of the decay constants constants (K_a

and K_c). The values for constants (K_a and K_c) were those that gave lowest the RMSE between the predicted and observed data values.

The two compartment model was not designed to allow interaction between (i.e. the transfer of C between) the labile pool (A) and the persistent SOM (C) pool over the period of decomposition. However it may not be necessary to consider C transfer between these pools in order to adequately predict CO_2 emissions from the decomposition of freshly collected soil, given the short period of time considered.

The equation for this two compartment model is as follows:

$$E_n = (C_n \times K_C) + (A_n \times K_a) \qquad \textbf{Eq. 6.2}$$

Where: En = Total CO_2-C evolved on any day "n" (mg C. g^{-1} dry soil weight. day^{-1}) and Cn and An are the size of the persistent SOM residue and labile pool on day N. Cn is calculated as:

$$C_n = C_{n-1} - ((C_{n-1} \times K_C) \qquad \textbf{Eq. 6.3}$$

and An is calculated as:

$$A_{n-1} - (A_{n-1} \times K_a) \qquad \textbf{Eq. 6.4}$$

The total C pool remaining in the soil at day N is Cn + An

For the incubation experiment n = 1 – 135 days.

The fit of the model compared to the accumulated CO_2-C measured during decomposition of the control soil is illustrated in Figure 6.3. The fast (A x K_a) and slow (C x K_C) pools provide a good fit of the model to the measured data. The value of K_a for the best fit model was 0.12. The K_c value for best fit of the two compartment model was 0.00025 of the initial size of pool C (Figure 6.3).

With these K_a and K_c values the two compartment model was able to explain 99.4 % of the variance in the CO_2 emissions from the freshly collected soil that would contribute to the "background" respiration of the soil alone in all incubations in which lotus and white clover shoot and root material would have an added effect.

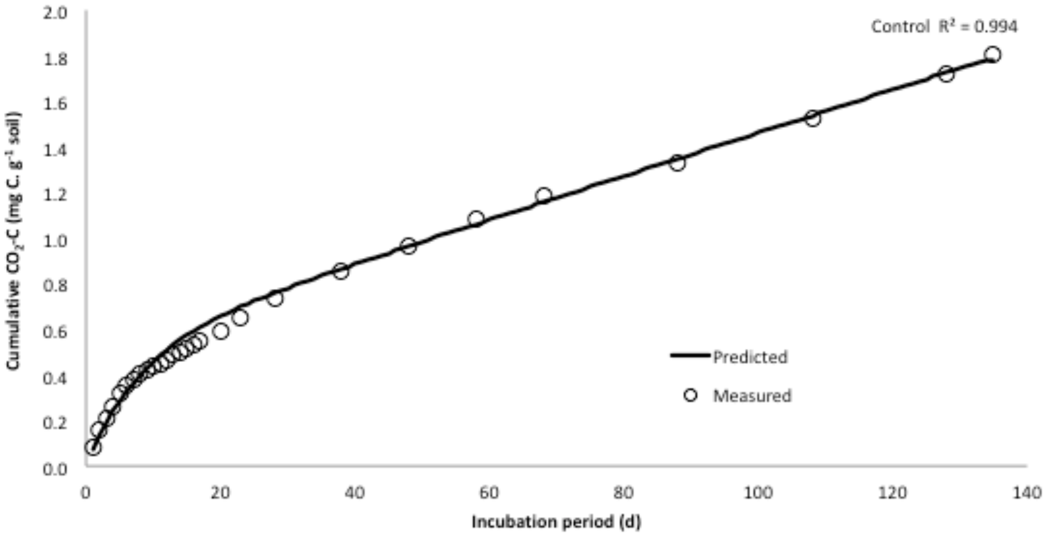


Figure 6.3 The two compartment (Eq. 6.2) dynamic model (solid line) fitted to the cumulative CO_2 emitted (respiration) during the incubation of freshly collected (control) Tokomaru (TK) soil.

Over the entire incubation period, this two compartment model was a very good fit (R^2 = 0.99) for the cumulative CO_2-C emitted from the freshly collected Tokomaru (TK) control soil; but it did not satisfactorarily fit (R^2 = 0.13) the early decomposition phase in instances where a fresh plant residue (e.g. LT shoots) was incorporated leading to a poor fit of the model over the entire incubation period (Figure 6.4). The added LT shoots increased the (A) pool by 2 mg C. g^{-1} dry soil. In this instance, rates of CO_2-C emitted accelerated dramatically over the early decomposition period, reflective of very labile C, the decomposition of which could not be modeled by the enlarged A pool with a K_a of 12 %.

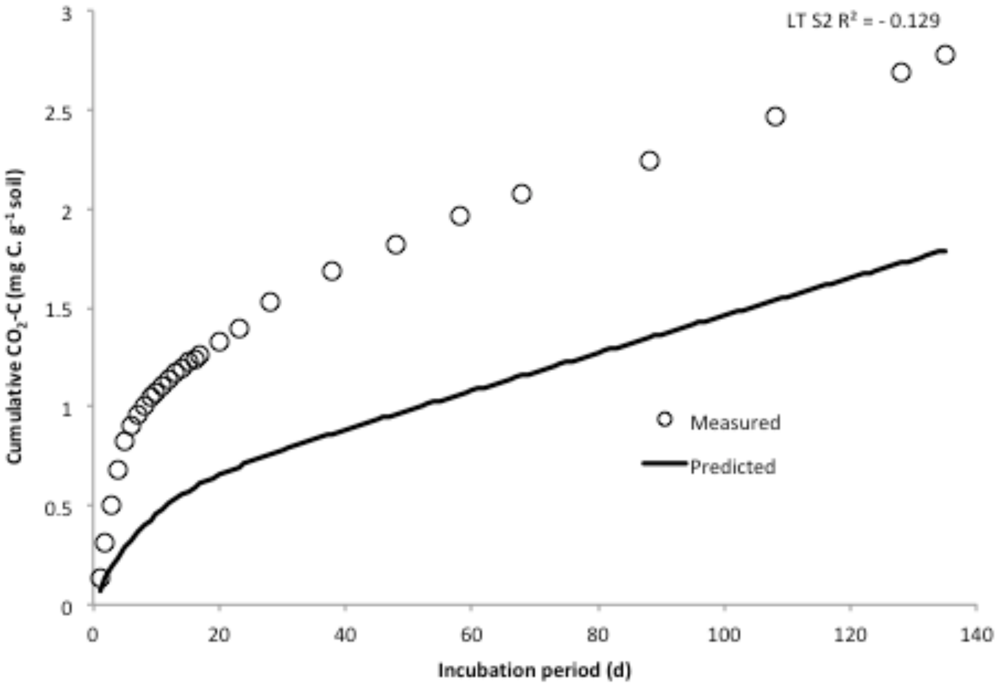


Figure 6.4 **The two compartment dynamic model (Eq. 6.2) fitted to the accumulated CO_2 emissions from the decomposition of freshly collected (control) soil amended with a fresh residue (LT shoot applied at 2 mg C. g^{-1} dry soil weight).**

A third compartment was therefore added to the model to reflect the fresh addition of plant residue. The rate constant for this compartment could not be satisfactorily controlled, over-estimating the CO_2-C emitted in the first 20 - 30 d of incubation and therefore the CO_2-C emitted by incubation end. It was decided that a fourth compartment was necessary to provide both a fast and slow decomposition constant for the fresh plant residue, noting that Pereira et al. (2017) also required a three compartment model to accurately describe the decomposition of fresh organic material added to soils.

6.2.4 Designing a four compartment decomposition model reflecting incorporation of fresh residues

This four compartment model was built to recognise that various components contribute to OM decomposition in soil, and that these different components have different decomposition rates. This model was built around the concept of a rumen based model, where digestabilty of plant material is key.

While the initial two compartment model provided for:

(i) Soil collected from the field which has a labile residue (A).
(ii) Soil collected from the field which also has persistent SOM (C).

This model takes the previous two compartments and adds two new compartments that effectively represent a fast and slow decomposition constant for fresh plant residue amendments (e.g. in this instance, fresh LT and WC shoots and roots). It recognises that:

(iii) Fresh plant residue will have components that decompose quickly, e.g. cellulose (B_f).
(iv) Fresh plant residue will also have components that decompose more slowly, e.g. lignin (B_s).

The model also runs on a daily time step based on incubation data as described above (Chapter 6.2.3).

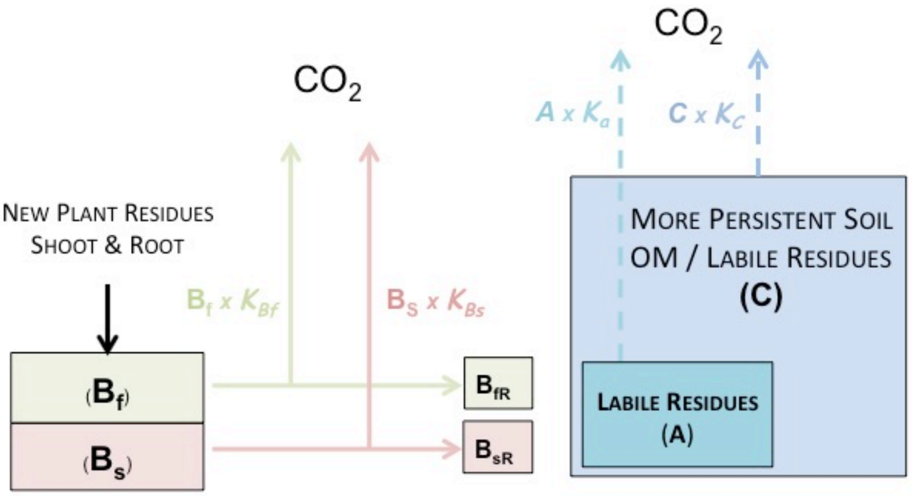


Figure 6.5 **Four compartment dynamic model developed to explain the behavior of fresh plant residue added to freshly collected soil, when incubated.**

<u>**The equation for this four compartment model is as follows:**</u>

$$\mathbf{E_n = (C_n \times K_C) + (A_n \times K_a) + (B_{fn} \times K_{Bf}) + (B_{sn} \times K_{Bs})} \qquad \textbf{Eq. 6.5}$$

Where: En= Total CO_2-C evolved on any day “n” (mg C. g^{-1} dry soil weight)
Where: n = 1 – 135 days.

The values for the decay constants K_a and K_c for the soil carbon pools A and C remained unchanged as in the two compartment model, simulating background CO_2 emissions from the soil alone. The decomposition constants (K_{Bf} and K_{Bs}) for the fresh plant material are related to the digestibility of the inputs. Low quality litter and dead roots will decay at a slower rate than more digestible inputs. This process of decomposition involves the utilisation of C to produce microbial biomass with an associated respiratory loss. For each plant species, the digestibility of both the root and shoot plant tissue is prescribed in the model, and the constants K_{Bf} and K_{Bs} change accordingly.

The fresh residue pools (B_f and B_s) were determined on any day (n) using the following formulas:

$$\mathbf{B_{fn} = B_{fn-1} - (B_{fn-1} \times K_{Bf})} \qquad \textbf{Eq. 6.6}$$

$$\mathbf{B_{sn} = B_{sn-1} - (B_{sn-1} \times K_{Bs})} \qquad \textbf{Eq. 6.7}$$

The decomposition constants K_{Bf} and K_{Bs} were estimated based on the best fit of the model to measured accumulated CO_2-C during the 135 d laboratory incubation of LT and WC shoot and root material. The model is illustrated in Figure 6.5 (see Chapter 6.3.1 for results). The fresh residue pools (B_f and B_s) decay independently of the soil pools and any undecayed residue (B_{fR} and B_{sR}) do not add to the soil pools C and A during the incubation experiment of n days. Of course on an annual basis they would.

A sensitivity analysis during the fitting procedure indicated that the constant K_{Bf} changes little in value between different types of residue, therefore the decision was made to hold K_{Bf} constant. The constant K_{Bf} was fixed at 0.14, and was assumed to be constant for all treatments irrespective of plant species or tissue form. The initial size (mg C. g^{-1} dry soil) of the fast decaying fresh residue pool (B_f at n=0) was variable and determined by least squares fitting of the model to the experimental data. The slow decaying pool size, B_s at n=0, was then determined as fresh plant C added – Bf (mg C. g^{-1} dry soil). The constant K_{Bs} was modified as required to obtain the best of fit the model to the measured CO_2-C data.

6.3 Results

6.3.1 A four compartment decomposition model reflecting incorporation of fresh residues

The decay rate of the fast (**A x K_a; B_{fn} x K_{Bf}**) and slow (**C x K_C; B_{sn} x K_{Bs}**) pools together provide a good fit of the soil plus residue model when fresh plant residues are added to the incubation chambers (Figure 6.6).

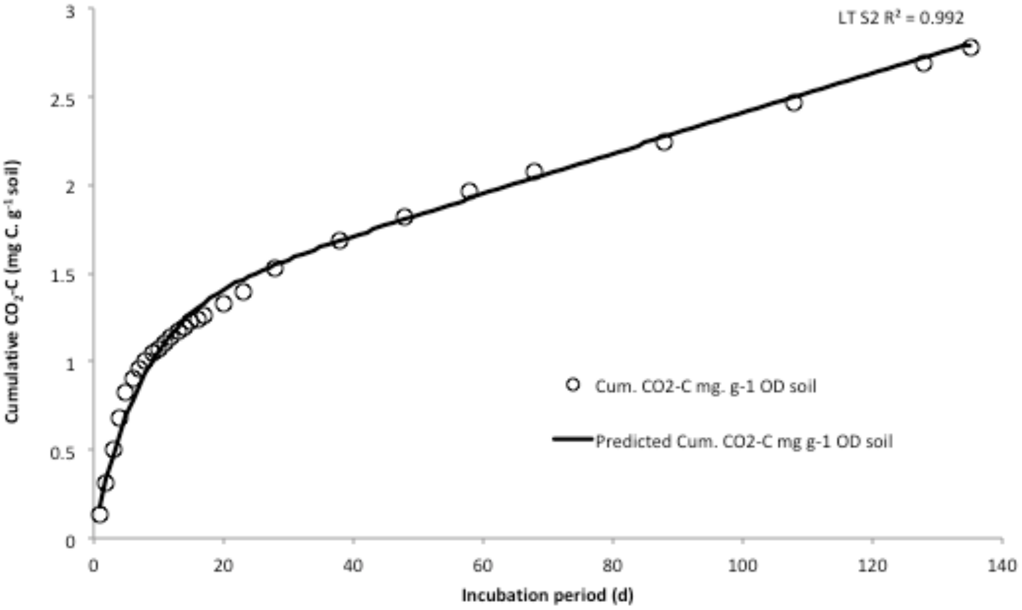


Figure 6.6 **The four compartment model fitted to the accumulated CO_2 emissions from the incubation of fresh Lotus shoot material, applied at 2 mg C. g^{-1} dry soil weight to freshly collected Tokomaru soil.**

By comparing Figures 6.3, 6.4 and 6.6 it is apparent that the persistent SOM (C) and labile soil C pools (A) contribute a large proportion (~ 58 %) of the CO_2-C lost over the incubation period, which is illustrated as the change in the soil and plant C pools in Figure 6.7. For example, the decay of fresh lotus shoot material (Bf + Bs) (Figure 6.6, 6.7) makes up the remaining 43 % of the CO_2 emitted. By holding K_{Bf} constant (Table 6.2 below), which controls the fast decay of the easily degraded plant material (Bf), then Bf is seen to vary for each plant material type ranging from 34 – 37 %, 54 – 57 %, 18 – 19 % and 9 – 12 % of the C added as fresh lotus shoot, white clover shoot, lotus root and white clover root material, respectively.

All decomposition models explained more than 97 % of the variation in the CO_2 emission data (Figure 6.8, Table 6.2). The simulated decay of each fresh C pool added and the soil C pools are illustrated in Figures 6.7 and 6.9. The remaining fresh carbon allocated to the slow fresh pool (B_s) had an apparent decay rate (K_{Bs}) with values ranging from 0.0015 – 0.0018, 100 fold slower than that set for the fast pool (K_{Bf} = 0.14). Whereas the fast pool (B_f) decomposed completely in approximately 40 days, approximately 80 % of the material in the slow pool (B_s) remained at the end of the incubation. This material would then contribute to a residue B_{sr} that would enter the slowly decomposing SOM pool C (Figure 6.5).

In both the fresh LT and WC shoot and root treatments the persistent SOM (C) pool contributes a large proportion of the CO_2-C lost over the incubation period when incubated at 2 mg C. g^{-1} dry soil weight in Tokomaru soil (Figure 6.9). However the proportion of C in the labile fraction (A, B) differs between fresh LT and WC shoot and root treatments. To fit the model to the shoot treatments, more C is partitioned into the shoot Bs pool (0.86 – 1.26 mg C. g^{-1} OD soil WC and LT); and less into the shoot Bf pool (0.74 – 1.14 mg C. g^{-1} OD soil LT and WC). Likewise, to fit the model to the root treatments, more C is partitioned into the root Bs pool (1.62 – 1.82 mg C. g^{-1} OD soil LT and WC) and less into the root Bf pool (0.18 – 0.38 mg C. g^{-1} OD soil WC and LT). For the fresh shoot treatments, more C is partitioned into the Bs pool for LT and into the Bf pool for WC compared to the other pools; whereas for the fresh root treatments, more C is partitioned into the Bs pool than the Bf pool for both WC and LT.

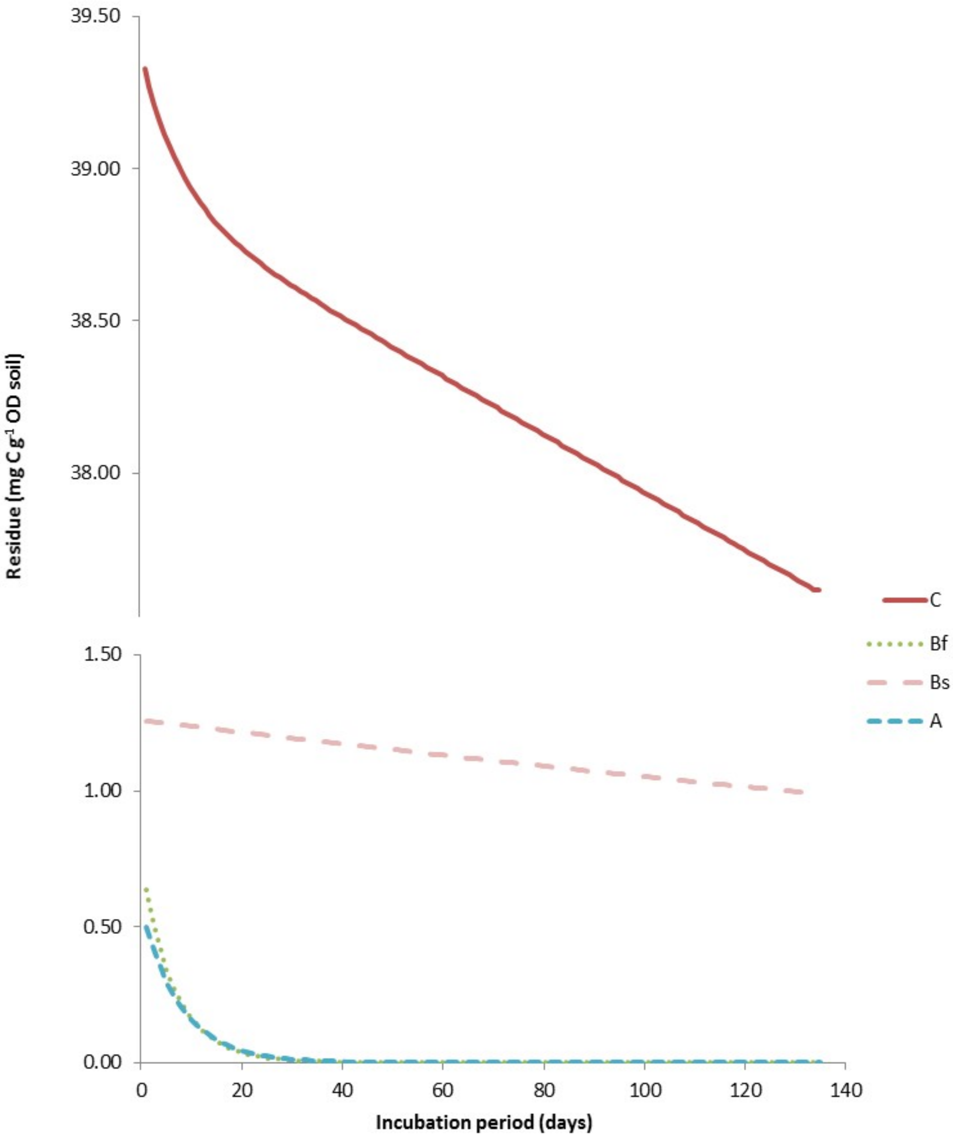


Figure 6.7 The fate of the soil C pools predicted by the four compartment model for the incubation of fresh LT shoot material, applied at 2 mg C. g^{-1} dry soil weight, in fresh Tokomaru soil.

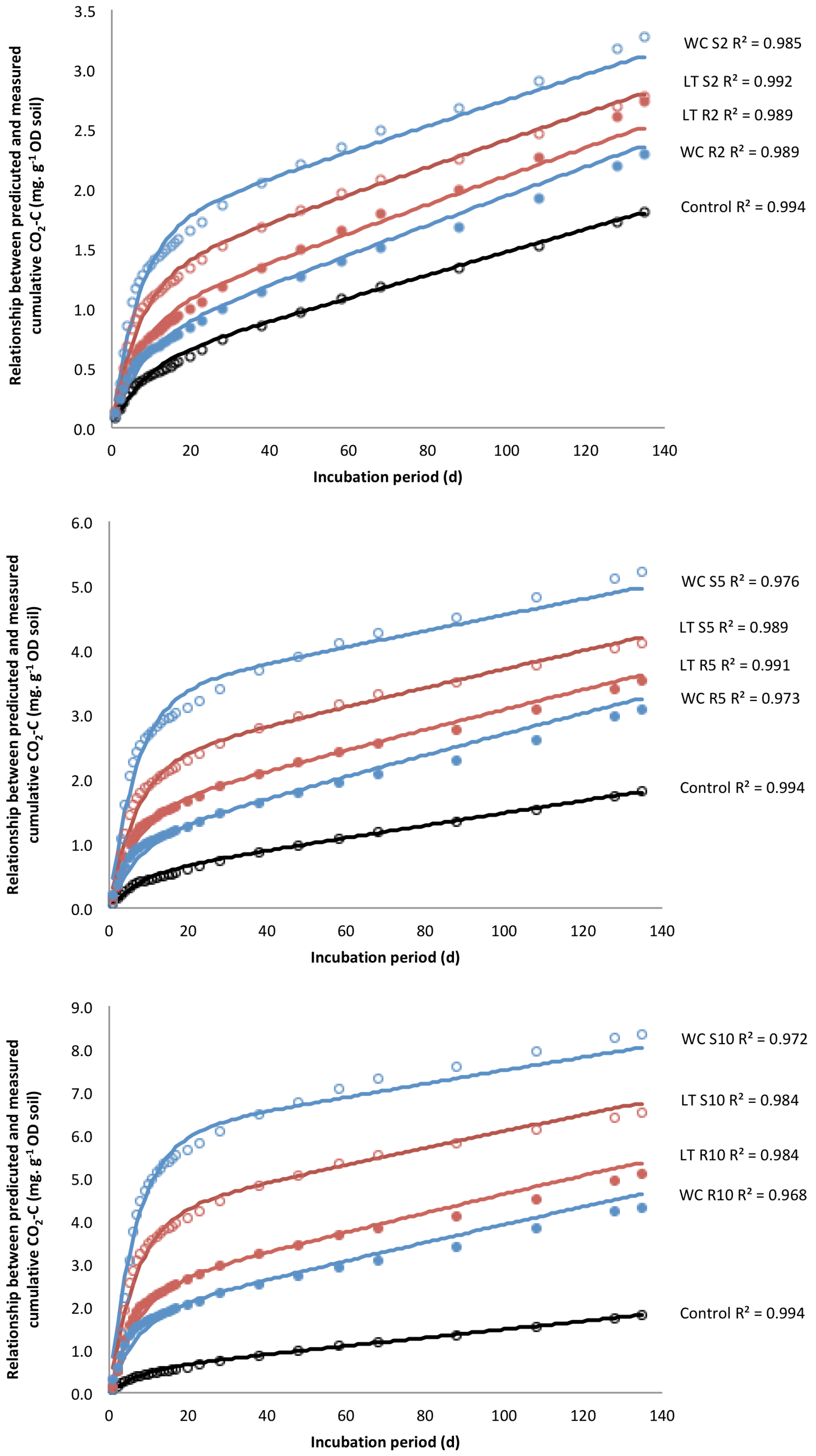


Figure 6.8 **The four compartment model (lines) fitted to the CO_2 emissions from the incubation of fresh Lotus (LT) and White Clover (WC) shoot (S) and root (R) plant material, incubated at 2 (*top*), 5 (*middle*) and 10 (*bottom*) mg C. g^{-1} dry soil weight (circle markers) in fresh Tokomaru soil. The R^2 values are presented for each model fit to the data.**

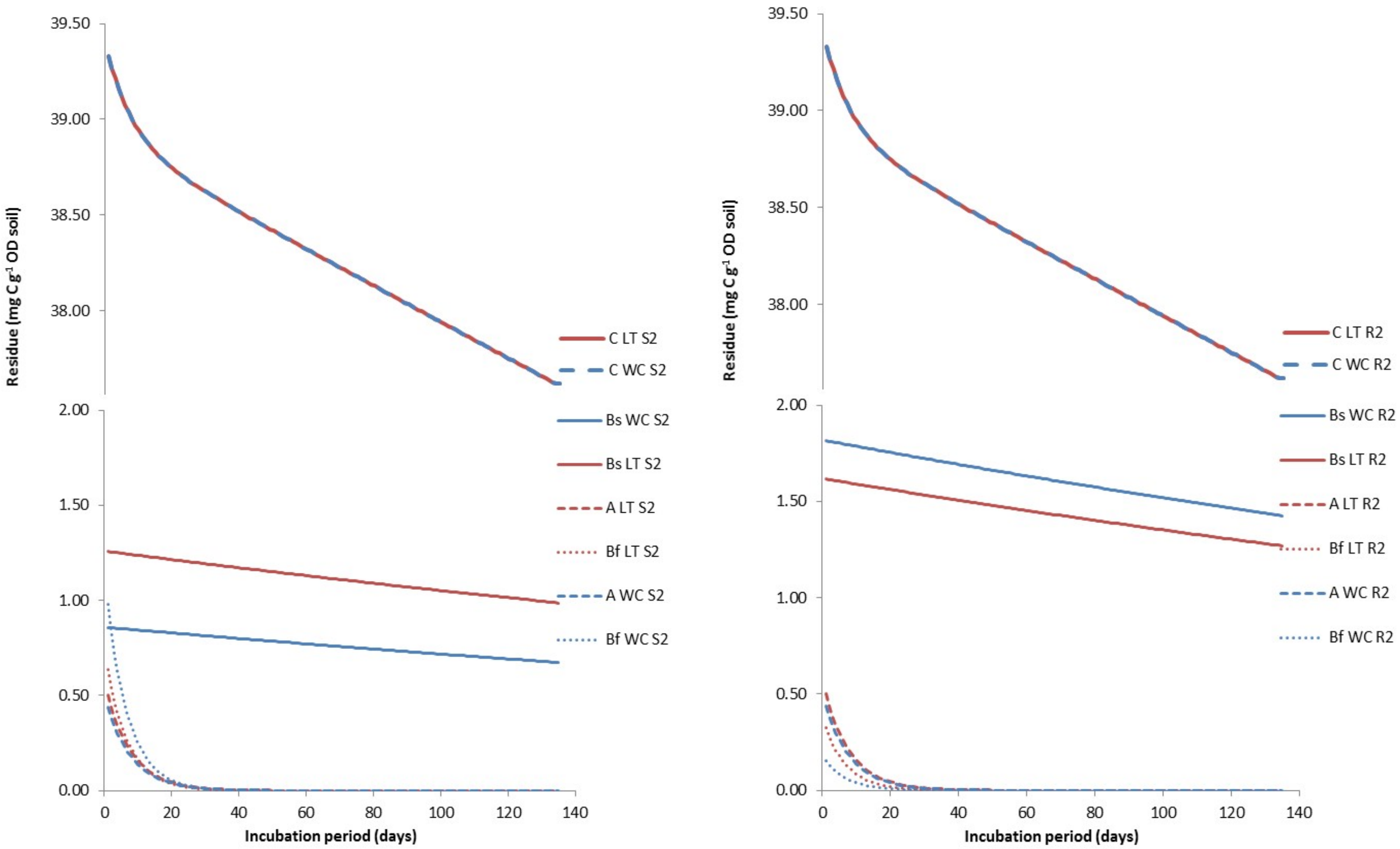


Figure 6.9 **The fate of the soil C pools predicted by the four compartment model for the incubation of fresh Lotus (LT) and White Clover (WC) shoot (S) (*left*) and root (R) (*right*) plant material, incubated at 2 mg C. g^{-1} dry soil weight in fresh Tokomaru soil.**

Table 6.2 Pools, constants and R^2 of LT and WC shoot and root material, incubated at 2, 5 and 10 mg C. g^{-1} dry soil weight.

	Large stable pool (mg C)	Amendment fast pool (mg C)	Amendment slow pool (mg C)	Labile soil pool (mg C. g^{-1} OD soil)	Fraction of labile soil pool evolved (mg CO_2-C. d^{-1})	Fraction of stable pool evolved (mg CO_2-C. d^{-1})	Fraction of fast pool evolved (mg CO_2-C. d^{-1})	Fraction of slow pool evolved (mg CO_2-C. d^{-1})	
	C	**B_f**	**B_s**	**A**	**K_a**	**K_C**	**K_{Bf}**	**K_{Bs}**	**R^2**
Control	39.40	-	-	0.50	0.12	0.00025	-	-	0.994
LT S2	39.40	0.74	1.26	0.50	0.12	0.00025	0.14	0.00180	0.992
LT S5	39.40	1.70	3.30	0.50	0.12	0.00025	0.14	0.00180	0.989
LT S10	39.40	3.55	6.45	0.50	0.12	0.00025	0.14	0.00180	0.984
WC S2	39.40	1.14	0.86	0.50	0.12	0.00025	0.14	0.00180	0.985
WC S5	39.40	2.78	2.23	0.50	0.12	0.00025	0.14	0.00150	0.976
WC S10	39.40	5.40	4.60	0.50	0.12	0.00025	0.14	0.00150	0.972
LT R2	39.40	0.38	1.62	0.50	0.12	0.00025	0.14	0.00180	0.989
LT R5	39.40	0.95	4.05	0.50	0.12	0.00025	0.14	0.00180	0.991
LT R10	39.40	1.81	8.19	0.50	0.12	0.00025	0.14	0.00180	0.984
WC R2	39.40	0.18	1.82	0.50	0.12	0.00025	0.14	0.00180	0.989
WC R5	39.40	0.50	4.51	0.50	0.12	0.00025	0.14	0.00180	0.973
WC R10	39.40	1.23	8.77	0.50	0.12	0.00025	0.14	0.00150	0.968

6.4 Discussion

6.4.1 A four compartment decomposition model

While the four compartment model fit designed here is really good at explaining the decomposition of added residues (R^2 > 0.968) there is room for improvement, especially over the period when peak CO_2 emission begin to decline (around T_{20}) and towards the second half of the incubation period (T_{70} - T_{135}) (Figure 6.8; Table 6.2). However, similar to the results presented in this thesis for lotus and clover shoots and root materials, Pereira et al. (2017) also found that the first 0 – 100 d defined the differences in the rate of decomposition of vetch, oats and clover residues. The creation of additional sets of data to validate the model would be useful, and this is something that should be considered in any related future work.

In 1985, Bosatta & Agren introduced the concept that decomposition is a continuum, with OM continually decreasing in quality (decomposability) as it decays. Jenkinson et al. (1990) discussed the mathematical complexity of such a model, as the amount of C in a soil (at steady-state conditions) then becomes the sum of the contribution from that year's input plus the remaining fraction from previous years' input. While Jenskinson (1990) makes the point that such a model would be incredibly difficult to validate, this concept does fit very nicely with the more recent theory of microbial turnover of C.

Every OM input has its own quality parameters, and as it undergoes decomposition there is not just a simple release of CO_2-C, but also the formation of secondary microbial decomposition products which are themselves decreased in quantity and quality. These products may undergo multiple stages of decomposition, becoming not only more microbially resistant themselves but eventually forming terminal decomposition products which, depending on the soil decomposition environment, may be chemically stabilised (Shen et al. 2018). In effect a microbial substrate is formed from the initial plant substrate and, as decomposition progresses (microbial succession), there is relatively far less of the original plant substrate and far more of the microbial substrate present, which is ultimately stabilised. It is known that biological, chemical and physical soil conditions impact on the fraction of organic residues that will remain in the soil system (Dungait et al. 2012a); by creating non-ideal conditions for microbial decomposition (Macias & Camps-Arbestain, 2010). However much is still to be learnt about the recalcitrance of soil organic matter fractions and the associations they form with mineral structures, when it comes to SOM and subsequent C sequestration (Rumpel & Chabbi, 2010). Recent studies suggest that the compounds that are being stabilised in soil and have the slowest decomposition rates are those that are microbially-derived during decomposition (Buurman et al. 2007; Suarez-Abelenda et al. 2011).

Whilst understanding and measurement of soil microbial populations and activities and the varied chemical and physical nature of SOM has improved, scientists have been unable to synthesise this knowledge into useful simulations of soil C stocks. As mentioned above most simulations of soil C stocks rely on more simple multicompartment models. The slow release of CO_2 from this successional decay of plant and microflora residues and minerally stabilised organic matter seems to be adequately modelled in this study by the allocation of fresh residue C to a slow pool (B_S) Figure 6. 5 with a slow decay rate (K_{BS},Table 6.2) and the slow decay of a large soil organic matter pool (C).

To be used in a predictive manner (not development and calibration as in this thesis) multi-compartmental decomposition models will require both physical and chemical composition of the incoming material to determine the allocation of C to fast or slow decomposing pools and the rate of decomposition. These models are more easily calibrated and validated against litter bag studies and laboratory or field incubations. However, this is time consuming and there could be real value in developing a model, where the C allocation can be predicted from physical and chemical analysis of the residue entering the soil.

6.4.2 Indices useful to partitioning plant material C into different decomposition pools.

There was a large difference in the total amount of CO_2-C emitted from each of the plant material decomposition treatments, similar to that found by Pereira et al. 2017. In this thesis it was found that the "fitted" proportion of fresh residue C that ended up in the slow fraction (B_s) was strongly related to the measured ME, lignin and Mn content of the initial plant material ($R^2 > 0.88$) (Figure 6.10). The higher the amount of ME present, the lower amount of C that was proportioned to the B_s pool. Conversely (and logically), the lower the amount of lignin and Mn present, the lower the amount of C allocated to that same slow pool (inverse relationships). In other words, there was a strong relationship between these plant chemical traits and the proportion of C into the labile and less labile fractions. There was little relationship between condensed tannin (CT) concentration and the allocation of C into the slow decay pool .

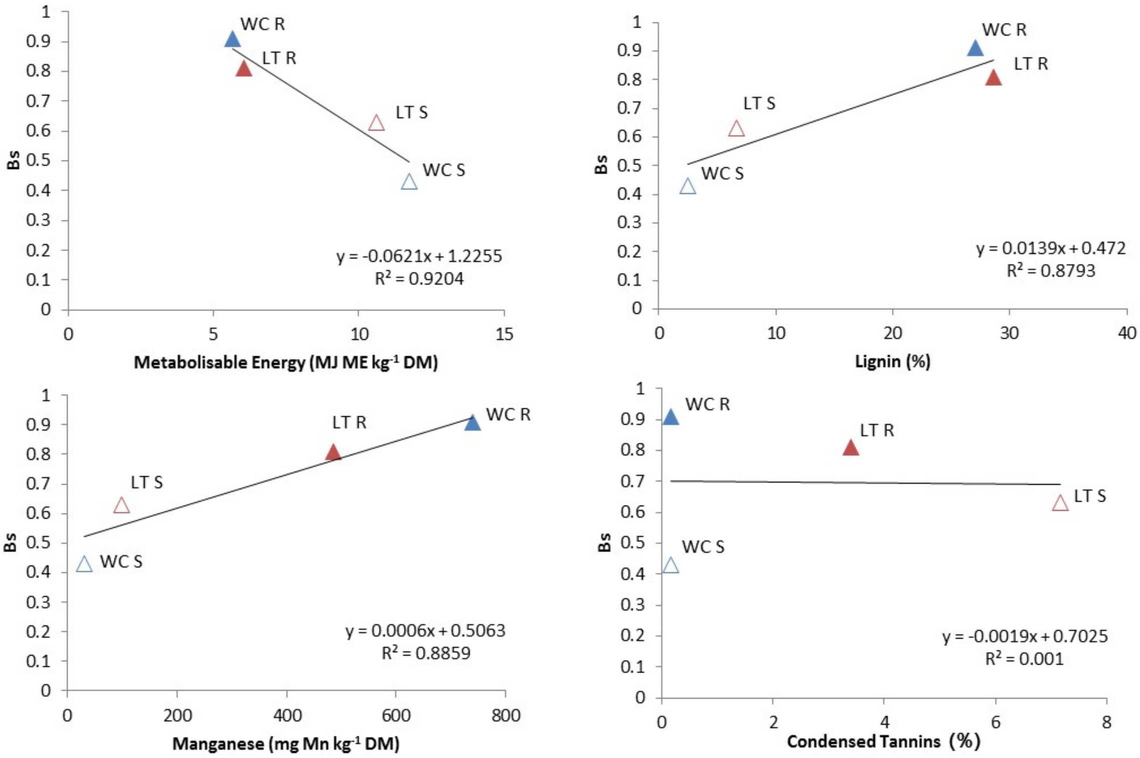


Figure 6.10 **Relationship between B_s and plant chemical attributes, ME, lignin, Mn and CT. Each data point represented is the average of data for 2, 5, 10 mg C. g^{-1} dry soil weight.**

Metabolisable energy is one form of plant analysis that is very common in New Zealand agricultural systems, as it is recognised as a key factor in driving farm system models and forms the basis of animal growth and milk production. Metabolisable energy is also particularly quick and easy to measure using VIS-NIR, making it an ideal candidate to be used as a quick test for more complex decomposition processes. The model fit is so strong that there is certain value in carrying out additional studies considering the use of ME to predict the proportion of fast and slow litter pools in this (or similar) models. Additional data would allow the model fit to be refined. These findings should be of particular interest to those running more complex models, especially given the ease of measurement.

Litter decay rates are often correlated with more labile compounds (e.g. N, cellulose), suggesting that interactions between lignin and more labile compounds is an important controlling factor over litter decomposition (see extended discussion in Chapter 2.2.6 and references therein). A strong relationship between lignin concentration and C allocation to a slow decaying pool is therefore to be expected (Figure 6.10). Moretto et al. (2001) discovered a significant negative correlation between C:N ratio and mass lost; as well as significant correlations between mass lost and lignin, N, P, lignin:N and lignin:P contents, for both leaf and root material. Johnson et al. (2007) showed that materials with a low C:N ratio or a low lignin:N ratio (e.g. alfafa) decomposed at a faster rate than organic material with higher ratios. Conversely, a study by Ghidey & Alberts (1993), failed to link a relatively lower C:N ratio in the corn roots to the speed of decomposition. The soybean roots in their study (substantially higher C:N ratio) decomposed faster than the corn roots. They also measured faster decay rates for buried shoot residue (corn and soybean) than for root material over both years of the 2-year study.

It is unclear why an increase in Mn concentration in the plant tissue is associated with partitioning more plant C into the slow Bs pool. In contrast, Mn concentration has more recently become known as an important factor in decomposition. It has a positive influence on lignin degradation, with results suggesting that higher Mn availability may promote fungal communities that are better adapted to decompose lignin. There is also a direct correlation between Mn concentration and litter decay rate; as Mn is a key part of the enzyme manganese peroxidase, used by white-rot fungi during lignin decomposition (Hofrichter, 2002; Hammel, 1997; Perez & Jeffries, 1992). Manganese may also promote lignin degradation by stabilizing and enhancing production of the lignin-degrading enzymes (Perez & Jeffries, 1992); therefore directly influencing the quantity and the quality of DOC release (Trum et al. 2015).

This role of Mn in decomposition has been particularly well documented in leaf litter studies across a variety of forest ecosystems; Berg et al. (1996) were the first researchers to show a positive correlation between the bioavailability, mobility and reactivity of Mn and litter decomposition rates. A subsequent detailed chemical imaging study of plant litter revealed that soil fungi redistribute Mn from fresh plant litter as reactive Mn at sites of active litter decomposition (Keiluweit et al., 2015). The formation of this reactive Mn directly correlates to oxidation byproducts of aromatic organic compounds. In other words, Mn concentration has a positive influence on lignin degradation, therefore further investigation is required before its role in the decomposition of plant materials containing higher Mn concentrations is clear.

6.5 Conclusions

Previous studies, reported in Chapters 3 to 5, have shown that plant species and management can have a large impact on CO_2-C evolved from a soil during incubation. The introduction of fresh shoot material to the incubation soil at the start of the study in Chapter 3, and likewise the shoot and root material added to the incubation in Chapter 5, are anticipated to have influenced the organic C cycling throughout the incubation period, and therefore the SOM chemical signature. CO_2 emission results from the shoot incubation experiment reported in Chapter 3 clearly show incomplete decomposition of the added plant C, particularly for *Lotus pedunculatus*. At the end of the incubation, careful sectioning of the soil zone to which the plant material had been added and total C analysis failed to show significant increases in soil C residues because of the large pool of existing soil C and high variation in its measurement.

Therefore it is important to be able to predict the fate of the added plant C. In this respect, the four compartment model developed provided a prediction of the decomposition of existing SOM and fresh plant material added to a soil incubation at differing rates. Validation is a key part of model integrity, and this model would certainly be improved by validation against additional sets of data. However further experimental work was outside of the time limit of the studies reported here.

There could be real value in developing a model, which incorporates a secondary microbial pool of C, particularly when considering the use of incubations as a technique to develop models which predict C behavior in soil over short term periods.

Plant chemical traits (ME, lignin, Mn) can be used in plant residue decomposition models to predict how different plant materials decay and release CO_2 from soil incubations. Similarly these measures may prove useful to determine model predictions of CO_2-C loss under pastoral soil management. In particular, there would be real value to be gained in considering the use of ME as a model predictor, especially considering the ready availability and relatively cheap cost of ME measurement.

Chapter 7

Conclusions and implications of research

7.1 Conclusions of thesis

Manipulation of soil C stocks may be the key to mitigating global climate change. Soil C stocks are known to contain roughly twice the amount of C than is currently held in the atmosphere (approximately 1450 Pg C) (Schimel, 1995); which means that even a small change, globally, in soil C sequestered can lead to a significant decrease (or increase) in atmospheric CO_2-C concentration. A review of the literature was undertaken to identify research gaps in CO_2 sequestration under temperate, grazed pasture systems. Current understanding about the influence of different plant species on C flows for legume-based hill pastures is scarce even though it represents more than 50 % of New Zealand's terrestrial ecosystem. This review suggested that there was potential to manipulate the C cycle in these pastoral systems, provided there was a solid understanding of the drivers that control the amount of C stored in those soils, and the fluxes of C in and out of the soil.

It is known that the amount of C in the soil system, at any point in time, is a direct result of the relative rate of C input versus the rate of C output. Increasing the rate of C input to the soil or increasing the longevity of C already in that soil may therefore lead to an outcome where the overall amount of soil C sequestered within the system is increased. This review indicated that the longevity of C could be manipulated by altering plant litter type and soil processes, and consequently total microbial biomass and community structure. From this it was surmised that there might be potential to exploit changes in plant traits or management of pastoral species to increase C longevity in the soil.

The research presented in this thesis aimed to quantify short term C loss as a result of pasture residue decomposition, identify management strategies that might conserve and perhaps increase soil C in pasture soils, and attempted to provide a simple decomposition simulation model to predict C loss from pasture soils. A glasshouse trial and a series of laboratory incubations were conducted as part of the study.

The glasshouse study was run for a period of two years to generate both shoot and root material of LT and WC. This plant tissue was chemically characterised, and formed the basis of the incubation decomposition studies conducted within the parameters of this thesis. It was clear from the incubation studies that the chemical differences in the WC and LT plant material had a direct influence on the rate of decomposition and the amount of residue C remaining after the incubation period. Plant shoot and root tissue type and chemistry also influenced the rate of decomposition and the amount of residue C remaining after a period of decomposition. Laboratory incubation techniques – method and rate of C application – also influenced the amount of residue C remaining after a period of decomposition.

7.2 Implications for C sequestration in soil

Within the parameters of the incubation described in Chapter 3, plant species (LT and WC) was seen to acount for 85 % of the variation in the rate of CO_2-C evolved from TK soil within the first 10 d of incubation. This early effect meant that there was a significant difference at incubation end (T_{125}). Despite different application techniques to the soil, LT shoot material released significantly less CO_2-C than that of WC, resulting in a higher amount retained in the soil. These results were consistent with the hypothesis that the high tannin and lignin content of LT compared to WC would influence the rate of decomposition in the soil, and prompted further investigation into the root material of the same species.

The results from the method of application of shoot litter to the incubation were consistent with other published reports; the conclusion from those being that moist plant material decomposes substantially faster than dry material, provided all other things are equal. Therefore it is fair to surmise that incubation studies which use pre-dried, ground plant material, particularly for ease of study, need to be aware that the results cannot be directly extrapolated to the field.

Chapter 4 simulated LT and WC decomposition in disturbed (D) and undisturbed (U) conditions in constrasting soil types, Pallic (TK) and Allophanic (EGT) soils. There was greater unexplained variation in the results of Chapter 4, compared to that of Chapter 3, as a result of the incubation design – with the root weight within each incubation chamber only an estimate of what was added due to the in situ nature of the incubation study. Overall WC roots were seen to decompose less over 157 d than LT roots, which was in contrast to the results from the shoot material in Chapter 3. Disturbance was seen to have an important effect in the early stages of decomposition (T_0 – T_{10}). All root incorporated in the soil slices was already dead, sprayed off by Roundup prior to incubation, so the disturbance effect seen was one of substrate access. Previous research by other authors commented on the fundamentally different physiological functions of roots versus shoots, resulting in different chemical composition and thus decomposition rates, so these results were not entirely unexpected. The key limitation with this study was the estimated amount of root C within each incubation chamber, which made the results difficult to interpret. While Chapter 3 made it clear that using freeze dried material can underestimate the amount of C that would be lost from fresh material in the field, Chapter 4 made it clear that the use of “fresh” roots in-situ can lead to uncertainities in estimation of total C present, and therefore limit understanding of the mechanisms at play.

The incubation study in Chapter 5 was designed to allow a comparison between the decomposition of LT and WC shoots and roots, to test any confounding factors arising from the rate of addition. The plant material in this chapter was added as freeze dried shoot and root material, at rates to simulate both residual shoot material but also the, potentially far greater, residual root material. The statistical analysis of the CO_2-C emitted during this incubation clearly showed that rate of application, plant part, and plant species (in that order) were the most significant factors influencing the decomposition rate. More C was conserved as undecomposed residue as the rate of C application increased, which was consistent with other published studies.

For both species considered, the degree of decomposition and emission of CO_2-C from root material was significantly lower than that of shoot material. By the end of the decomposition period, root material left between 54 and 75 % undecomposed residue in the soil, compared to 26 – 64 % of the shoot material. This was in line with other published studies which surmised this difference was due to the variation in chemical and structural traits between plant parts, as well as across species. As was also the case in Chapter 3, LT shoot material released significantly less CO_2-C than WC; and likewise with Chapter 4, WC root material released significantly less CO_2-C than LT. The importance of litter quality is well documented. Differences in the decomposition rate among the species LT and WC, despite the same growth and decomposition conditions, suggests that the composition of these species is what is influencing decomposition. Differing litter composition between shoot and root also influenced decomposition.

Across the three incubation chapters it was clearly demonstrated that plant species, plant part, soil type, method of application and rate of application all influence decomposition rates and total C sequestration – and that most of the influence comes from the early stages (around the first 10 d) of the incubation. Practically speaking, it would be immensely useful to be able to run a series of short incubations and use the results from these to predict long term decomposition outcomes. Other studies have shown that, although the decomposition of plant material in soils is extremely complex, relatively simple mathematical models can be used to describe the decomposition process. The results here highlight that particular attention should be paid to the relationship between the initial litter chemistry and the rate of decomposition, in order to predict the effect on soil C stocks.

Within Chapter 6, a two compartment model was created and fitted to the cumulative CO_2-C emitted during the incubation of freshly collected (control) TK soil. While the model was a very good fit for the control soil, it did not satisfactorily fit the decomposition phase in instances where a plant residue was incorporated. A third compartment was added to the model to reflect the fresh addition of plant residue. However the rate constant for this compartment over estimated the CO_2-C emitted, so a fourth compartment was added to provide both a fast and a slow decomposition constant for the fresh plant residue.

The four compartment model was designed around the concept of a rumen based model, where digestibility of plant material is key, and was built to recognise that various components contribute to OM decomposition in soil, and that these different components have different decomposition rates. There was a strong relationship ($R^2 > 0.88$) between plant traits (ME, lignin, Mn) in the initial plant material and subsequent decomposition. The proportion of C that ended up in the slow fraction of the model (Bs) was therefore based on this relationship. There was little relationship between CT concentrations and C fractions so these were not explored in the model.

Farming systems are forever evolving. Although many farmers are proactive when it comes to best management practices, they are ultimately very practical and there is always going to be a cost benefit analysis when it comes to implementing new technology and systems on-farm. The cost associated with increasing soil C can be high (Lam et al. 2013; Kirkby et al. 2011; Carlyle et al. 2010), and large increases in soil C are generally only detectable on a long timeframe (25 + years). On a purely C sequestration basis, a farmer may not see ample justification for maintaining high levels of SOM.

In 2015, Meyer et al. put forward the argument that, although C sequestration in soils is limited in its use as a mitigation strategy, the ecosystem benefits of SOM are evident - increased CEC and nutrient supply; increased soil structure, porosity and plant available WHC; and overall increased crop yields. They used a whole-farm system model to simulate pastures with high and low SOM across two climatic zones and two soil types; and estimated that, across the different sites, the increased pasture production associated with higher SOM was valued between AUD \$26 and \$95 ha^{-1}. Their results indicate substantial on-farm benefits from increased SOM and subsequent soil C sequestration, which would certainly tip the scales in favour of maintaining high levels of SOM on-farm.

Soil decomposition studies by themselves are time consuming, costly, and can be narrow in scope; limiting their usefulness as an on-farm tool. Soil models, on the other hand, may allow a quick snapshot into how a farming system might react to a change in management; in this case to either an increase in the quantity of C inputs to soil, or to a manipulation of the drivers of loss (i.e. the parameters of soil decomposition). They lend themselves to the development of an on-farm tool where farmers can input data on proposed changes to the system and, based on set parameters relating to the on-farm conditions (temperature, precipitation, soil type, pasture composition, etc.), receive a snapshot of how the system might react to such a change.

7.3 Future research potential

The research work described in this thesis was ultimately constrained by budget and the time period of study for a PhD thesis. However, there are a number of decomposition parameters that require further investigation, and consideration should be give to the following areas:

Incubation Studies

The decomposition studies herein used two soils (primarily one), both of which were silt loam soils; and used only legume pasture residues. Further studies using residues from other common pasture species and a wider range of soil types / textures may be considered as a means to understanding the decomposition pattern of different residues in different soil types in New Zealand. In particular, plant species that are of interest in current GHG reduction studies (such as plantain and brassicas) should be studied for their rate of decomposition (NZAGRC. 2022; Podolyan et al. 2020; de Klein et al. 2019).

It is very clear from the incubations conducted herein that there is continued scope to pursue pasture plants that will leave residues that are slow to decompose in soil, therefore increasing the equilibrium concentration of CO_2-C within the pasture soils. There is real potential for this to be a significant and effective GHG mitigation technique; as well as of practical advantage to farmers, as continued sequestration of C within their soils ultimately increases the quality of that soil.

The slow decay of white clover roots may be the reason why New Zealand legume based pastures have relatively high SOC. A move back to clover based N fixation rather than urea use on grass dominant pastures should be investigated as a pathway to greater C sequestration. A survey of soil C stocks on continuous clover-dominant pastures on System 1 dairy farms (DairyNZ. 2022a), could be initiated to determine if clover dominance leads to greater soil C stocks.

Hill country grasslands, both high and low fertility, account for a large area of New Zealand hill country that could potentially be used to sequester more C into. While New Zealand grassland topsoils are relatively close to the C saturation index, there is room to increase C concentrations in the subsoil of these soils. The concept of soil C saturation is that once the topsoil is enriched in C, new C inputs can no longer be protected and decomposition will speed up. Soil C saturation may only affect SOC absorbed onto soil mineral surfaces, or where soil aggregates form around it. The incubation studies reported in this thesis indicate that both WC root and LT high in lignin and low in ME leave greater residual C in soil after decomposition. The correlation between plant analysis and C decomposition rates suggests that the slow pool seen in the decomposition model developed is likely to be lignified material. Residues of lignified material may accumulate in soils despite the pool of mineral protected organic matter being saturated. Further plant residue decomposition studies could focus on soils that are close to apparent saturation and soils that are not. In the short term, lignified material may stay as lignin as it is stabilised by aggregation (as opposed to being chemically stabilised).

In order to accelerate soil C sequestration though pasture management in New Zealand there is a need for studies that will provide evidence that such change is possible. Long-term soil fertility trials, such as those conducted at the Ballantrae Hill Country Research Station have produced nearly 40 year old high fertility ryegrass-white clover pastures with high ME, maintaining high sheep stocking rates and low fertility browntop pastures with low ME, maintaining low sheep stocking rates. Soil samples taken from these trials should be subjected to soil C classification and decomposition studies to examine how pasture management may influence gain or loss of soil C stocks.

Modelling

While the four component model fit is really good at explaining the decomposition of added residues ($R^2 > 0.968$), there is still room for improvement, especially over the period when peak CO_2-C emission begins to decline. Although out of the scope of the work here, the creation of additional sets of data to validate the model would be useful, and should be considered in any related future work. There is increasing research evidence that points to minerally protected soil C as mainly seconday decomposition products resulting from bacterial and fungal decomposition. Therefore there could be real value in developing a model which incorporates a secondary microbial pool of C; particularly when considering the use of incubations as a technique to develop models which predict C behaviour in soil over short term periods.

Plant chemical traits (ME, lignin, Mn) could be used to determine model predictions of CO_2-C loss under pastoral soil management. In particular, there would be real value to be gained in considering the use of ME as a model predictor, especially considering the ready availability and relatively cheap cost of ME measurement. Metabolisable energy is one form of plant analysis that is very common in New Zealand agricultural systems, making it an ideal candidate to be used as a quick test for more complex decomposition processes. The model fit is so strong that there is certain value in carrying out additional studies considering the use of ME to predict the proportion of fast and slow litter pools in this (or similar) models. Incubation studies which focus on plant materials that vary in ME and lignin, particularly catch crops, would be a useful research addition. This may include, but is not limited to, oats, vetch, beans, mustard, and other cover crops (Table 7.1). Additional data would allow the model fit to be refined. These findings should be of particular interest to those running more complex models, especially given the ease of measurement.

Table 7.1 **Metabolisable energy of oats, faba beans, fodder beet, plantain, soya beans and vetch (ME. kg^{-1} DM).**

Species	Metabolisable Energy (ME. kg^{-1} DM)	Source
Oats	11.5	DairyNZ. 2022b
Faba beans	8.0 - 8.7	Kleinmans et al. 2019
Faba beans	11.8	FAR. 2012
Fodder beet	12.0 - 12.5	DairyNZ. 2022b
Plantain	11 - 12	DairyNZ. 2022b
Soya beans	12 - 12.9	DairyNZ. 2022b
Vetch	6.8 - 12.4	Matic, R. 2007

When hill country pastures are fertilised the result is a decrease in the prevalence of low-fertility species like browntop and increased prevalence of higher fertility species like white clover and ryegrass. Results obtained in this thesis indicate that shoot material with high ME and low lignin content will decomposes more completely leading to lower soil C pools, but shoot and root material with high lignin content and low ME may decompose more slowly leading to increased soil C. A complex model of pasture plant type, grazing animal behaviour and excreta quantity and quality would be required to explain what happens to the soil C as management changes low fertility to high fertility systems (or vice versa). To create this model, experiments would need to be set up to test the effect of plant traits like ME and lignin on both plant residue and excreta decomposition rates.

Any change in the management strategies on-farm will always have a flow on effect throughout the farming system, and an impact on the amount of CO_2-C sequestered in that soil system. The C stored below New Zealand farms is an incredibly valuable resource, both from a GHG mitigation perspective and a soil health perspective. There exists a huge potential for on-farm tools to be developed to enable farmers to manage this finite resource. This thesis is just one more step in that process.

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